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Hermansky-Pudlak Syndrome Type 3 in Ashkenazi Jews and Other Non–Puerto Rican Patients with Hypopigmentation and Platelet Storage-Pool Deficiency

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Hermansky-Pudlak syndrome (HPS), consisting of oculocutaneous albinism and a bleeding diathesis due to the absence of platelet dense granules, displays extensive locus heterogeneity. *HPS1* mutations cause HPS-1 disease, and *ADTB3A* mutations cause HPS-2 disease, which is known to involve abnormal intracellular vesicle formation. A third HPS-causing gene, *HPS3*, was recently identified on the basis of homozygosity mapping of a genetic isolate of HPS in central Puerto Rico. We now describe the clinical and molecular characteristics of eight patients with HPS-3 who are of non–Puerto Rican heritage. Five are Ashkenazi Jews; three of these are homozygous for a 1303+1G→A splice-site mutation that causes skipping of exon 5, deleting an *Rsa*I restriction site and decreasing the amounts of mRNA found on northern blotting. The other two are heterozygous for the 1303+1G→A mutation and for either an 1831+2T→G or a 2621-2A→G splicing mutation. Of 235 anonymous Ashkenazi Jewish DNA samples, one was heterozygous for the 1303+1G→A mutation. One seven-year-old boy of German/Swiss extraction was compound heterozygous for a 2729+1G→C mutation, causing skipping of exon 14, and resulting in a C1329T missense (R396W), with decreased mRNA production. A 15-year-old Irish/English boy was heterozygous for an 89-bp insertion between exons 16 and 17 resulting from abnormal splicing; his fibroblast *HPS3* mRNA is normal in amount but is increased in size. A 12-year-old girl of Puerto Rican and Italian background has the 3,904-bp founder deletion from central Puerto Rico on one allele. All eight patients have mild symptoms of HPS; two Jewish patients had received the diagnosis of ocular, rather than oculocutaneous, albinism. These findings expand the molecular diagnosis of HPS, provide a screening method for a mutation common among Jews, and suggest that other patients with mild hypopigmentation and decreased vision should be examined for HPS.

Introduction

Human disorders of hypopigmentation can be divided into ocular albinism (OA), which is generally X-linked, and oculocutaneous albinism (OCA), which is usually inherited in an autosomal recessive fashion (King et al. 2001). OCA1 (MIM 203100) results from deficiency of tyrosinase, the primary pigment-forming enzyme, whereas OCA2 (MIM 203200) and "brown OCA" are results of mutations in the *P* gene, whose function is currently under investigation. OCA3 (MIM 203290) results from mutations in *TYRP1*, the gene coding for another melanogenic protein, tyrosinase-related protein 1 (King et al. 2001). In addition, a variety of syndromes have hypopigmentation as one of their primary manifestations. These include Chediak-Higashi syndrome (MIM 214500), which is characterized by a fatal infectious diathesis and mild bleeding (Introne et al. 1999), Griscelli syndrome (MIM 214450), which is characterized by neurological and immunoproliferative involvement (Griscelli et al. 1978), and Hermansky-Pudlak syndrome (HPS [MIM 203300]; Hermansky and Pudlak 1959).

HPS is considered a disorder of the formation of intracellular vesicles, such as the melanosome in melanocytes, the dense body in platelets, and the lysosome in less-specialized cells (Shotelersuk et al. 1998; Huizing et al. 2000). This explains both the clinical manifestations of the disorder (Gahl et al. 1998) and its locus heterogeneity (Hazelwood et al. 1997; Oh et al. 1998), because many different gene products are required for vesicle formation. Patients with HPS exhibit various degrees of oculocutaneous albinism, characterized by con-

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genital nystagmus, decreased visual acuity, and hypopigmentation of the skin, hair, and irides (Simon et al. 1982; Summers et al. 1988; Toro et al. 1999; Iwata et al. 2000). Their platelets lack dense bodies, accounting for the loss of a secondary aggregation response and for subsequent bleeding of mucous membranes and soft tissues. The absence of dense granules in platelets examined by whole-mount electron microscopy confirms the diagnosis of HPS (Witkop et al. 1987). Lysosomal involvement in HPS is manifested by increased accumulation within intracellular vesicles of ceroid lipofuscin, an amorphous lipid-protein complex (Witkop et al. 1988). Some patients with HPS develop granulomatous colitis (Schinella et al. 1980; Mahadeo et al. 1991) and a fatal pulmonary fibrosis (Garay et al. 1979; Harmon et al. 1994; Brantly et al. 2000), in addition to the common manifestations of the disorder (Gahl et al. 1998).

