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Review

Long-term effects and significant adverse drug reactions (ADRs) associated with the use of gonadotropin-releasing hormone analogs (GnRHa) for central precocious puberty: a brief review of literature

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Summary. Central precocious puberty (CPP) is defined as an early pubertal development that occurs before the age of 9 years in boys and 8 years in girls. It results from premature activation of the hypothalamic-pituitary-gonadal axis. Gonadotropin-releasing hormone agonists (GnRHa) have been the gold standard therapy for CPP for more than 30 years. These compounds have a high affinity for the pituitary LHRH receptor and are resistant to enzymatic degradation. Through continuous stimulation, GnRHa inhibit the pulsatile secretion of gonadotropin, resulting in hormonal suppression, cessation of pubertal development, and normalization of growth and skeletal maturation rates. The goal of therapy is to halt pubertal progression and delay epiphyseal maturation that leads to improvement of final adult height. There are no widely accepted guidelines for how long to continue treatment with a GnRHa for CPP, and individual practice varies widely. Furthermore, conflicting results have been published on the long-term effects of GnRHa therapy in patients with CPP. Therefore, we reviewed the current literature focusing our attention on the long-term effects and the significant adverse drug reactions (ADRs) observed during treatment with GnRHa in patients with CPP. Our review may provide the necessary data to enable clinicians to administer GnRHa in the safest and most appropriate way. Further studies are necessary to identify the mechanisms of development of potential adverse drug reactions related to GnRHa therapy in CPP. (www.actabiomedica.it)

Key words: precocious puberty, gonadotropin-releasing hormone analogs, long-term effects, significant adverse drug reactions (ADRs), Hartwig and Siegel severity scale

Introduction

Precocious puberty (PP) is one of the most common reasons for referral to pediatric endocrinologists. PP is defined as the development of secondary sexual characteristics before the age of 8 years in females and 9 years in males (1-3). The overall incidence of sexual precocity is estimated to be 1:5,000 to 1:10,000, with the female-to-male ratio being approximately 10:1 (1).

Central precocious puberty (CPP) results from premature activation of the hypothalamic-pituitary-gonadal (HPG) axis) unlike peripheral precocious puberty, where the HPG axis is not involved.

Although the precise mechanisms triggering the onset of puberty are unclear, the earliest known biochemical change during puberty is increased production of kisspeptin produced by arcuate nucleus and anteroventral periventricular area of the hypothalamus.

This step is critical to puberty initiation. Neurokinin B and dynorphin from the same neurons stimulate and inhibit the release of kisspeptin respectively, and hence these kisspeptin, neurokinin and dynorphin neurons have now been recognized to be central to puberty initiation (1-4).

In females, CPP more frequently is idiopathic while in boys is more likely to be due to a pathological source (1-3). Risk factors for CPP include a history of international adoption, as well as congenital or acquired CNS insults. Several genetic syndromes are associated with CPP (4).

Apart from recognized genetic syndromes, from 5.2% to 27.5 % of cases have been reported to be familial and segregation analysis has suggested an autosomal dominant transmission with incomplete sex-dependent penetrance (4,5). Currently, mutations in the kisspeptin system, MKRN3, and DLK1 have been identified in sporadic and familial cases of CPP. In familial CPP, *MKRN3* defects were found in about 30% of families while in patients with apparently sporadic CPP, *MKRN3* defects were detected in about 8% of cases. In theses cases, genetic counselling should be considered in affected patients and their families (6).

The earliest clinical manifestation of central puberty in girls is usually breast development (thelarche), followed by pubic hair (pubarche). The pubertal growth spurt typically occurs during Tanner stage II-III, with the first menstrual period usually occurring at Tanner stage IV. In boys, the initial clinical sign of central puberty is testicular enlargement and the pubertal growth spurt happens later than in girls (7).

Gonadotropin-releasing hormone analogs (GnRHas) are the treatment of choice for children with CPP. Treatment aims to halt physical maturation, to prevent an early menarche, to retard skeletal maturation, to improve final adult height, to avoid psychosocial/behavioural sequelae, and to relieve the parents of the associated anxiety (8-11).

Good predictors of height outcomes include younger chronological age (CA), younger bone age (BA), greater height standard deviation score for CA at initiation of therapy (11-14) and a higher predicted adult height using Bayley-Pinneau tables (15). A suppression of luteinizing hormone (LH) to < 3 mIU/mL in patients on GnRHa therapy may be a reasonable target in patients on GnRHa therapy (16).

Although GnRHa therapy appears to be both well tolerated and effective in pediatric patients; there are no widely accepted guidelines for how long to continue treatment with a GnRHa for CPP. Individual practice varies widely among endocrinologists. Furthermore, conflicting results have been published on the long-term effects of GnRHa therapy in patients with CPP. These included a higher incidence of polycystic ovary syndrome (PCOs), changes in body composition, metabolic profiles and bone mineral density (16-21). Moreover, short term side effects such as headaches, hot flushes, mood swings and injection site reactions (rashes, bruising and sterile abscess formation) have been reported in the literature.

Therefore, we reviewed the current literature focusing on the long-term effects and the significant adverse drug reactions (ADRs) observed during treatment with GnRHa in patients with CPP. As long-term studies of male CPP patients are scarce, this review mainly addresses female CPP patients.

Gonadotropin releasing-hormone analogs (GnRHa)

First synthesized in 1980, GnRHa desensitize and down-regulate GnRH-receptors, suppress gonadotropin secretion, and eventually reduce gonadal hormones to pre-pubertal levels (9,11,22,23).

Basically, the native GnRH molecule is modified at least at the glycine 6 position, where it is substituted by another amino acid resulting in a super-agonistic effect. Prolonged exposure of the pituitary to a GnRHa paradoxically results in inhibition of gonadotropin secretion.

In 1986, the first long-term study of daily GnRHa treatment in 27 children (21 female and 6 male), treated for 2-4 years, showed a reduction of growth velocity to pre-pubertal levels, improved the advancement of skeletal maturation, and increased the predicted adult height (PAH) (24,25).

GnRHa are available as rapid-acting or long-term depot preparations. The long-acting preparations available include: leuprolide, triptorelin and goserelin,

given every 3-4weeks or as a long-acting depot at 10 to 12-weekly intervals. The monthly (leuprolide 3.75 mg or triptorelin 3.75 mg) or 3-month depot leuprolide 11.25 mg are the most common formulation used to treat CPP as they cause a steady release of the drug without relevant side effects (1-4,8).

All these preparations are synthetic analogues of naturally occurring gonadotropin releasing hormone (GnRH) which possess greater potency than the natural hormone.

All depot preparations are available as lyophilized powder along with separate reconstituting fluid in a composite syringe. It is important to inject the preparation immediately after re-constitution, to avoid solidification and injection failure. Injection should always be administered deep intramuscularly, preferably in the gluteal region.

In the United States a histrelin implant that causes pubertal suppression for more than a year has been approved and successfully used. While short-acting intranasal preparations such as nafarelin are available for daily administration, these are less efficient and there are significant difficulties with compliance, which limit their use substantially as a first-line treatment.

In Europe, triptorelin depot is widely used at 28-day intervals, even though some authors have reported shorter frequency intervals of administration (21- 26 days). It is usually administered at a dose of 3.75 mg (approximately 60-75 μg/kg) for children weighting more than 20 kg; and a half dose has been employed in patients weighting less than 20 kg. Some authors have used higher doses (100-120 μg/kg/21-25 days) (26).

Leuprolide depot is used at different doses in Europe (3.75 mg/28 days) and in the USA (7.5- 15 mg/28 days) (21,27). The dose of leuprolide required for gonadal suppression is unclear, with higher doses employed in the USA (7.5 mg monthly) compared to European countries (3.75 mg monthly). This was addressed in a trial comparing the effect of 7.5 mg leuprolide monthly against 11.25 mg and 22.5 mg, 3-monthly, in girls with gonadotropin dependent precocious puberty (28). The study demonstrated that at 6 months, greatest suppression was observed in the 22.5 mg group, but the effects were similar at 1 year. Thus, the initial use of higher-dose leuprolide may be worthwhile, particularly in girls weighing more than 30 kg.

Results on goselerin depot (10,8 mg, 3 monthly) are mainly from the United Kingdom and limited to girls (56 females and 6 males) (29).

Long-term Effects

a. Linear growth during the treatment

A recent consensus document of 30 experts from Europe, the USA and Canada concluded that the efficacy of GnRHa in increasing adult height is undisputed only in girls <6 years old with early-onset CPP (9) but does not improve final height in girls beyond 8 years of age, and there is only modest improvement in final adult height (FAH) in girls aged 6-8 years (30,31). Carel et al. (32) also pointed out that continuing GnRHa treatment beyond 11 yr of age in girls did not improve FAH and could may potentially decrease it.

During treatment with GnRHa, it is frequently observed that height velocity decreases, even below pre-pubertal levels. The effects of GnRHa treatment on the growth hormone (GH)-IGF axis remain controversial. To compensate for the reduced spontaneous or stimulated secretion of GH and IGF-1 during GnRHa therapy, it would be logical to add recombinant human GH (rhGH) in combination with GnRHa.

Several groups have studied the effect of the addition of rhGH to GnRHa in children with CPP. The overall analysis of the data failed to indicate any benefit of combined therapy, while " individual reports suggested that in specific instances combined therapy may be beneficial in preserving or reclaiming growth potential and improving adult height " (33).

Nevertheless, a recent meta-analysis searched randomized controlled trials (RCTs) and clinical controlled trials (CCTs) adopting GnRHa therapy and GnRHa plus rhGH combination therapy to treat CPP girls. A total of six RCTs (162 patients) and six CCTs (247 patients) were included. Compared to the GnRHa therapy group, "the combination therapy group achieved taller final height, greater progression of final height compared with target height and larger height gains. No severe adverse effects to treatment were reported" (34).

b. Weight changes during treatment

Several reports have demonstrated that treatment with GnRHa in patients with CPP was associated with an increase risk of obesity but others have not confirmed these observations.

In summary, to date the reported results on changes in the BMI values of CPP patients before, during and after treatment are inconsistent. Table 1 summarizes the data reported in the literature from 1991 to 2019. Therefore, long-term prospective controlled research is required to evaluate the weight changes in these subjects.

c. Metabolic changes

Currently there is a relatively little research concerning changes in body composition and metabolic profiles in CPP patients following GnRHa treatment. It seems that in the normal-weight group there are no

Table 1. Review of body mass index (BMI) changes before, during and after GnRHa treatment

Authors and references	Results
Kamp GA et al. J Clin Endocrinol Metab. 1991;72:301-7.	The increased BMI SDS during treatment seems to be a transient phenomenon.
Boot AM et al. J Clin Endocrinol Metabol. 1998;83:370–3.	The Authors performed dual-energy x-ray absorptiometry (DEXA) before and during treatment with GnRHa in girls with CPP and early puberty. Their findings showed that BMI SDS, fat mass, and percent of body fat for chronological age increased during GnRHa therapy.
Heger S et al. J Clin Endocrinol Metab. 1999;84:4583-90.	Many CPP patients were obese prior to GnRHa treatment but experienced no changes in BMI SDS following treatment. The BMI SDS before treatment correlated strongly with the BMI SDS after treatment discontinuation.
Palmert MR et al. J Clin Endocrinol Metab. 1999;84:4480-8.	Obesity occured at a high rate among children with CPP, but did not appear to be related to long term pituitary-gonadal suppression induced by GnRHa administration.
Feuillan PP et al. J Clin Invest. 2001; 24:734-6.	The increased BMI, at initial presentation and during therapy, persisted after discontinuation of therapy and progressed to frank obesity.
van der Sluis IM et al. J Clin Endocrinol Metab. 2002;87:506-12.	After an initial increase of percentage body fat during treatment, percentage body fat decreased and normalized within 1 yr after cessation of treatment.
Arrigo T et al. Eur J Endocrinol. 2004;150:533-7.	23.8% of CPP patients were obese prior to GnRHa treatment but experienced BMI decreases after at least 2 years of treatment.
Paterson WF et al. Clin Endocrinol (Oxf). 2004;61:626-34.	The mean BMI SD scores of CPP patients increased from 0.93 to 1.2. The frequency of overweight increased from 41% to 59%, and the frequency of obese patients increased from 28% to 39%.
Traggiai C et al. Eur J Endocrinol. 2005;153:463-4.	The Authors compared 29 ICPP girls with 45 healthy girls with normal onset puberty. Regarding BMI SDS few changes were observed during the first year of therapy, while an increasing trend was observed at the end of therapy and a complete recovery after 2.5 years of the end of therapy.
Pasquino AM et al. J Clin Endocrinol Metab. 2008;93:190-5.	CPP patients maintained their previous BMI SDS during treatment regardless of the overall increase in BMI after GnRHa treatment.

(continued)

Table 1 *(continued).* Review of body mass index (BMI) changes before, during and after GnRHa treatment

Authors and references	Results
Glab E et al. Pediatr Endocrinol Diabetes Metab. 2009;15:7-11.	No significant correlation between overweight and obesity at the end of treatment and the duration of the therapy, and with the duration of CPP before introduction of GnRHa therapy was observed.
Magiakou MA et al. J Clin Endocrinol Metab. 2010;95:109-17.	No difference in the BMI SDS between GnRHa-treated group and a nontreated group was observed. Therefore, it appears likely that GnRHa treatment is not associated with an increase in fat mass
Ko JH et al. Horm Res Paediatr. 2011;75: 174–9.	The Authors assessed the percentage of body fat with DEXA method, at baseline and after one year of GnRHa therapy in 121 Korean girls and concluded that GnRHa therapy does not increase the prevalence of obesity in girls with CPP.
Yoon JY et al. J Korean Soc Pediatr Endocrinol.2011;16:165- 71.	BMI z-score increased from 0.26 ± 1.03 to 0.4 ± 0.89 during a year of GnRHa treatment.
Wolters B et al. Horm Res Paediatr. 2012;78:304-11.	Patients who were normal-weight at the start of the GnRHa treatment, exhibited an increase in BMI z-score (0.08±1.02 at baseline vs. 0.40±0.85 at the end of treatment vs. 0.41±0.89 at 6-month follow-up). In the overweight group, there was an insignificant change in BMI z-score (2.01±0.69 at baseline vs. 2.03 ± 0.54 at the end of treatment vs. 1.9 ± 0.51 at 6 months after the end of treatment).
Lee SJ et al. Chonnam Med J. 2012; 48:27-31.	BMI z-score of a Korean girl with CPP significantly increased from 0.58± 1.18 to 0.96 ± 0.83, after 18 months of GnRHa treatment.
Sorensen K et al. Eur J Endocrinol. 2012;166:903-10.	A year of GnRHa treatment increased BMI from 18.1 to 18.6 kg/m2.
Karamizadeh Z et al. Acta Med Iran. 2013;51:41–6.	GnRHa therapy cause central obesity and hyperlipidemia. The maximum weight gain of was observed at sixth months of therapy.
Gillis D et al. J Pediatr. 2013; 163: 532–6.	34 girls with CPP treated with a GnRHa were evaluated before, and the end of treatment until menarche. Changes of BMI-SDS was not significant in neither group.
Anik A et al. Indian J Endocrinol Metab. 2015;19:267-71.	GnRHa treatment did not induce significant changes in BMI z-score for chronological age, but it increased BMI z-score for bone age. The percentage of overweight/ obese CPP patients increased from 59.4% to 65.7%, after a year of treatment.
Arani KS and Heidari F. Int J Endocrinol Metab. 2015 July; 13(3): e23085. DOI: 10.5812/ijem.23085v2	The prevalence of obesity was significantly different between study groups at baseline and at sixth and 12th months of therapy (P = 0.11, P = 0.068, and P = 0.052, respectively).
Chemaitilly W et al. Clin Endocrinol (Oxf). 2016;84:361-71.	Obesity was more prevalent at the last follow-up than at the completion of GnRHa or the puberty onset (37,7%, 22,6% and 20,8%, respectively, P = 0.03).
Park J et al. Ann Pediatr Endocrinol Metab.2017; 22:27-35.	GnRHa treatment increased BMI z-score within a year of treatment, regardless of the subject's obesity status.
Arcari AJ et al. J Pediatr Endocrinol Metab. 2019;32:181-6.	An increase of BMI in girls with normal weight was observed.

changes in insulin resistance, whereas a tendency to develop an insulin resistance was detected in patients who at the start of the treatment were overweight or obese (Table 2). Different diagnostic criteria, race/ethnicity, age at follow-up, and potential for bias make comparison of studies difficult, but concern continues for long-term endocrine and metabolic outcomes (35,36). Therefore, long-term prospective controlled research is required to evaluate the changes in obesity and insulin resistance in subjects with CPP.

d. Bone mineral density (BMD) and bone markers

Although suppression of ovarian activity has been associated with BMD reduction during GnRHa treatment (37), recent studies have shown no changes in bone mineralization among CPP patients who had received 3 years of GnRHa treatment (38). Antoniazzi et al. (39) reported that although the BMD decreased during GnRHa treatment, this was reversible and preventable with calcium supplementation. Furthermore, restoration of BMD after cessation of treatment has been also documented (26). As in normal girls and adolescents, exercise and adequate nutritional intake would be helpful for bone mass formation in CPP patients.

Regarding bone turnover markers in CPP patients, the expression of carboxy terminal telopeptide of type 1 collagen (ICTP), a bone resorption marker, and procollagen type 1 C-terminal propeptide (PICP), a bone formation marker, increased prior to GnRHa treatment but decreased during a 6-month treatment period and stabilized after treatment. Bone age-adjusted bone turnover markers were also normalized 2 years after treatment cessation. On the other hand, a report indicated that no changes in age- and bone age-adjusted BMD-SDS was observed during GnRHa treatment (40).

In brief, the long-term BMD studies in CPP patients proposed that although BMD levels decreased during GnRHa treatment, the bone mass was sufficiently preserved after treatment.

e. Menarche, menstrual cycles and polycystic ovary syndrome (PCOS)

Regarding reproductive function, studies indicate that menstruation occurs on average 16 months after the treatment of CPP is withdrawn (with a variation of 2 to 61 months). Regular ovarian cycles occur in 60% to 96% of the patients, and infertility has not been reported (9,26). However, there are concerns that PCOs may occur more often in those with CPP than in those with normal puberty (41). The reported frequencies vary and conflicting data on the long-term risk of developing PCOS in conjunction with CPP remain (Table 3).

PCOS is observed in 5%-10% of women of reproductive age and is characterized by anovulation, hyperandrogenism, and polycystic ovaries (42,43). Severe

Table 2. Review of the metabolic changes reported in patients with precocious puberty treated with GnRHa

Authors and references	Results
Taşcilar ME et al. Turk J Pediatr. 2011;53:27-33.	An exaggerated elevation in trunk fat mass and insulin resistance (IR) in GnRHa-treated ICPP children was observed.
Sorensen K et al. Eur J Endocrinol. 2012;166:903-10.	Fasting insulin, first phase insulin release and mean plasma insulin during oral glucose tolerance test in CPP patients increased after a 52-week period of GnRHa treatment, whereas whole body insulin sensitivity index decreased, indicating an insulin resistance.
Park J et al. Ann Pediatr Endocrinol Metab.2017;22:27-35.	No changes were observed in QUICKI and HOMA-IR within a year of treatment in the normal-weight girls with CPP.
Arcari AJ et al. J Pediatr Endocrinol Metab. 2019;32:181-6.	GnRHa did not affect BMI, insulin index and lipid profile. However, an increase of BMI in girls with normal weight was observed.

Table 3. Review of PCOS prevalence in girls with precocious puberty before, during or after treatment with GnRHa

Authors and references	Results
Boepple PA. In: Savage MO, Bourguignon J-P, Grossman AB, eds. Frontiers in paediatric neuroendocrinology. Oxford, London,Edinburgh, Cambridge, Carlton: Blackwell. 1994: pp. 23–9.	PCOS was reported in approximately half of the patients treated with GnRHa.
Bridges NA et al. Clin Endocrinol (Oxf) 1995; 42: 135-40.	The prevalence of PCOS among CPP patients was 24%, compared with 2% in an age-matched control group.
Lazar L et al. Eur J Endocrinol. 1995; 133: 403-6.	A significant number of girls with CPP develop PCO-like syndrome at a relatively young age.
Baek-Jensen AM et al. J Pediatr.1998; 132:105–8	The Authors did not observe PCOS during or after treatment with GnRHa.
Heger S et al. J Clin Endocrinol Metab.1999; 84:4583–90.	No increased incidence of PCOS in GnRHa-treated patients with CPP compared with the normal population was reported.
Chiavaroli V et al. Eur J Endocrinol. 2010;163:55-62.	The prevalence of PCOS and hyperandrogenemia was significantly higher in GNRHa-treated adolescents than in untreated adolescents (36 and 14.5% respectively, P=0.04; 56 and 23.6% respectively, P=0.01).
Magiakou MA et al. J Clin Endocrinol Metab. 2010;95:109-17.	21% of subjects evaluated between ages 16 and 32 had PCOS, using the National Institutes of Health criteria.

insulin-resistant obesity, premature adrenarche, and sexual precocity in childhood are some of the known risk factors of PCOS (43,44).

In summary, the prevalence of PCOS among CPP patients varies depending on the characteristics of the patients, durations of treatment and follow-up period, and differences in the PCOS diagnosis standards. It is unclear whether this association is due to the hyperinsulinemia or premature adrenarche already present at CPP onset or a result of an abnormal hormonal response to GnRHa treatment (45). A comparison with a control group of CPP patients through a long-term evaluation from diagnosis to post-treatment adulthood is needed to determine the causative factors of PCOS in patients treated for PPC (45).

f. Psychosocial changes

One of the most common concerns about PP in girls is the potential for adverse psychological consequences. Numerous studies have reported an association between early normal puberty and adverse psychological, behavioural, and social outcomes in girls (46-51).

Although many studies have examined early maturity or puberty, little is known about psychosocial changes in girls with CPP receiving treatment with GnRHa (Table 4). The available results are reassuring regarding concerns of adverse psychological consequences of early puberty in girls. However, long-term prospective studies are needed in order to further elucidate the psychological impact of PP on girls and their mothers.

Adverse Drug Reactions (ADRs) associated with the use of GnRHa

Bone pain, micturition problems, hypersensitivity (itching, skin rash, fever), gynecomastia, flushing, depression, easy and quick to anger, headache, nausea, muscle pain, joint pain, excessive sweating, fatigue, sleep disturbances, pain at the injection site, predisposition to hypertension, and thrombosis are the adverse

Table 4. Review of psychosocial changes in girls with precocious puberty before and during treatment with GnRHa

Authors and references	Results
Xhrouet-Heinrichs D et al. Acta Paediatr. 1997; 86:808–15.	Some behavioral and affective characteristics were observed in girls with PP. During treatment with long acting triptorelin, problematic behavior and functioning decrease slightly, particularly in the few girls showing breast regression.
Officioso A et al. J Pediatr Endocrinol Metab. 2000 Jul;13 Suppl 1:835-9.	Ten adolescent girls aged 14 years treated for ICPP were evaluated. All the adolescents had a negative body image compared with age-matched controls and expressed a strong inhibition of their femininity. Their poor body image was reflected by their low self-esteem.A psychological support was recommended.
Mul D et al. Acta Paediatr. 2001;90:965–71.	The psychological evaluation did not reveal any consistent abnormalities in adopted children with early puberty. Treatment with GnRHa with or without rhGH did not increase emotional and behavioural problems in adopted children, nor was their self-perception decreased.
Zheng F et al. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2008; 37: 289- 94.	The authors compared the psychological behavior of girls with ICPP before and after treatment by GnRHa. They found that the self-esteem scale, and body-esteem scale score in ICPP were significantly lower compared to controls (P <0.05).
Kim YJ et al. Ann Pediatr Endocrinol Metab 2013;18:173-8.	The psychological assessment did not exhibit a significant difference except with scores for sociability and behavior problems.
Choi MS et al. Ann Pediatr Endocrinol Metab. 2016; 21:155-60.	Patients with PP had distorted perception about their body image and breast development that seems to contribute to depression score.
Schoelwer MJ et al. Horm Res Paediatr. 2017; 88:347-53.	Girls with CPP completed psychological assessments at baseline and after 1 year along with their mothers. All girls were treated with GnRH analogs. Psychological measures were normal in all girls.

drug reactions (ADRs) observed in adults (52-57). In children, the available evidences show that GnRHa in general are safe and effective long- term (58,59). However, some significant ADRs in children treated with GnRHa for CPP have been reported (60).

ADRs are basically defined according to the World Health Organisation as: "any response to a drug which is noxious, and unintended, and which occurs at doses normally used in man for prophylaxis, diagnosis or therapy of disease, or for the modification of physiological function " (61).

Relevant studies indexed in Pubmed and Google Scholar were selected using the search terms: "precocious puberty/early puberty, GnRH analogue, GnRHa safety and adverse events". For the classification of ADRs severity we choose the Hartwig Siegel assessment scale (62).

a. Vaginal spotting/bleeding (Hartwig and Siegel severity scale: Level 1)

Continuous stimulation of the pituitary gland results in a short period of pubertal stimulation, followed by down regulation of GnRH receptors, pituitary desensitisation and reduced gonadotropin synthesis. Therefore, the first injection of GnRHa is associated with a transient surge in LH and FSH resulting in a transient increase in estradiol levels, which then rapidly drops following down regulation of GnRH receptor, usually within a fortnight (63).

This transient surge in estradiol may result in vaginal spotting/ bleeding, in a small number of female patients, following the first injection due to discontinuation of the estrogen support of the proliferative and stable endometrium.

Eight of the 28 (28.5%) girls, aged 6.5-11 years, with idiopathic CPP treated by Yeshaya et al. (64) every 28 days with an intramuscular depot GnRHa developed vaginal bleeding after GnRHa administration. Of these, prolonged vaginal bleeding of 11-13 days occurred in four girls, three recurrent episodes occurred in one during the second injection, and in one other girl the 4th episode occurred after 6 months of treatment. The episodes resolved spontaneously and necessitated no further treatment.

However, other researchers have suggested the use of an anti-androgen [cyproterone acetate, given (usually) for the first six weeks of therapy at a dose of 70 mg/m^2/day], or a prostanoid receptor antagonist or a co-injection of depot medroxy-progesterone acetate (MPA) with the first dose of GnRHa (65,66).

b. Local side effects (Hartwig and Siegel severity scale: Levels 1 and 3)

Local side effects, including pain at the injection site and flares usually are mild, although some may persist for several months and can leave significant scarring. Rare cases of subcutaneous nodules and sterile abscess (SAs) formation related to GnRHa, affecting the compliance with the treatment, have been observed (60, 67-69).

Lee et al (70) reported a prevalence of SAs formation in 4 out of 621 patients (0.6%) with CPP and early onset puberty, who were receiving monthly long-acting GnRHa (leuprolide acetate, triptorelin acetate) (70). In one patient, SAs occurred following leuprolide acetate depot therapy and also developed after a switching the treatment to triptorelin acetate depot. The fact that one patient had SAs formation following treatment with 2 different long-acting GnRHa can suggest that the cause could be attributed to the antibody formation against the same type of biodegradable polymers (lactic acid glycolic acid copolymer) present in the depot formulations (69,70).

c. Slipped capital femoral epiphyses (SCFE) (Hartwig and Siegel severity scale: Level 5)

Slipped capital femoral epiphyses (SCFE) occur mainly in boys in late childhood or adolescence. The incidence is 0.33/100,000 to 24.58/100,000 children 8 to 15 years of age. The single most significant risk factor for SCFE is obesity. Other risk factors include male sex, periods of rapid growth, and prior radiation therapy. The average age of onset is 11.2 years in females and 12.0 years in males. Approximately 25% (range: 8-50%) of cases are bilateral. Delay in the diagnosis of SCFE is associated with higher rates of complications, including femoral head osteonecrosis (71,72).

Five events of SCFE associated with GnRHa occurred in children during or shortly after the drug discontinuation (70,73,76). Inman et al. (76) suggested that a lack of adequate sex hormone exposure at a " critical period " of bone formation may result in a weakened epiphysis that becomes susceptible to slipping. In addition, the increase in growth velocity after

Table 5. Hartwig and Siegel severity scale

Level description
Level 1: An ADR occurred but required no change in treatment with the suspected drug.
Level 2: The ADR required that treatment with the suspected drug be held, discontinued, or otherwise changed. No antidote or other treatment requirement was required. No increase in length of stay
Level 3: The ADR required that treatment with the suspected drug be held, discontinued, or otherwise changed, and/or an antidote or other treatment was required. No increase in length of stay.
Level 4: Any level 3 ADR which increases length of stay by at least 1 day.
Level 5: Any level 4 ADR which requires intensive medical care.
Level 6: The adverse reaction caused permanent harm to the patient.
Level 7: The adverse reaction either directly or indirectly led to the death of the patient.

stopping GnRHa , subsequently results in a reduction of the shearing force needed for the displacement of the epiphysis.

d. Pseudotumor Cerebri (PTC) (Hartwig and Siegel severity scale: Levels 5)

Pseudotumor cerebri (PTC), also known as idiopathic intracranial hypertension, is a disorder with increased intracranial pressure and associated headaches, papilledema, vision changes, or pulsatile tinnitus in the setting of normal imaging and cerebrospinal fluid (CSF) studies. Children of both genders are affected equally before puberty (77). Males (aged 12 to 15 years) have an annual incidence of 0.8 per 100,000; females aged 12 to 16 years have an annual incidence of 2.2 per 100,000 (78). PTC can be classified as either primary (when there is no clear causal factor) or secondary to cerebral venous thrombosis or changes in the composition of the CSF. Proposed mechanisms involve the vascular, hormonal, and cellular systems. The first-line treatment is acetazolamide. The most concerning complication of PTC is permanent vision loss because of compression of the optic nerve secondary to elevated intracranial pressure (77).

Pseudotumor cerebri (PTC) secondary to use of leuprolide acetate is an extremely rare event with only two cases reported in the literature (79,80).

Summary of case 1 presentation reported in the literature (Reference 79)

A 9-year-old girl with PP was treated with leuprolide acetate (3.75 mg). After the 4th dose, she presented headache and hypertension (130-155/85-110 mmHg). There were no causes underlying the hypertension such as cardiac, renal, or endocrine. Neurological examination was normal except for bilateral papilledema. Cranial magnetic resonance imaging was normal and the orbital section of MRI revealed bilateral optic nerve enlargement. Cerebrospinal fluid (CSF) opening pressure was elevated. Triptorelin therapy was stopped and acetazolamide was started. The patient improved and the CSF pressure and fundoscopic examinations returned to normal (79).

Summary of case 2 presentation reported in the literature (Reference 80)

A 9-year-old girl with PP was treated with leuprolide acetate (3.75 mg). After 4 month, she complained of holocranial headache, transient visual obscuration followed by progressive visual loss. After 6 months, she persisted with holocranial headache and progressive visual loss associated with ocular deviation. Neuro-opthalmological examination revealed severe visual loss and bilateral papilledema. Cerebrospinal fluid (CSF) analysis showed opening pressure of 45 cm H2O. The most likely diagnosis was PTC associated with leuprolide acetate. Treatment was started immediately with oral acetazolamide and leuprolide was discontinued. Unfortunately, acetazolamide induced a metabolic acidosis. A ventriculoperitoneal shunt was performed to control intracranial pressure as an alternative to acetazolamide treatment. The follow-up of 18 months showed CSF pressure of 14 cm H2O, stabilization of visual acuity and resolution of papilledema (80).

e. Hypertension (HTN) (Hartwig and Siegel severity scale: Levels 5)

According to the instructions for GnRHa use, issued by the manufacturer, arterial hypertension is considered an infrequent complication (81).

Hypertension has been reported in girls with CPP (82-85) and in girls with gender dysphoria (86), likely due to loss of the vaso-protective properties of estrogens (87). The authors concluded that although estrogen depletion may play a role in the pathogenesis of triptorelin-induced HTN, this aspect should be further investigated. Furthermore, clinicians should be aware of the possibility, although rare, of HTN developing during triptorelin administration in childhood, specifically in patients at increased risk of HTN, such as those with Williams-Beuren syndrome (Table 6) (83).

f. Anaphylactic reactions (Hartwig and Siegel severity scale: Levels 5)

Anaphylaxis is defined by the European Academy of Allergy and Clinical Immunology (EAACI)

Table 6. Summary of patients with central precocious puberty developing arterial hypertension during GnRHa treatment

Authors and references	Results
Calcaterra V et al. Indian J Pediatr. 2013;80:884-885.	A 7-year-old girl with triptorelin-treated CPP, who developed reversible HTN with secondary concentric left ventricular hypertrophy, requiring transient antihypertensive therapy.
Siomou E et al. Pediatr Nephrol. 2014;29:1633–1636	A 10-year-old girl with a Williams-Beuren syndrome and CPP who developed HTN with triptorelin treatment. In that case, blood pressure totally normalized, without any anti-hypertensive medication once GnRHa was discontinued.
Palma L et al. J Pediatr Endocrinol Metab. 2018 Aug 8. pii: /j/jpem. ahead-of-print/jpem-2018-0210/ jpem-2018-0210.xml. doi: 10.1515 /jpem-2018-0210	A girl with CPP who developed HTN from treatment with GnRH-a (triptorelin). HTN subsided once triptorelin was interrupted. Consequently, the Authors hypothesized that the hypertension was related to triptorelin treatment.
Sifaki L et al. Front Pediatr. 2019 Mar 19;7:74. doi: 10.3389/ fped. 2019.00074.	A 10-year-old girl with CPP during treatment with triptorelin, developed an asymptomatic stage II HTN. Initial workup showed no renal, thyroid, or electrolytes abnormalities. A complete normalization of her blood pressure was obtained without any medication.

as a severe, life-threatening generalised or systemic hypersensitivity, characterised by its rapid onset with life-threatening airway, breathing and/ or circulatory problems (88).

The incidence of anaphylaxis in children worldwide varied widely, ranging from 1 to 761 per 100 000 person-years for total anaphylaxis and 1 to 77 per 100 000 person-years for food-induced anaphylaxis. Gender and ethnicity are demographic risk factors associated with anaphylaxis in children (89).

Whilst drug-induced anaphylaxis is more commonly reported in adulthood, less is known about the role of drugs in pediatric anaphylaxis. Antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) are the main elicitors in drug-induced anaphylaxis in children. Anaphylactic reactions to GnRHa agonists are exceedingly rare (90-92).

Summary of case 1 presentation reported in the literature (Reference 93)

An 8-year-old girl who was diagnosed with CPP was receiving triptorelin acetate treatment uneventfully for 6 months. To evaluate the efficacy of the treatment, an LH-RH stimulation test with gonadorelin acetate was planned. Within 3 min after intravenous administration of gonadorelin acetate, she lost consciousness and tonic seizures began in her hands and feet. She was immediately treated with epinephrine (0.01 mg/kg; 1:1000), high flow supplemental oxygen (6-8 L/min), IV diphenhydramine (1 mg/kg), and IV methylprednisolone (1 mg/kg). Her vital signs recovered within 30 min. When her medical history was more deeply investigated, her parents recalled that there were several skin reactions along with pruritus after a previous gonadorelin acetate injection. A skin test with gonadorelin acetate was planned during the follow-up, however, it could not be performed due to the unwillingness of her parents (93).

Summary of case 2 presentation reported in the literature (Reference 70)

An 8.4-year-old girl with CPP was treated with triptorelin acetate depot SC injected at four-week intervals. Immediately after the sixth injection, she developed dizziness, headache, whole body redness, and chest tightness. Subsequently, she lost consciousness. Blood pressure (BP) could not be measured at that time. All the above symptoms were relieved within several minutes without any particular treatment. Anaphylaxis was considered to have occurred (70).

In conclusion, although the occurrence of anaphylactic reactions to GnRHa are very rare, it can have serious practical implications. Therefore, clinicians should be aware of the potential association of GnRH analogs with systemic reactions, should recognize that recurrent anaphylaxis may occur due to the long half-life of these therapeutic agents in tissue and recommend GnRHa administration under proper conditions, even if there is no history of previous systemic hypersensitivity reactions.

Conclusions

Since 1981, GnRHa administration has been the standard treatment for CPP. GnRHa suppress LH and FSH and thereby induce a marked inhibition of gonadal activity. This treatment is generally considered to be safe and well tolerated in children and adolescents. The most commonly reported drug reactions were pain, swelling, and urticaria at the injection site. Most events were mild, and there was no interruption in study procedures from these ADRs. Nevertheless, whatever is the frequency of these side-effects, clinicians using these treatments should be aware of the possibility of significant local and general ADRs that can lead to treatment withdrawal in the most severe cases.

We hope that our review may provide the necessary data in order to enable clinicians to administer GnRHa in the safest and most appropriate way. Further studies are necessary to identify the mechanisms of development of potential adverse drug reactions related to GnRHa therapy in CPP and the potential risk of causing prolonged QT, as reported in adults (94).

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ORIGINAL CONTRIBUTION

Re-evaluation of the Dutch approach: are recently referred transgender youth different compared to earlier referrals?

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Abstract
The background of this article is to examine whether consecutively transgender clinic-referred adolescents between 2000 and 2016 differ over time in demographic, psychological, diagnostic, and treatment characteristics. The sample under study consisted of 1072 adolescents (404 assigned males, 668 assigned females, mean age 14.6 years, and range 10.1–18.1 years). The data regarding the demographic, diagnostic, and treatment characteristics were collected from the adolescents' files. Psychological functioning was measured by the Child Behaviour Check List and the Youth Self-Report, intensity of gender dysphoria by the Utrecht Gender Dysphoria Scale. Time trend analyses were performed with 2016 as reference year. Apart from a shift in sex ratio in favour of assigned females, no time trends were observed in demographics and intensity of dysphoria. It was found, however, that the psychological functioning improved somewhat over time (CBCL $\beta -0.396$, $p < 0.001$, 95% CI -0.553 to -0.240, YSR $\beta -0.278$, $p < 0.001$, 95% CI -0.434 to -0.122). The percentage of referrals diagnosed with gender dysphoria (mean 84.6%, range 75–97.4%) remained the same. The percentage of diagnosed adolescents that started with affirmative medical treatment (puberty suppression and/or gender-affirming hormones) did not change over time (mean 77.7%; range 53.8–94.9%). These findings suggest that the recently observed exponential increase in referrals might reflect that seeking help for gender dysphoria has become more common rather than that adolescents are referred to gender identity services with lower intensities of gender dysphoria or more psychological difficulties.

Keywords Adolescence · Gender dysphoria · Mental health · Clinical sample · Transgender

Introduction

Gender dysphoria (GD) is defined as a marked incongruence between a person's gender identity and the gender assigned at birth accompanied by psychological distress [1]. It is also the term for the diagnostic classification according to the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5). Not all people who experience feelings of GD might be diagnosed with DSM-5's 'Gender Dysphoria', because it is likely that they do not all seek assessment and gender affirmative treatment. For this reason and given the lack of systematic epidemiological studies, it is difficult to establish the prevalence of GD. Most former studies on prevalence of GD were based only on the numbers of individuals who are being treated at a transgender clinic. The prevalence in these studies in birth-assigned males ranges from 0.0004 to 0.0352% and in birth-assigned females from 0.0003 to 0.0066% [2]. In recent studies based on self-reported gender identity and GD, higher rates are reported, specifically in adolescents and younger adults [3]. E.g., the Williams institute, a public policy research institute on sexual orientation and gender identity, reports a survey-based prevalence of 0.7% of US adolescents who identify a transgender [4]. Another recent high school sample study that was held amongst 135.760 adolescents under the age of 21 years showed that 0.6% identified as the opposite gender and 3.3% identified as non-binary [5].

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While medical gender affirming treatment with hormones and surgeries has been an accepted treatment for adults with GD since the 1970s [6], more reluctance exists concerning medical interventions in adolescents with GD. Some argue that puberty suppression by means of GnRH analogues or affirming hormonal treatment should not be initiated before a person's physical puberty development is complete, because gender identity still may change during this phase of life, so adolescents should not make decisions regarding this subject [7]. Another point of concern is the lack of data on the long-term physical outcome and the signs from animal models that puberty suppression influences the brain development [8, 9].

Despite these reluctances, the introduction of supposedly fully reversible puberty suppression since the beginning of this century, to provide transgender adolescents who enter puberty with time to explore their gender identity, has rapidly become an accepted and widely prescribed medical intervention for adolescents with GD in Northern America (USA and Canada), and in some countries in Europe and Australia/New-Zealand [10, 11]. Puberty suppression was first introduced as a part of affirming treatment at the Center of Expertise on Gender Dysphoria in Amsterdam in The Netherlands, and therefore, GD treatment that includes puberty suppression is sometimes referred to as the 'Dutch Model' [12].

Few evaluative studies of this approach have been performed. Two studies on the first 55 adolescents treated at the Amsterdam clinic showed that behavioural and emotional problems and depressive symptoms decreased, while general functioning improved significantly during puberty suppression [13]. After gender affirming hormonal treatment and surgery, GD was alleviated and psychological functioning further improved [14]. A study on 201 adolescents who attended the transgender clinic in London found that puberty suppression in addition to psychological support was more helpful than psychological support on itself [15].

These results are promising. However, the participants were all adolescents who were referred to transgender clinics before the remarkable recent increase in the number of adolescent referrals [16, 17]. It remains, therefore, unknown whether the positive outcomes of early medical intervention also apply to adolescents who are referred in more recent years. There are several reasons why one could hypothesize that early referrals may differ from more recent registered adolescents. Due to the increased awareness of GD, it could well be that adolescents are referred at a younger age in recent years. In addition, the experimental character of medical treatment may first have attracted the most well-functioning group of adolescents with above average intelligence from stable families with a higher educational background able enough to support their off-spring to ask for a treatment that was still considered controversial in early years. The current flow of referrals might include families that are less well functioning in these aspects. The same may count with regard to co-occurring psychological problems. Finally, present referrals may have less extreme and non-binary forms of GD; a diagnosis of GD could be less likely in present referrals and a smaller percentage would start with medical treatment compared to the early years.

So far, one study has examined whether adolescents ($N = 203$) who were referred to a gender clinic in recent years were different from those in initial reports [18]. In contrast to initial studies, it was found that more birth-assigned females than birth-assigned males applied. Like the earlier studies, high rates of mental health difficulties were reported. Comparisons within the short 2.5 year period (2014–2016) of the first and second half of the adolescents showed that the age at first visit declined somewhat, while the presence of mental health difficulties did not change. Therefore, this study showed some changes over time, but the time span of the study seems too short to actual identify trends. Furthermore, the demographic and the psychological characteristics of the adolescents that were measured were very limited. For example, regarding psychological functioning, they only included the chart-reported mental health history and the Beck Depression Inventory [19].

To gain more insight in the possible change of characteristics of transgender adolescents, we conducted the present study in a large Amsterdam transgender clinic sample ($N = 1072$). The aim of this study is to examine whether there are time trends in demographic, psychological, diagnostic, and treatment characteristics in adolescents referred between 2000 and 2016.

Methods

Participants

The 1072 persons included in this study were all consecutively referred adolescents [404 assigned males, 668 assigned females, age 10.14–18.08 years (mean age 14.64, SD 2.19)] who registered at the Center of Expertise on Gender Dysphoria in Amsterdam, The Netherlands, between 2000 and 2016 and could be eligible for medical affirming interventions, including puberty suppression. See Table 1 for further demographic characteristics. Due to missing information, not all the parents' marital status and parents' educational level of the adolescents are known.

Procedure

Each adolescent followed the usual assessment, which consisted of several sessions with a psychologist/psychiatrist with the adolescent and the parents together and separate

Table 1 Demographic variables

Birth-assigned gender, *N* (%)	
Assigned males at birth	404 (37.7%)
Assigned females at birth	668 (62.3%)
Age at assessment in years, *M* (SD)	14.64 (2.19), range 10.14–18.08
Parents' marital status, *N* (%)	
Living with both biological parents	579 (54%)
Other	449 (41.9%)
Unknown	44 (4.1%)
Parents' educational level, *N* (%)	
Vocational educated	581 (54.2%)
Higher vocational and academic educated	322 (30%)
Unknown	169 (15.8%)
Full-scale IQ, *M* (SD)	99.15 (16.08), range 59–145
Diagnosis, *N* (%)	
Not diagnosed with a form of gender dysphoria	57 (5.3%)
Diagnosed with a form of gender dysphoria	908 (84.7%)
Unknown	107 (10%)

M mean, *SD* standard deviation

from each other [20]. A psycho-diagnostic assessment was part of the procedure. The recommended treatment plan was provided in a feed-back session.

Some adolescents remained undiagnosed, because the diagnostic process ended prematurely ($N = 107$). In a majority of the cases, the adolescents themselves ended the process due to a discontinued wish for medical treatment. In a minority of the cases, the psychologist decided to prematurely end the diagnostic trajectory due to psychological or social problems that seriously interfered with the diagnostic assessment.

Demographics

All demographic, psychological, diagnostic, and treatment characteristics were obtained from the adolescents' files. Four demographic measures were coded: (1) birth-assigned sex of the adolescent, (2) age at assessment, (3) parents' marital status, and (4) parents' educational level.

Marital status of the parents was categorized as either "living with both biological parents" or "all other categories" (e.g., single parent, divorced, widowed, and adopted). Educational level of the parents was categorized as either "vocational educated" or "higher vocational educated or academic educated". Traditionally, vocational education is education that focuses on preparing students to work in a trade or a craft, whereas higher vocational education and academic education focuses on higher learning and professional training.

Measures

Intelligence

During the psycho-diagnostic assessment, a Full-Scale IQ was measured by the Dutch version of the Wechsler Intelligence Scale for Children, or the Wechsler Adult Intelligence Scale, depending on the age of the assessed adolescent [21–23].

Child Behaviour Checklist and Youth Self-Report

The Child Behaviour Checklist (CBCL) and the Youth Self-Report (YSR) were administered during the diagnostic phase to assess a broad spectrum of behavioural and emotional problems in the adolescents. The CBCL was completed by the respective caregivers and the YSR was completed by the adolescents themselves [24].

Four main outcomes from the CBCL and the YSR were used: (1) the *T*-score for the Total Problem score; (2) the *T*-score for Internalizing problems; (3) the *T*-score for Externalizing problems; (4) the clinical range scores (> 90th percentile) for these three outcomes. The *T*-scores were calculated on the basis of the Dutch norms.

We further calculated the Peer Relation Scale and the Suicidality Scale. As in previous studies, the Peer Relation Scale was created by three items: "Does not get along with other kids" (Item 25), "Gets teased a lot" (Item 38), and "Not liked by other kids" (Item 48) [25]. The Suicidality Scale was based on two items: "Deliberately harms self or attempts suicide" (Item 18) and "Talks about killing self" (Item 91) [26].

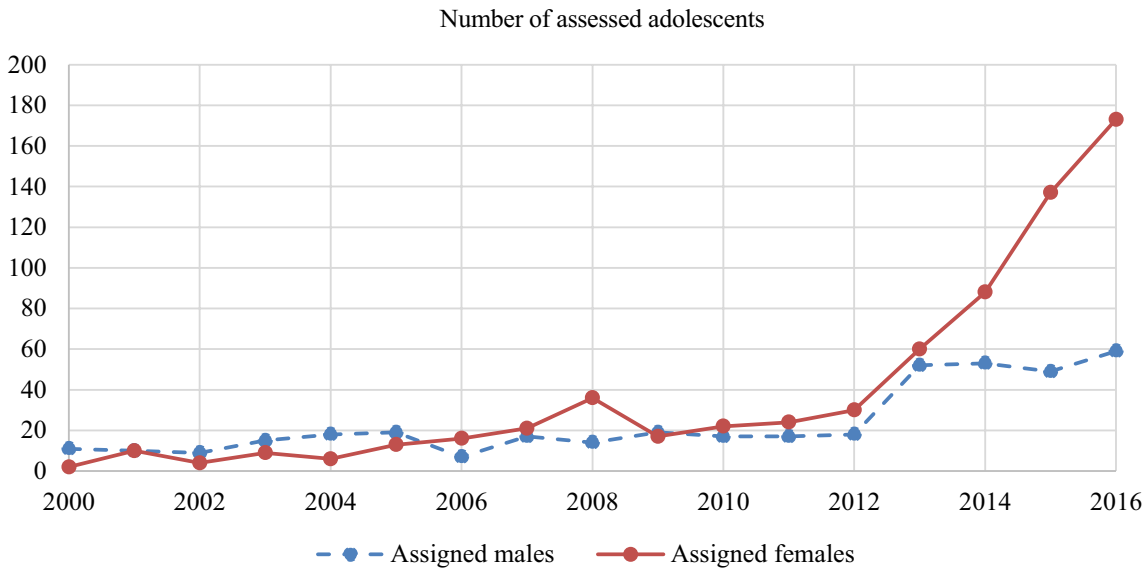

Fig. 1 Sex assigned at birth of assessed adolescents

Utrecht Gender Dysphoria Scale

The Utrecht Gender Dysphoria Scale (UGDS) was administered to measure the intensity of the GD. Answers on the statements can be given on a five-point scale ranging from strongly agree to strongly disagree. There are two different versions for birth-assigned males and females, respectively [27].

Transgender diagnoses and possible subsequent treatment

For a gender incongruence diagnosis, DSM criteria were followed by the examining psychologist or psychiatrist. Between 2000 and 2014, the DSM-IV-TR was used, and the DSM-5 is used since 2015 [1, 28]. The diagnosis of the adolescent was categorized as either diagnosed with a form of Gender Dysphoria, including Gender Identity Disorder, Gender Identity Disorder Not Otherwise Specified, Gender Dysphoria, Other Specified Gender Dysphoria, and Unspecified Gender Dysphoria or not diagnosed with any form of Gender Dysphoria.

For each participant that was diagnosed with a form of Gender Dysphoria, it was coded whether they started with medical treatment including puberty suppression and/or gender affirming hormones (in older adolescents).

Statistical analyses

All data analyses were performed using SPSS version 22. A significance level of $p < 0.05$ (two-tailed) was used. Independent *t* tests and Chi-square tests identified if sex assigned at birth was associated with the outcome variables. This variable was included when significant and reported as control variable in the regression analyses.

First, regression analyses with year of assessment as continuous variable were performed and second, if significant, regression analyses with year of assessment as categorical variable represented by dummy variables were carried out. Regarding the regression analyses, for continuous outcomes, linear regression analyses were used, while for the dichotomous outcomes, logistic regression analyses were used.

Results

Demographics

Regression analyses revealed that year of assessment was a significant predictor for sex assigned at birth (OR 1.110, $p < 0.001$, 95% CI 1.078–1.143). Regression analyses with time as a categorical variable also showed this shift in sex ratio, with birth-assigned females being overrepresented in later years compared to earlier years (Fig. 1).

The initial regression analyses regarding age at assessment showed a significant increase over time (β 0.061, $p < 0.001$, 95% CI 0.031–0.092), indicating that adolescents who were assessed more recently, were older at the time of intake compared to adolescents who were assessed in previous years. However, the regression analyses with time as a categorical variable showed fluctuations over the years, rather than a trend. Over time, the mean age at intake of the

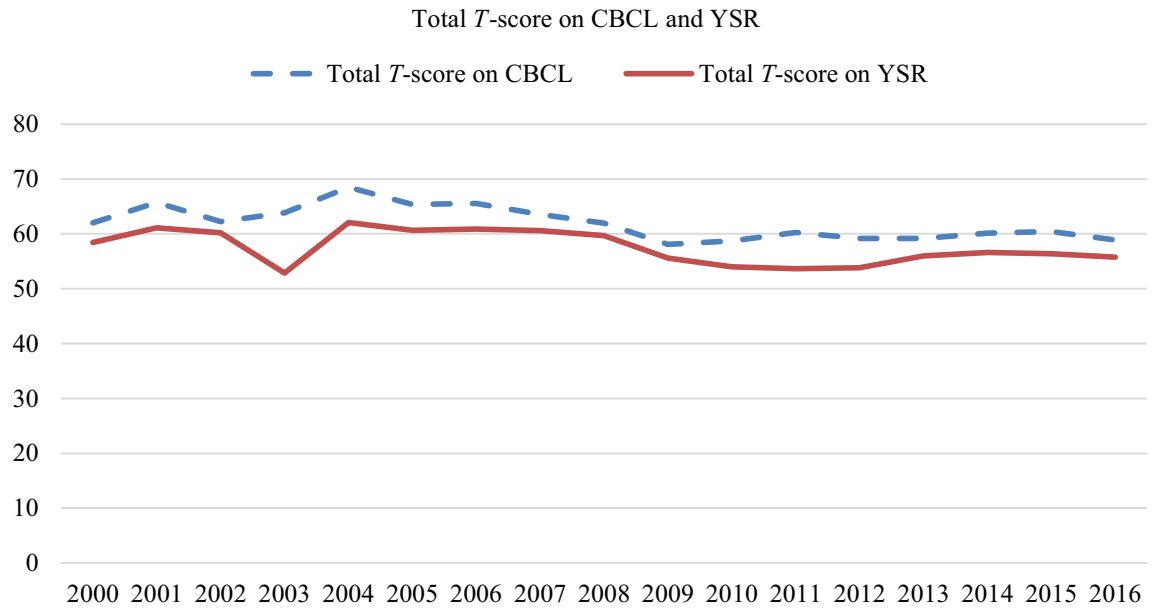

Fig. 2 Total *T*-score on CBCL and YSR of assessed adolescents

adolescents continued to vary between 14 and 15 years of age. No interaction was found with sex assigned at birth.

Initial analyses showed that the mean Full-Scale IQ of the adolescents became higher over time (β 0.366, p 0.004, 95% CI 0.116–0.617). Nonetheless, the analyses with time as a categorical variable showed that the mean Full-Scale IQ score of the adolescents remained within the average range each year and that this did not change over time. Our initial regression analyses further showed that most of the adolescents who were assessed at our clinic between 2000 and 2016 lived with both of their biological parents and that this remained the same over the years (OR 0.985, p 0.314, 95% CI 0.957–1.014). Furthermore, initial analyses demonstrated that the educational level of the parents of the adolescents increased significantly over time (OR 1.063, $p < 0.001$, 95% CI 1.026–1.100). In the subsequent analyses with time as a categorical variable, however, we found that most of the parents were vocational educated and that this did not change significantly over time. No interaction was found with sex assigned at birth.

Psychological functioning

A decreasing trend was found between 2000 and 2016 in the mean total *T*-score of the assessed adolescents on the CBCL ($\beta -0.396$, $p < 0.001$, 95% CI −0.553 to −0.240) and the YSR ($\beta -0.278$, $p < 0.001$, 95% CI −0.434 to −0.122). This decrease in the *T*-score was also found by the analyses with time as a categorical variable for both the CBCL and the YSR. A downward trend is seen until 2008, as shown in Fig. 2. No interactions were found with birth-assigned sex.

Initial analyses showed no trend over time in the mean internalizing total *T*-score on the CBCL ($\beta -0.100$, p 0.250, 95% CI −0.272 to 0.071) and the YSR ($\beta -0.011$, p 0.901, 95% CI −0.169 to 0.192). No interactions were found with birth-assigned sex.

The mean externalizing total *T*-score on the CBCL and YSR of the adolescents who applied in later years became significantly lower compared to the mean score of the adolescents who applied in early years (CBCL: $\beta -0.408$, $p < 0.001$, 95% CI −0.582 to −0.234 and YSR: $\beta -0.323$, $p < 0.001$, 95% CI −0.473 to −0.173). The decreasing trend in the mean externalizing total *T*-score persisted through the analyses with time as a categorical variable. No interactions were found with birth-assigned sex.

Initial analyses showed a significant decrease for the clinical range total *T*-scores on both parent and self-report (CBCL: OR 0.950, p 0.002, 95% CI 0.919–0.982 and YSR: OR 0.968, p 0.041, 95% CI 0.937–0.999). The more detailed analyses with time as a categorical variable contradicted this trend and showed fluctuation over the years rather than a decrease. No interactions were found with birth-assigned sex.

The clinical range internalizing total *T*-scores of the adolescents on the CBCL and the YSR showed no trend over time (CBCL: OR 0.986, p 0.377, 95% CI 0.954–1.018, YSR: OR 1.016, p 0.317, 95% CI 0.985–1.049). No interactions were found with birth-assigned sex.

In the clinical range externalizing total *T*-scores, a decreasing trend was found on both parent and self-report (CBCL: OR 0.922, $p < 0.001$, 95% CI 0.892–0.953 and YSR: OR 0.931, $p < 0.001$, 95% CI 0.897–0.966). A

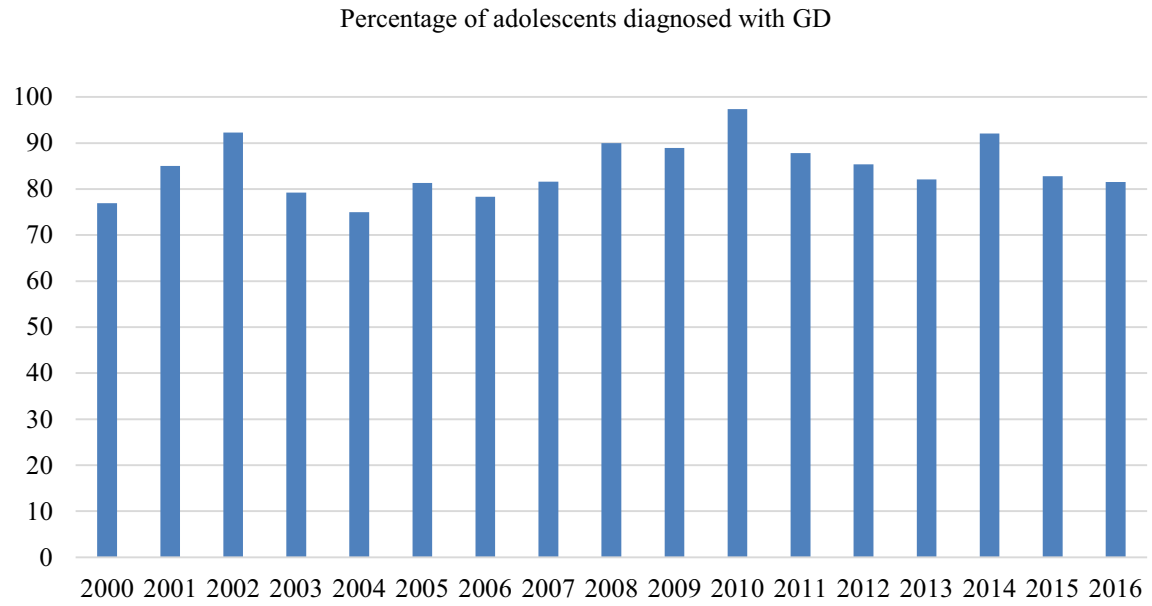

Fig. 3 Percentage of assessed adolescents diagnosed with a GD diagnosis

similar trend was seen in the subsequent analyses with time as a categorical variable. No interactions were found with birth-assigned sex.

Initial regression analyses showed a decreasing trend in the Peer Relation Scale on the CBCL and the YSR (CBCL: β −0.017, $p < 0.001$, 95% CI −0.024 to −0.010 and YSR: β −0.009, p 0.007, 95% CI −0.016 to −0.003), indicating that the quality of the peer relations of adolescents in more recent years was assessed better compared to the quality of the peer relations of adolescents in earlier years. Analyses with time as a categorical variable also showed this trend for the CBCL. On the YSR, however, this decreasing trend was less clear. No interactions were found with sex assigned at birth.

Initial regression analyses showed no trend in time in the score on the Suicidality Scale on the CBCL or the YSR (CBCL: β 0.007, p 0.273, 95% CI −0.005 to 0.019 and YSR: β 0.006, p 0.115, 95% CI −0.001 to 0.013). Again, no interactions were found with sex assigned at birth.

Linear regression analyses showed that the aforementioned decrease over time of the externalizing problems of the adolescents and the improvement of their peer relationships could explain the decrease in the mean total *T*-score on the CBCL.

Gender dysphoria intensity

The intensity of the feeling of dysphoria did not change over time for assigned males at birth (β 0.055, p 0.689, 95% CI −0.214 to 0.323) and assigned females at birth (β −0.015, p 0.835, 95% CI −0.159 to 0.129).

Diagnosis and medical treatment

Initial analyses showed a trend over time of an increasing percentage of adolescents who were diagnosed with a form of GD according to the DSM-IV and DSM-5 (OR 1.117, $p < 0.001$, 95% CI 1.060–1.178). In the subsequent analyses, however, we found that most of the adolescents were diagnosed with a form of GD and that this did not change significantly over time (Fig. 3). No interaction was found with sex assigned at birth.

The initial analyses regarding the actual start of medical treatment after one was diagnosed with GD, showed that over the years, an increasing percentage started with puberty suppression or gender-affirming hormones (OR 1.085, p 0.003, 95% CI 1.028–1.146). In the subsequent analyses, instead of a trend, a fluctuation over the years was seen (Fig. 4). No interaction was found with sex assigned at birth.

Discussion

The present study revealed that despite the sharp increase in the number of adolescent referrals to the Amsterdam transgender clinic in recent years, most demographic and psychological characteristics remained similar over time. Our results also showed that recent referrals were as often diagnosed with GD (mean 84.6%; range 75–97.4%) as early referrals and started just as often with medical treatment including puberty suppression and/or gender affirming hormone treatment (mean 77.7%; range 53.8–94.9%). Despite the fact that GD has become more well known, the mean age

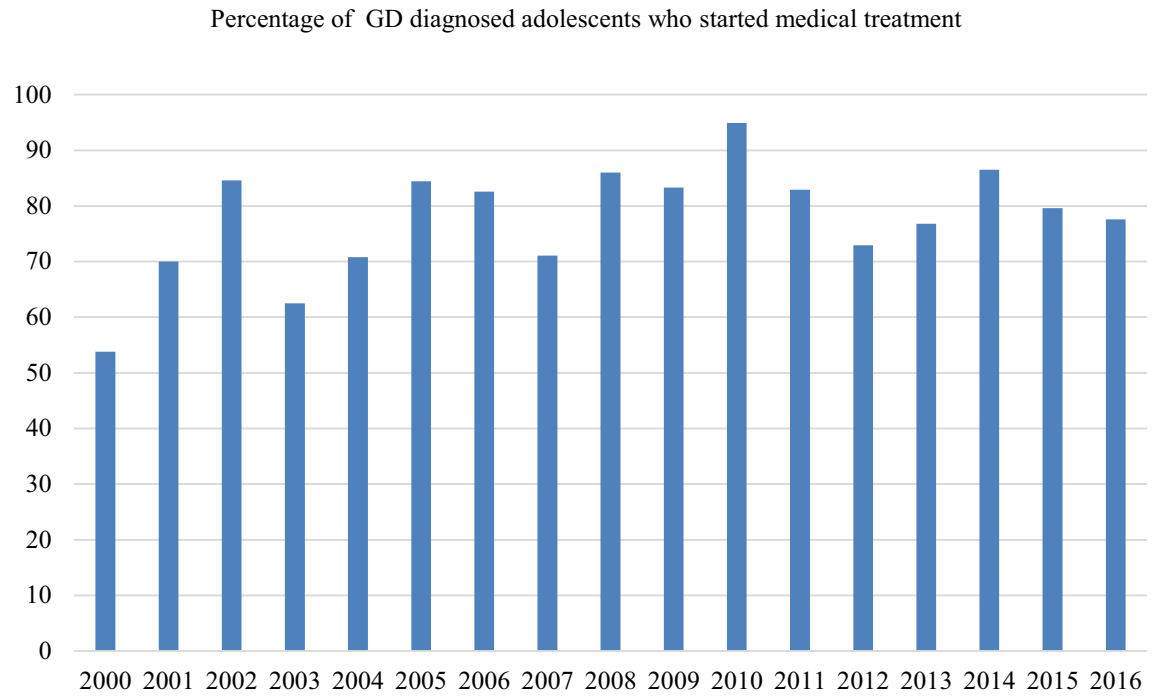

Fig. 4 Percentage of adolescents with a GD diagnosis who started with puberty suppression and/or gender affirming hormonal treatment

at intake of the adolescents did not change over the years. No trends in time were found on the Full-Scale IQ of the adolescents (around 100), parents' marital status (mostly lived with both biological parents) and parents' educational level (mostly practically educated). Therefore, contrary to our hypotheses, early referrals seemed just as good as a reflection of the general society as present referrals are.

In addition, although we hypothesized that is was possible that present referrals had more psychological problems, the opposite seems the case. Our analyses showed that psychological functioning of the referred adolescents improved somewhat over time. This could be explained by a subtle improvement in externalizing problems and better peer relations. It might be the case that it has become easier to openly identify as transgender in recent years, so that recent referrals do not have to stand up as fiercely for themselves as earlier referrals. In addition, they seem more accepted by peers.

Regarding the intensity of the experienced GD, our results showed that adolescents who are referred nowadays experience the same high-level of GD as early referrals did. Therefore, contrary again to what was expected, the percentage of adolescents who were diagnosed with a GD diagnosis did not change over time nor did the percentage of GD diagnosed adolescents who started medical treatment. From this, one might conclude that adolescents who are being referred nowadays are still looking for medical treatment, rather than support and counselling only.

The described findings have clinical implications for providing early medical interventions. Since the assessed adolescents are so similar on most relevant characteristics over the years, this provides confidence that early medical treatment may also be helpful for recent referrals. It is likely that previously found results regarding the effectiveness of the Dutch protocol that includes puberty suppression as part of a multidisciplinary approach [13, 14], can be generalized to the transgender adolescents who currently apply.

Next to our results regarding our hypotheses, in our demographic analyses, there was one changing trend. Although in early years, more assigned boys were referred to gender identity services [29], referred adolescents favour birth-assigned females since the mid-2000s [16, 30]. Our study showed that this shift in sex ratio is further skewed. In 2016, the ratio between birth-assigned males and birth-assigned females was 1:2.93, while in 2015 and 2014, it was 1:2.80 and 1:1.66, respectively. One suggested theory to explain this shift is that it is easier for birth-assigned females to be open about their transgender feelings, since they experience less stigma when they behave masculine than birth-assigned males who behave in a more feminine manner [16, 31–33]. Another possible factor that could play a role in this shift is the difference in pubertal onset between birth-assigned females and birth-assigned males. On average, puberty starts earlier in birth-assigned females than it does in birth-assigned males, which could mean that the feelings of dysphoria with one's body and physical characteristics

intensify earlier in birth-assigned females [16]. However, if this hypothesis was correct, one would expect that birth-assigned females would be referred at a younger age than birth-assigned males, and from our results, it appears that the mean age at intake did not differ between these two sexes.

Our study also provides a new insight into factors that have possibly contributed to the recent increase in the number of adolescent referrals in gender identity services. Since most characteristics remained similar, we suggest that GD might be more common than previously thought and the exponential increase in referrals is just a reflection thereof. The increased publicity and visibility may have helped more young people and their parents to recognize and come out for their transgender feelings, and they seem more likely to dare to seek assessment and treatment.

Our results should be seen in the light of some limitations. First of all, the adolescents in this study are part of a clinic-referred sample. Therefore, the drawn conclusions cannot be generalized to transgender identifying youth from the general population. It could be that outside of our gender identity clinic, changes over time have taken place that we did not observe. Second, some relevant characteristics could not be included in this study since data collection started many years ago when awareness lacked on these. For example, if the identity of the adolescents is exclusively masculine or feminine or that, e.g., non-binary, agender, or gender fluid, would better define their identity. The UGDS is not an ideal instrument to detect these gender identities. In the third place, there may be trends in recent years that we could not yet demonstrate with the data we have. Because the increase in referrals may reflect a catch-up of adolescents experiencing GD, it is likely that this increase will come to a plateau when the catch-up is complete. We would have to repeat the analyses at that point in time to identify any other trends. Finally, this study is a historical cohort study. Thus, we can describe the longitudinal trends we see, but we can only speculate about the causes for these trends.

Conclusion

Our study assured that adolescents referred to a long existing specialized transgender service did not show critical changes in key demographic, psychological, diagnostic, and treatment characteristics over 16 years with the exception of a shift in sex ratio. This may suggest that in the early years, only the tip of the iceberg of the actual number of transgender youth was presented to a transgender clinic and this iceberg has come to surface in recent years. In other words, it seems to be that the increase in the number of referrals is probably due to the fact that feelings of GD are more common than originally expected, rather than that the threshold to register at a transgender clinic has decreased in such extent, that a group of other adolescents is seen nowadays. This finding suggests that a larger group of adolescents who experience GD is able to profit from gender affirming treatment, including puberty suppression.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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Long-term health consequences of central precocious/early puberty (CPP) and treatment with Gn-RH analogue: a short update

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Abstract. *Background:* The relationship between precocious or early puberty and its treatment has received significant research attention, yielding diverse outcomes. This short review aims to comprehensively analyze and summarize research articles to elucidate the potential link between precocious or early pubertal onset (CPP) and crucial health factors. *Methods:* We conducted a systematic review of studies published from January 2000 to March 2023, sourced from databases of Medline, PubMed, Google Scholar and Web of Science. We assessed the relationship between CPP and final adult height (FHt), bone health, reproductive function, body mass index, metabolic and cardiovascular abnormalities, and increased cancer risk. *Results:* Upon reviewing and analyzing selected studies, the following key findings emerged: (a) treating CPP in girls before age 6-7 and in boys before age 9 improves FHt; (b) bone mineral density (BMD) decreases during GnRHa treatment but normalizes afterward, with no lasting effects on peak bone mass during puberty; (c) GnRH treatment does not negatively affect menstrual cycles; however, untreated CPP increases the risk of premature or early-onset menopause; (d) the incidence of PCOS/hyperandrogenemia may be slightly elevated in women with a history of CPP, but overall reproductive function remains largely unaffected; (e) earlier thelarche and menarche may enhance susceptibility to breast carcinogenesis; (f) CPP contributes to an increased risk of obesity and type 2 diabetes in both genders; (g) early menarche may slightly increase the risk of coronary heart disease and ischemic strokes and (h) early pubertal timing increases the risk of depression and anxiety disorders. *Conclusion:* Monitoring and early diagnosis of these conditions are of paramount importance for successful management. (www.actabiomedica.it)

Key words: Central precocious puberty (CPP), final adult height, bone health, reproductive function, breast cancer, metabolic abnormalities, cardiovascular risk

Introduction

Central precocious puberty (CPP) represents an abnormal acceleration of pubertal development, characterized by the premature emergence of secondary sexual characteristics, advanced skeletal maturation, and accelerated physical growth in affected children. CPP not only impacts the final adult height of affected individuals but can also give rise to psychological and behavioral issues, including fear and anxiety (1).

Currently, the gold standard for the treatment of CPP worldwide is the use of gonadotropin-releasing hormone analogs (GnRHa). These agents effectively suppress the hypothalamic-pituitary-gonadal (HPG) axis by desensitizing pituitary gonadotrophs, resulting in regression or stabilization of pubertal symptoms.

Moreover, treatment with GnRHa extends the growth period by reducing growth velocity to prepubertal levels and mitigating bone age advancement (2,3). Clinical and diagnostic evaluation of treatment efficacy involves Tanner staging, linear growth assessment, radiological assessment of bone age maturation, and biochemical analysis through luteinizing hormone (LH) and 17-β estradiol measurements (4,5).

Despite the well-established efficacy of GnRHa therapy, several questions surrounding its long-term consequences and potential side effects persist. Notably, concerns arise regarding its association with conditions such as obesity, metabolic syndrome, bone health, reproductive function, cardiovascular effects, and the risk of breast cancer.

Objectives and methods

In this study, we conducted a systematic review of pertinent literature, encompassing articles available in databases such as Medline, PubMed, Google Scholar, and Web of Science from 2000 to March 2023. Our aim was to explore the relationship between precocious/early puberty and the occurrence of various health-related outcomes, including bone disorders, metabolic abnormalities, reproductive function, and the risk of cancer. A total of 148 research papers, comprising meta-analyses, review articles, randomized controlled trials, and both prospective and retrospective studies, were meticulously reviewed and analyzed (Figure 1).

Results

Precocious/early onset of puberty (EP) in relation to final adult height (Fht)

A review of 24 studies showed that final adult height (FHt) gain in subjects on GnRH treatment varied between 2 and 8 cm. Although treatment of CPP before 6-7 years of age resulted in better gain in the FHt, Yang et al. (6) showed that GnRHa treatment can help to increase FHt even in patients diagnosed with EP after the age of 8 years (6-11).

A study compared data on post-GnRHa treatment course and FHt in 115 girls [22 diagnosed before chronological age of 6 yr; 38 between ages 6 and 8 yr; and 55 early fast puberty (EFP), between ages 8 and 9 yr] treated with GnRHa from Tanner stage

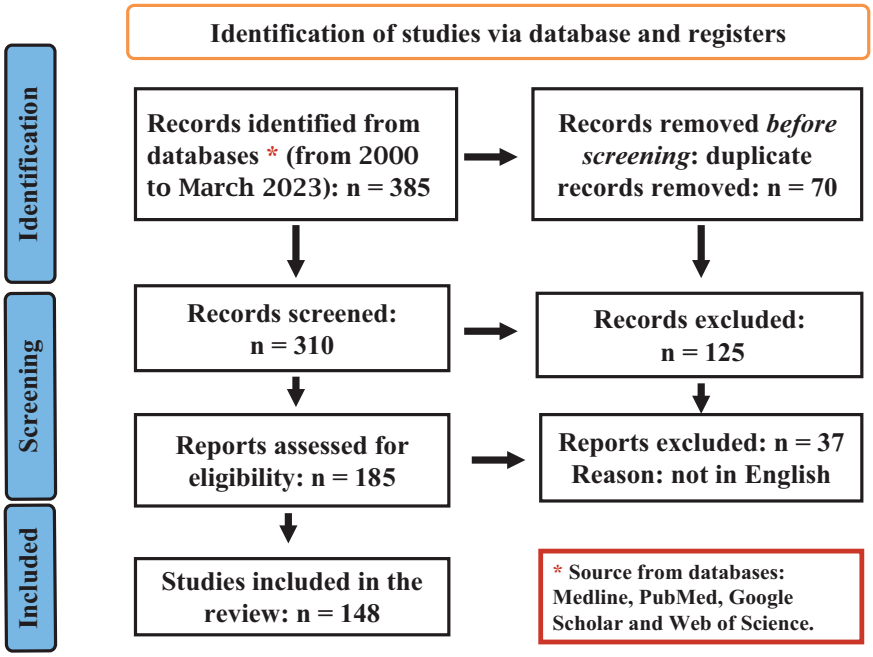

Figure 1. PRISMA chart for the review.

2-3 to chronological age 11-12 yr and bone age 12-12.5 yr. Height gain from cessation of therapy to FHt was greater, and time to epiphyseal fusion was longer in the younger CPP than in the older CPP (P: < 0.05), in those with early fast puberty (P: < 0.001) groups and those with smaller ovarian volume (OV) compared to those with larger OV at diagnosis (11,12).

On the other hand, in a small and long-term randomized control study, GnRHa did not have a significant effect on final adult height in girls with advanced puberty. Two studies by Lazar et al. (11) and Bouvattier et al. (13) analyzed the data of 63 girls and 30 girls respectively who were treated with GnRHa and compared them to untreated girls. They showed that the height gain was similar between the treated and untreated groups.

Pasquino et al reported a large variability of individual responses suggested that one should choose more parameters than increment in height, especially in girls with pubertal onset over 8 yr of age (14). Cheuiche et al. (15) recommended that treatment should be considered in girls before the age of 6 and boys before the age of 9 with progressive CPP, while in girls between 6 and 8 years the treatment decision is individualized.

Early onset of puberty in relation to adult bone health

Peak bone mass (PBM), shares similarities with pubertal timing (physiological variability, genetic influence and environmental aspects). Factors influencing pubertal timing significantly affect bone acquisition. Analysis of the 20 research articles revealed the following:

a. Epidemiological studies show that women with early menarche are at higher risk of premature and early menopause.
b. The Gothenburg Osteoporosis study demonstrates a negative association between age at peak height velocity (PHV) and bone mineral density (BMD) in young men (16). Age at PHV predicts fractures, independently of risk factors, such as: birth weight, childhood body mass index (BMI) and adult height. The National Health and Nutrition Examination Survey (NHANES) data reveals that late menarche ≥16 years is associated with lower lumbar spine (LS) BMD, even after adjusting for confounding factors (16,17).

   An age at menarche ≥16 years was associated with lower lumbar spine BMD compared to those with a menarche age ≤12 years, while another study found that late puberty was associated with increased risk for adult fracture (18,19). A longitudinal study in girls with CPP showed an increased BMD, but this advantage waned when corrected for their advanced bone age. In another study GnRHa treatment seemed to have no detrimental effect on BMD (20).
c. Longitudinal studies on British participants suggested that male subjects gained BMD faster than females and confirmed that late pubertal age is associated with persistently lower BMD in both genders (21). Studies analyzing GnRHa treatment in children with CPP revealed variable results. Some suggested a reversible reduction in BMD during treatment that improved when using calcium and vitamin D supplementations (22-25). Others find spontaneous restoration of bone mass 2-3 years after cessation of therapy and one study showed structural bone changes with treatment (20, 26-32).

Patients with CPP have increased expression of carboxy-terminal telopeptide of type 1 collagen (ICTP), a bone resorption marker, and procollagen type 1 C-terminal propeptide (PICP), a bone formation marker, before GnRHa treatment that decreases during a 6-month treatment period and stabilizes after treatment. Bone age-adjusted bone turnover markers normalized 2 years after treatment cessation (33,34).

In summary, some studies indicate associations between GnRHa treatment and decreased BMD while others emphasize good potential for recovery post-treatment cessation. Bone mineral density decreases during GnRHa treatment but recovers to normal afterward, and peak bone mass formation through bone mineral accretion during puberty is not affected.

Early/precocious puberty: Effects on menarche, menstrual status, and onset of menopause

The impact of GnRHa treatment on the timing of menarche, menstrual cycles, and onset of menopause in girls with early/precocious puberty is of clinical interest. Ten studies were reviewed and analyzed.

Three long-term studies demonstrated that pubertal reactions in CPP patients were restored within 1 year, with most recovering within 6 months after treatment discontinuation. After GnRHa treatment cessation, the onset of menarche required an average interval of 0.9-1.5 years, with menarche starting around 12.6-13.6 years old chronologically (27, 35-37).

In a Korean study (38), menarche began approximately 14 months post-GnRHa treatment, corresponding to 11.9 years in chronological age and 12.8 years in bone age (similar to normal girls' average age at menarche). The age at CPP diagnosis (< 6, 6-8, or 8-9) did not significantly impact the prepuberty period or onset of menarche post-treatment.

In another study, among CPP patients, GnRHa-treated individuals exhibited an average age at menarche of 12 years, showing a delay compared to the untreated group's age of 9.6 years (39).

A study reported regular menstrual cycles in the majority (82/87) of GnRHa-treated patients, with few (5/87) experiencing oligomenorrhea due to excessive exercise, which improved after exercise control (27). Other investigators found that in adulthood, 80% of CPP patients who received GnRHa treatment maintained regular menstrual cycles (39).

A long-term follow-up study reported that patients treated with GnRHa treated subjects presented regular menstrual cycles and no breast or uterine disorders, after an average of 12.5 years post-treatment (37).

A pooled study of postmenopausal women, from different countries, indicated that early menarche (≤11 years) is a risk factor for both premature or early menopause (<40 and 40–44 years, respectively) (40).

In addition, random-effect meta-analysis of six studies observed a pooled risk ratio (RR) estimate of 4.71 (95% CI 2.81–7.90) for the combined association of early menarche and nulliparity with premature menopause (41).

In summary, GnRHa treatment does not seem to negatively affect the history of menstrual cycles. Nevertheless, untreated early puberty increases the risk of premature or early-onset menopause, which can potentially be mitigated by GnRHa therapy.

The impact of GNRH analog treatment on reproductive function of girls with precocious/early puberty

When discussing GnRHa treatment for CPP, one of the common concerns raised by parents pertains to its potential long-term effects on fertility.

The development of signs and symptoms of polycystic ovarian syndrome (PCOS) in former CPP women is controversial. In a review article analysis of 14 studies focusing on reproductive function, PCOS morphology detected by ultrasound (US) was reported in 0–37% of treated CPP girls (median 2%) with different lengths of post-treatment follow-up (up to 20 years) (42). A similar percentage (2%) was reported by Heger et al. (43).

In contrast, another study involving 46 young women (average age 18.1 years) treated with GnRHa during childhood revealed that 32% had PCO, with 28% exhibiting clinical hyperandrogenism, and 48% showing biochemical hyperandrogenism (44).

Lazar et al. (45) reported that the clinical signs of hyperandrogenism (acne/hirsutism with oligomenorrhea) were more frequent in CPP women than in controls with normal puberty, matched for age and year of birth but not for BMI. The relative risk for the development of clinical hyperandrogenism with irregular menses was twofold higher in the untreated than the treated group. The reproductive outcome in early and mid-adulthood was normal in the majority of the patients studied. A high prevalence of fertility problems was present in the untreated CPP group only, suggesting that GnRHa therapy may have a protective effect on the reproductive outcome.

Another study indicated a significant correlation between GnRHa treatment and PCOS (P: 0.03). Specifically, 36% of girls previously treated with GnRHa for CPP developed PCOS, compared to 14.5% of untreated girls with CPP (46). However, contrasting findings were reported in other studies. No statistically significant difference in the prevalence of

PCOS was in 2751 women treated with GnRHa versus non treated subjects. The overall prevalence of PCOS was 19.6%. (47) Similarly, the overall prevalence of either clinical or laboratory hyper-androgenism was 29.4% and 33.3% for the treatment and non-treatment groups, respectively (48). Another report showed a significant incidence of PCOS in former CPP patients, with a lower prevalence of PCOS in GnRHa-treated girls than in nontreated girls [17.2% (n = 33) vs. 30.8% (n = 14)]. Elevated DHEAS and androstenedione concentrations occurred in 56% of those receiving GnRHa versus 23.6% among those who did not (49).

Four reports did not establish a clear link between CPP, whether treated or untreated, and the development of PCOS (46,50,51). A longitudinal study of 20 CPP patients who reached adulthood revealed that 7 patients achieved a total of 12 pregnancies, with normal childbirth experiences (52).

The PREFER (PREcocious puberty, FERtility) study prospectively analyzed fertility, via a series of questionnaires, in women treated during childhood with triptorelin (depot formulation) for CPP. Most pregnancies (84.4%, 95% CI [67.2–94.7%]) occurred within 1 year of trying to conceive, in line with the waiting time to pregnancy (WTP) for women without previous CPP (53).

Demographic data analysis of 214 CPP women (135 GnRHa-treated and 61 untreated) compared to controls with normal puberty showed that both treated CPP and control groups achieved spontaneous pregnancy at similar rates (90.4% vs. 90.2%). (53) However, untreated CPP subjects were associated with higher fertility problems and assisted fertilization rates (45).

In summary, while the incidence of PCOS/hyperandrogenemia may be slightly elevated in women with a history of CPP, their overall reproductive function remains largely unaffected.

The association between precocious/early puberty and the risk for breast cancer

A comprehensive analysis of 117 epidemiological studies, involving 118,964 women with invasive breast cancer and 306,091 controls, demonstrated that younger age at menarche correlated with an increased breast cancer risk and each year younger at menarche was associated with a 5% increase in relative breast cancer risk (54). Several other studies have confirmed these observations (55- 60).

Sister-matched case-control research involving 1,406 women diagnosed with breast cancer before the age of 50 years and 1,648 control subjects reaffirmed the relationship between older age at menarche and reduced young-onset of breast cancer risk (61). A study involving 1,811 pairs of female twins with breast cancer demonstrated that the twin with earlier puberty was more likely to receive an earlier diagnosis of breast cancer (62). A case-control study of 237 breast cancer cases and 237 age-matched controls found that early menarche (OR = 1.60, 95% CI: 1.08–2.38) significantly heightened breast cancer risk (63).

A prospective US cohort study on women aged 35–74 years without breast cancer history but with a diagnosed sister revealed that early pubertal development ages at thelarche and menarche was positively associated with breast cancer risk. A 30% higher risk of breast cancer compared to those without these risk factors (64,65).

In a Japanese study, age at menarche was somewhat more closely associated with the risk of progesterone receptor-negative than positive breast cancer (66). On the contrary, other cohort and meta-analysis studies did not find a relation between early puberty and the risk of breast cancer (66-70).

In summary, the collective evidence supports the notion that earlier ages at thelarche, and menarche may heighten susceptibility to breast carcinogenesis and may add another potential advantage to hormonal suppressive therapy.

The relationship between precocious/early puberty and obesity associated to metabolic abnormalities

The impact of early-onset puberty on the development of obesity, overweight and metabolic abnormalities has been the subject of extensive research with variable outcomes. (71,72).

The Gothenburg osteoporosis and obesity determinants study followed 579 subjects with early pubertal onset. The Authors demonstrate that early pubertal onset in males predicts a central fat mass distribution, while a predominantly subcutaneous obese phenotype is strongly predicted by a high prepubertal BMI (73).

The long-term community-based Bogalusa Heart Study revealed that girls with an history of early menarche presented a significant higher BMI, skinfold thickness, fasting insulin and insulin resistance (HOMA-IR) in childhood and adulthood (74).

A twin cohort analysis documented a strong heritability in the age at the onset of the pubertal growth spurt and adult height. These traits were found to be associated with childhood BMI and early adulthood stature due to shared genetic factors (75). A Finnish cohort study supported a strong genetic correlation between early puberty and higher childhood BMI, especially in girls (76).

A meta-analysis of 34 studies demonstrated that early menarche correlated with increased adult BMI, while late menarche correlated with decreased BMI (77). Another meta-analysis, including 28 studies, found that earlier age at menarche was associated with a higher risk of type 2 diabetes mellitus (T2DM) even after adjusting the results with adiposity (78).

A Swedish study of 30,697 men demonstrated that earlier pubertal onset was linked to a higher risk of early diabetes and early need for insulin therapy even after BMI adjustments, while late puberty correlated with reduced diabetes risk (79).

An analysis of the Nurses' Health Study cohorts showed that early menarche was associated with an increased risk of T2DM. Adiposity partly mediated this association (80).

A meta-analysis of 28 observational studies (N = 1,228,306) reported that without adjustment for adult adiposity, impaired glucose tolerance and T2DM risk was higher for early versus later menarche (78).

Moreover, in a UK Biobank study, it was reported that in women and men earlier puberty timing was associated with higher risks for angina, hypertension and T2DM (81).

A study performed in Brazil, involving 8,075 women, demonstrated that early menarche was linked to a higher risk of diabetes, even after controlling for socio-demographic factors and maternal diabetes (82).

A small study on girls with CPP reported worse lipid profiles and lower insulin sensitivity at diagnosis with further worsening of the metabolic profile during GnRHa treatment. Another report also showed increased insulin resistance during GnRHa treatment (83,84). However, a case-control study of the incidence of obesity and obesity-related metabolic outcomes in former CPP GnRHa-treated and -untreated women did not differ from the age-matched control group, reassuring the health status of adult former CPP women (45, 85).

Collectively, early puberty appears to contribute to an increased risk of obesity and T2DM in both men and women, often mediated by factors such as childhood BMI and genetic predisposition. Monitoring and early diagnosing of these conditions are important for successful management.

Early puberty in relation to coronary heart disease (CHD) and stroke

Early menarche is generally associated with an increased risk of coronary heart disease (CHD) and stroke. Six research articles were reviewed (86- 89). Early menarche increased the risk of ischemic stroke regardless of age at menopause and reproductive span in contrast to the associations between these factors and myocardial infarction (90).

In a population-based retrospective cohort study from the National Health Insurance Service database of Korea, including a total of 1,224,547 postmenopausal women, a U-shaped association between age at menarche and the risk of ischemic stroke (IS) was found, with a 16% higher risk in early menarche group (≤ 12 years). Women who had undergone early menarche and had a short reproductive span had the highest risk of IS, perhaps due to the combined effect of improper timing of estrogen production and insufficient estrogen exposure (91.92).

In summary, early menarche may increase the risk of coronary heart disease and ischemic strokes.

Psychological aspects of early puberty and treatment

Children with CPP may experience significant behavioral, social, and emotional problems. They may also face different social pressures of fitting in their body's development before peers.

Recent studies have found an increase of social anxiety, depressive and externalizing symptoms, and risk of psychopathology, in association with physical

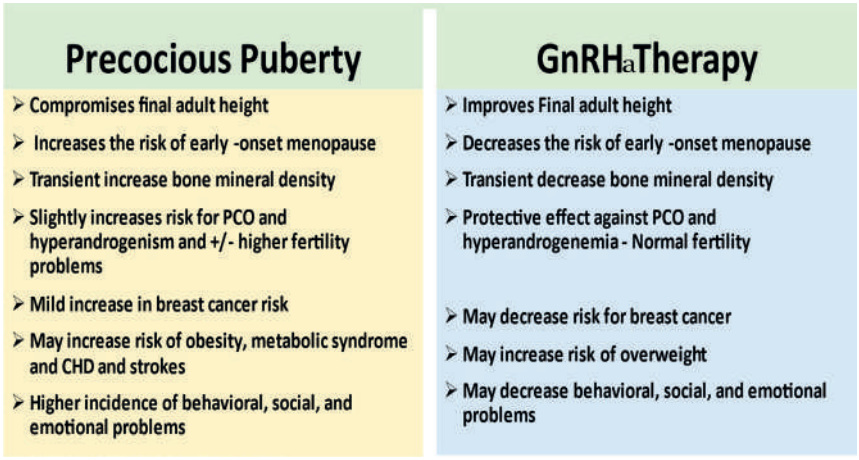

Figure 2. Consequences of precocious puberty in relation to GnRHa treatment.

changes secondary to early pubertal maturation, mainly in females (93-99). Therefore, these signs and symptoms should be taken in deep consideration as an additional indication for GnRHa treatment (100- 102).

In summary, findings across all studies revealed that early pubertal timing served as a transdiagnostic risk factor for depression and anxiety disorders, requiring GnRHa treatment in selected cases.

Conclusions

The therapeutic approach for the treatment of children with CPP using various GnRHa preparations has rapidly evolved. While the efficacy and safety of these formulations in suppressing puberty and slowing down the pubertal growth spurt appear to be acceptable, several unresolved questions persist regarding the clinical management of affected children. These areas of concern include the long-term effects of precocious/early puberty and the effects of GnRHa. Despite their relatively low risk, there is a need for vigilant and long-term monitoring of these patients. Therefore, any effort directed to establish the optimal strategy for monitoring treatment is imperative (Figure 2).

Conflict of Interest Statement: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Gonadal Suppressive and Cross-Sex Hormone Therapy for Gender Dysphoria in Adolescents and Adults

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Individuals with gender dysphoria experience distress associated with incongruence between their biologic sex and their identified gender. Gender dysphoric natal males receive treatment with antiandrogens and estrogens to become feminized (transsexual females), whereas natal females with gender dysphoria receive treatment with androgens to become masculinized (transsexual males). Because of the permanence associated with cross-sex hormone therapy (CSHT), adolescents diagnosed with gender dysphoria receive gonadotropin-releasing hormone analogs to suppress puberty. High rates of depression and suicide are linked to social marginalization and barriers to care. Behavior, emotional problems, depressive symptoms, and global functioning improve in adolescents receiving puberty suppression therapy. Gender dysphoria, psychological symptoms, quality of life, and sexual function improve in adults who receive CSHT. Within the first 6 months of CSHT, changes in transsexual females include breast growth, decreased testicular volume, and decreased spontaneous erections, and changes in transsexual males include cessation of menses, breast atrophy, clitoral enlargement, and voice deepening. Both transsexual females and males experience changes in body fat redistribution, muscle mass, and hair growth. Desired effects from CSHT can take between 3 and 5 years; however, effects that occur during puberty, such as voice deepening and skeletal structure changes, cannot be reversed with CSHT. Decreased sexual desire is a greater concern in transsexual females than in transsexual males, with testosterone concentrations linked to sexual desire in both. Regarding CSHT safety, bone mineral density is preserved with adequate hormone supplementation, but long-term fracture risk has not been studied. The transition away from high-dose traditional regimens is tied to a lower risk of venous thromboembolism and cardiovascular disease, but data quality is poor. Breast cancer has been reported in both transsexual males and females, but preliminary data suggest that CSHT does not increase the risk. Cancer screenings for individuals of both natal and transitioned sexes should occur as recommended. More long-term studies are needed to ensure that CSHT regimens with the best outcomes can continue to be prescribed for the transsexual population.

KEY WORDS gender dysphoria, cross-sex hormone therapy, transsexualism, puberty suppression, transsexual male, transsexual female, women's health.

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Hormone therapy has helped individuals alter their physical appearance toward a desired gender since the first half of the 20th century. The first professional organization devoted to the topic—the Harry Benjamin International Gender

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Dysphoria Association—was established in 1979 but is now known as the World Professional Association of Transgender Health (WPATH).[1, 2] The most recent WPATH standards of care were updated in 2011, and practice guidelines were developed by the Endocrine Society in 2009.[1, 2]

According to the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-V), individuals whose assigned gender at the time of birth is not the gender with which they identify or experience are considered to have gender dysphoria.[3] This designation was traditionally referred to as transgender or gender identity disorder. This updated term was intended to better characterize the experience of affected children, adolescents, and adults and to remove the pathology attached to gender nonconformity, a common expression of gender dysphoria that has been linked to social stigma. By using the term *gender dysphoria,* the focus is placed on the psychological distress caused by the discrepancy between natal sex and gender identity.[4] Available published research may use these terms interchangeably. For the purpose of this review and in accordance with currently accepted terminology, a transsexual male is an individual with the primary and/or secondary sex characteristics of a natal female who desires to take on male characteristics.[1] Alternatively, a transsexual female is an individual with the primary and/or secondary sex characteristics of a natal male who desires to take on female characteristics. Table 1 lists definitions of these and additional terms related to gender dysphoria and transsexualism.

The incidence and prevalence of gender dysphoria is currently unknown due to underreporting and variations in cultural expression of gender. The available data therefore come from studies of transsexual individuals who have received treatment for gender dysphoria in the form of cross-sex hormone therapy (CSHT) and/or sex reassignment surgery (SRS). From research conducted in 10 studies from eight predominantly European countries, the prevalence of transsexualism is estimated to be between 1 in 11,900 and 1 in 45,000 individuals for transsexual females and between 1 in 30,400 and 1 in 200,000 individuals for transsexual males.[1]

The diagnosis of gender dysphoria should be made by a mental health professional before medical interventions are considered.[2] Those seeking sex reassignment are advised to live as the desired sex for a minimum of 1 year before CSHT or surgical intervention is used. For those who are ready and eligible to initiate a gender transition, CSHT is the cornerstone of management. It involves the administration of androgen therapy for transsexual males and a combination of estrogens and antiandrogen therapy for transsexual females.[2] In adolescents, CSHT and SRS are commonly delayed until later in adolescence when informed decisions regarding the use of CSHT, which can cause irreversible physical changes, can be made.[5] Gonadotropin-releasing hormone (GnRH) analogs are used to block the release of sex hormones, thereby halting further pubertal development. It is important to note that the decision to withhold therapy is not a neutral option because of the potential psychological consequences associated with progressing puberty.[1] A review of the CSHT and puberty suppression therapies included in the transsexualism literature and recommended in guidelines are included in Table 2.

From a recent cohort study of individuals who sought sex reassignment between 1973 and 2003, after adjustment for psychiatric morbidity, the all-cause mortality rate in transsexual individuals was found to be significantly increased compared with age-matched controls (adjusted hazard ratio [HR] 2.8, 95% confidence interval [CI] 1.8–4.3).[8] Adjusted HRs for mortality were no longer significant when only the years 1989–2003 were evaluated (adjusted HR 1.9, 95% CI 0.7–5.0). Another recent study of 1331 transsexual individuals receiving care from a gender clinic between 1975 and 2009 reported significantly increased mortality in transsexual females (standardized mortality ratio 1.51, 95% CI 1.47–1.55) but not in transsexual males (standardized mortality ratio 1.12, 95% CI 0.87–1.42).[7] Increased mortality has been linked to suicide, acquired immunodeficiency syndrome (AIDS), cardiovascular disease, and substance abuse.[7, 8] Improved care is believed to be the factor responsible for the nonsignificant adjusted HR for mortality after the 1990s; however, both studies were conducted in countries where medical care for gender dysphoria is more widely available (Sweden and the Netherlands).[8]

Transsexual individuals experience frequent discrimination and social marginalization that puts them at risk for mental health problems, abuse or neglect, and economic hardship.[1] Major barriers to care include lack of access to health care providers knowledgeable about the needs of transsexual individuals, lack of available transgender medicine specialists to receive referrals

Table 1. Gender Dysphoria and Sex Reassignment Terminology

Terminology	Definition
Gender identity	A person's intrinsic sense of being male (a boy or a man), female (a girl or a woman), or an alternative gender (e.g., boygirl, girlboy, transgender, genderqueer, eunuch)[1]
Gender nonconformity	The extent to which a person's gender identity, role, or expression differs from the cultural norms prescribed for people of a particular sex[1]
Gender dysphoria (formerly known as gender identity disorder)	Discomfort or distress that is caused by a discrepancy between a person's gender identity and that person's sex assigned at birth (and the associated gender role and/or primary and secondary sex characteristics)[1]
Sex	Attributes that characterize biologic maleness or femaleness; the best known attributes include the sex-determining genes, the sex chromosomes, internal and external genitalia, and secondary sex characteristics[2]
Natal male or natal female	The sex associated with the individual at birth based on genes, sex chromosomes, genitalia, and/or secondary sex characteristics[1]
Transgender	A diverse group of individuals who cross or transcend culturally defined categories of gender. The gender identity of transgender people differs to varying degrees from the sex they were assigned at birth[1]
Transsexual male or transsexual female (formerly known as female-to-male individuals and male-to-female individuals, respectively)	Individuals who seek to change or who have changed their primary and/or secondary sex characteristics through feminizing or masculinizing medical interventions (hormones and/or surgery), typically accompanied by a permanent change in gender role[1]
Sex reassignment	The complete treatment procedure for those who want to adapt their bodies to the desired sex[2]
Cross-sex hormone therapy	Pharmacologic compounds or regimens designed to induce secondary sex characteristic development associated with the desired sex and to diminish characteristics associated with the natal sex[5]
Sex reassignment surgery	Surgery to change primary and/or secondary sex characteristics to affirm a person's gender identity[1]

from other health care providers, and lack of health insurance coverage for therapies and/or procedures.[1, 9] Barriers to care may also be as subtle as having a health care setting ill adapted to the needs of transsexual individuals. Examples can include assigning nonprivate hospital rooms by natal gender; providing limited options for selecting gender on intake forms (male/female); denying insurance reimbursement for disease screenings based on gender (e.g., no breast cancer screening for natal males); and failing to develop, disseminate, and enforce policies against gender discrimination.[9] Psychological distress is a product of the environment and not inherent to being transsexual or gender nonconforming, so it is important to be sensitive to the impact associated with the quality of care being provided.[1] The use of self-acquired hormone therapy and genital surgery performed outside of a health care setting are also widely reported, adding to the risk associated with barriers to care.[10]

The initial assessment process should be conducted by a mental health practitioner with knowledge of gender-nonconforming identities and the expression of those identities. Although this would seem to create a sizable barrier to care, any health care professional culturally competent in gender identity issues can assist a patient with accessing care and support. The ability to identify someone with gender dysphoria who is in crisis can have a significant impact on morbidity and mortality in these individuals. Pharmacotherapy should ideally be initiated in a comprehensive and coordinated primary care setting specializing in gender dysphoria (i.e., a transgender health clinic), but long-term monitoring for adverse effects related to CSHT can be facilitated by a variety of health care providers in a variety of primary care settings with access to expertise in transgender health for more complex issues.[1]

Expressions of gender nonconformity in response to gender dysphoria exist on a spectrum.[4] Some individuals opt for early initiation of CSHT followed by SRS involving augmentation mammoplasty, penectomy, orchiectomy, vaginoplasty, clitoroplasty, or vulvoplasty in transsexual females or subcutaneous mastectomy, hysterectomy, salpingo-oophorectomy, metoidioplasty, phalloplasty, penile/scrotal prosthesis implantation, vaginectomy, or scrotoplasty in transsexual males.[4] Others prefer to manage their gender dysphoria with drug therapy alone

Table 2. Hormone-Suppressive and Cross-Sex Hormone Therapy Regimens[2, 4, 6, 7]

Hormone-suppressive therapy	Mechanism of action	Practical experience
Leuprolide 3.75–7 mg i.m. every month Histrelin implant 50 μg/day released over a period of 12 mo Triptorelin 3.75 mg i.m. every month Goserelin acetate 3.8 mg s.c. every 4 wks	GnRH agonists initially increase FSH and LH release followed by a cessation of release of these pituitary hormones through a negative feedback mechanism that desensitizes the gonadotropic cells of the pituitary to endogenous GnRH stimulation; the result is a cessation of FSH and LH release from the pituitary, a cessation of testosterone and dihydrotestosterone release from the testes in males and a cessation of estradiol and estrone release from the ovaries in females	Primary indications include prostate cancer, endometriosis, and precocious puberty. Reproductive studies in animals involving GnRH agonist exposure have identified adverse events, and it is recommended that pregnancy be excluded before therapy initiation in reproductive-age females. Hot flashes or flushes occur in up to 40–77% of patients who receive GnRH agonists, but they are not often associated with discontinuation of therapy. Decreased bone density is associated with long-term GnRH agonist therapy in men, but most of this research involves women receiving this therapy for endometriosis. Loss in bone mineral density is minor but may not be completely irreversible on discontinuation. In women with endometriosis, norethindrone is used as "add-back" therapy to reduce the loss of bone mineral density. It is used in early adolescence to inhibit physical changes associated with puberty in males and females with gender dysphoria. It is also used in combination with estrogen therapy in transsexual females to block the effects of endogenous testosterone on physical appearance.
Spironolactone 100–200 mg/day p.o.	Weak inhibitor of testosterone synthesis and testosterone receptor binding; the inhibition of 17-α hydroxylase inhibits testosterone synthesis but can also result in an increase of progesterone through inhibition of the same enzyme; inhibits aldosterone in the distal renal tubules	It has been used for many cardiovascular and endocrine indications including hirsutism in women and precocious puberty in children. Dehydration is a concern in patients taking diuretics; hyperkalemia is a concern when combined with potassium supplements and other potassium-sparing medications. It is used in combination with estrogen therapy in transsexual women for the primary purpose of blocking effects of endogenous androgens on physical appearance.
Cyproterone acetate 50–100 mg/day p.o.	Cyproterone acetate is also an antiandrogen that works through competitive dihydrotestosterone antagonism at the level of target tissues; it also has progestational activity that results in an inhibition of LH and FSH release from the pituitary, thereby causing a decrease in testosterone concentrations	Primary indications include hirsutism and prostate cancer. It is not available in the United States but it has been used for decades in other countries for its androgen-blocking properties in transsexual women. It has also been used in combination with spironolactone.
Finasteride 5 mg/day p.o.	Inhibits 5α-reductase, which metabolizes testosterone to DHT in the prostate and other tissues; this inhibition leads to significant reductions in serum and tissue DHT	Indications for finasteride are benign prostatic hyperplasia and male pattern baldness. Use in transsexual females blocks the effects of endogenous testosterone, providing favorable effects on scalp hair loss, body hair growth, and skin consistency. Safety concerns include teratogenicity in pregnant women. Increased risk of high-grade prostate cancer has been reported due to its ability to lower PSA.

(continued)

Table 2. (continued)

Hormone-suppressive therapy	Mechanism of action	Practical experience
Cross-sex hormone therapy for transsexual females		
Estradiol valerate 2–6 mg/day p.o. 17β-estradiol 2–6 mg/day p.o.	Estrogen preparations are synthetic derivatives of estrogen hormones that are principally secreted by the ovarian follicles, adrenals, corpus luteum, placenta, and testes; estrogens play an important role in the reproductive, skeletal, and cardiovascular systems, as well as the central nervous system of natal women; estradiol is the most active endogenous estrogen, and ethinyl estradiol is the most potent of the synthetic estrogens, with 15–20 times more activity than estradiol; estradiol valerate has a duration of action of 14–21 days, and estradiol cypionate has a duration of action of 14–28 days; exogenous estrogens can elicit a variety of effects that are all the pharmacologic responses typically produced by endogenous estrogens; endogenous estrogens are essential for normal growth and development of the female sex organs and maintenance of secondary sex characteristics; the exact mechanism has not been established, but estrogen is known to contribute to the shaping of body contours and the skeleton	Indications for oral, transdermal, or topical preparations of estradiol are management of moderate to severe vasomotor symptoms associated with menopause and management of vulvar and vaginal atrophy. Transdermal and oral estradiol can be used in the treatment of female hypoestrogenism due to hypogonadism, castration, or primary ovarian failure. Use in transsexual females achieves two goals of CSHT: reduction of endogenous hormone levels and secondary sex characteristics of the natal sex, and the replacement of the endogenous sex hormones levels with the reassigned or desired sex by using hormone replacement using principles from the treatment of hypogonadal patients. Safety concerns include thromboembolism and cardiovascular complications; thus transsexual females are highly encouraged to undergo tobacco cessation to decrease overall risk. Metabolic screening should be conducted to determine risk of adverse effects associated with CSHT including hypertension, dyslipidemia, diabetes mellitus, osteoporosis, hepatic function, and prolactin levels to assess for prolactinoma.
Estradiol valerate 5–20 mg i.m. every 2 wks		Estradiol valerate is indicated for management of moderate to severe vasomotor symptoms associated with menopause as well as management of vulvar and vaginal atrophy, female hypogonadism and castration, and primary ovarian failure. Safety concerns include risk of adverse cardiovascular effects.
Estradiol cypionate 2–10 mg i.m. every week		Estradiol cypionate is indicated for management of moderate to severe vasomotor symptoms associated with menopause and female hypogonadism.

(continued)

Table 2. (continued)

Hormone-suppressive therapy	Mechanism of action	Practical experience
Cross-sex hormone therapy for transsexual males		
Testosterone undecanoate 160–240 mg/day p.o. Testosterone enanthate or cypionate 100–250 mg i.m. every 2–3 wks (or one-half the dose every week) Testosterone undecanoate 1000 mg i.m. every 12 wks Testosterone gel 1% 2.5–10 g/day Testosterone patch 2.5–7.5 mg/day	Testosterone is an androgen sex hormone responsible for the development of male growth and masculine characteristics; testosterone is primarily secreted by the testicles in natal males and is considered to be the principal endogenous androgen and naturally occurring androgenic anabolic steroid; through feedback inhibition of LH, exogenous administration of testosterone inhibits the release of endogenous testosterone; used for replacement or substitution purposes due to the absence of testicular hormone	Indications for testosterone preparations currently include treatment of men who lack or have low testosterone levels associated with a medical condition; these conditions include hypogonadism, male climacteric or andropause, delayed puberty, corticosteroid-induced hypogonadism and osteoporosis, and erectile dysfunction. Indications in females include inoperable carcinoma of the breast, postpartum breast pain and engorgement, and menopause. Testosterone is also used in male transsexuals, similar to its use in hypogonadal males. Safety concerns include increased red blood cell mass that can increase the risk for thromboembolic events; changes in serum lipid levels; hypercalcemia; cardiac dysfunction including edema with or without congestive heart failure; and renal and hepatic dysfunction. Testosterone is contraindicated in hormone-dependent tumors including known or suspected carcinoma of the prostate or breast. Increases in PSA levels have been seen in older males ($\geq$ 50 yrs of age) that could also increase the risk of prostate cancer. Reports of hepatocellular carcinoma have occurred in patients receiving long-term therapy with high doses of testosterone.

CSHT = cross-sex hormone therapy; DHT = dihydrotestosterone; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; i.m. = intramuscular; LH = luteinizing hormone; p.o. = orally; PSA = prostate-specific antigen; s.c. = subcutaneous.

or drug therapy plus a major or minor surgery. Goals of treatment should be highly individualized without presumptions about future needs or concerns.

This review summarizes the efficacy and safety surrounding the CSHT used to manage gender dysphoria in adolescents and adults. In addition, the safety and efficacy surrounding the medications used to inhibit endogenous hormone release in this population are discussed.

Pediatric Considerations

In children and adolescents with gender dysphoria, the process of identifying and managing the condition is markedly different. Cases of gender dysphoria that present during childhood persist into adulthood 6–27% of the time.[1] Presentation is heterogeneous in childhood, with some children exhibiting extreme gender-nonconforming behaviors accompanied by severe discomfort and other children showing less intense characteristics. However, adolescents with gender dysphoria have considerably higher rates of persistence, with the physical changes of puberty intensifying body aversion. Not all adolescents with gender dysphoria experience symptoms in early childhood, but those who do often present with more extreme gender nonconformity. The prevalence of gender dysphoria in adolescence is not currently known due to sampling difficulty associated with this population. Externalizing comorbidities such as anxiety and depression are common as is autism spectrum disorder in children and adolescents with gender dysphoria. For this reason, the diagnosis should be made by a mental health professional with child and adolescent developmental psychopathology training.[2] After children or adolescents have met the DSM-V criteria for gender dysphoria, according to practice guidelines, they must have experienced at least Tanner stage 2 puberty with an associated worsening of gender dysphoria.[2] In girls, Tanner stage 2 is characterized by the development of breast buds, areola enlargement, and sparse pubic hair.[11] In boys, Tanner stage 2 is associated with initial enlargement of the scrotum and testes and the appearance of sparse pubic hair at the base of the penis.[12] At this point, practice guidelines describe eligibility for puberty suppression therapy, assuming the child or adolescent will receive adequate social and psychological support during treatment.[2]

An approach used since at least the late 1990s in the Netherlands involves the administration of GnRH analogs to adolescents as a means to delay the onset of puberty. The primary purpose of this approach has been to alleviate the negative psychological effects associated with the onset of puberty in gender dysphoric adolescents. Current clinical practice guidelines advocate for the use of long-acting GnRH analogs for adolescents fulfilling readiness criteria for sex reassignment.[2] In addition to the psychological benefits, delaying puberty until CSHT can be started will likely result in more satisfactory physical outcomes.[13] Delaying pubertal development in males prevents the accentuation of brow, zygoma, and mandible bones; Adam's apple development and unwanted phallic growth are also prevented. Progression of the permanent changes in voice and hair growth patterns will also cease. When cross-gender hormone therapy is begun in the early stages of puberty, increases in breast tissue and testicular volume can regress.[14] When started later in adolescence, late stages of breast development, voice changes, and facial hair development will not regress completely. A premature fusion of the growth plates in response to estrogen administration to transsexual females results in a lower final adult height more consistent with the height of natal females. A delay in the fusion of the growth plate in transsexual males can conversely allow for an increased final adult height. Treatment with a GnRH analog is thought to be a diagnostic aid as well as a therapeutic intervention for this age group because stopping the progression of the physical changes of puberty would be expected to partially alleviate gender dysphoria symptoms in true gender dysphoria.[2] If the patient has a clear understanding of the expected outcomes from GnRH analog therapy, compliance is enhanced and adverse mental health effects can be prevented.

The puberty suppression approach, also called the Dutch protocol, is the most well studied approach in this population, especially with respect to psychological outcomes. The regimen used is triptorelin 3.75 mg subcutaneously or intramuscularly every 2 weeks for 4 weeks, then every 4 weeks thereafter.[14] Children present at a mean age of 10–12 years and receive GnRH analog therapy until age 16 years, which is the age at which Dutch children can consent to their own medical treatments and begin CSHT. However, a more recent retrospective study from British Columbia describes beginning CSHT at a mean age of 14.7 years (SD 2.2 yrs, range 13.3–22.3 yrs) in 37 transsexual females and a mean

age of 17.0 years (SD 1.6 yrs, range 13.7–19.8 yrs) in 45 transsexual males.[15] Adverse effects reported in association with GnRH agonist therapy included sterile abscess, leg pains, headaches, and weight gain.

The first prospective study of psychological outcomes in adolescents with a mean ± SD age at time zero of 13.65 ± 1.85 years, which was followed by a mean ± SD of 1.88 ± 1.05 years of puberty suppression therapy, showed a statistically significant improvement in behavior, emotional problems, and general functioning after puberty suppression.[16] Depression measured by using the Beck Depression Inventory was also significantly improved compared with baseline; however, mean scores at baseline were below clinical thresholds for depression (p=0.004). Gender dysphoria and body image scores did not improve after puberty suppression therapy compared with baseline. The clinical significance of these results is not clear, but previous research supports the finding that gender dysphoria does not improve significantly before CSHT and/or SRS. Because all children in this study received puberty suppression therapy, the benefits of drug therapy cannot be separated from the benefits of study participation. Treatment of gender dysphoria is multifaceted and includes psychotherapy, puberty suppression when appropriate, CSHT, and SRS, which have varying levels of benefit.

