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Skewed X-Chromosome Inactivation Is Common in Fetuses or Newborns Associated with Confined Placental Mosaicism

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Summary

The inactivation of one X chromosome in females is normally random with regard to which X is inactivated. However, exclusive or almost-exclusive inactivation of one X may be observed in association with some X-autosomal rearrangements, mutations of the *XIST* gene, certain X-linked diseases, and MZ twinning. In the present study, a methylation difference near a polymorphism in the X-linked androgen-receptor gene was used to investigate the possibility that nonrandom X inactivation is increases in fetuses and newborns that are associated with confined placental mosaicism (CPM) involving an autosomal trisomy. Extreme skewing was observed in 7 (58%) of 12 cases with a meiotic origin of the trisomy, but in none of 10 cases examined with a somatic origin of the trisomy, and in only 1 (4%) of 27 control adult females. In addition, an extremely skewed X-inactivation pattern was observed in 3 of 10 informative cases of female uniparental disomy (UPD) of chromosome 15. This may reflect the fact that a proportion of UPD cases arise by "rescue" of a chromosomally abnormal conceptus and are therefore associated with CPM. A skewed pattern of X inactivation in CPM cases is hypothesized to result from a reduction in the size of the early-embryonic cell pool, because of either poor early growth or subsequent selection against the trisomic cells. Since ~2% of pregnancies detected by chorionic villus sampling are associated with CPM, this is likely a significant contributor to both skewed X inactivation observed in the newborn population and the expression of recessive X-linked diseases in females.

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Introduction

Dosage compensation in mammals is achieved by the inactivation of one X chromosome in females during early embryogenesis (Lyon 1961). Although X inactivation in humans is normally considered to be random in the embryo proper, primary nonrandom inactivation can be caused by mutations at the *XIST* locus (Plenge et al. 1997), as has also been reported for *Xist* in mice (Penny et al. 1996). Secondary nonrandom inactivation resulting from a selective advantage or disadvantage of cells may result in nonrandom inactivation in carriers of X rearrangements or certain X-linked diseases (Migeon et al. 1981; Schmidt and Du Sart 1992). Furthermore, tissue-specific, nonrandom X inactivation may arise as a result of monoclonality (e.g., because of bone-marrow transplant) and is sometimes observed in association with MZ twinning (reviewed in Belmont 1996). Studies of artificial chimeras, combined with inferences of precursor-cell population size that are based on the frequency of X skewing within and between tissues, have led to the conclusions that in mouse (1) X inactivation has not yet occurred in the inner cell mass of the blastocyst, (2) $\geqslant$10–20 embryonic precursor cells must be present at the time that an X is designated to be inactivated, and (3) inactivation occurs prior to the differentiation of embryonic tissues. Therefore, X inactivation is assumed to be initiated in the embryonic ectoderm shortly after blastocyst implantation but may take several days to complete (Gardner and Lyon 1971; Nesbitt 1971; Tan et al. 1993).

Confined placental mosaicism (CPM) is detected in ~2% of viable pregnancies ascertained through chorionic villus sampling (CVS) at 10–12 wk gestation (Mikkelsen and Ayme 1987; Ledbetter et al. 1992; Wang et al. 1993). CPM refers to the presence of two karyotypically different cell lines (usually trisomic and diploid) in the placenta but only one cell line (usually diploid) in the fetus. Although many pregnancies with CPM progress to term uneventfully, some may result in spontaneous abortion, intrauterine growth retardation (IUGR), or perinatal morbidity (for review, see Kalousek 1994). Risk of abnormal outcome increases when high levels

of trisomy persist in term placenta and depends on the chromosome involved (Kalousek et al. 1991). CPM may have a meiotic origin, in which a trisomic chromosome is lost in an embryo/fetal progenitor cell of a trisomic conceptus, or may arise from somatic duplication of one chromosome within the placental cell lineage of a normal diploid conceptus. Previous studies have shown that the majority of the CPM for autosomes 9, 16, and 22 are meiotic in origin, whereas CPM for autosomes 2, 7, 8, 10, and 12 are predominantly somatic in origin (Robinson et al. 1997). Poor pregnancy outcome appears to be restricted to CPM of meiotic origin, which is also correlated with a high level of trisomy in the placental tissues, compared with somatic CPM conceptions.

