Am. J. Hum. Genet. 42:534–541, 1988

Cytogenetic and Molecular Analysis of Sex-Chromosome Monosomy

Terry Hassold,* Frances Benham,† and Mark Leppert‡

*Division of Human Genetics, Department of Pediatrics, Cornell University Medical College, New York; †Pediatric Research Unit, Guys Hospital Medical School, London; and ‡Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City

Summary

X chromosome– and Y chromosome–specific DNA probes were used to study different aspects of the genesis of sex-chromosome monosomy. Using X-linked RFLPs, we studied the parental origin of the single X chromosome in 35 spontaneously aborted and five live-born 45,X conceptions. We determined the origin in 35 cases; 28 had a maternal X (X^m) and seven had a paternal X (X^P). There was a correlation between parental origin and parental age, with the X^P category having a significantly reduced mean maternal age by comparison with the X^m group. Studies aimed at detecting mosaicism demonstrated the presence of a Y chromosome or a second X chromosome in three of 33 spontaneous abortions, a level of mosaicism much lower than that reported for live-born Turner syndrome individuals.

Introduction

We recently reported our observations on the inheritance of X-linked RFLPs in a small series of 10 45,X spontaneous abortions (Hassold et al. 1985). The results of that analysis showed the utility of using RFLPs to determine the parental source of the chromosome error, as we were able to specify the parent of origin of the single X in nine of 10 cases studied. Additionally, we identified cases in which the single X was maternally derived (X^m) and cases in which the single X was paternally derived (X^P), demonstrating that sex-chromosome monosomy resulting in spontaneous abortion can result from loss of either parental sex chromosome.

Since that time we have continued to use X chromosome– and Y chromosome–specific probes to study three aspects of the genesis of sex-chromosome monosomy: first, the parental origin of the single X chromosome; second, the relationship between parental origin and parental age; and third, the incidence of mosaicism, as detected by molecular probes, in cytogenetically defined nonmosaic 45,X conceptions.

In this paper we summarize our studies of 40 45,X conceptions, consisting of the original 10 cases and 30 cases studied since the time of the initial report. Our results indicate that the single X chromosome is usually X^m, both in live-born and in spontaneously aborted 45,X conceptions. Additionally, there is a correlation between parental origin and parental age, with the X^P cases having a significantly reduced mean maternal age by comparison with the X^m category. Our analysis of mosaicism indicates that some 45,X abortions are mosaics for a second sex chromosome, but the incidence of mosaicism is clearly lower than that observed among live-born 45,X conceptions.

Material and Methods

Ascertainment of Cases

From 1976 to 1985 we conducted a cytogenetic study of all spontaneous abortions occurring at a single hospital in Honolulu. The details of the study population and the methodology for the collection and culturing of the tissue samples have been described elsewhere (Hassold et al. 1980), and a summary of the cytogenetic observations recently has been presented by Jacobs et al. (in press). During the study period we identified 271 abortions with sex-

Received September 29, 1987.

Address for correspondence and reprints: Dr. Terry Hassold, Division of Medical Genetics, Emory University, 2040 Ridgewood Drive, N.E., Atlanta, GA 30322.

0002-9297/88/4204-0002$02.00

chromosome monosomy, of which 265 had an apparent nonmosaic 45,X chromosome constitution, two were 45,X/46,XX, one was 45,X/46,XY, and three had complex chromosome abnormalities including sex-chromosome monosomy. In 35 of these cases we obtained parental blood samples, making it possible to study the parental origin of the single X chromosome.

We also obtained blood samples from five girls with Turner syndrome who are being followed by the Division of Pediatric Endocrinology, New York Hospital–Cornell University Medical College. All five girls had been studied previously by other laboratories and been diagnosed as having a nonmosaic 45,X chromosome constitution. In three of the five cases we received blood samples from both parents, and in the two other cases we obtained a sample from one parent only.

Cytogenetic Analysis

Our routine cytogenetic evaluation of spontaneous abortions typically consisted of scoring four or five Q-banded cells/case. As one aim of the present study was an analysis of mosaicism, we augmented the original observations, using 2–4-year-old chromosome slides that were still available on 30 of the 35 abortions. The quality of the slides was rather poor, but, nevertheless, in each of the 30 cases we were able to increase to at least 10 the number of cells analyzed (table 1).

