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ORIGINAL INVESTIGATION

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A study of females with deletions of the short arm of the X chromosome

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Abstract We have undertaken a clinical and molecular study of 25 females with deletions of the short arm of the X chromosome. We have determined the deletion breakpoints, the parental origin and the activation status of the deleted X chromosomes. Genotype–phenotype correlations suggest that the presence of a single copy of the DFFRX gene, previously postulated as a gene involved in the ovarian failure seen in Turner syndrome, may be compatible with normal ovarian function, and that there may be a gene for Turner-like features located in distal Xp22.3.

Introduction

Deletions of the whole short arm of the X chromosome in females are associated with short stature, gonadal dysgenesis and the classic stigmata of Turner syndrome (Ferguson-Smith 1965). Patients with terminal Xp deletions have short stature and may have some somatic traits of Turner syndrome while gonadal function is generally preserved (Pfeiffer 1980; Wyss et al. 1982). The short stature has recently been shown to result from the deletion of a homeobox gene, SHOX, which escapes X-inactivation and is located in the pseudoautosomal region of Xp (Rao et al. 1997). Regions of both Xp and Xq are thought to carry genes involved in normal ovarian function, and recently it has been proposed that the gene DFFRX located in Xp11.4 may play such a role (Jones et al. 1996). The gene responsible for the other, somatic, features of the phenotype have not yet been identified. Studies of patients with deletions of Xp have led to suggestions that a gene (or genes) responsible for the stigmata of Turner syndrome may lie proximal to Xp11.2 (Hecht et al. 1980; Ferguson-Smith 1991) or along the length of Xp and in Xq21q26 (Wyss et al. 1982). However, there have been several cases described of females with small terminal deletions of Xp exhibiting some of the somatic features of Turner syndrome (e.g. Bartsch-Sandhoff et al. 1976; Massa et al. 1992; Meindl et al. 1993; Kuznetzova et al. 1994). Molecular studies of males with deletions of distal Xp have allowed the mapping of a number of genes, the deletions of which are associated with contiguous gene syndromes (Ballabio and Andria 1992), and recently it has been suggested that an imprinted, paternally expressed, gene involved in social-cognitive development is located on the X chromosome, either on Xp, proximal to the UBE1 locus at Xp11.23, or on Xq (Skuse et al. 1997).

We have undertaken a molecular study of 25 females with deletions of Xp in both mosaic and non-mosaic form. We have: (1) used detailed cytogenetic and molecular analysis to map the breakpoints of the deletions; (2) determined the parental origin of the abnormality; (3) studied the X-inactivation patterns; and (4) attempted genotype–phenotype correlations. Of particular interest was the presence or absence of somatic features of Turner syndrome, and the investigation of the potential role of the DFFRX gene in ovarian failure.

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Materials and methods

The study population consists of 25 females with deletions of Xp. Details of the females together with the original reason for their referral are shown in Table 1. The patients were referred for a variety of reasons, either with or without a clinical diagnosis of Turner syndrome. They were included in this study only when a deletion of the short arm of the X chromosome had been demonstrated. Patient 6 has been described previously (case 2, Al-Gazali et al. 1990; cell line 138, Schaefer et al. 1993). Females with i(Xq) were excluded from

Table 1 Details of the study population

Case number	ID	Karyotype	Ascertainment
1	88.4577	46,t(X;Y)(p22.31;q11.21)	Short stature
2	95.2697	46,X,t(X;Y)(p22.33;q12)	Turner syndrome
3	96.0284	46,X,t(X;Y)(p22.33;q12)	Mother of 95.2697
4	96.1693	46,X,del(X)(p22.31 or p22.32)	Developmental delay
5	95.2669	46,X,del(X)(p22.31)	Turner syndrome
6	86.1593	46,X,der(X)t(X;Y)(p22.3;q11.2)	Microphthalmia with linear skin defects
7	85.5142	46,X,del(X)(p22.1)	Short stature
8	94.6043	46,X,del(X)(p22.1)	Daughter of 85.5142
9	94.3570	45,X[8]/46,X.del(X)(p21.2)[72]	Mother of unexpected stillbirth
10	96.8407	45,X[45]/46,X,del(X)(p21.3)[5]	Turner syndrome
11	96.1123	46,X,psudic(X)(qter>p22.1::p11.2>qter)	Turner syndrome
12	95.3557	45,X[18]/46,X,del(X)(p22.11)[32]	Turner syndrome
13	95.4894	46,X,del(X)(p21.2)	Short stature
14	95.4247	45,X[33]/46,X,del(X)(p21.2)[17]	Turner syndrome
15	96.5509	46,X,der(X)del(X)(p21.2)inv(X)(p21.2q22.1)	Turner syndrome
16	95.1115	46,X,del(X)(p11.33)	Known del(X)
17	96.5786	46,X,del(X)(p11.33)	Mother of 95.1115
18	94.5158	45,X[8]/46,X,del(X)(p21.2)[22]	Mother of fetus with severe abnormalities
19	90.2284	46,X,del(X)(p11)	Short stature, some stigmata of Turner syndrome
20	96.0863	46,X,del(X)(p11.2)	Turner syndrome
21	96.8266	46,X,del(X)(p11.23)	Turner syndrome
22	95.2767	45,X[50]/46,X,del(X)(p11)[87]/46,X,i(Xq)[16]	Turner syndrome
23	95.2770	46,X,der(X)(qter>p11.2::q26>qter)	Turner syndrome
24	90.3806	45,X[45]/46,X,del(X)(p11)[55]	Short stature, infantile sexual characteristics
25	97.2052	45,X[33]/46,X,del(X)(p11.21)[17]	Turner syndrome

this study since they constituted a large relatively homogeneous group that was considered separately (James et al. 1997).

