X-chromosome gene dosage as a determinant of impaired pre and postnatal growth and adult height in Turner syndrome

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Abstract

Objective: Short stature is a key aspect of the phenotype of patients with Turner syndrome (TS). SHOX haploinsufficiency is responsible for about two-thirds of the height deficit. The aim was to investigate the effect of X-chromosome gene dosage on anthropometric parameters at birth, spontaneous height, and adult height (AH) after growth hormone (GH) treatment.

Design: We conducted a national observational multicenter study.

Methods: Birth parameter SDS for gestational age, height, and AH before and after GH treatment respectively, and height deficit with respect to target height (SDS) were classified by karyotype subgroup in a cohort of 1501 patients with TS: 45,X (36%), isoXq (19%), 45,X/46,XX (15%), XrX (7%), presence of Y (6%), or other karyotypes (17%).

Results: Birth weight, length (P < 0.0001), and head circumference (P < 0.001), height and height deficit with respect to target height (SDS) before GH treatment, at a median age of 8.8 (5.3–11.8) years and after adjustment for age and correction for multiple testing (P < 0.0001), and AH deficit with respect to target height at a median age of 19.3 (18.0–21.8) years and with additional adjustment for dose and duration of GH treatment (P = 0.006), were significantly associated with karyotype subgroup. Growth retardation tended to be more severe in patients with XrX, isoXq, and, to a lesser extent, 45,X karyotypes than in patients with 45,X/46,XX karyotypes or a Y chromosome.

Conclusion: These data suggest that haploinsufficiency for an unknown Xp gene increases the risk of fetal and postnatal growth deficit and short AH with respect to target height after GH therapy.

Introduction

Turner syndrome (TS) is a condition in which all or part of one X chromosome is absent from some or all cells, affecting approximately one in every 2500 liveborn girls (1). It is characterized by growth retardation, with short adult stature and gonadal dysgenesis. It may be associated with a large number of diseases and conditions: physical abnormalities, congenital cardiac/renal malformations, and acquired conditions that may occur at any time in the patient’s life (1, 2, 3, 4, 5, 6, 7, 8). Its genetic basis is highly variable, with diverse causal gene defects.
The most frequently observed karyotypes are 45,X (45–50%) and the mosaic karyotype of 45,X/46,XX (40%). Karyotypes with an isochromosome of X (isoXq or isoXp), Y chromosome, X ring chromosome, and deletions of the X chromosome are less frequent (9).

Short stature is the cardinal finding in girls with TS, affecting more than 95% of patients. This growth deficiency is already well established by the middle of the second trimester of gestation (10). In a French study of 160 patients, 45% of patients were found to have been small for gestational age (SGA), for length and/or weight (11). Height velocity decreases during childhood and the height deficit increases during adolescence due to the absence of a pubertal growth spurt, resulting in a spontaneous adult height (AH) approximately 20 cm shorter than mid-parental height (143 cm in France) (11, 12). Most clinical trials have shown that treatment with recombinant human growth hormone (GH) can yield a mean height gain of about 7 cm, varying with age at treatment initiation and treatment duration (13, 14).

Haploinsufficiency of the SHOX gene is responsible for about two-thirds of the height deficit observed in TS (15). This gene is located within the telomeric part of PAR 1 and escapes X chromosome inactivation (16). Thus, each individual has two functional copies of this gene, one inherited from each parent. Its main effect is to promote differentiation and to stop the proliferation of hypertrophic growth plate chondrocytes. Haploinsufficiency results in the excessive proliferation of these chondrocytes and a premature fusion of the growth plate (17, 18). SHOX gene function is dosage dependent. A heterozygous mutation can cause Léri–Weill dyschondrosteosis, resulting in a mean AH of −2.2 SDS (15). The loss of both alleles causes Langer syndrome, which is associated with a more severe height deficit (16). By contrast, the gain of additional copies is generally associated with tall stature, but the relationship is nonlinear (19, 20). Haploinsufficiency of other genes may also contribute to growth impairment in TS.