At least 100 Q-banded cells were scored on each of four girls with Turner syndrome. However, in one instance (X66) the blood sample we received was unsuitable for cytogenetic analysis, and in this case the cytogenetic results are those of the referring laboratory.

DNA Analysis

DNA from cultured fetal cells or frozen fetal tissue and from peripheral blood samples was prepared as described elsewhere (Hassold et al. 1985). DNA samples were digested with the appropriate restriction endonucleases, according to the conditions specified by the manufacturer (International Biotechnologies, Inc.), and the digested DNA was size fractionated overnight on 0.8%–1.2% agarose gels. The samples were then transferred to nylon membranes (Zetabind; AMF-Cuno, Inc.) by using the method of Southern (1975) and, following prehybridization, hybridized under standard conditions to nick-translated ^{32}P-labeled probes (Rigby et al. 1977) or probes that had been labeled using random primers (Feinberg and Vogelstein 1983).

Fifteen probes detecting X-chromosome RFLPs were used in the parental origin studies: p8(DXS1), p19-2(DXS3), L1.28(DXS7), p58-1(DXS14), X13 (DXS15), S21(DXS17), 52A(DXS51), St14(DXS52), 754(DXS84), St1(DXS86), pXG16(DXS92), pXG12(DXS94), C11(DXS144), pDP34(DXYS1), and pOTC (Willard et al. 1985).

In studies aimed at detection of Y-chromosome material, two hybridization probes were used: pDP-105 (DYZ4), which recognizes repeated sequences on both Yq and Yp and detects male-specific restriction fragments after digestion with any of several enzymes (Andersson et al. 1986; D. Page, personal communication), and Y97, which is derived from an alphoid centromeric repeat and detects a male-specific 5.5-kb fragment on an *Eco*RI digest (Stalvey and Erickson 1987). For pDP105, we hybridized at 42 C in 50% formamide and washed the filters at 55 C at a final stringency of 0.1%–0.5% SSC. For Y97, we hybridized at 50 C in 50% formamide and washed the filters at 65 C at a final stringency of 0.1% SSC.

Results

Detailed information on the 35 spontaneous abortions and five live borns is presented in table 1.

Analysis of Parental Origin

We were able to determine unambiguously the parental origin of the single X chromosome in 30 of the 35 spontaneous abortions and in four of the five live borns (fig. 1). Overall there were 27 cases (79.4%) in which the X was X^m and seven (20.6%) in which the X was X^p. In all but nine cases the decision was based on results from more than one probe, and in most cases at least three RFLPs were informative.

There were six cases in which we were unable to determine the origin of the single X. Three of these (K3288, K3383, and K3446) were mosaics and are discussed below. In two other cases involving spontaneous abortions (K2872 and K2915), there was no evidence of a paternally inherited allele, but both cases were heterozygous for those loci heterozygous in the mother (see example in fig. 2). As the DNA in these cases was isolated from cultured fetal cells, which are occasionally contaminated with maternal cells (Hassold et al. 1983), the most likely explanation for these cases is overgrowth of the fetal cells by

Table 1

Summary of Information on 35 Spontaneous Abortions and Five Live Borns with Sex-Chromosome Monosomy

