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Familial Skewed X Inactivation and X-Linked Mutations: Unbalanced X Inactivation is a Powerful Means to Ascertain X-Linked Genes That Affect Cell Proliferation

To the Editor:

We read with great interest the article by Pegoraro et al. (1997) in which they report familial skewed X inactivation associated with a submicroscopic deletion at Xq28. This deletion, which spans ~800 kb near the F8C locus, is transmitted in the expected X-linked fashion through females, but no males have the deletion. Although we agree that the skewed X-inactivation pattern in this family is attributable to the region around or included in the deletion, we disagree with their inter-

pretation of these results and with some assertions that may lead to confusion about the underlying basis for skewed X inactivation in this family.

The authors suggest that the simplest interpretation of their data is that this disorder behaves like an X dominant with lethality in the male fetus. All the evidence supports this hypothesis: The Xq28 deletion is lethal when present in hemizygous males and confers a proliferative disadvantage to cells of heterozygous females in whom the deleted chromosome is active, which explains why these females are clinically normal.

However, Pegoraro et al. (1997) considered the hypothesis of an X-dominant disorder with male lethality inconsistent with their data on the basis that the relevant locus cannot be a genetic or cell lethal, because, if so, affected males would die soon after fertilization. In fact, the time at which a male embryo carrying the deletion would become inviable, or at which the population of mutant cells in the female would be eliminated, depends on the nature of the mutant gene product—when it is needed, in which cells it is expressed, and how detrimental (or advantageous) it is in the presence of wild-type cells in the mosaic population. Clearly, a male with this small deletion might not be aborted before the pregnancy is recognizable; some males with macroscopic deletions of the X chromosome can survive until late gestation or term (Rosenberg et al. 1987; Cremers et al. 1988).

This family resembles those in which selection has been shown to underlie X inactivation. The familial skewing seen with mutations at the incontinentia pigmenti (IP) locus, in cytogenetically normal females, is also associated with recurrent abortion (Migeon et al. 1989; Parrish et al. 1996). However, IP heterozygotes do not eliminate their mutant population of cells as completely as do the heterozygotes in this family, and hence some IP heterozygotes may be clinically abnormal. Large differences in the growth of normal and abnormal cells can result in early loss of disfavored cells, whereas more subtle differences account for progressive loss during the lifetime of the heterozygote (Migeon et al. 1981, 1988). The familial skewed X inactivation associated with adrenoleukodystrophy was also mapped to Xq28, by tight linkage with the G6PD locus (Migeon et al. 1981), and was shown to be attributable to a proliferative advantage of the mutant gene.

Pegoraro et al. (1997) suggest two alternative novel hypotheses to explain their findings: (1) the deleted region in Xq28 is directly involved in the primary mechanisms of X inactivation; or (2) the region is directly responsible for skewing. In either case, the observed skewing is attributed to a gene in Xq28 that determines the choice of X chromosome to inactivate or the ability of an X to inactivate. As Pegoraro et al. (1997) note, there is no evidence in either mouse or human to support the existence of a such a gene within the deletion at Xq28. In addition, there is uncited evidence (discussed below) that argues against the existence of such a hypothetical locus in Xq28.

There may be multiple *trans*-acting factors involved in the initiation and maintenance of X inactivation. Observations of triploid embryos suggest that these are likely to reside on autosomes (discussed by Jacobs and Migeon 1989). There is no convincing evidence that factors responsible for *cis* inactivation lie outside the X chromosome–inactivation center (XIC) at Xq13.2. Studies of chromosomal deletions and translocations leading to deletions indicate that this segment is the site of the XIC (Therman et al. 1979; Mattei et al. 1981), and all X-linked genes shown to have an effect on choice (Xce) or on inactivation (XIST) lie within this region. Evidence that other regions of the X are unlikely to be relevant in the choice of X to inactivate comes from recent studies of the murine XIC region transfected into male embryonic stem cells, which indicate that the XIC region contains all the genetic information needed for *cis* inactivation of an X chromosome, including choice of inactive X (Lee et al. 1996; Lee and Jaenisch 1997).

None of the reported families with skewed patterns of X inactivation has been shown, convincingly, to involve the mechanisms of X inactivation. Most of them can be explained by mutations or cytogenetic deletions that adversely affect the proliferation of one of the cell populations produced by random X inactivation (reviewed in Belmont 1996). In light of this, it seems that relatively few cases of familial unbalanced X inactivation will be due to mutations causing primary nonrandom inactivation. Although mutations affecting the process of X inactivation are real possibilities, one expects that those severe enough to interfere with X inactivation would be embryonic lethals, because of the functional X disomy. Mutations in the inactivation machinery that are associated with normal phenotypes and two intact X chromosomes are most likely to be the ones that influence only the choice of X to inactivate and therefore would be at the XIST or an Xce-like locus or in other constituents of the XIC. However, even linkage to the XIC or with specific XIST alleles (Plenge et al. 1997) is not sufficient evidence that skewing is in fact attributable to these genes, because it could be due to a hitchhiker effect, resulting from mutations in a close neighbor with potential for cell selection.

We believe that familial skewing, which maps to the X chromosome but is unlinked to the XIC, most likely results from mutations that do not interfere with the inactivation machinery but that affect the proliferation of the cell populations produced following normal random X inactivation. Females who manifest X-recessive diseases provide a powerful means to detect unbalanced X inactivation (Migeon 1993). The corollary is that

skewed patterns of X inactivation, familial or not, provide a powerful means of ascertaining mutations that influence cell proliferation.

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Reply to Migeon and Haisley-Royster

To the Editor:

We thank Drs. Migeon and Haisley-Royster (1998 [in this issue]) for their interest in our research. We are, however, a bit puzzled by their letter to the editor, since they write that they disagree with the interpretation of our results yet then restate what was already written in our previously published article (Pegoraro et al. 1997).

The 50-member pedigree that we reported showed an X-linked dominant disorder with male lethality. There is no question of this fact, because we found a deletion mutation of Xq28 associated with skewed X inactivation and recurrent pregnancy loss (LOD = 6.92). The deletion included the factor VIII gene, yet there were no males from 50 females with factor VIII deficiency, again clearly proving that this family had an X-linked dominant disorder with male lethality.

Drs. Migeon and Haisley-Royster appear to wish to address two issues: (1) interpretation of the likely mechanisms that would cause X-inactivation skewing in the females in this family; and (2) transcriptional timing of the deleted gene or gene products in Xq28 and the observed effect on miscarriage detection. There is very little to disagree with in Drs. Migeon and Haisley-Royster's interpretation of our results; they suggest that a growth disadvantage is probably playing a role, which is precisely what we stated in our discussion. We, too, feel that growth disadvantage is the most likely mechanism causing skewing of X-chromosome inactivation. However, in the absence of characterization of the causative genes in Xq28, it seems unreasonable to dismiss the possibility that the gene(s) may actually be involved in the process of X inactivation. This is the least likely mechanism, but it does not seem to warrant exclusion from discussion.

The timing of transcription of the gene products in Xq28 undoubtedly affects when the miscarriage occurs. In fact, the issue of timing is central to the inferred ge-