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# Evidence for a Turner Syndrome Locus or Loci at Xp11.2-p22.1

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## Summary

**Turner syndrome is the complex human phenotype associated with complete or partial monosomy X. Principle features of Turner syndrome include short stature, ovarian failure, and a variety of other anatomic and physiological abnormalities, such as webbed neck, lymphedema, cardiovascular and renal anomalies, hypertension, and autoimmune thyroid disease. We studied 28 apparently nonmosaic subjects with partial deletions of Xp, in order to map loci responsible for various components of the Turner syndrome phenotype. Subjects were carefully evaluated for the presence or absence of Turner syndrome features, and their deletions were mapped by FISH with a panel of Xp markers. Using a statistical method to examine genotype/phenotype correlations, we mapped one or more Turner syndrome traits to a critical region in Xp11.2-p22.1. These traits included short stature, ovarian failure, high-arched palate, and autoimmune thyroid disease. The results are useful for genetic counseling of individuals with partial monosomy X. Study of additional subjects should refine the localization of Turner syndrome loci and provide a rational basis for exploration of candidate genes.**

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## Introduction

Turner syndrome is the phenotype associated with complete or partial monosomy X in human females. The phenotype consists of short stature, ovarian failure, and other variable features, such as webbed neck, aortic coarctation, high-arched palate, low-set ears, increased carrying angle of the elbow (cubitus valgus), hypoplastic nails, multiple pigmented nevi (moles), impaired glucose tolerance, hypertension, autoimmune thyroid disease, and other minor stigmata. The neurocognitive profile is characterized by selective nonverbal deficits usually without global impairment.

The pathogenesis of the Turner syndrome phenotype is complex. Most authors believe that growth retardation, ovarian failure, and other physical abnormalities are separate and distinct genetic effects. Growth failure may result from deficiency of X-linked gene(s), perhaps together with nonspecific effects of aneuploidy. Similarly, ovarian failure may be due to inadequate dosage of X-linked genes (Krauss et al. 1987) and/or incomplete meiotic-chromosome pairing (Burgoyne and Baker 1985). Webbed neck, aortic coarctation, and hypoplastic nails may all be secondary to in utero lymphedema (Lippe 1991). Alternatively, neural crest–cell defects may be the etiology of cardiovascular anomalies, as well as of multiple pigmented nevi (Miyabara et al. 1989, 1997). The pathogenesis of impaired glucose tolerance, hypertension, autoimmune thyroid disease, and cognitive deficits is obscure.

The task of identification of specific genes responsible for Turner syndrome may, at first glance, seem herculean, since the X chromosome encodes probably thousands of genes. However, there are some mitigating factors. Most X-linked genes are subject to X inactivation during early embryogenesis and are thus functionally haploid in both 45,X and 46,XX fetuses during critical developmental stages (Lyon 1961). These genes are thus unlikely to be

involved in the Turner syndrome phenotype, with the possible exception of ovarian failure, since the inactive X is reactivated during oogenesis. Furthermore, it appears that the Y chromosome supplies the second dose of critical Turner syndrome genes in normal males. Therefore, Turner syndrome genes are predicted to be X-Y homologous and to escape X inactivation (Zinn et al. 1993). There are presently only 18 such candidate genes, and this number is unlikely to increase substantially (Lahn and Page 1997). The challenge is to determine which of these genes, acting alone or in concert, cause the Turner syndrome phenotype.

One way of addressing this problem is to compare the phenotypes of individuals missing various portions of one sex chromosome. Previous cytogenetic and molecular studies suggest that most Turner syndrome physical features map to the short arms of the X and Y chromosomes (Ferguson-Smith 1965; Kalousek et al. 1979; Fryns et al. 1981; Goldman et al. 1982; Simpson 1988; Jacobs et al. 1990; Temtamy et al. 1992; Ogata and Matsuo 1995). The 2.6-Mb Xp-Yp pseudoautosomal region would seem likely to play a role in Turner syndrome: X- and Y copies of the region are identical, and all genes within the region appear to escape X inactivation (Rappold 1993). However, short stature is the only clinical finding consistently associated with deletions of just this region (Ballabio et al. 1989; Ogata et al. 1992*a*, 1992*b*; Ogata and Matsuo 1995).

Identification of genes or critical regions responsible for individual Turner syndrome features other than short stature (e.g., renal malformations, palate abnormalities, short fourth metacarpal, strabismus, lymphedema, etc.) has been problematic. Most studies have used only cytogenetic, not molecular, techniques to define X-chromosome abnormalities (Ferguson-Smith 1965; Kalousek et al. 1979; Fryns et al. 1981; Goldman et al. 1982; Simpson 1988; Jacobs et al. 1990; Temtamy et al. 1992; Ogata and Matsuo 1995). The precision and accuracy of cytogenetics may not be adequate for genotype correlations. Mosaicism, frequently present in Turner syndrome patients, may confound karyotype/phenotype associations. Literature reviews with large sample sizes are subject to interobserver variation in phenotypic evaluation. Last, the variability of Turner syndrome features, even among 45,X patients, necessitates statistical methodology for genotype/phenotype correlations.

