

## Mutation watch:

# PEX PLUS? Gene(s) for X-linked hypophosphatemia and deafness

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Three decades of work on X-linked hypophosphatemia in human and mouse reached fruition with the recent discovery of PEX, the “Phosphate regulating gene with homologies to Endopeptidases on the X chromosome” (HYP Consortium 1995; Strom et al. 1997). The mouse story began in 1966, when six males with shortened trunk and hindlimbs were discovered during a linkage experiment at The Jackson Laboratory (Eicher et al. 1976). Biochemical detective work identified hypophosphatemia as a critical defect, and administration of phosphate to the drinking water corrected many of the skeletal abnormalities and the dwarfing. Abnormal renal phosphate reabsorption accounts for the low level of phosphate in blood and the elevated level in urine. The mutation was inherited as an X-linked dominant and designated hypophosphatemia (*Hyp*).

In 1986, a second dominant X-linked hypophosphatemia mutation, *gyro* (*Gy*), was described (Lyon et al. 1986). In addition to hypophosphatemia and skeletal defects that closely resemble *Hyp*, *gyro* mice exhibit circling behavior due to degeneration of hair cells in the vestibular apparatus and cochlea. The neuroepithelial abnormalities of the inner ear also result in a hearing deficiency (Steel 1995). Because the phenotypes of *Hyp* and *Gy* are dominant, it was not possible to draw conclusions about possible allelism from the phenotype of the compound heterozygote. Evidence for very close linkage was obtained from a cross between *Gy* *+/+* *Hyp* females and normal males (Lyon et al. 1986). Among the 239 male offspring of this cross, one had normal blood phosphate levels and generated normal male offspring, indicating that he carried a recombinant *+* *+* chromosome. This recombination was interpreted as evidence that *Hyp* and *Gy* were mutations in distinct but closely linked and functionally related genes. However, recent molecular analysis found that *Hyp* and *Gy* are caused by independent mutations in the *Pex* gene that are separated by approximately 100 kb (Strom et al. 1997). The original recombination between *Gy* and *Hyp* was thus a rare example of intragenic recombination and reminds us that a single recombination does not provide a reliable estimate of genetic distance.

### Positional cloning of human PEX

Human X-linked hypophosphatemia, or Vitamin D-resistant rickets (MIM 307800), is a dominant disorder with an incidence of 1/20,000 characterized by a renal phosphate transport defect, hypophosphatemia, and elevated urinary phosphate. High-resolution genetic mapping was achieved by analysis of 258 family members from 20 kindreds (Rowe et al. 1996). A YAC contig spanning 3.3 Mb was generated, and an interesting application of the Southern blotting technique demonstrated the presence of Vitamin D receptor binding sites in one YAC that subsequently proved to contain the disease gene. Southern blotting of genomic DNA from 150 families and hybridization with radiolabeled cosmid DNA detected deletions in four families (HYP Consortium 1995). Cosmids from the deleted region were sequenced and used for cDNA

selection, resulting in a cDNA contig that overlapped the patient deletions. Database sequence comparisons demonstrated that the new gene, PEX, is a member of a subfamily of endopeptidases involved in activation and degradation of a variety of peptide hormones. Other family members include neutral endopeptidase (NEP), endothelin-converting enzyme 1 (ECE-1) and the Kell antigen. One interesting feature of PEX and related peptidases is the presence of a putative transmembrane domain near the N-terminus of the protein.

Subsequent isolation and sequencing of the mouse *Pex* cDNA revealed a predicted protein of 749 amino acids with 96% sequence identity to the human protein (Du et al. 1996; Strom et al. 1997). Northern blotting demonstrated a 6.6-kb transcript in calvaria and osteoblasts that was absent from *Hyp* tissues (Du et al. 1996). Detection of a low level of *Pex* transcript by RT-PCR suggested the possibility of a regulatory mutation in *Hyp*. However, when *Pex* cDNA fragments were used as probes on Southern blots of genomic DNA, large deletions were identified in both *Hyp* and *Gy* (Strom et al. 1997). The mutations are located at opposite ends of the cDNA, with loss of exons 1–3 in *Gy* and loss of exons 15–22 in *Hyp*. The *gyro* deletion was induced by X-radiation, but *Hyp* was a spontaneous mutation.

Although mutations in PEX have now been clearly associated with hypophosphatemia in human and mouse, the biochemical mechanism responsible for the clinical phenotypes remains unclear. One hypothesis is that PEX may be involved in cleavage of an as yet unidentified polypeptide hormone involved in phosphate transport. In addition to the medical benefits, the effort involved in positional cloning of HYP has been rewarded by the discovery of a novel metabolic pathway of fundamental biological importance.

### A new deafness gene?

Also remaining for future investigation are the inner ear defects in *gyro* mice that are also seen in some human patients with HYP. It has been suggested that the abnormal phosphate metabolism itself could be directly or indirectly responsible. The identification of the *gyro* deletion, which extends for  $\leq 40$  kb upstream of *Pex*, raises the alternative possibility that a second deleted gene could account for the inner ear phenotype (Strom et al. 1997). Consistent with this hypothesis, a human nonsyndromic deafness disorder (DFN6) was recently mapped to Xp22 close to PEX (del Castillo et al. 1996). In view of the genetic and molecular resources now available, we may expect the rapid identification of the locus or loci responsible for this inner ear disorder.

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