Cytogenetic analysis

Chromosomal analysis was carried out on peripheral blood lymphocytes from each proband and from both parents when available. Cytogenetically visible translocation and deletion breakpoints were determined after G-banding of metaphase chromosomes (Seabright 1971). The replication status of the abnormal X chromosome was determined by pulse labelling with 5-bromo-2′-deoxyuridine (BrdU) (Willard and Latt 1976). In two patients with very distal terminal Xp deletions it was not possible to determine X-inactivation status by conventional cytogenetic techniques as the deletions were too small for the X chromosomes to be distinguished from each other after incorporation of BrdU. A molecular cytogenetic technique was used in these patients. Lymphocyte cultures to which BrdU had been added 6 h prior to harvest were used to make slide preparations. The slides were R-banded and used for fluorescence in situ hybridisation (FISH) analysis with two probes: DXZ2, which maps to the X-centromeric alphoid repeat sequences, and zWXD2539, a yeast artificial chromosome (YAC) that hybridises to the subtelomeric region of the short arm of the X chromosome. Both probes were digoxigenin labelled by nick translation using the manufacturer's protocols (Boehringer) and 50–100 ng of labelled probe was resuspended in a hybridisation mixture containing 50% formamide and 2×SSC (w/v). Following denaturation of probe and target DNA, hybridisation was carried out at 37°C for 24–48 h. Following two 5-min stringent washes (1×SSC, 50% formamide) at 42°C the slides were incubated in rhodamine-conjugated anti-digoxigenin for 20 min at room temperature. The chromosomal DNA was counterstained using 0.05 mg/ml 4,6-diamidino-2-phenylindole (DAPI) suspended in an antifade solution (Vectra Laboratories). The slides were examined using an epifluorescent UV microscope equipped with the Pinkel no. 83 filter series. Metaphase images were captured using a cooled CCD camera and the digitised data analysed using Smartcapture (now Vysis) software. The late replication status of the X chromosomes was determined by processing the DAPI-stained metaphase spreads using computer-controlled hard files by which R-banding images were produced. The early replicating X was R-banded while the late-replicating, and thus inactive X, was uniformly pale. The normal X chromosome showed a tetramethylrhodamine isothiocyanate signal of YAC hybridisation on distal Xp while the chromosome in which the YAC signal was absent was the deleted Xp (data not shown).

Molecular cytogenetic analysis of the deletion breakpoints was undertaken for a few patients whose breakpoints were shown to lie in very proximal Xp. Cosmids ZXDA, DXS423E and UBE1, which map to proximal Xp loci, were labelled with biotin by standard techniques and FISH was performed on metaphase spreads as described by Pinkel et al. (1986). Deleted X chromosomes were scored either as containing a locus or being deleted for it.

Molecular analysis

DNA was extracted from peripheral blood, from the proband and both parents when available, by a salt-precipitation technique (Miller

et al. 1988). In some cases it was only possible to obtain samples of buccal cells from the parents. The parental origin of the normal X chromosome was determined by polymerase chain reaction (PCR) amplification of polymorphic microsatellite repeat sequences located in distal Xp, in a region deleted in the structurally abnormal X chromosome. All primer sequences are available on the Genome Database. Standard PCR conditions were used (Hudson et al. 1992). One primer of each pair was end-labelled and PCR products were visualised using a 6% denaturing polyacrylamide sequencing gel followed by autoradiography. The breakpoints of the deletions and translocations were mapped to the nearest polymorphic microsatellite repeat marker in the Généthon map of the human X chromosome (Dib et al. 1996). Breakpoints were assigned between the most proximal informative deleted marker and the most distal informative non-deleted marker.

Analysis of DFFRX

The copy number of the DFFRX gene was determined for subjects 14–24 by a quantitative fluorescent PCR assay. DNA from these subjects, two 46,XY males, four 46,XX females and two 45,X Turner female controls was quantified by duplicate spectrophotometric readings, and standardised to approximately 50 ng/μl. The optimum conditions for linear PCR amplification and gel resolution were 19 cycles with an annealing temperature of 60°C and a final extension of 60°C for 1 h.