Our goal in the current study was to improve the phenotype mapping of Turner syndrome. To this end, we assembled a cohort of 28 subjects with partial monosomy for Xp and carefully analyzed their phenotypes and chromosome abnormalities. We also applied a quantitative statistical method for testing the relationships between phenotypes and X-chromosome deletions. Our results provide evidence for the presence of one or more loci in Xp11.2-p22.1 that are involved in growth, ovarian function, and some but not all Turner syndrome physical abnormalities. These results provide a framework for identification of candidate Turner syndrome genes.

## Subjects and Methods

### *Subjects*

This study was approved by the human-studies committees at Thomas Jefferson University and University of Texas Southwestern Medical School. Informed consent and assent was obtained either from the participants or, in the case of minors, from a parent or legal guardian. Prospective subjects were identified on the basis of abnormal karyotypes that included partial Xp monosomy due to either a deletion or an unbalanced translocation. We excluded patients with any of the following: (1) known sex-chromosome mosaicism; (2) X isochromosomes, because of the high likelihood of mosaicism and the possible confounding effects of Xq and proximal Xp duplication (Wolff et al. 1996); (3) ring X chromosomes, since such patients are also likely to have mosaicism because of mitotic instability of rings; and/or (4) autosomal aneuploidy other than unbalanced X;autosome translocations.

### *Cytogenetic and Molecular Analyses*

Repeat karyotypes were obtained for subjects not evaluated cytogenetically during the preceding 2 years. At least 20 cells were examined for mosaicism, for each subject. A blood sample was drawn at the time of clinical evaluation, for molecular-cytogenetic studies. Lymphoblastoid cell lines were derived by standard methods (Gilbert 1995). DNA prepared from 1 ml of whole blood by means of a commercial kit (Promega) was tested for skewed X inactivation, by the androgen receptor–methylation assay (Allen et al. 1992).

We examined metaphase spreads from cultured peripheral blood leukocytes (PBLs), for the presence of either 45,X mosaicism or cryptic X translocations, using FISH with an X-centromere probe (DXZ1) and a whole X-chromosome paint. At least 50 metaphases were examined for each subject. We then mapped deletions by FISH, using single-copy Xp probes and metaphases prepared from PBLs and/or lymphoblastoid cell lines. Every probe detected two loci in >85% of metaphases from control 46,XX cells. Each hybridization included the DXZ1 centromere probe as a control for random X-chromosome loss during preparation of metaphase spreads. A marker was scored as not deleted if two loci were detected in most metaphases. A marker was scored as deleted if two loci were never detected in five or more metaphases. Hybridizations were repeated if necessary, so that at least five metaphases were examined. In no

case was the presence or absence of a marker on the deleted X chromosome ambiguous. Markers were tested sequentially until the closest loci flanking each breakpoint were determined.

### *Phenotype Evaluation*

The evaluation included a careful history and physical examination emphasizing abnormalities associated with Turner syndrome (webbed neck, lymphedema, increased carrying angle, etc.), anthropometric measurements, and appropriate laboratory tests. Twenty-two subjects were evaluated in person by one of us (J.L.R.). Two subjects (SW16 and SW105) were each seen by two geneticists. Four subjects (SW46, SW144, SW145, and SW146) were reported to have no Turner syndrome stigmata. Since we did not personally evaluate these four subjects, we did not include their palate or carrying-angle phenotypes in the statistical analyses.

Heights were measured with a stadiometer. Measured or reported parental heights were also recorded. Mid–parental height adjusted for the child's sex (i.e., target height) was calculated by the formula 0.5 × [father's height (cm) −13 + mother's height (cm)] (Tanner et al. 1970). In seven cases, the deletion was also present in the subject's mother. For these subjects we substituted the mothers' target height (based on mid–parental height of maternal grandparents) for her measured or reported height. Target height LOD score ($Z$) values were calculated from sex-adjusted mid–parental-height data obtained from the National Center for Health Statistics' growth-curve data (Hamill et al. 1979). Mid–parental height was not calculated for one subject, who was adopted.

Cardiac anomalies were documented by ultrasound. Renal anomalies were documented by ultrasound or IVP. Metacarpals were inspected radiographically. The diagnosis of ovarian failure was based on measurements of serum estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels. Ovarian failure was defined by castrate levels of FSH and LH for subjects $\geqslant$10 years of age. Ovarian function in girls <10 years of age was considered indeterminate, and their gonadotropin measurements were repeated periodically during the course of the study.

