Mutation Analysis of Patients with Hermansky-Pudlak Syndrome: A Frameshift Hot Spot in the *HPS* Gene and Apparent Locus Heterogeneity

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Summary

Hermansky-Pudlak syndrome (HPS) is a rare, autosomal recessive disorder in which oculocutaneous albinism, bleeding, and lysosomal ceroid storage result from defects of multiple cytoplasmic organelles—melanosomes, platelet-dense granules, and lysosomes. As reported elsewhere, we mapped the human *HPS* gene to chromosome segment 10q23, positionally cloned the gene, and identified three pathologic mutations of the gene, in patients from Puerto Rico, Japan, and Europe. Here, we describe mutation analysis of 44 unrelated Puerto Rican and 24 unrelated non–Puerto Rican HPS patients. A 16-bp frameshift duplication, the result of an apparent founder effect, is nearly ubiquitous among Puerto Rican patients. A frameshift at codon 322 may be the most frequent *HPS* mutation in Europeans. We also describe six novel *HPS* mutations: a 5′ splice-junction mutation of IVS5, three frameshifts, a nonsense mutation, and a one-codon in-frame deletion. These mutations define an apparent frameshift hot spot at codons 321–322. Overall, however, we detected mutations in the *HPS* gene in only about half of non–Puerto Rican patients, and we present evidence that suggests locus heterogeneity for HPS.

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Introduction

Hermansky-Pudlak syndrome (HPS; MIM 203300) is an autosomal recessive disorder characterized by tyrosinase-positive oculocutaneous albinism, a tendency to bleed, and a ceroid-lipofuscin lysosomal storage disease (Hermansky and Pudlak 1959). At the cellular level, HPS is associated with defects of multiple cytoplasmic organelles, including melanosomes, platelet-dense granules, and lysosomes (Hermansky and Pudlak 1959; Witkop et al. 1990), and it likely results from a defect of a protein required for the biogenesis, structure, or function of these various membrane-bound organelles. Life-threatening manifestations are frequent, and death typically results from restrictive lung disease (68%), hemorrhage (17%), or granulomatous colitis (15%), in patients aged 30–50 years (Witkop et al. 1990). There is no specific therapy for HPS, and treatment is usually limited to supportive care.

HPS is rare in most populations, but it is perhaps the most common single-gene disorder in Puerto Rico, where it occurs with an estimated frequency of ~1/1,800 persons (Witkop et al. 1990). The disorder is also frequent in a long-isolated village in the Swiss Alps (Lattion et al. 1983; Schallreuter et al. 1993). These two patient populations were instrumental to the mapping of the *HPS* gene to 10q23 (Fukai et al. 1995; Wildenberg et al. 1995) and to our eventual positional cloning of the gene (Oh et al. 1996). The human *HPS* gene consists of 20 exons that span ~30.5 kb (Bailin et al. 1997), and it encodes a 700–amino acid polypeptide that contains two apparent transmembrane domains but that has no evident homology to any other known proteins (Oh et al. 1996). Analysis of the mouse *Hps* gene (Feng et al.

1997; Gardner et al. 1997) demonstrated that human HPS is homologous to the murine *pale-ear* (*ep*) mutation, which produces a phenotype that is not unlike that of human HPS.

We described, elsewhere (Oh et al. 1996), three frameshifts in the *HPS* gene: a 16-bp duplication at codons 491–496, in Puerto Rican patients; a 1-bp duplication at codon 441, in a Japanese patient; and a 1-bp duplication at codons 322–324, in the Swiss patients and in an Irish HPS patient. Here, we describe mutation analyses of 44 unrelated Puerto Rican HPS patients, 24 unrelated non–Puerto Rican HPS patients, and a patient with isolated platelet-storage-pool deficiency. We show that the common Puerto Rican codon 491–496 frameshift is nearly ubiquitous among HPS patients from this Caribbean island. Among European HPS patients, the codon 322–324 frameshift appears to be most frequent. We also detected six novel mutations among the non–Puerto Rican HPS patients; these mutations help to define an apparent frameshift hot spot in the region of codons 321–324. Though we describe one single-codon deletion, we detected no missense mutations; this suggests that most amino acid substitutions might not result in the phenotype that is recognized as HPS.

Overall, we detected mutations in the *HPS* gene in only about half of non–Puerto Rican HPS patients. In addition, genetic mapping data exclude the *HPS* locus in several of the patients who lack detectable mutations. Together, these findings point toward locus heterogeneity for HPS, particularly among non–Puerto Rican patients.