The multiplex reaction contained two primer sets: the primers 5′-TCGAACCATTCGAACAGCTTCCTG-3′ and 5′-TCCATGAAGAC TTCATTCAGTCTT-3′ generated a product of 124 bp from the DFFRX gene (Jones et al. 1996); and the control primers 5′-ATAGG-TCATTGCTTCTTGCTGAT-3′ and 5′-TGAATTTTAATGGATTA-CCTAGGT-3′ generated a product of 194 bp from exon 4 of the DP5.2 gene on chromosome 5q (Groden et al. 1991). One primer from each pair was labelled with the phosphoramidite dye 6′-FAM (OSWEL, Southampton), to allow amplified products to be visualised on an ABI 377 and quantified using Genescan and Genotyper software (Perkin Elmer).

For each sample the quantified signal (as measured by peak height) from the DFFRX gene was divided by the peak height of the corresponding autosomal control product to compensate for variation in DNA concentration. This normalised value was divided by the normalised value of the mean male peak heights, such that, for non-mosiacs, a ratio of 2 indicated the presence of two copies of the DFFRX gene and a ratio of 1 indicated the presence of one copy. Results were confirmed by analysis of products from four separate PCR amplifications.

X-inactivation studies

A molecular technique was also employed to determine the X-inactivation status of the deleted X chromosomes: analysis of the methylation patterns at the HUMARA locus at Xq11 was performed according to the method described by Allen et al. (1992). PCR amplification of the polymorphic trinucleotide repeat sequence at the Androgen Receptor gene used undigested DNA from the proband and both parents and DNA from the proband digested with the methylation sensitive enzyme *Hpa*II. Enzyme digestion of the unmethylated site on the active X chromosome cuts the template DNA within the region amplified by the PCR primers. Therefore any PCR product detected represents the methylated, inactive X chromosome.

Clinical examination

Clinical examination of 17 females in the study population was undertaken by one of us (B. C.). A history and examination were undertaken according to a detailed protocol on each of the subjects. Particular note was made of any of the signs associated with a clinical diagnosis of Turner syndrome. For the remaining eight patients, clinical details were obtained by examining the medical records.

Results

The karyotypes of 25 females with a deletion of Xp in mosaic or non-mosaic form are shown in Table 1. The majority of individuals included in the study had a terminal deletion of Xp, although in four this involved a translocation between distal Xp and Yq. However, four patients did not have simple deletions of the short arm of the X. Patient 11 had a psudic(Xq) and was effectively deleted for Xp22.1pter and duplicated for both Xq and Xp11.2p22.1. Patient 15 had a complex karyotype, with a deletion from Xp21.2 to pter and a large pericentric inversion from p21.2 to q22.1. Patient 22 had a mosaic karyotype with three cell lines, the predominant one being a large deletion of Xp. Patient 23 appeared cytogenetically to have a terminal deletion of Xp; however, molecular analysis demonstrated that the deletion extended to Xp11.2, with concomitant duplication of Xq26qter material.

Three relatives of patients were included in the study population after cytogenetic analysis demonstrated that they carried the same structural rearrangement as the probands in their families: one mother, patient 3, was found to have a karyotype [46,X,t(X;Y)(p22.33;q12)] identical to that in her proband daughter, patient 2. A daughter, patient 8, had the same terminal deletion as her proband mother, patient 7. A second mother, patient 17, had a large terminal deletion of Xp, which was also present in her proband daughter, patient 16.

Results of the molecular analysis of the deletion breakpoints are shown in Fig. 1. The breakpoints ranged from Xp11 to Xp22.3 and there was no evidence of any hot spots for breakpoints. For the three cases where relatives had the same chromosomal rearrangement, molecular studies showed the breakpoints to be identical. Patient 23, with the complex rearrangement of her X chromosome, gave anomalous results with markers in proximal Xp. She was heterozygous at the polymorphic microsatellite locus DXS1199, but her abnormal chromosome gave no FISH signal with the probe DXS423E, which is proximal to DXS1199 according to the map of Nagaraja et al. (1997). The results obtained for patient 23 suggest that the order of this marker may be incorrect.

Parental origin of the X chromosomes

The results of the parental origin studies are shown in Table 2. In 19 of the 23 de novo deletions, the parental origin of the normal X chromosome was maternal and in 2 it was paternal. In only 2 cases, both of whom were mothers ascertained as a result of their daughters' deletions, were we unable to determine the parental origin of the normal X chromosome. In order to exclude uniparental disomy (UPD) of the X chromosomes, the parental origin of 24 of

