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ORIGINAL INVESTIGATION

The physical phenotype of girls and women with Turner syndrome is not X-imprinted

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Abstract Certain behavioral and metabolic aspects of Turner syndrome (TS) are attributed to X-chromosome genomic imprinting. To investigate the possible contribution of imprinting to the physical features of the TS phenotype in live-born individuals, we genotyped the single normal X-chromosome in subjects with TS who all underwent a comprehensive evaluation as part of the NIH genotype–phenotype protocol. All had physical examinations, auxological measurements and imaging of the renal and cardiovascular systems. Absolute height and height as a percent of predicted height was the same in X^M ($n = 56$) and X^P ($n = 23$) subjects that had reached final height and were not growth hormone treated. Interestingly, adult height was significantly correlated with maternal but not paternal heights in both X^M and X^P groups. Neck webbing was found in 35% of the X^M ($n = 133$) and 22% of the X^P ($n = 50$) groups ($P = 0.11$). Renal anomalies were present in 24% of X^M and 25% of X^P groups ($P = 0.9$). Bicuspid aortic valve was found in 26% of X^M and 24% of X^P groups ($P = 0.83$), and any cardiovascular anomaly (abnormal aortic valve, aortic coarctation, elongated transverse aortic arch, anomalous pulmonary venous connection, left superior vena cava) affected 55% of X^M and 52% of X^P groups. Thus, we found no evidence for X-linked genomic imprinting effects on stature or lymphatic, renal or cardiovascular development in TS. Our sample size was sufficient to exclude such effects within 95% confidence limits. We did demonstrate a selective maternal effect on final stature that was independent of X-chromosome origin, suggesting potential autosomal imprinting effects on growth revealed by X monosomy.

Keywords X-chromosome · Congenital heart disease · Aortic valve · Genomic imprinting · Stature · Renal defects · Neck webbing

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Introduction

Turner syndrome (TS) refers to females demonstrating a variety of phenotypic features due to loss of all or a significant part of one sex chromosome and affects ~1/2,000 live female births (Nielsen and Wohlert 1990). Affected individuals may be consistently 45,X or mosaic for 45,X and normal 46,XX or 46XY cell lines, or for 45,X and 46,X*abnX* cell lines. Major physical features include short stature, webbing of the neck, and congenital cardiovascular and renal defects (Lippe 1991). The short stature is the most constant feature of the syndrome, and is largely due to haplo-insufficiency for SHOX, a pseudoautosomal gene located in the p-terminal pseudoautosomal region (PAR1) (Blaschke and Rappold 2000). SHOX haplo-insufficiency may not account for all the TS deficit in stature, however (Ross et al. 2001).

The other major physical stigmata of TS are more variable in expression. Congenital cardiovascular defects include elongated transverse arch (ETA) of the aorta (~50%) (Ho et al. 2004), abnormal aortic valve and coarctation of the aorta, which affect approximately 20 and 10% of patients, respectively (Gotzsche and Krag-Olsen 1994; Ho et al. 2004; Mazzanti and Cacciari 1998; Prandstraller et al. 1999). Other defects include persistent left superior vena cava and partial anomalous venous return (~13% each) (Ho et al. 2004; Bechtold et al. 2004) with septal defects or other anomalies much less common. Renal anomalies including horseshoe kidney and reduplicated collecting systems are found in approximately 25% of patients, and neck webbing has been noted in about 25% as well. The reasons for such variability in phenotype expression are not clear. Phenotypic variability in some studies is at least partly due to study populations including less affected mosaic individuals with significant proportion of normal cells. However, considerable variability remains even in relatively pure 45,X populations. The genetic background obviously affects expression of certain traits, but a differential X-chromosome gene dosage effect based on parental, or genomic imprinting could also contribute to variability in some features of TS. Genomic imprinting is well documented on autosomal sites and disrupted imprinting has been implicated in disorders such as Beckwith–Weidman, Russell–Silver and Prader–Willi syndromes (Katia Delaval 2006).

