

A cytogenetic and molecular study of a series of 45,X fetuses and their parents

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Abstract

The parental origin of the single X chromosome in 10 45,X fetuses was studied using DNA restriction fragment length polymorphisms. In six the single X was maternal in origin, in one it was paternal, and in one the results were consistent with a paternal origin. Therefore the parental origin of the X in 45,X fetuses that survive to the second or third trimester of pregnancy is similar to that of spontaneous abortions and live births with a 45,X constitution. The mothers of two of the fetuses were themselves found to have an abnormal sex chromosome complement, but in neither case did it appear to be related to the chromosome abnormality in the fetus.

Approximately 15 out of every 1000 conceptions that survive long enough to be clinically recognised pregnancies have a 45,X chromosome constitution. The great majority are spontaneously aborted during the first trimester of pregnancy. Morphologically the majority of such abortions consist of a chorionic and amniotic sac with a cord which has at its end either a fragment of embryonic tissue or a small macerated embryo. A minority are ruptured sacs with no evidence of cord or fetal development.¹ A small proportion of 45,X conceptuses survive until later in pregnancy and present as second trimester abortions or stillbirths. The majority of such pregnancies have a well developed fetus with cystic hygroma and other clinical stigmata associated with Turner's syndrome. Fewer than 1 in every 100 45,X conceptuses survives to birth when they develop as females with gonadal dysgenesis and the classical features of Turner's syndrome.

The reasons why 45,X conceptions are associated with three such different phenotypes are not known. One possible explanation is that the parental origin

of the single X chromosome affects the phenotype and in most liveborn females the single X has a different parental origin from that of the majority of 45,X abortions or late fetal deaths. It was estimated from classic work using the X linked blood group gene Xg that approximately 80% of liveborn females with a 45,X constitution had a single X chromosome of maternal origin.² We recently confirmed these results using X linked restriction fragment length polymorphisms in a series of non-mosaic 45,X females with Turner's syndrome.³ We have also investigated the parental origin of a series of spontaneous abortions with a 45,X constitution and shown that this ascertainment group is similar to the liveborn, approximately 80% having a maternally derived X and 20% a paternally derived X.⁴ Thus, in these two groups it is clear that the parental origin of the single X is not responsible for the widely differing phenotypes. However, there is evidence from the 45,X spontaneous abortions that the parental origin of the X may affect the phenotype as those with a paternally derived X were more likely to have a small gestational sac with or without an embryo than those with a maternally derived X.⁴

To date there is no information on the origin of the single X chromosome in late fetal deaths or stillbirths with a 45,X constitution. The purpose of this communication is to report our observations on 10 45,X fetuses ascertained during the second trimester of pregnancy.

Material and methods

During the past two years 10 fetuses have been referred to us for routine cytogenetic examination and found to have a 45,X constitution (table 1). One of these was a second trimester missed abortion and the remaining nine were diagnosed as being grossly abnormal on ultrasound examination and the pregnancy subsequently terminated. It has been shown that such fetuses are very unlikely to survive to birth⁵ and therefore it is reasonable to consider them representative of late fetal deaths. Wherever possible, we obtained tissue for karyotyping and for DNA extraction from the fetus and a blood sample from both parents from which we set up cultures for cytogenetic analysis and also extracted DNA.

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Table 1 The study population.

ID No	Ascertainment	Father		Mother		Previous reproductive history
		Age	Ch	Age	Ch	
88/2326	US at 18 weeks: IUGR, cystic hygroma, and hydrocephalus. Pregnancy terminated.	32	N	26	N	Primigravida
89/486	US at 16 weeks: cystic hygroma, pleural effusion, ascites, and very little amniotic fluid. Pregnancy terminated.	31	N	29	N	1 spontaneous abortion
89/216	US at 17 weeks: cystic hygroma and ascites. Pregnancy terminated.	25	N	26	N	1 livebirth
88/3743	US at 17 weeks: cystic hygroma, hydrops, and umbilical hernia. Pregnancy terminated.	28	N	28	N	1 livebirth
89/1326	US at 16 weeks: cystic hygroma, oedema, and pleural effusions. Pregnancy terminated.	23	N	20	N	1 livebirth
89/328	US at 17 weeks: cystic hygroma and ascites. Pregnancy terminated.	29	NT	23	NT	Primigravida
89/1258	Missed abortion at 16 weeks: cystic hygroma and oedema.	37	N	30	47,XXX	1 livebirth and 2 intrauterine deaths
89/983	US at 17 weeks: cystic hygroma. Pregnancy terminated.	27	N	23	N	1 TOP
89/3395	US at 16 weeks: cystic hygroma and hydrops fetalis. Pregnancy terminated.	27	N	22	46,X,del(X)(q22.3-q26)	1 livebirth
90/491	US at 18 weeks: cystic hygroma, grossly abnormal fetus. Pregnancy terminated.	28	N	27	N	1 TOP

