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X Chromosome–Inactivation Patterns Confirm the Late Timing of Monoamniotic-MZ Twinning

To the Editor:

We recently reported, in the *Journal*, on the patterns of X-chromosome inactivation in female MZ twin pairs (Monteiro et al. 1998). The data supported the hypothesis that dichorionic (DC) and monochorionic (MC) MZ twin pairs differ in the timing of the twinning event, with respect to the onset of X inactivation. Specifically, DC-MZ twin pairs frequently exhibit dramatic differences, in X-inactivation patterns, between members of the pair, whereas MC-MZ pairs are highly correlated. The most reasonable interpretation of these results is that MC-MZ pairs undergo splitting after the X-inactivation event, whereas DC-MZ pairs must split before or around the time of X inactivation.

We noted in our report that, although MC-MZ twin pairs have X-inactivation patterns that are more highly correlated than those in DC-MZ pairs, they were not as closely correlated as were results of repeated assays of the same individual (Monteiro et al. 1998). We interpreted this as being reflective of heterogeneity in the timing of MC-MZ twinning, with some MC-MZ pairs splitting after—but close in time to—the onset of X inactivation. We further hypothesized that a subgroup of MC-MZ twins—namely monoamniotic-MC twin pairs (MA-MZ)—would have X-inactivation patterns that are even more closely correlated, since MA-MZ twins probably result from much later twinning events that take place many cell divisions after the X-inactivation event. We have now directly addressed this issue experimentally.

MA-MZ twinning is a relatively rare event, with only ~2% of MZ twins falling into this anatomic subgroup (Derom et al. 1995). Nevertheless, we have identified 11 female MA-MZ twin pairs, as part of the East Flanders Prospective Twin Study. The amniotic anatomy of each twin pair was assessed at birth, by examination of the placental structure (Loos et al. 1999). The absence of any septum in a well-preserved fetal sac, when one is able to peel off the amnion from the placental surface, indicates the presence of a single amniotic sac. The X-inactivation patterns in these twins were assessed by a *Hpa*II-PCR assay, which depends on methylation differences, in the androgen-receptor gene, between the inactive and active X chromosomes (Allen et al. 1992). In order to avoid the confounding factor of a shared placental blood supply in MC-MZ twins, the assessment of X inactivation was done by use of DNA isolated from buccal mucosa.

Figure 1 shows a comparison of the absolute difference in X-inactivation patterns, both among the various anatomic subtypes of MZ twin pairs and among patterns in repeated assays performed on the same individual. The data on DC-MZ twin pairs (mean X-inactivation difference 14.3%) and MC-MZ (mean X-inactivation difference 5.9%) are taken from our previous report (Monteiro et al. 1998) and are significantly different, with $P < .05$. (Note that all the "MC-MZ" pairs reported in figure 1 are diamniotic.) Strikingly, MA-MZ twin pairs exhibit identical X-inactivation patterns, at least to the limits of the assay used for this analysis, since the mean difference among MA-MZ pairs (2.1%) does not differ significantly from that observed when the assay is repeated twice on the same individual (3.7%). In contrast, the MA-MZ twin pairs are considerably more similar to one another than are diamniotic MC-MZ pairs, in a pairwise comparison ($P = .02$).

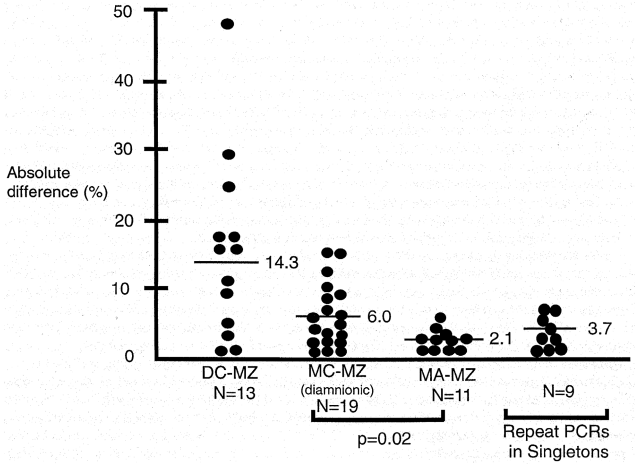

Figure 1 Summary of differences in X-inactivation patterns, among various subgroups of MZ twin pairs and among repeat assays of singletons. An *Hpa*II/PCR methylation assay for the androgen-receptor gene was performed on buccal mucosal DNA, as described elsewhere (Monteiro et al. 1998). The 19 diamniotic MC-MZ twins have been analyzed elsewhere (Monteiro et al. 1998). The repeat assays on singletons were done on buccal mucosal DNA; separate buccal samples were analyzed at different times (Monteiro et al. 1998).

