Oxandrolone in growth hormone-treated girls with Turner syndrome

Menke, L.A.

2

Efficacy and safety of oxandrolone in growth hormone-treated girls with Turner syndrome

Leonie A. Menke, Theo C.J. Sas, Sabine M.P.F. de Muinck Keizer-Schrama, Gladys R.J. Zandwijken, Maria A.J. de Ridder, Roelof J. Odink, Maarten Jansen, Henriëtte A. Delemarre-van de Waal, Wilhelmina H Stokvis-Brantsma, Johan J. Waelkens, Ciska Westerlaken, H. Maarten Reeser, A.S. Paul van Trotsenburg, Evelien F. Gevers, Stef van Buuren, Philippe H. DeJonckere, Anita C.S. Hokken-Koelega, Barto J. Otten, Jan M. Wit

Journal of Clinical Endocrinology and Metabolism 2010, 95:1151-60

Abstract

Context and objective: Growth hormone (GH) therapy increases growth and adult height in Turner syndrome (TS). The benefit to risk ratio of adding the weak androgen oxandrolone (Ox) to GH is unclear.

Design and participants: A randomized, placebo-controlled, double-blind, dose-response study was performed in ten centers in the Netherlands. One hundred thirty-three patients with TS were included in age group 1 (2-7.99 years), 2 (8-11.99 years), or 3 (12-15.99 years). Patients were treated with GH (1.33 mg/m^2/day) from baseline, combined with placebo (Pl) or Ox in low (0.03 mg/kg/day) or conventional (0.06 mg/kg/day) dose from the age of eight, and estrogens from the age of twelve years. Adult height gain (adult height minus predicted adult height) and safety parameters were systematically assessed.

Results: Compared with GH+Pl, GH+Ox 0.03 increased adult height gain in the intention-to-treat analysis (mean±SD, 9.5±4.7 *vs.* 7.2±4.0 cm, P=0.02) and per-protocol analysis (9.8±4.9 *vs.* 6.8±4.4 cm, P=0.02). Partly due to accelerated bone maturation (P<0.001), adult height gain on GH+Ox 0.06 was not significantly different from that on GH+Pl (8.3±4.7 *vs.* 7.2±4.0 cm, P=0.3). Breast development was slower on GH+Ox (GH+Ox 0.03, P=0.02; GH+Ox 0.06, P=0.05), and more girls reported virilization on GH+Ox 0.06 than on GH+Pl (P<0.001).

Conclusions: In GH-treated girls with TS, we discourage the use of the conventional Ox dosage (0.06 mg/kg/day) because of its low benefit to risk ratio. The addition of Ox 0.03 mg/kg/day modestly increases adult height gain and has a fairly good safety profile, except for some deceleration of breast development.

Introduction

Turner syndrome (TS) is a disorder in females that is caused by the complete or partial absence of the second sex chromosome. It is one of the most common chromosomal disorders, affecting approximately one in 2000 live-born girls (1). Untreated adult patients are on average 20 cm shorter than healthy women (2), mainly due to haploinsufficiency of the Short stature HomeobOX-containing (*SHOX*) gene (3). Growth hormone (GH) therapy increases adult height with 5 to 12 cm (4-6), and the addition of the weak androgen oxandrolone (Ox) may further increase adult height (7-9). However, in previous studies Ox dosages of 0.1 mg/kg/day or greater had to be lowered to 0.05 and 0.06 mg/kg/day (7-9) on the frequent findings of virilizing side effects and increased bone maturation. Although the recommended Ox dosage is nowadays 0.05 mg/kg/day or less (10), the efficacy and safety of such dosage is unclear. We hypothesized that, due to the effect of Ox on bone maturation, the optimal dosage with respect to final height gain could be lower than 0.06 mg/kg/day, and therefore performed a dose-response study. In this randomized, placebo-controlled, double-blind study we assessed the benefit to risk ratio of Ox at a low (0.03 mg/kg/day) and previously conventional dosage (0.06 mg/kg/day) in GH-treated girls with TS.

Methods

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 and 15.99 years; and a bone age younger than 12.00 years (11). 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 enrollment, 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) (12), they were randomized by a computer-generated schedule with a block size of six, 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 (Pfizer Inc., New York, NY) was used in age groups 1 and 2, and Humatrope (Eli Lilly, Indianapolis, IN) in age group 3. Ox/Pl was started at the age of eight after a number of years 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 and 16.0 years). In the absence of spontaneous puberty (Tanner breast stage < 2 (B2) (13)), estrogen therapy was started between the age of 12.0 and 12.99 (after a number of full years of GH therapy) in age groups 1 and 2, and at inclusion (*i.e.* between the age of 12 and 16 years) in age group 3. 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. Thereafter, patients were followed for two subsequent year-visits to measure growth after discontinuing GH+Ox/Pl.

Assessments

Two trained observers performed all half-yearly measurements during the total study period. The primary outcome was adult height gain (cm), defined as adult height (the last measured height after discontinuing GH+Ox/Pl) minus predicted adult height, calculated using the modified projected adult height method (mPAH) (14). Briefly, Lyon *et al.* (15) used longitudinal heights of untreated TS girls to modify the projected adult height (which assumes that adult height SDS is equal to height SDS at a younger age) into a regression equation predicting adult height in TS. Adapted to North European girls with TS (2), this equation is: mPAH = 146.95 + 6.37 x (-0.2 + 0.836 x height SDS at baseline) (14, 16). Height was measured at every visit using a Harpenden stadiometer. The mean of four measurements was expressed as SDS for healthy Dutch girls (17) and untreated Northern European girls with TS (2) using Growth Analyser (www.growthanalyser.org). To avoid overestimation of adult height SDS in patients who stopped growing at an earlier age than healthy peers or untreated girls with TS, reference data for the age of 21 instead of the actual age were used for calculating adult height SDS. Target height (cm) corrected for sex and secular trend was defined as: 0.5 x ($height_{maternal}$ + $height_{paternal}$ - 13) + 4.5 (17).

Secondary outcomes included the influence of age group on the effect of Ox, and the effect of Ox on: short-term height gain, adult height gain adjusted for bone age at start, safety parameters, pubertal development, bone maturation, and duration and costs of GH therapy. To assess bone maturation (Δbone age / Δcalendar age), one trained, and up until 2008 blinded investigator (L.A.M.) determined bone ages of the yearly made hand x-rays retrospectively and chronologically according to the Tanner and Whitehouse radius, ulna, short-bones score (11). Pubertal stages were assessed half-yearly according to Tanner (13) and expressed as SDS adjusting for age and sex (18). The cumulative amount of GH prescribed was multiplied by 44.32 euro/mg (Genotropin, www.fk.cvz.nl, 2009) to obtain cumulative costs of GH therapy.

