Organization and Nucleotide Sequence of the Human Hermansky-Pudlak Syndrome *(HPS)* Gene

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Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism, bleeding tendency, and lysosomal ceroid storage disease, associated with defects of multiple cytoplasmic organelles–melanosomes, platelet-dense granules, and lysosomes. HPS is frequently fatal and is the most common single-gene disorder in Puerto Rico. We previously characterized the human HPS cDNA and identified pathologic mutations in the gene in patients with HPS. The HPS protein is a novel apparent transmembrane polypeptide that seems to be crucial for normal organellar development. Here we describe the structural organization, nucleotide sequence, and polymorphisms of the human *HPS* gene. The gene consists of 20 exons spanning about 30.5 kb in chromosome segment 10q23.1-q23.3. One of the intervening sequences is a member of the novel, very rare class of so-called "AT-AC" introns, defined by highly atypical 5′ and 3′ splice site and branch site consensus sequences that provide novel targets for possible pathologic gene mutations. This information provides the basis for molecular analyses of patients with HPS and will greatly facilitate diagnosis and carrier detection of this severe disorder. *Key words: oculocutaneous albinism/AT-AC introns/RNA splicing. J Invest Dermatol 108:923–927, 1997*

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive, multisystem disorder characterized by the triad of tyrosinase-positive oculocutaneous albinism (OCA), a bleeding tendency, and a ceroid-lipofuscin lysosomal storage disease (Hermansky and Pudlak, 1959). Severe manifestations are frequent and may include progressive pulmonary fibrosis, granulomatous colitis, renal failure, and cardiomyopathy. As a result, HPS is frequently fatal; death usually occurs at age 30 to 50 or less as a result of restrictive lung disease, hemorrhage, or colitis (Witkop *et al*, 1990). At the cellular level, HPS is characterized by abnormalities of multiple cytoplasmic organelles–melanosomes, platelet dense granules, and lysosomes (Hermansky and Pudlak, 1959; Witkop *et al*, 1990) and thus has been thought to result from a defect of a protein required in common for the assembly, maturation, or structure of these different organelles.

Although generally rare, HPS is quite frequent in Puerto Rico, where it occurs with an incidence of 1 per 1800 (Witkop *et al*, 1990) and is the most common single-gene disorder, and in an isolated village in the Swiss Alps (Frenk and Lattion, 1982; Schallreuter *et al*, 1993). We previously localized the *HPS* gene to chromosome segment 10q23.1-q23.3 by linkage disequilibrium mapping in these two groups (Fukai *et al*, 1995), and others mapped the gene to 10q2 (Wildenberg *et al*, 1995). Recently, we identified the *HPS* gene by a positional cloning approach and characterized several different frameshift mutations in patients with HPS (Oh *et al*, 1996). The predicted human HPS protein is a highly conserved, ubiquitously expressed, 700-amino acid polypeptide with predicted molecular mass of 79.3 kDa that contains two potential transmembrane helices and has no sequence similarity to any known proteins. The HPS polypeptide may thus be a transmembrane protein, possibly located in melanosomes, lysosomes, and other organelles that are defective in HPS, although equally possibly located in subcellular structures involved in trafficking of proteins to these various organelles.

Here, we report the exon-intron organization of the human *HPS* gene and its nucleotide sequence, and we describe a number of polymorphisms of the gene and HPS polypeptide. Of particular interest, *HPS* is the first disease gene that contains one of the very rare "AT-AC" class of introns (Hall and Padgett, 1994). The results of this study should thus form the basis for DNA-based mutation analyses of patients with HPS, greatly improving ease and accuracy of diagnosis of this unusual disorder.

MATERIALS AND METHODS

Analyses of a Human *HPS* Genomic Clone We previously described a ~450-kb human *B*acterial *A*rtificial *C*hromosome (BAC)/*P*1 *A*rtificial *C*hromosome (PAC)/P1 clone contig that spanned the human *HPS* gene region (Oh *et al*, 1996). BAC 145M16 spanned the entire *HPS* gene and was thus used for direct DNA sequence analyses. DNA was prepared from 2-liter cultures of *Escherichia coli* by a modified alkaline lysis method, and 1 μg was used as template in each sequencing reaction using the SequiTherm cycle sequencing kit (Epicentre Technologies, Madison, WI) and a large series of oligonucleotide primers derived either from the human *HPS* cDNA sequence (Oh *et al*, 1996) or from apparent intervening sequences (IVSs). Most sequences were determined on both strands and have been confirmed numerous times by analyses of polymerase chain reaction (PCR) products amplified from genomic DNAs of normal individuals and patients with

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Abbreviations: HPS, Hermansky-Pudlak syndrome; IVS, intervening sequence.