A single case report described the long-term outcomes associated with puberty delay in a transsexual adolescent.[17] A 13-year-old natal girl received 4.9 years of triptorelin followed by intramuscular testosterone every 2–3 weeks and surgery 7 and 9 years later. By the age of 35 years, height and bone mineral density (BMD) were found to be higher than natal females and lower than natal males. The effects of pubertal suppression on future BMD and cognitive development in this population have not been studied systematically. To date, long-term outcome data are limited to psychological effects and a single study center. Psychological outcomes associated with an eventual gender transition following puberty suppression have also not yet been thoroughly explored.

Discussions surrounding the effects of cross-gender hormone therapy on future fertility should also occur with adolescents contemplating cross-gender hormone therapy. Ideally, education and consent should involve the parents, members of the adolescent's support group, and the referring mental health professional. No formally evaluated decision practice guidelines are currently available for this purpose. Cryopreservation of sperm or oocytes could be facilitated, but this would require spontaneous or induced gonadotropin release, which could also be associated with physical manifestations of puberty. Multiyear treatment with GnRH analog therapy should not compromise fertility; however, the effects of multiyear CSHT do not favor future reproductive function. Longitudinal reproductive clinical outcome data for this population are not currently available.[2]

Efficacy and Quality of Life of Cross-Sex Hormone Therapy

Psychological Functioning

A recent systematic review and meta-analysis of almost 30 trials of CSHT for gender dysphoria found significant improvement in psychological and physiologic symptoms as well as overall quality of life after starting therapy.[18] Of those evaluated, 80% (95% CI 68–89; 8 studies; I^2 = 82%) reported significant improvement in gender dysphoria, 78% (95% CI 56–94; 7 studies; I^2 = 86%) reported significant improvement in psychological symptoms, 80% (95% CI 72–88; 16 studies; I^2 = 78%) reported improvement in quality of life, and 72% (95% CI 60–81%; 15 studies; I^2 = 78%) reported significant improvement in sexual function.

Physical Appearance

Transsexual Females

The desired physical effects in transsexual females can include decreased spontaneous erections, reduced testicular size, increased ratio of body fat to muscle mass, and increased breast size, with adequate secondary sexual characteristics directly correlating to the individual's psychological wellness.[1, 19] Once CSHT is initiated, body fat redistribution, initial breast growth, decreased muscle mass, decreased testicular volume, and decreased spontaneous erections can occur within the first 6 months.[1] Full effects can take as little as 1 year for muscle mass changes or as long as 5 years for body fat redistribution.[1] Body and facial hair changes take the longest, with the onset occurring after 6–12 months of hormone therapy and the full effect taking more than 3 years.[1] Desired skeletal structure (e.g., chest, hips, face) and some aspects of voice can-

not be modified with CSHT when the transition occurs later in life.[5] Erections associated with sexual excitement will continue to occur, but spontaneous erections will fully or partially diminish.[20]

The development of breast tissue in transsexual females receiving estrogen therapy follows the same stages as those seen during natal female puberty; however, due to anatomic differences in the thorax of natal males, the appearance of the breast is often found to be unsatisfactory, thus precipitating the need for breast augmentation.[19] In a small retrospective case-control study of transsexual females receiving CSHT from a gender identity clinic or through self-acquisition (Internet or other health care providers), the need for augmentation surgery was not significantly associated with any particular type of estrogen therapy; nor was there an association with serum concentrations of estradiol, testosterone, dihydrotestosterone, sex hormone-binding globulin, luteinizing hormone (LH), or follicle-stimulating hormone.[19] Among antiandrogen therapies consisting of cyproterone, finasteride, dutasteride, GnRH analogs, or spironolactone, only spironolactone was associated with increased requests for breast augmentation (16% augmentation vs 6% no augmentation, p=0.025). The study reported an association between self-medication and requests for breast augmentation; however, the lack of randomization makes interpretation of this difference problematic. Two years after CSHT, another study reported that 70% of subjects were not satisfied with the extent of their breast development despite 40% achieving the size of a B cup bra.[21]

An older study that studied estrogen preparations, which are no longer recommended for gender dysphoria (conjugated estrogens and ethinyl estradiol), found no significant differences in penis length in transsexual females not yet undergoing SRS but found a significant difference in testicular size (25% decrease, $p<0.05$) after a mean of 15.6 months of follow-up (SD 12.9 months).[22] Patients in this study did not uniformly receive antiandrogen therapy, but those who did received an agent that is no longer recommended (medroxyprogesterone).

With respect to hair growth, after 1 year of combination estrogen and antiandrogen therapy, Ferriman and Gallwey scores, a validated scoring tool often used for hirsutism, significantly decreased from a median (interquartile range) of 21 (19–25) at baseline to 10 (8–13.8) ($p<0.001$).[23] Clinically, suppression of beard hair appearance lagged behind abdominal hair appearance, which can best be explained by the high hair density and diameter on the face.

Transsexual Males

In transsexual males, desirable characteristics can include deepening of the voice, growth of facial and body hair, cessation of menses, atrophy of breast tissue, decreased ratio of body fat to muscle mass, and clitoral enlargement. Within 6 months of CSHT, transsexual males will see increases in face and body hair, redistribution of body fat, cessation of menses, voice deepening, clitoral enlargement, and vaginal atrophy.[1, 24] Increases in muscle mass can take as long as 12 months to change.[1] Maximal effects on clitoral size and voice can take as long as 2 years, and maximal effects on hair growth, muscle mass, and body fat redistribution can take as long as 5 years.[1] Positive changes can be accompanied by negative effects associated with testosterone administration including acne and scalp alopecia.[1]

From limited data, maximal clitoral size was approximately 6 cm, and breast size did not significantly change after testosterone therapy.[22] Higher testosterone doses (250 mg every 2 wks) are associated with earlier physical changes compared with lower doses (125 mg every 2 wks or 250 mg every 3 wks), but outcomes are no longer significantly different after 6 months of CSHT. Higher and lower doses in these ranges result in concentrations similar to those of natal males.[24] Progestational agents can be added to testosterone therapy in transsexual males with continued uterine bleeding; however, specific agents and doses have not been adequately studied.[2]

Summary

Once CSHT is initiated, the desired effects of feminization and masculinization are both time and dose dependent.[1, 2, 24] Genital appearance is not changed greatly by estrogen therapy, but some transsexual females will be encouraged by small changes in testicular size.[22] CSHT is effective in promoting breast development in transsexual females but less effective in reducing male patterns of hair growth, especially with respect to facial hair. Transsexual males experience a relatively more rapid onset of changes in skin, hair growth, and menstrual patterns, but

they experience limited or no physical changes with respect to genital appearance from CSHT alone. Physical appearance is not well correlated with target hormone concentrations, but practice guidelines recommend target CSHT doses that are associated with serum hormone concentrations in the adult ranges for the desired sex.[2]

Sexual Function

Transsexual Females

In a small observational study of transsexual females who underwent SRS a minimum of 6 months before the study, general functioning scores were similar to a control group of Dutch and American women.[25] When compared with historical data from nontranssexual Dutch women, Female Sexual Function Index (FSFI) scores were significantly lower for arousal, lubrication, and pain ($p<0.05$). Neither testosterone concentrations nor estradiol concentrations correlated with FSFI scores.

In an observational study conducted in Belgium and the Netherlands, compared with natal females, transsexual females who had received SRS had similar rates of hypoactive sexual desire disorder diagnosis (33.9%) compared with premenopausal natal females (23.3%).[26] Sexual satisfaction, however, was significantly decreased compared with natal females ($p=0.002$) with no significant difference in general life and relationship satisfaction. Contrary to previous research in premenopausal natal females, sexual desire in transsexual females did not correlate with free or total testosterone concentrations ($r = -0.06$ to 0.1, $p>0.05$). A small pilot study of seven transsexual females reported improved sexual desire associated with administration of a 300 μg/day testosterone patch, with no reported adverse effects.[27]

Transsexual Males

Compared with the prehormone state, testosterone administration in transsexual males resulted in a statistically significant increase in desire ($p=0.0014$) and arousal ($p<0.0005$) after 12 months of CSHT in one study.[28] Relationship satisfaction and orgasm were unchanged in this evaluation, but frequency of sexual intercourse was correlated with testosterone concentrations ($p=0.039$, $\eta^2 = 0.054$). In a similar study, sexual desire was reported to be higher or much higher in 72.7% of transsexual males, with 25% reporting similar sexual desire and 1% reporting worse sexual desire.[29] Suppressed LH concentrations in transsexual males indicate that testosterone therapy is excessive, and low LH concentration was found to be associated with excessive sexual desire ($p=0.007$). Free and total testosterone concentrations were not found to be associated with measures of sexual desire in this study.

Summary

Transsexual females experience hypoactive sexual desire disorder at a rate similar to natal females but report increased concerns regarding desire, pain, and lubrication despite achieving goal estrogen concentrations. Further research is needed regarding the safety and efficacy of low-dose add-back testosterone administration for decreased sexual desire. Transsexual males experience improved sexual desire in response to testosterone therapy; however, similar to transsexual females, problems with sexual functioning do not always correlate with testosterone concentrations. Effects of sexual functioning on overall satisfaction with relationships and quality of life deserve further study.

Safety of Cross-Sex Hormone Therapy

Similar risk profiles are believed to be associated with CSHT when compared with hormone replacement therapy associated with the natal sex.[2] Unfortunately, long-term disease risk in untreated individuals or those receiving placebo is difficult to assess for ethical reasons.

Bone Health

Transsexual Females

Androgens play a key role in maintaining bone mass in aging males. Androgen deprivation for treatment of prostate cancer using GnRH analogs is associated with a loss of BMD.[2] However, estrogen in aging natal males correlates more with improved BMD and peak bone mass than testosterone.[2]

Transsexual females who have not undergone SRS and were treated with an antiandrogen and estrogens for 3 years or more showed an increase in BMD (difference in *T*-score lumbar spine = 0.7, $p=0.04$; difference in *T*-score femoral neck = 0.8, $p=0.01$) when adjusted for height and weight, with no significant change in markers of bone turnover.[30] This suggests that estrogen

activity is sufficient to maintain normal bone mass in transsexual females. Treatment with estrogens plus goserelin, a GnRH analog, for 24 months resulted in a significant increase in BMD at the lumbar spine and at the femoral neck.[31]

In transsexual females who have undergone SRS and are receiving CSHT with estrogen alone, BMD remains the same or is similar to untreated males.[32, 33] In a study of transsexual females who underwent SRS and were treated with cyproterone plus estrogens, BMD was significantly decreased (difference in *Z* score −1.1, $p<0.001$, at both the lumbar spine and femoral neck).[34] In this study, transsexual females had significantly lower testosterone levels but similar estrogen levels compared with the untreated male controls, leading to low levels of C-terminal telopeptides and procollagen 1 aminoterminal propeptide, markers of bone resorption and formation, respectively, which may have contributed to the lower measured BMD.

Transsexual Males

Multiple studies have found androgen therapy to be effective in preventing bone loss after oophorectomy in transsexual males by increasing cortical BMD compared with baseline in untreated age-matched females.[33, 35–37] This may be due to cortical bone having more androgen receptors than trabecular bone.[33]

Forty-five transsexual males who had not undergone SRS and had been treated with testosterone undecanoate 1000 mg intramuscularly every 12 weeks for 24 months had no change in BMD compared with baseline.[36]

Transsexual males who had undergone SRS and who received CSHT with testosterone had similar lumbar and femoral neck BMD results compared with untreated females.[33, 35, 37] Cortical thickness and tibial BMD were significantly increased compared to untreated females.[33, 37] A group of 19 transsexual males who had undergone SRS and were being treated with testosterone for 3.5 years had a significant decrease in BMD ($p=0.003$).[32] However, serum testosterone levels were below the normal range for males, suggesting that the patients may have had insufficient testosterone supplementation. Transsexual males who had undergone SRS and were treated with testosterone and letrozole, an aromatase inhibitor, showed significantly decreased BMD at the lumbar spine (difference in *T* score −0.9, $p=0.008$) compared with transsexual males treated with testosterone monotherapy, suggesting that the conversion of testosterone to estradiol is sufficient to maintain BMD.[38]

Summary

To our knowledge, no fracture data are available for transsexual males or females who are using CSHT; thus fracture risk in these individuals is unknown. The Endocrine Society guidelines recommend measuring BMD if the patient has risk factors for osteoporosis, especially if they have undergone SRS and stop hormone therapy.[2] Expert opinion recommends checking BMD at baseline and every 1–2 years if the patient has risk factors for fracture or stops taking hormone treatment.[5] Therefore, it is important to maintain normal hormone levels for their current gender to help prevent the loss of BMD.

Thromboembolic Disease

Transsexual Females

In epidemiological research, venous thromboembolism (VTE) rates have varied from 0–143 cases/10,000 patient-years in transsexual females.[7, 19, 39–43] Although the data are retrospective and subject to recall and reporting bias, the high rate in these studies can be at least somewhat explained by pre-1990 frequent use of oral ethinyl estradiol (50–100 μg/day), oral conjugated equine estrogens (5–10 mg/day), and self-procured estradiol (200–800 mg/mo) administered intramuscularly.[41, 42] In contemporary practice, estradiol is used more frequently in accordance with current guidelines.[2] In two of these studies of the same population of transsexual females receiving comprehensive care from a Dutch clinic, the rate of VTE decreased from 143 cases/10,000 treatment-years between 1972 and 1986[42] to 58 cases/10,000 treatment-years between 1975 and 1994,[41] which can potentially be explained by different estrogen formulations in later years. A more recent study on thrombophilia and venous thrombosis in transsexual females involving 866 treatment-years both before and after SRS found no cases of VTE with the use of transdermal estradiol combined with presurgery antiandrogen therapy despite discovering activated protein C resistance in 10% of the transsexual females.[40] One would expect to see 5 cases or more in this study if the rate of VTE were comparable to the previous rate of 58

cases/10,000 treatment-years, but more long-term data on the rate of VTE in patients receiving estradiol are needed.

Transsexual Males

A recently published retrospective observational study of transsexual males receiving testosterone esters described no cases of VTE associated with 496 treatment-years.[39] Another study found a 5.6% incidence of activated protein C resistance but no cases of VTE in 349 treatment-years of testosterone therapy.[40] The largest study of morbidity in transsexual males receiving testosterone therapy included 2418 treatment-years and identified one case of VTE for an incidence rate of 4 cases/10,000 treatment-years.[41]

Summary

A history of thromboembolism is a contraindication to combination hormonal contraception or hormone replacement therapy in natal females. Because gender dysphoria is associated with high morbidity and mortality if untreated, likely surpassing the mortality rate associated with VTE, it would make sense that the safety threshold would also be higher. A few cases of the use of estrogen therapy in transsexual females with a history of VTE were reported.[7] Oral anticoagulant therapy and hormone therapy have also been coadministered, including in a transsexual female patient homozygous for the factor V Leiden mutation.[40] The risk of thromboembolism does not seem to be increased in transsexual males receiving testosterone compared with natal females.

Cardiovascular Disease and Stroke

Transsexual Females

Continuous use of ethinyl estradiol in transsexual females is associated with an increased risk for cardiovascular disease mortality (4.1% vs 1.3% for continuous use of ethinyl estradiol vs former use of or never used ethinyl estradiol) even after adjustment for age and smoking history (HR 3.64, 95% CI 1.52–8.73, p=0.004).[7] One proposed mechanism for this increased thrombotic risk is through an inhibitory effect on tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor (PAI)-1. This has been documented in a small subset of transsexual females after 4 months of cross-gender hormone administration.[23] Inhibition of tPA and PAI-1 is less pronounced with transdermal estradiol. Oral ethinyl estradiol is believed to increase the hepatic clearance of tPA.

For the same population of transsexual females receiving care in the Netherlands, the rate of stroke was 7.5/10,000 treatment-years from 1975–1994 for a follow-up period of 7734 treatment-years.[41] This rate was similar to the rate that was identified from 1972–1986, which represented 1333 treatment-years.[42] More recent data show a stroke mortality rate of 2.7 cases/10,000 treatment-years during a follow-up period between 1975 and 2007 representing 18,678 treatment-years.[7] The standardized mortality ratio for stroke was 1.26 (95% CI 0.93–1.64) when compared with the general population of Dutch natal males of similar age.

Hypertension, an important risk factor for stroke, which was defined as a systolic blood pressure higher than 160 mm Hg or diastolic blood pressure higher than 95 mm Hg, did not occur at a rate greater than that associated with the age-matched general Dutch population in a large study of 7734 patient-years in transsexual females (incidence rate ratio 0.98, 95% CI 0.75–1.26).[41] In a Belgian study representing 473 hormone treatment years in transsexual females and 496 hormone treatment years in transsexual males, the transsexual females had a lower diastolic blood pressure (mean ± SD 77.1 ± 10.1 vs 81.3 ± 10.7 mm Hg) and mean arterial blood pressure (mean ± SD 93.0 ± 11.2 vs 95.8 ± 10.1 mm Hg) compared with transsexual males (p=0.002 and p=0.008, respectively).[39] In the same study group, a comparable number of transsexual males and females had self-reported elevated blood pressure and/or were being treated with antihypertensive medications during the study period. In a smaller Dutch study representing the observation period between 1972 and 1986 and 1333 treatment-years, 14 cases of hypertension (105/10,000 treatment-years) were identified in transsexual females.[42] In the observation period between 1975 and 1994, representing 7734 treatment-years for the same population, hypertension rates decreased in transsexual females (79/10,000 treatment-years) compared with earlier study periods.[41] The decrease in hypertension rates could have been related to a switch from conjugated estrogen–based and ethinyl estradiol–based regimens to transdermal estradiol–based and oral estradiol valerate–based regimens. A change in hyperten-

sion rates due to lifestyle changes secondary to awareness of risk factors and complications of hypertension cannot be ruled out.

Transsexual Males

For the same population of transsexual males receiving care in the Netherlands, in the longest follow-up period, no cases of stroke were reported between 1975 and 2007, representing 6866 treatment-years.[7] Conclusions regarding stroke risk cannot be made at this time because of the lack of available comparison data between transsexual males and the natal female general Dutch population. In the same study, the rate of ischemic heart disease was 1.45 cases/10,000 treatment-years, with a standardized mortality ratio of 1.19 (95% CI 0.39–2.74), indicating a rate similar to the age-matched general Dutch population.

Hypertension, again defined as a systolic blood pressure higher than 160 mm Hg or diastolic blood pressure higher than 95 mm Hg, did not occur at a rate greater than in the general Dutch population in a study of 2418 treatment-years in transsexual males (incidence rate ratio 0.84, 95% CI 0.43–1.47).[41] In the Belgian study described previously, the diastolic blood pressure and mean arterial blood pressure were significantly higher in transsexual males versus transsexual females.[39] In a smaller Dutch study representing the observation period between 1972 and 1986, three cases of hypertension were identified in transsexual males (68/10,000 treatment-years).[42] In the observation period between 1975 and 1994 in the same population, hypertension rates decreased slightly in transsexual males (50/10,000 treatment-years).[41]

Summary

Clinical practice guidelines recommend the monitoring of blood pressure, lipid levels, and serum glucose levels regularly with the management of cardiovascular risk factors as they emerge in transsexual males and females according to guidelines established for nontranssexual individuals.[2] The rate of cardiovascular disease mortality in transsexual females taking ethinyl estradiol was recently found to be higher compared with nontranssexual individuals.[7] Because ethinyl estradiol is no longer recommended, studies will need to be repeated with an adequate sample of patients who are taking the currently recommended estrogen regimens. Although testosterone therapy in transsexual males is associated with a less favorable lipid profile, from the limited data and low-quality research available, cardiovascular disease risk is not increased in transsexual males compared with natal females.[2]

Cancer

Transsexual Females

Epidemiological data suggest that the rate of cancer-related death is similar between transsexual females and natal males. A study of 966 transsexual females over 18,678 patient-years of follow-up identified 28 cases of malignant neoplasms for a standardized mortality ratio of 0.98 (95% CI 0.88–1.08). Most of the cancers identified involved lung or blood cancers that were believed to be linked to smoking and AIDS cases (16 deaths), respectively.[7]

Breast cancer in natal males accounts for less than 1% of all cancers in men.[44] Guidelines recommend the same breast cancer screening used for natal females due to the handful of reported cases in the literature of breast cancer in transsexual females.[2] Five cases of breast cancer in transsexual females receiving CSHT were reported in the literature.[45–48] Two cases of estrogen receptor–negative ductal carcinoma of the breast reported in the late 1980s and early 1990s were associated with 10 or more years of conjugated estrogen therapy (0.625 mg/day and 1.25 mg/day, respectively) in patients in their mid-30s who had undergone orchiectomy.[46, 47] One additional case from the Amsterdam gender clinic was also reported.[48]

A family history of breast or ovarian cancer is reported in 15–20% of natal males with breast cancer with the *BRCA2* gene identified as the most strongly associated mutation.[44] This mutation confers a lifetime risk of breast cancer of 5–10% in natal males compared with the general male population.[44] Although thousands of transsexual females have received estrogen therapy, the variability in the drug and dosage prescribed and the duration of therapy make risk estimations difficult.[48] In addition, cancer risk is correlated with age, making the limited duration of experience (30 yrs with the longest running programs) with these individuals a barrier to assessing risk.

Androgen deprivation results in a decrease in prostate volume, and estrogen exposure has not been linked to prostate hyperplasia or malignancy. Removal of the prostate is not considered

part of SRS. From epidemiologic research, orchiectomy before the age of 40 years results in a decreased risk of prostate hypertrophy or cancer.[48] The three cases of prostate cancer reported in the literature described transsexual females who began CSHT later in life (after age 50 yrs)[48] compared with the age when most individuals with gender dysphoria begin CSHT. Prostate cancer monitoring should be performed in accordance with screening recommendations for nontranssexual individuals.[2]

Transsexual Males

Although cancers of reproductive organs and breasts are common in natal females, and risk might be expected to increase or decrease in response to a cessation of gonadal function or the administration of CSHT, limited data suggest that cancer rates are similar between transsexual and nontranssexual individuals. One study evaluating 6866 treatment-years for 365 transsexual males found no cancer-related deaths for a calculated standardized mortality ratio of 0.99 (95% CI 0.65–1.44) for any type of cancer compared with individuals of similar age, natal sex, and country of residence (the Netherlands).[7] In another retrospective study of transsexual males that included 50 patients and 473 treatment-years, no cancer cases or cancer-related deaths were reported.[39]

Epidemiological evidence suggests an association between circulating androgen concentrations and pre- and postmenopausal breast cancer in natal females.[49] Other types of research, including in vivo, in vitro, and clinical evidence, suggest that androgens may confer a protective effect against breast cancer. Conclusions regarding long-term risk cannot be drawn at this point due to a lack of data on transsexual males who have been receiving CSHT for multiple decades or for the years of life that are associated with the highest risk for breast and gynecologic cancers.[48] Case reports suggest that the risk of breast cancer still exists, even after SRS.[50, 51] There are currently four case reports of breast cancer in transsexual males in the literature.[50–52] Ages at diagnosis were 33, 53, 27, and 42 years; before diagnosis, the duration of hormone therapy was 13, 5, 6, and 2.5 years, respectively. All but one of the four patients described in the literature continued androgen therapy concurrently or after cancer therapy. The mastectomy procedure performed in transsexual males can be nipple-sparing or involve a nipple reimplantation. One of the four cases described in the literature occurred in a transsexual male who had previously received nipple-sparing bilateral mastectomy.[50]

Total hysterectomy and bilateral salpingo-oophorectomy may be performed on some but not all transsexual males. Although the procedure is recommended to be laparoscopic, scarring and other surgery-related risks exist.[1] Currently, it is debated as to whether ovariectomy or hysterectomy are medically necessary to reduce the risk of ovarian and endometrial cancers, respectively. Ovarian cancer was reported in three transsexual males in the literature.[48] It was initially thought that transsexual males were at a higher risk for the development of polycystic ovarian syndrome. More recent research suggests that polycystic ovaries identified on ultrasound in transsexual males do not resemble polycystic ovaries seen in natal females with polycystic ovarian syndrome due to a higher number of atretic follicles present in transsexual males.[53] This was a small study, and confirmatory research is needed. Women with polycystic ovaries are not thought to be at a significantly greater risk for ovarian cancer compared with women without polycystic ovaries. Oophorectomy for the purpose of preventing ovarian cancer would not seem to be necessary at this point based on the currently available literature. The risk of endometrial cancer has been tied to endometrial hyperplasia seen when estrogen concentrations are high and progesterone concentrations are low (i.e., unopposed estrogen). In transsexual males receiving CSHT, estrogen concentrations remain in the physiologic range.[48] Progestin administration and/or yearly ultrasound examination of the uterus has been suggested in transsexual males as a means of preventing and mitigating the risk of endometrial cancer.

Summary

Based on the available research, cancer is not more prevalent in transsexual females or males compared with the general population. A more thorough understanding of the risks versus benefits of SRS on the incidence of cancer is sorely needed, but it does not seem at this time that SRS is required to mitigate the risk of cancer. The consequences of discontinuing hormone therapy in transsexual females and males diagnosed with an estrogen- or testosterone-responsive cancer are not clear from the perspective of

recurrence prevention but could present a significant problem in terms of mental well-being, which is likely why the limited case reports of cancer in transsexual individuals describe continuing hormone therapy after diagnosis.[51, 52, 54]

Conclusion

Gender dysphoria is well described in the literature, but comparative research on drug regimens with respect to efficacy and long-term safety is sorely needed. The most well-documented work in this population comes from established clinics in the Netherlands, but little data exist in other populations. For those individuals seeking sex reassignment, the range of care sought is highly variable, making outcomes research difficult and providing individualized care very important. Physical effects of CSHT can vary depending on the age at which treatment is initiated, making early diagnosis of gender dysphoria in adolescents and adults an important goal. Preliminary efficacy data on puberty suppression in adolescents prior to CSHT are promising, but long-term safety data are severely lacking.

The safety of CSHT depends on hormone type, route of administration, and dosage. Coincidental to a cessation of the use of higher doses and certain estrogen preparations, morbidity and mortality rates due to VTE and cardiovascular disease improved after the 1980s. In limited European populations, overall mortality has improved in recent decades as well. Adverse effects that remain of the highest concern in this population due to a lack of high-quality research include VTE, fractures, cardiovascular disease, stroke, and hormone-dependent cancers. Health care professionals who are aware of the risks associated with gender dysphoria undertreatment, the risks associated with self-acquisition of CSHT, and the potential adverse effects associated with CSHT for the purpose of monitoring can contribute to improved care in this population.

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TRANSGENDER HEALTH

Efficacy and Safety of Gonadotropin-Releasing Hormone Agonist Treatment to Suppress Puberty in Gender Dysphoric Adolescents

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ABSTRACT

Introduction: Puberty suppression using gonadotropin-releasing hormone agonists (GnRHas) is recommended by current guidelines as the treatment of choice for gender dysphoric adolescents. Although GnRHas have long been used to treat precocious puberty, there are few data on the efficacy and safety in gender dysphoric adolescents. Therefore, the Endocrine Society guideline recommends frequent monitoring of gonadotropins, sex steroids, and renal and liver function.

Aim: To evaluate the efficacy and safety of GnRHa treatment to suppress puberty in gender dysphoric adolescents.

Methods: Forty-nine male-to-female and 67 female-to-male gender dysphoric adolescents treated with triptorelin were included in the analysis.

Main Outcome Measures: Physical examination, including assessment of Tanner stage, took place every 3 months and blood samples were drawn at 0, 3, and 6 months and then every 6 months. Body composition was evaluated using dual energy x-ray absorptiometry.

Results: GnRHa treatment caused a decrease in testicular volume in 43 of 49 male-to-female subjects. In one of four female-to-male subjects who presented at Tanner breast stage 2, breast development completely regressed. Gonadotropins and sex steroid levels were suppressed within 3 months. Treatment did not have to be adjusted because of insufficient suppression in any subject. No sustained abnormalities of liver enzymes or creatinine were encountered. Alkaline phosphatase decreased, probably related to a slower growth velocity, because height SD score decreased in boys and girls. Lean body mass percentage significantly decreased during the first year of treatment in girls and boys, whereas fat percentage significantly increased.

Conclusion: Triptorelin effectively suppresses puberty in gender dysphoric adolescents. These data suggest routine monitoring of gonadotropins, sex steroids, creatinine, and liver function is not necessary during treatment with triptorelin. Further studies should evaluate the extent to which changes in height SD score and body composition that occur during GnRHa treatment can be reversed during subsequent cross-sex hormone treatment.

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Key Words: Gender Dysphoria; Gonadotropin-Releasing Hormone Agonist; Puberty Suppression; Gonadotropins; Sex Steroids

INTRODUCTION

Gender dysphoria is characterized by incongruence between the experienced gender and the sex assigned at birth. It was believed to be a rare phenomenon, but the number of individuals seeking advice and/or treatment at dedicated clinics is increasing.[1] Children can express a sense of belonging to the other sex at a very young age and might show gender role behavior typical of the experienced gender. However, studies have shown that in the children who met the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text*

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Revision criteria for gender identity disorder, the gender dysphoria persisted into adolescence only in a minority.[2] If gender dysphoria persists or worsens at the onset of puberty, then it is very likely that it will persist into adulthood.[2]

The Endocrine Society has issued a clinical practice guideline on the endocrine treatment of gender dysphoric individuals.[3] For adolescents, pubertal development of the natal sex can be very distressing. Once irreversible characteristics of the natal sex have developed, such as breasts in natal girls and a low voice and outgrowth of the Adam's apple and jaw in natal boys, it becomes more difficult for the individual to live in the experienced gender. Therefore, treatment with gonadotropin-releasing hormone agonists (GnRHas) to suppress puberty is recommended. This gives individuals time to carefully consider their wishes regarding gender-affirming treatment without the distress caused by the development of unwanted sex characteristics. From approximately 16 years of age, individuals can be treated with sex steroids to induce the sex characteristics consistent with the gender identity.[3]

Treatment with GnRHa has been shown to improve psychological well-being in several domains.[4] However, physical outcome has not been very well studied. The Endocrine Society guidelines describe that testicular volume decreases and slight development of sex characteristic regresses,[3] but little evidence is available to support these statements.[5] This makes it difficult to counsel individuals on what they can expect. In addition, it is recommended to measure gonadotropins and sex steroids every 3 months during treatment and monitor liver enzymes and renal function.[3] However, the necessity of these frequent measurements is uncertain. A consensus statement on the use of GnRHa in children states there is insufficient evidence for any specific short-term monitoring scheme.[6] GnRHas have been used for many years for the treatment of children with precocious puberty and no side effects on liver or kidney function have been reported, but adolescents might respond differently.

AIM

We set out to describe the changes in Tanner stage, testicular volume, gonadotropins, and sex steroids during GnRHa treatment of gender dysphoric adolescents to evaluate the efficacy of this treatment. In addition, we report on the yield of monitoring liver enzymes and renal function and on changes in body composition.

METHODS

Subjects and Protocol

Gender dysphoric adolescents seen at the Centre of Expertise on Gender Dysphoria at the VU University Medical Centre (Amsterdam Netherlands) from 1998 through 2009 were invited to participate in a study on brain development, brain functioning, growth, and metabolic aspects of their treatment. These adolescents were diagnosed as described in existing guidelines,[3] were eligible for treatment fulfilling *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* criteria for gender identity disorder,[7] had lifelong extreme gender dysphoria, were psychologically stable, and were living in a supportive environment. The design of the study was observational and prospective. Treatment consisted of intramuscular injections of the GnRHa triptorelin 3.75 mg (Decapeptyl-CR, Ferring Pharmaceuticals, Copenhagen, Denmark), at 0, 2, and 4 weeks, followed by injections every 4 weeks. Individuals were seen at 3-month intervals. The duration of treatment with GnRHa alone depended on when the individual reached the age at which cross-sex hormone therapy could be added. Only individuals who had been treated for at least 3 months were included in this study.

Fifty-five male-to-female (MtF) and 73 female-to-male (FtM) adolescents started treatment according to this protocol. Twelve subjects were excluded from analysis because no baseline data were available (n = 4), treatment duration was shorter than 3 months (n = 2), or they were already being treated with medication that affects the hypothalamus-pituitary-gonadal axis at baseline (an antiandrogen, n = 1; a GnRHa provided elsewhere, n = 2; or a progestin, n = 3). Data from 49 MtFs and 67 FtMs were analyzed. None of the subjects in this study discontinued the GnRHa treatment.

Ethical Approval

Medical ethical approval was granted by the local medical ethics committee and informed consent was obtained from all participants and their parents or guardians. The study was placed on the International Standard Randomized Controlled Trial Number register and ascribed the registration number ISRCTN 81574253 (http://www.controlled-trials.com/isrctn/).

MAIN OUTCOME MEASURES

Physical Examination

Tanner stage was determined by the same examiners at each visit and based on breast development in FtMs and testicular volume and genital development in MtFs.[8,9] Testicular volume was determined using a Prader orchidometer. Weight and height were measured using an electronic scale and a wall-mounted stadiometer (SECA, Hanover, MD, USA), with weight measured to the nearest 0.1 kg and height to the nearest 0.1 cm. Height SD score (SDS) was calculated using Dutch reference data[10] and body mass index (BMI) SDS was calculated using reference data from Cole et al.[11]

Laboratory Investigations

After 0, 3, and 6 months of treatment and every 6 months thereafter, blood was drawn for measurement of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, estradiol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, γ-glutamyl transferase, and creatinine. The

Table 1. Baseline characteristics and changes in anthropometric measurements and body composition during the first year of GnRHa treatment*

	MtF		*P* value	FtM		*P* value
Age (y), median (range)	13.6 (11.6–17.9)			14.2 (11.1–18.6)		NS
Tanner G and B stages, median (range)	4 (2–5)			4 (2–5)		NS
Menarche	N/A			49/65 (77%)		
	Start GnRHa	1 y GnRHa		Start GnRHa	1 y GnRHa	
Height (cm), mean (SD)	167.8 (7.5)	172.3 (6.5)	<.001	161.4 (8.4)	163.5 (7.9)	<.001
n	36	36		41	41	
Height SDS, mean (SD)	0.20 (1.0)	−0.04 (1.0)	<.001	−0.10 (1.1)	−0.25 (1.1)	<.001
n	36	36		41	41	
Weight (kg), mean (SD)	57.4 (11.1)	63.3 (11.9)	<.001	55.1 (14.7)	59.5 (14.4)	<.001
n	36	36		41	41	
BMI (kg/m^2), mean (SD)	20.3 (3.0)	21.2 (3.2)	<.001	21.0 (4.5)	22.1 (4.6)	<.001
n	36	36		41	41	
BMI SDS, mean (SD)	0.82 (1.1)	0.89 (1.2)	NS	0.68 (1.2)	0.84 (1.2)	.01
n	36	36		41	41	
Fat percentage (%), mean (SD)	22.4 (6.9)	26.8 (6.6)	<.001	25.0 (6.9)	29.5 (7.3)	<.001
n	26	26		27	27	
Lean body mass (%), mean (SD)	74.6 (6.4)	70.9 (7.3)	.001	71.5 (6.7)	67.7 (6.7)	<.001
n	26	26		27	27	
Testicular volume (ml), median (range)	13.5 (4–25)	8 (2.5–22.5)	<.001	N/A		
n	33	33				
AP (U/L), mean (SD)	303 (109)	216 (79)	<.001	215 (101)	168 (58)	<.001
n	19	19		21	21	
Creatinine (μmol/L), mean (SD)	70 (12)	66 (13)	NS	73 (8)	68 (13)	.01
n	28	28		29	29	

AP = alkaline phosphatase; B stage = breast stage; BMI = body mass index; FtM = female-to-male; G stage = genital stage; GnRHa = gonadotropin-releasing hormone agonist; MtF = male-to-female; N/A = not applicable; NS = not significant; SDS = SD score.
*Baseline characteristics are shown for all included patients. Where data at the start of treatment are compared with data after 1 year of treatment, results are shown of individuals from whom data were available at the two time points.

following assays were used: an immunometric assay for LH and FSH (Delfia, PerkinElmer, Wallac Oy, Finland; lower limit of quantification = 0.3 U/L for LH and 0.5 U/L for FSH), a radioimmunoassay for estradiol (Diasorin, Saluggia, Italy; lower limit of quantitation = 18 pmol/L), and a radioimmunoassay for testosterone (Siemens Medical Solutions USA, Malvern, PA, USA; lower limit of quantification = 1 nmol/L). The laboratory participates in external quality control and meets International Organization for Standardization 15189 criteria.

Dual-Energy X-Ray Absorptiometry

Fat mass, fat percentage, and lean body mass percentage were measured with dual-energy X-ray absorptiometry using a Hologic QDR 4500 scanner (Hologic Inc, Waltham, MA, USA).

Statistical Analysis

The statistical package used was SPSS 22 (SPSS Inc, Chicago, IL, USA). Changes in various laboratory parameters between two time points in participants with complete data were compared using paired-samples t-test if normally distributed and Wilcoxon signed rank test if not normally distributed. Correlations were analyzed using bivariate correlations. Results were considered statistically significant at a *P* value less than .05.

RESULTS

Anthropometry and Body Composition

Baseline characteristics are presented in Table 1. Height, weight, and BMI significantly increased during the first year of treatment in girls and boys. In MtFs, height SDS significantly decreased by 0.23 ± 0.23 in the first year of treatment (n = 36, $P < .001$), by 0.28 ± 0.14 in the second year (n = 21, $P < .001$), and did not change in the third year (n = 3). BMI SDS did not significantly change in the first year and increased by 0.13 ± 0.21 in the second year (n = 21, $P = .009$). In FtMs, height SDS decreased by 0.15 ± 0.23 SDS in the first year (n = 41, $P < .001$), by 0.13 ± 0.24 in the second year (n = 22, $P = .02$), and did not significantly change in the third year (n = 10). BMI SDS increased by 0.17 ± 0.41 in the first year (n = 41, $P = .01$) and did not significantly change

thereafter. The lean body mass percentage significantly decreased during the first year of treatment in MtFs and FtMs, whereas fat percentage significantly increased (Table 1). Absolute fat mass increased in MtFs and FtMs ($P < .001$).

Pubertal Development

Many subjects were in late puberty (Tanner stages 4 and 5) at the time GnRHa treatment was started. Only four FtMs were at Tanner breast stage 2 at baseline and in one of these individuals breast development regressed completely to Tanner breast stage 1 after 6 months of treatment with GnRHa. In the other FtMs, some unstimulated tissue remained palpable. In the FtMs who had had menarche, menses ceased, often after a withdrawal bleed. Testicular volume decreased during GnRHa treatment in 43 of 49 MtFs. One of the individuals who did not show a decrease had not adhered to the therapy. Three others had been treated for less than 1 year. In one individual, mean testicular volume had increased slightly from 20 mL at baseline to 22.5 mL at 12 months but decreased again to 20 mL at the next measurements. In 33 individuals from whom testicular volume measurements were available at baseline and at 12 months, testicular volume decreased from 13.9 ± 6.5 to 8.6 ± 4.7 mL ($P < .001$; Figure 1). The decrease was significantly correlated to the testicular volume at baseline ($P < .001$). During the second year of treatment, testicular volume did not significantly change in 17 individuals (mean = 8.2 ± 4.4 mL at 12 months and 8.4 ± 3.7 mL at 24 months, $P = .65$).

Gonadotropin and Sex Steroid Levels

Gonadotropins decreased within the first 3 months of treatment and did not change thereafter (Table 2). In MtFs, testosterone decreased from a median of 9.1 nmol/L (range < 1.0−34) at baseline to lower than 1.0 nmol/L (<1.0−1.1) at 3 months; in FtMs, estradiol decreased from a median of 123 pmol/L (23−1136) to 29 pmol/L (<18−73). Estradiol levels after 3 months of treatment were not as low in some FtMs who were in Tanner breast stages 4 and 5 at the start of treatment compared with those who were in Tanner breast stages 2 and 3 at baseline (Figure 2). However, these slightly higher estradiol levels under GnRHa treatment were not associated with clinical signs of insufficient suppression such as progressive breast development or uterine bleeding. GnRHa treatment did not have to be adjusted because of insufficient suppression in any subject. Gonadotropins and testosterone were not suppressed in only one MtF after 6 months but this individual had not adhered to the therapy. After 3 months of treatment, estradiol also significantly decreased in MtFs ($P < .001$). Sex steroid levels remained low thereafter.

Creatinine Levels

In MtFs, no creatinine levels above the upper limit of normal (ULN) were detected during 1 year of treatment. In four FtMs, creatinine was just above the ULN (91−94 μmol/L, with ULN = 90 μmol/l; two at baseline, two during treatment) but no progressive increase was seen. In individuals from whom creatinine levels were available at baseline and after 12 months of treatment, a significant decrease was seen in FtMs (73 ± 8 to 68 ± 13 μmol/L, n = 29, $P = .01$) but not in MtFs (70 ± 12 to 66 ± 13 μmol/L, n = 28, $P = .2$). There was no significant correlation between change in creatinine and change in lean body mass in either sex (data not shown).

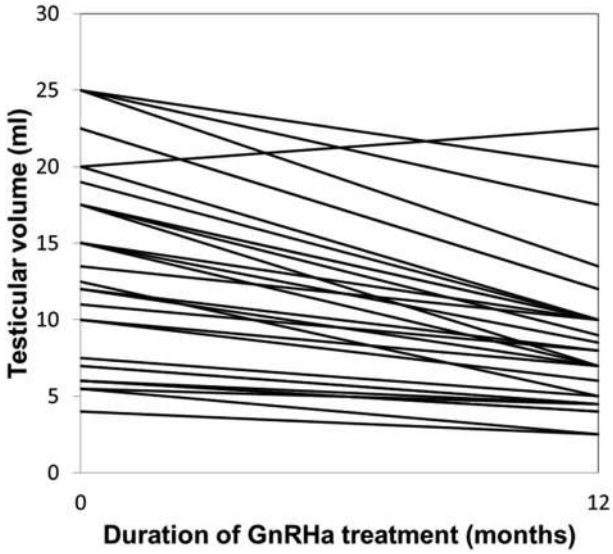

Figure 1. Change in testicular volume during the first year of gonadotropin-releasing hormone agonist (GnRHa) treatment. A decrease in testicular volume was observed in 32 of 33 natal boys from whom data were available at the two time points.

Liver Enzymes

γ-Glutamyl transferase was not elevated in any subject. Mild elevations of AST and ALT above the reference range were fairly common at baseline (AST and ALT were elevated in 8/39 respectively 4/39 MtFs and 3/61 respectively 1/59 FtMs) but were not more prevalent during treatment than at baseline. Only one MtF and one FtM had AST and/or ALT levels more than twice the upper limit of normal after 3 months of treatment, but these levels subsequently decreased without any change to the treatment. γ-Glutamyl transferase, AST, and ALT levels did not significantly change from baseline to 12 months of treatment.

In girls and boys, a significant decrease in alkaline phosphatase was observed during the first year of GnRHa treatment (Table 1). Individuals with a high alkaline phosphatase level at baseline showed the largest decrease. These were the individuals who were still growing as evident from the significant negative correlation between growth during the first year of treatment and the change in alkaline phosphatase (Spearman $\rho = -0.624$, $P < .001$; Figure 3).

DISCUSSION

Although GnRHa treatment has become the treatment of choice in gender dysphoric adolescents, few data are available on the efficacy and safety of this treatment in this population.

Table 2. Changes in gonadotropins and sex steroid levels during the first year of GnRHa treatment*

	Duration of GnRHa treatment (mo)			
	0	3	6	12
MtF				
LH (U/L)	1.7 (1.4, 49)	0.4 (0.4, 41)	0.4 (0.4, 44)	0.4 (0.3, 37)
FSH (U/L)	2.4 (1.4, 49)	<0.5 (0, 41)	<0.5 (0.1, 45)	<0.5 (0.1, 37)
T (nmol/L)	9.1 (8.7, 49)	<1.0 (0, 40)	<1.0 (0, 43)	<1.0 (0, 37)
E2 (pmol/L)	44 (35, 46)	25 (8, 37)	24 (7, 40)	26 (8, 34)
FtM				
LH (U/L)	3.0 (4.2, 64)	0.3 (0.3, 55)	0.4 (0.4, 58)	0.3 (0.3, 35)
FSH (U/L)	4.6 (2.1, 64)	1.3 (1.6, 55)	1.7 (1.4, 58)	1.7 (0.9, 35)
T (nmol/L)	<1.0 (0.4, 60)	<1.0 (0, 53)	<1.0 (0, 55)	<1.0 (0, 36)
E2 (pmol/L)	123 (118, 63)	29 (9, 53)	26 (12, 57)	30 (12, 37)

E2 = estradiol; FSH = follicle-stimulating hormone; FtM = female-to-male; GnRHA = gonadotropin-releasing hormone agonist; LH = luteinizing hormone; MtF = male-to-female; T = testosterone.
*Data are presented as median (interquartile range for the number of measurements available).

Therefore, the Endocrine Society recommends rather extensive safety monitoring.[3] We set out to evaluate the extent to which (early) pubertal physical changes can be reversed, the need for monitoring of gonadotropins and sex steroid levels, and the need for screening of liver and renal function.

Regression of early sex characteristics is difficult to quantify. In natal girls, Tanner breast stage 2 development can be expected to regress to Tanner stage 1, because glandular breast tissue can atrophy in the absence of estradiol. Few individuals presented at this early pubertal stage, but only one of the four who did showed complete regression of breast development. However, unstimulated tissue that remains palpable behind the nipple on examination is of little clinical relevance. In practice, this is not experienced as the presence of breasts by the individual and is not visible.

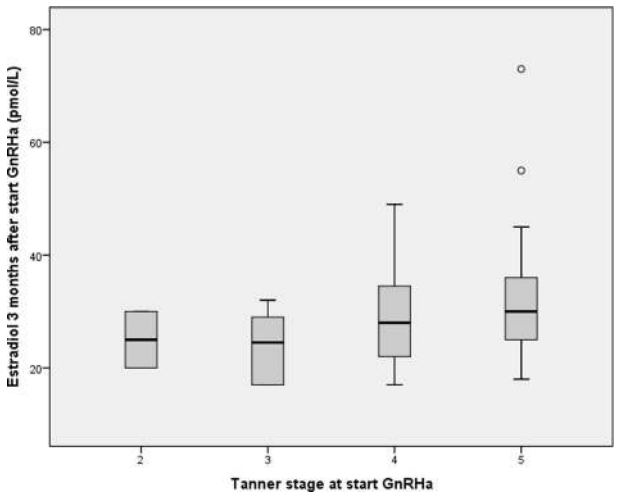

Figure 2. Estradiol levels in female-to-male individuals after 3 months of gonadotropin-releasing hormone agonist (GnRHa) treatment according to Tanner breast stage at the start of treatment. In general, estradiol was well suppressed, although levels were slightly higher in some individuals who were at Tanner stage 4 or 5 at the start of treatment.

In natal boys, testicular volume decreased in 88% of adolescents. This is in accordance with previous studies that have reported a decrease in testicular volume in boys treated with GnRHa for precocious puberty.[12,13] In adults, testicular volume also has been shown to decrease by approximately 50% during treatment when gonadotropins are suppressed with a long-acting androgen or a long-acting androgen in combination with a GnRHa.[14] Why testicular volume did not change despite adequately suppressed gonadotropins in several individuals from our cohort is unclear, although the limited duration of GnRHa treatment could partly account for this finding in some of these individuals.

Gonadotropins and sex steroids decreased within 3 months in all individuals, after which levels remained low in all but one

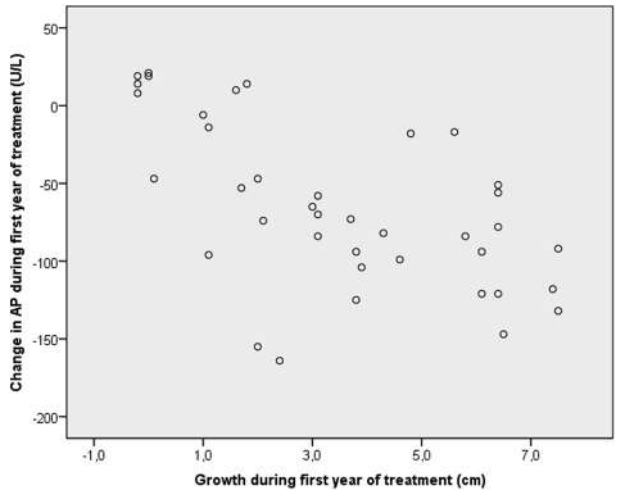

Figure 3. Correlation between the change in alkaline phosphatase (AP) and growth velocity during the first year of gonadotropin-releasing hormone agonist treatment. Alkaline phosphatase decreased in those who were still growing and changed little in those who had completed growth as evident from the significant negative correlation between growth during the first year of treatment and the change in alkaline phosphatase (Spearman $\rho = -0.624$, $P < .001$).

non-adherent individual. Therefore, it seems unnecessary to routinely monitor gonadotropins and sex steroids during treatment with triptorelin. Rather, these can be measured if there are clinical signs of inadequate suppression (ie, progressive breast development or increase of testicular volume).

In some FtMs who had (nearly) completed puberty before the start of treatment, estradiol levels did not decrease to levels as low as in those who started treatment in early puberty, but this was not associated with clinical signs of estrogen exposure such as uterine bleeding and treatment did not have to be adjusted. The FSH, LH, and estradiol levels we observed in natal girls were similar to or slightly lower than those found in girls treated with triptorelin because of central precocious puberty (CPP) or because of isolated growth hormone deficiency and early puberty.[15,16] In boys with CPP, median LH after 6 to 24 months of triptorelin treatment was 0.29 IU/L (range = 0.17−1.41), similar to what we observed and what has been reported in girls, but median FSH was lower than levels reported in girls with CPP (0.14 IU/L, range = 0.10−0.28).[12] Another small study reported undetectable FSH (<1.0 IU/L) in a boy with CPP on triptorelin, whereas FSH levels were not as well suppressed in four girls.[17] We also observed lower FSH levels during triptorelin treatment in natal boys compared with natal girls. Lower FSH levels also have been found in healthy boys compared with girls, prepubertally and during puberty, and in the basal state and in response to GnRH.[18] This might be due to sexually dimorphic development or programming of the hypothalamus-pituitary-gonadal axis, for example, by prenatal sex steroids.

Suppression of gonadotropins and estradiol by triptorelin was superior to that reported in gender dysphoric adolescents (natal girls) treated with the androgenic progestin lynestrenol.[19] Many adolescents reported metrorrhagia during lynestrenol treatment, especially during the first 6 months,[19] which was not observed during treatment with triptorelin.

None of the adolescents discontinued GnRHa treatment because of side effects. This is in agreement with the finding that GnRHa treatment is well tolerated by children and adolescents with CPP.[6] In a Canadian study, 1 of 27 individuals with gender dysphoria who were treated with GnRHa was reported to have stopped treatment owing to emotional lability.[20] Another developed sterile abscesses from leuprolide injections and therefore was switched to triptorelin, which was well tolerated[20]; this complication also has been observed in children treated for CPP but is very rare.[6] Although few adolescents seem to discontinue GnRHa treatment, side effects such as hot flushes are common, but these were not formally assessed in the present study.

We found small changes in BMI SDS in contrast to a previous study that did not report a change in BMI SDS in gender dysphoric adolescents treated with triptorelin.[21] From a combined analysis of several studies in children with CPP, it was concluded that long-term GnRHa treatment does not seem to cause an increase in BMI SDS.[6] We observed an increase in fat percentage and decrease in lean body mass percentage in boys and girls. Because no controls were included, it is unclear to what extent the changes in body composition can be attributed to the treatment, but studies in adults are in agreement with our findings. Healthy premenopausal women showed a decrease in fat-free mass in response to treatment with GnRH analogues,[22,23] but no change in fat mass.[23,24] In healthy men, GnRH analogue treatment was found to induce an increase in body fat and a decrease in lean mass.[25] Further studies are needed to determine the extent to which changes in body composition during GnRH analogue treatment are reversed during treatment with cross-sex hormones. In children who had been treated with GnRH analogues for CPP or to delay puberty during growth hormone treatment, body composition at adult height was comparable to that of controls.[26,27]

We did not identify any renal or hepatic complications of the treatment, and previous studies on GnRHa treatment in children with precocious puberty did not find such adverse effects.[6] Therefore, it does not seem necessary to routinely monitor these parameters. Alkaline phosphatase decreased during GnRHa treatment. This is most likely due to a decrease of the bone fraction rather than the liver fraction of alkaline phosphatase, because none of the other liver enzymes showed similar changes. This decrease is probably related to a change in growth velocity and lower bone mineral accrual, which are known determinants of alkaline phosphatase levels,[28] because we observed a decrease in height SDS and GnRHa treatment has been shown to result in a decrease in bone mineral density z-scores.[21]

This study has some weaknesses. First, the number of adolescents who presented in early puberty was small, which made it difficult to assess whether early pubertal changes regress under GnRHa treatment and whether prolonged puberty suppression is safe. Now that adolescents are finding their way to gender clinics at younger ages, we should increase our knowledge of the efficacy and safety of this treatment in early pubertal subjects. Second, no control group was included, so it remains unclear how changes in, for example, body composition and alkaline phosphatase during GnRHa treatment in the gender dysphoric adolescents differ from changes that normally occur during the progression of puberty. Ideally, future studies would include age-matched control subjects. Third, the safety parameters assessed in the present study were limited. Effects on bone mineral density and on executive function have been reported, but further studies are necessary.[21,29] These should explore the effects of cross-sex hormone treatment in individuals who have been treated with GnRH analogues to assess outcomes such as adult height and body composition.

CONCLUSIONS

We have shown that GnRHa treatment using triptorelin is effective in suppressing gonadotropins and sex steroids and results in a decrease in testicular volume and cessation of menstrual bleeding. Monitoring of creatinine and liver enzymes did not identify any pathology. Therefore, we propose that routinely

monitoring gonadotropins, sex steroid levels, renal function, and liver enzymes during GnRHa treatment using triptorelin is not necessary. Further studies will have to determine the extent to which changes in height SDS and body composition that were observed during GnRHa treatment can be reversed during subsequent cross-sex hormone treatment.

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Accelerated Versus Slowly Progressive Forms of Puberty in Girls with Precocious and Early Puberty. Gonadotropin Suppressive Effect and Final Height Obtained with Two Different Analogs

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ABSTRACT

Objectives: **To distinguish which children with precocious puberty (PP) and early puberty (EP) should be treated and which followed without therapy. To determine the effect of GnRH analog treatment on the final height of treated patients and compare the effect of two different analogs on gonadotropin suppression and final height.**

Study design: **Sixteen females with PP or EP with a mean chronological age (CA) of 8.8 ± 1.4 years and a mean bone age (BA) of 10.8 ± 1.3 years were treated for a mean of 2.7 ± 1.0 years with a GnRH analog (triptorelin or leuprolide acetate; group A), while 21 girls with a mean CA of 8.5 ± 1.0 years, a mean BA of 9.7 ± 1.4 years and a predicted adult height of >155 cm were followed without therapy (group B). Criteria for treatment were one of: a. predicted adult height (PAH) of <155 cm initially or at any time during follow up; b. PAH over 155 cm with a dramatic decrease in PAH over a 6-month follow-up period; c. advanced and rapidly progressing breast development for age (Tanner 3 before the age of 9 years).**

Results: **GnRHa therapy suppressed gonadotropins in group A, while gonadotropins increased gradually in group B. Height velocity (HV) decreased in group A, while it remained accelerated in group B; BA increased a mean of 1.7 ± 0.5 years in group A and 3.2 ± 0.3 years in group B. This resulted in a height increase in group A from a baseline PAH of 153.7 ± 1.2 cm to a final height (FH) of 160.9 ± 4.0 cm ($p < 0.001$), clearly above their target height (TH) of 157.7 ± 4.2 cm. The height of group B children did not change over time (164.1 ± 4.1 cm before therapy and 166.0 ± 6.0 cm at FH), both above their TH. The mean leuprolide acetate dose utilized in this study decreased during treatment, while both the initial and final triptorelin dose remained unchanged. Adequate gonadotropin suppression (peak level of LH and FSH of <2 IU/l after i.v. GnRH stimulation) was noted with both leuprolide acetate and triptorelin, although LH suppression was slightly more pronounced with triptorelin. BA advanced 1.8 ± 0.4 years during leuprolide acetate treatment and 1.5 ± 0.3 years with triptorelin, so that FH increased a mean of 5.5 ± 1.3 cm with leuprolide acetate and 8.7 ± 2.2 cm with triptorelin.**

Conclusions: **PAH of <155 cm before or during therapy, PAH of >155 cm with a dramatic decrease in predicted height over a 6-month follow-up period and/or advanced and rapidly progressing breast development in girls with PP or EP were useful parameters in deciding which patients to treat. GnRHa therapy suppressed gonadotropins, HV and bone maturation in children with an accelerated form of PP or EP, resulting in a significant height increase. Final height remained stable over time in untreated patients. Adequate gonadotropin suppression was noted with both analogs, although with the doses of analog used in our study, LH and BA suppression were more pronounced with triptorelin, resulting in a larger height gain.**

KEY WORDS

precocious puberty, early puberty, predicted adult height, final height, gonadotropin releasing hormone analog treatment, triptorelin, leuprolide acetate

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INTRODUCTION

Children with central precocious puberty (pubertal signs before the age of 8 years in females and 9 years in males) present with premature secretion of sex steroids which leads to an increase in their growth rates and in the progression of bone maturation. This in turn can shorten their growing period and result in decreased adult height. Gonadotropin releasing hormone analog (GnRHa) treatment in this group of patients has been shown to suppress gonadotropin and sexual steroid secretion, to slow bone age (BA) progression, and possibly to help preserve growth potential[1-6]. In addition to the classical progressing form of central precocious puberty, which generally requires GnRHa therapy, some children with idiopathic precocious puberty may present with a slowly progressive form which has been recently demonstrated to be benign, so that these patients can reach normal adult height and their genetically determined height potential without the need for treatment[7-11].

Early puberty (EP) (pubertal signs at an age of 8-9 years in females and of 9-10.5 years in males) can also be attenuated, clinical changes are sustained but slow, without a significant acceleration of growth or bone maturation, so that final height is not compromised and gonadotropin-suppressive therapy is not warranted. EP, however, may be accelerated in some children, so that patients achieve a state of sexual precocity for age, present with accelerated growth and bone maturation, and their adult height may be compromised; in this group of patients gonadotropin-suppressive therapy would seem warranted[12,13]. However, recent reports suggest that although suppression therapy in boys with EP may convert accelerated puberty into non-sustained slow puberty and probably prevent compromised final height[12], the rate of height gain and of bone maturation in treated and untreated girls with early puberty is similar, so that therapy had no effect on pubertal growth potential and suppressive therapy would only be advisable for those girls with psychological difficulties in coping with their early puberty[13].

The criteria for distinguishing slowly and rapidly progressing precocious puberty (PP) and EP have not been well established, so that the decision of whom and when to treat remains difficult. A predicted adult height (PAH) of 155 cm or less, luteinizing hormone (LH)/follicle stimulating hormone (FSH) peak ratio greater than 0.6, LH-predominant response to exogenous GnRH, and BA advance greater than 2 years above the chronological age (CA), are some of the criteria that have been used to decide whether gonadotropin suppressive therapy should be started in children with either PP or EP[9-11].

A number of different GnRH analogs are being utilized around the world. Depot preparations which require intramuscular injection every 3-4 weeks are used extensively and dosages vary among the different analogs. A comparison of the efficacy of two different long-acting analogs in suppressing gonadotropin secretion and affecting the final height (FH) of children with either PP or EP has, to our knowledge, not been previously performed.

In this study, we analyzed the levels of gonadotropins, the growth velocity (HV), BA maturation, and the FH of children with precocious and early puberty, who were either treated with a GnRH analog or followed without therapy. We also compared the results obtained with two different GnRHa in the treated patients. The aim of the study was to ascertain whether patients with precocious or early puberty with a rapidly progressive form of puberty may improve their FH following GnRHa therapy and whether children with a slowly progressive, attenuated, form could be followed off therapy without impairment of their growth potential.

PATIENTS AND METHODS

Thirty-seven females, 20 with true central precocious puberty (pubertal signs before the age of 8 years) and 17 with early puberty (pubertal signs at an age of 8-9 years) were included in the study. Patients were evaluated at our outpatient clinic and were either assigned into a GnRHa treatment group or were followed without therapy, according to the treatment criteria described below. Sixteen children with PP or EP with a mean CA of 8.8 ± 1.4 years and mean BA of 10.8 ± 1.3 years were treated for a mean of 2.7 ± 1.0 years with a GnRHa (group A), while the remaining 21 children with PP or EP,

mean CA of 8.5 ± 1.0 years and mean BA of 9.7 ± 1.4 years were followed for the same period of time without therapy (group B).

The decision to treat or not to treat was made after the initial evaluation and after 0.5 years or more of follow up and the children were then observed until they reached FH. The criteria for treatment were one of: a. PAH of 155 cm or less at initial or subsequent evaluations; b. PAH over 155 cm with a dramatic decrease in predicted height over a 6-month follow up period (at least 3 cm within a 6-month period with BA advancement to at least 2 years above CA); c. advanced and rapidly progressing sexual development for age (breast tissue Tanner stage 3 or more, with or without sexual hair, before the age of 9 years in a child who presented with no secondary sexual characteristics 6-12 months earlier). The decision not to treat at the time of initial evaluation was based on evidence of a slowly progressive puberty (breast tissue Tanner stage 2, with BA advance of <2 years above CA) with a PAH of >155 cm at the time of initial and subsequent evaluations.

The initial evaluation included determination of height, weight, pubertal stage, PAH, target height (TH), BA, baseline plasma LH, FSH, estradiol, and the gonadotropin response following i.v. administration of 100 μg GnRH. There was no evidence in any of our patients of an organic disorder of the central nervous system as assessed by magnetic resonance imaging or of adrenal or gonadal disease as assessed by ultrasound. Patients were evaluated every 3 months by physical examination; BA was calculated every 6-12 months, and a GnRH test to ascertain the degree of gonadotropin suppression was performed every 6 months in the patients treated with GnRHa, while basal gonadotropins and gonadal steroids were measured at the same interval in those patients being followed without therapy. Treated children received either triptorelin (D-Trp6-GnRH; Decapeptyl®, Ferring Pharmaceuticals) or leuprolide acetate (Depot Lupron®, Abbott Laboratories), both administered i.m. every 28 days; the dose of the analog was adjusted depending on the degree of gonadotropin suppression (for inadequate suppression as determined by a peak level of LH and FSH of >2 IU/l after i.v. GnRH stimulation).

We also compared the FH data and the degree of gonadotropin suppression of our eight leuprolide acetate-treated patients (mean CA of 8.9 ± 1.2 years, mean BA of 10.8 ± 1.8 years), with that of the eight patients receiving triptorelin (mean CA of 8.7 ± 1.0 years and mean BA of 10.9 ± 0.9 years). In addition to analyzing the combined data of patients with PP and EP, we also looked at the FH data of treated and untreated patients with either PP or EP separately.

Patients were considered to have reached FH when their BA was >15 years or when growth over a one-year period was <0.5 cm. Pubertal development was assessed according to Tanner. BAs were read by the same investigator (RL) according to the Greulich and Pyle method[14]; PAH was calculated using the Bayley and Pinneau method[15]. TH was calculated from mid-parental height[16]. Serum LH, FSH, estradiol and testosterone levels were determined by standard radioimmunoassay techniques as previously described[17].

Informed consent was obtained from the parents and the study was approved by the hospital ethics committee. Results are reported as means ± SD. Comparison between groups was made using Student's unpaired t-test or the Wilcoxon rank sum test for non-parametric data. The paired t-test was used as well to compare FH with initial PAH within the same group.

RESULTS

Children in groups A and B had a similar baseline CA, BA, BA/CA ratio, height, pubertal status, LH, FSH and LH/FSH ratio (Table 1). With GnRHa therapy, gonadotropin levels were suppressed significantly in group A patients ($p < 0.0003$ and <0.05, respectively, for LH and FSH) (Table 2), while baseline gonadotropins increased gradually in group B patients. HV decreased significantly in group A children from 8.7 ± 1.1 cm/yr before therapy to 4.6 ± 1.1 cm/yr during therapy, while it remained accelerated in group B patients during follow up; BA increased by a mean of 1.7 ± 0.5 years during the 2.7 ± 1.0 years of therapy in the treated group, while it increased 3.2 ± 0.3 years during this same period in the untreated group. This resulted in an increase in the height of group A

TABLE 1

Baseline clinical and laboratory characteristics of children with precocious or early puberty who were either treated with an GnRH analog (group A) or were followed without therapy (group B)

	Group A (n = 16)	Group B (n = 21)
CA (yr)	8.8 ± 1.4	8.5 ± 1.0
BA (yr)	10.8 ± 1.3	9.7 ± 1.4
BA/CA	1.2 ± 0.2	1.0 ± 0.6
Height velocity (cm/yr)	8.7 ± 1.1	8.5 ± 1.4
PAH (cm)	153.7 ± 1.2	164.1 ± 4.1
Target height (cm)	157.7 ± 4.2	162.2 ± 8.4
Peak LH (IU/l)	9.4 ± 4.3	7.1 ± 4.3
Peak FSH (IU/l)	9.1 ± 3.8	8.5 ± 4.2
LH/FSH	1.2 ± 0.9	1.0 ± 0.8

CA = chronological age; BA = bone age;
PAH = predicted adult height

patients from a baseline PAH of 153.7 ± 1.2 cm to a FH of 160.9 ± 4.0 cm ($p < 0.001$), clearly above their TH of 157.7 ± 4.2 cm. The height of group B children did not change over time (PAH 164.1 ± 4.1 cm before therapy, and 166.0 ± 6.0 cm at FH, both above their TH of 162.2 ± 8.4 cm) (Table 2).

Patients receiving leuprolide acetate were treated for a total of 2.8 ± 0.7 years, while the total treatment period for patients treated with triptorelin was 2.5 ± 0.6 years. The initial dose of leuprolide acetate utilized was 238 ± 63.4 μg/kg and this dose decreased at the end of treatment to 215 ± 91.7 μg/kg; the triptorelin dose utilized decreased only slightly over time from a mean baseline dose of 62.0 ± 5.4 to 59.3 ± 5.6 μg/kg at the end of the treatment period. Analysis of the degree of gonadotropin suppression of group A patients revealed that leuprolide acetate and triptorelin were both effective in suppressing the gonadotropin secretion of our patients (from a peak LH of 8.1 ± 4.3 and 10.8 ± 5.3 IU/l before therapy to a peak LH of 1.4 ± 0.5 and 0.7 ± 0.5 IU/l during leuprolide acetate or triptorelin treatment ($p < 0.0003$ and < 0.001, respectively) and from a peak FSH level of 7.7 ± 3.3 and 10.7 ± 2.5 IU/l before gonadotropin suppressive therapy to a peak FSH concentration of 1.8 ± 0.8 and 1.2 ± 0.8 IU/l during either leuprolide acetate or triptorelin ($p < 0.007$ and < 0.01, respectively). BA advanced a total of 1.8 ± 0.4 years over the 2.8 year treatment period in the leuprolide acetate treated group, while it advanced a mean of 1.5 ± 0.3 years in the triptorelin treated patients over 2.5 years of treatment. FH increased a mean of 5.5 ± 1.3 cm over PAH in the leuprolide acetate treated patients, while it increased 8.7 ± 2.2 cm in the triptorelin treated group.

We then analyzed FH data of patients with PP and EP separately. Eight children with PP treated

TABLE 2

Predicted adult height (PAH), peak LH and FSH at initial presentation, and final height (FH), peak LH and FSH at last evaluation, as well as target height (TH) of treated (group A) or untreated (group B) children with precocious and early puberty

	At initial presentation			At last evaluation			
	PAH (cm)	Peak LH (IU/l)	Peak FSH (IU/l)	FH (cm)	Peak LH (IU/l)	Peak FSH (IU/l)	TH (cm)
Group A (n=16)	153.7±1.2	9.4±4.3	9.1±3.8	160.9±4.0*	1.0±0.9**	1.4±0.4***	157.7±4.2
Group B (n=21)	164.1±4.1	7.1±4.3	8.5±4.2	166.0±6.0			162.2±8.4

* $p < 0.001$, PAH vs FH in group A; ** $p < 0.0003$, peak LH at initial presentation vs peak LH at last evaluation on GnRHa;
*** $p < 0.05$, peak FSH at initial presentation vs peak FSH at last evaluation on GnRHa.

TABLE 3

Predicted adult height before treatment, final height and target height of GnRHa treated and untreated children with either precocious puberty or early puberty

	Predicted adult height (cm)	**Final height** (cm)	**Target height** (cm)
Precocious puberty			
Treated children	153.6 ± 1.3	162.6 ± 4.7*	157.4 ± 4.5
Untreated children	166 0 ± 7.3	168.5 ± 9.9	164.5 ± 8.7
Early puberty			
Treated children	153.1 ± 1.8	159.5 ± 4.7**	158.3 ± 4.0
Untreated children	162.5 ± 5.5	163.8 ± 7.9	160.1 ± 7.9

* $p < 0.01$, PAH vs FH in PP; ** $p < 0.05$, PAH vs FH in EP.

with GnRHa improved their final height significantly; it increased from a PAH of 153.6 ± 1.3 cm before therapy to a FH of 162.6 ± 4.7 cm ($p < 0.01$), well above their TH of 157.4 ± 4.5 cm. Twelve untreated children with PP reached a FH that was similar to their PAH before therapy and above their TH (baseline PAH 166 ± 7.3 cm, FH 168.5 ± 9.9 cm, TH 164.5 ± 8.7 cm) (Table 3).

Similar results were seen in eight children with EP treated with GnRHa, so that a significant improvement in final height over the PAH before therapy was noted (baseline PAH 153.1 ± 1.8 cm, FH 159.5 ± 4.7 cm [$p < 0.05$], TH 158.3 ± 4.0 cm), while nine untreated children with EP reached a FH that was similar to their initial PAH, without the need of therapy (initial PAH 162.5 ± 5.5 cm, FH 163.8 ± 7.9 cm, TH 160.1 ± 7.9 cm) (Table 3).

DISCUSSION

In children with precocious or early puberty, rapid pubertal development can lead to a state of premature sexual development which in turn can have psychological consequences. Premature exposure of the growth plates to sex steroids can shorten the growing period and lead to a decrease in the final height of these patients. GnRHa therapy is intended to block the pituitary-gonadal axis, slow bone maturation, and preserve growth potential[1-6].

Both PP and EP can be attenuated, so that clinical changes are sustained but slow without a significant acceleration of growth or bone maturation; FH is not compromised and gonadotropin-suppressive therapy is not warranted. It may, however, be accelerated in some children, so that they achieve a state of sexual precocity for age, present with accelerated growth and bone maturation, and their adult height may be compromised. GnRHa therapy would seem to be warranted in these patients[7-13]. The criteria for distinguishing slowly and rapidly progressing PP and EP have not been well established, so that the decision of whom and when to treat remains difficult.

In our study, gonadotropin suppressive therapy seemed not to be needed in children with the slowly progressing form of puberty, as adult height was spontaneously preserved in both a combined group of children with PP and EP and in patients with either PP or EP analyzed separately, who reached a FH similar to their PAH at the beginning of follow up and to their TH, without the need for gonadotropin suppressive therapy. In contrast, our patients with a more accelerated form of pubertal development (with either a limited PAH before therapy, an acceptable PAH before therapy which suffered a dramatic decrease over several months of follow up, or with inappropriate sexual development for age before the age of 9 years) seemed to benefit

from GnRHa treatment; their FH improved over their PAH before therapy with an apparent preservation of their height potential, as FH exceeded their TH.

The criteria we used to decide which patients to follow and which to treat, allowed us to select two distinct groups of patients. On the one hand we had an untreated group which was not severely affected by their sexual precocity and whose height prognosis at initial evaluation was not impaired (>155 cm) and did not change during the follow-up period; in this group rapid bone maturation was accompanied by an appropriate acceleration in growth, so that FH was not compromised. In contrast, the group of treated children had a limited mean PAH at baseline (<155 cm) or during follow up; HV decreased during therapy but was accompanied by a significant deceleration in bone maturation which allowed for an improvement in FH. One must, however, remember that BA standards are based on children who enter puberty at an average age, and that in those children entering puberty at an age younger than average a rapid acceleration and progressive advancement of BA occurs. As a result, as explained by Kelnar and Stanhope in a recent commentary[18], PAH at the start of GnRHa treatment can be misleadingly low so that any treatment effect on FH may be overestimated. Double-blind, placebo-controlled prospective trials will have to be performed in the near future to determine the adult height benefit of therapeutic intervention in children with precocious puberty.

There is controversy in the literature on whether GnRHa therapy will result in an increase in the FH of children with central PP or EP. Adan *et al.*[9] in a recent publication found that treatment with GnRHa for nearly 3 years in girls with idiopathic central precocious puberty produced a mean height increase of only 3-4 cm between the PAH and FH, but probably prevented a further decrease in growth potential. Kelnar and Stanhope[18] concluded that a critical review of the evidence suggested that any improvement in height prognosis in children with central precocious puberty treated with GnRHa therapy is, at best, marginal. Lazar *et al.*[13] recently suggested that treatment with a GnRHa affected only the pace of puberty in girls with PP, as both treated and untreated girls had a similar height gain and bone maturation rate at each pubertal stage, so that therapy had no effect on pubertal growth potential. However, in our study in girls with precocious or early puberty, we detected a clear improvement in the FH of patients treated with a GnRHa, with a probable preservation of their height potential, as their FH exceeded their TH. Our results are similar to those of Kreiter *et al*[7] who concluded that GnRHa therapy preserved the height potential in girls with idiopathic true precocious puberty with an initially impaired height prognosis, and to those of Carel *et al.*[19] who recently reported a restoration of genetic TH or 5-10 cm increase in FH in girls treated for at least 2 years with triptorelin for central PP. Paul *et al.*[6] found the mean height deviation from TH to be significantly less in treated females (-1 SD) than in untreated ones (-2.4 SD), and Oerter *et al*[4] found the mean proximate adult height for treated girls to be significantly greater than the pre-treatment PAH (by 5.2 ± 8.4 cm), but below TH. In boys with either central PP or EP, Lazar *et al.*[12] found that suppression therapy converted accelerated puberty into non-sustained slow puberty and probably prevented compromised FH.

The emotional and social implications of sexual precocity for age for many girls and their parents also need to be taken into account when considering suppressive therapy with a GnRHa. Development of secondary sexual characteristics early in life may be embarrassing for the child and may lead to poor socialization, and behavioral, emotional and school-related problems. Issues such as menstrual hygiene, promiscuous sexual behavior and sexual abuse in an emotionally or mentally immature girl are of particular concern to parents of girls with precocious puberty. On the other hand, when deciding whether to begin parental therapy with an analog one must also consider the psychosocial problems related to prolonged therapy and the need for frequent evaluation and testing which may themselves create the poor self image and maladjustment often seen with chronic disease.

Both triptorelin and leuprolide acetate seem to adequately suppress gonadotropin secretion and to contribute to an increase in the FH of patients with PP and EP. In our study, triptorelin treatment

suppressed gonadotropins slightly more effectively than leuprolide acetate, and this may account for the slower bone maturation and for the larger increase in FH of patients treated with this analog; our findings, however, probably reflect the dose of analog we administered. The dose of triptorelin used in different studies varies, but some authors have suggested an initial dose of 60 μg/kg which can then be adjusted according to gonadotropin suppression and the clinical progression of puberty[19,20]; our mean initial dose of triptorelin was 62 μg/kg, while the mean dose at the end of the study was 59.3 μg/kg, both close to this recommended dose. The suggested initial dose of depot leuprolide acetate is 300 μg/kg, with subsequent dose adjustments according to the clinical progression and gonadotropin suppression[1,2]; we used a somewhat lower than recommended mean initial dose of 238 μg/kg and this dose decreased even further to 215 μg/kg at the end of therapy. Leuprolide acetate in our country comes only in 3.75 mg vials and the cost of analog therapy frequently has to be covered by the patient and not by private or public insurance; in an attempt to reduce the cost of treatment we were often hesitant to increase the dose of this analog from 3.75 mg to 7.5 mg or from 7.5 mg to 11.25 mg, and this apparently resulted in the use of a somewhat lower than ideal dose of this analog. Triptorelin, on the other hand, with its 3.75 mg vial, allows for the treatment of almost any size child with the use of a single vial.

In conclusion, our study confirms that both children with precocious puberty and early puberty may present with either a slow, attenuated form of the disease which enables them to preserve their height potential without suppressive therapy or an accelerated form in which children achieve a state of sexual precocity for age, present with accelerated growth and bone maturation, and in which their adult height may be compromised. In the latter group, gonadotropin suppression would seem to be warranted as FH improved over PAH before therapy with an apparent preservation of their height potential, as FH exceeded TH. PAH of <155 cm before or during therapy, a dramatic decrease in predicted height over a 6-month follow-up period with a BA advancement to at least 2 years above CA and/or advanced and rapidly progressing breast development in girls with PP or EP were useful parameters in deciding which patients to treat.

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Effects of Combined Gonadotropin-Releasing Hormone Agonist and Growth Hormone Therapy on Adult Height in Precocious Puberty: A Further Contribution*

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ABSTRACT

Out of 35 girls with idiopathic central precocious puberty (CPP) treated with gonadotropin-releasing hormone agonist (GnRHa) (depot-triptorelin) at a dose of 100 μg/kg every 21 days i.m. for at least 2-3 years whose growth velocity fell below the 25th percentile for chronological age (CA), 17 received growth hormone (GH) in addition at a dose of 0.3 mg/kg/week, s.c., 6 days per week, for 2-4 years. The other 18, matched for bone age (BA), CA and duration of GnRHa treatment, who showed the same growth pattern but refused GH treatment, remained on GnRHa alone, and were used as a control group to evaluate GH efficacy. No patient was GH deficient. Both groups discontinued treatment at a comparable BA (mean ± SD): BA 13.4 ± 0.6 in GnRHa plus GH group vs 13.0 ± 0.5 years in the GnRHa alone group. The 35 patients have reached adult height (i.e. growth during the preceding year was less than 1 cm, with a BA of over 15 years). Patients of the group treated with GH plus GnRHa showed an adult height (161.2 ± 4.8 cm) significantly higher (p <0.001) than pre-treatment predicted adult height (PAH) calculated according to tables either for accelerated girls (153.2 ± 5.0 cm) or for average girls (148.6 ± 4.3 cm). The adult height of the GnRH alone treated group (156.6 ± 5.7) was not significantly higher than pre-treatment PAH if calculated on Bayley and Pinneau tables for accelerated girls (153.9 ± 3.8 cm), whilst it remained significantly higher if calculated on tables for average girls (149.6 ± 4.0 cm) (p <0.001). The gain between pre-treatment PAH and final height was 8.2 ± 4.8 cm according to tables for accelerated girls and 12.7 ± 4.8 cm according to tables for average girls in patients treated with GH plus GnRHa; while in patients treated with GnRH alone the gain calculated between pre-treatment PAH for accelerated girls was just 2.3 ± 2.9 cm and 7.1 ± 2.7 cm greater than pre-treatment PAH for average girls. The difference between the gain obtained in the two groups (about 6 cm) remained the same, however PAH was calculated. The addition of GH to GnRHa in a larger cohort of patients with CPP with a longer follow-up confirms the safety of the combined treatment and the still significant but more variable gain in the group with the combined treatment, probably due to the larger number of patients analyzed. Caution is advised in using such an invasive and expensive treatment, and there is need for further studies before widespread clinical use outside a research setting.

KEY WORDS

central precocious puberty, growth velocity, GnRH analogues, growth hormone

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INTRODUCTION

Gonadotropin-releasing hormone agonists (GnRHa) are considered the treatment of choice for children with central precocious puberty (CPP)[1-5]. During this therapy the secretion of gonadotropins and the consequent production of sex steroids are suppressed. Therefore GnRHa arrest the development of sexual characteristics, and decrease either growth velocity (GV) or bone maturation (BA)[1-5], and consequently should improve adult height[2,6-20]. However, in some patients GV decrease is so marked that the expected improvement in predicted adult height (PAH) is not achieved[21,22]. Therefore some investigators suggest, on the basis of several studies on growth hormone (GH)-IGF-I axis function in this subset of patients with CPP[23-28], the use of GH in combination with GnRHa[28-32].

The aim of this study was to evaluate the efficacy of GH in addition to GnRHa on final height in comparison with GnRHa alone using a previously reported cohort of patients[30] increased by those who have since completed the previous study protocol. Thus, we report here the results obtained in 35 girls with idiopathic CPP who have reached adult height, 17 treated with combined therapy and 18 treated with GnRHa alone.

PATIENTS AND METHODS

Thirty-five females with idiopathic CPP, diagnosed according to the classic criteria[1], treated with GnRHa (depot-triptorelin) at a dose of 100 μg/kg every 21 days i.m. for at least 2-3 years, showed a GV decrease below the 25th percentile for chronological age (CA). Seventeen girls agreed to receive GH in addition to GnRHa, at a dose of 0.3 mg/kg/week, s.c., 6 days per week, for 2-4 years. The other 18, matched for BA, CA and duration of GnRHa treatment, who showed the same growth pattern but refused GH treatment, remained on GnRHa alone, and were used as a control group to evaluate GH efficacy. Auxological data before, during and at the end of treatment for the two groups are shown in Tables 1 and 2, respectively.

No patient had any evidence of progressive organic central nervous system disorders by magnetic resonance imaging (MRI). All patients were euthyroid; GH secretory status was studied at the time of growth deceleration. No patient was GH deficient (peak of GH >10 ng/ml).

This study was approved by the ethics committee of our institution; written consent was obtained from parents of children who received GH.

Both groups of patients were examined at the start of treatment and every 6 months during and after discontinuation of therapy. At each evaluation, height was measured three times by the same observer with a Harpenden's stadiometer. Pubertal staging was calculated by the standards of Marshall and Tanner[33]. BA was determined according to the method of Greulich and Pyle[34] by the same two observers. Predicted adult height (PAH) was calculated according to the method of Bayley and Pinneau[35] twice for each patient, as follows: 1) as suggested by Bayley and Pinneau, using the tables for accelerated girls when BA was advanced for CA by 1 year or more, and using the tables for average girls when BA was within 1 year of CA; 2) as suggested by Kauli *et al.*[13] using the tables for average girls for all patients, disregarding advanced bone age. Target height (TH) was calculated using measured parental height adjusted for the child's sex according to Tanner *et al.*[36].

Plasma samples for determination of sex steroid levels were obtained every 6 months and gonadotropins were evaluated every 6 months after i.v. administration of 100 μg of luteinizing hormone releasing hormone (LHRH); the LHRH stimulation test was performed on day 20 after GnRHa injection. Pelvic ultrasound, to evaluate uterine and ovarian volumes, was performed every 6 months.

Blood screening to assess metabolic, hepatic, renal, hematological, and thyroid function were also performed at each evaluation. An oral glucose tolerance test (OGTT) was performed every 12 months in the patients treated with GH, to evaluate any possible insulin resistance[37].

Both groups discontinued treatment at a comparable BA and CA: BA was 13.4 ± 0.6 years in the GnRHa plus GH group vs 13.0 ± 0.5 years in the GnRHa alone group, and CA 13 ± 1.3 vs 12.5 ± 1.0 years, respectively. GH was discontinued contemporaneously with GnRHa, regardless of the current criteria for withdrawal (i.e. GV less than 2 cm/year and BA >14 years). GnRHa were discontinued

according to the following criteria: 1) BA <13 years; 2) GV less than 2 cm/year in the previous year; 3) a satisfactory attained height; 4) the psychological need of each girl to progress in sexual development.

The 35 patients have reached adult height (i.e. growth during the preceding year was less than 1 cm, with a BA of over 15 years).

Hormonal assays

Circulating levels of LH, follicle stimulating hormone (FSH), and estradiol were assayed in duplicate, using commercial kits. LH and FSH levels was determined by an immunoradiometric assay (Maiaclone, Serono Biodata, Milan, Italy); estradiol was measured by radioimmunoassay (RIA) (DPC, Los Angeles, CA); GH was measured in duplicate by polyclonal RIA (Sorin Biomedica, Vercelli, Italy).

Statistical analysis

Data are expressed as means ± SD, unless otherwise stated. Statistical analysis of the results was assessed using Student's t-test, paired and unpaired as required. A p value less than 0.05 was considered significant.

RESULTS

Plasma FSH and LH peaks after LHRH test were suppressed during treatment, both in the GnRHa + GH treated group (LH 0.5 ± 0.45 vs 26.9 ± 19.8 IU/l, FSH 1.5 ± 0.76 vs 12.1 ± 2.7 IU/l, both $p < 0.05$) and in the GnRHa alone treated group (LH 0.55 ± 0.44 vs 24.1 ± 13.8 IU/l, FSH 1.71 ± 1.2 vs 15.9 ± 7.9 IU/l, both $p < 0.05$).

After discontinuation of therapy, peak LH rose to 15.8 ± 6.3 and FSH peak to 10.0 ± 8.5 IU/l within 1 year in the combined group, and peak LH rose to 14.2 ± 5.9 and FSH peak to 11.54 ± 7.71 IU/l within a similar period in the group treated with GnRHa alone ($p < 0.05$).

In the GH treated group, no adverse effects were observed either regarding lipid and glucose metabolism (glucose/insulin basal ratio greater than 6[37]) or abnormal advancement in BA.

On treatment, pelvic ultrasound showed reduced ovarian volume in both groups and no ovarian cysts were observed in girls treated with GH, as previously reported by others[38]. Ovarian volumes were reduced from 3.2 ± 1.3 to 2.3 ± 1.22 ml ($p < 0.05$) during treatment in the GnRHa + GH group; similarly, in the group treated with GnRHa alone, ovarian volumes during therapy reduced from 2.35 ± 1.26 to 1.74 ± 0.68 ml ($p < 0.05$).

The uterine length remained unchanged during treatment in both groups (from 4.6 ± 1.0 to 4.5 ± 0.6 cm in the GnRHa + GH treated group, and from 4.4 ± 0.8 to 4.3 ± 0.4 cm in the GnRHa alone group). After 6-12 months ovarian volumes and uterine length increased to pubertal values. In both groups menarche occurred about 6-18 months (average 1 year) after discontinuation of treatment.

In the group treated with GnRHa plus GH, adult height was 161.2 ± 4.8 cm, strongly significantly higher ($p < 0.001$) than pretreatment PAH, either calculated as accelerated girls (153.2 ± 5.0 cm) with a gain of 8.2 ± 4.8 cm (range 2.2-17 cm) or as average girls (148.6 ± 4.3 cm) with a gain of 12.7 ± 4.8 cm (range 4.4-20.6 cm). Target height (157.4 ± 4.9 cm) was significantly exceeded ($p < 0.05$) (Table 1).

In the group treated with GnRHa alone, adult height was 156.6 ± 5.7 cm, not significantly higher than pretreatment PAH if calculated as accelerated girls (153.9 ± 3.8 cm) with a gain of just 2.3 ± 2.9 cm (range -1.2 to 5.9), and significantly ($p < 0.001$) higher than pretreatment PAH calculated as average girls (149.6 ± 4.0 cm) with a gain of 7.1 ± 2.7 cm (range 2.5-12.3 cm) (Table 2). Target height (157.2 ± 6.0 cm) was not significantly exceeded.

DISCUSSION AND CONCLUSIONS

The effect of GnRHa on the growth axis is still not clear[24-27]; it generally results in GV decrease at prepubertal values, but sometimes the GV deceleration is so marked as not to allow any improvement of pre-treatment PAH. For this reason the addition of GH has been suggested by some authors, though clear evidence of GH-IGF-I axis dysfunction has never been reported[27-31].

Our previous study on 20 patients with CPP[30] and striking GV deceleration showed that the

TABLE 1

Auxological data of 17 patients with central precocious puberty treated with gonadotropin-releasing hormone agonist (GnRHa) plus GH

	At diagnosis	At start of GnRHa	At start of GnRHa + GH	At end of GnRHa +GH	At adult height
CA (yr)	6.6 ± 1.0	8.3 ± 1.6	9.9 ± 1.3	13.0 ± 1.3	16.4 ± 2.4
BA (yr)	9.5 ± 1.2	11.0 ± 1.4	12.1 ± 0.8	13.4 ± 0.6	16.0 ± 1.0
PAH accelerated tables B&P[35] (cm)	156.2 ± 4.5	153.2 ± 5.0	154.2 ± 5.3	165.3 ± 7.3**	
PAH average tables B&P (cm)	150 ± 4.5	148.6 ± 4.3	150.5 ± 5.1	163.1 ± 6.7**	
Adult height (cm)	161.2 ± 4.8**				
Target height (cm)	157.4 ± 4.8*				
Gain (cm) **(difference between adult height and pre-treatment PAH accelerated tables B&P)**	8.2 ± 4.8				
Gain (cm) **(difference between adult height and pre-treatment PAH average tables B&P)**	12.7 ± 4.8				

Values are means ± SD. CA = chronological age; BA = bone age; PAH = predicted adult height.
** $p < 0.001$ vs start of GnRHa; * $p < 0.05$ vs adult height.

TABLE 2

Auxological data of 18 patients with central precocious puberty treated with gonadotropin-releasing hormone agonist (GnRHa) alone

	At diagnosis	At start of GnRHa	At start of GH in the other group (Table 1)	At end of GnRHa	At adult height
CA (yr)	6.0 ± 2.0	7.9 ± 0.8	9.9 ± 0.9	12.5 ± 1.0	15.4 ± 1.7
BA (yr)	8.4 ± 2.9	10.7 ± 1.2	12.0 ± 0.8	13.0 ± 0.5	15.6 ± 1.1
PAH accelerated tables B&P[35] (cm)	158.7 ± 5.4	153.9 ± 3.8	154.9 ± 4.6	160.6 ± 5.3**	
PAH average tables B&P (cm)	151.8 ± 5.6	149.6 ± 4.0	152.3 ± 5.0	158.2 ± 5.3**	
Adult height (cm)	156.6 ± 5.7**				
Target height (cm)	157.2 ± 6.0				
Gain (cm) **(difference between adult height and pre-treatment PAH accelerated tables B&P)**	2.3 ± 2.9				
Gain (cm) **(difference between adult height and pre-treatment PAH average tables B&P)**	7.1 ± 2.7				

Values are means ± SD. CA = chronological age; BA = bone age; PAH = predicted adult height.
* $p < 0.05$, ** $p < 0.001$ vs start of GnRHa and vs PAH average at start of GnRHa.

addition of GH to GnRHa led to a significant gain on adult height compared with pre-treatment PAH in ten girls, versus a less significant gain obtained in the ten girls treated with GnRHa alone. The present study on a larger number of patients, with the addition of those who had in the meantime completed the study protocol (seven treated with combined GnRHa plus GH, and eight treated with GnRH alone) confirms a significant gain in adult height obtained with the co-administration of GH. However, a greater variability of response was observed, probably due to the greater number of patients analyzed.

GH, given at a dose nowadays considered as replacement therapy, did not advance bone maturation, nor did it alter either metabolic parameters or ovarian morphology, as ovarian cysts did not appear during treatment or after discontinuation. That a prompt recovery of hypothalamic-pituitary-gonadal axis activity occurred in both groups was shown by the response of LH and FSH to GnRH stimulation, as well as increased ovarian and uterine volumes 1 year after the withdrawal of therapy.

In conclusion, our study on a larger number of patients with a longer follow-up period than in the previous study[30] confirms the safety of co-administration of GH and GnRHa on metabolic parameters and the hypothalamic-pituitary-gonadal axis, but the true efficacy of the addition of GH to GnRHa therapy is still questionable. This combined therapy leads to a gain in adult height versus pre-treatment PAH that is greater than the gain obtained with GnRHa alone (the difference between the gains obtained in the two groups being around 6 cm). However, we recommend caution regarding such an invasive and expensive treatment, and there is need for further studies before widespread clinical use outside a research setting.

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ORIGINAL ARTICLE

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Long-term efficacy and safety of gonadotropin-releasing hormone analog treatment in children with idiopathic central precocious puberty: A systematic review and meta-analysis

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Abstract

Objective: To investigate the long-term efficacy and safety of gonadotropin-releasing hormone analog (GnRHa) treatment in children with idiopathic central precocious puberty (CPP).

Method: The protocol was registered with International Prospective Register of Systematic Reviews (CRD42018102792). PubMed, EMBASE and the Cochrane Library were searched for eligible comparative and single-arm studies.

Results: We identified a total of 98 studies that included 5475 individuals. The overall risk of bias of the eligible studies ranged from critical to moderate. The overall quality of evidence for each outcome ranged from very low to moderate. Evidence-based comparative studies showed that GnRHa treatment increase final adult height (FAH, cm; studies = 4, n = 242; mean difference [MD] = 4.83; 95% confidence interval [CI], 2.32 to 7.34; I^2 = 49%) and decrease body mass index (BMI, kg/m^2; studies = 3, n = 334; MD = −1.01; 95% CI, −1.64 to −0.37; I^2 = 0%) in girls with idiopathic CPP compared with no treatment. The incidence of polycystic ovary syndrome (PCOS) did not significantly differ with and without GnRHa treatment (studies = 3, n = 179; risk ratio = 1.21; 95% CI, 0.46 to 3.15; I^2 = 48%). The evidence for other long-term outcomes was very weak to deduce the effects of GnRHa treatment. Further, limited evidence is available on its effects in boys.

Conclusion: Compared with no treatment, evidence indicates that GnRHa treatment increase FAH and decrease BMI in girls with idiopathic CPP. GnRHa treatment did not evidently increase the risk of PCOS. However, evidence regarding other key long-term outcomes (such as infertility and malignant or metabolic diseases) was considered very weak to suggest the benefits or side effects of GnRHa treatment. Additional high-quality evidence is needed before firm conclusions can be drawn.

KEYWORDS
central precocious puberty, gonadotropin-releasing hormone analog, meta-analysis, systematic review

1 | INTRODUCTION

Central precocious puberty (CPP) results from premature activation of the hypothalamic–pituitary–gonadal axis (HPGA) and is commonly characterized by the early development of pubertal biochemical and physical features before 8 years of age for girls and 9 years of age for boys.[1,2] CPP is a rare condition and has an estimated overall prevalence of approximately 1 per 5000–10,000 children, with a five- to 10-fold higher incidence in girls than in boys.[3–6] CPP can be classified into idiopathic CPP (ICPP) and secondary CPP; the latter is including genetic causes(familial CPP, chromosomal abmormalities), central nervous system abnormalities (hypothalamic hamartomas, cysts, central nervous system granulomas, hydrocephalus, septo-optic hypoplasia), secondary to chronic exposure to sex steroid hormones (late treatment of simple virilizing congenital adrenal hyperplasia, following resection of tumours secreting sex steroid hormones, testotoxicosis, McCune-Albright syndrome) or endocrine disruptors..[7] ICPP is the most frequent form of CPP, accounting for approximately 90% cases of CPP in girls and 25%–60% in boys.[8–10] Although the exact mechanism underlying the development of ICPP is not well understood, several potential metabolic, genetic and epigenetic explanations have been considered.[11–15] CPP is associated with a lower final adult height (FAH), potential sexual abuse, increased risk of psychological disturbances and increased risk of developing cardiovascular diseases and reproductive tract cancers.[16,17]

Gonadotrophin-releasing hormone analog (GnRHa) is a synthetic peptide drug that is modelled based on human hypothalamic gonadotropin-releasing hormone (GnRH), which is designed to act on the anterior pituitary.[7] GnRHa interacts with the GnRH receptor and stimulates the synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the initial phase of administration ('flare up'). Sustained release of GnRHa suppresses the production of FSH and LH, which in turn suppress the production of sex hormones by the gonads.[7] Several pharmaceutical formulations of GnRHa, such as buserelin, histrelin, leuprorelin, triptorelin and goserelin, are available and used clinically.[18,19] The choice of drug and duration of treatment depend on the unique growth and development needs.[19,20] GnRHa has been a treatment choice for CPP since the mid-1980s, and its effects on HPGA suppression has been generally recognized.[19,21,22] However, the long-term efficacy and safety of GnRHa treatment remain unclear, and some studies have reported contradictory findings.[3]

Several studies have reported that GnRHa may improve FAH in girls with CPP[3,23–26]; this is particularly true if they were diagnosed before the age of 6 years and treated with GnRHa from Tanner stage 2–3 to chronological age 11–12 years and bone age 12–12.5 years.[27] However, the effects of GnRHa treatment are unknown in girls diagnosed between 6 and 8 years of age.[3] Regarding body mass index (BMI), several studies have found that GnRHa treatment did not lead to an increased risk of weight gain.[28–30] Among these studies, Corripio et al[30] reported an increase in weight based on BMI standard deviation score (SDS). In terms of its effect on the reproductive system, GnRHa treatment was not confirmed to be harmful to ovarian function or fertility.[31] There was no clear difference in the incidence of androgen excess or polycystic ovary syndrome (PCOS) between children with CPP treated with GnRHa and those in the healthy comparison group.[31–33] However, the effects of GnRHa treatment on bone mineral density (BMD), glucose and lipid metabolism, and psychological status remain unclear.[19,20,34,35] Therefore, we conducted this systematic review and meta-analysis to evaluate the long-term efficacy and safety of GnRHa treatment in children with ICPP.

2 | METHODS

2.1 | Registration

The protocol for this review was registered with the International Prospective Register of Systematic Reviews (CRD42018102792). This article has been prepared according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines.[36]

2.2 | Literature search and study selection

We searched PubMed, EMBASE and the Cochrane Library in November 2019, without placing any limitations on language or publication year. The detailed search strategies were developed by an information specialist and are presented in the Online Supplementary Materials. Two reviewers (LH and WW) independently screened the search results based on the following inclusion criteria: (a) prospective or retrospective comparative studies and single-arm studies; (b) participants with ICPP (as defined in the original study) with the onset of secondary sex characteristics before 8 years of age in girls and before 9 years of age in boys; and (c) studies that reported long-term (defined as a duration of ≥6 months) outcomes in participants who received GnRHa (any type of dosage regimen) compared with participants who received no treatment/placebo or GnRHa plus growth hormone (GH; any type of dosage regimen). We excluded studies that enrolled participants with negative results in the GnRH stimulation test and those with non-idiopathic CPP (such as isosexual precocious puberty, familial male-limited precocious puberty, or familial precocious puberty). Studies in which the participants were diagnosed with a brain tumour, trauma, infection, macrophage activation syndrome, congenital adrenal hyperplasia or GH deficiency were also excluded. Any disagreement during screening was resolved by discussion and, when necessary, with assistance from a third reviewer (YL).

2.3 | Outcome measures

The primary outcomes were as follows: FAH, which is considered the final adult stature of an individual when the bone age

is ≥15 years and/or the rate of growth in height is <1 cm/year in the past year (or within ≥2 years after a girl has experienced menarche); target height (TH), which is calculated using the height of the individual's parents (as defined in the original study); BMI and risk of being overweight/obese (being overweight is defined as a BMI above the 85th percentile or 25–29.9 kg/m^2 and obesity as a BMI above the 95th percentile or >30 kg/m^2); and the incidence of PCOS among girls and androgen excess among boys. PCOS is defined as a syndrome of ovarian dysfunction along with the cardinal features hyperandrogenism and polycystic ovary (PCO) morphology. The secondary outcomes included menstrual parameters (such as age at menarche and regularity of menstruation), growth velocity (GV), insulin-like growth factor 1 (IGF-1) level, BMD, glucose and lipid metabolism, insulin resistance parameters and psychological state.

2.4 | Data extraction and risk of bias assessment

Two reviewers (LH and WW) independently extracted qualitative and quantitative data using a standard data collection form. The risk of bias of the included studies was assessed according to the study design. Randomized controlled trials (RCTs) were assessed using the risk of bias tool from the Cochrane Handbook for Systematic Reviews of Interventions.[37] Non-randomized comparative studies were assessed using the 'Risk Of Bias In Non-randomized Studies - of Interventions' (ROBINS-I) tool.[38] Single-arm studies were rated as having a high risk of bias. Disagreements were resolved by discussion or by consulting with the third reviewer (XPL) when necessary.

2.5 | Statistical analysis

Separate analyses were performed based on single-arm studies and comparative studies. Regarding single-arm studies, qualitative and quantitative data are summarized to provide a comprehensive description of the phenotype of the participants and the primary reasons for treatment. Meta-analyses were performed for comparative studies. We estimated risk ratios (RRs) with 95% confidence intervals (CIs) for dichotomous outcomes, and mean differences (MDs) with 95% CIs for continuous outcomes. We employed a random-effects model for all meta-analyses using the R software,[39] and we performed separate analyses based on sex. The outcome data derived from comparative studies and single-arm studies were combined if there was no clinical and methodological heterogeneity present. To explore clinical heterogeneity, we planned to perform a priori subgroup analysis on primary outcomes based on the age of onset (<6 vs ≥6 years of age) as well as the type of GnRHa used. However, due to insufficient data and wide CIs for most treatment estimates, we did not perform additional sensitivity analyses. Statistical heterogeneity was estimated by I^2 and χ^2 statistics (substantial statistical heterogeneity was defined as I^2 ≥ 50% with a p-value of <.1 in the χ^2 test).

3 | RESULTS

3.1 | Search results

A total of 3515 hits were identified from searching the electronic databases. After assessing their eligibility, 98 studies with 105 references were included in this systematic review. The detailed reasons for exclusion are illustrated in the PRISMA study selection flow diagram (Figure 1).

3.2 | Included studies

The 98 included studies enrolled a total of 5475 participants (98.5% were girls). All references for the included studies are presented in the Supplementary Material. The sample size of the included studies ranged from 6 to 333. No RCTs were identified. Among the 98 included studies, 18 were randomized comparative studies (n = 1303) and the remaining 81 (n = 4172) were single-arm studies. Antoniazzi 2000 employed both comparative and single-arm study designs, thereby accounting for both non-randomized comparative and single-arm studies. The average age of CPP onset ranged from 4.5 to 8 years, and the average age of GnRHa treatment initiation ranged from 5 to 9.31 years. Various formulations of GnRHa were used in the included studies such as leuprorelin, triptorelin, buserelin, goserelin, deslorelin and histrelin. Thirteen studies (n = 1047) compared GnRHa treatment with no treatment, and six studies (n = 310) compared GnRHa treatment with GnRHa plus GH. The treatment duration ranged from 3 months to 5 years for all included studies. Additional study details are presented in Table S1.

3.3 | Quality assessment of included studies

Among the 18 comparative studies, none received low risk of bias scores across all domains. Based on ROBINS-I, 10 (55.6%) studies (Liang 2015, Poomthavorn 2011, Antoniazzi 2000, Shiasi Arani 2015, Colmenares 2014, Gyon 2015, Lanes 2004, Léger 2000, Magiakou 2010, and Pucarelli 2003) were judged to have an overall moderate risk of bias. Six (33.3%) studies (Faienza 2017, Swaiss 2017, Antoniazzi 2000, Bridges 1995, Jung 2014, and Yuan 2011) were judged to have a critical risk of bias because they selected participants based on either the intervention they received or the prediction of FAH. Two (11.1%) studies (Lazar 2014 and Lazar 2015) were judged to have a critical risk of bias with regards to the selection of participant domains as well as an overall critical risk of bias. Following our protocol that was established a priori, the 81 single-arm studies were regarded to have a high risk of bias. The summary of our assessment of risk of bias for

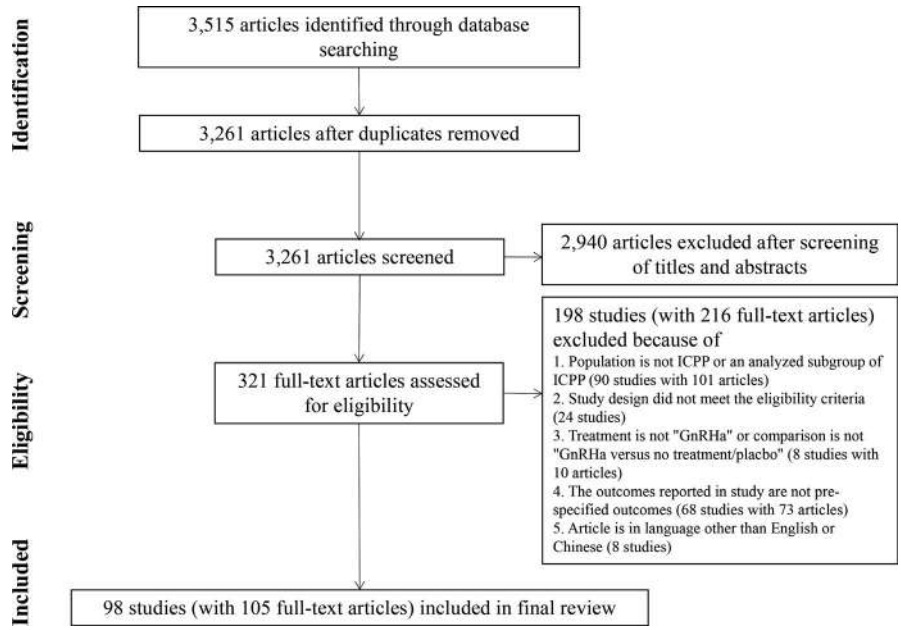

FIGURE 1 PRISMA study selection flow diagram

comparative studies is presented in Table S2. Following the consideration of inconsistency and indirectness, the overall quality of evidence for each outcome ranged from very low to moderate.

3.4 | Results of single-arm studies

Among the 81 single-arm studies (*n* = 5316), 47 included non-specified CPP patients (*n* = 2527) and 34 included ICPP patients (*n* = 2789). A total of 130 males and 5903 females were included in 80 studies, and one study (Comite 1986) did not report information on sex. The age of onset of ICPP ranged from 4.5 to 8 years, and the age at which the patients first received treatment ranged from 5 to 9.31 years. The included participants were treated with leuprolide in 26 studies, buserelin in one study, decapeptyl (including triptorelin) in 34 studies, histrelin in two studies, nafarelin in one study, non-specific GnRHa treatment in 10 studies, and a combination of these drugs in the remaining seven studies. The duration of treatment ranged from 3 months to 5 years (Table S1).

Among the 81 studies, 12 (Nabhan 2007, Borges 2015, Lin 2017, Lazar 2007, Antoniazzi 2000, Antoniazzi 2003, Baumann 2001, Carel 1999, Chen 2009, Gillis 2013, Kempers 2002, and Ying 2017) (*n* = 485) reported the average TH and FAH of girls (Table S4). In six studies (Borges 2015, Lin 2017, Lazar 2007, Carel 1999, Chen 2009, and Gillis 2013), the mean FAH of girls exceeded their TH (Table 1). One retrospective study (Lazar 2007) investigated the posttreatment height gain against the age of onset.

Four studies reported average BMI (*n* = 72), and eight studies reported average BMI-SDS (*n* = 300) in girls with ICPP after GnRHa treatment (Table S4).

The age at menarche was reported in 11 studies (*n* = 615), and all 11 studies reported the time to menarche after discontinuation of treatment. Further, 26 studies reported GV, 8 reported IGF-1 level, five reported BMD, 6 reported glucose and lipid indices, and three reported insulin resistance parameters. There were no remarkable findings in relation to the secondary outcomes (including GV, IGF-1 level, BMD, glucose and lipid indices, and insulin resistance parameters; Table S4, S6 and S7).

Five studies reported psychological outcomes, including cognitive functioning and emotional reactivity (Baumann 2001, Menk 2017, Schoelwer 2017, Wojniusz 2016, and Zheng 2008). Meta-analysis was not performed because the included studies used different scales. In general, GnRHa-treated CPP girls did not significantly differ in their cognitive or psychosocial functioning from age-matched controls.

Five single-arm studies evaluated boys with ICPP, and the descriptive results regarding FAH, BMI, GV and IGF-1 based on single-arm studies are presented in Table S5. The results were similar to those of girls, although the sample size of each study was very small (*n* = 8–13).

3.5 | Meta-analysis of comparative studies

All comparative studies included girls with ICPP (Table 2; Table S3).

3.6 | Adult height improvement

Five studies compared GnRHa treatment with no treatment (Faienza 2017, Swaiss 2017, Poomthavorn 2011, Antoniazzi 2000, and Lanes 2004). The results of these studies demonstrated that girls treated with GnRHa reached their TH, whereas most girls without treatment did not reach their TH. In addition, FAH (cm)

TABLE 1 Height (cm) reported in single-arm studies

Study ID	Sample size (*n*)	Sex	Characteristics at presentation/initiation of therapy						GnRHa	FAH, cm, Mean (SE)	TH, cm, Mean (SE)
			Pubertal stage	CA, years, Mean (SD)	BA, yeas, Mean (SD)	BA minus CA, years, Mean (SD)	Height SDS at CA, Mean (SD)	PAH, cm, Mean (SD)			
Antoniazzi 2000	71	Female	NR	7.0 (1.3)	9.8 (1.4)	BA/CA: 1.4 (0.3)	1.5 (1.7)	155.5 (7.0)	Triptorelin	158.4 (0.69)	161.5 (0.82)
Antoniazzi 2003	21	Female	Breast and pubic hair stage ≥2	7.28 (1.14)	8.82 (1.04)	NR	129.9 (6.8) cm	153.3 (4.8)	Leuprorelin	160.5 (1.18)	160.8 (1.37)
Baumann 2001	19	Female	NR	5.8 (2.2)	NR	NR	NR	NR	Buserelin or triptorelin	160.9 (1.62)	161.8 (1.33)
Borges 2015	54	Female	NR	NR	8.3 (2.3)	1.7 (1.1)	1.05 (1.03)	NR	Leuprorelin	162 (1.64)	158 (1.02)
Carel 1999	58	Female	NR	7.5 (1.3)	10.1 (1.5)	NR	2.4 (1.5)	156.4 (6.3)	Triptorelin	161.1 (0.77)	160.1 (0.58)
Chen 2009	26	Female	NR	7.8 (0.7)	11.2 (0.9)	NR	NR	151.5 (5.6)	Non-specific	158 (0.78)	155.3 (0.86)
Gillis 2013	23	Female	Breast stage ≥3 (16/23, 70%) Pubic hair stage ≥3 (4/23, 17%)	8.4 (0.3)	10.0 (0.3)	1.7 (0.2)	0.99 (0.26)	155.2 (1.9)	Triptorelin	157.9 (1.70)	160.8 (0.75)
Gillis 2013	11	Female	Breast stage ≥3 (10/11, 91%) Pubic hair stage ≥3 (4/11, 36%)	8.7 (0.3)	10.4 (0.4)	1.7 (0.3)	0.89 (0.26)	156.8 (2.6)	Histrelin	161.1 (2.00)	160.1 (0.97)
Lazar 2007	22	Female	tanner stage 2 to 3	6.4 (1.2)	NR	2.5 (0.8)	1.3 (0.8)	154.6 (6.6)	Triptorelin	162.8 (1.07)	159.3 (1.07)
Lazar 2007	38	Female	tanner stage 2 to 3	7.5 (0.6)	NR	2.5 (0.9)	1.2 (0.8)	153.7 (6.7)	Triptorelin	157.9 (0.83)	157.8 (0.84)
Lin 2017	43	Female	NR	8.76 (1.32)	NR	BA/CA: 1.20 (0.13)	135.91 (9.30) cm	NR	Leuprorelin	158.98 (0.83)	157.8 (0.53)
Nabhan 2009	26	Female	Breast development (Tanner) 2.6 (0.8)	7.2 (2.0)	10.1 (2.2)	2.9 (1.2)	NR	158.5 (6.8)	Leuprorelin	152.6 (1.27)	164 (1.12)
Kempers 2002	17	Female	NR	6.4	NR	NR	NR	NR	Triptorelin	166.2 (2.12)	168.8 (1.98)
Ying 2017	101	Female	NR	8.4 (0.84)	10.6 (0.53)	NR	137.7 (6.26) cm	153.1 (5.37)	Non-specific	157 (0.48)	157.7 (0.38)

Abbreviations: BA, bone age; CA, chronological age; FAH, final adult height; GnRHa, gonadotropin-releasing hormone analog; *n*, number; NR, not reported; PAH, predicted adult height; SDS, Standard deviation score; TH, target height.

