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Mutational Analysis of the PEX Gene in Patients with X-Linked Hypophosphatemic Rickets

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Summary

X-linked hypophosphatemic rickets (HYP) is a dominant disorder characterized by renal phosphate wasting and abnormal vitamin D metabolism. PEX, the gene that is defective in HYP and is located on Xp22.1, is homologous to members of the neutral endopeptidase family. However, the complete coding sequence of the PEX cDNA, the structure of the PEX gene, and the role that PEX plays in phosphate transport remain unknown. We determined the genomic structure of the published PEX gene, which was found to be composed of 18 short exons, and demonstrated that the genomic organization of PEX shares homology to members of the family of neutral endopeptidases. Primer sets were designed from the intron sequence, to amplify each PEX exon from genomic DNA of HYP patients. Mutations in PEX were identified in 9/22 unrelated HYP patients, confirming that defects in PEX are responsible for HYP. The mutations detected included three nonsense mutations, a 1-bp deletion leading to a frameshift, a donor splice-site mutation, and missense mutations in four patients. Although the entire PEX gene has not been identified and some mutations may have been missed, the lack of detection of mutations in the remaining 13 patients, especially in 1 patient who has an apparently balanced, de novo 9;13 translocation, implies that there may be other loci involved in the generation of the HYP phenotype.

Introduction

X-linked hypophosphatemic rickets (HYP) is a dominant disorder and is the most common inherited form of rickets in the United States (Rasmussen and Tenenhouse 1989). HYP is characterized by a defect in renal phosphate transport, leading to phosphate wasting and hypophosphatemia, and by abnormal 1,25-dihydroxyvitamin D metabolism. Manifestations of HYP include rickets in children, short stature, and osteomalacia.

HYP was localized to Xp22.1 by linkage analysis in some families (Read et al. 1986; Thakker et al. 1987; Econs et al. 1992, 1994; Francis et al. 1994; Rowe et al. 1994). PEX (phosphate-regulating gene with homologies to endopeptidases, on the X chromosome) was identified recently, via positional cloning, as the gene defective in HYP (HYP Consortium 1995). Large deletions in four HYP patients led to the localization of PEX, and point mutations were described in three additional patients (HYP Consortium 1995). The complete coding region of PEX was not identified, including the translation start and stop sites.

PEX is homologous to members of the family of neutral endopeptidases, including neutral endopeptidase (NEP) (D'Adamio et al. 1989), endothelin-converting enzyme-1 (ECE-1) (Xu et al. 1994), and the Kell antigen (Lee et al. 1991). Neutral endopeptidases are characterized by a short N-terminal tail, a single transmembrane domain, and a long C-terminal region containing the catalytic domain and a zinc-binding motif. The known endopeptidases cleave proteins in the process of creating an active or an inactive form (D'Adamio et al. 1989; Shipp et al. 1989; Xu et al. 1994). The homology to endopeptidases suggests that PEX activates or inactivates a protein that goes on to regulate phosphate transport. However, the tissues of PEX expression and the role that PEX plays in phosphate transport have not been elucidated.

Recently, a 2,975-bp mouse Pex cDNA was identified, containing a start and stop site and a 2,247-bp open reading frame (Du et al. 1996). No defect was identified in mouse Hyp, the mouse homologue of HYP, mapping to the mouse X chromosome (Eicher et al. 1976; Meyer et al. 1980). Mouse Pex is homologous to human PEX, NEP, and ECE-1. The identity between mouse Pex and human PEX is 91% on the nucleotide level and 95% on the amino acid level (Du et al. 1996). Mouse Pex shows 70% similarity to human NEP and 67% similarity to human ECE-1 (Du et al. 1996). The open reading frame of mouse Pex is very similar in size to those of NEP (2,250 bp) (D'Adamio et al. 1989) and ECE-1 (2,274 bp) (Xu et al. 1994).