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Table I. Primer Pairs Used to Amplify *HPS* Exon Segments

Exon[b]	PCR Primers	PCR Product Size (bp)	PCR Conditions[a]
1 (promoter and 5′-URT)	5′-GGAAACCTGGGTTCTGAGCCTG 5′-GGAGTGGGTGAGGCTAGGAGT-3′	375	1
2 (5′-UTR)	5′-CACGCAGCCCTTCTCCAGCTT-3′ 5′-ATTCCATCTGCCTCCCAAAG-3′	281	4
3	5′-TCAGACTGAGCTCATAGATG-3′ 5′-TATGGTTCCTTCCTATCCAC-3′	229	1
4	5′-CCCTTGATATCAAGGCCTGATCT-3′ 5′-TCAAGCTGAGGGAAGAGGAAC-3′	273	2
5	5′-CTGCAGAGACCAGGGCAGGT-3′ 5′-CACACGCTGCCTGGCCCAGC-3′	279	5
6	5′-CTGAGACTGTGGTCCTTTGGT-3′ 5′-CCCTCTGACCTGACAGCTTC-3′	243	3
7	5′-AGGGCTTGGAGTGGGCAAGGTT-3′ 5′-TAGGGGAGTGAGAAGGCCAG-3′	280	3
8	5′-TTAACCTGCCTGCCCCGTCT-3′ 5′-AGGCAGGTTTTCTGAACTAC-3′	228	2
9	5′-AGATAGGAGCTAACCAGCCT-3′ 5′-AAAGGCCTGGTCGCCTGCAGAG-3′	205	3
10	5′-GGGACAGGGTTGAGCCAGCT-3′ 5′-CGCAGCTAGCTATTGTTTCC-3′	172	3
11	5′-GGTACTTACATCGTTATGTT-3′ 5′-CCAATACTCACTGCGGCATCT-3′	149	1
12	5′-CTTGTGGTTCACAGATAGGGCC-3′ 5′-GGAAGACGTGCCAACCCTAAG-3′	303	2
13	5′-CTTAGGGTTGGCACAGTCTTC-3′ 5′-ACCGGATGTACCCTGCTGCTAG-3′	312	2
14	5′-CACTGGAAATATGGTCGGGATG-3′ 5′-TGCTGATAAATGAAGGGCAG-3′	210	1
15	5′-GATGGTCCACAAAGGACGAG-3′ 5′-GCGTGAAGGAAGTACGGGCC-3′	268	2
16	5′-GGGTGGCCAGGGCAGGGTCT-3′ 5′-AAGAGGCTTGTTGGGCTGGCT-3′	171	3
17	5′-CGTCCCTAGCTCCTGGCTTGT-5′ 5′-AAGGGCTACCCAATATTGGCT-3′	249	2
18	5′-AAGGCCTGGGAGAGTGGTGAT-3′ 5′-GAGTTACAGAGCGGGAAGTG-3′	215	2
19	5′-AGCCCGGTGCGTCTGACTCAG-3′ 5′-AATGGGGGCATCTGTCCCCAG-3′	195	2
20-a	5′-GCCACAGCCTCACTCCTGAAT-3′ 5′-AGACAGGAGGCTCTCGTCAT-3′	293	1
20-b (3′-UTR)	5′-CACCCTTGCTTGCCACTGTT-3′ 5′-CCTACCACACTGGGTAATCCT-3′	467	2
20-c (3′-UTR) & poly(A) site	5′-TAAAGCGATGGCACCTCCGT-3′ 5′-AAGACATCAAAGCCTGGGCT-3′	264	3

[a] PCR conditions are 94°C for 4 min, followed by 30 cycles of 94°C for 30 sec, T°C for 1 min, and 72°C for 1 min, followed by 72°C for 10 min., where T = 55 (1); 58 (2); 62 (3); 65 (4); or 68 (5).
[b] UTR, untranslated region.

HPS, using primer pairs derived from the IVSs flanking each exon **(Table I)**. DNA sequences were compiled and analyzed by computer using the DNASTAR (Madison, WI) software package.