ID No.	Cytogenetic Analysis	No. of Cells Analyzed	No. of Cells with Chromosome Counts of			Gestational Age (Days)	Parental Age (Years)		Parental Origin of Single X Chromosome
			44	45	46		Father	Mother	
Spontaneous abortions:									
K2525	45,X	13		13		87	29	29	Maternal (1)[a]
K2739	45,X	10		10		130	35	32	Paternal (2)
K2808	45,X	14		14		88	32	32	Maternal (2)
K2812	45,X	10	2	8		81	22	20	Paternal (1)
K2872	45,X	14	1	13		86	32	24	Unknown, maternal contamination
K2915	45,X	15		15		114	34	30	Unknown, maternal contamination
K2937	45,X	12		12		116	37	34	Maternal (1)
K2954	45,X	12		12		77	30	27	Maternal (3)
K3021	45,X/46,X,+16	21		12	9	69	34	34	Maternal (5)
K3034	46,X,+22	9		1	8	83	37	36	Maternal (6)
K3052	45,X	13	2	11		88	34	33	Maternal (2)
K3075	45,X	10		10		86	18	18	Paternal (1)
K3081	45,X	14	3	11		103	49	30	Paternal (3)
K3090	45,X	10	2	8		98	25	20	Maternal (3)
K3113	45,X	13	2	11		87	36	31	Maternal (5)
K3125	45,X	8	2	6		71	35	32	Maternal (4)
K3129	45,X	5		5		80	22	19	Maternal (5)
K3135	45,X	12	1	11		52	25	25	Paternal (3)
K3206	45,X	11		11		87	42	26	Maternal (3)
K3231	45,X	11	1	10		85	34	24	Maternal (2)
K3245	45,X	10		10		89	39	31	Maternal (3)
K3253	45,X	11		11		84	49	40	Maternal (1)
K3255	45,X	10	3	7		91	31	31	Maternal (4)
K3261	45,X	6	4	2		87	32	27	Maternal (3)
K3281	45,X	11		11		92	33	23	Maternal (8)
K3288	45,X/46,XX	13		9	4	85	36	37	Mosaic (based on cytogenetics, DNA)
K3299	45,X	12		12		86	18	18	Paternal (1)
K3331	45,X	11		11		83	32	31	Maternal (4)
K3340	45,X	4		4		87	34	33	Maternal (3)
K3362	46,X,+13	10			10	74	34	30	Maternal (2)
K3370	45,X	10	2	8		100	29	26	Maternal (1)
K3383	45,X	18	2	15	1(46,X,+10)	81	25	26	Mosaic (based on DNA)
K3430	45,X	12		12		82	32	32	Maternal (1)
K3442	45,X	12	1	11		119	32	40	Maternal (4)
K3446	45,X	13	4	9		107	...	25	Mosaic (based on DNA)
Live borns:									
X49	45,X	120	1	119		...	...	28	Unknown, consistent with maternal on 10 probes
X54	45,X	100		100		...	29	26	Maternal (3)
X63	45,X	100	1	99		...	19	18	Maternal (2)
X66	45,X	20		20		...	20	15	Paternal (1)
X131	45,X	100		100		...	28	24	Maternal (4)

[a] No. of RFLPs giving results on parental origin.

