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INVITED EDITORIAL
Breaking Away from Home

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Duplications have been instrumental in shaping the evolving genome. Duplications typically arise from unequal crossing-over during meiosis between misaligned homologous chromosomes, a process catalyzed by the presence of repetitive elements. The recombination products of such an event are a duplication at the site of the exchange and a reciprocal deletion. What Hodes et al. (2000 [in this issue]) have discovered is an intriguing class of duplications in which the duplicated region has popped up far away from home base (fig. 1).

Duplication of the Dosage-Sensitive Proteolipid Protein Gene Causes Pelizaeus-Merzbacher Disease

The duplicated region encompasses the X-linked gene encoding proteolipid protein (PLP), which is the most abundant protein of the myelin sheath in the central nervous system. Mutations at the *PLP* gene locus result in a loss of myelin that translates into neurological problems of psychomotor developmental delay, nystagmus, spastic paraplegia, dystonia, and cerebellar ataxia (reviewed in Hudson [2000]). The severity of myelin loss is dependent on the particular *PLP* mutation and can range from early lethal forms of Pelizaeus-Merzbacher disease (PMD [MIM 312080]) to the mildly affected allelic disorder known as "spastic paraplegia type 2" (SPG2 [MIM 312920]). A duplicated *PLP* gene, arranged in tandem with the normal *PLP* gene at Xq22, is the culprit in the majority of patients with PMD (Wang et al. 1997; Sistermans et al. 1998; Woodward et al. 1998; Inoue et al. 1999; Mimault et al. 1999). There is no one "hotspot" for recombination in this part of the X chromosome, since both the size of the duplication and the end points of the duplication vary widely in PMD. The extent of the duplicated region has been estimated to be 500–1,650 kb, and it includes the entire *PLP* locus (Woodward et al. 1998). No simple correlation exists between the size of the duplication and the severity of the disease. The *PLP* duplication is almost always present in the mothers of affected boys, and, in a majority of cases, the mutation can be traced to the maternal grandfather, suggesting that the rearrangements originated within male germ cells (Inoue et al. 1999; Mimault et al. 1999). The tendency toward homozygosity of polymorphic markers across the duplicated region suggests that the duplicated alleles are probably derived from the same chromosome and that, therefore, the duplication occurred as an intrachromosomal event. In toto, the features of the typical duplication in PMD that emerge from haplotype inspection and FISH analysis point to the phenomenon of unequal sister-chromatid exchange in male meiosis accounting for the majority of *PLP* duplications. This mechanism pertains to duplications of another X-linked gene: the Duchenne muscular dystrophy gene (Hu et al. 1990). Heterozygosity of markers has been noted in haplotype analysis of only one family with PMD (Woodward et al. 1998), leaving open the possibility that interchromosomal rearrangements of maternal origin may also occur, albeit infrequently.

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Breaking into the Genome

The atypical *PLP* duplications in the three families with PMD that were examined by Hodes et al. (2000 [in this issue]) defy categorization. Their most striking attribute is their ability to break into the genome at secondary sites in the X chromosome. Instead of the tandem nature of the duplication usually observed in FISH analysis of patients with PMD (Inoue et al. 1999), in these families, the extra copy of *PLP* resides many megabases away from the Xq22 home base. In the case of one family, no karyotypic abnormalities can be detected on the X chromosome in which an extra *PLP* segment on the short arm (Xp22) coexists with the *PLP* region on the long arm (Xq22) (fig. 1). In another family, the additional segment has invaded the Xq26 region, again in the absence of microscopically detectable changes in the X chromosome. However, the carrier mother in this family is a somatic mosaic; half of her cells have three copies of *PLP* (one normal X chromosome and one X chromosome carrying the Xq26 duplication), whereas the

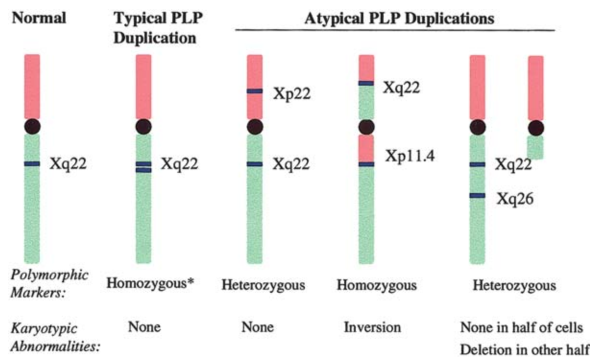

Figure 1 Duplications of the *PLP* locus on the X chromosome that result in PMD/SPG2. A single X chromosome is schematized with the short arm (*pink*), the long arm (*green*), and the position of the *PLP* locus at Xq22 (denoted by a bar). The asterisk indicates that all but one of the "typical" *PLP* duplications display homozygosity for the polymorphic markers in the *PLP* region. The exact breakpoints of the insertions, inversion, or deletion are not known. For the carrier mother in which the duplicated copy of *PLP* is at Xq26, half of her cells contain one normal X chromosome and one chromosome with two *PLP* genes (at Xq22 and Xq26), whereas the other half of her cells contain one normal X chromosome and one X chromosome that has been deleted for much of the long arm, including the Xq22 region. Adapted from the results of Hodes et al. (in this issue).

