Letters to the Editor

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Highly Skewed X-Chromosome Inactivation Is Associated with Idiopathic Recurrent Spontaneous Abortion

To the Editor:

Recurrent spontaneous abortion (RSA) is a major health concern for women, affecting one in every 100 couples wishing to have children (Stephenson 1996). It has been estimated that 37%–79% of those couples will not receive an explanation for their pregnancy losses, adding to their emotional burden (Hatasaka 1994; Stephenson 1996). Inherited causes of recurrent miscarriage are often assumed, but the presumed high degree of genetic heterogeneity and lack of a carrier phenotype have made genetic studies impossible. Similarly, X-linked recessive lethality has long been proposed for RSA, but the sex of abortuses is generally unknown, and the high population prevalence of pregnancy loss makes the ascertainment of X-linked pedigrees problematic (Motulsky and Vogel 1997, pp. 139–141).

We have recently shown that carriers of X-linked recessive lethal traits may have the "molecular phenotype" of skewed X-chromosome inactivation (Pegoraro et al. 1997). Our model predicts that extreme skewing of X inactivation occurs during embryonic development in the female (asymptomatic) carrier, secondary to cell-autonomous selection against cells in which the abnormal X chromosome is active. All male (XY) conceptions of the carrier that receive the abnormal X chromosome would be spontaneously aborted. The miscarriage rate of female carriers of such traits would be expected to increase from an estimated population rate of ~15% to ~40% (15% + 25% for X-linked recessive recurrence risk). We recently reported a 70-member pedigree that validated this hypothesis: a maternally inherited trait, which caused sole use of the paternally derived X chromosome in female carriers, was associated with a 32% spontaneous abortion rate, whereas noncarriers in the same family all showed the 15% population rate ($P < .05$; Pegoraro et al. 1997).

To test the hypothesis that X-linked lethal traits are a significant cause of RSA, we recruited women who had experienced at least two miscarriages in the absence of any cytogenetic, anatomic, infectious, immunologic, or hormonal abnormalities known to cause RSA (table 1). A priori, "skewed X chromosome inactivation" was defined as preferential use of one allele in ⩾90% of peripheral leukocytes. This value was selected because we proposed that this level of skewing represents negative selection strong enough to be associated with RSA but not such a rare event as to go unobserved in our case group. Genomic DNA extracted from peripheral lymphocytes was assayed for X inactivation at the androgen receptor (HUMARA) locus (Pegoraro et al. 1994).

A total of 48 women who met the diagnostic criteria for enrollment were assayed for X inactivation, 7 (14.6%) of whom were found to show highly skewed X inactivation (table 1 and fig. 1). In contrast, in the age-matched control group, comprising women from the same demographic region with no known history of pregnancy loss, only 1 (1.5%) of 67 exhibited similar X-inactivation skewing (⩾90%) with the same assay system (table 1). This finding is statistically significant ($P < .01$, one-tailed Fisher's exact test). The distribution of X-inactivation ratios for both cases and controls is shown in figure 2.

Although the frequency of skewed X inactivation in the control women is lower than that observed by Naumova et al. (1996), this finding remains significant in comparison with the frequency observed in X-inactivation controls in other published reports (table 1). Plenge et al. (1997) found that in 115 unrelated control-group women, 4 (3.5%) showed skewed X inactivation ⩾90%. When this result is compared with our case population, the association remains statistically significant ($P < .02$, one-tailed Fisher's exact test). In a study of the effect of aging on patterns of X inactivation, Gale et al. (1997) found that 3 (3.2%) in 94 control-group women in the cohort including the age range of our cases and controls (17–50 years) showed skewed X inactivation ⩾90%. Again, our case group shows a statistically significant increase in the frequency of highly skewed X inactivation when compared with this control group ($P < .02$, Fisher's one-tailed exact test).

Table 1

X Inactivation in Women with RSA of Unknown Cause

	No. (%) with X Inactivation		
	Skewed ≥90%	Random	Total
RSA cases	7 (14.6)	41	48
Controls	1 (1.5)	66	67[a]
Plenge et al. (1997)	4 (3.5)	111	115[b]
Gale et al. (1997)	3 (3.2)	91	94[b]

Note.—Women with RSA of unknown cause have a statistically significant increased frequency of skewed X inactivation, compared with control-group women. RSA cases were women who had undergone an extensive series of diagnostic tests to rule out known causes of recurrent pregnancy loss. The tests performed were as follows: cytogenetic—parental and abortus karyotyping; anatomic—hysterosalpingogram; infectious—cervical cultures for mycoplasma, ureaplasma, gonnococcus, and chlamydia; immunologic—anticardiolipin antibody, antinuclear antibody, and lupus anticoagulant; and hormonal—serum progesterone, late luteal-phase endometrial biopsy, and thyroid-stimulating hormone.

[a] $P < .01$.

[b] $P < .02$.

The excess of women with idiopathic RSA observed in our study who showed highly skewed X inactivation suggests that ~15% of women with RSA may be carriers of X-linked cell-autonomous lethal traits. However, there are two potential confounding variables that merit further discussion: the mechanism of selection in peripheral leukocytes and the effect of aging on X inactivation.