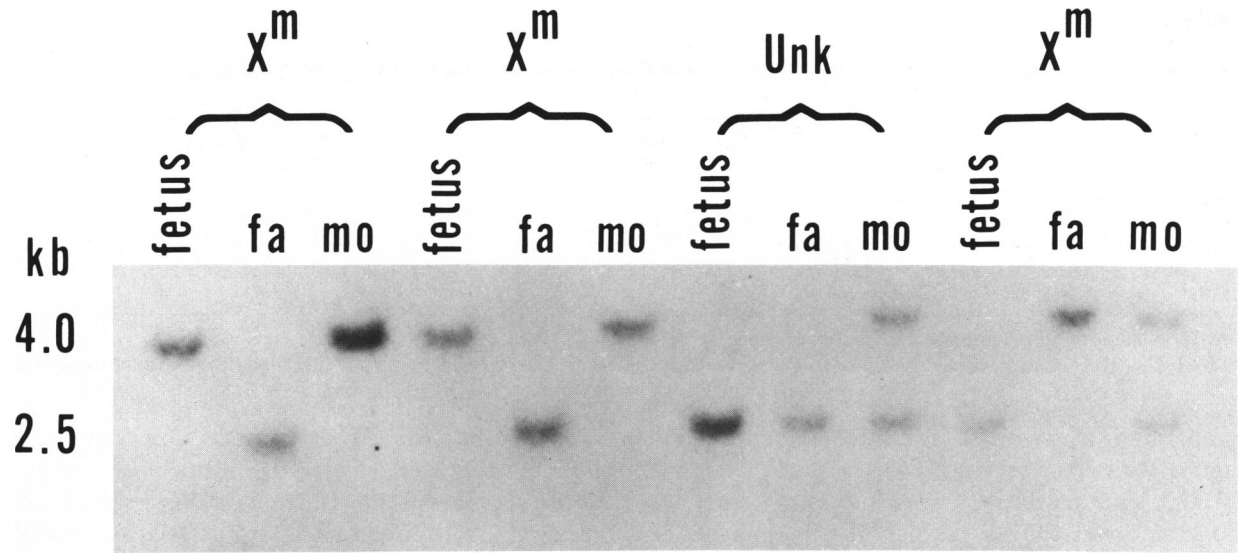
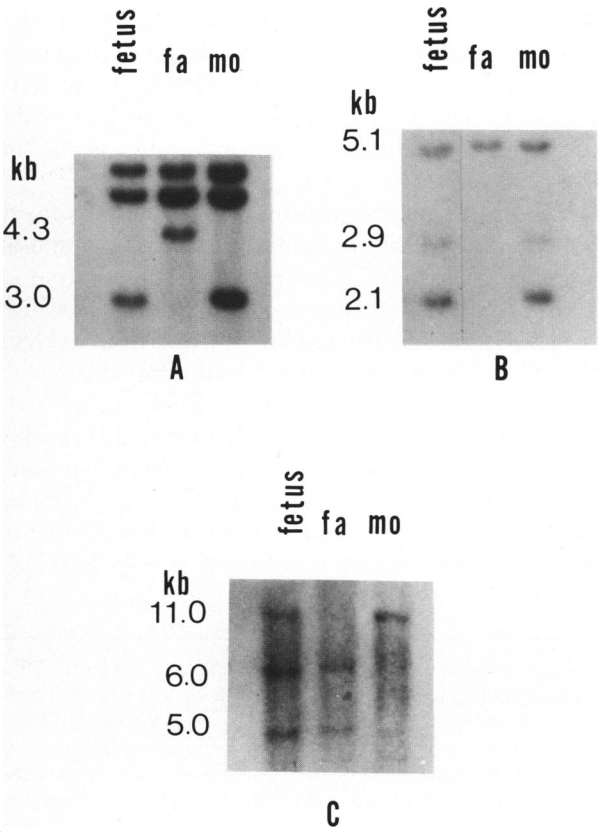


Figure 1 Analysis of the parental origin of the single X chromosome in four 45,X spontaneous abortions (K3034, K3331, K3383, and K3442), using *Msp*I-digested DNA samples probed with p58-1. In three cases the X is maternally derived, and in the other case the origin cannot be determined.



maternal cells, with the extracted "fetal" DNA being largely, if not exclusively, maternal in origin.

In the final case (X49) we had blood only from the patient and the mother. In an analysis of nine different loci (DXS1, 3, 7, 15, 52, 86, 92, and 144 and DXYS1) we never identified an allele in the patient that was not also present in the mother. Thus, while we cannot formally exclude the father as the parent of origin of the single X, a consideration of (1) the allelic frequencies of the 10 loci and (2) the genotypes of the mother and daughter makes this a highly unlikely possibility ($P = .013$). Therefore, if we assume the X chromosome in this case to be X^m, the total

Figure 2 Use of X-linked RFLPs to distinguish between maternal contamination and mosaicism. *A* and *B*, Analysis of K2915 and parents, using *Taq*I-digested DNA samples probed with (*A*) C11 or (*B*) 19.2. From the results with C11 it is clear that the fetus did not inherit the 4.3-kb paternal allele. However, when 19-2 is used, the fetus has both the upper (5.1-kb) and lower (2.9 + 2.1–kb) alleles, indicating the presence of two X chromosomes. As both X's must be maternally derived, the case presumably represents maternal contamination. *C*, Analysis of K3383 and parents, using *Bgl*II-digested samples probed with St1. The fetus has inherited an allele from each parent, and thus, even though we were unable to detect a second cell line on cytogenetic analysis (table 1), the fetus must be mosaic for a second X chromosome.

Table 2

Summary of Information on Gestational Age and Maternal Age by Parent of Origin of Single X Chromosome[a]

Population	Mean ± SD Maternal Age (Years)		Mean ± SD Gestational Age (Days)	
	45,X^p	45,X^m	45,X^p	45,X^m
Spontaneous abortions	23.8 ± 6.1 (6)	29.6 ± 5.5 (21)	89.7 ± 25.8 (6)	89.4 ± 11.3 (21)
Live borns	15 (1)	24.0 ± 4.3 (4)	...	...
Total	22.6 ± 6.5 (7)	28.7 ± 5.6 (25)	...	...