All adverse events reported by the patient, parent, or medical doctor were registered. Virilizing adverse events included voice deepening, clitoral enlargement, or an increase in body hair, all being previously identified as Ox-related virilization (7-9, 19). These events were included in the analysis if a girl reported virilization at least twice (*i.e.* either the same complaint at two visits or two complaints at one visit) or if

the girl decided to discontinue Ox/Pl because of the event. Dynamap blood pressure (BP) monitoring was performed half yearly. The latter three of four measurements were averaged and expressed as SDS adjusting for age, gender and height (20). Blood samples were taken at starting GH, after six months, yearly, and six months after discontinuing GH+Ox/Pl. Determinations included plasma IGF-I, IGF binding protein (IGFBP)-3, glycosylated hemoglobin (HbA1c), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), free T_4, and TSH. IGF-I, IGFBP-3, and HbA1c levels were determined in a central laboratory. IGF-I was measured by RIA from 1991 to 2000, an immunometric technique on an Advantage chemiluminescense system from 2000 to 2006 (Nichols Institute Diagnostics, San Juan Capistrano, CA), and an immunometric technique on an IMMULITE 1000 Analyzer from 2006 to 2008 (Siemens Medical Solutions Diagnostics, Los Angeles, CA), which produced identical results. IGFBP-3 was measured using a chemoluminescence based immunometric technique (IMMULITE 2000, Siemens Medical Solutions). IGF-I and IGF-I to IGFBP-3 molar ratio were transformed into SDS using reference levels for healthy Dutch children (21). HbA1c levels were measured using a dedicated automatic high pressure liquid chromatography analyzer (DIAMAT from 1991 to 1997, and VARIANT from 1997 to 2008, Bio-Rad Laboratories, Inc., Edgemont, CA). Both methods produced identical results (upper normal assay limit < 6.6%).

Statistical analyses

We estimated that 15 patients per dosage and age group were needed to achieve a power of 80% to detect a difference (P=0.05, two sided) in first-year height velocity of 2 cm with an assumed SD of 2.6. Intention-to-treat analyses were performed and differences in adult height gain were also assessed by a per-protocol analysis. Safety parameters were assessed in a modified intention-to-treat analysis, in which patients who refused to start Ox/Pl therapy were excluded. When Ox/Pl was discontinued before discontinuing GH, the moment at which GH was discontinued was identified as at discontinuing GH+Ox/Pl. Differences between dosage groups were tested by linear regression using two dummies (for GH+Ox 0.03 and GH+Ox 0.06) and differences in proportions by Pearson χ^2 tests and Fisher's exact tests. Means were compared with zero by a one-sample *t* test. Differences in change of outcome variables during

the study period were assessed by repeated-measurements analysis. Models were fitted with a different intercept and slope per dosage group and a random intercept and slope per patient. To assess the influence of the age groups, interaction terms between age groups and dosage groups were used.

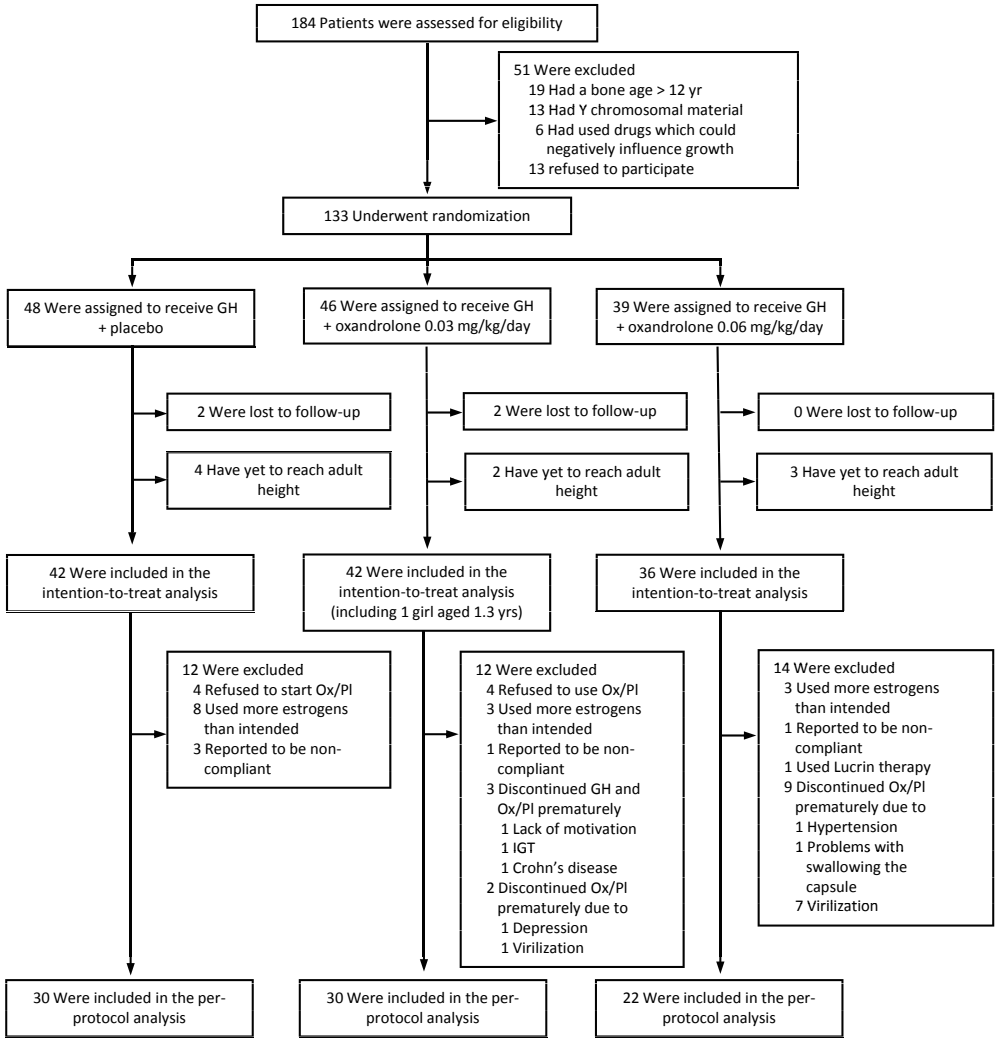

Figure 1. Enrollment, randomization and analysis of the patients.

Ox/Pl, oxandrolone/placebo; IGT, impaired glucose tolerance. Some patients were excluded from the per-protocol analysis for more than one reason, the sum of these numbers may therefore not equal the total number of patients in these boxes.

Results

Patient characteristics

Fig. 1 shows the 133 patients that were randomized. Four patients were lost to follow-up and nine patients were still treated when the analysis started in May 2008. Of the 120 patients included in the intention-to-treat analysis, the parents of eight girls refused Ox/Pl because of fear of side effects and/or satisfaction with growth. After excluding all protocol-violators, 82 patients (68%) were left for the per-protocol analysis.

Adult height gain

Baseline data were similar between the dosage groups (Table 1). Fig. 2 shows the height SDS before, during and after discontinuing GH+Ox/Pl therapy. The two-year increase in height SDS was significantly greater on GH+Ox 0.03 and 0.06 than on GH+Pl (P<0.001 for both comparisons), but differences decreased before reaching adult height. Fig. 3 shows the individual heights of the 120 girls at starting GH therapy, as well as after reaching adult height. Adult height gain, measured 1.9±0.8 years after discontinuing GH, was greater than zero in each dosage group (P<0.001 for all comparisons) (Fig. 4). Compared with GH+Pl, it was 2.3 cm greater on GH+Ox 0.03 (95% confidence interval (CI), 0.4 to 4.2, P=0.02), and 1.2 cm greater on GH+Ox 0.06 (95% CI, -0.8 to 3.2, P=0.3) (Fig. 4A). Similar results were obtained when leaving out the eight patients that did not start Ox/Pl therapy (data not shown), and when analyzing the increase in height SDS from baseline to adulthood (Table 2). When correcting for bone age at starting GH therapy, the difference in adult height gain compared with GH+Pl was 1.8 cm on GH+Ox 0.03 (P=0.05), and 1.0 cm on GH+Ox 0.06 (P=0.3). In the per-protocol analysis, adult height gain was 3.1 cm greater on GH+Ox 0.03 (95% CI, 0.5 to 5.6, P=0.02) and 2.2 cm greater on GH+Ox 0.06 (95% CI, -0.6 to 4.9, P=0.1) (Fig. 4B).

