Evidence for a cryptic 46,XX cell line in a 45,X/46,X,psu idic(Xq) patient with normal reproduction

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Abstract

Gonadal dysgenesis resulting in primary infertility is one of the most common features of Turner syndrome. There have been a number of cases described of pregnancy in 45,X subjects, but whether or not the fertility is associated with a 46,XX cell line in the germ cells is not known. We describe a 45,X/46,X,psu idic(Xq) female with normal fertility, in whom a cryptic 46,XX cell line was found in the germ cells.

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The first publication of reproduction in a 45,X female was that of Bahner *et al*¹ who reported a short female who was otherwise phenotypically unremarkable. She had a 45,X constitution in skin fibroblasts and bone marrow, and gave birth to a normal son. Subsequently, King *et al*² reported a patient with short stature and minimal features of Turner syndrome who had a 45,X karyotype in leucocytes and biopsies from skin, uterus, and both ovaries and who gave birth to a normal son. A further eight cases of apparently non-mosaic 45,X females who had successfully reproduced were summarised by Wray *et al*.³ Four of the 10 required therapeutic intervention for infertility, while six conceived without difficulty. There are many reports of pregnancy in patients with a mosaic sex chromosome constitution and a number resulted in live births with congenital abnormalities or chromosome aberrations or both. However, it is not at all clear how many of these patients were ascertained because of the abnormal offspring, and thus no accurate information is available on the outcome of pregnancies in females with a sex chromosome abnormality.

The observation that 45,X mice were fertile, although their litter size and reproductive life span were both reduced compared with 46,XX sibs,⁴ led to speculation as to whether in man the pure 45,X state was, in exceptional circumstances, compatible with fertility or alternatively whether all such women who reproduce are in reality cryptic mosaics with a normal cell line in untested tissues including the ovaries.

The availability of molecular probes makes it possible to test whether offspring of 45,X females are indeed the products of 45,X germ cells. If this is the case, the offspring would inherit from the mother all the DNA markers

present on the single maternal X. Alternatively, if maternal markers that are not present on the single maternal X are seen in the offspring, they provide evidence for the presence of a second cell line and thus for cryptic mosaicism involving the germ cells.

We report a female who presented to us for prenatal diagnosis. She had a 45,X non-mosaic karyotype in peripheral blood cells, but skin fibroblasts showed the presence of a 46,X,psu idic(Xq)(qter→p21::p21→qter) cell line, while molecular analysis of her 46,XX fetus showed that a normal 46,XX cell line must have been present in the maternal germ cells.

Case report

The proband was born in 1967 and was first investigated at the age of 10 for short stature and, at the age of 19, she was told that she had Turner syndrome. At that time chromosome studies were carried out on peripheral blood and she was said to have "97% 45,X cells and 3% 46,X, + marker". This test was done in another laboratory and further details are not available. Chromosome studies were repeated in 1991 in yet another laboratory when 50 cells from peripheral blood were all found to be 45,X. At that time she was 14 weeks pregnant and she subsequently delivered a normal male child.

The proband presented to us for prenatal diagnosis in April 1996 when she was aged 29. At that time we repeated her blood karyotype and found it to be 45,X in 50 cells studied. We did prenatal diagnosis on a CV sample and showed the fetus to have a 46,XX chromosome constitution. A normal female child was delivered in October 1996. Placental samples were sent to us for confirmation, but cytogenetic studies were not possible as they were fixed in formalin.

The patient was examined by one of us (BC). On clinical examination she was of short stature (147 cm). She had a high arched palate, a short philtrum, and a low posterior hair line, but no webbing. She had puffy fingers and toes, short fingers with bilateral camptodactyly of her fifth fingers, and short fourth metacarpals. Her toe nails arose from the nail bed at an angle to the plane of the distal phalanx, typical of Turner syndrome. She did not exhibit ptosis, wide carrying angle, or multiple pigmented naevi. Apart from the minor signs mentioned above she was normal. Menarche was at 14 years and her menses have since been regular

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Table 1 Cytogenetic observations

Subject	Tissue	Results
Patient	Blood	45,X [50]
	Blood: BrdU cultures scanned for late synthesising X chromosomes	45,X[148]/46,X, psu idic(Xq)[2]
	Skin fibroblasts	45,X [83]/46,X, psu idic(Xq) (qter→p21::p21→qter) [17]
Fetus	Chorionic villi	46,XX[50]

and normal. Pelvic ultrasound showed polycystic ovaries.