Webbed neck included redundant skin. Since lymphedema can resolve over time, this feature was considered positive either if it was present on exam or if a history of lymphedema was documented in medical records. Palate abnormalities were noted by inspection and included an inverted U shape or an "ogival" form characterized by a narrow vault with bulging of the lateral alveolar ridges. Subjects were photographed with elbows maximally extended and supinated, to measure carrying angle. Miscellaneous Turner syndrome features (strabismus, multiple pigmented nevi, congenitally dislocated hips or scoliosis, and abnormal nails) were clinically assessed. Thyroid abnormalities were documented by measurement of serum thyroxine (T4) and thyroid-stimulating hormone levels, as well as by thyroid-antibody titers.

### *Statistical Analysis*

Our approach to phenotype mapping of Turner syndrome, a chromosome disorder, was analogous to association studies for Mendelian or complex traits (Lander and Schork 1994). We analyzed subjects' deletions with a set of markers distributed along the short arm of the X chromosome. For each marker, we divided subjects into two groups: those with or without deletion of that marker. Each trait was considered as either a dichotomous variable (e.g., ovarian failure [yes or no]) or a numerically continuous variable (e.g., height).

*Dichotomous variables.*—For each marker, we compared the proportion of individuals with a given trait, in the group with the marker deleted, versus the proportion of individuals with the same trait, in the group without the marker deleted. The comparison between the proportions was based on a simple $Z$ value for the difference of two binomial proportions. We did not use Yates's correction for continuity, which is often suggested for binomial probabilities with small sample sizes (expected frequencies less than five), in order to avoid missing associations.

*Numerically continuous variables.*—We used an unpaired $t$-test to compare the mean of all individuals with the marker deleted versus the mean of all individuals without the marker deleted, for each marker. The strength of the association was estimated from the $Z$ value $t$ score. The $P$ value associated with a specific test would be appropriate for testing one specific marker. However, we tested a series of markers. Thus, the proper $P$ value is actually the observed $P$ value times the number of markers tested. Furthermore, because we analyzed multiple traits, we applied an additional Bonferroni correction for simultaneous multiple inferences. Therefore, the $P$ value that we reported for a specific marker (of a set of $m$ markers) and a specific trait (of a set of $k$ traits) equals the observed $P$ value times $mk$. For our tests (one tailed) at $\alpha = .05$, comparing six markers and five traits, the threshold required for an observed $P$ value to be considered statistically significant was $P < .0017$ ($Z_{.0017} = 2.93$; $t_{.0017,25} = 3.2$).

## Results

### *Subjects*

Subjects who participated in this study are listed in table 1, along with karyotype results from clinical rec-

**Table 1**

**Reported Karyotypes and Chronological Ages of Subjects at the Time of Evaluation**

| Subject | Karyotype | Age (years) |
|---|---|---|
| SW16 | 46,X,der(X)t(X;1)(p11;q44)mat | 17.4 |
| SW46 | 46,X,del(X)(p21) | 33.7 |
| SW71 | 46,X,del(X)(p11.2) | 20.2 |
| SW74 | 46,X,del(X)(p11.21) | 13.2 |
| SW75 | 46,X,del(X)(p11.3) | 12.6 |
| SW80 | 46,X,del(X)(p11.4) | 13.0 |
| SW85 | 46,X,del(X)(p11.2) | 45.2 |
| SW86 | 46,X,der(X)t(X;X)(p11;q24) | 10.7 |
| SW92 | 46,X,del(X)(p11.23) | 4.5 |
| SW93 | 46,X,del(X)(p11.23) | 10.1 |
| SW96 | 46,X,del(X)(p21.2) | 12.9 |
| SW97 | 46,X,del(X)(p21.2) | 12.9 |
| SW103 | 46,X,del(X)(p11.2) | 1.3 |
| SW105 | 46,X,der(X)t(X;1)(p11;q44)mat | 25.4 |
| SW106 | 46,X,del(X)(p22.3) | 7.0 |
| SW109 | 46,X,del(X)(p11.1) | 45.9 |
| SW111 | 46,X,del(X)(p11.21) | 15.2 |
| SW112 | 46,X,del(X)(p11.2) | 10.7 |
| SW122 | 46,X,del(X)(p21.2) | 16.2 |
| SW144 | 46,X,del(X)(p22.12p22.33) | 35.8 |
| SW145 | 46,X,del(X)(p22.12p22.33)mat | 14.2 |
| SW146 | 46,X,del(X)(p22.12p22.33)mat | 12 |
| SW151 | 46,X,del(X)(p11.2) | 7 |
| SW157 | 46,X,del(X)(p22.31p22.33) | 41.5 |
| SW161 | 46,X,del(X)(p11.23) | 8.6 |
| SW174 | 46,X,der(X)t(X;A)(p22.3;p11.2)mat[a] | 21.9 |
| SW175 | 46,X,der(X)t(X;A)(p22.3;p11.2)[a] | 39.7 |
| SW190 | 46,(X),del(X)(p22.1) | 40 |

[a] A = acrocentric chromosome positive for NOR staining and beta satellite but not alpha satellite sequences by FISH.

ords and chronological age at the time of evaluation for this study. Subjects were Caucasian (23), African American (2), Hispanic (2), and Asian (1) and had an age range of 1.3–45.2 years. SW16/105, SW96/97, and SW145/146 were sib pairs. SW144 was the mother of SW145 and SW146; SW175 was the mother of SW174. Other subjects were unrelated.