US=ultrasound. N=normal. NT=not tested. IUGR=intrauterine growth retardation.

Solid tissue cultured from the fetus and parental lymphocytes were processed by standard techniques. The chromosomes were G or Q banded and subsequently observed by direct microscopic examination. DNA samples were extracted by a salt precipitation method,⁶ digested with the appropriate restriction enzymes (BCL), separated by gel electrophoresis, and transferred to Hybond nylon membranes (Amersham). The filters were then hybridised⁷ with random primed ³²P labelled DNA probes.⁸ All 45,X samples were tested with a Y centromere probe and at least one other Y specific probe to look for cryptic mosaicism. The 45,X fetuses and parents of the remaining eight families were tested with four X chromosome probes, namely DXS278, DXS255, DXYS1, and DXS52, selected to span the greater part of the X chromosome (see Jacobs *et al*³ for the details). Information from these probes was used to establish the parental origin of the single X chromosome.

Results

Nine of the 10 fetuses were found to have a 45,X chromosome constitution while cytogenetic observations were unsuccessful in one (89/1258). However, DNA studies showed fetus 89/1258 to have a single X and no Y chromosome and the chromosome constitution was therefore considered to be 45,X. In one case (89/328) no parental blood samples were obtained but chromosomes were examined in the parents of the remaining nine cases. The chromosomes of all nine fathers appeared normal, but two of the mothers were found to be chromosomally abnormal. The mother of

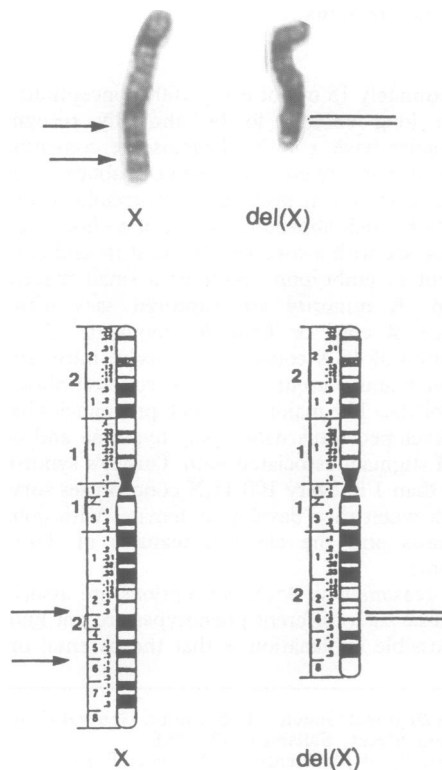


Figure 1 Partial GTG banded karyotype of X chromosomes, showing deleted (X)(q22.3→q26) on the right. Arrows indicate breakpoints.

Table 2 Results of X and Y probe analysis of 45,X fetuses.

No	X probes				Y probes				Final result
	DXS278	DXS255	DXYS1	DXS52	DYZ3	ZFY	DXYS1	DYS136	
89/2326	M	M	NI	NT	-	-	-	-	45,X(M)
89/486	M	M	M	NI	-	-	-	-	45,X(M)
89/216	M	M	NI	NT	-	-	-	-	45,X(M)
88/3743	Cons with P	Cons with P	NT	NT	-	-	-	-	45,X (cons with P)
89/1326	M	M	M	M	-	NT	-	-	45,X(M)
89/328	NT	NT	NT	NT	-	-	NT	NT	45,X
89/1258	M	M	NI	M	-	-	-	-	45,X(M)
89/983	M	M	NI	M	-	-	-	-	45,X(M)
89/3395	NT	NT	NT	NT	-	NT	NT	NT	45,X
90/491	P	NI	NI	P	-	NT	-	-	45,X(P)

- =negative. M=maternal. P=paternal. NI=not informative. NT=not tested.