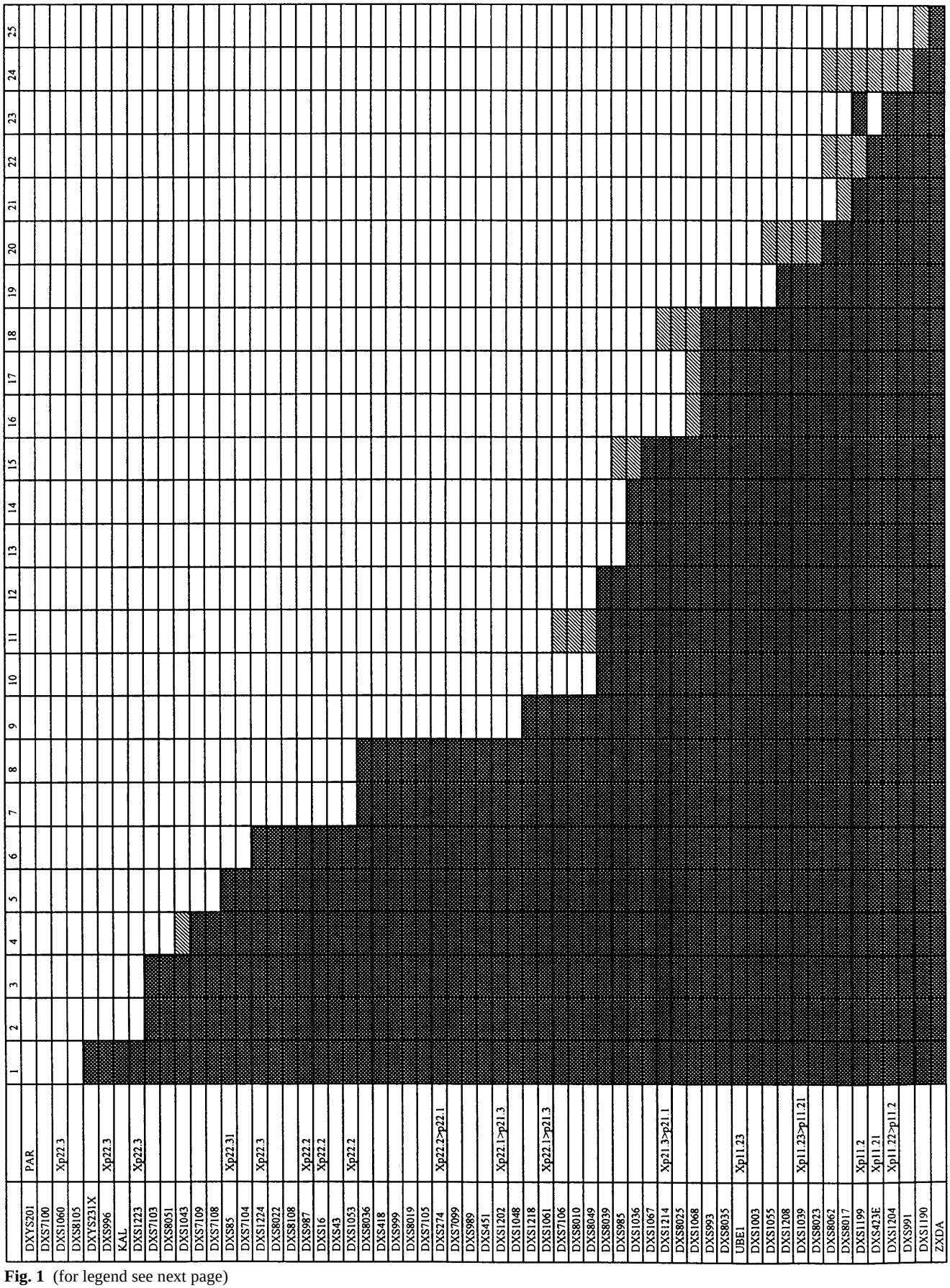

Fig. 1 (for legend see next page)

Table 2 Parental origin and activation status of the deleted X chromosomes. (*BrdU* 5-bromo-2′-deoxyuridine, *N/I* non-informative)

Case	Cytogenetic breakpoint	Origin of deletion	Deletion	BrDU	HUMAR
1	p22.31	Paternal	De novo	t(X;Y) inactive 18/55, active 32/55	t(X;Y) active in >50%
2	p22.33	Maternal	Familial	t(X;Y) inactive 70/105, active 35/105	t(X;Y) active in <50%
3	p22.33	?	?	t(X;Y) inactive 59/67, active 8/67	t(X;Y) active in ~90%
4	p22.31 or p22.32	Paternal	De novo	del(X) inactive 20/49, active 29/49	del (X) active in ~80%
5	p22.31	Paternal	De novo	del(X) inactive 9/30, active 21/30	del (X) active in ~50%
6	p22.3	Paternal	De novo	Not done as only lymphoblastoid cell line available	Not done
7	p22.12	paternal	De novo	del(X) inactive	del (X) inactive
8	p22.12	Maternal	Familial	del(X) inactive	del (X) inactive
9	p21.2	Paternal	De novo	del(X) inactive	del (X) inactive
10	p21.3	Paternal	De novo	del(X) inactive	del (X) inactive
11	p22.1	Paternal	De novo	psudic(X) inactive	psudic(X) inactive
12	p22.11	Paternal	De novo	del(X) inactive	del (X) inactive
13	p21.2	Paternal	De novo	del(X) inactive	del (X) inactive
14	p21.2	Paternal	De novo	del(X) inactive	del (X) inactive
15	p21.2	Paternal	De novo	del(X) inactive	Not done
16	p11.33	Maternal	Familial	del(X) inactive	Not done
17	p11.33	?	?	del(X) inactive	Not done
18	p21.2	Paternal	De novo	del(X) inactive	N/I
19	p11	Paternal	De novo	del(X) inactive	del (X) inactive
20	p11.2	Paternal	De novo	del(X) inactive	Not done
21	p11.23	Paternal	De novo	del(X) inactive	del (X) inactive
22	p11	Paternal	De novo	del(X) inactive	del (X) inactive
23	p11.2	Paternal	De novo	del(X) inactive	Not done
24	p11	Maternal	De novo	del(X) inactive	del (X) inactive
25	p11.21	Maternal	De novo	del(X) inactive	del (X) inactive