The effect of karyotype on growth in TS patients remains a matter of debate, and most studies to date have concerned small series of patients. Some authors have reported more severe spontaneous growth deficits in patients with monosomy of the short arm of the X chromosome than in those with disomy (11, 21, 22, 23, 24, 25, 26). Others found no significant difference in height between several karyotype subgroups (27, 28, 29). AH after GH treatment has been evaluated as a function of karyotype in only one study, and the results obtained were not significant (30).

In this study, we evaluated auxological data at birth, spontaneous postnatal growth, and AH after GH treatment as a function of karyotype subgroup, in a large cohort of patients with TS.

Patients and methods

Patients

This national observational multicenter study included all patients (n=1536) with TS diagnosed up to January 2013 and followed within the French National Rare Disease Network. TS was diagnosed prenatally in 254 patients (17%) and at birth in 220 (14%). Median age (25–75th percentile) at diagnosis for the 1038 patients diagnosed postnatally was 10.0 (6.1–13.3) years. Patients were classified into six subgroups on the basis of karyotype: 45,X (n=549, 36%), isoXq (n=280, 19%), 45,X/46,XX (n=221, 15%), XrX (n=106, 7%), presence of Y (n=87, 6%), and other karyotypes (n=258, 17%). The karyotype was unknown for 35 patients.

Protocol

Growth and karyotype data were collected from medical records, on standardized data collection forms completed retrospectively for the time of TS diagnosis and time points at 5-year intervals thereafter. Birth length (BL), birth weight (BW), and birth head circumference were assessed for patients with a known gestational age (GA) at birth (n=1037). Spontaneous growth during childhood was evaluated in patients treated with GH, immediately before the initiation of this treatment (n=1075). Patients were considered to have reached AH if they were over the age of 18 years, and GH therapy had been stopped and if they were over the age of 17 years and had been on adult combined estrogen and progesterone therapy for more than 1 year after the end of GH therapy (n=527). A flow chart summarizing this information is shown in Fig. 1. Patients received a daily subcutaneous injection of open-label GH at an initial median dose of 0.048 (0.040; 0.054) mg/kg per day, in accordance with current guidelines (http://www.has-sante.fr/portail/upload/docs/application/pdf/pnds_turner_web.pdf, 31)). Puberty began at a median age of 12.0 (11.2; 12.5) years in patients with spontaneous puberty. Median age at the initiation of estrogen therapy was 14.0 (12.9; 15.3) years in patients with induced puberty. The total duration of GH treatment for patients who had achieved AH was 5.8 (3.6; 8.5) years.
The study protocol was approved by the Paris Nord Ethics Review Committee for Biomedical Research Projects (CEERB) (N° 812–029).

**Methods**

The genetic analyses carried out included standard karyotype analyses of more than 20 cells or fluorescence in situ hybridization with about 100 studied cells in 829 patients, karyotype analyses of <20 cells in 243 patients, and the type of analysis carried out was unknown for 464 patients. Birth measurements and postnatal height are expressed as SDS for GA and chronological age, respectively, based on the normative data for the general population at birth (32) and, thereafter, for the general (33) and TS populations (12). SGA was defined as a BL or weight of ≤−2 SDS or less. Target height was calculated as mid-parental height minus 6.5 cm (34). The height deficit was calculated as the difference between the child’s height in SDS and the target height in SDS.

**Statistical analysis**

Data are expressed as medians (25th–75th percentiles) or numbers (%). The five well-defined karyotype subgroups (45,X, isoXq, 45,X/46,XX, XrX, and presence of Y) were compared in Kruskal–Wallis tests for continuous variables with a non-Gaussian distribution, one-way ANOVA or generalized linear models for continuous variables with a Gaussian distribution, or χ²-tests for categorical variables. The patients with other karyotypes (n=258) were not included in the analyses due to the large heterogeneity of this subgroup. The ‘global test’ was used to determine whether there was at least one significant difference between the groups. Comparisons for height and for distance to target height before GH treatment, as a function of karyotype, were adjusted for age. Comparisons for AH and distance to target height after GH treatment, as a function of karyotype, were adjusted for known confounding factors, such as the dose and duration of GH treatment. Bonferroni correction was applied to adjust the significance threshold for multiple comparisons. All tests were two-tailed, and values of $P<0.01$ were considered statistically significant. The analysis was performed with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