At the blastocyst stage of development, the majority of cells contribute to extraembryonic lineages, whereas only three to five cells of the inner cell mass are progenitors of the embryo proper (Gardner and Lyon 1971). Thus, the dichotomy between high levels of trisomy in placenta and the absence of trisomic cells in the fetus may be achieved by chance incorporation of only disomic cells into the few embryo/fetal progenitors, such that only those with primarily disomic embryo precursor cells survive (Kalousek and Dill 1983). There might also be a mechanism to preferentially allocate aneuploid cells to extraembryonic lineages, as occurs for polyploid cells in mouse chimeras made with a mix of polyploid and diploid cells (James et al. 1995; Everett and West 1996). In either case, X inactivation would likely be random in the embryo proper, since it is not expected to occur until several cell divisions later, when the pool of embryo/fetal progenitor cells has increased in size (Nesbitt 1971). Alternatively, the pool of embryo/fetal progenitors in CPM cases at the time of X inactivation may be severely restricted, as a consequence of either poor early growth or extreme selection against trisomic cells after X inactivation has occurred. An effective reduction in embryonic progenitors at the time of X inactivation should be associated with increased incidence of nonrandom (skewed) X inactivation.

To determine whether the effective embryo precursor pool might be reduced in pregnancies associated with significant placental mosaicism, X-inactivation status was evaluated in 28 cases of CPM, 22 of which were informative at the androgen-receptor (AR) locus. A risk for trisomy in embryonic progenitors would be expected to be limited to the cases showing a meiotic origin of the trisomic cell line in the placenta, since "somatic" CPM is thought to arise in either the trophoblast or chorionic lineages after differentiation. Therefore X-inactivation results were analyzed separately for CPM cases of meiotic origin and CPM cases of somatic origin and were compared. Uniparental disomy (UPD), the inheritance of the two copies of a chromosome from the same parent, may sometimes be associated with placental mosaicism; therefore, X skewing in cases of UPD was analyzed also.

Methods

Ascertainment of Cases and Origin of Trisomy

A description of ascertainment, cytogenetic analysis, and DNA extraction of CPM cases has been given in a report by Robinson et al. (1997). In brief, most pregnancies were ascertained through mothers undergoing CVS for advanced maternal age and in which a trisomic cell line was identified. In most cases the abnormal cell line was absent from amniotic fluid and fetal blood. However, a few cases were also included if the level of trisomy in amniotic fluid was low (<12%) and/or no indication of trisomy mosaicism in blood was present. In these cases, it was assumed that the trisomy detected in cultured amniotic fluid was due to placenta contamination. Additionally, some cases in which ascertainment initially was due to an abnormal triple-screen result, ultrasound abnormality (usually IUGR), maternal anxiety, previous aneuploidy, mosaic amniocentesis, or IUGR noted at birth were included in the study. Ascertainment of UPD 15 is as reported previously (Robinson et al. 1993).

The origin of trisomy had been determined previously in many of the CPM cases tested (Robinson et al. 1997). In six cases however, no molecular data were available, and we inferred origin in the following manner: Since CPM for chromosomes 16 and 22 is almost always meiotic (Robinson et al. 1997), case 93.73, which involves CPM 16 and a high level of trisomy in placenta samples, and case 93.122, which involves CPM 22, were classified as "probably meiotic." Case 94.36 involves trisomy 8 mosaicism, which is likewise almost always somatic in origin. CPM 2 is also generally somatic, particularly if the level of trisomy in the placenta is low, as it was for case 93.83. In the two remaining cases of unknown origin—cases 92.77 (CPM 17) and 92.20 (CPM 20)—a probable somatic origin was inferred on the basis of low levels of trisomy and confinement of the trisomic lineage to just the chorion of the placenta.

Our control adult females consisted of 11 mothers of CPM cases, 10 mothers of UPD cases, and 7 females who were either mothers of normal babies or random adults. We do not know the maternal ages in many of these cases. However, the ages of the random adults were 24–38 years, most CPM mothers ascertained in our studies were 35–45 years of age, UPD is associated with increased maternal age, and at least one UPD mother was >50 years of age when a blood sample was taken for DNA analysis. Thus we estimate that most of our controls were 30–50 years of age.