To begin the analysis of the PEX gene in a large cohort

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of patients and to begin a genotype-phenotype analysis of mutations at the locus, we determined the location of each PEX exon of the published PEX cDNA and designed primers, from the intron sequence, to amplify each exon. Twenty-two HYP patients were investigated for mutations in PEX, by SSCP analysis of each exon, and mutations were identified in nine patients. Somewhat surprisingly, mutations were not detected in the majority of HYP patients, and these patients may serve as a clue to other genes involved in phosphate transport.

Patients and Methods

Patient Population

Twenty-two unrelated individuals diagnosed with HYP were included in the study and were obtained from the Endocrine Clinic at Children's Hospital in Boston and from the Pediatric Endocrine Clinic at Massachusetts General Hospital in Boston and at the Yale University Medical Center in New Haven. Fourteen individuals had a family history of HYP. Eight individuals appeared to have sporadic HYP, since they had no affected family members. After they gave informed consent, blood was collected and DNA was extracted from 45 affected individuals and 74 unaffected family members. Cytogenetic analyses were performed on six patients. If possible, a male was chosen for the mutational analysis; in 12 families, only females were available. Eight unaffected individuals, mostly spouses of affected individuals, with no family history of rickets, served as controls for the initial SSCP analysis. In order to test the possibility that a mutation was a polymorphism, DNA from an additional 96 unaffected, unrelated individuals was used for SSCP analysis, and 48 of these individuals were tested by use of the amplification-refractory mutation system (ARMS).

Cloning the PEX Gene

RNA was prepared, by use of Trizol reagent (Gibco-BRL), from discarded bone obtained after an orthopedic procedure on a patient without rickets. Reverse-transcriptase PCR was performed on 2 μg of total bone RNA by use of Superscript II (Gibco-BRL). The cDNA was amplified by PCR, by use of PFU DNA polymerase (Stratagene), a forward primer designed from 1–21 bp, and a reverse primer from 1,890–1,910 bp of the published PEX cDNA (all sequences in this study are numbered according to the published PEX cDNA sequence [HYP Consortium 1995]). PCR was performed in a thermocycler (MJ Research) by use of the following parameters: 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min 30 s (these PCR parameters were used for all reactions in this study, unless otherwise indicated). A second round of PCR was performed by use of a forward primer at 41–60 bp and a reverse primer at 1,865–1,886 bp, which were designed with an *Xba* and an *Xho* restriction-enzyme cut site, respectively, on the 5′ end. The PCR products were purified by use of the Wizard PCR Preps DNA Purification System (Promega), were digested with *Xba* and *Xho*, and were cloned into Bluescript (Stratagene). The cDNAs were sequenced by use of six primers designed from the PEX cDNA (the sequences for the primers and the oligonucleotides used in this study are available by request). All sequencing in this study was performed on either an ABI 373 or an ABI 377 sequencer.

PEX Intron-Exon Border Analysis

An EMBL3 phage library was constructed (Clontech) from yeast DNA containing YAC A0472, which spans the HYP region (Francis et al. 1994). Two hundred twelve clones were determined to contain human DNA, and these clones were screened, by hybridization using 15 radiolabeled oligonucleotides designed from the PEX cDNA. Exon 6 also was used as a probe to screen the phage library and was amplified radioactively by PCR from genomic DNA by use of the primers P3E23-A3F and P3E23-A3R (HYP Consortium 1995). A second screening of the phage library was performed by use of radiolabeled cDNA from exons 2–5 and exons 15–16.