HPS can be caused by mutations in any one of several genes, reflecting, in part, the situation in mice, where 15 different genes cause hypopigmentation and platelet storage-pool deficiency (Swank et al. 1998; Wilson et al. 2000). In humans, mutations in *HPS1*, which codes for a 79.3-kD cytoplasmic protein of unknown function (Oh et al. 1996), cause HPS-1 disease (MIM 604982). This disorder is common in northwestern Puerto Rico, where ~400 people are homozygous for a 16-bp duplication in *HPS1* (Witkop et al. 1990). All patients with HPS-1 are at increased risk of developing pulmonary fibrosis (Gahl et al. 1998; Brantly et al. 2000).

Mutations in *ADTB3A* cause human HPS-2 disease (MIM 603401), which affects only three known individuals in two families (Shotelersuk et al. 2000; Huizing et al. 2001*b*). Patients with HPS-2 have mild oculo cutaneous albinism, a mild bleeding diathesis, persistent neutropenia, and recurrent childhood infections. *ADTB3A* codes for the β3A subunit of adaptor complex–3 (AP-3), a coat protein that facilitates the formation of vesicles of lysosomal lineage from the trans-Golgi network (Simpson et al. 1996; Dell'Angelica et al. 1997*a*, 1997*b*; Simpson et al. 1997). This function suggests that all types of HPS may result from abnormal vesicle formation.

Recently, a third HPS-causing gene, *HPS3* (MIM 606118), was isolated using a combination of homozygosity mapping, genome database searches, northern blot analysis, and sequencing applied to patients within an isolate of HPS from central Puerto Rico (Anikster et al. 2001). *HPS3*, on chromosome 3q24, has 17 exons and a 3,015-bp open reading frame that codes for a 1,004–amino acid protein of unknown function. Central Puerto Rican patients with HPS exhibit a founder effect; all are homozygous for a 3,904-bp deletion removing the first exon of *HPS3* and >2 kb of upstream sequence (Anikster et al. 2001).

We now describe HPS-3 disease and *HPS3* mutations in eight non–Puerto Rican patients. HPS-3 disease manifests clinically with mild OCA, absent platelet dense bodies, and little or no pulmonary disease. We document *HPS3* mutations that include four splice-site mutations, a C1329T missense mutation, and an 89-bp insertion, with variable degrees of mRNA expression on northern blots. In addition, a 1303+1G→A splice-site mutation was evident in the homozygous state in three Ashkenazi Jewish patients and in the heterozygous state in two other Ashkenazi Jewish patients. Using a diagnostic assay based on restriction enzymes, we found this mutation in one of 235 DNA samples from unaffected Ashkenazi Jews. These findings suggest that consideration be given to screening for mutations in *HPS3* among patients with OCA—or even those with OA who have any history of a bleeding diathesis.

Methods

Patients and Cells

All patients were enrolled in a protocol approved by the National Institute of Child Health and Human Development (NICHD) institutional review board to study the clinical and molecular aspects of HPS. Informed consent was obtained from either the patient or the patient's parents. The diagnosis of HPS was based on documentation of OCA (i.e., decreased visual acuity, nystagmus, and some degree of hypopigmentation relative to family members) and the absence of platelet dense bodies on whole-mount electron microscopy (fig. 1). Patient numbers correspond to a master file of all NICHD patients with HPS. Primary cultures of skin fibroblasts, obtained from a 4-mm punch biopsy, were grown in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum containing 100 U/ml penicillin and 0.1 mg/ml streptomycin.

Electron Microscopy of Platelet Dense Bodies

Platelet-rich plasma, prepared from fresh citrated blood, was placed on copper grids and treated as described elsewhere (Witkop et al. 1987; Hazelwood et al. 1997). The grids were air dried and examined using a Philips model 301 electron microscope.

PCR Amplification and Sequencing

Standard PCR amplification procedures were used (Sambrook et al. 1989), with an annealing temperature of 58°C for all primers. Automated sequencing was performed on a Beckman CEQ 2000, using the CEQ Dye Terminator Cycle Sequencing kit, according to the manufacturer's protocols.

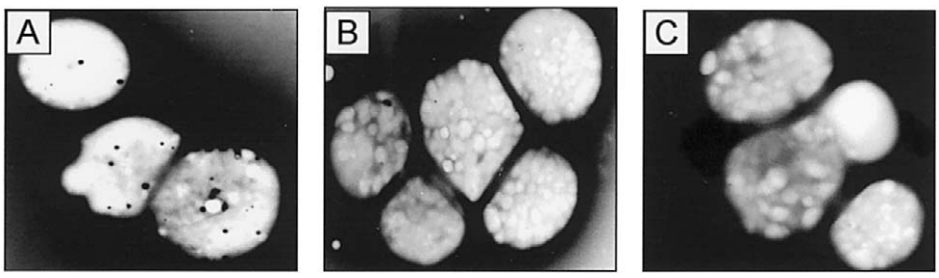

Figure 1 Whole-mount electron micrographs of platelet dense bodies. *A*, Normal platelets showing two to six dense bodies per platelet. *B*, Platelets of patient 91, which are devoid of dense bodies. *C*, Platelets of patient 100, which are totally lacking dense granules.

