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Determination of the Parental Origin of Sex-Chromosome Monosomy Using Restriction Fragment Length Polymorphisms

TERRY HASSOLD,[1] ERIKA KUMLIN,[2] NORMA TAKAESU,[1] AND MARK LEPPERT[2]

SUMMARY

The parental origin of the single X chromosome in sex-chromosome monosomy was evaluated by comparing restriction fragment length polymorphisms (RFLPs) of 10 spontaneous aborted 45,X conceptions with those of their parents. Seven X-linked marker loci were used, and we were able to specify the origin of the X in nine cases, with six being maternally and three paternally derived. These results demonstrate the efficiency of the technique and show that the single X chromosome in 45,X spontaneous abortions can be derived from either parent.

INTRODUCTION

Sex-chromosome monosomy is the most commonly observed chromosome abnormality in humans, occurring in approximately 1%–2% of all clinically recognizable pregnancies [1]. Rarely, 45,X conceptions survive to term and have the clinical features of Turner syndrome. However, more than 99% of all 45,X's are eliminated before birth [2], a much higher level of fetal death than that observed for trisomies 13, 18, and 21, even though the phenotypic abnormalities in live-born 45,X's are much less severe than those in live-born autosomal trisomies. The reason for the high in utero lethality of this condition is not known, but it could be related to the mechanism of origin of the abnormal-

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[1] Department of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, HI 96822.

[2] Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City, UT 84132.

ity. The in utero survival of triploid conceptions is affected by the parental origin of the additional haploid set, with digynic triploids aborting much earlier than those resulting from dispermy [3], and it is conceivable that the parental origin of the single X in 45,X conceptions might also affect the phenotype. Alternatively, the timing of the error leading to monosomy may differ between live-born and spontaneously aborted 45,X conceptions. It has been suggested that most, if not all, live-born 45,X individuals arise from a mitotic error in an early zygote with a normal cell line being present, even if undetected [2]. In contrast, mosaicism is very rarely observed among 45,X spontaneous abortions, suggesting that they result from an error at, or before, fertilization.

Information on the parental origin of the X chromosome in sex-chromosome monosomy is only available from live-born individuals, where studies of the Xg blood group indicate the presence of a paternal X (X^p) in approximately one-fourth of cases and a maternal X (X^m) in the remainder [4]. However, Xg is not expressed in cultured cells, and other suitable X-linked markers have not previously been available. Therefore, there are no comparable data on the origin of the single X chromosome in spontaneously aborted 45,X conceptions.

The recent identification of RFLPs on all human chromosomes, including the X, now makes it possible to study the origin of this abnormality in aborted, as well as in live-born 45,X conceptions. We have used this approach to study the parental origin of a series of 45,X conceptions ascertained among spontaneous abortions. Our results demonstrate the efficiency of the technique and show that the single X chromosome can be dervied from either parent.

MATERIALS AND METHODS

Ascertainment of Tissue Samples

We are conducting a cytogenetic survey of spontaneous abortions occurring in a large maternity hospital in Honolulu. The composition of the study population and methodology for the collection, culture, and cytogenetic examination of the samples has been described [5]. Upon receipt of a sample, tissue was minced and seeded into 3–4 tissue-culture dishes and cultured for cytogenetic analysis. Additionally, if extra fetal material was available, a portion of the sample was frozen in liquid nitrogen for direct extraction of DNA. Following identification of a sex-chromosome monosomy, DNA was isolated from the frozen tissue or, if we had received insufficient fetal material to both culture and freeze the tissue, cultures were maintained until there were a sufficient number of cells for DNA extraction. In seven of the ten 45,X abortions studied, DNA was obtained directly from the frozen tissue, and in the remaining three cases, cultured cells were used.

Isolation of Human DNA

DNA from cultured fetal cells and from parental peripheral blood samples was extracted with phenol and chloroform:isoamyl alcohol (24:1) as described [6]. Additionally, upon receipt of fetal material of at least 0.2 g wet weight, the tissue was transferred to a cryotube and placed in liquid nitrogen for freezing. Following removal from liquid nitrogen and before being thawed out, the tissue was placed into a cold mortar and ground to a fine powder with a pestle. The powdered tissue was then transferred to a centrifuge tube containing tissue lysis buffer, 1% sarkosyl and 200 mcg/ml proteinase K, and the sample incubated overnight at 4°C with gentle shaking. Following incubation, DNA was extracted as described above. We routinely obtained yields in excess of 400 mcg DNA/g of wet tissue using this technique.

