Oxandrolone in growth hormone-treated girls with Turner syndrome

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The effect of the weak androgen oxandrolone on psychological and behavioral characteristics in growth hormone-treated girls with Turner syndrome

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Abstract

The weak androgen oxandrolone (Ox) increases height gain in growth-hormone (GH) treated girls with Turner syndrome (TS), but may also give rise to virilizing side effects. To assess the effect of Ox, at a conventional and low dosage, on behavior, aggression, romantic and sexual interest, mood, and gender role in GH-treated girls with TS, a randomized, placebo-controlled, double-blind study was conducted. 133 patients were treated with GH (1.33 mg/m^2/day) from baseline, combined with placebo (Pl), Ox 0.03 mg/kg/day, or Ox 0.06 mg/kg/day from the age of eight, and with estrogens from the age of twelve. The child behavior checklist (CBCL), Junior Dutch Personality Questionnaire (DPQ-J), State-subscale of the Spielberger's State-Trait Anger Scale, Romantic and Sexual Interest Questionnaire, Mood Questionnaire, and Gender Role Questionnaire were filled out before, during, and after discontinuing Ox/Pl. The changes during Ox/Pl therapy were not significantly different between the dosage groups. In untreated patients, the mean CBCL total (P=0.002) and internalizing (P=0.003) T scores, as well as the mean DPQ-J social inadequacy SD score (SDS) (P=0.004) were higher than in reference girls, but decreased during GH+Ox/Pl therapy (P<0.001, P=0.05, P<0.001, respectively). Whereas the mean total (P=0.01) and internalizing (P<0.001) T score remained relatively high, the mean social inadequacy SDS became comparable with reference values. We conclude that in GH-treated girls with TS, Ox 0.03 mg/kg/day or 0.06 mg/kg/day does not cause evident psychological virilizing side effects. Problem behavior, frequently present in untreated girls with TS, decreases during therapy, but total and internalizing problem behavior remain increased.

Introduction

Turner syndrome (TS) is a disorder in females, caused by the partial or complete absence of the second sex chromosome. It is one of the most common chromosomal disorders, affecting approximately 1 in 2000 live-born girls (Gravholt et al., 1996). The main characteristics are gonadal dysgenesis and short stature. Other possible features are congenital heart disease, renal anomalies and a number of dysmorphic features, including webbed neck, cubitus valgus and lymphedema of the hands and feet (Sybert and McCauley, 2004). Because the ovaries usually start to involute within 4 or 5 months of gestation (Singh and Carr, 1966), the majority of patients is infertile, has diminished ovarian estrogen and androgen production (Apter et al., 1982; Gravholt et al., 1999), and needs estrogen replacement therapy to induce pubertal maturation. Untreated adult patients are on average 20 cm shorter than healthy women (Rongen-Westerlaken et al., 1997), mainly due to haploinsufficiency of the Short stature HomeobOX-containing (SHOX) gene (Rao et al., 1997). Even though patients are not growth hormone (GH) deficient, GH therapy increases adult height, and the addition of the weak androgen oxandrolone (Ox) to GH may further increase height (Menke et al., in press). Ox is a synthetic, nonaromatizable anabolic androgenic steroid with the chemical name 17β-hydroxy-17α-methyl-2-oxa-5α-androstane-3-one. It is derived from testosterone, in which a carbon atom (at position 2 in the phenanthrene nucleus) is replaced by an oxygen atom. In comparison with testosterone, Ox has a high anabolic to androgenic ratio (10:1) (Fox et al., 1962). Although several safety parameters of Ox have been assessed in GH-treated girls with TS, possible effects on psychological functioning have not been studied yet.

Little is known about the psychological effects of androgen administration in young girls. Conventionally, androgenic effects are divided in organizational and activational, resulting from antenatal and postnatal androgen exposure, respectively (Arnold and Breedlove, 1985). Antenatally raised androgen levels may exert long lasting effects on brain development, resulting in more male-typical behavior and preferences (Hines, 2008). Activational effects occur only in the presence of the hormone, and are mainly expressed in the area of aggression (Sato et al., 2008), sexuality (Braunstein, 2006), mood, and well being (Davison and Davis, 2003).

In a previous report, we showed that compared with placebo, Ox in a low dosage (0.03 mg/kg/day) increases adult height gain in GH-treated girls with TS, and has an acceptable safety profile, while Ox in a conventional dosage (0.06 mg/kg/day) does not significantly increase adult height gain, and frequently results in virilizing side effects (Menke et al., in press). In the present article, we describe the effect of Ox on behavior, aggression, romantic and sexual interest, mood, and gender role by analyzing the questionnaires that were completed yearly during this randomized, placebo-controlled, double-blind study.

Methods

Study setting and participants

Participants were recruited in ten pediatric endocrine centers in the Netherlands from December 1991 to June 2003. Inclusion criteria were: a karyotype associated with TS (except for cytogenetical evidence of Y-chromosomal material); a calendar age between 2.00-15.99 years; and a bone age younger than 12.00 years (Tanner et al., 1983). Exclusion criteria were: growth failure due to other causes; use of drugs that could interfere with growth; and previous GH, sex hormone, or androgen therapy. The study was performed in accordance with the World Medical Association Declaration of Helsinki and approved by the ethics committee of each participating center. Before enrolment, written informed consent was obtained for each patient.

Treatment

Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). After stratification for calendar age and height SD score (SDS) (Ranke et al., 1988), they were randomized by a computer-generated schedule, and blindly assigned to receive, orally at bedtime after reaching the age of 8 years, Ox 0.03 mg/kg/day (Ox 0.03) (S.p.A., Milano, Italy), Ox 0.06 mg/kg/day (Ox 0.06), or a similar appearing placebo (Pl). The capsules were manufactured and distributed by one hospital pharmacy. All patients and doctors were blinded for the allocation of the patients, and will remain so until the last patient will finish the study. Only the independent pharmacist (dr. C.M.A. Rademaker), the statistician (M.A.d.R.), and data

analyst (L.A.M., from 2008 onward) saw unblinded data, but none of them had any contact with the participants. From baseline onward, biosynthetic human GH (1.33 mg/m^2 body-surface/day, at 1 m^2 equivalent to 46 µg/kg/day) was administered subcutaneously at bedtime. Genotropin^R (Pfizer Inc, New York, USA) was used in age groups 1 and 2, and Humatrope^R (Eli Lilly, Indianapolis, USA) in age group 3. Ox/Pl was started at the age of eight after a number of complete years of GH therapy (i.e. at their main 'year-visit') in age group 1, and at inclusion in age groups 2 and 3 (i.e. between the age of 8.0-16.0 years). In the absence of spontaneous puberty (Tanner breast stage < 2 (B2) (Marshall and Tanner, 1969)), estrogens were started at the age of 12.0-12.99 after a number of complete years of GH therapy in age groups 1 and 2, and at inclusion in age group 3 (i.e. between the age of 12.0 and 16.0 years). 17-ß-estradiol was prescribed in age groups 1 and 2, and ethinyl-estradiol in age group 3 (5 and 0.05 µg/kg/day orally, increased to 10 and 0.1 µg/kg/day after two years, respectively). When ethinyl-estradiol became unavailable after March 2002, 17-ß-estradiol was also prescribed in age group 3. Cyclic progesterone was added after at least two years of estrogen therapy. Doses were adjusted every six months, and GH+Ox/Pl were stopped when height velocity was less than 1 cm per six months, or when patients decided to stop because they were satisfied with their height.

Assessments

From September 1993 onwards, a psychological examination was performed at starting Ox/Pl, three to six months thereafter, and yearly up until six months after discontinuation of Ox/Pl. The patients were seen by two trained observers, who performed all measurements during the total study period. Demographic characteristics were assessed yearly by a questionnaire that was completed by the parents. Questions included the age of the parents and possible siblings, and educational level of the patient and parents. Educational level was divided into special (i.e. a school for children with hearing, learning and/or behavioral difficulties), low (elementary school, lower level of secondary school or vocational training), middle (medium and higher level of secondary school, or medium level of vocational training), and high (higher level of vocational training, or university).