Phage DNA was prepared for sequencing following growth, polyethylene glycol precipitation, and isolation of phage particles on a cesium gradient (Sambrook et al. 1989). Phage DNA was prepared from phage particles by use of the Qiagex lambda purification system (Qiagen). Two micrograms of phage DNA was sequenced by use of PEX cDNA primers. In some cases, phage DNA was sequenced by use of intron primers designed from the first-pass sequence to sequence across the exon and into the intron on the other side. Intron-exon borders were identified by comparison of genomic sequence to cDNA sequence and by analysis of splice sites. On the basis of the intron sequence, primers were designed for each exon, except exons 6 and 15, to amplify the exon (table 1). All primers are ≥35 bp from the exon, except for the primer exon 7R, which is 22 bp from the exon. Several intron-exon borders were determined by sequencing of the long-range PCR products, obtained by PCR amplification of the intron between adjacent exons by use of genomic DNA, the *TaKaRa LA Taq* enzyme system (PanVera), and a longer extension time for PCR.

Sequence Comparisons

GenBank BLAST (basic local alignment search tool) was used to identify protein-sequence homology between PEX, NEP, and ECE-1. Nucleotide-sequence comparisons were performed by use of GAP (Genetics Computer Group). Intron-exon borders were considered

Table 1

PEX Exons and Primers Used to Amplify Each Exon

Exon	Location[a] (bp)	Exon Size (bp)	Forward Primer[b] (5′ to 3′)	Reverse Primer[b] (5′ to 3′)	Product Size (bp)
1	1–11	111	AGCCACCAAACCACGAAAAG	CCCTATACGTGTGTTATGAC	269
2	112–180	69	GCGTATGTTTCCGAGGGTG	CTGTCTTCTCTTCCACTTCC	214
3	181–342	162	GTGCTTGTCATTAATCCTATGA	CTATAAAGTGTATCACCAAACC	237
4	343–429	87	GGCACCATATGTGGGTGGA	TAATAGAACTCTAAAGGATAGGG	196
5	430–656	227	ATTCTAGTGTGCTGATCCAGT	CCAGGATGGCACCCCAAAA	354
6	657–725	69	CATCACTCTTGTTAACATGG	GGCCTTAGAACTAATGGGC	208
7	726–842	117	CAAACATATTTCCTAAGAAGCTA	GAGATTTTCAGTGAAGACTTAC	196
8	843–926	84	CAGTAAGAAATGGTTGCACTTG	ACAGAGATGAAATCCAATCCC	207
9	927–1072	146	TTCTTACTTGCTACCTAACCG	CCCTATGACTCTTTTGATGTAA	325
10	1073–1166	94	CTGCAGAGCATCAGATATTGA	CTGGATCAGTTAATATATGGAC	232
11	1167–1295	129	GGGTGTGCAGTGTTTTGTAG	GGCCACTACCACCTGGAAG	336
12	1296–1397	102	ACCTCGACTGAAGCTTCGTG	CTCTGGGGTCATTCAGAGTC	279
13	1398–1475	78	GGGCGCATTTCTACATCTTG	GCTAGGACTTGGGTTAGTTG	239
14	1476–1579	104	GCTCCTTCCTATGCTGAAGT	CTAAAGAGACTCCGCTTCTC	250
15[c]	1580–1638	59	...	...	
16	1639–1693	55	CCAGGTACTCATCATTGAATC	CCATGGCTTCTTTCTGCTGA	181
17	1694–1761	68	GCTCTGAGATTCATGCTTGTA	CAATTAACATCCATATAGTCAAC	213
18	1762–1892	131	GAGGGGAAAGGAAAGATGAAT	CACGCTTTGAGAACCAATGG	294

[a] Based on the published PEX cDNA sequency (HYP Consortium 1995).
[b] All primers are >35 bp from the intron-exon border, except for the exon 7 reverse primer.
[c] Primers currently are not available for exon 15. Exon 15 could be longer, since the 5′ splice site has not been determined definitely.

to be conserved if the borders were in the same position when the two homologous amino acid and nucleotide sequences were compared. An amino acid residue was considered to be conserved between PEX, NEP, and ECE-1 if the same amino acid was in the same position in each of the three sequences. Nucleotide comparisons between human PEX and mouse Pex were performed by use of Sequencher (Amersham).