Hybridization Probes

Seven hybridization probes were used to evaluate the parental origin of the single X chromosome:

(1) Phage S21—[7]. This probe reveals X-linked *Taq*I RFLP alleles of 2.7 and 2.5 kilobases (kb), with respective allelic frequencies of .35 and .65. The human insert has been mapped to Xq213-Xq220.

(2) Plasmid 52A—[7]. This probe reveals an X-linked *Taq*I RFLP with two alleles, one 1.3 kb and the other with bands at 0.6 and 0.7 kb. The two alleles are approximately equal in frequency. The human insert has been mapped to Xq27.

(3) Plasmid L1.28—[7]. This probe reveals X-linked *Taq*I RFLP alleles of 10.2 and 9.0 kb, with respective frequencies of .68 and .32. The human insert has been mapped to Xp11-Xp13.

(4) Plasmid DX13—[7]. This probe reveals X-linked *Bgl*II RFLP alleles of 6.0 and 2.5 kb, with respective frequencies of .55 and .45. The human insert has been mapped to the terminal end of the long arm of the X.

(5) Plasmid 8—[8]. This probe reveals X-linked *Taq*I RFLP alleles of 14.2 and 8.0 kb, with respective frequencies of .84 and .16. The human insert has been mapped to Xql.

(6) Plasmid 58—[8]. This probe reveals an *Msp*I RFLP with X-linked alleles 3.7 and 2.8 kb, each occurring in approximately equal frequency. The human insert maps to Xpl.

(7) Plasmid DXYSl—[9]. This probe reveals X-linked *Taq*I RFLP alleles 11.5 and 10.0 kb, in addition to a 14.0-kb Y-specific fragment. The X-linked alleles occur in approximately equal frequency. The human insert hybridizes to Yp and to Xq13-21.

Restriction Enzyme Digestion, Electrophoresis, and Filter DNA Hybridization

Human DNA samples were digested with the restriction endonucleases *Taq*I, *Msp*I, and *Bgl*II according to the conditions specified by the supplier (New England Biolabs, Beverly, Mass.), electrophoresed overnight on 0.5%–1.2% agarose gels, transferred by the method of Southern [10] to a nylon membrane (Magna 66, Micron Separations, Honeoye Falls, N.Y.), and, after prehybridization, hybridized to the appropriate nick-translated ^{32}P-labeled probe [11].

RESULTS

DNA from 10 monosomic spontaneous abortions and their parents were evaluated using the seven marker loci (table 1). We were able to unambiguously determine the origin of the single X in nine of the 10 cases, with six being maternally derived and three paternally derived; analyses of two of the cases are illustrated in figure 1. In seven cases (K2739, K2808, K2954, K3052, K3081, K3090, and K3113), the decision on parental origin was based on information from two or more markers, while in the remaining two instances, only one marker was informative. All seven of the marker loci were informative in at least some of the cases, and there were no inconsistent results for any of the 10 cases that were evaluated.

Heterozygosity was not detected among the 45,X samples in any of the analyses, and in all comparisons, there was a clear difference in dosage between the 45,X's and the 46,XX maternal samples.

DISCUSSION

Our present study demonstrates the utility of the RFLPs in determining the parental origin of de novo chromosome abnormalities, as it was possible to