Emotional and behavioral problems

Potential behavioral, emotional, and social problems were investigated using a parent report and a self-report questionnaire. The Dutch version of the Child Behavior Checklist (CBCL) was completed by one of the parents (Achenbach, 1991; Verhulst et al., 1996). The CBCL consists of eight syndrome scales that are summed into an internalizing problem score (representing overcontrolled symptoms such as excessive worrying), an externalizing problem score (representing undercontrolled symptoms such as aggressive behavior or hyperactivity), and a total problem score (computed by summing almost all items). Computerized scoring was done and raw scores were converted into T scores using Dutch norms. According to the CBCL manual, T scores above 63 (>90th percentile) were considered to indicate problem behavior.

Self-reported emotional and behavioral problems were assessed using the inadequacy, social inadequacy, and recalcitrance scales of the Junior Dutch Personality Questionnaire (DPQ-J) (Luteijn et al., 1989). The Inadequacy scale measures anxieties, vague somatic complaints, and feelings of inadequacy; the Social Inadequacy scale measures social anxiety and avoidance of social contacts; and the Recalcitrance scale measures rebelliousness, distrust and the willingness to manage one's own affairs. Scores above the 95th percentile were considered to indicate problem behavior. Standard deviation scores were calculated using Dutch reference data (9-16 years) for age and gender (Luteijn et al., 1989).

Aggressive feelings and behavior

The aggression subscale of the CBCL was completed by the parents to measure aggressive behavior of the patients (Achenbach, 1991). According to the CBCL manual, T scores greater than 70 (>97th percentile) were considered to indicate problem behavior. In addition, the Dutch version of the State-subscale of the Spielberger's State-Trait Anger Scale (STAS) was completed to measure aggressive feelings of the patients themselves (Spielberger, 1980; van der Ploeg et al., 1982). At home, seven questions were scored from 1 (never) to 5 (very frequently), and the mean was used for the analysis.

Romantic and sexual interest

The self-developed Romantic and Sexual Interest Questionnaire (RSQ) was completed at home, at five consecutive days from the age of 12.00 years onwards. The following six statements were scored from 1 (never) to 10 (very frequently): "how much did you today …romantically fantasize about boys?; …fantasize about dating a boy?; … have amorous feelings?; …feel butterflies or other tingling feelings?; …fantasize about having a boyfriend?; …fantasize about making love with a boy?."

Mood

Mood was assessed by the self-developed Mood Questionnaire (MQ). At five consecutive days at home, four positive mood items (being cheerful, being fit/full of energy, being relaxed/at ease, and getting along well with others), and six negative mood items (being tired, nervous, bad-tempered, aggressive, sad, and being cheerful and sad in an unstable way) were scored by the patient from 1 (never) to 5 (very frequently). For the analysis, the mean scores of the four positive items, and the mean score of the six negative items were used.

Gender role behavior and preferences

The self-developed Gender Role Questionnaire (GRQ) was completed by the patients at home. It consisted of four questions: "Did you have much interest in playing sportive games this week (note a score from 1 to 10)?," "Did you have much interest in rough- and tumble play this week (note a score from 1 to 10)?," "Which profession would you prefer in the future?," and "Who was your favorite friend this week?." The raw scores of the interest in sportive games and rough-and-tumble play were used in the analysis, with higher scores indicating higher energy expenditure, considered a more masculine characteristic. The favorite profession was scored as "masculine" vs. "neutral or feminine" by one blinded observer (LAM). To test inter-observer agreement, the same was done by a second observer (MV). The percentages of patients that noted a masculine future profession, and that noted a male favorite friend, respectively, were used for the analysis.

Statistical analysis

We performed a modified intention-to-treat analysis including only patients who took at least one dose of the study medication. Patients who were not capable of completing the questionnaires because of a developmental delay were also excluded. When Ox/Pl was discontinued before GH, the moment GH was discontinued was identified as "at discontinuing GH+Ox/Pl". Values for untreated girls with TS were obtained using the baseline values of girls from age groups 2 and 3 because these groups, in contrast to age group 1, were not treated with GH before starting Ox/Pl. Inter-observer agreement was analyzed using the inter-observer coefficient KAPPA, and the internal consistency (i.e. the consistency between the questions of self-composed questionnaires assumed to measure the same parameter) by Cronbach's α. Differences between dosage groups were tested by linear regression using two dummies (for groups GH+Ox 0.03 and GH+Ox 0.06), and differences in percentages by Pearson χ^2 tests and Fisher's exact tests. Means were compared with means of the reference group by a one-sample *t* test. Difference in change of outcome variables during the study were assessed by repeated measurements analyses. For continuous outcome variables, linear mixed models were fitted with different intercept and slope per dosage group and a random intercept and slope per patient. For dichotomous outcome variables Generalized Estimating Equations logistic regression was applied with independent working correlation matrix using SPSS version 17.0. Results are presented as means±SD and differences assessed by repeated measurements analyses as means, SE. A P-value less than 0.05 was considered significant.

Results

Patient characteristics

Fig. 1 shows the 133 patients that were randomized. Twenty-seven girls were excluded from the analysis: four were non-compliant and lost to follow-up, nine were still treated when the analysis started, eight refused to start Ox/Pl therapy when they reached the age of eight, two had mental retardation. and four did not take part in the psychological examinations because they were included before the psychological examinations commenced in September 1993. These patients had the

same baseline characteristics as the 106 patients that were included in the analysis (data not shown).

Table 1 shows the characteristics of the patients, which were similar between the three dosage groups. Baseline and treatment characteristics per age group are shown in Supplementary Table 1. The patients started GH at a mean age of 9.2±3.6 years, and Ox/Pl at a mean age of 10.4±2.3 years. They were treated with GH for 6.1±2.8 years, and with Ox/Pl for 4.7±1.6 years. GH+Ox/Pl was discontinued at a mean age of 15.3±1.4 years; the last psychological examination was performed 0.6±0.2 years thereafter. A total of 3407 questionnaires (82%) were completed and returned.

Emotional and behavioral problems

Child Behavior Checklist

In the untreated patients at baseline (i.e. patients from age groups 2 and 3), the CBCL was indicating total problem behavior in 23% (13/56), externalizing problem behavior in 13% (7/56), and internalizing problem behavior in 25% (14/56) of the patients. Compared with the mean T score of the reference population (50.0±10.0), the scores of the total and internalizing problem scales were significantly higher (55.0±11.1, P=0.002 and 54.3±10.4, P=0.003, respectively), while the externalizing problem scale was comparable (51.8±10.1, P=0.2) (Supplementary Fig. 1). The baseline values of the three scales appeared somewhat lower in age group 2 than in age group 3 (Supplementary Fig. 1). Fig. 2 shows the mean scores of the problem scales before, during, and after discontinuing Ox/Pl therapy for the patients from age groups 1, 2 and 3 together. At starting Ox/Pl, the scores of the total, externalizing and internalizing problem scale were higher than those of the reference population (55.8±10.8, P<0.001; 52.6±10.5, P=0.02; and 54.4±10.1, P<0.001, respectively) (Fig. 2). During Ox/Pl therapy, the T scores of the three problem scales decreased (mean, SE: -0.8/yr, 0.2, P<0.001; -0.7/yr, 0.2, P<0.001; and -0.4/yr, 0.2, P=0.05, respectively) (Fig. 2). At discontinuing Ox/Pl therapy, the mean externalizing problem T score had become similar to that of the reference population (49.7±11.0, P=0.6), whereas the total and internalizing problem T scores were still higher (52.7±10.9, P=0.01; 53.8±10.4, P<0.001, respectively) (Fig. 2). The decrease of the T scores was not significantly different between the dosage groups.