Material and Methods

Patient Samples and Mutation Analyses

Blood samples were collected from 44 unrelated Puerto Rican HPS patients and selected relatives, according to a protocol approved by the University of Wisconsin Medical School Human Subjects Committee. High–molecular-weight genomic DNA was prepared from peripheral blood by use of the Puregene kit (Gentra Systems). PCR analysis of the Puerto Rican 16-bp codon 491–496 duplication was carried out exactly as described elsewhere (Oh et al. 1996).

For most of the 25 unrelated non–Puerto Rican patients (table 1), DNA was prepared from peripheral blood samples as described above; although in some cases DNA was prepared from cell or tissue samples by use of the QIAamp Tissue Kit (Qiagen). DNA segments that spanned exons of the *HPS* gene were amplified by PCR, as described elsewhere (Bailin et al. 1997), and were screened for mutations by means of nonradioactive simultaneous SSCP/heteroduplex (HDX) analyses, performed with mutation-detection electrophoresis gels (AT Biochem), also as described elsewhere (Lee et al. 1995). Samples from individuals who were homozygous and/or heterozygous for all known polymorphisms (Bailin et al. 1997) were included as controls for specific exons, as relevant. PCR products that exhibited aberrant SSCP/HDX patterns were reamplified in duplicate, purified by use of the QIAEX II kit (Qiagen), and sequenced directly by use of the Sequitherm Cycle Sequencing Kit (Epicentre Technologies).

Genotype Analysis

High–molecular-weight DNA from relevant family members was genotyped (see Oh et al. [1996] for a detailed map) by means of four polymorphic microsatellite markers that span ~200 kb across the *HPS* gene region of 10q23—*D10S2437–D10S110/D10S184–HPS–D10S2436–D10S2435* (*D10S110* and *D10S184* define the same polymorphism)—in accordance with standard procedures (Dracopoli et al. 1994). One primer of each pair was end-radiolabeled with ^{32}P, and the PCR products were analyzed by denaturing gel electrophoresis and autoradiography. Alleles were assigned by visual inspection.

Results

Mutation Analysis of Puerto Rican HPS Patients

We described elsewhere a 16-bp frameshift duplication that involves codons 491–496, in exon 15 of the *HPS* gene, in patients from Puerto Rico (Oh et al. 1996). Results of direct PCR-based tests for this frameshift in a total of 44 unrelated Puerto Rican HPS proposita (and in a great many more affected relatives) indicated that all were homozygous for this mutation (data not shown). However, we did not detect this mutation in a patient of mixed Puerto Rican/Dominican parentage (patient 24; table1). Moreover, SSCP/HDX screening failed to detect any apparent abnormality of the *HPS* gene in this patient. Thus, HPS is genetically nearly homogeneous in Puerto Rico; it results from homozygosity for the *HPS* codon 491–496 frameshift.

Mutation Analysis of Non–Puerto Rican HPS Patients

We found apparently pathologic mutations of the *HPS* gene in 10 of the 24 unrelated non–Puerto Rican HPS patients we studied. These data are summarized in table 1. Patients 1 and 2, described elsewhere (Oh et al. 1996), represent the Swiss HPS group and an unrelated Irish-German patient, respectively. All of these individuals were homozygous for a 1-bp frameshift duplication, in a poly(C) tract, at codons 322–324 (T322insC; fig. 1*A*). However, these patients were divergent for intragenic polymorphisms that flank this mutation on both sides; this finding suggests that this frameshift probably arose independently in these two groups (Oh et al. 1996). Patient 3 was also homozygous for the T322insC frame-

Table 1

Results of Mutation Analysis of the *HPS* Gene in Non–Puerto Rican HPS Patients

Patient No.	Mutations[a]	Ethnic Origin(s)	Consanguinity[b]	HPS Phenotype	Comment(s)
1	T322insC/ T322insC	Swiss	+	Mild	Oh et al. (1996)
2	T322insC/ T322insC	Irish/German	−	Mild	Oh et al. (1996)
3	T322insC/ T322insC	French	−	Typical	
4	T322insC/E666X	Scottish	−	Mild	
5	E397delC/ E397delC	American (USA)	−	Severe	Coriell Cell Repository (GM13958)
6	E397delC/ G321delG	Ukrainian	−	Typical	
7	A441insA/ A441insA	Japanese	+	Typical	Oh et al. (1996)
8	T322delC/ T322delC	Japanese	+	Severe	
9	IVS5, +5G→A/ IVS5, +5G→A	Japanese	+	Typical	
10	ΔI55/ΔI55	Afghan	+	Very mild	
11	ND	Dutch	−	Typical	
12	ND	Dutch	−	Typical	Gerritsen et al. (1977)
13	ND	Dutch	−	Typical	
14	ND	Dutch	−	Typical	Atypical platelet-storage-pool deficiency
15	ND	Finnish	+	Mild	
16	ND	British	−	Typical	Ellis et al. (1995)
17	ND	Portuguese	−	Typical	
18	ND	Brazilian	−	Typical	
19	ND	Belgian	−	Typical	
20	ND	Japanese	+	Typical	
21	ND	American (USA)	−	Typical	
22a	ND	Canadian	+	Typical	
22b	ND	Canadian	−	Typical	First cousin, once removed, of 22a
22c	ND	Canadian	−	Typical	First cousin of 22a; first cousin, once removed, of 22b
23	ND	Canadian	+	Atypical	HPS, frequent infections, and cyclic neutropenia
24	ND	Puerto Rican/ Dominican	−	Typical	
25	ND	French	−	Platelet-storage-pool deficiency	