the 25 deleted X chromosomes was confirmed by the use of microsatellite repeat markers located proximal to the deletion breakpoint. In all cases the parental origin of the deleted X was consistent and opposite to that of the normal X, i.e. no cases of UPD(X) were detected. Thus the great majority of deletions occurred on the paternal chromosome, while the only two de novo deletions that were maternal in origin involved the two most proximal breakpoints. Thus in terms of parental origin and breakpoints these very proximally deleted Xps resemble i(Xq) (James et al. 1997).

X-inactivation studies

Results of cytogenetic X-inactivation studies are shown in Table 2. In all cases where the breakpoint was in or proximal to Xp22.1 the deleted X was late replicating in all cells observed. In those patients with X;Y translocations whose breakpoints were in Xp22.3 the derived X was early replicating and therefore active in a proportion of cells. In two cases of terminal deletions with breakpoints in Xp22.3, a molecular cytogenetic technique was used to determine X-inactivation status. In patient 4, the deleted X chromosome was active in 29/49 cells and inactive in 20/49 cells. In patient 5, simultaneous analysis of FISH signals and late-replicating pattern showed that the deleted X chromosome was early replicating and thus active in 21/30 cells and late replicating and thus inactive in 9/30 cells.

Molecular studies of X-inactivation using the HUMARA locus were undertaken on 19/25 patients. The results are also shown in Table 2. In patient 18, the results were uninformative as the proband was homozygous at this locus. In all other cases where the breakpoint in Xp was proximal to Xp22.3, there was non-random X-inactivation, with the deleted X being preferentially inactivated. In the five cases tested with breakpoints in Xp22.3, there was evidence of both the normal X and abnormal X being active in various proportions of cells, and the results obtained with cytogenetic and molecular techniques were equivalent in all cases except one. In patient 3, cytogenetic analy-

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Fig. 1 Molecular definition of the breakpoints in 25 cases of del(Xp). The markers for which the patient was heterozygous are shaded in *black*. Non-informative markers are *hatched*. Deleted markers are *unshaded*

Table 3 Results of quantitative fluorescent polymerase chain reaction assay for copy number of the DFFRX gene. (*N/A* not applicable)

Case no.	Karyotype	Ovarian failure	DFFRX peak height	Autosomal gene peak height	Normalised peak height	Copy no. of DFFRX[a]
14	45,X[33]/46,X,del(X)(p21.2)[17]	N	1344	1395	0.96	1.33
15	46,X,der(X)del(X)(p21.2)inv(X)(p21.2q22.1)	N	1108	822	1.35	1.88
16	46,X,del(X)(p11.33)	N	1004	697	1.44	2
17	46,X,del(X)(p11.33)	N	995	806	1.23	1.71
18	45,X[8]/46,X,del(X)(p21.2)[22]	N	1113	1138	1	1.39
19	46,X,del(X)(p11)	N	478	617	0.77	1.07
20	46,X,del(X)(p11.2)	N	650	795	0.82	1.14
21	46,X,del(X)(p11.23)	[b]	877	1272	0.69	0.96
22	45,X[50]/46,X,del(X)(p11)[87]/46,X,i(Xq)[16]	Y	670	854	0.78	1.08
23	46,X,der(X)(qter>p11.2::q26>qter)	Y	580	701	0.83	1.15
24	45,X[45]/46,X,del(X)(p11)[55]	Y	541	726	0.75	1.04
2 male controls	46,XY	N/A	501	697[c]	0.72[c]	1
4 female controls	46,XX	N	1734.5[c]	1266[c]	1.37[c]	1.9[c]
2 Turner controls	45,X	Y	700.5[c]	996.5[c]	0.70[c]	0.98[c]

[a] DFFRX copy number was determined by the ratio of DFFRX normalised peak height and mean normalised male peak height
[b] Ovarian function not known. Patient <10 years
[c] Results shown are mean results obtained for controls

sis demonstrated that the translocated X was active in only 8/67 cells whereas the molecular technique suggested that there was a significant majority of cells (~90%) where the translocated X chromosome was active. The reasons for this discrepancy are not obvious. The cytogenetic results are based only on the T-lymphocytes stimulated to divide by phytohaemagglutinin, while molecular results are based on total DNA extracted from the blood. However it seems surprising for the T-lymphocytes to have such a different pattern of X inactivation from the polymorphs, which form the greater component of nucleated cells in the blood.