Bone maturation was greater in both GH+Ox groups than on GH+Pl (GH+Ox 0.03, P=0.007; GH+0.06, P<0.001) (Table 2). When corrected for bone age at starting GH, the duration of GH therapy was shorter on GH+Ox 0.03 and 0.06 (-0.4 years and -0.8 years, P=0.06 and P=0.001, respectively), and the cumulative costs of GH were

lower (-10,100±6,100 and -13,500±6,300 euro, P=0.1 and P=0.03, respectively) than on GH+Pl (mean cumulative costs, 161,200±59,500 euro).

Supplemental Table 1 shows the baseline and clinical data per age group and per dosage group. In the intention-to-treat analysis, mean adult height gain in age groups 1, 2, and 3 was 9.4±4.4, 6.0±3.6, and 5.8±2.6 cm on GH+Pl; 10.4±5.8, 9.0±4.4, and 8.6±3.0 cm on GH+Ox 0.03; and 10.0±3.7, 9.0±4.8, and 5.4±4.7 cm on GH+Ox 0.06, respectively. Among the three age-groups, no statistically significant difference in the effect of Ox on adult height gain was found.

Pubertal development

Puberty started spontaneously in 28 girls (mean age at B2, 11.1±1.0 years). In the remaining 92 girls, estrogen therapy was started at a mean age of 12.8±0.9 years. In the years thereafter, breast stage SDS of the girls that had started Ox/Pl (modified intention-to-treat analysis, n=112) increased less on GH+Ox 0.03 and 0.06 than on GH+Pl (during first 2 years, P=0.05 and 0.1, respectively; until discontinuing GH+Ox/Pl, P=0.02 and 0.05, respectively) (Table 2). Breast stage SDS at discontinuing Ox/Pl was lower on GH+Ox 0.03 than on GH+Pl (P=0.01) and although it caught up after discontinuing Ox and increasing estrogen dosages, it was still lower on GH+Ox 0.03 than on GH+Pl after discontinuing GH+Ox/Pl (P=0.04) (Table 2). Pubic hair stage SDS increased significantly more on GH+Ox 0.03 and 0.06 than on GH+Pl during the first two years of Ox/Pl (P=0.008 and 0.003, respectively), but the increase during the total duration of Ox/Pl therapy was not significantly different between the dosage groups (Table 2). Mean pubic hair stage SDS at discontinuing Ox/Pl was, however, significantly greater on GH+Ox 0.06 than on GH+Pl (P=0.003).

Table 1. Characteristics per dosage group.*

Characteristic	GH+Pl (N = 42)	GH+Ox 0.03 (N = 42)	GH+Ox 0.06 (N = 36)
Age at starting GH – yr	9.4±3.8	8.5±4.0	9.1±3.5
Bone age at starting GH – yr	9.0±3.4	8.1±3.6	8.8±3.4
Height at starting GH (ref: healthy Dutch girls) – SDS	-3.0±0.8	-3.0±0.7	-2.9±0.7
Height at starting GH (ref: untreated girls with TS) – SDS	0.5±1.0	0.3±0.9	0.5±0.9
Predicted adult height (mPAH) – cm	148.4±5.5	147.2±4.9	148.1±4.9
Karyotype 45,X – n (%)†	24 (57)	17 (41)	16 (44)
Karyotype other than 45,X – n (%)†	18 (43)	25 (60)	20 (56)
Target height – SDS	-0.1±0.8	0.1±1.0	0.0±0.8
Age at starting Ox/Pl – yr§	10.9±2.3	10.2±2.5	10.2±2.2
Puberty developed spontaneously – n (%)	9 (21)	10 (24)	9 (25)
Puberty induced – n (%)	33 (79)	32 (76)	27 (75)
Age at starting estrogens (if puberty was induced) – yr¶	12.9±1.0	12.8±0.9	12.7±0.9
Age at B2 (if puberty developed spontaneously) – yr	11.0±1.2	11.1±1.2	11.0±0.6
Breast stage at starting estrogens or at B2 – SDS‡‖	-1.5±1.1	-1.5±1.0	-1.3±1.0
Pubic hair stage at starting Ox/Pl – SDS‖	-0.8±1.0	-0.5±1.1	-0.2±1.3
Systolic blood pressure at starting Ox/Pl – SDS‖	1.1±1.2	1.2±1.0	1.4±1.2
Diastolic blood pressure at starting Ox/Pl – SDS‖	0.3±0.9	0.5±0.8	0.4±0.7
IGF-I at starting Ox/Pl in age group 1 (already using GH) – SDS§‖	0.3±1.1	0.7±1.4	1.3±0.9
IGF-I at starting GH+Ox/Pl in age groups 2 and 3 – SDS‖	-1.2±1.1	-1.2±0.6	-1.1±1.1
IGF-I to IGFBP-3 ratio at starting Ox/Pl in age group 1 – SDS§‖	-0.2±1.1	-0.3±1.3	0.3±1.0
IGF-I to IGFBP-3 ratio at starting GH+Ox/Pl in age groups 2 and 3 –SDS§‖	-1.1±1.0	-0.9±0.6	-1.0±1.0

* Values are expressed as means±SD, unless otherwise indicated.
† Percentages may not total 100 because of rounding.
‡ If puberty was induced, the moment at starting estrogens was used; if puberty developed spontaneously, the moment at Tanner breast stage 2 was used.
§ Ox/Pl therapy was started between the age of 8.0 to 8.99, after a number of years of GH therapy in age group 1, and at inclusion (*i.e.* between the age of 8 and 16 years) in age groups 2 and 3. Because (according to the protocol) GH and Ox were not started at the same time in age group 1, the age at starting GH is not equal to the age at starting Ox.
¶ Estrogen therapy was started between the age of 12.0 to 12.99 (after a number of years on GH therapy) in age groups 1 and 2, and at inclusion (*i.e.* between the age of 12 and 16 years) in age group 3.
‖ For the analysis of the safety parameters, the 8 patients that did not start Ox/Pl were excluded.