TABLE 2 Characteristics of comparative studies—GnRHa vs no treatment

				Characteristics at presentation/initiation of GnRHa							
Study ID	**Sample size (*n*)**	**Sex**	**GnRHa**	**Pubertal stage**	**CA, years, Mean (SD)**	**BA, yeas, Mean (SD)**	**BA minus CA, years, Mean (SD)**	**Height SDS at CA, Mean (SD)**	**HV, , Mean (SD), SDS**	**PAH, cm, Mean (SD)**	**TH, cm, Mean (SD)**
Antoniazzi 2000	40	Female	Buserelin; triptorelin	Breast stage ≥2	7.7 (0.9)	10.2 (1.1)	NR	2.1 (0.5)	2.3 (0.5)	152.9 (6.6)	155.5 (5.3)
Arani 2015	110	Female	Triptorelin	NR	7.46 (1.02)	8.96 (1.66)	NR	0.62 (1)	NR	156.31 (7.61)	158.06 (4.75)
Bridges 1995	54	Female	Buserelin or goserelin	NR	NR	NR	NR	NR	NR	NR	NR
Colmenares 2014	37	Female	Triptorelin	Tanner stage 2 to 3	7.4 (1.3)	8.7 (2.1)	NR	2.8 (1.2)	1.6 (2.1)	SDS: 0.3 (2.3)	NR
Faienza 2017	50	Female	Triptorelin	Breast development (Tanner B2 or above)	7.0 (0.6)	10.1 (1.6)	NR	Height SDS/BA: −1.2 (0.8)	8.1 (1.5) cm/year	158.4 (3.6)	160.8 (4.7)
Lanes 2004	20	Female	Triptorelin or leuprorelin	NR	8.8 (1.4)	10.8 (1.3)	BA/CA: 1.2 (0.2)	NR	8.7 (1.1) cm/year	153.6 (1.3)	157.4 (4.5)
Lazar 2014	235	Female	Triptorelin	Breast Tanner stage 2 with or without sexual hair	8.1 (1.0)	NR	NR	NR	NR	NR	NR
Lazar 2015	142	Female	Triptorelin	Breast Tanner stage 2 with or without sexual hair	8.3 (0.9)	NR	NR	NR	NR	NR	NR
Léger 2000	26	Female	Triptorelin	Tanner stage 2 to 3	7.6 (1.1)	9.2 (1.9)	NR	NR	0.9 (1.2)	157.7 (6.6)	161.3 (4.7)
Magiakou 2010	47	Female	Triptorelin	Breast stage 3 pubic hair stage 2	Median 7.92	Median 10	NR	Median 0.66	NR	Median 151.53	NR
Poomthavorn 2011	58	Female	Triptorelin or leuprorelin	NR	8.5 (1.0)	11.1 (1.7)	2.7 (1.1)	1.5 (1.0)	9 cm/year	155.3 (6.7)	155.8 (4.1)
Swaiss 2017	50	Female	Triptorelin	NR	7.11 (0.7)	10.1 (1.6)	2.8 (1.3)	131.3 (9.2) cm	NR	158.5 (10.8)	163.9 (5.7)
Yuan 2011	134	Female	Non-specific	NR	8.16 (0.76)	9.78 (1.24)	NR	0.54 (0.96)	NR	SDS: −0.41 (1.38)	158.29 (3.81)

Abbreviations: BA, bone age, CA, chronological age, FAH, final adult height, GnRHa, gonadotropin-releasing hormone analog; HV, height velocity, *n*, number, NR, not reported, PAH, predicted adult height, SDS, Standard deviation score, TH, target height.

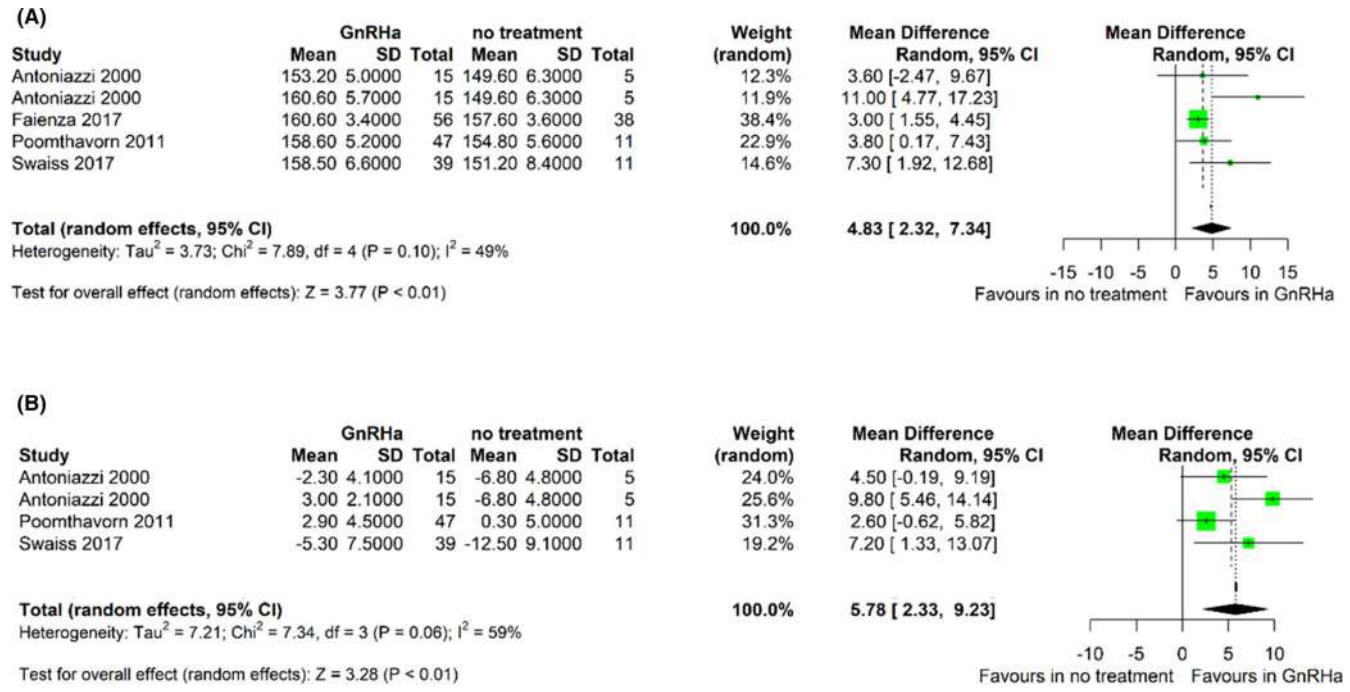

FIGURE 2 Forest plots of gonadotropin-releasing hormone analog treatment compared with no treatment for height outcomes [Colour figure can be viewed at wileyonlinelibrary.com]

was greater in girls treated with GnRHa than in those who were not treated (studies = 4, *n* = 242; MD = 4.83; 95% CI, 2.32 to 7.34; I^2 = 49%; Figure 2A). The participants of the study by Lanes 2004 (not included in the meta-analysis) were assigned to the intervention group based on their predicted height, and the girls with a predicted height of <155 cm received GnRHa treatment. The average FAH of the participants in the intervention group was not significantly different from that of the participants in the no-treatment group.

The difference between FAH and TH (FAH minus TH, cm) was larger in the GnRHa group than in the no-treatment group (studies = 3, *n* = 148; MD = 5.78; 95% CI, 2.33 to 9.23; I^2 = 59%; Figure 2B).

Five studies (Liang 2015, Gyon 2015, Bridges 1995, Jung 2014, and Pasquino 1996) were included in this comparison (Table S3). All girls in both GnRHa and GnRHa plus GH groups (Liang 2015, Gyon 2015, Jung 2014, and Pasquino 1996; *n* = 168) reached their TH. No significant difference was found in FAH or FAH minus TH after treatment between the groups.

3.7 | BMI

Six studies compared GnRHa treatment with no treatment and reported relevant outcomes on weight (Poomthavorn 2011, Shiasi Arani 2015, Colmenares 2014, Yuan 2011, Lazar 2015, and Arcari 2016). When participants reached their FAH, the pooled BMI level was lower in the GnRHa group treatment than in the no-treatment group (BMI (kg/m^2): studies = 3, *n* = 334; MD = −1.01; 95% CI, −1.64 to −0.37; I^2 = 0%; Figure 3A and BMI-SDS: studies = 3, *n* = 285; MD = −0.51; 95% CI, −0.75 to −0.28; I^2 = 13%; Figure 3B). The proportion of girls who were overweight or obese was similar between the two groups (studies = 3, *n* = 289; RR = 0.95; 95% CI, 0.66 to 1.38; I^2 = 58%; Figure 3C).

3.8 | Menarche and Menstrual irregularity

Four studies (Faienza 2017, Lazar 2014, Léger 2000, and Lazar 2015) reported that girls who received GnRHa treatment did not experience early menarche, and the average age at menarche ranged from 12 to 13 years. Results showed that girls who received GnRHa treatment experienced menarche later than those who did not (studies = 4, *n* = 458; MD = 1.18; 95% CI, 0.77 to 1.58; I^2 = 94%; Figure 4B). Two studies (Liang 2015 and Gyon 2015) (*n* = 125) showed that the GnRHa group experienced menarche at a younger age than the GnRHa plus GH group (MD = −0.35; 95% CI, −0.62 to −0.09; I^2 = 0%).

3.9 | Fertility and PCOS

Only one study (Lazar 2014) reported that the proportion of pregnancies was lower in the GnRHa (triptorelin) group than in the no-treatment group (*n* = 235; RR = 0.63; 95% CI, 0.50 to 0.80). However, among pregnant women (*n* = 108), the proportion requiring ovulation induction and/or in vitro fertilization was significantly lower in the GnRHa (triptorelin) group than in the no-treatment group

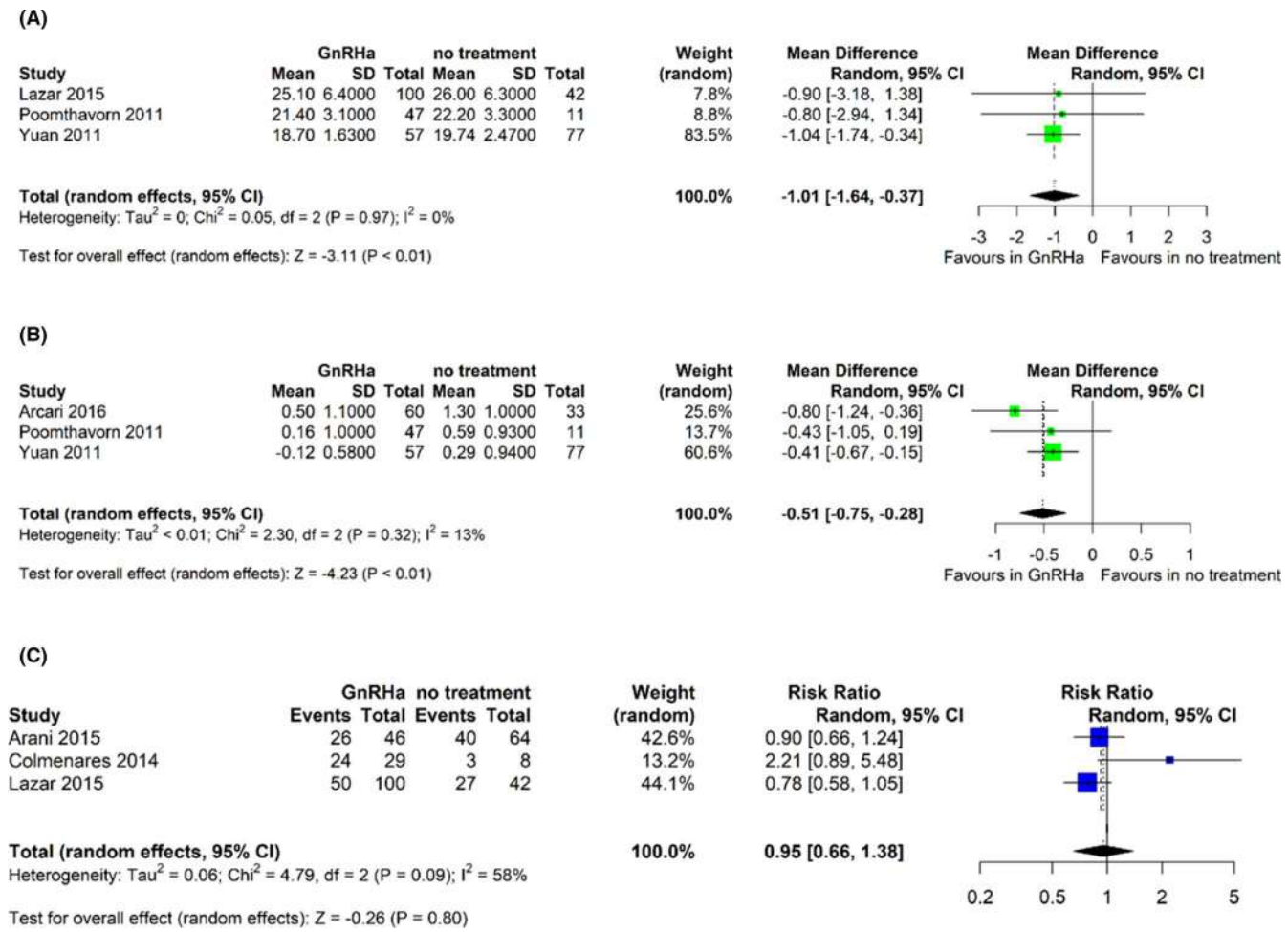

FIGURE 3 Forest plots of gonadotropin-releasing hormone analog treatment compared with no treatment for body mass index [Colour figure can be viewed at wileyonlinelibrary.com]

(RR = 0.33; 95% CI, 0.15 to 0.75). There was no clear difference in the incidence of early miscarriages or preeclampsia between the two groups (RR = 1.07; 95% CI, 0.32 to 3.58).

Individual studies showed more oligomenorrhoea and higher adrenal androgen levels (Faienza 2017) and reduced ovarian volume, LH:FSH ratio and Ferriman-Gallwey score (Magiakou 2010) in GnRHa-treated girls. However, overall the meta-analysis showed there was no significant difference between the GnRHa and no-treatment groups (studies = 3, n = 179; RR = 1.21; 95% CI, 0.46 to 3.15; I^2 = 48%) (Figure 4A). Bridges 1995 (n = 29) showed that there was no significant difference in the incidence of PCOS between GnRHa and GnRHa plus GH groups.

3.10 | Malignant diseases

Only one study (Lazar 2015; n = 142) reported only one patient had acute lymphoblastic leukaemia in the GnRHa group. No significant difference in the incidence of malignant diseases during young adulthood (around 30 years) between GnRHa and no GnRHa groups.

4 | DISCUSSION

In this systematic review, we aimed to determine the long-term efficacy and safety of GnRHa treatment in children with ICPP. Current evidence is mainly focused on girls with ICPP, and the overall quality of evidence for each studied outcome was found to range from very low to moderate. The main findings of our meta-analyses showed that compared with no treatment, GnRHa treatment improved the FAH of girls by increasing FAH by ≥2.32 cm. The average FAH of girls after GnRHa treatment was closer to their TH, if not more than their TH. The impact of GnRHa treatment on girls with different ages of CPP onset remains unclear due to insufficient evidence. In addition, the follow-up results (average follow-up: 3 years, range: 6 months to >20 years) revealed that GnRHa treatment might not lead to strong side effects such as risk of overweight/obesity and of PCOS, other malignancies, and metabolic syndromes. Although BMI levels were shown to increase slightly at the start of GnRHa treatment (particularly in girls with a normal baseline BMI status), girls who received treatment had lower BMI levels (reduced by ≥0.28 kg/m^2) than those who did not in adulthood. Furthermore, BMI levels did not significantly exceed the normal range, which indicated that

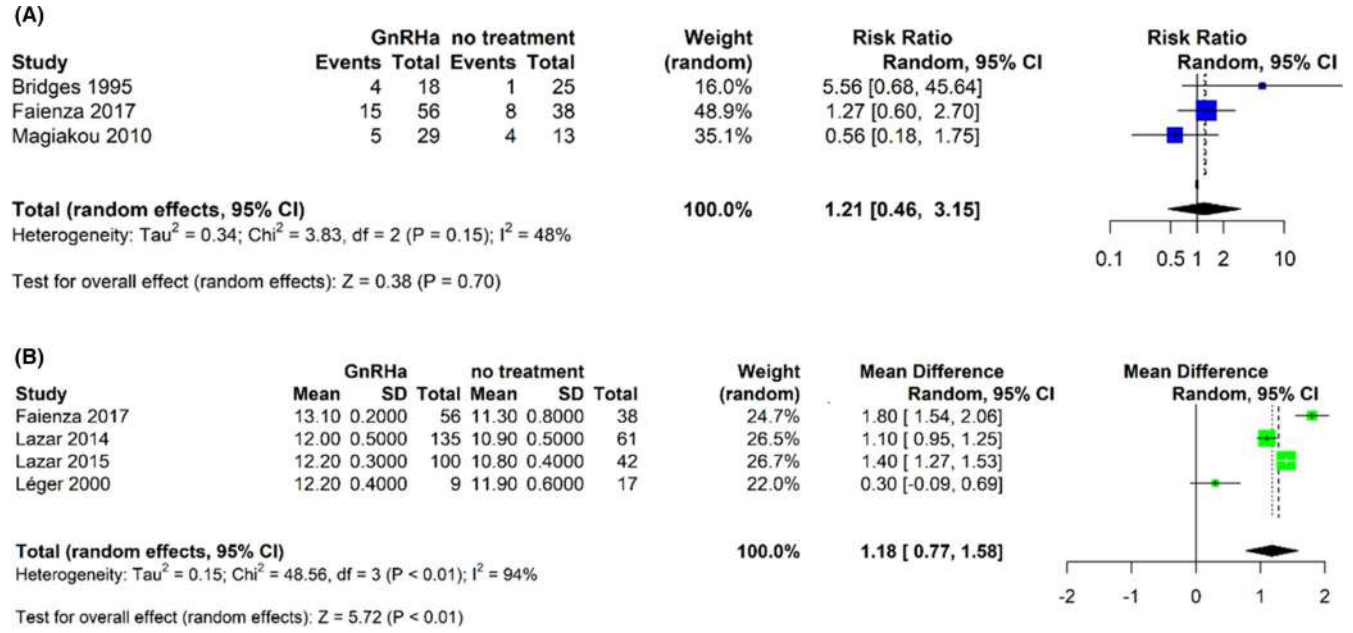

FIGURE 4 Forest plots of gonadotropin-releasing hormone analog treatment compared with no treatment for reproductive issues [Colour figure can be viewed at wileyonlinelibrary.com]

GnRHa treatment is less likely to increase the risk of overweight/obesity. GnRHa treatment may reduce the risk of early menstruation, and the average age at menarche was 1 year older than that in girls who did not receive treatment. There was no significant difference in the incidence of PCOS between the GnRHa and no-treatment groups. In addition, the prevalence of malignant diseases was low among women with former ICPP and in healthy controls. The evidence regarding fertility was obtained from only one study (Lazar 2014; *n* = 235); among the pregnant women with former ICPP, more women experienced spontaneous pregnancy in the GnRHa group than in the no-treatment group. Furthermore, GnRHa did not increase the risk of early miscarriage. Bone densitometric parameters were within the normal range for the respective sex and age groups before and after GnRHa treatment, and GnRHa treatment did not increase the risk of metabolic diseases such as diabetes and hyperlipidemia.

Early evidence has indicated that precocious puberty may lead to certain psychological or social problems, which are considered to bother parents and may affect the clinical treatment of CPP.[40] However, according to the results of the included studies, GnRHa treatment did not worsen the cognitive, psychological and social problems of children with ICPP and has the potential to reduce problems in some children, which was consistent with recent evidence.[41,42]

Several of the outcomes in the present review showed substantial heterogeneity (I^2 > 50%) and one possible source may be the use of different drugs of GnRHa treatment. In addition, the small sample size may have contributed to the heterogeneity.

Our findings are somewhat consistent with those of a previous systematic review[3] that explored the long-term outcomes of GnRHa treatment in children with CPP. Guaraldi 2016[3] reported that GnRHa treatment appeared to improve FAH in girls with CPP and had no clear negative impact on BMI, risk of PCOS, or BMD. However, only the PubMed database was searched in this review. Another network meta-analysis is currently assessing the efficacy and safety of GnRHa treatment.[43] Although the present review did not predefine the exact population as Gu 2019,[43] a similar conclusion was reached.

4.1 | Strengths and limitations

The strengths of this systematic review include the creation of comprehensive search strategies to identify all relevant published studies and the use of sound methodology, which involved use of two reviewers to independently select studies and extract data. The latter strength minimizes the risk of performance bias in conducting the systematic review. However, our work also has some limitations. The results generated from pooling data of single-arm studies had a high level of statistical heterogeneity; thus, it was not possible to infer and draw meaningful conclusions from these meta-analyses. Furthermore, bias in the selection of participants is a major concern in several of the included comparative studies. The treatment regimen of GnRHa and the dropout rates were not well described in most of the comparative studies, which may exaggerate the magnitude of the estimated effects of meta-analysis. Treatment duration has been suggested as a contributing factor to improved FAH in the literature. However, all of the included comparative studies reported treatment duration of 2–5 years, which limited the conduction of subgroup analysis. Furthermore, a substantial level of statistical heterogeneity was evident for some outcomes such as the differences between FAH and TH and age at menarche. Therefore, our results should be interpreted with

caution. Moreover, the current evidence cannot be directly applied to boys with CPP due to the lack of data on this population. Further research, particularly large-scale RCTs (multicenter) or high-quality comparative studies with an adequate sample size, follow-up rate and duration, including both girls and boys, are required before firm conclusions can be drawn. In addition, it will be important to explore the main influencing factors on the long-term effects of GnRHa treatment.[44]

5 | CONCLUSION

Compared with no treatment, the current evidence indicates that GnRHa treatment improve the FAH of girls with ICPP, thus allowing them to meet or exceed their TH. GnRHa treatment also reduce the BMI levels of participants compared with BMI of those treated with placebo. Furthermore, GnRHa did not appear to increase the risk of PCOS. However, evidence regarding other predefined key outcomes, such as infertility, malignancy and metabolic diseases, is very weak to indicate the benefits or side effects of GnRHa treatment.

AUTHOR CONTRIBUTION STATEMENT

Xiaoping Luo: protocol development, manuscript review and revision. Yan Liang: study selection and data collection. Ling Hou: study selection and data collection. Wei Wu: study selection and data collection. Yanqin Ying: data analysis and partial review drafting. Feng Ye: partial review drafting.

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CONFLICT OF INTEREST

The authors have nothing to disclose.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article or in the data repositories listed in references.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Long-Term Observation of 87 Girls with Idiopathic Central Precocious Puberty Treated with Gonadotropin-Releasing Hormone Analogs: Impact on Adult Height, Body Mass Index, Bone Mineral Content, and Reproductive Function

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Objective: We assessed in a retrospective unicenter study the impact of treatment with GnRH analogs (GnRHa) on adult height (AH), body mass index (BMI), bone mineral density (BMD), and reproductive function in girls with idiopathic central precocious puberty (ICPP).

Patients: Eighty-seven ICPP patients were treated with GnRHa for 4.2 ± 1.6 yr (range 3–7.9) and observed for 9.9 ± 2.0 yr (range 4–10.6 yr) after discontinuation of treatment; to estimate the efficacy better, 32 comparable ICPP untreated girls were analyzed.

Results: AH was 159.8 ± 5.3 cm, significantly higher than pretreatment predicted AH (PAH) either for accelerated or for average tables of Bayley and Pinneau. The gain in centimeters between pretreatment PAH and AH was 5.1 ± 4.5 and 9.5 ± 4.6 cm, respectively. Hormonal values and ovarian and uterine dimensions, reduced during treatment, increased to normal after 1 yr without therapy. Age of menarche was 13.6 ± 1.1 yr with an interval of 0.9 ± 0.4 yr after therapy. Menstrual pattern was normal. Six girls became pregnant and delivered normal offspring. BMI SD score for chronological age increased, but not significantly, before, during, and after therapy. BMD at discontinuation of treatment was significantly lower and increased to control values after gonadal activity resumption.

Conclusions: GnRHa treatment in ICPP is safe for the reproductive system, BMD, and BMI and helpful in reaching AH close to target height; however, the variability of individual responses suggests that one choose more parameters than increment in height, especially in girls with pubertal onset over 8 yr of age. (***J Clin Endocrinol Metab*** **93: 190–195, 2008)**

For more than 20 yr (1), GnRH analogs (GnRHa) have been used in the treatment of central precocious puberty (CPP). The question of adult height (AH) improvement is still controversial, although a considerable number of CPP subjects treated with GnRHa for many years have reached AH. Long-term observations during and after discontinuation of therapy and follow-up studies of big cohorts of CPP patients are reported (2–14). In this unicenter retrospective study on a group of 87 girls affected by idiopathic central precocious puberty (ICPP) treated with GnRHa and observed for several years after discontinuation of treatment, we evaluated the impact on AH, body mass index (BMI), bone mineral density (BMD), and reproductive function.

Subjects and Methods

Subjects

Eighty-seven girls with ICPP were treated with GnRHa for 4.2 ± 1.6 yr (range 3–7.9) and observed for 9.9 ± 2.0 yr (range 4–10.6) after discontinuation of treatment (Tables 1 and 2).

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Abbreviations: AH, Adult height; BA, bone age; BMD, bone mineral density; BMI, body mass index; CA, chronological age; CPP, central precocious puberty; GnRHa, GnRH analogs; ICPP, idiopathic CPP; MRI, magnetic resonance imaging; PAH, predicted adult height; PAH-BP, PAH using tables for accelerated girls; PAH-BPav, PAH using tables for average girls; SDS, SD score; TH, target height; vBMD, volumetric BMD.

TABLE 1. Clinical and auxological characteristics of CPP patients at the start and end of treatment and AH

Parameter	Treated group (n = 87)	Untreated group (n = 32)
Before treatment		
CA at first observation (yr)	6.5 ± 1.5	6.8 ± 1.6
BA at first observation (yr)	9.1 ± 2.3	9.1 ± 1.0
CA at start of treatment (yr)	8.4 ± 1.5	8.3 ± 1.2
BA at start of treatment (yr)	11.1 ± 1.6	11.2 ± 1.4
Height velocity before treatment (cm/yr)	8.2 ± 1.8	
BMI (kg/m^2)	18.5 ± 2.4	
BMI SDS for CA	0.39 ± 0.8	
Height SDS-BA	−1.2 ± 0.8	−1.1 ± 0.6
Height (cm)	134.8 ± 9.3	136.0 ± 8.9
PAH-BPav (cm)	150.0 ± 5.1	151.0 ± 3.9
PAH-BP (cm)	154.2 ± 5.2	155.1 ± 4.3
At end of treatment		
CA (yr)	12.6 ± 1.0	
BA (yr)	13.1 ± 0.5	
BMI (kg/m^2)	21.7 ± 3.1	
BMI SDS for CA	0.41 ± 0.9	
Height SDS-BA	−0.2 ± 0.8[a]	
Height (cm)	153.8 ± 5.0	
PAH-BPav (cm)	160.6 ± 5.9[a]	
PAH-BP (cm)	162.8 ± 6.6[a]	
Duration of treatment (yr)	4.2 ± 1.6	
At adult height		
CA (yr)	16.1 ± 2.2	16.3 ± 2.7
BA (yr)	16.0 ± 1.5	17.7 ± 2.7
BMI (kg/m^2)	22.9 ± 3.8[b]	
BMI SDS for CA	0.44 ± 1.0	
Height SDS-BA	−0.5 ± 0.9[a]	−1.3 ± 1.0[c]
Adult height (cm)	159.8 ± 5.3[d]	154.4 ± 5.9[e]
Target height (cm)	157.6 ± 4.7	158.5 ± 4.8
ΔAH-PAH-BPav at start (cm)	9.5 ± 4.6	3.0 ± 6.0[c]
ΔAH-PAH-BP at start (cm)	5.1 ± 4.5	0.6 ± 4.5[c]
ΔAH-final height (cm)	5.6 ± 2.6	
ΔAH-TH (cm)	2.4 ± 5.2	−4.3 ± 5.7[c]

Values are the mean ± SD.

[a] $P < 0.001$.

[b] $P < 0.01$ *vs.* start of GnRHa.

[c] $P < 0.001$.

[d] $P < 0.01$, AH *vs.* TH.

[e] $P < 0.01$, treated group *vs.* nontreated group.

Diagnosis of CPP was made according to the following classical criteria: 1) onset of breast development (stage B2 or above according to Tanner) before 8 yr of chronological age (CA), 2) pubertal LH response (>7 IU/liter) to GnRH stimulation test, 3) increment of height velocity and advancement of bone age (BA) by at least 1 yr over CA, 4) uterine length greater than 3.5 cm and ovarian volumes greater than 1.5 cm^3 at ultrasound. No evidence of hypothalamic-pituitary organic lesions at magnetic resonance imaging (MRI) allowed us to classify as idiopathic the CPP of these girls. However, throughout the years, MRI was repeated to confirm the initial findings. We repeated MRI in the youngest subjects and those with particularly progressive clinical picture before treatment without following a rule in number and frequency.

Personal history, rate of pubertal progression, and consequent psychological problems were evaluated since the first observation. CA at initial evaluation was 6.5 ± 1.5 yr (range 1.2–7.9), BA was 9.1 ± 2.3 yr (range 2–11). Although CA at initial evaluation was generally older than CA at appearance of the first signs of puberty because this is reported by relatives and generally quite not sufficiently documented, we decided not to consider for statistical evaluation CA at onset of puberty but CA at first observation.

The initial evaluation included measuring height, pubertal stage, BA, basal plasma estradiol levels, and LH and FSH responses to GnRH test. In girls presenting with pubic hair as first sign of puberty and striking advancement of BA, an ACTH iv test was performed to evaluate basal and after stimulation 17α-hydroxyprogesterone and testosterone levels to exclude the possibly underlying coexistence of nonclassical congenital adrenal hyperplasia.

GnRHa treatment was undertaken after an observation period of at least 6 months to rule out transient or slowly progressive forms of CPP. Patients were treated with depot triptorelin (D-TRP6-LHRH) at a dose of 100–120 μg/kg every 21–25 d im. Cyproterone acetate was given orally at the dose of 100 mg/d, divided into two administrations, for 21 d before and 21 d after the first GnRH analog injection to prevent any stimulatory effect by gonadotropins during this flare-up period. The dose was reduced to 50 mg/d the last week.

CA at the start of therapy was 8.4 ± 1.5 yr (range 1.7–9.5), BA was 11.1 ± 1.6 yr (range 3–12), respectively.

Height, weight, BA, pubertal staging, and LH and FSH levels after standard GnRH test were evaluated every 6 months during treatment to assess the suppression of the pituitary-gonadal axis. The dose of GnRHa was adjusted to maintain complete suppression of the pituitary-gonadal axis, demonstrated by GnRH test and after the change of body weight along treatment.

The girls discontinued treatment at a CA of 12.6 ± 1.0 yr (range 10.2–13.5) and at a BA of 13.1 ± 0.5 yr (range 12–14.2).

During the observation period subsequent to the cessation of therapy, all the girls reached AH. AH was considered to be reached when during the preceding year growth was less than 1 cm with a BA of over 15 yr.

BMI of each subject was calculated before, during, and after treatment (even after more than 5 yr) to verify significant changes.

BMD was evaluated at discontinuation of therapy and yearly afterward.

As to reproductive function, during treatment, FSH and LH levels, uterine length, and ovarian volumes at ultrasound were evaluated every 6 months. After discontinuation of treatment, the resumption of menarche, menstrual cycles, underachievement of pregnancy, and birth of a fetus were documented.

To estimate the treatment efficacy better, we analyzed 32 contemporary untreated girls comparable with those treated (Table 1). These patients had refused GnRHa treatment for several reasons, although continuing to remain under observation.

Methods

At each evaluation, height was measured three times by the same observer with a Harpenden's stadiometer. Pubertal staging was calculated by the standards of Marshall and Tanner (15). BA was determined according to the atlas of Greulich and Pyle (16) by the same two observers. Predicted adult height (PAH) was calculated according to the method of Bayley and Pinneau (17) twice for each patient, as follows: the tables for accelerated girls, in which BA is advanced for CA by 1 yr or more (PAH-BP) and the tables for average girls, in which BA is within 1 yr of CA (PAH-BPav), which was used in all the patients as suggested by Kauli *et al.* (18) also the tables for average girls, disregarding how advanced BA was, in each girl.

Target height (TH) was calculated as midparental height adjusted for sex (minus 6.5) (19).

BMI was calculated as weight (kilograms)/height (square meters) and was expressed in SD score (SDS) for CA, according to Cacciari *et al.* (20)

BMD was measured by dual-energy x-ray absorptiometry in the lumbar spine at the L2-L4 level, a site that provides by a measure of integral (cortical plus trabecular) bone, with a QDR 4500 densitometer (Hologic, Bedford, MA). The values were corrected by the vertebral surface scanned and expressed as BMD (grams per square centimeter). Dual-energy x-ray absorptiometry-derived data were used to calculate lumbar spine volumetric BMD (vBMD), expressed in grams per cubic centimeter,

TABLE 2. Clinical and auxological characteristics of group 1 (CA ≤ 7 yr at first observation) and group 2 (CA > 7 yr at first observation) at diagnosis, the start, discontinuation of treatment, and AH

Parameter	Treated		*P* value
	≤7 yr (n = 44)	>7 yr (n = 43)	
Before treatment			
CA at first observation (yr)	5.6 ± 1.6	7.5 ± 0.3	
BA at first observation (yr)	8.1 ± 2.6	10.1 ± 1.3	
CA at start (yr)	7.7 ± 1.6	9.1 ± 0.8	
BA at start (y)	10.4 ± 1.8	11.7 ± 0.9	
Height SDS-BA	−1.03 ± 0.8	−1.34 ± 0.7	NS
Height (cm)	133.0 ± 10.3	137.5 ± 5.5	
PAH-BPav (cm)	150.3 ± 5.4	149.7 ± 4.3	NS
PAH-BP (cm)	155.2 ± 5.7	153.2 ± 4.5	NS
TH (cm)	157.1 ± 5.4	158.0 ± 3.9	NS
At end of treatment			
CA (yr)	12.4 ± 1.05	12.8 ± 1.02	NS
BA (yr)	13.0 ± 0.5	13.2 ± 0.5	NS
Height SDS-BA	−0.04 ± 0.8	−0.42 ± 0.8	<0.05
Height (cm)	154.7 ± 4.6	152.8 ± 5.4	NS
PAH-BPav (cm)	162.0 ± 6.1	158.5 ± 6.4	<0.05
PAH-BP (cm)	164.6 ± 6.5	161.1 ± 5.8	<0.05
Treatment (yr)	4.7 ± 1.8	3.7 ± 1.0	<0.005
At AH			
CA (yr)	16.2 ± 2.6	15.8 ± 2.0	NS
BA (yr)	15.9 ± 1.5	15.9 ± 1.5	NS
Height SDS-BA	−0.24 ± 0.9	−0.68 ± 0.8	<0.05
AH (cm)	160.9 ± 5.6	158.6 ± 4.8	NS
ΔAH-PAH-BPav at start (cm)	10.4 ± 4.7	8.6 ± 4.4	NS
ΔAH-PAH-BP at start (cm)	5.6 ± 4.6	4.9 ± 4.3	NS
ΔAH-final height (cm)	5.8 ± 2.7	5.5 ± 2.5	NS
ΔAH-TH (cm)	4.0 ± 5.1	0.75 ± 4.8	<0.01

Data are expressed as mean ± SD.

taking the vertebral body as an ellipsoid cylinder and dividing bone mineral content obtained by lateral scan (in grams) by body vertebral volume (in cubic centimeters), calculated (p × width/2 × depth/2 × height) to reduce the confounding effect of bone size (21).

Statistical analysis

Data are expressed as the mean ± SD, unless otherwise stated. Statistical analysis of the results was assessed using Student *t* test, paired and unpaired if required. Correlations between two parameters were determined by Pearson's correlation coefficient analysis. $P < 0.05$ was considered significant.

Results

At first observation, mean CA was 6.5 ± 1.5 and BA 9.1 ± 2.3 yr; at the start of treatment, CA was 8.4 ± 1.5 and BA 11.1 ± 1.6 yr, height was 134.8 ± 9.3 cm, and BMI 18.5 ± 2.4 kg/m^2. AH, reached after GnRHa treatment for a duration of 4.2 ± 1.6 yr, was 159.8 ± 5.3 cm. Because pretreatment PAH was 154.2 ± 5.3 cm (BP accelerated) and 150.1 ± 5.1 (BP average), the gain obtained with treatment on AH was 5.1 ± 4.5 and 9.5 ± 4.6 cm, respectively. Nevertheless, AH was well above TH ($P < 0.01$). Regression analysis between AH and several parameters (Table 3) showed a positive correlation with TH, height at the initiation and end of treatment, and PAH before and at the end of treatment and no correlation with duration of treatment, in agreement with other authors.

To investigate whether growth results could be influenced by the age at onset of puberty, we divided our patients into two groups: group 1 with CA younger than 7 yr (n = 44) and group 2 with CA older than 7 yr (n = 43). No significant difference was

TABLE 3. Factors associated with AH (centimeters) in girls treated with GnRHa for precocious puberty

	r	*P* value
TH	0.411	<0.05
CA at first observation (yr)	−0.268	<0.05
Height at the start of treatment (SDS)	0.588	<0.001
PAH-BP at the start of treatment (cm)	0.558	<0.001
PAH-BPav at the start of treatment (cm)	0.425	<0.01
Duration of treatment	0.252	NS
Height at the end of treatment (SDS)	0.588	<0.001
Growth velocity at the end of treatment (cm/yr)	0.533	<0.001
PAH-BP at the end of treatment (cm)	0.881	<0.001
PAH-BPav at the end of treatment (cm)	0.558	<0.001
ΔAH-height at the end of treatment (cm)	0.361	<0.001

found between the two groups as to AH or the gain in centimeters over PAH (Table 2).

The comparison between AH of the 87 treated girls and 32 ICPP comparable untreated girls, who served as control group, although not randomized, showed that the untreated subjects had a significant loss in terms of centimeters *vs.* treated girls' AH (5.4 cm) *vs.* their TH (4.3 ± 5.7 cm; $P < 0.01$) and *vs.* their average PAH (about 6 cm) and accelerated PAH (about 5 cm; $P < 0.001$; Table 1).

Because BMI in children is age related, either considering the whole group or considering the two groups with onset before or after 7 yr of age, during treatment a marked increase was observed. However, as at the beginning of treatment, BMI SDS for CA was 0.39 ± 0.8, at discontinuation 0.41 ± 0.9, and many years after 0.44 ± 1.0; no significant difference (P = NS) was found. Not all the patients were overweight or obese (14.3 and 9.1%, respectively, at the start of therapy and 11.7% for both categories either at discontinuation of treatment or several years after at AH).

We observed that, besides individual data, on the whole BMI increased, although remaining in the same centile or SDS throughout treatment. Furthermore, patients who were overweight or obese at the end of treatment were in the same position of the beginning. Regression analysis showed BMI SDS for CA at the end of treatment positively correlated with BMI SDS for CA at the start of treatment ($P < 0.001$; r = 0.332).

BMD was evaluated in 66 of 87 patients. At discontinuation of treatment, mean BMD lumbar spine was 0.82 ± 0.01 g/cm^2 and mean vBMD was 0.135 ± 0.03 g/cm^3; both values were significantly lower ($P < 0.001$) than in controls (1.001 ± 0.11 g/cm^2 and 0.143 ± 0.03 g/cm^3, respectively).

At complete resumption of gonadal activity, mean BMD lumbar spine increased to 1.000 ± 0.11 g/cm^2, not significantly different from controls (1.015 ± 0.11 g/cm^2); similarly, mean vBMD increased to 0.165 ± 0.01 g/cm^3, not significantly different from controls (0.166 ± 0.02 g/cm^3).

Plasma FSH and LH peaks after the LHRH test were suppressed during treatment significantly lower than pretreatment (peak LH 0.6 ± 0.7 *vs.* 24.2 ± 28.3 IU/liter, peak FSH 1.6 ± 1.0 *vs.* 13.2 ± 7.1 IU/liter, both $P < 0.005$); by 1 yr after therapy, peak LH arose back to 30.3 ± 16.0 and FSH to 11.5 ± 11.9 IU/liter ($P < 0.005$). Estradiol basal levels (26.9 ± 5.5 pg/ml) during treatment were significantly lower than pretreatment (8 ± 2.8 pg/ml; $P < 0.001$) and arose to 64.9 ± 13.6 pg/ml 1 yr after therapy withdrawal ($P < 0.001$).

Ovarian volumes, reduced from 2.8 ± 1.3 to 1.9 ± 1.0 cm^3 during treatment, increased to 5.4 ± 3.2 cm^3 ($P < 0.001$), and uterine length, unchanged during treatment (4.6 ± 0.8 cm), increased to 6.7 ± 0.9 cm ($P < 0.001$), both already after 1 yr without therapy. Menarche appeared at the age of 13.6 ± 1.1 yr after withdrawal of GnRHa at 0.9 ± 0.4 yr (range 0.3–2 yr). The history of menstrual pattern showed that 82 patients had regular menses; the remaining five showed oligomenorrhea due to intensive sport activity, which within 2–3 yr resolved after decrease of intensive exercise. Six girls (one of them twice) became pregnant and delivered normal offspring (Figs. 1 and 2).

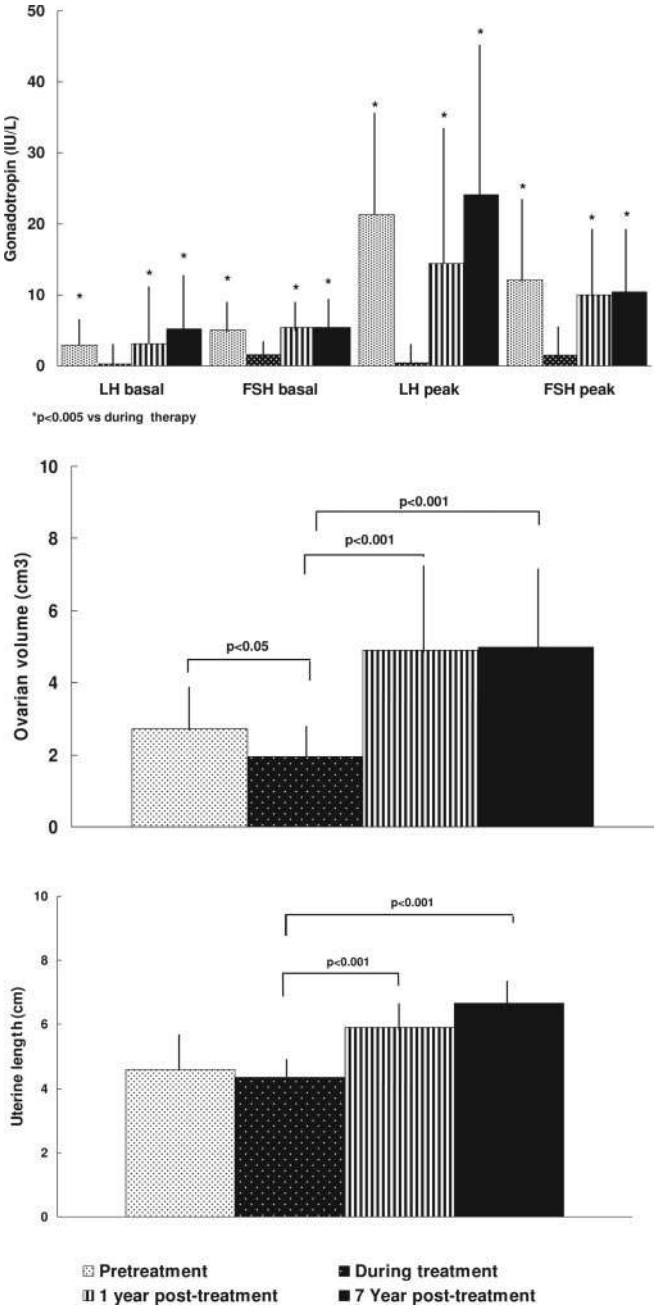

FIG. 1. LH and FSH basal and post-GnRH stimulated levels. Ovarian volume and longitudinal uterine length before treatment, during treatment, and at 1 and 7 yr after treatment in ICPP girls.

Discussion

ICPP is the most frequent cause of CPP in girls aged 6–8 yr (11, 22). Because these patients represent a relatively homogeneous population, it allows a more accurate evaluation of the impact on AH due to the use of GnRHa than in organic CPP.

Our 87 patients, as a whole, reached or overcame TH, and their AH increased significantly *vs.* pretreatment PAH (8, 9). The comparison of AH obtained in treated girls *vs.* AH of the untreated control group shows that in the latter AH is shorter about 5 cm, significantly shorter than 4 cm *vs.* their TH and has no

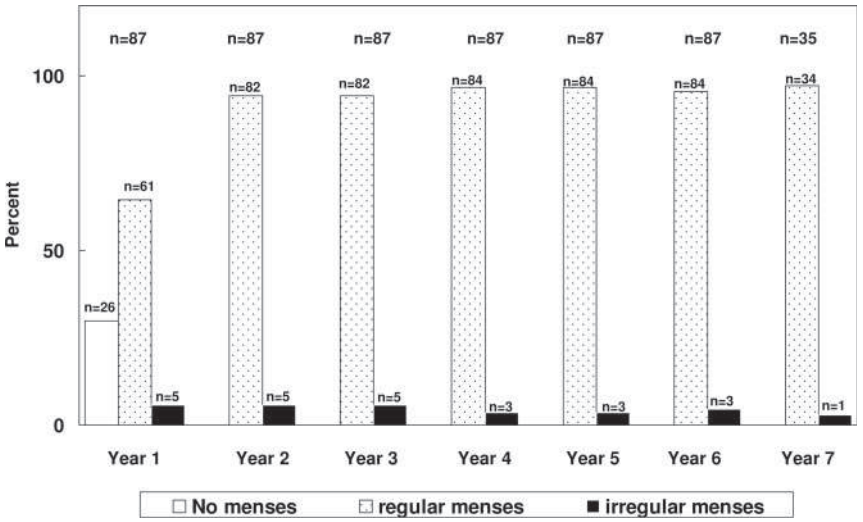

FIG. 2. Patterns of menses at 1, 2, 3, 4, 5, 6, and 7 yr after therapy in girls with ICPP.

significant gain *vs.* their average PAH and no gain *vs.* accelerated PAH.

Our results (7) confirm that there is no significant difference between the gain on AH over PAH pretreatment obtained in girls with onset of puberty less than 7 yr and those with onset over 7 yr. The division in the two groups below and over 7 yr is justified by the fact that in Italy the cutoff of 8 yr in girls is still maintained for the diagnosis of precocious puberty (23). Of course, more striking results are obtained in younger children, younger than 5–6 yr, in whom the potential height should be restored in the range of TH, in view of a severe loss in AH. The extreme variability observed in the growth response of these patients to GnRHa suggests that other factors besides auxological results should be considered when deciding on whether a patient should be treated.

A debated point is still the BMI pattern during and after treatment. Some authors (13, 24, 25) reported a significant increase all along the observation, others (26) even a reduction during the first period. In our cohort, which had a lesser number of overweight or obese children in comparison with other cohorts reported (8, 13, 24), we observed that, besides individual data, on the whole BMI increased, although remaining in the same centile or SDS throughout treatment. Furthermore, patients who were overweight or obese at the end of treatment were in the same position as at the beginning. In conclusion, GnRHa did not result in a significant BMI increment.

As to the bone mineral content, ovarian activity suppression was previously demonstrated to be the cause of BMD reduction, already 1 yr after the beginning of treatment (27–30). We observed, some years after the cessation of therapy, at AH and complete resumption of ovarian activity, that mineral content was totally restored and peak bone mass reached, leading to the conclusion that GnRHa inhibits the acquisition of mineral content in the bone during therapy, but mineral content is restored after therapy (8, 31–33).

No relevant side effects (rash, anaphylaxis) were observed (34). The reactivation of the hypothalamo-pituitary-gonadal axis was prompt and similar for all the patients, as either gonadotropin and estrogen levels or completion of uterine and ovarian development; menarche appeared around 1 yr after the end of treatment with regular cycles and six pregnancies with normal offspring, as observed by other authors (8, 13, 14, 24, 35–38).

Because treatment leads to reduction of height velocity, together with bone maturation, in turn influenced by hormonal extragonadal (adrenal), nutritional, and genetic factors and height prediction should be considered with caution for the inaccuracy of methods (13, 14, 39), the increment of statural growth with a gain of some centimeters on AH cannot be reasonably considered the aim of GnRHa therapy. The rate of pubertal progression, psychological problems depending on personal sensitivity, and the age of onset well below 7 yr, in which the loss of linear growth for years is unavoidable, seem to be the main factors for deciding to treat girls affected by ICPP with GnRHa.

Furthermore, our experience suggests not to establish fixed rules (BA, CA, height velocity slow-down) for discontinuation of therapy. It is better to consider each individual with respect to height satisfaction, compliance, and quality of life, including the need to sexually develop contemporaneously with their peers.

In conclusion, GnRH treatment in ICPP is safe and reversible for the reproductive system, BMD, and BMI. As to growth, it seems to be helpful in reaching an AH close to TH, but the variability of individual response suggests that one choose other parameters than increment in height, especially in girls with pubertal onset over 8 yr of age.

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Reproductive Axis after Discontinuation of Gonadotropin-Releasing Hormone Analog Treatment of Girls with Precocious Puberty: Long Term Follow-Up Comparing Girls with Hypothalamic Hamartoma to Those with Idiopathic Precocious Puberty

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ABSTRACT

Although the GnRH agonist analogs have become an established treatment for precocious puberty, there have been few long term studies of reproductive function and general health after discontinuation of therapy. To this end, we compared peak LH and FSH after 100 μg sc GnRH, estradiol, mean ovarian volume (MOV), age of onset and frequency of menses, body mass (BMI), and incidence of neurological and psychiatric problems in 2 groups of girls: those with precocious puberty due to hypothalamic hamartoma (HH; n = 18) and those with idiopathic precocious puberty (IPP; n = 32) who had been treated with deslorelin (4–8 μg/kg·day, sc) or histrelin (10 μg/kg·day, sc) for 3.1–10.3 yr and were observed at 1, 2, 3, and 4–5 yr after discontinuation of treatment. The endocrine findings were also compared to those in 14 normal perimenarcheal girls. There were no differences between the HH and IPP groups in age or bone age at the start of treatment, at the end of treatment, or during GnRH analog therapy. We found that whereas the peak LH level was higher in HH than in IPP girls before (165.5 ± 129 *vs.* 97.5 ± 55.7; $P < 0.02$) and at the end (6.8 ± 6.0 *vs.* 3.9 ± 1.8 mIU/mL; $P < 0.05$) of therapy, this difference did not persist at any of the posttherapy time points. LH, FSH, and estradiol rose into the pubertal range by 1 yr posttherapy in both HH and IPP. However, the mean posttherapy peak LH levels in both HH and IPP groups tended to be lower than normal, whereas the peak FSH levels were not different from normal, so that the overall posttherapy LH/FSH ratio was decreased compared to that in the normal girls (HH, 2.7 ± 0.3; IPP, 2.6 ± 0.1; normal, 5.2 ± 4.8; $P < 0.05$). The MOV was larger in HH than IPP at the end of treatment (3.7 ± 3.5 *vs.* 2.0 ± 1.2 mL; $P < 0.05$) and tended to increase in both groups over time to become larger than that in normal girls by 4–5 yr posttherapy (HH, 14.9 ± 12.9; IPP, 7.6 ± 2.2; normal, 5.4 ± 2.5 mL; $P < 0.05$). Whereas the onset of spontaneous menses varied widely in both groups, once menses had started, the HH group had a higher incidence of oligomenorrhea. Pelvic ultrasonography revealed more than 10-mm hypoechoic regions in 4 HH patients, 15 IPP patients, and 3 normal girls, all of whom were reporting regular menses. Live births of normal infants were reported by 2 HH and 2 IPP patients, and elective terminations of pregnancy were reported by 1 HH and 2 IPP patients. BMI was greater than normal in HH and IPP both before treatment and at all posttherapy time points and tended to be higher in the HH patients. Marked obesity (BMI, +2 to +5.2 SD score) was observed in 5 HH and 6 IPP patients, 1 of whom had a BMI of +2.5 SD score and developed acanthosis nigricans, insulin resistance, and hyperglycemia. Seizure disorders developed during GnRH analog therapy in 5 HH and 1 IPP patient, and 2 additional HH girls developed severe depression and emotional lability posttherapy. Although the mean anterior-posterior dimension of the hamartoma was larger in the HH patients with seizure than in those who were seizure free (1.7 ± 1.2 *vs.* 0.9 ± 0.4 cm; $P < 0.05$), no change in hamartoma size was observed either during or after therapy, and no patient has reported the onset of a seizure disorder posttherapy. Other than a tendency toward a larger MOV, a higher incidence of oligomenorrhea, obesity, and frequency of neurological disorders, recovery of the reproductive axis after GnRH analog therapy was not markedly different in HH compared to IPP. Continued follow-up of these patients may determine whether the decreased LH responses and increased BMI in both groups compared to those in normal girls remain clinically significant problems. (*J Clin Endocrinol Metab* **84:** 44–49, 1999)

THE ASSOCIATION of hypothalamic hamartoma (HH) with precocious puberty is well established (1, 2). Whereas children with HH often present at an earlier age and with higher gonadotropin responses after administration of exogenous GnRH than do children with idiopathic precocious puberty (IPP), their clinical response to treatment with the long acting GnRH agonist analogs has not differed from that of patients with IPP (3–5). In addition, most, but not all (6), imaging studies have indicated that hamartoma size remains stable during and after treatment (7), and short term follow-up studies have to date not reported a higher frequency of delayed menses, infertility, or other neuroendocrine complaints in girls with HH (5, 8, 9).

To assess the long term recovery of the pituitary-gonadal axis after discontinuation of GnRH analog therapy and to determine whether patients with hamartoma might be at increased risk for posttherapy reproductive disorders, we compared our findings in girls with precocious puberty and HH to those in girls with a diagnosis of IPP. To control for biases that could overestimate the incidence of adverse effects because one group was treated longer or began treatment at an earlier age, we selected a subgroup of IPP patients

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for whom age at the start of therapy (and hence the duration of therapy) was not different from that for the group of patients with HH. Patients were treated with GnRH analog for 3.1–10.3 yr (mean, 6.8 ± 1.9 yr) and were followed for 3–8 yr after treatment was stopped.

Subjects and Methods

Eighteen girls had precocious puberty due to HH, and 32 had IPP. All began GnRH analog treatment at less than 7 yr of age and had been observed for at least 3 yr after discontinuation of therapy. There were no significant differences between the girls with HH and those with IPP in mean ± SD chronological age (CA; 4.3 ± 1.6 *vs.* 4.5 ± 1.7 yr) or bone age (BA; 8.5 ± 2.3 *vs.* 8.8 ± 3.1 yr) at the start of therapy, in the length of GnRH analog treatment (7.2 ± 2.1 *vs.* 6.6 ± 1.8 yr), or in CA (11.3 ± 0.8 *vs.* 11.1 ± 0.8 yr) and BA (12.8 ± 1.2 *vs.* 12.4 ± 1.2 yr) at discontinuation of treatment.

The diagnosis of hamartoma was based on the finding of an isodense, pedunculated, nonenhancing mass in the area of the mammillary bodies using computed tomography and/or magnetic resonance imaging. Patients with nonhamartomatous intracranial masses (*i.e.* glioma or astrocytoma) or with gonadotropin-dependent puberty secondary to a gonadotropin-independent process (*i.e.* congenital adrenal hyperplasia, familial male precocious puberty, or McCune-Albright syndrome) were not included in this analysis.

Patients were treated with either deslorelin (D-Trp^6,Pro^9,NEt-LHRH; 4–8 μg/kg·day, sc; 46 patients) or histrelin (D-His[Bzl]^6,Pro^9,Net-LHRH; 10 μg/kg·day; 4 patients). Therapy was discontinued in most cases at the age when normal puberty would be expected (10–12 yr), but earlier (8–9 yr) in 2 patients (1 HH and 1 IPP) at the request of the family and patient. After discontinuation of treatment, girls were evaluated at 1- to 2-yr intervals at the Clinical Center at the NIH. Immunoreactive LH and FSH were measured (10) from 0–120 min after the iv administration of 100 μg GnRH at 0900 h. Serum estradiol (E_2) was measured (11) at 0 min. Ovarian structure and dimensions were assessed using pelvic ultrasonography, and mean ovarian volume (MOV) was estimated using the formula: MOV = volume of left ovary + volume of right ovary/2. The frequency of menses was determined from monthly diaries maintained by the patient and her family. Because menses were frequently irregular, the in-hospital endocrine evaluations were performed without regard to the phase of the menstrual cycle.

The body mass index (BMI) was calculated from weight (kilograms)/height (meters)^2, and the SD score was calculated from that of normal children using published standards (12).

For comparative purposes, we also measured LH and FSH (after administration of 100 μg GnRH), E_2, MOV, and BMI in 14 normal, postmenarcheal girls, aged 13.0–15.5 yr (mean, 14.0 ± 0.9), with breast pubertal stages IV–V.

Statistical analyses

Hormonal and MOV findings are presented as the mean ± SD of all patients in each group evaluated at baseline (yr 0, before discontinuation of GnRH treatment) and at the 1, 2, 3, and 4–5 yr points thereafter. Data from 4 and 5 yr visits were evaluated as the mean of yr 4 and 5, when both time points were available. Endocrine data from two HH patients were not available after 2 yr; hence, they have been excluded from the hormonal analysis.

Comparisons of hormonal parameters (gonadotropin and estrogen levels and ovarian volumes) and BMI were made using the *t* test. The frequency of menstrual bleeding was classified for each year as follows: 1, oligomenorrhea (menses at 60- to >150-day intervals); 2, irregular (every 35–60 days); and 3, regular (every 25–35 days). Comparisons between groups were made using the χ^2 statistic. When a diary was unavailable for a posttherapy year, the patient was excluded from that year's analysis of menses.

Results

LH and FSH after GnRH

Before the start of GnRH analog therapy, the HH group had significantly higher levels of peak GnRH-stimulated LH (165.5 ± 129 *vs.* 97.5 ± 55.7 mIU/mL; $P < 0.02$) and a higher peak LH/FSH ratio (5.4 ± 2.8 *vs.* 3.6 ± 2.4; $P < 0.05$) than the IPP group. At discontinuation of treatment (0 yr), the mean peak LH and FSH levels were also greater in HH than in IPP, but the groups did not differ in this respect at any of the subsequent posttherapy time points (Fig. 1, A and B). By 1 yr posttherapy, peak LH and FSH levels in both HH and IPP girls had entered the range for normal pubertal stage IV–V girls (13). However, the mean peak LH in both HH and IPP tended to be lower than that in normal girls at all time points, whereas the peak FSH levels were comparable to normal, so that the mean peak LH/FSH ratio for posttherapy yr 1–5 in both HH and IPP was lower than that in normal girls (HH, 2.7 ± 0.3; IPP, 2.6 ± 0.1; normal, 5.2 ± 4.8; $P < 0.05$).

Serum E_2, pubertal stage, and ovarian volume and structure

By 1 yr after stopping therapy and at all the subsequent time points, E_2 levels had risen from near or below the assay detection limit in both HH and IPP and were not different from normal (Fig. 1C). Breast development was pubertal stage III in 78% of HH and 79% of IPP girls at 0 yr, and had progressed to stage V in 53% of HH and 71% of IPP by 2 yr, in 81% of HH and 90% of IPP by 3 yr, and in all patients by 4–5 yr posttherapy.

The MOV was larger in HH than in IPP at discontinuation of therapy and at 4–5 yr, but was not significantly different at the intermediate time points. However, MOV tended to increase progressively in both groups over the first 3 yr after treatment was stopped and was significantly greater than that in the normal girls in HH at 3 yr and those in both HH and IPP at 4–5 yr posttherapy (Fig. 1D). Small (<1.0-cm) hypoechoic regions resembling follicles were observed in at least 1 posttherapy ultrasound study in 10 HH and 16 IPP girls and in 2 or more studies in 7 HH and 13 IPP girls. Larger, well circumscribed, more than 1.0-cm hypoechoic areas resembling cysts were observed at least once in 4 HH and 16 IPP girls and recurred once in 2 HH and 4 IPP girls. One IPP girl had recurrent, isolated, unilateral, 1.0- to 4.8-cm hypoechoic, cyst-like areas in both right and left ovaries at each yearly ultrasound study. Although the ultrasound appearance in this patient was suggestive of polycystic ovarian syndrome (14), she reported regular menses, she was not androgenized, and her LH levels were normal at baseline (4.2–6.8 mIU/mL) and after GnRH treatment (30.9–96.5 mIU/mL).

Two normal girls had multiple, bilateral hypoechoic areas, 0.2–1.0 cm in diameter. One asymptomatic normal girl had a large, unilateral hypoechoic area 5.0 × 8.0 cm in diameter, on a single ultrasound study.

Menses

The time interval between the discontinuation of GnRH analog and the start of spontaneous menses varied widely in both groups and was not significantly different in patients with HH (20.5 ± 16.3; range, 0–60 months) and those with IPP (17.6 ± 11.0; range, 5–61 months). One HH patient began menstruating before discontinuation of therapy despite a

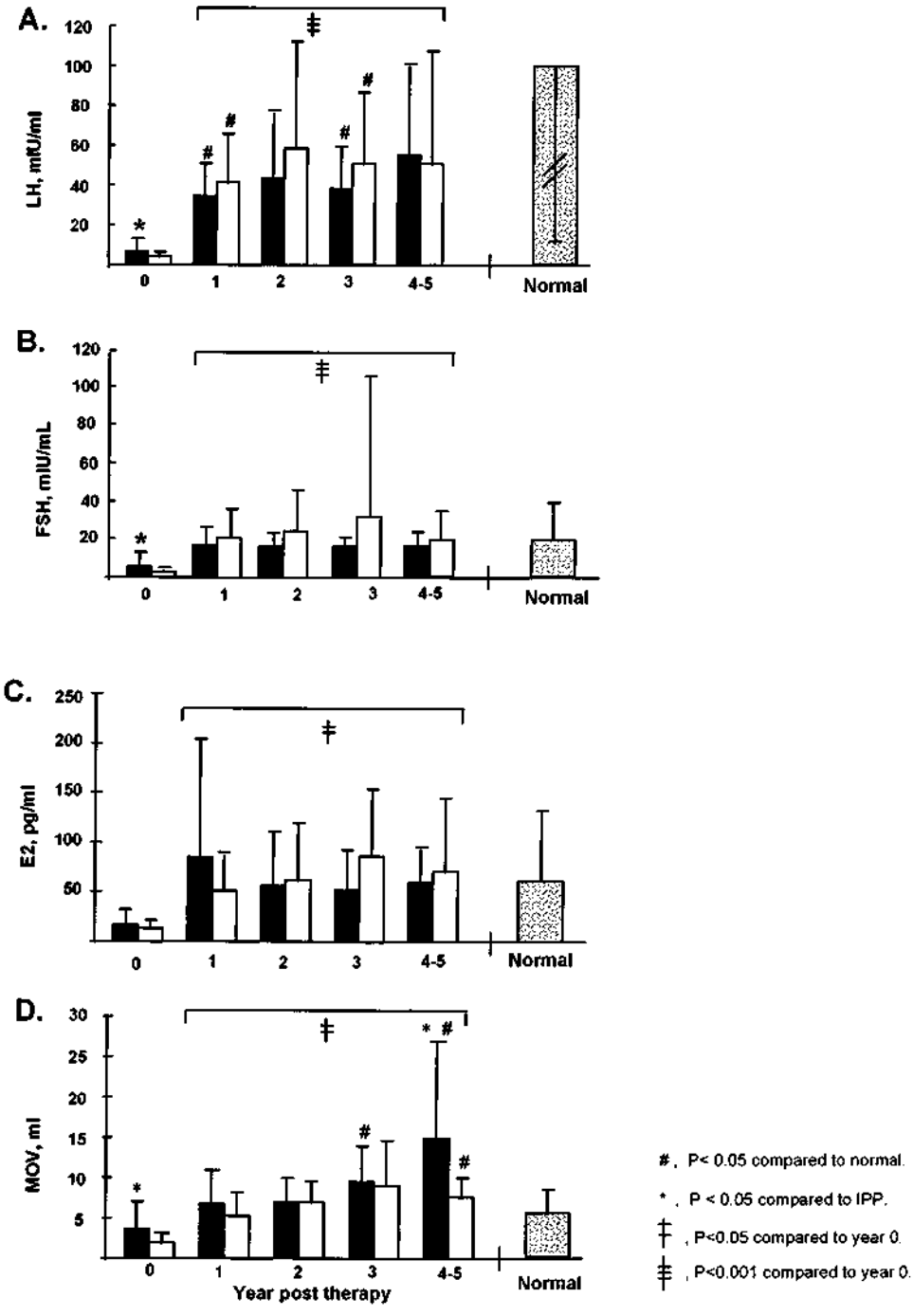

FIG. 1. Mean ± SD GnRH-stimulated LH (A) and FSH (B), E_2 (C), and MOV (D) at the end of GnRH analog therapy (yr 0) and at 1, 2, 3, and 4–5 yr posttherapy in girls with HH (*solid bars*), girls with IPP (*open bars*), and normal perimenarcheal girls (*textured bars*). *, $P < 0.05$ compared to IPP; #, $P < 0.05$ compared to normal girls; ‡, $P < 0.05$ compared to yr 0; ⁜, $P < 0.001$ compared to yr 0.

2-fold increase in the dose of GnRH agonist and apparently good compliance, and 1 other HH patient had persistent primary amenorrhea, although she had withdrawal bleeding after a 10-day challenge with medroxyprogesterone acetate at 4 yr posttherapy. There were no significant differences between the groups in the mean CA (13.4 ± 1.9 *vs.* 12.5 ± 0.7 yr) or BA (14.6 ± 1.3 *vs.* 13.8 ± 1.0 yr) at the onset of spontaneous menses. The number of girls reporting regular menses tended to increase in both groups over the 4 yr of follow-up; however, in the subset of girls who had begun spontaneous menses, a greater percentage of HH than IPP girls reported oligomenorrhea during yr 2 [4 of 13 (30%) *vs.* 0 of 24 (0%); $P < 0.005$] and yr 3 [3 of 13 (23%) *vs.* 1 of 31 (3%); $P < 0.05$] posttherapy (Fig. 2). The MOV was not significantly different in patients reporting oligomenorrhea *vs.* those with regular menses (7. 8 ± 2.9 *vs.* 5.6 ± 2.5 mL).

Seven girls (three HH and four IPP) have reported pregnancies; five pregnancies resulted in normal, live infants (one IPP girl was pregnant twice), two pregnancies were terminated electively, and one ended in spontaneous abortion in a girl with insulin-dependent diabetes mellitus (Table 1).

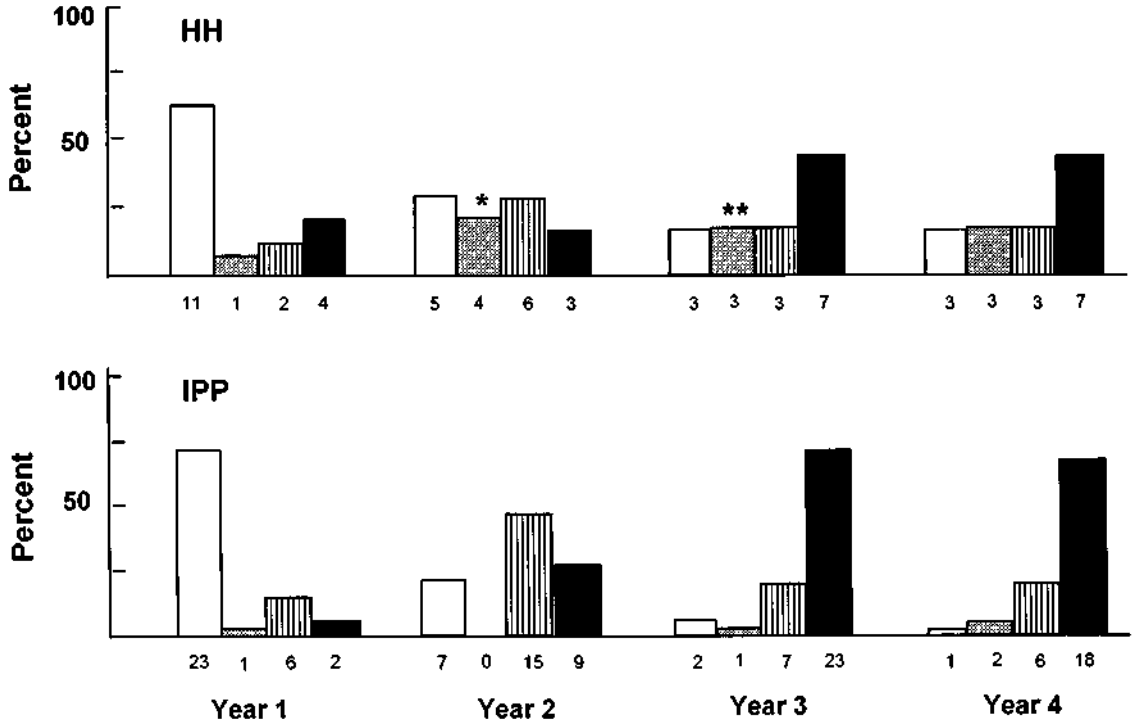

FIG. 2. Patterns of menstrual bleeding at 1, 2, 3, and 4 yr posttherapy in girls with HH (*upper panel*) and IPP (*lower panel*). *Column height* denotes the percentage of girls reporting no menses (□), oligomenorrhea (▨), irregular menses (▥), and regular menses (■). Figures *below each column* denote the number of patients. *, $P < 0.005$, HH *vs.* IPP; **, $P < 0.05$, HH *vs.* IPP.

TABLE 1. Diagnosis, duration of deslorelin therapy, time (months) posttherapy, age (years) at onset of menses, menstrual pattern, time (years) posttherapy, age (years) at end of gestation, and outcome in girls reporting pregnancy after discontinuing deslorelin treatment for precocious puberty

Patient no.	Diagnosis	Duration of therapy (yr)	Onset of menses: time, (months), post-treatment (age, years)	Menstrual pattern[a]	Conception: time, yr post-tx (age, years)	Outcome
1	HH	8.9	13 (12.4)	3	8.0 (19.4)	Normal female infant
2	HH	7.5	24 (12.4)	3	7.3 (17.7)	Abortion (elective)
3	HH	5.5	30 (13.0)	2	9.1 (19.5)	Normal male infant
4	IPP	6.0	16 (12.5)	2	4.7 (15.9)	Abortion (elective)
5	IPP	5.8	6 (12.6)	2	9.0 (20.9)	Normal female infant
6	IPP	5.1	13 (11.9)	3	6.0 (16.9)	Abortion (spontaneous)[b]
7	IPP	4.0	24 (12.5)	3	5.8 (16.3)	Normal male infant
					6.8 (17.3)	Normal male infant

[a] 3, Regular menses; 2, irregular menses.
[b] Patient was diabetic and receiving insulin therapy.

BMI (Fig. 3)

As expected, at the start of GnRH analog therapy, the mean BMI SD score of both HH (1.6 ± 1.2) and IPP (1.2 ± 1.3) girls was greater than that of normal girls of comparable age. At the end of therapy and at all the posttherapy time points, the mean BMI of both HH and IPP groups continued to exceed that of the normal girls, and marked obesity (BMI, 28.9–42.0, +2.0 to +5.2 SD score) was observed in five HH and in six IPP girls. Elective cosmetic breast reduction was performed in one HH and one IPP patient. The BMI and the incidence of obesity tended to be greater in HH than that in IPP, although this increase was not significantly different due to the wide variability among patients. Acanthosis nigricans (without clinically apparent insulin resistance) was observed in three HH girls with BMI values of +5.0, +3.0, and +0.9. Insulin resistance and hyperglycemia requiring treatment with oral agents and, eventually, insulin, was observed during and after GnRH analog therapy in one IPP patient who continued to menstruate regularly, but had mild hirsutism (BMI, +2.3) and reported a spontaneous abortion (see above).

Neurological abnormalities

Episodes of seizure, which were first noticed during the course of GnRH agonist therapy, were reported by 5 of 18 HH patients. Four patients were between 9 and 10 yr of age, and 1 was 11 yr. Anticonvulsants were used in all patients and were continued during and after discontinuation of GnRH analog therapy; however, 2 of these patients have been seizure free after discontinuation of anticonvulsant treatment. Diagnoses were reported as gelastic epilepsy in 1 girl and as

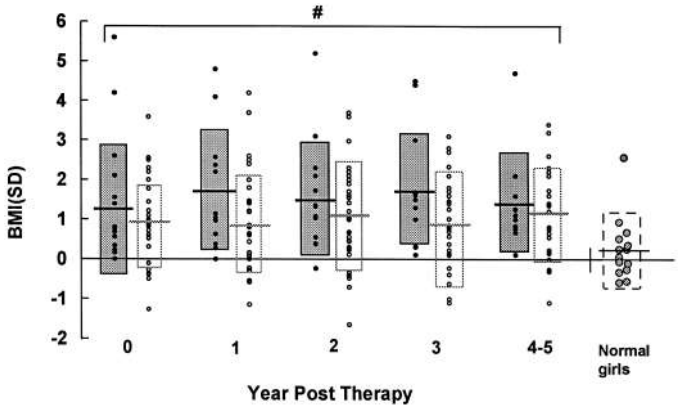

FIG. 3. Mean ± SD BMI SD score of girls with HH (*shaded bars*) and IPP (*open bars*) at the end of GnRH analog therapy and at 1, 2, 3, and 4–5 yr posttherapy. #, $P < 0.04$ compared to normal girls (*broken bar*).

complex partial seizure in 4 girls. No family has yet reported the onset of a seizure disorder after discontinuation of GnRH analog therapy. Emotional lability, depressive behavior, and mood swings without a diagnosis of seizure were reported in 2 additional HH patients after GnRH treatment was stopped. Whereas the mean hamartoma size was larger in HH girls reporting seizures than in those who were seizure free (maximum anterior-posterior diameter, 1.7 ± 1.2 *vs.* 0.9 ± 0.4 cm; $P < 0.05$) no girl had a documented change in the size of her hamartoma either during or after GnRH analog therapy.

A disorder diagnosed as benign nocturnal seizure of childhood developed during GnRH analog treatment in one IPP patient at 8 yr of age and was treated with anticonvulsants for 3 yr. This girl has been seizure free after discontinuation of GnRH analog.

Discussion

The GnRH agonist analogs have been used to treat many diverse reproductive system disorders, including precocious puberty. Initial clinical trials of these compounds appear to confirm their effectiveness and safety in suppressing gonadal activation and to document the reversibility of their effects in both adults and children. However, concerns have recently been expressed and are now widely disseminated via electronic media (15) that the GnRH analogs may have long lasting adverse effects on reproductive function as well as on physical and mental well-being. At present, there are few long term studies that address these issues objectively, particularly with regard to children with precocious puberty, many of whom are treated for long periods of time and from a very early age. To this end, we undertook to compare two groups of treated patients: girls with hypothalamic hamartoma and girls with idiopathic precocious puberty. We found that the reproductive axis appeared to have returned to normal in both patient groups by 4–5 yr after discontinuation of GnRH analog, but that the HH patients tended to have a higher incidence of irregular menses, obesity, and neurological and behavioral problems.

The pattern of relatively increased peak baseline LH and increased peak GnRH-stimulated LH/FSH ratio that we observed in the HH patients at initial presentation did not reemerge over the 5-yr posttreatment period. We did observe a significantly higher peak LH and increased MOV in the HH group at the end of treatment, and one such patient, with a MOV of 15.9 mL, who denied noncompliance, reported menarche and had a pubertal peak LH (28.8 mIU/mL) at 0 yr. Although these findings suggest resistance to GnRH analog or a higher pituitary gonadotropin reserve in HH, compliance may have been playing a major role despite the assertions of the patient and her family.

Although our findings confirm previous reports (5) that gonadotropin responses return to the normal range by 12 months after discontinuation of GnRH analog treatment, they also reveal that the peak GnRH-stimulated LH/FSH ratio after treatment tends to be low compared to that of normal girls. This may represent a blunted sensitivity to GnRH stimulation similar to that observed in normal women during the very early follicular phase of the menstrual cycle (16). However, it is not clear whether this is a consequence of prolonged suppression of the pituitary gonadotropes or represents the natural history of early maturation of the hypothalamic-pituitary-gonadal unit.

A previous study (7) indicated that ovarian volumes in girls who have stopped GnRH analog therapy return to pretreatment levels by 3 months and remain constant thereafter. However, these initial reports reflect findings in girls who were older (7.8 ± 1.1 yr) at the start of treatment and were treated for a shorter period of time (3.5 ± 0.9 yr) than those in the present groups. Our findings that ovarian volumes tend to increase progressively over the first 3 posttreatment years and were often larger than normal by 3 yr posttherapy suggest that recovery of the suppressed gonad of girls treated for longer periods of time may be a more gradual process, and that a complete picture of the effects of therapy may only emerge after several years have passed. The somewhat greater age and bone age at menarche, and the greater interval between discontinuation of therapy and menarche that we found in our patients compared to those reported by other observers (8) may also reflect the longer period of gonadal suppression and/or the early age when treatment was instituted. Nevertheless, it is reassuring that the mean age of menarche in our patients was comparable to that in normal girls and that seven patients have demonstrated fertility.

As has been noted by others (17), our patients' ovaries were larger than those of normal postpubertal girls (18) and were comparable in size to those of cycling mature women (19, 20). Hypoechoic, cyst- or follicle-like areas were detected in most patients at one or more posttherapy time points and in three normal girls. Whereas irregular menses and obesity were common complaints in our patients, none of the girls with persistent hypoechoic regions in their ovaries had the elevated baseline or GnRH-stimulated gonadotropin levels that would suggest true polycystic ovarian syndrome. Although acanthosis nigricans was observed in three HH patients, two of whom had oligomenorrhea and moderate to marked obesity, none of the three had clinical diabetes mellitus. Conversely, the IPP girl with insulin-dependent diabetes and mild hirsutism was having regular menses. Although we did not measure androgen levels in our patients, elevated adrenal androgen responses have been observed by

other groups (21) after GnRH analog treatment of IPP, and continuing follow-up will be needed to determine whether a fully developed polycystic ovary syndrome may eventually develop in any of these girls.

The timing of sexual maturation in normal females is linked to body fat content. Obese girls have earlier menarche than thin girls. Not surprisingly, as we and others (22) have reported, precocious puberty is also associated with increased body mass both at initial presentation and during GnRH analog treatment. Our current data show that this condition persists after discontinuation of therapy and progresses to frank obesity (BMI, $>+2$SD score) in many girls, more frequently in those with HH. Although it is tempting to implicate the hypothalamic abnormality in this trend toward obesity, there have been, to the best of our knowledge, only anecdotal reports (23) of an association between HH and extreme weight gain. Those who care for girls with precocious puberty may need to address this issue early in view of the morbidity that accompanies persistent obesity (24, 25).

HH has a known association with gelastic epilepsy (26, 27); however, only one HH patient carried this diagnosis, whereas four had partial complex seizure. In the HH patients as a group, seizures were associated with larger hamartomas, all seizures were first observed during GnRH analog treatment, and three of the six girls have been able to discontinue anticonvulsant therapy after stopping GnRH analog. Although it is not possible to determine from our data whether GnRH analog treatment delayed or, possibly, accelerated the onset of seizure in these patients, the lower incidence of seizure disorder in the IPP patients [1 of 32 girls; 3%, *vs.* 0.5% in normal children (28)] suggests that the preexisting neurological abnormality, rather than GnRH analog therapy, played the major roll.

Clinical trials using GnRH analogs to treat precocious puberty have not included untreated placebo control groups. Although the choice of an uncontrolled study design is understandable, it will be difficult to learn whether altered gonadotropin and gonadal androgen levels, changes in ovarian volume and structure, increased body weight, and neurological complications are a result of therapy or inevitable manifestations of the primary process. It is hoped that some or all of these issues will be resolved as these patients are followed throughout adulthood.

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Original Article

Effect of gonadotropin-releasing hormone agonist therapy on body mass index and growth in girls with idiopathic central precocious puberty

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ABSTRACT

Objective: The study aimed to assess the effect of gonadotropin-releasing hormone (GnRH) agonist therapy on body mass index (BMI) and growth in girls diagnosed with idiopathic central precocious puberty (CPP). **Materials and Methods:** Hospital records of 32 girls with idiopathic CPP who have been receiving GnRH agonist therapy for at least 12 months were retrospectively reviewed and auxological, clinical and laboratory parameters of the patients were recorded. BMI, body mass index standard deviation score (BMI SDS) for chronological age body mass index standard deviation score (CA-BMI SDS), BMI SDS for bone age body mass index standard deviation score (BA-BMI SDS), ratios of obesity and overweight were assessed before treatment and on the 12th month of therapy in patients diagnosed with idiopathic CPP. **Results:** The study comprised of 32 girls diagnosed with idiopathic CPP. BMI values showed statistically significant increase in the 1st year of treatment (19.16 ± 2.8 vs. 20.7 ± 3.4, $P = 0.001$). Despite a mild increase in CA-BMI SDS in the 1st year of treatment versus before treatment, it was no statistically significant (1.0 ± 0.8 vs. 1.1 ± 0.9, $P = 0.061$). However, significant increase was observed in BA-BMI SDS in the 1st year of treatment versus before treatment (0.8 ± 0.7 vs. 0.4 ± 0.8, $P < 0.001$). Before treatment, 37.5% (12/32) of the patients were overweight and 21.9% (5/32) were obese, whereas in the 1st year, 34.4% (11/32) of the patients were overweight and 31.3% were obese ($P = 0.001$). **Conclusion:** Whilst 1/3 of the cases diagnosed with idiopathic CPP were overweight and obese at the time of diagnosis, GnRH agonist therapy caused statistically significant weight gain in patients diagnosed with CPP. Therefore, these patients should be closely monitored and weight control should be provided by diet and exercise programs in the course of treatment.

Key words: Body mass index, central precocious puberty, gonadotropin-releasing hormone agonist

Introduction

Idiopathic central precocious puberty (CPP) is defined as development of secondary sex characteristics before the age of 8 in girls and 9 in boys due to early activation of gonadotropin-releasing hormone (GnRH)-secreting neurons without the presence of an organic reason.[1,2] GnRH agonist therapy used in the treatment of CPP inhibits stimulating effects of endogenous GnRH by desensitizing hypophyseal gonadotropic cells and thus acceleration in bone maturation and early puberty is suppressed. This therapy delays the onset of puberty and leads to delay in menarche and provides an increase in final stature.[3,4]

Many previous studies evaluating the effect of GnRH agonist therapy on anthropometric parameters in CPP patients particularly investigated the effect on adult height, however, effect on body weight has been rarely investigated.[5] Nevertheless, some studies demonstrated positive and negative effects of GnRH agonist therapy on weight gain.[6,7] It has been also demonstrated that weight gain continues to increase after discontinuation of therapy and may lead to obesity.[6] Considering the importance of nutrition and weight gain on moving the onset of puberty to earlier, this likely side-effect becomes

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more important.[8-10] Results of the studies investigating the effect of GnRH agonist therapy on body weight are quite variable. Although some studies have demonstrated weight gain resulted from GnRH agonist therapy,[6,7,11,12] some studies concluded that therapy has no effect on body mass index (BMI).[13,14] Unlike these studies, two studies demonstrated a decrease in BMI with GnRH agonist therapy.[8,15] In the present study, we aimed to investigate the effect of GnRH agonist therapy on height gain and BMI in girls diagnosed with CPP.

Materials and Methods

Hospital records of 32 girls with idiopathic CPP who have been receiving GnRH agonist therapy for at least 12 months were retrospectively reviewed and auxological, clinical and laboratory parameters of the patients were recorded. Eligibility criteria were: (a) breast development before the age of 8 years, (b) presence of pubertal growth spurt, (c) bone age at least 1 year advanced than the chronological age, (d) peak luteinizing hormone (LH) ≥5 IU/L chemiluminescent microparticle immunoassay (CMIA) method after exogenous intravenous administration of luteinizing-hormone releasing hormone (LHRH) (gonadorelin 100 μg), (e) absence of history for hypothalamic/hypophyseal disease suggestive of organic CPP and normal brain magnetic resonance imaging, (f) suppressed gonadotropins and sex steroids in the course of treatment, or (g) premature menarche (≤10 years of age).

All patients have undergone a standard LHRH test. Both basal and peak LH and follicle stimulating hormone (FSH) levels were recorded. Serum LH, FSH and estradiol levels were studied by CMIA method (Abbott Architect i2000, USA). Measurable lowest limits for LH, FSH and estradiol were 0.1 IU/L, 0.6 IU/L and 20 pmol/L, respectively. All patients were treated with leuprolide (Lucrin Depot®; Takeda Pharmaceutical, Japan) or triptorelin (Decapeptyl Depot®; Ferring Pharmaceuticals, Kiel, Germany), which are depot forms of GnRH agonist, at a dose of 3.75 mg regardless of body weight and administered through intramuscular route every 28 days. Suppression of hypothalamus-pituitary-gonad axis was checked every 3 months and adequate suppression was considered in patients with clinically paused or regressed pubertal signs and a serum LH level < 0.4 IU/L and estradiol level <20 pg/ml before GnRH agonist injection.[16] The height was measured by Harpenden stadiometer of 0.1 cm sensitivity and the body weight was measured by SECA scale of 0.1 kg sensitivity. BMI was calculated using weight (kg)/height^2 (m) formula. BMI was assessed according to the data of Center for Disease Control. Cases with a BMI percentile of 85-95 were considered overweight and >95 were considered obese. Weight, height, BMI and bone ages of the patients were recorded before treatment and on the 12th month of treatment. Height standard deviation score (SDS) was calculated both for bone age height standard deviation score and for chronological age height standard deviation score. Likewise, body mass index standard deviation score (BMI SDS) was calculated both for bone age body mass index standard deviation score (BA-BMI SDS) and for chronological age body mass index standard deviation score (CA-BMI SDS). Bone age was assessed by Greulich-Pyle method,[17] whereas sexual maturation was assessed by Marshall-Tanner method.[18] Predicted adult height (PAH) was calculated according to Bayley-Pinneau method.[19] Targeted adult height was calculated by subtracting 6.5 to the mean of parental heights.

Statistical analysis

Statistical analyses were performed by Statistical Package for Social Sciences version 19.0 (Inc., Chicago, IL, USA). All data were presented as mean ± standard deviation and paired *t*-test was used for the comparison of data. In the case of longitudinal comparisons of the same parameter, repeated-measures ANOVA was performed. A *P* value of less than 0.05 was considered statistically significant.

Results

The mean age at the onset of patients' complaints was 7.6 ± 1.2 years, whereas the mean age at the onset of treatment was 8.5 ± 1.2 years. The mean pre-treatment bone age was 2.7 ± 1.1 years higher than the chronological age (10.9 ± 1.2). At the time of diagnosis, height SDS for chronological age was 1.3 ± 1.1 and BMI SDS was 1.0 ± 0.8, whereas BMI SDS for bone age was 0.4 ± 0.8. Before treatment, mean PAH was 156.0 ± 9.2 cm and targeted height was 159.4 ± 5.4 cm. Mean basal LH and FSH levels were 1.6 ± 1.1 and 4.5 ± 1.9 respectively; mean stimulated peak LH, FSH and LH/FSH ratio were 18.8 ± 13.6, 15.7 ± 3.2 and 1.1 ± 0.6, respectively [Table 1].

It was observed that the difference between bone age and chronological age was decreased from 2.7 ± 1.1 years to 1.9 ± 1.1 years in the 1st year of treatment. No difference

Table 1: Characteristics of treated patients before treatment

Parameters	Mean±SD
Age at the onset of complaints (year)	7.6±1.2
Age at the beginning of treatment (year)	8.5±1.2
Bone age at the time of diagnosis (year)	10.9±1.2
Target height	159.4±5.4
Basal LH (IU/L)	1.6±1.1
Basal FSH (IU/L)	4.5±1.9
Peak LH (IU/L)	18.8±13.6
Peak FSH (IU/L)	15.7±3.2
Peak LH/Peak FSH (IU/L)	1.1±0.6

LH: Luteinizing hormone, FSH: Follicle stimulating hormone, SD: Standard deviation

between the pre-treatment height SDS for chronological age and height SDS in the 1st year of treatment was observed (1.3 ± 1.1 vs. 1.3 ± 1.1, $P = 0.477$). However, height SDS for bone age showed statistically significant increase in the 1st year of treatment versus before treatment (−1.1 ± 1.0 vs. −0.4 ± 1.1, $P < 0.001$). Although PAH was increased up to 158.9 ± 7.0 cm in the 12th month of treatment versus 156.0 ± 9.2 cm before treatment, this increase was not statistically significant ($P = 0.113$). Despite tendency toward increment in CA-BMI SDS in the 1st year of treatment versus before treatment, this increase as well was no statistically significant (1.0 ± 0.8 vs. 1.1 ± 0.9, $P = 0.061$). Nevertheless, statistically significant increase was observed in BA-BMI SDS in the 1st year of treatment versus before treatment (0.4 ± 0.8 vs. 0.8 ± 0.7, $P < 0.0001$) [Table 2].

Before treatment, 37.5% (12/32) of the cases were overweight and 21.9% (7/32) were obese, whereas in the 1st year, 34.4% (11/32) were overweight and 31.3% (10/32) were obese ($P = 0.001$) [Figure 1].

Discussion

The present study investigated the effect of GnRH agonist therapy on BMI in a relatively homogeneous group of girls with idiopathic CPP. Significant increase was observed in BMI SDS for bone age in the 1st year of treatment.

Table 2: Anthropometric data before treatment and in the first year of treatment

Parameters	Before treatment	1st year of treatment	*P**
BMI (kg/m²)	19.2±2.8	20.7±3.4	<0.001
CA-BMI SDS	1.0±0.8	1.1±0.9	0.061
BA-BMI SDS	0.4±0.8	0.8±0.7	<0.001
CA-Height SDS	1.3±1.1	1.3±1.1	0.477
BA-Height SDS	−1.1±1.0	−0.4±1.1	<0.001
PAH (cm)	156.0±9.2	158.9±7.0	0.113
BA (year)	10.9±1.2	11.3±1.3	0.031

*Paired sample *t* test, data were presented as mean±SD, CA-BMI SDS: Chronological age body mass index standard deviation score, BA-BMI SDS: Bone age body mass index standard deviation score, CA-BMI SDS: Chronological age height standard deviation score, CA-Height SDS: Chronological age height standard deviation score, BA-Height SDS: Bone age height standard deviation score, PAH: Predicted adult height, BA: Bone age

CPP is a clinical condition characterized by accelerated growth, advancement in bone age and increment in sex steroids, which may lead to premature menarche and loss in final height unless treated. GnRH agonists used for the treatment of CPP provides an increase in final adult height by inhibiting pubertal progression and advancement in bone age.[3,4] Nevertheless, there is no randomized controlled study investigating the effect of GnRH agonist therapy on adult height. Many studies have compared pre-treatment PAH with final adult height. Pasquino *et al*. retrospectively evaluated 87 girls diagnosed with idiopathic CPP and treated with GnRH agonist for 3-8 years and found 9.5 ± 4.6 cm increase in adult height as compared with pre-treatment PAH calculated according to Bayley-Pinneau method. Bone age at the time of diagnosis, age at the beginning of treatment and therapy duration are the factors that influence adult height.[20] Whereas, mean height gain is 9-10 cm in girls that have been treated before the age of 6 years, it is 7.2 ± 5.3 cm in those treated between 6 years and 8 years.[5] Likewise, the present study as well found that PAH has been increased to 158.9 ± 7.0 from 156.0 ± 9.2 at the time of diagnosis. Weise *et al*. evaluated growth rate in 100 girls treated for CPP and found that growth rate for chronological age (height velocity) has decreased below normal values in the course of treatment (−1.6 ± 1.7 SDS) and that growth rate is inversely proportional to duration of exposure to high estrogen before treatment. Nevertheless, they found the growth rate for bone age to be normal, even increased, in girls aged less than 10 years and height SDS for bone age to be increased after treatment.[21] In the present study, height SDS for chronological age showed no change in the 1st year of treatment versus before treatment (1.3 ± 1.1 SDS). However, height SDS for bone age has been increased to -0.4 ± 1.1 SDS in the 1st year of treatment from -1.1 ± 1.0 before treatment and this increment was statistically significant.

In the literature, results of the studies evaluating the effect of GnRH agonist therapy on BMI are conflicting. There are studies demonstrating that GnRH agonist therapy increases BMI SDS,[6,14,22-24] as well as studies expressing just the opposite, reporting that GnRH agonist therapy

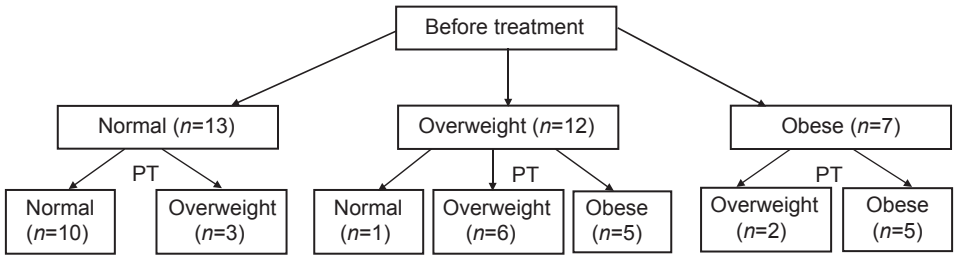

Figure 1: Change in body weight of the patients in the 1st year of treatment versus before treatment (PT: Post-treatment)

has no significant effect on BMI SDS and obesity.[8,10,25] The reason of this inconsistency between the studies is not clear. Likely causes of this inconsistency include different age, sex and body weight of study participants at the onset of GnRH agonist therapy. Oostdijk *et al*.[26] and Ko *et al*.[27] reported that BMI SDS was increased both for bone age and for chronological age in CPP patients receiving GnRH agonist therapy. Increase in BMI SDS with GnRH agonist therapy is mostly seen in children who are overweight before treatment.[14] Nonetheless, Wolters *et al*. found that BMI SDS was increased in children with normal body weight before GnRH agonist therapy and that unlike control group, BMI SDS remained stable in children who were overweight before treatment.[28] In the present study, it was observed that BA-BMI SDS was significantly increased in the 1st year of treatment versus before treatment and that CA-BMI SDS was also increased in the 1st year of treatment versus before treatment, but the difference was not statistically significant. Whilst obesity was not observed in the 1st year of treatment in the group with normal body weight before treatment, obesity was observed in 5 of 12 children who were overweight before the treatment.

It has been reported that obesity is more common in girls with CPP.[25] Studies conducted in different geographical regions of our country found the prevalence of overweight to be 9.9-14.3% and obesity to be 1.6-7.8% in children.[29] In the present study, 37.5% of the cases were overweight and 21.9% were obese before treatment so as to corroborate that overweight-obesity is frequent in cases with precocious puberty and was consistent with the literature. It is not clear whether weight gain leads to precocious puberty or pubertal development leads to weight gain. It is known that an adequate amount of leptin is required for the initiation of puberty and leptin initiates pubertal development by increasing gonadotropin secretion.[30] Nevertheless, gonadal steroids secreted in case of precocious puberty also cause increment in body fat.[31] In the light of this information, the present study failed to explain weight gain in cases in which synthesis of sex steroids was suppressed with GnRH agonist therapy. It will also be important to determine in future studies whether the subjects with CPP had elevated BMI SDS before the onset of precocious pubertal development or only after exposure to a pubertal gonadal steroid milieu.

Limitations of the present study are: (a) Absence of a control group due to ethical concern; (b) absence of information about BMI SDS values after discontinuation of therapy; (c) probability of different therapy responses among patients due to the use of standard dose of GnRH agonist (regardless of body weight) and thus absence of standardization; (d) unavailability for the auxologic data of the parents.

In summary, the height SDS for bone age significantly increased during GnRH agonist treatment in our patients and the PAH was also increased after treatment. Furthermore, the BMI SDS for bone age increased significantly. It is difficult to determine whether increased BMI is a result of therapy or is an expected manifestation of the primary process. Preventive measures, such as increased physical activity, can be introduced to minimize possible alterations in body weight and a long-term follow-up study is required to elucidate whether GnRH agonist treatment in Turkish girls with CPP affects adult obesity.

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Long-term effects of GnRH agonist treatment on body mass index in girls with idiopathic central precocious puberty

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Abstract

Introduction: Studies evaluating effects of gonadotropin-releasing hormone agonist (GnRHa) on weight and body-mass-index (BMI) in girls with idiopathic central precocious puberty (iCPP) include short-term effects. The aim of this study is to investigate changes in BMI during and 2 years after completion of GnRHa to determine the factors that may impact BMI in girls with iCPP.

Methods: Medical files of 138 girls who completed GnRHa were evaluated. All patients had weight and height measurements at the beginning and end of treatment, and 111 patients had anthropometric measurements 2 years after the completion of treatment.

Results: In the beginning, 82 (59.4%) had normal weight (NW), 42 (30.4%) were overweight (OW), and 14 (10.2%) were obese (OB). Analysis of BMI-standard deviation score (SDS) in the whole group showed an overall increase during GnRHa treatment (0.92 ± 0.74 vs. 1.20 ± 0.51, $p < 0.001$). Changes in BMI-SDS (ΔBMI-SDS) during GnRHa differed between NW and OW/OB (0.45 ± 0.31 vs. 0.03 ± 0.20, $p < 0.001$). BMI-SDSs of both groups returned to baseline scores (or initial levels) 2 years after the completion of treatment. Two factors affecting ΔBMI-SDS in multiple linear regression analyses were baseline BMI and Δheight-SDS, both correlated negatively with ΔBMI-SDS.

Conclusions: The present study is one of the studies evaluating BMI change over a long period of time in girls with CPP. Although BMI-SDS increased during GnRHa in NW girls, it was reversible in follow-up after treatment. However, BMI-SDS did not change during and in follow-up in OW/OB girls. Conserving BMI-SDS in OW/OB girls may be related to the fact that weight management programs were recommended for these patients. Dietary recommendations should be provided for children with NW who undergo GnRHa, as is the case for OW patients.

Keywords: body mass index (BMI); BMI-SDS; central precocious puberty; GnRHa.

Introduction

There are many studies investigating the effect of gonadotropin-releasing hormone agonist (GnRHa) on final height in girls with central precocious puberty (CPP); however, studies evaluating the impact of this treatment on other anthropometric parameters, such as body mass index (BMI) are scarce [1]. Previous studies analyzing the effects of GnRHa on body weight and BMI provided conflicting results. Most studies in girls with CPP indicate that BMI of these girls is higher than age-matched controls at the time of diagnosis [2]. Some studies report an increase in body weight under GnRHa [3–6]; however, other studies suggest that BMI does not change [2, 7–9] and moreover, it may even decrease during treatment [10, 11]. Patients with CPP due to a hypothalamic hamartoma show an increase in BMI under GnRHa [3]. A few recent studies suggest that the effect of GnRHa on BMI depends on the patient's BMI at the time of diagnosis; the BMI of patients with normal weight (NW) and that of overweight (OW) and obese (OB) patients are affected differently [12]. The aim of this study is to investigate changes in BMI during and 2 years after completion of GnRHa treatment and to determine the factors that may impact BMI in girls with idiopathic CPP (iCPP).

Materials and methods

The medical files of 138 girls who completed GnRHa (leuprolide acetate) for iCPP were evaluated retrospectively. Age at diagnosis, bone age, body weight, height, pubertal stage, basal estradiol levels, and basal and stimulated gonadotropin levels were recorded. Girls who had breast development (Tanner stage 2 or higher) before the age of 8, acceleration of growth velocity, a bone age of at least 1 year greater than the chronological age, and a peak LH level of ≥5 IU/L in GnRH

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stimulation test were defined as CPP [13–15]. Pituitary magnetic resonance imaging (MRI) was performed on all cases. Cases with no pathology on MRI were considered as idiopathic, and were included in the study. Patients with CPP due to organic causes were excluded, given the possible presence of comorbid conditions such as hypothalamic obesity that may influence their BMI. Patients were excluded from the analysis if they had any additional condition that might affect BMI and onset of puberty (e.g. hypothyroidism, growth hormone deficiency, and congenital adrenal hyperplasia). The GnRH test was performed as previously described [16]. Commercial kits (ARCHITECT System, Abbott Laboratory Diagnostics, Abbott Park, IL, USA) with immunochemiluminometric assay (ICMA) method were used to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol levels. The lowest measurable levels of FSH, LH, and estradiol assays were 0.3 IU/L, 0.07 IU/L, and 10 pg/mL, respectively.

The patients received 3.75 mg leuprolide acetate every 28 days. In the third month of treatment, a GnRH stimulation test was performed and a peak LH level of <2 IU/L was defined to indicate suppression of the hypothalamo-pituitary-gonad axis [17–19]. The dose of leuprolide acetate was increased to 7.5 mg/28 days in patients with a peak LH of ≥2 IU/L, and the GnRH stimulation test was repeated in the third month to ensure all patients had a peak LH of <2 IU/L. All patients were followed with clinical and hormonal evaluations every 6 months until the end of treatment, and their pubertal stage, body weight, height, basal LH, and estradiol levels were recorded. The GnRH stimulation test was also repeated when necessary, and peak LH levels were recorded. As markers of sufficient hormonal control, basal LH measurement <0.6 IU/L and basal estradiol <10 pg/mL and GnRH-stimulated LH peak <2 IU/L were targeted during the depot GnRHa treatment [17–19]. The patients received GnRHa for a mean period of 29.9 ± 9.2 months.

All patients underwent weight and height measurements at the beginning and the end of treatment; 111 of 138 girls also underwent weight and height measurements 2 years after the completion of treatment. The body weight was measured as kilograms using a digital weighing scale (SECA, 769) with a sensitivity of 0.1 kg. The height was measured in meters with the patients in the standing position using a stadiometer (Harpenden, Holtain Ltd., Crymych, Dyfed, UK) with a sensitivity of 0.1 cm. All the measurements were made by a nurse trained in auxology. The height-SDSs were calculated using Centers for Disease Control (CDC) charts with respect to the chronological age. The BMI was calculated using the formula weight in kilograms/height in meters squared. BMI-SDSs were calculated using the lambda-mu-sigma (LMS) method [20]. Marshall and Tanner staging was used to determine the pubertal stage [21]. The bone age was determined using the Greulich and Pyle method [22]. CDC percentile curves were used for BMI assessment, and those with a BMI percentile of ≥10 and <85 were defined as NW, those with a BMI of ≥85 and <95 percentile were defined as OW, and those with a BMI of ≥95 percentile were defined as OB. At the beginning of treatment, 82 girls were NW, 42 were OW, and 14 were OB. The weight status of the patients prior to and after GnRHa treatment are shown in Figure 1. Auxological and hormonal data of the patients in the three BMI groups prior to GnRHa treatment are compared to each other. The number of patients in the OB group was small, and the calculated minimum sample size for significance was 34. Thus, OB and OW groups were combined for further longitudinal analysis of the changes in BMI-SDS and height-SDS during and 2 years after GnRHa treatment.

The change in BMI-SDS (ΔBMI-SDS) during treatment was defined as BMI-SDS at the end of treatment minus BMI-SDS at the beginning of treatment. The changes in the height-SDS during treatment (Δheight-SDS) was calculated by subtracting the height-SDS at the beginning of treatment from the height-SDS at the end of treatment. The factors that affected the change in BMI-SDS under GnRHa were assessed with a multivariate linear regression analysis. The dependent variable was ΔBMI-SDS and the independent variables were age, baseline BMI-SDS, duration of GnRHa treatment, and pubertal stages. The effect of Δheight-SDS during treatment on ΔBMI-SDS is also evaluated using a separate model.

The study protocol was approved by Hacettepe University Ethics Committee (Approval Number: 16969557-922, Project Number: GO 19/458-42). The requirement for informed consent was waived due to the retrospective nature of the study.

Statistical analysis

All statistical analyses were carried out using SPSS 21.0 for Windows software package (IBM Corp. Armonk, NY, USA). Normality was tested using the Kolmogorov-Smirnov test. Descriptive statistics were shown as mean ± standard deviation. Clinical and laboratory data of predefined BMI groups were compared using the analysis of variance (ANOVA) and Tukey's test for parametric variables. The change in each parameter over time was compared using repeated measures ANOVA test. For pairwise comparisons, the Bonferroni adjustment was used with a p-value of 0.017. The difference between categorical variables was tested using Pearson's chi-square (χ^2) and Fisher's exact tests. The factors affecting BMI-SDS change over time were assessed with multivariate linear regression analyses. The pubertal stages were scored according to Tanner and included in the multivariate analyses as 1 through 5. A p-value of less than 0.05 was considered statistically significant.

Sensitivity analysis was carried out using G*Power program. Since the number of patients in the OW group was small, the group number was chosen to be 2 for sensitivity analysis i.e. OW/OB groups were considered as a single group. When α error probability is chosen to be 0.05, and power (1-β err probability) is 0.80, three measurements in two groups, with group means and number of patients equal to 0.42

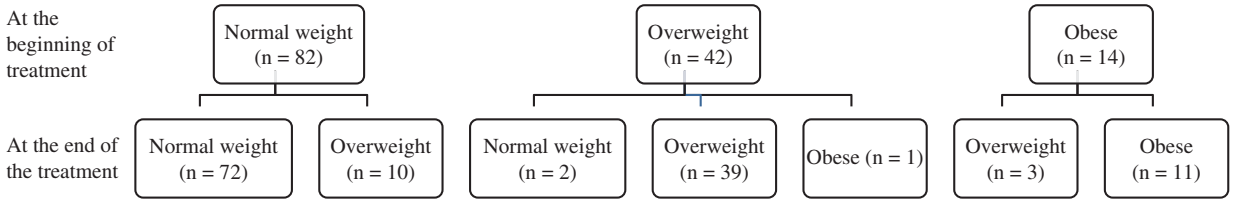

Figure 1: The status of the patients who were normal weight, overweight, or obese prior to and after GnRHa treatment.

and 66, and 1.66 and 45 (respectively in NW and OW/OB), standard deviation of 0.6, and for an effect size of 0.409, the minimum sample size was 34. Patient numbers in both groups i.e. NW vs. OW/OB exceeded this number, suggesting that group sizes were powerful enough to detect a difference as long as OW and OB children were considered to be together, analysis was carried out against the NW group.

Results

Auxological and hormonal data prior to GnRHa treatment

Auxological and hormonal data prior to GnRHa is presented in Table 1. The mean age of the patients at the beginning of treatment was 8.5 ± 1.0 years, and their mean bone age was 10.7 ± 0.9 years. At the beginning of treatment, 82 (59.4%) had NW, 42 (30.4%) were OW, and 14 (10.2%) were OB. There was no patient with a low body weight (BMI <10th percentile). Eighty-four patients (60.9%) were at Tanner stage 2, 41 (29.7%) at Tanner stage 3, and 13 (9.4%) at Tanner stage 4. Pubertal stages, basal LH, basal estradiol levels, and peak stimulated LH levels were higher in OW and OB girls in comparison to girls with NW.

Changes in BMI-SDS and height-SDS during and in follow-up after GnRHa treatment

Analysis of BMI-SDS in the whole group showed an overall increase during GnRHa treatment. The BMI-SDS was 0.92 ± 0.74 at the beginning of treatment and increased to 1.20 ± 0.51 at the end of treatment ($p < 0.001$). However, the change in BMI-SDS during treatment varied between different BMI groups. In the NW group, the BMI-SDS increased significantly during treatment (0.42 ± 0.54 at the beginning of treatment vs. 0.87 ± 0.33 at the end of the treatment). On the other hand, OW and OB patients maintained their BMI-SDS with no significant change in BMI-SDS during treatment. ΔBMI-SDS during treatment differed between NW and OW/OB patients (0.45 ± 0.31 vs. 0.03 ± 0.20, $p < 0.001$) (Table 2). The BMI-SDSs of both groups returned to baseline scores (or initial levels) 2 years after the completion of treatment (Table 2).

Overall, the mean height-SDS decreased significantly during GnRHa treatment. However, change in height-SDSs also varied between the different BMI groups. Patients who had NW initially showed a significant decrease in mean height-SDS at the end of treatment. However, the OW/OB group preserved their height-SDS at the end of GnRHa treatment. ΔHeight-SDS during treatment differed between the NW and OW/OB patients (−0.14 ± 0.47 vs. −0.01 ± 0.40) (Table 2). The mean height-SDS returned to baseline in both groups (NW and OW/OB) 2 years after the completion of GnRHa treatment (Table 2).

The factors affecting ΔBMI-SDS over time are shown in Table 3. Two factors affecting ΔBMI-SDS in multiple linear regression analyses were baseline BMI and Δheight-SDS, both correlated negatively with ΔBMI-SDS.

Discussion

In the present study, girls with iCPP showed a marked increase in their BMI at the end of GnRHa treatment when

Table 1: Auxological and hormonal data of the patients prior to GnRHa treatment.

	All patients (n = 138)	Normal weight (NW) (n = 82) (59.4%)	Overweight (OW) (n = 42) (30.4%)	Obese (OB) (n = 14) (10.2%)	p-Value[a]
Age at diagnosis, years	8.5 ± 1.0	8.6 ± 0.9	8.4 ± 0.6	8.3 ± 0.5	0.150
Bone age (BA), years	10.7 ± 0.9	10.7 ± 0.9	10.6 ± 0.8	10.6 ± 0.8	0.422
BMI-SDS	0.92 ± 0.74	0.42 ± 0.54[b,c]	1.33 ± 0.32[b,d]	2.15 ± 0.10[c,d]	<0.001[e]
Height-SDS	1.29 ± 0.70	1.13 ± 0.60	1.50 ± 0.58	1.55 ± 0.61	0.991
Duration of treatment, months	29.9 ± 9.2	28.8 ± 10.8	31.2 ± 7.2	32.4 ± 6.0	0.234
Pubertal stages		[b,c]	[b,d]	[c,d]	<0.001[e]
Tanner stage 2	84 (60.9%)	60 (73.2%)	20 (47.6%)	4 (28.6%)	
Tanner stage 3	41 (29.7%)	16 (19.5%)	18 (42.9%)	7 (50.0%)	
Tanner stage 4	13 (9.4%)	6 (7.3%)	4 (9.5%)	3 (21.4%)	
Basal FSH, IU/L	4.7 ± 2.3	4.5 ± 2.2	4.8 ± 2.3	5.5 ± 2.5	0.121
Basal LH, IU/L	1.3 ± 0.9	1.2 ± 0.8[c]	1.4 ± 0.9[d]	1.9 ± 1.2[c,d]	<0.001[e]
Basal Estradiol, pg/mL	34.4 ± 11.0	30.8 ± 10.6[b,c]	38.2 ± 10.8[b]	42.4 ± 14.4[c]	<0.001[e]
Peak LH in GnRH test, IU/L	12.7 ± 6.0	11.6 ± 5.2[b,c]	13.5 ± 6.5[b,d]	16.6 ± 9.2[c,d]	<0.001[e]

[a]One-way ANOVA test; [b]NW vs. OW, [c]NW vs. OB, [d]OW vs. OB, $p < 0.001$; [e]Multiple comparisons with post hoc Tukey.

Table 2: Changes in BMI-SDS and height-SDS during and long after GnRHa treatment.

	At the beginning of treatment	At the end of the treatment	Two years after completion of treatment	p-Value[a]
All patients (n:111)				
BMI-SDS	0.92 ± 0.74[b,c]	1.20 ± 0.51[b,d]	0.90 ± 0.62[c,d]	<0.001
Height-SDS	1.28 ± 0.57[b,c]	1.19 ± 0.64[b,d]	1.27 ± 0.51[c,d]	<0.001
Normal weight (n:66)				
BMI-SDS	0.42 ± 0.54[b,c]	0.87 ± 0.33[b,d]	0.40 ± 0.48[c,d]	<0.001
Height-SDS	1.13 ± 0.49[b,c]	0.99 ± 0.64[b,d]	1.10 ± 0.44[c,d]	<0.001
Overweight/obese (n:45)				
BMI-SDS	1.66 ± 0.48	1.69 ± 0.53	1.65 ± 0.52	0.205
Height-SDS	1.49 ± 0.75	1.48 ± 0.63	1.51 ± 0.61	0.098

[a]Change in each parameter over time was compared using a repeated measures ANOVA test, (n:111). [b]At the beginning of the treatment vs. at the end of the treatment, $p < 0.001$. [c]At the beginning of treatment vs. 2 years after completion of treatment $p > 0.05$. [d]At the end of the treatment vs. 2 years after completion of treatment, $p < 0.001$.

Table 3: Assessment of the factors affecting ΔBMI-SDS under GnRHa treatment using a multivariate linear regression analysis.

