SEX CHROMOSOME GENETICS '99
The X Chromosome and Recurrent Spontaneous Abortion: The Significance of Transmanifesting Carriers

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Recurrent spontaneous abortion (RSA) is a surprisingly prevalent disorder, affecting an estimated 1 of every 100 couples wishing to have children (Stephenson 1996). The diagnostic evaluation of RSA is extensive, with many different etiologies, each causing a small proportion of the total cases. The etiologies can be grouped into five categories: anatomic, infectious, hormonal, immunologic, and genetic. The diverse nature of the causal factors generally obligates a collaborative diagnostic effort, bringing together gynecologists, reproductive endocrinologists, geneticists, and other infertility specialists. Despite the thorough (and expensive) diagnostic workup (Wolf and Horger 1995), it has been estimated that the specific cause of RSA remains unknown in 37%–79% of affected women (Hatasaka 1994; Stephenson 1996).

It has been assumed that a large proportion of idiopathic RSA is genetic in origin. To date, however, the standard genetic evaluation consists solely of parental and abortus karyotyping. This identifies parental defects, such as balanced translocations, that can cause RSA (Neri et al. 1983), and it ascertains fetal aneuploidy, a common cause of spontaneous abortion that intrinsically has little recurrence risk (Guerneri et al. 1987; Eiben et al. 1990). As a result, the published total "genetic" contribution to RSA is essentially the frequency of translocations in the cohort of women with RSA: ~3% (Stray-Pedersen and Stray-Pedersen 1984; Stephenson 1996). This is quite likely an underestimation, since subcytogenetic defects are almost certainly a significant cause of RSA.

The hypothesis that either autosomal or X-linked recessive lethal traits could cause RSA is not novel. In fact, such models are a natural extension of Haldane's (1935) publication on mutation selection balance in recessive diseases. Identification of recessive lethal traits has been difficult, since heterozygous carriers of such traits would appear phenotypically normal. A subset of lethal traits may cause an increased frequency of spontaneous abortion in carriers if the hemizygous trait produces a clinically detectable pregnancy. Extended pedigrees in which women exhibit frequent spontaneous abortion are readily available but are difficult to analyze, because of the high population prevalence of spontaneous abortion and the assumed extensive genetic and etiologic heterogeneity. Although a method for clinically ascertaining carriers of autosomal recessive lethal defects remains problematic, it is possible that carriers of X-linked recessive lethal traits manifest the molecular phenotype of nonrandom (skewed) X chromosome inactivation. The logic underlying this hypothesis will be illustrated herein.

Received February 3, 1999; accepted for publication February 16, 1999; electronically published March 12, 1999.

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0002-9297/99/6404-0004$02.00

Nonrandom X Inactivation

The X chromosome is unique in that it undergoes transcriptional silencing (inactivation) to achieve sex-independent dosage equilibrium of X-linked genes. X inactivation occurs early in embryogenesis, at the 64- to 100-cell stage. It is thought to be a stochastic event; that is, each cell is equally likely to inactivate either the maternal or paternal allele, and the allele "choice" is not influenced by neighboring cells. Therefore, in normal females, approximately half the cells transcribe genes on the maternal X chromosome.

Given this feature of X chromosome inactivation, it is unusual to find women with nonrandom (skewed) X inactivation, that is, the preferential use of either the paternal or maternal allele in a preponderance of that female's cells. When this phenomenon is observed, it is frequently associated with a defect on one of the X chromosomes. The most extreme example occurs in individuals with X:autosome balanced translocations, in which the translocated X is found to be active in most cells. This phenomenon results from improper postzygotic dosage that is apparently lethal to those cells with the normal X chromosome active. At the time of X inactivation in such a case, it is presumed that many cells, in

fact, randomly inactivate the X-chromosome–carrying autosome-derived material. However, this results in persistent disomic dosage of genes on the portion of the X chromosome that was translocated to the autosome, in addition to partial inactivation of autosomal material. This partial disomic X chromosome dosage and monosomic autosome dosage is deleterious to the cells, causing cell-autonomous death or growth disadvantage, yielding highly skewed X inactivation in the female by the time of birth. It is pertinent to emphasize that the skewed X inactivation is due to selection against those cells with improper X dosage and not to a primary defect in the process of X inactivation (Schmidt and Du Sart 1992).

This model of cell selection during embryonic development, which yields skewed X inactivation in an X: autosome translocation carrier, predicts that functional hemizygosity for even a single vital X-linked gene would also lead to skewed X inactivation in a female carrier. Such a carrier may be spared any clinical phenotype, but, as a result of selection against those cells missing the vital gene, she would manifest the molecular phenotype of skewed X inactivation. Hemizygous male progeny of the carrier of such a gene would not be viable and would spontaneously abort some time after conception. As such, it is possible that female carriers of X-linked recessive lethal traits will show skewed X inactivation and may show RSA. Since these women would abort half of all male embryos, their risk of spontaneous abortion increases from a population risk of 15% to a combined risk of up to 40% (15% + 25%), if we assume that carriers of X-linked lethal traits do not constitute a major proportion of all females who experience single pregnancy losses.