Laboratory investigations

CYTOGENETIC ANALYSIS
The cytogenetic observations were all made using standard techniques and the results are shown in table 1. When the second cell line was seen in skin fibroblasts, the blood was re-examined after the addition of BrdU at the end of the culture period in order to scan for the presence of the late synthesising psu idic(Xq). A further 150 cells were examined and two were found with a psu idic(Xq). Thus, although this abnormal chromosome must be present in low levels in the blood, it was not seen on routine cytogenetic examination of blood leucocytes.

MOLECULAR ANALYSIS
Samples of blood, buccal cells, urine, and skin fibroblasts were obtained from the proband. A sample of blood was obtained from her husband and placental samples were taken at the time of delivery of her daughter. DNA was extracted from all the tissues by a salt precipitation technique.⁵ Molecular analysis of the X chromosomes was by amplification of polymorphic microsatellite repeat sequences located along the length of the chromosome using standard PCR techniques.⁶ Details of the primer sequences used are available on the Genome Data Base. PCR primers were labelled in one of two ways. One primer of the sets for DXS996, DXS999, DXS1068, DXS993, DXS990, DXS1047, DXS984, and

DXS998 was labelled with a fluorescent tag, and the results were analysed using an ABI 377 automated sequencer. For all the other loci, one primer was radioactively end labelled, the PCR products were separated on a 6% denaturing polyacrylamide gel and visualised using autoradiography. The status of 22 highly polymorphic X linked loci was tested on DNA samples from blood, urine, and skin fibroblasts. Unfortunately, the limited amount of DNA available from the mouth brushes only allowed us to test 11 loci from this source and, of the 11, six were successful. Eighteen of the loci spanned Xp while four were on Xq. The results are shown in table 2, with the paternal and maternal contributions to the fetus being separately distinguished. As expected from the cytogenetic results, the patient's blood appears hemizygous at all tested loci, as did the mouth brush, urine, and fibroblast DNA for all short arm probes distal to DXS8025. However, a number of short arm probes proximal to DXS8025 and long arm probes were clearly heterozygous. We know that in skin fibroblast DNA this must reflect the 17% of cells with a psu idic(Xq), and we interpret our results on mouth brush and urine DNA as showing that a cell line with a 46,X,psu idic(Xq) must also be present in these tissues. Furthermore, the breakpoint on Xp in the psu idic(Xq) must be localised in Xp21 between locus DXS997 and locus DXS8025, as they separate a region of hemizygosity from one of heterozygosity. This observation is in good agreement with the cytogenetic localisation of the breakpoint.

When the maternal contribution to the fetus is considered, a very different picture emerges. For a number of informative loci from DXS8025 to qter the fetus has inherited alleles that are identical to those on the mother's abnormal, not her normal X. These loci include DXS8025, DXS993, DXS8035, DXS8054, DXS8023, DXS991, DXS990, and DXS984. When the nine loci on distal Xp are considered, it can be seen that seven have