[a] ND = none detected.

[b] A plus sign (+) indicates presence, and a minus sign (−) indicates absence.

shift and was homozygous for the polymorphism haplotype found in the patient of Irish-German origin. Patient 4 was a compound heterozygote for the T322insC frameshift, again associated with this same polymorphism haplotype, and for a novel nonsense mutation, E666X (fig. 1*B*).

Patient 5 was homozygous for a novel frameshift, E397delC (fig. 1*C*), and patient 6 was a compound heterozygote for the E397delC frameshift and for another novel frameshift, G321delG, a 1-bp deletion in a poly(G) tract, at codons 320–321 (fig. 1*D*). Patient 7, described elsewhere (Oh et al. 1996), was homozygous for a frameshift, A441insA (fig. 1*E*). Patient 8 was homozygous for another novel frameshift, T322delC (fig. 1*F*), that involves the same poly(C) tract involved in the T322insC frameshift.

Patient 9 was homozygous for a novel mutation within the 5′ splice consensus of IVS5, +5G→A (fig. 1*G*). We identified an apparent 5′ splice-consensus mutation, IVS5 +5G→A, in a Japanese HPS patient. The 5′ splice-junction consensus for primates is $A_{58}G_{78}/g_{100}t_{100}a_{57}a_{71}g_{84}t_{47}$, where G is the most conserved nucleotide at position +5 (Shapiro and Senepathy 1987), and substitutions at IVS nucleotide 5 can radically alter RNA splicing patterns (Treisman et al. 1983; Cheng et al. 1984; Highsmith et al. 1990; Zielenski et al. 1995). We