Status of DFFRX gene in patients with proximal Xp breakpoints

It was possible to determine the number of copies of the DFFRX gene present in all the patients tested (patients 14–24). The results are shown in Table 3, and are consistent with the location of the gene being between the markers DXS77 and DXS556 (Jones et al. 1996). The nearest polymorphic markers that we have tested are DXS8035, DXS993 and DXS1068 (Fig. 1). In cases 14–18, with breakpoints distal to DXS993, two copies of the DFFRX gene were present. Two of these cases (patients 14 and 18) were mosaics with a 45,X cell line, and this was reflected in a ratio of peak heights that was lower than in the non-mosaic patients in this category. In all cases (19–24) with breakpoints proximal to DXS993, only one copy of the DFFRX gene was present.

Clinical features

Details of the clinical features of the patients are summarised in Table 4.

Discussion

The classic phenotype of Turner syndrome includes short stature, infertility and variable somatic stigmata. The full phenotype is associated with the deletion of a stature gene in the pseudoautosomal region of distal Xp and of one or more genes thought to be in proximal Xp. It is hypothesised that these genes escape X-inactivation, and haploinsufficiency in patients with monosomy X results in the full Turner phenotype. The mapping of Turner syndrome genes in individuals with deletions of Xp is complicated by the frequent presence of a 45,X cell line, and by the fact that several of the clinical signs of Turner syndrome are difficult to assess or quantify, e.g. ptosis, low posterior hairline and ovarian function in young children. Previous such studies have failed to reach a consensus on their locations. We have studied 25 females with deletions of Xp in an attempt to determine the location of genes responsible for the Turner syndrome phenotype. Apparently non-mosaic karyotypes were seen in 17 of the 25 cases.

Short stature

Of the 25 patients, 23 had short stature consistent with a diagnosis of Turner syndrome. The short stature results from the deletion of the SHOX gene in the pseudoautosomal region of Xp (Rao et al. 1997). This region was apparently deleted in all our patients. Two of the subjects (patients 15 and 23) were not short for their families. Both of these girls had complex rearrangements of their X chromosomes rather than simple terminal deletions, but this fact does not provide an obvious explanation for their stature. All of the rest plotted on Turner charts close to their parents' mid parental centile on standard charts.

Ovarian function

It has been suggested that the presence of two copies of the DFFRX gene may be required for normal fertility (Jones et al. 1996). We tested 11 patients with breakpoints in proximal Xp for the presence of one or two copies of the DFFRX gene. We found two copies to be present in patients 14–18, all of whom had normal ovarian function, and one copy in patients 19–24. In patients 19 and 20, apparently normal ovarian function was seen in association with the presence of a single copy of DFFRX. Patient 19 was on ethinyl oestradiol between the ages of 10 years 7 months and 13 years 6 months. Irregular menstrual periods began at 13 years 4 months and continued for at least 1 year after exogenous oestrogen was discontinued. Gonadotrophin levels were normal at 13 years 10 months, and pelvic ultrasound showed normal ovarian morphology at 14 years 2 months. Patient 20 experienced menarche at the age of 16 years, having taken oestrogen for 4 years. Pelvic ultrasound at the age of 16 years 9 months showed ovaries of 17×12×6 mm and 23×17×8 mm, both of which appeared to contain follicles. Both patients 19 and 20 have an apparently non-mosaic 46,X,del(Xp) karyotype in peripheral blood but we cannot exclude the possibility that there is a 46,XX cell line in the ovaries. However this seems unlikely because detected 46,XX/46,X,del(X) mosaicism is extremely rare; we have never identified such a mosaic among approximately 5000 abnormal karyotypes diagnosed in our laboratory. Therefore from our results it seems that haploinsufficiency for DFFRX may not be responsible for the ovarian failure in Turner syndrome.

Somatic stigmata of Turner syndrome

The somatic stigmata of Turner syndrome are highly variable and their assessment may be subjective. Ogata and Matsuo (1995) suggest that they may be divided into four groups: (1) anomalies attributable to lymphatic obstruction, (2) skeletal anomalies, (3) cardiovascular and renal anomalies and (4) miscellaneous anomalies.

Anomalies due to lymphatic obstruction

Neck webbing is one of the more commonly described features of the classical Turner syndrome phenotype. In the review of Turner syndrome features undertaken by Ogata and Matsuo (1995), 49% of 45,X individuals had neck webbing. None of the patients with deletions of Xp who were examined for this study had a webbed neck, nor was it described in the case notes of any of the eight patients for whom clinical data were abstracted. Neck webbing is less commonly described in individuals with a 46,X,i(Xq) karyotype (Ogata and Matsuo 1995), suggesting that a gene (or genes) responsible for this feature may reside on Xq or very proximal Xp. This suggestion is compatible with the results of this study. Six of the 18 patients examined were found to have a low posterior hairline. The most distal breakpoint in a patient with this sign was Xp22.31. Congenital oedema was described in two cases with breakpoints in Xp22.3, but not in any of the other cases, even those with breakpoints in very proximal Xp. Of the non-mosaic patients with terminal deletions, the presence of ptosis was limited to those with breakpoints proximal to Xp11.2.