**Results**

**Anthropometric parameters at birth**

Median GA at birth was 39 (38–40) weeks, and 10% of the patients with TS were born premature (Table 1). Premature birth appeared more likely for patients with XrX and isoXq karyotypes, but median GA did not differ significantly between karyotype subgroups. Overall, BL SDS was more strongly affected than BW SDS ($−1.16$ vs $−0.90$ SDS) ($P<0.0001$), and the frequency of SGA was 24% for BL and 19% for BW (24 vs 19%; $P<0.0001$). Each of the anthropometric parameters (BL, BW, and birth head circumference, SDS for GA) was found to differ significantly between karyotype subgroups (Table 1). Despite high levels of variability, patients with XrX and isoXq displayed more severe growth retardation at birth, for BW, BL, and head circumference, than patients with other karyotypes. Consequently, the prevalence of SGA was higher among patients with XrX and isoXq, in whom it affected one-third of patients, than among patients with other karyotypes, in whom its frequency range was 13–26%, depending on the karyotype subgroup and the parameter (BL or BW) considered (Fig. 2).

**Spontaneous postnatal growth before GH treatment**

At the time of evaluation, at a median age of 8.8 (5.3; 11.8) years, height and distance to target height (SDS) were...
significantly associated with karyotype subgroup after adjustment for age and correction for multiple testing ($P$ < 0.0001). Patients with XrX, isoXq, and, to a lesser extent, 45,X karyotypes were more strongly affected than patients with mosaicism or the presence of a Y chromosome (Table 2, Fig. 3A).

**AH after GH treatment**

At the time of AH evaluation, at a median age of 19.3 (18.0; 21.8) years, after adjustment for the duration and dose of GH treatment and for multiple testing, AH deficit was greater in patients with XrX, isoXq, and, to a lesser extent, 45,X karyotypes than in patients with mosaicism or the presence of a Y chromosome (Table 2, Fig. 3A).

**Discussion**

This study, one of the largest cohorts of TS patients ever studied, clearly demonstrated an association between karyotype subgroup and spontaneous prenatal and postnatal growth. The median rates of preterm birth and SGA were consistent with previous studies, although higher rates of SGA have been reported (11). However, there appeared to be a slight excess of premature neonates among our patients with XrX and isoXq, and these patients with XrX and isoXq were not included in the analyses due to the heterogeneity of the karyotypes observed.

GA < 37 weeks.

**Table 1** Clinical characteristics at birth of patients with TS ($n = 1501$) and by karyotype subgroup. Data reported in bold highlight differences.