Mutational Analysis of PEX

SSCP analysis was performed on each PEX exon by PCR amplification using 30 ng of patient DNA and the intron primers. The published primers (P3E23-A3F and P3E23-A3R) (HYP Consortium 1995) were used to amplify exon 6. PCR products were separated by electrophoresis on 0.5 × MDE gels (FMC Bioproducts), both with and without glycerol (10%). For females, and some males, aberrantly migrating bands were cut out of the gel and eluted in 100 μl of H_2O for 6–12 h. Five microliters of the eluted band were used for a second round of PCR amplification using the primers used for SSCP. In several cases, the apparently normal allele also was excised, eluted, and amplified by PCR. In some males, exons were amplified by PCR using 120 ng of genomic DNA. In all cases, the PCR products were purified by use of the Wizard PCR Preps DNA Purification System (Promega) and were sequenced. Sequence comparisons were performed by use of Sequencher. ARMS was used, as per the protocol described elsewhere (Little 1995), on 48 unaffected, unrelated individuals with no history of rickets, to determine if several missense mutations represented polymorphisms.

Results

Cytogenetic Analysis

One HYP patient, with no family history of rickets, was referred with a 9;13 translocation. Cytogenetic analysis revealed her karyotype to be 46,XX,t(9;22)(q22;q14). The translocation was apparently balanced and de novo; cytogenetic analysis on the unaffected parents revealed that neither parent carries the translocation. No visible chromosomal abnormalities were seen on the cytogenetic analysis of five additional patients.

PEX cDNA

On the sequencing of PEX cDNAs from bone, two of the base pairs were found to differ from the published sequence and were confirmed by sequencing of the genomic DNA (data not shown). Nucleotide 1081 is a C, not an A, changing residue 360 from an aspartic acid to an alanine; and nucleotide 1200 is a T, not an A, changing residue 400 from an arginine to a tryptophan.

Identification of PEX Intron-Exon Borders

Eighty-eight genomic clones from the phage library constructed from YAC A0472 hybridized to probes from the PEX cDNA. Fifteen clones formed a partial

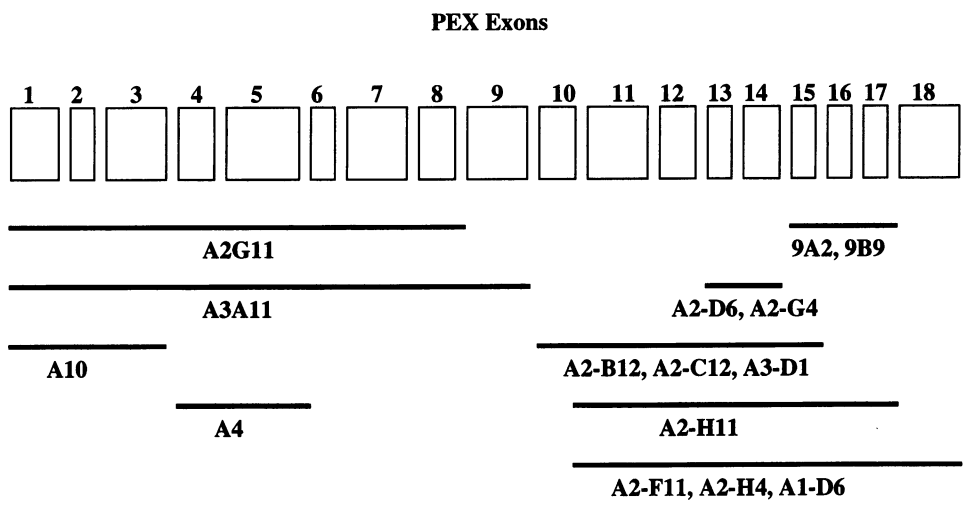

Figure 1 Phage contig spanning the PEX gene. The relative size of each PEX exon is indicated by the size of the box. The length of the introns is not known, and thus the introns are not drawn to scale. The number of PEX exons spanned by each clone is based on the location of PEX cDNA probes hybridizing to clones from the phage library and on the results of sequencing of the clones by use of PEX primers. The sequences of the exons have been deposited in GenBank (accession nos. U81167–U81183).

contig across PEX (fig. 1). A gap may exist between exons 9 and 10, although the clones were not sequenced in their entirety and may overlap in the intron. We have been unable to sequence the clones containing exon 15 by using exon 15 cDNA primers to determine the intron-exon borders of this exon.