Recent studies have reported imprinting effects in select aspects of the Turner phenotype (Chu et al. 1994; Hamelin et al. 2006; Skuse et al. 1997; Van et al. 2006). Chu et al. (1994) found that the parental origin of the single normal X-chromosome has an impact on stature and cardiovascular defects and Skuse et al. (1997) noted an effect on social behavior. We recently demonstrated that women with a single maternal X-chromosome (X^M) were prone to excessive visceral adiposity and dyslipidemia (Van et al. 2006). Therefore in the present study we genotyped the single normal X-chromosome in our TS protocol study participants and compared the outcomes of auxology and renal and cardiovascular imaging in X^M versus X^P groups.

Patients and methods

Study subjects

Girls and women with TS were participating in an ongoing intramural NICHD protocol at the NIH Clinical Research Center. Parents or guardians of study subjects and adult subjects signed informed consents and minor subjects signed informed assents approved by the NICHD Institutional Review Board. Criteria for entry into the TS study include a 50-cell karyotype by G-banding, with >70% of cells showing absence or abnormality of the second sex chromosome in girls/women aged 7–70 years. All subjects were off estrogen and growth hormone treatment during the study. We recruited study subjects through NICHD (http://www.turners.nichd.nih.gov/ProtFrNewProtocols.html) and Turner Syndrome Society, USA Website announcements.

Genotyping

We obtained DNA samples from peripheral lymphocytes of study subjects with TS and from peripheral lymphocytes or saliva of their biological parents, when available. The DNA was prepared from saliva using the Oragene DNA Collection Kit (Genetek, Ottowa, Ont). The parental origin of the single normal X was determined by comparing proband and parental DNA polymorphisms using PCR amplification of X-alleles as described (Van et al. 2006) using eight highly polymorphic microsatellite markers distributed along the chromosome: DXS987, DXS1001, DXS1047, DXS1214, DXS1060, DXS1226, DXS8091 (Applied Biosystems ABI PRISM® Linkage Set v2.5). Additional markers including DXS1061, DXS1223, DXS993, and DXS1068 were genotyped as needed to determine the parental origin of the missing X segment in subjects with partial X deletions.

Phenotyping

Each study subject had a detailed physical examination by a study investigator including specific documentation with medical photography on the presence of neck webbing. Height and weight for each subject were measured by NIH Clinical Research Center (CRC) nurses using a SRScale® with a height rod. Predicted adult height based upon familial genetic potential was calculated as follows: $[(\text{f-ht-cm} - 13) + (\text{m-ht-cm})]/2$. Renal anatomy was determined by transabdominal ultrasound. Cardiovascular anatomy was determined by standard two-dimensional echocardiography and magnetic resonance imaging as previously described (Ho et al. 2004; Loscalzo et al. 2005).

Statistics

Continuous data are expressed as means with standard error. Comparisons between group means were by one-way ANOVA with Fisher's protected least significant

difference. Comparisons between proportions used the exact unconditional method for the difference of two proportions. We analyzed the influence of the parental origin of the intact X-chromosome on the final height by using a general linear model with mixed independent variables taking into account age and the parental height. Analyses were performed using Stat View for Windows, version 5.0.1 (SAS Institute Inc., Cary, NC), and StatAct 4 for Windows (2000), Cytel Software Corporation, Cambridge, MA.

Results

Our study group included 50 individuals with X^P and 133 with X^M. The karyotype distributions for each group are in Table 1.

We compared height in subjects 16 years of age and older that had not received growth hormone. (All the patients 16 years of age and up, not getting GH treatment, were on full dose estrogen treatment, and had ceased growth.) Mean heights were nearly identical in the two groups (Table 2). Heights expressed as a percent of predicted adult height and as a percent of maternal height were also equal for the two groups (Table 2). This was true for the whole groups and for 45,X only groups as well (not shown). We found a complex relation between mature TS height and parental heights. Heights in the both X^M and X^P groups were significantly correlated with maternal but not with paternal heights (Fig. 1). The correlation was apparently stronger in the X^P compared with the X^M group (Fig. 1a, b).

We analyzed the influence of the parental origin of the intact X-chromosome on the final height by using a multiple regression model in a group of women and girls with TS who had completed their growth (age ≥ 16), and who had never received GH treatment. Parental origin of the X-chromosome, age, maternal and paternal heights were used as independent variables. This model had $R^2 = 0.22$ and $P = 0.0014$. Parental origin of the X-chromosome had no effect on final height (least squares means of height for X^M ($n = 61$) 147.02 ± 1.03 cm, LSM height for X^P ($n = 27$) 146.90 ± 1.53 cm, $P = 0.950$). However, age (F-ratio 6.20, $P = 0.015$) and maternal height (F-ratio 6.39, $P = 0.014$) were significantly associated with the final height, but the paternal height was not (F-ratio 1.62, $P = 0.207$).