DNA Studies

When possible, blood samples from CPM cases were obtained from both parents, and fetal/newborn DNA was obtained from either cord blood obtained either at term or, in cases of fetal demise, fetal tissues. Tissue from trisomic material was obtained from either CVS samples or term placentas. Blood samples from UPD cases were obtained during infancy or childhood, for the purpose of confirmation of the clinical diagnosis of Prader-Willi syndrome or Angelman syndrome. DNA was extracted according to standard protocols. Both diagnosis of UPD and DNA typing of parents and child/fetus were performed by PCR amplification of highly polymorphic microsatellite markers, as described elsewhere (Robinson et al. 1993).

AR-Gene Methylation Assay

Inactivation status of the X was assessed by methylation at the AR locus. In somatic tissues there is a strong correlation between X inactivation and the methylation status of the CpG islands associated with genes (reviewed in Gartler and Riggs 1983). Previous studies by Allen et al. (1992) have specifically shown that methylation at the human AR locus is correlated with X inactivation in somatic tissues. CAG repeats in the first exon of the gene are closely linked to *Hpa*II restriction sites. *Hpa*II is a methylation-sensitive enzyme that cuts only the unmethylated (in this case, the active) X. Therefore, amplification of *Hpa*II-digested DNA by use of primers that flank the CAG and *Hpa*II site will amplify only the copy on the inactive X. Heterozygosity at this locus is estimated to be 90%, with 20 alleles.

One microgram of each DNA sample were digested with 10 U *Hpa*II in a total volume of 10 μl. For each sample, a control with 1 μg DNA and enzyme buffer (but no enzyme) was prepared. DNA samples from males (usually from the father's blood) were also digested, as a control for complete *Hpa*II digestion. Incubation was performed overnight at 37°C. One microliter of the digested or control sample was used for amplification. Amplification of the control (undigested) sample was used to determine whether preferential amplification of alleles had occurred. PCR was performed in a total volume of 25 μl containing 200 μM dNTP, 0.005 U *Taq* polymerase, 1.5 mM $MgCl_2$, 2.5 μl 10 × *Taq* buffer, and 0.28 μM each primer. Primer sequences were as follows: forward primer, 5′ GCT GTG AAG GTT GCT GTT CCT CAT 3′; reverse primer, 5′ TCC AGA ATC TGT TCC AGA GCG TGC 3′. PCR cycling conditions were 95°C for 3 min (initial denaturation); 95°C for 45 s, 60°C for 30 s, and 72°C for 30 s, for 28 cycles;, and 72°C for 7 min (final extension). An equal volume of urea-loading buffer was added to the PCR products. A maximum amount of 10 μl was loaded onto a 5% polyacrylamide/50% urea gel and was electrophoresed for 1 h 20 min–1 h 40 min at 50 W. Bands were visualized by silver staining.

In the male control samples, no amplification should have occurred in the digested sample after PCR, since males have only an active X, which therefore should be unmethylated and sensitive to digestion by *Hpa*II and should elude amplification by the AR primers. Therefore, the absence of a band on the polyacrylamide gel after amplification of the digested male samples was taken as an indication that complete digestion by *Hpa*II had occurred. However, this is only an indirect way of ensuring complete digestion by *Hpa*II. It should be noted that subsequent PCR amplification of DNA that has not been digested to completion may lead to underestimation of skewing. In our study, the male control DNA was completely digested, as judged on the basis of the lack of any PCR product in the digested sample.

Estimation of Degree of Skewing

Polyacrylamide gels were dried between two pieces of cellophane or on blotting paper and were scanned by an Apple Color OneScanner Dispatcher. NIH Image, obtained on-line (http://rsb.info.nih.gov/nih-image), was used to analyze the band intensities. Degree of skewing was defined as the band intensity of the most intense allele, relative to the total intensity of both alleles. Background levels were taken into account by setting the peak base to the average intensity reached on either side of the main peaks. The areas under the peaks corresponding to each band, from both a *Hpa*II-digested sample and an undigested sample, were determined. Values for the digested samples were normalized with those for the undigested samples, to account for preferential allele amplification. Thus, degree of skewing was calculated as (Bd1/Bu1)/(Bd1/Bu1 + Bd2/Bu2), where Bd1 represents the band intensity of the more intense *Hpa*II-digested allele, Bd2 represents the band intensity of the fainter *Hpa*II-digested allele, and Bu1 and Bu2 are the corresponding bands from the undigested samples. Accurate quantification of allele ratios is not really possible without controlling for the facts that (*a*) the PCR amplification is always in the linear range and (*b*) development of gels is constant from gel to gel, with little or no background, such that there is linearity with respect to band intensity. We found a linear correlation when we performed amplification of the AR for mixtures of DNA samples from two individuals homozygous for different AR alleles, using ratios of DNA that were within a range of 1:10–10:1, with our usual DNA concentrations. However, there is uncontrollable sample-to-sample variation in both amplification intensity and gel-exposure levels. Nonetheless, there should be no bias induced from these fluctuations, since maternal controls, meiotic