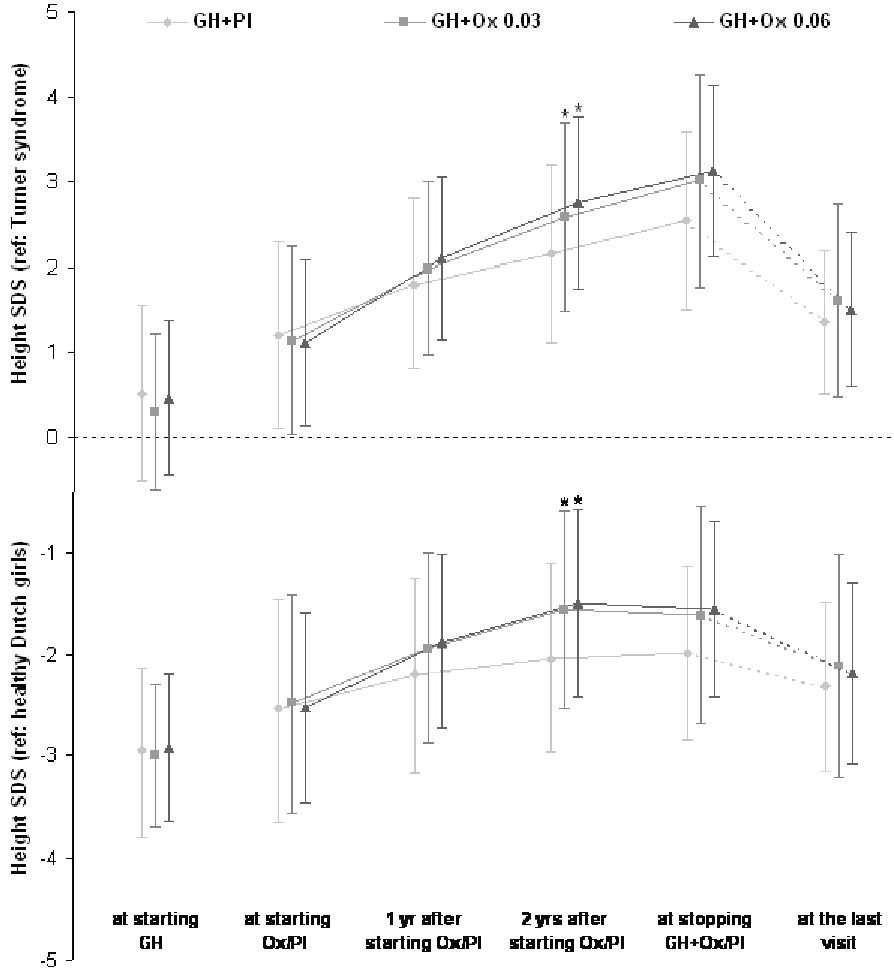

Figure 2. Height SDS during the study compared with untreated Northern European girls with Turner syndrome (upper part) and healthy Dutch girls (lower part).

Symbols represent means±SD; Ox/Pl, oxandrolone/placebo. Note that Ox/Pl therapy was started between the age of 8.0 to 8.99, after a number of years of GH therapy in age group 1, and at inclusion (*i.e.* between the age of 8 and 16 years) in age groups 2 and 3. Height SDS at starting GH therefore reflects untreated values, whereas height SDS at starting Ox/Pl includes values from girls that had already been treated with GH. Height SDS at the last visit was calculated using reference values for 21-year-old girls. The asterisk indicates that mean first two-year increase in height SDS was greater than on GH+Pl ($P<0.001$).

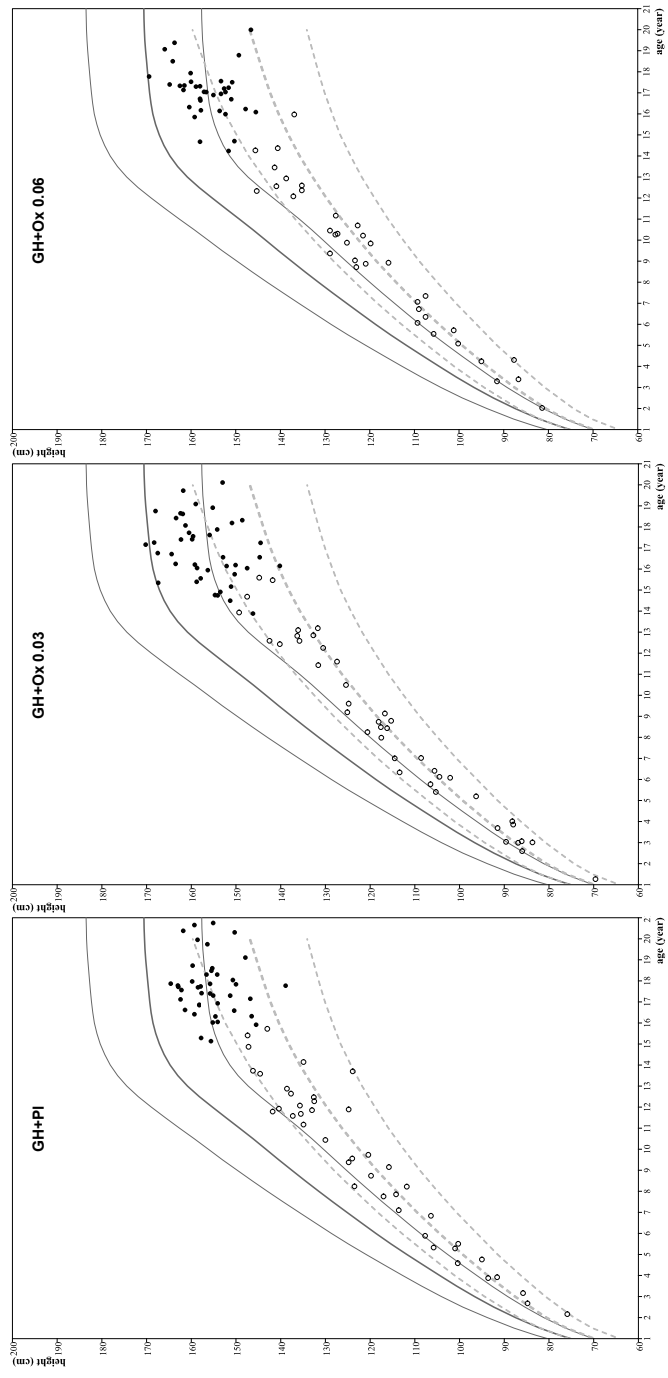

Figure 3. Individual baseline and adult heights plotted in the growth charts of healthy Dutch girls and untreated girls with Turner syndrome. Lines represent mean±2SD of the age references of healthy Dutch girls (uninterrupted lines) and untreated Northern European girls with Turner syndrome (dashed lines). Open circles indicate height at baseline (*i.e.* at starting GH therapy) and filled circles indicate adult height.

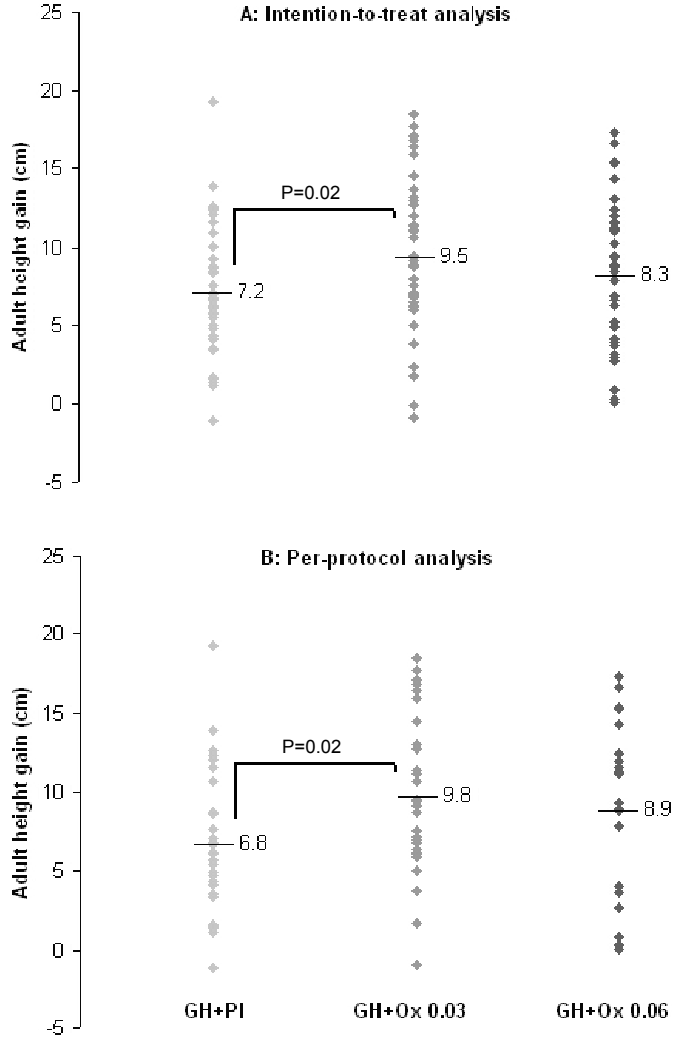

Figure 4. Adult height gain in the intention-to-treat analysis (A) and per-protocol analysis (B). Diamonds represent adult height gain (adult height minus predicted adult height) of the individual patients; lines represent mean adult height gain per dosage group. Mean adult height gain was greater than zero in each dosage group (P<0.001 for all comparisons in both analyses). Compared with GH+Pl, adult height gain was greater on GH+Ox 0.03 (P=0.02 both in the intention-to-treat analysis and per-protocol analysis), and not significanlty greater on GH+Ox 0.06.