Determination of IVS Positions and Sizes Exon–IVS junctions were identified by comparing the *HPS* genomic sequence with that of *HPS* cDNA (Oh *et al*, 1996). Exon contents of all relevant components of the BAC/PAC/P1 contig were determined by PCR using primer pairs derived from the adjacent IVSs **(Table I)**, followed by agarose gel electrophoretic analysis of the PCR products. The sizes of all IVSs were determined by long-range PCR using the Expand High Fidelity PCR system (Boehringer Mannheim, Indianapolis, IN), primer pairs derived from the 5′ and 3′ flanking exons **(Table I)**, and human genomic DNA and *HPS* BAC 145M16 as templates. PCR conditions were 94°C for 4 min, followed by 30 cycles of 94°C for 20 s, either 62°C or 68°C for 1 min, and 68°C for 3 to 5 min, followed by 72°C for 10 min, and PCR products were analyzed by agarose gel electrophoresis.

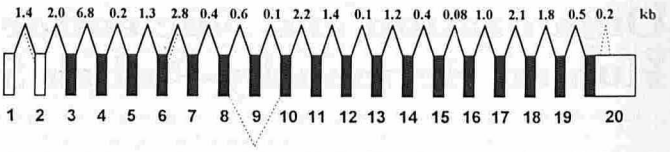

Figure 1. Organization of the human *HPS* gene. *Boxes* denote exons as numbered below. Exons 1 and 2 (□) are entirely noncoding. The sizes of the IVSs (kb) are shown. . . . . Indicate alternative RNA splices

Primer Extension Analysis of the 5′ End of Human *HPS* mRNA Polyadenylated mRNA was isolated from cultured human FME melanoma cells (Tveit *et al*, 1980) using the FastTrack kit (InVitrogen, San Diego, CA). Primer extension analysis was carried out using a 20-mer primer, 5′-GAGGCCCACGTACCGGATCG-3′, derived principally from exon 1. The primer was 5′-end labeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase and purified using a Bio-Rad Biospin P6 column (Bio-Rad, Richmond, CA). Radiolabeled primer (5×10^5 cpm) was hybridized to 10 μg poly(A) mRNA for 20 min at 58°C, and primer extension analysis was carried out using the Primer Extension System (Promega, Madison, WI) essentially as described (Kingston *et al*, 1989).

Identification and Analyses of *HPS* Gene Polymorphisms Nonpathologic polymorphisms of the *HPS* gene were identified in the course of mutation analyses of patients with HPS by PCR-based single-strand conformation polymorphism/heteroduplex screening and subsequent DNA sequence analysis as described previously (Lee *et al*, 1995). Allele frequencies were determined by PCR amplification of the corresponding *HPS* exon segment from DNA of unrelated normal individuals and single-strand conformation polymorphism/heteroduplex or restriction enzyme cleavage analysis.

RESULTS AND DISCUSSION

Organization and Nucleotide Sequence of the Human *HPS* Gene DNA sequence analysis of BAC 145M16 using primers derived from the *HPS* cDNA sequence (Oh *et al*, 1996) showed that the *HPS* gene consists of 20 exons **(Fig 1)**. The positions of the 19 IVSs and the DNA sequence at each IVS-exon boundary are shown in **Table II**. The 20 exons range in size from 50 to 180 nucleotides (nt), except for the 3′-terminal exon 20, which can span from 695 to 1525 nt, depending on which of two principal alternative mRNA polyadenylation sites is utilized (*c.f.* Oh *et al*, 1996, Fig. 4). Exons 1 and 2 are noncoding, corresponding to the 5′-untranslated region of *HPS* mRNA; the translational initiation codon is thus located exactly at the beginning of exon 3. The entire 9730-bp DNA sequence determined has been deposited in the GenBank DNA sequence database (accession numbers U79121-U79136).

To estimate the sizes of the 19 IVSs of the *HPS* gene, we carried out PCR amplification from human genomic DNA and from BAC 145M16. In every case the sizes of the PCR products amplified from genomic and BAC DNA templates were identical. In some cases we also determined the complete DNA sequence spanning the IVS. As indicated in **Fig 1**, the 19 IVSs ranged in size from 84 bp to about 6.8 kb. The total size of the human *HPS* gene is thus about 30.5 kb.