<table>
<thead>
<tr>
<th>Karyotype Subgroup</th>
<th>All ($n = 1501$)*</th>
<th>XrX ($n = 106$)</th>
<th>IsoXq ($n = 280$)</th>
<th>45,X ($n = 549$)</th>
<th>45,X/46,XX ($n = 221$)</th>
<th>Presence Y ($n = 87$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>39 (38-40)</td>
<td>39 (38-40)</td>
<td>39 (38-40)</td>
<td>39 (38-40)</td>
<td>39 (38-40)</td>
<td>39 (38-40)</td>
<td>0.40</td>
</tr>
<tr>
<td>N</td>
<td>1037</td>
<td>77</td>
<td>200</td>
<td>371</td>
<td>153</td>
<td>55</td>
<td>0.04</td>
</tr>
<tr>
<td>Prematurity (%)</td>
<td>108 (10%)</td>
<td>12 (16%)</td>
<td>28 (14%)</td>
<td>28 (8%)</td>
<td>15 (10%)</td>
<td>3 (6%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Birth weight (g) (SDS)</td>
<td>2770 (2460; 3120)</td>
<td>2560 (2215; 2790)</td>
<td>2690 (2340; 3005)</td>
<td>2790 (2500; 3100)</td>
<td>2970 (2580; 3300)</td>
<td>2800 (2505; 3173)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N</td>
<td>1037</td>
<td>77</td>
<td>200</td>
<td>371</td>
<td>153</td>
<td>55</td>
<td>0.04</td>
</tr>
<tr>
<td>Birth length (cm) (SDS*)</td>
<td>47.0 (45.0; 48.0)</td>
<td>46.0 (44.0; 47.0)</td>
<td>46.0 (45.0; 48.0)</td>
<td>47.0 (45.0; 48.0)</td>
<td>48.0 (46.0; 49.3)</td>
<td>47.0 (46.0; 48.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N</td>
<td>1003</td>
<td>75</td>
<td>197</td>
<td>360</td>
<td>151</td>
<td>147</td>
<td>54</td>
</tr>
<tr>
<td>Birth head circumference (cm) (SDS*)</td>
<td>33.0 (32.0; 34.0)</td>
<td>32.5 (32.0; 33.3)</td>
<td>33.0 (31.5; 33.5)</td>
<td>33.0 (32.0; 34.0)</td>
<td>34.0 (32.0; 34.5)</td>
<td>33.3 (33.0; 34.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N</td>
<td>706</td>
<td>59</td>
<td>130</td>
<td>253</td>
<td>110</td>
<td>34</td>
<td>34</td>
</tr>
</tbody>
</table>

*SDS for GA, N, available data. Differences between groups were evaluated by ANOVA, Kruskal-Wallis, or $\chi^2$-tests, as appropriate. Patients with other karyotypes ($n = 258$) were not included in the analyses due to the heterogeneity of the karyotypes observed.

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**Figure 2**

Small for gestational age (%)

- **XX**
- **XrX**
- **IsoXq**
- **45,X**
- **45,X/46,XX**
- **With Y**

Growth parameters were adjusted for birth weight and length. $P$ values were obtained in one-way ANOVA tests (with post hoc Bonferroni correction for multiple comparisons). $P$ values < 0.01 were considered statistically significant.
patients also displayed a significantly more severe growth deficit, affecting BL, BW, and head circumference (expressed as SDS for GA). Previous studies have reported different effects on birth anthropometric data in TS patients (24, 26, 27), and some of these studies have even reported conflicting findings (11, 21, 22, 23), but not all studies (27, 28, 29, 30, 31, 32) and have even reported conflicting results on the association with karyotype subgroup (27), probably due to the limited number of subjects studied. In two studies, BW was shown to be more affected in patients monosomic for the SHOX gene than in patients disomic for this gene, while the SHOX gene was more strongly affected in patients with pseudoautosomal region (38). Some non-p pseudoautosomal region (38). Some non-pseudoautosomal genes have been shown to undergo incomplete inactivation, and some of these candidate genes are likely to contribute to the observed growth phenotype in TS. The effect of the SHOX gene on height is well documented, for both fetal and postnatal growth. However, little is known about the contribution of other genes to growth. It is likely that both fetal and postnatal growth in TS are influenced by the contributions of multiple genes, including the SHOX gene and other genes on the X chromosome, as well as other factors such as environment and lifestyle. The precise role of each gene in the growth phenotype of TS remains to be determined, and further studies are needed to identify the genetic basis of this complex condition.
Further studies are required to determine the causal variants underlying the association between karyotype subgroup and anthropometric parameters in TS, but our observations seem to reflect, at least partly, incomplete dosage compensation for the Xp chromosome. They also suggest that haploinsufficiency for, as yet unidentified, Xp genes increases the risk of a larger AH deficit with respect to TH both before and after GH treatment in patients with TS.

The strengths of this observational cohort study with extensive national coverage include the large number of patients with TS for whom comprehensive data were available. P values < 0.01 were considered statistically significant (Bonferroni correction).

*Patients with other karyotypes (n = 258) were not included in the analyses due to the heterogeneity of the karyotypes observed.