	First model[a]				Second model[b]			
	B	SE	β	p-Value	B	SE	β	p-Value
Constant	−0.109	0.136		0.425	−1.596	0.035		<0.001
Age	0.133	0.016	0.359	0.148	0.215	0.004	0.577	0.241
Baseline BMI-SDS	−0.263	0.011	−0.804	<0.001				
ΔHeight-SDS[c]					−2.036	0.017	−1.229	<0.001
Duration of treatment	−0.015	0.001	−0.475	0.068	−0.003	0.001	−0.085	0.138
Pubertal stages	−0.024	0.012	−0.065	0.056	−0.002	0.003	−0.006	0.463

[a]First model: Dependent variable, ΔBMI-SDS; Independent variables, Age, baseline BMI-SDS, duration of GnRHa treatment, and pubertal stages; r2, 0.609. [b]Second model: Dependent variable, ΔBMI-SDS; Independent variables, Age; Δheight-SDS, duration of GnRHa treatment, and pubertal stages; r2, 0.809. [c]ΔHeight-SDS, Height-SDS at the end of treatment – Height-SDS at the beginning of treatment.

compared to their baseline BMI. There are several studies in literature indicating an increased risk of obesity in girls under GnRHa treatment [3–5, 23]. Lee et al. showed that the BMI z-score increased from 0.58 ± 1.18 to 0.96 ± 0.83 in the 18th month of GnRHa treatment in girls with CPP [6]. On the other hand, there are studies reporting no significant change in BMI z-score during, at the end, or following the completion of GnRHa treatment [2, 7–9]. There are also studies reporting a decrease in BMI under GnRHa treatment [10]. Most studies investigating the effects of GnRHa treatment on BMI involve a small number of participants [2–5, 7–9, 23]. In the present study, we report the findings of 138 girls with iCPP which is one of the largest series in literature providing follow-up data after the completion of treatment.

With respect to the baseline BMI-SDS groups, while OW/OB patients maintained their BMI-SDS, those with NW had an increase in BMI-SDS. Concurring with the findings of the present study, there are several other studies suggesting that patients in different BMI groups respond to GnRHa treatment in different ways in terms of BMI change. Wolters et al. reported that the BMI z-score of patients with NW increased from 0.08 ± 1.02 to 0.40 ± 0.85 during treatment, although the OW group had no significant change in BMI z-scores (2.01 ± 0.69 vs. 2.03 ± 0.54) [12]. In addition to BMI changes during the treatment, we also reported BMI changes 2 years after the completion of treatment. In the present study, the BMI-SDSs of all study groups (all the patients, NW, OW/OB) returned to baseline levels 2 years following the completion of treatment. An increase in BMI-SDS during GnRHa treatment in NW patients was reversible after the discontinuation of treatment, so it can be assumed that GnRHa does not lead to a permanent increase in BMI-SDS.

Similar to the present study, Arcari et al. categorized patients according to pre-treatment BMI status and evaluated the effect of GnRHa on BMI at 1 and 2 years of treatment in 117 girls with iCPP [24]. They reported that significant increases in BMI-SDS at the first and second year of treatment were observed in NW girls compared

to baseline BMI-SDS. OW girls had a significant increase in BMI-SDS only in the first year of treatment while OB girls did not display any change in BMI-SDS during treatment. The authors also compared BMI-SDS of 60 girls who reached adult height to that of 33 untreated iCPP girls and showed a significant decrease in BMI-SDS at adult height compared to baseline and compared to BMI-SDS at adult height of untreated girls. This study is one of the few studies that divides the cases into three groups according to the pre-treatment BMI and gives the follow-up of these three groups during and long after treatment such as at adult height. In this study, similar to ours, it was shown that weight gain during GnRHa treatment was different between pre-treatment BMI groups and these changes were reversible after the cessation of GnRHa. It was also shown that the patients had lower BMI-SDSs at adult height compared to the baseline BMI-SDSs.

Lazar et al., in 2015, evaluated the BMI of girls with iCPP who were treated or not treated with GnRHa in long term at 3rd and 5th decades of life [25]. This is the longest period in literature in which BMI changes are evaluated after cessation of GnRHa in girls with CPP. The weight status of all women with former CPP whether treated or untreated resembled that of the general population from late adolescence to early-mid adulthood despite their above average BMI at the onset of puberty. Longitudinal analysis showed an increase in BMI percentiles during GnRHa treatment followed by a decrease from cessation of treatment to late adolescence to young adulthood.

The different weight change responses to treatment in different studies may be attributed to variations in age groups, genders, and baseline body weights. Arrigo et al. reported a decrease in BMI under GnRHa treatment, although the mean BMI z-score in their study was >1 and most of the patients in that study were OW [10]. Concurring with the findings in that study, no increase in BMI was noted among OW patients in the present study.

Among the factors affecting the change in BMI-SDS under GnRHa treatment, the significant factors were the baseline BMI-SDS and change in height-SDS during treatment. Both of these factors were correlated negatively with the change in BMI-SDS. In the studies by Park et al. and Yang et al., the change in BMI-SDS was also negatively correlated with the baseline BMI-SDS [26, 27].

It is yet not clear why NW patients experience an increase in BMI-SDS during GnRHa treatment while OW/OB patients do not. The change in growth velocity could be a factor in this inconsistency, and we found that patients with NW had a significantly lower height-SDS at the end of the treatment compared to their baseline levels ($p < 0.001$). However, OW/OB children maintained their height-SDS under GnRHa treatment. The baseline height-SDSs were representative of a period of accelerated growth due to the effect of sex steroids. GnRHa treatment is expected to decrease growth velocity [28], which returns to that seen in the prepubertal period under GnRHa treatment, and the discontinuation of this treatment leads to an increase in sex steroids, resulting in an increase in growth velocity. The fact that height-SDS is high at the beginning of GnRHa treatment, decreases under treatment, and increases again after the completion of treatment supports this view. That said, the expected decrease in growth velocity in the OW/OB children was not significant, and these patients had similar height-SDS levels before, during, and after GnRHa treatment. It is well known that OB children grow at a greater velocity than their peers [29, 30], although they reach a similar final height related to their genetic potential [31, 32]. This may be the reason why the height-SDS of children with NW was found to decrease during GnRHa treatment, while the height of OW/OB did not decrease. The decrease in the height-SDS of children with NW might result in a relative and transient increase in BMI-SDS. Change in height-SDS during treatment was found to be significantly negatively correlated with change in BMI-SDS under GnRHa treatment and this finding may also support this hypothesis. One of the possible reasons for the lack of increase in the BMI-SDS of OW/OB patients under GnRHa treatment may be the weight management programs and diet and exercise recommended to these cases.

Childhood obesity is reported to be a marker of morbidity and mortality in adulthood, and obesity has been reported to be more common in girls with CPP [33]. Overweight and obesity rates were quite high in the present study, the rates of obesity and overweight being 10.2% and 30.4%, respectively, prior to treatment. These rates were also significantly higher than those reported for the Turkish population. The rates of obesity and overweight in this age group are reported to be 1.6–7.5% and 7.8–14.3%, respectively, in healthy children [34–38]. Given the metabolic and cardiovascular effects of obesity, these cases should be monitored closely and preventive measures should be taken. As CPP patients with NW are at greater risk of weight gain under GnRHa treatment than those who are OW or OB, all patients with CPP should be informed about weight management, and lifestyle changes should be implemented. Patients who are OW or OB should undergo weight management programs at the time of diagnosis, and those with NW should be provided such measures once they gain weight.

The present study has several limitations, the most significant of which is its retrospective design. In general, all

OW and OB cases are provided dietary plans, but because of the retrospective design of the study, it is not known if all of the patients were given similar dietary recommendations. While the sample size is larger than in previous studies, the number of patients with obesity was relatively low when compared to the other groups. Accordingly, the BMI-SDS and height-SDS of the OW and OB patients were assessed together.

Conclusions

The present study is one of the studies evaluating BMI change over a long period of time in girls with CPP. Although BMI-SDS increased during GnRHa treatment in NW girls, it was reversible in follow-up after the completion of treatment. However, BMI-SDS did not change during and in follow-up in OW or OB girls. Conserving BMI-SDS in OW or OB girls with CPP during treatment may be related to the fact that body weight management programs were generally recommended and applied on these patients. Another reason for the difference in BMI change during GnRHa treatment between NW and OW/OB girls may be the difference in growth velocity of these two groups. Dietary recommendations should be provided for children with NW who undergo GnRHa treatment for CPP, as is the case for OW patients.

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Long-Term Outcomes of Treatments for Central Precocious Puberty or Early and Fast Puberty in Chinese Girls

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Context: Gonadotropin-releasing hormone analogues (GnRHa) and recombinant human growth hormone (rhGH) have been widely used to treat idiopathic central precocious puberty (CPP) or early and fast puberty (EFP). However, large-scale studies to evaluate the treatment effects on final adult height (FAH) are still lacking.

Objective: To assess the effects of long-term treatment for CPP/EFP on FAH and its main influencing factors.

Design and Setting: Retrospective, multicenter observational study from 1998 to 2017.

Participants: Four hundred forty-eight Chinese girls with CPP/EFP received GnRHa and rhGH treatment (n = 118), GnRHa alone (n = 276), or no treatment (n = 54).

Main Outcome Measures: FAH, target height (Tht), and predictive adult height (PAH).

Results: The height gain (FAH–PAH) was significantly different among the GnRHa and rhGH treatment, GnRHa alone, and no treatment groups (P < 0.05; 9.51 ± 0.53, 8.07 ± 0.37, and 6.44 ± 0.91 cm, respectively). The genetic height gain (FAH–Tht) was 4.0 ± 0.5 cm for the GnRHa + rhGH group and 2.0 ± 0.27 cm for the GnRHa group, while the control group reached their Tht. In addition, 5 critical parameters derived from PAH, bone age, and Tht, showed excellent performance in predicting which patients could gain ≥5 cm (FAH–PAH), and this was further validated using an independent study.

Conclusions: The overall beneficial effect of GnRHa + rhGH or GnRHa on FAH was significant. The control group also reached their genetic target height. Clinicians are recommended to consider both the potential gains in height and the cost of medication. **(*J Clin Endocrinol Metab* 105: 705–715, 2020)**

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Central precocious puberty (CPP) is defined as the development of secondary sexual characteristics before 8 years of age in girls and 9 years of age in boys and is caused by premature activation of the hypothalamic–pituitary–gonadal axis (1). Early and fast puberty (EFP) is characterized by signs of puberty in girls between 8 and 9 years of age and a fast transition from Tanner I stage to the next stage within 6 months, accompanied by accelerated growth and bone maturation (2). Children with CPP/EFP tend to exhibit temporarily rapid acceleration in growth due to increased sex hormone production or exposure, leading to premature closure of the growth plate and a shorter final adult height (FAH) (1). Rapid progression of the secondary sexual characteristics in children can also cause poor social adaptability, psychological stress, and emotional disorders (3). In addition, recent studies indicated that early menarche or male puberty were associated with gynecological, breast, or testicular cancer (4). Recent epidemiologic studies demonstrated that the incidence and prevalence rates of CPP are high and increased steeply from 2004 to 2014 (5–8).

Gonadotropin-releasing hormone analogues (GnRHa) have been used as the first-line drug for CPP treatment for over 40 years and can significantly improve the FAH by suppressing bone maturation and pubertal development to stabilize or regress secondary sexual characteristics (9–14). Although GnRHa treatment is safe overall, several studies have reported that it can affect body weight (BW) and bone mineral density (15–17). The decrease in growth velocity (GV) might further impair the predicted adult height (PAH) (18). Therefore, combined recombinant human growth hormone (rhGH) and GnRHa therapy is recommended to compensate for the reduction of GH during GnRHa treatment (19, 20). Oostdijk et al. first reported obvious improvement of PAH after 18 months of treatment with the combined GnRHa and rhGH in 3 female cases of CPP with low GV (21). This approach was further verified by Volta et al. (22) and others (23), who showed that the combined treatment could help CPP patients with low GV to obtain a greater final height. However, the Pediatric Endocrine Society and the European Society for Pediatric Endocrinology suggested in 2009 that the addition of rhGH should not be recommended as a routine treatment procedure due to the lack of large-scale randomized controlled trials to evaluate the efficacy of the combined therapy (11).

In the present study, we reported a multicenter retrospective study including a total of 488 Chinese girls diagnosed with CPP or EFP who received treatment with GnRHa alone, combined GnRHa and rhGH treatment, or no treatment. The participants were followed between 1998 and 2017 until they reached their FAH, and their medical records were collected and carefully reviewed. The aims of this work were to answer (i) whether the treatment has an effect on the FAH of girls with CPP/EFP and (ii) whether we predict which patients will benefit most from GnRHa alone or GnRHa + rhGH therapy?

Materials and Methods

Participants

The study design is illustrated in Fig. 1. The retrospective study was conducted by reviewing the medical records of 448 girls with CPP or EFP between 1998 and 2017 from 12 medical centers in China. According to the National Consensus Statement in China (24), the participants were diagnosed with CPP when secondary sexual characteristics including breast buds and/or genitalia Tanner stage II with or without sexual hair appeared before the age of 8 years. The participants were diagnosed with EFP when pubertal signs started to develop between 8 and 9 years of age and progressed rapidly from one stage to the next within 6 months with accelerated growth and bone maturation. In both the CPP and EFP patients, the peak luteinizing hormone (LH) concentration after GnRH stimulation reached 5.0 IU/L or greater and increased ovarian and uterine volumes were observed on pelvic ultrasonography. The exclusion criteria were hypothalamic–pituitary congenital

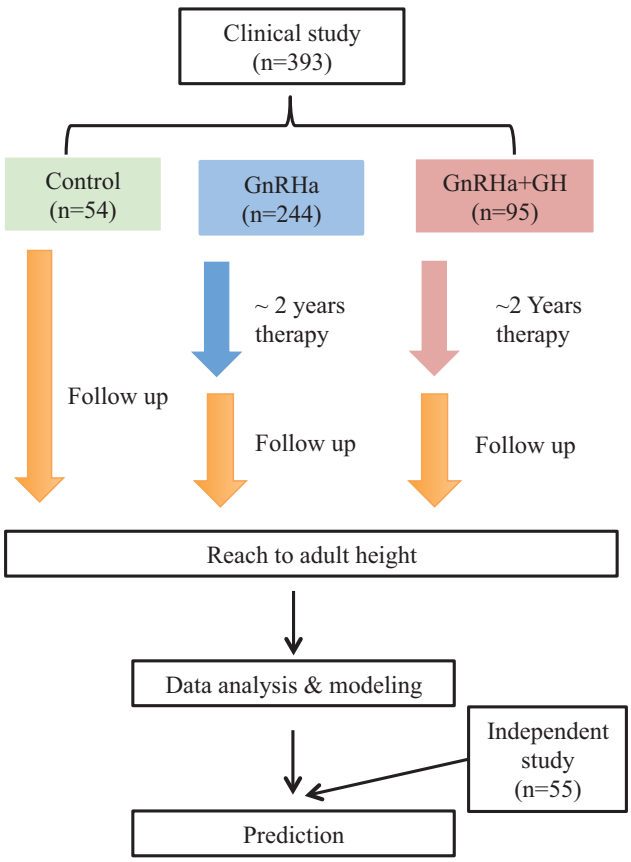

Figure 1. Illustration of the current study design.

malformations, tumors, McCune Albright syndrome, infections, infiltrative and inflammatory disorders, and iatrogenic or traumatic injuries; other types of abnormal growth and development including Turner syndrome, hypothyroidism, etc.; and previous or current use of other medication.

Treatment and grouping

A total of 448 patients were recruited from 12 children's hospitals in China, of which one hospital was randomly selected to perform an independent validation study. Specifically, a total of 393 patients who received GnRHa alone (n = 244), GnRh + rhGH (n = 95), or no treatment (n = 54) were from 11 children's hospital and used for the training data set. A total of 55 patients who received GnRHa (n = 32) or GnRHa + rhGH (n = 23) were from an additional hospital and used for the validation data set. GnRHa was administered monthly at a dose of 75 to 150 μg/kg (ie, 1.875 mg for BW <20 kg, 2.5 mg for BW 20–30 kg, and 3.75 mg for BW > 30 kg). For the combined GnRHa and rhGH treatment, GnRHa was administered as aforementioned, and rhGH was injected subcutaneously at a dose of 0.1 to 0.2 U/kg/day. RhGH treatment was discontinued at the age of 11 to 11.5 years old, at the bone age (BA) of 12 to 12.5 years old, or until the patient's GV slowed to <4 cm/year. Of note, in the training data set, 55 patients in the combined treatment group received GnRH alone first and rhGH was added later when their GV was below –1 standard deviation score (SDS). All patients were followed-up until they reached their FAH. The study protocol was reviewed and approved by the Institutional Review Board of the Children's Hospital of Zhejiang University.

Medical records

All participants were followed-up by their corresponding endocrinologists. The chronological age (CA), height, BW, BA, dosage, and FAH of each patient were recorded at the beginning of the study and at follow-up appointments. BA was measured using the Greulich and Pyle Atlas (25). The parents' heights were also recorded. Body height was measured using a standardized protocol in 12 clinical centers with an accuracy of ±0.1 cm. The genetic target height (Tht, sum of parents heights/2–6.5 cm), body mass index (BMI), calculated-age based on height (HA), calculated-height based on BA (BH), PAH at the beginning (PAH1) and end of treatment (PAH2), the GV at the 1st and 2nd year of treatment (GV1 and GV2, respectively), the loss in height potential ($PAHSDS_{Tht}$, (PAH1–Tht)/5.4), height gain (FAH–PAH1), and genetic height gain (FAH–Tht) were calculated. The HtSDS was calculated as follows: HtSDS = (height–average height of the same population)/

Table 1. Clinical Characteristics of all Participants in This Study

	Control	GnRHa	GnRHa + GH	*P*
Before treatment				
CA1 (years)	8.4 ± 0.12	8.4 ± 0.06	8.7 ± 0.1	0.109
Weight1 (kg)	30.5 ± 0.74	30 ± 0.35	29.5 ± 0.53	0.489
Height1 (cm)	134.4 ± 0.79	133.75 ± 0.4	131.3 ± 0.7	0.165
BMI1 (kg/m^2)	16.98 ± 0.3	16.66 ± 0.14	17.14 ± 0.22	0.537
BA1 (years)	11 ± 0.16	10.6 ± 0.07	11 ± 0.14	0.693
Father height (cm)	170 ± 0.71	170 ± 0.33	168 ± 0.5	0.165
Mother height (cm)	159 ± 0.6	158 ± 0.29	156 ± 0.49	**0.023**
Tht (cm)	157.5 ± 0.57	157 ± 0.24	156 ± 0.42	**0.048**
BA1–CA1 (years)	2.3 ± 0.12	2 ± 0.05	1.8 ± 0.09	**0.025**
PAH1 (cm)	151 ± 0.94	152 ± 0.42	150.81 ± 0.66	0.415
Height1/BH1	0.93 ± 0.01	0.93 ± 0	0.93 ± 0	0.492
HA1/BA1	0.85 ± 0.01	0.86 ± 0.01	0.84 ± 0.01	0.281
HtSDS1	0.55 ± 0.12	0.48 ± 0.06	–0.02 ± 0.09	**<0.001**
HtSDS_BA1	–1.85 ± 0.15	–1.72 ± 0.07	–1.79 ± 0.11	0.642
After treatment				
CA2 (years)		10.5 ± 0.06	11.1 ± 0.11	**<0.001**
BA2 (years)		12 ± 0.05	12 ± 0.1	**0.003**
Height2 (cm)		145.7 ± 0.39	149.5 ± 0.67	**<0.001**
1st GV (cm)	5.35 ± 0.19	5.85 ± 0.09	6.4 ± 0.24	**0.003**
2nd GV (cm)	5 ± 0.15	5 ± 0.09	6.2 ± 0.17	**<0.001**
GnRHa treatment (years)		2 ± 0.05	2.5 ± 0.08	**<0.001**
$PAHDSD_{Tht}$	–1.09 ± 0.19	–1 ± 0.07	-0.86 ± 0.12	0.537
PAH2 (cm)		157.81 ± 0.39	160.36 ± 0.53	**0.003**
HtSDS2		0.3 ± 0.06	0.2 ± 0.09	0.999
HtSDS_BA2		–0.83 ± 0.06	–0.43 ± 0.08	**0.001**
FAH (cm)	158 ± 0.55	159.7 ± 0.28	160 ± 0.51	0.174
FAH–PAH1 (cm)	6.44 ± 0.91	8.07 ± 0.37	9.51 ± 0.53	**0.048**
FAH–Tht (cm)	0 ± 0.61	2 ± 0.27	4 ± 0.5	**<0.001**
PAH2–PAH1 (cm)		5.92 ± 0.36	9.25 ± 0.62	**<0.001**

Numbers 1 and 2 indicate the time points before and after treatment, respectively. Values represent median ± standard error of the mean (SEM). *P*-values were adjusted for multiple comparisons using the false discovery rate method.

Abbreviations: BA, bone age; BH, calculated-height based on bone age; BMI, body mass index; CA, chronological age; FAH, final adult height; FAH–PAH1, height gain; FAH–Tht, genetic height gain; GnRHa, gonadotropin-releasing hormone analogue; GH, growth hormone; GV, growth velocity; HA, calculated-age based on height; PAH, predictive adult height; SDS, standard deviation score; Tht, target height.

standard deviation for height in the same population. The HtSDS_BA was calculated by: HtSDS_BA = (height–average height at the same BA)/standard deviation for height in the same population.

Data analysis

A nonparametric univariate analysis (ie, Mann Whitney U test) was applied to compare the differences in clinical parameters between any 2 groups. The Kruskal–Wallis test was used for multigroup comparisons. Values of *P* were adjusted for multiple comparisons using a false discovery rate (FDR), and an adjusted *P* value less than 0.05 was considered statistically significant. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the performance of each clinical parameter and the cut-off value for the AUC was greater than 0.8. Logistic regression models were obtained to identify significant and independent

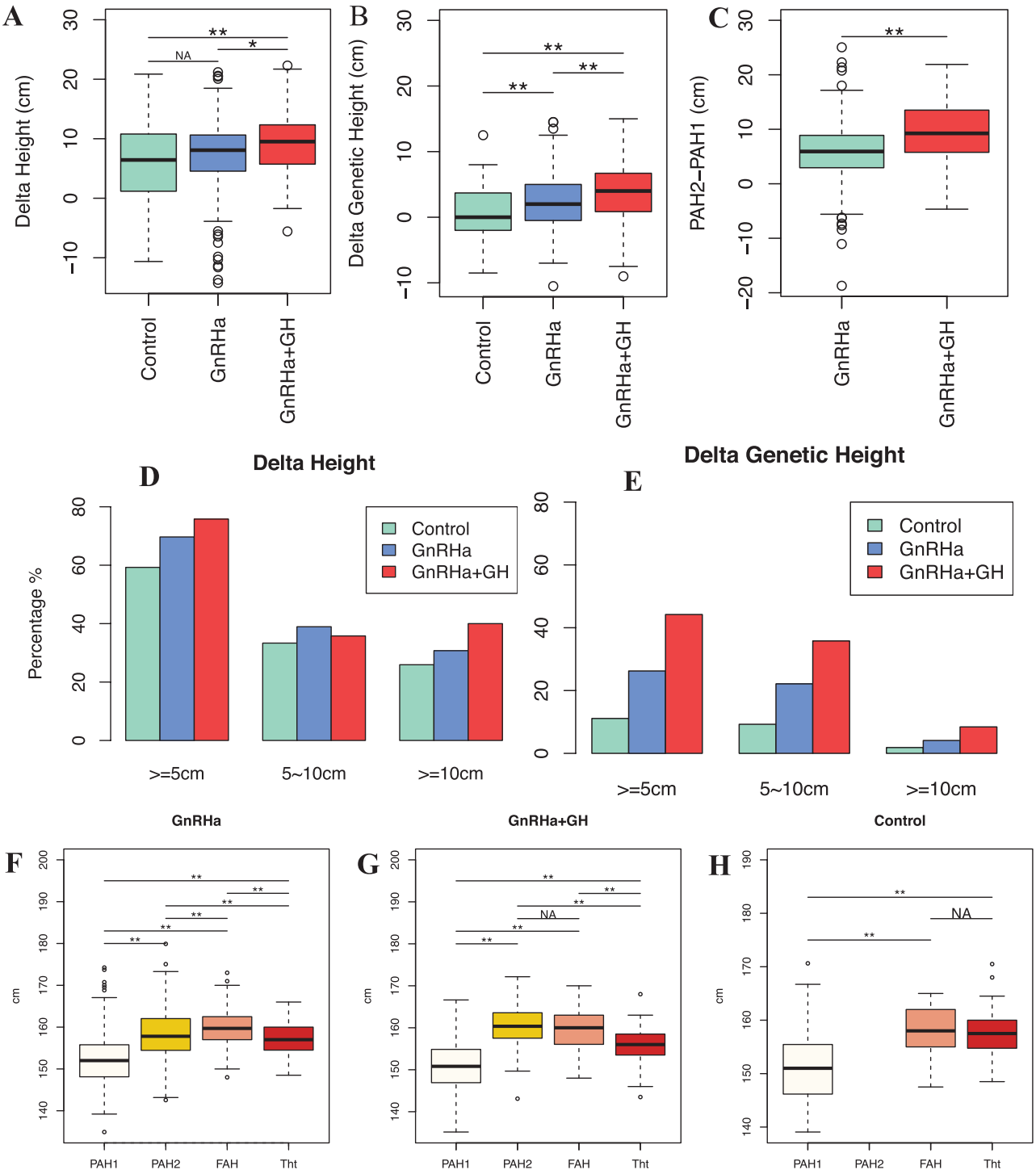

Figure 2. (A–C) Box plots of delta height, delta genetic height, and PAH2–PAH1 in three/two groups. (D–E) Bar plots of the percentage of patients who had received ≥5-cm, 5–10-cm, and ≥10-cm height gains. (F–H) Box plot of PAH1, PAH2, FAH, and genetic height in three different groups.

contributing factors and were further applied to predict the treatment outcomes of GnRHa only or GnRHa + rhGH in patients from the validation group. Lasso regression analysis with 10-fold cross validation was applied to enhance the predictive accuracy and model interpretation. All statistical analyses were performed and all graphics were prepared using the R packages (R version 3.4.0).

Results

Growth indexes of patients before and after treatment

As shown in Table 1, most of the baseline parameters were comparable between the 3 groups, including CA, BA, height, weight, BMI, father height, HA1/BA1, Height1/BH1, PAH1, $PAHSDS_{Tht}$, and HtSDS_BA (all $P > 0.05$). After treatment, the patients of the GnRHa + rhGH group were 0.6 years older and 3.8 cm taller as compared to those in the GnRHa group because the combined treatment was administered over a longer time. However, the PAH and GV had progressed obviously in the GnRHa + rhGH group at the end of the 1st and 2nd years of treatment ($P < 0.05$).

The FAH appeared slightly higher in the GnRHa + rhGH group after treatment (Table 1); however, the delta height (FAH–PAH1), delta genetic height (FAH–Tht), and difference in PAH before and after treatment (PAH2–PAH1) were significantly greater in the GnRHa + rhGH group than in the GnRHa group ($P < 0.05$). These indexes in the GnRHa group were also higher than those in the control group ($P < 0.05$; Fig. 2A-C). Upon division of the FAH–PAH1 and FAH–THt differences into 3 categories (≥5 cm, 5–10 cm, and ≥10 cm), we observed that the GnRHa + rhGH group showed the highest percentage of participants in each subgroup, followed by the GnRHa group (Fig. 2D,E). In addition, the control group only reached their Tht, whereas both the GnRHa + GnRHa and rhGH groups were significantly taller than their Tht ($P < 0.05$; Fig. 2F–H).

Factors influencing the effects of GnRHa treatment

The patients were divided into 2 subgroups based on their height gain after treatment: the satisfactory

Table 2. Comparison of Patients Who Did (Yes) or Did Not (No) Achieve a Satisfatory Outcome with a ≥5-cm Height Gain after GnRHa Treatment

	No	Yes	*P*	FC	ROC
$PAHDSD_{Tht}$	0.12 ± 0.12	–1.33 ± 0.06	**<0.001**	-11.04	**0.883**
PAH1 (cm)	157.14 ± 0.7	149.72 ± 0.37	**<0.001**	0.95	**0.868**
HtSDS_BA1	–0.92 ± 0.11	–2 ± 0.06	**<0.001**	2.18	**0.852**
Height1/BH1	0.96 ± 0	0.92 ± 0	**<0.001**	0.96	**0.85**
HA1/BA1	0.92 ± 0.01	0.83 ± 0.01	**<0.001**	0.90	**0.836**
PAH2–PAH1 (cm)	2.07 ± 0.65	6.98 ± 0.35	**<0.001**	3.38	**0.807**
BA1–CA1 (years)	1.56 ± 0.1	2.2 ± 0.05	**<0.001**	1.41	0.744
BA1 (years)	10 ± 0.16	10.8 ± 0.06	**<0.001**	1.08	0.662
1st GV (cm)	5.5 ± 0.17	6 ± 0.11	**0.002**	1.09	0.638
BA2 (years)	11.7 ± 0.11	12 ± 0.05	**0.006**	1.03	0.621
HtSDS1	0.65 ± 0.1	0.4 ± 0.06	**0.008**	0.61	0.617
Height1 (cm)	135.4 ± 0.79	133 ± 0.44	**0.013**	0.98	0.609
GnRHa treatment (years)	2 ± 0.07	2 ± 0.06	**0.014**	1.00	0.604
HtSDS_BA2	–0.68 ± 0.1	–0.89 ± 0.07	**0.021**	1.31	0.601
Weight1 (kg)	30.5 ± 0.65	29.4 ± 0.41	**0.031**	0.96	0.594
PAH2 (cm)	158.19 ± 0.72	157.58 ± 0.46	**0.031**	1.00	0.594
FAH (cm)	158 ± 0.52	160 ± 0.33	**0.037**	1.01	0.59
2nd GV (cm)	4.7 ± 0.19	5 ± 0.1	0.055	1.06	0.583
FAH–Tht (cm)	1.75 ± 0.49	2.5 ± 0.32	0.078	1.43	0.576
CA2 (years)	10.4 ± 0.11	10.6 ± 0.08	0.158	1.02	0.562
BMI1 (kg/m^2)	17.08 ± 0.23	16.57 ± 0.18	0.179	0.97	0.559
HtSDS2	0.48 ± 0.11	0.26 ± 0.07	0.211	0.55	0.554
Mother height (cm)	158 ± 0.53	158 ± 0.34	0.588	1.00	0.474
Tht (cm)	158.25 ± 0.45	157 ± 0.28	0.828	0.99	0.513
Father height (cm)	170 ± 0.59	170 ± 0.39	0.896	1.00	0.507
Height2 (cm)	146.55 ± 0.73	145.45 ± 0.47	0.896	0.99	0.507
CA1 (years)	8.4 ± 0.11	8.4 ± 0.06	0.957	1.00	0.502

Numbers 1 and 2 indicate the time points before and after treatment, respectively. Values represent median ± standard error of the mean, ordered by *P* value. FC represents fold change. *P*-values were adjusted for multiple comparisons using the false discovery rate method. *P* values or ROC values highlighted with bold indicated statistically differential variables of interest in this study.

Abbreviations: BA, bone age; BH, calculated-height based on bone age; BMI, body mass index; CA, chronological age; FAH, final adult height; FAH–PAH1, height gain; FAH–Tht, genetic height gain; GnRHa, gonadotropin-releasing hormone analogue; GH, growth hormone; GV, growth velocity; HA, calculated-age based on height; PAH, predictive adult height; SDS, standard deviation score; Tht, target height.

outcome group (height gain ≥5 cm; n = 170) and fair outcome group (height gain <5 cm; n = 74). As shown in Table 2, CA, BMI, and Tht were similar between the groups at baseline. However, patients in the satisfactory treatment group demonstrated significant differences in $PAHSDS_{Tht}$, PAH, HtSDS, Height1/BH1, HA1/BA1, height, weight, BA, and BA–CA as compared to those in the fair treatment group (Table 2). Moreover, the satisfactory treatment group showed a significantly faster growth rate at the end of the 1st year treatment, especially for PAH2–PAH1, in comparison to the fair treatment group (P < 0.05; Table 2).

The ROC curve analysis indicated that 5 baseline parameters (ie, $PAHSDS_{THt}$, PAH1, HtSDS_BA1, Height1/BH1, HA1/BA1, and PAH2–PAH1) showed excellent performance in predicting the treatment effect of GnRHa therapy (AUC > 0.8, Table 2). Among these, $PAHSDS_{THt}$, PAH1, and HA1/BA1 were further identified as independent factors by logistic regression analysis and validated via a lasso regression analysis method (P < 0.05; Fig. 3A). Then, a predicted score formula was obtained: Ypred = 53.81–1.44 × $PAHSDS_{THt}$ + 27.73 × HA1/BA1–0.5 × PAH1. The AUC for the predicted Y score was 0.907, and the optimal cut-off value was 0.54 (Fig. 3B). The equation was applied to predict the treatment effect in 32 patients who received GnRHa treatment in the validation data set. Patients in the validation data set had similar growth indexes with the training data set (Table 3). As a result, a total of 20 out of 22 (90.9%) patients who achieved a ≥5-cm gain in height and 8 out of 10 patients (80%) who achieved a <5-cm gain in height were correctly identified (Fig. 3C).

Factors influencing the effect of GnRHa + rhGH treatment

In the GnRHa + rhGH group, 40 girls were treated with both GnRHa + rhGH initially, and the remaining 55 girls received GnRHa only at first with the addition of rhGH later (Table 4). Similarly to the previously

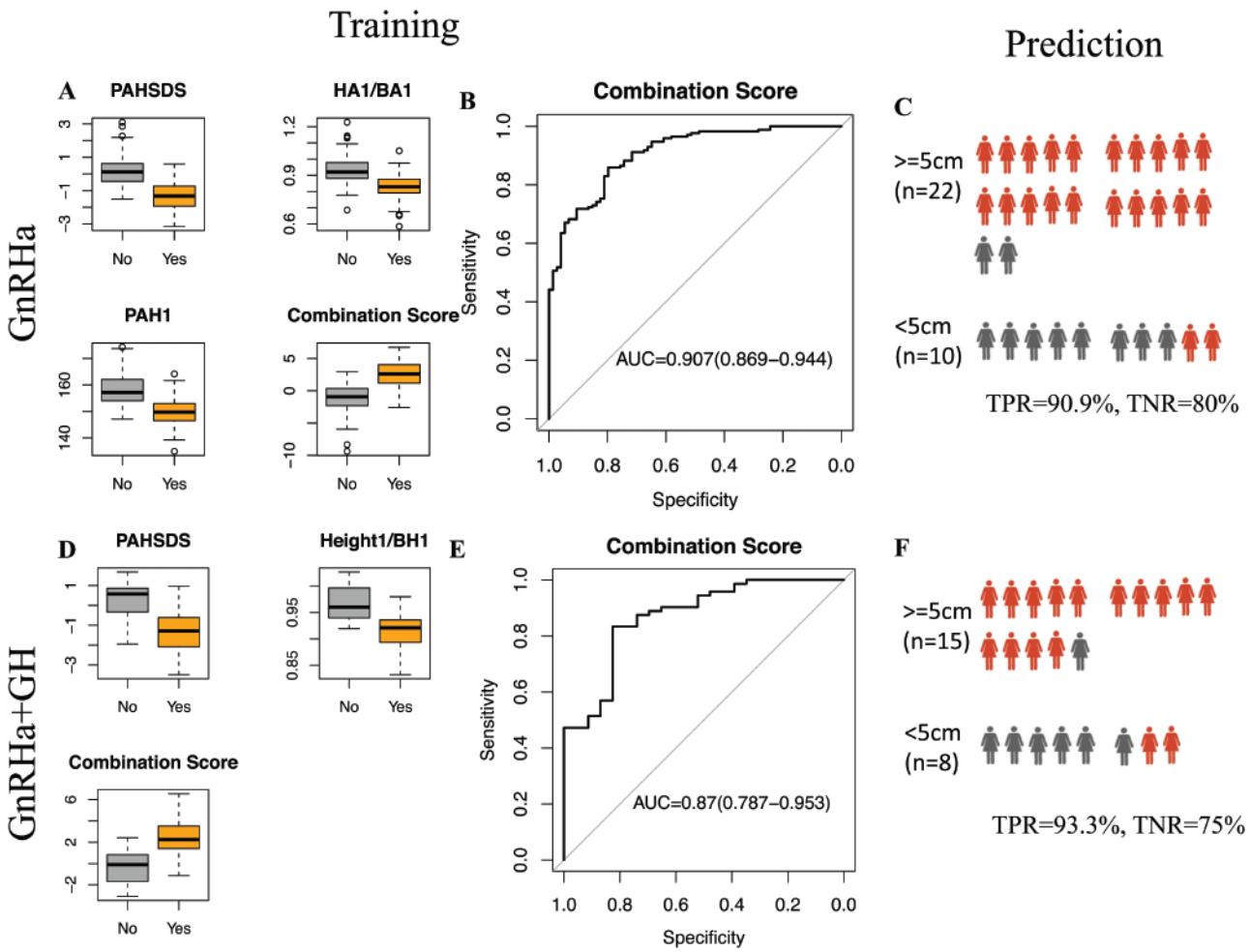

Figure 3. (A) Box plots of $PAHSDS_{THt}$, HA1/BA1, PAH1, and predicted score in 2 subgroups of GnRHa-treated patients. YES or NO indicates whether patients achieved a satisfactory outcome or not. (B) ROC curves for a combination score from the GnRHa model. (C) Results for prediction of the therapeutic effects of GnRHa in an independent study. (D) Box plot of Height1/BH1 in YES and NO groups of GnRHa + rhGH-treated patients. (E) ROC curves for Height1/BH1. (F) Results for prediction of the therapeutic effects of GnRHa + rhGH in an independent study.

described analysis, the patients in the GnRHa + rhGH group were also divided into 2 subgroups based on their height gain after treatment: a satisfactory outcome group (height gain ≥5 cm; n = 72) and fair outcome group (height gain <5 cm; n = 23). As shown in Table 5, both groups had similar values for CA, height, and Tht at baseline and were treated over a similar period of treatment and with a similar dosage of rhGH. The patients in the satisfactory treatment group tended to be older, taller, and heavier and to have a larger BMI at baseline than those in the fair treatment group. Nevertheless, Height1/BH1, PAH1, $PAHSDS_{Tht}$, HA1/BA1, BA1, and BA1–CA1 in the satisfactory treatment group were significantly different from those in the fair treatment group (Table 5). In addition, the growth rate was slightly faster in the satisfactory treatment group at the end of the 1st and 2nd treatment years, and the PAH2–PAH1 difference was significantly larger in the satisfactory treatment group than in the fair treatment group ($P < 0.05$; Table 5).

ROC curve analysis identified six parameters (PAH2–PAH1, PAH1, Height1/BH1, $PAHSDS_{THt}$, HtSDS_BA1, PAHSDSTHt, and HA1/BA1) that showed excellent performance (AUC > 0.8) in predicting the therapeutic effect of GnRHa + rhGH treatment (Table 5). Of these, $PAHSDS_{Tht}$ and Height1/BH1 were identified as independent factors using a logistic regression model and validated using lasso regression analysis ($P < 0.05$; Fig. 3D). Then, a predicted score formula was obtained: Ypred = 33.12–34.44 × Height1/BH1–0.65 × $PAHSDS_{THt}$. The ROC curve analysis showed that AUC for the predicted Y score was 0.870, and the optimal cut-off value of Ypred score was 1.02 (Fig. 3E). The equation was applied to predict the treatment effect in 23 patients who received GnRHa + rhGH treatment in the validation data set. A total of 14 out of 15 (93.3%) patients who achieved a ≥5-cm gain in height and 6 out of 8 patients (75%) who achieved a <5-cm gain in height were correctly identified (Fig. 3F).

Discussion

GnRHa has been used clinically for CPP treatment to restore genetic growth potential for over 40 years, because it prevents premature bone maturation and pubertal development. However, to the best of our knowledge, a large-scale multicenter study has not yet been performed on the long-term effects of GnRHa alone or

Table 3. Clinical Characteristics in the Training and Testing Datasets

	Training	Testing	P
CA1 (years)	8.4 ± 0.04	8.5 ± 0.15	0.656
Weight1 (kg)	30 ± 0.27	30 ± 0.78	0.829
Height1 (cm)	133.5 ± 0.32	135.05 ± 0.73	0.356
BMI1 (kg/m^2)	16.83 ± 0.11	16.81 ± 0.3	0.996
Father height (cm)	170 ± 0.26	170 ± 0.65	0.656
Mother height (cm)	158 ± 0.23	158 ± 0.53	0.998
BA1 (years)	10.7 ± 0.06	11 ± 0.16	0.356
PAH1 (cm)	151.65 ± 0.33	151.35 ± 0.8	0.996
CA2 (years)	10.5 ± 0.19	10.6 ± 0.13	0.356
BA2 (years)	12 ± 0.05	12.3 ± 0.1	0.128
Height2 (cm)	146.5 ± 0.35	148.4 ± 0.71	0.356
FAH (cm)	159.3 ± 0.23	158.5 ± 0.62	0.569
1st GV (cm)	5.9 ± 0.09	5.8 ± 0.27	0.702
2nd GV (cm)	5 ± 0.08	5.25 ± 0.21	0.996
Tht (cm)	157 ± 0.2	157.5 ± 0.44	0.865
GnRHa treatment (years)	2 ± 0.04	2 ± 0.12	0.478
FAH–PAH1 (cm)	7.91 ± 0.29	7.32 ± 0.94	0.478
BA1–CA1 (years)	2 ± 0.04	1.9 ± 0.11	0.998
$PAHDSD_{Tht}$	-0.98 ± 0.06	-1.2 ± 0.16	0.996
FAH–Tht (cm)	2.5 ± 0.23	2 ± 0.69	0.435
PAH2 (cm)	159.04 ± 0.32	157.83 ± 0.71	0.656
PAH2–PAH1 (cm)	6.7 ± 0.32	5.93 ± 0.79	0.570
Height1/BH1	0.93 ± 0	0.93 ± 0	0.996
HA1/BA1	0.85 ± 0	0.84 ± 0.01	0.998
HtSDS1	0.37 ± 0.04	0.53 ± 0.12	0.765
HtSDS2	0.13 ± 0.04	0.41 ± 0.13	0.478
HtSDS_BA2	-1.77 ± 0.05	-1.84 ± 0.13	0.996
HtSDS_BA1	-0.52 ± 0.04	-0.75 ± 0.11	0.356

Abbreviations: BA, bone age; BH, calculated-height based on bone age; BMI, body mass index; CA, chronological age; FAH, final adult height; FAH–PAH1, height gain; FAH–Tht, genetic height gain; GnRHa, gonadotropin-releasing hormone analogue; GH, growth hormone; GV, growth velocity; HA, calculated-age based on height; PAH, predictive adult height; SDS, standard deviation score; Tht, target height.

Table 4. Comparison of Subgroups Based on Treatment Order for GnRHa + rhGH Therapy

	Group 1	Group 2	*P*
rhGH treatment (years)	3 ± 0.11	1 ± 0.1	**<0.001**
BA1–CA1 (years)	1.2 ± 0.11	2.2 ± 0.08	**<0.001**
$PAHDSD_{Tht}$	-0.19 ± 0.16	-1.52 ± 0.14	**<0.001**
HtSDS_BA1	-1.49 ± 0.13	-2.09 ± 0.13	**<0.001**
Height1/BH1	0.94 ± 0.01	0.92 ± 0	**<0.001**
PAH1 (cm)	153.45 ± 0.86	149.08 ± 0.82	**<0.001**
HA1/BA1	0.86 ± 0.01	0.82 ± 0.01	**<0.001**
FAH-PAH1 (cm)	7.31 ± 0.69	11.25 ± 0.7	**<0.001**
PAH2-PAH1 (cm)	6.93 ± 0.89	10.36 ± 0.73	**0.003**
BA1 (years)	10 ± 0.23	11 ± 0.11	**0.025**
FAH–Tht (cm)	5 ± 0.6	3.25 ± 0.78	0.107
HtSDS_BA2	-0.25 ± 0.1	-0.49 ± 0.12	0.233
Mother height (cm)	156 ± 0.67	156.5 ± 0.72	0.340
FAH (cm)	160 ± 0.63	160 ± 0.79	0.340
PAH2 (cm)	160.73 ± 0.68	160.15 ± 0.81	0.340
CA1 (years)	8.8 ± 0.16	8.6 ± 0.1	0.467
CA2 (years)	11.3 ± 0.17	10.9 ± 0.13	0.467
Height2 (cm)	150.6 ± 1.04	149.1 ± 0.84	0.467
1st GV (cm)	6.7 ± 0.36	6 ± 0.32	0.467
HtSDS1	-0.1 ± 0.12	0.13 ± 0.14	0.467
Height1 (cm)	130.8 ± 1.05	132 ± 0.91	0.655
GnRHa treatment (years)	2.8 ± 0.12	2.1 ± 0.11	0.655
Tht (cm)	155.5 ± 0.59	156.25 ± 0.59	0.656
Weight1 (kg)	29 ± 0.83	30.25 ± 0.64	0.783
BA2 (years)	12 ± 0.17	12 ± 0.1	0.799
BMI1 (kg/m^2)	17.24 ± 0.32	17.13 ± 0.3	0.890
Father height (cm)	170 ± 0.77	168 ± 0.65	0.907
HtSDS2	0.24 ± 0.12	0.19 ± 0.14	0.924
2nd GV (cm)	6.2 ± 0.24	6.2 ± 0.23	0.970

Group 1 represents patients treated with GhRHa and rhGH initially. Group 2 represents patients who were treated with GhRHa alone initially and then GnRHa with rhGH after 6–12 months. *P* values or ROC values highlighted with bold indicated statistically differential variables of interest in this study. Abbreviations: BA, bone age; BH, calculated-height based on bone age; BMI, body mass index; CA, chronological age; FAH, final adult height; FAH–PAH1, height gain; FAH–Tht, genetic height gain; GnRHa, gonadotropin-releasing hormone analogue; GH, growth hormone; GV, growth velocity; HA, calculated-age based on height; PAH, predictive adult height; SDS, standard deviation score; Tht, target height.

GnRHa + rhGH on the final height of girls with CPP/EFP. Additionally, the effect of the GnRHa treatment on adult height has been controversial. The present study compared the long-term outcomes of GnRHa alone and GnRHa + rhGH treatment in 393 girls with CPP/EFP in the training data set and 55 girls in the validation data set from 12 medical centers. The adult height gains were 9.51 ± 0.53 cm, 8.07 ± 0.37 cm, and 6.44 ± 0.91 cm for the GnRHa + rhGH, GnRHa, and control groups, respectively, which were consistent with previous reports of height gains between 2.0 and 9.8 cm (26–29). For genetic height gains, the patients who received GnRHa treatment achieved an average increase of 2.0 ± 0.27 cm, and those who received GnRHa + rhGH treatment achieved an average increase of 4.0 ± 0.5 cm. In comparison, the girls in the control group (no treatment) reached their Tht, which appeared to be slightly lower than that in the 2 treatment groups. The parents of some patients from the control group refused treatment due to concerns about potential side effects and/or high cost; other patients who showed slow progression of CPP during the follow-up period were not recommended for treatment because they could achieve a normal adult height without medical intervention (30). Notably, the selection of these patients as controls may reduce the differences with the other 2 treatment groups in terms of FAH.

We also explored the main influencing factors for height gains and established regression models to assist clinicians in determining optimal treatment methods for CPP/EFP patients. Several factors have been reported to affect FAH–PAH1 or FAH in the girls with CPP/EFP. Patients who received GnRHa treatment at a younger age were found to be most likely to gain more height and achieve a higher adult height (26, 29). However, other studies did not show correlations between FAH and age or between height gain and age at initial treatment (31, 32). In addition to CA, other influencing factors associated with height gain or FAH include the degree of BA advancement, height SDS at the beginning or end of treatment, BMI, GV during treatment, and treatment duration (11, 13). Kauli et al. suggested that children with a BA <12 years are more likely to benefit from GnRHa treatment (33). In the present study, a panel of

Table 5. Comparison of Two Groups Who Did (Yes) or Did Not (No) Achieve a Satisfatory Outcome with a ≥5-cm Height Gain after GnRHa + rhGH Treatment

	No	Yes	*P*	FC	ROC
PAH2–PAH1 (cm)	2.12 ± 1.03	10.89 ± 0.57	**<0.001**	5.15	**0.87**
PAH1 (cm)	155.36 ± 1.07	150 ± 0.65	**<0.001**	0.97	**0.854**
Height1/BH1	0.96 ± 0.01	0.92 ± 0	**<0.001**	0.96	**0.856**
HtSDS_BA1	–1.06 ± 0.17	–1.97 ± 0.1	**<0.001**	1.86	**0.859**
$PAHDSD_{Tht}$	0.57 ± 0.23	–1.29 ± 0.12	**<0.001**	-2.25	**0.839**
HA1/BA1	0.89 ± 0.02	0.83 ± 0.01	**<0.001**	0.93	**0.833**
BA1–CA1 (years)	1 ± 0.17	2 ± 0.09	**<0.001**	2.00	0.798
BA1 (years)	9 ± 0.35	11 ± 0.11	**<0.001**	1.22	0.781
BA2 (years)	11 ± 0.27	12.3 ± 0.09	**0.008**	1.12	0.704
CA1 (years)	8 ± 0.26	8.8 ± 0.09	**0.016**	1.10	0.688
Weight1 (kg)	27 ± 1.05	30.5 ± 0.58	**0.024**	1.13	0.676
Height2 (cm)	147.2 ± 1.42	150.6 ± 0.72	**0.024**	1.02	0.675
CA2 (years)	10.8 ± 0.24	11.2 ± 0.12	**0.030**	1.04	0.667
BMI1 (kg/m^2)	15.97 ± 0.36	17.35 ± 0.26	**0.039**	1.09	0.66
HtSDS1	0.22 ± 0.15	–0.04 ± 0.11	0.133	-0.16	0.622
Height1 (cm)	130.3 ± 1.73	132 ± 0.72	0.169	1.01	0.611
FAH (cm)	157 ± 0.94	160.15 ± 0.6	0.216	1.02	0.6
1st GV (cm)	5.9 ± 0.36	6.7 ± 0.3	0.216	1.14	0.599
2nd GV (cm)	6.1 ± 0.27	6.2 ± 0.2	0.434	1.02	0.569
FAH-Tht (cm)	4 ± 1.14	4 ± 0.55	0.666	1.00	0.545
GnRHa treatment (years)	2.96 ± 0.14	2.25 ± 0.1	0.708	0.76	0.537
GH treatment[a] (years)	3 ± 0.16	2.9 ± 0.15	0.408	0.97	0.574
GH treatment[b] (years)	1.37 ± 0.71	1.14 ± 0.66	0.154	0.83	0.619
PAH2 (cm)	160.04 ± 0.83	160.52 ± 0.65	0.738	1.00	0.537
Father height (cm)	170 ± 0.8	168 ± 0.62	0.787	0.99	0.528
Mother height (cm)	156 ± 1.16	156 ± 0.54	0.792	1.00	0.525
Tht (cm)	156 ± 0.82	155.75 ± 0.49	0.979	1.00	0.502
HtSDS2	0.18 ± 0.15	0.24 ± 0.11	0.979	1.34	0.505
HtSDS_BA2	–0.45 ± 0.12	–0.43 ± 0.09	0.979	0.95	0.505

Numbers 1 and 2 indicate the time points before and after treatment, respectively. Supercase letters a and b indicate two different patterns of combined therapy: GnRHa and rhGH initially and rhGH after 6–12 months of GnRHa therapy, respectively. Values represent median ± standard error of the mean, ordered by *P* value. *P* values were adjusted for multiple comparisons using the false discovery rate method. *P* values or ROC values highlighted with bold indicated statistically differential variables of interest in this study.

Abbreviations: BA, bone age; BH, calculated-height based on bone age; BMI, body mass index; CA, chronological age; FAH, final adult height; FAH–PAH1, height gain; FAH–Tht, genetic height gain; GnRHa, gonadotropin-releasing hormone analogue; GH, growth hormone; GV, growth velocity; HA, calculated-age based on height; PAH, predictive adult height; SDS, standard deviation score; Tht, target height.

5 critical parameters (ie, $PAHSDS_{Tht}$, PAH1, Height1/BH1, HA1/BA1, and HtSDS_BA1) showed excellent performance in predicting a >5 cm height gain in patients with either GnRHa or GnRHa + rhGH treatment. Our results demonstrated that patients who had a more advanced BA, a larger difference between baseline PAH and Tht (ie, $PAHSDS_{Tht}$), or a smaller PAH, Height/BH, HA/BA, and more significant height loss (ie, HtSDS_BA) were more likely to benefit from the treatment.

Notably, our study identified that the patients who received the combined GnRHa and rhGH treatment gained more height, with a higher percentage of these patients achieving ≥5-cm height gains, compared with patients who received GnRHa treatment alone or no treatment. However, not all of the patients who received combined therapy achieved satisfactory height gains. Meanwhile, the cost for combination treatment was very expensive, and the treatment was not yet covered by health insurance in China. For example, a patient was diagnosed with CPP at 8.5 years old with a weight of 30 kg. If she could achieve a 5-cm net height gain after receiving GnRHa injection monthly and was checked up every 6 months for 2 years, the average cost of medication, lab tests, and transportation would have been approximately $1064 per centimeter of height gain. If she received the combination therapy for 2 years, including check ups every 3 months, then the cost would be $5968 per centimeter of height gain. Therefore, both patients and clinicians must weigh the potential height gain with the high cost of treatment. The Pediatric Endocrine Society and the European Society for Pediatric Endocrinology suggested that rhGH should not be recommended as routine treatment due to the lack of large-scale randomized clinical trials evaluating the efficacy of the combined treatment (11). In agreement with this recommendation, we do not recommend use of rhGH as a routine therapy, but our results indicate that the combined treatment may be beneficial for female CPP/EFP patients with low predicted heights and a greater degree of BA advancement.

This long-term retrospective follow-up study (up to 20 years) of 448 patients with CPP/EFP from multiple centers presented data for growth curves and FAHs, which were not presented in many previous studies. Our study has provided strong evidence for the long-term effects of GnRHa or GnRHa + rhGH treatment on adult heights in Chinese female children. Because this was a retrospective study without random treatment assignment, some patients who had mild CPP progression were included in the control group, and a prospective, randomized, and double-blind clinical trial should be conducted in the future. In addition, the current study included only girls with CPP/EFP, and an analysis of the long-term effects of GnRHa or GnRHa + rhGH treatment in boys with CPP/EFP is still needed.

Conclusions

The order of treatments in terms of their overall long-term therapeutic effects on adult height (height gains and percentage of individuals achieving ≥5-cm-gains) was GnRHa + rhGH > GnRHa > Control. However, the treatment outcomes showed some variation among individuals, meaning that not all patients achieved satisfactory height gains from either GnRHa monotherapy or GnRH + rhGH treatment. Five growth indices, $PAHSDS_{THt}$, PAH, Height/BH, HA/BA, and HtSDS_BA, were identified as important factors that may influence the final therapeutic outcome. Namely, the therapy may be beneficial to female CPP/EFP patients with a low PAH and a greater degree of BA. Finally, in agreement with the Pediatric Endocrinology Society and the European Society for Pediatric Endocrinology, the combination of GnRHa and rhGH is not recommended as a routine therapy due to the high cost and lack of large-scale randomized controlled trials demonstrating its safety and efficacy.

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ORIGINAL ARTICLE

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Long-term efficacy and safety of gonadotropin-releasing hormone analog treatment in children with idiopathic central precocious puberty: A systematic review and meta-analysis

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Abstract

Objective: To investigate the long-term efficacy and safety of gonadotropin-releasing hormone analog (GnRHa) treatment in children with idiopathic central precocious puberty (CPP).

Method: The protocol was registered with International Prospective Register of Systematic Reviews (CRD42018102792). PubMed, EMBASE and the Cochrane Library were searched for eligible comparative and single-arm studies.

Results: We identified a total of 98 studies that included 5475 individuals. The overall risk of bias of the eligible studies ranged from critical to moderate. The overall quality of evidence for each outcome ranged from very low to moderate. Evidence-based comparative studies showed that GnRHa treatment increase final adult height (FAH, cm; studies = 4, n = 242; mean difference [MD] = 4.83; 95% confidence interval [CI], 2.32 to 7.34; I^2 = 49%) and decrease body mass index (BMI, kg/m^2; studies = 3, n = 334; MD = −1.01; 95% CI, −1.64 to −0.37; I^2 = 0%) in girls with idiopathic CPP compared with no treatment. The incidence of polycystic ovary syndrome (PCOS) did not significantly differ with and without GnRHa treatment (studies = 3, n = 179; risk ratio = 1.21; 95% CI, 0.46 to 3.15; I^2 = 48%). The evidence for other long-term outcomes was very weak to deduce the effects of GnRHa treatment. Further, limited evidence is available on its effects in boys.

Conclusion: Compared with no treatment, evidence indicates that GnRHa treatment increase FAH and decrease BMI in girls with idiopathic CPP. GnRHa treatment did not evidently increase the risk of PCOS. However, evidence regarding other key long-term outcomes (such as infertility and malignant or metabolic diseases) was considered very weak to suggest the benefits or side effects of GnRHa treatment. Additional high-quality evidence is needed before firm conclusions can be drawn.

KEYWORDS
central precocious puberty, gonadotropin-releasing hormone analog, meta-analysis, systematic review

1 | INTRODUCTION

Central precocious puberty (CPP) results from premature activation of the hypothalamic–pituitary–gonadal axis (HPGA) and is commonly characterized by the early development of pubertal biochemical and physical features before 8 years of age for girls and 9 years of age for boys.[1,2] CPP is a rare condition and has an estimated overall prevalence of approximately 1 per 5000–10,000 children, with a five- to 10-fold higher incidence in girls than in boys.[3–6] CPP can be classified into idiopathic CPP (ICPP) and secondary CPP; the latter is including genetic causes(familial CPP, chromosomal abmormalities), central nervous system abnormalities (hypothalamic hamartomas, cysts, central nervous system granulomas, hydrocephalus, septo-optic hypoplasia), secondary to chronic exposure to sex steroid hormones (late treatment of simple virilizing congenital adrenal hyperplasia, following resection of tumours secreting sex steroid hormones, testotoxicosis, McCune-Albright syndrome) or endocrine disruptors..[7] ICPP is the most frequent form of CPP, accounting for approximately 90% cases of CPP in girls and 25%–60% in boys.[8–10] Although the exact mechanism underlying the development of ICPP is not well understood, several potential metabolic, genetic and epigenetic explanations have been considered.[11–15] CPP is associated with a lower final adult height (FAH), potential sexual abuse, increased risk of psychological disturbances and increased risk of developing cardiovascular diseases and reproductive tract cancers.[16,17]

Gonadotrophin-releasing hormone analog (GnRHa) is a synthetic peptide drug that is modelled based on human hypothalamic gonadotropin-releasing hormone (GnRH), which is designed to act on the anterior pituitary.[7] GnRHa interacts with the GnRH receptor and stimulates the synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the initial phase of administration ('flare up'). Sustained release of GnRHa suppresses the production of FSH and LH, which in turn suppress the production of sex hormones by the gonads.[7] Several pharmaceutical formulations of GnRHa, such as buserelin, histrelin, leuprorelin, triptorelin and goserelin, are available and used clinically.[18,19] The choice of drug and duration of treatment depend on the unique growth and development needs.[19,20] GnRHa has been a treatment choice for CPP since the mid-1980s, and its effects on HPGA suppression has been generally recognized.[19,21,22] However, the long-term efficacy and safety of GnRHa treatment remain unclear, and some studies have reported contradictory findings.[3]

Several studies have reported that GnRHa may improve FAH in girls with CPP[3,23–26]; this is particularly true if they were diagnosed before the age of 6 years and treated with GnRHa from Tanner stage 2–3 to chronological age 11–12 years and bone age 12–12.5 years.[27] However, the effects of GnRHa treatment are unknown in girls diagnosed between 6 and 8 years of age.[3] Regarding body mass index (BMI), several studies have found that GnRHa treatment did not lead to an increased risk of weight gain.[28–30] Among these studies, Corripio et al[30] reported an increase in weight based on BMI standard deviation score (SDS). In terms of its effect on the reproductive system, GnRHa treatment was not confirmed to be harmful to ovarian function or fertility.[31] There was no clear difference in the incidence of androgen excess or polycystic ovary syndrome (PCOS) between children with CPP treated with GnRHa and those in the healthy comparison group.[31–33] However, the effects of GnRHa treatment on bone mineral density (BMD), glucose and lipid metabolism, and psychological status remain unclear.[19,20,34,35] Therefore, we conducted this systematic review and meta-analysis to evaluate the long-term efficacy and safety of GnRHa treatment in children with ICPP.

2 | METHODS

2.1 | Registration

The protocol for this review was registered with the International Prospective Register of Systematic Reviews (CRD42018102792). This article has been prepared according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines.[36]

2.2 | Literature search and study selection

We searched PubMed, EMBASE and the Cochrane Library in November 2019, without placing any limitations on language or publication year. The detailed search strategies were developed by an information specialist and are presented in the Online Supplementary Materials. Two reviewers (LH and WW) independently screened the search results based on the following inclusion criteria: (a) prospective or retrospective comparative studies and single-arm studies; (b) participants with ICPP (as defined in the original study) with the onset of secondary sex characteristics before 8 years of age in girls and before 9 years of age in boys; and (c) studies that reported long-term (defined as a duration of ≥6 months) outcomes in participants who received GnRHa (any type of dosage regimen) compared with participants who received no treatment/placebo or GnRHa plus growth hormone (GH; any type of dosage regimen). We excluded studies that enrolled participants with negative results in the GnRH stimulation test and those with non-idiopathic CPP (such as isosexual precocious puberty, familial male-limited precocious puberty, or familial precocious puberty). Studies in which the participants were diagnosed with a brain tumour, trauma, infection, macrophage activation syndrome, congenital adrenal hyperplasia or GH deficiency were also excluded. Any disagreement during screening was resolved by discussion and, when necessary, with assistance from a third reviewer (YL).

2.3 | Outcome measures

The primary outcomes were as follows: FAH, which is considered the final adult stature of an individual when the bone age

is ≥15 years and/or the rate of growth in height is <1 cm/year in the past year (or within ≥2 years after a girl has experienced menarche); target height (TH), which is calculated using the height of the individual's parents (as defined in the original study); BMI and risk of being overweight/obese (being overweight is defined as a BMI above the 85th percentile or 25–29.9 kg/m^2 and obesity as a BMI above the 95th percentile or >30 kg/m^2); and the incidence of PCOS among girls and androgen excess among boys. PCOS is defined as a syndrome of ovarian dysfunction along with the cardinal features hyperandrogenism and polycystic ovary (PCO) morphology. The secondary outcomes included menstrual parameters (such as age at menarche and regularity of menstruation), growth velocity (GV), insulin-like growth factor 1 (IGF-1) level, BMD, glucose and lipid metabolism, insulin resistance parameters and psychological state.

2.4 | Data extraction and risk of bias assessment

Two reviewers (LH and WW) independently extracted qualitative and quantitative data using a standard data collection form. The risk of bias of the included studies was assessed according to the study design. Randomized controlled trials (RCTs) were assessed using the risk of bias tool from the Cochrane Handbook for Systematic Reviews of Interventions.[37] Non-randomized comparative studies were assessed using the 'Risk Of Bias In Non-randomized Studies - of Interventions' (ROBINS-I) tool.[38] Single-arm studies were rated as having a high risk of bias. Disagreements were resolved by discussion or by consulting with the third reviewer (XPL) when necessary.

2.5 | Statistical analysis

Separate analyses were performed based on single-arm studies and comparative studies. Regarding single-arm studies, qualitative and quantitative data are summarized to provide a comprehensive description of the phenotype of the participants and the primary reasons for treatment. Meta-analyses were performed for comparative studies. We estimated risk ratios (RRs) with 95% confidence intervals (CIs) for dichotomous outcomes, and mean differences (MDs) with 95% CIs for continuous outcomes. We employed a random-effects model for all meta-analyses using the R software,[39] and we performed separate analyses based on sex. The outcome data derived from comparative studies and single-arm studies were combined if there was no clinical and methodological heterogeneity present. To explore clinical heterogeneity, we planned to perform a priori subgroup analysis on primary outcomes based on the age of onset (<6 vs ≥6 years of age) as well as the type of GnRHa used. However, due to insufficient data and wide CIs for most treatment estimates, we did not perform additional sensitivity analyses. Statistical heterogeneity was estimated by I^2 and χ^2 statistics (substantial statistical heterogeneity was defined as I^2 ≥ 50% with a p-value of <.1 in the χ^2 test).

3 | RESULTS

3.1 | Search results

A total of 3515 hits were identified from searching the electronic databases. After assessing their eligibility, 98 studies with 105 references were included in this systematic review. The detailed reasons for exclusion are illustrated in the PRISMA study selection flow diagram (Figure 1).

3.2 | Included studies

The 98 included studies enrolled a total of 5475 participants (98.5% were girls). All references for the included studies are presented in the Supplementary Material. The sample size of the included studies ranged from 6 to 333. No RCTs were identified. Among the 98 included studies, 18 were randomized comparative studies (n = 1303) and the remaining 81 (n = 4172) were single-arm studies. Antoniazzi 2000 employed both comparative and single-arm study designs, thereby accounting for both non-randomized comparative and single-arm studies. The average age of CPP onset ranged from 4.5 to 8 years, and the average age of GnRHa treatment initiation ranged from 5 to 9.31 years. Various formulations of GnRHa were used in the included studies such as leuprorelin, triptorelin, buserelin, goserelin, deslorelin and histrelin. Thirteen studies (n = 1047) compared GnRHa treatment with no treatment, and six studies (n = 310) compared GnRHa treatment with GnRHa plus GH. The treatment duration ranged from 3 months to 5 years for all included studies. Additional study details are presented in Table S1.

3.3 | Quality assessment of included studies

Among the 18 comparative studies, none received low risk of bias scores across all domains. Based on ROBINS-I, 10 (55.6%) studies (Liang 2015, Poomthavorn 2011, Antoniazzi 2000, Shiasi Arani 2015, Colmenares 2014, Gyon 2015, Lanes 2004, Léger 2000, Magiakou 2010, and Pucarelli 2003) were judged to have an overall moderate risk of bias. Six (33.3%) studies (Faienza 2017, Swaiss 2017, Antoniazzi 2000, Bridges 1995, Jung 2014, and Yuan 2011) were judged to have a critical risk of bias because they selected participants based on either the intervention they received or the prediction of FAH. Two (11.1%) studies (Lazar 2014 and Lazar 2015) were judged to have a critical risk of bias with regards to the selection of participant domains as well as an overall critical risk of bias. Following our protocol that was established a priori, the 81 single-arm studies were regarded to have a high risk of bias. The summary of our assessment of risk of bias for

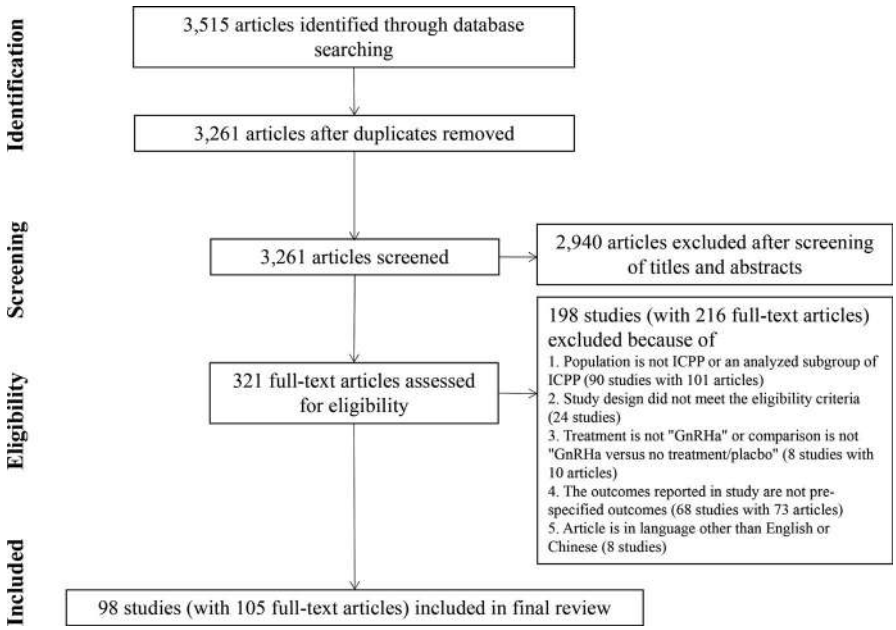

FIGURE 1 PRISMA study selection flow diagram

comparative studies is presented in Table S2. Following the consideration of inconsistency and indirectness, the overall quality of evidence for each outcome ranged from very low to moderate.

3.4 | Results of single-arm studies

Among the 81 single-arm studies (*n* = 5316), 47 included non-specified CPP patients (*n* = 2527) and 34 included ICPP patients (*n* = 2789). A total of 130 males and 5903 females were included in 80 studies, and one study (Comite 1986) did not report information on sex. The age of onset of ICPP ranged from 4.5 to 8 years, and the age at which the patients first received treatment ranged from 5 to 9.31 years. The included participants were treated with leuprolide in 26 studies, buserelin in one study, decapeptyl (including triptorelin) in 34 studies, histrelin in two studies, nafarelin in one study, non-specific GnRHa treatment in 10 studies, and a combination of these drugs in the remaining seven studies. The duration of treatment ranged from 3 months to 5 years (Table S1).

Among the 81 studies, 12 (Nabhan 2007, Borges 2015, Lin 2017, Lazar 2007, Antoniazzi 2000, Antoniazzi 2003, Baumann 2001, Carel 1999, Chen 2009, Gillis 2013, Kempers 2002, and Ying 2017) (*n* = 485) reported the average TH and FAH of girls (Table S4). In six studies (Borges 2015, Lin 2017, Lazar 2007, Carel 1999, Chen 2009, and Gillis 2013), the mean FAH of girls exceeded their TH (Table 1). One retrospective study (Lazar 2007) investigated the posttreatment height gain against the age of onset.

Four studies reported average BMI (*n* = 72), and eight studies reported average BMI-SDS (*n* = 300) in girls with ICPP after GnRHa treatment (Table S4).

The age at menarche was reported in 11 studies (*n* = 615), and all 11 studies reported the time to menarche after discontinuation of treatment. Further, 26 studies reported GV, 8 reported IGF-1 level, five reported BMD, 6 reported glucose and lipid indices, and three reported insulin resistance parameters. There were no remarkable findings in relation to the secondary outcomes (including GV, IGF-1 level, BMD, glucose and lipid indices, and insulin resistance parameters; Table S4, S6 and S7).

Five studies reported psychological outcomes, including cognitive functioning and emotional reactivity (Baumann 2001, Menk 2017, Schoelwer 2017, Wojniusz 2016, and Zheng 2008). Meta-analysis was not performed because the included studies used different scales. In general, GnRHa-treated CPP girls did not significantly differ in their cognitive or psychosocial functioning from age-matched controls.

Five single-arm studies evaluated boys with ICPP, and the descriptive results regarding FAH, BMI, GV and IGF-1 based on single-arm studies are presented in Table S5. The results were similar to those of girls, although the sample size of each study was very small (*n* = 8–13).

3.5 | Meta-analysis of comparative studies

All comparative studies included girls with ICPP (Table 2; Table S3).

3.6 | Adult height improvement

Five studies compared GnRHa treatment with no treatment (Faienza 2017, Swaiss 2017, Poomthavorn 2011, Antoniazzi 2000, and Lanes 2004). The results of these studies demonstrated that girls treated with GnRHa reached their TH, whereas most girls without treatment did not reach their TH. In addition, FAH (cm)

TABLE 1 Height (cm) reported in single-arm studies

Study ID	Sample size (*n*)	Sex	Characteristics at presentation/initiation of therapy						GnRHa	FAH, cm, Mean (SE)	TH, cm, Mean (SE)
			Pubertal stage	CA, years, Mean (SD)	BA, yeas, Mean (SD)	BA minus CA, years, Mean (SD)	Height SDS at CA, Mean (SD)	PAH, cm, Mean (SD)			
Antoniazzi 2000	71	Female	NR	7.0 (1.3)	9.8 (1.4)	BA/CA: 1.4 (0.3)	1.5 (1.7)	155.5 (7.0)	Triptorelin	158.4 (0.69)	161.5 (0.82)
Antoniazzi 2003	21	Female	Breast and pubic hair stage ≥2	7.28 (1.14)	8.82 (1.04)	NR	129.9 (6.8) cm	153.3 (4.8)	Leuprorelin	160.5 (1.18)	160.8 (1.37)
Baumann 2001	19	Female	NR	5.8 (2.2)	NR	NR	NR	NR	Buserelin or triptorelin	160.9 (1.62)	161.8 (1.33)
Borges 2015	54	Female	NR	NR	8.3 (2.3)	1.7 (1.1)	1.05 (1.03)	NR	Leuprorelin	162 (1.64)	158 (1.02)
Carel 1999	58	Female	NR	7.5 (1.3)	10.1 (1.5)	NR	2.4 (1.5)	156.4 (6.3)	Triptorelin	161.1 (0.77)	160.1 (0.58)
Chen 2009	26	Female	NR	7.8 (0.7)	11.2 (0.9)	NR	NR	151.5 (5.6)	Non-specific	158 (0.78)	155.3 (0.86)
Gillis 2013	23	Female	Breast stage ≥3 (16/23, 70%) Pubic hair stage ≥3 (4/23, 17%)	8.4 (0.3)	10.0 (0.3)	1.7 (0.2)	0.99 (0.26)	155.2 (1.9)	Triptorelin	157.9 (1.70)	160.8 (0.75)
Gillis 2013	11	Female	Breast stage ≥3 (10/11, 91%) Pubic hair stage ≥3 (4/11, 36%)	8.7 (0.3)	10.4 (0.4)	1.7 (0.3)	0.89 (0.26)	156.8 (2.6)	Histrelin	161.1 (2.00)	160.1 (0.97)
Lazar 2007	22	Female	tanner stage 2 to 3	6.4 (1.2)	NR	2.5 (0.8)	1.3 (0.8)	154.6 (6.6)	Triptorelin	162.8 (1.07)	159.3 (1.07)
Lazar 2007	38	Female	tanner stage 2 to 3	7.5 (0.6)	NR	2.5 (0.9)	1.2 (0.8)	153.7 (6.7)	Triptorelin	157.9 (0.83)	157.8 (0.84)
Lin 2017	43	Female	NR	8.76 (1.32)	NR	BA/CA: 1.20 (0.13)	135.91 (9.30) cm	NR	Leuprorelin	158.98 (0.83)	157.8 (0.53)
Nabhan 2009	26	Female	Breast development (Tanner) 2.6 (0.8)	7.2 (2.0)	10.1 (2.2)	2.9 (1.2)	NR	158.5 (6.8)	Leuprorelin	152.6 (1.27)	164 (1.12)
Kempers 2002	17	Female	NR	6.4	NR	NR	NR	NR	Triptorelin	166.2 (2.12)	168.8 (1.98)
Ying 2017	101	Female	NR	8.4 (0.84)	10.6 (0.53)	NR	137.7 (6.26) cm	153.1 (5.37)	Non-specific	157 (0.48)	157.7 (0.38)

Abbreviations: BA, bone age; CA, chronological age; FAH, final adult height; GnRHa, gonadotropin-releasing hormone analog; *n*, number; NR, not reported; PAH, predicted adult height; SDS, Standard deviation score; TH, target height.

TABLE 2 Characteristics of comparative studies—GnRHa vs no treatment

				Characteristics at presentation/initiation of GnRHa							
Study ID	**Sample size (*n*)**	**Sex**	**GnRHa**	**Pubertal stage**	**CA, years, Mean (SD)**	**BA, yeas, Mean (SD)**	**BA minus CA, years, Mean (SD)**	**Height SDS at CA, Mean (SD)**	**HV, , Mean (SD), SDS**	**PAH, cm, Mean (SD)**	**TH, cm, Mean (SD)**
Antoniazzi 2000	40	Female	Buserelin; triptorelin	Breast stage ≥2	7.7 (0.9)	10.2 (1.1)	NR	2.1 (0.5)	2.3 (0.5)	152.9 (6.6)	155.5 (5.3)
Arani 2015	110	Female	Triptorelin	NR	7.46 (1.02)	8.96 (1.66)	NR	0.62 (1)	NR	156.31 (7.61)	158.06 (4.75)
Bridges 1995	54	Female	Buserelin or goserelin	NR	NR	NR	NR	NR	NR	NR	NR
Colmenares 2014	37	Female	Triptorelin	Tanner stage 2 to 3	7.4 (1.3)	8.7 (2.1)	NR	2.8 (1.2)	1.6 (2.1)	SDS: 0.3 (2.3)	NR
Faienza 2017	50	Female	Triptorelin	Breast development (Tanner B2 or above)	7.0 (0.6)	10.1 (1.6)	NR	Height SDS/BA: −1.2 (0.8)	8.1 (1.5) cm/year	158.4 (3.6)	160.8 (4.7)
Lanes 2004	20	Female	Triptorelin or leuprorelin	NR	8.8 (1.4)	10.8 (1.3)	BA/CA: 1.2 (0.2)	NR	8.7 (1.1) cm/year	153.6 (1.3)	157.4 (4.5)
Lazar 2014	235	Female	Triptorelin	Breast Tanner stage 2 with or without sexual hair	8.1 (1.0)	NR	NR	NR	NR	NR	NR
Lazar 2015	142	Female	Triptorelin	Breast Tanner stage 2 with or without sexual hair	8.3 (0.9)	NR	NR	NR	NR	NR	NR
Léger 2000	26	Female	Triptorelin	Tanner stage 2 to 3	7.6 (1.1)	9.2 (1.9)	NR	NR	0.9 (1.2)	157.7 (6.6)	161.3 (4.7)
Magiakou 2010	47	Female	Triptorelin	Breast stage 3 pubic hair stage 2	Median 7.92	Median 10	NR	Median 0.66	NR	Median 151.53	NR
Poomthavorn 2011	58	Female	Triptorelin or leuprorelin	NR	8.5 (1.0)	11.1 (1.7)	2.7 (1.1)	1.5 (1.0)	9 cm/year	155.3 (6.7)	155.8 (4.1)
Swaiss 2017	50	Female	Triptorelin	NR	7.11 (0.7)	10.1 (1.6)	2.8 (1.3)	131.3 (9.2) cm	NR	158.5 (10.8)	163.9 (5.7)
Yuan 2011	134	Female	Non-specific	NR	8.16 (0.76)	9.78 (1.24)	NR	0.54 (0.96)	NR	SDS: −0.41 (1.38)	158.29 (3.81)

Abbreviations: BA, bone age, CA, chronological age, FAH, final adult height, GnRHa, gonadotropin-releasing hormone analog; HV, height velocity, *n*, number, NR, not reported, PAH, predicted adult height, SDS, Standard deviation score, TH, target height.

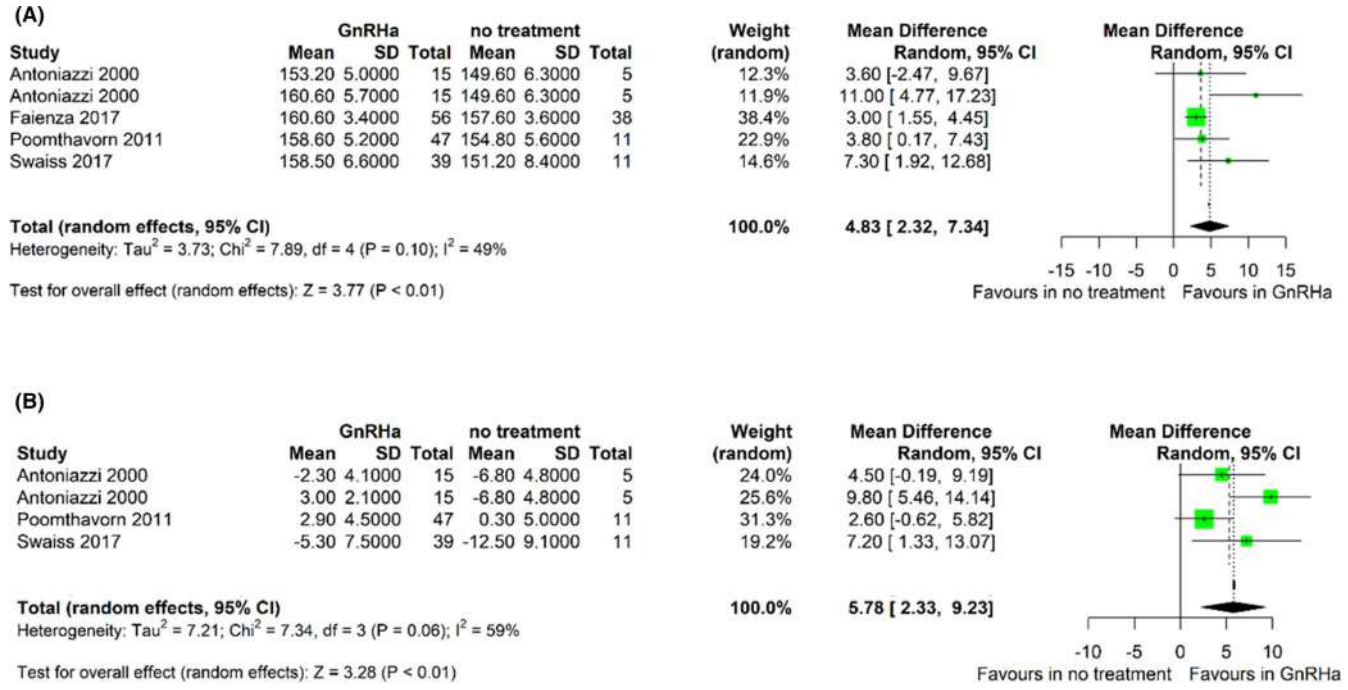

FIGURE 2 Forest plots of gonadotropin-releasing hormone analog treatment compared with no treatment for height outcomes [Colour figure can be viewed at wileyonlinelibrary.com]

was greater in girls treated with GnRHa than in those who were not treated (studies = 4, *n* = 242; MD = 4.83; 95% CI, 2.32 to 7.34; I^2 = 49%; Figure 2A). The participants of the study by Lanes 2004 (not included in the meta-analysis) were assigned to the intervention group based on their predicted height, and the girls with a predicted height of <155 cm received GnRHa treatment. The average FAH of the participants in the intervention group was not significantly different from that of the participants in the no-treatment group.

The difference between FAH and TH (FAH minus TH, cm) was larger in the GnRHa group than in the no-treatment group (studies = 3, *n* = 148; MD = 5.78; 95% CI, 2.33 to 9.23; I^2 = 59%; Figure 2B).

Five studies (Liang 2015, Gyon 2015, Bridges 1995, Jung 2014, and Pasquino 1996) were included in this comparison (Table S3). All girls in both GnRHa and GnRHa plus GH groups (Liang 2015, Gyon 2015, Jung 2014, and Pasquino 1996; *n* = 168) reached their TH. No significant difference was found in FAH or FAH minus TH after treatment between the groups.

3.7 | BMI

Six studies compared GnRHa treatment with no treatment and reported relevant outcomes on weight (Poomthavorn 2011, Shiasi Arani 2015, Colmenares 2014, Yuan 2011, Lazar 2015, and Arcari 2016). When participants reached their FAH, the pooled BMI level was lower in the GnRHa group treatment than in the no-treatment group (BMI (kg/m^2): studies = 3, *n* = 334; MD = −1.01; 95% CI, −1.64 to −0.37; I^2 = 0%; Figure 3A and BMI-SDS: studies = 3, *n* = 285; MD = −0.51; 95% CI, −0.75 to −0.28; I^2 = 13%; Figure 3B). The proportion of girls who were overweight or obese was similar between the two groups (studies = 3, *n* = 289; RR = 0.95; 95% CI, 0.66 to 1.38; I^2 = 58%; Figure 3C).

3.8 | Menarche and Menstrual irregularity

Four studies (Faienza 2017, Lazar 2014, Léger 2000, and Lazar 2015) reported that girls who received GnRHa treatment did not experience early menarche, and the average age at menarche ranged from 12 to 13 years. Results showed that girls who received GnRHa treatment experienced menarche later than those who did not (studies = 4, *n* = 458; MD = 1.18; 95% CI, 0.77 to 1.58; I^2 = 94%; Figure 4B). Two studies (Liang 2015 and Gyon 2015) (*n* = 125) showed that the GnRHa group experienced menarche at a younger age than the GnRHa plus GH group (MD = −0.35; 95% CI, −0.62 to −0.09; I^2 = 0%).

3.9 | Fertility and PCOS

Only one study (Lazar 2014) reported that the proportion of pregnancies was lower in the GnRHa (triptorelin) group than in the no-treatment group (*n* = 235; RR = 0.63; 95% CI, 0.50 to 0.80). However, among pregnant women (*n* = 108), the proportion requiring ovulation induction and/or in vitro fertilization was significantly lower in the GnRHa (triptorelin) group than in the no-treatment group

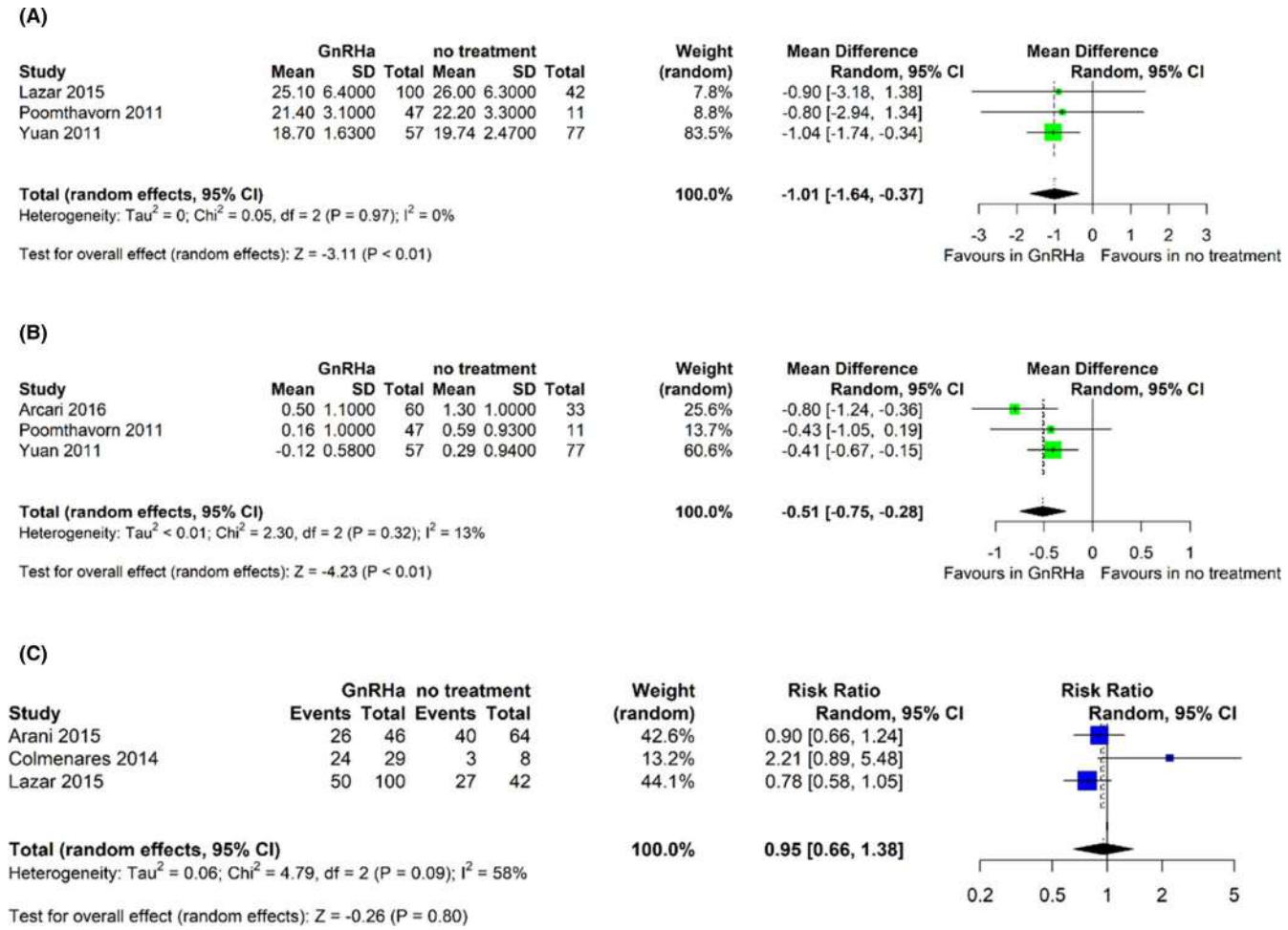

FIGURE 3 Forest plots of gonadotropin-releasing hormone analog treatment compared with no treatment for body mass index [Colour figure can be viewed at wileyonlinelibrary.com]

(RR = 0.33; 95% CI, 0.15 to 0.75). There was no clear difference in the incidence of early miscarriages or preeclampsia between the two groups (RR = 1.07; 95% CI, 0.32 to 3.58).

Individual studies showed more oligomenorrhoea and higher adrenal androgen levels (Faienza 2017) and reduced ovarian volume, LH:FSH ratio and Ferriman-Gallwey score (Magiakou 2010) in GnRHa-treated girls. However, overall the meta-analysis showed there was no significant difference between the GnRHa and no-treatment groups (studies = 3, *n* = 179; RR = 1.21; 95% CI, 0.46 to 3.15; I^2 = 48%) (Figure 4A). Bridges 1995 (*n* = 29) showed that there was no significant difference in the incidence of PCOS between GnRHa and GnRHa plus GH groups.

3.10 | Malignant diseases

Only one study (Lazar 2015; *n* = 142) reported only one patient had acute lymphoblastic leukaemia in the GnRHa group. No significant difference in the incidence of malignant diseases during young adulthood (around 30 years) between GnRHa and no GnRHa groups.

4 | DISCUSSION

In this systematic review, we aimed to determine the long-term efficacy and safety of GnRHa treatment in children with ICPP. Current evidence is mainly focused on girls with ICPP, and the overall quality of evidence for each studied outcome was found to range from very low to moderate. The main findings of our meta-analyses showed that compared with no treatment, GnRHa treatment improved the FAH of girls by increasing FAH by ≥2.32 cm. The average FAH of girls after GnRHa treatment was closer to their TH, if not more than their TH. The impact of GnRHa treatment on girls with different ages of CPP onset remains unclear due to insufficient evidence. In addition, the follow-up results (average follow-up: 3 years, range: 6 months to >20 years) revealed that GnRHa treatment might not lead to strong side effects such as risk of overweight/obesity and of PCOS, other malignancies, and metabolic syndromes. Although BMI levels were shown to increase slightly at the start of GnRHa treatment (particularly in girls with a normal baseline BMI status), girls who received treatment had lower BMI levels (reduced by ≥0.28 kg/m^2) than those who did not in adulthood. Furthermore, BMI levels did not significantly exceed the normal range, which indicated that

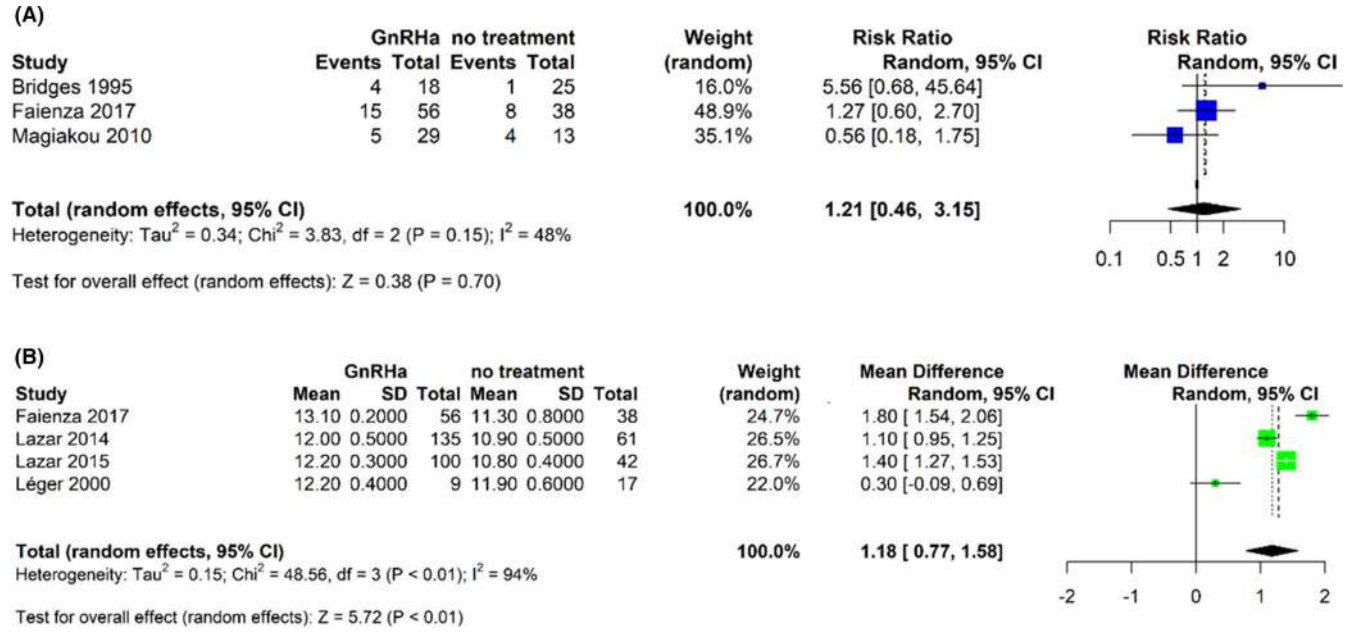

FIGURE 4 Forest plots of gonadotropin-releasing hormone analog treatment compared with no treatment for reproductive issues [Colour figure can be viewed at wileyonlinelibrary.com]

GnRHa treatment is less likely to increase the risk of overweight/obesity. GnRHa treatment may reduce the risk of early menstruation, and the average age at menarche was 1 year older than that in girls who did not receive treatment. There was no significant difference in the incidence of PCOS between the GnRHa and no-treatment groups. In addition, the prevalence of malignant diseases was low among women with former ICPP and in healthy controls. The evidence regarding fertility was obtained from only one study (Lazar 2014; $n = 235$); among the pregnant women with former ICPP, more women experienced spontaneous pregnancy in the GnRHa group than in the no-treatment group. Furthermore, GnRHa did not increase the risk of early miscarriage. Bone densitometric parameters were within the normal range for the respective sex and age groups before and after GnRHa treatment, and GnRHa treatment did not increase the risk of metabolic diseases such as diabetes and hyperlipidemia.

Early evidence has indicated that precocious puberty may lead to certain psychological or social problems, which are considered to bother parents and may affect the clinical treatment of CPP.[40] However, according to the results of the included studies, GnRHa treatment did not worsen the cognitive, psychological and social problems of children with ICPP and has the potential to reduce problems in some children, which was consistent with recent evidence.[41,42]

Several of the outcomes in the present review showed substantial heterogeneity ($I^2 > 50\%$) and one possible source may be the use of different drugs of GnRHa treatment. In addition, the small sample size may have contributed to the heterogeneity.

Our findings are somewhat consistent with those of a previous systematic review[3] that explored the long-term outcomes of GnRHa treatment in children with CPP. Guaraldi 2016[3] reported that GnRHa treatment appeared to improve FAH in girls with CPP and had no clear negative impact on BMI, risk of PCOS, or BMD. However, only the PubMed database was searched in this review. Another network meta-analysis is currently assessing the efficacy and safety of GnRHa treatment.[43] Although the present review did not predefine the exact population as Gu 2019,[43] a similar conclusion was reached.

4.1 | Strengths and limitations

The strengths of this systematic review include the creation of comprehensive search strategies to identify all relevant published studies and the use of sound methodology, which involved use of two reviewers to independently select studies and extract data. The latter strength minimizes the risk of performance bias in conducting the systematic review. However, our work also has some limitations. The results generated from pooling data of single-arm studies had a high level of statistical heterogeneity; thus, it was not possible to infer and draw meaningful conclusions from these meta-analyses. Furthermore, bias in the selection of participants is a major concern in several of the included comparative studies. The treatment regimen of GnRHa and the dropout rates were not well described in most of the comparative studies, which may exaggerate the magnitude of the estimated effects of meta-analysis. Treatment duration has been suggested as a contributing factor to improved FAH in the literature. However, all of the included comparative studies reported treatment duration of 2–5 years, which limited the conduction of subgroup analysis. Furthermore, a substantial level of statistical heterogeneity was evident for some outcomes such as the differences between FAH and TH and age at menarche. Therefore, our results should be interpreted with

caution. Moreover, the current evidence cannot be directly applied to boys with CPP due to the lack of data on this population. Further research, particularly large-scale RCTs (multicenter) or high-quality comparative studies with an adequate sample size, follow-up rate and duration, including both girls and boys, are required before firm conclusions can be drawn. In addition, it will be important to explore the main influencing factors on the long-term effects of GnRHa treatment.[44]

5 | CONCLUSION

Compared with no treatment, the current evidence indicates that GnRHa treatment improve the FAH of girls with ICPP, thus allowing them to meet or exceed their TH. GnRHa treatment also reduce the BMI levels of participants compared with BMI of those treated with placebo. Furthermore, GnRHa did not appear to increase the risk of PCOS. However, evidence regarding other predefined key outcomes, such as infertility, malignancy and metabolic diseases, is very weak to indicate the benefits or side effects of GnRHa treatment.

AUTHOR CONTRIBUTION STATEMENT

Xiaoping Luo: protocol development, manuscript review and revision. Yan Liang: study selection and data collection. Ling Hou: study selection and data collection. Wei Wu: study selection and data collection. Yanqin Ying: data analysis and partial review drafting. Feng Ye: partial review drafting.

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CONFLICT OF INTEREST

The authors have nothing to disclose.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article or in the data repositories listed in references.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Central Precocious Puberty – Management and Long-term Outcomes

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Summary

Central precocious puberty (CPP) results from premature re-activation of the gonadotropic axis. CPP is much more common in girls than in boys and is idiopathic in most cases. In boys, precocious puberty is more likely to be linked to hypothalamic lesions (≈40%). Recent studies have implicated the inactivation of *MKRN3* gene in 'idiopathic' CPP. Gonadotropin-releasing hormone agonists are the standard treatment for progressive CPP.

Keywords

Precocious puberty, treatment, outcome

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Precocious puberty (PP) is defined as the onset of clinical signs of puberty before age 8 years in girls and 9.5 years in boys. However, the onset of puberty may be subject to constitutional (genetics, ethnicity) environmental (secular trends, adoption, absence of the father and possible exposure to oestrogenic endocrine-disrupting chemicals) and nutritional (body mass index) variations,[1–3] with implications for the definition of precocious puberty. The signs of puberty include breast development in girls and testicular enlargement in boys (testicular volume greater than 4 ml or testicular length greater than 25 mm).

PP leads to the progressive development of secondary sexual characteristics, together with the development of pubic hair, and an acceleration of growth velocity and bone maturation, resulting in premature fusion of the growth plates, potentially responsible for adult height deficit. It may have consequences for growth and psychosocial development. PP may be caused by central or peripheral mechanisms.[1]

Central PP (CPP), which is much more common in girls than in boys,[4] results from premature reactivation of the hypothalamo-pituitary-gonadal axis and pulsatile gonadotropin-releasing hormone (GnRH) secretion, with a hormonal pattern similar to that of normal puberty. CPP may be due to hypothalamic lesions but is idiopathic in most cases, particularly in girls[1]. Recent studies have implicated the activation of Kisspeptin and its receptor and the inactivation of Makorin ring finger 3 (*MKRN3*) genes in "idiopathic" CPP[5–6]. *MKRN3* is an imprinted gene located on the long arm of chromosome 15, with a potentially inhibitory effect on GnRH secretion. *MKRN3* gene defects have been identified as a cause of paternally transmitted familial CPP, but such defects do not underlie maternally transmitted CPP and are rarely involved in sporadic forms.

Premature sexual maturation is a frequent cause for referral. Clinical evaluation is generally sufficient to reassure the patients and their families, but premature sexual maturation may reveal severe conditions, and thorough evaluation is therefore required to identify its cause and potential for progression so that appropriate treatment can be proposed. If a non-progressive form of PP is suspected, it is recommended to wait a few months and then to reassess the patient, to avoid unnecessary treatment. The heterogeneity of CPP, in terms of its clinical presentation and definition, can be accounted for by the gradual nature of the transition to puberty. Indeed, in many girls with idiopathic CPP, puberty progresses very slowly and may even be regressive, resulting in an unchanged predicted final stature and a normal adult height close to parental target height.[7,8] Therapeutic abstention is appropriate in most cases, because puberty progresses slowly, with the menarche occurring, on average, 5.5 years after the onset of clinical signs of puberty and normal adult height relative to parental target height being reached. However, in some cases (about one-third of subjects), final stature prognosis may worsen during the progression of puberty, in parallel with the emergence of evident biological signs of oestrogenisation. Clinical assessments should therefore be systematically carried out in children for whom no treatment is justified at the initial assessment, at least until the age of 9 years, to identify girls subsequently requiring treatment to block precocious puberty.[1,8,9]

In both sexes, the central cause of precocious puberty is demonstrated by an increase in pituitary gonadotropin levels. Indeed, the mechanism of precocious puberty involves premature activation of the hypothalamic-pituitary-gonadal axis, with the initiation of pulsatile luteinising hormone (LH) secretion and an increase in the secretion of pituitary gonadotropins, both in basal conditions and after stimulation

with LH-releasing hormone (LHRH). Before the onset of puberty, the follicle-stimulating hormone (FSH) peak is greater than the LH surge. During and after puberty, the LH surge predominates. In cases of central precocious puberty, basal serum LH concentration is usually ≥0.3 IU/l and LH concentration after stimulation is ≥5 IU/l.[1,10] Oestrogenic impregnation is assessed on pelvic ultrasound scans, which may show an oestrogenised appearance of the uterus (length ≥35 mm).[11] Central magnetic resonance imaging focusing on the hypothalamic region is required in most cases of CPP.[1,12]

GnRH agonists (GnRHa) are the standard treatment for progressive CPP.[9,12] Such treatment results in the regression or stabilisation of pubertal symptoms and decreases in growth velocity and bone age advancement. The factors affecting height outcome include initial patient characteristics and treatment duration. After the cessation of GnRHa therapy, generally at an age of about 11 years, biological and clinical signs of puberty reappear within months, with most girls achieving menarche, with menstrual ovulation cycles, during the following year.[9,13,14] PP associated with the presence of a hypothalamic lesion may progress to gonadotropin deficiency. The available data indicate that long-term GnRHa treatment does not seem to cause or aggravate obesity or have repercussions for body composition, bone mineral density, fertility and metabolic or cancer comorbidities. General health status is not different from that of women with normal puberty.[14,15] However, data concerning psychosocial outcomes are scarce,[15,16] and studies of this aspect are required. ■

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Influence of Gonadotropin Hormone Releasing Hormone Agonists on Interhemispheric Functional Connectivity in Girls With Idiopathic Central Precocious Puberty

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Purpose: The pubertal growth suppressive effects of gonadotropin hormone releasing hormone agonists (GnRHa) are well-known, although it remains unclear if long-term GnRHa treatment influences the brain function of treated children. The present study investigated the differences in the homotopic resting-state functional connectivity patterns in girls with idiopathic central precocious puberty (ICPP) with and without GnRHa treatment using voxel-mirrored homotopic connectivity (VMHC).

Methods: Eighteen girls with ICPP who underwent 12 months of GnRHa treatment, 40 treatment-naïve girls with ICPP, and 19 age-matched girls with premature thelarche underwent resting-state functional magnetic resonance imaging using a 3T MRI. VMHC method was performed to explore the differences in the resting-state interhemispheric functional connectivity. The levels of serum pubertal hormones, including luteinizing hormone (LH), follicular-stimulating hormone, and estradiol, were assessed. Correlation analyses among the results of clinical laboratory examinations, neuropsychological scales, and VMHC values of different brain regions were performed with the data of the GnRHa treated group.

Results: Significant decreases in VMHC of the lingual, calcarine, superior temporal, and middle frontal gyri were identified in the untreated group, compared with the control group. Medicated patients showed decreased VMHC in the superior temporal gyrus, when compared with the controls. Compared to the unmedicated group, the medicated group showed a significant increase in VMHC in the calcarine and middle occipital gyrus. Moreover, a positive correlation was observed between basal LH levels and VMHC of the middle occipital gyrus in medicated patients.

Conclusions: These findings indicate that long-term treatment with GnRHa was associated with increased interhemispheric functional connectivity within several areas

responsible for memory and visual process in patients with ICPP. Higher interhemispheric functional connectivity in the middle occipital gyrus was related to higher basal LH production in the girls who underwent treatment. The present study adds to the growing body of research associated with the effects of GnRHa on brain function.

Keywords: gonadotropin-releasing hormone agonists, idiopathic central precocious puberty, hypothalamic-pituitary-gonadal (HPG) axis, luteinizing hormone, functional magnetic resonance imaging, voxel-mirrored homotopic connectivity

INTRODUCTION

Central precocious puberty (CPP) is defined as the occurrence of secondary sexual characteristics before 8 years of age in girls, due to the premature activation of the hypothalamic-pituitary-gonadal (HPG) axis (1–3). The term "idiopathic CPP" (ICPP) refers to cases without central nervous system lesions, such as hypothalamic tumors or other detected lesions. ICPP accounts for the majority of cases of CPP (4). Gonadotropin releasing hormone analogs (GnRHa) are most commonly and effectively used for the management of CPP. Long-acting GnRHa function by decreasing the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and consequently, the production of sex steroids. GnRHa have wide applications in the management of several gynecological diseases and for treating gender dysphoria (5).

It is thought that puberty represents a second organizational period in brain development, which features dramatic fluctuations in pubertal hormone levels (6, 7). Recent study has reported that alterations in the amplitude of low-frequency fluctuation in the superior temporal gyrus and right superior frontal gyrus in girls with early HPG-axis activation, and higher prolactin (PRL) levels were associated with increased activity in the right superior frontal gyrus (8). Earlier studies have revealed the effects of the premature activation of the HPG axis on brain structure and function (8, 9). However, the influence of GnRHa on the underlying processing of brain networks is still poorly understood, when used as standard hormone suppression treatment for CPP.

To date, only a few studies have explored changes in brain function following GnRHa treatment in humans, but these studies have mainly focused on young women and adolescents with gender dysphoria. For instance, one study reported that cerebral blood flow decreased in the dorsolateral prefrontal cortex and inferior parietal and temporal lobes in young women after GnRHa treatment (10). However, both resting-state fMRI (RS-fMRI) and diffusion tensor imaging studies in adolescents with gender dysphoria (irrespective of whether they were transmen or transwomen) failed to find a significant effect of GnRHa on brain development (11, 12). Prospective trials with pubertal girls are needed to elucidate the effects of GnRHa on brain function development.

Although GnRHa therapy is clearly beneficial in stabilizing pubertal symptoms and improving adult height in patients with CPP (2, 13), studies with young women suggest that GnRHa may have negative effects on memory (14, 15), eliciting concern regarding the potential psychosocial outcomes of GnRHa administration during adolescence (16). To date, evidence regarding the deleterious effects of GnRHa on the cognitive, behavioral, and social functions in patients with CPP has not been found (17, 18).

RS-fMRI is a non-invasive technique for investigating intrinsic brain activation patterns, without a task-related bias. Voxel-mirrored homotopic connectivity (VMHC) is an effective method to evaluate interhemispheric functional connectivity (FC) using fMRI data. Studies on normal development using homotopic RSFC showed region-specific developmental trajectories across the lifespan of the individual (19). Therefore, VMHC is an appropriate and relatively novel approach for exploring the effects of GnRHa on brain function development in girls with CPP.

To best of our knowledge, no study has examined the effects of GnRHa on cognition and brain function with regard to the interhemispheric RSFC changes in patients with CPP. This study examined interhemispheric RSFC in ICPP girls who had not received GnRHa therapy (unmedicated group), those who had been treated with GnRHa for about 12 months (medicated group), and age-matched girls with premature thelarche (control group). The aim of the present study was to investigate changes in VMHC following GnRHa treatment for 12 months in girls with ICPP. We also explored the endocrine mechanism by which GnRHa affects brain function. There is no hypothesis regarding the effect of GnRHa on brain functional connectivity, as it has not been investigated in patients with CPP.

METHODS

Participants

A total of 77 participants were enrolled in this study. Fifty-eight girls diagnosed with ICPP and 19 girls diagnosed with premature thelarche, before the age of 8 years (13 patients had been first diagnosed at other hospitals), were recruited from the Child Healthcare Department of the Second Affiliated Hospital of Wenzhou Medical University. The diagnostic criteria for ICPP included: (1) Tanner stage of breast development $\geq$ 2, (2) the skeletal age of patients was greater than their chronologic age, (3) normal brain and pituitary MRI, and (4) peak LH levels $\geq$ 5IU/L

Abbreviations: GnRHa, gonadotropin releasing hormone analog; CPP, central precocious puberty; VMHC, voxel-mirrored homotopic connectivity; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; E2, estradiol; HPG, hypothalamic pituitary gonadal; FC, functional connectivity; CBCL, parent-rated Child Behavior Checklist; WISC-CR, Wechsler Abbreviated Scale of Intelligence for Children-Chinese Revised; STG, Superior temporal gyrus; MFG, Middle frontal gyrus; MOG, Middle occipital gyrus.

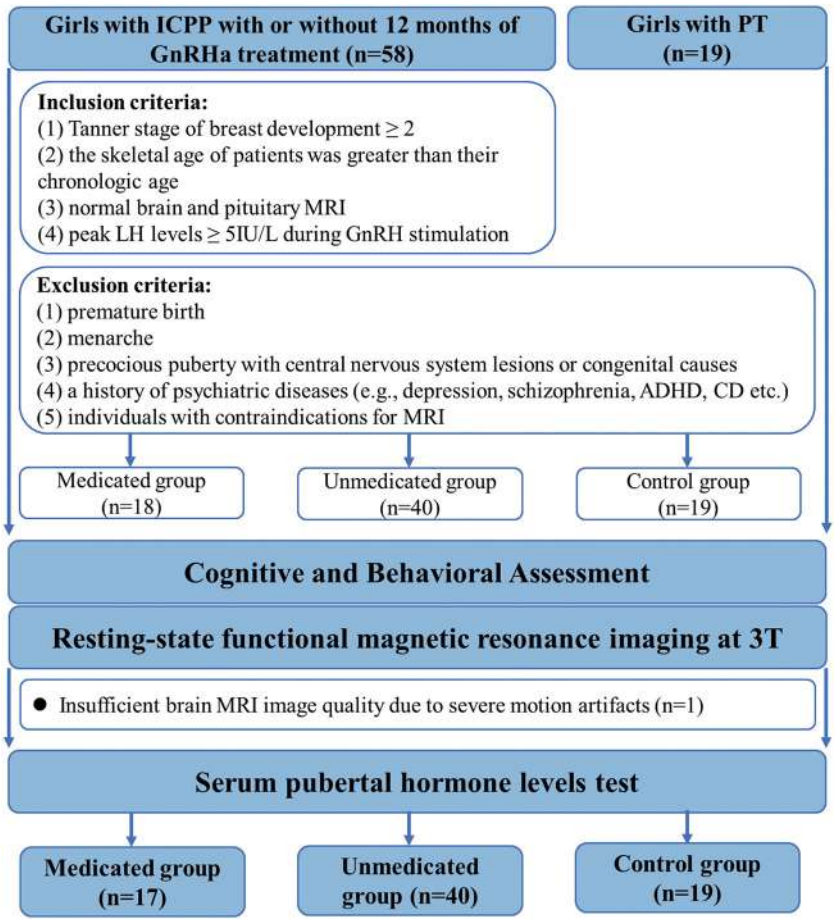

FIGURE 1 | Flowchart for selecting the study population. GnRHa, gonadotropin releasing hormone analogs; LH, luteinizing hormone.

during gonadotropin hormone releasing hormone (GnRH) stimulation (2). The exclusion criteria for all groups included: (1) premature birth, (2) menarche, (3) precocious puberty with central nervous system lesions or congenital causes, (4) a history of psychiatric diseases [e.g., depression, schizophrenia, attention deficit hyperactivity disorder (ADHD), conduct disorder (CD) etc.] and (5) individuals with contraindications for MRI (**Figure 1**).