Table 1

X Inactivation in Fetal and Extraembryonic Tissues of Meiotic and Somatic CPM

			Fetal Tissue			Extraembryonic Tissue		
Case	Abnormal Cell Line	Origin of Trisomy	Source	Degree of Skewing (%)	Inactive Allele	Source	Degree of Skewing (%)	Inactive Allele
94.25	47,+2	Meiotic, maternal	Blood	100	Maternal	Chorionic villi	56	
91.53	47,+7	Meiotic, maternal	Blood	73	Not determined	Amnion	54	
						Chorion	76	
CPM16-36	47,+16	Meiotic, maternal	Brain	100	Maternal	Amnion	58	
			Spleen	100	Maternal			
			Kidney	79	Maternal			
			Skin	51				
94.21	47,+16	Meiotic, maternal	Blood	100	Paternal	Chorionic villi	59	
95.28	47,+16	Meiotic, maternal	Diaphragm	100	Paternal			
91.71	47,+16	Meiotic, maternal	Blood	70	Paternal	Amnion	52	
						Chorion	92	Paternal
						Chorionic villi	92	Paternal
91.55	47,+16	Meiotic, maternal	Blood	83	Paternal	Amnion	77	
						Chorion	60	
						Chorionic villi	55	
93.43	47,+16	Meiotic, maternal	Intestine	100	Paternal			
93.73	47,+16	Probably meiotic[a]	Blood	100				
90.9	47,+16	Meiotic, maternal	Blood	74				
93.122	47,+22	Probably meiotic[a]	Blood	76				
95.66	47,+22	Meiotic, maternal	Blood	100	Maternal	Amnion	59	
						Chorionic villi	86	Maternal
91.54	47,+2	Somatic	Blood	62				
92.29	47,+2	Somatic	Blood	74				
93.83	47,+2	Probably somatic[b]	Blood	60				
92.81	47,+2	Somatic	Blood	73				
95.21	47,+3	Somatic	Blood	75		Chorionic villi	68	
91.85	47,+8	Somatic	Blood	70		Chorionic villi	89	Paternal
94.36	47,+8	Probably somatic[c]	Blood	50				
93.92	47,+12	Somatic	Blood	51				
92.77	47,+17	Probably somatic[b]	Blood	82				
92.20	47,+20	Probably somatic[b]	Blood	57				

[a] Trisomy 16 CPM or trisomy 22 CPM is usually meiotic; high levels of trisomy in these cases are consistent with this conclusion (see text).
[b] Cases 93.83, 92.77, and 92.20 show low levels of trisomy, confined to the chorion.
[c] Case shows low-level trisomy 8 mosaicism in amniotic fluid; however, mosaic trisomy 8 is usually somatic (see text).

CPM, and somatic CPM were run side-by-side under same conditions and on the same gels. In addition, *Hpa*II digestion and amplification of many samples were repeated on separate gels, to test for reproducibility of digests, and reported values in such cases are the average of two readings from two gels. It should be emphasized that the focus of this study is on identification of extremely skewed values (i.e., close to 100% inactivation of one allele), which should not be affected by fluctuations in band quantitation, since, in these cases, there was complete disappearance of one band after sample digestion.

Table 2

Extreme Skewing of X Inactivation

Sample	No. with Skewing ⩾90%/Total No.
Cases:	
Meiotic CPM (newborn)	7/12 (58%)*
UPD (age 0–15 years)	3/11 (27%)
Controls:	
Somatic CPM (newborn)	0/10
Adult females[a]	1/27 (4%)

[a] Mothers of CPM, UPD, and healthy newborns.
* $P = .005$ compared with somatic CPM and $P = .0003$ compared with control adult females.