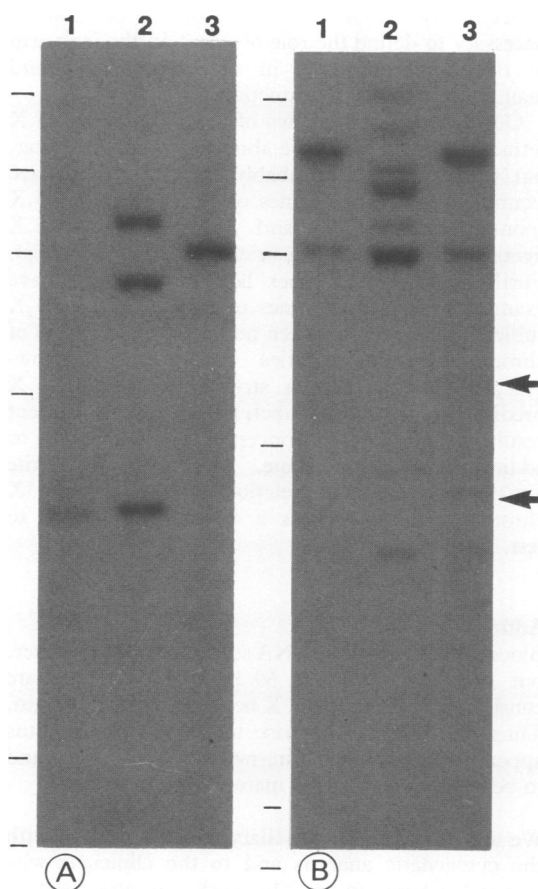


Figure 2 Southern blot analysis of two 45,X fetuses. Lane 1 proband, lane 2 mother, lane 3 father. (A) Analysis of patient 89/1258 with probe DXS255. The fetus has inherited its single X from its mother. The mother in this case can also be seen to have a 47,XXX karyotype. (B) Analysis of patient 90/491 with the probe DXS278. The fetus in this case can clearly be seen to have inherited its single X from its father. Size markers are λ HindIII. Arrows indicate Y specific bands.

89/1258 had a 47,XXX constitution in all 30 cells examined from a lymphocyte culture, while the mother of 89/3395 had a 46,X,del(X)(q22.3-q26) karyotype in all 50 cells examined from a lymphocyte culture (fig 1). Her mother had a normal chromosome constitution but her father was not available. As it is unlikely that a male would be viable and normal with a large deletion of one of his X chromosomes, it seems reasonable to assume that the deletion in the mother of 89/3395 is a de novo mutation. The deletion carrier was clinically unremarkable and had had a healthy male child before the 45,X pregnancy. Unfortunately, she declined to provide a further blood sample and therefore it was not possible either to determine the parental origin of the single X chromosome in the 45,X conceptus or to determine the precise extent of the deletion using molecular probes.

No evidence for Y chromosome material was found in any of the 10 fetuses. Because of the lack of DNA from the parents of 89/3395 and 89/328 no further studies were carried out on these families. However, the parental origin of the remaining eight fetuses was determined using the four X linked probes. As can be seen from table 2, in six cases the single X was clearly maternal in origin, in one case clearly paternal in origin, and in the remaining case the observations were consistent with a paternal origin, the DNA from the mother being degraded. Interestingly enough, in the 45,X whose mother had a 47,XXX constitution the single X was maternal in origin. This is illustrated in fig 2A where it can be seen that the mother has three different alleles for the multiallelic probe M27 β , the father a different allele, and the fetus a single band identical to one of the mother's.