Most of the *HPS* IVSs obey the so-called "GT-AG rule," which describes exon–IVS junctions in protein-encoding genes. *HPS* IVS16, which is about 1.0 kb in size, is strikingly different, however, with the dinucleotide AT occurring at its 5′ end and AC at its 3′ end **(Fig 2)**. *HPS* IVS16 therefore is a rare member of a newly discovered class of so-called "AT-AC introns" (Jackson, 1991; Hall and Padgett, 1994; Wu and Krainer, 1996), defined by the occurrence of these atypical dinucleotides at their 5′ and 3′ ends, respectively. AT-AC introns are characterized also by somewhat longer, highly conserved, 5′ and 3′ splice site and branch site consensus sequences; the 5′ splice site consensus is /ATATCCT, the 3′ splice site consensus is CAC/, and the branch site consensus sequence is TCCTTAAC (Hall and Padgett, 1996; Tarn and Steitz,

Table II. Exon-Intron Boundaries of the Human *HPS Gene*

5′..Exon	1	..AGTGAGgtagggtccc..	IVS1	...gcatttgcagGACCAG..	Exon2..
				1	
..Exon	2	..GCCAAGgtagatgatc..	IVS2	...tccctggcagATGAAG..	Exon3..
				MetLys	
		39		40	
..Exon	3	..GAAGAGgtgagtgcag..	IVS3	...atttttgcagCTCCCT..	Exon4..
		GluGlu		LeuPro	
		85		86	
..Exon	4	..CACCTGgtgagtctat..	IVS4	...gtgcctgcagTTTGGA..	Exon5..
		HisLeu		PheGly	
		133		133	
..Exon	5	..AAAGGAgtgagtcttc..	IVS5	...ctgcccccagGCTGCG..	Exon6..
		LysGl		uLeuAr	
		169		170	
..Exon	6	..GTGGAGgtgactactg..	IVS6	...ccctggctagGCCCTG..	Exon7..
		ValGlu		AlaLeu	
		223		223	
..Exon	7	..CTCTAGgtgagctcaa..	IVS7	...gtgtctgcagCCACAG..	Exon8..
		SerSe		rHisSe	
		256		257	
..Exon	8	..ATTCAGgtctcagggc..	IVS8.	.ccctgcacagCCTTCC..	Exon9..
		IleGln		ProSer	
		289		290	
..Exon	9	..GAGACGgtgtgtgctg..	IVS9	...tccattccagGAGACA..	Exon10..
		GluThr		GluThr	
		313		313	
..Exon	10	..GCTCAGgtgaggaccg..	IVS10	..tggcctgcagGTAGCA..	Exon11..
		SErG		lySer	
		329		330	
..Exon	11	..CTTCAGgtgagtttct..	IVS11	..tcctctgtagATAGCA..	Exon12..
		LeuGln		IleAla	
		385		386	
..Exon	12	..ACCAGGgtaggagagc..	IVS12	..ttaccctcagAGCCCC..	Exon13..
		ThrArg		SerPro	
		445		446	
..Exon	13	..ATTCAGgtgagaccca..	IVS13	..ttccttccagAGCACC..	Exon14..
		IleGln		SerTGhr	
		466		466	
..Exon	14	..CTGGGAgtgagtggtg..	IVS14	..tcgtgctcagGCTGCT..	Exon15..
		TrpGl		uLeuLe	
		511		511	
..Exon	15	..CATGCGgtaagtgcag..	IVS15	..ccctcggcagGGAGAA..	Exon16..
		MetAr		gGluLy	
		533		533	
..Exon	16	..GGTGTCatatcctgac..	IVS16	..agaccttcacCTACCT..	Exon17..
		ValSe		rTyrLe	
		581		582	
..Exon	17	..ACTAAGgtaaccattt..	IVS17	..ttttccccagGTCTGG..	Exon18..
		ThrLys		ValTrp	
		619		620	
..Exon	18	..GACATGgtaggtgagg..	IVS18	..ccctcctcagGGGTAC..	Exon19..
		AspMet		GlyTyr	
		647		647	
..Exon	19	..CTACAGgtgacgctgc..	IVS19	..tcctccccagGAAGCT..	Exon20..3′
		TyrAr		gLysLe	

[a] Note. Codon enumeration is according to Oh *et al* (1996). *Uppercase,* exon sequences; *lowercase,* IVSs. All sequences have been deposited in the GenBank database, accession numbers U79121–U79136.