Adverse events and virilization

During the study, 2030 adverse events were reported, none of which were considered GH related. Twenty-three girls reported a total of 40 virilizing adverse events (Table 2), and more girls on GH+Ox 0.06 than on GH+Pl reported virilization (P<0.001). One girl on GH+Ox 0.03 and seven on GH+Ox 0.06 (*vs.* zero on GH+Pl, P=0.005) discontinued Ox because of virilization. After discontinuing Ox/Pl, two girls (from groups GH+Ox 0.03 and 0.06) still reported hirsutism, and one girl (from group GH+Ox 0.06) still reported having a low voice. However, hirsutism relieved in three girls, subjective voice deepening relieved in two girls (all from group GH+Ox 0.06), and clitoral size appeared less in two girls (from group GH+Ox 0.03). The other complaints of virilization were not reported anymore.

Blood pressure

Mean systolic and diastolic BP (Tables 1 and 2) was significantly higher than in healthy girls, at both starting and discontinuing GH+Ox/Pl (P<0.001 for all comparisons). During Ox/Pl, systolic BP SDS tended to decrease somewhat more on GH+Ox 0.06 than on GH+Pl (P=0.06), whereas changes in diastolic BP SDS were not significantly different between the dosage groups (Table 2).

Biochemical evaluation

Compared with GH+Pl, mean IGF-I SDS just before discontinuing GH+Ox tended to be greater on GH+Ox 0.03 an 0.06 (corrected for values at starting Ox/Pl, P=0.09 and 0.05, respectively), and the proportion of patients with an IGF-I greater than 2 SDS at least once during therapy was greater (GH+Ox 0.03, P=0.04; GH+Ox 0.06, P=0.06) (Table 2). The change of IGF-I SDS as well as IGF-I to IGFBP-3 ratio during the first year of Ox/Pl therapy was, however, not significantly different between the dosage groups (Table 2).

After starting Ox/Pl, five girls had intermittently elevated ASAT and/or ALAT levels (Table 2), and five girls (three on GH+Ox 0.03 and two on GH+Ox 0.06) developed hypothyroidism and started thyroxine supplementation.

One girl, who already had an impaired glucose tolerance at baseline, had impaired glucose tolerance and an elevated HbA1c (7.7%) after two years of GH+Ox 0.03

therapy. She therefore discontinued GH+Ox, after which HbA1c and glucose levels returned to normal. The HbA1c levels of all other girls remained normal, and none of the girls developed diabetes mellitus type 1 or 2.

Discussion

In the past two decades, several trials suggested that the addition of Ox to GH positively affected adult height in girls with TS (7-9). However, these studies enrolled smaller numbers of patients, were neither randomized nor placebo controlled, and had to lower their Ox starting dosages (≥ 0.1 mg/kg/day) on the frequent finding of virilizing adverse events. Our randomized, placebo-controlled, double-blind study shows that GH combined with Ox at a previously not studied low dosage (0.03 mg/kg/day) moderately increases adult height gain and has an acceptable safety profile except for a small deceleration in breast development. The addition of the previously conventional Ox dosage (0.06 mg/kg/day) does not significantly increase adult height gain and causes virilization in a large proportion of patients.

During the first two years after starting Ox/Pl, the increase in height SDS on GH+Ox 0.03 and 0.06 was significantly greater than on GH+Pl. Adult height gain on GH+Ox 0.06 was, however, smaller than on GH+Ox 0.03, which may be explained by the relatively frequent premature discontinuation of Ox 0.06 because of virilization, and the increase in bone maturation with increasing Ox dosages. In contrast, the growth-promoting effect of GH+Ox 0.03 outweighed the increase in bone maturation, resulting in an increased adult height gain compared with GH+Pl.

Table 2. Outcome variables per dosage group.*

Outcome	GH+Pl (N = 42)	GH+Ox 0.03 (N = 42)	GH+Ox 0.03 *vs.* GH+Pl *P Value*	GH+Ox 0.06 (N = 36)	GH+Ox 0.06 *vs.* GH+Pl *P Value*
Age at discontinuing GH – yr	15.8±1.2	15.2±1.4	0.03	14.9±1.3	0.005
Age at discontinuing Ox/Pl – yr	15.2±1.2	14.4±1.8	0.03	13.9±1.7	0.001
Duration of GH therapy – yr	6.4±3.1	6.7±3.3	0.6	5.8±2.8	0.4
Duration of Ox/Pl therapy – yr	5.0±1.5	4.8±1.6	0.6	4.2±1.7	0.05
Bone maturation during GH therapy – yr/yr	0.9±0.2	1.0±0.2	0.007	1.1±0.3	<0.001
Age at last visit – yr†	17.7±1.4	16.9±1.5		17.0±1.2	
Adult height – cm†	155.6±5.4	156.7±7.2		156.5±5.8	
Adult height (reference: healthy Dutch girls) – SDS †‡	-2.3±0.8	-2.1±1.1		-2.2±0.9	
Adult height (reference: untreated girls with TS) – SDS †‡	1.4±0.8	1.5±1.1		1.5±0.9	
Adult height gain – cm§	7.2±4.0	9.5±4.7	0.02	8.3±4.7	0.3
Delta height (from starting GH therapy to adult height) – SDS	0.8±0.7	1.2±0.7	0.02	1.0±0.8	0.3
Patients in per-protocol analysis – n (%)†	30 (71)	30 (71)		22 (61)	
Adult height gain, per-protocol analysis – cm§	6.8±4.4	9.8±4.9	0.02	8.9±5.4	0.1
Pubertal stage					
Change in breast stage during first two years of estrogen therapy – SDS/yr, SE¶‖	0.18, 0.07	-0.01, 0.07	0.05	0.04, 0.07	0.1
Change in breast stage from starting estrogen therapy until discontinuing GH+Ox/Pl – SDS/yr, SE¶‖	0.22, 0.06	0.01, 0.06	0.02	0.05, 0.06	0.05
Breast stage at discontinuing GH+Ox/Pl – SDS ¶	-0.8±1.0	-1.4±1.0	0.01	-1.1±1.0	0.1
Breast stage after discontinuing GH+Ox/Pl – SDS ¶	-0.4±0.8	-0.8±0.9	0.04	-0.3±0.9	0.7
Breast stage after discontinuing GH+Ox/Pl – median (range)¶†	5 (4 - 5)	4 (2 - 5)		5 (3 - 5)	
Change in pubic hair stage during first two years of Ox/Pl therapy – SDS/yr, SE¶	-0.07, 0.08	0.24, 0.08	0.008	0.28, 0.08	0.003
Change in pubic hair stage from starting Ox/Pl until discontinuing GH+Ox/Pl – SDS/yr, SE¶	-0.06, 0.05	-0.05, 0.05	0.9	-0.02, 0.05	0.6
Pubic hair stage at discontinuing GH+Ox/Pl – SDS ¶	-0.9±1.1	-0.8±0.9	0.6	-0.3±0.8	0.003