There is consensus that not all patients with CPP need medical intervention. Medical treatment is indicated when the natural course of the disease is likely to result in adult short stature, early menarche, or severe psychological problems in the affected children; specific treatment to postpone menarche or to reduce psychological stress is not recommended (20, 21). Eventually, in accordance with our departmental policy, the prospect of GnRHa therapy was discussed with the patients and their parents, especially with regard to the risk of adult short stature. Based on acceptance or refusal of GnRHa therapy, all patients were categorized into treated and untreated groups.

Eighteen patients who received GnRHa therapy were categorized into the treated group. The medicated group received triptorelin (3.75 mg) or leuprorelin every 4 weeks, subcutaneously or intramuscularly (mean duration ± standard deviation, 12.71 ± 2.91 months). Treatment regimens were adjusted according to each patient's condition. During the treatment course, the dosage was halved for 2 patients and the type of medication was changed for 2 others.

Forty girls with ICPP who did not receive GnRHa therapy comprised the ICPP group. One reason for refusal of GnRH treatment was the potential side effects such as local erythema, hyperlipidemia, central obesity, temporary vaginal bleeding, osteoporosis, and infertility (22, 23) Another reason for refusal was that the expected adult height was almost equal to the predicted adult height.

Nineteen girls with premature thelarche comprised the control group. Premature thelarche was diagnosed on the basis of isolated breast development and a peak LH level <5 IU/L on GnRH stimulation test (20). Because girls with advanced pubertal stage present increased prevalence of behavioral problems compared to those with normal pubertal stage, we selected girls with isolated, premature thelarche as our "controls," thereby controlling for the effect of early development of secondary sexual characteristics (7) and isolating the effects of pubertal suppression.

The duration of illness was defined as the number of months between the scan and first diagnosis, which was based on medical records maintained at our hospital or other (previous) hospitals. Radiographs of the left hand and wrist were used to evaluate the skeletal age. The pubertal stage (Tanner scale) of all participants was assessed by inspection and palpation by a pediatric endocrinologist. All patients were pre-menarcheal and were classified as at least Tanner stage 2 of breast development.

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University. We obtained informed written consent from the appointed proxies for all patients. After initial selection, o participant from the medicated group was excluded from further MRI data analysis, due to the presence of several movement artifacts on the image.

Cognitive and Behavioral Assessment

The full-scale intelligence quotient (IQ) was assessed using the Wechsler Abbreviated Scale of Intelligence for Chinese Children-Revised (WISCC-R). Behavioral assessment was performed using the parent-reported Child Behavior Checklist (CBCL) (24).

Hormonal Assays and Gonadotropin Hormone Releasing Hormone Stimulation Test

The GnRH stimulation tests were performed at 8:00 a.m. for all patients. Blood samples were evaluated using electrochemiluminescence immunoassay. Serum LH and FSH levels were measured at 0, 30, and 60 min. E2 was measured at 0 and 30 min after gonarelin injection (2.5 μg/kg, maximum dose: 100 μg). The maximum measured levels of serum LH, FSH, and E2 were regarded as the peak LH, FSH, and E2 levels, respectively. Hormone levels measured at 0 min were treated as baselines. Hormone concentrations were expressed according to minimum detection values (assay sensitivity = 0.2 IU/L). A peak LH level $\geq$ 5 IU/L after GnRH stimulation indicated HPG-axis stimulation (25). A suppressed luteinizing hormone after GnRHa administration (peak LH $\leq$ 3 IU/L) indicates that the treatment was having the desired effect (23).

Magnetic Resonance Imaging Acquisition

MRI was performed 1–2 days prior to the GnRH stimulation examination. Structural and functional MRI was performed using a 3.0 T GE Healthcare-Signa HDxt 3T MRI scanner (General Electric, Milwaukee) with an eight-channel phase array head coil. We used noise-reducing headphones and sponge pads to minimize head movement. The high-resolution 3-dimensional structural image data was collected using the whole brain spoiled gradient echo sequence [repetition time = 8.88 ms; echo time = 4.02 ms; inversion time = 900 ms; flip angle = 15°; matrix size =256 × 256; field-of-view = 256 × 256 mm; slice thickness =1 mm, 160 slices]. Participants were instructed to lie quietly and keep their eyes closed for the resting-state functional scan. The images were acquired with a gradient echo-planar imaging sequence (repetition time = 2,500 ms, echo time = 40 ms, flip angle = 90, slice thickness = 4 mm, slice gap = 4 mm, matrix size = 64 × 64, and field-of-view = 256 × 256, 34 slices). The scan time was 6 min.

Functional Magnetic Resonance Imaging Image Preprocessing

All preprocessing was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and Data Processing Assistant for RS-fMRI (http://www.restfmri.net) for the RS-fMRI data. The first 10 frames were discarded to account for hemodynamic delay. Preprocessing comprised slice-timing correction for interleaved acquisitions, head motion correction (head motion was <2.5-mm translation in the x, y, or z directions, or <2.5° of angular rotation along the three axes), spatial registration of the high resolution structural T1 images for each participant, and adjustment of the time series of the images by removing the linear drift. Normalized images were smoothed at 6-mm full width, at half maximum of the isotropic Gaussian kernel. Several nuisance signals, including the 6 motion parameters, white matter (WM), and cerebrospinal fluid (CSF) were regressed for temporal correction. Subsequently, the RS images were bandpass filtered between 0.01 and 0.08 Hz to reduce low-frequency drift, and physiological high-frequency respiratory and cardiac noise. Segmentation of the anatomical image into gray matter, WM, and CSF, and normalization of the anatomical and functional images to the standard Montreal Neurological Institute (MNI) brain space (voxel size = 3 mm^3) were performed.

Voxel-Mirrored Homotopic Connectivity Analysis

We averaged spatially normalized T1 images of the 76 participants to generate a mean normalized T1 image, to obtain a left-right hemisphere symmetric brain template and its left-right mirror version was used to make the template (26). Subsequently, each individual T1 image was normalized to standard MNI space using the symmetrical template. Pearson's correlation was performed for the time-series of each voxel and that of its symmetrical interhemispheric counterpart, resulting in individual VMHC maps. The VMHC map from each individual participant was transformed into a Z-values map, using Fisher's transformation for group level analysis.

Statistical Analysis

The comparison analysis for demographic, psychometric measures, and basal pubertal hormone levels was performed using a one-way analysis of variance (ANOVA). The resulting significant measures underwent pairwise *t*-tests (2-tailed) between groups, with the exception of comparisons between the patient groups. The Holm–Bonferroni method was used to correct for multiple comparisons on *post-hoc* pairwise tests. Two-sample *t*-tests were used to compare the peak hormone levels and duration of illness between the medicated and unmedicated groups. Given that the distribution of hormonal data was negatively skewed (Kolmogorov–Smirnov test, $P < 0.05$), the hormonal data underwent logarithmic transformations to improve its normality. The logarithmic transformations of the hormone levels were used in subsequent analyses.

Individual VMHC maps were entered into a voxel-wise one-way ANOVA, to examine the differences in the interhemispheric FC among the groups. Independent samples *t*-tests were performed for significant ANOVA measures, to evaluate pair-wise differences among the three groups. Significant differences in VMHC among the three groups were defined at the voxel level as $P < 0.001$. The cluster threshold was set at > 25 voxels and $P < 0.05$ corrected by AlphaSim multiple comparison correction. Partial correlation analysis was conducted to assess the relationships between the VMHC values (that had significant difference in the structural volume among the different groups) and clinical variables that were different among the different groups by controlling for age during screening. Moreover, a correlation analysis was performed between VMHC and age in each group, to further exclude age from the results. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 25.

TABLE 1 | Demographics and neuropsychological data.

	Study group[a]		
		Patient group[b]	
	Control ($n = 19$)	Medicated ($n = 17$)	Unmedicated ($n = 40$)
Demographic and clinical data			
Age, y	9.9 (0.5)	10.1 (0.6)	10.0 (0.5)
Height, cm	136.8 (5.1)	140.9 (6.0)	140.9 (5.0)*
Weight, kg	31.1 (5.4)	34.7 (5.1)	35.6 (5.6)*
BMI	16.5 (2.1)	17.4 (1.8)	17.9 (2.3)*
IQ (WASI-CR)	105.6 (11.4)	104.8 (10.8)	106.9 (13.0)
CBCL (total)	14.1 (14.0)	10.3 (11.3)	12.1 (13.6)
Pubertal hormone data			
Basal LH, IU/L	0.35 (0.48)	0.41 (0.62)	2.22 (1.71)**
Basal FSH, IU/L	3.45 (1.19)	1.63 (1.27)	5.97 (6.07)**
Basal E2, IU/L	35.09 (17.19)	21.52 (10.17)	52.80 (27.10)**
Peak LH, IU/L	NA	3.01 (7.67)	27.63 (14.32)**
Peak FSH, IU/L	NA	3.73 (4.63)	13.91 (5.93)**
Peak E2, IU/L	NA	23.88 (10.20)	55.21 (26.53)**
Duration of illness, month[c]	NA	37.48 (9.88)	38.47 (9.21)
GnRHa use			
Duration, month	NA	12.7 (2.9)	NA
Dose, mg	NA	46.2 (10.8)	NA
GnRHa type at mri scan, No. of participants			
Triptorelin	NA	3	NA
Leuprorelin	NA	14	NA

CBCL, the parent-rated Child Behavior Checklist; E2, estradiol; FSH, follicle-stimulating hormone; GnRHa, gonadotropin releasing hormone analogs; LH, luteinizing hormone; NA, not applicable; WISC-CR, Wechsler Abbreviated Scale of Intelligence for Children-Chinese Revised.

[a] *All data is expressed as mean (SD).*

[b] *Medicated means girls with ICPP who had been treated for a number of months with gonadotropin releasing hormone analogs; unmedicated means girls with ICPP who were not were received GnRH analog treatment at the time of assessment.*

[c] *Duration of illness is defined as the number of months between the time of scan and first diagnosis, which was based on medical records from our hospital or other previous hospitals.*

**P < 0.05; **P < 0.005.*

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RESULTS

Clinical Characteristics and Levels of Hypothalamic-Pituitary-Gonadal Axis Hormones

The demographic characteristics of the participants and clinical information are shown in **Table 1**. Significant group differences were found in height [$F_{(2,76)} = 4.387$, $P = 0.016$] and weight [$F_{(2,76)} = 4.395$, $P = 0.016$]. *Post-hoc* independent-sample *t*-tests demonstrated that participants in the medicated [$t_{36} = 4.109$, $P = 0.021$] and unmedicated [$t_{(59)} = 4.111$, $P = 0.006$] groups were significantly taller, compared to the control group. There were no differences in height between the medicated and unmedicated groups [$t_{47} = 0.001$, $P = 0.999$]. The difference between the medicated and control groups showed a trend toward higher weight [$t_{36} = 3.571$, $P = 0.053$]. Participants in the unmedicated group weighed significantly more than the controls [$t_{59} = 4.457$, $P = 0.004$].

Significant group differences in basal LH levels [$F_{(2,76)} = 18.800$, $P < 0.001$], basal FSH levels [$F_{(2,76)} = 6.038$, $P = 0.004$], and basal E2 levels [$F_{(2,76)} = 12.985$, $P < 0.001$] were observed. *Post-hoc* independent-sample *t*-tests demonstrated that the medicated and control groups showed significantly lower basal LH levels compared with those of the unmedicated group (both $P < 0.001$). Basal LH levels did not differ between the controls and medicated ($P > 0.05$). The medicated ($P < 0.001$) and control groups ($P = 0.016$) showed significantly lower basal E2 levels compared with those of the unmedicated group. Basal

TABLE 2 | Regions of group differences in voxel-mirrored homotopic connectivity.

Region[a]	Brodmann area	*T*-value	Cluster size, mm^3	MNI coordinates		
				x	y	z
Unmedicated patient group < control group						
Lingual	18	−4.3620	41	±6	−66	−3
Calcarine	18	−4.4243	78	±18	−93	9
STG	13	−3.6222	24	±57	−39	15
MFG	10	−4.0389	27	±33	45	15
Medicated patient group < control group						
STG	42	−5.1543	56	±63	−18	9
Unmedicated patient group < medicated patient group						
Calcarine	23	−5.6288	175	±9	−78	9
MOG	19	−3.9338	40	±30	−81	21

STG, Superior temporal gyrus; MFG, Middle frontal gyrus; MOG, Middle occipital gyrus; MNI: Montreal Neurological Institute.

[a] *The control group compared with the unmedicated patient group, the control group compared with the medicated patient group, the medicated group compared with the unmedicated patient group.*

E2 levels did not differ between the control and medicated groups ($P > 0.05$). The medicated group ($P = 0.004$) showed significantly lower basal FSH levels compared with those of the unmedicated group. Basal FSH levels did not differ between the control and medicated groups, as well as the control and unmedicated groups (both $P > 0.05$). Moreover, two-sample t-tests showed that the medicated showed a significantly lower peak LH level, peak FSH level, and peak E2 level compared with the unmedicated group (both $P < 0.001$). However, no significant group differences were found in age [$F_{(2,76)} = 1.099$, $P = 0.339$], body-mass index [$F_{(2,76)} = 2.450$, $P = 0.093$], PRL [$F_{(2,76)} = 0.680$, $P = 0.712$], or cortisol [$F_{(2,76)} = 0.801$, $P = 0.670$].

Interhemispheric Connectivity Differences

The between-group interhemispheric connectivity comparisons are shown in **Table 2** and **Figure 2**. First, comparison between the control and untreated groups revealed significant reductions in VMHC in the unmedicated group within the lingual gyrus, calcarine gyrus, superior temporal gyrus, and middle frontal gyrus (MFG). No areas of increased VMHC were observed in the unmedicated group, when compared with the control group. Second, compared to the control group, the medicated group showed significant decrease in VMHC in the superior temporal gyrus. VMHC was not higher in any of the regions in the medicated group, when compared to that in the control group. Finally, the medicated group showed a significant increase in VMHC in the calcarine and middle occipital gyrus (MOG) compared with that in the unmedicated group. No significant decreases in VHMC were observed in the medicated group, when compared to the unmedicated group.

Correlations Between Pubertal Hormones and Different Brain Regions

Correlation analysis revealed a significant positive correlation between the VMHC values in the MOG and the basal LH levels in the medicated group ($r = 0.597$, $p = 0.011$) (**Figure 3**). It remained significant after controlling for age ($r = 0.546$, $p = 0.044$). In the control group, the VMHC values in the MOG were negatively correlated with both basal ($r = -0.714$, $p = 0.004$) and peak E2 levels ($r = -0.716$, $p = 0.004$), after controlling for

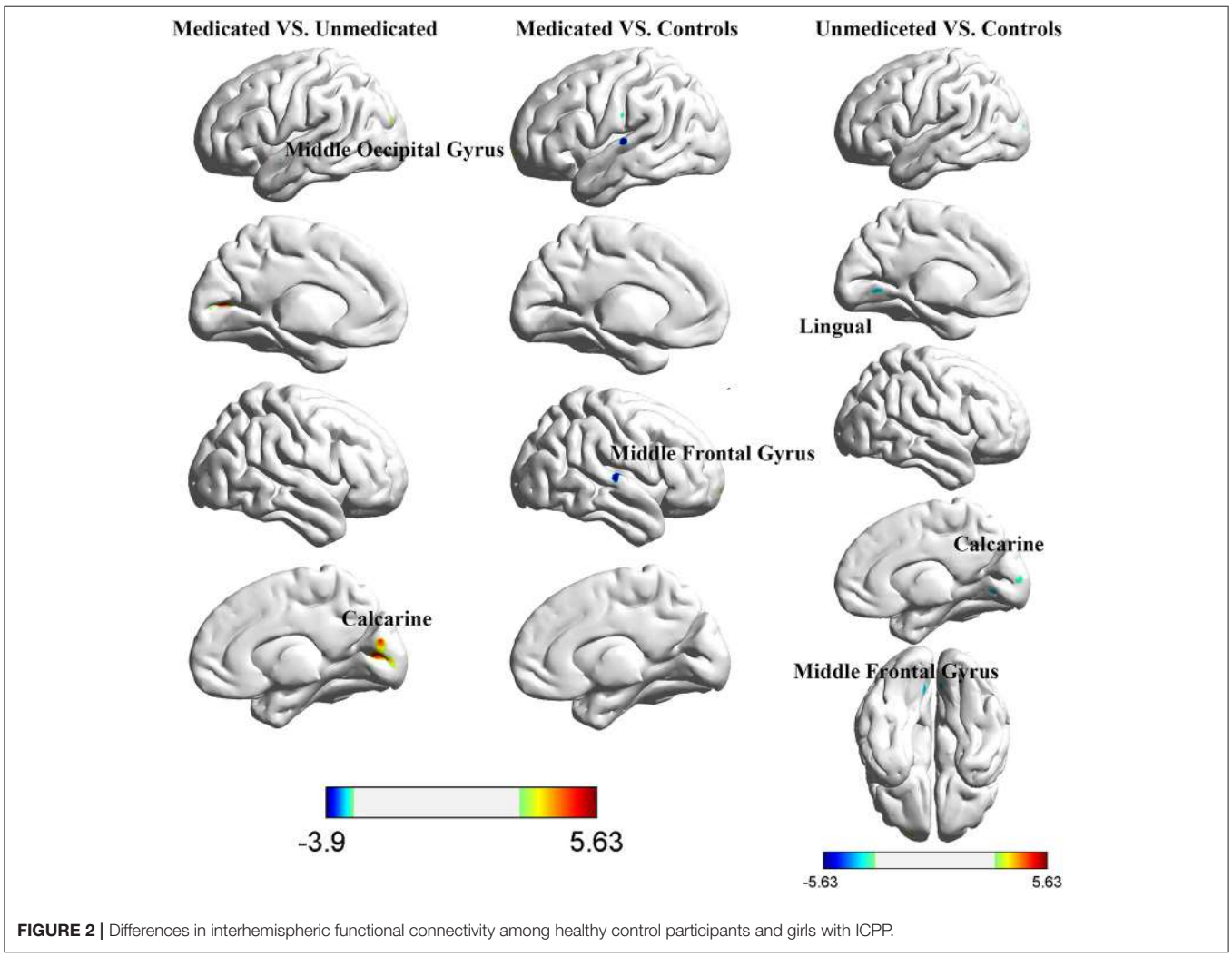

FIGURE 2 | Differences in interhemispheric functional connectivity among healthy control participants and girls with ICPP.

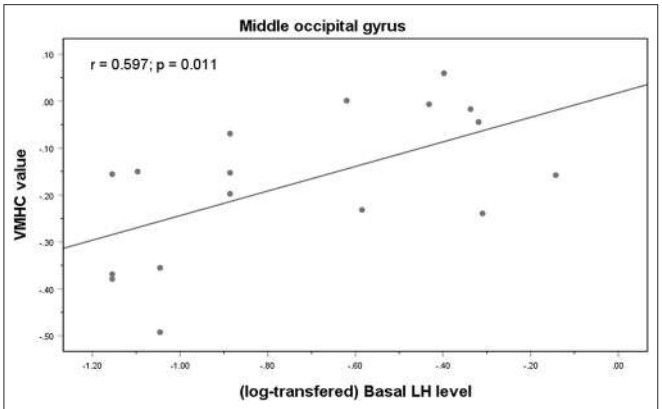

FIGURE 3 | Correlations between VMHC values in the middle occipital gyrus and basal LH levels in the medicated CPP patients.

the participants' ages during scanning. There was a significant negative correlation between VMHC values in the superior temporal gyrus and basal FSH levels after controlling for age ($r = -0.365$, $p = 0.026$) in the unmedicated group. No significant correlation was observed between VMHC and the CBCL scores in the medicated group ($p > 0.05$).

Additional correlation analyses were performed due to the differences in height and weight among the three groups. Partial correlations analysis was conducted between VMHC values (where there was a significant difference in structural volume among the groups) and clinical variables, which differed among the groups, after controlling for age at scanning, height, and weight. The positive correlation between the VMHC values in the MOG and basal LH levels in the medicated group remained significant ($r = 0.591$, $p = 0.043$). The VMHC values in MOG remained negatively correlated with basal ($r = -0.774$, $p = 0.003$) and peak E2 levels ($r = -0.755$, $p = 0.005$) in the control group.

DISCUSSION

To the best of our knowledge, this is the first study to demonstrate the effects of long-term GnRHa treatment on brain function in ICPP. Our study had three noteworthy findings. First, both ICPP groups showed significantly reduced VMHC within the superior temporal gyrus compared with that in the control group. Moreover, unmedicated patients also exhibited decreased VMHC within the MFG, MOG, and calcarine gyrus, when compared with the control group. Second, significant increases in VMHC in the calcarine gyrus and MOG were observed in the medicated group compared with the unmedicated group. Third, a significant positive correlation was observed between VMHC in the MOG and basal LH concentrations in the medicated group. Our findings contribute preliminary evidence for the potential endocrine mechanism underlying the effects of GnRHa on brain function in patients with ICPP.

Present study reported the unmedicated patient group showing reduced VMHC in the MFG compared with control group. The MFG is a hub of default mode network (DMN) which is involved in high-order cognitive and emotional behavior regulation (27, 28). Consistent with this findings, our previous structural MRI studies have observed reductions of cortical thickness in the prefrontal cortex of ICPP patients compared to controls (9). Moreover, recent fMRI study has reported altered amplitude of low frequency fluctuation in right frontal gyrus in girls with reactivated HPG axis (8). Decreased interhemispheric connectivity in MFG may suggest intrinsic dysfunctions of the DMN in patients with ICPP.

We observed increased VMHC in the calcarine gyrus and MOG in the medicated group, when compared to the unmedicated group. In addition to visual function (29), primary visual cortex and the occipital gyrus are also responsible for working memory processing (30, 31). Multiple fMRI studies found that visual working memory information can be decoded from early visual cortex during retention (32–34). Makovski et al. explored the role of the visual cortex in retaining visual working memory information using transcranial direct current stimulation reporting that the occipital cortex are involved in working memory consolidation (35). Especially, working memory is one of the main cognitive side effects of GnRHa (36, 37). Increased homotopic connectivity within the occipital cortex which influence the development of girls' working memory in GnRHa treated ICPP may be the crucial neural mechanism of GnRHa's working memory dysfunction.

Previous studies mainly investigated the influence of short-term GnRHa on brain activity in young women, suggesting altered brain activity in the frontal and temporal cortices which belong to default mode network (38–40). There is a possible reason for this inconsistent brain region results, given the differences in duration of treatment and age between the participants of earlier studies and our study. Handa and McGivern (41) posited that visual processing was related to higher-order cognition. Specifically, small deviations in visual processing may have long-term effects on the abstract visualization or conscious recall aspect of higher cognition (42). Thus, the lack of frontal and temporal results in present study may be attributed to the modulation of the visual network prior to default mode network by GnRHa. Besides, the effects of GnRHa on the brain regions of primary systems, including the visual system may become apparent only after long-term treatment (40).

The neuroendocrinological basis of the effect of GnRHa on human brain functional development remains unclear. This preliminary study found a positive correlation between basal LH concentration and MOG VMHC values in the medicated group. Our experimental design made it impossible to differentiation between the direct effects of GnRHa treatment and the secondary effects of LH and FSH suppression and/or E2 secretion. LH and sex steroids could mediate neurogenesis and synaptogenesis (43, 44). These hormones are known to have permanent organization and temporary activation effects on brain maturation (45, 46). Therefore, we speculate that LH might specifically regulate interhemispheric FC by enhancing the neural activity or synaptic coupling in these regions. Our findings can probably be attributed to the indirect effects of GnRHa. GnRHa receptors were recently discovered to be located on neurons within the

human brain (47), which provides evidence for the direct effects of GnRHa treatment. Future studies on GnRHa receptors could help to identify the direct and indirect effects of GnRHa and further elucidate the exact mechanism underlying the effects of GnRHa treatment on brain function.

The present study has some limitations. Its design was cross-sectional and thus, considerable differences in interhemispheric connectivity may have existed already between the untreated and treated patients, prior to treatment. There are several reasons, which prove the improbability of these differences. First, the possibility of other diseases affecting brain development was ruled in all participants, who were well-matched for variables such as age, hand preference, IQ, and the course of illness. Second, the relatively long mean duration of GnRHa treatment (12.7 months) made the effect of treatment more significant and credible. Third, the age range of the participants was narrow (9.0–11.4 years) and VMHC values which showed differences among groups were not correlated with age. Thus, it is credible that our results were largely attributable to the treatment. Furthermore, there are existing asymmetries in brain. We tried to mitigate this phenomenon by using a symmetric template and smoothing the functional data. Finally, the sample size used in the present study was relatively small, and future studies with larger sample sizes are required to further substantiate our findings.

This study provided preliminary evidence for the effects of long-term GnRHa therapy on brain function in girls with ICPP. The present study demonstrated that long-term GnRHa therapy is associated with higher interhemispheric connectivity in several areas responsible for memory and visual process and this effect may be associated with LH levels. Longitudinal clinical studies that will compare multimodal neuroimaging (functional and structural MRI) parameters and cognition among GnRHa treated patients with ICPP and age-matched controls with typical development are needed in future to validate the present findings and support the hypothesis that pubertal hormone suppression influences cognition, brain function, and brain structures during the developmental stages.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

ZY designed and supervised the study. TC, WY, HG, DY, LZ, and XX collected the data. TC and YF collected the participants. TC and WY integrated the data. XL and TC preprocessed and analyzed the data. TC drafted the manuscript. XX, WY, and TC discussed the results and commented on the manuscript.

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Long-term effects of gonadotropin-releasing hormone analogs in girls with central precocious puberty

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Gonadotropin-releasing hormone analogs (GnRHa) are widely used to treat central precocious puberty (CPP). The efficacy and safety of GnRHa treatment are known, but concerns regarding long-term complications are increasing. Follow-up observation results after GnRHa treatment cessation in female CPP patients up to adulthood showed that treatment (especially <6 years) was beneficial for final adult height relative to that of pretreated or untreated patients. Puberty was recovered within 1 year after GnRHa treatment discontinuation, and there were no abnormalities in reproductive function. CPP patients had a relatively high body mass index (BMI) at the time of CPP diagnosis, but BMI standard deviation score maintenance during GnRHa treatment seemed to prevent the aggravation of obesity in many cases. Bone mineral density decreases during GnRHa treatment but recovers to normal afterwards, and peak bone mass formation through bone mineral accretion during puberty is not affected. Recent studies reported a high prevalence of polycystic ovarian syndrome in CPP patients after GnRHa treatment, but it remains unclear whether the cause is the reproductive mechanism of CPP or GnRHa treatment itself. Studies of the psychosocial effects on CPP patients after GnRHa treatment are very limited. Some studies have reported decreases in psychosocial problems after GnRHa treatment. Overall, GnRHa seems effective and safe for CPP patients, based on long-term follow-up studies. There have been only a few long-term studies on GnRHa treatment in CPP patients in Korea; therefore, additional long-term follow-up investigations are needed to establish the efficacy and safety of GnRHa in the Korean population.

Key words: Precocious puberty, Gonadotropin-releasing hormone, Follow-up studies

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Introduction

If not diagnosed and treated at an early stage, central precocious puberty (CPP) can compromise final adult height, cause incongruity between psychological and physical development, and may also trigger psychological problems arising from early menarche. Gonadotropin-releasing hormone analogs (GnRHa) have been widely used for more than 30 years to solve these problems in patients with CPP[1]. Although the safety and efficacy of GnRHa are known, the treatment dosage and duration and point of cessation vary among different countries and ethnic groups, and the reported results are inconsistent because of many variables, including genetic height, pubertal traits, and treatment response. An analysis of Korean CPP patients from 2006–2010 conducted by the Health Insurance Review and Assessment Service showed an average 44.4% annual increase in patients diagnosed with CPP and a 4.5-fold increase in patients treated with drug therapy during the last 5 years[2]. Likewise, concerns about the long-term side effects of GnRHa treatment are increasing along with the rapid increase in patients diagnosed with CPP and receiving

subsequent treatment. However, the long-term effects of GnRHa treatment remain controversial. This paper reviewed long-term data up to adulthood from both international and Korean studies regarding final adult height, reproductive function, body composition, polycystic ovary syndrome (PCOS), and bone and psychological outcomes in girls with CPP after GnRHa treatment. As long-term studies of male CPP patients are scarce, this paper only addressed female CPP patients.

Final height

The effect of GnRHa treatment on final height (FH) gain is well known, but the degree of height gain varies among studies. This variance may result from differences in the adult height prediction methods, which include comparing adult heights between GnRHa treated and untreated groups or comparing the target height with predicted adult height (PAH) before and after treatment according to the Bayley–Pinneau method. In addition, differences in height gain may be induced by the delayed onset of treatment after diagnosis, duration of treatment, and age at the start of treatment.

Regarding the timing of CPP treatment discontinuation, Carel et al.[3] reported that the final adult height increased when GnRHa treatment was discontinued at 11 years of age but decreased when GnRHa treatment was continued beyond that age. According to bone age standards, 12–12.5 years is the suggested appropriate age at which to discontinue treatment in order to reach the maximum adult height[4]. However, the psychological states of the patients and their families, as well as the proposed age for recovery of the optimal adult height and bone age, must be considered before deciding whether or not to discontinue treatment.

Long-term investigations have reported results from objective evaluations of the effects of GnRHa treatment on final adult height. Klein et al.[5] reported the effect of GnRHa treatment on final adult height based on a sample of 98 males and females patients with growth cessation after GnRHa treatment. In that study, the final adult height increased significantly relative to the PAH before treatment after GnRHa treatment in 80 female subjects, although not sufficiently to reach the target height; 18 male subjects also had similar outcomes. However, as these patients achieved their target heights when they started treatment within 2 years of diagnosis, the authors concluded that rapid treatment after early diagnosis and, by extension, long-term treatment would be beneficial for achieving a maximum final adult height.

Some studies show that the effect on final adult height differs depending on the onset of CPP treatment. GnRHa treatment is known to maximally affect final adult height when initiated before 6 years of age[6] but the effect is decreased after age 6 years[7]. However, Carel et al.[3] compared the effects of starting treatment before or after age 6 years on the FHs of CPP patients. The results indicated that the under 6 group and older group had height gains of 5.3±7.2 cm and 4.5±5.3 cm, respectively, compared to the PAH, and that the FH gain therefore had no relationship with the onset of puberty or the age of treatment onset. On the other hand, in a study that stratified patients by age and compared their height gains after treatment, the age 6–8 groups and age 8–10 groups had final adult heights shorter than the PAH at the time of treatment discontinuation, but the age below 6 group had similar final adult heights relative to the PAH at the time of treatment discontinuation[8]. Also, a recent study evaluated a group of patients aged 16–32 years who had previously received GnRHa treatment and found no difference in the FH between subjects who had begun receiving treatment at approximately 8 years and other subjects who had not received any treatment[9]. It appears that patients experience the benefit of height gain from GnRHa treatment only if treatment is initiated before age 6. In other words, patients can have a longer period of growth following long-term treatment, allowing the acquisition of genetic height.

However, in a study of CPP patients who had received treatment 10 years earlier at an average age of 8.4 years, the adult height increased significantly compared to the PAH before treatment, and the patients reached or exceeded their target heights. Also, when the treated group was compared with the untreated group, the adult height of the untreated group was an average of 5.4 cm shorter than that of the treated group and 4.1 cm shorter than the target height[10]. Currently, children enter puberty at younger ages and therefore some argue that the diagnostic standard of CPP in females should accordingly be lower than age 8. However, GnRHa treatment is still needed to increase the FH in CPP patients under the age of 8 years.

Currently, there are few long-term studies on the final adult heights of CPP patients in Korea. Kwon et al.[11] reported that after tracking CPP patients who had received an average of 2.5 years of GnRHa treatment for approximately 3.4 years, the PAH increased by approximately 5 cm in 2 years and approximately 6–7 cm in 3 years after treatment[11]. Another study showed that the near adult height increased by approximately 5.1 cm, compared with the PAH in CPP patients after GnRHa treatment[12]. A long-term study involving Korean CPP patients is needed to examine the effect of treatment on final adult height gains.

In CPP, posttreatment growth evaluation is often performed using the difference between the FH and PAH according to the Bayley–Pinneau method. One study reported that when calculating the PAH, using the average table from the Bayley–Pinneau method reduced potential measurement errors from PAH overestimation when compared with using the accelerated table

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from the Bayley–Pinneau method[13]. However, in a recent study, there was no significant difference in the change in FH (ΔFH)-PAH of the untreated group when the values were calculated using either the accelerated or the average table from the Bayley–Pinneau method; in the GnRHa-treated group, the average table ΔFH-PAH (ΔFH-PAHav) was significantly higher than the accelerated table ΔFH-PAH (ΔFH-PAHacc)[9] (Table 1). Therefore, it is more appropriate to use the accelerated table when evaluating the FH in CPP patients after treatment. However, instead of evaluating the PAH at a certain point, periodic height, growth rate, pubertal progress, and bone age evaluations should be conducted so that optimal height gain can be achieved.

In a study of factors affecting FH gain, early diagnosis and treatment were found to be essential because bone age progression and treatment delay are known to adversely affect height gain. Furthermore, the degree of bone age progression, decrease in growth velocity, and low PAH at treatment cessation have been acknowledged as factors that negatively affect FH acquisition[14].

In the case of rapidly progressive precocious puberty, early GnRHa treatment is necessary; however, as the adult height can reach the target height without GnRHa treatment in slowly progressive CPP, side effects resulting from unnecessary GnRHa treatment should be considered[15,16].

Time of menarche after treatment discontinuation and reproductive function

Long-term studies on the recovery of reproductive function in CPP patients of more than 6–20 years are being reported. In CPP patients, pubertal reactions were recovered within 1 year and mostly within 6 months after treatment discontinuation. An average interval of 0.9–1.5 years was required for the onset of menarche after treatment cessation, and menarche started at approximately 12.6–13.6 years old in chronologic age. In a Korean study, Baek et al.[12] reported that menarche began at approximately 14 months post-GnRHa treatment, which was 11.9 years in chronologic age and 12.8 years in bone age; these ages are similar to the average age at menarche among normal girls in Korea (12.6 years). When patients were stratified according to their age at CPP diagnosis (age under 6 groups, age 6–8 groups, and age 8–9 groups), the repuberty period and onset of menarche after treatment were similar regardless of the age at diagnosis[8,10,17]. These results demonstrate that older CPP patients may have a shorter final adult height than younger patients because of the shorter period of time remaining for height gain after GnRHa treatment.

Among CPP patients, the average age at menarche in the GnRHa-treated group was 12 years, a significant delay when compared with the age of 9.6 years in the untreated group[9]. Therefore, untreated CPP can cause psychological problems due to early menarche. These studies demonstrated that the average age at menarche after GnRHa treatment was similar to the age at menarche in normal, healthy girls.

Menstrual cycles and pregnancy outcomes were analyzed to evaluate reproductive function. Pasquino et al.[10] reported that among 87 GnRHa-treated patients, 82 had regular menses, 5 had oligomenorrhea caused by excessive exercise but recovered after controlling their exercise, and 6 achieved pregnancy and normal childbirth. Neely et al.[17] reported that among 20 CPP patients who had reached adulthood, 80% had normal posttreatment menstrual cycles and 7 patients achieved a total of 12 pregnancies; these patients experienced normal childbirth and a number of miscarriages similar to that of the general population. Heger et al.[18] investigated 34 patients (average age, 23.6 years) who were followed up for an average of 12.5 years after treatment and wished to become pregnant. Twelve of these

Table 1. Height outcomes in girls with central precocious puberty who received gonadotropin-releasing hormone agonist therapy

Source	No.	Onset of treatment (yr)	Treatment duration (yr)	PAH at the start of treatment (cm)	Target height (cm)	Adult height (cm)	Height gain (ΔFH–PAH) (cm)
Carel et al.[3] (1999)	58	7.5±1.3	3.7±1.5	156.4±6.3	160.1±4.4	161.1±5.9	4.8±5.8
Klein et al.[5] (2001)	80	5.4±1.9	5.7±2.1	149.3±9.6	163.7±5.6	159.8±7.6	9.8±9.0
Pasquino et al.[10] (2008)	87 (GnRHa treated)	8.4±1.5	4.2±1.6	154.2±5.2* 150.0±5.1†	157.6±4.7	159.8±5.3	5.1±4.5 9.5±4.6
	32 (untreated)	8.3±1.2		155.1±4.3* 151.0±3.9†	158.5±4.8	154.4±5.9	0.6±4.5 3.0±6.0
Magiakou et al.[9] (2010)	33 (GnRHa treated)	7.9 (6.4–10.8)	2.8 (1–5.2)	158.1* (144.8–169.7) 151.5† (140.3–163.0)		158.5 (145–168.5)	–1.7 (–10.1 to 11.9) 7.0 (–3.0 to 15.8)
	14 (untreated)	8.0 (6.8–10.0)		160.3* (147.4–176.8) 154.3† (144.7–169.4)		161.5 (142.5–170)	–1.2 (–6.8 to 10.4) 3.3 (–2.8 to 15.6)

Values are presented as mean±standard deviations or median (range).
PAH, predicted adult height calculated according to the method of Bayley and Pinneau; FH, final adult height; GnRHa, gonadotropin-releasing hormone analogs.
*PAH calculated using the accelerated tables from the Bayley-Pinneau method. †PAH calculated using the average tables from the Bayley-Pinneau method.

patients became pregnant. The patients had regular menses cycles and did not have any breast or uterine disorders. Therefore, these results demonstrate that the reproductive function of women treated for CPP does not different from that of normal, healthy women.

Obesity and metabolic syndrome

Genetic factors, nutritional status, obesity, and other environmental factors contribute to the onset of puberty. In particular, a certain level of lipid accumulation in the body is required for the onset of puberty, and adipocyte-secreted leptin stimulates the release of gonadotropin-releasing hormone from the pituitary gland, thereby increasing sex hormone secretion. Breast development and menarche are known to be accelerated in women with high BMI values[19]. Furthermore, the risks of metabolic syndrome such as adult obesity, cardiovascular disease, and diabetes were reported to be higher in patients with early menarche[20]. In other words, childhood obesity may induce early puberty and early menarche and may lead to metabolic problems during adulthood. Likewise, there is increasing interest in changes in BMI or metabolic risk factors in CPP patients before and after GnRHa treatment.

To date, the reported results of research on changes in the BMI values of CPP patients before and after treatment are inconsistent. Paterson et al.[21] reported that the mean BMI SD scores (SDS) of CPP patients increased from 0.93 to 1.2, the frequency of overweight patients increased from 41% to 59% of all patients, and the frequency of obese patients increased from 28% to 39%. Arrigo et al.[22] reported that 23.8% of their CPP patients were obese prior to GnRHa treatment but experienced BMI decreases after at least 2 years of treatment. Another study showed that GnRHa treatment did not aggravate obesity, as CPP patients maintained their previous BMI SDS during treatment regardless of the overall increase in BMI after GnRHa treatment[10,23]. Many CPP patients were obese prior to GnRHa treatment but experienced no changes in BMI SDS following treatment, and the BMI SDS before treatment correlated strongly with the BMI SDS after treatment discontinuation[24]. From a comparison of a GnRHa-treated group and a nontreated group among adult CPP patients, Magiakou et al.[9] reported no difference in the BMI SDS between the 2 groups. Furthermore, as no difference in body fat mass via dual-energy x-ray absorptiometry was found between the 2 groups, it appears likely that GnRHa treatment is not associated with an increase in fat mass.

Research on the metabolic risk factors following GnRHa treatment in CPP patients is limited. In a previous study, reduced insulin sensitivity and lipid disorder incidence were observed in CPP patients at diagnosis, and metabolic disorders were aggravated in these patients during a 1-year GnRHa treatment period[25]. Another report indicated that increased insulin resistance after GnRHa treatment could lead to metabolic problems in CPP patients[26]. Yet another study showed that despite a lack of change in the BMI SDS during GnRHa treatment period, CPP patients had a 2-fold increase in total fat mass compared with a normal population after reaching their FH after treatment[23]. Currently, there is a relatively low amount of research concerning changes in body composition and metabolic profiles in CPP patients following GnRHa treatment, but adequate education concerning lifestyle and diet after treatment is needed in addition to medical treatment during the GnRHa treatment period.

Bone mineral density and bone markers

During the pubertal growth spurt, children experience improved bone mineral accretion, and almost half of the adult peak bone mass (PBM) is accumulated during this period. Genetic factors, hormonal status (growth hormone/insulin-like growth factor-1, sex steroids), nutrition, and physical activities are factors that influence PBM[27]. Estrogen in particular is well known as an important factor in bone mineralization and development. Reduced bone mass consequent to decreased estrogen levels along with a high occurrence of osteoporosis in menopausal women was reported in adults who had received GnRHa treatment[28]. Therefore, there is growing concern that missing the critical PBM stage might have adverse effects on adult bone, as the estrogen concentration is decreased during GnRHa treatment. In an earlier study, bone mineral density (BMD) decreased in CPP patients during GnRHa treatment[29]. In a study comparing BMD between a GnRHa-treated group and GnRHa plus calcium supplementation-treated group, Antoniazzi et al.[30] reported that although the BMD decreased during GnRHa treatment, this was reversible and preventable with calcium supplementation. However, another study reported a normal BMD in CPP patients who had reached their FH after GnRHa treatment. Regarding bone turnover markers in CPP patients, the expression of carboxy terminal telopeptide of type 1 collagen (ICTP), a bone resorption marker, and procollagen type 1 C-terminal propeptide (PICP), a bone formation marker, increased prior to GnRHa treatment but decreased during a 6-month treatment period and stabilized after treatment. Bone age-adjusted bone turnover markers were also normalized 2 years after treatment cessation. Furthermore, a report indicated that no changes in age- and bone age-adjusted BMD SDS was observed during GnRHa treatment[31]. A study conducted in Korea observed no changes in bone maturation among CPP patients who had received 3 years of GnRHa treatment[32]. In another study, bone mineral accumulation was suppressed during GnRHa treatment; however, the bone mineral

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content was recovered and PBM was sufficiently achieved when the adult height was reached after treatment discontinuation[10].

The varying BMD findings after GnRHa treatment might be explained by differences in age, treatment duration, treatment dosage, BMD area of measurement, and BMD evaluation method. However, to summarize the long-term BMD studies in CPP patients, although BMD levels decreased during GnRHa treatment, the bone mass was sufficiently preserved after treatment and treatment seemed to have no adverse effects on bone. As in normal girls and adolescents, exercise and adequate nutritional intake would be helpful for bone mass formation in CPP patients.

Polycystic ovary syndrome

PCOS is observed in 5%–10% of women of reproductive age and is characterized by anovulation, hyperandrogenism, and polycystic ovaries[33,34]. Severe insulin-resistant obesity, premature adrenarche, and sexual precocity in childhood are some of the known risk factors of PCOS. The cause of PCOS is not certain, but it may be induced by neuroendocrine system abnormalities that could cause abnormal luteinizing hormone (LH) pulsation and secretion, gonadal steroidogenesis disorders, or hyperinsulinemia[35].

Among the many causes of PCOS, hypersecretion of LH relative to follicle-stimulating hormone is especially similar in mechanism to activation of the hypothalamic–pituitary axis in patients with CPP, and some researchers have explained that CPP precedes PCOS through neuroendocrine dysregulation[36]. Exaggerated adrenarche was observed in 55% of cases at CPP diagnosis, and premature adrenarche was diagnosed via the hypersecretion of 17-hyderoxyprognenolone after adrenocorticotropic hormone stimulation. As 40% of patients diagnosed with CPP also had PCOS during the postmenarcheal period, the risk of PCOS might have increased because of the inherent premature adrenarche in CPP patients. In CPP patients, PCOS is known to usually develop within 0.5–4 years after menarche[37].

The prevalence of PCOS among CPP patients varies depending on the characteristics of the patients, durations of treatment and follow-up period, and differences in the PCOS diagnosis standards. Furthermore, a report stated that the prevalence of PCOS among CPP patients was 24%, compared with 2% in an age-matched control group[38]. On the other hand, Magiakou et al.[9] reported that GnRHa treatment had no influence on the occurrence of adult PCOS; rather, the authors concluded that ovarian dysfunction was more likely to occur in GnRHa-untreated patients. Franceschi et al.[26] reported the prevalence rate of PCOS in 46 adult women who were previously diagnosed with CPP and received GnRHa treatment. PCOS was diagnosed according to the Rotterdam and Androgen Excess Society definitions in 32% and 30% of the patients, respectively. That study reported that ovarian hyperandrogenism and polycystic ovaries occurred in CPP regardless of GnRHa treatment. However, the evaluation was limited by the lack of a GnRHa-untreated control group. Chiavaroli et al.[39] conducted a study on PCOS and compared a GnRHa-treated group with a GnRHa-untreated group among patients with early puberty (8–10 years of age). The authors reported higher prevalence rates of PCOS and hyperandrogenemia in the GnRHa-treated group than in the untreated group and that GnRHa treatment appeared to be an independent risk factor for PCOS occurrence.

Unlike previous studies, recent studies have reported a higher incidence of PCOS among CPP patients, but it is unclear whether this is due to the hyperinsulinemia or premature adrenarche already present at CPP onset or a result of an abnormal hormonal response to GnRHa treatment. A comparison with a control group of CPP patients through a long-term evaluation from diagnosis to posttreatment adulthood is needed to determine the causative factors of PCOS.

Psychosocial problems

Early pubertal timing in adolescents tends to result in severe emotional problems, antisocial behavior, or conflict with parents[40]. Adolescents who experienced menarche at an early age are known to be at risk for several problems such as excessive drug or alcohol intake, sexual contact at an early age, and increased psychosomatic problems during menstrual periods[41,42]. However, there are very few studies on the psychosocial problems of CPP patients after GnRHa treatment. A study evaluated the behavior and self-esteem of CPP patients before and during a 2-year GnRHa treatment period and reported that although the patients had been very concerned about physical differences from their peers, they recovered slightly from loneliness and behavior disorders during treatment[43]. Mul et al.[44] reported that there were no emotional or behavioral problems in early puberty patients at the start of or during treatment and there was also no influence on self-perception. In my study, the externalization problem, total behavior, thought, and attention problem scores were higher in CPP patients than in a normal control group, but there were no clinically meaningful behavioral problems[45]. Body image self-perception had an influence on emotional problems, regardless of a good response to treatment, in CPP patients with pubertal suppression after GnRHa treatment (not submitted). The patient's self-perceived body image, rather than physical improvement after GnRHa treatment, may play a more significant psychological role, and therefore psychological support should be provided during GnRHa treatment.

Conclusions

Long-term follow-up results obtained after GnRHa treatment indicated improvements in adult height. This treatment was largely reported to be effective, especially in patients who were diagnosed with CPP younger than 6 years of age and had received treatment, and GnRHa treatment did not seem to have a particularly adverse effect on reproductive function or bone growth.

However, proper treatment should be preceded by an accurate diagnosis and assessment of the ongoing disease process, as in some cases a normal adult height can be reached through the slow advancement of puberty without treatment. As mentioned above, the adequate GnRHa treatment dosage and systematic and consistent follow-up evaluation methods should be proposed in addition to an analysis of the cause behind the increase in CPP diagnoses and CPP treatment. A multicenter, large-scale research study of CPP patients in Korea is needed to obtain long-term follow-up data on the effects of CPP treatment on adult height, reproductive function, bone, and psychological aspects.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Original research

Family planning preferences in transgender youth in an urban multi-disciplinary gender clinic

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ABSTRACT

Background: Known barriers to family planning in the transgender population include low utilization of cryopreservation and decisional regret. There is growing data on the risk of infertility with GAHT, and on to what degree transgender adolescents feel informed about fertility and family planning options.
Objective: Assess preferences regarding options for family planning and fertility preservation in transgender adolescents treated with GAHT in a pediatric endocrinology gender clinic. The goal is to enhance patient education about potential effects of GAHT on fertility and options for family planning.
Methods: Forty one adolescents aged 10 years and older treated with GAHT in an urban outpatient pediatric endocrinology clinic were surveyed over a 6-month period from January to June 2022. Survey questions were multiple choice, Likert scale, and open-ended. Participants were at least 10 years of age, actively followed in the clinic, and receiving GAHT at time of enrollment.
Results: Forty one participants completed the survey. Four (10 %) expressed interest in discussing family planning with their provider. Eighteen (45 %) were open to discussion in the future; 16 (39 %) were not interested at all. 12 (30 %) participants were planning for future parenthood, and 16 (40 %) participants were undecided. Of those interested in parenthood 7 (53.8 %) planned to adopt or foster. Barriers to family planning expressed included financial concerns, potential need to pause GAHT, and social stigma of transgender parenthood. Twenty (50 %) participants recalled prior family planning discussion with their endocrinologist.
Conclusion: Family planning discussions may not be optimally impactful given that 50 % of participants did not recall the conversations. Family planning is a lower priority in this population as most desired to postpone discussion with their provider despite choosing treatment that could influence fertility. It is essential to identify methods to engage transgender youth in discussions related to family planning during GAHT.

Introduction

Gender diversity occurs when an individual identifies with a gender identity different from the sex assigned at birth. Gender diverse, gender incongruent, gender expansive, and transgender are examples of terminology included under this umbrella. Many individuals who identify as transgender seek care in the form of gender affirming hormone therapy (GAHT) to ameliorate gender dysphoria by developing secondary sexual characteristics more in line with their gender identity. GAHT refers to the use of gonadotropin releasing hormone analogs (GnRHa), testosterone, and estrogen. These treatments have been shown to support both the mental and physical well-being of gender diverse individuals, including decreasing the incidence of suicidal ideation and completed suicide, a significant cause of mortality in gender diverse individuals [1]. The World Professional Association for Transgender Health (WPATH) and the Endocrine Society recommend that healthcare providers discuss with patients and families the risk for decreased fertility and infertility both prior to and during medical and surgical therapies pursued for transition [2,3]. In the 2017 Clinical Practice Guidelines for the Treatment of Gender-Dysphoric/Gender-Incongruent

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Persons the Endocrine Society noted the dearth of validated decision aids to assist providers in this discussion, and in the decision-making process regarding the future fertility of individuals considering gender-affirming treatment [3]. This remains true today.

The long-term effects of GAHT on fertility are still sub-optimally understood, as research in this area is still growing. There have been multiple studies assessing the safety profile of GnRHa therapy in children with central precocious puberty (CPP). A consensus statement based on review of the current literature found no evidence of significant impairment in future gonadal function related to the use of GnRHa in females with CPP [4]. Studies in males with CPP do not suggest significant negative impact on future gonadal function, however paternity data has not been published [4]. The suppressive effect of long term clinical use of GnRHa on gonads of gender diverse individuals is reversible once treatment is discontinued; this has been demonstrated in both individuals with ovaries and those with testes, with more robust data in the former [3].

There is a growing body of evidence regarding fertility of transmasculine individuals undergoing treatment with testosterone therapy. A small prospective study found that testosterone use in transmasculine individuals led to anovulation [5]. A retrospective cohort study by Ghofranian demonstrated that transmasculine individuals with history of testosterone use discontinued prior to fertility treatment were able to successfully undergo oocyte procurement and cryopreservation. These individuals were subsequently able to become pregnant with resulting live births [6]. There are also several case reports demonstrating effective oocyte retrieval in individuals with ovaries treated with GnRHa therapy and long term testosterone [7,8] including a transgender individual assigned female at birth who was able to achieve pregnancy after a two month period of testosterone cessation [9].

It has been shown that treatment with both GnRHa and estrogen affects the morphology and quantity of sperm [10–13]. In a study published by Barnard, transgender individuals assigned male at birth underwent semen analysis. One participant who had discontinued GnRHa therapy demonstrated azoospermia for five months after treatment cessation. Another participant treated with spironolactone and estradiol demonstrated azoospermia four months after discontinuation of therapy, then subsequently elected to undergo orchiectomy [10]. In a study by Jiang histological evaluation was performed of post-orchiectomy gonads from 72 individuals assigned male at birth treated with estrogen for at least one year. One-hundred fourteen (81 %) of these were found to contain preserved germ cells, and 57 (40 %) demonstrated ongoing spermatogenesis, with no significant difference related to the duration of GAHT [11].

The most common method for individuals who opt to begin GAHT or undergo gonadectomy to safeguard the ability to have genetically related children is through gamete or embryo cryopreservation [2]. Existing research suggests that many transgender individuals either are unable or choose not to utilize this option. In a retrospective review of patients followed in a single-center pediatric endocrinology clinic only two of the 72 (3 %) patients who received counseling about family planning prior to GAHT chose to undergo cryopreservation [14]. Another retrospective chart review of 105 transgender adolescents found that of the 13 individuals (12 %) who had a formal consultation for fertility preservation prior to initiating GAHT, only five elected to pursue cryopreservation [15].

Fertility preservation in prepubertal children and those just beginning puberty is currently limited to the preservation of gonadal tissue [3]. At this time prospective data is limited regarding the use of this technique in this population for both ovarian and testicular tissues [3]. GnRHa therapy may limit successful procurement of viable gametes which may influence an early pubertal patient's choice on when to initiate GAHT.

Multiple studies have identified a variety of barriers that may preclude transgender and gender diverse individuals from choosing parenthood. Common themes include cost of treatment, hesitancy to pause GAHT, social stigma related to being a transgender parent, general lack of knowledge, and gender dysphoria worsened by pregnancy in individuals assigned female at birth [16]. It is more common for adult transgender individuals to voice a desire to have genetically related children compared to adolescents [2].

Decisional regret related to lack of fertility preservation following GAHT or GAS is a worry for both providers and many parents of transgender individuals. A study utilizing the Decision Regret Scale to assess regret related to family planning found that transgender individuals who voiced indecision about family planning goals demonstrated moderate-to-severe regret. Additionally, 37 % of study participants reported inadequate counseling regarding family planning, a common theme across several studies [16].

The Pediatric and Adolescent Gender Education (PAGE) Program, a multi-disciplinary clinic in Louisville, Kentucky, was created to provide gender-affirming care for children and youth with gender diversity. Members of the team include board certified pediatric and adult endocrinologists, an adolescent medicine physician, endocrine registered nurses, behavioral and mental health specialists, and licensed clinical social workers. Services offered include both medical and mental healthcare.

Based on the current paucity of information regarding knowledge of options for fertility preservation and family planning in gender diverse adolescents, we sought to expand the existing literature in this area. The primary objective of this study was to determine knowledge of family planning options in the transgender adolescent population followed in the PAGE Program, with the goal of applying the results to provide more equitable and comprehensive care. Secondary objectives included identifying barriers that may limit gender diverse adolescents from achieving family planning goals and developing mechanisms to overcome these barriers.

Materials and methods

This cross-sectional pilot study assessed participants during their regularly scheduled appointments in the PAGE Program from January 2022 to June 2022. Surveys were completed on paper and lacked any identifiable patient information. Institutional Review Board approval was obtained prior to participant enrollment. The study goals and objectives were discussed with participants and guardians when applicable. Participant and/or guardian consent and participant assent as indicated was obtained prior to survey administration. Participants were offered the opportunity to withdraw or not participate during the process. Inclusion criteria included participant age of least 10 years, an established patient in the PAGE Program, and on GAHT at time of enrollment. Exclusion criteria included non-English speaking participants and those with mental or physical limitations that would inhibit participation.

Surveys assessed demographic information including age, sex assigned at birth, gender identity, sexual attraction, and current level of satisfaction with their transition process. Question types varied and included multiple choice and open-ended. Multiple choice questions included an "other" option to be used when appropriate. Open-ended questions assessed participants' definitions of parenthood, reasons for or against desiring a future family, and whether and how their transgender identity affected their desire for parenthood. Surveys also assessed whether participants recalled discussing family planning with their providers, if so whether this was helpful, and in what ways providers could assist them with future family planning goals. The survey is available in Appendix A.

Standard descriptive statistics was used for data interpretation including the calculation of means where appropriate such as the age of the participants and Likert scale values. Descriptive phenomenology was used to extrapolate themes using an inductive analytic approach from the open answer responses of study participants utilizing key words or phrases. Representative quotes for the most common themes are

reported in the results. Power calculation was not performed due to the investigational and pilot nature of this initial study. Data regarding the total number of participants approached and the number who declined are not available.

Results

Forty-one participants completed the survey; one participant was later excluded from data analysis based on inclusion/exclusion criteria as this individual was not actively taking GAHT at the time of survey completion. Data related to specific pubertal timing of GAHT initiation was not available. The average age of participants was 17.8 years with a standard deviation of 1.8 years. The participant age ranged from 14 to 22 years. Twenty-one (52.5 %) participants identified as male and nine (22.5 %) identified as female. The remainder (25 %) self-identified as non-binary. Only 3 (7.5 %) participants reported treatment with a combination of testosterone and GnRHa. Most masculine-identifying participants (60 %) reported treatment with testosterone alone. In contrast, nine of the twelve (75 %) transfeminine participants reported treatment with a combination of estrogen and GnRHa. Only 3 (7.5 %) of participants reported treatment with estrogen alone. A single participant, a self-identified transmasculine individual, reported treatment with GnRHa alone.

Regarding sexual attraction, 22 (55 %) participants reported attraction to both male and female genders or identified as pansexual. Our study population reported overall satisfaction with the transition process ranging from "very unsatisfied" (1) to "very satisfied" (5), with an average satisfaction rating of 3.8 and a standard deviation of 1. See Table 1 for demographic information.

Twelve (30 %) participants reported interest in creating a family. Of these, seven (58.3 %) mentioned the possibility of fostering or adopting. Only one participant reported a desire for biologically related children.

Table 1
Participant demographics (N = 41)*.

Factor	Avg (SD)
Participant Age	17.8 (1.8)
Range	14–22
Sex Assigned at Birth	**N (%)**
AMAB	12 (30)
AFAB	28 (70)
Self-Identified Gender	
Male	21 (52.5)
Female	9 (22.5)
Non-Binary	10 (25)
Sexual Attraction	
Male	5 (12.5)
Female	9 (22.5)
Both	16 (40)
Pansexual	6 (15)
Asexual	1 (2.5)
Unsure	3 (7.5)
GAHT Use	
Testosterone Only	24 (60)
Testosterone and GnRHa	3 (7.5)
Estrogen Only	3 (7.5)
Estrogen and GnRHa	9 (22.5)
GnRHa alone	1 (2.5)

*1 participant was excluded for analysis as they were not on GAHT at time of survey.
AMAB (Assigned Male at Birth).
AFAB (Assigned Female at Birth).
GAHT (Gender Affirming Hormone Therapy).
GnRHa (Gonadotropin Releasing Hormone Agonist).

Twelve (30 %) participants did not desire future children, and 16 (40 %) were undecided.

Many distinct themes were identified regarding reasons for or against desire for parenthood. Several participants stated that regardless of their gender identity, they had already decided whether they desired a future family. See Table 2 for identified parenthood themes.

> *"I've never been particularly keen on having children, so being transgender has not affected me very much."*
> *"It [being a transgender person] hasn't affected my parenthood goals as I have always wanted to foster/adopt."*

Another theme related to the lack of desire to have children. Responses varied from personal distaste for children, a desire for independence, disinterest in childcare responsibilities, and prioritization of professional goals. Some participants voiced a lack of emotional capacity to care for and raise a child effectively. This is demonstrated by a selected quote below:

> *"I'm not sure if I am emotionally mature enough for children."*

Three participants articulated concern for worsening dysphoria during pausing of GAHT as a factor in family planning goals. Themes related to finances were more related to the cost of childcare, rather than cryopreservation, implantation, or surrogacy. One participant raised the concern of social stigma related to being a transgender parent:

> *"I am somewhat afraid that if at some point trans people were targeted/persecuted that any child I have wouldn't be safe."*

Reasons given for desiring parenthood involved providing an inclusive home, forming a family unit, and wishing to instill their values in children. Some participants reported that GAHT made them feel more inclined to form a family and voiced a desire to give back to children in the foster care system. Examples include:

> *"Increase in self-confidence and positive mood has me thinking about possibly adopting in my future."*

Table 2
Parenthood Planning (N = 41)*.

Factor	N (%)
Yes	12 (30)
Biological	1 (8.3)
Adopt/Foster	7 (58.3)
Undecided how	4 (10)
No	12 (30)
Undecided	16 (40)
Reasons for Parenthood	
Creating a family unit	3 (7.5)
Partners' desire for a family	1 (2.5)
Providing and inclusive home	3 (7.5)
Instilling their values	2 (5)
Reasons against Parenthood	
Financial	3 (7.5)
Worsening dysphoria	3 (7.5)
Concern about GAHT effects on a fetus	1 (2.5)
Concern for emotional stability as a parent	5 (12.5)
Social stigma	1 (2.5)
Other**	11 (27.5)
Transgender identity has not affected family planning goals	15 (37.5)
Underwent Cryopreservation***	1 (2.5)

*1 participant was excluded for analysis as they were not on GAHT at time of survey.
**Disinterest in children/Need for independence/Excessive responsibility.
***A single AMAB individual underwent sperm cryopreservation
AMAB (Assigned Male at Birth).
GAHT (Gender Affirming Hormone Therapy).

"I want to be a parent because I want to be able to carry my family forward another generation and to be a parent that my father never could be."
"I want to have a family and raise children to be good people."
"… I would like to foster LGBT + children in need."

Half of participants recalled discussion related to family planning with their provider. Discussions when recalled were rated from "not helpful" (1) to "very helpful" (5). Scoring ranged from 2 to 5 with only one participant rating the discussion as "unhelpful" (2). The mean response was 3.96 with a standard deviation of 0.9. Four participants (10 %) reported interest in further discussion and 18 (45 %) stated they would be interested in the future. Sixteen participants (40 %) reported no interest at all. The most common feedback was that providers simply introduce the subject. Other themes included desire for general education and local resources:

"Just talk to me about it."
"Guide me to the proper family counselors and/or adoption agencies."
"Maybe offer a way to take parenting classes and learn how being a parent would affect me."

Table 3 demonstrates perceived participant-physician family planning discussions.

Discussion

Our results reveal that many study participants were undecided about their plans for children in the future. This is concerning, as current evidence suggests that the individuals most at risk for decisional regret are those who were undecided prior to beginning GAHT [16]. This same study also found that participants who knew they either did or did not desire future children tended to be resolute, experiencing less regret than their undecided counterparts [16].

Our study demonstrates that many participants prioritize adoption and fostering over having a biological child. Only one of the 12 participants (8.3 %) who endorsed a desire to have a family wished to have a genetically related child. Only one participant who was assigned male at birth chose to undergo fertility preservation prior to beginning GAHT with sperm cryopreservation. Existing data shows overall low rates of cryopreservation in this population. A systematic review of 10 studies by Baram found the preservation uptake rates of transgender youth were quite variable, ranging from 9.6 to 81.8 % in individuals with testes and 0–16.7 % in those with ovaries [17]. It should be noted that the higher reported rates of preservation appear to be outliers given low study power. This review also observed low uptake rates in transgender adults, similar to those seen in transgender youth [17]. As the process of oocyte procurement, preservation, fertilization, and implantation is more involved and more financially prohibitive than sperm cryopreservation, this can be a significant barrier for fertility preservation for individuals with ovaries.

Table 3
Family planning discussions (N = 41*).

Recalled Family Planning Discussions	N (%)
Yes	20
No	20
Helpfulness of discussion**	3.96 (0.8)
Desire to discuss Family Planning	
Interested in discussion	4 (10)
Not interested in discussion	16 (40)
Will be interested in the future	18 (45)
Undecided	2 (5)
How a provider can be more helpful	
Provider to mention family planning	10 (40)
Education about GAHT effects on fertility	2 (5)
Education on available options	4 (10)
Local resources***	5 (12.5)

*1 participant was excluded for analysis as they were not on GAHT at time of survey.
**Mean based on Likert Scale from 1 to 5 with 1 being "Not Helpful and 5 being "Very Helpful".
***Adoption agencies, support groups, parenting classes.
GAHT (Gender Affirming Hormone Therapy).

Interestingly, most of our participants did not report gender dysphoria or social stigma as major contributors to their family planning goals. Some participants with ovaries did voice hesitation over halting therapy to become pregnant, citing their concerns for worsening dysphoria and potential side effects of testosterone on a fetus. One articulated preference for their *cis*-female partner to carry the pregnancy. Both a participant with ovaries and one with testes reported fearing for the safety of both themselves and their future children due to being a transgender parent. Additional reasons given for a lack of desire for parenthood included disinterest in children, desire for independence, and weight of the responsibility of having a child.

Reasons for desiring a future family included a desire to instill their own personal values and create a core family unit. Six participants (15 %) emphasized the importance of establishing an inclusive and accepting home. Many of those who voiced a desire to foster or adopt other LGBTQ + children mentioned their own personal experience as a reason, wishing to give their own children a better childhood than they themselves had experienced.

While data are sparse there is some evidence that transgender individuals are less likely to have children than their cisgender counterparts [18]. Transgender individuals choose to have children in a variety of ways including adoption and fostering. A literature review performed in 2014 by Stotzer investigating the prevalence and characteristics of transgender parenting found that 64 % of cisgender individuals reported parenthood compared to 38 % of transgender respondents [19]. This review also found that transfeminine individuals were less likely to have children compared to those identifying as transmasculine (34 % and 50 % respectively) [19]. Higher parenting rates correlated with older age at time of transition; this was partially due to prior heterosexual relationships which resulted in biological children prior to transition [19]. There is little data on the relative breakdown of rates for adoption, fostering, and having biological children [19].

Adoption was the most common method in our study participants who voiced interest in parenthood, with seven participants (58.3 %) preferring this option. Legal protection against discrimination because of sexual orientation/gender identity minorities regarding adoption and fostering varies state-by-state, both in degree and scope. Legislation has been introduced on a national level with the potential to reduce or restrict the ability of transgender individuals to adopt or foster [20]. These legal implications highlight the importance of advocacy, both locally and nationally to support the right of transgender individuals to become parents.

Of note, fifteen (37.5 %) of our study participants reported that their gender identity did not affect their family planning goals. This is contrary to several previous studies that found gender dysphoria, financial barriers, and social stigma were obstacles to parenthood. One common reason given was personal disinterest in forming a family. The destigmatization of transgender individuals and the increase in medical treatment availability and acceptance may be changing how this population views parenthood.

Surprisingly only 50 % of our study population recalled having had a family planning discussion with their healthcare provider. In the era of electronic medical records (EMR), our clinic templates include documentation of family planning discussion as a data point. Despite documented discussions about the potential effects of GAHT on future fertility, many study participants did not recall these conversations. It is known that medical information recall is widely variable. Research on this topic shows that patients recall 20–40 % of information provided

during a medical encounter [21,22]. This raises the questions of whether current methods of discussion are unmemorable, or whether patients lack recall because they do not value the information discussed at the time of presentation. Regardless of whether they recalled the discussions many participants (45 %) stated a desire to postpone discussion of family planning until a later time, with a similar number preferring to forgo discussion entirely (40 %).

Fertility preservation is also an area of research interest for pediatric oncologists and reproductive endocrinologists. There has been significant advancement in the study of fertility preservation in children undergoing treatment for cancer. It is well known that both chemotherapy and radiation, particularly when targeted to the pelvic area and gonads, can result in impaired fertility [23]. At this time pre-pubertal gonadal preservation is experimental. Many interventions are based around gonadal shielding during radiation therapy. Most fertility preservation is performed after puberty onset using similar methods to those described above [23]. The American Society of Clinical Oncology has published guidelines recommending that all providers discuss treatment risks of infertility, options for fertility preservation, and referral to fertility experts as soon as possible [24].

Similar to providers caring for the transgender population, oncologists are not always able to refer patients to fertility specialists. Barriers to care include but are not limited to lack of knowledge of fertility preservation options, personal discomfort discussing fertility, and assuming families cannot afford preservation techniques [23]. This population also falls victim to inadequate or complete lack of insurance coverage for fertility preservation [23]. Interestingly, current data suggests that only about 50 % of parents recalled fertility discussions with their child's oncologist, and when they did approximately a third of them expected normal fertility [25]. Efforts are underway to improve the efficacy of fertility conversations and overcome barriers in this population. Suggested methods include delaying initiation of therapy when appropriate and facilitating andrology laboratory visits for patients with sperm [23]. The formation of multi-disciplinary cancer survivorship clinics in pediatric tertiary care centers is another way in which providers are combatting the barriers faced by these patients [26,27]. This technique could be adapted for multi-disciplinary gender clinics.

We did not include parental perception in this study; this would be a beneficial area of interest for future research. Existing research suggests that parents may have more concerns related to the ramifications of GAHT on fertility and parenthood priority compared to adolescents [28,29]. Strang developed the Transgender Youth Fertility Attitudes Questionnaire (TYFAQ) which surveys parents as well as patients [28]. Similarly to our study population, very few participants voiced interest in having genetically related children. Future investigation could include evaluation of the patient-parent relationship and may potentially yield additional information on how to best meet the needs of transgender youth.

There are some limitations to our study. Our survey was not designed to differentiate between desire for biological or non-biological children; the responses from the open-ended questions provided additional information about this difference. Our study was designed to assess patient preferences related to family planning, rather than document discussions from the medical record. As such we did not include data from EMR chart review to verify documented family planning discussions. Future studies could include comparison of patient recall of fertility discussions with medical record documentation. If a discrepancy is found, subsequent research could include development of novel methods to emphasize the importance of family planning discussions.

Our study did not collect data on the timing of GAHT related to pubertal or Tanner stage. This limits our study's ability to assess a difference in family planning preferences of our population related to GAHT initiation and pubertal timing. Family planning options related to gamete viability vary based on timing of GAHT initiation [3]. There are also surgical considerations for the timing of GnRHa therapy that may also play a role in fertility planning preferences. For example, GnRHa therapy in individuals with testes early in puberty can impact the phallic length, which may pose potential surgical challenges if vaginoplasty is desired in the future [30].

Another potential limitation is lack of data about anti-androgen use in our population. This could potentially be related to self-reported responses to the question assessing the type of hormone therapies prescribed. Study participants may have considered only estradiol, testosterone, and GnRHa therapy, rather than spironolactone in their responses. Currently there is insufficient data regarding whether or how anti-androgen treatment plays a role in family planning discussions.

Our study has several strengths. The use of multiple choice, Likert scale, and open-ended questions allowed space for our participants to explain their answers and extrapolate their thoughts. Similarly, allowing for open-ended responses and feedback on specific ways to better conduct family planning discussions provides pragmatic guidance for quality improvement. Assessing the resources our participants believed would be helpful provides future direction for improving the effectiveness of fertility discussions.

One novel feature of our questionnaire was the inclusion of several qualitative inquiries about what respondents felt parenthood meant to them. Assessing reasons for choosing parenthood may inform future research and ongoing discussions between patients and providers. Documentation of demographic information, transition satisfaction, and percentage of recalled family planning conversations may allow our center to track and monitor success of future interventions.

Our findings emphasize the importance of prioritizing conversations about future fertility with patients who begin their GAHT journey undecided about parenthood goals. We recognize this may be a burden for transgender individuals, particularly adolescents who must consider these decisions at such an early stage of life. These conversations carry more weight in the transgender community, as cisgender adolescents are overall less likely to receive medical treatments with potential to affect fertility. Partnership between providers, patients, and their families is of utmost importance when discussing how treatments may affect their human experience.

Conclusion

Transgender and gender diverse youth require a multi-disciplinary approach to family planning that is not yet routinely available in the United States. Novel techniques to address inequality in this population are needed, as this population faces unique barriers not experienced by their cisgender peers. Only 50 % of our participants recalled having had family planning discussions with their providers prior to initiating GAHT. Our study highlights the necessity of focused provider attention to patient education regarding family planning options, as often family planning is not a priority for this population.

As many transgender individuals who do desire children voice a preference for adoption and fostering, providers can assist patients with parenthood planning by providing local resources, including information about adoption and foster agencies. Advocacy for safe and cost-effective fertility preservation options is necessary to level the playing field, providing truly equitable opportunities for this population to create biological families when desired. Advocacy to protect the rights of transgender individuals seeking parenthood, both locally and on a national level, should be a priority for all those who care for this population.

CRediT authorship contribution statement

Ryan Conard: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lisal Folsom:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

PAGE Family Planning Questionnaire.

1. **How old are you?**
2. **What sex were you assigned at birth?**

A. Male B. Female

3. **What is your gender identity? (Circle your answer. Please feel free to elaborate)**

A. Male B. Female C. Unsure C. Other:_____

4. **Multiple Choice: I am attracted to… (Circle your answer. Please feel free to elaborate)**

A. Males B Females C. Both D. Neither E. Unsure F. Other: _____

5. **Do your plans for your transition include any type of hormone therapy? If so, which hormone(s)?**

A. Testosterone B. Estrogen C. Puberty Blockers D. None E. Undecided

6. **Do you plan to have gender affirming surgery? (Circle your answer)**

A. Yes B. No C. Undecided

7. **As of today how satisfied are you with your transition progress?**

1 *(Very Unsatisfied)* 2 3 4 5 (*Very satisfied)*

8. **Do your plans for your future family include having children in any way?**

A. Yes B. No C. Undecided

9. **Explain what it means to you to be a parent.**
10. **What are some reasons your future does or does not include parenthood?**
11. **Describe how being transgender has affected your parenthood goals.**
12. **Have any medical providers ever discussed family planning with you?**

A. Yes B. No

13. **If so, was this helpful?**

1 *(Very Helpful)* 2 3 4 5 (*Very Helpful)*

14. **What can your medical providers do to help you make decisions about family planning?**
15. **Are you interested in learning more about ways in which you can start a family?**

A. Yes B. Yes, but I am not ready for that at this time C. No D. Unsure

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Access, barriers, and decisional regret in pursuit of fertility preservation among transgender and gender-diverse individuals

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Objective: To query transgender and gender-diverse individuals on their desire for fertility preservation, perceived barriers to access care, and decisional regret.
Design: Cross-sectional.
Setting: Not applicable.
Patient(s): A total of 397 gender-diverse individuals undergoing intake to the University of California Los Angeles Gender Health Program from January 2018 to March 2019. Seventy participated in a follow-up survey from September to October 2019 clarifying reproductive desires or intentions.
Intervention: Multiple-choice questionnaire.
Main Outcome Measure(s): Perceived barriers to access fertility preservation and decisional regret surrounding choice to pursue fertility preservation as measured with the use of the validated Decision Regret Scale (scored 0 to 100).
Result(s): Barriers to accessing care were primarily cost of treatment (36%), discontinuation/delay of hormonal therapy (19%), or worsening of gender dysphoria with treatment/pregnancy (11%). Respondents indicated that their family planning goals were addressed by primary care providers and/or medical endocrinologists (multiple responses allowed), but 37% stated that their family planning goals were not adequately addressed. Those who had made a firm decision to pursue or not pursue fertility treatment had mild decisional regret. Moderate-to-severe decisional regret was noted in those who were undecided regarding the pursuit of fertility perseveration before transition and in those who were interested in referral to reproductive endocrinology.
Conclusion(s): Consultation with a reproductive endocrinologist may reduce decisional regret as well as clarify perceived barriers to fertility preservation in transgender and gender-diverse individuals interested in fertility preservation. (Fertil Steril® 2021;115: 1029–34. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Transgender, gender-diverse, fertility preservation, family planning, decisional regret

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Professional organizations, such as the American Society for Reproductive Medicine (ASRM), the World Professional Organization for Transgender Health, and the Endocrine Society, recommend that all transgender individuals be offered counseling regarding the impact of medical treatment or surgery on fertility before initiation of transition and the option to pursue fertility preservation, including oocyte, sperm, or embryo cryopreservation. Furthermore, the ASRM Ethics Committee suggests that transgender and gender-diverse individuals have similar family planning desires and that access to fertility services should be comparable to that of their cisgender counterparts (1). However, access to fertility consultation and preservation has not been widely studied in this population. Previous studies have focused on a transmale population instead of the

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broader gender-diverse group (2, 3).

One such study demonstrated that transgender men and gender-nonbinary individuals (n = 172) perceived financial and psychologic barriers to family planning, including cost of using cryopreserved gametes and difficulties in the adoption process (2). Another study (n = 50) revealed that a significant percentage of transgender men (37.5%) would have considered cryopreservation of gametes had the technique been available at the time of transition (3). A multicenter study in Germany with 189 participants showed that although 76.1% of transgender women and 76.6% of transgender men thought of fertility preservation before medical transition, only 9.6% and 3.1%, respectively, had followed through with preservation (4).

Given the overall low rate of fertility preservation noted in transgender populations, we were interested to see if our multidisciplinary university-based population differed from previously described international populations regarding access to care, perceived barriers, and interest in family building (2, 5). Previous studies focused on select populations of transgender individuals (i.e., transfemale or transmale cohorts) outside of the United States. We consider the gender-diverse population to be heterogeneous and therefore we sought to include all those who identified as transgender and gender diverse. To our knowledge, there have been no large studies to describe fertility desires among transgender and gender-diverse individuals in the United States. In addition, we sought to evaluate the decisional regret surrounding fertility preservation in transgender individuals, which has not been formally studied with a validated tool. We hypothesized greater level of regret among transgender and gender-diverse individuals who were not offered consultation with a reproductive endocrinologist or had chosen not to undergo fertility preservation.

MATERIALS AND METHODS

The University of California Los Angeles (UCLA) Gender Health Program (GHP) is a multidisciplinary medical, surgical, and behavioral health team that supports transgender and gender-diverse individuals before, during, and after their transitions. Patients were referred by medical professionals, mental health professionals, insurance companies, and friends, as well as self-referred. Our initial cross-sectional survey used intake demographic data acquired by a care coordinator at the time of care establishment with the GHP; this included demographics, desired specialty services, and reproductive life goals, including desire for referral to reproductive endocrinology and interest in family planning. The study proposal was reviewed by the institutional review board and determined to be exempt. Intake data for all individuals who enrolled in the GHP from January 2018 to March 2019 were obtained. The data were coded and deidentified. Descriptive statistics were then performed. Logistic regression was used to identify predictors of interest in fertility preservation.

After examining intake information, we subsequently created an anonymous electronic survey with the use of Qualtrics to ask follow-up questions pertaining to reproductive intentions within this population. This study proposal was reviewed by the institutional review board and determined to be exempt. The survey was disseminated to GHP members who had previously indicated willingness to participate in research, were at least 18 years of age, and spoke English. This follow-up survey was disseminated from September 2019 to October 2019. The mean time frame from the initial intake survey to completion of the follow-up study was 12 months. Descriptive statistics were then performed. Decisional regret was measured with the use of the Decision Regret Scale, a validated five-item scale with total scores from 0 to 100 (6). A score of 0 is considered to indicate no regret, 1–24 mild regret, and ≥25 moderate to severe regret (7). Decisional regret has been shown to have positive correlation with decisional conflict surrounding health care decisions (7).

TABLE 1

Demographics.

	Intake survey		Follow-up survey	
Characteristic	**n**	**%**	**n**	**%**
Age, y				
<18	31	7.8%	–	–
18–25	128	32.2%	15	21.4%
26–35	111	28.0%	19	27.1%
36–50	49	12.3%	16	22.9%
>50	31	7.8%	14	20.0%
Chose not to respond	47	11.8%	6	8.6%
Assigned documented sex at birth				
Female	187	47.1%	24	34.3%
Male	152	38.3%	46	65.7%
Intersex	1	0.3%	–	–
Chose not to disclose	57	14.4%	–	–
Gender identity				
Female	100	25.2%	9	12.9%
Male	74	18.6%	13	18.6%
Transfemale	49	12.3%	24	34.3%
Transmale	76	19.1%	8	11.4%
Gender queer	9	2.3%	1	1.4%
Gender nonbinary	43	10.8%	9	12.9%
Gender fluid	6	1.5%	2	2.9%
Chose not to disclose	40	10.1%	4	5.7%
Sexual orientation				
Lesbian	10	2.5%	8	13.1%
Gay	10	2.5%	5	8.2%
Bisexual	14	3.5%	12	19.7%
Straight	22	5.5%	18	29.5%
Queer	13	3.3%	17	27.9%
Chose not to disclose	328	82.6%	1	1.6%
Race				
American Indian or Alaska Native	3	0.8%	–	–
Asian	13	3.3%	8	12.1%
Black or African-American	8	2.0%	3	4.5%
White or Caucasian	76	19.1%	43	65.2%
Hispanic or Latino	24	6.0%	4	6.1%
Native Hawaiian or Pacific Islander	2	0.5%	–	–
Unknown	2	0.5%	–	–
Other	5	1.3%	7	10.6%
Chose not to disclose	264	66.5%	1	1.5%
Started medical hormonal transition				
Yes	163	41.1%	62	88.6%
No	120	30.2%	8	11.4%
Chose not to disclose	114	28.7%	–	–

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RESULTS

Intake Survey

Intake data from a total of 397 individuals were included in the initial cross-sectional analysis. The average age of individuals who had established care at the GHP was 29 ± 12.4 years. Gender assignments at birth were female (n = 187), male (n = 152), intersex (n = 1), and declined answer (n = 57; Table 1). Forty-eight individuals (12.1%) stated that they had reproductive life planning goals. Twelve respondents (3%) had previously undergone fertility preservation, of whom 11 were assigned male and one assigned female at birth. Forty-eight (12.1%) also stated that they desired fertility preservation, but only eight (2%) endorsed presenting to the GHP requesting referral for fertility preservation (Table 2). Additional demographics are listed in Table 1. Neither gender identity, race/ethnicity, age as a continuous variable, nor age category (in 5-year increments from 20 to 40 years) was predictive of desire for fertility preservation.

Follow-Up Study

Of the 145 patients who agreed to be contacted for research, 70 responded to our online survey (48.3% response rate). The average age was 38.5 ± 16.8 years. Of the respondents, 46 (66%) were assigned male at birth and 24 (34%) were assigned female at birth. Gender identities included male (n = 8), female (n = 13), gender nonbinary (n = 8), transmale (n = 8), transfemale (n = 23), gender fluid (n = 2), and other (n = 6). A majority of the population was white (65.2%), employed (62.1%), and had completed at least high school (97.0%).

Self-Reported Family Planning Intentions

Forty-nine (70%) did not have children. Of the 21 who had children, 13 had biological children (65%), two had adopted (10%), and five had both biological and adopted children (25%). Of the forty-nine who did not have children, nine (18.4%) were interested in future biological children, and 13 (26.5%) were unsure (Supplemental Table 1, available online at www.fertstert.org). Thirteen (18.6%) stated that having biologically related children was very or somewhat important, and 30 (42.8%) stated that this was very or somewhat unimportant. The remaining 25 (35.8%) felt neutral about having their own biological child. Thirty (44%) were not planning to adopt, 27 (40%) were unsure, and 11 (17%) were planning to adopt.

Family Planning Counseling and Pursuit of Fertility Preservation

Respondents indicated that primary care providers and medical endocrinologists were the primary sources of information surrounding family planning; multiple responses were allowed (Supplemental Table 2, available online at www.fertstert.org). A large percentage of patients (37%) stated that medical professionals did not adequately address their family planning goals.

Forty-five respondents (66%) stated that they had no intention to pursue fertility preservation, and 18 (27%) were undecided. One individual (1%) had intention to pursue and four (6%) had already completed fertility preservation. Notably, of those who had completed fertility preservation, all had performed sperm cryopreservation.

Perceived barriers to accessing care among all respondents are listed in Table 3. Barriers (multiple responses allowed) were primarily cost of treatment (36%), discontinuation/delay of hormonal therapy (19%), or worsening of gender dysphoria with treatment/pregnancy (11%). Another 13% stated that there was no perceived barrier to accessing fertility preservation.

Those who had made a firm decision to pursue or not pursue fertility treatment had mild decisional regret (mean score 9.7 ± 18.2, median 0, interquartile range [IQR] 0-15). Moderate-to-severe decisional regret was seen in those who had not yet undergone fertility preservation and were undecided on their decision to pursue it (mean score 36.4 ± 20.8, median 42.5, IQR 18.8-50; Table 4). Eighteen (33%) stated that they would accept a consultation with a reproductive endocrinologist for fertility preservation counseling if offered. Moderate to severe decisional regret pertaining to fertility preservation was also noted among this group (mean score 35.6 ± 26.1, median 42.5, IQR 8.8–51.3).

DISCUSSION

In the large multidisciplinary UCLA GHP, only 2% of the patients who established care requested referral for reproductive

TABLE 2

Intake survey.

Question and answers	n	%
Reproductive life planning		
Do you have reproductive life planning goals?		
Yes	48	12.1%
No	153	38.5%
Choose not to disclose	196	49.4%
Have you undergone fertility preservation?		
Yes	12	3.0%
No	211	53.1%
Choose not to disclose	174	43.8%
Do you have fertility preservation desires?		
Yes	48	12.1%
No	168	42.3%
Choose not to disclose	181	45.6%
Patient goals at time of referral (select all that apply)		
Establish primary care	219	55.2%
Initiate hormones for gender transition	157	39.5%
Manage maintenance of hormones	92	23.2%
Referral for surgical services	191	48.1%
Referral for fertility services	8	2.0%
Referral for other health concerns	19	4.8%
Behavioral health services	52	13.1%
Ask insurance questions	14	3.5%
Other	25	6.3%

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TABLE 3

Perceived barriers to fertility preservation (select all that apply).

Barrier	n	%
No desire for fertility	37	53%
Cost of treatment/lack of insurance coverage	25	36%
Discontinuation/delay of hormonal therapy	13	19%
Potential worsening of gender dysphoria with treatment/pregnancy	11	16%
No barriers perceived	9	13%
Lack of social support	8	11%
Other	6	9%
Invasiveness of procedures	5	7%

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endocrinology at entry. However, in a subset of patients responding to a follow-up survey about reproductive desires and intentions, 33% stated that they would be interested in consultation with a reproductive endocrinologist for fertility preservation counseling if offered. This disconnect may be explained by a focus on transition at the time of establishing care at the GHP over reproductive planning. Another explanation may be difference in mean age between the population in the intake data and the subsequent follow-up survey (26 years vs. 38.5 years). The latter group may have had more time to consider their stance on fertility preservation or may have found that priorities had shifted at a later stage of life. In addition, the subset that responded to the follow-up survey about reproductive interest may have different reproductive goals as consistent with a selection bias. However, desire for fertility preservation was not shown to be dependent on any individual factors, including gender identity, race/ethnicity, or age.

Barriers to pursuing fertility preservation were primarily cost of treatment, lack of insurance coverage, discontinuation or delay of hormonal therapy, and potential worsening of gender dysphoria with treatment or pregnancy. There is increasing prevalence of state legislation mandating coverage for fertility preservation for patients facing medical diagnoses that may affect fertility (8). Legislation such as this may prove to benefit this population by alleviating a financial barrier. In addition, there is a significant difference in impact on treatment course for those who pursue fertility preservation before or after initiation of medical transition. For example, in transmales who have already started testosterone, the recommended interval off testosterone before fertility preservation varies but has traditionally been ~3 months (9). In contrast, transmales who have not yet initiated testosterone would experience treatment delay for 2–3 weeks while undergoing oocyte cryopreservation (9,10). In the absence of evidence-based recommendations for a prescribed interval off testosterone therapy before initiating fertility treatment, a delay of less than 3 months may be reasonable. Furthermore, a random-start approach to fertility preservation, as is used in oncofertility patients, may be an avenue for minimizing time to initiate medical transition. This strategy allows patients to proceed immediately to a stimulation cycle without waiting for a menstrual bleed, both decreasing time to transition and potentially, reducing gender dysphoria (11). Consultation with a reproductive endocrinologist before transition would allow for thorough counseling on options for treatment with the goal of reducing decisional regret and perceived barriers. While it is encouraging that 63% had family planning addressed by their medical professionals, there is room for improvement to ensure that the remaining 37% feel that they receive adequate counseling. A reproductive endocrinologist may be able than other medical providers to provide more detailed counseling into the specifics surrounding fertility preservation techniques.

Decisional regret was noted to be moderate to severe among those who had not yet undergone fertility preservation and were unsure if they would in the future. Moderate-to-severe regret as measured by the Decisional Regret Scale has been associated with higher decisional conflict, lower satisfaction with a decision, fear of adverse physical health outcomes, and greater anxiety levels (7). It is notable that those who were interested in a consultation with a reproductive endocrinologist also expressed moderate-to-severe regret. We infer from this data that undergoing a consultation with a fertility specialist may decrease overall decisional regret. Some of our subjects had made a decision regarding the pursuit of fertility preservation, whereas the decision

TABLE 4

Decision regret surrounding fertility preservation decision.

		Decisional Regret Scale (0–100)[a]				
Prior Fertility	Mean age, y	0	1–24	≥25	Mean ± SD	Median (IQR)
No, and I have no plans to	42	28	11	6	9.6 ± 18.2	0 (0–15)
No, and I am undecided	33	2	3	13	36.4 ± 20.8	42.5 (18.75–50)
No, but I plan on it	19	0	1	0	10 ± N/A	10 (N/A)
Yes	29	1	3	0	8.8 ± 8.5	7.5 (1.25–17.5)
Open to consultation with REI						
Yes	30	3	3	12	35.8 ± 26.1	42.5 (8.75–51.25)

Note: IQR = interquartile range; REI = reproductive endocrinology and infertility; SD = standard deviation.
[a] Score 0 = no regret; 1–24 = mild regret; ≥25 = moderate to severe regret.

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making was in process for others. For those in whom the decision was in process, it may be difficult to interpret results from the Decisional Regret Scale, which evaluates completed decisions. The Decisional Conflict Scale, which evaluates a decision that has yet to be made, may be more appropriate in this subgroup. However, it is worth noting that a higher decisional regret score has been associated with higher decisional conflict (7). Nevertheless, we propose that the Decisional Conflict Scale be used in future studies given that the decision to pursue fertility preservation may be ongoing and fluid for individuals who have not undergone gonadectomy (12).

Strengths of the present study include uniformity in services and consultations offered to all patients enrolled at the GHP at the time of intake. Our study population of 397 transgender and gender-diverse individuals is the largest U.S. population studied for interest in referral to reproductive services. In addition, our patient demographics from the follow-up study are largely reflective of the transgender population in the United States based on the 2015 Behavioral Risk Factor Surveillance System survey (13). In that survey, 199,113 respondents in 22 states were contacted and asked, "Do you consider yourself to be transgender?" Of those respondents, 764 identified as transgender (239 transgender men and 369 transgender women); 56.4% of the transgender individuals identified as white, 49.7% were employed, and 74.8% had completed at least high school. This is comparable to our follow-up survey population wherein 65.2% were white, 62.1% employed, and 97.0% had completed at least high school.

A limitation to our study includes the variation in demographics between the intake survey of 397 respondents and the follow-up study of 70 respondents, including mean age, which may decrease external validity. However, these data may be skewed, as a large number of patients chose not to disclose demographic information in the intake survey, such as sexual orientation. We speculate that our vulnerable population felt uncomfortable revealing this information over the telephone to an unknown entity. The percentage of respondents who chose to disclose demographic information in the follow-up survey increased (Table 1), which may indicate a willingness to answer more sensitive questions among the cohort electing to participate in research. In addition, the size of our sample population decreased from 397 to 70 for the follow-up survey after we introduced an opt-in function to be contacted for research. Given the vulnerability of this patient population, we felt that it was important to have permission before contacting patients, even through anonymous surveys. This response bias is a limitation we felt comfortable with in having to respect the privacy of these individuals.

CONCLUSION

Given our findings, we propose that transgender and gender-diverse individuals interested in fertility preservation undergo a consultation with a reproductive endocrinologist to reduce regret surrounding the decision to or not to undergo fertility preservation and clarify perceived barriers. Larger studies, including those evaluating decisional conflict, are needed to better understand the family planning and fertility preservation goals of this population.

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Acceso, barreras, y arrepentimiento de la decisión en la búsqueda de la de preservación de la fertilidad entre individuos transgénero y de género diverso.

Objetivo: Consultar individuos transgénero y de género diverso sobre su deseo de preservación de la fertilidad, barreras percibidas al acceso a la atención, y arrepentimiento de la decisión.

Diseño: Transversal.

Escenario: No aplica

Paciente(s): Un total de 397 individuos de género diverso llevando a cabo el uso del Programa de Salud de Género de la Universidad de California , Los Angeles desde enero 2018 a marzo 2019. Setenta participaron en una encuesta de seguimiento desde septiembre a octubre de 2019 aclarando intenciones o deseos reproductivos.

Intervención(es): Cuestionario de opción múltiple.

Medida(s) de resultado principal: Barreras percibidas al acceso a la preservación de la fertilidad y arrepentimiento de la decisión alrededor de la opción de buscar preservación de la fertilidad medidos con el uso de Escala de Arrepentimiento Decisión validada (puntuación 0 a 100).

Resultado(s): Las barreras para el acceso a la atención fueron principalmente coste del tratamiento (36%), discontinuidad/retraso de terapia hormonal (19%), o empeoramiento de la disforia de género con tratamiento/ embarazo (11%). Los encuestados indicaron que sus metas de planificación familiar fueron abordadas por proveedores de atención primaria y/o endocrinólogos (múltiples respuestas permitidas), pero 37% declararon que sus metas de planificación familiar no fueron adecuadamente abordadas. Aquellos que habían tomado una decisión firme de búsqueda o no búsqueda de tratamiento de fertilidad tuvieron arrepentimiento de decisión medio. Arrepentimiento de decisión moderado a severo se observó en aquellos estaban indecisos con respecto a la búsqueda de preservación de la fertilidad antes de la transición y en aquellos que estaban interesados en ser remitidos a endocrinología reproductiva.

Conclusión(es): La consulta con un endocrinólogo reproductivo podría reducir el arrepentimiento de la decisión así como aclarar barreras para la preservación de la fertilidad percibidas por individuos transgénero y de género diverso interesados en preservación de la fertilidad.

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FERTILITY PRESERVATION

Decision regret, and other mental health outcomes, following fertility preservation in the transgender individual compared to the cisgender woman

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Abstract

Purpose This study aimed to (1) determine differences in depression, anxiety, body image, quality-of-life (QOL), and decision regret scale (DRS) scores in transgender individuals undergoing fertility preservation (FP) compared to those who decline and (2) determine if DRS score following FP varies between transgender individuals and cisgender women.

Methods Sixteen transgender birth–assigned (BA) females and 13 BA males, undergoing FP consultation at an academic center between January 2016 and November 2019, were compared to each other and cisgender cohorts with pre-existing data: 201 women undergoing elective oocyte cryopreservation (EOC) between 2012 and 2016 and 44 women with cancer undergoing FP between 1993 and 2007. Outcomes included demographics; validated scales for depression, anxiety, body image, QOL (see below) in the trans cohort; DRS score in all three cohorts.

Results Of 29 transgender individuals participating, 10 BA females (62%) and 12 BA males (92%) underwent FP. Beck Depression Inventory II, Hospital Anxiety and Depression Scale, Body Image Scale for Transsexuals, Satisfaction with Life Scale, Short Form Health Survey-36, and DRS scores were not significantly different between trans individuals who underwent FP and those who declined. On univariate modeling, regret was significantly lower in transpeople undergoing FP compared to those who did not (OR 0.118, $p=0.03$). BA female and BA male transpatients undergoing FP reported DRS median scores 5 (mean 9) and 7.5 (mean 15), respectively, both were not significantly different from cisgender women ($p=0.97$, $p=0.25$) nor from each other ($p=0.43$).

Conclusions Depression, anxiety, body image, and QOL, in a group of individuals presenting for FP consultation, appear similar between transpeople undergoing FP and not, while regret is significantly lower in those choosing FP. FP is an option for transgender individuals without significant differences in regret compared to cisgender women.

Keywords Transgender · Fertility preservation · Decision regret

Introduction

Transgender individuals, those who identify with a gender different from sex assigned at birth, desire to have children for the same reasons as their cisgender counterparts: intimacy, nurturance, and family [1]. While the right to procreate has traditionally been granted to heterosexual, fertile couples, the advent of assisted reproductive technologies (ART) has revolutionized reproductive rights for many including transgender persons, who are now a growing group seeking reproductive care and fertility preservation (FP) [2, 3].

Fertility preservation allows one to take definitive steps to improve the odds of biological reproduction when predicted to have significant infertility [4]. The World Professional Association for Transgender Health (WPATH), American Society of Reproductive Medicine (ASRM), and Endocrine Society all recommend counseling trans individuals on options for fertility prior to medical or surgical therapy [5]. FP for postpubertal transmen mainly involves controlled ovarian stimulation for oocyte

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or embryo cryopreservation, and ejaculation or extraction with sperm cryopreservation for postpubertal transwomen [2].

Gender-affirming medical treatments in people with gender dysphoria have notably documented increased quality-of-life (QOL), decreased depression, and anxiety [6–9]. In those seeking gender-affirming surgeries, significantly improved QOL, body image, and overall psychiatric functioning have been reported [10–13]. Studies report the prevalence of regret following gender-affirming surgery as extremely low (1%) [10, 14]. While medical and surgical therapy in gender affirmation has been associated with improved mental health, little is known about the effect of FP on mental health outcomes in transgender people.

Regret has been evaluated in other cisgender populations following FP, namely, women with cancer undergoing medically indicated FP prior to chemotherapy or radiation and those undergoing elective oocyte cryopreservation (EOC) [15, 16]. Cancer survivors pursuing FP experience significantly less regret and greater QOL than those who do not [15]. In an observational study of cisgender women undergoing EOC, decision regret scale scores were reported with a mean of 10 (range 0–100, > 25 indicating moderate to severe regret) with reduced regret associated with receiving adequate information and anticipated probability of achieving a live birth [16]. After a qualitative study noted that genital exams and hormonal treatments can trigger dysphoria and gender incongruence in transmen undergoing FP, a cross-sectional study of 70 transpeople reported on overall decisional regret surrounding the choice to pursue FP [17, 18]. However, comparisons of regret between trans versus cisgender individuals following FP or between trans people undergoing FP versus not undergoing FP have never been reported in the existing literature and would be important to elucidate..

The objectives of this study were (1) to determine differences in depression, anxiety, body image, quality-of-life (QOL), and decision regret scale (DRS) score in transgender individuals who undergo FP compared to those who do not and (2) to determine if DRS score following FP varies between transgender individuals and cisgender women. We posited the hypothesis that transpeople who underwent FP have lower DRS scores than those who do not and that transdiverse patients undergoing FP do not have a significantly different DRS score compared to cisgender women undergoing FP.

Materials/methods

Populations

Cohort 1: Transgender individuals

Following Institutional Review Board approval, all transgender individuals, both birth-assigned males and females, 13 years of age or older, presenting for FP consultation at the University of California, San Francisco Center for Reproductive Health between January 2016 to November 2019, were identified and retrospectively contacted by telephone and follow-up email for study participation. Those desiring participation provided consent with their electronic signatures and were sent a secure link to a web-based survey administered through the RedCap platform (Vanderbilt University). Email and phone reminders were sent to ensure completion 1 week after expressing desire to participate. Minors less than 18 years of age required parental consent and were encouraged to complete the survey with their own responses. The survey was intended to capture data from both people who underwent FP and those who decided against it following initial consultation.

Queried items included basic demographics and the following standardized scales: Beck Depression Inventory II (BDI-II), Hospital Anxiety and Depression Scale (HADS) (anxiety component only), Body Image Scale for Transsexuals (BIS), Satisfaction with Life Scale (SLS), Short Form Health Survey (SF-36), and Decision Regret Scale. A final free text box was provided for trans respondents to address any specific details pertinent to decision regret they desired to share that were not addressed in the questionnaire.

Cohort 2: Cisgender women undergoing elective oocyte cryopreservation

The first comparison cohort of cisgender women was pulled from existing data obtained at our same institution previously; those undergoing elective oocyte cryopreservation were retrospectively recruited from 2012 to 2016. Excluded individuals included those who froze eggs for medical indications (i.e., a cancer or gender dysphoria diagnosis anticipating potentially gonadotoxic therapy), underwent oocyte donation, or intended to pursue in vitro fertilization but did not have available sperm on the day of egg retrieval. Participants provided electronic consent and completed an online survey with demographics, validated decision regret scale, and additional questions specific to the experience of oocyte cryopreservation.

Cohort 3: Cisgender women with cancer undergoing medically indicated oocyte cryopreservation

The second cisgender female comparison cohort was also pulled from pre-existing data from a study at our academic institution. These women received cancer diagnoses, between 1993 and 2007 at 18–40 years of age, and were recruited through the California Cancer Registry (CCR). Women with non-gynecologic cancer diagnoses (leukemia, Hodgkin disease, non-Hodgkin lymphoma, breast cancer, or gastrointestinal) were contacted between January and

September 2010. An initial contact letter was sent to potential participants describing the survey, followed by a second mailing that included a consent form, the written survey, and link to an online survey. Women were encouraged to complete the paper or online versions. Those without response within 3 weeks received a telephone follow-up call and a reminder postcard with the online survey link. DRS raw scores were converted to a 100-point scale to allow comparison to other cohorts.

Validated scales used

BDI-II is a 21-item multiple choice inventory to screen for depression in normal populations [19, 20]. Each of the 21 items is rated on a 4-point scale ranging from 0 to 3, with total scores ranging from 0 to 63, where scores of 0–13 represent "minimal" depression, 14–19 are "mild," 20–28 are "moderate," and 29–63 are "severe" [20].

The HADS questionnaire consists of seven questions each for anxiety and depression to diagnose and track progression of symptoms pertinent to each disorder [21]. HADS scoring ranges from 0 (low) to 3 (highest anxiety/depression) for each item, and total subscale score > 8 out of 21 denotes considerable anxiety or depression (0–7 normal, 8–10 mild, 11–14 moderate, 12–21 severe) [21, 22]. Only the anxiety component was used in this study, as depression was formally assessed with the BDI-II.

BIS allows quantification of body image satisfaction in those with gender dysphoria through a list of 30 body features, where satisfaction is rated on a 5-point Likert scale [23]. Higher scores reflect higher body dissatisfaction, with six subscales reported as means: social and hair, head and neck, muscularity and posture, hip, breasts, genitalia [23, 24].

The SWLS is a 5-item scale intended to measure satisfaction with life as a whole, where participants strongly disagree (score of 1) to strongly agree (score of 7) to five statements [25]. Summing the items leads to a final score ranging from 5 to 35 (low–high satisfaction) [25]. It is one of the most commonly-used scales for measuring well-being [26].

SF-36 is the most widely used measure of quality-of-life (QoL) in research, used in over 4000 publications [27]. Adapted as a short form from the original 149-item Medical Outcomes Study, the RAND-36 ranges from 0 to 100, where higher scores define more favorable health states [28]. Items are averaged to create scores for each of eight health domains: physical functioning, bodily pain, limitations due to physical health, limitations due to emotional problems, emotional well-being, social functioning, energy, and general health perceptions [28]. Only the emotional well-being score was used in this study. SF-36 has been used in several studies assessing the QoL of transgender individuals on gender-affirming hormone therapy [29–32].

The DRS is a validated, 5-item scale measuring level of regret regarding a specific medical decision, namely, fertility preservation [33]. Scores range from 0 (absent regret) to 100 (extreme regret) with scores interpreted as follows: 0, no regret; 1–25, mild regret; and > 25, moderate to severe regret [33].

Statistics

A power calculation to determine necessary sample size in the transgender cohort was completed using available data on cisgender decision regret scores from elective oocyte cryopreservers. A 150% increase of the known decision regret score available for cisgender people undergoing EOC (mean 10.1, SD 14.99) would represent a clinically meaningful difference suggesting a change in qualitative regret from mild to moderate (10.1 to 25). A sample size of 16 total transgender individuals would be required to achieve an 80% power at a 5% significance level [34]. Twenty-two total trans individuals (10 BA females and 12 BA males) who underwent FP were included in this study.

Statistical analysis was performed using STATA version 16.1 (Stata Corp., College Station, TX). Descriptive statistics were used for demographic variables, chi-square test for comparison of categorical variables, and Wilcoxon rank-sum (Mann–Whitney) test for comparison of continuous nonparametric variables. Following conversion of DRS scores to a dichotomous outcome, where scores > 25 indicated moderate to severe regret, univariate logistical regression models were completed to determine variables associated with higher regret. Statistical significance was considered at $p < 0.05$.

Results

Response rates

Cohort 1

A total of 60 transdiverse patients were contacted for study inclusion; 32 were birth-assigned (BA) females and 28 were BA males. Nineteen BA females signed consents with 16 (50%) completing the survey and 17 BA males consented with 13 (46%) reaching survey completion (Fig. 1). Fourteen BA females (88%) identified with the male gender, 1 (6%) identified as gender non-binary, and 1 (6%) identified as nonconforming. Eleven BA males (85%) identified with the female gender and 2 (15%) as genderqueer.

Of the 16 BA-female participants, 10 (63%) chose to undergo oocyte cryopreservation, while 12 (92%) of 13 BA males chose to undergo sperm cryopreservation. Common reasons for declining FP included need to be off of gender-affirming therapy (5/7, 71%), not desiring biological

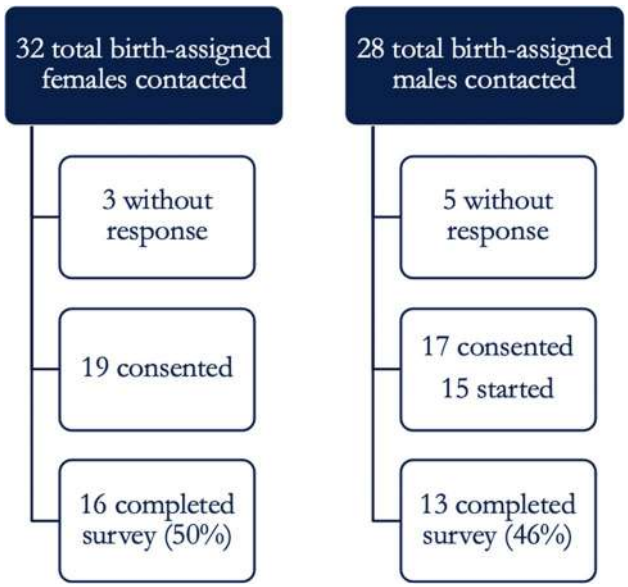

Fig. 1 Cohort I of transgender individuals contacted with response rates

children (3/7, 43%), and fear of dysphoria from transvaginal ultrasounds in BA females (2/6, 33%). Follow-up interval between FP and survey submission averaged 1.5 years in trans patients. Only one of ten BA females (10%) undergoing FP have since returned to use their frozen eggs to embryo creation, and ultimately live birth in a cis-gender female partner. There were two of 12 BA males (17%) with sperm cryo who have since returned to use their sperm.

There were five teenagers less than 20 years surveyed (range 15.4–19.2), with three (60%) proceeding with FP. All surveyed teenagers were BA females.

Cohort 2

Of the 503 survey invitations e-mailed to cisgender women having FP, 16 were undeliverable and 487 were successfully delivered. A total of 201 cisgender participants (41%) completed the survey. None of these participants identified as transgender. Average follow-up interval between FP and survey completion was 2 years.

Cohort 3

Of the 4112 women with cancer contacted, 1686 (41%) completed the survey. Nineteen respondents reported their gender as transgender (1) or other (18) and were removed from cis-cohort inclusion. Forty-seven patients (2.8%) reported freezing eggs or embryos, while 1639 (97.2%) did not undergo FP. Of the 47 who self-identified as female and froze gametes, three patients lacked complete raw data for DRS scores. A total of 44 patients met study inclusion as they identified as cisgender women, froze gametes, and completed all components of the DRS score.

Demographics

Demographics of respondents by sex assignments at birth are presented in Table 1. Respondents assigned female at birth were younger, more likely to be multiracial, less educated, and less likely to have FP compared to those assigned male at birth. All teenage participants were assigned female at birth. Trans BA females who underwent FP were similar in age and education but more likely to be single (6/10, 60%), when compared to those who did not undergo FP (1/6, 17% single). All four partnered transmale patients who underwent FP had cisgender female partners. Of the five partnered transmale patients who did not undergo FP, three had partners assigned to a female sex with transgender or gender nonconforming identities, and two had cisgender male partners.

Demographics of transgender patients undergoing FP compared to cisgender cohorts with either elective oocyte cryopreservation or FP due to cancer are presented in Table 2. Trans individuals were significantly younger than the cisgender elective oocyte cryopreservation (EOC) group by about 9 years but similar in age to the cisgender cancer group. Trans participants were significantly less likely to have a post-graduate degree compared to all cisgender counterparts. They were significantly more likely to be partnered

Table 1 Demographics of birth-assigned female versus male respondents. GAT = gender affirming treatment (medical); GAS = gender affirming surgery

	Assigned female at birth ($N = 16$)	Assigned male at birth ($N = 13$)
Mean age (years, range)	25.5 (15.4–39.9)	31.2 (21.8–47.04)
Ethnicity		
African-American	2	0
Asian	7	3
Caucasian	11	8
Hispanic	0	2
Multiracial	5	0
Education (%)		
Some college or less	11 (69%)	4 (31%)
College graduate	0	6 (46%)
Post graduate	5	2 (15%)
Had partner	9 (56%)	8 (62%)
Married (%)	3 (19%)	4 (31%)
Had history of GAT	13 (81%)	13 (100%)
Had GAS	11 (69%)	7 (54%)
Ever sexually active	12 (75%)	10 (77%)
Had Fert Pres	10 (63%)	12 (92%)

or married compared to the EOC group but less likely to be partnered or married compared to the cancer FP cohort.

Mental health outcomes in trans cohort

Validated mental health outcomes are presented for BA females who underwent FP compared to those who declined FP (Table 3). BA females undergoing FP had mean scores reflecting minimal depression (7.7, SD 7.75), mild regret (9, SD 10.22), normal level of anxiety (4.7, SD 3.86), and neutral satisfaction with life (20.6, SD 8.54). While no significance levels were reached in all evaluated parameters, BA females who declined FP had higher depression, regret, and anxiety scores that approached significance and were qualitatively higher than their counterparts who had FP.

Corresponding scores are also depicted for BA males (Table 4) who underwent FP compared to those who declined; however, no *p*-values were computed as only one participant in this group declined FP. BA males who underwent FP also had minimal depression (9.92, SD 7.70), mild regret (15, SD 17.19), normal anxiety (5.67, SD 3.39), and neutral satisfaction with life scores (20.17, SD 6.82).

Decision regret

Of the 22 transpeople who underwent FP, nine (41%) had a DRS score of 0, no regret at freezing gametes. Ten (45%) reported minimal regret with DRS score ranges 1–25, and three (14%) reported moderate to severe regret. The highest score reported in this group was 50, from a BA female. Of the seven transpeople who declined FP, two (29%) had no regret, one (14%) had minimal regret, and four (57%) had moderate-severe regret. Highest score in nonfreezers was 75, from the only BA male participant who declined FP. Any free texted answers at the end of the survey were reviewed qualitatively to determine relevance to study limitations or potential future study directions.