Results

In total, 28 cases of CPM were tested, 22 of which were informative at the AR locus. Twelve informative cases were classified as meiotic in origin, and 10 were of probable somatic origin of the trisomic lineage. Overall, a higher degree of skewing was observed in the meiotic CPM samples than in the somatic CPM samples (tables 1 and 2; also see fig. 1). Specifically, extreme

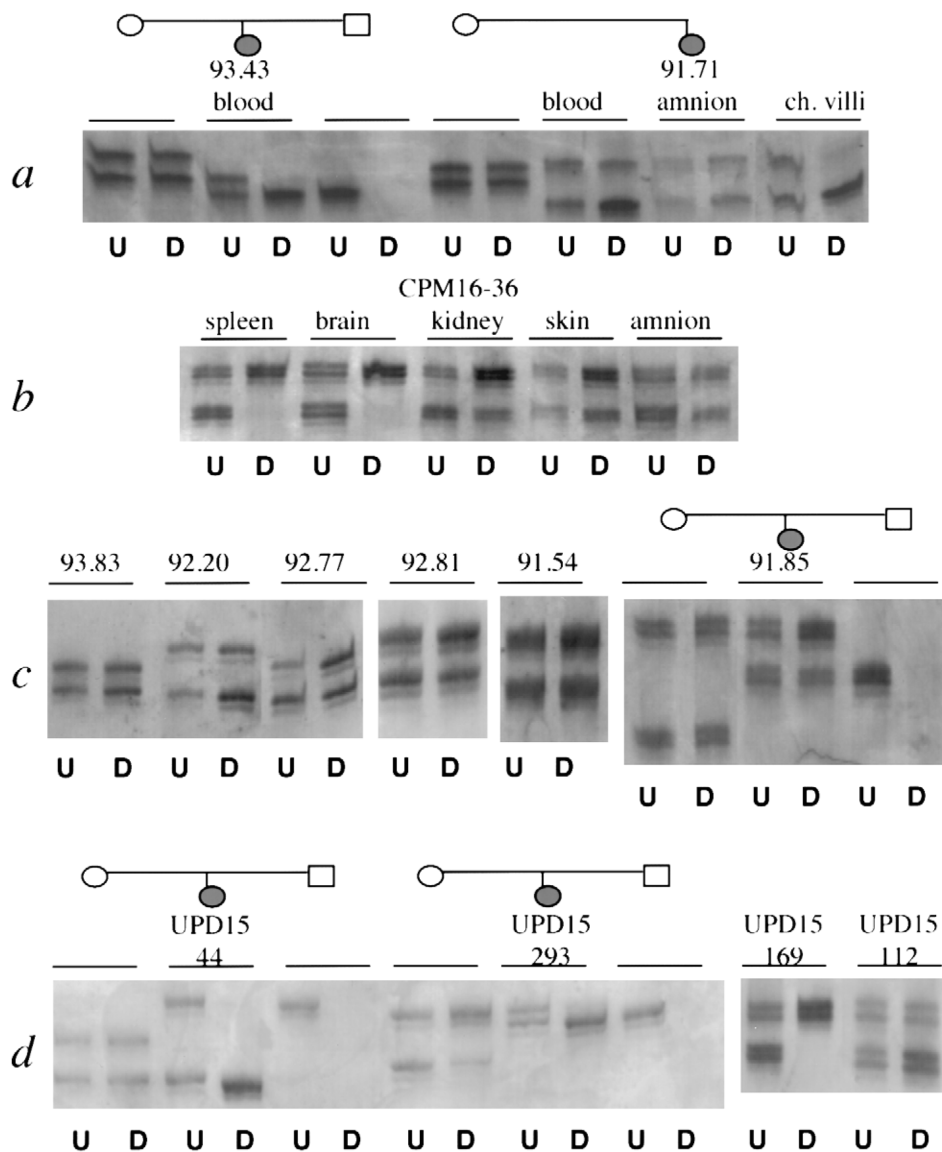

Figure 1 AR methylation analysis. U = undigested; and D = digested with *Hpa*II. *a,* Example of meiotic CPM cases. In the pedigree on the left, the paternal allele is preferentially inactivated in the blood of case 93.43; X inactivation appears to be random in the mother; and *Hpa*II digestion is complete in the father, as indicated by a lack of product in the digested paternal sample. In the pedigree on the right, the amnion displays random X inactivation, whereas the blood shows moderate skewing and the chorionic villi show extreme skewing with preferential inactivation of the paternal allele. *b,* Extreme skewing observed in some but not all fetal tissues sampled from case CPM16-36. Digests were repeated for verification, with the same result. *c,* AR methylation analysis in somatic CPM cases. Random X inactivation (e.g., <75% skewing) is seen in each case. *d,* AR methylation analysis in UPD 15 cases. Prader-Willi syndrome patients with maternal UPD 15 (UPD15-44, UPD15-293, and UPD15-169) show extremely skewed X inactivation, whereas the Angelman syndrome patient with paternal UPD 15 (UPD15-112) does not.