Table 1. Characteristics and treatment per dosage group.*

Characteristic		GH+Pl (N=35)	GH+Ox 0.03 (N=37)	GH+Ox 0.06 (N=34)
Age at starting GH – yr		9.7±3.6	9.1±3.9	8.8±3.5
Height at starting GH – SDS†		-2.9±0.7	-3.0±0.7	-2.9±0.7
Age at starting Ox/Pl – yr		10.7±2.3	10.3±2.5	10.1±2.1
Age at starting estrogens or at Tanner stage B2 – yr‡		12.3±1.3	12.3±0.8	12.0±0.8
Karyotype, 45,X – no. (%)		20 (57)	13 (35)	14 (41)
Karyotype, other – no. (%)§		15 (43)	24 (65)	20 (59)
Puberty developed spontaneously – no. (%)		9 (26)	8 (22)	9 (27)
Duration of GH therapy – yr		6.1±3.0	6.1±2.9	6.0±2.7
Duration of Ox/Pl therapy – yr		5.0±1.5	4.7±1.6	4.3±1.7
Age at discontinuing GH+Ox/Pl – yr		15.8±1.2	15.2±1.5	14.9±1.3
Age at last visit – yr		17.8±1.4	17.1±1.5	17.0±1.2
Adult height – SDS†¶		-2.2±0.73	-2.1±1.1	-2.2±0.9
Caretakers – no. (%)‖	Biological parents	30 (86)	31(84)	30 (88)
	One biological parent	5 (14)	5 (14)	4 (12)
	Other	0 (0)	1 (3)	0 (0)
Parental age at birth of patient – yr	Maternal	29.1±4.4	28.6±5.1	26.9±4.0
	Paternal	31.5±4.5	32.3±6.8	30.2±5.0
Brothers and sisters – median no. (range)		2 (1-4)	1 (0-6)	1 (1-4)
Educational level at age 14 – no. (%)‖**	Special	7 (20)	3 (8)	7 (21)
	Low	18 (51)	21 (57)	20 (59)
	Middle	10 (29)	13 (35)	7 (21)
Parental educational level – no. (%)††	Special	0 (0)	2 (3)	1 (2)
	Low	27 (39)	25 (34)	23 (36)
	Middle	29 (41)	29 (40)	29 (45)
	High	14 (20)	17 (23)	11 (17)

* Values are expressed as means ±SD, unless otherwise indicated; no statistical tests were applied.

† Height was measured using a Harpenden stadiometer; the mean of four measurements was used for analysis, and expressed as standard deviation scores (SDS) for healthy Dutch girls (Fredriks et al., 2000).

‡ If puberty was induced, the moment at starting estrogens was used; if puberty developed spontaeously, the moment at Tanner breast stage 2 was used.

§ These consisted of the following karyotypes: mosaic (45,X/46,XX, n=8); isochromosome (45,X/46,X,i(Xq), n=12; 46,X,i(Xq), n=9; 45,X/46,XX/46,X,i(Xq), n=1; 46,X,Xp-/46,X,i(Xq), n=1); deletions (46,X,del(X), n=3; 45,X/46,X,del(X), n=3, 46,XXq-(q13-qter), n=1); trisomy X (45,X/47,XXX, n=4; 45,X/46,XX/47,XXX, n=1; 45,X/46,X,i(Xq)/47,XXX, n=1);

ring chromosome (45,X/46,X,r(X), n=9; 45,X/46,XX/46,X,r(X), n=1); marker chromosome (45,X/46,X+mar, n=4); and a translocation karyotype (46,X,+der,t(X;13)(q13;q12.3), n=1)

¶ Adult height was defined as the last measurement after discontinuing GH. To prevent adult height from being overestimated in patients who stopped growing at an earlier age than the reference group, reference data for the age of 21 rather than the actual age were used in calculating adult SDS.

‖ Percentages may not total 100 because of rounding.

** Because not all patients had started vocational training by the end of the study, the educational level at the age of 14 was used. Inevitably, none of the girls was educated at a high educational level in this analysis.

†† Parental educational level was unknown in one parent from a girl on GH+Ox 0.03, and four parents from four girls on GH+Ox 0.06.

Junior Dutch Personality Questionnaire

In the untreated patients at baseline, the inadequacy score was above the normal range in 2% (1/56), the social inadequacy score in 13% (7/56), and the recalcitrance score in 4% (2/56) of the patients. Mean social inadequacy SDS was higher than that of the reference population (0.4±1.0 vs. zero, P=0.004), while the mean inadequacy and recalcitrance SDS were not significantly different (-0.1±0.9, P=0.4 and -0.2±0.9, P=0.2, respectively) (Supplementary Fig. 2). Fig. 2 shows the mean SDS of the problem scores before, during, and after discontinuing Ox/Pl therapy of the patients from age groups 1, 2 and 3 together. At starting Ox/Pl, the social inadequacy score was higher than zero (0.5±0.9, P<0.001), whereas the mean inadequacy and recalcitrance SDS were not significantly different from zero (0.1±0.9, P=0.4 and 0.1±1.0, P=0.4, respectively) (Fig. 2). During Ox/Pl therapy, inadequacy, social inadequacy, and recalcitrance SDS lowered (mean, SE: -0.07/yr, 0.02, P=0.007; -0.09/yr, 0.03, P<0.001; -0.07/yr, 0.03, P=0.007, respectively) (Fig. 2). At discontinuing Ox/Pl, the mean social inadequacy SDS had become comparable with zero (-0.1±1.1, P=0.6), whereas the mean inadequacy and recalcitrance SDS had become lower than zero (-0.3±0.9, P=0.03 and -0.3±1.2, P=0.01, respectively) (Fig. 2). The decrease of the scores was not significantly different between the dosage groups.

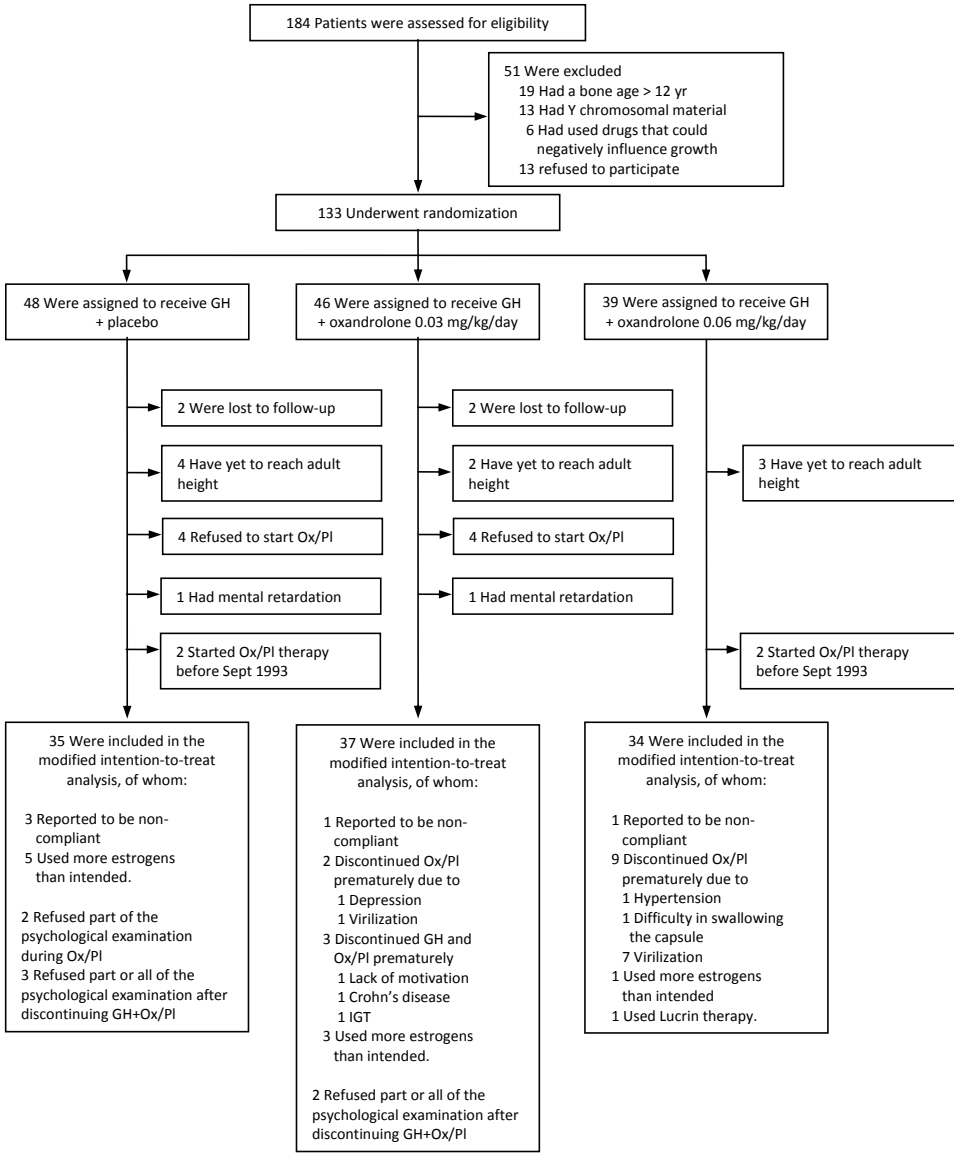

Figure 1. Enrollment, randomization and analysis of the patients.

Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). GH, growth hormone; Ox/Pl, oxandrolone/placebo; IGT, impaired glucose tolerance; the patients who started Ox/Pl therapy before September 1993 (i.e. when the psychological examinations commenced) never took a psychological examination.

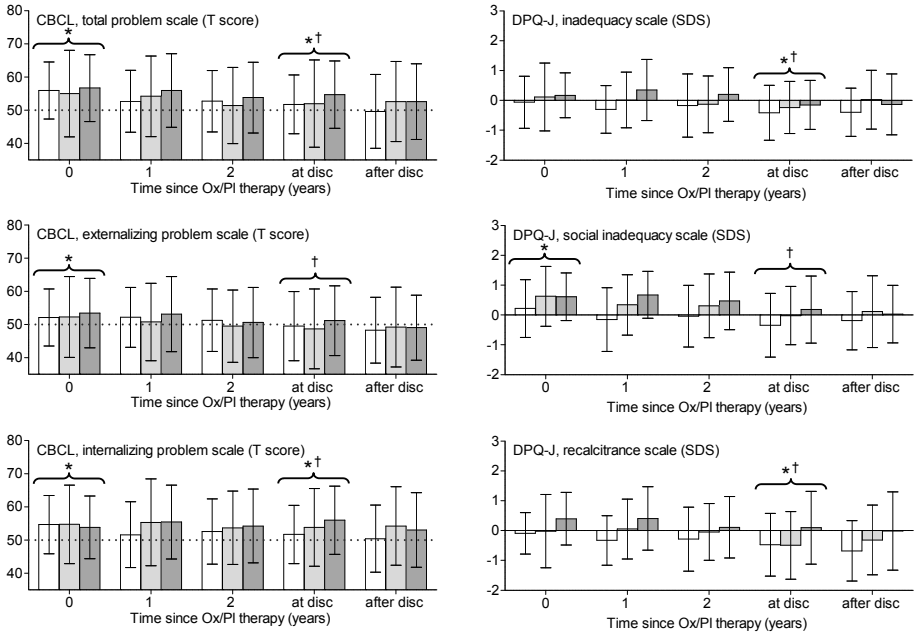

Figure 2. Problem behavior in patients given GH+Pl (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars).

Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD; CBCL, Child Behavior Checklist; DPQ-J, Junior Dutch Personality Questionnaire; disc, discontinuing GH+Ox/Pl; *, P<0.05 compared with reference mean (i.e. CBCL, T score, 50; DPQ-J, 0 standard deviation score (SDS)); †, P<0.05 change during Ox/Pl therapy. The dotted line represents the mean T score of the Dutch reference population. The changes during Ox/Pl therapy and the values at discontinuing GH+Ox/Pl were not significantly different between the dosage groups.

Aggressive feelings and behavior

In the untreated patients at baseline, the mean parental-rated CBCL aggression T score was 55.6±7.0, and the mean patient-rated STAS score (scale 1-5) was 1.2±0.5 (Supplementary Fig. 3). During Ox/Pl therapy, the CBCL aggression T-score decreased (mean, SE: -0.3/yr, 0.1, P=0.01), while the STAS score remained constant (Fig. 3). Differences in change between the dosage groups during Ox/Pl therapy were not significant.

Romantic and sexual interest

The internal consistency of the five RSQ questions concerning romantic interest was high (Cronbach's α, 0.89), allowing us to combine them in measuring romantic interest. In the untreated patients at baseline, the mean romantic interest score (scale 1-10) was 1.7±1.3, and the mean sexual interest score was 1.1±0.3 (Supplementary Fig. 4). During Ox/Pl therapy, romantic and sexual interest increased (mean, SE: 0.19/yr, 0.06, P=0.005; 0.11/yr, 0.04, P=0.01, respectively) (Fig. 3), without significant differences between the dosage groups.

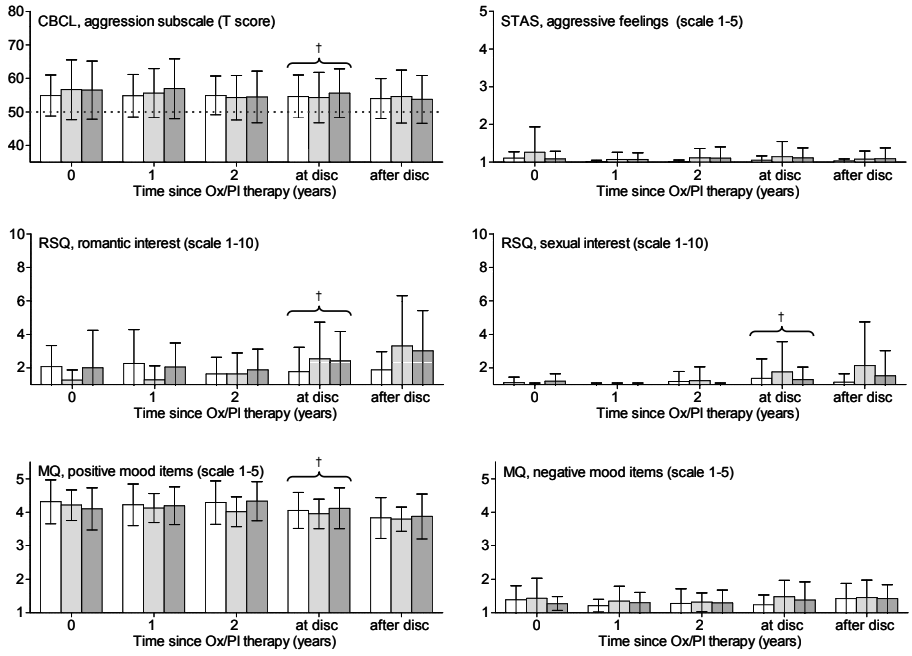

Figure 3. Aggression, romantic and sexual interest, and mood in patients given GH+Pl (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars).

Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD; CBCL, Child Behavior Checklist; disc, discontinuing GH+Ox/Pl; MQ, Mood Questionnaire; RSQ, Romantic and Sexual interest Questionnaire; STAS, State-subscale of the Spielberger's State-Trait Anger Scale; †, P<0.05 change during Ox/Pl therapy. The dotted line represents the mean T score of the Dutch reference population. The changes during Ox/Pl therapy and the values at discontinuing GH+Ox/Pl were not significantly different between the dosage groups.

Mood

In the untreated patients at baseline, the mean positive mood score (scale 1-5) was 4.2±0.6 and the mean negative mood score was 1.3±0.5 (Supplementary Fig. 5). The mean positive score lowered during Ox/Pl therapy (mean, SE: -0.04/yr, 0.02, P=0.02), while the mean negative score remained constant (Fig. 3). Differences in change between the dosage groups during Ox/Pl therapy were not significant.

Gender role

Mean interest (scale 1-10) in sportive games and rough-and-tumble play in the untreated patients at baseline was 5.2±2.8 and 1.3±0.5, respectively (Supplementary Fig. 6). During Ox/Pl therapy, the interest in sportive games increased (mean, SE: 0.28/yr, 0.06, P<0.001), while the interest in rough-and-tumble play remained constant (Fig. 4).