As shown in Table 2, renal anomalies were detected in 24% of X^M and 25% X^P ($P = 0.9$). Neck webbing was present in 35% of X^M and 22% of X^P subjects ($P = 0.11$). An abnormal aortic valve was detected in 26% of X^M and 24% of X^P. Any major cardiovascular anomaly, including bicuspid aortic valve, aortic coarctation, anomalous pulmonary veins, left superior vena cava or ETA of the aorta affected 55% of X^M and 52% of X^P groups. Results were similar when only "pure" 45,X subjects were included in the analyses. For example, 22/72 or 31% of 45, X^M subjects had renal anomalies versus 6/17 or 35% of 45,X^P subjects had these renal defects, $P = 0.71$. Likewise, 42/72 or 58% of 45, X^M subjects had cardiovascular defects (as defined in

Table 1 Karyotype distribution in X^M versus X^P groups

X^M	Karyotype	X^P
72/133(54%)	45,X	17/50 (34%)
17/133(13%)	45,X/46,XiXq	13/50 (26%)
10/133(8%)	45,X/46,XrX	3/50 (6%)
8/133(6%)[a]	45,X/46,XX	5/50 (10%)[a]
3/133(2%)	46,XdelXp	1/50 (2%)
11/133(8%)	46,XiXq	4/50 (8%)
1/133(1%)	46,XderX	0
3/133(2%)	45X/46,XY	0
4/133(3%)	46,XdelXq	0
4/133(3%)	45,X/47,XXX	2/50 (4%)
4/133(3%)	45,X/46,XdelXq	5/50 (10%)
1/133(1%)	45,X/46,Xdel (Xp)	0

The karyotypes were done on at least 50 lymphocytes by high resolution G-banding

[a] Less than 15% of cells were 46,XX

Table 2 Effects of X-chromosome parental origin on Turner phenotype

Stature	X^P (23)	X^M (56)	P
Height (SE), cm	147.4 (1.8)	147.1 (1.1)	0.89
% Predicted height[a]	89.3 (0.7)	89.4 (0.9)	0.98
% Maternal height	90.0 (0.8)	90.1 (0.8)	0.99

Developmental anomalies	X^P (50)	X^M (133)	P	CI[f]
Renal anomaly[b]	12/50 (24%)	33/133 (25%)	0.92	−0.18, 0.15
Webbed neck	11/50 (22%)	47/133 (35%)	0.11	−0.30, 0.03
Abnormal aortic valve[c]	12/50 (24%)	33/128[e] (26%)	0.83	−0.19, 0.14
Any CVS defect[d]	26/50 (52%)	71/128[e] (55%)	0.69	−0.22, 0.13

Stature was evaluated in subjects >15 years that had reached final height and had never been treated with GH. Developmental anomalies were evaluated in all subjects age 7 and older

[a] Predicted height based on average of maternal and (paternal height: 13 cm)

[b] Renal anomalies included single or horseshoe kidney and duplication of collecting system

[c] Includes bicuspid and partially fused aortic valves

[d] Cardiovascular (CVS) includes abnormal aortic valve, aortic coarctation, partial anomalous pulmonary venous connection, left superior vena cava, elongated transverse aortic arch