Comparisons for height and distance to target height before GH treatment, as a function of karyotype, were adjusted for age. Comparisons for height and distance to target height after GH treatment, as a function of karyotype, were adjusted for the dose and duration of GH treatment.

Table 3: Adult height after the cessation of GH treatment in patients with TS (n = 527) and according to karyotype subgroup. Data reported in bold highlight differences.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>All (n = 527)</th>
<th>XrX (n = 48)</th>
<th>IsoXq (n = 109)</th>
<th>45,X (n = 224)</th>
<th>45,X/46,XX (n = 47)</th>
<th>Presence Y (n = 36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at the evaluation of adult height (years)</td>
<td>N</td>
<td>19.3 (18.0; 21.8)</td>
<td>18.9 (17.7; 20.9)</td>
<td>19.5 (18.3; 21.8)</td>
<td>19.3 (18.0; 21.9)</td>
<td>20.1 (18.3; 22.8)</td>
<td>20.1 (18.4; 24.1)</td>
</tr>
<tr>
<td>Adult height (SDS based on the general population (34))</td>
<td>N</td>
<td>-2.05 (−2.95; −1.20)</td>
<td>-2.32 (−3.09; −1.59)</td>
<td>-2.23 (−2.98; −1.38)</td>
<td>-2.05 (−2.94; −1.34)</td>
<td>-2.05 (−2.77; −1.00)</td>
<td>-1.34 (−2.23; −0.98)</td>
</tr>
<tr>
<td>Adult height (cm)</td>
<td>N</td>
<td>152 (147; 156)</td>
<td>150 (146; 155)</td>
<td>151 (146; 156)</td>
<td>152 (147; 156)</td>
<td>152 (148; 158)</td>
<td>156 (151; 158)</td>
</tr>
<tr>
<td>Adult height deficit with respect to target height (SDS based on the general population (34))</td>
<td>N</td>
<td>1.75 (1.04; 2.54)</td>
<td>2.23 (1.17; 2.78)</td>
<td>1.94 (1.41; 2.92)</td>
<td>1.74 (1.04; 2.47)</td>
<td>1.54 (0.85; 2.28)</td>
<td>1.07 (0.68; 1.93)</td>
</tr>
<tr>
<td>Adult height deficit with respect to target height (cm)</td>
<td>N</td>
<td>9.5 (5.5; 14.2)</td>
<td>12.5 (6.3; 15.5)</td>
<td>10.8 (8.0; 16.5)</td>
<td>9.5 (5.5; 13.6)</td>
<td>8.8 (4.5; 12.5)</td>
<td>6.0 (2.0; 10.5)</td>
</tr>
</tbody>
</table>

N, available data. P values < 0.01 were considered statistically significant (Bonferroni correction).

Differences between groups were evaluated in Kruskal-Wallis tests.

Adjusted for the dose and duration of GH treatment; adjusted analyses were carried out with general linear models.
collected, making it possible to evaluate infrequent karyotype subgroups, such as the rare XrX subgroup, and to adjust for major confounding factors, including all major birth measurements, according to GA, height deficit with respect to target height, and duration and dose of GH treatment. However, this study also had several inherent limitations, due to its observational and retrospective nature. Karyotyping was carried out on blood samples, but there remains some debate about whether exclusive monosomy X applies to all tissues, as some degree of mosaicism has been observed. However, it is widely accepted that routine karyotype analyses on 20 cells are sufficient to detect mosaicism down to levels of about 5% (9). The sensitivity of the method used to detect mosaicism in our patients should therefore have been adequate. It was also not possible to control for the effects of potential concomitant morbidities inherently associated with TS. However, we believe that such factors were correctly managed, at least postnatally, and that they were thus unlikely to have a major effect on our estimates of association. The relationships described are therefore plausible.

In conclusion, our study provides the first evidence, to our knowledge, of a link between karyotype subgroup and phenotypic variation in both spontaneous fetal and postnatal growth, and growth after GH, in individuals with TS. This association provides insight that could guide further studies to improve our understanding of the pathophysiological pathways, other than that involving the SHOX gene, underlying short stature in TS.

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