The agreement between the two investigators who scored the preferred profession as masculine vs. neutral or feminine was high (inter-observer coefficient KAPPA, 0.85), showing that the scoring method was consistent. In the untreated patients at baseline, 11.4% of patients preferred a masculine future profession, and none of the patients had a male best friend. In age group 1, 2, and 3 combined, the percentage of patients preferring a masculine future profession remained constant during Ox/Pl therapy, while the percentage having a male favorite playmate decreased (P=0.04) (Fig. 4). Differences in the number of patients with masculine preferences during Ox/Pl therapy were not significantly different between the dosage groups.

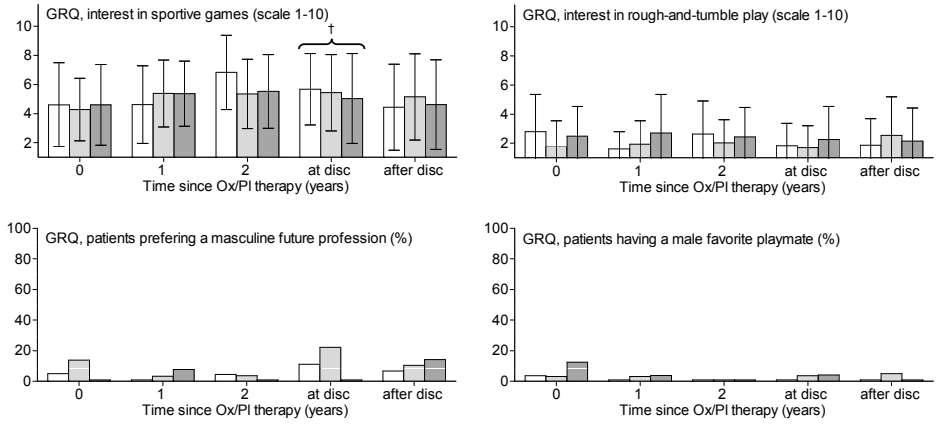

Figure 4. Gender role in patients given GH+Pl (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars).

Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD (upper panel) and percentages (lower panel); disc, discontinuing GH+Ox/Pl; GRQ, Gender Role Questionnaire; †, $P<0.05$ change during Ox/Pl therapy. The changes during Ox/Pl therapy and the values at discontinuing GH+Ox/Pl were not significantly different between the dosage groups.

Discussion

This randomized, placebo-controlled, double-blind study shows that Ox at a dosage of 0.03 mg/kg/day or 0.06 mg/kg/day does not significantly affect behavior, aggression, romantic and sexual interest, mood, and gender role in GH-treated girls with TS. Problem behavior, frequently present in untreated girls with TS, decreased during therapy, but total and internalizing problem behavior remained increased.

The percentage of untreated patients with total, externalizing, and internalizing problem behavior (23%, 13%, 25%, respectively) was relatively high compared with the reported data on healthy girls (9%, 8%, and 8-10%, respectively (Verhulst et al., 1996)). These results are in line with several other studies showing that total (Rovet, 1993; Skuse et al., 1994; Ross et al., 1996), externalizing (Rovet, 1993; Boman et al., 2000) and internalizing (Rovet, 1993) problem behavior are often increased in children and adolescents with TS. In our study, problem behavior decreased during

therapy, but total and internalizing problem behavior remained more prominent than in the reference population. These data imply that that patients kept suffering from overcontrolled symptoms (such as anxiety and depression (Skuse et al., 1994; Lagrou et al., 1998), and social withdrawnness (Lagrou et al., 1998; Boman et al., 2000)). Because, to our best knowledge, no longitudinal behavioral data are available of untreated girls with TS who are followed until adult height, it is unclear whether the decrease of internalizing problems may reflect the natural development of girls with TS (Lagrou et al., 1998), or may be the result of therapy. However, because the T scores at baseline of age group 3 were not lower, but even somewhat higher than those of age group 2 (Supplemental Fig. 1), a natural developmental effect seems unlikely. Previous longitudinal reports in patients with TS also showed that internalizing and externalizing problem behavior decreased during GH therapy (Siegel et al., 1998), and that total and externalizing problem behavior decreased during estrogen therapy (Ross et al., 1996).

Apart from the parents, the patients also reported more problematic psychological functioning themselves. When looking at the untreated patients at baseline, mean social anxiety and avoidance of social contacts were greater, and a greater proportion of patients reported problems above the normal range (13% vs. 5% of the reference population (Luteijn et al., 1989)). During therapy, social anxiety scores diminished, whereas recalcitrant behavior and feelings of inadequacy became less than in the reference group. These results are in line with the understanding that girls with TS report more social problems than healthy girls (Lagrou et al., 1998), and that GH and/or estrogen therapy may improve some aspects of psychosocial functioning. Longitudinally assessed self-perception improved during estrogen (Ross et al., 1996) and/or GH therapy (Rovet and Holland, 1993; Lagrou et al., 1998), and a cross-sectional study suggested that GH and estrogen therapy had positive effects of on health-related quality of life and self-reported psychosocial functioning (Van Pareren et al., 2005; Bannink et al., 2006). In addition, in a randomized GH trial, a positive correlation was found between growth rate and the girls' perceptions of being intelligent, attractive and popular; having more friends, and experiencing less teasing (Rovet and Holland, 1993). These data were however contradicted by other studies, in which no correlation between growth response and psychosocial

functioning was found (Lagrou et al., 1998), and height and estimated height gain were not associated with quality-of-life scores (Carel et al., 2005).

Several other psychological parameters changed during Ox/Pl therapy. Although the patients reported aggressive feelings to remain constant, the parents reported a significant decrease in aggressive behavior. A previous report also showed a decrease in parental-rated aggression during three years of estrogen therapy in 12- to 16-year-old girls with TS (Ross et al., 1996). Furthermore, romantic interest increased during Ox/Pl therapy. This rise may have been age-related and may also have been influenced by estrogen therapy. A previous study found that the age at first sexual intercourse was influenced by the age at which puberty was induced (Carel et al., 2006). We subjectively felt that the girls scored rather low on romantic and sexual interest. Although possibly influenced by embarrassment towards the observers, the low scores likely reflect a characteristic of women with TS, who are known to have a delayed pattern of dating and initiation of sexual activities (Pavlidis et al., 1995). In addition, positive mood scores decreased during Ox/Pl therapy, whereas negative mood scores remained constant. Although the girls noted relatively high positive mood scores, depressive symptoms are frequently reported in TS, and tend to increase during adolescence (Lagrou et al., 1998). However, no evidence of depression was found in adolescents with TS who had been treated with GH and who had started estrogen replacement therapy to induce puberty at an appropriate age (Van Pareren et al., 2005). Finally, the interest in sportive games increased during Ox/Pl therapy. We hypothesize that several factors may have resulted in this effect, including age-related effects, a positive effect of GH+Ox/Pl on height and muscularity (Ari et al., 2006), as well as a positive effect of estrogen therapy on physical fitness (Gravholt et al., 1997). Apart from the interest in sportive games, the gender related preferences of the patients appeared typically feminine. Interest in rough-and-tumble play was low, preferred future professions were predominantly feminine, and best friends were almost exclusively girls. These results are consistent with previous findings that patients with TS have typical female interests and gender role behaviors (Downey et al., 1987).

Several contributors are thought to underlie the psychosocial characteristics in patients with TS. The potential presence of physical anomalies (such as short

stature and neck webbing), concomitant illnesses, repeated hospitalization, parental overprotection, and infertility may all play a part. The specific neurocognitive deficits in TS, resulting from abnormal expression of one or more X chromosome genes, are however regarded the main underlying cause (Ross et al., 2000). Some of these deficits result from estrogen insufficiency, and may be somewhat reversible with treatment (Ross et al., 2000), and also androgen insufficiency may play a role. Because the ovaries normally produce up to 50% of the circulating androgens in females, androgen levels are subnormal in both adolescent (Apter et al., 1982) and adult patients with TS (Gravholt et al., 1999).

