Analysis of the origin of Turner's syndrome using polymorphic DNA probes

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Abstract

Thirty-four families with a child or fetus with Turner's syndrome were studied using a series of polymorphic DNA probes. Analysis of the origin of the normal X chromosome was possible in all cases. In 16 families with 45,X (four fetuses and 12 livebirths), the observed X was maternal in each case, indicating a preferential loss of the paternal sex chromosome at, or before, conception. In the remaining 18 families with a variety of karyotypes, but especially in those where the child had an isochromosome of Xq or a ring X, there was again a strong tendency for the normal X to be maternal. Analysis of parental ages was performed with known origin of each abnormality, but no evidence for an increased or decreased parental age effect was detected.

Turner's syndrome is one of the most common chromosomal abnormalities with an estimated frequency at conception of 1.5%. Most affected fetuses are, however, spontaneously aborted and the residual birth frequency is 1 in 2500 to 5000 females.¹ In a large consecutive study the majority of fetuses were found to have monosomy X (45,X) with 45,X/46,XX in the remainder, whereas in the same study, among liveborn Turner's syndrome patients, 53% were 45,X, 14.8% were mosaic 45,X/46,XX, 9% were 46,Xi(Xq)/45,X, 4.9% were mosaic 47,XXX/45,X, and the remaining 9.7% showed five other karyotypes (46,XXq-, 46,XXp-, mosaic 46,XX/45,X, + frag, mosaic 45,X/46,Xr(X), and mosaic 48,XXXX/45,X).²

Early analyses of the origin of Turner's syndrome used the Xg blood group, but these studies were not

applicable to cultured cells from affected fetuses, were only informative in a proportion (31%) of families, and were biased in that informative families were more frequent for 45,X^m than for 45,X^p.^{3,4} The development of multiple polymorphic DNA markers for the X chromosome offers an alternative approach which should avoid these difficulties. Hassold *et al*^{5,6} used this alternative approach to study 40 families with Turner's syndrome where the majority were spontaneously aborted fetuses, and were able to identify the parental origin in 35 (88%) of their families. We describe our results from a study of 34 families in which the majority were live born and where the origin of the abnormality has been determined for a variety of karyotypic abnormalities.

Patients and methods

ASCERTAINMENT OF CASES

Families for this study were recruited from the records of the West of Scotland Regional Genetics Service and with the assistance of the United Kingdom Child Growth Foundation.

CYTOGENETIC ANALYSIS

Chromosomal analysis was undertaken using standard cytogenetic techniques on peripheral blood lymphocytes or cultured products of conception. Two families where the proband had Turner's syndrome but 45,X/46,XY (that is, a paternal defect) were excluded from DNA analysis.

DNA ANALYSIS

DNA was extracted from cultured fetal cells in the case of an affected fetus or from peripheral blood lymphocytes of affected children and of both parents. DNA samples were digested with the appropriate restriction enzymes under the conditions specified by the manufacturers. The resulting fragments were fractionated on 0.8% agarose gels and Southern blotted onto Hybond-N (Amersham) followed by hybridisation with radiolabelled DNA probes. The probes used were St14.1 (DXS52), L1.28 (DXS7), XJ1.1 (DXS206), p198 (MIC2), L754 (DXS84), pERT87.1 (DXS164), p99.6 (DXS41), F8C, 781

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Table 1 Parental origin of the normal X chromosome.

Ped No	Clin stat	Mat age	Pat age	Karyotype	Parental origin of normal X (informative probe)
7751	F	24.4	25.8	45,X	Maternal (St14.1)
7376	F	NA	NA	45,X	Maternal (St14.1)
7780	F	19.3	NA	45,X	Maternal (St14.1)
7962	F	27.5	30.7	45,X	Maternal (St14.1)
1125	LB	27.5	28.5	45,X	Maternal (St14.1)
2395	LB	33.7	34.2	45,X	Maternal (St14.1)
4278	LB	NA	NA	45,X	Maternal (St14.1)
4769	LB	23.4	25.7	45,X	Maternal (St14.1)
5212	LB	31.5	29.5	45,X	Maternal (St14.1)
8437	LB	37.6	41.7	45,X	Maternal (St14.1)
9018	LB	27.5	26.3	45,X	Maternal (St14.1)
9020	LB	33.5	34.1	45,X	Maternal (St14.1)
9039	LB	17.7	NA	45,X	Maternal (St14.1)
9051	LB	26.9	30.3	45,X	Maternal (St14.1)
9447	LB	NA	NA	45,X	Maternal (St14.1)
9533	LB	NA	NA	45,X	Maternal (754)
0618	LB	28.7	29.2	46,Xi(Xq)	Maternal (p19B)
9378	LB	22.5	27.6	46,Xi(Xq)	Maternal (St14.1)
3515	LB	22.3	25.2	46,Xi(Xq)	Paternal (St14.1)
7398	LB	29.8	28.9	45,X/46,Xi(Xq)	Paternal (87.1)
9019	LB	25.3	26.8	45,X/46,Xi(Xq)	Maternal (St14.1)
9029	LB	26.8	30.5	45,X/46,Xi(Xq)	Maternal (St14.1)
9288	LB	30.3	35.6	45,X/46,Xi(Xq)	Maternal (p20)
1241	LB	30.5	28.5	45,X/46,Xr(X)	Paternal (782)
1349	LB	20.5	23.5	45,X/46,Xr(X)	Paternal (XJ1.1)
3171	LB	27.7	30.3	45,X/46,Xr(X)	Maternal (St14.1)
3927	LB	22.5	24.3	45,X/46,Xr(X)	Maternal (St14.1)
5879	LB	32.7	36.1	45,X/46,Xr(X)	Maternal (L128)
8438	LB	24.2	26.5	45,X/46,Xr(X)	Maternal (99.6)
9017	LB	26.6	24.6	45,X/46,Xr(X)	Maternal (F8A)
9316	LB	NA	NA	45,X/46,Xr(X)	Maternal (F8A)
3805	LB	19.4	23.3	45,X/46,XX	Paternal (St14.1)
9401	LB	31.3	30.4	45,X/46,XX	Paternal (St14.1)
3517	LB	26.7	26.7	46,XXp-	Paternal (754)