Note.—Numbers in parentheses are number of cases.

[a] Excluding all known sex-chromosome mosaics and cases with chromosome abnormalities in addition to sex-chromosome monosomy.

number of 45,X's with X^m is 28 (80%) and the total number with X^p is seven (20%).

Table 2 summarizes information on maternal age and gestational age by the parent of origin of the single X chromosome. There was no association between parental origin and gestational age of the spontaneous abortions, but the mean maternal age of the seven 45,X^p cases was significantly reduced in comparison with that of the 45,X^m category ($t = 2.45$; $P < .05$).

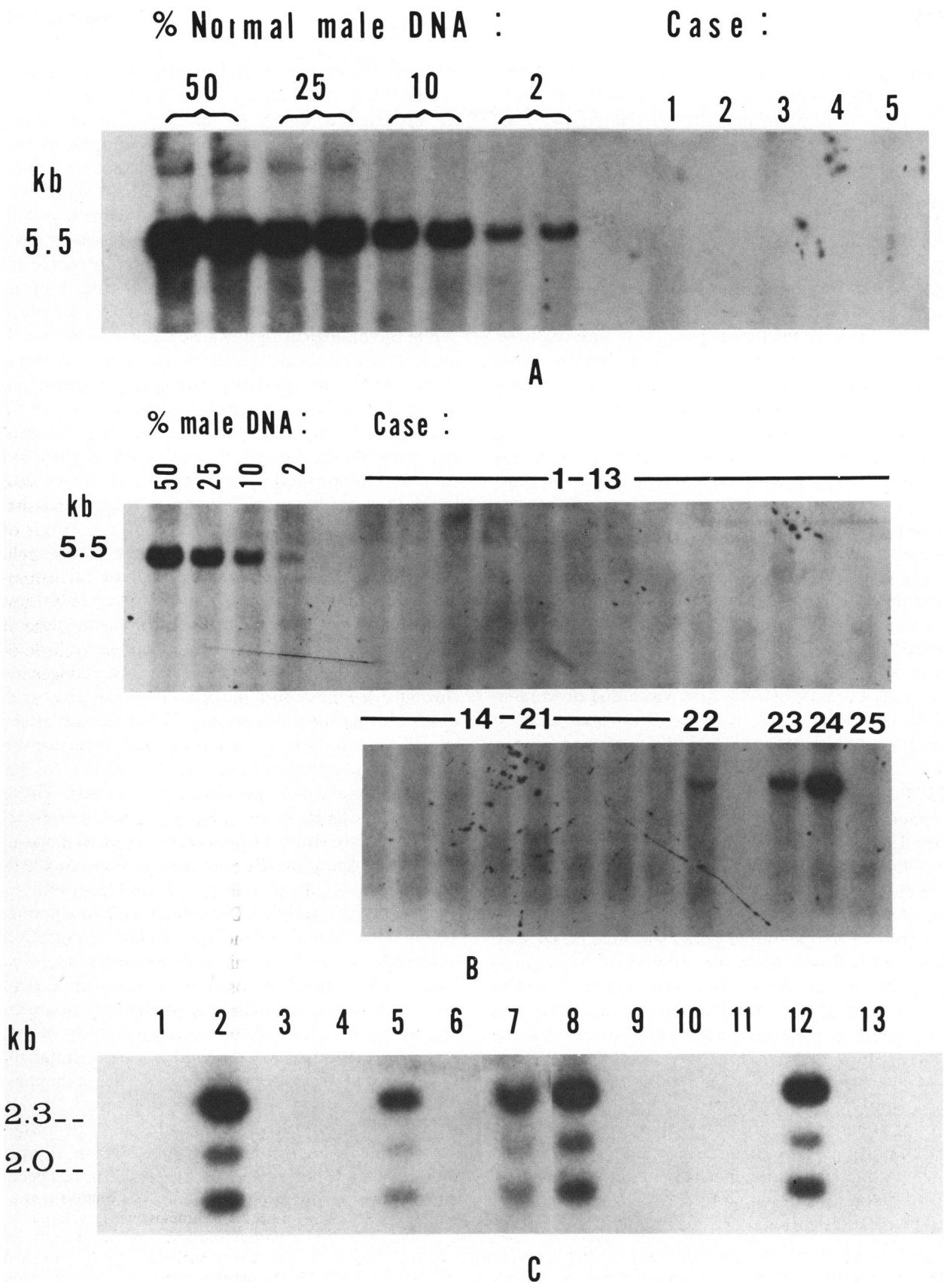
Analysis of Mosaicism

A summary of the cytogenetic observations on mosaicism is given in table 1. We counted 10 or more cells on 30 of the 35 spontaneous abortions, and thus, for these cases, we could exclude an ~25% level of mosaicism with 95% confidence (Hook 1977). We identified two mosaics, one a mosaic for trisomy 16 (K3021) and one a mosaic for a normal female cell line (K3288). In the latter case, an analysis of Q-banded chromosome heteromorphisms showed the markers of the two fetal cell lines to be identical and to be different from those of the mother; thus, the fetal 46,XX line represented a product of conception and was not the result of maternal contamination. We scored 100 or more cells on four of the five live-born 45,X girls, and 20 cells were scored on the fifth girl. There was no evidence for a second cell line in any of the five girls.

In addition to the cytogenetic observations, all 45,X^m cases were analyzed for the presence of Y chromosome–specific DNA. For this analysis samples were hybridized to Y97 or pDP105, as described in Material and Methods. The test samples were routinely compared with a panel of controls consisting of artificial mixtures of differing proportions of normal male and female DNA. Using this approach, we were able to detect clearly levels of Y-chromosome mosaicism of 1%–2% with both Y97 and pDP105 (fig. 3). From figure 3, it is clear that one of the spontaneous abortions (K3446) was Y-chromosome positive, even though there was no cytogenetic evidence for a second cell line. However, we were unable to detect a Y-bearing cell line in any of the remaining spontaneous abortions or in any of the live-born girls.