### *Molecular Cytogenetics*

Markers spaced at ~10-Mb intervals along the 60-Mb X-chromosome short arm (table 2) were tested initially. Additional markers were then tested to resolve clustered breakpoints but were not included in statistical analyses. DXYS14, situated <100 kb from the Xp/Yp subtelomeric repeats, distinguished terminal versus interstitial deletions.

The molecular-cytogenetic breakpoints are depicted graphically in figure 1. Karyotypes were generally accurate. We did find that subjects SW144, SW145, and SW146 had either a terminal Xp deletion or a cryptic X translocation, rather than an interstitial Xp deletion. Conversely, subject SW190 had an interstitial Xp deletion that was thought, on the basis of karyotyping, to be terminal. The largest group of breakpoints clustered within a few megabases of the X centromere. We were able to resolve several cytogenetically similar breakpoints by using molecular markers. For example, the Xp11.2 breakpoints of SW71, SW85, and SW103 could be distinguished by our FISH probes.

The X-inactivation pattern in peripheral blood leukocytes from 21 subjects was also examined, by means of the androgen-receptor gene–methylation assay (see Subjects and Methods). This assay measures the presence of methylated cytosine residues flanking a highly polymorphic trinucleotide repeat in the X-linked androgen-receptor gene. The presence of methylation reflects inactivation of an androgen-receptor allele and its chromosome. Twenty subjects were informative for the androgen-receptor polymorphism. Of these 20, 18 showed completely skewed inactivation, with evidence for methylation of only one androgen-receptor allele (presumably the allele borne by the deleted X chromosome, because

**Table 2**

**Molecular-Cytogenetic Markers**

| Locus[a] | Position[b] (Mb) | Band | Probe | Source |
|---|---|---|---|---|
| DXYS14 | 0 | Xp22.33 | c11D2 | G. Rappold |
| DXS7470 | 9 | Xp22.32 | b9P2 | Research Genetics |
| PDHA1 | 20 | Xp22.13 | cPDH3 | H. Dahl |
| DXS274 | 23 | Xp22.13 | ICRFc104A0359 | RLDB (Zehetner and Lehrach 1994) |
| GK | 33 | Xp21.3 | ICRFc104E07137 | RLDB |
| DXS1110 | 40 | Xp11.4-p21.1 | b227D11 | Research Genetics |
| SYN1 | 47 | Xp11.3 | cSY11 | T. Südhof |
| SYP | 50 | Xp11.23 | c38-10 | T. Südhof |
| DXS423E | 54 | Xp11.22 | ICRFc100H0164 | RLDB |
| ZXDB | 58 | Xp11.21 | ICRFc104A07135 | RLDB |
| DXZ1 | 60 | Xcen | pBAMX7 | H. Willard |

[a] Data from markers that are underlined were used for statistical analyses.
[b] From the Sixth International Workshop on X Chromosome Mapping (Nelson et al. 1995).