Discussion

We found six second trimester fetuses with a 45,X constitution to have a single maternally derived X, while one of the remaining two was consistent with and one had a paternally derived X. Thus, in the

limited number of cases studied by us, the parental origin of the single X chromosome in 45,X conceptuses presenting in the second trimester of pregnancy as missed abortions or grossly abnormal fetuses appears no different from the parental origin of 45,X spontaneous abortions⁴ or 45,X livebirths.³ In all three populations, approximately 80% of the 45,X subjects have a maternal X and 20% a paternal X. Thus the parental origin of the X does not seem to be responsible for the very different phenotypes shown by 45,X conceptuses.

Previous studies have shown that sex chromosome monosomy is associated with a reduced maternal age and that this reduction is confined to those cases in which the maternal X chromosome is absent.^{3,4} This observation suggests that some 20% of sex chromosome monosomy results from a non-disjunctional mechanism that is more frequent among younger than older women. Non-disjunction at a paternal meiotic division is unlikely to be the major cause of the remaining 80% in which the paternal sex chromosome is absent, because cytogenetic studies of human spermatozoa provide no evidence of an unusually high incidence of non-disjunction of the sex chromosomes.^{9,10} It seems probable that most sex chromosome monosomy results from postzygotic loss of a paternal sex chromosome. Such a mechanism is consistent with observations in the mouse where most 45,X cases arise from loss of a paternal X or Y chromosome after fertilisation.¹¹

In our small series we found two of nine mothers tested to have a sex chromosome abnormality. In one, a 47,XXX female, we were able to show that the error resulting in the 45,X fetus was loss of a paternal X chromosome and therefore unrelated to the maternal abnormality. Furthermore, as the mother had three different alleles for at least one X linked marker, M27 β , she herself must have been the result of a non-disjunctional meiotic error in her mother, who in turn must have been heterozygous for M27 β . It has been shown that over 90% of 47,XXX females result from an error in a maternal meiotic division, so the mother of 89/1258 conforms to the general rule.¹² There are anecdotal reports of a number of chromosomally abnormal offspring born to 47,XXX females¹³ and, on the basis of these, it has been suggested that 47,XXX women may be at an increased risk of producing chromosomally abnormal offspring. Now that the methodology exists for studying the parental origin of such chromosomally abnormal children it would seem salutary to do so. Many ascertained through chromosomally abnormal offspring may, like our case, be no more than chance observations.

The second maternal chromosome abnormality was an X(q22.3→q26) deletion, but we were not able to determine the relation of the deletion to the 45,X conception. There are very few large interstitial deletions of Xq published, although it may be that

many deletions classified as terminal are in reality interstitial. We can find reference to only one other deletion similar to that seen in the mother of 89/3395. Krauss *et al*¹⁴ described a family in which a del(Xq) was segregating in three generations and in which four of five carrier females had premature ovarian failure and the remaining one irregular menses. Nevertheless, four of the five female carriers had reproduced successfully. Their patients, like ours, showed none of the clinical features of Turner's syndrome. Our patient is aged 22, and thus it is too early to know whether she too will have premature ovarian failure. It is interesting that large deletions of the so-called critical region of the long arm of the X chromosome, thought to be necessary in double dose for normal ovarian function,¹⁵ are compatible with ovulation and fertility. It is clear that careful cytogenetic and molecular definition of a series of Xq deletions is necessary to define the role of genes on the long arm of the X chromosome in the development and maintenance of ovarian function.

Our observation that two of nine mothers of 45,X fetuses had a chromosome abnormality is surprising, but we regard it as probably fortuitous. We have examined the chromosomes of 35 mothers of 45,X spontaneous abortions and 31 mothers of 45,X livebirths and all have been chromosomally normal. Furthermore, many other laboratories must have examined the chromosomes of the parents of 45,X subjects and there has been no report of an excess of chromosome abnormalities among them. However, it may be that a structurally abnormal X predisposes to non-disjunction of the X bivalent resulting in an excess of conceptuses with a missing or additional X chromosome. The rarity of fertile females with a large deletion of one of their X chromosomes makes this a difficult hypothesis to test.

Addendum

Blood was obtained for DNA studies from the mother, but not the father, of 89/3395. The results are consistent with the single X being of maternal origin. Thus, the error giving rise to the 45,X conceptus appears to involve the paternal sex chromosome and to be independent of the maternal deletion.

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