These data strongly support the hypothesis that MC twinning occurs during a relatively broad time frame following X inactivation. Some MC pairs apparently result from splitting quite soon after commitment to X inactivation, leading occasionally to measurable X-inactivation differences between the members of the pair, as much as 15% (fig. 1). In contrast, when twinning occurs late, leading to MA anatomy, members of the twin pair have virtually identical patterns of X inactivation. This conclusion depends on the assumption that roughly equal numbers of cells go to each embryo after splitting (Monteiro et al. 1998). Clearly, highly asymmetric splitting cannot explain MA twinning events,

even though twins in this subgroup are more likely to exhibit birth-weight differences (C. Derom, unpublished data). Of course, asymmetric splitting might occur occasionally but result in one or both fetuses being nonviable. Interestingly, there is marked female predominance in the MA-twin group, with a sex proportion of .23 (Derom et al. 1988). The reasons for this are unclear. It appears that female embryos are relatively delayed in early embryonic development (Pergament et al. 1994). Thus, female embryos could be somewhat less mature at the time of formation of the amnion, and thus splitting of female embryos may be more compatible with survival at this stage. The delay in early female development has been ascribed to the absence of a Y chromosome (Pergament et al. 1994). However, the process of X inactivation, since it may occur when there are $\leqslant 10$ cells in the embryo (Puck et al. 1992; Monteiro et al. 1998), might itself contribute to a slight delay in early female-embryo development.

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Smita Chitnis,[1] Catherine Derom,[2] Robert Vlietinck,[2] Robert Derom,[2] Joanita Monteiro,[1] and Peter K. Gregersen[1]
[1]*Division of Biology and Human Genetics, North Shore University Hospital, New York University School of Medicine, Manhasset; and* [2]*Center for Human Genetics, Katholieke Universiteit, Leuven, Belgium*

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Address for correspondence and reprints: Dr. Peter K. Gregersen, Division of Biology and Human Genetics, North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030. E-mail: peterg@nshs.edu

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Refinement of the Locus for Autosomal Recessive Retinitis Pigmentosa (RP25) Linked to Chromosome 6q in a Family of Pakistani Origin

To the Editor:

"Retinitis pigmentosa" (RP) is the term used to define a clinically and genetically heterogeneous group of retinal degenerations primarily affecting the rod photoreceptors. RP is characterized by progressive loss of vision, initially manifesting as night blindness and reduction in the peripheral visual field and later involving loss of central vision (Bird 1995). Ophthalmoscopic examination typically reveals pigmentary disturbances of the mid-peripheral retina. RP may be inherited as an autosomal recessive, autosomal dominant, digenic, or X-linked trait. Autosomal recessive RP (arRP) accounts for ~20% of all cases of RP, whereas sporadic RP, which is presumed to be recessive in most cases, accounts for a further 50% (Jay 1982).

Mutations causing arRP have been found in the genes encoding rhodopsin (Rosenfeld et al. 1992), in the α and β subunits of rod phosphodiesterase (Huang et al. 1995; McLaughlin et al. 1995), in the α subunit of the cyclic-GMP gated–channel protein (Dryja et al. 1995), and in the genes *RPE65* (Gu et al. 1997), *RLBP1* (Maw et al. 1997), *ABCR* (Martinez-Mir et al. 1998) and *TULP1* (Banerjee et al. 1998; Hagstrom et al. 1998). In addition, genetic linkage studies have identified arRP loci at 1q31-q32.1 (van Soest et al. 1994; Leutelt et al. 1995), 2q31-q33 (Bayes et al. 1998), and 16p12.1-p12.3 (Finckh et al. 1998). Recently, linkage of arRP to a region on chromosome 6q has been reported in several Spanish families (Ruiz et al. 1998). All the above are reference at the RetNet website.

We studied 20 members of a three-generation consanguineous Pakistani family in which RP segregated as an autosomal recessive trait (fig. 1). This pedigree contained 12 affected individuals. Examination of all af-