Univariate logistic regression modeling of all trans respondent dichotomous DRS scores (> 25 for moderate to severe regret) identified that those who froze gametes had a

Table 2 Demographics of trans vs cisgender respondents undergoing fertility preservation. Percentages are out of available data points, as some respondents did not answer all questions. *P*-values are in comparison to the transgender FP cohort

	Trans FP (*N* = 22)	Cis EOC (*N* = 201)	Cis cancer FP (*N* = 44)	All Cis FP (*N* = 2fs45)
Age, y (SD)	27.83 (7.78)	36.45 (3.08) *p* < 0.0001	27.70 (5.91) *p* = 0.99	34.88 (5.03) *p* < 0.0001
Education				
Some college or less	10 (48%)	1 (0.5%)	7 (17%)	8 (3%)
College graduate	6 (29%)	44 (22%)	22 (52%)	66 (27%)
Post graduate	5 (24%)	156 (78%) *p* < 0.001	13 (31%) *p* = 0.03	169 (70%) *p* < 0.001
Had partner (%)	11 (52%)	49 (24%) *p* = 0.01	33 (75%) *p* = 0.03	82 (34%) *p* = 0.12
Married (%)	5 (24%)	2 (1%) *p* < 0.001	15 (34%) *p* = 0.001	17 (7%) *p* = 0.20

Table 3 Standardized mental health scores for transgender birth–assigned females

	Assigned female at birth underwent FP (*N* = 10)	Assigned female at birth declined FP (*N* = 6)	*p*-value
Depression (SD) [BDI-II]	7.7 (7.75)	14 (6.07)	0.08
Decision Regret (SD) [CRS]	9 (10.22)	25 (21.45)	0.10
Anxiety (SD) [HADS]	4.7 (3.86)	8 (4.86)	0.11
Body Image (SD) (BIS)	2.90 (0.94)	2.92 (0.39)	0.66
Satisfaction with Life (SD) [SWLS]	20.6 (8.54)	22 (7.62)	0.79
Emotional QOL (SD) [SF-36]	69.2 (16.55)	62 (10.35)	0.44

BDI-II, Beck Depression Index — 0–13 = minimal; 14–19 = mild; 20–28 = moderate; 20–63 = severe. *DRS*, Decision Regret Scale — 0 = no regret; 1–25 = mild; > 25 = moderate to severe. *HADS*, Hospital Anxiety and Depression Score — only anxiety component. 0–7 = normal; 8–10 = borderline; 11–21 = abnormal. *BIS*, Body Image Scale — range 1–5. 1 = very satisfied; 5 = very dissatisfied. *SWLS*, Satisfaction with Life Scale — 5–9 = extremely dissatisfied; 10–14 = dissatisfied; 15–19 = slightly dissatisfied; 20 = neutral; 21–25 = slightly satisfied; 26–30 = satisfied; 31–35 = extremely satisfied. *SF-36*, Short From-36 — range 0–100. Higher score more favorable health state

Table 4 Standardized mental health scores for transgender birth–assigned males

	Assigned male at birth underwent FP ($N=12$)	Assigned male at birth declined FP ($N=1$)
Depression (SD) [BDI-II]	9.92 (7.70)	3
Decision Regret (SD) [CRS]	15 (17.19)	75
Anxiety (SD) [HADS]	5.67 (3.39)	0
Body Image (SD) (BIS)	2.96 (0.55)	2.72
Satisfaction with Life (SD) [SWLS]	20.17 (6.82)	35
Emotional QOL (SD) [SF-36]	63.33 (17.71)	92

BDI-II: Beck Depression Index — 0–13 = minimal; 14–19 = mild; 20–28 = moderate; 20–63 = severe. DRS: Decision Regret Scale — 0 = no regret; 1–25 = mild; > 25 = moderate to severe. HADS: Hospital Anxiety and Depression Score — only anxiety component. 0–7 = normal; 8–10 = borderline; 11–21 = abnormal. BIS: Body Image Scale — range 1–5. 1 = very satisfied; 5 = very dissatisfied. SWLS: Satisfaction with Life Scale — 5–9 = extremely dissatisfied; 10–14 = dissatisfied; 15–19 = slightly dissatisfied; 20 = neutral; 21–25 = slightly satisfied; 26–30 = satisfied; 31–35 = extremely satisfied. SF-36: Short From-36 — range 0–100. Higher score more favorable health state

significantly lower DRS score than those who did not (OR 0.118, $p=0.03$, 95% CI 0.017–0.816). Age, education, presence of partners, and marriage status were not associated with higher regret in trans individuals.

Comparisons of transgender individuals' DRS scores to cisgender persons who underwent EOC and FP due to cancer are shown in Table 5. Mean DRS score for BA males was 15 (SD 17.19), for BA females was 9 (SD 10.22), and for all trans respondents was 12.27 (SD 14.45). Comparison means for the cisgender EOC group was 10.10 (SD 14.99), and the cancer FP group was 15.45 (SD 23.17). *p*-values reflect that means noted in each trans FP group were not significantly different from levels reported in both cisgender cohorts. All means qualitatively fell into the mild regret category.

Discussion

Transpeople undergoing FP have increased recently as awareness and education efforts expand, and barriers to care are critically evaluated and addressed [35, 36]. FP in transpeople may present additional challenges that could worsen dysphoria: cessation of gender-affirming treatments leading to further dysphoria, psychological challenge of acquiring a semen specimen in transfemales, the possibility of transvaginal imaging in transmales, and inaccurate pronoun usage or use of dysphoric anatomical terms such as "vagina," "penis," or "ovaries" [2, 4]. It is imperative to study mental health outcomes following a procedure such as FP, with the potential consequence of worsening dysphoria in the transgender patient.

Although case reports and observational studies reporting on clinical outcomes in gender-diverse individual undergoing FP are increasing, mental health outcomes surrounding FP are largely unaddressed [3, 37, 38]. Armuand et al. reported on the experiences of transmen who underwent FP using qualitative thematic analysis of interviews; however, no quantitative analyses were conducted [17]. The only existing study to report on quality-of-life measures in transpeople following FP was a small observational pilot that reported transmasculine people experienced more healthy days compared to transfeminine people according to the CDC health-related QoL survey; this was presented as a conference abstract and not formally reviewed at the time of this publication [39]. Our study is the first to evaluate various mental health outcomes,

Table 5 DRS score by gender–Cis FP by cohort compared to all trans people and birth-assigned females

Trans cohorts	DRS scores by gender cohort			
	Trans FP	Cis elective FP ($N=192$)	Cis cancer FP ($N=44$)	All Cis FP ($N=236$)
All Trans Patients with FP, N = 22 (SD)	12.27 (14.45)	10.1 (14.99) $p=0.31$	15,045 (23.17) $p=0.90$	11.10 (16.89) $p=0.39$
All birth-assigned women with FP, N = 10 (SD)	9.00 (10.22)	10.1 (14.99) $p=0.89$	15.45 (23.17) $p=0.74$	11.10 (16.89) $p=0.97$

including decision regret, following FP in transgender individuals and compare regret to transgender counterparts who deferred FP as well as to cisgender women.

It is important to acknowledge that the motive for FP can present variations to comparative analysis. Cisgender women with cancer diagnoses face a potential loss of reproductive capacity with gonadotoxic treatments, while cisgender women undergoing EOC exercise their reproductive choice to delay childbearing [40]. While it is not known whether testosterone therapy in transmales definitely poses a gonadotoxic environment, professional societies recommend FP in transpeople prior to initiation of gender-affirming treatments, suggesting closer alignment with cisgender cancer patients receiving gonadotoxic chemotherapy [1, 41]. Analysis was conducted to assess for potential differences amongst people with ovaries who, despite different motivations and likely mental and emotional experiences, underwent the same physical process. We, unfortunately, did not have a similar cohort of cisgender males undergoing sperm cryopreservation to compare with trans women who underwent FP (see limitations).

Survey response rates were similar in both cisgender groups (41%) and higher in the transgender group (46–50%). Mean age of 27.8 years in trans individuals seeking FP was comparable to cisgender cancer patients undergoing FP, but significantly younger than cisgender women undergoing EOC. All of the teenagers evaluated in our study were birth-assigned females, contributing to the younger age in BA females compared to males. Consistent with age, cisgender women having EOC or FP related to cancer had a higher tendency to hold both college and post-graduate degrees. Transpatients are more likely to be partnered or married compared to cisgender elective preservers but less likely to have partners or be married compared to cancer preservers. It is interesting to note that almost all birth-assigned males (92%) completed the FP process of sperm cryopreservation, compared to only 62% of birth-assigned females completing oocyte cryopreservation. This discrepancy is likely due in large part to the relative ease and low cost of cryopreserving sperm as compared to oocytes.

Our study uses standardized scores for depression, anxiety, body image, satisfaction with life, and emotional quality-of-life and reassuringly, found no significant differences between individuals assigned female at birth who underwent FP compared to those who declined FP. There was a trend towards higher depression and anxiety scores amongst BA females who declined FP compared to those who had FP, which we hope to further elucidate in future studies with a larger sample size. All noted mean scores in transmasculine individuals who underwent FP were consistent with minimal depression, normal anxiety, and neutral satisfaction with life. This is an important finding to add to the existing literature as it suggests that FP does not appear to worsen mental health outcomes.

Decision regret is in the mild range for all transgender individuals who underwent FP. Transmen who chose not to undergo FP were interestingly more likely to be partnered and with either a cisgender male or a genderdiverse individual, compared to partnered transmen who underwent FP all had cisgender female partners. Of the transmen who underwent FP, only one has since returned to use their frozen eggs to create embryos with donor sperm for transfer into their cis-gender female partner, with successful live birth. It is possible that people with a clear plan to use their gametes, those with a partner actively desiring pregnancy, may have less decisional regret. Univariate logistic regression indicated that those transpeople who completed FP had significantly lower decision regret compared to those who did not. This is consistent with known studies in cancer cohorts linking FP to lower regret as well as a study reporting on DRS scores in a group of trans and gender-diverse individuals [15, 18]. The latter cross-sectional study of 70 trans-respondents reported that those who made a firm decision to pursue or not pursue fertility treatment had mild regret, while those who were undecided regarding FP had moderate-to-severe regret [18]. The study also noted that for those where the decision for FP was in process, it may be difficult to interpret DRS results as these scores pertain to completed decisions [18]. Trans individuals in our study who answered "no" to the statement "I decided to freeze my eggs or sperm" could certainly have been still contemplating their final decision, therefore having higher decisional conflict consistent with higher regret scores [42]. Decision regret in transpeople undergoing FP does not appear to be significantly different from cisgender people undergoing FP.

DRS scores can present an overly simplified version of regret. Regret may not be a single construct but rather exist on multiple levels [43]. Process regret reflects self-blame for making a hasty or uninformed decision, role regret presents if the individual feels they have taken a passive role in the decision-making process, and outcome regret occurs when the consequence of a decision is poorer than an alternate outcome would have been [43]. Interestingly, in the freetext portion of our study, one BA male respondent noted "while I did not regret the decision to freeze my sperm, I regretted the manner in which I had to undergo ejaculation to obtain a specimen. I wish I had been informed of other methods to extract sperm instead." While DRS scores represent easy, quantitative values used for comparisons, they may neglect some of the nuanced aspects of regret as a whole.

Strengths of our study include the use of validated instruments to assess mental health parameters, including decision regret, representing the first study to publish on various psychological outcomes following FP.

Additionally, our study is the first to report regret following FP in transpatients compared to a respective cisgender cohort.

Our study does have several limitations. There was a limited survey response rate, though it was higher in the transgender group compared to cisgender cohorts. Methodology was retrospective in nature, allowing the introduction of recall bias. Our comparison cohort was a cisgender female group; we did not have an available cisgender male cohort undergoing sperm cryopreservation to whom we could compare our transgender BA males, who may have different levels of regret given the differences in the FP process. Our comparative cisgender cohorts were surveyed at an earlier point in time, due to the presence of existing data, and compared to more recent data obtained from our growing trans population. While it is possible that timing at which data were obtained may have had an effect on the overall outcome, the follow-up intervals between time of FP and surveys were similar in both cohorts. Our power calculation representing the necessary sample size in the transgender cohort assumed a clinically meaningful change in DRS score of 150%, which is high. To allow a smaller increment rise in DRS score, a larger sample size would be necessary and is the goal of future studies as we continue our efforts in this emerging field.

While our study evaluated trans BA male freezing sperm and BA female freezing oocytes, the comparison cisgender group consisted only of women. It would be interesting to note decision regret in transgender females compared to cisgender males freezing sperm, whether as sperm donors or for medically indicated reasons such as cancer. The length of time on gender-affirming therapy and the relationship to decision regret after FP would be an interesting topic to further explore. The cisgender group included retrospective data only; hence, we were not able to evaluate those who presented for consultation and chose not to undergo FP, which we were able to capture this in our trans group. It would be interesting to evaluate if all people, regardless of gender, who decline FP tend towards more regret in the future. Similarly, evaluating mental health scores using validated instruments both prior to and following FP in a longitudinal cohort study would reveal the role of FP in changing mental health outcomes. Larger, multicenter trials addressing mental health outcomes are necessary to corroborate the findings reported here.

Conclusion

In conclusion, choosing to undergo FP, in a group of individuals presenting for FP consultation, does not seem to significantly alter depression, anxiety, body image, satisfaction with life, or emotional QOL in trans individuals. Transpeople who undergo FP have lower regret than those who do not. Mean levels of decision regret for transgender individuals undergoing FP are not different from cisgender women undergoing elective oocyte cryopreservation or cancer-related FP. Hence, fertility preservation is a viable and safe option for transgender individuals seeking genetic children without significant changes in regret compared to cisgender women.

Data Availability The data that support the findings of this study are available on request from the corresponding author, VS.

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Fertility preservation for transgender adolescents and young adults: a systematic review

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BACKGROUND: Many transgender individuals choose to undergo gender-affirming hormone treatment (GAHT) and/or sex reassignment surgery (SRS) to alleviate the distress that is associated with gender dysphoria. Although these treatment options often succeed in alleviating such symptoms, they can also negatively impact future reproductive potential.

OBJECTIVE AND RATIONALE: The purpose of this systematic review was to synthesize the available psychosocial and medical literature on fertility preservation (FP) for transgender adolescents and young adults (TAYAs), to identify gaps in the current research and provide suggestions for future research directions.

SEARCH METHODS: A systematic review of English peer-reviewed papers published from 2001 onwards, using the preferred reporting items for systematic reviews and meta-analyses protocols (PRISMA-P) guidelines, was conducted. Four journal databases (Ovid MEDLINE, PubMed Medline, Ovid Embase and Ovid PsychINFO) were used to identify all relevant studies exploring psychosocial or medical aspects of FP in TAYAs. The search strategy used a combination of subject headings and generic terms related to the study topic and population. Bibliographies of the selected articles were also hand searched and cross-checked to ensure comprehensive coverage. All selected papers were independently reviewed by the co-authors. Characteristics of the studies, objectives and key findings were extracted, and a systematic review was conducted.

OUTCOMES: Included in the study were 19 psychosocial-based research papers and 21 medical-based research papers that explore fertility-related aspects specific for this population. Key psychosocial themes included the desire to have children for TAYAs; FP discussions, counselling and referrals provided by healthcare providers (HCPs); FP utilization; the attitudes, knowledge and beliefs of TAYAs, HCPs and the parents/guardians of TAYAs; and barriers to accessing FP. Key medical themes included fertility-related effects of GAHT, FP options and outcomes. From a synthesis of the literature, we conclude that there are many barriers preventing TAYAs from pursuing FP, including a lack of awareness of FP options, high costs, invasiveness of the available procedures and the potential psychological impact of the FP process. The available medical data on the reproductive effects of GAHT are diverse, and while detrimental effects are anticipated, the extent to which these effects are reversible is unknown.

WIDER IMPLICATIONS: FP counselling should begin as early as possible as a standard of care before GAHT to allow time for informed decisions. The current lack of high-quality medical data specific to FP counselling practice for this population means there is a reliance on expert opinion and extrapolation from studies in the cisgender population. Future research should include large-scale cohort studies (preferably multi-

Key words: transgender / gender dysphoria / gender reassignment / sex reassignment / fertility preservation / cryopreservation / oocyte cryopreservation / sperm cryopreservation / systematic review

Introduction

Gender dysphoria is a condition in which a person experiences internal psychological conflict due to incongruence of his/her gender assigned at birth and the gender with which he/she identifies (Coleman *et al.*, 2012). The estimated prevalence of adults who identify as 'transgender' or 'gender nonconforming' in the USA is 0.6% of the population (Flores *et al.*, 2016; Crissman *et al.*, 2017; Herman *et al.*, 2017). Based on these data, it has been estimated that 0.7% of youth aged 13 to 17 years identify as transgender (Rafferty, 2018); estimations that are much higher than those previously made (Coleman *et al.*, 2012; Arcelus *et al.*, 2015) and which may differ according to definition (Collin *et al.*, 2016) and geographical location (T'Sjoen *et al.*, 2019). Despite an increasing focus on transgender health research, gender nonconforming youth and young adults remain an underserved population that is seen more often by health care professionals generally and in fertility clinics (Arcelus *et al.*, 2015; Wiepjes *et al.*, 2018).

Many, though not all, transgender adolescents and young adults (TAYAs) choose to undergo treatment to alleviate the distress associated with gender dysphoria (see Table I for glossary of terms) (Meriggiola and Gava, 2015a, 2015b; T'Sjoen *et al.*, 2019). Treatment of transgender persons should be individual and multidisciplinary and consist of medical treatment aimed at suppression of assigned gender sexual characteristics, gender-affirming hormone treatment (GAHT) and/or sex reassignment surgery (SRS). While these treatment options often help alleviate the symptoms of gender dysphoria, decrease depression and increase self-esteem (Gorin-Lazard *et al.*, 2013), there are significant fertility risks that should be considered prior to commencing the transition process. SRS is irreversible and most often leads to sterility. The effect of GAHT on fertility appears to be somewhat unpredictable in terms of its possible long-term negative impact on fertility preservation (FP) outcomes and future reproductive potential (Hembree *et al.*, 2009; Hembree *et al.*, 2017).

Psychosocial studies have reported that many transgender individuals are eager to medically transition, and some may even choose to start GAHT during their adolescence (Auer *et al.*, 2018). Due to the young age of these individuals, their future of having biological children may not yet be a concern (Wierckx *et al.*, 2012a); however, it is likely that some may develop a desire to have biological children later in life. The decision to transition early may cause these individuals to later regret undergoing these treatments and/or procedures without considering

Table I Glossary of terms.

Term	Definition
Cisgender[a]	A term for a person whose gender identity matches the gender they were assigned at birth; someone who is not trans.
Cisnormative/cisnormativity[a]	The assumption, in individuals or in institutions, that everyone is cisgender (not trans).
Gender dysphoria	A mental state involving an internal conflict between one's gender assigned at birth and the gender with which he/she/they identify.
Gender identity[b]	A person's intrinsic sense of being male (a boy or a man), female (a girl or woman) or an alternative gender (e.g. boygirl, girlboy, transgender, genderqueer and eunuch) (Bockting, 1999; Stoller, 1964).
Heteronormative/Heteronormativity[a]	The assumption, in individuals or in institutions, that everyone is heterosexual.
Non-binary	One's gender is not exclusively male or female.
Transgender[b]	Adjective to describe a diverse group of individuals who cross or transcend culturally defined categories of gender. The gender identity of transgender people differs to varying degrees from the sex they were assigned at birth (Bockting, 1999).
Transgender man (FtM)[b]	Adjective to describe individuals assigned female at birth who are changing or who have changed their body and/or gender role from birth-assigned female to a more masculine body or role.
Transgender woman (MtF)[b]	Adjective to describe individuals assigned male at birth who are changing or who have changed their body and/or gender role from birth-assigned male to a more feminine body or role.
Transition[b]	Period of time when individuals change from the gender role associated with their sex assigned at birth to a different gender role. For many people, this involves learning how to live socially in another gender role; for others this means finding a gender role and expression that are most comfortable for them. Transition may or may not include feminization or masculinization of the body through hormones or other medical procedures. The nature and duration of transition are variable and individualized.
Transphobia[a]	Fear or hatred of trans people; discrimination directed toward people who are or are presumed to be trans.
Transsexual[b]	Adjective (often applied by the medical profession) to describe individuals who seek to change or who have changed their primary and/or secondary sex characteristics through feminizing or masculinizing medical interventions (hormones and/or surgery), typically accompanied by a permanent change in gender role.

[a]Definition copied verbatim from James-Abra *et al.*, (2015).
[b]Definition copied verbatim from the World Professional Association for Transgender Health *Standards of Care* Version 7 by Coleman *et al.* (2012).

the fertility risks and future limitations to their family building plan (De Sutter *et al.*, 2002; Wierckx, *et al.*, 2012b).

Several organizations, including the World Professional Association for Transgender Health (WPATH), American College of Obstetrics and Gynecology (ACOG), the American Society of Reproductive Medicine (ASRM) and the Endocrine Society, have issued guidelines recommending that all transgender individuals receive counselling prior to transitioning on the possible negative impacts of GAHT and SRS on fertility and the subsequent available FP options (Coleman *et al.*, 2012; Ethics Committee of the ASRM, 2015; Committee on Adolescent Health Care of the ACOG, 2017; Hembree *et al.*, 2017; Rafferty, 2018).

The mainstay of FP medical options includes oocyte cryopreservation, embryo cryopreservation and ovarian tissue cryopreservation (OTC) for transgender men (FtM) and sperm cryopreservation and testicular tissue cryopreservation (TTC) for transgender women (MtF) (James-Abra *et al.*, 2015; Martinez, 2017). It should be noted that TTC (Picton *et al.*, 2015) and spermatogonial stem cell (SSC) cryopreservation (Sato *et al.*, 2011; Vassena *et al.*, 2015; Sun *et al.*, 2018) are generally considered experimental. OTC is not readily accessible in some countries (Lee *et al.*, 2006; Loren *et al.*, 2013; Zarandi *et al.*, 2018). Embryo cryopreservation for single individuals may not be an acceptable option because of the use of donor gametes to create embryos. Thus, oocyte and sperm cryopreservation are the most commonly utilized FP options for these individuals (James-Abra *et al.*, 2015). The young age of TAYAs pursuing GAHT and lack of high-quality data create a dilemma when counselling patients regarding fertility risk and FP (see Table II for an overview of FP options).

The reproductive needs of TAYAs have largely been ignored by medical practitioners (Hunger, 2012), though these individuals may be very interested in their future potential of having children. While some studies suggest that TAYAs are concerned with the idea of postponing GAHT in order to preserve their fertility (Armuand *et al.*, 2017; Bartholomaeus and Riggs, 2019), there are several other factors that may prevent TAYAs from pursuing FP. TAYAs may not be aware of the fertility risks of undergoing GAHT without receiving proper FP counselling (Coleman *et al.*, 2012). Given the oocyte cryopreservation process is an expensive medical procedure, cost can be a major barrier preventing FtM TAYAs from pursuing FP (James-Abra *et al.*, 2015). Several countries, such as Canada (Government of Ontario, 2017) and Sweden (Armuand *et al.*, 2017), cover some or all of the cryopreservation costs for individuals diagnosed with gender dysphoria; however, TAYAs who lack knowledge regarding FP may not even be aware of these subsidies. It is crucial to explore the attitudes, knowledge and beliefs of healthcare providers (HCPs), given the position they are in to discuss with TAYAs the fertility risks associated with GAHT and to inform them of FP options. It is also important to explore the attitudes, knowledge and beliefs of the parents/guardians of TAYAs.

Table II Fertility preservation options for TAYAs.

	Method	Procedure	Considerations	Application
MtF transgenders	Sperm cryopreservation	• Masturbation • Assisted ejaculation (vibratory stimulation or electro-stimulation	• Clinically available • Post-pubertal	• Female partner: use for IUI, IVF or IVF/ICSI and ET, depending on sperm quality • Male/no partner: need for donor oocytes and surrogacy
	Surgical sperm retrieval	• Percutaneous sperm aspiration/extraction	• Clinically available • Invasive • Post-pubertal	• Female partner: IVF/ICSI and ET • Male/no partner: need of donor oocytes and surrogacy
	Testicular tissue cryopreservation and spermatogonium cell cryopreservation	• Surgical biopsy of testicular tissue • Can potentially be done at SRS	• Experimental • Pre- or post-pubertal	• Requires in vitro maturation • Female partner: IVF/ICSI and ET • Male/no partner: need of donor oocyte and surrogacy
FtM transgenders	Embryo cryopreservation	• COH and oocyte retrieval with fertilization of mature oocytes and cryopreservation of embryos	• Clinically available • Invasive • Post-pubertal • Need of sperm	• Male partner: use of partner's sperm, need for surrogate if no uterus • Female/no partner: need for donor sperm. Embryo can be transferred into partner's uterus
	Oocyte cryopreservation	• COH and oocyte retrieval with cryopreservation of mature oocytes	• Clinically available • Invasive • Post-pubertal • No partner required	• Male partner: use of partner's sperm, need for surrogate if no uterus • Female/no partner: need for donor sperm. Embryo can be transferred into partner's uterus
	In Vitro Maturation (IVM)	• Retrieval of immature oocytes with or without mild ovarian stimulation • Ex vivo collection of immature oocytes at the time of SRS	• In vivo: clinically available, invasive • Ex vivo-experimental • No partner required	• Male partner: use of partner's sperm, need for surrogate if no uterus • Female/no partner: need for donor sperm. Embryo can be transferred into partner's uterus
	Ovarian tissue cryopreservation	• Surgical excision of ovarian tissue for cryopreservation • Can be performed at the time of SRS	• Clinically available • Pre- and post-pubertal • No need for COH • No partner required	• Re-transplantation of cryopreserved tissue and possible IVF (clinically proven) • In vitro maturation and use of partner's or donor sperm (experimental at this stage)

COH: controlled ovarian stimulation; ET: embryo transfer; FP: fertility preservation; SRS: sex reassignment surgery; TAYAs: transgender adolescents and young adults.

Since many transgender individuals are often adolescents during the time of GAHT, their parents/guardians may have a significant impact on the FP decision-making process.

This systematic review aims to examine both the psychosocial and medical aspects of FP in TAYAs. First, to better understand the facilitators and barriers for TAYAs to undergo FP, we examine the attitudes, knowledge and beliefs of TAYAs, their parents/guardians and HCPs on FP. Second, we conduct a review of current data regarding the risks of GAHT, FP options and outcomes specific to this population. In addition, we identify future research directions that are vital to improve awareness and knowledge of FP for TAYAs.

Methods

Ethical approval

This study was a systematic review of aggregated published data so no formal ethical approval was required.

Population and outcomes

A comprehensive review of studies evaluating all aspects of FP among TAYAs, both psychosocial and medical, was conducted. The population was TAYAs, their guardians and HCPs. For the psychosocial review, the primary outcomes were FP counselling and utilization rates; the secondary outcomes were TAYAs' desire to have children, as well as the attitudes, knowledge and beliefs of TAYAs, their parents and HCPs. For the medical review, the primary outcomes were complications and GAHT effects on testes and ovaries (semen parameters, number of mature oocytes, testicular/ovarian morphology, hormone levels and ultrasound appearance), and secondary outcomes were pregnancy and live birth rates after use of preserved gametes.

Search strategy

This systematic review of the literature was undertaken in accordance to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (Moher *et al.*, 2009). The protocols for

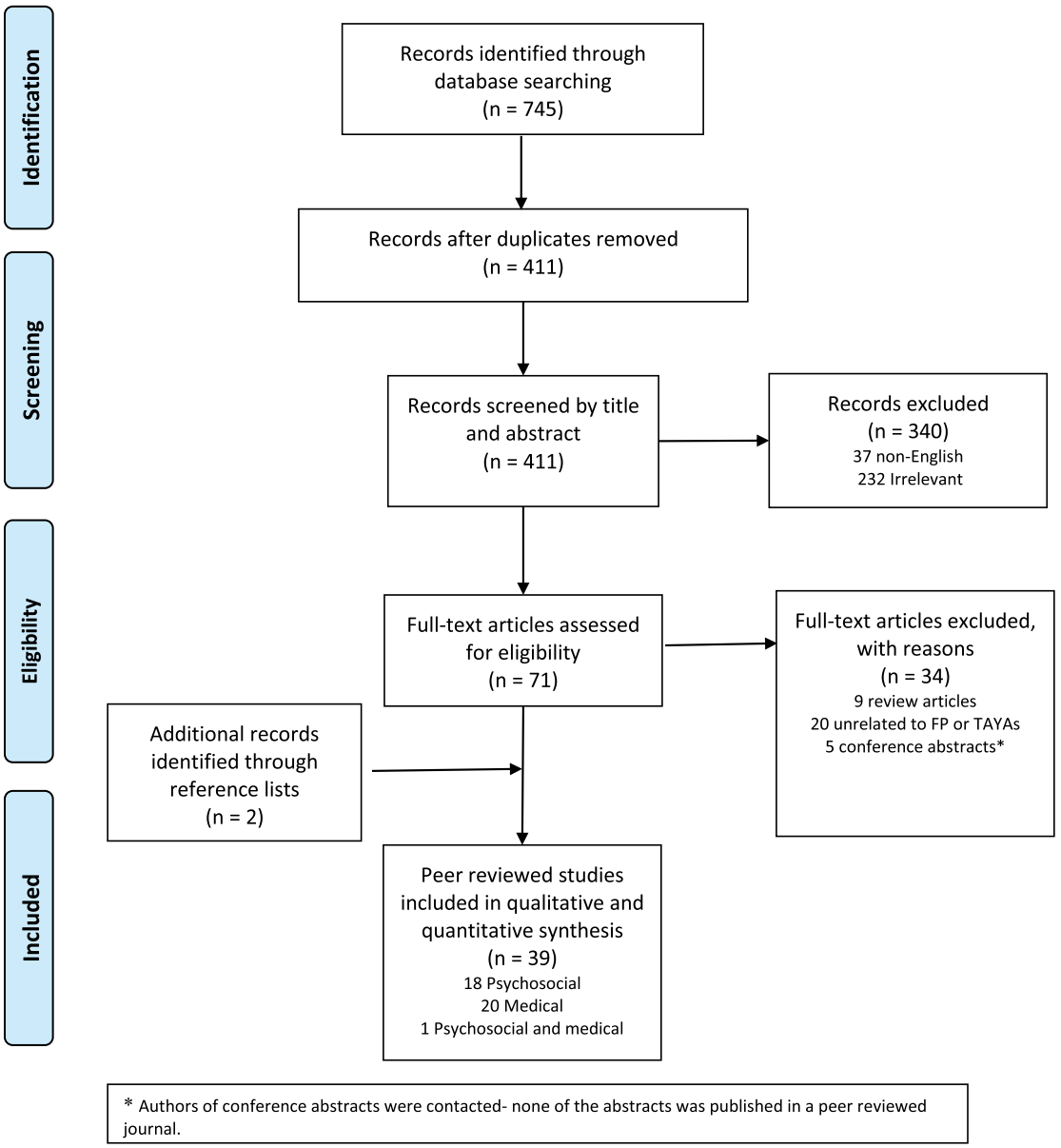

Figure 1 PRISMA 2009 flow diagram for a systematic review of fertility preservation for transgender adolescents and young adults. TAYAs: transgender adolescents and young adults, FP: fertility preservation

searching and assessing the literature were determined prior to the start of the literature search.

Information sources

The search strategy was developed by the research team in consultation with an experienced research librarian. A comprehensive literature search using four electronic databases (Ovid MEDLINE®, PubMed® Medline, Ovid Embase® and Ovid PsychINFO®) was conducted to identify all relevant studies relating to the study topic. Examples of search keywords included 'transgender', 'transwomen', 'trans-women', 'transmen', 'trans-men', 'transsexual*', 'gender dysphoria', 'sex reassignment', 'gender affirming', 'gender reassignment' or 'gender transitioning' and 'fertility preservation' or 'reproductive technologies' (see Supplementary Table SI for all the search terms and search strategy). The search was conducted using appropriate controlled vocabularies and a combination of subject headings and generic terms. The final search was conducted on 22 March 2019 to ensure inclusion of all relevant studies.

Study selection

Given that FP for TAYAs is a relatively recent practice, we included studies published from 2001 onward. Only peer-reviewed papers,

articles written in English and studies involving human subjects were included. Exclusion criteria were articles in the form of abstracts, clinical reviews, clinical overviews, opinion pieces, editorial letters, media reports and theses/dissertations. The bibliographies of the included studies were cross-checked and hand searched for further references to ensure comprehensive coverage of all relevant papers.

Study screening

All selected abstracts following the first search ($n = 745$) were screened to eliminate duplicates and were subsequently reviewed independently by three authors (S.B., S.A.M. and S.Y.) based on the PRISMA 2009 guidelines using the inclusion and exclusion criteria referenced above. As shown in the PRISMA flow diagram (Figure 1), 340 articles were excluded based on title and abstract screening, 71 full text articles were assessed for eligibility, of which 19 were included for the psychosocial analysis and 19 were included for the medical analysis (one of which is included in both). Of those excluded articles, 9 were review papers, 20 were deemed unrelated to FP in TAYAs and 5 were conference abstracts that were not published in a peer-reviewed journal as confirmed by the corresponding authors. Two additional articles were added to the medical review after snowballing the references of articles identified (for a total of 21 publications). Disagreements regarding inclusion or exclusion were resolved first by discussion and deliberation and, if needed, by the senior author (C.L.L.). See Tables III–V for a summarized description of the studies.

Quality assessment

Quality assessment was conducted using a modified checklist by Downs and Black (1998). Two common modifications were made to better suit the type of studies included and to aid in coding. This resulted in a 27-item checklist for assessment of the methodological quality. The criteria covered in the Downs and Black checklist included quality of reporting, internal validity (bias and confounding), power, as well as external validity. Each study received a score between 0 and 27. Since many of the categories in Downs and Black (1998) are not applicable for studies with a small sample size, small case series and case reports with a sample size of $\leq$5 were automatically appraised as poor quality without a formal assessment. Quality assessment was performed independently by the co-authors (S.A.M. and S.Y. for the psychosocial review; S.B. and C.L.L. for the medical review) and an experienced statistician. Differences in rating were resolved through discussion until consensus was reached.

Data extraction

Two data extraction spreadsheets were developed and agreed upon between the co-authors. The selected studies were comprehensively assessed; all relevant data were extracted and entered into the spreadsheets by S.A.M. and S.Y. for the psychosocial review and by S.B. for the medical review and cross-checked by C.L.L. Information selected included author details, year of publication, country of study, study objectives, sample size, data collection time period, methodology, sample characteristics, outcomes and findings. Disagreements were resolved by discussion and if needed by the senior author (C.L.L.).

Results

Sample description

The 19 papers selected for the systematic review of psychosocial aspects include chart reviews (4), prospective interviews (1), retrospective interviews (4) and cross-sectional questionnaires (10), from seven countries: Australia (3), Belgium (2), Canada (3), Germany (1), Netherlands (1), Sweden (2) and the USA (7) (Table II). Among the 19 papers, 6 US papers (Johnson *et al.*, 2016; Chen *et al.*, 2017; Nahata *et al.* 2017; Kyweluk *et al.*, 2018; Chen *et al.*, 2019; Tishelman *et al.*, 2019) and 3 Australian papers (von Doussa *et al.*, 2015; Riggs and Bartholomaeus, 2018; Bartholomaeus and Riggs, 2019) were published by teams comprising overlapping co-authors. The sampling groups of these papers are FtM TAYAs (two), MtF TAYAs (two), both MtF and FtM TAYAs (nine), both MtF and FtM TAYAs as well as their parents (two) and HCPs (four). Data collection sites in these studies include fertility clinics (two), medical centers (one), pediatric transgender clinics (six), gender clinics (one) and organizations and groups (nine). Two sets of papers presented different sub-data sets from the same project that examined either the views of TAYAs (Riggs and Bartholomaeus, 2018; Bartholomaeus and Riggs, 2019) or HCPs (Chen *et al.*, 2019; Tishelman *et al.*, 2019). The topics discussed in the papers include the desire to have children; FP discussions, counselling and referrals; FP utilization; attitudes, knowledge and beliefs of TAYAs, HCPs and the parents of TAYAs and barriers to accessing FP (Supplementary Table SII).

The 21 papers selected for the systematic review of medical FP aspects include prospective cohort studies (5), observational cohort studies (1), observational case control studies (1), retrospective chart reviews (10), case series (3) and case reports (1) from nine countries: Belgium (3), Canada (1), France (1), Germany (2), Italy (1), Japan (1), Netherlands (2), Thailand (1), USA (8) and USA/Belgium (1) (Tables IV and V). Studies evaluated both MtF and FtM FP-related aspects. Among topics discussed in the selected papers are GAHT effects on testis and ovaries, effect on spermatogenesis and oocytes and FP outcomes.

The quality assessment using the Downs and Black (1998) checklist is shown in Tables III–V (see Supplementary Tables SIII and SIV for the full quality assessment). The average score was 12.16 (range 6–18) for the psychosocial papers and 12.76 (range 8–18) for the medical papers. Papers exploring MtF TAYAs had an average score of 11.66, whereas those exploring FtM TAYAs had a higher average score of 14, yet four of the case series papers (Wallace *et al.*, 2014; Broughton and Omurtag, 2017; Maxwell *et al.*, 2017; Chen *et al.*, 2018) with a sample size of $\leq$5 were not rated, as discussed above.

Desire to have children

Eight papers discussed the desire of TAYAs to have children, with two studies focused on MtF TAYAs (De Sutter *et al.*, 2002; Brik *et al.*, 2019), one study on FtM TAYAs (Wierckx *et al.*, 2012b) and five studies on both groups (von Doussa *et al.*, 2015; Auer *et al.*, 2018; Riggs and Bartholomaeus 2018; Strang *et al.*, 2018; Chiniara *et al.*, 2019;). Between one-third and two-thirds of TAYAs desire to have children sometime in their lifetime, biological or otherwise, and changes of parenthood desire were noted throughout the transition process (Strang *et al.*, 2018). Before undergoing GAHT, the majority (65.4% MtF, 53% FtM) of TAYAs indicated that having children was

Table III Description of studies examining the FP psychosocial aspects in TAYAs.

Author (Year), Country	Study design	Sample size and group(s)	Age range, mean age (years)	Data collection site(s)	Data collection period	Objectives	D&B score
Armuand et al. (2017) Sweden	Prospective qualitative; semi-structured interview	n = 15 FtM	Age range 19–35, mean age 25	Single site involving a university hospital-based IVF clinic	Mar 2014 to Dec 2015	To explore the experiences of FtM in oocyte cryopreservation.	12
Auer et al. (2018) Germany	Cross-sectional quantitative; self-constructed questionnaire	n = 189 transgender people (99 MtF and 90 FtM)	Age range 26–51 (MtF); ages 20–37 (FtM)	Multi-sites involving four different medical centers	Nov 2013 to Oct 2016	To examine how the desire for children and the use of FP options vary among transgender women and transgender men in different transitioning stages.	18
Bartholomaeus & Riggs (2019) Australia	Qualitative analysis on comments provided in a self-constructed questionnaire	n = 295 transgender and non-binary adults	N/A	Convenience sample via Internet, organizations and groups	Jan to Feb 2018	To understand transgender and non-binary individuals' experiences with HCPs in FP.	14
Brik et al. (2019) Netherlands	Retrospective chart review	n = 35 MtF adolescents	Mean age 15	Single site involving a pediatric gender clinic at a medical center	June 2011 to Aug 2017	To examine the frequency of fertility information provision and FP discussion, and the factors associated with the use of FP.	17
Chen et al. (2017) USA	Retrospective chart review	n = 105 transgender adolescents	Not clearly specified	Single site involving a pediatric gender clinic at a children hospital	July 2013 to July 2016	To examine the FP utilization by transgender adolescents in a pediatric gender clinic.	13
Chen et al. (2019) USA	Cross-sectional quantitative; self-constructed questionnaire	n = 202 HCPs	N/A	Convenience sample at transgender health conferences	Aug to Nov 2017	To examine fertility knowledge, practice behaviours, and perceived barriers to fertility care among transgender HCPs.	14
Chiniara et al. (2019) Canada	Cross-sectional quantitative; self-constructed questionnaire	n = 79 transgender adolescents and 73 parents	Age range 12–18	Single site involving a pediatric gender clinic at a children hospital	Not specified	To investigate the views of young people with gender dysphoria and their parents on FP, reproductive and life priorities.	16
De Sutter et al. (2002) Belgium	Cross-sectional quantitative; self-constructed questionnaire	n = 121 MtF	70% were ages 30–50	Convenience sample via Internet, organizations and groups	April 2002 to Sept 2002	To explore how trans women feel about sperm banking prior to commencing any medical treatment.	10
James-Abra et al. (2015) Canada	Retrospective qualitative; semi-structured interview	n = 11 (9 trans people and their partners).	Age range 26–45	Convenience sample via Internet and by mail	Dec 2010 to Aug 2011	To explore the experiences of trans persons who sought assisted reproduction services.	12
Johnson et al. (2016) USA	Cross-sectional quantitative; self-constructed questionnaire	n = 12 HCPs	N/A	Convenience sample involving a multidisciplinary gender and sex diversity fertility working group	2015	To understand the perspectives and priorities of HCPs who care for gender and sex diverse patients.	6

(Continued)

Table III *(Continued)*

Author (Year), Country	Study design	Sample size and group(s)	Age range, mean age (years)	Data collection site(s)	Data collection period	Objectives	D&B score
Jones et al. (2016) Canada	Retrospective chart review	n = 14 (11 MtF and 3 FtM)	MtF: Age range 20–40, mean age 26; FtM: Age range 33–39, mean age 36	Single site involving a university-affiliated IVF clinic	Jan 2010 to May 2014	To identify all transgender patients referred for FP at one university-affiliated fertility clinic.	13
Kyweluk et al. (2018) USA	Retrospective qualitative; semi-structured interview	n = 18 (6 MtF and 12 FtM)	Age range 15–24	Single site involving a pediatric gender clinic at a children hospital	Dec 2016 to Aug 2017	To explore transgender youth's perspective on family building possibilities and imagined parenthood futures via FP technologies.	10
Nahata et al. (2017) USA	Retrospective chart review	n = 73 (23 MtF and 50 FtM adolescents)	Age range 9–18, median 15	Single site involving a pediatric gender clinic at a children hospital	Jan 2014 to Aug 2016	To examine the rates of fertility counselling and utilization of FP among a cohort of adolescents with gender dysphoria.	17
Payne & Erbenius (2018) Sweden	Retrospective qualitative; semi-structured interview	n = 7 HCPs and transgender advocates	N/A	Snowball sample involving professionals from different organizations	2015 to 2017	To explore the perspective of professionals on the changing practice and remaining obstacles of transgender fertility care.	3
Riggs & Bartholomaeus (2018) Australia	Cross-sectional quantitative; self-constructed questionnaire	n = 409 trans people (131 FtM, 97 MtF, and 181 non-binary or agender)	Age range 18–72, mean age 29	Convenience sample via Internet, organizations and groups	Jan to Feb 2018	To understand the FP decision making process of Australian transgender and non-binary individuals.	11
Strang et al. (2018) USA	Cross-sectional quantitative; standardized questionnaire	n = 51 trans youth and their parents (10 MtF, 14 FtM, 1 non-binary person, and 26 parents)	Not clearly specified	Single site involving an academic children's hospital	Not specified	To assess the attitudes of transgender youth and parents regarding the potential impact of GAHT on fertility and FP options.	11
Tishelman et al. (2019) USA	Qualitative analysis on comments provided in a self-constructed questionnaire.	n = 110 HCPs	N/A	Convenience sample via organizations and groups	Aug to Nov 2017	To examine provider perceptions of practice behaviors and barriers related to fertility counselling, FP and family building among transfer patients.	8
von Doussa et al. (2015) Australia	Retrospective qualitative; semi-structured interview	n = 13 transgender adults	Age range 23–62, mean age 47	Convenience sample via organizations and groups	Not specified	To explore the views and experiences of transgender adults on parenthood, and/or the ways they have negotiated parenthood with their transgender identity.	9
Wierckx et al. (2012) Belgium	Cross-sectional quantitative; self-constructed and standardized questionnaires	n = 50 FtM	Age range 22–54, mean age 37	Single site involving an academic hospital	Nov 2009 to Apr 2010	To provide information on the reproductive wishes of transsexual men after sex reassignment surgery.	17

HCPs: health care providers; D&B: Downs and Black (1998), GAHT: gender-affirming hormone treatment.

Table IV Description of studies examining FP medical aspects in MtF TAYAs.

Author (Year), Country	Study design	Sample size and group(s)	Mean age ± SD (range) (years)	Study period (years)	GAHT	Treatment median length and range	Objectives	Main outcomes	D&B score
Adeleye et al. (2019) USA	Retrospective cohort study	n = 28 (18—no prior GAHT use, 3—previous use, 5—current use)	(18–39.9)	2012–2018	Estrogen (oral 2–6 mg/transdermal 300 mcg/conjugated estrogen 0.625 mg/d) plus Spironolactone 50/100 mg BID/ Finasteride 2.5–5 mg/ micronized Progesterone 100 mg	30 months for current users 42 months for prior use (mean discontinuation 4.4 months)	To describe semen parameters in MtF TAYAs in the presence or absence of GAHT	Semen parameters	18
Hamada et al. (2015) USA/Belgium	Retrospective cohort study	n = 29	28.4 ± 1.1 (21–41)	2003–2011	None	N/A	To describe semen parameters in MtF TAYAs referred to sperm banking	Semen parameters	9
Jindarak et al. (2018) Thailand	Retrospective analysis	n = 173	26.09 ± 5.37	2000–2015	Oral contraceptive/ Estrogen ± antiandrogen/ Spironolactone	8.51 ± 4.67 years	To identify spermatogenesis abnormalities in MtF TAYAs at the time of SRS To analyze the association between level of infertility and duration of GAHT	Testicular histology and spermatogenesis	10
Jones et al. 2016 Canada	Retrospective case series	n = 11	28.5 (20–40)	2010–2014	Recorded for 6/11, medications not specified	0.33 months	To define the population of transgenders referred to a fertility clinic for FP	Semen parameters	12
Kent et al. (2018) USA	Retrospective chart review	n = 135	30 (18–76)	2014–2017	Spironolactone ± Estrogen ± Finasteride ± Progesterone	5 years (1–57 years)	To examine post orchiectomy specimens of MtF TAYAs in order to evaluate the testicular effects of GAHT on a microscopic level and malignant changes	Testicular histology and spermatogenesis	11
Leavy et al. (2017) Germany	Prospective cohort study	n = 9 (7 controls)	26–52		Estrogen (estradiol gel/oral) ± GnRH analogue/antiandrogen/ progestin/progesin	24 months (1–6 years)	To assess the effects of estrogen treatment on the functional morphology of the testis and on spermatogenesis in MtF TAYAs	Testicular cellular morphology and function	8

(Continued)

Table IV (Continued)

Author (Year), Country	Study design	Sample size and group(s)	Mean age ± SD (range) (years)	Study period (years)	GAHT	Treatment median length and range	Objectives	Main outcomes	D&B score
Li et al. (2018) USA	Retrospective analysis	n = 78 (compared to 141 healthy cisgender samples)	TAYA: 24.1 ± 7.6 Control: 36.0 ± 9.4	2006–2016	None	N/A	To assess the incidence of MtF TAYAs seeking sperm cryopreservation compared to cismen and compare semen parameters between the two populations	Semen parameters and cryosensitivity analysis	16
Matoso et al. (2018) USA	Retrospective cohort	n = 50 (99 specimens) compared with 15 controls	33 (21–63)	2015–2018	Estrogen + Spironolactone/ Finasteride/ Progesterone	6 months–20 years	To study the histologic and immunohistochemical finding in orchiectomy specimens from MtF TAYAs	Testicular histology	10
Schneider et al. (2015) Germany	Multicenter prospective cohort study	n = 108 (22—stopped GAHT 6 weeks prior to SRS; 51—stopped 2 weeks prior to SRS and 35—continued GAHT)	42	2012–2014	Cyproterone acetate (10-100 mg)/ Spironolactone ± Estrogen		To evaluate the effectiveness of GAHT. To investigate blood hormone levels and testicular histology on the day of SRS	Hormone levels; testicular histology	11

D&B:Downs and Black (1998); GAHT: gender-affirming hormone treatment; SRS: sex reassignment surgery.

important to them. By contrast, only about a quarter (22% MtF, 25% FtM) expressed a desire to have children during the GAHT process, although 7 in 10 TAYAs (70% MtF, 69% FtM) recalled that having children had been an important issue for them prior to the transitioning (Auer *et al.*, 2018). In regard to the importance of genetic relatedness, 12% (Strang *et al.*, 2018), 34% (Riggs and Bartholomaeus, 2018) and 50% (De Sutter *et al.*, 2002) of TAYAs preferred to have their own biological children, yet 9% (7 out of 79) would be frustrated if they could not have a biological child (Chiniara *et al.*, 2019). Almost half (48%) of TAYAs acknowledged that their feelings about having biological children could change in the future (Strang *et al.*, 2018).

FP discussion, counselling and referrals

Nine papers reported the findings related to FP discussion, counselling and referrals (von Doussa *et al.*, 2015; Armuand *et al.*, 2017; Chen *et al.*, 2017; Nahata *et al.*, 2017; Riggs and Bartholomaeus, 2018; Brik *et al.*, 2019; Bartholomaeus and Riggs, 2019; Chen *et al.*, 2019; Chiniara *et al.*, 2019). The process of FP discussion and referrals was described in three out of the four retrospective chart review studies conducted in pediatric gender clinics (Chen *et al.*, 2017; Nahata *et al.*, 2017; Chiniara *et al.*, 2019). Patients diagnosed with gender dysphoria were referred to pediatric endocrinologists who specialize in the care of TAYAs. During counselling sessions, both endocrinologists and other HCPs used a multidisciplinary team approach to meet with patients and families to discuss the risks and benefits of GAHT and the available FP options. Fertility discussion was incorporated in the clinic protocol, in that all TAYAs received a routine FP discussion through verbal counselling and provision of written information prior to commencing GAHT. Subsequent referrals for FP consultation were made by in-house social workers or practice nurses for patients interested in pursuing cryopreservation. Of these four studies, three showed a very high frequency of FP counselling before initiating GAHT, with discussion rates of 100% (79 out of 79) (Chiniara *et al.*, 2019), 98.6% (72 out of 73, mean age 16.5 years) (Nahata *et al.*, 2017) and 91% (32 out of 35, mean age 14.8 years) (Brik *et al.*, 2019). By contrast, only 13 out of 105 TAYAs (12.4%, mean age 16.5 years) received a formal FP consultation prior to initiating GAHT in the Chen *et al.* (2017) study.

Apart from the studies conducted at gender clinics, low discussion rates of the potential negative impacts of GAHT on future biological parenthood and available FP options were reported (von Doussa *et al.*, 2015; Riggs and Bartholomaeus, 2018; Bartholomaeus and Riggs, 2019). Some TAYAs had to initiate an FP discussion and asked for an FP referral, as opposed to being referred for FP counselling routinely as a standard protocol (Armuand *et al.*, 2017; Chen *et al.*, 2017). In an Australian study by Riggs and Bartholomaeus (2018), 68.2% (210 out of 308, mean age 28.5 years) of transgender participants did not recall receiving FP counselling or advice, and only 22.7% (70 out of 308) reported having such discussion. Among those who were counselled about FP, positive experiences were often reported when the endocrinologist was also a fertility specialist (James-Abra *et al.*, 2015), the HCPs were sensitive to and knowledgeable about transgender health care (von Doussa *et al.*, 2015; Armuand *et al.*, 2017) and the counselling was provided in trans-friendly clinic environments (James-Abra *et al.*, 2015; Auer *et al.*, 2018).

Table V Description of studies examining FP medical aspects in FtM TAYAs.

Author (Year), Country	Study design	Sample size	Mean age ± SD (range)	Study period	GAHT	Treatment median length (weeks/months ±SD) + range	Objectives	Main outcomes	D&B score
Broughton et al. (2017) USA	Case series	n = 3	31 (30–32)	2015–2016	Case 1: Testosterone depot (IM); Cases 2&3: none	26 months—discontinued for 3 months prior to FP	To describe one institutional experience with different clinical scenarios	Cycle description and results	
Caanen et al. (2015)) Netherlands	Observational prospective cohort study	n = 22	22.4 ± 6.8	2010–2012	Testosterone transdermal gel and aromatase inhibitor + GnRH analogue	24 weeks	To investigate the influence of androgenic treatment on AMH levels in FtM TAYAs	Hormone levels	15
Caanen et al. (2017) Netherlands	Prospective, observational, case control study	n = 56 (controls = 60)	22.8 (19.6–26.3)	2014–2015	Testosterone IM or transdermal	29.5 months	To identify the effect of long-term testosterone treatment on ovarian morphology, determined by 3D TVU in adult FtM	Polycystic ovarian morphology of 3D TVU and hormone levels	18
Chen et al. (2018) USA	Case series	n = 5	16.4 (14–18)		None	N/A	To report the feasibility of oocyte cryopreservation in FtM adolescents prior to initiating GAHT	Oocyte cryopreservation cycle results and parameters	
De Roo et al. (2017) Belgium	Prospective cohort study	n = 40	24.3 ± 6.15	2013–2015	IM/oral/transdermal testosterone	58.18 weeks ± 26.57 weeks	To investigate ovarian histology in FtM TAYAs and the possibility of ex-vivo harvesting of COC	Ovarian histology, oocyte in vitro maturation and spindle analysis	12
Grynberg et al. (2010) France	Retrospective cohort	n = 112	28.9 ± 0.9 (21–53)	2000–2006	Androgen treatment (mode not specified)	3.7 ± 0.6 years (2–9 years)	To describe the histological changes observed in the genital tract of FtM TAYAs	Ovarian, uterine and breast histology	12
Ikeda et al. (2013) Japan	Retrospective case control study	n = 11 (compared to 10 controls)	33.18 ± 5.99 (26–42)	2007–2011	Testosterone IM	38 months (17 months-14 years)	To examine the effect of high doses of exogenous androgen treatment on ovarian morphology	Ovarian histology, physical and biochemical parameters	14
Lierman et al. (2017) Belgium	Prospective cohort study	n = 16	24.1 ± 6.1		Testosterone (IM or transdermal gel)	53.6 ± 21 weeks	To assess vitrification and thawing survival of IVM oocytes harvested from COCs at the time of SRS	Oocyte maturation, cryopreservation survival and spindle analysis	13
Loverro et al. (2016) Italy	Prospective cohort study	n = 12	28 ± 6.58 (20–32)	2011–2013	Oral + IM testosterone	31.9 ± 14.3 months	To determine histologic and steroid receptor changes in the ovary, endometrium and myometrium om MtF TAYAs at the time of SRS	Histologic analysis on the uterus and ovaries	13

(Continued)

Table V Description of studies examining FP medical aspects in FtM TAYAs.

Author (Year), Country	Study design	Sample size	Mean age ± SD (range)	Study period	GAHT	Treatment median length (weeks/months ±SD) + range	Objectives	Main outcomes	D&B score
Maxwell et al. (2017) USA	Case series	n = 3	17–32		None	N/A	To present 3 cases of FtM TAYAs who underwent oocyte cryopreservation prior to initiation of GAHT	Oocyte preservation cycle results and pregnancy outcomes	
Tack et al. (2016) Belgium	Retrospective analysis	N = 45	15.1	2010–2015	Progestin monotherapy for 6 months followed by Progestin + testosterone IM	24 months (12.6 progestin alone + 11.4 P + T)	To analyze the impact of consecutive treatment with Progestin monotherapy and in combination with Testosterone on physical characteristics, safety, metabolic parameters and hormone levels in FtM TAYAs	Patient and treatment characteristics, treatment safety and hormone levels	15
Wallace et al. (2014) USA	Case report	n = 1	17		none	N/A	To report a case of oocyte cryopreservation for a FtM transgender	Oocyte preservation cycle results	

AMH: anti Müllerian hormone; COC: cumulus oocyte complex; PCOM: polycystic ovary morphology; TVU: transvaginal ultrasound; 3D TVU: 3-dimensional transvaginal ultrasound; D&B:Downs and Black (1998).

FP utilization

A total of 10 papers discussed FP utilization for TAYAs; however, the results of each paper differ in uptake rates ranging from 9.6% to 81.8% for MtF TAYAs, from 0% to 16.7% for FtM TAYAs and from 2.7% to 7% for both groups (Wierckx *et al.*, 2012b; Jones *et al.*, 2016; Armuand *et al.*, 2017; Chen *et al.*, 2017; Nahata *et al.*, 2017; Auer *et al.*, 2018; Kyweluk *et al.*, 2018; Riggs and Bartholomaeus, 2018; Brik *et al.*, 2019; Chiniara *et al.*, 2019). FP utilization rates among transgender adolescents were reported in four studies, and all showed high discrepancy between FP counselling and FP uptake rates. A Canadian study found that none of 79 transgender adolescents chose to pursue FP despite all being counselled about the potential negative impact of GAHT on fertility (Chiniara *et al.*, 2019). Of the 98.6% (72 out of 73) transgender adolescents who underwent a formal FP counselling in the Nahata *et al.* (2017) study, two MtF adolescents attempted sperm banking (2.7%) with one able to cryopreserve sperm successfully. Of the 13 in 105 TAYAs (12.4%) who received a formal FP consultation between 2013 and 2016 in the Chen *et al.* (2017) study, only 5 (4 Mtf and 1 FtM) chose to undergo FP (4.8%), all before initiating GAHT. A recent study by Brik *et al.* (2019) on a Dutch adolescent cohort reported a relatively high FP consultation rate, with 32 out of 35 (91%) of MtF TAYAs receiving formal FP consultation and 12 (34%) attempting FP, 9 of which successfully cryopreserved sperm. Finally, Kyweluk *et al.* (2018) interviewed 18 TAYAs on their views on FP. The study found that the majority of MtF TAYAs (66.7%, 4 out of 6) completed sperm banking after receiving FP consultation, yet only 16.7% (2 out of 12) FtM TAYAs proceeded with oocyte cryopreservation.

Other available studies examining transgender adult groups also showed low FP uptake rates. In the Auer *et al.* (2018) study, 9.6% of MtF adults cryopreserved their sperm, and 3.1% of FtM adults cryopreserved their oocytes (age not reported). In the Riggs and Bartholomaeus (2018) study, 28 (53% Mtf, 18% FtM and 29% non-binary/agender) out of 398 (7%) young adults underwent FP (mean age of 25.3 years). Of those individuals, 68% underwent FP prior to initiating GAHT.

Conversely, studies involving participants referred for FP consultation at fertility centers found higher FP utilization rates compared to other studies. Of the 15 FtM young adults who underwent oocyte cryopreservation in the Armuand *et al.* (2017) study, 7 (46.7%) FtM young adults initiated GAHT prior to undergoing FP, while 3 (20.0%) initiated GAHT after completing the process (ages 19–35 years). In the Jones *et al.* (2016) retrospective chart review study between 2010 and 2014 at an IVF clinic, 14 transgender patients (11 MtF, 3 FtM, mean age 28.5 years) received FP counselling, the majority of whom were first seen after initiating GAHT. Of the 11 MtF patients, 9 underwent sperm cryopreservation with an uptake rate of 81.8%. Of these nine patients, only one had so far used the cryopreserved sperm for fertility treatment that also led to a successful pregnancy. For various reasons, none of the three FtM patients underwent oocyte cryopreservation.

Attitudes/knowledge/beliefs of TAYAs

Five papers discussed the attitudes, knowledge and beliefs of TAYAs regarding FP (De Sutter *et al.*, 2002; Wierckx *et al.*, 2012b; Johnson *et al.*, 2016; Kyweluk *et al.*, 2018; Riggs and Bartholomaeus 2018). The studies found that some TAYAs did not pursue FP due to the lack of

awareness of their FP options. Of the TAYAs who did not undertake FP in the Riggs and Bartholomaeus (2018) study, over two-thirds (68%) indicated they were not informed by HCPs about their options to preserve fertility, and 317 out of 335 (94.6%) of participants were affirmative that FP should be offered routinely to all TAYAs.

Similarly, 77.1% of FtM TAYAs had not considered oocyte cryopreservation due to the lack of awareness of this as an option during GAHT, and 37.5% stated that they would have considered freezing their oocytes if this option was available to them prior to commencing GAHT (Wierckx *et al.*, 2012b). If sperm cryopreservation had been offered to MtF participants in the De Sutter *et al.* (2002) study, 51% indicated that they would have seriously thought about this FP option. This study also found a correlation between age and FP attitudes; over two-thirds (67%) of participants below age 40 years would have chosen sperm cryopreservation, compared to just over one-third (35%) of participants over age 40 years (De Sutter *et al.*, 2002). In a provider needs assessment survey, both a fertility questionnaire and a decision aid tool were recommended as methods to improve TAYAs' knowledge regarding fertility issues concerning transgender individuals and available FP options (Johnson *et al.*, 2016).

Attitudes/knowledge/beliefs of HCPs

Seven papers discussed the attitudes, knowledge and beliefs of HCPs regarding FP for TAYAs (James-Abra *et al.*, 2015; von Doussa *et al.*, 2015; Armuand *et al.*, 2017; Payne and Erbenius, 2018; Bartholomaeus and Riggs, 2019; Chen *et al.*, 2019; Tishelman *et al.*, 2019). Overall, TAYAs found that HCPs appeared to lack knowledge and expertise on managing the fertility needs of transgender people. Accordingly, they felt the need to educate themselves about transgender-related health services and fertility matters (James-Abra *et al.*, 2015). Some HCPs made incorrect assumptions when working with TAYAs, such as utilizing the incorrect name, pronoun or gender (James-Abra *et al.*, 2015; Armuand *et al.*, 2017; Chen *et al.*, 2017). Other HCPs failed to acknowledge the individuals' gender identities by perceiving them through a cis-normative lens (James-Abra *et al.*, 2015). Some HCPs failed to initiate a discussion on the reproductive risks of medically transitioning, and most did not discuss FP options or make FP referrals (James-Abra *et al.*, 2015; von Doussa *et al.*, 2015). Some patients had feelings of regret, upon reflection, of feeling that HCPs rushed them into undergoing medical transition procedures without considering their reproductive and FP needs (von Doussa *et al.*, 2015). In addition to lacking FP knowledge for TAYAs, it was reported that some HCPs treated transgender patients with disrespect and discrimination. This included participants being denied access to FP information and services by fertility clinic staff after disclosing their transgender identities (James-Abra *et al.*, 2015).

Of those participants who had positive experiences with HCPs, many had attended 'transgender-friendly' clinics and benefited from environments where HCPs used gender-neutral terminology and were educated about transgender-related health services (James-Abra *et al.*, 2015). Latest data on HCPs' fertility-related knowledge found that 94.6% (191 out of 202) of HCPs agreed with WPATH's recommendation of FP discussion and referral prior to commencing GAHT, and 91% (183 out of 202) 'always' or 'often' discussed the negative impact of GAHT on future fertility with TAYAs (Chen *et al.*, 2019). Positive attitudinal shift to the provision of transgender reproductive care in fertility clinics was noted when HCPs made efforts to un-learn cis-normative ways of thinking about family building (Payne and Erbenius, 2018).

Attitudes/knowledge/beliefs of the parents/guardians of TAYAs

Two papers discussed the attitudes, knowledge and beliefs of the parents/guardians of TAYAs regarding FP (Strang *et al.*, 2018; Chiniara *et al.*, 2019). In a transgender youth fertility attitudes questionnaire pilot trial (Strang *et al.*, 2018), nearly all parents (25out of 26) emphasized the importance of educating TAYAs on the fertility risks associated with GAHT. All but one parent stated that they would be angry if HCPs neglected to tell them that GAHT might impact their child's reproductive potential. Approximately 65% of parents indicated a wish that their child would have children at some point in their life. However, only 20.8% of parents stated that they would be disappointed if their child could not have biological children. Also, 52% of parents desired discussions about their child's FP options. While 54.2% of parents expressed a wish that their child would at least consider FP, only 29.1% desired that their child would undergo FP (Strang *et al.*, 2018).

Barriers to accessing FP

A total of 11 papers discussed the barriers to accessing FP, with 7 papers discussing the barriers for both MtF and FtM TAYAs (James-Abra *et al.*, 2015; von Doussa *et al.*, 2015; Jones *et al.*, 2016; Chen *et al.*, 2017; Nahata *et al.*, 2017; Kyweluk *et al.*, 2018; Riggs and Bartholomaeus, 2018), 2 papers discussing the barriers specifically for MtF TAYAs (De Sutter *et al.*, 2002; Brik *et al.*, 2019) and 2 other papers discussing the barriers specifically for FtM TAYAs (Wierckx *et al.*, 2012b; Armuand *et al.*, 2017). While these barriers often differed based on the transitioning gender, both groups were negatively impacted by HCPs' poor attitudes and inadequate knowledge of transgender reproductive care, with a variety of negative experiences being encountered by TAYAs. Many TAYAs never received FP counselling or written documents, thus preventing them from pursuing FP (James-Abra *et al.*, 2015; von Doussa *et al.*, 2015; Riggs and Bartholomaeus, 2018). Some TAYAs reported that their HCPs discriminated against them by denying them access to FP information and services (James-Abra *et al.*, 2015), mis-gendering them, treating them disrespectfully (Chen *et al.*, 2017) and assuming that TAYAs were not interested in biological parenthood (von Doussa *et al.*, 2015).

Another major barrier for both MtF and FtM TAYAs was the concern of stopping or delaying the start of GAHT for FP when they already found it stressful to cope with gender dysphoria (Nahata *et al.*, 2017; Chiniara *et al.*, 2019). Prior to undergoing FP, individuals who have started GAHT must discontinue treatment for several months to allow sperm production or ovulation to resume. For FtM TAYAs specifically, they must discontinue GAHT until menstruation resumes (Armuand *et al.*, 2017). Of those who undertook FP in the Riggs and Bartholomaeus (2018) study, the majority (57.7%) had purposely delayed their GAHT to allow time for FP. For some TAYAs, the urgency to proceed with GAHT overrode the desire to pursue FP (Nahata *et al.*, 2017).

For MtF TAYAs, less FP counselling was needed compared to FtM, and they were usually briefly informed of the sperm cryopreservation process to determine whether the procedure was the right decision for them. The main barrier for MtF TAYAs to FP was the psychological

inability to masturbate to produce a semen sample for freezing (De Sutter *et al*., 2002; Nahata *et al*., 2017). Many MtF TAYAs who already had difficulties masturbating due to their gender dysphoria found it even more difficult to masturbate in a medical setting. Some chose not to undergo FP because the mental burden of masturbating to produce a sperm sample was too great (De Sutter *et al*., 2002; Brik *et al*., 2019). Some MtF TAYAs could not utilize the FP service because the fees associated with sperm banking, and long-term storage was beyond their financial reach (Nahata *et al*., 2017; Kyweluk *et al*., 2018).

For FtM TAYAs, FP consultation provided by a fertility specialist was required for informed decisions regarding the medical procedures involved in oocyte cryopreservation; this included a detailed discussion about the time estimated to complete the process, the costs associated with the procedure, the medications utilized for ovarian stimulation, a description of the oocyte retrieval process and information about cryopreservation and storage (Jones *et al*., 2016). Some FtM TAYAs indicated that the referral wait time to a fertility center was long and stressful (Armuand *et al*., 2017). Since oocyte cryopreservation is expensive, cost was a significant barrier for FtM TAYAs wanting to preserve oocytes but who were unable to afford it (Jones *et al*., 2016; Chen *et al*., 2017). Some declined FP referrals knowing that the FP process was financially prohibitive to them despite their future parenthood desire (Nahata *et al*., 2017; Kyweluk *et al*., 2018; Riggs and Bartholomaeus, 2018). The process to cryopreserve oocytes includes invasiveness and risks associated with the oocyte retrieval process and the psychological stress of undergoing transvaginal ultrasound monitoring, all of which could further intensify gender dysphoria (Jones *et al*., 2016; Chen *et al*., 2017).

MtF TAYAs–related fertility concerns and effect of GAHT on fertility

Nine papers evaluated fertility-related aspects among MtF TAYAs and the effects of MtF GAHT on fertility (Table IV): four evaluated semen parameters (Hamada *et al*., 2015; Jones *et al*., 2016; Li *et al*., 2018; Adeleye *et al*., 2019) and five evaluated the effect on testicular histology and/or spermatogenesis (Schneider *et al*., 2015; Leavy *et al*., 2017; Jindarak *et al*., 2018; Kent *et al*., 2018; Matoso *et al*., 2018).

GAHT effects on semen parameters

A single retrospective study evaluated semen parameters in the presence (past and current) or absence of GAHT (Adeleye *et al*., 2019) in 69 samples produced by 28 patients. They found significantly reduced semen parameters among those with current GAHT, compared to both past and no treatment. Discontinuation of GAHT prior to semen cryopreservation was associated with improved parameters, although a trend towards diminished semen parameters remained. The small number of past and current GAHT users in this study ($n = 8$) makes it difficult to draw firm conclusions. One case series presented outcomes of nine MtF TAYAs, with a mean age of 26.4 years, who cryopreserved a median of two sperm samples, most performed 2 weeks apart. Of the 11, 6 had their GAHT duration recorded, and of these, the mean duration was 0.33 months. Median semen parameters for this cohort were within the normal reference range for the first sample, but the parameters were lower for the second sample (Jones *et al*., 2016).

Two studies that evaluated semen parameters in MtF TAYAs with no prior GAHT both found an increased incidence of semen abnormalities (Hamada *et al*., 2015; Li *et al*., 2018). Hamada *et al.* (2015) evaluated semen parameters in 29 MtF TAYAs, revealing a high proportion of abnormalities such as oligospermia, teratospermia and asthenospermia. The total motile count was abnormal in almost 75% of this cohort. Li *et al.* (2018) compared semen parameters of cryopreserved sperm from healthy cisgender and healthy MtF TAYAs. Although the transgender population in this study was younger than its cisgender counterparts and none received GAHT, they found the MtF TAYA group to have poorer semen quality for most semen parameters, particularly a higher proportion of oligospermia, and MtF TAYAs' sperm to be more cryosensitive.

GAHT effects on testicular morphology and spermatogenesis

Five studies (Jindarak *et al*., 2018; Kent *et al*., 2018; Leavy *et al*., 2017; Matoso *et al*., 2018; Schneider *et al*., 2015) evaluated the effects of GAHT on testicular histology and spermatogenesis, with diverse results. Median duration of GAHT in these studies ranged between 6 months and 8.5 years. GAHT consisted of either estrogen treatment alone or in combination with an anti-androgen or GnRH analogue. Schneider *et al.* (2015) evaluated serum hormone levels and testicular histology in 108 MtF TAYAs undergoing SRS. Prior to SRS, all patients received a combination of anti-androgens and estrogen or estrogen alone. Based on each clinic's usual practice, treatment was discontinued either 2 weeks or 6 weeks before SRS or not at all. Found to have normal spermatogenesis were 24% of histologic specimens, with the remainder revealing different degrees of impairment: 24% meiotic arrest, 35% spermatogonial arrest, 14.8% Sertoli cell only and 1.8% tubular shadows. Ongoing GAHT-induced feminized hormone blood levels as opposed to a virilized profile when GAHT is discontinued, yet no correlation between spermatogenesis and treatment protocol was observed (Merklinghaus *et al*., 2015; Schneider *et al*., 2015).

Three more recent studies found a much lower incidence of normal spermatogenesis among the samples, ranging between 0% and 11% normal (Jindarak *et al*., 2018; Kent *et al*., 2018; Matoso *et al*., 2018). The most common abnormality in all studies was maturation arrest ranging between 35% and 100%. Jindarak *et al.* (2018) examined samples from 173 MtF TAYAs undergoing SRS and found that in this cohort the proportion of normal spermatogenesis was 11%, with the rest being maturation arrest (36.4%), hypospermatogenesis (26%), Sertoli cell only (20.2%) and seminiferous hyalinization (6.4%). Kent *et al.* (2018) evaluated a cohort of 135 MtF TAYAs in which 79% had no evidence of spermatogenesis, 21% had some degree of spermatogenesis and only 4% had normal spermatogenesis. Similarly, the pathology in most of those with impaired spermatogenesis was maturation arrest. They also found a trend (not statistically significant) towards poorer spermatogenesis with longer duration of therapy. Matoso *et al.* (2018) evaluated samples from 50 MtF TAYAs and found abnormal spermatogenesis in all samples, with maturation arrest at either the spermatogonia (80%) or primary spermatocyte stage (20%).

Two of the studies also evaluated signs of malignancy, with no specimens showing malignant or premalignant changes (Kent *et al*., 2018; Matoso *et al*., 2018). Two of the studies (Leavy *et al*., 2017; Matoso *et al*., 2018) described other gross and histologic changes within orchiectomy samples from SRS, mainly changes in size and weight, decreased seminiferous tubule diameter, Sertoli and Leydig cell number and appearance and presence of vacuoles and fibrosis. Leavy *et al.* (2017) further investigated prolonged estrogen therapy effects on the functional morphology of the testis and found it exerts both

inhibiting and stimulatory effects depending on the testicular cell type. Their findings suggest that a combination of either estrogen and GnRH analogue or estrogen and anti-androgens inhibit spermatogenesis to a higher degree than estrogen alone. Of note in this study, one of the specimens treated for an extensive time period resulted in spermatocytes, spermatids and even spermatozoa being present in the tubules, suggesting the possibility of drug resistance.

FP outcomes in MtF TAYAs

Our search did not yield any large-scale studies regarding usage rates of cryopreserved sperm, live birth rates or pregnancy outcomes specific to the transgender population. Two case reports (Jones *et al.*, 2016; Broughton and Omurtag, 2017) outline the feasibility of the process. One presented nine MtF TAYAs with a mean age of 26.4 years who cryopreserved sperm obtained from fresh ejaculate after masturbation. The median number of banking events per patient was two. The mean number of straws frozen was 27.1. Only one of the nine had used their cryopreserved sperm for IVF-ICSI followed by frozen embryo transfer, resulting in a viable pregnancy (Jones *et al.*, 2016). The second case report presented three cases of individuals who utilized their cryopreserved gametes, one of which obtained an FP consult and cryopreserved four sperm samples prior to initiating GAHT at the age of 25 years. Samples were later used with her cisgender partner for IUIs, and later a cycle of IVF-ICSI, though all attempts were unsuccessful. Semen parameters in this case were reported as normal (Broughton and Omurtag, 2017). No studies on the use of surgical sperm retrieval (SSR) or TTC among MtF TAYAs were identified.

FtM TAYA related fertility concerns and effect of GAHT on fertility

Eight studies evaluated the effects of GAHT on fertility hormone levels, ovarian morphology and histology, as well as oocytes, with conflicting results (Grynberg *et al.*, 2010; Ikeda *et al.*, 2013; Caanen *et al.*, 2015; Loverro *et al.*, 2016; Tack *et al.*, 2016; Caanen *et al.*, 2017; De Roo *et al.*, 2017; Lierman *et al.*, 2017) (Table V).

GAHT effect on hormone levels and ultrasound appearance

Two studies measured and reported conflicting anti-müllerian hormone (AMH) levels: one reported a decrease in AMH level (Caanen *et al.*, 2015) in FtM TAYAs receiving GAHT, while the other reported no change (Tack *et al.*, 2016). Caanen *et al.* (2015) evaluated various hormone levels after 8 weeks of GnRH analogue treatment (considered the first sample) and again after 16 weeks of androgenic treatment. They found significant reductions in AMH and sex hormone-binding globulin, significant increases in total testosterone, androstenedione and FSH levels, and stable levels of inhibin B, LH and estradiol. Tack *et al.* (2016) evaluated 45 FtM TAYAs who had received GAHT for over 6 months (Progestin followed by Progestin plus testosterone, mean duration 12.6 months and 11.4 months, respectively) with no changes found in AMH level and suppression of both FSH and LH.

One study evaluated sonographic ovarian changes in FtM TAYAs taking GAHT (testosterone with or without GnRH analogue) for a median duration of 29.5 months, compared to a group of cisgender controls. The prevalence of polycystic ovary morphology based on 3-dimensional ultrasound did not differ between the FtM TAYAs and the control group, nor was there a difference within the TAYA group between those who received GnRH analogue and those who did not (Caanen *et al.*, 2017).

GAHT effects on ovarian histology

Four studies evaluated the effect of GAHT on ovarian histology. Median length of GAHT in these studies ranges between 58 weeks to 3.7 years (Grynberg *et al.*, 2010; Ikeda *et al.*, 2013; Loverro *et al.*, 2016; De Roo *et al.*, 2017). In two papers (n = 124) the majority of ovarian specimens' gross morphology and histology (79–82%) resembled that of polycystic ovary syndrome (PCOS) (Grynberg *et al.*, 2010; Loverro *et al.*, 2016). Two of the studies also evaluated endometrial histology. In one, the majority of samples exhibited proliferative endometrium (10/12) (Loverro *et al.*, 2016) and in the other study there were two distinct patterns: proliferative (54/112) and atrophic (50/112) (Grynberg *et al.*, 2010). Other studies evaluating ovarian histology revealed normal follicular distribution compared with controls (Ikeda *et al.*, 2013; De Roo *et al.*, 2017). One of the studies found a higher proportion of atretic follicles within the FtM TAYA group compared with age and BMI-matched controls (Ikeda et al., 2013). Among the histologic changes described within FtM TAYAs' ovaries were a thicker ovarian cortex, more hyperplastic collagen, ovarian stromal hyperplasia and stromal luteinization. No correlation was observed between hormone levels and follicle number or GAHT duration (De Roo *et al.*, 2017).

IVM in FtM TAYAs

Two studies (De Roo *et al.*, 2017; Lierman *et al.*, 2017) evaluated the option of IVM of oocytes from cumulus-oocyte complexes (COCs) retrieved from MtF TAYAs' ovaries at the time of SRS. De Roo *et al.* (2017) studied 40 transgender males with mean age of 24.3 years and mean androgen exposure of 58 weeks. The mean number of COCs obtained was 37.51 per person (median 27 per person). COC number correlated positively with AMH level, but no other hormone levels. Of the 1313 COCs collected, 34% matured via IVM, 94% of which showed a normal appearing spindle as a marker of oocyte functionality. Similarly, Lierman *et al.* (2017) collected 680 COCs at the time of SRS of 16 FtM TAYAs, and 38% of oocytes matured via IVM: the mean age of this group was 24.1 years and mean duration of GAHT was just over 1 year (53.6 weeks). Lierman *et al.* (2017) further analyzed IVM oocyte survival post-vitrification by dividing the oocytes into two groups: one was fixed immediately and the other vitrified and thawed before fixation. There was a 68% survival rate with 87% and 92% normal appearing spindles in the fresh and vitrified groups, respectively. Of note, the median duration of GAHT in both of these studies was relatively short.

Our search did not yield studies that evaluated the reversibility of GAHT effects on ovarian tissue or oocyte yield upon treatment cessation or studies evaluating the effect of different regimens and longer duration of GAHT.

FP outcomes in FtM TAYAs

There are no large-scale reports of FP outcomes among FtM TAYAs. Four reports presented data about FtM TAYAs pursuing FP before or after initiating GAHT (Wallace *et al.*, 2014; Broughton and Omurtag, 2017; Maxwell *et al.*, 2017; Chen *et al.*, 2018). One is a case series detailing one adolescent and two adults (ages 17–32 years) who pursued oocyte cryopreservation prior to initiating GAHT, with the number of cryopreserved mature oocytes ranging between 13 and 45. In two of the cases, patients returned to use their cryopreserved

oocytes (after 5–8 years) with their cisgender partner and donor sperm. Both resulted in delivery of healthy twins (Maxwell *et al*., 2017). Another case report described cryopreservation of 35 mature oocytes in a 17-year-old FtM TAYA (Wallace *et al*., 2014). In both papers, ovarian stimulation was achieved by either antagonist or low dose agonist protocols.

Chen *et al.* (2018) published a small series of five cases of oocyte preservation among FtM youth (ages 14–18 years) before initiating GAHT. All five completed ovarian stimulation and oocyte retrieval and cryopreservation without serious adverse side effects or complications. The mean number of oocytes retrieved was 18.2 (range 11–28), of which 14.2 (8–25) were mature and were cryopreserved.

One paper reported on individuals who had already initiated GAHT. Broughton and Omurtag (2017) presented two cases of use of IVF in FtM TAYAs. The first was a 30-year old FtM who initiated testosterone treatment 26 months earlier. He was urged to stop treatment for 3 months before attempting IVF, followed by 2-week down regulation with oral contraceptives. After ovarian stimulation, 13 mature oocytes were retrieved and fertilized with donor sperm. Two blastocysts transferred to his partner's uterus resulted in pregnancy and one additional blastocyst was cryopreserved. The second was a case of a 32-year old gender non-binary patient and female partner. Both underwent ovarian stimulation and oocyte retrieval, and fertilization with donor sperm. The cycle yielded 16 oocytes, nine fertilizations, and five blastocysts were cryopreserved. Later in a frozen cycle, two blastocysts, one from each partner, were transferred into the cisgender partner's uterus resulting in a twin pregnancy. In this case, GAHT has yet to be initiated prior to IVF.

No publications were identified that discussed OTC for the transgender population.

Discussion

To our knowledge this is the first published systematic review synthesizing the current state of knowledge on FP for TAYAs. We analyzed the data from all relevant papers that explored the attitudes, knowledge and beliefs of TAYAs, their parents/guardians and HCPs regarding FP for TAYAs, as well as major barriers preventing TAYAs from utilizing an FP service. We also systematically analyzed all the relevant papers that explored GAHT utilization and reproductive effects and outcomes of FP in TAYAs from the medical perspective in order to assist HCPs in providing FP counselling for TAYAs.

Other than one paper (De Sutter *et al*., 2002), all papers included in this study were published between 2010 and 2018, suggesting that this is an emerging field of research. Prior to this past decade, FP services for TAYAs, particularly for FtM TAYAs, were not easily accessible. Oocyte cryopreservation was deemed an experimental procedure by the ASRM until 2012, though this is no longer the case due to recent advances in oocyte freezing technology, namely the introduction of the vitrification technique (Practice Committees of the ASRM and SART, 2013b; Practice Committees of the ASRM and SART, 2014). With regard to psychosocial aspects of FP among TAYAs, the reported sample sizes were very small, with 16 of the 30 papers having sample sizes under 20. The small sample sizes are problematic, potentially undermining the reliability of the source and inaccurately characterizing the population of TAYAs. The paucity of published research studies, the fact that all but one of the papers was published within the last decade and the very small sample sizes all suggest the need for continued research on this topic. Furthermore, quality assessment of the included papers reveals poor to fair quality, mostly linked to the studies' methodologies, such as lack of a control group and small sample sizes, again supporting the need for better quality research in this field.

Barriers to accessing FP service

The literature makes it evident that many TAYAs wish to have children at some point in their lives (De Sutter *et al*., 2002; Wierckx *et al*., 2012b; Auer *et al*., 2018; Strang *et al*., 2018). It is essential for HCPs to begin FP counselling and discussions prior to the initiation of GAHT. Many TAYAs agreed that their desire to have biological children may change in the future (Strang *et al*., 2018). FP counselling and discussions are a vital way for TAYAs to learn about their reproductive options for future parenthood. Although some research suggests that the rates of TAYAs who receive FP counselling and subsequent referrals to a fertility specialist have increased over time (Armuand *et al*., 2017), no overall chronological trends were uncovered based on this review. Also, no trends were observed in the different countries represented by the studies. As expected, the rates of FP counselling and referrals were highest among studies conducted in dedicated hospital-based pediatric gender clinics.

We believe FP counselling should be the standard of care for all TAYAs considering utilizing GAHT and/or considering undergoing SRS. Unfortunately, in most of the papers that discussed FP counselling, FP discussions rarely occurred (Wierckx *et al*., 2012a; James-Abra *et al*., 2015; Armuand *et al*., 2017; Chen *et al*., 2017; Nahata *et al*., 2017; Riggs and Bartholomaeus, 2018), resulting in many TAYAs lacking awareness of the reproductive risks of GAHT and of their FP options. Several TAYAs stated that if they had been aware of their fertility options, they would have seriously considered undergoing FP (Wierckx *et al*., 2012b). Some individuals even regretted undergoing medical transitioning without first being aware of their FP options (von Doussa *et al*., 2015).

This review demonstrates the limited knowledge that HCPs have about FP for TAYAs. In order for TAYAs to have better access to FP information, HCPs must be more knowledgeable on the reproductive needs of these individuals in order to educate them. Since the language of transgender identity is constantly evolving, it is imperative for HCPs to stay up-to-date with the latest vocabulary for respectful communication. It cannot be stressed enough that all HCPs should be more sensitive toward TAYAs, treating them with respect by using the correct names, genders and pronouns to establish a transgender-friendly clinical environment.

More research is needed to better understand the attitudes of the parents/guardians of TAYAs toward FP. Although two articles mentioned the attitudes of the parents/guardians, only one pilot trial article thoroughly examined their opinions and beliefs (Strang *et al*., 2018). Studies found that parents/guardians who supported their child's decision to transition were more involved in the FP process when the child was a minor, including accompanying the minor to counselling sessions and FP procedure appointments (Chen *et al*., 2017; Maxwell *et al*., 2017) while conflicts can arise when a child's and their parents'/guardians' wishes for FP are in dissent. As many TAYAs are minors when they begin the transition process, parents/guardians are often very involved in the process, which points to the need for

more studies to understand the parents'/guardians' impact on the FP decision-making process.

While some barriers preventing TAYAs from pursuing FP are difficult to eliminate, such as the invasiveness of the oocyte retrieval process, others can be mitigated. For instance, as many TAYAs choose not to pursue FP due to the need to discontinue GAHT, it is important that knowledge translation and decisions regarding FP procedures take place prior to the initiation of GAHT. By pursuing FP before commencing the medical transition process, TAYAs avoid the burden of interrupting GAHT to allow sperm production or ovulation to resume. Some individuals are concerned with the affordability of the FP process so HCPs should be aware of any funding assistance that is available through insurers, government, or charities, and be able to relay this information to their patients. Furthermore, for MtF TAYAs who have concern about masturbation to produce a semen sample, HCPs should offer alternative approaches, such as electroejaculation or sperm extraction procedures.

Reproductive effects of GAHT in MtF TAYAs

This review brings to light the paucity of data available in the literature on the reproductive effects of GAHT and outcomes of FP in this population. Current recommendations from professional medical associations recommend that FP counselling take place before commencing any GAHT (Coleman *et al.*, 2012; Ethics Committee of the ASRM, 2015; Hembree *et al.*, 2017) and support medical therapy initiation soon after diagnosis is established, as early as Tanner stage II (gender affirming medical management in adolescents consists of puberty suppression by a GnRH agonist, Cyproterone acetate or Spironolactone, followed by cross-gender hormonal therapy to induce puberty at age 16 years). This paradigm, while assisting to alleviate gender dysphoria symptoms and improve mental health and general well-being (Gorin-Lazard *et al.*, 2013), creates a dilemma for the reproductive HCPs, as there are currently few high-quality data to rely upon when counselling transgenders and their guardians about the extent and reversibility of fertility risks by GAHT. The methods available for FP in young adolescents are restricted and depend on the individual's sexual development.

Most available data point to some degree towards an effect of GAHT on both testis and ovaries, yet the extent and reversibility of that effect and the lag time needed from cessation of treatment to recovery has not yet been thoroughly explored. In MtF TAYAs, only a single study (with a small sample size) evaluated semen parameters with past, current and no prior GAHT. Discontinuation of GAHT prior to semen cryopreservation was associated with improved parameters, though a trend towards worsening semen parameters remained (Adeleye *et al.*, 2019). Some studies reported an increased incidence of semen abnormalities among MtF TAYAs who have not yet started GAHT (Hamada *et al.*, 2015; Li *et al.*, 2018). The etiology of this observation is unknown yet could potentially be explained by the effect of psychological stress (Eskiocak *et al.*, 2006; Nargund, 2015), increased heat (due to tight undergarments and high positioning of the testes) (Li *et al.*, 2013; Rao *et al.*, 2015), undisclosed GAHT or genetic polymorphism.

Studies on the effect of GAHT on testicular histology and spermatogenesis, including small older observational studies, reviewed by Schneider *et al.* (2017), yield highly variable results, from normal spermatogenesis to complete azoospermia. While some found normal spermatogenesis in almost a quarter of the samples (Schneider *et al.*, 2015), most reported much lower proportions (Jindarak *et al.*, 2018; Kent *et al.*, 2018; Matoso *et al.*, 2018). These variations may represent individual sensitivity to treatment regimens or variations in treatment regimen and duration. One study revealed a trend, albeit not statistically significant, towards poorer spermatogenesis with longer duration of therapy (Kent *et al.*, 2018). In another study, in which GAHT was discontinued for a variable period of time before SRS, no correlation between spermatogenesis and treatment protocol was observed (Schneider *et al.*, 2015). One study even found that longer duration of GAHT was associated with an increased likelihood of resumption of spermatogenesis (Leavy *et al.*, 2017). The authors proposed decreased responsiveness of androgen receptors or increased elimination of exogenous hormones as possible explanations for this phenomenon. Due to the small sample size and heterogeneity of the studies, no conclusions can be drawn regarding dose, regimen and treatment duration effects or the nature of the specimens acquired at the time of SRS. No study evaluated the reversibility of GAHT's reproductive effects.

FP options and outcomes for MtF

These results do support the consensus of many organizations that FP should be discussed, and carried out if desired, prior to commencement of GAHT. However, if treatment is started at an early age, the modes by which to do so are few. Semen cryopreservation of specimens obtained by masturbation is an established method of FP for post-pubertal individuals and has been used for decades. It is easy, reliable and relatively inexpensive. However, some transgender individuals find masturbation psychologically distressing or experience erectile and ejaculatory dysfunction as a result of GAHT (Hamada *et al.*, 2015). For those individuals, electro-stimulation or penile vibratory stimulation may be appropriate but may not be readily available (Sonksen and Ohl, 2002; Kafetsoulis *et al.*, 2006). SSR, although more invasive and costly, is an option when ejaculation is not, or in cases of severe oligospermia or azoospermia. For young pre-pubertal transgenders, the only feasible option is TTC, which is still considered experimental and has not yet been proven successful in humans. When counselling about TTC, both the patient and guardian need to be aware that it is relying on the emerging technologies of tissue reimplantation or SSC *in vitro* expansion or SSC IVM (Picton *et al.*, 2015; Gassei and Orwig, 2016).

To date, there are only case reports or small case series on the use of cryopreserved sperm and outcomes of such pregnancies in TAYAs (Broughton and Omurtag 2017; Jones *et al.*, 2016). Usage of cryopreserved sperm in MtF TAYAs requires the use of either a partner's or a surrogate's uterus. Although uterine transplantation has emerged as a viable option, it is still considered experimental (Practice Committee of the ASRM, 2018), and there are several anatomical, hormonal, fertility and obstetric considerations that require consideration for this option in TAYAs (Brannstrom *et al.*, 2015; Testa *et al.*, 2018; Ejzenberg *et al.*, 2019; Jones *et al.*, 2019). There are currently no studies comparing or reporting outcomes from different methods of sperm collection in transgenders. There are also no studies comparing outcomes, with and without cessation of GAHT, in those who have already started treatment to guide HCPs as to whether they should recommend stopping treatment prior to collection or not and, if so, for how long.

The effect of GAHT on ovaries is controversial. Older observational studies on testosterone effect on ovarian histology reported mainly an

ovarian histology resembling that of PCOS (Futterweit and Deligdisch, 1986; Spinder *et al*., 1989; Pache *et al*., 1991). Recent studies, including those in the current review, yield more conflicting results; some still report a phenotype similar to PCOS (Grynberg *et al*., 2010; Loverro *et al*., 2016), and others report normal follicular count (Ikeda *et al*., 2013; Caanen *et al*., 2017; De Roo *et al*., 2017). One paper reported no correlation between duration of GAHT and follicle count, but the mean duration of testosterone treatment was relatively short (58 weeks) (De Roo *et al*., 2017).

There are currently no studies to guide clinicians when counselling FtM TAYAs who have already started GAHT about whether testosterone treatment should be discontinued prior to treatment and for how long. In the only published case report (Broughton and Omurtag, 2017), the authors elected to stop testosterone treatment for a period of 3 months, basing their decision on data pertaining to other medications with teratogenic effects, such as Methotrexate. They also elected to use ICSI for fertilization due to the possible effect of testosterone on the oocytes; however, there are little data to support this, and spontaneous pregnancies while on testosterone treatment do occur (Light *et al*., 2014; Obedin-Maliver and Makadon, 2016; Light *et al*., 2018). In a cross-sectional survey evaluating the experiences of 41 FtMs with respect to pregnancy and fertility, 61% of respondents reported taking GAHT prior to pregnancy, 68% discontinued treatment in order to achieve pregnancy, 80% regained menses within 6 months and 20% conceived while still amenorrheic from testosterone treatment (Light *et al*., 2014). The possible teratogenic effect of testosterone highlights the importance of consulting transgenders about contraception while taking GAHT until pregnancy is desired.

FP options and outcomes for FtM

The FP process itself may be challenging for FtM TAYAs. The process of ovarian stimulation and oocyte retrieval is both invasive and costly (Inhorn *et al*., 2018). With advances in oocyte cryopreservation and live birth rates approaching those of fresh oocytes (Cobo *et al*., 2008a,b, 2013, 2017), oocyte cryopreservation is the leading option for FtM TAYA in place of embryo cryopreservation, which requires the use of either a partner's or donor's sperm. Transgenders undergoing oocyte cryopreservation may find the process distressing as it involves the need to temporarily stop GAHT before commencing the process and increases estrogen levels due to ovarian stimulation, which may aggravate gender dysphoria symptoms (Armuand *et al*., 2017). In addition, treatment is most often taking place in a setting that is predominantly female patient oriented and involves the need to undergo serial transvaginal ultrasound examinations (Belaisch-Allart *et al*. 1991; Wittmaack *et al*. 1994; Rosen *et al*., 2008). Among the options to alleviate some of the distressful parameters of the process is the use of transabdominal ultrasound and aromatase inhibitors to minimize estradiol levels during ovarian stimulation. Studies on breast cancer patients attempting FP have consistently proven this method's effectiveness without compromising outcomes (Oktay *et al*., 2015).

For younger transgenders and when ovarian stimulation is not acceptable, OTC is the only option. Data from other populations, mainly FP related to malignancy and conditions predisposing to primary ovarian insufficiency, is promising, and there are reports of live births from cryopreserved ovarian tissue of individuals who were pre-pubertal or peri-pubertal at the time of the procedure (Donnez, 2015; Demeestere, 2015; Jensen *et al*., 2017a; Jensen *et al*., 2017b; Javed *et al*., 2018; Donnez and Dolmans 2018). OTC is very appealing to transgenders since it does not require ovarian stimulation and can potentially be carried out at the time of SRS, though not all institutions have an established protocol. OTC most often involves re-transplantation of the cryopreserved tissue into the individual from which it was harvested, which is less than ideal for transgenders as it involves cessation of GAHT with accompanying gender dysphoria symptoms and the possible need for future ovarian tissue removal upon completion of their family.

Two papers report outcomes of IVM of oocytes collected from ovaries exposed to GAHT at the time of SRS (De Roo *et al*., 2017; Lierman *et al*., 2017). In both these studies, the number of COCs retrieved was higher than reported previously for other indications (Segers *et al*., 2015; Ye, 2016). This is mainly because both ovaries are procured and prepared, and gonadotrophin is downregulated by androgen treatment. The number of COCs retrieved also correlates with age and AMH level (Yin *et al*., 2016). Maturation and survival rates were comparable to those published in the literature (Shalom-Paz *et al*., 2010; Abir *et al*., 2016; Fasano *et al*., 2017). These results seem very promising, albeit the option is not yet clinically available (Fasano *et al*., 2017). One also needs to keep in mind the median testosterone treatment duration in both studies was relatively short (53–58 weeks), and the effect of longer treatment duration has still to be thoroughly investigated. Also, oocyte potency in these studies was evaluated by the oocytes' spindle appearance (Mandelbaum *et al*., 2004; Coticchio *et al*., 2009); however, fertilization, embryo yield and quality have yet to be explored. Previous studies on utilization of IVM oocytes show that these oocytes yield lower pregnancy rates and higher pregnancy loss rates (Practice Committee of the ASRM, 2013; Kedem *et al*., 2018).

Limitations

Owing to the nature of this review and topic, we did not limit our search to specific study types in order to include all the relevant literature. We did decide to limit our search to papers published after 2001. However, since FP in general and FP in TAYAs are a relatively recent practice, we believe this did not exclude papers of high importance. It is also worth noting that relevant publications do exist, mainly on the effect of GAHT on histology, and were explored by the authors after snowballing through the references of included papers and relevant reviews. The search was also limited to publications in English.

Conclusion

Upon synthesizing the current body of research literature, it is evident that FP for TAYAs is not yet a standard practice and there are many barriers preventing TAYAs from pursuing FP. Contributing factors include the absence of FP discussion and counselling initiated by HCPs, often due to lack of knowledge. FP counselling for TAYAs is far from straightforward because there is little data in the literature about the reproductive effects of GAHT. As a result, most TAYAs lack awareness of the following: FP options; costs, including those that may be off-set by various programs; invasiveness of the procedures and the potential psychological impact of going through the process. The literature suggests that FP counselling should begin as early as possible prior to undergoing GAHT, allowing time for informed decisions to

be made. FP counselling and support services should be the standard of care. Research should continue in this area to rectify the current issue with a lack of high-quality medical data that results in current FP practice relying primarily on expert opinion and extrapolation from cisgender population studies. Future research should also include the following: large-scale cohort studies, preferably multi-centered; longitudinal studies of TAYAs throughout the entire FP process; qualitative studies of the parents/guardians of TAYAs; and psychosocial studies evaluating the effectiveness of different strategies to improve the attitudes, knowledge and beliefs of HCPs. Lastly, as fertility clinics only continue to encounter TAYAs who are seeking FP services, it is crucial to apply specific sensitivity training for medical personnel to make the FP process both more accessible and less stressful for TAYAs.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

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ORIGINAL ARTICLE *Reproductive biology*

Histological study on the influence of puberty suppression and hormonal treatment on developing germ cells in transgender women

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STUDY QUESTION: Can transgender women cryopreserve germ cells obtained from their orchiectomy specimen for fertility preservation, after having used puberty suppression and/or hormonal treatment?

SUMMARY ANSWER: In the vast majority of transgender women, there were still immature germ cells present in the orchiectomy specimen, and in 4.7% of transgender women—who all initiated medical treatment in Tanner stage 4 or higher—mature spermatozoa were found, which would enable cryopreservation of spermatozoa or testicular tissue after having used puberty suppression and/or hormonal treatment.

WHAT IS KNOWN ALREADY: Gender affirming treatment (i.e. puberty suppression, hormonal treatment, and subsequent orchiectomy) impairs reproductive function in transgender women. Although semen cryopreservation is generally offered during the transition process, this option is not feasible for all transgender women (e.g. due to incomplete spermatogenesis when initiating treatment in early puberty, in case of inability to masturbate, or when temporary cessation of hormonal treatment is too disruptive). Harvesting mature spermatozoa, or testicular tissue harboring immature germ cells, from orchiectomy specimens obtained during genital gender-affirming surgery (gGAS) might give this group a chance of having biological children later in life. Previous studies on spermatogenesis in orchiectomy specimens showed conflicting results, ranging from complete absence of germ cells to full spermatogenesis, and did not involve transgender women who initiated medical treatment in early- or late puberty.

STUDY DESIGN, SIZE, DURATION: Histological and immunohistochemical analyses were performed on orchiectomy specimens from 214 transgender women who underwent gGAS between 2006 and 2018. Six subgroups were identified, depending on pubertal stage at initiation of medical treatment (Tanner stage 2-3, Tanner stage 4-5, adult), and whether hormonal treatment was continued or temporarily stopped prior to gGAS in each of these groups.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All transgender women used a combination of estrogens and testosterone suppressing therapy. Orchiectomy specimen sections were stained with Mayer's hematoxylin and eosin and histologically analyzed to assess the Johnsen score and the ratio of most advanced germ cell types in at least 50 seminiferous tubular cross-sections. Subsequently, immunohistochemistry was used to validate these findings using spermatogonia, spermatocytes or spermatids markers (MAGE-A3/A4, γH2AX, Acrosin, respectively). Possibilities for fertility preservation were defined as: preservation of spermatozoa, preservation of spermatogonial

stem cells or no possibilities (in case no germ cells were found). Outcomes were compared between subgroups and logistic regression analyses were used to assess the association between the duration of hormonal treatment and the possibilities for fertility preservation.

MAIN RESULTS AND THE ROLE OF CHANCE: Mature spermatozoa were encountered in 4.7% of orchiectomy specimens, all from transgender women who had initiated medical treatment in Tanner stage 4 or higher. In 88.3% of the study sample orchiectomy specimens only contained immature germ cells (round spermatids, spermatocytes or spermatogonia, as most advanced germ cell type). In 7.0%, a complete absence of germ cells was observed, all these samples were from transgender women who had initiated medical treatment in adulthood. Cessation of hormonal treatment prior to gGAS did not affect the presence of germ cells or their maturation stage, nor was there an effect of the duration of hormonal treatment prior to gGAS.

LIMITATIONS, REASONS FOR CAUTION: Since data on serum hormone levels on the day of gGAS were not available, we were unable to verify if the transgender women who were asked to temporarily stop hormonal treatment 4 weeks prior to surgery actually did so, and if people with full spermatogenesis were compliant to treatment.

WIDER IMPLICATIONS OF THE FINDINGS: There may still be options for fertility preservation in orchiectomy specimens obtained during gGAS since a small percentage of transgender women had full spermatogenesis, which could enable cryopreservation of mature spermatozoa via a testicular sperm extraction procedure. Furthermore, the vast majority still had immature germ cells, which could enable cryopreservation of testicular tissue harboring spermatogonial stem cells. If maturation techniques like *in vitro* spermatogenesis become available in the future, harvesting germ cells from orchiectomy specimens might be a promising option for those who are otherwise unable to have biological children.

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Key words: transgender women / orchiectomy / fertility preservation / testicular tissue / spermatogenesis / hormonal treatment / azoospermia / germ cells

Introduction

Gender dysphoria refers to the distress experienced by people with an incongruence between their sex assigned at birth and their gender identity (APA, 2013). People assigned male at birth with a female gender identity are referred to as transgender women.

Many transgender women seek medical treatment to avoid (further) masculinization and induce feminization, and hereby align their physical characteristics with their gender identity. The preferred treatment protocol depends on the person's age at time of start of medical treatment. For adolescents (<18 years), treatment can be initiated when a person reaches puberty (Tanner stage 2 or higher, determined by the development of secondary sex characteristics). It aims to suppress further pubertal development by administration of a gonadotropin-releasing hormone agonist (GnRHa) which reversibly inhibits the production of sex hormones. Hereby, adolescents have more time to explore options and to live in the experienced gender before deciding whether or not to proceed with additional, sometimes irreversible, treatments. At the age of approximately 16 years, treatment can be supplemented with estrogens to induce the development of female secondary sex characteristics (Hembree *et al.*, 2017). For transgender women presenting at adult age (≥18 years), treatment usually does not consist of a phase of hormone suppression only, but immediately involves a combination of anti-androgens and estrogens, to achieve feminization. The combination of testosterone suppressing therapy and estrogen supplementation is referred to as gender affirming hormonal treatment (GAHT). Transgender women of 18 years or older who have used GAHT for at least one year, can opt for genital gender-affirming surgery (gGAS) if no surgical contraindications are present. gGAS may comprise vaginoplasty, gender confirming vulvoplasty or bilateral orchiectomy, depending on the desires of the individual (van der Sluis *et al.*, 2021).

The use of testosterone suppressing therapy results in a severely impaired reproductive function, since spermatogenesis—the differentiation of spermatogonial stem cells into spermatozoa—requires adequate levels of intratesticular testosterone (Adeleye *et al.*, 2019). This reproductive loss is permanent after gGAS. Although gender affirming treatment significantly improves quality of life, reproductive loss may be an unwanted consequence (Auer *et al.*, 2018; Chen *et al.*, 2018; Vyas *et al.*, 2020). Therefore, it is important that (future) desire for biological children and the options for fertility preservation are discussed and offered prior to the start of medical treatment (Hembree *et al.*, 2017).

The currently available option for fertility preservation in transgender women is cryopreservation of spermatozoa from a semen sample, obtained through ejaculation. Cryopreservation of surgically obtained spermatozoa through testicular sperm extraction (TESE) may serve as an alternative for those who are unable to ejaculate or in case of azoospermia (Wallace *et al.*, 2014).

A complicating factor for contemporary fertility preservation in transgender female adolescents is the requirement of complete spermatogenesis, which only develops from Tanner stage 3 onwards, under the influence of increasing intratesticular testosterone levels. If puberty suppression is started in Tanner stage 2, full spermatogenesis is usually not present yet and therefore preservation of spermatozoa is not possible (Brik *et al.*, 2019). The equipoise of commencing medical treatment to avoid progression of puberty and delaying treatment to enable semen cryopreservation as only option for biological children may be stressful, as puberty is accompanied by irreversible and often unwanted physical changes such as a lowering of the voice and facial hair growth. Severe genital dysphoria may pose another barrier for fertility preservation, since semen cryopreservation requires masturbation which is non-negotiable for some young transgender women (Brik *et al.*, 2019). In addition, TESE, the currently available alternative to

obtain spermatozoa for cryopreservation requires invasive procedures including surgery and (general) anesthesia.

For transgender women, cryopreservation of germ cells harvested from testicular tissue obtained during gGAS may serve as an alternative to keep the option for genetically related offspring open. How these germ cells can be used for procreation depends on their maturation phase. Spermatozoa can directly be used for ART. However, the use of immature germ cells relies on the feasibility of maturation techniques outside the human body, such as *in vitro* spermatogenesis. Unfortunately, complete *in vitro* spermatogenesis has only been successfully demonstrated in mouse models and is still unsuccessful in humans (Sato *et al.*, 2011). If *in vitro* spermatogenesis becomes available in the future, cryopreservation of testicular tissue containing spermatogonial stem cells might be a promising option for fertility preservation in those who are otherwise unable to retain the possibility of having genetically related offspring.

Currently, limited data are available on the effect of GAHT on testicular histology and the most advanced germ cell type that can be harvested from testicular tissue obtained at time of gGAS. Previous studies conducted on this topic showed varying proportions of hyalinization of seminiferous tubules as well as conflicting results regarding spermatogenesis, ranging from a complete absence of germ cells to full spermatogenesis (Schneider *et al.*, 2017; Matoso *et al.*, 2018). Moreover, none of these studies focused on people who initiated medical treatment in early puberty.

The primary aim of this study is to evaluate the influence of puberty suppression and/or GAHT on exocrine testicular function, by determining the most advanced germ cell type in orchiectomy specimens obtained during gGAS. We aim to compare the outcome between people who started medical treatment as adult (≥18 years) and those who started as adolescent in early puberty (Tanner stage 2-3) or late puberty (Tanner stage 4-5). In addition, we will assess the influence of discontinuation of medical treatment 4 weeks prior to gGAS in each of these groups, and the association between the duration of hormonal treatment and the possibilities for fertility preservation. Hereby, we will get insights in the options for fertility preservation in orchiectomy specimens obtained during gGAS after having used puberty suppression and/or hormonal treatment.

Materials and methods

Study population and clinical data collection

For this study, we used orchiectomy specimens of transgender women who underwent bilateral orchiectomy combined with vaginoplasty at the Center of Expertise on Gender Dysphoria of Amsterdam UMC between 2006 and 2019. All participants provided written permission for the use of their body material and clinical data for research purposes. The Ethical Review Board of the Amsterdam UMC, location VUMC provided approval for conducting this study (METC2014322).

A total of 788 transgender women were identified. Data on medical history, age and Tanner stage at start of medical treatment, documented hormone use, date of gGAS, alcohol consumption, smoking, drug use, BMI at time of gGAS and last known serum hormone levels before gGAS, were collected from the medical files. Transgender women were categorized according to age and Tanner stage at initiation of medical treatment (Tanner stage 2-3, Tanner stage 4-5 or ≥18 years). Transgender women operated before 2017 discontinued GAHT 4 weeks prior to surgery, because of a presumed increased risk of perioperative thrombosis. As evidence suggested this risk is negligible, GAHT is continued in the perioperative period since July 2017. Six subgroups were created based on Tanner stage/age at start of medical treatment and continuation/discontinuation of GAHT prior to gGAS. People with an unknown age or Tanner stage at time of initiation of medical treatment were excluded. Other exclusion criteria were hard drug use, cryptorchidism, a medical history of receiving chemotherapy or genetic disorders which can all possibly impair spermatogenesis. Lastly, since the vast majority used estrogens combined with either triptorelin, or cyproterone acetate, people who used estrogen monotherapy and those who used spironolactone as anti-androgenic treatment were excluded to create a homogeneous study population. A maximum number of 80 transgender women were enrolled per group as this was deemed sufficient to answer the study questions. A random sample was drawn from groups that exceeded 80 individuals using STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA). In total, 263 transgender women were selected for inclusion in the study cohort.

Testicular tissue preparation and analysis

Preparation for histology

Testicular tissue was obtained from the biobank of the Pathology Department of Amsterdam UMC, where orchiectomy specimens, obtained during gGAS, were stored after histopathological analysis for clinical purposes. Upon arrival at the Pathology Department, the orchiectomy specimens were fixed in 4% w/v paraformaldehyde and embedded in paraffin. For this study, seven slices of 5 µm thickness of one testicle were sectioned and mounted on microscope slides. From one slide of each specimen paraffin sections were deparaffinized and subsequently stained with Mayer's hematoxylin and eosin, and at least one other slide was used for immunohistochemistry to confirm germ cell subtypes.

Histological analysis

Histological examination was conducted using a bright field microscope (Olympus BX41, OM Digital Solutions Americas, Bethlehem, PA, USA). From each specimen, at least 50 seminiferous tubules per slide were analyzed to assess spermatogenesis by determining the most advanced germ cell type from each seminiferous tubular cross-section based on their location within the tubule and nuclear morphology. The Modified Johnsen's scoring system was used to assign a score to each tubule, and per slide a mean Johnsen's score was calculated. The Modified Johnsen's scoring system involves a 10-point Likert scale where score 1 corresponds to complete sclerosis without recognizable seminiferous epithelium, and score 10 implies the presence of more than 10 elongated spermatids without immature and apoptotic cells in the lumen (Supplementary Table SI) (Johnsen, 1970).

After assessment of spermatogenesis, overall testicular histology was assessed including the presence of a lumen in the seminiferous tubules and rate of seminiferous tubule hyalinization. The lumen was categorized as open, half-open or absent. Hyalinization was defined as a

hyaline area separating the peritubular layer from the basal membrane of the seminiferous tubule.

Preparation for immunohistochemistry

In order to validate our findings, a second slide of each specimen was analyzed using immunohistochemistry. The primary antibodies were chosen based on the most advanced germ cell type that was identified during histological analysis, or on uncertainty regarding the presence of a germ cell type.

For the detection of spermatogonia, slides were stained for spermatogonial marker MAGE-A3/A4 using mouse monoclonal Anti-Mage A3/A4 antibody (clone 57B; Merck Millipore, Germany). Endogenous peroxidase activity was inactivated with 0.3% H_2O_2/phosphate-buffered saline (PBS) for 10 min at room temperature in the dark. Non-specific binding sites were then blocked with Superblock (ScyTek Lab, USA) for 1 h at room temperature in a humid slide box. Sections were subsequently incubated overnight at 4°C with Anti-Mage A3/A4 antibody diluted 1:2000 in BrightDiluent (Immunologic, the Netherlands). The next day, all slides were washed three times with PBS followed by 30 min incubation with Powervision goat-anti Mouse/Rabbit poly-horseradish peroxidase (DPVO110HRP, Immunologic, the Netherlands) secondary antibody at room temperature. After washing, the signal was visualized using Bright-DAB (3,3′-diaminobenzidine, Immunologic, the Netherlands) after which the sections were counterstained with Mayer's hematoxylin. Finally, after dehydration in increasing ethanol concentrations and xylene, the slides were encapsulated with glass coverslips using Entellan® (Merck Millipore, Germany) for further microscopic analysis.

For the detection of spermatocytes, slides were stained for γH2AX using mouse monoclonal Anti-phospho-Histone H2A.X (Merck Millipore, Germany) antibody. Antigen retrieval was carried out by boiling tissue sections in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH = 9.0). The buffer was first heated until boiling in the microwave for 3 min at maximum Watt. After cooling down for 2 min at room temperature, the buffer was heated again in the microwave for 12 min at minimum Watt. Non-specific binding sites were blocked with 5% bovine serum albumin (BSA)/PBS/0.5% Triton X-100. This was followed by overnight incubation at 4°C with Anti-phospho-Histone H2A.X diluted 1:150 in 1% BSA/PBS/0.05% Tween. After incubation of the primary antibody, the same steps were performed as for the detection of spermatogonia with MAGE-A3/A4.

For the detection of round spermatids and spermatozoa, slides were stained for the presence of their Acrosin cap using rabbit polyclonal Acrosin antibody (ThermoFisher, PA5-61804). Antigen retrieval was carried out by boiling tissue sections in 0.01 M sodium citrate buffer (tri-sodium citrate dihydrate $Na_3C_6H_5O_7.2H_2O$, pH 6.0). The buffer was first heated until boiling in the microwave for 3 min at maximum Watt. After cooling down for 2 min at room temperature, the buffer was heated again in the microwave for 10 min at minimum Watt. Subsequently, the buffer was cooled down for 10 min at room temperature and placed under running tap water. After these steps, a standard immunohistochemical preparation protocol was followed, as described above.

For all three antibodies, slides with testicular tissue from a prostate cancer patient with normal spermatogenesis served as a positive control. Negative controls were carried out by replacing the first antibody by isotype IgG (Supplementary Fig. S1).

Immunohistochemical analysis

The immunohistochemically stained slides were examined using a bright field microscope (Olympus BX41) and assessed on the presence of the specifically targeted germ cell type. Outcome was then used to validate the Modified Johnsen's scoring of the histologically analyzed slide of that same specimen. Results from the immunohistochemically stained slides were preferred if there was a difference between the two.

Statistical analyses

After completion of histological and immunohistochemical analyses, results were linked to clinical data and descriptive analyses were conducted for the total cohort and the six subgroups. Data are presented as means (SD) when normally distributed, as medians with interquartile ranges (IQRs) when non-normally distributed, or as numbers with percentage.