Sequencing of the phage clones spanning PEX revealed that the published PEX cDNA is encoded by 18 exons ranging from 55 bp to 226 bp in length (table 1). The exon encoding the last 24 bp has not been identified. The previously reported exons 1 and 2 (HYP Consortium 1995) correspond to exons 6 and 7, respectively, in our study and were renamed. PCR primers were designed from the intron sequence surrounding each exon, except for exons 6 and 15, to specifically amplify the exon. The PCR products were 195–353 bp in length (table 1).

The intron sequences of exon 15 remain unknown, despite attempts to sequence phage clones spanning exon 15. The location of the 5′ border of exon 15 is based on the location of the known donor consensus sequence of exon 14. The location of the 3′ end of exon 15 is based on where the genomic and cDNA sequences around exon 16 diverge. Given the small size (59 bp) of exon 15, it seems unlikely that exon 15 is more than one exon.

Sequence Comparisons

Protein-sequence comparisons confirmed the strong homology of PEX to NEP and to ECE-1 (data not shown). Twelve intron-exon borders are conserved between PEX and NEP and seven between PEX and ECE-1 (fig. 2). Like NEP and ECE-1, PEX contains a short N-terminal tail, a single N-terminal hydrophobic region characteristic of a transmembrane domain, and a highly conserved zinc-binding motif at the 3′ end (fig. 2). The ECE-1 coding region continues for 354 bp from the end of the region of homology to PEX (1,868 bp) to the stop codon; NEP continues for 345 bp from the end of the region of homology to PEX exon 18 (1,886 bp) to the stop codon; and mouse Pex continues for 324 bp from the end of the region of homology to human PEX (1,916 bp) to the stop codon.

Mouse Pex cDNA was compared to >500 bp of sequence obtained from sequencing of the 5′ intron-exon border of human PEX exon 1; 410 bp of the sequence was 5′ of the first base pair of the published PEX cDNA. From −16 bp to −1 bp of the human sequence was found to be identical to mouse Pex cDNA, and the methionine at −7 bp of the human sequence was in the same position as the start methionine of mouse Pex. There is a stop codon in the same position in the human and the mouse sequences, at −43 bp of the human sequence, and within a 75-bp region showing >85% identity between the human and the mouse sequences. These findings suggest that the methionine at −7 bp in human PEX is the start of the open reading frame. The primers used to amplify exon 1 include the presumed start methionine.

PEX Mutational Analysis in HYP Patients

Seventeen PEX exons were analyzed by SSCP analysis of PCR products derived from 22 patient DNA samples.