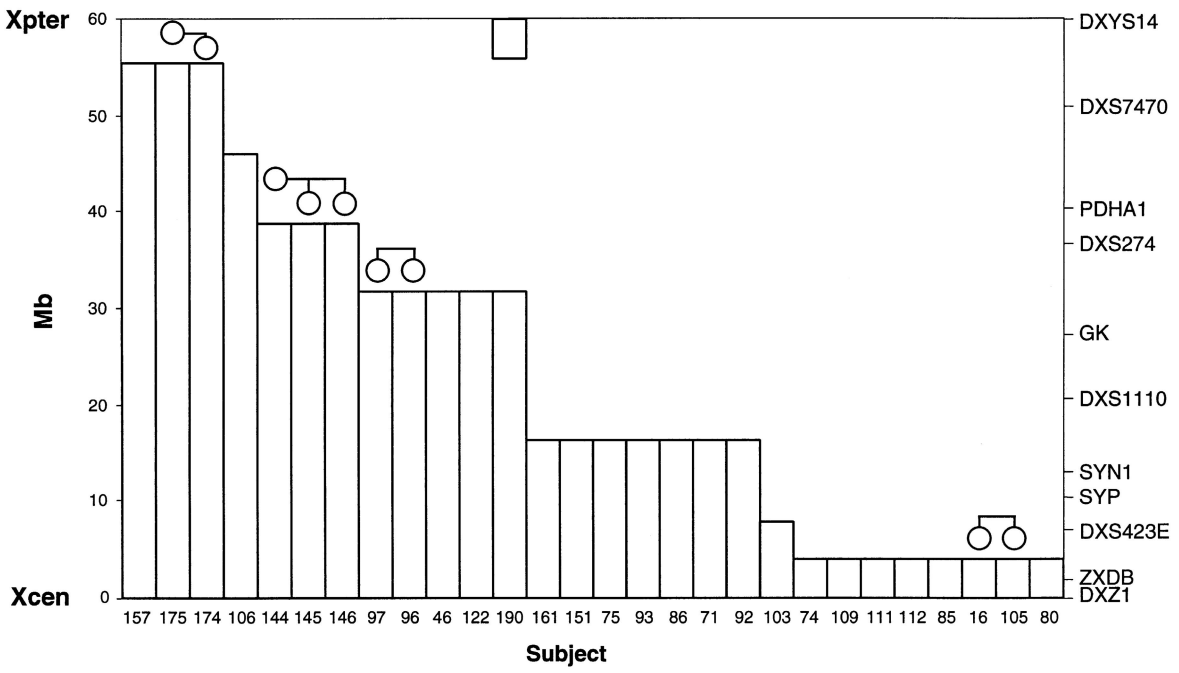


**Figure 1** Graphic representation of deletions. Bars represent material present in deleted X chromosomes. Positions of molecular-cytogenetic markers are indicated.

of selection against cells in which the normal X is inactivated [Migeon 1998]) (data not shown). The two subjects without complete skewing, SW174 and SW175, had a very small deletion.

*Phenotype Mapping*

Table 3 shows the presence or absence in our subjects of five Turner syndrome–associated phenotypes: short stature, high-arched palate, increased carrying angle, thyroid antibodies, and ovarian failure. All subjects were evaluated as described in Subjects and Methods. In general, but not always, the phenotype was similar in relatives with the identical deletion. For example, ovarian failure was apparently absent in SW97 and was present in her sister, SW96. Siblings SW16 and SW105 were discordant for thyroid autoimmunity and the presence of a high-arched palate. Phenotypic features that we did not personally evaluate or that were not objectively documented in medical records were not scored. Missing data were not used in statistical analyses.

Certain Turner syndrome–associated abnormalities were present in so few subjects that we did not attempt statistical analysis of the loci involved. These included horseshoe kidney (1 subject), aortic coarctation (0), webbed neck (0), and lymphedema (2). Other minor Turner syndrome stigmata, including multiple pigmented nevi, short 4th or 5th metacarpal, abnormal nails, strabismus, congenitally dislocated hips, and scoliosis, were seen in a number of subjects but showed no apparent relationship to the size or position of deletions (data not shown). We focused our statistical analysis on the following five phenotypic traits: short stature, high-arched palate, increased carrying angle, thyroid antibodies, and ovarian failure.

We tested the association between these traits and the presence or absence of specified Xp markers (DXYS14, DXS7470, PDHA1, GK, DXS1110, and SYP) that are spaced at ~10-Mb intervals (Nelson et al. 1995). Table 4 shows the results for stature (adjusted for mid–parental or target height). No comparison was made for the most distal marker (DXYS14), since only one subject did not have this marker deleted. Subjects with deletion of DXS7470 or PDHA1 tended to have lower mean heights than were seen in subjects not deleted for these markers, although the differences were not statistically significant. By contrast, the mean stature of subjects deleted for GK was almost 2 SDs less than that of subjects not deleted for this marker. This difference was statistically significant. No breakpoints fell between GK and DXS1110, so the statistical results for these two markers were identical. The decrease in mean stature SDs that was associated with deletion of SYP, although still statistically significant, was less than that associated with deletion of GK/DXS1110.

We performed similar analyses for ovarian failure, abnormal palate, and autoimmune thyroid disease. In these

**Table 3**

**Phenotypic Data**

| Subject | SD from Mid–Parental Height[a] | Palate[b] | Carrying Angle[b] | Thyroid Antibodies | Ovarian Failure?[a] |
|---|---|---|---|---|---|
| SW75 | −2.2 | High arched | Markedly increased | Absent | No |
| SW74 | −3.3 | High arched | Mildly increased | Present | No |
| SW93 | −3.5 | High arched | Mildly increased | Present | Yes |
| SW97 | −.4 | High arched | Mildly increased | Absent | No |
| SW86 | −1.2 | High arched | Mildly increased | Absent | Yes |
| SW106 | −1.6 | Normal | Markedly increased | Absent | ND |
| SW96 | −1.2 | Normal | Mildly increased | Absent | Yes |
| SW109 | −4.7 | High arched | Mildly increased | Absent | Yes |
| SW175 | −1.82 | Normal | Mildly increased | Absent | No |
| SW174 | −.8 | Normal | Normal | Absent | No |
| SW161 | −3.3 | High arched | Mildly increased | Present | ND |
| SW111 | −3.3 | High arched | Mildly increased | Present | Yes |
| SW112 | −3.9 | Ogival | Mildly increased | Present | Yes |
| SW46 | −.3 | (Normal) | (Normal) | Absent | No |
| SW85 | −3.4 | Ogival | Mildly increased | Absent | Yes |
| SW122 | −2.7 | Normal | Mildly increased | Absent | Yes |
| SW151 | −2.5 | High arched | Normal | Present | ND |
| SW103 | −2.7 | Ogival | Mildly increased | Absent | Yes |
| SW146 | −.4 | (Normal) | (Normal) | Absent | No |
| SW144 | −.6 | (Normal) | (Normal) | Absent | No |
| SW145 | −1.4 | (Normal) | (Normal) | Absent | No |
| SW71 | −2 | Normal | Markedly increased | Absent | Yes |
| SW157 | −.9 | Normal | Mildly increased | Absent | No |
| SW190 | .8 | Normal | Mildly increased | Absent | No |
| SW105 | −3.3 | Normal | Normal | Present | Yes |
| SW16 | −3.5 | High arched | Normal | Absent | Yes |
| SW92 | −2.4 | Ogival | Mildly increased | Present | Yes |
| SW80 | ND | High arched | Markedly increased | Present | Yes |