Progress of spermatogenesis, determined by the presence of the most advanced germ cell type per orchiectomy specimen, was used as main outcome measurement (no germ cells, spermatogonia, spermatocytes, round spermatids or spermatozoa). Secondary outcome measurements included mean Johnsen score per orchiectomy specimen, the degree of hyalinization and presence of a lumen.

To assess the possibilities for fertility preservation three categories were defined: preservation of spermatozoa; preservation of spermatogonial stem cells for those with round spermatids, spermatocytes or spermatogonia as most advanced germ cell type; and no possibilities for those with a complete absence of germ cells. Outcome was expressed as proportion with 95% confidence interval (95% CI) and compared between people who started medical treatment as an adult (>18 years) and those who started as adolescent in early puberty (Tanner stage 2-3) or late puberty (Tanner stage 4-5) (Newcombe, 1998). Since some categories contained no observations, we were not able to perform statistical tests. Therefore, differences between groups are shown in a figure. To assess the effect of cessation of GAHT prior to surgery, Fisher's exact tests were used to compare outcome within each pubertal stage at initiation of medical treatment. The significance level was set at $P < 0.05$, and all tests were two-sided.

Lastly, logistic regression analyses were performed to assess the association between the duration of medical treatment and the possibility for preservation of spermatozoa, as well as the possibility for preservation of spermatogonial stem cells. Since the duration of medical treatment prior to gGAS, as well as progress of spermatogenesis both might be dependent on the age at start of medical treatment, a correction was performed for this factor. Odds ratios (ORs) with 95% CI were calculated.

All statistical analyses were performed using STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA).

Results

Initially, 263 transgender women were selected for inclusion in the study cohort. A total of 35 individuals were excluded when, upon preparation for analysis of the orchiectomy specimens, it became evident that for these transgender women no tissue was stored at the Pathology department of Amsterdam UMC. Another 14 transgender

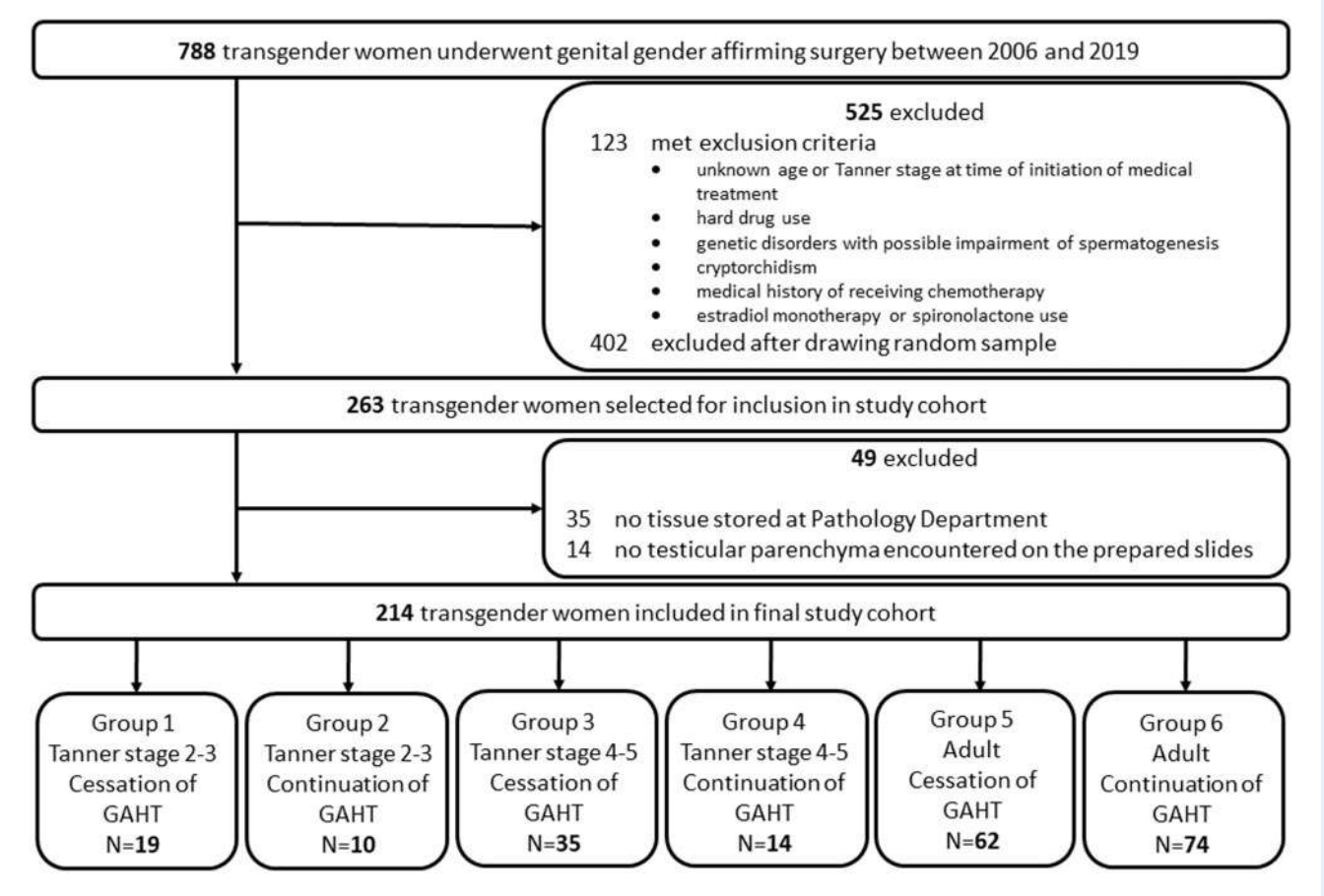

Figure 1. Study flowchart. GAHT, gender affirming hormonal treatment.

women were excluded because no testicular parenchyma was encountered on the prepared slides. Therefore, the final cohort consisted of 214 transgender women divided into 6 subgroups (Fig. 1).

Characteristics at time of gGAS are presented in Table I. Mean age at gGAS was 29.6 years (SD 12.4) and was lower in people who started medical treatment in adolescence compared to those who started medical treatment in adulthood. Since adolescents started medical treatment with puberty suppressive therapy and had to wait until reaching the age of 18 years before being able to undergo gGAS, prior medical treatment duration was longer in the adolescent subgroups compared to those who initiated treatment at adult age. Different estradiol formulations were prescribed, including estradiol patches (50–150 µg/24 h twice weekly), estradiol gel (0.75–3.0 mg daily) and oral estradiol valerate or hemihydrate (2–6 mg daily). Testosterone suppressing therapy consisted of triptorelin injections (3.75 mg i.m./s.c. every 4 weeks or 11.25 mg i.m. every 12 weeks) for those who initiated treatment as adolescent, and cyproterone acetate (25–100 mg daily) for those who initiated treatment as adult. The last known serum hormone levels, median 189 days (IQR 96–340) before gGAS, showed that testosterone was adequately suppressed (median 0.7 nmol/l, IQR 0.5–1.0) and estradiol levels were in the female range (median 193 pmol/l, IQR 120–307). Furthermore, LH and FSH levels were suppressed. In transgender women with a cessation of GAHT 4 weeks prior to gGAS, estradiol levels were lower and testosterone and LH levels were higher, compared to those who continued GAHT until gGAS.

In 10 transgender women (4.7%) some seminiferous tubules contained full spermatogenesis, all of whom had initiated medical treatment in Tanner stage 4 or higher and it occurred in both the group that had continued GAHT until gGAS and in the group that had discontinued four weeks prior to gGAS (Table II, Fig. 2E). Complete absence of germ cells was encountered in 15 transgender women (7.0%) (Fig. 2A), all of whom had initiated medical treatment in adulthood. Also, mean Johnsen's scores were lowest in the adult cohort. In the subgroup of transgender women who initiated medical treatment in Tanner stage 2 or 3, all specimens showed immature germ cells of which spermatogonia were most commonly observed (60–79%) (Fig. 2B–D). Supplementary Table SII shows the Modified Johnsen's score for each individual separately.

Hyalinization of seminiferous tubules was observed in 161 orchiectomy specimens (75.2%) and was most common in the adult subgroup (Fig. 3E and F). An open or half-open lumen of the seminiferous tubule was encountered in 8.4% and 25.2% of the orchiectomy specimens (Fig. 3A and B), respectively. The complete absence of a lumen was most common in those who initiated treatment in Tanner stage 2 or 3 (Fig. 3C).

Table I Baseline characteristics at time of genital gender affirming surgery (gGAS).

	Total (n = 214)	Adolescent Tanner stage 2-3 (n = 29)		Adolescent Tanner stage 4-5 (n = 49)		Adult (n = 136)	
		Cessation of GAHT (n = 19)	Continuation of GAHT (n = 10)	Cessation of GAHT (n = 35)	Continuation of GAHT (n = 14)	Cessation of GAHT (n = 62)	Continuation of GAHT (n = 74)
Age (years)—mean (SD)	29.6 (12.4)	19.0 (1.5)	19.6 (1.9)	19.7 (1.2)	19.3 (0.7)	34.5 (12.3)	36.2 (12.2)
Alcohol							
Drinker—% (n)	44 (82)	43 (6)	30 (3)	60 (18)	21 (3)	56 (26)	35 (26)
Non-drinker—% (n)	56 (106)	57 (8)	70 (7)	40 (12)	79 (11)	44 (20)	65 (48)
Unknown—n	26	5	0	5	0	16	0
Smoking							
Smoker—% (n)	7 (12)	0	0	8 (2)	0	22 (10)	0
Non-smoker—% (n)	93 (171)	100 (15)	100 (10)	92 (24)	100 (14)	78 (34)	100
Unknown—n	31	4	0	9	0	18	0
Cannabis use							
Yes—% (n)	3 (5)	0	0	4 (1)	7 (1)	6 (2)	1 (1)
No—% (n)	97 (166)	100 (15)	100 (10)	96 (24)	93 (13)	94 (31)	99 (73)
Unknown—n	43	4	0	10	0	29	0
BMI (kg/m^2)—mean (SD)	23.1 (3.3)	22.0 (3.3)	23.2 (2.8)	21.6 (3.6)	20.9 (3.6)	23.9 (2.9)	23.8 (3.0)
Mean duration of medical treatment (years)˜—(SD)	3.3 (2.0)	5.9 (1.4)	6.8 (1.3)	4.1 (1.8)	2.8 (0.6)	2.8 (1.9)	2.3 (1.2)
Testosterone suppression							
Triptorelin injections—% (n)	36 (78)	100 (19)	100 (10)	100 (35)	100 (14)	0	0
Cyproterone acetate—% (n)	64 (136)	0	0	0	0	100 (62)	100 (74)
Estrogen supplementation							
Transdermal formulation—% (n)	25 (54)	11 (2)	10 (1)	0	0	40 (25)	35 (26)
Oral formulation—% (n)	75 (160)	89 (17)	90 (9)	100 (35)	100 (14)	60 (37)	65 (48)
Serum hormone levels before gGAS—Median (IQR)^							
Testosterone (nmol/)	0.7 (0.5–1.0)	1.0 (0.8–1.0)	0.6 (0.5–0.8)	1.0 (0.6–1.2)	0.6 (0.5–1.1)	0.7 (0.5–1.0)	0.5 (0.5–0.8)
Estradiol (pmol/l)	193 (120–307)	95 (43–332)	160 (141–392)	120 (82–220)	222 (100–281)	219 (130–282)	237 (151–341)
LH (U/l)	0.1 (0.1–0.3)	0.2 (0.1–0.4)	0.3 (0.2–0.5)	0.3 (0.2–0.4)	0.2 (0.2–0.4)	0.1 (0.1–0.3)	0.1 (0.1–0.1)
FSH (U/l)	0.2 (0.1–0.5)	0.2 (0.1–0.5)	0.4 (0.4–0.5)	0.2 (0.1–0.5)	–	0.3 (0.1–0.5)	0.8 (0.1–3.0)

GAHT, gender affirming hormone treatment; IQR, interquartile range.
˜Including GnRH agonist use, if applicable.
^Data were available for 201 (testosterone and LH), 200 (estradiol), and 53 (FSH) transgender women, respectively.

When comparing the options for fertility preservation, we found that for some transgender women it would still have been possible to harvest mature spermatozoa from testicular tissue obtained during gGAS (Fig. 4). This was the case for 4% (95% CI 2–8) of the adult subgroup and 10% (95% CI 4–22) of adolescents in the Tanner stage 4-5 subgroup, compared to 0% in the Tanner stage 2-3 subgroup. For 100% of people in the Tanner stage 2-3 subgroup, 90% (95% CI 78–96) of people in the Tanner stage 4-5 subgroup and 85% (95% CI 78–90) of the adult subgroup, preservation of testicular tissue containing spermatogonial stem cells would have been their only option for fertility preservation. Furthermore, for 11% (95% CI 7–17) of the adult subgroup no options for fertility preservation would have been available, compared to 0% of the two adolescent subgroups. No statistically significant differences were found between those who had continued GAHT until gGAS and those with four weeks cessation of GAHT prior to gGAS.

Lastly, logistic regression analyses showed no association between the duration of GAHT and the possibility for preservation of spermatozoa (OR 0.75, 95% CI 0.47–1.18) or spermatogonial stem cells (OR 1.03, 95% CI 0.81–1.31).

Discussion

The results of our study imply that there may be options for fertility preservation for transgender women who are unable to pursue semen cryopreservation, by using testicular tissue from orchiectomy specimens obtained during gGAS. In a small percentage of transgender

Table II Results of histological and immunohistochemical analyses of orchiectomy specimens.

	Total (n = 214)	Adolescent Tanner stage 2–3 (n = 29)		Adolescent Tanner stage 4–5 (n = 49)		Adult (n = 136)	
		Cessation of GAHT (n = 19)	Continuation of GAHT (n = 10)	Cessation of GAHT (n = 35)	Continuation of GAHT (n = 14)	Cessation of GAHT (n = 62)	Continuation of GAHT (n = 74)
Spermatozoa	4.7 (10)	0 (0)	0 (0)	6 (2)	22 (3)	6 (4)	1 (1)
Round spermatids	0.5 (1)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)	0 (0)
Spermatocytes	21.5 (46)	21 (4)	40 (4)	31 (11)	14 (2)	23 (14)	15 (11)
Spermatogonia	66.3 (142)	79 (15)	60 (6)	63 (22)	64 (9)	61 (38)	70 (52)
No germ cells	7.0 (15)	0 (0)	0 (0)	0 (0)	0 (0)	8 (5)	14 (10)
Mean Johnsen's score—(SD)	2.5 (0.8)	2.6 (0.3)	2.7 (0.4)	2.8 (0.8)	3.2 (1.4)	2.5 (0.8)	2.3 (0.6)
Hyalinization	75.2 (161)	47 (9)	40 (4)	63 (22)	79 (11)	76 (47)	92 (68)
Lumen							
Open	8.4 (18)	0 (0)	0 (0)	3 (1)	22 (3)	18 (11)	4 (3)
Half-open	25.2 (54)	26 (5)	10 (1)	31 (11)	14 (2)	26 (16)	26 (19)
Absent	66.4 (142)	74 (14)	90 (9)	66 (23)	64 (9)	56 (35)	70 (52)

Data are % (n) unless stated otherwise.

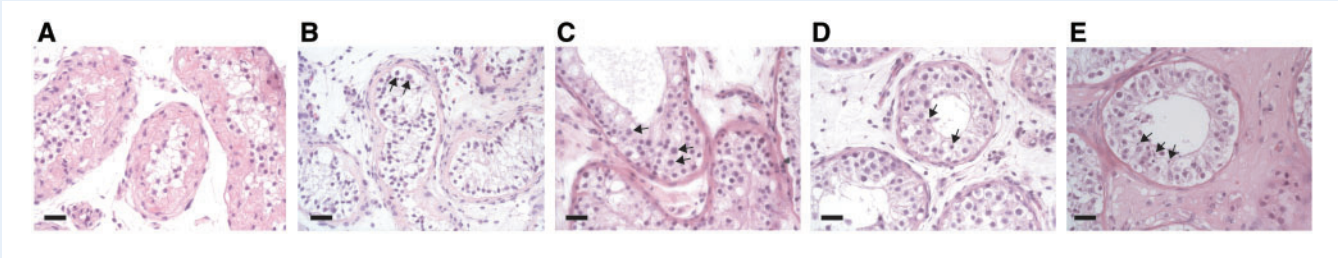

Figure 2. Orchiectomy specimens with their most advanced germ cell type. (**A**) No germ cells present. (**B**) Spermatogonia. (**C**) Spermatocytes. (**D**) Round spermatids. (**E**) Spermatozoa. Arrows indicate most advanced germ cells. Bar represents 20 µm.

women who initiated medical treatment in Tanner stage 4 or higher, complete spermatogenesis was observed in the orchiectomy specimen. For this group, it would theoretically be possible to perform TESE and cryopreserve the harvested spermatozoa from this specimen. Furthermore, the vast majority of transgender women still had immature germ cells in their orchiectomy specimen. This is the first study to report on people who initiated medical treatment in Tanner stage 2-3, and it was found that in 100% of their orchiectomy specimens immature germ cells were present. If maturation techniques like *in vitro* spermatogenesis become available in the future, cryopreservation of testicular tissue containing spermatogonial stem cells might be a promising option for this group to retain the possibility to have biological children. A complete absence of germ cells was only observed in transgender women who commenced GAHT as adult. Cessation of GAHT prior to gGAS did not affect the possibilities for fertility preservation, neither was there an effect of the duration of GAHT prior to gGAS.

Although some previous studies have been conducted on the influence of GAHT on spermatogenesis and testicular architecture, this is the first study taking age and pubertal stage at time of initiation of medical treatment into account. Between 1970 and 1990, several small studies were conducted reporting on 4–11 transgender women per study (Rodriguez-Rigau *et al.*, 1977; Lu and Steinberger, 1978; Payer *et al.*, 1979; Sapino *et al.*, 1987; Schulze, 1988; Venizelos and Paradinas, 1988). Therefore, no strong conclusions could be drawn, but results showed high proportions of tubular hyalinization and reduced spermatogenesis in all transgender women. The first large cohort study on this topic was performed in 2015 and assessed orchiectomy specimens of 108 transgender women from three clinics with different preoperative treatment protocols (6 weeks, 2 weeks or no discontinuation of GAHT prior to gGAS) (Schneider *et al.*, 2015). Their results on testicular histology and spermatogenic state were highly heterogeneous and did not show a relation with treatment strategy. Remarkably, a high number of transgender women (24% of

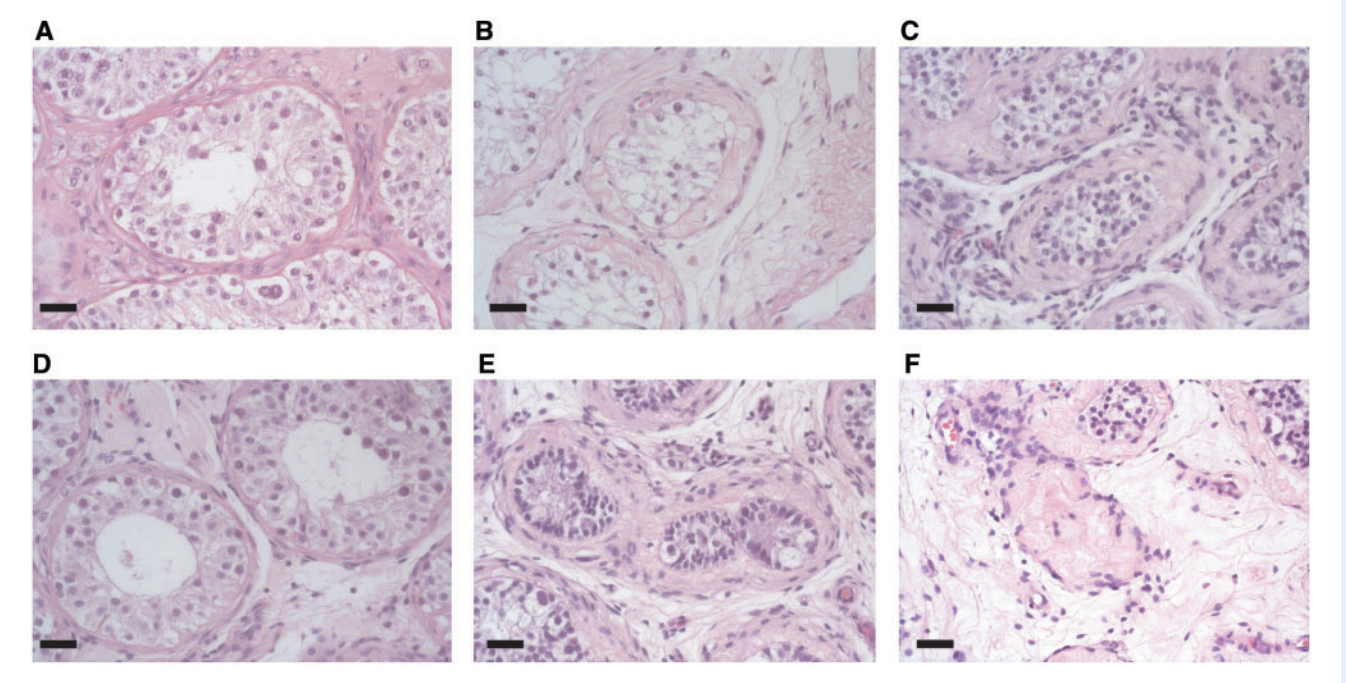

Figure 3. Different aspects of lumen and degrees of hyalinization of seminiferous tubules. (**A**) Open lumen. (**B**) Half-open lumen. (**C**) Absent lumen. (**D**) No hyalinization. (**E**) Mild hyalinization. (**F**) Severe hyalinization. Bar represents 20 µm.

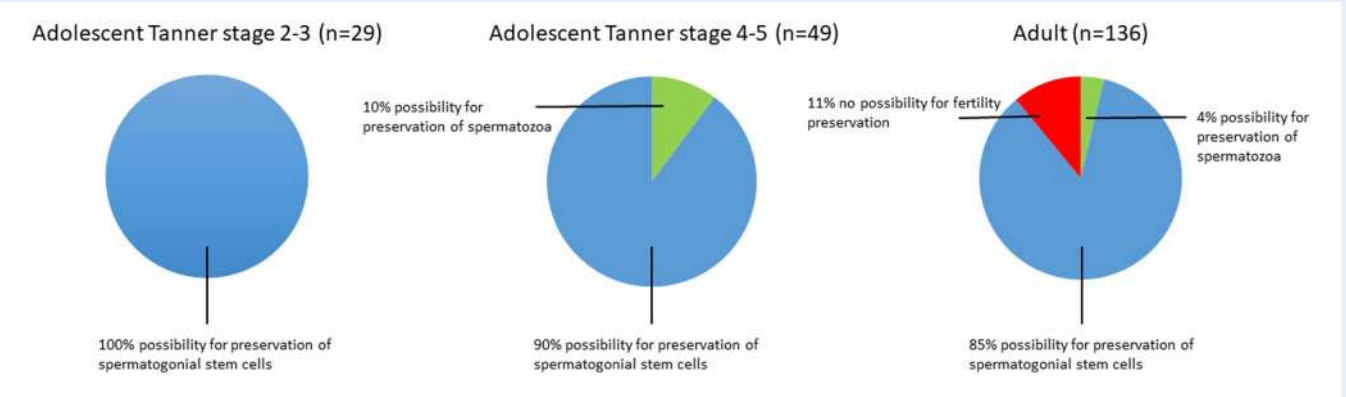

Figure 4. Comparison of the possibilities for fertility preservation between people who started medical treatment as adolescent in early puberty (Tanner stage 2-3) or late puberty (Tanner stage 4-5), and those who started as an adult (>18 years).

their study cohort) had complete spermatogenesis at time of gGAS. This finding was confirmed by Jiang *et al.* (2019) who even observed complete spermatogenesis in 40% of the 72 included transgender women. However, several other recent studies found lower percentages of complete spermatogenesis ranging from 0% to 11% of the study cohort (Jindarak *et al.*, 2018; Kent *et al.*, 2018; Matoso *et al.*, 2018; Vereecke *et al.*, 2021). It must be noted that hormonal and preoperative treatment protocols vary considerably within, and between, the different studies conducted on this topic. Therefore, for the current study, it was decided to only include transgender women who used estradiol in combination with testosterone suppressing therapy (triptorelin when initiated in adolescence, cyproterone acetate when initiated in adulthood), and to report results for those who continued GAHT until gGAS separate from those who discontinued four weeks prior to gGAS.

Since a study performed by Vereecke *et al.* (2021) also adhered strict in- and exclusion criteria that are similar to those in our adult subgroup, their results allow for the most accurate comparison. In addition, their method of analysis using immunohistochemistry to determine the most advanced germ cell type is similar to our study. In their

cohort of 97 transgender women, 12.4% had a complete absence of germ cells which is in line with the observed 11% in our cohort. However, none of their orchiectomy specimens showed complete spermatogenesis, as opposed to 4% of orchiectomy specimens in the adult subgroup of our cohort. Vereecke *et al.* (2021) also assessed the relationship between serum hormone levels and spermatogenic state in their cohort. They found that higher serum testosterone levels were associated with more advanced maturation, and higher serum estradiol levels were associated with a lower number of spermatogonia. However, the hormone levels were not measured on the day of gGAS, but at the last visit in the outpatient clinic 91.0 (57.5–152.5) days before surgery (Vereecke *et al.*, 2021). In contrast, Schneider *et al.* (2015) did collect serum and intratesticular testosterone levels on the day of gGAS but did not find an obvious correlation with spermatogenic state. In our gender identity clinic, hormone levels are not determined on the day of gGAS and laboratory results from the last visit in the outpatient clinic likely do not adequately reflect hormonal status during gGAS because of the preoperative cessation of GAHT 4 weeks prior to surgery. It was therefore decided not to assess this relationship in our cohort.

An interesting observation in the current study is that testicular histology and spermatogenesis seemed more negatively affected by GAHT in the adult subgroup compared to the adolescent subgroups, despite the lower mean duration of medical treatment in the former prior to gGAS. A higher percentage of hyalinization of the seminiferous tubules was observed in the adult subgroup, as well as a complete absence of germ cells in 15 orchiectomy specimens. The difference between the adult subgroup and the adolescent subgroups might be explained by age, lifestyle (a higher percentage of smokers and alcohol drinkers), higher dosages of estradiol or the use of cyproterone acetate instead of GnRHa as testosterone suppressing therapy. Whereas GnRHa only leads to inhibition of gonadotropin secretion, cyproterone acetate also has progestative effects and acts as a direct antagonist of the androgen receptor. It hereby inhibits the influence of androgens on the androgen-dependent organs, among which the testes. The latter might have more profound and irreversible effects on testicular tissue. Because of unwanted side-effects of cyproterone acetate (e.g. increased risk for meningioma), transgender women commencing GAHT in our clinic above the age of 18 years now receive GnRHa as testosterone suppressing therapy instead of cyproterone acetate. The potential consequence of irreversible infertility might be an extra reason to not prescribe cyproterone acetate anymore. In a future study, it would be interesting to assess if differences in testicular histology and spermatogenesis between adults and adolescents are still observed when they both receive GnRHa as testosterone suppressing therapy.

Cessation of GAHT prior to gGAS did not affect the possibilities for fertility preservation. In our study, the preoperative cessation of GAHT involved a period of four weeks, whereas the differentiation of spermatogonial stem cells into spermatozoa generally takes 10–12 weeks (Muciaccia *et al.*, 2013). Therefore, the period of cessation was most likely not long enough to influence the options for fertility preservation. If transgender women would be willing to discontinue GAHT for at least 12 weeks prior to gGAS, this might positively influence the chances of finding mature spermatozoa in the orchiectomy specimen. Moreover, they could even consider an attempt for cryopreservation of spermatozoa from a semen sample, obtained through ejaculation. However, it is unknown if spermatogenesis can recover if GAHT is stopped and how much time is needed for this purpose. Furthermore, it should not be underestimated that cessation of GAHT will result in increased testosterone levels which is likely to have negative physical and psychological consequences, and that masturbation is often not an option in transgender women due to severe genital dysphoria. A disadvantage of spermatozoa that are harvested from testicular tissue, is that they are not suitable for a minimally invasive and inexpensive IUI and can only be used for ICSI (Ombelet *et al.*, 2014). In addition, such ICSI treatments using surgically obtained spermatozoa are not always successful, since the cumulative ongoing pregnancy rate per cycle has been reported to be 22.8% and the live birth rate 22.3% (Meijerink *et al.*, 2016). Therefore, cryopreservation of a semen sample prior to initiation of GAHT remains the preferred method of fertility preservation in transgender women and harvesting germ cells from orchiectomy specimens might only be considered an alternative in those for whom this is not an option.

The lumina of the seminiferous tubules in those who initiated medical treatment in Tanner stage 2-3 were all either half-open, or absent. This observation might be explained by the immaturity of testicular tissue in early puberty, since an open lumen develops parallel to the development of spermatogenesis under the influence of increasing intratesticular testosterone levels. The fact that germ cells were encountered in all orchiectomy specimens from transgender women who initiated medical treatment as adolescent, is reassuring. Decision-making about fertility can be very difficult for adolescents since their intellectual, emotional and social immaturity may impede assessment and prediction of future desires regarding fertility and family planning. A recent study among transgender youth showed that 67% of young transgender women expressed a desire for future parenthood, but only 7% indicated to be frustrated if biological parenthood would not be feasible (Chiniara *et al.*, 2019). Another study, however, reported that 48% of transgender adolescents acknowledged that their desires regarding parenthood might change over time (Strang *et al.*, 2017). Reduced levels of gender dysphoria and improved mental health might result in an improved capability to establish romantic relationships and consider future family building. Our observation that immature germ cells remain present in testicular tissue during GAHT suggest that transgender adolescents still have potential options for fertility preservation after initiation of treatment by cryopreserving testicular tissue from orchiectomy specimen obtained during gGAS.

Cryopreservation of testicular tissue containing spermatogonial stem cells is mostly offered to pre-pubertal boys with cancer, prior to undergoing gonadotoxic therapies such as chemo- and radiotherapy, but some clinics also offer this option to transgender adolescents (Pang *et al.*, 2020). In the absence of complete spermatogenesis, the purpose of spermatogonial stem cell preservation in cisgender adolescents is to transplant these cells back into the testes years later, via injection into the rete testis space that is contiguous with all seminiferous tubules. Spermatogonial stem cells have the potential to colonize the testicular niche and regenerate spermatogenesis (David and Orwig, 2020). However, re-transplantation is not a feasible option for transgender women, as they will most likely use lifelong GAHT and many will undergo bilateral orchiectomy. Therefore, spermatogonial stem cell preservation will only be a viable method for fertility preservation in transgender women when other options for maturation become available, such as de novo testicular morphogenesis or *in vitro*

spermatogenesis. Although these techniques are successful in animal models, they are still experimental and far from the clinical realm (Pelzman et al., 2020). Continuing research in this area will hopefully make these techniques available so that transgender adolescents, who are otherwise unable to have genetically related children, will be able to retain this possibility by cryopreserving testicular tissue containing spermatogonial stem cells. Furthermore, future research should focus on how GAHT influences the quality of germ cells and the safety of using cells harvested from orchiectomy specimens, for reproductive techniques. Lastly, it is important to examine how transgender women feel about fertility preservation options in orchiectomy specimens obtained during gGAS.

A limitation of this study is the lack of data on serum hormone levels on the day of gGAS. We were therefore unable to verify if the transgender women who were asked to temporarily stop hormonal treatment four weeks prior to surgery actually did so, and if people with complete spermatogenesis were compliant to treatment. However, the last known serum testosterone levels before gGAS were suppressed in all participants. Furthermore, despite our efforts to create a homogeneous study population, by excluding people who used estrogen monotherapy and those who used spironolactone as anti-androgenic treatment, participants still used varying formulations of estrogens and switched between different formulations over time. We were therefore unable to assess if different estrogen formulations have different effects on testicular histology and spermatogenesis. Strengths of our study include the large sample size of 214 transgender women, and the creation of six subgroups to allow for comparison between different preoperative protocols before gGAS and pubertal stage at initiation of medical treatment. Hereby, this study provides novel information about the influence of starting medical treatment in early puberty on testicular function, and its consequences for the possibilities for fertility preservation at time of gGAS. This is relevant because we are seeing a global increase of the number of referrals of adolescents to gender identity clinics (Handler et al., 2019; Kaltiala et al., 2020). At the same time, there is increasing controversy over the provision of GAHT to adolescents, with the negative effect on fertility often cited as an argument for limiting adolescents' access to gender-affirming care (The Economist, 2020). Our observation that the spermatogonial stem cell pool is still intact in people who initiated GAHT during adolescence is therefore valuable information in this debate.

Conclusion

Counseling of transgender women about the effect of medical treatment on fertility and the currently available options for fertility preservation remains essential. However, for some transgender women with a wish for fertility preservation, there are barriers that prevent the use of semen cryopreservation. For example, some initiate medical treatment in early puberty before the development of complete spermatogenesis, some are unable to masturbate, and some feel that a temporary cessation of GAHT would be too psychologically and physically disruptive. The results of this study show that there may still be options for fertility preservation using orchiectomy specimens obtained during gGAS. In a small percentage of transgender women who initiated medical treatment in Tanner stage 4 or higher, spermatozoa could have been harvested from the orchiectomy specimen at time of gGAS. In addition, the vast majority (>85%) of transgender women in our cohort could still opt for cryopreservation of testicular tissue harboring spermatogonial stem cells. A complete absence of germ cells was only observed in a small number (7%) of transgender women in our cohort, who all commenced GAHT as adult. The possibilities for fertility preservation seem irrespective of preoperative cessation of GAHT and the duration of GAHT prior to gGAS.

Initiation of medical treatment in early pubertal adolescents (Tanner stage 2-3) limits the ability to retrieve mature spermatozoa that can directly be used for assisted reproductive techniques. However, if maturation techniques like *in vitro* spermatogenesis become available in the future, harvesting germ cells from orchiectomy specimens might be a promising option for those who are otherwise unable to have biological children.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

Part of the data underlying this article are available in the article and in its online supplementary material, the rest of the data will be shared on reasonable request to the corresponding author.

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Authors' roles

I.d.N.—conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript. C.L.M.—conception and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for intellectual content. A.M.—analysis and interpretation of data, critical revision of the manuscript for intellectual content. Y.S.—acquisition of data, analysis and interpretation of data, drafting of manuscript. E.M.H.—acquisition of data, drafting of manuscript. W.B.v.d.S.—acquisition of data, critical revision of the manuscript for intellectual content. S.E.H.—analysis and interpretation of data, critical revision of the manuscript for intellectual content. M.d.H.—conception and design, analysis and interpretation of data, critical revision of the manuscript for intellectual content. J.H.—conception and design, analysis and interpretation of data, critical revision of the manuscript for intellectual content. A.M.M.v.P.—conception and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for intellectual content. N.M.v.M.—conception and design, analysis and interpretation of data, critical revision of the manuscript for intellectual content. All authors approved the final version of the manuscript.

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Conflict of interest

None.

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Family Planning and Fertility Counseling Perspectives of Gender Diverse Adults and Youth Pursuing or Receiving Gender Affirming Hormone Therapy

Eric Walton, Sina Abhari, Vin Tangpricha, Cameron Futral, and Akanksha Mehta

OBJECTIVE To describe family planning and fertility counseling perspectives of reproductive-age gender diverse adults and youth pursuing gender affirming hormone therapy.

MATERIALS AND METHODS This was a cross sectional survey study of gender diverse adults and youth pursuing or receiving gender affirming hormone therapy. The primary outcomes of interest were parental desire and priorities for fertility preservation.

RESULTS Fifty-seven individuals (46 adults and 11 youths) completed the survey; 51% were transgender women, 35% were transgender men, and 14% identified as non-binary. 32 participants expressed interest in (n = 15, 26%) or uncertainty about (n = 18, 32%) future parenthood. 48% of participants had considered gamete cryopreservation, but only 7% each previously completed or planned to pursue this fertility option; 67% cited cost as a barrier. Participants with interest in or uncertainty about future parenthood were more likely to consider cryopreservation (P <.001) or stopping hormones for fertility preservation (P <.001). 58% of respondents reported discussing fertility preservation with a health care provider with lower rates among youth participants (P = .017). From a family planning perspective, 58% of respondents described counseling as adequate; 23% described it as inadequate and 19% reported not receiving any counseling. Participants who endorsed strong or uncertain parental desire were more likely to report inadequate counseling (P = .016).

CONCLUSION Gender diverse individuals interested in or undecided about future parenthood were more likely to consider cryopreservation and report inadequate family planning counseling. Therefore, current counseling practices may be insufficient and referral to a fertility specialist should be considered. UROLOGY 171: 244–250, 2023. © 2022 Elsevier Inc.

Transgender individuals increasingly engage with the healthcare system for gender affirming hormone therapy (GAHT) to help align physical characteristics with gender identity.[1] GAHT appears to negatively impact fertility potential. Individuals assigned male at birth (AMAB) demonstrate variable levels of altered spermatogenesis and abnormal semen parameters after receiving GAHT.[2-4] For individuals assigned female at birth (AFAB), exogenous testosterone does not appear to decrease ovarian reserve[2,5]; it may be associated with changes in ovarian morphology, however, more recent research challenges these findings.[2,6] Transgender youth must consider fertility preservation options carefully as pre-pubertal suppression of puberty may limit gonad development and fertility preservation options.[7] As a result, best practice guidelines recommend discussion of fertility preservation and family planning prior to the initiation of GAHT.[8-10]

Transgender adults report variable interest in future biologically related children ranging from 21% to 54%.[11] Biologically related children may be less important to transgender youth with 12%-36% expressing interest.[11-13] Gamete cryopreservation is frequently considered by transgender individuals, but less than 10% of adults or youth complete fertility preservation. The most common barrier to fertility preservation is cost[11,14,15]; some barriers

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may disproportionately affect individuals AFAB.[16] Another barrier to fertility preservation is adequate counseling with studies reporting up to 50% of adults and 80% of youth do not receive counseling prior to initiating GAHT.[13,17-19] Literature evaluating the perceived quality of family planning counseling is sparse, but 1 study found that 37% of participants at a gender health program did not think they received adequate information.[20]

To better describe perspectives about fertility preservation and family planning in the transgender population of the United States, we surveyed adults and youth pursuing GAHT at an endocrinology clinic about fertility-related care. We assessed desires for future parenthood and obstacles to parenthood. Furthermore, we evaluated for the presence and adequacy of family planning counseling.

MATERIALS AND METHODS

This cross-sectional survey study utilized an anonymous non-validated online survey built on the REDCap electronic data capture platform. The study was conducted at a single academic medical center in the adult and pediatric endocrinology clinics. Individuals referred to these clinics between February 2021 and May 2022 for GAHT were screened for eligibility. Only individuals no older than 55 years of age were eligible for participation. Individuals unable to provide informed consent and vulnerable populations were excluded. This study was reviewed and approved by an Institutional Review Board.

Participant recruitment and informed consent occurred in-person during a clinic visit or post-hoc via telephone (only for adults). At the conclusion of a clinic visit (or after conclusion for telephone recruitment), eligible individuals were offered the opportunity to participate. Informed consent to use de-identified data was verbal for adults and written for youth. Participants were then given a QR code and/or emailed a link to the REDCap survey. The survey was completed at a time and location convenient to the participant.

The REDCap survey was divided into 3 sections: demographics, family planning, and fertility preservation. We included a screening question to ensure the survey was not completed more than once by a single individual. In the family planning section, we asked about current children, desires for future children, and obstacles to parenthood. Gender affirming care, priorities related to cryopreservation, and participant experiences with family planning counseling were addressed in the fertility preservation section. The survey instrument is available as a supplemental document.

Our primary measures of interest were the desire for future parenthood and interest in fertility preservation among transgender individuals. In addition, we assessed obstacles to future parenthood and the adequacy of family planning counseling in this cohort. Survey responses were compared according to age group, sex assigned at birth, and self-described parental desire. Descriptive statistics were calculated for continuous variables. Univariate t-tests were used to analyze continuous data and chi-square tests of independence were used for categorical data. Missing data points were excluded from analyses. All statistical analyses were completed using SPSS® software (IBM SPSS, Armonk, NY).

RESULTS

Subject Demographics

During the study period, 112 individuals presented for GAHT; 11 were not eligible for this study due to age. The remaining individuals were approached and 80 consented to participate. Ultimately, 57 gender diverse persons (71% of those consented) seeking GAHT completed the online survey – 46 adults and 11 youths. Table 1 displays characteristics of survey respondents. Respondents AFAB were younger and more likely to identify as non-binary compared with their AMAB counterparts. Overall, 83% of respondents reported receiving GAHT, and 67% had been on this therapy for at least 6 months. Transgender youth were less likely to be on GAHT. Only 18% reported a history of

Table 1. Characteristics of gender diverse survey respondents recruited from an endocrinology clinic stratified by age group and sex assigned at birth

Characteristic	All (n = 57)	Youth (n = 11)	Adult (n = 46)	*P*	AMAB (n = 31)	AFAB (n = 26)	*P*
Age, years							
Median	23	15	25	<.001	29	20	<.001
Interquartile range	18-31	13-15	21-35		20-40	17-23	
Sex assigned at birth				.18	–	–	–
Male (AMAB)	31 (54)	4 (36)	27 (59)		–	–	
Female (AFAB)	26 (46)	7 (64)	19 (41)		–	–	
Gender identity				.21			<.001
Female	29 (51)	3 (27)	26 (57)		29 (94)	0 (0.0)	
Male	20 (35)	6 (55)	14 (23)		0 (0.0)	20 (77)	
Non-binary	8 (14)	2 (18)	6 (13)		2 (6.5)	6 (23)	
Ethnicity				.42			.11
White	41 (72)	9 (82)	32 (70)		25 (81)	16 (62)	
Non-white	16 (28)	2 (18)	14 (30)		6 (19)	10 (39)	
Relationship status				.80			.45
Partner(s)	26 (46)	5 (50)	21 (46)		13 (42)	13 (52)	
No partner	30 (53)	5 (50)	25 (54)		18 (58)	12 (48)	
Current GAHT	47 (83)	4 (36)	43 (96)	<.001	26 (87)	21 (81)	.55
History of GAS	10 (18)	1 (9.1)	9 (20)	.40	6 (20)	4 (15)	.65
Current biologic child	4 (7.0)	0 (0.0)	4 (8.9)	.31	4 (13)	0 (0)	.053

AFAB, assigned female at birth; AMAB, assigned male at birth; GAHT, gender affirming hormone therapy; GAS, gender affirming surgery.

gender affirming surgery with no difference noted between age groups or sex assigned at birth. Few participants had an existing biologically related child. Those who did were exclusively AMAB. The most common type of health insurance was employer or union sponsored coverage (67%), including as a dependent; no respondents reported being uninsured.

Parental Desire and Access to Fertility Preservation

Table 2 displays family planning considerations respondents were asked about in the survey.: 26% expressed interest in future parenthood, 32% were undecided, and 42 % were not interested. Those with strong or uncertain parental desire were most likely to consider a future biologically related child important. All these respondents were adults (n = 17, 38%, $P = .015$). None of the participants with current children expressed interest in future parenthood.

Although 48% of respondents had considered gamete preservation, only 7.0% previously completed this fertility option and only another 7.0% planned to pursue it. Notably, those who were interested in or undecided about future parenthood reported higher interest in gamete cryopreservation but did not have statistically significant higher planning or completion rates. They were, however, more willing to stop GAHT for cryopreservation. Importantly, 67% of respondents cited cost as a barrier to cryopreservation with higher rates among those with strong or uncertain parental desire. Respondents interested in a future biologically related child were also more likely to consider cryopreservation (82% vs 33%, $P < .001$), stop GAHT for cryopreservation (69% vs 16%, $P < .001$), and cite cost as a barrier (88% vs 61%, $P = .040$). Compared with individuals who do not consider a future biologically related child important, this group planned or completed cryopreservation at a statistically significant higher rate (31% vs 7.7%, $P = .024$). The most common preferred alternative family planning option was adoption (86%).

There were some important differences between AMAB and AFAB respondents. All participants who completed cryopreservation were AMAB (n = 4, 13%, $P = .058$). In contrast, AFAB respondents were more likely to cite cost as a barrier (84% vs 57%, $P = .029$) to cryopreservation and list the complexity of the medical process as an obstacle to future parenthood (42% vs 16%, $P = .029$). They were also more willing to stop GAHT for fertility preservation (46% vs 18%, $P = .025$).

Table 3 displays reasons that may prevent future parenthood selected by survey participants. There were 32 individuals either interested in future parenthood or undecided; these participants more frequently cited financial status and the complexity of the medical process as reasons that may prohibit future parenthood. In contrast, 24 individuals expressed no parental desire; they more frequently identified current parenthood as a reason they may avoid future parenthood. Participants who considered a future biologically related child important also frequently listed complexity of the medical process (59% vs 15%, $P < .001$) as a reason that may prevent parenthood. However, financial status was not a statistically significant barrier for this group (53% vs 31%, $P = .12$). Only 49% of respondents felt that society views LGBTQ parenting as acceptable, however, most (61%) also indicated that societal views have no influence on their family planning choice and only 2 cited this as a barrier to future parenthood. Of note, these 2 individuals also expressed interest in future parenthood.

Family Planning Counseling

Only 58% of survey respondents reported discussing fertility preservation with a health care provider. This was less common among transgender youth than adults (27% vs 67%, $P = .017$). Figure 1 displays how participants described the adequacy of the fertility and family planning counseling they have received from health care providers: 58% of respondents described counseling as adequate, 23% described it as inadequate, and 19% reported not receiving counseling. Responses to this question re-demonstrate a lower rate of counseling among transgender youth. AFAB respondents also reported lower rates of adequate counseling. Participants interested in or undecided about future parenthood reported inadequate counseling more frequently than those without parental desire who endorsed adequate and absent counseling at higher rates. This pattern was not reflected between participants who do and do not consider a future biologically related child important.

COMMENTS

In this study of transgender youth and adults seeking GAHT with endocrinology providers, we found that 58% of participants were interested (26%) or undecided (32%) about future parenthood. There was no difference in

Table 2. Responses to family planning survey questions by 57 gender diverse patients recruited from an endocrinology clinic stratified by future parental desire

		Interest in Future Parenthood — n (%)			
Family Planning Consideration	Total — n (%)	*Interested (n =15)*	*Undecided (n = 18)*	*Not Interested (n = 24)*	*P*
Current biologic child	4 (7.0)	0 (0)	0 (0.0)	4 (17)	.057‡
Future biologic child*	17 (30)	7 (50)	9 (50)	1 (4.2)	.001†
Gamete cryopreservation					
Considered	27 (48)	10 (71)	14 (78)	3 (13)	<.001
Plan to pursue	4 (7.0)	3 (21)	1 (6.3)	0 (0.0)	.051
Completed	4 (7.0)	0 (0.0)	3 (18)	1 (4.2)	.13
Plan or completed	8 (14)	3 (21)	4 (24)	1 (4.2)	.16
Willing to stop GAHT	17 (30)	9 (69)	8 (44)	0 (0.0)	<.001
Cited cost as a barrier	38 (67)	13 (93)	15 (83)	10 (44)	.002†

GAHT, gender affirming hormone therapy.
* Rated a future biologically related child as important.
† Statistical significance greater when interested/undecided groups combined.
‡ Association statistically significant when interested/undecided groups combined.

Table 3. Reasons preventing future parenthood selected by 57 gender diverse survey respondents recruited from an endocrinology clinic stratified by degree of parental desire

Reasons Preventing Future Parenthood	Total — n (%)	Interest in Future Parenthood — n (%)			P
		Interested (n = 15)	*Undecided (n = 18)*	*Not Interested (n = 24)*	
Financial status	21 (37)	8 (53)	9 (50)	4 (17)	.026*
Lack of desire	20 (35)	0 (0.0)	5 (28)	15 (63)	<.001
Complicated medical process	16 (28)	7 (47)	8 (44)	1 (4.2)	.003*
Current parenthood	6 (11)	0 (0.0)	0 (0.0)	6 (25)	.010*
LGBTQ parenting not accepted	2 (3.5)	2 (13)	0 (0.0)	0 (0.0)	.055
Personal gender identity	1 (1.8)	0 (0.0)	1 (5.6)	0 (0.0)	.33
Other†	13 (23)	4 (27)	5 (28)	4 (17)	.64

* Statistical significance greater when interested/undecided groups combined.
† Includes: age (too young/too old), biological barriers, psychological barriers, preference for adoption, complexity of the adoption process.

parental desire between adult and youth respondents. We found that transgender individuals with strong or uncertain parental desire considered fertility preservation at high rates and were more likely to report inadequate family planning counseling. Regardless of parental desire, few participants completed cryopreservation. Our findings add to the ongoing characterization of family planning desires among transgender persons. Importantly, we demonstrated that many gender diverse people of reproductive age are undecided about future parenthood while pursuing GAHT and would benefit from further counseling.

A recent multi-center survey-based study of 80 American transgender adults reported similar rates of parental desire as our study. They found a mean desire for parenthood of 59.9% among respondents.[17] A web-based study of 543 transgender adults living in Belgium found that 23.6% of AMAB and 39.6% of AFAB respondents had current or future parental desire; 7.9% and 9.3% were undecided, respectively.[18,19] The median age of that cohort was 43 years for AMAB and 24 years for AFAB participants compared with 23 years for our study of transgender adults and youth. Most studies of transgender youth comment on desire for future biologically related children, however, 1 Canadian study did find that 66% of participants expressed desire for any future parenthood.[21] Our findings regarding parental desire are very similar to these studies that show a little more than half of transgender persons have interest in future parenthood.

Studies of gender diverse adults indicate parental desire for biologically related children ranges from 21% to 54%.[11] Most studies report low desire (<20%) for future biologically related children among transgender youth,[11,12] however, 36% of adolescents in 1 study expressed interest.[13] In our study, a future biologically

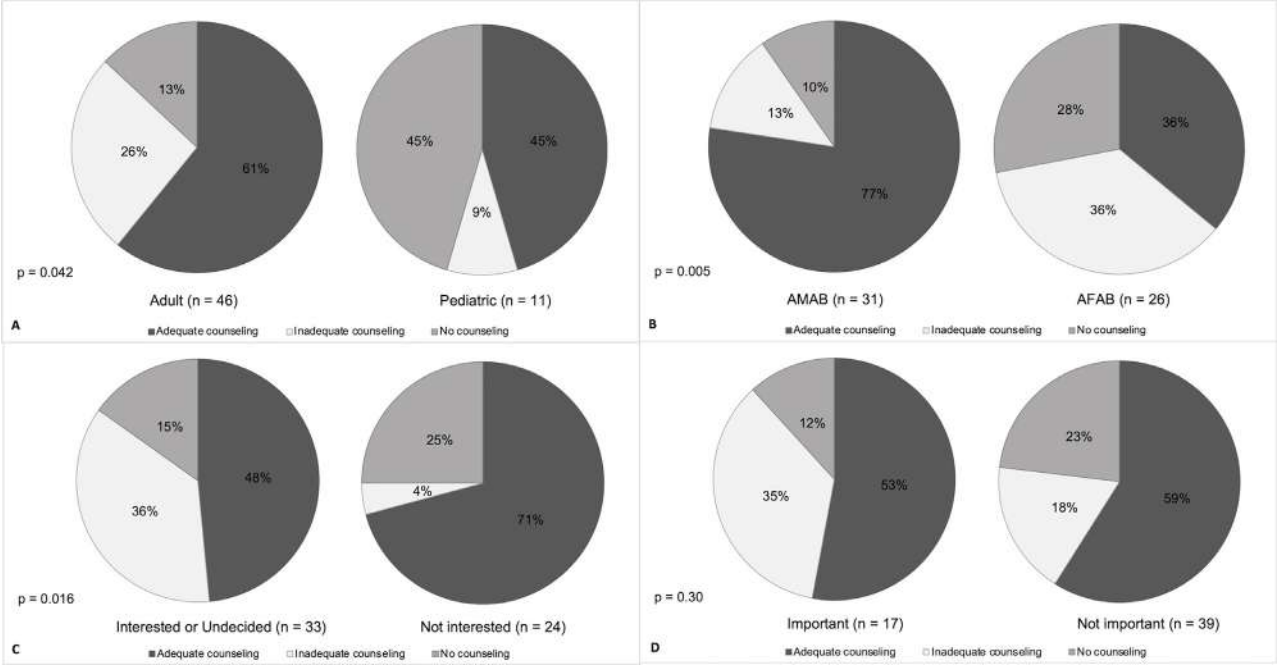

Figure 1. Description of health care provider fertility and family planning counseling by 57 gender diverse survey respondents recruited from an endocrinology clinic. (**A**) by age group. (**B**) by sex assigned at birth. (**C**) by interest in future parenthood. (**D**) by importance of future biologically related child. AFAB, assigned female at birth; AMAB, assigned male at birth.

related child was important to 30% of participants, similar to prior studies. All respondents who placed importance in a future biologically related child were adults, consistent with recent observations that biologically related children may not be important to transgender youth. Adoption appears to be the preferred alternative with more than 70% of respondents endorsing it in this study and the existing literature.[13,21] Furthermore, there was significant variability in desire for a biologically related child depending on parenting intentions: 50% among undecided and interested vs 4.2% among not interested. This is particularly important given evidence for the fertility impact of GAHT and our finding that one third of individuals presenting for GAHT have not solidified their intentions for future children.

While 48% of respondents in our study considered gamete cryopreservation, less than 10% either planned to pursue or previously completed fertility preservation. As expected, participants who expressed strong or uncertain parental desire were much more likely to consider cryopreservation (>70%) compared with those not interested in parenthood. Importantly, they did not pursue or complete fertility preservation at statistically significant higher rates. Our results are consistent with other published results: 1 study found that 37.5% of transgender men considered gamete cryopreservation and another found that 51% of transgender women would have considered fertility preservation if it had been offered.[11] In contrast, rates of cryopreservation are usually less than 5%.[11,22] Similarly, several studies identify that less than 10% of transgender youth in North America complete fertility preservation with higher rates among transgender women.[14,15] Interestingly, studies out of Australia and the Netherlands found rates of sperm cryopreservation among AMAB gender diverse youth between 38% and 62%, possibly related to lower costs.[23]

Cessation of GAHT is an important consideration for gender diverse individuals pursuing cryopreservation as the impacts on fertility potential are at least partially reversible when stopped.[2] Prior studies indicate that stopping GAHT is a barrier to fertility preservation for 20%-25% of transgender adults,[17,20] but willingness to stop GAHT increases with self-reported parental desire.[17] From the youth perspective, only 3% indicate willingness to delay GAHT for fertility preservation.[12] In our study only 32% of respondents expressed willingness to stop GAHT for fertility preservation; however, respondents with interest in or uncertainty about future parenthood expressed a much higher willingness (69% and 44%, respectively) to stop hormones. We also found that individuals AFAB may be more willing to stop GAHT. This finding accentuates the importance of appropriately timed counseling prior to the initiation of GAHT, which would eliminate the need to consider stopping hormone therapy.

Transgender individuals include cost as a barrier to fertility preservation as frequently as 39-60% of the time.[16,17,20,22] We had similar findings in our study with 67% of survey respondents citing cost as a barrier to gamete cryopreservation and higher rates among those with strong or uncertain parental desire. Fewer respondents (37%) endorsed their current financial circumstance as an obstacle to future parenthood. Notably, individuals AFAB (compared with AMAB) were more likely to consider cost a barrier to fertility preservation. This association did not persist when considering the financial implications of future parenthood. As such, cost may influence parental desire separate from the price of fertility preservation. Other commonly endorsed reasons that may prevent future parenthood included lack of desire (35%) and the complexity of the medical process (28%) with complexity more frequently listed by individuals AFAB. Our findings corroborate the obstacles to fertility preservation listed in recent studies, as well as the greater influence of medical complexity on individuals AFAB.[16,20]

Professional societies recommend counseling gender diverse individuals about the fertility consequences and family planning options prior to the initiation of GAHT.[8-10] Despite universal knowledge of the potential fertility implications of GAHT, only 58% of our cohort reported discussing fertility preservation with a health care provider with lower rates among youth. These findings are consistent with counseling rates reported in the adult and pediatric literature[13,17-19]; they indicate an opportunity to improve fertility counseling for gender diverse persons. Comparatively, research indicates clinicians providing transgender care perceive they perform counseling at a higher rate (80%-90%).[24] While self-reported provider-level barriers in that study were low, 15%-20% of clinicians providing transgender care reported lack of knowledge about fertility implications and preservation options as an impediment to well-informed discussions with patients.

Few studies assess the perceived adequacy of family planning counseling received by gender diverse individuals. In our study, 58% of respondents described counseling as adequate; 23% described it as inadequate and 19% reported not receiving any counseling whatsoever. Adequacy of counseling varied with age, sex assigned at birth, and parental desire. Adults reported adequate counseling and youth reported no counseling at higher rates, respectively. AMAB individuals also reported higher rates of adequate counseling than their AFAB counterparts. Of note, AFAB individuals were younger in this study, which may explain some of this variability. Perhaps most important, individuals pursuing GAHT with strong or uncertain parental desire reported significantly higher rates of inadequate counseling. These data suggest that current counseling paradigms may be insufficient for those most at risk of GAHT impacting future family planning. Referring this subset of people to a fertility specialist could improve the counseling they receive.

Our study is not without limitations. It was conducted at an academic endocrinology clinic among gender diverse persons of reproductive age presenting for GAHT using a non-validated survey instrument, which means the results may not be generalizable to the larger transgender population.

For example, not all gender diverse individuals choose to medically or surgically transition. This decision could influence their response to questions related to fertility-related care. Furthermore, our survey did not query participants who provided counseling, when that counseling occurred (ie, before or after GAHT), or which provider (ie, our institution vs another institution) they were considering when answering the survey. Thus, our survey may reflect participant perception of counseling provided by our institution, which we hypothesize may be an overestimate due to the specialized nature of our clinic, and limits measurement of counseling adequacy prior to initiating GAHT. Furthermore, participants in this study were more insured than most descriptive studies of transgender persons[25] and we likely underestimated the influence of cost on decision making. As a cross-sectional survey-based study the influence of response bias must be considered. Approximately 20% of eligible people declined to participate in the study and 30% of consented participants did not complete the online survey; it is difficult to predict how their responses would change the results of this study. Response bias may have also contributed to the age difference between AMAB and AFAB individuals. Despite these limitations, many of our results uphold findings from population-level studies. Our findings are also limited by a small sample size, especially for transgender youth and non-binary persons. This likely made our study underpowered to highlight small-to-moderate differences between these groups and the general cohort. Finally, due to the cross-sectional design we could not assess how responses changed over time. For example, we could not measure whether differences between youth and adults remained constant or narrowed as adolescents mature.

CONCLUSION

Gender diverse individuals interested in or undecided about future parenthood constitute a sizable portion of individuals presenting for GAHT. Current family planning counseling practices appear to be insufficient for educating and supporting transgender people undergoing hormonal gender transition, especially those interested in future parenthood. As such, all persons presenting for gender affirming care should be queried about future parental desire to guide clinician attention to counseling and referral to fertility specialists.

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SUPPLEMENTARY MATERIALS

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Pubertal Suppression in Early Puberty Followed by Testosterone Mildly Increases Final Height in Transmasculine Youth

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Abstract

Context: Treatment for transmasculine youth (TMY) can involve testosterone treatment and is sometimes preceded by gonadotropin-releasing hormone agonist (GnRHa) treatment for puberty blockade. GnRHas can increase final height in birth-assigned females with central precocious puberty. Maximizing final adult height (FAH) is an important outcome for many TMY.

Objective: Our objective was to determine how GnRHa treatment before testosterone impacts FAH.

Methods: Retrospective cohort study at 5 US transgender health clinics. Participants were 32 TMY treated with GnRHas in early to midpuberty before testosterone (GnRHa + T group) and 62 late/postpubertal TMY treated with testosterone only (T-only group).

Results: The difference between FAH minus midparental target height (MPTH) was +2.3 ± 5.7 cm and −2.2 ± 5.6 cm in the GnRHa + T and T-only groups, respectively ($P < .01$). In the GnRHa + T group, FAH was 1.8 ± 3.4 cm greater than predicted adult height (PAH) ($P < .05$) and FAH vs initial height (IH) z-score was 0.5 ± 1.2 vs 0.16 ± 1.0 ($P < .05$). After adjusting for patient characteristics, each additional month of GnRHa monotherapy increased FAH by 0.59 cm (95% CI 0.31, 0.9 cm), stage 3 breast development at start of GnRHa was associated with 6.5 cm lower FAH compared with stage 2 (95% CI −10.43, −2.55), and FAH was 7.95 cm greater in the GnRHa + T group than in T-only group (95% CI −10.85, −5.06).

Conclusion: Treatment with GnRHa in TMY in early puberty before testosterone increases FAH compared with MPTH, PAH, IH, and TMY who only received testosterone in late/postpuberty. TMY considering GnRHas should be counseled that GnRHas may mildly increase their FAH if started early.

Key Words: transgender youth, final adult height, GnRHa, gender-affirming care, testosterone, transmasculine

Abbreviations: AFAB, assigned female at birth; BMI, body mass index; FAH, final adult height; GAHT, gender-affirming hormone therapy; GnRHa, gonadotropin-releasing hormone agonist; IH, initial height; MPTH, midparental target height; PAH, predicted adult height; T, testosterone; TMY, transmasculine youth.

An increasing number of youth are identifying as transgender and gender diverse. Gender-affirming hormone therapy (GAHT) has been shown to improve psychological well-being and significantly reduce gender dysphoria and adverse mental health outcomes [1-3]. Pubertal suppression with gonadotropin-releasing hormone agonists (GnRHas) can offer benefit by preventing further progression of secondary sex characteristics while allowing patients time to explore their gender further and to decide whether to pursue GAHT. GnRHas are sometimes recommended for management of gender dysphoria and may be started as early as the first sign of puberty, or in Tanner stage 2. Individuals may be on GnRHa monotherapy for years prior to initiating GAHT [4, 5]. Many transgender and gender-diverse youth who start GnRHas eventually choose to allow puberty to progress with GAHT, rather than their endogenous sex hormone production [6]. For transmasculine youth (TMY) on GnRHas,

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pubertal induction is accomplished using testosterone, which is often started while continuing GnRHa treatment for some time. Testosterone is commonly started at a low dose and increased gradually over 1 to 3 years to eventually reach testosterone serum levels in the adult male normal range [5].

Adult height may be an important physical characteristic for transmasculine individuals in particular [7]. On average, adult females in the United States are about 13 cm shorter than adult males, and a height at the 50th percentile for an adult female is at the third percentile for an adult male based on the Centers for Disease Control and Prevention (CDC) growth charts for ages 2-20 years [8, 9]. Adult height is determined by multiple factors, including chromosomal sex, pubertal timing, genetics, nutritional status, etc. Therefore, final adult height (FAH) is an important consideration when discussing the impact of GAHT.

Since the 1980s, the use of GnRHas has been shown to slow the rate of bone age advancement and increase FAH in individuals assigned female at birth (AFAB) treated for central precocious puberty [10-15]. However, the effect on final height in those with borderline central precocious puberty (ages 6-8 years) or early–normal puberty timing is not as clear [16]. Studies performed using GnRHas in AFAB children with short stature but normal pubertal development showed a minimal increase in FAH [17, 18]. However, a meta-analysis looking at adult height in girls with early puberty treated with GnRHas vs after spontaneous pubertal growth spurt showed that treatment with GnRHas did not lead to a significant difference in final height [19].

A key difference between the studies using GnRHas for height augmentation in presumed cisgender females undergoing estrogen-mediated puberty is that TMY on GnRHas may then opt for testosterone-mediated pubertal development. Since males on average have a higher peak pubertal growth velocity and FAH than females [20], we were interested in determining whether using GnRHas to slow bone age progression and allow growth plates to remain open followed by testosterone administration would result in greater adult height than otherwise expected. A recent study by Willemsen et al [21] demonstrated a possible mild increase in FAH in those treated with GnRHas prior to testosterone compared with midparental target height (MPTH) and predicted adult height (PAH) based on bone age height predictions. In this study we aimed to determine whether GnRHas prior to testosterone increased FAH compared with their initial height (IH) z-score, MPTH, and PAH, as well as compared with a control group of TMY treated with testosterone without preceding GnRHas.

Materials and Methods

Study Design and Participants

This is a retrospective cohort study involving multiple institutions in the United States: Children's National Hospital as the coordinating center, Children's Hospital of Philadelphia, Chase Brexton Health Center, Ann & Robert H. Lurie Children's Hospital of Chicago, and Boston Children's Hospital. Some patients who were seen at Children's National had visits at MedStar Washington Hospital Center in Washington, DC, in addition to Children's National Hospital. Institutional Review Board approval was obtained from each institution except for Chase Brexton, which was overseen by the Institutional Review Board at the University of Maryland. This study involved TMY, defined for purposes of this study as AFAB individuals who identify along a masculine spectrum and may include nonbinary individuals. Participants were selected after query of the electronic medical records for all patients seen in at least 1 of the participating clinics between January 1, 2008, and June 15, 2020, using diagnostic codes ICD-10 F64.0, F64.9, and E34.9 or ICD-9 302.85. Eligible participants were AFAB youth who received testosterone with or without preceding GnRHa and reached FAH. FAH was defined as a growth velocity ≤1.5 cm/year, 3 years postmenarche, or a bone age ≥15 years. For the data analysis, participants were separated into 2 groups: those who received GnRHas prior to testosterone (GnRHa + T group) and those who received gender-affirming testosterone therapy alone (T-only). The GnRHa + T group included only those who were in Tanner stage 2 or 3 breast development (or confirmed premenarche if puberty staging was unclear at the time of GnRHa initiation), and therefore had remaining growth potential, and for whom biologic parents' heights were available in order to calculate MPTH. All subjects who met inclusion criteria for the GnRHa + T group were included. Those in the T-only group had to be at least 15 years old at the start of treatment, and therefore had completed most of their growth by the time testosterone was initiated and served as a treatment-naïve control group. There was a surplus of patients in the T-only group at all sites, so all eligible subjects were deidentified and a subset was randomly selected by the statistician in a 2:1 ratio for T-only:GnRHa + T participants. All patients on growth-altering medications, such as systemic glucocorticoids, or with any history of a growth-altering disorder, such as precocious puberty, growth hormone deficiency, or an advanced or delayed baseline bone age, were excluded. Patients on stimulant medications were included in both groups as has been done in previous studies investigating height in this population [22].

Data Collection

Manual chart review was performed for each participant. Study data were collected in REDCap electronic data capture tools hosted at the Children's Research Institute, part of Children's National Hospital [23, 24]. Data collected for the initial visit (visit right before GnRHa or testosterone was initiated, depending on group) included race/ethnicity, height, weight, body mass index (BMI), age and tanner stage (if known), medication name, formulation and frequency of administration, and, if performed, bone age X-ray and interpretation (all were read using Gruelich and Pyle standards) [25]. PAHs from bone age X-rays were calculated using the Bayley and Pinneau method [26]. The same information was collected from subsequent clinic visits until FAH was achieved. Due to the variability of height measurements in the medical records, the average of all the heights recorded after the FAH was reached was calculated and this was used as the FAH for data analysis purposes. Any height measurement that was either greater or equal to 3 cm above or below all other measurements was excluded as an outlier when calculating the average FAH. One participant in the GnRHa + T group did not have a final height recorded in the medical record, so they were contacted via telephone to report their current height. For consistency, the z-score for all heights including the FAH was calculated based on the CDC 2-20 year growth chart for girls [9]. MPTH was calculated for all participants in the GnRHa + T group and for participants in the T-only group, when possible, using the following formula: [(father's height − 13 cm) + mother's height]/2 [27]. The MPTH was obtained from the medical record if that information was recorded, and if not a member

Table 1. Participant characteristics

	GnRHa + T (n = 32)	T- only (n = 62)	*P* value
Initial height (cm)	153.8 ± 7.9 (32)	164.1 ± 6.8 (62)	<.001
Initial height (z-score)	0.16 ± 1.0 (32)	0.21 ± 1.0 (62)	.8
BMI (at initial visit; Z-score)	−0.14 ± 1.2 (32)	0.71 ± 1.1 (62)	.001
Age at start of GnRHa (years)	12.3 (11.5, 13.2) (32)	—	n/a
Bone age at the start of GnRHa (years)	11.3 ± 1.2 (17)	—	n/a
Length of time on GnRH before starting testosterone (years)	1.9 ± 0.7 (32)	—	n/a
Age at start of testosterone (years)	14.3 ± 0.8 (32)	16.8 (15.8, 17.8) (62)	n/a
Breast Tanner stage at time of starting GnRHa (n = 25)			
2	12 (48%)	—	n/a
3	13 (52%)	—	n/a
Race			
White	25 (78.1%)	49 (79%)	1.00
Other	7 (21.9%)	13 (21%)	
Black/African American	0	3	
Asian or PI	1	1	
American Indian or Alaskan	0	1	
Other/Unknown	6	8	
Ethnicity			
Hispanic	3 (9%)	5 (8%)	1.00
Formulation of GnRHa (n = 30)			
Implants	23 (76.6%)	—	n/a
Injections	7 (23.3%)	—	n/a
Formulation of testosterone at initiation			
Testosterone cypionate/enanthate intramuscular injection	11 (34.4%)	9 (14.5%)	n/a
Testosterone cypionate/enanthate subcutaneous injection	18 (56.2%)	53 (85.5%)	n/a
Testosterone gel (topical)	3 (9.4%)	0 (0%)	n/a
Initial starting dose of injectable testosterone			
Testosterone cypionate/enanthate intramuscular injection (mg)	50 (37.5, 50) (7)	25 (25, 25) (9)	n/a
Frequency (days) Range (days)	30 (21, 30) 7-30	7 (7, 7) 7-14	
Testosterone cypionate/enanthate subcutaneous injection (mg)	25 (16, 25) (14)	26 (25, 50) (53)	n/a
Frequency (days) Range (days)	7 (7, 7) 7-30	7 (7, 14) 7-14	

Mean ± SD (n) are presented for variables which are normally distributed and median (25th percentile, 75th percentile) (n) are reported for variables which are not normally distributed.
Abbreviations: T, testosterone; BMI, body mass index; GnRHa, gonadotropin-releasing hormone agonist; PI, Pacific Islander.

of the study team called or emailed the participant/family for that information. The participant/parents verbally consented to participation in the study when contacted. A waiver of written consent was obtained for this group and a waiver of consent was obtained for those who did not need to be contacted.

Statistical Analysis

Data are presented as mean (±SD) for data that are normally distributed and median (25th percentile, 75th percentile) for data that are not normally distributed. R statistical software was used for analyses. A paired t-test was used to compare FAH with MPTH, PAH, and z-score at different time points in the GnRHa + T group, and a 2-sample t-test was used to compare the difference in FAH between the GnRHa + T vs T-only groups. Fisher's exact test was used to compare race and ethnicity distribution between groups. A multivariable linear regression model was used to examine the effect of IH, BMI, racial group, Tanner stage at GnRHa initiation, length of time on GnRHa monotherapy, and GnRHa formulation on final height in GnRHa + T group. Another multivariable linear regression model was used to examine the effect of receiving either only testosterone or GnRHa + T on final height after adjusting for all the patient attributes. A sensitivity analysis was conducted to determine whether including the participant whose final height was attained via self-report changed the outcomes by rerunning the analyses with and without this participant and no significant change was found in any of the results (data not shown), so the participant was included in all analyses.

Results

Participant Characteristics

Table 1 shows the participant characteristics for both groups: GnRHa + T (n = 32) and T-only (n = 62). Most participants in

Table 2. Height outcomes

	GnRHa + T (n = 32) Mean ± SD (n)	T- only (n = 62) Mean ± SD (n)	*P* value
FAH (cm)	166.6 ± 7.5 (32)	164.7 ± 6.7 (62)	.25
FAH (z-score)[a]	0.5 ± 1.2 (32)	0.21 ± 1.0 (62)	.26
FAH in those who had a MPTH (cm)	—	162.1 ± 7.4 (26)	n/a
FAH in those who had a MPTH (z-score)	—	−0.18 ± 1.1 (26)	n/a
FAH in those who had a bone age X-ray (cm)	168.8 ± 7.1 (18)	—	n/a
FAH in those who had a bone age X-ray (z-score)	0.84 ± 1.1 (18)	—	n/a
Age when near-FAH first reached (years)	15.9 ± 1.1 (31)	—	n/a
Duration of testosterone before near-FAH reached (years)	1.7 ± 1.1 (30)	—	n/a
MPTH (cm)	164.2 ± 5.7 (32)	164.3 ± 6.3 (26)	.96
MPTH (z-score)	0.14 ± 0.88 (32)	0.15 ± 1.0 (26)	.97
PAH (cm)	167.0 ± 6.9 (18)	—	n/a
PAH (z-score)	0.57 ± 1.1 (18)	—	n/a
ΔFAH – MPTH (cm)	2.3 ± 5.7	−2.2 ± 5.6 (26)	<.01
ΔFAH – MPTH (z-score)	0.3 ± 0.9	−0.3 ± 0.9 (26)	<.01
ΔFAH – PAH (cm)	1.8 ± 3.4 (18)	—	n/a
ΔFAH – PAH (z-score)	0.28 ± 0.52 (18)	—	n/a

Abbreviations: GnRHa, gonadotropin-releasing hormone agonist; T, testosterone; FAH, final adult height; MPTH, mid-parental target height; PAH, predicted adult height
[a]Based on 20 year old on the CDC girl's growth chart.

both groups self-identified as white and of non-Hispanic race and ethnicity. In the GnRHa + T group, the mean height and age at initiation of therapy with GnRHas were 153.8 ± 7.9 cm and 12.3 (11.5, 13.2) years, respectively. For the T-only group, the IH and age at initiation of testosterone were 164.1 ± 6.8 cm and 16.8 (15.8, 17.8) years, respectively. The heights and age of initiation of initial therapy were different due to the design of the study, but there was no difference between the IH z-scores in each group (0.16 ± 1.0 vs 0.21 ± 1.0, $P = .8$).

At the start of GnRHas in the GnRHa + T group, participants had Tanner stage 2 (48%) or Tanner stage 3 (52%) breast development; 76.6% (n = 23) were treated with a GnRHa implant, and 23.3% (n = 7) received injectable formulations of GnRHas. They remained on GnRHa monotherapy for an average of 1.9 ± 0.7 years, and thus the average age of initiation of testosterone in the GnRHa + T group was 14.3 ± 0.8 years. In both groups, when testosterone was initiated, the majority received subcutaneous injections (GnRHa + T group: 18/32 (56%); T-only group: 53/62 (85%)). Table 1 shows the average starting dose and intervals for each type of testosterone and group.

FAH in the GnRHa + T Group Is Increased Compared With the T-only Group After Controlling for Other Patient Characteristics

The FAH in the GnRHa + T group (166.6 ± 7.5 cm) was on average 1.9 cm taller than the T-only group (164.7 ± 6.7 cm), but did not statistically differ ($P = .25$; Table 2). Similarly, the mean height z-score in the GnRHa + T group of 0.50 ± 1.2 compared with the T-only group of 0.21 ± 1.0 did not statistically differ ($P = .26$). The multivariable linear regression model demonstrated that average final height was 7.95 cm less in the T-only group than in GnRHa + T group after adjusting for IH, age of starting testosterone, initial BMI, and racial group (β = −7.95, 95% CI −10.85, −5.06).

FAH Is Increased Compared With MPTH in the GnRHa + T Group and not in the T-only Group.

The MPTH and MPTH z-scores between groups did not statistically differ ($P = .96$ and .97, respectively; Table 2). The mean FAH for the GnRHa + T group was 2.3 cm taller than their MPTH (166.6 ± 7.5 cm vs 164.2 ± 5.7 cm $P < .05$; Fig. 1). These correspond to mean height z-scores of +0.5 (FAH) and +0.14 (MPTH) ($P < .05$). In the T-only group participants for whom MPTH was available, the difference between FAH was not significantly different from MPTH (162.1 ± 7.4 cm vs 164.3 ± 6.3 cm, $P = .06$; Fig. 1). The difference between FAH and MPTH in the GnRHa + T group of 2.3 ± 5.7 cm was statistically higher than the difference between FAH and MPTH in the T-only group of −2.2 ± 5.6 cm ($P < .01$; Table 2). Similarly, the difference in z-scores between the FAH and MPTH in the GnRHa + T group was statistically significantly higher than the difference in z-scores between FAH and MPTH in the T-only group (0.3 ± 0.9 vs −0.3 ± 0.9, $P < .01$; Table 2).

FAH is Increased Compared With PAH based on Bone age in the GnRHa + T Group

Eighteen participants in the GnRHa + T group had a bone age X-ray done prior to initiating treatment with GnRHas. PAH based on the X-rays averaged 167.0 ± 6.9 cm, corresponding to a z-score of 0.6 ± 1.1 (Table 2). The mean FAH for these 18 participants was of 168.8 ± 7.1 cm, corresponding to a z-score of 0.8 ± 1.1. When compared with PAH and PAH z-score, both the FAH and FAH z-score were significantly higher ($P = .04$ for both comparisons; Table 2 and Fig. 2).

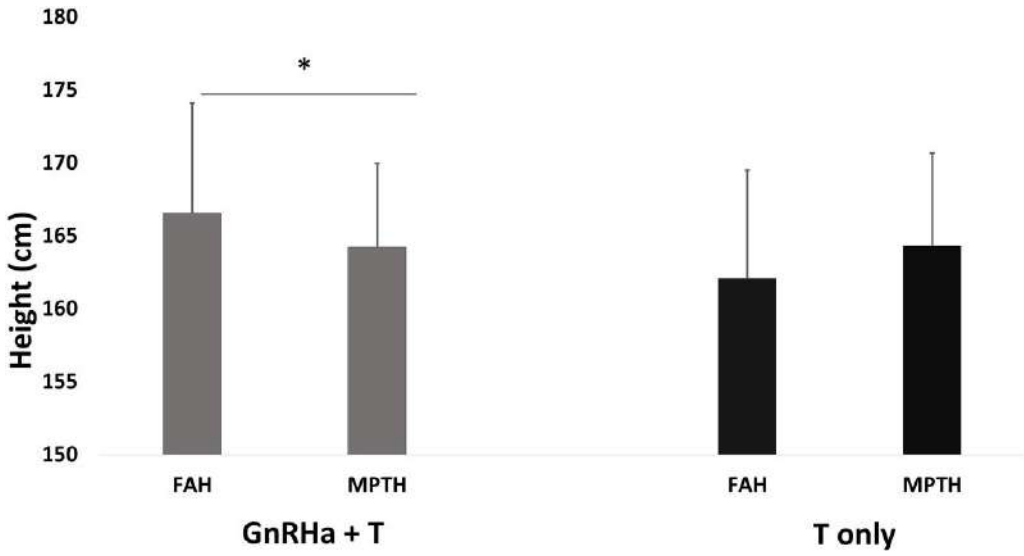

Figure 1. Difference in final adult height (FAH) vs midparental target height (MPTH) in the gonadotropin-releasing hormone agonist plus testosterone (GnRHa + T) group (n = 32) and the testosterone only (T-only) group (n = 26) (cm). Gray bars are used for the GnRHa + T group and black bars are used for the T-only group. *$P < .05$.

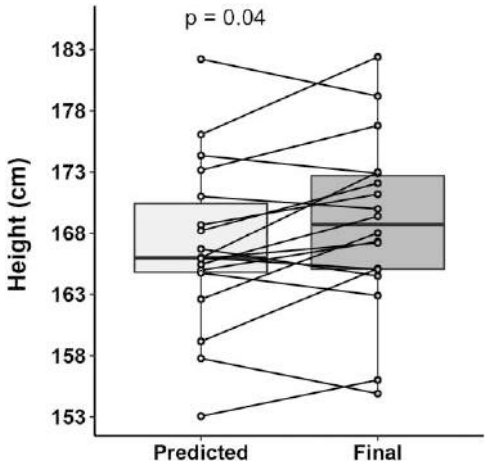

Figure 2. Difference in height (cm) between final adult height (FAH) and predicted adult height (PAH) in the gonadotropin-releasing hormone agonist plus testosterone (GnRHa + T) group with bone age X-ray results (n = 18). The light gray box represents the PAH with a median (25th percentile, 75th percentile) = 165.99 cm (164.80, 170.43). The dark gray box represents the median (25th percentile, 75th percentile) = 168.71 cm (165.06, 172.70) for FAH. Data are normally distributed. Paired t-test compared PAH mean ± SD of 167.0 ± 6.9 cm with FAH mean ± SD 168.8 ± 7.1 cm, $P = .04$.

FAH z-Score Is Higher at the end of Treatment With GnRHa and Testosterone Compared With Initial Height z-Score

Height z-score at the start of treatment in the GnRHa + T group was 0.16 ± 1, decreased to −0.1 ± 1 over the course of GnRHa monotherapy, and then with testosterone initiation increased to 0.5 ± 1.2 by the time FAH was reached (Fig. 3). There was a statistically significant difference between all 3 timepoints (IH vs start of testosterone height, $P < .05$, start of testosterone vs FAH, $P < .001$ and IH vs FAH, $P < .05$).

Taller Height at Baseline, Earlier Stage of Puberty at GnRHa Initiation, and Longer GnRHa Monotherapy Were Associated With Increased FAH in the GnRHa + T Group

Table 3 shows the results of a multivariable linear regression model performed to examine the effects of different variables on FAH in the GnRHa + T group. With 1 cm taller IH there was a 1.16 cm increase in the FAH (β = 1.16, 95% CI 0.86, 1.46). Tanner stage 3 breast development at the initiation of GnRHa therapy was associated with a mean FAH 6.49 cm lower than those who started in Tanner stage 2 puberty (β = −6.49, 95% CI −10.43, −2.55). One month increase in duration of GnRHa use prior to testosterone increased final height by 0.59 cm after adjusting for all other covariates (β = 0.59, 95% CI 0.31, 0.88). The other variables including initial BMI z-score, formulation of GnRHas, and race did not impact final height outcome in this group. The age of initiation of testosterone was not associated with final height outcomes in either group (data not shown).

Discussion

GAHT improves body satisfaction and physical appearance congruence with gender identity in transgender and gender diverse youth [1, 3]. While some research has focused on increasing height for cisgender boys in the United States [28-30], at this time there are fewer published data on height outcomes for transmasculine individuals. Still, adult height is a common concern raised by transmasculine patients and families in clinic settings [31]. Our results show that treatment with GnRHas prior to testosterone in early-pubertal TMY was associated with mildly increased FAH compared with what would be expected without intervention. The strongest evidence for this is that FAH was increased compared with MPTH by 2.3 cm, a difference not seen in the group who started on testosterone after growth was near complete. Additionally, the FAH z-score was statistically significantly increased compared with IH z-score. FAH was also mildly

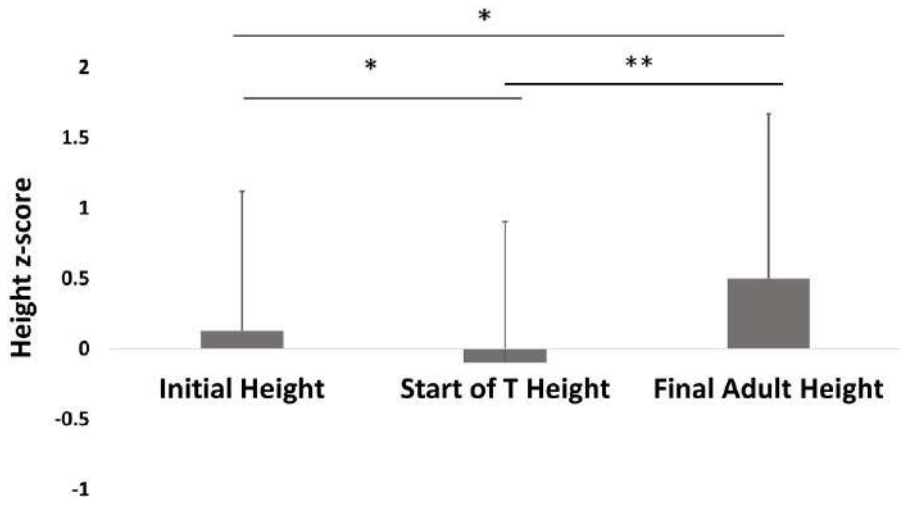

Figure 3. Height z-scores in the gonadotropin-releasing hormone agonist plust testosterone (GnRHa + T) group at start of GnRHas, start of testosterone, and at final height measurement. $*P < 0.05$, $**P < .001$.

Table 3. Estimates from multivariable linear regression model to examine the effect of variables on final height (in cm) in GnRHa + T (adjusted $R^2 = 0.77$) (n = 22 after excluding the missing data)

Variables	Estimate	95% CI
Initial height (cm)	**1.16**	**(0.86, 1.46)**
BMI initial (Z-score)	−1.29	(−2.86, 0.29)
Tanner stage initial (ref = stage 2; n = 9)		
Stage 3 (n = 12)	**−6.49**	**(−10.43, −2.55)**
Duration on GnRHa before testosterone (months)	**0.59**	**(0.31, 0.88)**
Formulation at initiation (ref = implants; n = 15)		
Injections (n = 6)	−2.39	(−6.54, 1.76)
Race group (ref = "Other," n = 4)		
White (n = 17)	−2.71	(−7.28, 1.86)

Bolded results are statistically significant.

increased compared with PAH at start of treatment in the GnRHa + T group. These results contribute to the small accumulating knowledgebase on how gender-affirming interventions might affect growth and height potential [21, 22, 32-35]. However, the FAH z-score of 0.5 on the CDC girls' growth chart still only translates to a z-score of −1.43 or a height in the eighth percentile on the CDC boys' growth chart.

These results are similar to those described in a recent article by Willemsen et al, who found that GnRHa + T treatment increased final height by 3.9 ± 6 cm compared with MPTH [21]. It is important to note that the standard error for MPTH is ±10 cm [27], indicating that the FAH in our study is still within a possible range of expected heights for a family, but the fact that both our study and Willemsen et al resulted in increased FAH compared with MPTH makes it more likely to be a true increase. On the other hand, a small study of 10 trans boys treated with GnRHas followed by testosterone found no difference between FAH and MPTH or PAH based on bone age [35]. PAH based on bone age provides a more accurate estimation of someone's adult height than MPTH [25]. Willemsen et al found a FAH 3.0 ± 3.6 cm taller than the PAH in their group. In our study, due to variation in clinic practices, only about half of the participants had a bone age X-ray performed before GnRHa initiation. In those who had a bone age X-ray, the mean FAH was increased by 1.8 ± 3.4 cm compared with mean PAH (Table 2 and Fig. 2). This increase is similar to the difference between FAH and MPTH for each participant (2.3 ± 5.7 cm) in the GnRHa + T group, adding more evidence that GnRHa + T may increase final height. However, since bone age X-rays were not done for every subject, it may be a biased sample (ie, bone ages may have been completed more frequently in patients whose families or clinicians had more concerns about height potential).

Another supporting factor for GnRHa and testosterone's positive impact on height is the fact that the group treated with GnRHas before testosterone was found to have a 7.95 cm taller final height on average than the T-only group after adjusting for IH, age of starting testosterone, initial BMI, and racial group in a multilinear regression model. Willemsen et al also describe a difference (3 cm) between their pubertal compared with postpubertal groups, but they found no significant difference between the difference in FAH vs MPTH between pubertal vs postpubertal groups, suggesting the difference in final height between their groups may not be due to the intervention. Our results did demonstrate a difference between the 2 groups, where the GnRHa + T group had a greater difference in FAH compared with MPTH than the T-only group (2.3 ± 5.7 vs −2.2 ± 5.6, $P < .01$). This might argue that starting GnRHas even in later stages of puberty could modestly increase final height compared with those who never received GnRHas. However, we did not have MPTH data on every participant in the T-only group which may have influenced our results.

Notably, differences in our study's findings may be accounted for by the difference in the age and pubertal development at the time of introduction of gender-affirming medications. The highest percentage of subjects in the Willemsen et al study were breast Tanner 3 or 4 at initiation of GnRHas (71%), whereas the majority of our cohort were Tanner 2 or 3 breast development (at least 78%, those without Tanner stages were premenarchal so presumed Tanner 3 or less). Additionally, the mean bone age for the pubertal group in the Willemsen study was 12.4 ± 1 years and in our study was 11.3 ± 1.1 years. Therefore, our cohort had more

growth potential than the Dutch cohort, despite similar ages at initiation of GnRHas. Further supporting the benefit on height of starting GnRHas in earlier stages of puberty, our study found that those who started in Tanner 2 had a significant increase in their final height (by about 6.5 cm) compared with those who started in Tanner 3 (Table 3). Although Willemsen et al found an increase in FAH compared with MPTH and PAH, they did not find significant change between FAH and IH z-scores, which differs from our study, which found an increase in FAH z-score compared with IH z-score in the GnRHa + T group (0.5 ± 1.18 vs 0.16 ± 0.99, $P < .05$). These findings suggest that the earlier puberty stage and earlier bone age at which GnRHas are started, the more potential benefit they may have on final height. However, in contrast with our results, the study of 10 trans boys by Ciancia et al also only included those in Tanner stage 2 or 3 but still found no difference in their FAH compared with PAH or MPTH. FAH z-score was higher than their IH z-score but it is unclear whether this change is significant [35].

The mechanism for which FAH was increased in the group who received GnRHas prior to testosterone is unclear. Treatment with GnRHa delays bone maturation and studies have shown it can increase FAH compared with predicted height in children with central precocious puberty [10-15]. However, studies have shown that when these medications have been used alone in children (mostly AFAB) with normally timed puberty, results have ranged from a mild increase to no significant effect on final height [16-18, 36]. As such, it is not a recommended intervention for treatment of short stature alone [16]. What is unique about the transgender population compared with these prior studies is that puberty is being induced or continued with testosterone, rather than estrogen. Therefore, it is plausible that the positive effect on height not only stems from delaying the bone age advancement by GnRHas, but also may be augmented by a more robust growth spurt from testosterone than they would have had from estrogen. Testosterone leads to maturation and eventual closure of growth plates primarily through aromatization to estrogen [37], therefore there are other factors that influence the difference between adult male and female height. These might include a direct androgen effect, genes involved in skeletal growth that differ between people with XX and XY sex chromosomes, timing of pubertal onset, and tempo of pubertal progression [8]. On average, puberty in assigned males at birth starts later than AFAB and the growth spurt also occurs later within puberty compared with typical female puberty allowing about an additional 2 years of growth, which contributes to taller male height on average [8]. Using GnRHas early in puberty can mimic this pattern by delaying puberty and therefore bone maturation. Treatment with GnRHas decreases growth velocity, and it has been shown to decrease growth velocity even below prepubertal levels in late pubertal gender-diverse adolescents [22], further suggesting that starting GnRHas earlier in puberty has much more benefit for growth rather than later when some of the growth spurt may have already occurred. This delay in puberty decreases the height z-score at the time of testosterone initiation, as seen in our study as well as Willemsen et al and Ciancia et al [21, 35]. However, if there is enough growth potential left, initiating testosterone may lead to robust growth spurt leading to a significant increase in height z-score between the height at testosterone initiation and FAH as seen in our study (Fig. 3), suggesting it may be the testosterone effect on the pubertal growth spurt that is contributing most to the increased FAH. We do not fully understand to what degree the increase peak growth velocity in typical male puberty compared with typical female puberty is influenced by testosterone, genetics, or other factors and more data are needed to determine the starting testosterone dose or if a specific dose escalation protocol is most beneficial for height augmentation.

Our study has several limitations. This was retrospective data from multiple institutions and therefore there was variability in treatment regimen, height recordings, puberty staging, and bone age interpretation. However, the fact that our data were collected from multiple institutions in the United States is also a strength as it may be more generalizable to other gender clinics in the United States. Also, typical for patients presenting to US gender clinics, most of the participants identified as white and non-Hispanic, limiting generalizability to other populations [38]. Additionally, it is debatable and subjective how much of a clinical difference a height increase of 2.3 cm (FAH compared with MPTH) makes, and individual response is widely variable (Fig. 2). The average height gain from growth hormone treatment for those with idiopathic short stature is about 5 cm [39], but youth and families regularly choose this course of treatment and the burden of daily injections, sometimes at great out of pocket expense, and, therefore, an increase of 2.3 cm in final height may be very meaningful to many individuals.

In conclusion, we found that treatment with GnRHas in early puberty to midpuberty followed by testosterone initiation when growth potential remains may mildly increase FAH for TMY when compared with IH, MPTH, PAH, and those treated with testosterone alone after growth is near complete. Although the overall effect appears to be small, the potential increase in final height can be discussed when counseling youth and caregivers about the impact of GnRHa use prior to testosterone on final height. Further studies in larger samples are needed to understand the mechanism behind this height increase and whether specific testosterone treatment regimens and timing impact FAH.

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Disclosures

None.

Data Availability

Datasets are not publicly available but may be made available upon reasonable request.

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TRANSGENDER HEALTH

ORIGINAL RESEARCH

Changes in Adrenal Androgens During Puberty Suppression and Gender-Affirming Hormone Treatment in Adolescents With Gender Dysphoria

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ABSTRACT

Introduction: Gender-affirming hormone treatment is known to affect adrenal androgen levels in adult individuals with gender dysphoria (GD). This may be clinically relevant because the adrenal gland plays a critical role in many different metabolic processes.

Aim: This study aims to assess the effects of gonadotropin-releasing hormone analogs (GnRHa) treatment and gender-affirming hormone treatment on adrenal androgen levels in adolescents with GD.

Methods: In this prospective study, dehydroepiandrosterone-sulfate (DHEAS) and androstenedione values were measured every 6 months during 2 years of GnRHa treatment only, and 2 years of GnRHa combined with gender-affirming hormone treatment (estradiol or testosterone) in 73 transgirls and 54 transboys. To determine trends in adrenal androgen levels a linear mixed model was used to approximate androgen levels.

Main Outcome Measures: DHEAS and androstenedione levels were the main outcome measures.

Results: DHEAS levels rose in transboys during GnRHa treatment, which may represent the normal increase during adolescence. In transgirls no change in DHEAS levels during GnRHa treatment was found. Gender-affirming hormone treatment did not affect DHEAS levels in either sex. In transboys androstenedione levels decreased during the first year of GnRHa treatment, which may reflect reduced ovarian androstenedione synthesis, and rose during the first year of gender-affirming hormone treatment, possibly due to conversion of administered testosterone. In transgirls androstenedione levels did not change during either GnRHa or gender-affirming hormone treatment.

Clinical Implications: No deleterious effects of treatment on adrenal androgen levels were found during approximately 4 years of follow-up.

Strengths & Limitations: This is one of the largest cohort of adolescents with GD, treated using a uniform protocol, with standardized follow-up. The lack of a control group is a limitation.

Conclusion: The changes in androstenedione levels during GnRHa and gender-affirming hormone treatment in transboys may not be of adrenal origin. The absence of changes in androstenedione levels in transgirls or DHEAS levels in either sex during gender-affirming hormone treatment suggests that gender-affirming hormone treatment does not significantly affect adrenal androgen production. **Schagen SEE, Lustenhouwer P, Cohen-Kettenis PT, et al. Changes in Adrenal Androgens During Puberty Suppression and Gender-Affirming Hormone Treatment in Adolescents With Gender Dysphoria. J Sex Med 2018;15:1357–1363.**

Key Words: Gender Dysphoria; Dehydroepiandrosterone-Sulfate; Androstenedione; Testosterone; Estradiol; Gonadotropin-Releasing Hormone Analog

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INTRODUCTION

Gender dysphoria (GD) is the distress associated with an incongruence between the sex assigned at birth and the gender identity. The cause of this incongruence is presumed to involve a complex interaction among biological, social, and cultural factors.[1] Adolescents with a diagnosis of GD according to *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* criteria who have entered puberty (Tanner stage $\geq$2) can be treated with gonadotropin-releasing hormone analogs (GnRHa) to suppress endogenous sex hormone production and prevent the development of unwanted sex characteristics.[1] Such treatment is recommended by the Endocrine Society and the World Professional Association for Transgender Health as standard of care.[1,2] If GD persists and the adolescent meets eligibility criteria in accordance with Endocrine Society guidelines, treatment with gender-affirming hormones can be started.[1] These include either estrogens, given to transfemales (individuals assigned male at birth with a female gender identity) or testosterone, given to transgender males (individuals assigned female at birth with a male gender identity). One follow-up study has shown that such treatment during adolescence, followed by gender-affirming surgery, results in good psychological outcome in young adults (at an average of 20.7 years).[3] However, limited data are available on the safety and side effects of this treatment and this remains an important concern.[4,5]

The adrenal gland produces various androgens, in addition to mineralocorticoids, glucocorticoids, and catecholamines. The most important adrenal androgens are dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), and androstenedione. After hydrolysis from DHEAS, DHEA is thought to exert its effects after conversion in peripheral tissues and the gonads to androstenedione, testosterone, dihydrotestosterone, and/or estradiol.[6] The adrenal androgens are involved in pubarche during childhood/adolescence. Their role in adult life is not fully understood but DHEA(S) is thought to have effects on insulin sensitivity, lipid metabolism, leptin production by adipocytes, body temperature regulation, and on the brain and the immune system.[6] Given this multitude of effects, it is important to evaluate adrenal androgen levels during hormonal treatment in adolescents with GD even though it is currently difficult to predict what the clinical impact is of changes in adrenal androgen levels on future health.

In adults, changes in serum levels of adrenal steroids have been observed during gender-affirming hormone treatment.[7] An increase of adrenal androgens was seen in transmen treated with testosterone. Adrenal androgen levels (DHEAS and androstenedione) decreased in transwomen treated with ethinyl-estradiol and the anti-androgen cyproterone acetate. In a more recent study, Mueller et al[8] have shown similar changes in middle-aged transwomen who received a combination of GnRHa and 17-beta estradiol.[8] The cause of these changes is not completely clear yet; both effects on the adrenal gland and on the testes have been proposed.[8] Normally adrenal androgen levels rise during adolescence.[9–11] They reach a peak at age 20–30 years and decrease thereafter.[9–12] This suggests that adrenal synthesis and/or metabolism of androgens differs between adolescents and adults. Therefore, GnRHa and gender-affirming hormone treatment may have different effects in adolescents as compared to adults.

As part of a larger study initiated to investigate the efficacy and safety of GnRHa and gender-affirming hormone treatment in adolescents with GD, we aimed to assess the effects of these treatments on DHEAS and androstenedione serum levels. This should add to the scarce data available on the safety and side effects of this treatment in adolescents.

METHODS

Subjects

127 adolescents with GD (54 transgirls [assigned male at birth] and 73 transboys [assigned female at birth]) treated between 1998 and 2009 at the VU University Medical Center in Amsterdam were included in a prospective study on brain development, brain functioning, growth, and metabolic aspects in the clinical management of adolescents with GD. Adolescents were included if they fulfilled the criteria for gender identity disorder according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* and the criteria for treatment according to the Endocrine Society.[13] There were no exclusion criteria.

Treatment Protocol

Treatment consisted of intramuscular injections of the GnRHa triptorelin (3.75 mg) (Decapeptyl-CR; Ferry B.V., Hoofddorp, The Netherlands) at 0, 2, and 4 weeks, followed by injections every 4 weeks. Individuals were seen at 3-month intervals. The duration of treatment with GnRHa alone depended on when the individual reached the age at which gender-affirming hormone therapy could be added (approximately 16 years). In transgirls gender-affirming hormone treatment consisted of daily oral doses of 17-beta estradiol, starting at 5 μg/kg/d. Doses were increased by 5 μg/kg/d every 6 months until the maintenance dose of 2 mg/d was reached. In transboys 25 mg/m^2 of testosterone esters (Sustanon; Aspen Pharma Trading Limited, Dublin, Ireland) were administered every 2 weeks through intramuscular injections. Doses were increased every 6 months by 25 mg/m^2 per 2 weeks until the maintenance dose of 125 mg per 2 weeks (or 250 mg per 4 weeks) was reached. If GnRHa treatment was started after the age of 16 years (and endogenous puberty had been completed), gender-affirming hormone treatment was usually added after 3 to 6 months and increased more rapidly. Transgirls were treated with 1 mg 17-beta estradiol daily for 6 months, followed by the maintenance dose of 2 mg; transboys were treated with 75 mg testosterone esters per 2 weeks for 6 months, followed by the maintenance dose of 125 mg per 2 weeks.

Main Outcome Measures

Laboratory Investigations

Blood was drawn at baseline and every 6 months during GnRHa treatment and gender-affirming hormone treatment for measurement of androstenedione, DHEAS, and testosterone. Androstenedione serum levels were determined by a coated-tube radioimmunoassay (Diagnostic Systems Laboratories Inc, Webster, TX, USA) and had a limit of quantification of 0.5 nmol/L. DHEAS serum levels were determined by radioimmunoassay Coat-A-Count (Siemens Medical Solutions, Malvern, PA, USA) and had a limit of quantification of 0.2 μmol/L. Testosterone levels were measured using a radioimmunoassay (Siemens Medical Solutions) (lower limit of quantification 1 nmol/L).

Statistical Analysis

Software (SPSS Statistics, Version 20; IBM Corp, Armonk, NY, USA) was used for all analyses reported in this study. Independent samples *t* tests were performed to compare group means if data were normally distributed and if sample sizes were large enough to detect statistical effects (n > 30). Otherwise nonparametric Mann-Whitney *U* tests were performed. Pearson and Spearman correlation tests were performed to determine associations between variables. For several subjects no laboratory data were available for certain time points, so to increase statistical power a linear mixed model analysis was performed to analyze changes in adrenal androgen levels during treatment. Restricted maximum likelihood was used as the method of estimation and the model was unstructured due to random variance. Main outcomes were the effects of time on the dependent variables DHEAS and androstenedione with significant effects defined as $P < .05$. Estimated marginal means were calculated to approximate DHEAS and androstenedione levels at baseline, after 1 and 2 years of GnRHa treatment alone, at the start of gender-affirming hormone treatment, and after 1 and 2 years of combined GnRHa and gender-affirming hormone treatment.

Ethics and Informed Consent

This study was approved by the hospital ethics committee and registered (International Standard Randomized Controlled Trial Number 81574253). All subjects and their parents/caretakers provided verbal informed consent.

RESULTS

Baseline characteristics are shown in Table 1. Individuals had been treated with GnRHa for approximately 2 years on average before gender-affirming hormone treatment was started, but this varied widely between subjects.

Effect of Treatment on DHEAS Levels

DHEAS levels were within the normal range in all subjects before and during treatment. In transboys DHEAS levels significantly increased during the first 2 years of GnRHa treatment only from 4.21 ± 0.30 to 5.67 ± 0.44 μmol/L (mean ± SE; $P < .001$), whereas in transgirls a non-significant increase of DHEAS was found ($P = .062$) (Figure 1). To study if these changes were related to the treatment, we performed analysis on subgroups of the cohort. 1 subgroup started treatment at age 12−14 years and the other at 14−16 years of age. We compared DHEAS and androstenedione levels after 2 years of GnRHa treatment of adolescents aged 12−14 years old at baseline to baseline serum levels of those aged 14 to 16 years (untreated group of similar age). In transboys aged 12−14 years at baseline (n = 17), DHEAS levels after 2 years of GnRHa treatment were comparable to baseline levels of untreated transboys aged 14−16 years (n = 18) (4.65 ± 1.92 μmol/L vs 3.94 ± 1.66 μmol/L; $P = .303$). In transgirls aged 12−14 years at baseline (n = 20) mean DHEAS levels after 2 years of GnRHa treatment were significantly higher than mean DHEAS levels of untreated transgirls aged 14−16 years (n = 14) (5.41 ± 1.86 μmol/L vs 4.14 ± 1.98 μmol/L; $P = .047$).

During gender-affirming hormone treatment DHEAS levels did not significantly change in transboys or transgirls (Figure 2). Body mass index as a covariate did not have a significant effect on the DHEAS levels ($P = .350$ and $P = .309$ for transgirls and transboys, respectively). DHEAS levels during treatment were not correlated to testosterone levels in transboys or estradiol levels in transgirls.

Table 1. Baseline characteristics

	Transgirls, n = 54	Transboys, n = 73
Age at GnRHa start, y, mean ± SD (range)	14.0 ± 1.6 (11.6 − 17.9)	14.3 ± 2.0 (11.5 − 18.6)
Weight, kg, mean ± SD	57.4 ± 12.7	56.8 ± 14.3
BMI, mean ± SD	20.2 ± 2.2	21.0 ± 3.8
Tanner G/B stage, median (range)	4 (2−5)	4 (2−5)
Menarche, yes/no		57/16
Mean testicular volume, mL, mean ± SD (range)	14.7 ± 6.8 (4−25)	
P stage, median (range)	3 (2−6)	5 (2−5)
GnRHa treatment, mo, mean ± SD	22.8 ± 11.8	24.5 ± 15.3
Age at GAH start, y, mean ± SD (range)	16.3 ± 1.2 (13.9 − 18.9)	16.8 ± 1.1 (13.6 − 19.5)

BMI = body mass index; GAH = gender-affirming hormone treatment; G/B = Tanner genital/breast; GnRHa = gonadotropin-releasing hormone analogs; P = Tanner pubic hair.

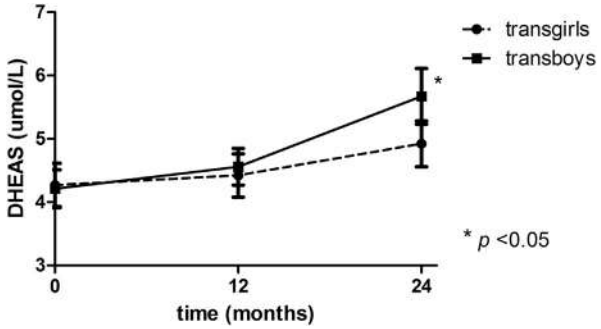

Figure 1. Dehydroepiandrosterone-sulfate (DHEAS) levels (μmol/L) during gonadotropin-releasing hormone analog treatment.

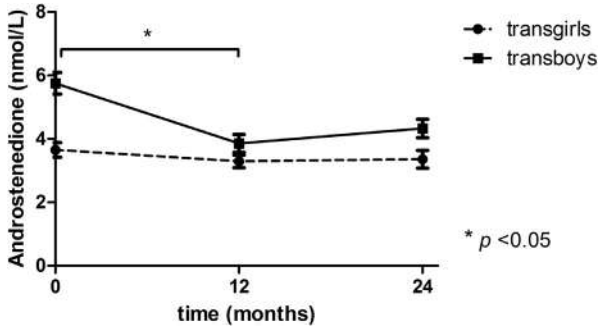

Figure 3. Androstenedione levels (nmol/L) during gonadotropin-releasing hormone analog treatment.

Effect of Treatment on Androstenedione Levels

In 5 of 67 transboys androstenedione levels were elevated at baseline, up to 11.6 nmol/L (reference range 2−9 nmol/L). After 2 years of GnRHa treatment androstenedione levels remained above the reference range in 2 of these 5 transboys and had increased to above the reference range in 1 additional transboy. All transgirls had normal androstenedione levels before and during treatment.

Androstenedione trends were different between the sexes (Figure 3). In transboys a significant decrease in androstenedione levels occurred in the first year of treatment (from 5.74 ± 0.34 nmol/L to 3.86 ± 0.28 nmol/L; $P = .01$), after which they stabilized during the second year of treatment. In transgirls androstenedione levels did not significantly change during GnRHa treatment.

In transboys androstenedione levels in individuals aged 12−14 years at baseline (n = 17) were significantly lower after 2 years of treatment with GnRHa compared to baseline levels of individuals aged 14−16 years (n = 18) (4.76 ± 1.76 nmol/L vs 6.86 ± 2.96 nmol/L; $P = .016$). In transgirls aged 12−14 years at baseline (n = 21) mean androstenedione levels after 2 years of GnRHa treatment were comparable to baseline levels of individuals aged 14−16 years (n = 14) (3.86 ± 1.52 nmol/L vs 3.96 ± 1.33 nmol/L).

During gender-affirming hormone treatment androstenedione levels in transboys rose during the first year of testosterone treatment ($P = .017$) and then stabilized (Figure 4). In transgirls androstenedione levels did not significantly change. Body mass index did not influence androstenedione levels during treatment ($P = .155$ and $P = .786$ for transgirls and transboys, respectively). In transboys androstenedione levels were significantly correlated to testosterone levels during treatment with testosterone ($P < .001$) (Figure 5).

From 4 transmen data were available after ovariectomy, when GnRHa treatment had been stopped. No consistent changes in androstenedione levels after ovariectomy/cessation of GnRHa were seen.

DISCUSSION

In order to increase knowledge on the safety of treatment this study aimed to assess whether GnRHa and gender-affirming hormones affect adrenal androgen production in adolescents with GD as was seen in adults during gender-affirming hormone treatment. At baseline, several transboys had mildly elevated androstenedione levels. There was no clinical suspicion of congenital adrenal hyperplasia but perhaps polycystic ovarian syndrome (PCOS) may explain the increased androstenedione levels in some adolescents. However, no further investigations were performed to investigate or exclude congenital adrenal hyperplasia or PCOS, which is a limitation of the study. Some studies have reported that PCOS is more

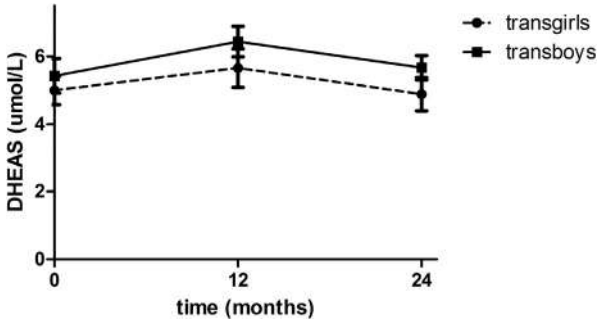

Figure 2. Dehydroepiandrosterone-sulfate (DHEAS) levels (μmol/L) during gonadotropin-releasing hormone analog and gender-affirming hormone treatment.

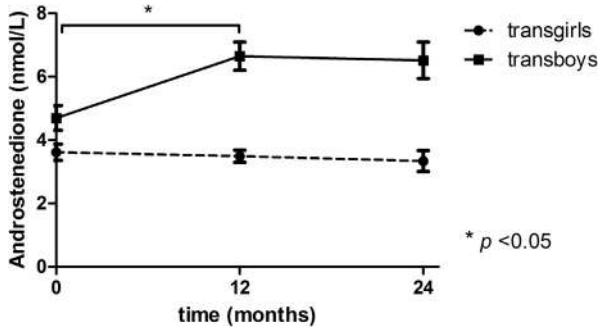

Figure 4. Androstenedione levels (nmol/L) during gonadotropin-releasing hormone analog and gender-affirming hormone treatment.

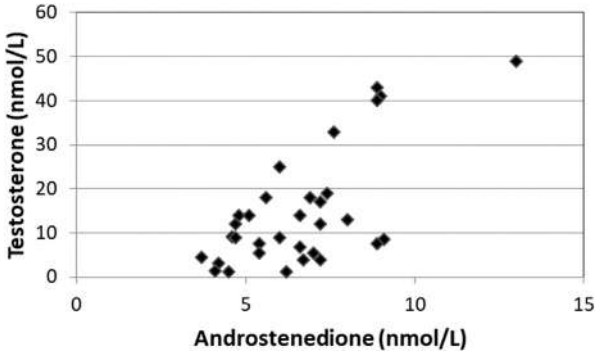

Figure 5. Correlation between serum testosterone (nmol/L) levels and serum androstenedione (nmol/L) levels in transboys treated with testosterone.

commonly found among transmen than in the female population.[14,15]

DHEAS levels were found to rise during GnRHa treatment in transboys. This rise may not actually be caused by treatment, but may be the normal increase seen during adolescence, because DHEAS levels after 2 years of treatment with GnRHa were comparable to levels from untreated individuals of a comparable age. In transgirls DHEAS levels showed a similar increasing trend although the increase was not significant. However, after 2 years of treatment with GnRHa, levels were higher than those of untreated transgirls of a similar age. An explanation might be that individuals who started treatment at a younger age had matured slightly earlier with earlier rise of adrenal androgen levels compared to those who presented later. A third of the adolescents who started treatment at age 12−14 years had advanced puberty, Tanner genital stage 4 or 5. However, we cannot directly compare the timing of pubertal maturation to the group who started at age 14−16 years as no data on their pubertal development 2 years earlier (when they were 12−14 years old) are available. It seems unlikely that GnRHa treatment influences adrenal DHEAS synthesis based on previous studies, which found no change in DHEAS levels during GnRHa treatment in elderly men with benign prostate hyperplasia,[16] and no change in either DHEAS or cortisol levels in hyperandrogenemic women during GnRHa treatment, whose response to adrenocorticotropic hormone was also unaffected.[17]

In transboys androstenedione levels decreased during the first year of GnRHa treatment and androstenedione levels after 2 years of treatment were significantly lower compared to those of untreated individuals of a similar age. This decrease is probably due to reduced ovarian androgen production. If the decrease were due to decreased adrenal androgen production, one may have expected that DHEAS levels also decreased during GnRHa treatment, which was not the case. In addition, no change in androstenedione levels was observed in transgirls, which also pleads against an effect of GnRHa on adrenal androstenedione production. In transgirls androstenedione levels after 2 years of treatment were comparable to those of untreated subjects of a similar age.