We also examined all samples for heterozygosity for X-linked RFLPs, a condition indicative of mosaicism for a second X chromosome. From analysis of DNA samples of sex-chromosome mosaics in which the level of mosaicism was known, and from analysis of artificial mixtures of DNA from individuals with different genotypes for the probes St14 and DXYS1, we had previously determined that we could typically detect a 10% level of mosaicim (T. Hassold, unpublished observations). Analysis of the 45,X samples led to the detection of two mosaics (fig. 2), one of which (K3288) had been identified as a 45,X/46,XX

Figure 3 Use of Y chromosome–specific probes to detect Y-chromosome mosaicism. *A*, *Eco*RI-digested samples probed with Y97. Control lanes consist of mixtures of normal male:female DNA samples, corresponding to 50%, 25%, 10%, and 2% male DNA. Total/lane = 5 mcg DNA. Lanes 1–5 = 45,X liveborn individuals. None of the cases exhibit the 5.5-kb Y-specific fragment; therefore, there is no evidence for Y-chromosome mosaicism in these cases. *B*, *Eco*RI-digested samples probed with Y97, with the same controls used as above. Lanes 1–22 = 45,X spontaneous abortions. Only one sample (lane 22, K3446) shows evidence for Y-chromosome mosaicism. Lane 23 = liveborn individual with 45,X/46,X mar Y karyotype. Lane 24 = normal male. Lane 25 = normal female. *C*, *Msp*I-digested samples probed with pDP105. Lanes 1, 4, 7, 10, and 11 = 45,X spontaneous abortions. Lanes 2, 5, 8, and 12 = normal males. Lanes 3, 6, 9, and 13 = normal females. Only the normal males and one spontaneous abortion (lane 7, K3446) give positive results.


% Normal male DNA :
Case :
50
25
10
2
1 2 3 4 5
kb
5.5
A
% male DNA :
Case :
50 25 10 2
1-13
kb
5.5
14-21
22
23 24 25
B
kb
1 2 3 4 5 6 7 8 9 10 11 12 13
2.3--
2.0--
C

mosaic by cytogenetic analysis. In the second case (K3383) there was no cytogenetic evidence for sex-chromosome mosaicism, although one cell with an extra chromosome 10 was identified.