Skeletal anomalies

There were no consistent breakpoints associated with short neck, high arched palate, or increased carrying angle. These features were seen in some of the patients and the deletion breakpoints in the non-mosaic cases ranged from Xp22.3 toXp11. The most distal breakpoint associated with markedly short metacarpals was Xp22.31.

Cardiovascular and renal anomalies

None of the patients with breakpoints distal to Xp11 had any cardiovascular problems; however, breakpoints in or proximal to Xp11 were associated with aortic valve abnormalities in one of the four non-mosaic patients (1/8 total).

Definite renal problems were present in four patients, all of whom had breakpoints proximal to Xp21.3. A further patient with a possible renal abnormality (a single attack of pyelonephritis) had a breakpoint at Xp22.3.

Miscellaneous problems

Only two patients (2 and 3) with excess melanocytic naevi had breakpoints distal to Xp22.11, and these (mother and daughter) gave a history of a lot of sun exposure and had other skin features of sun damage. In contrast the majority (7/8) of non-mosaic patients with breakpoints proximal to Xp22 had excess naevi. Five of the 17 examined subjects had clear evidence of disease, possibly of autoimmune origin. In four of the five cases, the deletion breakpoints were proximal to Xp11.4.

Table 4 (for legend see next page)

Case	Cyto-genetics	Age at exam. (years)	SS	OF	WN	LPH	Oed	Pto	SN	HAP	ICA	SM	Cardio vascular	Renal	AImD	Naevi	Other
1	t(X;Y)	24.25	Y	N	N	N	N	N	N	(Y)	Unilateral	N	N	–	N	N	Up-slanting palpebral fissures
2	t(X;Y)	5.80	Y	–	N	N	Y	N	N	N	N	N	N	–	N	Y[a]	Convergent strabismus
3	t(X;Y)	48.90	Y	N	N	(Y)	N	N	N	N	N	N	N	(Y)	N	Y[a]	Short distal phalanx of thumbs. Schizoid disorder
4	del(Xp)	2.30	Y	–	N	(Y)	N	N	N	N	N	(Y)	N	–	N	N	Autism; hypertelorism; bat ears; mongoloid slant; preauricular pit; alternating strabismus
5	del(Xp)	12.60	Y	N	N	(Y)	Y	N	Y	(Y)	Bilateral	Y	N	–	Alopecia areata	N	Divergent strabismus
6	t(X;Y)	–	Y	–	N	N	N	N	N	–	N	N	N	N	–	–	Microphthalmia with linear skin defects
7	del(Xp)	24.50	Y	N	N	N	N	N	N	(Y)	N	Y	N	–	N	N	
8	del(Xp)	1.70	Y	–	N	(Y)	N	N	(Y)	N	N	N	N	–	N	N	
9	Mosaic	–	Y	–	–	–	–	–	–	–	–	–	–	–	–	–	
10	Mosaic	7.10	Y	–	N	(Y)	N	Y	N	(Y)	N	N	N	VUR	N	Y	Hypertelorism;triangular lower face; bat ears
11	Psudic	15.90	Y	N	N	N	?	N	N	N	N	Y	N	Crossed ectopia	N	Y	Long cylindrical nose, reminiscent of VCF
12	Mosaic	–	Y	–	N	N	N	N	–	–	–	N	–	–	–	N	Short sight, glue ear
13	del(Xp)	5.60	Y	–	N	N	N	N	N	(Y)	N	N	N	N	N	Y	
14	Mosaic	20.40	Y	N	N	N	N	Y	N	N	N	Y	N	N	N	Y	Hypertelorism; cleft palate
15	Rearranged	10.90	?	N	N	(Y)	N	Y	Y	N	N	N	N	Renal scarring	N	Y	Hirsuit; divergent strabismus
16	del(Xp)	33.00	Y	N	N	N	N	N	N	N	N	Y	N	–	N	Y	Seizures
17	del(Xp)	63.80	Y	N	–	–	N	N	–	(Y)	N	(Y)	N	–	Colitis	N	
18	Mosaic	–	Y	N	N	–	–	–	(Y)	–	N	–	–	–	–	–	
19	del(Xp)	14.60	Y	N	N	N	N	N	N	Y	N	N	AS?	–	N	Y	Strabismus
20	del(Xp)	16.90	Y	N	N	–	–	Y	(Y)	Y	N	(Y)	N	N	Thyroi-ditis	Y	Synophrys; short philtrum; single central incisor; convergent strabismus
21	del(Xp)	<10.0	Y	–	N	Y	N	N	Y	Y	–	Y	Bicuspid aortic valve	N	– –	Y –	
22	Mosaic	–	Y	(Y)[b]	N	–	–	–	–	–	–	–	–	–	Hypothy-roidism	N	
23	Rearranged	14.25	?	(Y)[c]	N	(Y)	N	Y	(Y)	(Y)	Unilateral	(Y)	N	Ectopic kidney	Thyroi-ditis	Y	VCF-like facies
24	Mosaic	–	Y	Y	–	Y	–	–	Y	Y	–	Y	–	–	–	Y	
25	Mosaic	–	Y	Y	N	Y	Y	N	–	–	–	–	N	N	–	Y	