other cells have a single copy of *PLP* (one normal X chromosome together with an X chromosome deleted for Xq22 and distal regions) (fig. 1). This deleted X chromosome may represent an intermediate in the successive breakage-and-reunion events that directed the relocation of a duplicated copy of *PLP.* In both the Xp22 and the Xq26 cases, haplotype analysis indicated an interchromosomal rearrangement. Therefore, the duplications in these cases arose from recombination between two X chromosomes in a female. An additional family displayed an inversion involving portions of the short and long arms of the X chromosome in which copies of the *PLP* gene were found at each end of the inverted segment. Such a large duplication (which is considered to be "submicroscopic" by the usual karyotype analysis but is on the order of 100 kb) has not been found at the termini of other inversions.

Sequencing of the breakpoints of the duplication will reveal whether repetitive elements have spawned or contributed to these bizarre relocation events. Interspersed repetitive elements (LINES and SINES [long and short interspersed nuclear elements, respectively]) account for more than one-fourth of the genome, and the illicit pairing of these and other repeated regions underlies many unequal crossovers (reviewed by Jurka [1998]). Also, target sites for double-strand breaks mediated by transposases may be present at the breakpoints, as characterized by Lupski et al. for the recombination hotspots responsible for duplications in Charcot-Marie-Tooth disease type 1A and the reciprocal deletions in hereditary neuropathy with liability to pressure (Lupski 1998; Reiter et al. 1998). The flanking sequences of the *PLP* gene that could not break away from home base at Xq22 will also shed light on the possible mechanism of duplication, since these sequences will indicate whether this region of the genome was subjected to a chromosome-breakage event. Does the process begin with the usual scenario for the generation of duplications—that is, with a misalignment of two X chromosomes, followed by chromosome breakage? Perhaps, during the alignment of two X chromosomes, only one of the two chromosomes was subjected to breakage at sites flanking the *PLP* region, and the liberated Xq22 segment dropped into an available site (also created by a chromosome-breakage event) of the neighboring X chromosome. The behavior of the duplications is reminiscent of transposable elements, in their ability to copy themselves and move to a new site; moreover, transposable elements can mobilize genomic segments, transferring them to new locations in the genome. However, the comparatively large size and nonuniformity of these PMD-causing duplications is without precedent in the transposon world (reviewed by Jurka [1998]). While transposition events would need only a single breakage-and-reunion event (at the site of insertion), any chromosome-misalignment model must account for the occurrence of at least three DNA-breakage events.

How Fluid Is the Genome?

This new class of duplications discovered in PMD offers another glimpse at the instability of the human genome. A major question is how frequently duplications, including these novel kinds of duplications, are generated. *PLP* is in the special class of genes in which the level of expression is tightly controlled by the number of gene copies, so that a duplication readily presents with a disease phenotype. As a structural protein of the myelin sheath, *PLP* is synthesized coordinately with the other myelin proteins that are assembled into the myelin sheath in strict proportions. Duplication of *PLP,* like duplication of a myelin counterpart (*PMP-22*) in the peripheral nervous system, destroys the balance of myelin proteins and creates the dysmyelinating disorders of PMD and Charcot-Marie-Tooth disease type 1A, respectively (Lupski 1998; Hudson 2000). Since only a fraction of genes are in this dosage-sensitive category, many gene duplications may arise yet may be phenotypically silent. Therefore, as pointed out by Hodes et al. (2000 [in this issue]), these events may occur more frequently than is suspected but may not be detected. Another issue that would affect the frequency of these

events is whether the X chromosome is more susceptible to this novel type of duplication. Could the features of this chromosome that make it the only chromosome subject to condensation/inactivation be a factor in these duplications?

Dropping into a new neighborhood can have a detrimental impact on the residents of the neighborhood. Either by means of an outright disruption of a coding region or by breaking up critically positioned regulatory sites, the interloping duplication can disturb gene expression. Position effects have been documented in a number of human disorders (reviewed by Kleinjan et al. [1998]). The fluidity of the human genome means that sequencing of the human genome is only the start for genetic diagnosis. Future efforts will need to focus on the organization of the genome in individuals, not just on the sequences of disease-related genes. The novel PMD duplications are a harbinger for the real work to come in the assessment of mutations contributing to human disease.

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Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PMD [MIM 312080] and X-linked SPG2 [MIM 312920])

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