1996). *HPS* IVS16 conforms well to these consensuses, /ATATCCT, CAC/, and ACCTT$^{A}/_{G}$AC, respectively, and it is tempting to speculate that these sequences represent unique targets

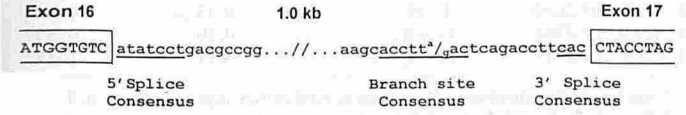

Figure 2. Splice junction sequences of IVS16. *Uppercase,* exon sequences; *lowercase,* IVS16 sequences. The AT-AC consensus 5′ and 3′ splice and branch site sequences (Hall and Padgett, 1996; Tarn and Steitz, 1996) are shown.

for mutations that result in HPS. There is no apparent functional relationship among genes containing AT-AC introns, and in each case the gene contains additional IVSs that conform to the GT-AG rule. This raises a problem for removal of AT-AC introns from such "mongrel" pre-mRNAs, solved by their excision by a novel spliceosome that contains U11, U12, and U5 snRNPs (Hall and Padgett, 1996; Tarn and Steitz, 1996).

We previously found (Oh *et al*, 1996) that almost half of the *HPS* cDNAs studied lacked a 99-base segment, encoding 33 amino acids, which corresponds precisely to exon 9. Exon 9 thus is either included or excluded from *HPS* mRNA by alternative splicing. We identified three additional rare alternative splices by analysis of *HPS* cDNAs and expressed sequence tag database entries. Use of an alternative 3′ splice site for IVS1, located 9 nt 5′ to the major 3′

```
                                                    TATA
cataggttcaacacaaaactcctaatgtgcactccactcccaaaatagattgagaactcctataaattccagcgttggcactctgggggcaactgagccc 100

ttctgtgcgctatgtcacctccccaaaggtcaggagagcagtcacaaaaggacagctctagccccagaacttcagttcattagcaaagcctgaggttccg 200

gggcatggggaggagagaagggatcagctgaggagccaggaaacctgggttctgagcctggttcggaccctcagcaagttatttcttctctctgcgcctc 300

              TATA                              TFIID/TBP                                                SP1
cgtttcctcttctgtaaaatggggcatgggcaaggcctggcaggggcggggctcgaacccgcggaaggcgacggcggctccggccctcccggggcgggc 400
                                                  ↑
  USF                    f(alpha)-f(epsilon)
ggtcacgtgcgcgatcacgcggtcctaccccggaaagcgcgcccgggctcctgcaggcgGGGCGCTGTGCGCG
    ↑         ↑
```

Figure 3. Nucleotide sequences of the promoter and exon 1 of the *HPS* gene. *Uppercase*, exon sequences; *lowercase*, 5′ flanking sequences. *Numbers* denote nucleotide positions. ↑ and ↑ indicates approximate mRNA 5′ starts based on primer extension. Very high probability transcriptional motifs are indicated; many others were identified with less certainty.

splice site, would result in the addition of 9 bases (CATTTGCAG) in the 5′ untranslated region. Use of an alternative 5′ splice site for IVS6, located 43 nt 3′ to the major 5′ splice site, would result in a frameshift. And in one expressed sequence tag (N44027) there appears to have been an additional splice of a minor 194-nt intron entirely within the 3′ untranslated region, removing nt 175–369 of the 3′ untranslated region. We reported previously that the major *HPS* mRNA is ~3.0 kb in size, with minor 3.9 and 4.4-kb mRNAs (Oh *et al*, 1996). We have since identified an additional major 1.5 kb mRNA on northern blots; however, this mRNA species is detected only by a probe derived from the 5′ portion of *HPS* cDNA (data not shown), and indirect evidence suggests that this signal may result from cross-hybridization to an unrelated mRNA.

The transcriptional initiation site of human *HPS* mRNA was characterized by comparison of the *HPS* genomic DNA sequence with the 5′-end sequences of a number of *HPS* cDNAs (Oh *et al*, 1996) and by primer extension analysis of poly(A) mRNA from human melanoma. The farthest upstream *HPS* cDNA 5′ end occurred at nt 458 of the *HPS* genomic sequence shown in **Fig 3**. Primer extension analysis, using a 20-mer oligonucleotide primer derived from exon 1, yielded a major extension product of approximately 139 nt **(Fig 4)**, consistent with an mRNA 5′ start at about nt 356. Minor primer extension products sized approximately 81 nt and 90 nt would correspond to mRNA 5′ termini at about nt positions 414 and 405, respectively. These data are consistent with the approximately 3.0 kb size of *HPS* mRNA and the position of this primer in the full-length *HPS* cDNA sequence (Oh *et al*, 1996). Analysis of the human *HPS* genomic sequence upstream of the cDNA end at position 458 **(Fig 3)** revealed no apparent 3′ splice sites, and computer-aided (NNPP-Eukaryotic, TSSW, TESS, TSSG) promoter analyses of this sequence identified two TATA-box transcriptional motifs, at nt positions 61 and 315, with predicted transcriptional starts at about nt 91 and 342; the latter is in reasonably good agreement with the primer extension result. A large number of additional putative transcriptional motifs were predicted in this region, although none associated with genes specifically expressed in melanocytes. These findings suggest that this region most likely contains the functional *HPS* gene transcriptional promoter.