During 2 years of gender-affirming hormone treatment DHEAS levels were stable in both sexes. These findings are in contrast to observations in adults. In adults the administration of high doses of ethinylestradiol in combination with cyproterone acetate to transwomen resulted in a decrease of DHEAS levels (among other adrenal hormones).[7] This change may partly be due to the effect of cyproterone acetate, which was also shown to reduce DHEAS levels in adolescents,[18] or the supraphysiological dose of estrogens. A combination of 17-beta estradiol and GnRHa treatment also resulted in a decrease of DHEAS in adult transwomen.[8] The higher dose of estradiol (6 mg vs a maximum of 2 mg in the current study) may explain the different findings from the current study, or the GnRHa may have a different effect in adults compared to adolescents. Administration of testosterone to transmen or cisgender women resulted in an increase of DHEAS levels that was accompanied by a decrease of DHEA.[7,19,20] It was hypothesized that testosterone might decrease metabolism of DHEAS to DHEA.[20] In contrast, another study in adults found lower DHEAS levels in transmen treated with testosterone compared to cisgender women.[21] In the current study no effect of testosterone on DHEAS was found, maybe due to the difference in the dose of testosterone (higher in adults) or due to a difference in response between adolescents and adults.

Androstenedione levels did not change in transgirls during estrogen treatment, but increased in transboys during the first year of testosterone treatment. Previous studies have reported similar changes in androstenedione levels during testosterone administration.[20,22] Theoretically the rise in androstenedione could be of ovarian, adrenal, or other origin. Studies have found androgen receptors in human adrenal tissue,[23,24] which makes a direct effect of testosterone conceivable. However, if testosterone had a stimulatory effect on adrenal androgen synthesis one might have expected a rise in DHEAS levels, which was not observed. In addition, in adult women the adrenocortical response to adrenocorticotropic hormone was not affected by testosterone treatment.[20] It seems unlikely that the rise in androstenedione levels is of ovarian origin as GnRHa treatment was continued during testosterone treatment (until gonadectomy) so that ovarian steroidogenesis was suppressed. In addition, an increase in androstenedione was also observed during testosterone administration to women who had undergone bilateral gonadectomy[20] and we did not observe a consistent change in androstenedione levels after ovariectomy. The most likely source of increased androstenedione levels during testosterone treatment in transboys seems conversion of the administered testosterone to androstenedione, possibly in the liver.[25] Alternatively this conversion may take place in erythrocytes, which have been shown to express the enzyme 17-beta hydroxy steroid dehydrogenase and convert testosterone into androstenedione.[26] The increase in red cell mass that occurs in response to testosterone may result in increased conversion.[4] The significant correlation between testosterone and androstenedione levels during

testosterone treatment supports the hypothesis that testosterone is converted into androstenedione. In the presence of testosterone levels in the normal male adult range the increase of androstenedione levels, still within the normal range in the large majority of adolescents, may not have clinical implications.

In adult transwomen combined GnRHa and 17-beta estradiol treatment was shown to result in a decrease of androstenedione levels.[8] The authors state that this may not necessarily reflect an influence on adrenal function but may be the effect of reduced testicular steroid synthesis. The fact that these results are different from the current study may be due to the higher dose of estradiol used in the adult transwomen or perhaps to a different response in adults compared to adolescents.

This study has some limitations. No control group was included, which made it difficult to distinguish between normal changes with increasing age and treatment effects. In addition, cortisol production was not assessed although this is an essential marker of adrenal function. However, the design of the study, with monitoring using fasting blood samples, was not suitable for the assessment of changes in cortisol production.

CONCLUSION

In conclusion, we found a decrease of androstenedione levels in transboys during GnRHa treatment, likely due to reduced ovarian androstenedione production, and a rise in androstenedione levels during testosterone treatment, possibly due to direct conversion. No changes in androstenedione were observed in transgirls and DHEAS levels remained stable during gender-affirming hormone treatment in both sexes. These findings suggest that gender-affirming hormone treatment does not significantly affect adrenal androgen production in adolescents during treatment.

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The effect of GnRH analogue treatment on bone mineral density in young adolescents with gender dysphoria: findings from a large national cohort

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Abstract

Background: More young people with gender dysphoria (GD) are undergoing hormonal intervention starting with gonadotropin-releasing hormone analogue (GnRHa) treatment. The impact on bone density is not known, with guidelines mentioning that bone mineral density (BMD) should be monitored without suggesting when. This study aimed to examine a cohort of adolescents from a single centre to investigate whether there were any clinically significant changes in BMD and bone mineral apparent density (BMAD) whilst on GnRHa therapy.

Methods: A retrospective review of 70 subjects aged 12–14 years, referred to a national centre for the management of GD (2011–2016) who had yearly dual energy X-ray absorptiometry (DXA) scans. BMAD scores were calculated from available data. Two analyses were performed, a complete longitudinal analysis (n = 31) where patients had scans over a 2-year treatment period, and a larger cohort over the first treatment year (n = 70) to extend the observation of rapid changes in lumbar spine BMD when puberty is blocked.

Results: At baseline transboys had lower BMD measures than transgirls. Although there was a significant fall in hip and lumbar spine BMD and lumbar spine BMAD Z-scores, there was no significant change in the absolute values of hip or spine BMD or lumbar spine BMAD after 1 year on GnRHa and a lower fall in BMD/BMAD Z-scores in the longitudinal group in the second year.

Conclusions: We suggest that reference ranges may need to be re-defined for this select patient cohort. Long-term BMD recovery studies on sex hormone treatment are needed.

Keywords: BMI; bone mineral density; gender dysphoria; GnRHa treatment; sex steroids.

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Introduction

Puberty triggers the development of the secondary sexual characteristics that reinforce gender identity [1]. It also triggers rapid growth within the adolescent individual, stimulating a rise in bone mineral density (BMD). Bone mass is greatly increased during puberty and it is known that the accumulation of bone mass can differ between sex and anatomical site [2].

In gender dysphoria (GD), a person is not able to equate their perceived gender with their birth-registered sex. It is an increasingly common phenomenon [3]. This incongruity can be a stressful and distressing experience for adolescents and often worsens at the onset of puberty. The introduction of gonadotrophin releasing hormone analogue (GnRHa) treatment to induce a pubertal blockade, allowing the adolescent to reflect on this disparity without unwanted progress in pubertal development is now a recommended treatment option [3].

It is not fully appreciated how this arrest of puberty affects the development of bone mass and bone density, although some studies with small cohort sizes have found that BMD can decrease [4, 5]. Similarly it is unknown how this therapeutic pubertal blockade should affect ongoing management as there are no international guidelines for the surveillance of bone health in young people with GD [6]. Reference ranges are available for children and adolescents, but when an adolescent is found to have low BMD, the indications for treatment are not clear [7].

We conducted a retrospective analysis of bone health in young adolescents with GD referred from across the UK and Ireland to our Early Intervention clinic at University College London Hospital (UCLH) to try to investigate whether there is any significant loss of BMD and bone mineral apparent density (BMAD), a size adjusted value incorporating body size measurements using UK norms in growing adolescents

for up to 3 years of GnRHa treatment prior to the consideration of cross-sex hormone (CSH) treatment. We particularly wished to investigate whether there was a significant drop after 1 year of GnRHa following abrupt sex hormone withdrawal, and whether any predictors could be identified to warn about potentially harmful changes in BMD.

Materials and methods

Subjects

Seventy subjects with GD aged 12–14 years of white Caucasian origin were referred to our Early Intervention programme national endocrine clinic at UCLH between 2011 and 2016 [3]. This included 31 transgirls (birth-registered males identifying as female) and 39 transboys (birth-registered females identifying as male). They had all been seen and assessed by the Gender Identity Development Service multi-disciplinary psychosocial health team at our partner Tavistock-Portman National Health Service (NHS) Trust for at least four assessments over a minimum of 6 months [3]. All subjects by definition had entered puberty to consider blockade. All but two of the transboys were postmenarchal. Fifty-seven percent of the transgirls were in early puberty (G2–3 and testicular volume >4 mL) and 43% were in late puberty (G4–5). Adequate vitamin D status and calcium intake were ensured. After full clinical assessment, subjects were offered treatment with GnRHa for at least 1 year or ongoing until they reached the age of 16 years when CSH treatment could be considered [3]. All eligible subjects with adequate data were included in this analysis.

At the start and then annually during treatment during scheduled clinic visits, the subjects underwent a dual energy X-ray absorptiometry (DXA) scan over the lumbar spine L1–L4 and the femoral neck using a Hologic Discovery QDR series model 010-1549 (Hologic Inc, Bedford, MA, USA). Thirty-one subjects had three scans, baseline and 2 subsequent years on GnRHa treatment, and 70 (an additional 39) had two scans, baseline and after 1 year on GnRHa treatment.

BMD variables

BMD for lumbar spine and femoral neck (hip) was reported as absolute areal values (g/cm^2) and as Z-scores for birth sex and age. Only the BMD values and Z-scores were produced by the internal software associated with this commercially available equipment. As this areal measurement does not allow for the growth of an adolescent or their ethnicity, BMAD volumetric values in g/cm^3 and Z-scores were calculated from the formulae proposed by Crabtree et al. from the ALPHABET study using UK norms for Caucasian subjects [8]. Hip BMAD Z-scores were not calculated as there were no reference ranges provided as per the International Society for Clinical Densitometry [8].

Statistical analysis

We conducted two analyses – one assessing pure longitudinal changes in BMD and BMAD (n = 31) in those having three serial DXA scans, and one to extend the observations in the first year on GnRHa treatment in an additional 39 subjects (total n = 70). SPSS Version 24 (Chicago, IL, USA) was used. As the data followed a normal distribution, paired t tests were used. A p value less than 0.05 was considered statistically significant.

Ethical considerations

Specific ethics consideration was not required as this was a retrospective evaluation of routine clinical data. All data were analysed and are presented anonymously.

Results

In the longitudinal analysis there were 31 subjects who had three DXA scans (10 transgirls and 21 transboys). Z-scores were lower in transboys than transgirls at each site and both showed a significant drop after 1 year (Table 1), thereafter levelling off but there was no change in BMD for the lumbar spine or hip, or lumbar spine BMAD absolute values over the course of the 3 years. We also note a gradual increase in height and weight over this period, with transgirls having a larger increase in BMI, and transboys a greater increase in height (Table 1).

Seventy subjects had at least two DXA scans (31 transgirls and 39 transboys). This analysis was performed to investigate the first year changes with greater power. BMD and BMAD Z-scores showed a fall of similar magnitude in this larger cohort with similar transboy/transgirl baseline differences although BMD Z-scores were higher in this larger group, and there was no significant change in absolute BMD in the spine or hip values, nor lumbar spine BMAD over this first treatment year (actually, a small but non-significant increase) (Table 2).

Discussion

This is the largest study to date on the effects of puberty blockade using GnRHa on a national cohort of early pubertal transgender adolescents. The annual DXA scans took place because of the concern that removal of sex hormone secretion in puberty might have a detrimental effect on bone mineralisation.

We have shown a progressive fall in BMD and BMAD Z-scores, most rapid in the first year on treatment, demonstrating that the usual pattern of accruing bone mass according to age does not happen when puberty is halted. The longitudinal analysis additionally showed that there were no changes in absolute values in either BMD

Table 1: Changes in BMD and BMAD over 3 years.

Characteristic Mean, SD	Scan 1	Scan 2	Scan 3	p-Value[a]	p-Value[b]	p-Value[c]
Transgirls, n	10	10	10			
Age, year	13.0 (1.1)	14.5 (1.2)	15.8 (1.3)			
Height, cm	160.3 (5.4)	163.4 (5.7)	165.1 (5.7)			
Weight, kg	66.4 (14.6)	76.1 (19.4)	82.9 (30.5)			
BMI, kg/m^2	25.8 (5.3)	28.2 (7.1)	30.5 (8.6)			
Hip BMD, kg/m^2	0.920 (0.116)	0.913 (0.099)	0.910 (0.125)	0.338	0.402	0.944
Hip Z-score	0.45 (0.781)	−0.344 (0.752)	−0.600 (1.059)	0.002	0.002	0.498
Spine BMD, kg/m^2	0.867 (0.141)	0.866 (0.126)	0.878 (0.130)	0.952	0.395	0.202
Spine BMD Z-score	0.130 (0.972)	−0.650 (1.182)	−0.890 (1.075)	0.001	0.000	0.203
Spine BMAD, g/cm^3	0.240 (0.027)	0.238 (0.029)	0.240 (0.030)	0.588	0.865	0.355
Spine BMAD Z-score	0.486 (0.809)	−0.097 (1.00)	−0.279 (0.93)	0.000	0.000	0.228
Transboys, n	21	21	21			
Age, year	12.9 (3.0)	14.3 (3.3)	15.6 (3.5)			
Height, cm	159.0 (35.8)	165.3 (36.7)	168.7 (37.5)			
Weight, kg	49.8 (17.1)	54.4 (17.5)	59.5 (19.6)			
BMI, kg/m^2	19.4 (5.9)	20.7 (7.9)	20.9 (6.6)			
Hip BMD, kg/m^2	0.766 (0.215)	0.774 (0.203)	0.773 (0.197)	0.913	0.604	0.314
Hip Z-score	−1.075 (1.145)	−1.633 (0.899)	−1.779 (0.816)	0.000	0.001	0.201
Spine BMD, kg/m^2	0.695 (0.220)	0.711 (0.205)	0.731 (0.209)	0.107	0.058	0.056
Spine BMD Z-score	−0.715 (1.406)	−1.610 (1.462)	−2.000 (1.384)	0.000	0.000	0.035
Spine BMAD, g/cm^3	0.195 (0.058)	0.195 (0.054)	0.198 (0.055)	0.835	0.433	0.316
Spine BMAD Z-score	−0.361 (1.439)	−1.007 (1.347)	−0.913 (1.318)	0.000	0.001	0.332

Scan 1 at baseline and scans 2 and 3 on GnRHa treatment in the pure longitudinal cohort n = 31. Data are expressed as mean unless p value is given a–scan 1 vs. scan 2; b–scan 1 vs. scan 3; c–scan 2 vs. scan 3. BMAD, bone mineral apparent density; BMD, bone mineral density; BMI, body mass index.

measured sites or in lumbar spine BMAD between the scans over the 3-year period (see Table 1). It is recognised that BMD is influenced by the height and weight of the patients. Therefore, we calculated lumbar spine BMAD as well to try and account for these variables [8].

The 2-year analysis corroborated the initial treatment year finding with larger subject numbers. Importantly, there was also no significant change in absolute BMAD after the first year after being on the treatment, in contrast to the fall in Z-score values, suggesting acute sex hormone withdrawal does not cause a direct loss of calcium, but the longer term effects of slow accrual are unknown. The majority of the transboys being in mid-late puberty and were still completing their growth spurt, whereas many of the transgirls were at an early stage of puberty where rapid height acceleration had not yet occurred, and the tendency to gain additional truncal body fat is well recognised. It is surprising therefore that the baseline Z-scores are lower in the transboys. This had been noted previously in transmen in their 20s [9]. Whether this is a small cohort-size effect is unclear, but extension of these observations is important. The extent to which puberty had started did not seem to influence the results, given bone mineralisation is a long-term process. Neither did the increase in body fat, most noticeably in the transgirls, appear to influence BMD or BMAD changes, unless it was this weight gain which was exhibiting a bone-protective effect.

Currently, patients with GD undergoing a DXA scan have their BMD compared with references from their contemporaries who have not undergone pubertal blockade. Multiple studies have shown that BMD increases with age as adolescents progress through puberty [10]. Reference ranges for BMD shift throughout puberty as shown by Crabtree et al. [8]. Abnormality is typically a Z score of –2 or lower [11], but this is not the sole definition of low bone mass in children, nor is this criterion a recognised predictor of later fracture risk. Our analyses of Z-scores alongside BMD/BMAD values shows that although there is no significant change in BMD/BMAD, there are significant falls in the Z-scores after 1 year and less so subsequently. The GnRHa treatment is interrupting the rapidity of bone size increase, so whether the Z-scores remain a valid comparator is debatable, although height continues to increase. The observations of Klink et al. [4] and Vlot et al. [5] in older-aged cohorts also demonstrating no significant change in absolute BMD, have led us to propose that it may be clinically inappropriate to compare these subjects' BMD with that of contemporaries who have not

Table 2: Changes in BMD and BMAD at baseline (scan 1) and after 1 year of GnRHa treatment (scan 2) in the full cohort n = 70.

Characteristic Mean, SD	Scan 1	Scan 2	p-Value
Transgirls, n	31	31	
Age, year	13.2 (1.4)	14.4 (1.5)	
Height, cm	161.0 (8.0)	163.7 (8.1)	
Weight, kg	64.7 (17.1)	70.3 (21.2)	
BMI, kg/m^2	24.8 (5.3)	26.1 (6.9)	
Hip BMD, kg/m^2	0.894 (0.118)	0.905 (0.104)	0.571
Hip Z-score	0.157 (0.905)	−0.340 (0.816)	0.002
Spine BMD, kg/m^2	0.860 (0.154)	0.859 (0.129)	0.962
Spine BMD Z-score	−0.016 (1.106)	−0.461 (1.121)	0.003
Spine BMAD, g/cm^3	0.235 (0.030)	0.233 (0.029)	0.459
Spine BMAD Z-score	0.859 (0.154)	−0.228 (1.027)	0.000
Transboys, n	39	39	
Age, year	12.6 (1.0)	13.8 (1.1)	
Height, cm	158.4 (9.5)	163.3 (8.7)	
Weight, kg	51.0 (13.7)	56.2 (13.4)	
BMI, kg/m^2	20.1 (4.1)	21.4 (5.4)	
Hip BMD, kg/m^2	0.772 (0.137)	0.785 (0.120)	0.797
Hip Z-score	−0.863 (1.215)	−1.440 (1.075)	0.000
Spine BMD, kg/m^2	0.694 (0.149)	0.718 (0.124)	0.006
Spine Z-score	−0.395 (1.428)	−1.276 (1.410)	0.000
Spine BMAD, g/cm^3	0.196 (0.035)	0.201 (0.033)	0.074
Spine BMAD Z-score	−0.186 (1.230)	−0.541 (1.396)	0.006

p-Values are for scan 1 vs. scan 2. BMAD, bone mineral apparent density; BMD, bone mineral density; BMI, body mass index; GnRHa, gonadotropin-releasing hormone analogue.

had pubertal blockade as the bone development in the GD subjects has been halted in comparison to those of their age group. It could be possible to derive a similar approach as in the UK RCPCH growth charts where separate growth centiles for prepubertal children are shown, differentiating height and weight from those entering puberty. We propose that a measure along these lines needs to be produced for those in whom puberty is blocked (https://www.rcpch.ac.uk/resources/uk-who-growth-charts-childhood-puberty-close-monitoring-cpcm-chart).

The NHS in England recently produced clinical guidelines regarding gender reassignment (https://www.england.nhs.uk/wp-content/uploads/2017/04/gender-development-service-children-adolescents.pdf), and there are also international guidelines suggested by Hembree et al. [6]. Within these there is a discussion regarding the protocol for sex reassignment, and there are currently accepted treatment regimens. However, there is room for institutional variation when monitoring the patients. The recent guidelines produced by NHS England suggests "concerns about the client's physical health such as low bone mineral density" as a stopping criteria (https://www.england.nhs.uk/wp-content/uploads/2017/04/gender-development-service-children-adolescents.pdf) leading to the assumption that there will be BMD monitoring, although the method by which this is done is not stipulated. This is due to the fact that long-term effects of GnRHa and CSH treatments are largely unknown [12].

The findings support the suggestion that yearly DXA scans may be unnecessary and that considerations should be made regarding the frequency of these scans as we have shown that there is very little actual change in absolute BMD or BMAD. Yearly DXA scans can be a large expense to service providers and are a source of exposure to ionising radiation, albeit in a low dose. To ensure optimum bone health in transgender adolescents, follow-up using DXA scans are still necessary as we do not know the full extent of mineralisation catch-up once on CSH treatment, though they may not be needed annually. Checking the BMD of subjects more frequently may only be required if there are risk factors for osteoporosis in accordance with the practice guidelines suggested by the Endocrine Society [6]. It has been noted that eventual bone mass was not affected when evaluated in patients with central precocious puberty (CPP), and it has also been shown that bone mass accrual resumes when CSH are introduced although the full extent may vary [12]. This further supports our suggestion for new reference ranges when monitoring the BMD of subjects with GD.

This study is not without its limitations. Firstly, these results have come from a retrospective analysis of clinical scans which were not acquired for the sole purpose of this study. As such, there are instances where there are gaps in the data due to the measurements not being recorded at the precise annual interval. Although 70 is a large sample size for the first year data, the pure longitudinal data set is smaller (n = 31) but still to date the largest published from a single centre. Although trends could be masked by the small sample size, the two modes of analysis produced similar findings.

In conclusion, although there is an immediate drop in BMD and BMAD Z-scores, we have shown that absolute BMD and BMAD does not change substantially over a 3-year period in transgender adolescents on GnRHa treatment whilst consideration is being made whether to start CSH treatment or not. The need for such frequent DXA scans, which produce exposure to ionising radiation and can have significant financial implications for healthcare providers warrants further study, as does knowing whether early pubertal scans are of any value at all in predicting adult bone density and any potential osteoporosis risk.

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TRANSGENDER HEALTH

Physical changes, laboratory parameters, and bone mineral density during testosterone treatment in adolescents with gender dysphoria

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ABSTRACT

Introduction: Current treatment guidelines for adolescents with gender dysphoria recommend therapy with gonadotropin-releasing hormone agonists (GnRHa) and testosterone in transgender males. However, most evidence on the safety and efficacy of testosterone is based on studies in adults.

Aim: This study aimed to investigate the efficacy and safety of testosterone treatment in transgender adolescents.

Methods: The study included 62 adolescents diagnosed with gender dysphoria who had started GnRHa treatment and had subsequently received testosterone treatment for more than 6 months.

Main Outcome Measure: Virilization, anthropometry, laboratory parameters, and bone mineral density (BMD) were analyzed.

Results: Adolescents were treated with testosterone for a median duration of 12 months. Voice deepening began within 3 months in 85% of adolescents. Increased hair growth was first reported on the extremities, followed by an increase of facial hair. Acne was most prevalent between 6 and 12 months of testosterone therapy. Most adolescents had already completed linear growth; body mass index and systolic blood pressure increased but diastolic blood pressure did not change. High-density lipoprotein (HDL) cholesterol and sex hormone binding globulin significantly decreased, but hematocrit, hemoglobin, prolactin, androstenedione, and dehydroepiandrosterone sulfate significantly increased, although not all changes were clinically significant. Other lipids and HbA1c did not change. Vitamin D deficiency was seen in 32−54% throughout treatment. BMD *z*-scores after 12 to 24 months of testosterone treatment remained below *z*-scores before the start of GnRHa treatment.

Clinical Implications: Adolescents need to be counseled about side effects with potential longer term implications such as increased hematocrit and decreased HDL cholesterol and decreased BMD *z*-scores. They should be advised on diet, including adequate calcium and vitamin D intake; physical exercise; and the use of tobacco and alcohol to avoid additional risk factors for cardiovascular disease and osteoporosis.

Strengths & Limitations: Strengths are the standardized treatment regimen and extensive set of safety parameters investigated. Limitations are the limited duration of follow-up and lack of a control group so some of the observed changes may be due to normal maturation rather than to treatment.

Conclusion: Testosterone effectively induced virilization beginning within 3 months in the majority of adolescents. Acne was a common side effect, but no short-term safety issues were observed. The increased hematocrit, decreased HDL cholesterol, and decreased BMD *z*-scores are in line with previous studies. Further follow-up studies will need to establish if the observed changes result in adverse outcomes in the long term. **Stoffers IE, de Vries MC, Hannema SE. Physical Changes, Laboratory Parameters, and Bone Mineral Density During Testosterone Treatment in Adolescents with Gender Dysphoria. J Sex Med 2019;16:1459−1468.**

Key Words: Transgender; Gender Dysphoria; Gender Incongruent; Testosterone; Adolescent; Androgen; Bone Mineral Density

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INTRODUCTION

Gender dysphoria involves an incongruence between a person's sex assigned at birth and the gender with which the person self-identifies. The request for medical and mental health care by young people with gender dysphoria has increased. The largest Dutch gender identity clinic in Amsterdam found that the annual number of people seeking care had increased 20-fold from 34 in 1980 to 686 in 2015.[1] They estimated that, in the Netherlands, 1 in 9,000 adolescents (12−18 years old) are transgender girls (assigned male sex at birth) who had sought care, and 1 in 4,800 are transgender boys (assigned female sex at birth).[1]

Developing secondary sex characteristics of the sex assigned at birth can be very distressing for transgender persons.[2,3] Therefore, treatment with gonadotropin-releasing hormone agonists (GnRHa) to inhibit puberty is offered to adolescents in whom gender dysphoria has been diagnosed; who have no psychological, medical, or social problems that may interfere with treatment; and who can consent to this treatment.[4] GnRHa treatment gives individuals time to explore their gender identity and carefully consider their wishes regarding gender-affirming treatment. In addition, inhibiting the development of characteristics such as breasts or a low voice makes it easier for an individual to live in the affirmed gender. When gender dysphoria persists and the adolescent can consent to partially irreversible gender-affirming therapy, treatment with gender-affirming hormones can be started.[4] Transgender girls may receive therapy with estrogen to develop female secondary sex characteristics.[4,5] Transgender boys may receive testosterone treatment, administered intramuscularly (i.m.) or subcutaneously (s.c.), to initiate virilization.[4] Physical changes such as increased musculature and facial and body hair, as well as deepening of the voice, will occur, although no data are available on the timing of these changes in adolescents.[4,6,7] A well-known side effect of testosterone is acne.[8]

Besides changes in physical characteristics, gender-affirming hormones induce changes in several laboratory parameters. A meta-analysis of data in adult transgender men using testosterone treatment found an increase of triglycerides and low-density lipoprotein (LDL) cholesterol at certain time points after the start of testosterone treatment and a decrease of high-density lipoprotein (HDL) cholesterol at all time points.[9] A systematic review reported an increase of hemoglobin and hematocrit,[10] although limited data are available in adolescents. Jarin et al[11] retrospectively analyzed changes in metabolic parameters in 72 transgender male adolescents (up to 22 years old) and confirmed changes in HDL cholesterol and hematocrit found in adults, but limitations of this study included missing data and the use of many different treatment regimens. Tack et al[6] also reported an increase in hematocrit and, in contrast to the study by Jarin et al, an increase of liver enzymes in 25 transgender boys. Only 2 studies with partial overlap between the studied populations have reported on bone mineral density (BMD) *z*-scores during GnRHa and testosterone treatment. BMD *z*-scores declined during GnRHa treatment, and, although they increased during subsequent testosterone treatment, there was incomplete catch-up, as *z*-scores were still lower than pretreatment values at age 22 years after a median testosterone treatment duration of 5.4 years.[12,13]

The current study aimed to provide data on virilization during testosterone treatment of transgender boys and on the timing of changes, because no such information is currently available. In addition, changes in height, body mass index (BMI), blood pressure, a comprehensive set of laboratory parameters, and BMD were investigated in order to add to the very limited body of evidence on the efficacy and safety of this treatment.

METHODS

Subjects

Adolescents were eligible for this retrospective study if they had been diagnosed with gender dysphoria and had received testosterone therapy for a minimum of 6 months (or had had their 6-month visit, which was sometimes scheduled just before 6 months of treatment) between November 2010 (when the clinic first started) and August 2018. Sixty-four adolescents were eligible, 2 of whom declined participation and were excluded.

Treatment Protocol

Individuals had been assessed by mental health professionals to confirm the diagnosis of gender dysphoria according to *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition, criteria[14]; to exclude psychological, medical, or social problems that might interfere with treatment; and to determine if they were able to consent to treatment. All had been treated with a GnRHa (Decapeptyl-CR; 3.75 mg every 4 weeks s.c.) to inhibit puberty for at least 6 months, prior to the start of gender-affirming treatment. Adolescents who desired gender-affirming hormone treatment and had no psychological, medical, or social problems that might interfere with treatment were eligible for testosterone treatment from the age of 15 to 16 years. Testosterone (Sustanon; 250 mg) treatment consisted of intramuscular injections; the dose was increased every 6 months using the following schedule: 25 mg/m^2/2 wk, 50 mg/m^2/2 wk, and 75 mg/m^2/2 wk, leading up to a standard adult dose of 125 mg every 2 weeks. For those who were ≥16 years old at the start of GnRHa treatment, the testosterone dose was increased more rapidly, beginning with 75 mg/2 wk, which was increased to 125 mg/2 wk after 6 months. When they were on an adult dose, some individuals chose to use intramuscular testosterone 250 mg every 3 to 4 weeks rather than 125 mg every 2 weeks. If individuals wished to switch to transdermal treatment because of a dislike of injections or problems with arranging the administration of injections, for example, they were switched to testosterone gel using an equivalent dose (standard adult dose 50 mg once daily, adjusted based on serum testosterone levels). Written informed consent for treatment was obtained from adolescents and if <16 years of age from their parents, too.

Clinical Evaluation

The clinical effects of testosterone were evaluated every 3 months (±1.5 months). Adolescents were asked about acne and whether it required treatment, hair growth and shaving, lowering of the voice, and (absence of) menses. During physical examinations, attention was paid to acne, hair growth, and voice pitch. Observations noted in the medical files were classified into different categories. Acne was scored as no acne, a few papules, acne, or acne requiring treatment. Hair growth was subdivided into facial hair (none, mild, moderate, ie, requiring shaving or advanced, ie, beard growth), abdominal hair (none, mild, moderate, or advanced), and hair on the extremities (typical feminine, mildly increased, moderate, or typical masculine). If an increase of hair growth in a certain area was described in the medical file, then the hair growth score in that area was increased by 1 category compared to the previous visit. Voice pitch was scored as unchanged, breaking, or low/masculine. Two pediatric endocrinologists carried out all of the assessments, and each individual was assessed by the same endocrinologist throughout treatment.

Height was measured using a wall-mounted stadiometer, and weight was measured using a digital floor scale. Height standard deviation scores (SDS) were calculated using Dutch reference data,[15] and BMI SDS were calculated using reference data from Cole et al.[16] Blood pressure was measured using a Dinamap automated device (Critikon Corp.; Tampa, FL).

Laboratory Investigations

Laboratory investigations were performed every 6 months (±3 months). Follicle-stimulating hormone (FSH), luteinizing hormone (LH), free thyroxine (FT4), dehydroepiandrosterone sulfate (DHEAS), and testosterone were measured by a Modular Analytics E170 (Roche Diagnostics, Mannheim, Germany) electrochemiluminescence immunoassay (ECLIA). The FSH and LH assays have a detection limit of 0.1 U/L; both have a total assay coefficient of variation (CV) of <5%. The testosterone assay has a detection limit of 0.1 nmol/L; for testosterone and FT4, the total assay variations were <3.5%. Thyroid-stimulating hormone (TSH) and prolactin were measured by a DELFIA solid-phase, 2-site, time-resolved fluoroimmunometric assay (Perkin Elmer; Groningen, the Netherlands). The TSH assay has a detection limit of 0.01 mU/L, an intra-assay CV of 1−2%, and an interasssay CV of 3−4%. The prolactin assay has a detection limit of 0.03 μg/L, an intra-assay CV of 4−6%, and an interassay CV of 5.5−7.2%. Estradiol was measured using radioimmunoassay (Diasorin; Saluggia, Italy) until 2014, after which the Modular Analytics E170 ECLIA assay was used which has a lower limit of detection of 18 pmol/L and an interassay CV of 2.1%. A conversion factor was determined to make concentrations measured using the radioimmunoassay comparable to those measured with the ECLIA assay. Androstenedione was measured using a radioimmunoassay (Diagnostic Systems Laboratories Inc; Webster, TX) until November 2017 and after that by liquid chromatography with tandem mass spectrometry, which gave comparable results. Sex hormone binding globulin (SHBG) was measured using an Immulite immunometric assay (Siemens Healthcare Diagnostics; Tarraytown, NY) until November 2017 and after that by the Modular Analytics E170 ECLIA assay.

Table 1. Population characteristics*

Characteristic	Value
Number of adolescents	62
Age at start of GnRHa (y), median (range)	16.5 (11.8–18.0)
Age at start of testosterone (y), median (range)	17.2 (14.9–18.4)
Duration of GnRHa therapy (mo), median (range)	8 (3–39)
Duration of testosterone therapy (mo), median (range)	12 (5–33)
Smoker, n (%)	
Yes	10 (16)
No	50 (79)
Menarche before start of GnRHa, n (%)	
Yes	57 (91)
No	3 (5)
Tanner stage at start of GnRHa, n (%)	
B3	6 (10)
B4	7 (11)
B5	49 (78)
Testosterone dose schedule, n (%)	
Regular	22 (35)
Fast	40 (65)

Fast = faster dose increase, used in those ages ≥16 y at the start of treatment (see methods); GnRHa = gonadotropin-releasing hormone agonist; Regular = regular pubertal induction scheme.
*Missing data are not shown.

Radiological Investigations

BMD at the lumbar spine (LS) and neck area of the left and right hip was evaluated with dual-energy x-ray absorptiometry (DXA) using a Hologic Discovery A scanner (Tromp Medical BV; Castricum, the Netherlands) before the start of GnRHa treatment and before the start of testosterone therapy (unless this was within a year of the previous DXA scan), and then every 1 to 2 years. BMD *z*-scores were calculated using female reference data from the Bone Mineral Density in Childhood Study[17]; for those >16 years of age, reference data from the Third National Health and Nutrition Examination Survey for the neck area of the hip and Hologic adult reference data for the LS were used. Bone mineral apparent density (BMAD) was calculated and *z*-scores determined for the lumbar spine and left femoral neck as described by Ward et al.[18] Because reference values are provided for up to 17 years of age, reference values for 17-year-olds were used for those aged >17 years.[18] Around the age of 18 years, adolescents were referred to an adult clinic elsewhere.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 23 (IBM Corp.; Armonk, NY). Data were verified for normal distribution using the

Shapiro-Wilk test. Normally distributed values are expressed as mean and standard deviation, whereas non-normally distributed values are expressed as median and interquartile range unless stated otherwise. Differences between time points were analyzed by paired samples *t*-test for normally distributed data and by the Wilcoxon signed-rank test for data that were not normally distributed. Results were considered statistically significant if $P < .05$.

Ethical Approval

The study was part of an observational study on the effects of hormonal treatment in adolescents with gender dysphoria which was approved by the local medical ethical committee.

RESULTS

Sixty-two individuals were included. They had been treated with GnRHa for a median duration of 8 months (range 3−39) before they began testosterone treatment at a median age of 17.2 years (range 14.9−18.4) (Table 1). Median duration of follow-up during testosterone treatment was 12 months (range 5−33). No one discontinued testosterone therapy.

Virilization

Increased hair growth was observed within 3 months of testosterone treatment. Hair on the extremities had increased in all by 12 months. Facial hair gradually increased during the first year of treatment (Figure 1) and was present in all after 15 months. After 1 year, 79% of the individuals for whom data were available had some abdominal or chest hair, but even after 2 years of treatment not all individuals had abdominal hair. Deepening of the voice was observed within 3 months in 46 of 54 (85%) individuals for whom data were available and within 6 months in all individuals (Figure 1). Virilization progressed more rapidly in those in whom the testosterone dose was increased more rapidly (Figure 1).

At the start of testosterone treatment 16% (n = 10) of adolescents had acne. After 6 months, this number had increased to 35% (n = 22), and 1 individual used topical treatment for acne. At 12 months, the prevalence was highest; 49% (n = 18) had acne not requiring treatment, and another 11% (n = 4) did use topical treatment for acne. After 15 months, the prevalence of acne decreased.

All adolescents experienced amenorrhea during treatment except for 3 individuals. One of these individuals was non-compliant with the GnRHa and testosterone for a period, which could explain the menstrual bleeding. Another had administered the GnRHa a few days late, but the cause was unclear for the third individual, who had mild bleeding for 1 to 2 days.

Height, BMI, and Blood Pressure

Most adolescents had completed linear growth before the start of treatment; only 5 individuals grew more than 2 cm during the follow-up period. BMI significantly increased during the first 6 months from 22.4 ± 3.4 kg/m^2 to 23.2 ± 3.0 kg/m^2 (n = 46; $P < .001$), but BMI SDS did not change (see Table 2). Systolic blood pressure increased from 118 mm Hg (114−125) to 124 mm Hg (118−132) during the first 6 months of testosterone treatment (n = 47; $P = .003$), after which it did not change. However, the systolic blood pressure after 6 months of testosterone was not significantly different from that before the start of GnRHa treatment. The diastolic blood pressure did not show any significant changes during 2 years of follow-up.

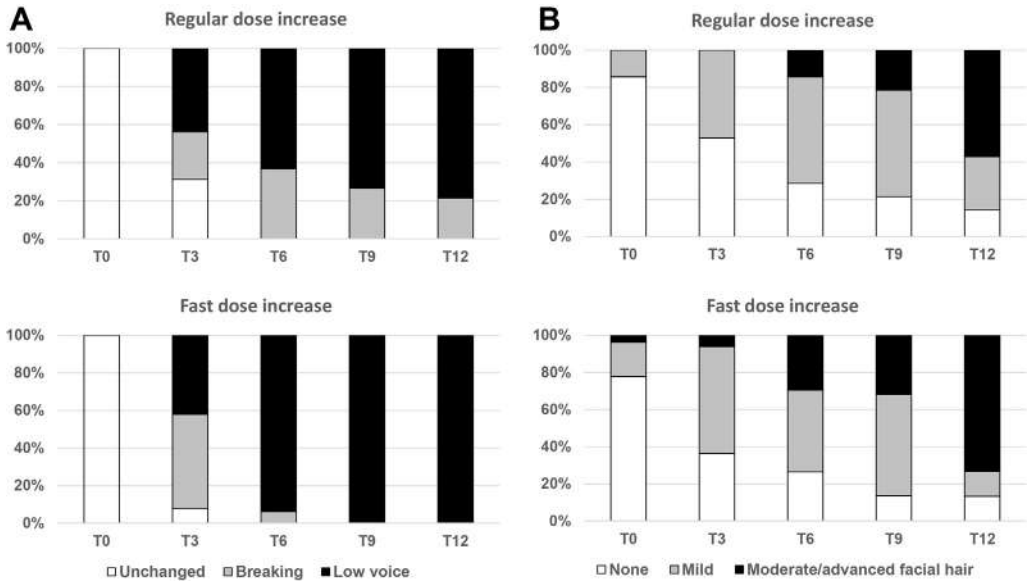

Figure 1. Changes in voice (A) and facial hair growth (B) after 0 to 12 months of testosterone treatment in the group with a regular (upper panel) and with a fast (lower panel) dose increase schedule. Percentages indicate how many of the adolescents from whom data were available at each time point fall into a particular category of voice or hair growth.

Table 2. Clinical measurements and bone mineral density at start of GnRHa therapy (G0) and during 24 mo of testosterone treatment (T0–T24)*

	Time point				
Variable	G0 (n = 62)	T0 (n = 62)	T6 (n = 62)	T12 (n = 37)	T24 (n = 15)
Height (cm)	167.1 ± 6.9	168.2 ± 6.2	169.8 ± 6.0†	169.7 ± 6.0†	167.8 ± 5.3‡
Height standard deviation score					
Male	−1.3 ± 1.2	−1.7 ± 0.9	−1.6 ± 0.9	−1.7 ± 0.8)	−1.8 ± 0.8
Female	−0.1 ± 1.0	−0.2 ± 1.0	0.1 ± 0.9	0.0 ± 0.9)	0.1 ± 0.8
Weight (kg)	61.7 ± 10.5	63.4 ± 11.0	66.7 ± 9.7†	67.2 ± 8.0‡	65.3 ± 10.6
BMI (kg/m^2)	21.5 (19.7–23.8)	21.8 (19.5–25.0)	22.6 (20.7–25.3)†	23.0 (21.2–25.3)	22.9 (20.7–26.5)
BMI standard deviation score					
Male	0.68 ± 1.0	0.58 ± 1.1	0.87 ± 0.9	0.84 ± 0.8	0.56 ± 1.2
Female	0.47 ± 1.0	0.40 ± 1.0	0.71 ± 0.9	0.70 ± 0.8	0.25 ± 1.1
Blood pressure (mm Hg)					
Systolic	124 (115–129)	118 (114–126)	124 (118–132)‡	126 (119–133)†	126 (117–129)‡
Diastolic	68 (65–73)	72 (66–77)	72 (65–76)	72 (66–77)	74 (63–76)
BMD§ (g/cm^2)					
Lumbar spine	0.96 ± 0.11	0.90 ± 0.11‖	0.94 ± 0.10‖	0.95 ± 0.09	0.95 ± 0.11
Left hip	0.84 ± 0.11	0.76 ± 0.09‖	0.83 ± 0.12¶	0.81 ± 0.08	0.86 ± 0.09
Right hip	0.84 ± 0.11	0.77 ± 0.08‖	0.84 ± 0.11‖	0.82 ± 0.08	0.85 ± 0.11
BMD *z*-score#					
Lumbar spine	0.02 ± 1.00	−0.81 ± 1.02‖	−0.67 ± 0.95‖	−0.66 ± 0.81¶	−0.74 ± 1.17¶
Left hip	−0.19 ± 1.04	−1.07 ± 0.85‖	−0.62 ± 1.12‖	−0.93 ± 0.63¶	−0.20 ± 0.70¶
Right hip	−0.16 ± 1.00	−0.97 ± 0.79‖	−0.54 ± 0.96‖	−0.80 ± 0.69¶	−0.31 ± 0.84¶

BMD = bone mineral density.
BMI = body mass index; GnRHa = gonadotropin-releasing hormone agonist.
*Data are presented as mean ± standard deviation or as median (interquartile range). Due to differences in the number of individuals from whom data are available at the different time points, significant changes may not be apparent from the data shown in the table although they are significant when paired values are compared.
†$P < .001$ compared to T0.
‡$P < .05$ compared to T0.
§BMD during testosterone treatment was also compared to values before the start of any treatment (G0).
‖$P < .001$ compared to G0.
¶$P < .05$ compared to G0.
#BMD *z*-scores were calculated using female reference values.

Bone Mineral Density

At the start of testosterone treatment, BMD at the LS and hips as well as BMD *z*-scores were lower than those at the start of GnRHa treatment (n = 18; all $P < .001$) (Table 2). After the start of gender-affirming therapy, all parameters increased. BMD measured after 12 to 24 months of testosterone therapy was no longer significantly different from BMD at the start of GnRHa treatment. However, BMD *z*-scores after 12 to 24 months of testosterone therapy remained lower than pretreatment values (LS, −0.77 ± 0.95 vs −0.18 ± 0.76, $P < .001$; left hip, −0.72 ± 0.75 vs −0.16 ± 0.77, $P < .001$; right hip, −0.66 ± 0.74 vs −0.14 ± 0.74, $P = .003$; n = 17).

Similar results were found when BMAD was calculated to take into account the effects of bone size. BMAD of the LS and left hip after 12 to 24 months of testosterone treatment was not significantly different from that at the start of GnRHa treatment (LS, 0.24 ± 0.02 vs 0.25 ± 0.02, $P = .11$; left hip, 0.32 ± 0.04 vs 0.33 ± 0.03, $P = .24$; n = 17). BMAD *z*-scores, on the other hand, remained lower than pretreatment values although only significantly so at the LS (LS, −0.57 ± 0.76 vs −0.16 ± 0.68, $P = .001$; left hip, −0.08 ± 0.79 vs 0.14 ± 0.60, $P = .07$; n = 17). The use of male reference values resulted in higher BMAD *z*-scores but similar treatment effects (male *z*-scores after 12 to 24 months of testosterone compared to before GnRHa: LS, −0.34 ± 0.87 vs 0.24 ± 0.91, $P < .001$; left hip, 0.01 ± 1.06 vs 0.24 ± 0.80, $P = .17$; n = 17).

Laboratory Investigations

The hematocrit significantly increased during the first year of testosterone treatment from 0.422 (0.407–0.434) to 0.466 (0.448–0.479) (n = 34; $P < .001$) (Table 3). This occurred more rapidly in the group in whom the testosterone dose was increased more rapidly (Figure 2). The maximum value observed during the 2-year follow-up was 0.536 L/L, and 6 individuals reached a value exceeding 0.5 L/L. All 6 had used the fast testosterone dose increase schedule; the testosterone doses at the time when a hematocrit of

Table 3. Laboratory investigations at the start of GnRHa therapy (G0) and during 24 mo of testosterone treatment (T0–T24)*

	Time Point				
Variable	G0 (n = 62)	T0 (n = 62)	T6 (n = 62)	T12 (n = 37)	T24 (n = 15)
Creatinine (umol/L)	63 ± 9	62 ± 7	70 ± 9†	74 ± 10†	81 ± 10†
ALP (U/L)	86 (71–118)	102 (78–136)	115 (102–147)†	112 (88–143)†	81 (69–98)
Total cholesterol (mmol/L)	4.03 (3.69–4.77)	4.47 (3.84–5.01)	4.10 (3.63–4.79)†	4.25 (3.55–4.86)	4.56 (3.36–5.39)
HDL cholesterol (mmol/L)	1.47 ± 0.34	1.59 ± 0.34	1.34 ± 0.25†	1.36 ± 0.29†	1.29 ± 0.27‡
LDL cholesterol (mmol/L)	2.33 (1.97–2.88)	2.48 (1.97–2.74)	2.45 (1.98–3.04)	2.51 (1.87–3.06)	2.24 (1.97–3.40)
Triglycerides (mmol/L)	0.74 (0.54–1.09)	0.75 (0.63–1.07)	0.83 (0.56–1.10)	0.76 (0.63–0.99)	0.79 (0.51–1.05)
Hb (mmol/L)	8.6 (8.0–9.0)	8.5 (8.0–8.8)	9.1 (8.7–9.5)†	9.5 (9.1–9.9)†	9.5 (8.9–10.2)‡
Ht (L/L)	0.416 (0.390–0.435)	0.413 (0.393–0.428)	0.442 (0.424–0.461)†	0.466 (0.447–0.479)†	0.453 (0.443–0.483)‡
LH (U/L)	5.3 (2.8–8.4)	0.4 (0.2–0.5)	0.3 (0.2–0.5)‡	0.2 (0.1–0.5)‡	1.3 (0.1–17.4)
FSH (U/L)	4.8 ± 2.8	2.6 ± 1.1	1.1 ± 0.8†	0.8 ± 0.6†	14.4 ± 19.9‡
E2 (pmol/L)	216 (107–482)	<18 (<18–27)	45 (22–86)†	65 (31–104)†	22 (<18–94)
PRL (μg/L)	13.0 (9.1–17.3)	8.6 (6.6–12.5)	10.8 (8.3–14.5)‡	12.8 (8.9–15.9)‡	13.6 (11.2–16.3)
SHBG (nmol/L)	50.3 ± 18.8	51.1 ± 20.1	28.8 ± 12.7†	25.9 ± 10.0†	29.4 ± 11.3‡
Testosterone (nmol/L)	1.2 (0.7–1.5)	0.7 (0.5–1.1)	12.3 (6.4–19.1)†	17.5 (11.3–22.7)†	13.9 (7.3–48.9)‡
A4 (nmol/L)	5.5 (3.8–7.9)	3.5 (2.4–4.8)	3.5 (2.3–5.9)	5.1 (3.5–6.4)‡	4.5 (2.0–8.0)
DHEAS (umol/L)	5.74 ± 2.57	6.34 ± 2.78	8.71 ± 4.42†	8.77 ± 3.72†	8.62 ± 4.54‡
Vitamin D (nmol/L)	36 (24–54)	58 (41–72)	49 (36–60)†	57 (46–75)	51 (33–81)
Vitamin D < 50 nmol/L (%)	74	37	54	32	40

A4 = androstenedione; ALP = alkaline phosphatase; DHEAS = dehydroepiandrosterone sulfate; E2 = estradiol; FSH = follicle-stimulating hormone; GnRHa = gonadotropin-releasing hormone agonist; Hb = hemoglobin; HDL = high-density lipoprotein; Ht = hematocrit; LDL = low-density lipoprotein; LH = luteinizing hormone; PRL = prolactin; SHBG = sex hormone binding globulin.

*Data are presented as mean ± standard deviation or median (interquartile range). Due to differences in the number of individuals from whom data are available at the different time points, significant changes may not be apparent from the data shown in the table although they are significant when paired values are compared.

†$P < .001$ compared to T0.

‡$P < .05$ compared to T0.

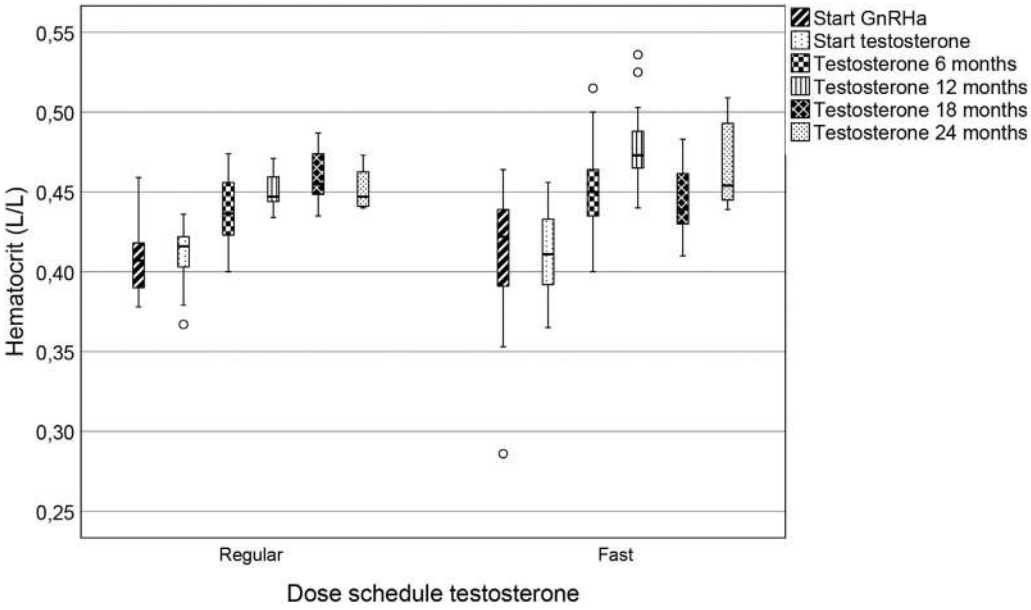

Figure 2. Changes in hematocrit during 24 months of testosterone treatment in groups with a regular or a fast dose increase schedule.

>0.5 L/L was observed were 75 mg/2 wk (n = 1), 125 mg/2 wk (n = 4), and 250 g/4 wk (n = 2) (1 individual had 2 measurements > 0.5 L/L). The hematocrit normalized without intervention or change of therapy in all 5 individuals for whom follow-up measurements were available.

Total cholesterol significantly decreased during the first 6 months, from 4.59 ± 0.92 mmol/L to 4.24 ± 0.92 nmol/L (n = 39; $P = .001$) but then slightly increased again and did not differ significantly from baseline after 12 and 24 months of testosterone therapy. HDL cholesterol significantly decreased during the first 6 months, from 1.58 ± 0.28 mmol/L to 1.33 ± 0.24 mmol/L (n = 39; $P < .001$) and remained unchanged thereafter. LDL cholesterol and triglycerides did not change during follow-up. One individual who already had elevated LDL cholesterol levels at the start of GnRHa therapy was diagnosed with familial hypercholesterolemia; after initiation of treatment with simvastatin, cholesterol levels normalized.

The liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase showed no significant changes. Alkaline phosphatase showed a significant increase from 102 U/L (80−134) to 115 U/L (102−143) during the first 6 months (n = 55; $P < .001$). Creatinine continued to rise throughout the 2 years of treatment, whereas ureum and hemoglobin A1c (HbA1c) showed no significant changes. Vitamin D deficiency was seen in 37% of individuals at the start of testosterone treatment and in similar percentages during the following 2 years (Table 3).

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As expected, serum testosterone levels increased from 0.7 nmol/L (0.6−1.1) to a median level of 12.3 nmol/L (6.9−19) after 6 months (n = 53; $P < .001$), whereas SHBG levels decreased from 50.6 ± 19.6 nmol/L to 28.8 ± 12.9 nmol/L (n = 39; $P < .001$). At 6 months, testosterone levels were significantly higher in those treated with the fast dose increase schedule compared to the regular schedule, with a median of 15.9 nmol/L (9.2−19.6) compared to 7.8 nmol/L (4.3−14.2) ($P = .007$). At 12 months, the difference was no longer significant. Estradiol levels increased from <18 pmol/L (<18−27) to 45 pmol/L (22−86) (n = 42; $P < .001$), and gonadotropins, already suppressed with GnRHa, showed a further decrease (Table 3).

Prolactin levels, which had declined during GnRHa treatment, rose during the first 6 months of testosterone therapy from 7.7 μg/L (6.5−11.7) to 10.6 μg/L (8.5−13.8) (n = 38; $P = .007$) and stabilized thereafter at levels similar to those before the start of any treatment; all values remained within the female reference range (upper limit, 23.3 μg/L), but in 9 out of 41 individuals prolactin was above the male reference range (upper limit, 15.0 μg/L). Androstenedione levels did not change during the first 6 months but increased from 4.2 nmol/L (2.9−5.4) to 5.2 nmol/L (3.7−6.3) (n = 31; $P = .017$) after 12 months of testosterone treatment. DHEAS levels rose from 6.34 ± 2.78 umol/L to 8.71 ± 4.42 during the first half year of testosterone treatment (n = 41; $P < .001$). No significant changes in TSH or FT4 were observed.

DISCUSSION

This study is the first to report detailed information on the virilizing effects of testosterone treatment in trangender boys. The onset of increased hair growth within 3 months of therapy with a further increase beyond 1 year of treatment is similar to findings in adults.[19] Change of voice was also noted within the

first 3 months of therapy in the great majority of adolescents. This is in accordance with studies in adults that measured the largest drop of speaking fundamental frequency within the first 3 months of testosterone treatment.[20,21]

Acne was a common side effect and most prevalent at 6 to 12 months of testosterone use. Previous studies among adolescents and adults found that the prevalence and severity of acne were greatest at 6 months of treatment and had decreased at 12 months.[6,19] This slightly earlier peak may be because of the use of higher starting doses of testosterone. In the long term, most individuals (94%) were reported to have no or mild acne.[19]

The increase in BMI observed in the first 6 months of testosterone treatment was similar to findings in previous studies among adolescents and adults.[6,10,11] In contrast to the study by Tack et al,[6] which showed that BMI SDS increased during testosterone treatment, possibly related to a gain in muscle mass, the current study did not find any significant change in BMI SDS, indicating that the increase in BMI found in the current study was similar to that observed in the general population during adolescence. The rise in systolic blood pressure found during the first 6 months of testosterone treatment is similar to findings of some previous studies in adolescents and adults, although other studies found no changes in blood pressure.[10,11,22] The small change does not seem to be of clinical relevance, as systolic blood pressure after 6 months of testosterone was similar to pretreatment values.

The significant decrease in BMD and BMD *z*-scores after initiation of GnRHa therapy is in line with previous findings.[12,13] BMD returned to baseline values after 12 to 24 months of testosterone treatment, but BMD *z*-scores remained significantly below values before the start of GnRHa treatment, indicating that during the entire treatment period less bone mineral accrual occurred than in peers. When BMAD was calculated to take into account bone size, *z*-scores were higher, but these may have been overestimated, especially at 12 to 24 months of treatment, because no reference values are available for adults so reference values for 17-year-olds were used for older adolescents.[18] Whether it is more appropriate to use male or female references to calculate *z*-scores after 12 to 24 months of testosterone treatment is unclear. Further catch-up of bone mineral accrual may still occur, although Klink et al[12] found that BMD *z*-scores at age 22 years, after a median of 5.4 years of testosterone treatment, were still lower than pretreatment values. To optimize bone health, adolescents are advised to exercise regularly and maintain a sufficient intake of calcium and vitamin D. Vitamin D deficiency was common, and, although vitamin D was prescribed to those with insufficient serum levels, one-third to one-half of adolescents still had vitamin D levels below 50 nmol/L at subsequent visits, possibly due to non-compliance.

Testosterone stimulates erythropoiesis, and erythrocytosis is a known adverse effect of testosterone treatment.[4] The current study shows an increase of hematocrit, similar to findings from previous studies in transgender adolescents.[11] Throughout the course of this study, no patient had a hematocrit exceeding 0.54 L/L, which is the level at which discontinuation of testosterone treatment is recommended to let the hematocrit decrease to a safe level.[23] However, levels above the normal male range (ie, >0.5 L/L) were encountered in several individuals. All of these adolescents had been treated using the fast dose increase schedule, suggesting that the tempo at which the testosterone dose is increased may influence the risk of erythrocytosis. If these findings are confirmed in future studies, then this may alter treatment recommendations. Although the hematocrit normalized without intervention, monitoring is important because erythrocytosis is associated with an increased risk of neuro-occlusive or cardiovascular events.[4,23]

Another possible risk factor for cardiovascular disease is the decrease in HDL cholesterol that was observed in the current study, which has also been found in a previous study in adolescents and in a meta-analysis of studies among adults.[9,11] This meta-analysis also concluded that testosterone treatment results in an increase of LDL cholesterol and triglycerides, in contrast to the findings in adolescents. What the changes in lipid profile mean for the future cardiovascular health of transgender adolescents is uncertain. Not enough data were available regarding cardiovascular events, such as myocardial infarction or stroke, or mortality for meaningful assessment of these outcomes in the meta-analysis.[9] Long-term follow-up studies of individuals who begin testosterone treatment in adolescence are necessary to assess cardiovascular outcomes in this population.

Some previous studies reported an increase of ALT and/or AST during testosterone treatment,[6,22] but others did not.[11] No changes in these liver enzymes were seen in the current study. Testosterone levels increased with treatment but varied widely because the time between the last testosterone injection and blood sampling varied. Although suppressed with GnRHa, LH and FSH showed a further decrease during testosterone treatment. At 24 months of treatment, some adolescents had undergone a gonadectomy and/or had stopped GnRHa treatment, which resulted in an increase of gonadotropins. Because of the lack of a control group it is uncertain if the significant increase of DHEAS observed during testosterone treatment was due to therapy or to normal maturation of the adrenal gland during adolescence. A previous study of transgender adolescents treated with testosterone did not find a significant change in DHEAS, but in adults an increase has also been described.[24,25] Azziz et al[25] hypothesized that testosterone might decrease the metabolism of DHEAS to DHEA, resulting in higher DHEAS levels. Alongside DHEAS, androstenedione levels also increased in the current study, stabilizing after the first year of therapy. This is similar to the results from Schagen et al,[26] who also described a plateau after 1 year and hypothesized that the increased androstenedione levels might be due to direct conversion of testosterone. Alternatively, testosterone might have a direct stimulatory effect on adrenal androgen synthesis, as the androgen receptor is expressed in human adrenal tissue.[27]

Prolactin levels significantly increased during the first 6 months of testosterone treatment in line with a previous study that described a rise of prolactin from 16.5 ng/mL to 28.1 ng/mL in transgender adolescents, but this was in a very small group (n = 5) and the change was not statistically significant.[11] In contrast, studies in adult populations have shown a decrease of prolactin levels during testosterone therapy.[28] These different findings may be due to the fact that in the current study adolescents had been treated with a GnRHa prior to the start of testosterone treatment. During GnRHa treatment, prolactin had decreased, similar to findings among cisgender females treated with a GnRHa,[29] likely related to the decrease in estradiol, which is known to have a stimulatory effect on prolactin.[30] During subsequent testosterone treatment, prolactin levels returned to pretreatment values along with a rise in estradiol due to aromatization of testosterone. The changes of prolactin levels do not seem of clinical concern, as prolactin levels remain within the female reference range. A previous study in adolescents described a significant decrease of FT4 during testosterone treatment, but we found no such change.[6] This different finding may be due to the prior use of lynestrenol vs GnRHa treatment to suppress puberty, as the authors describe an initial rise of FT4 during lynestrenol treatment.[6]

The strengths of this study are the group size and the standardization of treatment and follow-up, although no systematic scoring system was used to classify acne and hair growth so our observations cannot be directly compared to findings from others. No control group was included which made it difficult to distinguish between normal changes with increasing age and treatment effects. The duration of follow-up did not allow us to study outcomes beyond 2 years of testosterone treatment.

CONCLUSION

Testosterone treatment using a dosing schedule as recommended by current guidelines effectively induces virilization in transgender boys, with increased hair growth and voice change noted within 3 months of therapy in the majority. No short-term safety issues were observed in the current study. However, the increased hematocrit (in some instances above the normal range), the decreased HDL cholesterol, and possibly the elevation of systolic blood pressure may increase the risk of cardiovascular events. In addition, the decrease in BMD *z*-scores during GnRHa treatment with incomplete catch-up after 12 to 24 months of testosterone treatment raises concern about bone health in the long term. Adolescents should be counseled about these concerns and advised about factors known to influence cardiovascular and bone health, such as diet (including adequate calcium and vitamin D intake), physical exercise, smoking, and alcohol. Studies following transgender males treated with testosterone from adolescence into adulthood will have to establish whether or not the treatment is safe in the long term.

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темпы роста детей больных СКдЛ часто оцениваются в сравнении с темпами роста здоровых детей, что приводит к ошибкам диагностики.

В отсутствии генотипирования диагноз СКдЛ определяется рядом клинических критериев, таких как нарушения строения лицевого черепа и минимум один критерий, относящийся к развитию, поведению или росту. Причиной низкорослости, ассоциированной с СКдЛ, часто является дефицит ГР или резистентность периферических тканей к гормону. Вместе с тем лишь в нескольких исследованиях предоставлены данные о терапевтическом эффекте применения ГР у детей, больных СКдЛ.

В нашей работе представлен случай заболевания СКдЛ девочки 4 лет 11 мес, направленной на обследование к эндокринологу по причине отставания в росте. Из анамнеза известно, что у девочки наблюдаются отставание в речевом развитии и регургитация легочного клапана. На момент осмотра дефицит роста составлял –2,32SDS, скорость роста –3SDS за предыдущий год, отставание костного возраста на 2,5 года от хронологического, масса тела и окружность головы определялись менее 5-й перцентили для соответствующего возраста. Также определялись: синофриз, дистрофия ротовой полости, микрогнатия и тонкая верхняя губа, опущенные уголки рта, гипертрихоз, систолический шум в проекции легочного клапана, частичное переразгибание в локтевом суставе.

Нарушения развития лицевого черепа и наличие двух основных критериев служили подтверждением диагноза СКдЛ. Эндокринологическое обследование выявило низкорослость, сопровождающуюся дефицитом гормона роста (ГР) по данным двух стимуляционных проб с уровнем ГР ниже 10 нг/мл. Пациентке было назначено лечение соматотропином в дозе 0,04 мг/кг/сут, в результате которого отмечалась прибавка росте на 3,5 см за первые 6 мес терапии (скорость роста –2,7 SDS).

Данный клинический случай предполагает, что дети со своевременным обследованием и выявлением СКдЛ, сопровождающимся дефицитом роста, являются подходящими кандидатами для терапии препаратами соматотропина с целью улучшения конечного роста.

КЛЮЧЕВЫЕ СЛОВА

Синдром Корнелия де Ланге, дефицит гормона роста.

★ ★ ★

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EFFECT OF SUPPRESSION OF PUBERTY AND CROSS-SEX HORMONE THERAPY ON BONE TURNOVER MARKERS AND BMAD IN TRANSGENDER ADOLESCENTS

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Background. Puberty is highly important for the accumulation of bone mass. Bone turnover and bone mineral density can be affected in transgender adolescents when puberty is suppressed by gonadotropin-releasing hormone analogues (GnRHa), followed by treatment with cross-sex hormone therapy (CSHT).

Objective. To investigate the effect of GnRHa and CSHT on bone turnover markers (BTMs) and bone mineral apparent density (BMAD) in transgender adolescents.

Material and methods. Thirty four female-to-males (FtMs) and 22 male-to-females (MtFs) were divided into a young and old pubertal group, based on the bone age of 14 years in the FtMs and 15 years in the MtFs. All patients received GnRHa triptorelin. CSHT was prescribed in incremental doses from the age of 16 years. FtMs received testosterone ester mixture and MtFs were treated with 17-β estradiol. BTMs P1NP, osteocalcin and ICTP and the BMD of lumbar spine (LS) and femoral neck (FN) were measured at three time points. Furthermore, BMAD and Z-scores were calculated.

Results. P1NP and 1CTP decreased during GnRHa treatment, indicating decreased bone turnover. Osteocalcin showed an aberrant pattern. A low BMAD Z-score of both FN and LS was observed in the MtFs at start of GnRHa treatment. The decrease in bone turnover upon GnRHa treatment was accompanied by an unchanged BMAD of both FN and LS, however BMAD Z-scores of predominantly the LS decreased. Twenty-four months after CSHT the BTMs P1NP and ICTP were even more decreased. During CSHT BMAD Z-scores increased and returned towards normal, especially of the LS.

Conclusion. Suppressing puberty by GnRHa leads to a decrease of BTMs in transgender adolescents. The increase of BMAD and BMAD Z-scores predominantly in the LS as a result of treatment with CSHT is accompanied by decreasing BTM concentrations after 24 months of CSHT. Therefore, the added value of evaluating BTMs seems to be limited and DEXA-scans remain important in follow-up of transgender adolescents.

KEYWORDS

Bone turnover, BMAD, transgender, adolescence, GnRHa, cross-sex hormones.

ВЛИЯНИЕ ПОДАВЛЕНИЯ ПУБЕРТАТА И ЗАМЕСТИТЕЛЬНОЙ ГОРМОНАЛЬНОЙ ТЕРАПИИ НА МАРКЕРЫ ФОРМИРОВАНИЯ И РЕМОДЕЛИРОВАНИЯ КОСТНОЙ ТКАНИ И BMAD У ТРАНСГЕНДЕРНЫХ ПОДРОСТКОВ

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Введение. Пубертат является очень важным периодом в процессе формирования костной массы. Процессы ремоделирования и формирования минеральной плотности костной ткани у трансгендерных подростков могут быть изменены на фоне проводимой терапии аналогами гонадотропин-рилизинг-гормона (GnRHa), а также на фоне заместительной гормональной терапии (CSHT).

Цель исследования — изучить влияние терапии GnRHa и CSHT на маркеры ремоделирования (BTMs) и формирования минеральной плотности костной ткани (BMAD) у трансгендерных подростков.

Материал и методы. 34 женщины-мужчина (FtMs) и 22 мужчины-женщина (MtFs) были разделены на группы раннего и позднего пубертатного периода, согласно костному возрасту, в 14 лет у FtMs и 15 лет у MtFs. Все пациенты получали терапию трипторелин GnRHa. CSHT была назначена в возрастающей дозе от настоящего возраста до

16 лет. FtMs получали смесь сложных эфиров тестостерона и MtFs получали 17-β-эстрадиол. Было проведено трехкратное определение BTMs P1NP, остеокальцина, ICTP и BMD поясничного отдела позвоночника (LS) и шейки бедра (FN).Также были определены BMAD и Z-критерий.

Результаты. P1NP и 1CTP снизились во время проведения терапии GnRHa, замедлились процессы формирования костной ткани. Также заметно изменились показатели Остеокальцина. По данным BMAD на момент старта терапии GnRHa в группеMtFs выявлено снижение Z-критерия во всех исследуемых отделах (FN, LS). На фоне терапии GnRHa отмечено замедление процессов ремоделирования костной ткани, тогда как по данным BMAD изменений в FN и LS не выявлено. Однако в LS наблюдалось снижение Z-критерия. При динамическом осмотре через 24 мес после старта CSHT было выявлено более выраженное снижение BTMs, P1NP и ICTP. Во время проведения CSHT по данным BMAD выявлено повышение Z-критерия, а в некоторых случаях, даже возвращение в норму, наиболее выраженные изменения наблюдались в LS.

Выводы. Задержка пубертатного периода на фоне терапии GnRHa приводит к снижению BTM у трансгендерных подростков. Увеличение BMAD и BMAD Z-критерия, особенно в LS отделе, рассматривался как результат Терапии CSHT, при этом сопровождается снижением BTM через 24 мес. Проведение денситометрии и BTMS является весьма важным диагностическим методом эффективности терапии у трансгендерных подростков.

КЛЮЧЕВЫЕ СЛОВА

Ремоделирование костной ткани; минеральная плотность костной ткани, транссексуализм, подростковый возраст, GnRHa; заместительная гормональная терапия.

Original Article

Bone mineral density increases in trans persons after one year hormonal treatment: a multicenter prospective observational study†

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Abstract

Sex steroids are important determinants of bone acquisition and bone homeostasis. Cross-sex hormonal treatment (CHT) in transgender persons can affect bone mineral density (BMD). The aim of this study is to investigate in a prospective observational multicenter study the first-year effects of CHT on BMD in transgender persons. 231 transwomen and 199 transmen were included who completed the first year of CHT. Transwomen were treated with cyproterone acetate and oral or transdermal estradiol, transmen received transdermal or intramuscular testosterone. A dual-energy X-ray absorptiometry was performed to measure lumbar spine (LS), total hip (TH), and femoral neck (FN) BMD before and after one year CHT. In transwomen, an increase in LS (+3.67%, 95% confidence interval (CI) 3.20 to 4.13%, p<0.001), TH (+0.97%, 95% CI 0.62 to 1.31%, p<0.001), and FN (+1.86%, 95% CI 1.41 to 2.31%, p<0.001) BMD was found. In transmen, TH BMD increased after one year CHT (+1.04%, 95% CI 0.64 to 1.44%, p<0.001). No changes were observed in FN BMD (-0.46%, 95% CI -1.07 to 0.16%, p=0.144). The increase in LS BMD was larger in transmen ≥50 years (+4.32%, 95% CI 2.28 to 6.36%, p=0.001) compared with transmen <50 years (+0.68%, 95% CI 0.19 to 1.17%, p=0.007). In conclusion, BMD increased in transgender persons after one year CHT. In transmen of postmenopausal age, the LS BMD increased more than in younger transmen, which may lead to the hypothesis that the increase in BMD in transmen is the result of the aromatization of testosterone to estradiol.

Key words: sex steroids, DXA, transgender, osteoporosis, cross-sex hormonal treatment

Introduction

Gender dysphoria (GD) is defined as the sufferance related to an incongruence between one's experienced and one's assigned gender of a duration of at least 6 months.[1] Persons who experience gender-related distress might desire gender-affirming treatment with sex steroids. Sex steroids are also important determinants of bone acquisition and bone homeostasis.

In natal men, testosterone stimulates the process of periosteal apposition, leading to a greater cortical bone size and wider bones than in women.[2,3] Men with aromatase deficiency have been found to have lower bone mass, indicating that estrogen is an important regulator of bone acquisition in men.[4–6] However, the effect of testosterone on bone mineral density (BMD) is less clear.

In natal women, estrogen inhibits periosteal apposition but stimulates endosteal bone formation.[7] Loss of estrogen at menopause leads to an increased osteoclastic activity and therefore accelerated bone loss.[8,9] In women with polycystic ovary syndrome with hyperandrogenism, an increased trabecular BMD has been found, even after adjustment for body mass.[10] In addition, women with complete androgen insensitivity syndrome (46,XY karyotype) have been found to have lower BMD[11], which might indicate that testosterone also regulates BMD in women.

Cross-sex hormonal treatment (CHT) in transgender persons causes changes in gonadal hormone levels in order to achieve desired body changes. Transwomen (male-to-female transgenders) receive estrogen and anti-androgens, transmen (female-to-male transgenders) are treated with testosterone. While a few studies have investigated the effects of CHT on BMD, most of these studies were cross-sectional or had small sample sizes with inconclusive or contradictory results.[12–19] The aim of this study is therefore to investigate, in a large multicenter prospective cohort, whether CHT influences BMD in transwomen and transmen during the first year of treatment.

Methods

Study design and population

This study is part of the European Network for Investigation of Gender Incongruence (ENIGI) study, a multicenter prospective observational study performed for its endocrine part in Ghent (Belgium), Oslo (Norway), Florence (Italy), and Amsterdam (the Netherlands).[20,21] Trans persons of 18 years and older who started CHT between 2010 and April 2016 after a confirmed GD diagnosis[1] were asked to participate in the study. Exclusion criteria were prior cross-sex hormone use, psychological vulnerability, the occurrence of protocol deviations (e.g. the use of gonadotropin-releasing hormone agonist or spironolactone), or insufficient knowledge of the native language. For the present analysis, only persons who completed the first year of CHT were included. Because the participating centers used different types of dual-energy X-ray absorptiometry (DXA) scanners (Ghent and Amsterdam: Hologic Discovery A; Florence: Hologic Delphi A; Oslo: Lunar), non-comparable BMD values were obtained. Consequently, only persons with BMD measurements performed in either Ghent or Amsterdam were included in the analyses. Persons who did not have a baseline DXA scan or had a baseline DXA scan outside the window of three months before or one month after the start of CHT were excluded. In addition, persons who did not have a follow-up DXA scan or had a follow-up scan before 10 or after 14 months after the start of CHT were excluded.

All trans persons were treated according to the Standards of Care Guidelines of the World Professional Association for Transgender Health (WPATH).[22] Transwomen received cyproterone acetate (50 to 100 mg daily) combined with oral estradiol valerate (2 to 4 mg daily) or an estradiol patch (50 to 100 µg twice a week). Transmen were treated with testosterone gel (50 mg daily), intramuscular testosterone esters (250 mg every two weeks), or intramuscular testosterone undecanoate (1000 mg every 12 weeks).

This study was conducted in accordance with the Declaration of Helsinki and the overall study protocol was approved by the Ethical Review Board of the Ghent University Hospital, Belgium. In

the other participating centers, approval for participation was also obtained of the local ethical committees. Study participants gave informed consent according to institutional guidelines.

Clinical data collection

During the first year of treatment, trans persons visited the outpatient endocrine unit once every three months, where they reported their medical history, medication use, smoking habits (in cigarettes per day), and alcohol use (in units per week). Physical examination was performed by measuring body weight (in kilograms) and height (in meters) in indoor clothing without shoes.

Biochemical assessment

Blood samples were drawn at baseline, after three months, and after 12 months of CHT, all after overnight fasting. In Amsterdam, estradiol was measured using a competitive immunoassay (Delfia, PerkinElmer, Finland) with an inter-assay coefficient of variation (CV) of 10-13% and a lower limit of quantitation (LOQ) of 20 pmol/L until July 2014. After July 2014, estradiol was measured using a LC-MS/MS (VUmc, Amsterdam, the Netherlands) with an inter-assay CV of 7% and a LOQ of 20 pmol/L. For conversion of the Delfia values, the formula LC-MS/MS = 1.60*Delfia–29 was used. Testosterone was measured using a radioimmunoassay (RIA) (Coat-A-Count, Siemens, USA) with an inter-assay CV of 7-20% and a LOQ of 1 nmol/L until January 2013. Thereafter, testosterone was measured using competitive immunoassay (Architect, Abbott, USA) with an inter-assay CV of 6-10% and a LOQ of 0.1 nmol/L. The RIA values were converted to the competitive immunoassay values. For testosterone levels below 8 nmol/L, the formula Architect = 1.1*RIA+0.2 was used; for testosterone levels above 8 nmol/L, the formula Architect = 1.34*RIA–1.65 was used. 25-hydroxyvitamin D (25(OH)D) was measured using LC-MS/MS as described previously.[23]
In Ghent, estradiol was measured using a E170 Modular (Gen II, Roche Diagnostics, Germany) until 19th March 2015. Thereafter, estradiol was measured using a E170 Modular (Gen III, Roche Diagnostics, Germany), with an inter-assay CV of 3.2% and a LOQ of 25 pg/mL (92 pmol/L). For

conversion of estradiol values measured before 19th March 2015, the formula Gen III =6.687940+0.834495*Gen II was used. E170 Modular (Roche Diagnostics, Germany) was used to measure testosterone (Gen II) and 25(OH)D, with an inter-assay CV of 2.6% and a LOQ of 10 ng/dL (0.4 nmol/L) for testosterone, and an inter-assay CV of 6.7% and a LOQ of 5 ng/mL (12.5 nmol/L) for 25(OH)D.

Bone mineral density

DXA was performed at baseline and after one year CHT. In both Ghent and Amsterdam, a Hologic Discovery A was used (Hologic Inc., Bedford, MA, USA). In Ghent, Software Version 12.7.3.1 was used. In Amsterdam, the Software Version was updated from 13.3 to 13.5.3 in July 2015. The Hologic Statement of Equivalency allowed for comparison of absolute BMD values between the different scan dates and times. Absolute BMD values were obtained for lumbar spine (LS, L1–L4), non-dominant total hip (TH), and femoral neck (FN).

Statistical analysis

The baseline characteristics of both transwomen and transmen are reported as medians (interquartile range, IQR) or percentages. Differences between included and excluded persons were analyzed using independent t-tests (or Wilcoxon rank-sum test in case of non-normal distribution) or chi-square tests. Difference between BMD values obtained in Amsterdam and Ghent were analyzed using an independent t-test. Data were log-transformed before further analysis in case of non-normal distribution. For all analyses, individuals with missing values were excluded. Percentage changes in LS, TH, and FN BMD after one year of CHT were calculated for every person and as these were normally distributed continuous variables, linear regression analyses were performed to generate the mean percentage changes with corresponding 95% confidence intervals (CI) and p-values.

Analyses were stratified for age groups (18–20, 21–29, 30–49, and ≥50 years) in order to stratify for accrual of peak bone mass, peak bone mass, age-related decrease in bone mass, and, in transmen, for postmenopausal state. Analyses were stratified for the use of vitamin D supplementation, as persons with vitamin D deficiency were treated with vitamin D supplementation. For analyses on different estradiol or testosterone administration routes, only transmen and transwomen who used the same administration route and dose during the entire year were included. Difference between age groups, vitamin D supplementation, or administration routes were analyzed using linear regression analyses with percentage change in BMD as outcome variable and age groups, vitamin D supplementation, or administration routes as categorical independent variable, respectively. In order to adjust for possible mediating factors, linear regression analyses were performed between percentage change in BMD and change in body weight, and between percentage change in BMD and change in cigarette and alcohol use. The change in alcohol or cigarette use was calculated as the percentage difference in number of cigarettes or units of alcohol between baseline and the 12-month visit. To investigate the influence of the serum concentrations of sex steroids on BMD change, linear regression analyses were performed between the change in BMD and the mean serum estradiol or testosterone levels after three to 12 months of CHT, and were analyzed separately for Amsterdam and Ghent, as no conversion formulas were available for the sex steroid assays. Sensitivity analyses were performed by repeating the analyses after exclusion of all persons with comorbidities or medication use with possible influence on BMD. Analyses were performed with STATA Statistical Software (Statacorp, College Station, Texas, USA), version 13.1.

Results

General characteristics

The flowchart of the inclusion of participants in both centers is shown in Figure 1. In total, 231 transwomen and 199 transmen were included for analyses. The baseline characteristics are shown in Table 1. Except for a younger age in excluded transmen (median age 22 years, IQR 20 to 27) compared to included transmen (median age 24 years, IQR 21 to 31, difference p=0.004), no baseline differences were found in weight, BMI, ethnicity, smoking habits, alcohol use, estradiol levels, testosterone levels, and vitamin D levels between persons excluded and included for analyses.

Bone mineral density

The mean time between two DXA scans was 12 months (range 10 to 14 months). No differences in baseline BMD and 12 month BMD between the two included centers were found (Table 2).
In transwomen, one year CHT increased BMD of the LS (+3.67%, 95% CI 3.20 to 4.13%, p<0.001), TH (+0.97%, 95% CI 0.62 to 1.31%, p<0.001), and FN (+1.86%, 95% CI 1.41 to 2.31%, p<0.001). In transmen, one year CHT increased LS and TH BMD (+0.86%, 95% CI 0.38 to 1.35%, p=0.001, and +1.04%, 95% CI 0.64 to 1.44%, p<0.001, respectively). No changes were observed in FN BMD (-0.46%, 95% CI -1.07 to 0.16%, p=0.144) (Figure 2).

Effects of weight change on BMD change

Transwomen had a mean weight increase of 2.4 kg (95% CI 1.5 to 3.2 kg, p<0.001). The gain in LS BMD did not change after adjustment for change in body weight (+3.64%), but an attenuation of TH (+0.65%) and FN (+1.52%) BMD change was found. Transmen had a mean weight increase of 2.0 kg (95% CI 1.2 to 2.8 kg, p<0.001). After adjustment for change in body weight, the mean increase of LS (+0.90%) and FN (-0.87%) BMD did not change, but an attenuation of TH (+0.86%) BMD change was found.

Effects of change in cigarette or alcohol use on BMD change

52.1% and 27.5% of the transwomen who either smoked cigarettes or drank alcohol at baseline quit smoking and stopped using alcohol, respectively. Adjusting the analyses for percentage change in cigarette or alcohol use did not change the results of LS (+3.80%), TH (+0.92%), or FN (+1.91%) BMD change. Of the transmen, 45.6% and 28.7% who either smoked cigarettes or drank alcohol at baseline quit smoking and stopped using alcohol, respectively. No changes were observed in LS (+0.93%), TH (+1.24%), and FN (-0.50%) BMD change after adjustment for change in cigarette or alcohol use.

The effect of age on BMD change

As shown in Figure 3, the change in LS, TH, or FN BMD in transwomen did not vary in different age groups, except for a larger increase in LS BMD in transwomen of 18 to 20 year compared to transwomen of 30 to 49 years. In transmen, LS BMD increased more in persons ≥50 years (+4.32%, 95% CI 2.28 to 6.36%, p=0.001) compared to persons below 50 years (+0.68%, 95% CI 0.19 to 1.17%, p=0.007). The geometric mean estradiol levels increased from 14 pmol/L to 150 pmol/L (+949%, 95% CI 304 to 2629%, p<0.001) in persons ≥50 years, compared to no increase in persons below 50 years (158 pmol/L to 194 pmol/L; +22%, 95% CI -2 to 53%, p=0.078).

The effect of vitamin D supplements on BMD change

As shown in Figure 4, LS and FN BMD increased more in transwomen who used vitamin D supplements compared to those who did not use supplements. No differences were found in TH BMD change. In transmen, no differences in LS, TH, and FN BMD change were found between persons with or without vitamin D supplementation.

Correlation of BMD change with sex hormone levels

After three to 12 months of CHT, the estradiol levels of transwomen in Amsterdam were correlated with LS (per 100 pmol/L: +0.95%, 95% CI 0.34 to 1.56%, p=0.003), TH (per 100 pmol/L: +0.48%, 95% CI 0.04 to 0.93%, p=0.034), and FN (per 100 pmol/L: +0.83%, 95% CI 0.31 to 1.36%, p=0.002) BMD change. The estradiol levels after three to 12 months in transwomen in Ghent were correlated with LS (per 100 pmol/L: +0.87%, 95% CI 0.27 to 1.47%, p=0.005), but not with TH (per 100 pmol/L: +0.40%, 95% CI -0.12 to 0.92%, p=0.126) or FN (per 100 pmol/L: +0.09%, 95% CI -0.67 to 0.85%, p=0.814) BMD change. In transmen in Amsterdam and Ghent, estradiol levels after three to 12 months were not correlated with BMD change. As testosterone levels after three to 12 months were suppressed in transwomen, no correlation analyses could be performed. In transmen, testosterone levels after three to 12 months were not correlated with LS, TH, and FN BMD change.

The effect of estradiol or testosterone administration routes on BMD change

LS, TH, or FN BMD change did not differ between transdermal estradiol or oral estradiol valerate use in transwomen (Figure 4). Serum estradiol levels were comparable between transdermal estradiol (ref) and oral estradiol valerate (difference -7 pmol/L, 95% CI -50 to 36 pmol/L, p=0.754). In transmen, no differences in LS and TH BMD change was observed between testosterone gel, testosterone esters, or testosterone undecanoate. FN BMD change was higher in testosterone undecanoate compared to testosterone gel (Figure 4). Testosterone levels were comparable between testosterone gel (ref) and testosterone undecanoate (-3.5 nmol/L, 95% CI -12.9 to 5.9 nmol/L, p=0.459), while testosterone esters provided higher testosterone levels than testosterone gel (+13.0 nmol/L, 95% CI 6.2 to 19.9 nmol/L, p<0.001).

Sensitivity analyses

Repeating the analyses after exclusion of all persons with bone influencing medication (use of diuretics (n=6), anti-epileptics (n=3), antidepressants (n=58), antipsychotics (n=9), corticosteroids

(n=15), or bisphosphonates (n=1)) or comorbidities (eating disorder (n=4), alcohol abuse (n=4), thyroid disease (n=5), diabetes mellitus (n=5), gastro-intestinal disease (n=8), malignancy (n=2)) did not change the effect sizes (data not shown).

Discussion

This study showed that after one year CHT the mean BMD increased in both transwomen and transmen, especially in lumbar spine and in transwomen. In transmen of postmenopausal age, the LS BMD increased more than in younger transmen. In both transwomen and transmen, the change in BMD could not be completely explained by a change in body weight, a change in cigarette or alcohol use, or by vitamin D supplementation.

The larger increase in BMD in transmen of postmenopausal age as compared to the other age groups may be the result of decreased bone resorption due to higher levels of estradiol after aromatization of testosterone to estradiol, as serum estradiol levels were low at baseline and largely increased during CHT. These findings may lead to the hypothesis that the increase in bone mineral density in older transmen is the result of aromatization of testosterone to estradiol and therefore an increase in estradiol levels, instead of direct effects of testosterone. This is in line with Finkelstein et al.[24] who demonstrated that effects of hypogonadism on bone in men are mainly due to estrogen deficiency and not to testosterone deficiency. In addition, the results are compatible with findings in older natal men, whose BMD is better correlated with bioavailable estradiol levels than with other sex steroid measures.[25] However, it is not known whether these findings can be extrapolated to transmen. The larger increase in LS BMD in transwomen of 18 to 20 years compared to transwomen between 30 to 49 years could be explained by the fact that the youngest group has not reached the peak bone mass yet and therefore the increase in BMD is larger.

In both transwomen and transmen, the TH BMD increase attenuated after adjustment for change in body weight, indicating that the increase in hip BMD is partly mediated by an increase in body weight. No differences in BMD change were observed between administration routes of estradiol or testosterone, except a small difference in FN BMD change between testosterone gel and testosterone undecanoate. However, the groups of testosterone administration routes were small and the analysis might not have enough power to detect small differences. No differences were observed in mean estradiol levels between transdermal estradiol or estradiol valerate. Testosterone esters

gave higher testosterone levels compared to testosterone gel and testosterone undecanoate. However, testosterone ester therapy results in highly fluctuating serum testosterone levels and since blood determination was independent of the last injection of testosterone esters, no representative testosterone levels were obtained from persons using testosterone esters. The current using dose of testosterone esters is thought to be similar to the dose of testosterone gel and testosterone undecanoate.[26]

Previously, small prospective studies were performed to investigate the change in BMD in transgender persons after one year CHT. For transwomen, our results are comparable to most other studies, as an increase in LS BMD was found in transwomen treated with anti-androgens and estrogens[18,27,28] or treated with estrogens and gonadotropin-releasing hormone agonists[13,29]. One prospective study did not find a change in BMD in 12 transwomen.[30] Although the same results were found in most other studies, our results cannot be generalized to all parts of the world, as the included transwomen used cyproterone acetate and this is not approved for use in the United States. We found different results for the change in BMD in transmen than described in literature. While previous prospective studies did not find a change in LS BMD after one year CHT[14,19,27,28,30], we found a small increase in the total group, and even a larger increase in the postmenopausal subgroup. This difference might be due to the small sample sizes of other studies and consequently lack of power to allow these studies to detect any differences. In addition, a subgroup analysis of the change in BMD in postmenopausal transmen has not been described before, as transmen included in previously mentioned studies were between 18 to 47 years[19], 16 to 39 years[28], and 20 to 46 years[30] of age.

This study is a large multicenter prospective study, including transwomen and transmen with a wide range of age. All trans persons were treated according to a defined treatment protocol and measurements were performed at baseline and during follow-up. Only a small percentage of participants was lost to follow-up (8.5%). There are also some limitations to our study. First, due to the multicenter aspect of this study, the BMD of participants was measured using different DXA

devices. In order to compare the baseline values, Oslo and Florence were excluded for the present analyses and only Ghent and Amsterdam were included. Each individual had a baseline and follow-up BMD measurements on the same DXA device and no differences were observed between Ghent and Amsterdam. Between the study centers, different laboratory assays were used and within one study center, the assay was adjusted when more accurate assays became available. Conversion formulas within one center allowed for comparison of the data. However, as no conversion formulas between the centers were available, the analysis had to be stratified by center. The associations found within both centers were similar, which allows for higher generalizability of the results. Second, the study was performed during regular patient care. Data about smoking habits, alcohol use, medication use, and comorbidities were self-reported and were collected during their outpatient clinic visits. It is possible that some medication use or comorbidities were not reported, including the use of additional testosterone or estradiol preparations next to the prescribed sex hormones. Third, no data about physical exercise or calcium intake was available. Persons with low baseline BMD were advised about factors of positive influence on BMD, including exercise, calcium intake, and vitamin D supplements. Therefore, we cannot prove that the increase in BMD is solely a result of CHT. Due to ethical and practical reasons, it is not possible to add a placebo group to this study. Fourth, as this study is only performed in trans persons without a control group, we cannot rule out that passing time is partly an explanation for the change in BMD. However, as most persons had already reached the age of peak bone mass achievement at the time of inclusion in the study, the natural course of BMD is to decrease over time. Lastly, for the present analyses we only assessed BMD using a DXA scan, which does not provide any information about bone geometry. Further research is needed into whether changes in bone geometry can be found after CHT. However, in regular patient care BMD is also assessed using a DXA scan, therefore this study may be valuable for clinical practice.

One study reported a lower BMD in transwomen at the start of CHT compared with age-matched control men.[31] Hormonal treatment may influence the achievement of higher BMD on short term.

A healthier lifestyle including more exercise and vitamin D exposure may also contribute to this change in BMD. Therefore, with regard to the clinical practice, monitoring bone quality in trans persons is relevant.

In conclusion, an increase in BMD in both transwomen and transmen after one year CHT was found. For further research, it is desirable to investigate alterations in BMD after long-term CHT, to monitor bone turnover markers, and to add other imaging modalities in order to gain more insight of the actual changes in bone metabolism due to sex steroid therapy.

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Table 1. Baseline characteristics of transwomen and transmen

	Transwomen (n=231)	Transmen (n=199)
Age, years (IQR)	28 (23 to 42)	24 (21 to 31)
Ethnicity (% Caucasian)	97.4	94.0
BMI, kg/m^2 (IQR)	22.5 (20.5 to 26.1)	23.9 (21.3 to 28.8)
Tobacco use (% yes)	23.5	29.3
- Cigarettes per day (IQR)	10 (5 to 12)	10 (5 to 15)
Alcohol use (% >7 units per week)	6.1	4.6
Biochemical results Amsterdam (IQR) *		
- Estradiol levels, pmol/L	105 (82 to 133)	169 (61 to 377)
- Testosterone levels, nmol/L	18.5 (14.0 to 23.0)	1.3 (1.0 to 1.7)
- 25(OH) vitamin D levels, nmol/L	38 (24 to 57)	54 (31 to 77)
Biochemical results Ghent (IQR) #		
- Estradiol levels, pmol/L	114 (95 to 135)	155 (114 to 301)
- Testosterone levels, nmol/L	19.0 (13.5 to 22.2)	1.1 (0.7 to 1.3)
- 25(OH) vitamin D levels, nmol/L	34 (22 to 52)	54 (36 to 72)

Data are expressed as median (interquartile range) or percentages. * Transwomen n=135, transmen n=137; # Transwomen n=93, transmen n=41. Abbreviations: IQR=interquartile range; BMI=body mass index.

Table 2. Baseline and one-year BMD in lumbar spine, total hip, and femoral neck for transwomen and transmen, stratified per center

	Amsterdam (n=137)	**Ghent (n=94)**	**Total (n=231)**	**p-values**
Transwomen				
Lumbar spine BMD				
- Baseline	0.966 (0.138)	0.983 (0.143)	0.972 (0.140)	0.362
- One-year	1.001 (0.137)	1.015 (0.143)	1.007 (0.139)	0.470
Total hip BMD				
- Baseline	0.938 (0.133)	0.939 (0.135)	0.938 (0.134)	0.942
- One-year	0.948 (0.133)	0.946 (0.138)	0.947 (0.135)	0.905
Femoral neck BMD				
- Baseline	0.798 (0.124)	0.799 (0.133)	0.799 (0.128)	0.960
- One-year	0.814 (0.127)	0.811 (0.135)	0.813 (0.130)	0.834
Transmen				
	Amsterdam (n=155)	**Ghent (n=44)**	**Total (n=199)**	**p-values**
Lumbar spine BMD				
- Baseline	1.030 (0.124)	1.022 (0.114)	1.028 (0.121)	0.705
- One-year	1.039 (0.126)	1.027 (0.108)	1.037 (0.122)	0.554
Total hip BMD				
- Baseline	0.954 (0.113)	0.945 (0.130)	0.952 (0.116)	0.646
- One-year	0.963 (0.114)	0.958 (0.129)	0.962 (0.117)	0.815
Femoral neck BMD				
- Baseline	0.837 (0.116)	0.833 (0.112)	0.836 (0.115)	0.805
- One-year	0.831 (0.116)	0.834 (0.117)	0.832 (0.116)	0.908

Numbers represent absolute bone mineral density in g/cm^2 (standard deviation). Independent t-tests were performed between the Amsterdam and Ghent data. Abbreviations: BMD=bone mineral density.

Figure 1. Flow chart of the inclusion of participants in the present study. Abbreviations: DXA=dual-energy X-ray absorptiometry.

Figure 2. Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen after one year cross-sex hormonal treatment. Data represent mean baseline and mean one-year BMD with standard deviation. Percentage changes in BMD were calculated for every person and as these were normally distributed continuous variables, linear regression analyses were performed to generate the mean percentage changes with corresponding 95% confidence intervals (CI), which are shown in the legends on the right. * $p \leq 0.001$. Abbreviations: BMD=bone mineral density.

Figure 3. Change in bone mineral density of lumbar spine, total hip, and femoral neck, in transwomen and transmen, stratified for age groups. Each individual bar represent the mean increase in bone mineral density with 95% confidence interval. The age distribution in transwomen is: 18–20 (n=36), 21–29 (n=88), 30–49 (n=83), and ≥50 (n=24) years; in transmen: 18–20 (n=56), 21–29 (n=87), 30–49 (n=46), and ≥50 (n=10) years. Difference between these age groups were analyzed using linear regression analyses, with percentage change in BMD as outcome variable and age groups as categorical dependent variable. * $p \leq 0.001$; ** $0.001 < p \leq 0.01$; *** $0.01 < p \leq 0.05$. Abbreviations: LS=lumbar spine; TH=total hip; FN=femoral neck; BMD=bone mineral density.

Figure 4. Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen, stratified for vitamin D supplementation or administration routes, respectively. Linear regression analyses with change in BMD as outcome variable and vitamin D supplementation or administration route as independent variable were performed. Only persons using the same dose and administration route during the entire year were included for analyses. *

p≤0.001; *** 0.01<p≤0.05. Abbreviations: LS=lumbar spine, TH=total hip, FN=femoral neck, BMD=bone mineral density, T=testosterone.

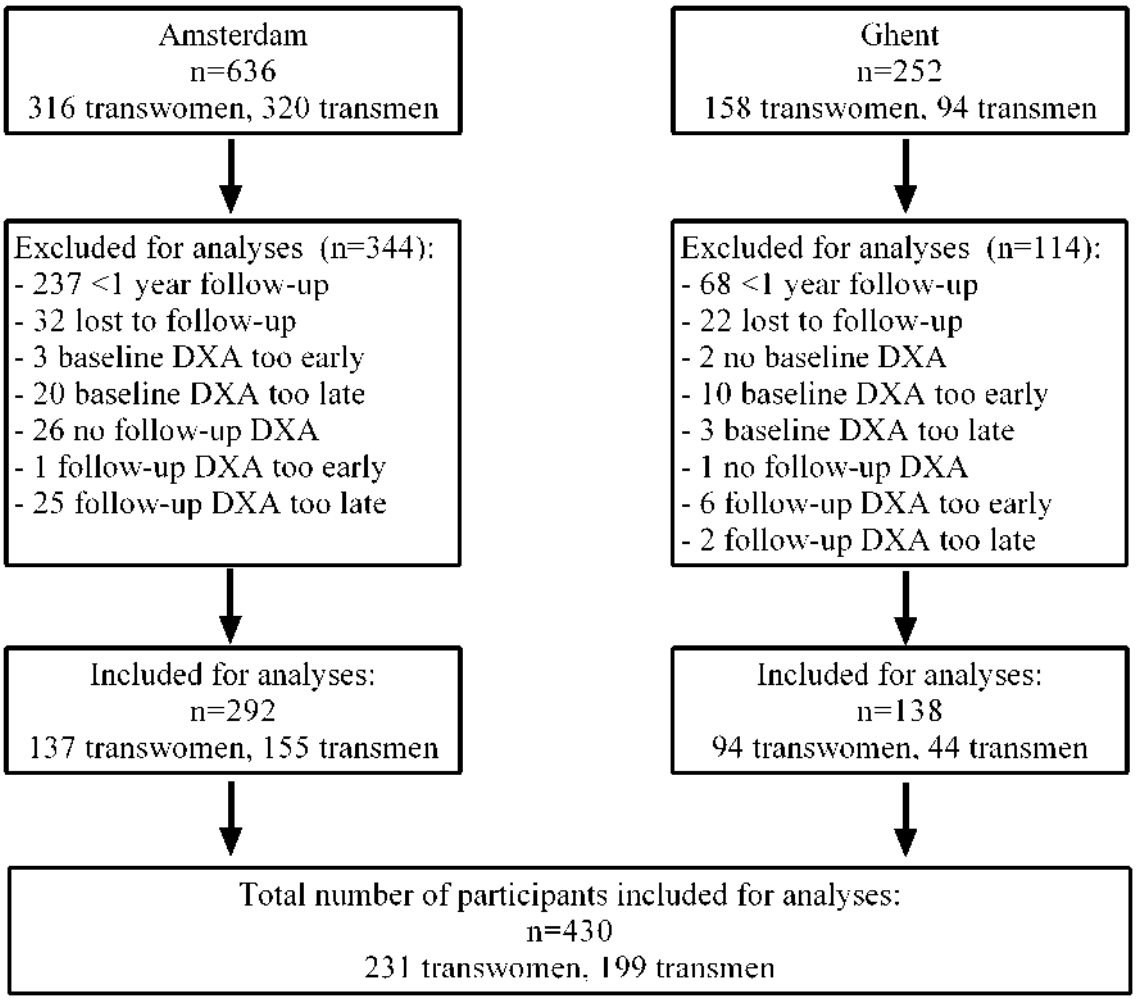


Figure 1

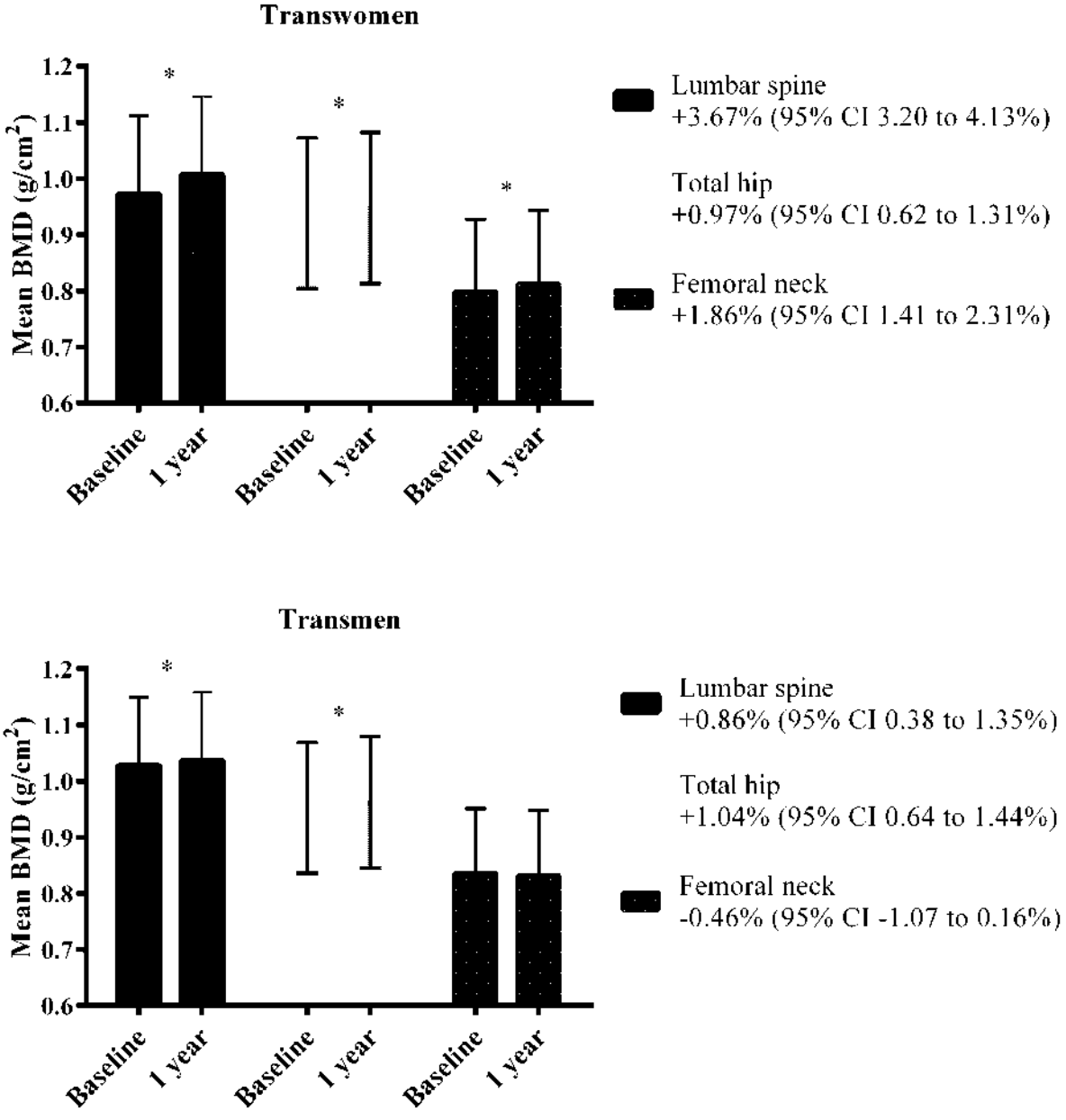


Figure 2

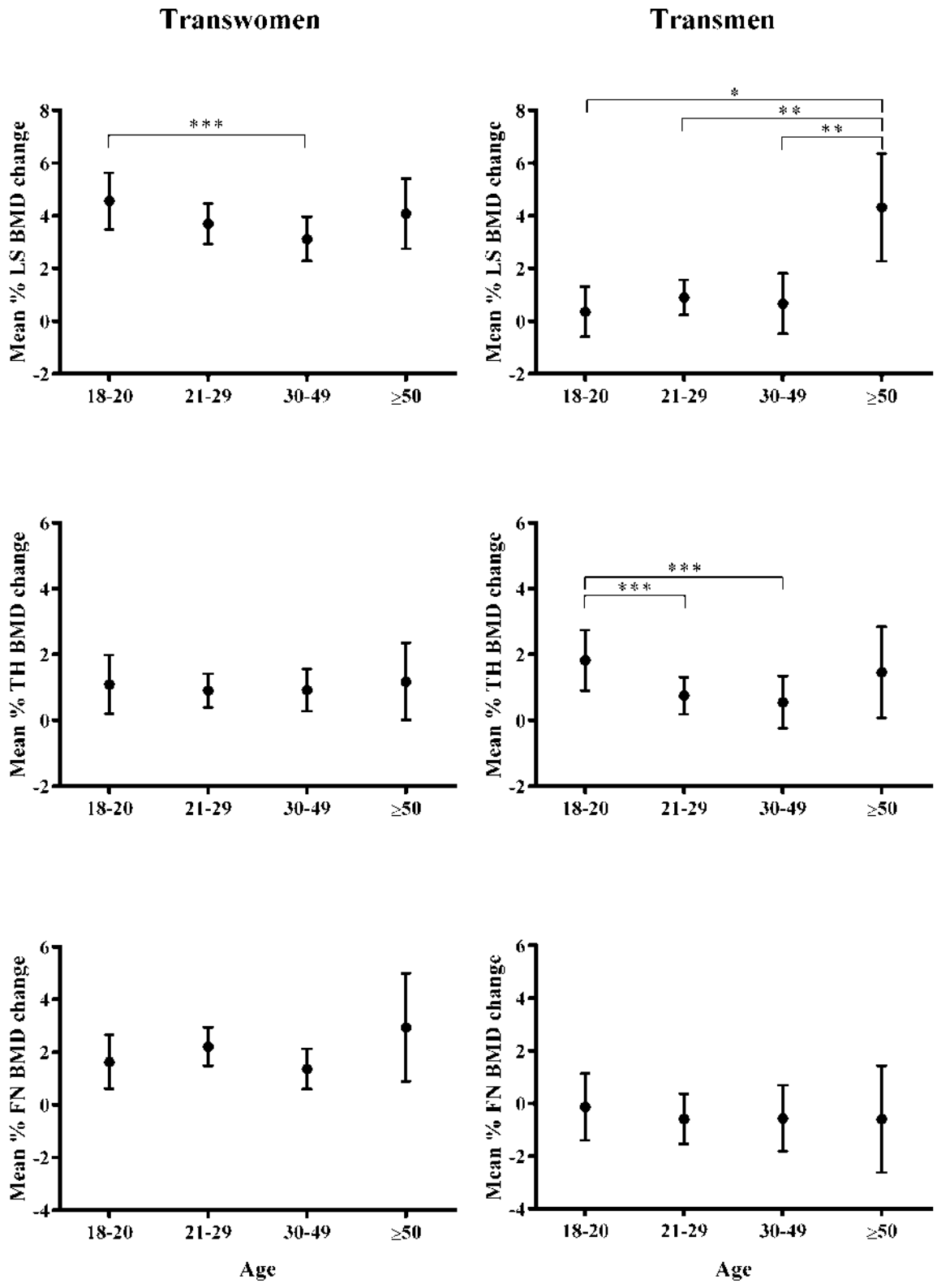
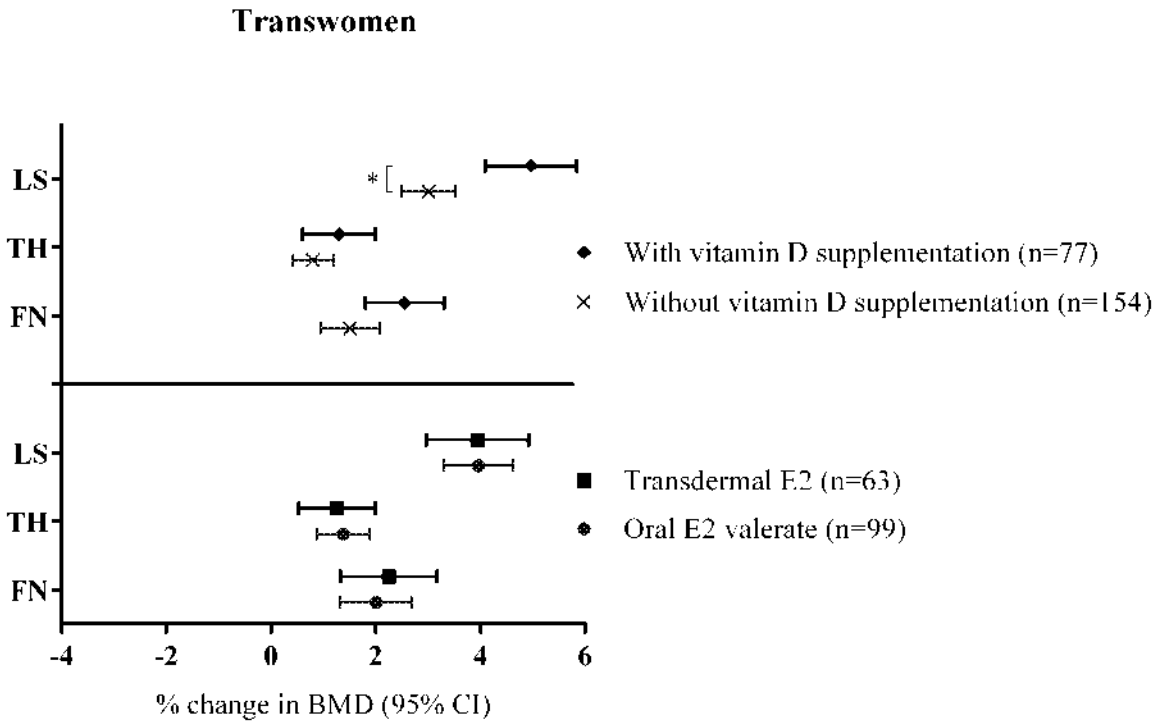


Figure 3



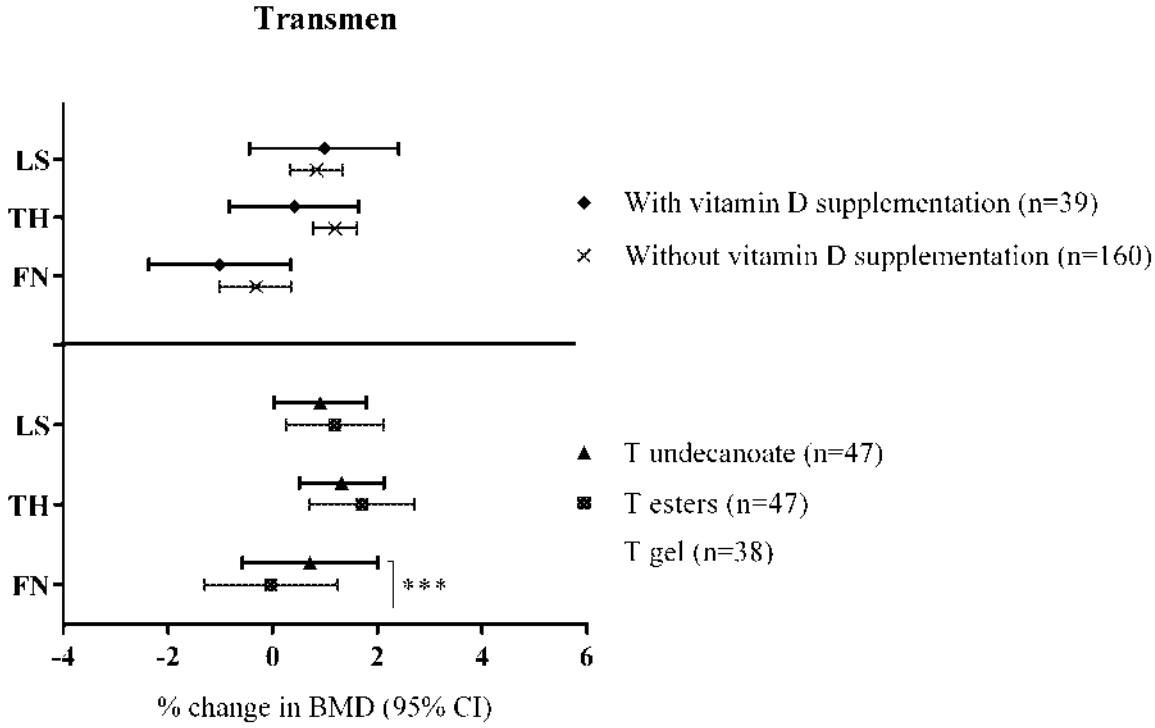


Figure 4