(>90%) skewing was observed in the majority (7/12) of the meiotic cases, but in none of the 10 somatic cases (P = .005) and in only 1 of 27 control females (P = .0003; Fisher's exact test). In most cases blood was used for the analysis, although other fetal tissues were used in three of the meiotic cases. We do not know what degree of skewing to expect in fetal tissues in humans; however, distributions of skewing of X inactivation have been calculated for multiple tissues in mouse and have not been found to be significantly different from that observed in blood (Nesbitt 1971). Exclusion of the data from fetal tissues does not alter the finding of significantly increased skewing in cases associated with meiotic CPM. No preferential inactivation of the maternal allele versus the paternal allele was observed in the skewed cases.

Table 3

X Inactivation in Female UPD Cases Ascertained Postnatally

Case	Type (Origin) of UPD	Degree of Skewing (%)	Allele Inactivated
UPD15-2	Maternal UPD 15 (meiotic)	59	Not determined
UPD15-5	Maternal UPD 15 (meiotic)	87	Maternal
UPD15-40	Maternal UPD 15 (meiotic)	50	Not determined
UPD15-44	Maternal UPD 15 (somatic)	98	Maternal
UPD15-60	Maternal UPD 15 (somatic)	64	Not determined
UPD15-116	Maternal UPD 15 (meiotic)	76	Not determined
UPD15-169	Maternal UPD 15 (meiotic)	100	Maternal
UPD15-209	Maternal UPD 15 (meiotic)	54	Not determined
UPD15-293	Maternal UPD 15 (meiotic)	94	Paternal
UPD15-112	Paternal UPD 15 (somatic)	65	Not determined
SRS11	Maternal UPD 7 (somatic)	59	Not determined

In our control adult females consisting of mothers of CPM, UPD, and normal births, 33% (9/27) were found to be moderately (75%–90%) skewed; however, only one case (a mother of a CPM 22) showed skewing >90% (allele ratios 9:1). In a study of newborn blood, nonrandom inactivation with >75% skewing (allele ratios 3:1) was observed in ~9% of samples and >90% skewing was observed in <2% of samples (Busque et al. 1996). The level of skewing in blood increases with age, however, and extreme (>90%) skewing was observed in 4.5% of women 28–32 years of age and in 23% of women >60 years of age. In a separate study of adult women (ages not specified), ~20% showed >80% skewing and 9% showed >90% skewing, consistent with the results given above (Naumova et al. 1996). Our adult control values are consistent with these published results. Using adult women as a control group is expected to be conservative for testing the hypothesis that there is increased skewing in newborn blood of CPM cases. Extraembryonic tissues, when available, were rarely skewed, even in two cases showing 100% skewing in fetal tissue. When examined, X inactivation in the amnion, which is of both embryonic and extraembryonic origin, always appeared to be random when this test was used.

In the present study, two of seven samples with meiotic UPD 15 showed extreme skewing, and one showed moderate skewing (table 3 and fig. 1*d*), consistent with the idea that a subset of such cases may lose the paternal chromosome postzygotically and are in fact associated with trisomy CPM. One case of UPD 15 attributed to somatic duplication of the maternal chromosome 15 also showed extreme skewing. Somatic UPDs are also likely to be associated with CPM. However, in this case the abnormal cell line may be, for example, a monosomy rather than a trisomy 15. One case of meiotic UPD 7, ascertained through the presence of Russell-Silver syndrome, showed random X inactivation.

Discussion

X inactivation is believed to occur randomly in somatic tissue, and extreme skewing is expected to be rare except in females with X rearrangements, X-linked diseases, or mutations in the *XIST* locus. In a prospective study of newborn blood, extreme (>90%) skewing was observed in <2% of samples (Busque et al. 1996). The level of skewing increases with age, yet extreme skewing was observed in only 4.5% of women 28–32 years of age in the same study and in only 1 (4%) of the 27 women in our own study. Our observation of an extremely skewed pattern of X inactivation in 58% of fetal tissue or newborn blood samples from CPM cases with a meiotic origin of the trisomy deviates significantly from these previously observed rates of extreme skewing.