[a] ND = not determined (see text).
[b] Phenotypes in parentheses were by report only and were not used in statistical analyses.

cases, the phenotypes were considered as dichotomous variables. The results are shown in tables 5–7. There was statistically significant evidence for a locus or loci for gonadal dysgenesis and abnormal palate in the same interval spanning GK–DXS1110. The data also indicate a possible locus for autoimmune thyroid disease within the same interval, although the strength of this association did not achieve statistical significance. By contrast, we did not find any association between increased carrying angle and deletion of any marker (data not shown). Figure 2 shows a graphic summary of the associations between these five traits and the presence or absence of the six markers.

## Discussion

Our key finding is that a locus (or loci) for short stature, ovarian failure, and high-arched palate maps to one region of the X chromosome, Xp11.2-p22.1. Our data also suggest a possible locus for autoimmune thyroid disease in this same region, although this result was not statistically significant. The critical region comprises approximately one-fifth of the X chromosome and is consistent with previous inferences from karyotype/phenotype correlations (Ferguson-Smith 1965; Fraccaro et al. 1977; Kalousek et al. 1979; Fryns et al. 1981; Goldman et al. 1982; Simpson 1988; Jacobs et al. 1990; Massa et al. 1992; Temtamy et al. 1992; Ogata and Matsuo 1995).

Our data suggest that a stature determinant is situated between markers PDHA1 (Xp22.1) and SYP (Xp11.2). Weaker associations between stature and deletion of markers that are more distal (e.g., DXS7470) or more proximal (e.g., SYP) can be explained by the fact that deletions were contiguous and spanned more than one interval. For example, all subjects missing SYP were also missing the markers, GK/DXS1110, that were maximally associated with short stature. Conversely, not every subject missing GK/DXS1110 was also missing SYP. Those subjects missing GK/DXS1110 but not SYP would weaken the association between stature and SYP.

A more distal short-stature gene, *SHOX,* has recently been identified in the Xp-Yp pseudoautosomal region (Rao et al. 1997). Only one of our subjects was not deleted for the pseudoautosomal region, and she had normal stature. FISH with a cosmid probe (not shown)

**Table 4**

**Mean ± SD *Z*-Score for Mid–Parental Height or Target Height**

| Marker | Position | Mean ± SD *Z*-Score for Parent-Adjusted Height (*n*), for Deleted Marker | Mean ± SD *Z*-Score for Parent-Adjusted Height (*n*), for Marker Not Deleted | Difference in Means | *t* | df |
|---|---|---|---|---|---|---|
| DXYS14 | 0 | −2.20 ± 1.25 (26) | +.80 ± .00 (1) | 3.00 | ... | |
| DXS7470 | 9 | −2.21 ± 1.38 (24) | −1.17 ± .55 (3) | 1.04 | 1.27 | 25 |
| PDHA1 | 20 | −2.23 ± 1.41 (23) | −1.28 ± .50 (4) | .96 | 1.33 | 25 |
| GK | 33 | −3.01 ± .86 (15) | −.94 ± .89 (12) | 2.07 | 6.13 | 25 |
| DXS1110 | 40 | −3.01 ± .86 (15) | −.94 ± .89 (12) | 2.07 | 6.13 | 25 |
| SYP | 50 | −3.51 ± .58 (8) | −1.49 ± 1.11 (19) | 2.02 | 4.82 | 25 |

confirmed the presence of two copies of *SHOX* in this subject. By comparison, the mean height SDs of subjects missing DXYS14 (*SHOX*) was −2.2.

Interestingly, the association of short stature and deletion of Xp22.1-p11.2 was not statistically significant when stature was not adjusted for mid–parental height (data not shown). Other studies have shown that correlation with mid–parental height is preserved in Turner syndrome (Brook et al. 1977). Thus, this haploinsufficient effect of Xp loci can be modified by genetic background. Subjects with the largest Xp deletions had height deficits essentially equivalent to that typically seen in 45,X Turner syndrome, suggesting that Xq loci play little or no role in growth failure. However, most of our subjects were ascertained because of short stature, and therefore the effect of Xp deletions on stature may be overestimated.