Discussion

The results of the present study reaffirm the usefulness of RFLPs in determining the parental origin of chromosome abnormalities. In our series of 40 45,X cases we were able to specify the origin of the single X in 35 cases, to document previously unrecognized mosaicism in two instances, and to confirm cytogenetically detected mosaicism in one instance. Only two cases were uninformative, and both involved spontaneous abortions in which there was no fetal DNA available for study, the fetal cultured cells apparently having been overgrown by maternal cells. Therefore, we were able to determine parental origin in each of the 38 cases in which appropriate material was available for analysis.

The results also provide additional evidence for the predominance of paternal sex-chromosome loss in the genesis of sex-chromosome monosomy. In both the spontaneous abortions and live borns, 80% of the cases were of this type, a value that agrees well with an earlier estimate of 77% that was based on studies of the Xg blood group in Turner syndrome patients and parents (Sanger et al. 1977; Tippett and Sanger 1985). The consistency of these observations suggests that the origin of monosomy is similar for spontaneously aborted and live-born 45,X conceptions—and that it typically involves loss of the father's X or Y chromosome.

Our analysis of parental age indicates that there is an interaction between parental origin and parental age, as the mean maternal age of the $45,X^p$ class was significantly lower than that of the $45,X^m$ group. There have been several cytogenetic studies of spontaneous abortions in which an inverse age effect has been observed for sex-chromosome monosomy (see, e.g., Warburton et al. 1980), and our results indicate that the effect is confined to those cases in which the maternal X chromosome is absent (i.e., the X^p group). If confirmed, our observation suggests that at least a portion of sex-chromosome monosomy results from a nondisjunctional mechanism that is more frequent among younger than older women.

The mean gestational ages were virtually identical for the $45,X^p$ and $45,X^m$ spontaneous abortions, suggesting that the parental source of the single X has no effect on the extent of fetal development. However, in a review of the routine hospital pathology reports, we observed a significant difference in the morphological appearance of the tissue samples of the two types of abortions. Specifically, tissue samples from four of the six X^p abortions were noted as containing small gestational sacs (greatest diameter <5.0 cm) with embryos 1.0–2.5 cm in greatest diameter, but sacs with or without fetuses were not noted in any of 21 X^m abortions for which pathology reports were available ($P = .0017$ by Fisher's exact test). While the biological significance of this difference is unclear, recent evidence from the mouse demonstrates that the parental origin of a gene(s) may affect its expression (see, e.g., Cattanach and Kirk 1985), and therefore our observation may reflect an interaction between the parental source of the X chromosome and some aspect of development. However, this is clearly a preliminary observation, which must be confirmed on a larger body of data.

Finally, the results of both the cytogenetic and molecular analyses demonstrate that mosaicism is much less common among spontaneous abortions than among live borns with sex-chromosome monosomy. In our spontaneous-abortion survey as a whole, we observed cytogenetically detectable sex-chromosome mosaicism in three (1.1%) of 271 cases of sex-chromosome monosomy. The molecular studies led to detection of two additional sex-chromosome mosaics among 32 putative nonmosaics (6.3%) for which fetal DNA was available for study. Thus, using a combination of cytogenetic and molecular techniques, we detected sex-chromosome mosaicism in 7.4% of spontaneous abortions with a 45,X cell line.

By comparison, the incidence of mosaicism among all newborn infants with a 45,X cell line, irrespective of phenotype, is ~82% (Hook and Hamerton 1977). Furthermore, this value may well be an underestimate by comparison with that of the present study because the newborn studies consisted solely of cytogenetic analysis of blood, whereas the present analysis consisted of cytogenetic and molecular examination.

Thus, there is little doubt that the level of mosaicism is much higher among live-born than among spontaneously aborted 45,X conceptions, as has been noted by other investigators (Hook and Warburton 1983). This suggests that the extraordinarily high in utero lethality of the 45,X condition is largely restricted to those cases lacking a second cell line and

implies that most, if not all, live-born 45,X's are mosaics (Hook and Warburton 1983). This suggestion is supported by recent molecular analyses that have detected Y-chromosome mosaicism among Turner syndrome individuals previously classified as being "pure" 45,Xs on cytogenetic examination (Muller et al. 1987). Continued use of X chromosome– and Y chromosome–specific probes to study mosaicism will determine the extent to which the presence of a second cell line affects survival of 45,X conceptions.