[e] Inadequate cardiovascular studies on five X^M subjects

[f] Confidence interval on the difference (X^P minus X^M) of proportions

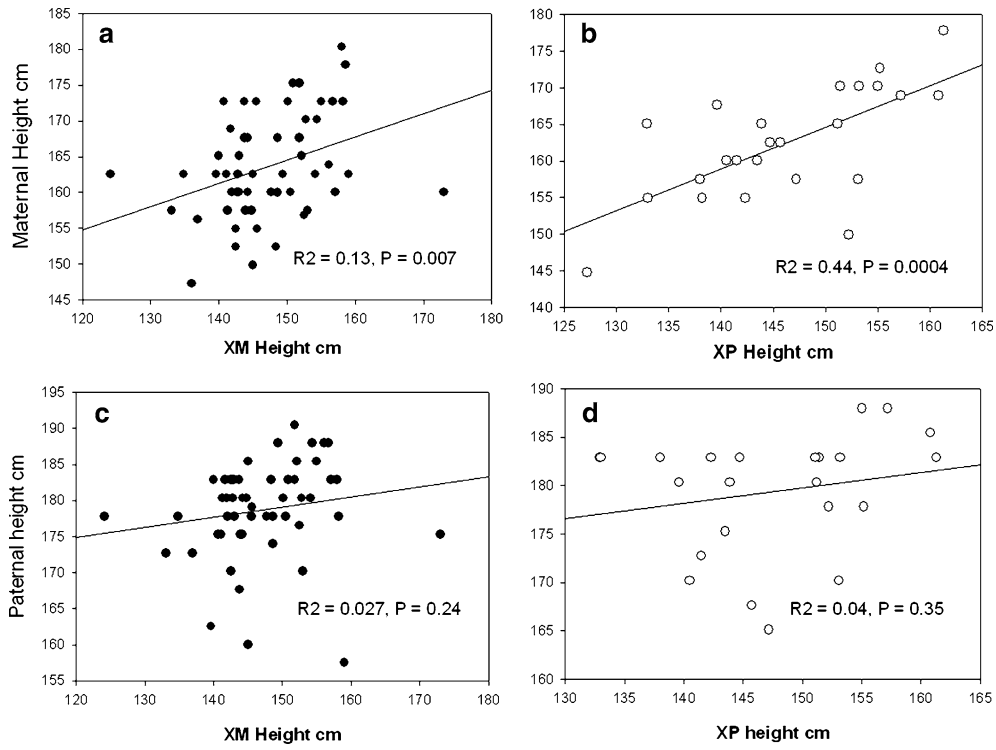

Fig. 1 Correlation between parental height and mature Turner syndrome daughters' height. X^M subjects (*solid symbols*) with maternal (**a**) and paternal (**c**) heights. X^P subjects (*open symbols*) versus maternal (**b**) and paternal (**d**) heights

Table 2) versus 11/17 or 65% of pure 45, X^P subjects, $P = 0.63$.

Discussion

Having genotyped relatively large number of girls and women with TS with respect to the parental origin of the intact X-chromosome, and evaluated their physical features using state of the art imaging, this study demonstrates with a fairly high degree of confidence that X-imprinting does not impact stature or the prevalence of major renal and cardiovascular defects. Previous studies have reported varied results concerning the relation of anatomical TS features and parental origin of the intact X-chromosome, Mathur et al. (1991) found no difference in height, neck webbing or prevalence of renal and cardiovascular anomalies in X^M and X^P groups but they had only seven X^P subjects. Chu et al. (1994) pooled data from all known sources and reported no difference in renal but an increased prevalence of cardiovascular defects and neck webbing in X^M subjects. The present study confirms uniformity of renal effects, but in contrast to Chu et al., we find very similar frequencies of cardiovascular effects in X^M and X^P groups. The likely explanation for the different results is that all the prior studies had very small X^P groups, and relied solely upon echocardiography to detect cardiovascular anomalies. With small sample size and limited diagnostic methodology, the estimates of cardiovascular anomalies in the X^P groups in early studies may not have been very accurate. Our study had a much larger group of X^P individuals and used both echocardiography and magnetic resonance angiography, which adds a great deal to the accuracy and comprehensiveness of cardiovascular imaging (Ho and Prince 1998). We did note a slightly higher prevalence of neck webbing among X^M subjects, but this was not statistically significant. The webbing represents the residua of fetal distension of blind-ended jugular lymphatics forming cystic hygromata, and has been correlated with the presence of cardiovascular defects such as aortic coarctation and bicuspid aortic valve (Loscalzo et al. 2005).