Data from previous studies indicate that ovarian failure is frequent in women with deletions of either Xp or Xq. Some have interpreted this finding to imply that the primary Turner syndrome ovarian defect involves meiotic-chromosome pairing (Ogata and Matsuo 1995). To the contrary, autopsy studies have indicated that germ cells are deficient in 45,X fetuses even prior to the pachytene stage, when chromosomes pair (Speed 1986). Our data suggest a phenotypic difference between distal deletions, associated with preserved ovarian function, and proximal deletions, associated with ovarian failure. The correlation is imperfect, since at least one subject missing almost all of Xp still had functioning ovaries. Until long-term follow-up studies have been conducted, we cannot rule out the possibility that our subjects with preserved ovarian function will suffer premature menopause. Another limitation of our data is that ovarian failure, defined as castrate levels of gonadotropins, cannot be reliably determined in young children, such as were three of our subjects.

One candidate gene within the critical region for ovarian failure is *ZFX*, which maps to Xp21.2. *ZFX* encodes a transcription factor of unknown function (Page et al. 1987); inactivation of the orthologous gene in mice causes growth retardation and reduced germ-cell number (Luoh et al. 1997). The hypothesis that *ZFX* haploinsufficiency contributes to ovarian failure could be tested by mutational analysis of 46,XX women with premature ovarian failure.

The localization of a high arched–palate determinant to Xp11.2-p22.1 was unexpected. High-arched palate, seen in many chromosome disorders, is generally considered a nonspecific aneuploidy effect. It may be that haploinsufficiency of one or a few genes is the major etiologic factor for high-arched palate associated with Turner syndrome, as appears to be the case for short stature. By contrast, there does not appear to be a major Xp locus for increased carrying angle of the elbow. The absence of a locus for cubitus valgus was surprising, since this classic trait is a relatively specific feature of monosomy X. It is possible that a gene(s) outside the critical region—for example, *SHOX*—contributes to this trait. Our finding that some Turner syndrome traits were associated with deletion of the critical region whereas others were not argues that the associations were not due to occult 45,X mosaicism.

Autoimmune thyroid disease is present in approximately one-third of our 45,X Turner syndrome patients (J. L. Ross, unpublished observation). This phenotype may be difficult to assess, because antibodies tend to develop with increasing age, and different antibodies (peroxidase, microsomal, or thyroglobulin) are measured by various laboratories. Our data suggest that hap-

**Table 5**

**Ovarian-Failure Trait**

| Marker | Position | No. with Trait/No. without Trait, for Deleted Marker | No. with Trait/No. without Trait, for Marker Not Deleted | Delta Proportion | *Z* |
|---|---|---|---|---|---|
| DXYS14 | 0 | 14/10 | 0/1 | .583 | .123 |
| DXS7470 | 9 | 14/8 | 0/3 | .636 | 1.463 |
| PDHA1 | 20 | 14/8 | 0/3 | .636 | 1.463 |
| GK | 33 | 12/2 | 2/9 | .675 | 2.971 |
| DXS1110 | 40 | 12/2 | 2/9 | .675 | 2.971 |
| SYP | 50 | 8/1 | 6/10 | .514 | 2.065 |

**Table 6**

**High Arched–Palate Trait**

| Marker | Position | No. with Trait/No. without Trait, for: Deleted Marker | Marker Not Deleted | Delta Proportion | Z |
|---|---|---|---|---|---|
| DXYS14 | 0 | 15/8 | 0/1 | .652 | .264 |
| DXS7470 | 9 | 15/6 | 0/3 | .714 | 1.753 |
| PDHA1 | 20 | 15/5 | 0/4 | .750 | 2.263 |
| GK | 33 | 14/2 | 1/7 | .750 | 3.130 |
| DXS1110 | 40 | 14/2 | 1/7 | .750 | 3.130 |
| SYP | 50 | 8/1 | 7/8 | .422 | 1.633 |

loinsufficiency of a locus in Xp11.2-p22.1, perhaps the same locus as that causing growth retardation, ovarian failure, or high-arched palate, also contributes to immune dysregulation. One speculative hypothesis is that these features are all manifestations of selective impairment of cellular growth during development.

Other typical Turner syndrome features, such as aortic coarctation or webbed neck, that are relatively common in 45,X patients were notably absent from our subjects. Lymphedema has been reported in 60% of 45,X newborns but in only 10% of Turner syndrome patients with other karyotypes (Sybert 1995). In our series, only one subject each had lymphedema or horseshoe kidney. One reason for the paucity of these features could be that deletion of Xq is required for their complete expression.