Our findings however show that the weak androgen Ox, at a dosage of 0.03 or 0.06 mg/kg/day does not significantly alter behavior, aggression, romantic and sexual interest, mood, and gender role in GH-treated girls with TS. No increase in aggression proneness and feelings was found, which confirms the conviction that androgens increase this characteristic when given at very high doses only (Sato et al., 2008). Furthermore, no effect of Ox on romantic and sexual interest was found. Although testosterone appears to improve sexual desire in adult women with TS (Zuckerman-Levin, 2009), testosterone levels in adult patients were not correlated with any aspect of sexual function (Sheaffer et al., 2008), confirming that androgens in the low physiological range have little impact on sexuality (Goldstat et al., 2003). In addition, we did not find an effect of Ox on positive or negative mood variables. Although not the aim of our study, a previous pilot study in adult women with TS found that testosterone therapy increased quality of life and well-being (Zuckerman-Levin, 2009), possibly by compensating the androgenic insufficiency in TS. Finally, we did not find an effect of Ox on gender role preferences, which is in line with the expectation that gender role is not so much influenced by postnatal androgen therapy (Hines, 2008).

Although we can not exclude that Ox may affect the studied parameters in ways that are too subtle to detect by questionnaires, our study shows that no obvious psychological side effects are expected in girls with TS when treated with Ox at a dosage of 0.03 or 0.06 mg/kg/day. Our study furthermore shows that several of the studied parameters increased or decreased over time. We hypothesize that an age-effect, the normal development in TS, as well as an effect of GH and/or estrogen

therapy may all underlie some of these changes. Although the data on the possible effect of GH and/or estrogen are promising, it is unknown whether the scores may have also been influenced by the fact that the questionnaires were completed repeatedly over several years (i.e. a repeated testing effect), or by the intense clinical care that the patients received throughout the study. One should furthermore bear in mind that the self-reported questionnaires may have been influenced by the tendency of TS women to under-report their problems (Lagrou et al., 1998). In our view however, these possible effects would have been similar in the three dosage groups, and would therefore not have influenced our findings on the effect of GH+Ox vs. GH+Pl.

Our analysis was performed according to the intention to treat principle: we analyzed all patients, including those who violated the protocol. According to the consort statement, an intention-to-treat analysis prevents bias caused by the loss of participants (www.consort-statement.org). A common modification of the intention to treat analysis (including only patients who take at least one dose of the study medication) was used to prevent under-estimation of the possible psychological adverse effects. Four patients were lost to follow-up and were excluded because we were unable to obtain their data. A further nine patients had yet to reach adult height, and were therefore not included. These patients were evenly distributed across the three dosage groups, their baseline characteristics were similar to those of the analyzed patients, and they had no apparent psychological problems, Despite the exclusion of these patients, we therefore consider the conclusions of our study generalizable to other girls with TS in whom Ox therapy may be considered.

We conclude that Ox at a dosage of 0.03 mg/kg/day or 0.06 mg/kg/day does not cause evident psychological virilizing side effects in the area of behavior, aggression, romantic and sexual interest, mood, and gender role in GH-treated girls with TS. Problem behavior is frequently present in untreated girls with TS, but seems to decrease during therapy. Total and internalizing problem behavior however remains increased.

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Supplementary Table 1 Characteristics and treatment per age group and dosage group.*

Characteristic	GH+Pl (N = 10)	GH+Ox 0.03 (N = 13)	GH+Ox 0.06 (N = 13)	GH+Pl (N = 15)	GH+Ox 0.03 (N = 12)	GH+Ox 0.06 (N = 13)	GH+Pl (N = 10)	GH+Ox 0.03 (N = 12)	GH+Ox 0.06 (N = 8)
Age at starting GH – yr	4.9±1.7	4.8±1.8	5.2±1.6	10.4±1.4	9.3±1.2	9.8±0.8	13.5±1.3	13.5±1.2	13.2±1.3
Height at starting GH – SDS†	-2.8±0.7	-2.8±0.8	-2.8±0.7	-2.7±0.8	-2.9±0.5	-2.9±0.6	-3.3±0.6	-3.4±0.7	-3.2±0.9
Age at starting Ox/Pl – yr	8.5±0.3	8.2±0.2	8.3±0.2	10.4±1.4	9.3±1.2	9.8±0.8	13.5±1.3	13.5±1.2	13.2±1.3
Age at starting estrogens or at B2 – yr‡	11.4±1.4	11.9±0.9	11.6±0.8	12.4±0.7	12.4±0.3	12.2±0.5	13.4±1.3	12.9±0.6	12.6±0.6
Karyotype, 45,X – no. (%)§	5 (50)	6 (46)	6 (46)	9 (60)	4 (33)	5 (39)	6 (60)	3 (25)	3 (38)
Karyotype, other – no. (%)§	5 (50)	7 (54)	7 (54)	6 (40)	8 (67)	8 (62)	4 (40)	9 (75)	5 (63)
Puberty developed spontaneously – no. (%)	4 (40)	2 (15)	6 (46)	4 (27)	4 (33)	2 (15)	1 (10)	2 (17)	1 (13)
Duration of GH therapy – yrs	9.9±2.1	9.2±2.4	8.8±1.9	5.0±1.5	5.5±1.2	5.1±1.1	3.8±0.8	3.5±0.7	3.0±0.7
Duration of Ox/Pl therapy – yrs	6.3±0.7	5.4±1.7	5.0±1.7	5.0±1.5	5.2±1.5	4.6±1.6	3.8±0.8	3.5±0.7	2.8±0.9
Age at discontinuing GH+Ox/Pl – yr	14.8±0.8	14.1±0.9	14.0±0.9	15.4±0.7	14.8±0.8	14.9±0.7	17.3±0.9	16.9±0.7	16.3±1.3
Age at last visit – yr	16.4±0.9	16.0±1.1	16.1±1.0	17.8±1.0	16.9±1.1	17.2±0.7	19.1±1.2	18.4±1.1	18.2±0.9
Adult height – SDS†¶	-2.3±0.8	-2.3±1.5	-2.4±0.8	-2.2±0.8	-2.2±0.9	-2.1±0.9	-2.1±0.5	-1.8±0.8	-2.0±1.1

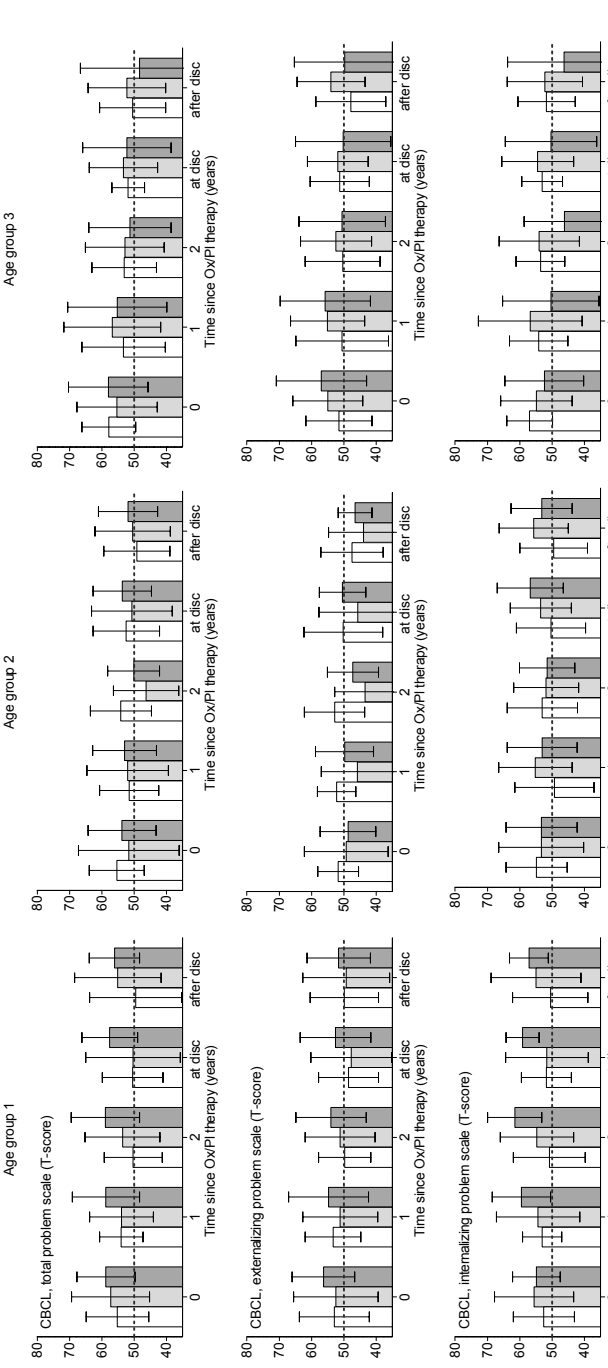
* Values are expressed as means±SD, unless otherwise indicated.