TABLE 1

RESULTS OF ANALYSES OF THE PARENTAL ORIGIN OF THE SINGLE X CHROMOSOME IN 45,X CONCEPTIONS, USING SEVEN X-LINKED RFLPS

ID NO.	PROBE-ENZYME	ALLELES OF: Mother	Father	Fetus	PARENTAL ORIGIN OF THE SINGLE X CHROMOSOME	
K2808	DXYS1–*Taq*I	2,2	1,Y	2	Maternal	
	S21 – "	2,2	1	2	"	
	52A – "	1,2	1	1	Uninformative	
	L1.28 – "	1,1	1	1	"	Maternal
	8 – "	1,1	1	1	"	
	58 –*Msp*I	1,1	1	1	"	
	DX13 –*Bgl*II	1,1	1	1	"	
K2812	DXYS1–*Taq*I	1,2	1,Y	1	Uninformative	
	S21 – "	1,1	1	1	"	
	52A – "	1,1	1	1	"	
	L1.28 – "	1,1	1	1	"	Unknown
	8 – "	1,1	1	1	"	
	58 –*Msp*I	1,2	1	1	"	
	DX13 –*Bgl*II	2,2	2	2	"	
K2937	DXYS1–*Taq*I	1,2	1,Y	1	Uninformative	
	S21 – "	1,2	1	1	"	
	52A – "	1,1	1	1	"	
	L1.28 – "	1,1	2	1	Maternal	Maternal
	8 – "	1,1	1	1	Uninformative	
	58 –*Msp*I	1,2	1	1	"	
	DX13 *Bgl*II	1,2	1	1	"	
K2954	DXYS1–*Taq*I	1,2	1,Y	2	Maternal	
	S21 – "	1,1	1	1	Uninformative	
	52A – "	1,1	1	1	"	
	L1.28 – "	1,1	2	1	Maternal	Maternal
	8 – "	1,1	1	1	Uninformative	
	58 –*Msp*I	1,2	2	1	Maternal	
	DX13 –*Bgl*II	1,1	1	1	Uninformative	
K2739	DXYS1–*Taq*I	1,1	1,Y	1	Uninformative	
	S21 – "	1,1	1	1	"	
	52A – "	2,2	1	1	Paternal	
	L1.28 – "	1,2	1	1	Uninformative	Paternal
	8 – "	1,1	1	1	"	
	58 –*Msp*I	1,1	2	2	Paternal	
	DX13 *Bgl*II	1,1	1	1	Uninformative	
K3090	DXYS1–*Taq*I	1,1	1,Y	1	Uninformative	
	S21 – "	1,2	1	1	"	
	52A – "	1,1	2	1	Maternal	
	L1.28 – "	...	1	1	Uninformative	Maternal
	8 – "	...	1	1	"	
	58 –*Msp*I	1,2	2	1	Maternal	
	DX13 –*Bgl*II	2,2	1	2	"	
K3113	DXYS1–*Taq*I	1,1	1,Y	1	Uninformative	
	S21 – "	1,1	1	1	"	
	52A – "	1,2	2	1	Maternal	
	L1.28 – "	1,2	1	2	"	Maternal
	8 – "	1,1	2	1	"	
	58 –*Msp*I	1,1	2	1	"	
	DX13 –*Bgl*II	2,2	1	2	"	

Table 1 continued on next page

TABLE 1 *(continued)*

ID NO.	PROBE-ENZYME	ALLELES OF: Mother	Father	Fetus	PARENTAL ORIGIN OF THE SINGLE X CHROMOSOME	
K3052	DXYS1–*Taq*I	1,2	1,Y	2	Maternal	
	S21 – "	1,1	1	1	Uninformative	
	52A – "	1,1	2	1	Maternal	
	L1.28 – "	2,2	2	2	Uninformative	Maternal
	8 – "	1,1	1	1	"	
	58 –*Msp*I	1,1	1	1	"	
	DX13 –*Bgl*II	2,2	2	2	"	
K3075	DXYS1–*Taq*I	1,1	1,Y	1	Uninformative	
	S21 – "	1,1	2	2	Paternal	
	52A – "	2,2	2	2	Uninformative	
	L1.28 – "	1,1	1	1	"	Paternal
	8 – "	1,1	1	1	"	
	58 –*Msp*I	2,2	2	2	"	
	DX13 –*Bgl*II	1,2	2	2	"	
K3081	DXYS1–*Taq*I	1,2	2,Y	2	Uninformative	
	S21 – "	1,1	2	2	Paternal	
	52A – "	2,2	1	1	"	
	L1.28 – "	1,1	1	1	Uninformative	Paternal
	8 – "	1,1	2	2	Paternal	
	58 –*Msp*I	1,2	1	1	Uninformative	
	DX13 –*Bgl*II	2,2	2	2	"	

determine the origin of the single X in nine of the ten 45,X's. Furthermore, each of the seven marker loci that were used are only two-allele systems and significant improvement in the efficiency of the technique will occur with the identification and use of multiallelic loci. This will be particularly important for autosomal trisomies in which the determination of parental origin using a two-allele system depends on demonstration of a dosage effect.

The results of our analyses indicate that it is at least as likely to be the paternal, as the maternal, sex chromosome that is missing in spontaneously aborted 45,X conceptions. While these are very preliminary observations, they are consistent with results of Xg blood group analysis among live-born 45,X individuals in which an estimated 77% of cases result from loss of the paternal sex chromosome. Additionally, in the mouse, the only other species that has been appropriately studied, 39,X mice are much more likely to result from paternal than from maternal sex-chromosome loss [12].