Acknowledgments

We wish to express our deep gratitude to Drs. Maria New and Madeleine Harbison for providing samples from Turner syndrome individuals and their parents; to Drs. G. Bruns, D. Drayna, R. Erickson, L. Kunkel, S. Latt, J. L. Mandel, D. Page, P. Pearson, and P. Szabo for making available probes used in this study; and to Norma Takaesu, Emerson Whittington, Chris Kobryn, and Teresa Tusie for providing expert technical assistance. This work was supported by National Institutes of Health grants HD21341 and BRSGS07 RR05396, by The Spastics Society of Great Britain, and by The Generation Trust.

References

Andersson, M., D. C. Page, and A. de la Chapelle. 1986. Chromosome Y–specific DNA is transferred to the short arm of X chromosome in human XX males. Science 233:856–858.

Cattanach, B., and M. Kirk. 1985. Differential activity of maternally and paternally derived chromosome regions in mice. Nature 311:374–376.

Feinberg, A. O., and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6–13.

Hassold, T., N. Chen, J. Funkhouser, T. Jooss, B. Manuel, J. Matsuura, A. Matsuyama, C. Wilson, J. Yamane, and P. A. Jacobs. 1980. A cytogenetic study of 1,000 spontaneous abortions. Ann. Hum. Genet. 44:151–178.

Hassold, T., E. Kumlin, N. Takaesu, and M. Leppert. 1985. Determination of the parental origin of sex-chromosome monosomy using restriction fragment length polymorphisms. Am. J. Hum. Genet. 37:965–972.

Hassold, T., S. D. Quillen, and J. A. Yamane. 1983. Sex ratio in spontaneous abortions. Ann. Hum. Genet. 47:39–47.

Hook, E. B. 1977. Exclusion of chromosomal mosaicism: tables of 90%, 95%, and 99% confidence limits and comments on use. Am. J. Hum. Genet. 29:94–97.

Hook, E. B., and J. L. Hamerton. 1977. The frequency of chromosome abnormalities detected in consecutive newborn studies. Pp. 63–79 *in* E. B. Hook and I. H. Porter, eds. Population cytogenetics: studies in humans. Academic Press, New York.

Hook, E. B., and D. Warburton. 1983. The distribution of chromosomal genotypes associated with Turner's syndrome: livebirth prevalence rates and evidence of diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. Hum. Genet. 64:24–27.

Jacobs, P. A., T. Hassold, A. Henry, D. Pettay, and N. Takaesu. Trisomy 13 ascertained in a survey of spontaneous abortions. J. Med. Genet. (in press).

Muller, U., T. Donlon, S. Kunkel, M. Lalande, and S. Latt. 1987. Y-190, a DNA probe for the sensitive detection of Y-derived marker chromosomes and mosaicism. Hum. Genet. 75:109–113.

Rigby, P. W. J., M. Dieckmann, C. Rhoades, and P. Berg. 1977. Labelling DNA to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. 113:237–251.

Sanger, R., P. Tippett, J. Gavin, P. Teesdale, and G. L. Daniels. 1977. Xg groups and sex chromosome abnormalities in people of northern European ancestry: an addendum. J. Med. Genet. 14:210–213.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

Stalvey, J., and R. P. Erickson. 1987. An improved method for detecting Y chromosomal DNA. Hum. Genet. 76: 240–243.

Tippett, P., and R. Sanger. 1985. Source of single X in XO Turner syndrome: a comment. Hum. Genet. 70:92.

Warburton, D., J. Kline, Z. Stein, and M. Susser. 1980. Monosomy X: a chromosomal anomaly associated with young maternal age. Lancet 1:167–169.

Willard, H. F., M. Skolnick, P. L. Pearson, and J. L. Mandel. 1985. Human gene mapping by recombinant DNA techniques. Human Gene Mapping 8. Cytogenet. Cell Genet. 40:420–423.