NA=not available, F=fetus, LB=livebirth.

(DXS120), and p20 (DXS324).⁷ The initial screening was performed using St14.1. Those families uninformative with this probe, or with chromosomal rearrangements, were subsequently screened with further short and long arm probes. In addition, the cytogenetically non-mosaic patients were screened with Y chromosome specific probes, GMGY1 (DYS12) which maps to the long arm of the Y chromosome, and GMGY7 (DYS58) which is a short arm probe.

PARENTAL AGE ANALYSIS

Parental ages were compared with those of the Scottish population as follows. For each maternal age, its residual was computed as: (maternal age-mean maternal age in population)/(standard deviation of maternal age in population) where the population means and standard deviations were computed from the published tables of the Registrar General Scotland for the corresponding year of birth. The method therefore allows for fluctuations in the population parental age distributions over the period of the study. Under the null hypothesis that the maternal ages of cases are a random sample from the population, the residuals have an expected mean of zero, and an expected standard deviation of 1, so that conventional

tests can be applied. Paternal ages were treated similarly. The parental ages of fetuses were incremented by 0.5 years to correspond (approximately) to expected dates of delivery.

Results

The parental origin of the normal X chromosome was determined in all families studied (table 1). This table includes 27 families briefly described previously.⁸ Overall there were 27 subjects (79.4%) in which the normal X was maternally derived (X^m) and seven (20.6%) with a paternally derived X (X^p). In this study children (12) and fetuses (four) with 45,X always had a maternal X, indicating loss of a paternal sex chromosome at, or before, conception. This maternal excess was also present, albeit less marked, for the other karyotypes found in liveborn Turner's syndrome patients. For isochromosome Xq (mosaic and non-mosaic), the normal X was maternal in five and paternal in two and for ring X the normal X was maternal in six and paternal in two. Maternal and paternal ages were available for 29 and 27, respectively, and gave mean residuals of 0.03 years and 0.01 years, not significantly different from the zero values expected under the null hypothesis. No significant

Table 2 Analysis of the origin of Turner's syndrome using polymorphic DNA probes.

	Normal X paternal (X ^P)	Normal X maternal (X ^m)	Reference
45,X children	1 9 7 0	4 20 14 12	5 6 9 10 Present study
Total	17	50	
45,X fetuses	6 0	24 4	5 6 Present study
Total	6	28	
46,Xi(Xq)	3 6 1	2 2 2	11 12 Present study
Total	10	6	
45,X/46,Xi(Xq)	3 1	4 3	9 Present study
Total	4	7	
45,X/46,XX	2	0	Present study
45,X/46,r(X)	4 2	6 6	9 Present study
Total	6	12	
46,XXp-	1	0	Present study

deviations from the null values were obtained when the families were grouped by karyotype or by parental origin, though in most groups the numbers were insufficient to give much force to this conclusion. The use of several X polymorphic probes and Y specific probes (but not X or Y centromeric probes) in cytogenetically non-mosaic patients did not show any evidence for cryptomosaicism.

Discussion

The utility of DNA analysis for the investigation of sex chromosome abnormality was shown in this study where the origin of the normal X was determined in every family. Table 2 compares our findings with those of others using DNA analysis. Overall, using DNA analysis, in 45,X (fetuses and livebirths) the normal X is maternal in 77.2%. For fetuses with 45,X, 82.4% have X^m and for livebirths 74.6% have X^m, a difference which is not statistically significant ($\chi^2=0.76$). The comparable figures derived using Xg studies in 80 informative liveborn females with Turner's syndrome and corrected for bias were 77% X^m and 23% X^P.^{3,4} Non-disjunction at paternal meiosis could account for the high number of apparently non-mosaic 45,X subjects with no paternal sex chromosome but this is not reflected in the frequency of sperm with no sex chromosome,^{13,14} nor with a frequency of 47,XXY and 47,XXX

comparable with Turner's syndrome in early pregnancy. A preponderance of paternal sex chromosome loss has also been found in comparable studies into the origin of 39,X mice and these errors are attributable to loss of the paternal X or Y following entry of the sperm into the egg.¹⁵ The human paternal sex chromosome could also be lost at this stage to result in 45,X. Later loss during an embryonic mitotic cell division would produce the most common type of mosaicism in both fetuses and live births with Turner's syndrome (45,X/46,XX). Overall for other karyotypes, using DNA analysis, the normal X is maternal in 52% but the numbers for individual karyotypes are still relatively small.

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