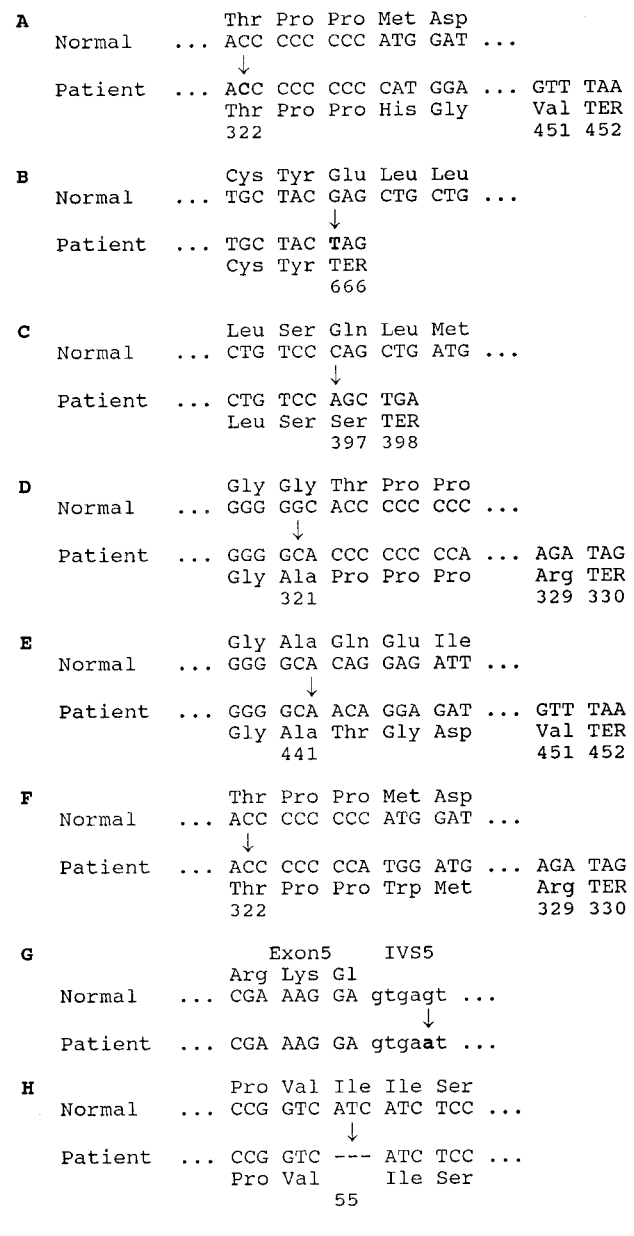

Figure 1 Mutations of the *HPS* gene in non–Puerto Rican HPS patients. *A,* T322insC mutation, patients 1–4. *B,* E666X mutation, patient 4. *C,* E397delC mutation, patients 5 and 6. *D,* G321delG mutation, patient 6. *E,* A441insA mutation, patient 7. *F,* T322delC mutation, patient 8. *G,* IVS5, +5G→A mutation, patient 9. *H,* ΔI55 mutation, patient 10.

failed to detect this mutation in 30 unrelated Asian individuals. Furthermore, patient 9 was also homozygous for three nonpathologic polymorphisms of the *HPS* gene—T99T (q = .23 in Asians), P491R (q = .23 in Asians), and R603Q (q = .83 in Asians)—that we have described elsewhere (Bailin et al. 1997). We sequenced the exon 5 PCR product from five unrelated Asian individuals who were heterozygous for all three of these

Table 2

Genotyping of Inbred Non–Puerto Rican HPS Patients for Markers That Immediately Surround the *HPS* Gene

Patient No.	Marker(s)			
	D10S2437	*D10S110/D10S184*	*D10S2436*	*D10S2435*
15	1,3	2,2	1,1[a]	2,4
20	1,1	1,1	2,2	8,8
22a	1,3	1,2	1,2	1,2
23	1,3	1,2	1,1	1,9

Note.—The *HPS* gene is located between markers *D10S110/D10S184* and *D10S2436* (*D10S110* and *D10S184* define the same polymorphism).

[a] Uninformative marker.

polymorphisms, and none of them carried the 5′ splice-consensus mutation (data not shown). Thus, this mutation is not a common nonpathological polymorphism.

Patient 10 was homozygous for a deletion of three bases (ATC) at a direct repeat at codons 55–56, which results in an in-frame deletion, ΔI55 (fig. 1*H*). HPS patients 11–24 and patient 25 (who has isolated platelet-storage-pool deficiency) had no detectable mutations of the *HPS* gene.

Homozygosity Testing and Genetic Linkage Analyses of Non–Puerto Rican HPS Patients

Our inability to detect *HPS* gene mutations in more than half of the non–Puerto Rican HPS patients suggested the possibility of locus heterogeneity for the disorder. Four of the patients (15, 20, 22, and 23) in whom we found no *HPS* mutations were inbred and, thus, were suitable for homozygosity-by-descent tests, performed by means of polymorphic markers that span an ~200-kb interval that embeds the *HPS* gene in 10q23 (*D10S2437–D10S110/D10S184–HPS–D10S2436–D10S2435;* see Oh et al. [1996] for a detailed map). In addition, the extended families of patients 12 (non-inbred) and 22 (inbred) were sufficiently large to allow tests for genetic linkage to these markers.

As shown in table 2, patient 20, whose parents were first cousins, was homozygous at all four of these markers; nevertheless, DNA sequencing of almost the entire *HPS* gene failed to detect any pathologic mutations in this patient. Thus, patient 20 probably either has an *HPS* gene mutation outside the regions amplified by PCR or is homozygous for this region on the basis of chance (P = .0625).

In contrast, patient 15, whose parents were distantly related, was heterozygous at *D10S2435* and *D10S2437*. Patient 22a, whose parents were first cousins, was heterozygous for all four markers. Patient 23, whose parents were second cousins, was heterozygous at *D10S2437, D10S110/D10S184,* and *D10S2435*. Ge-

netic linkage analysis of the families of patients 12 and 22 excluded linkage to these four markers (minimum LOD scores were $-\infty$ at $\theta = .00$ and -4.282 at $\theta = .00$, respectively), and genotypes of the family of patient 23 were inconsistent with linkage to all four markers (data not shown). Together, these results argue strongly that HPS—in at least patients 12, 15, 22a, and 23—does not result from mutations in the 10q23 *HPS* locus.

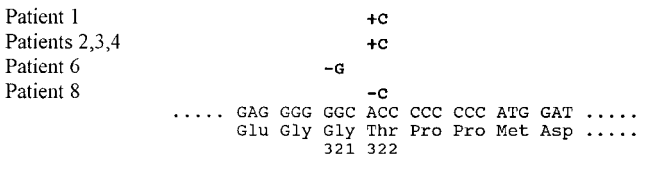

Figure 2 A frameshift hot spot at codons 321–322 of the *HPS* gene.