Extraembryonic tissues, when available, were rarely skewed, even in two cases showing 100% skewing in fetal tissue, implying that a primary tendency to skewing (i.e., skewing due to a mutation in the *XIST* gene) is unlikely in these cases. It should be noted, however, that the reliability of the AR-methylation assay for X inactivation has not been thoroughly examined in extraembryonic tissues, and, therefore, the results for these samples must be interpreted with caution. A result suggestive of nonrandom X inactivation was observed in some samples of chorion (villus stroma or chorionic plate), including one case showing a tendency to inactivate the maternal allele. Although there is evidence for preferential inactivation of the paternal X in human trophoblast, studies of inactivation in chorion generally show random inactivation (Migeon et al. 1985; Harrison 1989; Mohandas et al. 1989; Goto et al. 1992). In addition, studies of the distribution of trisomic cells in the placenta of CPM cases show large site-to-site variation in the distribution of trisomic cells, indicating that the development of the placenta is quite clonal (Henderson et al. 1996). This clonality could be reflected by skewed

X inactivation in individual placental biopsies and may depend on the size or location of the sample taken. Further studies of CPM and normal placentas, taking biopsies in a controlled manner, are therefore necessary to determine whether X inactivation is altered in any way in placentas of CPM cases.

Some cases of UPD have been ascertained subsequent to the finding of CPM for the involved chromosome and are inferred to arise from "rescue" of a trisomic zygote through somatic chromosome loss (Cassidy et al. 1992; Purvis-Smith et al. 1992; Kalousek et al. 1993). However, diagnosis of UPD 15, associated with Prader-Willi and Angelman syndromes, is normally made postnatally, and it is not known what proportion might be associated with CPM. Given the high rate of aneuploidy in human gametes, it is also possible that the majority of maternal UPD 15 results from a fertilization of a disomy 15 oocyte with a nullisomy 15 sperm (for discussion, see Robinson et al. 1991). In the present study, 3 of 10 samples with UPD 15 showed extreme skewing, which would be consistent with a proportion of UPD arising in association with CPM and would suggest that X-linked recessive disease may be more common in such patients.

Several explanations may be proposed to explain the occurrence of a skewed pattern of X inactivation in embryonic tissues of meiotic CPM cases. First, it is possible that designation of an X to be inactivated occurs prior to (or at the time of) the trisomic rescue event. All subsequent cells derived from the disomic cell would then be expected to have the same X inactivated; hence one would expect to see 100% skewing of X inactivation in the derivative cell line. Although this is an attractive hypothesis, it is unlikely that X inactivation could occur prior to the event generating the mosaicism. If it is assumed that the somatic loss occurred only once, then it must have occurred prior to the differentiation of chorion from embryonic progenitors in the inner cell mass of the early blastocyst, since both chorion and embryo typically show disomic cells. Although results of one study suggest that the X-counting mechanism may possibly occur at the morula stage, well before the process of X inactivation itself (Migeon et al. 1996), the actual commitment of a particular X to be inactivated must occur at a later stage of development (Gardner and Lyon 1971; also see Brown and Robinson 1997). It is believed that only three to five cells of the developing blastocyst actually contribute to the resulting fetus (Gardner and Lyon 1971), and the level of skewing in the normal female population would be expected to be much greater if X inactivation were to occur at or before this stage of development. Furthermore, in case CPM16-36, extreme skewing was observed in some but not all tissues (fig. 1*b*) implying that more than one disomic cell was present when X inactivation occurred.

It is more probable that X inactivation occurs multiple cell divisions after the trisomic rescue. To explain the observed skewing in meiotic CPM cases, we therefore assume a reduction, at the time of X inactivation, in the number of cells present that will ultimately contribute to the fetus. A reduction in the total number of cells at the time of X inactivation has been proposed as an explanation for increased X skewing in some female MZ twins (Goodship et al. 1996). It has been hypothesized that onset of tissue differentiation in MZ twins might be dependent on the time since fertilization, rather than on the cell mass. If this hypothesis is true, poor early growth up to the blastocyst stage may similarly result in reduced cell mass at the time of X inactivation in CPM cases. In addition, it has been hypothesized that twinning may be caused by mosaicism for a genetic abnormality (Hall 1996) and that CPM may result from a twinning event caused by repulsion of the trisomic from disomic cells, with subsequent death/resorption of the trisomic twin (Kalousek 1993).