Girls with virilization during Ox/Pl – n (%)¶	2 (5)	6 (16)	0.3	15 (42)	<0.001
Subjective voice deepening¶†	1 (3)	3 (8)		9 (25)	
Hirsutism¶†	1 (3)	5 (13)		12 (33)	
Mild clitoromegaly¶†	0 (0)	4 (11)		5 (14)	
Girls discontinuing Ox/Pl due to virilization – n (%)¶	0 (0)	1 (3)	1.0	7 (19)	0.005
Blood pressure					
Change in systolic blood pressure during Ox/Pl – SDS/yr, SE¶	0.01, 0.03	-0.05, 0.03	0.2	-0.08, 0.03	0.06
Systolic blood pressure at discontinuing GH+Ox/Pl – SDS ¶	1.1±1.1	0.8±1.2	0.3	1.0±1.4	0.8
Change in diastolic blood pressure during Ox/Pl – SDS/yr, SE¶	-0.02, 0.02	-0.04, 0.02	0.5	-0.03, 0.02	0.7
Diastolic blood pressure at discontinuing GH+Ox/Pl – SDS ¶	0.5±0.8	0.2±0.8	0.3	0.4±1.1	0.7
IGF-I					
First-year change during Ox/Pl, age group 1 – SDS, SE¶††	-0.30, 0.45	0.29, 0.40	0.3	-0.23, 0.41	0.9
First-year change during Ox/Pl, age groups 2 and 3 – SDS, SE¶††	1.82, 0.27	2.25, 0.29	0.3	2.21, 0.30	0.3
At discontinuing GH+Ox/Pl – SDS¶	0.8±1.2	1.3±1.1	0.09‡‡	1.4±0.8	0.05‡‡
Patients > 2 SDS at least once during Ox/Pl – n (%)¶	15 (39)	24 (63)	0.04	22 (61)	0.06
IGF-I to IGFBP-3 ratio					
First-year change during Ox/Pl, age group 1 – SDS, SE¶††	-0.58, 0.53	0.13, 0.48	0.3	-0.31, 0.49	0.7
First-year change during Ox/Pl, age groups 2 and 3 – SDS, SE¶††	1.08, 0.25	1.06, 0.26	1.0	1.22, 0.27	0.7
At discontinuing GH+Ox/Pl – SDS¶	0.0±0.9	0.4±1.2	0.1‡‡	0.5±1.0	0.2‡‡
Intermittently elevated ASAT/ALAT – n (%)¶**	3 (8)	0 (0)		2 (6)	

* Values are expressed as means±SD, unless otherwise indicated.
† No statistical tests were applied.
‡ Adult height SDS was calculated using reference values for 21-year-old girls.
§ Defined as adult height minus predicted adult height.
¶ In the analysis of the safety parameters, the 8 patients that had not started Ox/Pl were excluded.
‖ If puberty was induced, the moment at starting estrogens was used; if puberty developed spontaneously, the moment at Tanner breast stage 2 (B2) was used.
** Defined as elevated aspartate aminotransferase (ASAT > 60 U/l) and/or alanine aminotransferase levels (ALAT > 50 U/l) at two or more visits during and/or after discontinuing GH+Ox/Pl therapy.
†† Ox/Pl therapy was started after a number of years of GH therapy in age group 1, and at baseline in age groups 2 and 3.
‡‡ Corrected for values just before starting Ox/Pl therapy.

The exact mechanism by which Ox increases bone growth and maturation is uncertain. A recent report showed that bone growth *in vitro* was not influenced by Ox, suggesting that Ox may mainly influence the growth plate in an indirect way (22). Ox does not appear to increase GH secretion (23), but it may increase growth by increasing IGF-I (presumably by increasing insulin-induced hepatic GH receptors) (24), by suppressing IGFBP-I (an inhibitor of IGF-I) (24), and/or by increasing free estrogen levels due to an Ox-induced decrease in SHBG (24, 25). Testosterone is thought to increase bone maturation primarily via the aromatase-induced local conversion to estradiol (25, 26). The increase in bone maturation due to the nonaromatizable androgen Ox, however, shows that androgens may also influence bone maturation either directly, or indirectly via other pathways (27).

We found that the addition of Ox to GH therapy delayed breast development to some extent. Although breast stage SDS caught up after discontinuing GH+Ox and increasing estrogen dosages, it was still lower on GH+Ox 0.03 than on GH+Pl. Particularly nonaromatizable androgens (such as Ox and dihydrotestosterone) are known to inhibit the stimulatory effect of estrogens on the mammary gland (28). This inhibitory effect may possibly be overcome by increasing estrogen dosages, although a further acceleration of bone maturation would then perhaps eliminate the positive effect of Ox on adult height.

Several girls on GH+Ox 0.06 reported virilization, and about half of them decided to discontinue Ox for that reason. The finding that also some girls on GH+Pl complained of virilization reflects that part of the reported virilization may be regarded physiological and/or that patients tended to report virilization because they knew this could be a consequence of Ox. Whereas hirsutism and clitoromegaly seem to regress after discontinuing Ox (9, 19), voice deepening appears irreversible (29).

Several other adverse events were scarce. As expected in adolescents with TS, a few patients developed hypothyroidism (30). Although Ox at higher dosages may elevate liver enzymes in non-TS patients (31), we did not find such an effect at the dosages we studied. We furthermore found that the addition of Ox did not increase diastolic BP, whereas systolic BP even tended to decrease on GH+Ox 0.06 *vs.* GH+Pl. One patient discontinued GH+Ox 0.03 because of an increased HbA1c and an impaired

glucose tolerance, after which HbA1c and glucose levels returned to normal. Patients with TS are at an increased risk of developing insulin resistance, and GH therapy, especially when combined with Ox ≥ 0.06 mg/kg/day, may increase this risk (24, 32, 33). This effect, however, appeared to be reversible after discontinuation of therapy (34). IGF-I levels were more frequently increased on GH+Ox than on GH+Pl, whereas the increase in IGF-I levels and IGF-I to IGFBP-3 ratio (an indicator of free IGF-I) was not significantly different between the dosage groups. Previous studies also showed conflicting results regarding the effect of Ox on IGF-I levels and IGF-I to IGFBP-3 ratio (35, 36).

The addition of Ox is not the only strategy to increase adult height gain in TS. Adult height gain may also be augmented by increasing GH dosages, rather than adding Ox to GH therapy (6). Increasing GH doses would, however, increase IGF-I levels (6) as well as costs, whereas our data show that Ox may lower the GH-associated costs. A further argument in favor of adding Ox is that it may result in a more physiological hormonal status, considering the androgen-insufficient state of untreated girls and women with TS (37, 38). Another strategy to increase adult height gain is to start GH therapy at a relatively young age. Our finding that mean adult height gain on GH+Pl was 9.4, 6.0, and 5.8 cm in age groups 1, 2, and 3, respectively, confirms that an early diagnosis and start of GH therapy positively influences adult height gain (6).

Our study has some limitations. First, although it included a follow-up period of 1.9±0.8 years after discontinuation of GH+Ox/Pl, a longer follow-up would be needed to assess long-term safety. Second, we did not study quality of life and well-being. We hypothesize that the observed delay in breast development may negatively affect these parameters, whereas the increase in height during therapy as well as the decrease in duration of GH therapy (*i.e.* subcutaneous injections) may have some positive effects. Compensating the androgenic insufficiency in TS may also have some positive effects, similar to the effect on well-being observed in androgen-treated adult patients with TS (39). Finally, no standardized scoring system was used in the assessment of the virilizing adverse events. Consequently, the reported virilization may underestimate the actual occurrence of virilization. Because we were unable to compare the girls with healthy girls in puberty, it is additionally unclear whether the reported virilization should be regarded as genuine virilization or a normalization

from an androgen-insufficient state. The relatively great number of patients that discontinued Ox 0.06 due to virilization, however, suggests that this dosage indeed results in virilization.