† Height was measured using a Harpenden stadiometer; the mean of four measurements was used for analysis, and expressed as SDS for healthy Dutch girls (Fredriks et al., 2000).

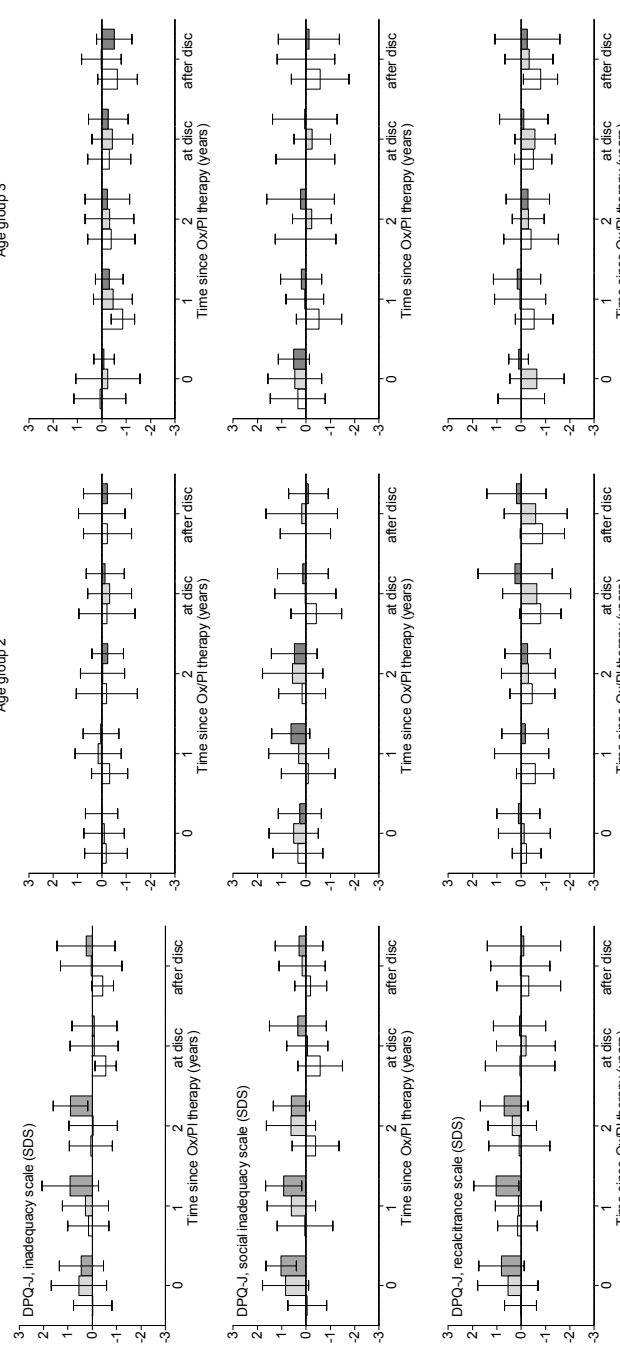
‡ If puberty was induced, the moment at starting estrogens was used; if puberty developed spontaeously, the moment at Tanner breast stage 2 was used.

§ Percentages may not total 100 because of rounding.

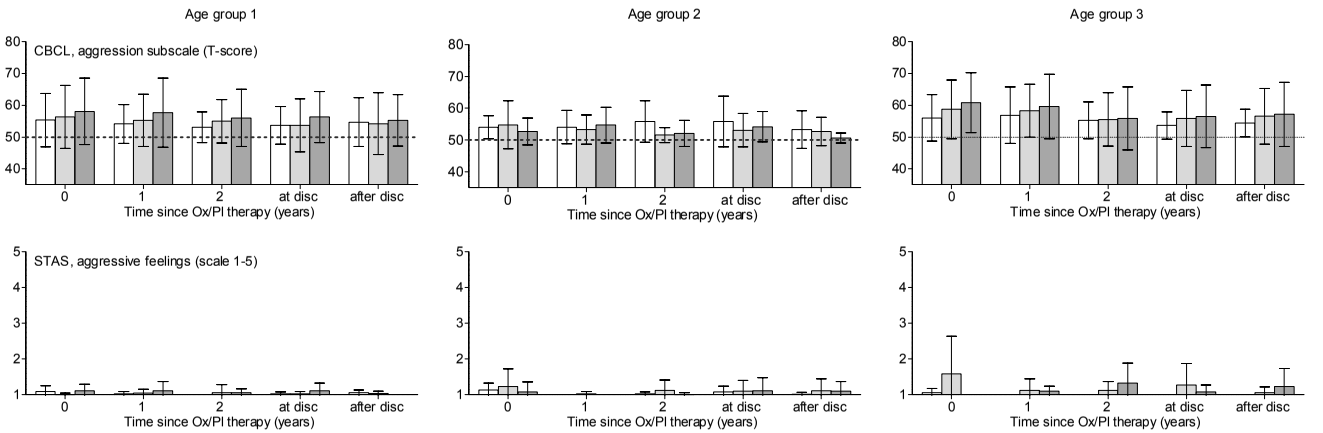
¶ Adult height was defined as the last measurement after discontinuing GH. To prevent adult height from being overestimated in patients who stopped growing at an earlier age than the reference group, reference data for the age of 21 rather than the actual age were used in calculating adult SDS.



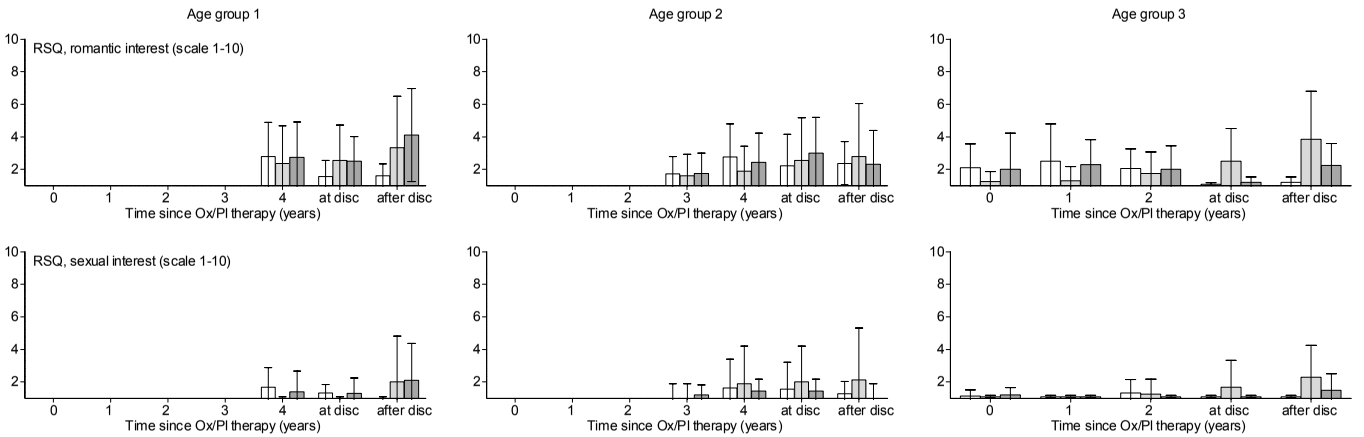
Supplementary Figure 1 Parental-rated emotional and behavioral problems per age group on GH+Pl (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars). Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD; CBCL, Child Behavior Checklist; disc, discontinuing GH+Ox/Pl. The dotted line represents the mean T score of the Dutch reference population.