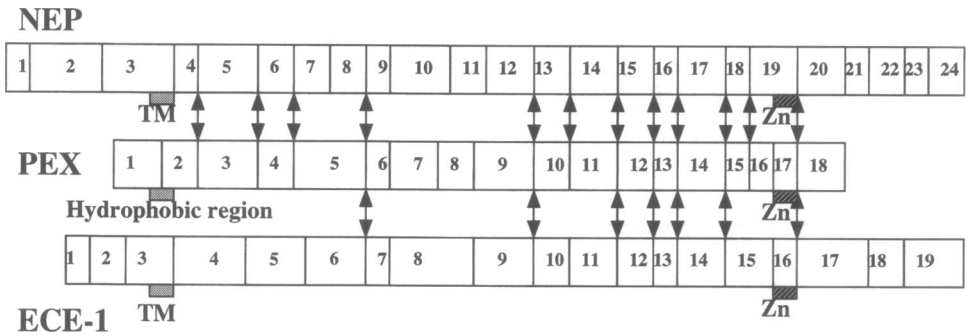

Figure 2 Comparison of the intron-exon borders of PEX, NEP, and ECE-1. The size of each box reflects the relative size of the PEX exon. Exon borders conserved between PEX, NEP, and ECE-1 are indicated by arrows. The location of the transmembrane domains (TM) of NEP and ECE-1 and the hydrophobic region of PEX are indicated by a dotted bar (▨). The zinc-binding motif (Zn) is indicated by a hatched bar (■). The GenBank/EMBL accession numbers used were J03779, M26606–M26628, and P08473, for NEP; Z35307, X91922–X91939, and P42892, for ECE-1; and U60475, for PEX.

Nine mutations were detected (table 2). In familial cases, mutations were confirmed by SSCP analysis and sequencing of affected and unaffected family members.

Three nonsense mutations were detected. A female (B42) with apparently sporadic HYP had a 823T→A transversion in one allele of exon 7, altering codon 274 from a leucine to a stop. A male (D31) with familial HYP had a transition in exon 1 at 51C→T, changing codon 17 from an arginine to a stop. The mutation obliterated a *Taq*I site and was confirmed by restriction digestion (data not shown). The third patient (G51), another male with familial HYP, had a transition in exon 8 at 864C→T, changing codon 288 from an arginine to a stop (fig. 3). A 1-bp deletion, 1518A in codon

Table 2

Mutations in Nine Patients with HYP

Patient and Mutation Type	Type of HYP	Exon	Nucleotide Location[a] (bp)	Nucleotide Change[a]	Amino Acid[a]	Result	Effect[b]	Verification[c]
Nonsense:								
B42 (female)	Sporadic	7	823	T→A	274	Leu→stop	Truncated protein	...
D31 (male)	Familial	1	51	C→T	17	Arg→stop	Truncated protein	Restriction site altered
G51 (male)	Familial	8	864	C→T	288	Arg→stop	Truncated protein	SSCP and sequencing
Frameshift:								
E3-10 (male)	Familial	14	1518	Deletion	506	510 stop	Truncated protein	SSCP and sequencing
Splice Site:								
C21 (male)	Familial	14	Donor splice site	4-bp deletion	...	Aberrant splicing	Exon skipping	SSCP and sequencing
Missense:								
K41 (male)	Familial	3	247	G→A	82	Cys→Tyr	Alters conserved Cys	SSCP
L41 (female)	Familial	7	748	T→C	249	Phe→Ser	Abnormal protein	SSCP,
		7	752	G→A	250	Met→Ile	Abnormal protein	sequencing, and ARMS analysis
P21 (female)	Sporadic	5	489	C→T	163	Arg→Cys	Abnormal protein	...
V1 (female)	Sporadic	17	1729	G→T	576	Gly→Val	Alters Gly next to zinc-binding domain	ARMS analysis

[a] Based on the published PEX cDNA sequence (HYP Consortium 1995).
[b] The effect of the mutation on the protein is assumed and has not been proved.
[c] SSCP and sequencing, to verify the mutations, were performed on affected and unaffected family members.

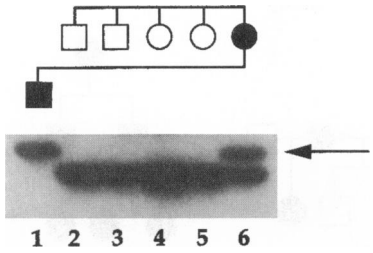

Figure 3 SSCP analysis of exon 8 in family G. Exon 8 was amplified, by PCR, from genomic DNA, and the products were separated, by electrophoresis, on a 10% glycerol gel. Normal bands from exon 8 in unaffected maternal uncles (lanes 2 and 3) and aunts (lanes 4 and 5) are shown. In the hemizygous male patient (lane 1), the exon 8 band is shifted upward. In the patient's heterozygous affected mother (lane 6), both the normal band and the shifted band (indicated by the arrow) can be seen. Sequencing of the excised shifted band in the affected mother demonstrated a C→T mutation, changing an arginine to a stop (results not shown).