We conclude that in GH-treated girls with TS, Ox in a conventional dose (0.06 mg/kg/day) has limited efficacy, and gives rise to virilizing side effects. We therefore discourage its use. The addition of low-dose Ox (0.03 mg/kg/day) modestly increases adult height gain and has a fairly good safety profile, except for a small deceleration in breast development. In patients considering this deceleration less important than the increment in height gain, Ox 0.03 mg/kg/day may be added to GH to increase height.

Acknowledgements

We thank all patients and parents for their valuable participation. We thank Karin Rademaker, Boudewijn Bakker, Sophie van Koningsbrugge, Janneke Baan and Sander Spaans for their technical assistance; Jan Van den Broeck, Bart Boersma and Arne van Teunenbroek† for their help in preparing the protocol; and research assistants Esther de Beus and Saskia Willemse-de Vries for their extensive and dedicated work on data collection and administration.

Disclosure summary

This investigator-initiated study was funded by Pfizer and Eli Lilly. Preliminary data of this report have been presented at the 46th and 47th Annual Meeting of the European Society of Pediatric Endocrinology (September 2007 and 2008) and at the KIGS investigators meeting in April 2008. LAM and SdMK-S have received lecture fees and travel and accomodation payments from Pfizer, TCS has received lecture fees and travel and accomodation payments from Pfizer and Novo Nordisk, and EFG has received a honorarium and travel payment from Pfizer. HMR receives funding for the pediatric endocrine fellows in his department from Novo Nordisk and Ipsen. JMW received consulting fees, honoraria, and lecture fees from Pfizer and Lilly; and has served on an advisory board for Pfizer and Lilly. All other authors declare that they have no conflict of interest.

References

1. Stochholm K, Juul S, Juel K, Naeraa RW, Gravholt CH 2006 Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. J Clin Endocrinol Metab 91:3897-3902
2. Rongen-Westerlaken C, Corel L, van den Broeck J, Massa G, Karlberg J, Albertsson-Wikland K, Naeraa RW, Wit JM 1997 Reference values for height, height velocity and weight in Turner's syndrome. Swedish Study Group for GH treatment. Acta Paediatr 86:937-942
3. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M, Nordsiek G, Heinrich U, Breuning MH, Ranke MB, Rosenthal A, Ogata T, Rappold GA 1997 Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet 16:54-63
4. Ranke MB, Lindberg A, Ferrandez Longas A, Darendeliler F, Albertsson-Wikland K, Dunger D, Cutfield WS, Tauber M, Wilton P, Wollmann HA, Reiter EO 2007 Major determinants of height development in Turner syndrome (TS) patients treated with GH: analysis of 987 patients from KIGS. Pediatr Res 61:105-110
5. Stephure DK 2005 Impact of growth hormone supplementation on adult height in turner syndrome: results of the Canadian randomized controlled trial. J Clin Endocrinol Metab 90:3360-3366
6. Van Pareren YK, de Muinck Keizer-Schrama SM, Stijnen T, Sas TC, Jansen M, Otten BJ, Hoorweg-Nijman JJ, Vulsma T, Stokvis-Brantsma WH, Rouwe CW, Reeser HM, Gerver WJ, Gosen JJ, Rongen-Westerlaken C, Drop SL 2003 Final height in girls with turner syndrome after long-term growth hormone treatment in three dosages and low dose estrogens. J Clin Endocrinol Metab 88:1119-1125
7. Nilsson KO, Albertsson-Wikland K, Alm J, Aronson S, Gustafsson J, Hagenas L, Hager A, Ivarsson SA, Karlberg J, Kristrom B, Marcus C, Moell C, Ritzen M, Tuvemo T, Wattsgard C, Westgren U, Westphal O, Aman J 1996 Improved final height in girls with Turner's syndrome treated with growth hormone and oxandrolone. J Clin Endocrinol Metab 81:635-640

8. Rosenfeld RG, Attie KM, Frane J, Brasel JA, Burstein S, Cara JF, Chernausek S, Gotlin RW, Kuntze J, Lippe BM, Mahoney CP, Moore WV, Saenger P, Johanson AJ 1998 Growth hormone therapy of Turner's syndrome: beneficial effect on adult height. J Pediatr 132:319-324
9. Stahnke N, Keller E, Landy H 2002 Favorable final height outcome in girls with Ullrich-Turner syndrome treated with low-dose growth hormone together with oxandrolone despite starting treatment after 10 years of age. J Pediatr Endocrinol Metab 15:129-138
10. Bondy CA 2007 Care of girls and women with Turner syndrome: A guideline of the Turner Syndrome Study Group. J Clin Endocrinol Metab 92:10-25
11. Tanner JM, Whitehouse RH, Cameron JS, Marshall W, Healy M, Goldstein H 1983 Assessment of skeletal maturity and prediction of adult height (TW2 method). 2nd ed. London: Academic Press; 54-71
12. Ranke MB, Stubbe P, Majewski F, Bierich JR 1988 Spontaneous growth in Turner's syndrome. Acta Paediatr Scand Suppl 343:22-30
13. Marshall WA, Tanner JM 1969 Variations in pattern of pubertal changes in girls. Arch Dis Child 44:291-303
14. van Teunenbroek A, Stijnen T, Otten B, de Muinck Keizer-Schrama S, Naeraa RW, Rongen-Westerlaken C, Drop S 1996 A regression method including chronological and bone age for predicting final height in Turner's syndrome, with a comparison of existing methods. Acta Paediatr 85:413-420
15. Lyon AJ, Preece MA, Grant DB 1985 Growth curve for girls with Turner syndrome. Arch Dis Child 60:932-935
16. Karlberg J, Abertsson-Wikland K, Naeraa RW, Rongen-Westerlaken C, Wit JM 1993 Reference values for spontaneous growth in Turner girls and its use in estimating treatment effects. In: Hibi I, Tkano K, eds. Basic and clinical approach to Turner syndrome. 1st ed. Amsterdam: Elsevier Science Publishers B.V.; 83-92
17. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-323
18. van Buuren S, Ooms JC 2009 Stage line diagram: an age-conditional reference diagram for tracking development. Stat Med 28:1569-1579

19. Naeraa RW, Nielsen J, Pedersen IL, Sorensen K 1990 Effect of oxandrolone on growth and final height in Turner's syndrome. Acta Paediatr Scand 79:784-789
20. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2004 The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 114:555-576
21. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-176
22. Chagin AS, Vannesjo J, Savendahl L 2009 Androgen receptor modulation does not affect longitudinal growth of cultured fetal rat metatarsal bones. Horm Res 71:219-227
23. Massarano AA, Brook CG, Hindmarsh PC, Pringle PJ, Teale JD, Stanhope R, Preece MA 1989 Growth hormone secretion in Turner's syndrome and influence of oxandrolone and ethinyl oestradiol. Arch Dis Child 64:587-592
24. Haeusler G, Schmitt K, Blumel P, Plochl E, Waldhor T, Frisch H 1996 Insulin, insulin-like growth factor-binding protein-1, and sex hormone-binding globulin in patients with Turner's syndrome: course over age in untreated patients and effect of therapy with growth hormone alone and in combination with oxandrolone. J Clin Endocrinol Metab 81:536-541
25. Chagin AS, Savendahl L 2009 Genes of importance in the hormonal regulation of growth plate cartilage. Horm Res 71 Suppl 2:41-47
26. Chagin AS, Chrysis D, Takigawa M, Ritzen EM, Savendahl L 2006 Locally produced estrogen promotes fetal rat metatarsal bone growth; an effect mediated through increased chondrocyte proliferation and decreased apoptosis. J Endocrinol 188:193-203
27. Vottero A, Pedori S, Verna M, Pagano B, Cappa M, Loche S, Bernasconi S, Ghizzoni L 2006 Final height in girls with central idiopathic precocious puberty treated with gonadotropin-releasing hormone analog and oxandrolone. J Clin Endocrinol Metab 91:1284-1287
28. Labrie F 2006 Dehydroepiandrosterone, androgens and the mammary gland. Gynecol Endocrinol 22:118-130
29. Andersson-Wallgren G, Albertsson-Wikland K 1994 Change in speaking fundamental frequency in hormone-treated patients with Turner's syndrome--a longitudinal study of four cases. Acta Paediatr 83:452-455