In Belmont's (1996) review of X inactivation and mechanisms of skewing, it was hypothesized that some individuals showing skewed X inactivation in blood samples (peripheral leukocytes) may exemplify somatic selection for a subset of hematopoietic cells. Such selection may or may not be cell-autonomous lethal, since a modest growth disadvantage may result in pronounced skewing over extended time. However, because we show an association between a lethal phenotype (RSA) and highly skewed X inactivation, we believe our hypothesis is also likely, that is, a subset of women with highly skewed X inactivation are carriers of cell-autonomous lethal traits. Such lethal traits could be subcytogenetic deletions, as reported by Pegoraro et al. (1997), or single-gene mutations, either of which would result in RSA.

Since reports of the effect of aging on X inactivation have shown an increased frequency of skewed X inactivation in older women, we need to exclude the possibility that our observed association was caused by an age effect (Busque et al. 1996; Gale et al. 1997). The group of reproductive-age women (17–50 years) studied by Gale et al. (1997) show a statistically significant lower frequency of highly skewed X inactivation, when compared with our case group. The distribution of ages among the controls in the population studied by Gale et al. is not significantly different from the distribution of ages among our cases and controls. Furthermore, the seven women with idiopathic RSA and highly skewed X inactivation in our case group are distributed throughout this range (mean age in case group is 34.8 ± 6.0 years; the ages of case women with highly skewed X inactivation are 28, 37, 39, 40, 41, 42, and 46 years). Thus, we feel it is unlikely that our observed association is caused by an age effect.

Future efforts will be directed at expanding the patient populations studied, with the use of both positive (RSA of known cause) and negative (multiple live-born children in the absence of any spontaneous abortions) control groups. This type of study will enable ascertainment of larger pedigrees cosegregating skewed X inactivation and pregnancy loss, leading to the identification of specific gene loci causing RSA. In such families, the molecular phenotype of skewed X inactivation should permit the genetic mapping of these loci.

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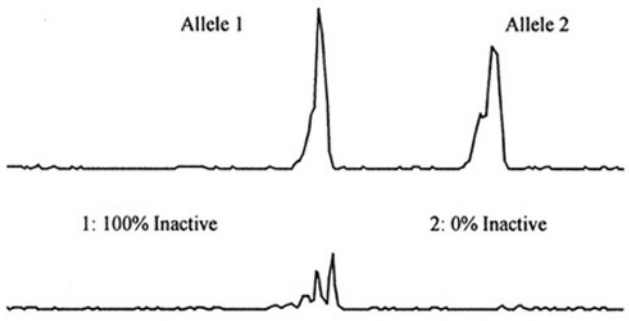

Figure 1 Skewed X inactivation in women with RSA. Genomic DNA samples from women with RSA pregnancy loss were subjected to PCR amplification of the highly polymorphic HUMARA locus,with fluorescent primers. A gravida 5 para 0 (G5P0) woman is heterozygous at this locus (*upper trace*). Digestion of genomic DNA with methylation-sensitive restriction enzymes prior to PCR at the HUMARA locus permits accurate quantitation of X-inactivation patterns (*lower trace*). The G5P0 woman shows complete (100%) skewing. X-inactivation analysis at the HUMARA locus was performed as described elsewhere (Pegoraro et al. 1994). Use of the highly polymorphic HUMARA locus afforded 90.6% (48/53) of individuals informative for the X-inactivation assay.

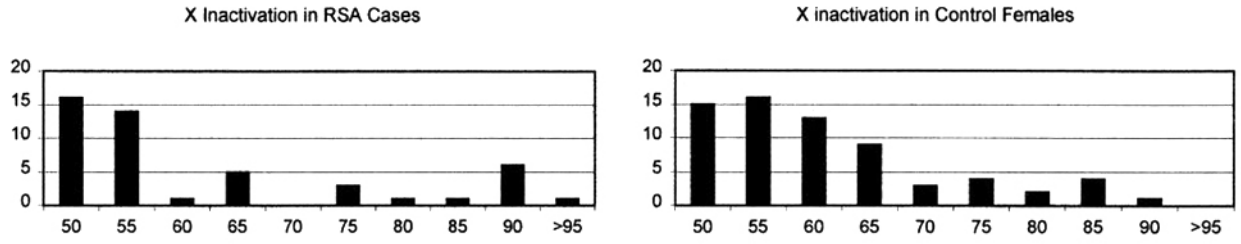

Figure 2 Frequency (*vertical axis*) of X inactivation (*horizontal axis*) in RSA cases ($n = 47$ [*left histogram*]) and controls ($n = 67$ [*right histogram*]). Women with RSA show a statistically significant abundance of highly skewed X-inactivation values, compared with control women. The X-inactivation values, which are reported as the percentage of activity of the more active allele; thus the data range is 50%–100%, inclusive. Although other groups have found the frequency of skewed X inactivation among controls to be closer to 10%, these studies use methodologically different assays, such as digestion with *Hha*I (Naumova et al. 1996). These specific methodological differences appear to yield distributions significantly different from those obtained in the present study and in studies published elsewhere (Busque et al. 1996; Gale et al. 1997; Pegoraro et al. 1997; Plenge et al. 1997).

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