It should be noted, however, that the frequency of skewing in MZ twins has been estimated to be 18% (Goodship et al. 1996), which is lower than that observed in the CPM cases in the present study. Another possible factor that may contribute to skewing in CPM cases would occur if the majority of embryo progenitors were trisomic but selection against these cells early in development resulted in only one or a few disomic cells actually contributing to the resulting embryo/fetus. This hypothesis does not require a reduction in the total number of cells present at the time of differentiation. Consistent with the hypothesis that selection and high cell death rate in the pool of fetal precursor cells can occur early in development is the extremely skewed X-inactivation seen in X-autosome translocations, which is attributed to severe selection against cells that inactivate the translocated X (Zabel et al. 1978).

Interestingly, a reduction in embryo-precursor pool size can also be induced by maternal administration of mitomycin-C in the mouse (Tam and Snow 1981; Snow 1985). The embryos exhibit catch-up growth and are of normal size, despite early growth delay. In these mice, various abnormalities were seen, which were attributed to the higher than usual rate of cell division during the catch-up phase. There seemed to be especially poor development of germ cells. CPM has been identified only since 1983, and thus fertility of identified cases is unknown; however, if X-inactivation skewing is a sign of small embryo-precursor size, skewed cases may also be at increased risk of fertility problems. Also, a recent study has suggested that, even if development of the germ cells is not impaired by early growth problems, reduction in fertility could occur, since germ-cell aneuploidy may be present in some CPM cases (Stavropoulos 1997).

It should be emphasized that CPM may arise through

multiple mechanisms and that a reduction in embryo-precursor size may be common only for certain trisomies (e.g., 16 and 22). Among CPM cases, chromosome-specific differences are seen in the distribution of trisomic cells within the placenta (i.e., trisomy either confined to trophoblast or chorion or present in both) (Godsen et al. 1995) and in the relative frequency of meiotic versus somatic origin of the trisomy (Robinson et al. 1997). One would expect that differential chromosome involvement in CPM could be also influenced by the efficiency with which any potential selection against specific trisomies occurs in embryonic lineages. Depending on the involved chromosome, some trisomies have been shown to be poorly tolerated in some mouse somatic tissues (Gropp et al. 1983). Conversely, in mouse chimeras artificially constructed by use of trisomy 16 and normal disomic blastocysts, viability was reduced when the karyotype of placenta and fetal cells differed compared with that of chimeras with fully trisomic placenta and fetus, indicating that, for some chromosomes, a dichotomy between placental and fetal karyotype may not be well tolerated, thereby resulting in selection against CPM (Bogart and Miyabara 1990). For human trisomy 13 and trisomy 18, it has been shown that survival to term is not possible unless the trisomy is lost from placental trophoblast (Harrison et al. 1993), indicating that, for these chromosomes, it may be the placenta and not the developing embryo/fetus itself that does not tolerate the trisomy. Unfortunately, data on humans suffer from so much ascertainment bias that the relative frequency with which CPM for any particular chromosome occurs, relative to its frequency in generalized mosaicism, cannot be accurately calculated, and it is difficult to predict how selection might act on different mosaic trisomies during embryonic development.

In summary, a high rate of a skewed pattern of X inactivation has been observed in cases of meiotic CPM. It is hypothesized that this results from a reduction in the effective embryonic-progenitor cell pool, because of poor growth and/or selection against trisomic cells in early embryogenesis. Consequently, abnormal pregnancy outcomes associated with CPM may be due, in part, to either a high rate of cell death in early embryonic development or, potentially, to a rapid catch-up growth phase. Furthermore, the results of the present study would predict that X-linked diseases should occur at increased frequency in female pregnancies associated with CPM or UPD. The true population incidence of CPM is unknown and could be either (*a*) higher than the 2% ascertained through CVS, because of incomplete detection, or (*b*) lower than that frequency, because CVS samples are biased by increased maternal age. Nonetheless, since only 2% of female newborns show extreme skewing of the X, CPM is likely a major factor contributing to skewed X inactivation in the newborn population.

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