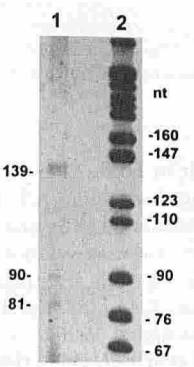

Figure 4. *HPS* primer extension analysis of human melanoma poly(A) mRNA. A 5′ end-radiolabeled 20-mer oligonucleotide complementary to the farthest upstream *HPS* cDNA (see *Materials and Methods*) was used to prime reverse transcription of the RNA. *Lane 1*, primer extension products; *lane 2*, molecular size standard (radiolabeled pBR322 DNA digested with *Hae*III plus *Bst*NI).

DNA Sequence Polymorphisms of the Human *HPS* Gene

By comparison of the *HPS* cDNA and genomic sequences, and in the course of mutation analyses of the *HPS* gene in patients with HPS (Oh *et al*, 1996; our unpublished data), we identified a total of 15 nonpathologic DNA sequence polymorphisms of the gene. Primers and conditions used for these analyses are shown in **Table I**, providing the basis for DNA-based diagnosis of HPS. As shown in **Table III**, we determined approximate allele frequencies for each polymorphism in specific human populations. Many of these polymorphisms are apparently silent and thus are unlikely to affect either function or expression of the *HPS* gene. Four polymorphisms, however, result in amino acid substitutions: Gly283Trp, Pro491Arg, Arg603Gln, and Val630Ile. The first three of these substitutions are nonconservative; the Val630Ile substitution is

Table III. Nonpathologic Polymorphisms of the Human *HPS* gene

Description[a]	Polymorphism	Allele Frequency[b] Caucasians (n = 28)	Allele Frequency[b] Asians (n = 30)
5′ UTR, nt 156	C→A	0.32	0.33
T99T	ACC→ACT	0.04	0.23
G283W	GGG→TGG	0.04	0
IVS11, +13	T→C	0	0.03
IVS14, +7, +8	GG→CT	0.25	0.40
P491R	CCC→CGC	0.11	0.23
IVS16, −15	A→G	0.25	0.40
S566S[c]	TCG→TCA	0	0
R603Q	CGG→CAG	0.89	0.83
V630I[d]	GTC→ATC	0	0
IVS19, +9	A→G	0	0.03
3′ UTR, nt 2581	C→T	0.64	0.83
3′ UTR, nt 2633	T→C	0.35	0.60
3′ UTR, nt 2761	G→A	0.39	0.57
3′ UTR, nt 3015[e]	G→C	ND	ND

[a] Nucleotide (nt) numbers refer to human *HPS* cDNA sequence (Oh *et al*, 1996).
[b] The indicated number of unrelated chromosomes from each group were studied.
[c] The S566S polymorphism has only been observed in a Portugese HPS patient.
[d] The V630I polymorphism has only been observed distal to a frameshift in Swiss patients with HPS (Oh *et al*, 1996).
[e] ND, not determined. This nt residue is G in the *HPS* cDNA (Oh *et al*, 1996) *versus* C in BAC 145M16.

conservative, but thus far has only been observed distal to a frameshift mutation in a subset of HPS patients and thus may not ever occur in the HPS polypeptide. Comparison of the human *HPS* cDNA sequence (Oh *et al*, 1996) with expressed sequence tags apparently corresponding to partial rat (GenBank H34001) and mouse (W29855) *HPS* cDNA sequences, as well as to that of full-length mouse *Hps* cDNA (our unpublished data), demonstrates that amino acid residues Pro491 and Val630 have been conserved during mammalian evolution; thus, these polymorphic substitutions at these residues may not be entirely without consequences for HPS function. These results will be of considerable assistance to interpretation of data obtained during DNA-based diagnoses of HPS.

NOTE ADDED IN PROOF

We have recently characterized the mouse *Hps* gene and shown it to consist of 19 exons, with IVSs corresponding to those in the human gene, except in the 5′ untranslated region. The mouse *Hps* gene corresponds to the murine *pale-ear* (*ep*) locus (Feng *et al*, 1997).

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