Table 2 Molecular results

Locus	Cytogenetic location	cM from Xpter	Husband	Proband				Placenta	
				Blood	M/B	Urine	Skin fibs	Pat allele	Mat allele
DXS996	Xp22.3		2,-	1,-	1,-	F	1,-	2	1
KAL	Xp22.12		2,-	1,-	F	1,-	1,-	2	3
DXS999	Xp22.1	28	1,-	2,-	F	F	2,-	1	1
DXS451	Xp22.1→p21		2,-	3,-	3,-	3,-	3,-	2	1
DXS1048		42	1,-	2,-	NT	2,-	2,-	1	3
DXS7106			1,-	2,-	NT	2,-	2,-	1	3
DXS8039		44	2,-	1,-	F	1,-	1,-	2	1
DXS1036			1,-	2,-	NT	2,-	2,-	1	3
DXS997	Xp21	49	1,-	2,-	NT	2,-	2,-	1	3
DXS8025		57	1,-	2,-	NT	2,(3)	2,(3)	1	3
DXS1068		57	1,-	1,-	1	1	1	1	1
DXS993		69	2,-	2,-	F	1,2	(1),2	2	1
DXS8035		72	2,-	1,-	NT	1,3	1,3	2	3
DXS8054		76	1,-	3,-	NT	2,3	(2),3	1	2
DXS1055		83	1,-	2,-	NT	2	2	1	2
DXS8023		86	2,-	1,-	NT	1,2	1,(2)	2	2
DXS8017	Xp11.21	86	2,-	1,-	NT	1	1	2	1
DXS991	Xp11	88	2,-	1,-	NT	1,2	1,()	2	2
DXS990	Xq		1,-	3,-	2,3	2,3	2,3	1	2
DXS1047	Xq		1,-	2,-	2	2	2	1	2
DXS984	Xq		1,-	3,-	F	(2),3	(2),3	1	2
DXS998	Xq		2,-	1,-	1,2	1,2	1,2	2	1

NT=not tested. F=analysis failed. (2)=weak allele intensity. ()=allele not demonstrable.

Figure 1 Examples of autoradiographs from (A) locus DXS1048 on distal Xp outside the region encompassed by the structurally abnormal X chromosome, and (B) locus DXS8035 on proximal Xp included in the structurally abnormal chromosome. Bld=peripheral blood DNA, U=urine DNA, Fib=skin fibroblast DNA.

inherited a "maternal" allele that is not present in any of the tested maternal DNA samples. These include KAL, DXS999, DXS451, DXS1048, DXS7106, DXS1036, and DXS997 (fig 1). We interpret these results as showing that the mother must have had a second normal X in her ovary and that this cryptic X has contributed at least 15 of the 22 tested loci to the normal maternal X chromosome of her female offspring. Furthermore, it must have been this cryptic normal X that, at some time early in embryogenesis, gave rise to the abnormal psu idic(Xq), because the Xp loci proximal to DXS997 and the Xq loci are the same in the fetus and the abnormal psu idic(Xq).

Discussion

Whether or not the proband merits the appellation of Turner syndrome is moot; clinically she is not a Turner, merely a short female. However, she was reported as having a pure 45,X constitution on routine cytogenetic analysis of 50 peripheral blood leucocytes on two separate occasions. She was only investigated further because of the discrepancy between her essentially normal phenotype and her karyotype. Because of the observation of an unusual psu idic(Xq) in skin fibroblasts and molecular evidence that this chromosome was also present in cells from her buccal mucosa and urine, we scanned a further 150 cells from

blood cultures labelled with BrdU to look for a late synthesising chromosome and detected two cells containing the psu idic(Xq). Thus, the patient is clearly a mosaic with widespread presence of the cell line with the structurally abnormal X chromosome. The abnormal chromosome might well explain the patient's relative phenotypic normality. A patient has been described with a mosaic 45,X/46,X,del(X)(pter→p22.2::p11.3→qter) karyotype who transmitted the structurally abnormal X chromosome to her female child.⁷

Comparison of a series of X linked polymorphic markers from the father, a number of different tissues of the mother, and the placenta of the 46,XX pregnancy showed that the patient must have a normal 46,XX cell line in her germ cells and, furthermore, that the cryptic second X chromosome had given rise to the psu idic(Xq). Thus, the patient is a 45,X/46,X,psu idic(Xq)/46,XX mosaic and it seems reasonable to attribute her normal fertility to the 46,XX cell line. The availability of highly polymorphic molecular probes should now allow investigation of all offspring of 45,X females and thus provide direct evidence as to whether or not human female germ cells with a 45,X constitution are capable of giving rise to viable gametes.

Note added in proof

Since submission of this manuscript, we have received samples from the proband's son. Molecular analysis shows that his X chromosome is derived from the two normal maternal X chromosomes between which two recombinational events were detected.

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