[a] Mother and daughter, patients 2 and 3, had both suffered excessive exposure to the sun; [b] 3rd centile for timing of puberty; [c] Elevated gonadotropin assays.
Multicystic ovaries measuring 28×18×24 mm on the left and 23×8×12 mm on the right. Menses twice yearly. Spontaneous onset of puberty achieving Tanner stage 4 for breast development at age 14 years and pubic hair 4 at 13 years

It was of interest that two patients (patients 2 and 5) with deletion breakpoints at Xp22.3 were originally referred with a clinical diagnosis of Turner syndrome. In patient 2, the translocated X chromosome was inherited in an apparently identical form from her mother. Clinical re-examination of patient 2 failed to detect any of the features of Turner syndrome other than short stature. Although she had excess naevi, this could be explained by exposure to the sun. However, patient 5 did exhibit a number of the somatic features of Turner syndrome. Some of these features have been reported in females with such distal deletions of Xp on several occasions (e.g. Bartsch-Sandhoff et al. 1976; Massa et al. 1992; Meindl et al. 1993; Kuznetzova et al. 1994). Although these observations initially seem incompatible with the general view that the somatic features of Turner syndrome result from haploinsufficiency of genes located in proximal Xp or in Xq, a hypothesis that would account for both mechanisms can be developed as follows. It is possible that there is a gene (or genes) subject to X-inactivation and associated with some Turner-like features, located in Xp22.3, distal to the region for microphthalmia with linear skin defects (MLS). The Turner phenotype associated with 45,X would not result from deletion of this gene since a single active copy is all that is required for normal function. Patients with Xp deletions proximal to Xp22.3 are not functionally nullisomic for this gene because of biased X-inactivation. Deletion of distal Xp22.3 is compatible with the deleted X chromosome remaining active, and hence mosaic nullisomy for this gene. The severity of somatic features seen in the patient could then be determined by the degree and tissue specificity of X-inactivation. In all five cases of deletions with breakpoints in Xp22.3, the structurally abnormal X chromosome was active in a proportion of cells as demonstrated by both cytogenetic and molecular techniques. This mechanism of disease aetiology has been proposed for MLS (Wapenaar et al. 1993; Lindsay et al. 1994), although the X-inactivation status of those cases of MLS associated with deletions of Xp has not been determined in the majority of cases. Features of Turner syndrome have not been described in MLS patients; however, it is likely that the severity of the MLS phenotype masks any "soft" Turner features.

Conclusion

The apparently normal ovarian function seen in two cases of females with a single copy of the DFFRX gene suggests that the ovarian failure seen in cases of 45,X Turner syndrome may result from a mechanism other than haploinsufficiency of DFFRX. The absence of neck webbing in any of the patients studied suggests that the gene for this feature may lie on Xq or very proximal Xp. There were no consensus breakpoints for the other stigmata associated with lymphatic abnormalities, skeletal abnormalities or cardiac and renal abnormalities. The presence of excess melanocytic naevi is associated with breakpoints proximal to Xp22. There is some evidence for the presence of a gene for some of the soft features of Turner syndrome, located in Xp22.3, and subject to X-inactivation. In cases of distal terminal deletions, the deleted X may remain active in a proportion of cells, resulting in a Turner-like phenotype dependent on the degree of mosaicism and the tissue specificity.

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Table 4 Clinical features in 25 patients with deletions of Xp. Excess naevi refers to the finding of a greater number of melanocytic naevi occurring on a subject than would be considered normal for age. The signs of short 4th and 5th metacarpals (*SM*) and low posterior hairline (*LPH*) were determined by clinical impression. Ptosis (*Pto*) was defined as increased upper lid visible with the face in the vertical plane and eyes neutrally open. Wide spacing of nipples is only a useful characteristic if there is little or no breast development as the measurements become uninterpretable after puberty due to variation in breast size and shape. Carrying angle (*ICA* increased carrying angle) was measured with the arm fully extended and supinated. It was expressed in degrees of deviation from straight. Greater than 22 degrees is considered abnormal in women. All three subjects in this study with wide carrying angles were unable fully to extend and supinate the affected arm(s). Evidence of functional ovarian tissue (*OF* ovarian failure) was obtained from history of spontaneous and full puberty; unassisted fertility; records of ovarian imaging studies (ultrasound); and gonadotropin levels not being elevated. Evidence of cardiovascular and renal disease was based on clinical findings and records of imaging where available. Oedema (*Oed*) was defined as a confident history of neonatal oedema or persistent signs of peripheral oedema. In a number of instances these signs were equivocal. Clinical details on patients 9, 10, 12, 18, 22, 24, and 25 were obtained from their case notes. [*SS* short stature, *WN* webbed neck, *SN* short neck, *HAP* high arched palate, *AImD* autoimmune disease, *AS* aortic stenosis, *VUR* vesico uretenic reflux, *VCF* velocardiofacial syndrome, *Y* yes, *N* no, *(Y)* mild]

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