The consistency of these observations indicates that the mechanism of origin of sex-chromosome monosomy differs from that of virtually all other human aneuploidies, including those involving an additional sex chromosome. Both the 47,XXY and 47,XXX conditions are known to be associated with increasing maternal age, implying maternal nondisjunction, and for the XXY's, there is direct evidence from Xg blood group analyses that the error most commonly occurs in oogenesis. Additionally, analyses of chromosome heteromorphisms in autosomal trisomies show the additional chromsome to be maternally de-

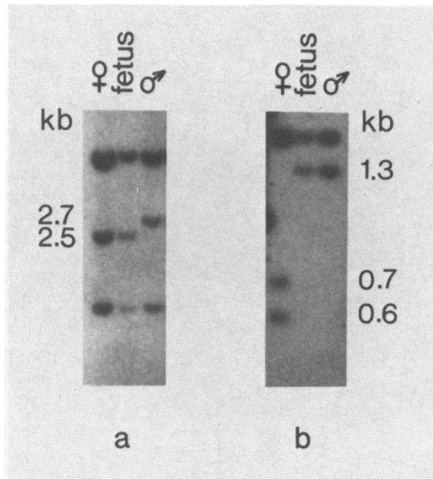

FIG. 1.—45,X abortuses in which the parental origin of the single X could be determined. *a*, K2808: DNA samples were digested with *Taq*I and hybridized to S21. The alleles of the mother are 2,2, the father 1, and the fetus 2; therefore, the X is maternal in origin. *b*, K2739: DNA samples were digested with *Taq*I and hybridized to 52A. The alleles of the mother are 2,2, the father 1, and the fetus 1; therefore, the X is paternal in origin.

rived in the vast majority of cases, regardless of the age of the woman or the chromosome involved. This holds for single and double trisomies and for mosaics as well as for nonmosaic conditions [13]. Autosomal monosomies are observed exceedingly rarely and, consequently, are poorly characterized. However, there is an apparent age effect for the condition, with the mean maternal age of five spontaneously aborted monosomies being 32.7 yrs. ([5, 14–16] and T. Hassold, unpublished observation, 1985), suggesting a predominance of maternal meiotic errors.

Thus, 47,XYY and sex-chromosome monosomy are the only human aneuploid conditions in which maternal meiotic errors do not predominate. The XYY's presumably result from paternal meiotic nondisjunction, but the mechanism(s) associated with sex-chromosome monosomy are much less obvious. Based on frequencies of sex-chromosome abnormalities among live borns and spontaneous abortions, Ford [17] argued that meiotic nondisjunction can account for only a small proportion of all 45,X conceptions. This is consistent with maternal-age data, as sex-chromosome monosomy does not increase with advancing age and may, in fact, be associated with an inverse maternal-age effect (e.g., [18]). Furthermore, cytogenetic studies of human spermatozoa provide no evidence of an unusually high incidence of paternal nondisjunction involving the sex chromosomes [19, 20].

Therefore, it is possible that sex-chromosome monosomy typically results from the post-meiotic loss of a sex chromosome. This would be consistent with data from the mouse, in which the preponderence of paternal errors has been attributed to loss of a paternal X or Y following sperm entry into the egg [12]. There is also evidence of a post-meiotic etiology among live borns with Turner

syndrome since at least 25% of all individuals with a 45,X cell line carry a chromosomally normal line [2]. In fact, Hook and Warburton [2] suggested that all live-born 45,X's are mosaics for a normal cell line in some organ or tissue, with the high in utero lethality of the 45,X condition being due to the absence of a normal line in almost all such conceptions.

If mosaicism or the lack of it does form the basis for the difference between live-born and spontaneously aborted 45,X conceptions, it could be that the mechanism leading to monosomy is the same for these two categories but that the timing of the error differs. For example, the random loss of either sex chromosome early in development in chromosomally normal zygotes should result in an appropriate 2:1 ratio of $45,X^m$:$45,X^p$ mosaics, since any 45,Y's would be quickly eliminated. A similar error occurring at the time of fertilization would also generate more $45,X^m$ than $45,X^p$ embryos, but in this instance, the abnormality would be carried in all cells and, presumably, would be associated with prenatal elimination.

Continued analysis of RFLPs in spontaneously aborted and live-born 45,X conceptions should help resolve these and other questions concerning the etiology of the condition. For example, analysis of RFLPs provides a new approach to the detection of mosaicism, and, in fact, Y-specific sequencies have recently been demonstrated in a putative nonmosaic 45,X live born [9]. In our present study, mosaicism was not apparent in any of the ten 45,X abortions, but we made no systematic attempt to evaluate different tissues for the presence of a second allele. Further analyses along these lines are intended and should help to determine whether or not the presence or absence of a normal cell line differentiates spontaneously aborted from live-born 45,X conceptions.

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