506 of exon 14, was detected in a male (E3-10), which resulted in a frameshift and a stop in codon 510. One splice-site mutation was detected: a male (C21) had a 4-bp deletion of +3 to +6 bp (5′-GTGAGT-3′; underlined) of the splice donor consensus sequence of exon 14.

Four patients had missense mutations. A male (K41) with familial HYP had a transition in exon 3, at 247G→A, changing a cysteine, conserved between PEX, NEP, and ECE-1, at codon 82 to a tyrosine. A female (P21) with apparently sporadic HYP had a 489C→T in exon 5, changing codon 163 from an arginine to a cysteine. Another female (V1) with apparently sporadic HYP had a 1729G→T mutation in exon 17, changing a glycine, conserved between PEX, NEP, and ECE-1, at codon 576, next to the zinc-binding motif, to a valine. This mutation was not detected by ARMS analysis of 48 unaffected individuals. Finally, a female (L41) with familial HYP had the unusual finding of two transitions that were 4 bp apart in exon 7: 748T→C changes codon 249 from a phenylalanine to a serine, and 752G→A changes codon 250 from a methionine to an isoleucine (fig. 4). The two mutations were both in one allele, as demonstrated by sequencing of both alleles. SSCP analysis of 96 unaffected individuals, as well as ARMS analysis of both mutations in 48 unaffected individuals, was normal. One explanation for the finding of two mutations so close together is a rearrangement, although study of this region so far has revealed nothing to suggest that a rearrangement has occurred. No mutations were detected among the remaining patients, including the female with apparently sporadic HYP and a balanced 9;13 translocation.

Discussion

The gene structure and the intron-exon borders are reasonably conserved between PEX, NEP, and ECE-1 (fig. 2). The published PEX cDNA does not contain a start site or a stop site. Our finding that the coding regions of NEP and ECE-1 continue for 345 bp and 354 bp, respectively, 3′ of the region of homology to PEX and that mouse Pex cDNA continues for 324 bp 3′ of the end of the published human PEX cDNA suggests that 350 bp remain unidentified between the 3′ end of the PEX cDNA and the stop codon. At the 5′ end, we appear to have identified the start methionine at −7 bp, which is within the primers used to amplify exon 1. Thus, the 1,916-bp published PEX cDNA, which we have screened for mutations in HYP patients, contains ~85% of the coding region of PEX.

The PEX mutations described so far, in our study and in the initial report (HYP Consortium 1995), are unique, suggesting that there is no hot spot for mutations. We detected point mutations and small deletions that are predicted to alter the PEX protein structure and function. The three nonsense mutations and the frameshift mutation, which leads to a stop codon downstream, presumably result in a truncated protein. The splice-donor-site deletion is expected to result in exon skipping. One missense mutation alters a conserved cysteine, which is predicted to be involved in disulfide-bond formation and in protein folding. Another missense mutation alters a conserved glycine next to the zinc-binding domain and may interfere with the catalytic activity of the protein. The effect of the arginine-to-cysteine change is unclear. Finally, the two missense mutations that are 4 bp apart, which were not seen in 96 normal controls, alter two adjacent amino acids, and the effect on the protein is unclear.

No large deletions were detected in our study. Unlike for the previous report (HYP Consortium 1995), Southern blot analysis was not performed in our study, owing

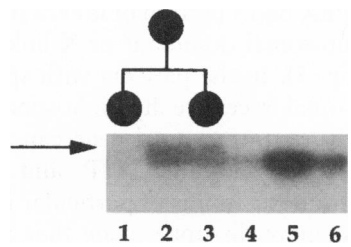

Figure 4 SSCP analysis of exon 7 in family L. Exon 7 was amplified, by PCR, from genomic DNA, and the products were separated, by electrophoresis, on a 10% glycerol gel. Lanes 4–6 show the bands corresponding to the normal exon 7 in unaffected individuals. Lanes 1–3 show the bands corresponding to exon 7 in the heterozygous affected patient, her mother, and her sister, respectively; a normal band and an upper shifted band (indicated by the arrow) also are seen. Sequencing of the excised normal band, representing the normal allele, and of the affected band, representing the mutant allele, from lane 3, demonstrated that both the T→C and the G→A mutations were present in one allele (results not shown).