Discussion

W previously mapped the *HPS* gene to chromosome 10q23, by means of linkage disequilibrium analysis of patients from two inbred groups, one from Puerto Rico and the other from an isolated village in the Swiss Alps (Fukai et al. 1995). We subsequently studied additional patients from Puerto Rico to more finely localize and, eventually, to positionally clone the *HPS* gene (Oh et al. 1996). We have now studied a total of 44 unrelated HPS patients from Puerto Rico, and we have found that all are homozygous for a 16-bp frameshift duplication, which strongly indicates a founder effect in this island population. Among non–Puerto Rican HPS patients, a frameshift, T322insC, which occurs in a tract of cytosines, appears to be the most common *HPS* gene mutation. In fact, haplotype analysis performed with intragenic polymorphisms suggests that this mutation has arisen at least twice in northern Europe (Oh et al. 1996). The occurrence of two additional frameshifts, G321delG and T322delC, in the immediate vicinity strongly indicates that the codon 321–322 region constitutes a hot spot for small duplications and deletions (fig. 2). This is further supported by our observation of the T322delC frameshift as a spontaneous inactivating mutation of the human *HPS* cDNA in a heterologous yeast expression system (data not shown).

It seems surprising that we have not yet identified missense substitutions of the *HPS* gene in any HPS patients, although we previously described three nonpathologic amino acid polymorphisms, G283W, P491R, and R603Q (Bailin et al. 1997). Likewise, both of the two extant *ep* mutations of mice result in frameshifts (Feng et al. 1997). It may be that the phenotype that results from amino acid substitutions in the HPS protein either is clinically very mild or is so different from that of classic HPS that this diagnosis is not generally made in individuals who carry such mutations. In this regard, the patient in whom we found a single-codon deletion of I55/I56 may be instructive. This patient has a very mild clinical phenotype that includes congenital nystagmus, ocular albinism, and bruising associated with deficient platelet-dense granules, but the patient has normal skin and hair pigmentation. This patient is still an infant, and it will thus be of interest to observe the phenotype as the child grows and develops.

In more than half of the non–Puerto Rican HPS patients, and in the one patient with isolated platelet-storage-pool deficiency, we found no apparent abnormalities of the *HPS* gene. This low mutation-detection frequency seemed surprising, and it suggested to us the possibility of either a high frequency of occult mutations or locus heterogeneity for HPS. Likewise, the absence of the codon 491–496 frameshift in an HPS patient of mixed Puerto Rican/Dominican ancestry also suggested the existence of either allelic heterogeneity or locus heterogeneity, even in Puerto Rico. To distinguish between these possibilities, we carried out homozygosity analysis of four inbred HPS patients in whom we had detected no mutations. If these patients were homozygous-by-descent for occult *HPS* mutations, they should also be homozygous for the polymorphic markers we tested that immediately flank the gene on both sides. However, three of these patients were heterozygous for these markers; this finding apparently excluded the *HPS* locus in these cases. Furthermore, genetic linkage analysis of the extended family of one of these patients, as well as in another, non-inbred family, showed no evidence for linkage. Together, these results strongly support our hypothesis of locus heterogeneity for HPS. A similar conclusion was recently reached by Hazelwood et al. (1997).

If there is a second *HPS* locus, what might it be? In the mouse, there are at least 14 different loci that produce phenotypes similar to that of human HPS (Bennett 1993), some of which might thus constitute candidate homologues. We (Feng et al. 1997) and others (Gardner et al. 1997) have already shown that human *HPS* is homologous to the mouse *ep* locus, located in the homologous region of murine chromosome 19. We previously considered another mouse mutant, *ruby eye* (*ru*), as a possible *HPS* homologue, partly on the basis of its phenotypic similarity to human HPS (Fukai et al. 1995) and its location on chromosome 19. However, if human-mouse synteny in this region has been conserved, our genotype data would probably exclude the human *ru* homologue as a likely second HPS locus, since the mouse *ep* and *ru* loci are located only 1.3 cM apart (O'Brien et al. 1994). Perhaps a better candidate for a second human HPS locus would be the homologue to the mouse *light ear* (*le*) locus. *Light ear* is located on mouse chromosome 5, near *Pdeb* and *Gus*, although the precision

of this localization is not high. The human *Pdeb* homologue (*PDE6B*) is located at 4p16.3, and the *Gus* homologue (*GUSB*) is located at 7q22; these locations can now be tested by homozygosity analysis of inbred non–chromosome 10 HPS patients.

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