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. A small proportion of 45,X conceptions 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 conceptions 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. 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. 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. 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.

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 birth 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.
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, digested with the appropriate restriction enzymes (BCL), separated by gel electrophoresis, and transferred to Hybond nylon membranes (Amersham). The filters were then hybridised with random primed $^{32}$P labelled DNA probes. 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\textsuperscript{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 cyrogenetic 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.](image-url)
Table 2  Results of X and Y probe analysis of 45,X fetuses.

<table>
<thead>
<tr>
<th>No</th>
<th>DXS278</th>
<th>DXS255</th>
<th>DXYS1</th>
<th>DXS52</th>
<th>DYZ3</th>
<th>ZFY</th>
<th>DXYS1</th>
<th>DYS136</th>
<th>Final result</th>
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<tr>
<td>89/2326</td>
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<td>M</td>
<td>NI</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45,X(M)</td>
</tr>
<tr>
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<td>M</td>
<td>M</td>
<td>M</td>
<td>NI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45,X(M)</td>
</tr>
<tr>
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<td>M</td>
<td>M</td>
<td>NI</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45,X(M)</td>
</tr>
<tr>
<td>88/3743</td>
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<td>P</td>
<td>NT</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
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<td>45,X (cons with P)</td>
</tr>
<tr>
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<td>M</td>
<td>M</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
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<td>45,X(M)</td>
</tr>
<tr>
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<td>NT</td>
<td>NT</td>
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<td>45,X(M)</td>
</tr>
<tr>
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</tr>
<tr>
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<td>45,X(M)</td>
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<td>NT</td>
<td>NT</td>
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<td></td>
<td>45,X</td>
</tr>
<tr>
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<td>NI</td>
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<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45,X(P)</td>
</tr>
</tbody>
</table>

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

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 M27β, 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 sponta-
neous abortions or 45,X livebirths. Furthermore,
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. This
observation suggests that some 20% of sex chromo-
some 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
spermatocytes provide no evidence of an unusually high
incidence of non-disjunction of the sex chromo-
somes. It seems probable that most sex chromo-
some 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.

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, M27β, she herself must have been the result of
a non-disjunctional meiotic error in her mother,
who in turn must have been heterozygous for M27β.
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.
There are anecdotal reports of a number of chromo-
somally abnormal offspring born to 47,XXX females
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,
ilike our case, be no more than chance observations.

The second maternal chromosome abnormality was
an Xq(22.3→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 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, 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. How-
ever, 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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