30. Livadas S, Xekouki P, Fouka F, Kanaka-Gantenbein C, Kaloumenou I, Mavrou A, Constantinidou N, Dacou-Voutetakis C 2005 Prevalence of thyroid dysfunction in Turner's syndrome: a long-term follow-up study and brief literature review. Thyroid 15:1061-1066
31. Orr R, Fiatarone Singh M 2004 The anabolic androgenic steroid oxandrolone in the treatment of wasting and catabolic disorders: review of efficacy and safety. Drugs 64:725-750
32. Haeusler G, Frisch H 1992 Growth hormone treatment in Turner's syndrome: short and long-term effects on metabolic parameters. Clin Endocrinol (Oxf) 36:247-253
33. Stahnke N, Stubbe P, Keller E 1992 Recombinant human growth hormone and oxandrolone in treatment of short stature in girls with Turner syndrome. Horm Res 37 Suppl 2:37-46
34. Wilson DM, Frane JW, Sherman B, Johanson AJ, Hintz RL, Rosenfeld RG 1988 Carbohydrate and lipid metabolism in Turner syndrome: effect of therapy with growth hormone, oxandrolone, and a combination of both. J Pediatr 112:210-217
35. Haeusler G, Frisch H, Schmitt K, Blumel P, Plochl E, Zachmann M, Waldhor T 1995 Treatment of patients with Ullrich-Turner syndrome with conventional doses of growth hormone and the combination with testosterone or oxandrolone: effect on growth, IGF-I and IGFBP-3 concentrations. Eur J Pediatr 154:437-444
36. Rosenfeld RG, Hintz RL, Johanson AJ, Sherman B, Brasel JA, Burstein S, Chernausek S, Compton P, Frane J, Gotlin RW, et al. 1988 Three-year results of a randomized prospective trial of methionyl human growth hormone and oxandrolone in Turner syndrome. J Pediatr 113:393-400
37. Apter D, Lenko HL, Perheentupa J, Soderholm A, Vihko R 1982 Subnormal pubertal increases of serum androgens in Turner's syndrome. Horm Res 16:164-173
38. Gravholt CH, Svenstrup B, Bennett P, Sandahl Christiansen J 1999 Reduced androgen levels in adult turner syndrome: influence of female sex steroids and growth hormone status. Clin Endocrinol (Oxf) 50:791-800
39. Zuckerman-Levin N 2007 Androgen replacement therapy in Turner syndrome. Horm Res 68 (suppl 1):18

Supplemental Table 1. Baseline and adult height data per age group and dosage group.*

	Age group 1			Age group 2			Age group 3		
	GH+PI (n = 15)	GH+Ox 0.03 (n = 18)	GH+Ox 0.06 (n = 13)	GH+PI (n = 15)	GH+Ox 0.03 (n = 12)	GH+Ox 0.06 (n = 13)	GH+PI (n = 12)	GH+Ox 0.03 (N = 12)	GH+Ox 0.06 (n = 10)
Age at starting GH – yr†	5.1±1.8	4.6±1.7	5.2±1.6	10.4±1.4	9.3 ±1.2	9.8±0.8	13.6±1.2	13.5±1.2	13.3±1.2
Bone age at starting GH – yr†	5.2±2.2	4.6±2.1	5.0±2.1	10.7±1.5	9.4±1.2	9.8±1.0	11.7±1.2	12.1±0.8	12.5±0.7
Height SDS at starting GH†									
Ref: healthy Dutch girls	-2.7±0.6	-2.8±0.7	-2.8±0.7	-2.7±0.8	-2.9±0.5	-2.9±0.6	-3.5±0.9	-3.4±0.7	-3.2±0.8
Ref: untreated girls with TS	0.0± 0.8	-0.2±0.9	-0.1±0.8	0.8±1.0	0.3±0.6	0.5±0.7	0.8±1.1	0.9±0.9	1.1±0.8
Karyotype – no. (%)†‡									
45,X	9 (60)	10 (56)	6 (46)	9 (60)	4 (33)	5 (39)	6 (50)	3 (25)	5 (50)
Other	6 (40)	8 (44)	7 (54)	6 (40)	8 (67)	8 (62)	6 (50)	9 (75)	5 (50)
Target height SDS†	0.1±0.9	0.2±1.1	-0.2±0.9	-0.2±0.8	0.1±0.9	-0.2±0.5	-0.1±0.7	0.0±1.0	0.6±0.9
Duration of GH therapy – yr†	9.9±1.9	9.8±2.6	8.8±1.9	5.0±1.5	5.5±1.2	5.1±1.1	3.6±0.8	3.5±0.7	2.9±0.7
Duration of Ox/PI therapy – yr†	6.4±0.7	5.5±1.7	5.0±1.7**	5.0±1.5	5.2±1.5	4.6±1.6	3.6±0.8	3.5±0.7	2.7±0.8
Age at discontinuing GH – yr†	15.0±0.9	14.2±0.8	14.0±0.9	15.4±0.7	14.8±0.8	14.9±0.7	17.2±0.8	16.9±0.7	16.2±1.2
Age at last visit – yr †	16.6±0.8	15.9±1.0	16.1±1.0	17.8±1.0	16.9±1.1	17.2±0.7	19.0±1.2	18.4±1.1	18.0±0.9
Adult height – cm†	155.1±5.2	155.3±8.7	155.3±5.5	156.1±5.4	156.6±6.0	157.1±5.8	155.6±6.1	159.2±5.2	157.1±6.4
Adult height SDS†¶									
Ref: healthy Dutch girls	-2.4±0.8	-2.4±1.3	-2.4±0.8	-2.2±0.8	-2.2±0.9	-2.1±0.9	-2.3±0.9	-1.8±0.8	-2.1±1.0
Ref: Northern European girls with TS	1.3±0.8	1.3±1.4	1.3±0.9	1.4±0.8	1.5±0.9	1.6±0.9	1.4±1.0	1.9±0.8	1.6±1.0
Adult height gain – cm‖	9.4±4.4	10.4±5.8	10.0±3.7	6.0±3.6	9.0±4.4	9.0±4.8	5.8±2.6	8.6±3.0	5.4±4.7
Patients in per-protocol analysis – no. (%)†	9 (60)	10 (56)	7 (54)	13 (87)	9 (75)	9 (69)	8 (67)	11 (92)	6 (60)
Adult height gain, per-protocol – cm‖	9.8±5.4	12.8±5.8	11.2±3.9	5.8±3.7	8.4±4.7	9.3±5.6	5.0±2.8	8.3±3.0	5.7±5.8

* Plus-minus values are means ±SD.
† No statistical tests were applied.
‡ Percentages may not total 100 because of rounding.
¶ Adult height SDS was calculated using reference values for 21-year-old girls.
‖ Defined as adult height minus predicted adult height.