A trait that is regulated by X-linked genomic imprinting should be differentially expressed in eukaryotic males and females, since males are monosomic for X^M while females with random X-inactivation are typically 50:50 mosaic for X^M and X^P cells. For example, boys are at considerably greater risk for deficient social cognition and autistic spectrum disorders than girls. Skuse et al. (1997) have suggested there could be an X-linked gene promoting the development of good social

skills imprinted on X^M and expressed preferentially from X^P—a proposition supported by the finding of poor social skills in TS girls with X^M versus X^P chromosomes and possibly a greater incidence of autism in X^M girls (Donnelly et al. 2000). Normal 46,XY men have a two to threefold increased risk of death from coronary vascular disease compared with 46,XX women. The increased cardiac risk is associated with the preferential accumulation of intra-abdominal or visceral fat in men whereas women usually concentrate fat in subcutaneous depots in hips and thighs. The visceral adiposity leads directly to dyslipidemia, insulin resistance and enhanced atherogenesis. We have shown that the tendency for preferential visceral fat accumulation and atherogenic lipid profile correlates with X^M genotype in TS suggesting that imprinted X-linked genes or loci influence differences in regional fat deposition and lipid metabolism between men and women (Van et al. 2006). It seems unlikely on biological grounds that X-chromosome imprinting would be critically involved in the embryonic development of lymphatic, cardiovascular and renal systems, since these are not sexually dimorphic. It seems more likely that pseudoautosomal or other X–Y common genes are involved in lympho-vascular and renal development, with haplo-insufficiency having a significant impact in some monosomic individuals, with diverse genetic backgrounds contributing to the variable expression of developmental defects.

We found no apparent effect of the parental origin of the single normal X-chromosome on adult stature in TS. We did find a strong correlation between maternal but not paternal height on stature in both X^M and X^P groups. This confirms earlier observations on young girls with TS. Salerno and Job (1987) reported that the height of 64 girls with TS was strongly correlated with the mother and weakly with the father. Chu et al. (1994) reported that heights of X^M but not X^P girls were correlated with their mother, and that neither TS group heights correlated with the paternal height. Hamelin et al. (2006) recently reported imprinting effects on growth hormone responsiveness in girls with TS. Similar to our findings for adults, they reported no height difference at baseline between X^M ($n = 35$) and X^P ($n = 19$) groups with average age of 10 years. Also similar to our data, baseline heights for both X^M and X^P correlated with maternal but not paternal heights. This study did find a difference in response to GH, with the X^M group ($n = 26$) increasing height by 1.1 SD and the X^P group ($n = 9$) by 0.8 SD ($P < 0.05$). One caveat in this latter observation is that ~25% of the X^M group were mosaic girls with an unspecified level of normal cells, while the very small X^P group was entirely 45,X. Thus it is possible that growth was slightly better in the X^M group not so much because they had a single maternal X-chromosome but because they had two normal X-chromosomes in some cells.

Thus, our study confirms in adults' previous observations that height in girls with TS correlates with maternal but not paternal height. This is quite extraordinary, since height is normally equally dependent of both parental factors (Li et al. 2004). Chu et al. (1994) noted this pattern for X^M but not X^P girls, but the very small sample size of the latter group provided little opportunity to detect any correlation. With larger sample sizes, Hamelin et al. (2006) and we found that both X^M and X^P group heights correlated with maternal but not paternal heights. Our study showed a larger effect for the X^P group, while in young girls the effect seems greater in the X^M group (Chu et al. 1994; Hamelin et al. 2006). It is likely that subject age affects the outcome of these studies, since the association between child and parental heights is greatest for adults (Li et al. 2004). Given the varying observations on X^M and X^P growth patterns in young girls with TS, and the report of an enhanced response to GH in X^M girls (Hamelin et al. 2006), the role for an X-imprinting effect on growth patterns in TS remains unclear. The fact that there is no difference in height between X^M and X^P groups in childhood (Hamelin et al. 2006) or adulthood (the present study) suggests that any X-imprinted growth-promoting effect is very slight. It is clear, however, based on consistent observations in this and three prior studies (Chu et al. 1994; Hamelin et al. 2006; Salerno and Job 1987), that the genetic contribution to height attainment in girls with TS is largely if not entirely maternal. The explanation for this unclear, but as a matter of speculation, it is possible that biallelic expression of sex chromosome loci is required to effectuate paternal effects on growth.

In conclusion, in this largest most comprehensive study to date, we find no evidence for X-imprinting of the major physical features in TS, i.e., short stature and renal and cardiovascular defects. Thus, there is no apparent clinical indication for investigation of X-chromosome parental origin in individuals with TS.

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