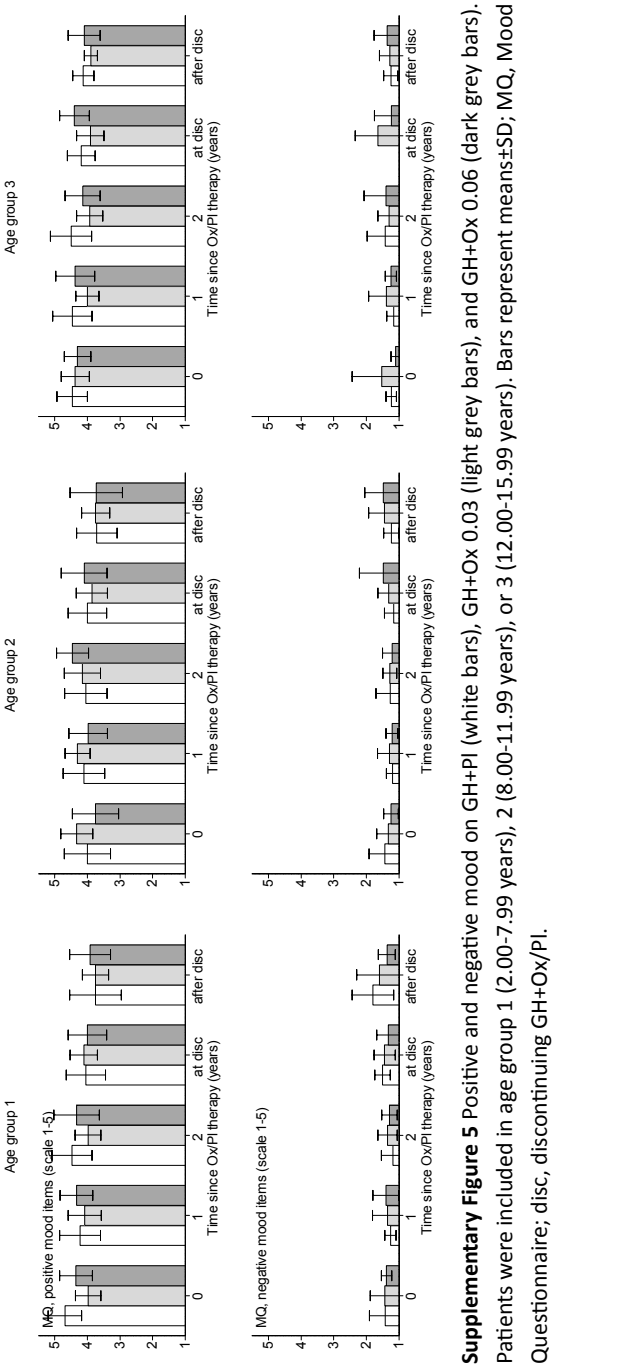
Supplementary Figure 2 Self-reported emotional and behavioral problems per age group on GH+PI (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars). Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD; DPQ-J, Junior Dutch Personality Questionnaire; disc, discontinuing GH+Ox/PI.



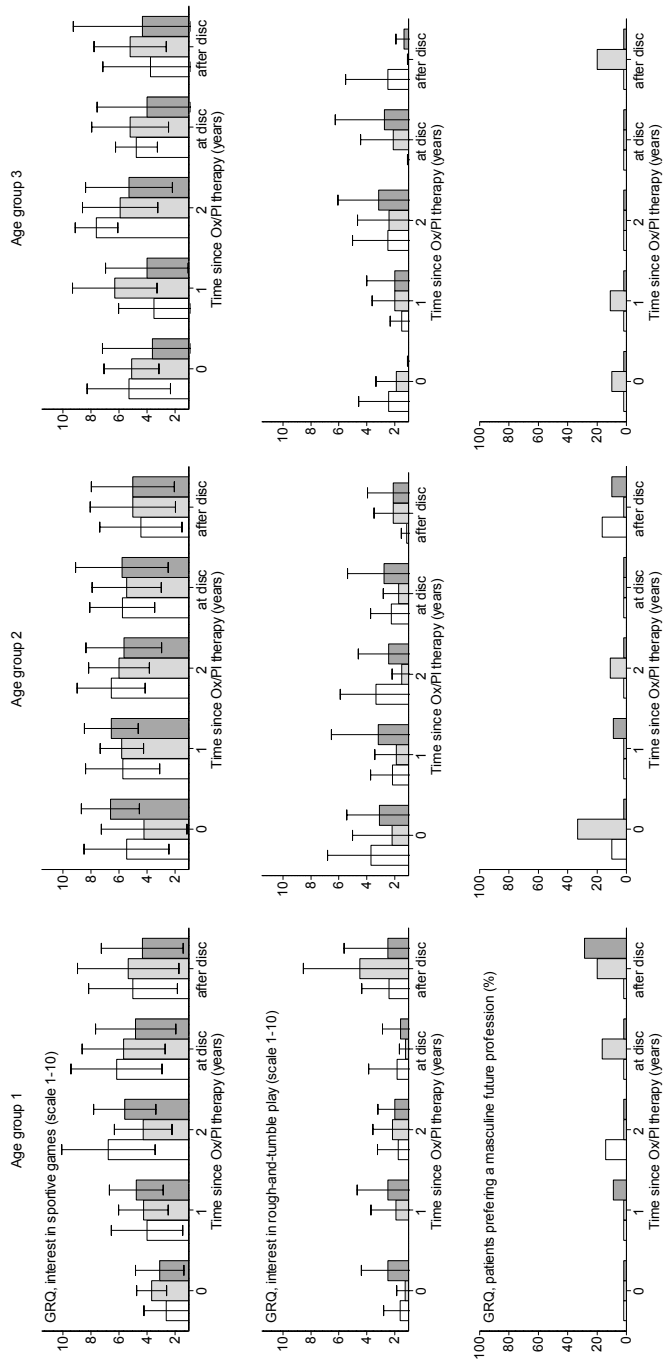
Supplementary Figure 3 Aggression per age group on GH+PI (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars). Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD; CBCL, Child Behavior Checklist; STAS, State-subscale of the Spielberger's State-Trait Anger Scale; disc, discontinuing GH+Ox/PI. The dotted line represents the mean T score of the Dutch reference population.

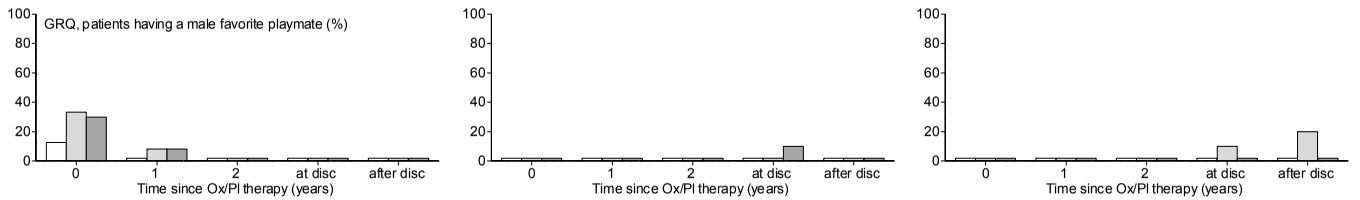


Supplementary Figure 4 Romantic and sexual interest on GH+Pl (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars). Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD; RSQ, Romantic and Sexual interest Questionnaire; disc, discontinuing GH+Ox/Pl.



Supplementary Figure 5 Positive and negative mood on GH+PI (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars). Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD; MQ, Mood Questionnaire; disc, discontinuing GH+Ox/PI.


Age group 1
Age group 2
Age group 3
GRQ, interest in sportive games (scale 1-10)
GRQ, interest in rough-and-tumble play (scale 1-10)
GRQ, patients prefering a masculine future profession (%)
Time since Ox/PI therapy (years)
at disc
after disc



Supplementary Figure 6 Gender role per age group on GH+Pl (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars). Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). Bars represent means±SD (upper panel) and percentages (lower panel). disc, discontinuing GH+Ox/Pl; GRQ, Gender Role Questionnaire.