X-Linked Dominant Disorders

The phenotypic expression and familial segregation of certain disorders solely in females has supported the hypothesis that some diseases are inherited as X-linked dominant diseases. Implicit in that mode of inheritance is male lethality, thus explaining the absence of affected males. For example, incontinentia pigmenti type II (IP2; MIM 308310), X-linked dominant chondrodysplasia punctata (MIM 302960) and Rett syndrome (MIM 312750) are strongly suggestive of this type of inheritance. In other disorders, such as fetal akinesia (MIM 300073) and X-linked pterygium (MIM 312150), the disease phenotype is either clinically similar to autosomal syndromes or is so rare that X-linked lethality can only be suggested on the basis of a sex bias in the few observed cases.

The X-inactivation patterns in IP2 and Rett syndrome yield interesting insights into the possible mechanisms of male lethality in X-linked dominant disorders. IP2 is a disease mapped to Xq28 and displays characteristic skin, retinal, and central nervous system findings. Affected females show highly skewed X inactivation both in cultured fibroblasts and in peripheral blood, probably indicating strong selection against those cells expressing the lethal IP2 allele (Migeon et al. 1989). A report of a Klinefelter (47, XXY) male with IP2 supports this interpretation; as in XX females, the second X chromosome allowed for cell-autonomous selection against mutant alleles (Garcia-Dorado et al. 1990). More recently, the natural history of a 46, XY pregnancy was described in which a fetus diagnosed with IP2 by CVS was spontaneously aborted at 20 weeks (Devriendt et al. 1998). It is now proposed that male lethality arises from absence of a vital gene in hematopoetic or immunologic development, which is concordant with the finding of highly skewed X inactivation in the peripheral blood of carrier females (Roberts et al. 1998). In contrast, in Rett syndrome, a disorder characterized by severe autism and purposeless hand movements, skewed X inactivation is not observed in affected patients (Camus et al. 1996; Sirianni et al. 1998). Instead, nonpenetrant carriers show exclusive use of the normal X chromosome and are, therefore, clinically protected from the disease by the preferential inactivation of the affected allele (Schanen et al. 1997; Sirianni et al. 1998). This contrast suggests that, in Rett syndrome, cells in which the abnormal allele is expressed survive but cause abnormal brain development in the symptomatic carrier; in IP2 patients, on the other hand, the abnormal allele is lethal to those cells expressing it. In either case, the mutation likely confers lethality to the hemizygous male.

Nonrandom X Inactivation as a Mendelian Trait

There is a growing body of literature on the inheritance or familial clustering of skewed X chromosome inactivation in the absence of any carrier phenotype. Skewed X inactivation can be classified as either primary or secondary (Puck and Willard 1998). Primary nonrandom X inactivation includes those cases in which skewed X inactivation is the result of a defect in the *process* of X inactivation—that is, either a defect in the regulation of X inactivation-specific transcript (*XIST*) or in another undiscovered gene that mediates X inactivation (Lee and Jaenisch 1997). Conversely, secondary skewed X inactivation arises because of secondary selection against those cells that have a deleterious mutation on the active X chromosome (Migeon 1998).

Although progress has been made in the functional characterization of *XIST* (Penny et al. 1996; Willard 1996; Panning et al. 1997), there are only two reported familial cases of primary nonrandom X inactivation. Both of these families carry a C→G mutation in the *XIST* minimal promoter, which confers preferential inactiva-

tion of the X chromosome in *cis* (Plenge et al. 1997). It is possible that cases of primary skewed X inactivation are indeed rare, but the relative scarcity of characterized cases may also be explained by the fact that *XIST* encodes a structural RNA whose critical sequence features remain largely unelucidated (Clerc and Avner 1998; Marahrens et al. 1998). Moreover, the fact that families segregating such mutations show no clinical phenotype in either hemizygous males (normal viability) or heterozygous females suggests that primary nonrandom X inactivation is unlikely to be associated with RSA.

Pegoraro et al. (1997) reported the largest characterized family manifesting secondary skewed X inactivation. In a five-generation family with 50 informative females, 16 showed highly skewed (≥95:5) X-chromosome inactivation. This family was originally identified via an apparent manifesting carrier of Duchenne muscular dystrophy (DMD; MIM 310200). However, Pegoraro et al. showed that the locus responsible for the skewed X inactivation molecular phenotype was linked to a marker at Xq28, rather than to the *DMD* locus at Xp21. Subsequently, a relatively large deletion (~500 kb) was found in distal Xq28, spanning the region of linkage. There was complete concordance between deletion carriers and highly skewed X inactivation. In this large pedigree, the deletion clearly causes selection against those cells with the deletion carrying X active. Interestingly, those females carrying the deletion had a statistically significant ($P < .02$) increased rate of spontaneous abortion (40%) compared with their siblings who did not carry the deletion (15%). This increase in the rate of spontaneous abortion was due to loss of hemizygous male embryos that inherited the deletion. This pedigree represents the first characterized family segregating X-linked recessive lethality, with females manifesting the X-linked dominant molecular trait of skewed X inactivation. Both effects are due to the cell-autonomous selection against a lethal trait.