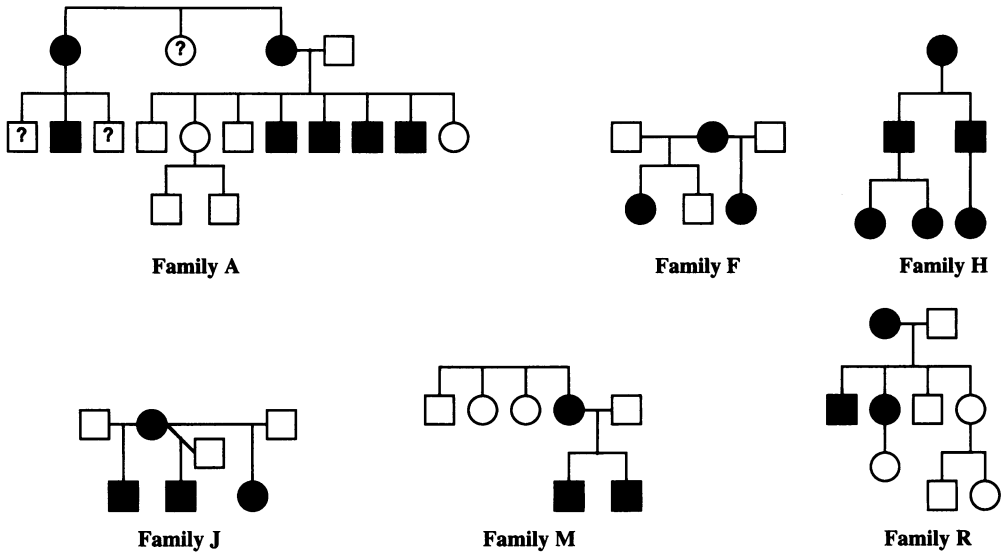

Figure 5 Six familial HYP pedigrees in which no mutation in PEX was detected. Affected males and females are represented by blackened squares and blackened circles, respectively, and unaffected males and females are represented by unblackened squares and unblackened circles, respectively. A question mark (?) indicates individuals who have not been tested for HYP, and thus the phenotype is not known for certain.

to lack of sufficient quantities of DNA. However, SSCP analysis should reveal deletions in hemizygous males. Bands were seen in all males, for all the exons.

No PEX mutation was detected in 13/22 patients. Reasons why a mutation could have been missed include the presence of a deletion in a heterozygous female, mutations in the unidentified 15% of PEX or in exon 15 (only 59 bp), or mutations that were not detected by SSCP. However, it seems unlikely that all 13 patients have mutations that were missed, and some of these patients may harbor mutations in other genes. Autosomal dominant hypophosphatemic rickets has been described elsewhere (Bianchine et al. 1971; Econs and McEnery 1995). In our study, the familial cases without mutations in PEX had a pattern of inheritance consistent with either autosomal dominant or X-linked dominant inheritance (fig. 5). In the patients with sporadic HYP, neither autosomal recessive hypophosphatemia nor a new mutation in an autosomal gene can be ruled out. The patient with sporadic HYP and a de novo 9q22;13q14 translocation is of particular interest, since the breakpoint may disrupt a gene that results in the HYP phenotype. Studies are being performed in the familial patients without mutations in PEX, to determine if the HYP phenotype is linked to 9q22 or to 13q14. In addition, an analysis is ongoing to determine if there are any phenotypic differences between patients with and patients without mutations in PEX.

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