We considered the possibility that imprinting or X inactivation might play a role in the phenotypic variability of our subjects. Imprinting could confound apparent relationships between deletions and phenotypes, unless the parental origins of the deleted chromosomes were factored into analyses. However, several previous reports, including a recent study describing a putative imprinted Turner syndrome cognitive locus (Skuse et al. 1997), indicate that imprinting plays no role in Turner syndrome physical features (Mathur et al. 1991). In other studies, we also failed to find any phenotypic differences due to parent-of-origin effects in 30 45,X subjects (J.L. Ross, H. Kushner, A.R. Zinn, unpublished data). Therefore, imprinting is unlikely to be important in the present study of Turner syndrome physical features. X-inactivation differences are also unlikely to have contributed to phenotypic variability of our subjects. All subjects tested, except for two with very small deletions, showed completely skewed inactivation, as is the general rule for partial monosomy X (Belmont 1996). The deletions in these two related women are 5–9 Mb in size (B. Franco, personal communication), which is consistent with inferential data from clinical manifestations of X-linked disorders in female carriers, which suggest that terminal Xp deletions $\leqslant$~15 Mb in size do not cause highly skewed X inactivation (Schaefer et al. 1993).

We approached phenotype mapping of Turner syndrome with the hypothesis that at least some features are due to haploinsufficiency of discrete loci on the X chromosome (Epstein 1988). Although this approach had been tried previously, earlier studies lacked precise phenotype descriptions, accurate molecular-cytogenetic definition of deletions, and sufficient numbers of subjects to permit statistical analysis. In our study, we attempted to address each of these shortcomings. One limitation to our data is that breakpoints were not uniformly distributed: 8 of 28 subjects were missing >80% of Xp. This distribution may reflect clinical ascertainment through the presence of major Turner syndrome features, which are more likely with larger deletions. Alternatively, there could be a propensity for the X chromosome to break near the centromere, because of repetitive sequences or other chromatin features.

Because of the difficulties in data collection, our statistical analysis also has limitations. First, the number of subjects in each comparison (deleted and undeleted) is small. Second, since some of our subjects are related, the statistical independence assumption underlying the *t* and binomial-comparisons tests does not hold. However, we did not eliminate redundant or minimally informative markers—for example, 6K/DXS1110 or DXYS14—so our correction for multiple inferences was conservative. As more subjects become available, we will not have to include related individuals, and the power to detect phenotypic differences between groups thereby will be increased.

Our data should be helpful for genetic-counseling in cases of partial monosomy X, heretofore a prognostic quandary. The results also provide the basis for future investigations aimed at molecular identification of Turner syndrome genes. The availability of additional subjects should allow us to refine the map location of Xp genes associated with specific traits and to determine whether the associations are manifestations of haploinsufficiency for a single gene or gene cluster. Guided by this information, mutational analysis of karyotypically normal individuals with Turner syndrome traits may re-

**Table 7**

**Autoimmune Thyroid–Disease Trait**

| Marker | Position | No. with Trait/No. without Trait, for: Deleted Marker | Marker Not Deleted | Delta Proportion | Z |
|---|---|---|---|---|---|
| DXYS14 | 0 | 9/18 | 0/1 | .333 | .389 |
| DXS7470 | 9 | 9/16 | 0/3 | .360 | .607 |
| PDHA1 | 20 | 9/15 | 0/4 | .375 | .909 |
| GK | 33 | 9/7 | 0/12 | .563 | 2.745 |
| DXS1110 | 40 | 9/7 | 0/12 | .563 | 2.745 |
| SYP | 50 | 5/4 | 4/ 15 | .345 | 1.393 |

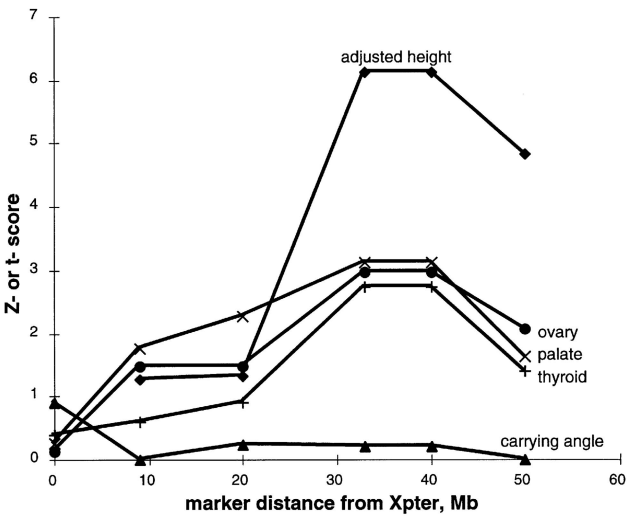


**Figure 2** Strength of associations between deleted markers and five phenotypic traits: adjusted height, ovarian failure (ovary), high-arched palate (palate), thyroid autoantibodies (thyroid), and increased carrying angle of the elbow (carrying angle). A score of "0" indicates that there is no association. Scores >~3 are statistically significant (see Subjects and Methods).

veal the culprit genes. The statistical approach that we have used should be useful for phenotype mapping not only of Turner syndrome but also of other aneuploidy syndromes, such as trisomy 21, for which accurate molecular-cytogenetic and phenotypic data are available (Epstein et al. 1991; Korenberg et al. 1994).

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