There are several other reports of familial skewed X inactivation. Naumova et al. (1996) presented a pedigree in which nonrandom X inactivation appeared to be inherited through the paternal line. Moreover, in this family the paternal allele conferred preferential inactivation in *trans*. Unfortunately, this pedigree was too small to permit genetic mapping. Recently, Parolini et al. (1998) reported an 8-year-old manifesting carrier of Wiskott-Aldrich syndrome with nonrandom X inactivation favoring expression of the mutant paternal allele. The girl's mother and maternal grandmother also showed nonrandom X inactivation, which led the authors to suggest that there is a primary defect of X inactivation in this family. However, it is also possible that, as in the family presented in Pegoraro et al. (1997), this family carries an X-linked recessive lethal trait in the maternal line, which causes preferential expression of the paternally derived disease allele in *trans*. We propose that such "trans-manifesting carriers," in whom the expression of a recessive disease allele is enforced by cell-autonomous selection against the other X chromosome, may represent a significant proportion of the cases of X-linked recessive disease in females. The mutations responsible for skewed X inactivation were not identified in either of the families reported by Naumova et al. (1996) or Parolini et al. (1998)

The Association between Idiopathic RSA and X Lethal Loci

If, as we have argued, a subset of female carriers of X-linked recessive lethal traits are genetically predisposed to spontaneous abortion, and if such carriers can reliably be ascertained via their skewed pattern X chromosome inactivation, it becomes possible to test the hypothesis that X-linked recessive lethal traits are a significant cause of RSA in the general population. Intrinsic to this argument are the assumptions that the trait is cell autonomous—that is, that it causes death or growth disadvantage to the cells with the mutant X active and that hemizygous males survive at least until the pregnancy is clinically observable through a positive bHCG test.

To test this hypothesis, we have initiated a case-control study wherein we compare the frequency of highly skewed X chromosome inactivation in women with two or more unexplained spontaneous abortions to the frequency in female controls (Lanasa et al. 1998). The women characterized with idiopathic RSA have undergone a complete evaluation to rule out any of the known causes of RSA described above (Stephenson 1996). The controls are women from the same demographic region, with no known history of spontaneous abortion; furthermore, the cases and controls are age-distribution matched, so that the distribution of ages between the two groups is the same. Defining skewed X inactivation as preferential use of one X chromosome in ≥90% of peripheral leukocytes, we have found 7 (14.6%) of 48 to have skewed X inactivation. In contrast, only 1 (1.5%) of 68 control females exhibit this extent of nonrandom X inactivation. This finding is statistically significant, with $P < .01$ (Fisher's exact test, one-tailed).

The frequency of nonrandom X inactivation is somewhat lower (1.5%) in our control group than has been reported previously. Other groups have estimated that the frequency of skewed inactivation (at the level of ≥90% silencing of one copy of the chromosome) as 3.2% (Gale et al. 1997) or 3.5% (Plenge et al. 1997) in women of the same age in the population at large. Although case-control comparisons across studies may be perilous, it is interesting to note that, even when these higher estimates of skewing frequency are used for the control group, the frequency we observe in our group

of RSA-affected women remains significant at the level of $P < .05$ (Fisher's exact test, one-tailed).

Although the results presented here are clearly preliminary, it is interesting to speculate about the frequency of X-linked recessive lethal traits in the general population. Given a frequency of idiopathic RSA of 1 in 250 in the general population, and an affection rate of ~1 in 7 in our case population, the population prevalence of X-linked lethals leading to RSA could be as high as 1 in 1,750. In fact, our ascertainment methodology will miss a large number of carriers, since, on average, a carrier would have to become pregnant five times to show two spontaneous abortions. Furthermore, there is great selective pressure against such traits. As X-linked recessive lethal traits can be passed on only to daughters, the carrier frequency should be halved in each generation. If 1 in 1,750, in fact, approximates the carrier frequency, then the new mutation rate must be 1 in 3,500. Since the highest-known single-gene–mutation rate is that of dystrophin at 1 in 10,000, a mutation rate of 1 in 3,500 indicates extensive genetic heterogeneity. This is consistent with the hypothesis that there are a significant number of vital genes on the X chromosome.

RSA is a major women's health concern. As the application of molecular genetics to RSA advances, it will be possible to begin characterizing those genes that cause spontaneous abortion in the recessive state. The X chromosome inactivation assay affords a methodology by which female carriers of X-linked recessive lethal defects can be identified. Over time, then, by assembling familial pedigrees, the individual causative genes can be identified. The X-inactivation assay should become an important diagnostic tool in the clinical evaluation of women with RSA, as secondary skewed X inactivation will be the common denominator by which carriers of X-linked recessive lethal traits can be identified.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for IP2 [MIM 308310], X-linked dominant chondrodysplasia punctata [MIM 302960], Rett syndrome [MIM 312750], fetal akinesia [MIM 300073], X-linked pterygium [MIM 312150], and DMD [MIM 310200]).

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