Northern Blot Analysis

RNA (20 mg), which was isolated from human fibroblasts by use of Trizol reagent (Gibco-BRL-Life Technologies), was electrophoresed on a 1.2% agarose/3% formaldehyde gel and was blotted onto a Nytran nylon membrane (Schleicher and Schuell) in the presence of 20 × sodium chloride (3 M)/sodium citrate (0.3 M). Northern blots of human immune system and cancer cell lines were purchased from Clontech. All blots were hybridized and washed, as described elsewhere (Hazelwood et al. 1997). The probe was a [^{32}P]-dCTP (DuPont/New England Nuclear) random primer that was labeled and prepared from human *HPS3* cDNA, using forward primer 5′-TCCTTACCTCATGTGGGCTATC-3′ (nt 1179–1200) and reverse primer 5′-TTCAAGTGAAGTGCAAGCTCGGT-3′ (nt 2341–2319). The same set of filters was also probed for the β-actin gene.

Screening for HPS-1 and HPS-2

Screening for the northwest Puerto Rican 16-bp duplication in the *HPS1* gene (GenBank accession number U65676) was performed by amplifying exon 15 of *HPS1* genomic DNA (forward primer 5′-GATGGTCCACAAAGGACGAG-3′ and reverse primer 5′-GCGTGAAGGAAGTACGGGCC-3′) and analyzing the products on a 2% agarose gel (Oh et al. 1996; Hazelwood et al. 1997). Mutation detection for the entire *HPS1* gene was performed on genomic DNA by amplifying and sequencing each exon, as described elsewhere (Bailin et al. 1997).

Screening for defects in *ADTB3A* (GenBank accession number U91931) was performed by western blot analysis of protein extracts of the patients' fibroblasts and the use of antibodies (generously provided by M. Robinson, Cambridge, U.K.) against the β3A subunit of AP-3 to detect defects in protein expression. Cells that showed aberrant expression of β3A protein subsequently had their cDNA or genomic DNA sequenced to detect defects in *ADTB3A* (Shotelersuk et al. 2000; Huizing et al. 2001*b*).

Screening for Mutations in HPS3

A multiplex PCR-amplification assay was performed on genomic DNA to screen for the common central Puerto Rican deletion in *HPS3* (Anikster et al. 2001). Primer pairs (nucleotide numbering according to *HPS3* genomic DNA [GenBank accession number AF375663]) across the deletion (nt 86–106, 5′-GGTGTTGTTTAGAGATGCAGA-3′; nt 4639–4616, 5′-GCATAGCCACCAGCTTTTGCAACG-3′) and within the deletion (nt 2581–2604, 5′-CGTGAACTCCACGTTGAGATGTCA-3′; nt 2977-2954, 5′-CGTTCTGACAATTCATCATCTATC-3′) were employed using PCR conditions, as described elsewhere (Anikster et al. 2001).

For patients with no Puerto Rican ancestry, cDNA was PCR amplified in three overlapping fragments and was analyzed both on 1% agarose gels and by direct sequencing. The following *HPS3* cDNA (GenBank accession number AY033141) primer pairs were used: fragment 1, 5′-CGGACGTCGGGATGGTGCAGC-3′ (nt 130–150) and 5′-GCAAAAATTGTGGCGTGAGTAC-3′ (nt 1290–1269); fragment 2, 5′-TCCTTACCTCATGTGGGCTATC-3′ (nt 1179–1200) and 5′-TTCAAGTGAAGTGCAAGCTCGGT-3′ (nt 2341–2319); and fragment 3, 5′-CCTCGGCTGTTGATTCAACAGA-3′ (nt 2177–2298) and 5′-CTCCTCTACTTGTACTTTGGCA-3′ (nt 3234–3213). We confirmed mutations found on cDNA by amplifying the exon in which the mutation was detected. Primer pairs and conditions for amplifying each *HPS3* exon on genomic DNA have been described elsewhere (Anikster et al. 2001).

Assays for Specific Mutations

Demonstration of the splice-site mutation resulting in exon 14 skipping (which was observed in patient 26, patient 91, and the family of patient 91) involved cDNA amplification, using forward primer 5′-CCTCGGCTGTTGATTCAACAGA-3′ (nt 2177–2298) and reverse primer 5′-ACCACTACACGGTAAGGTAAGC-3′ (nt 3335–3314). Illustration of the missense mutation in the other allele of patient 91 was provided by PCR ampli-

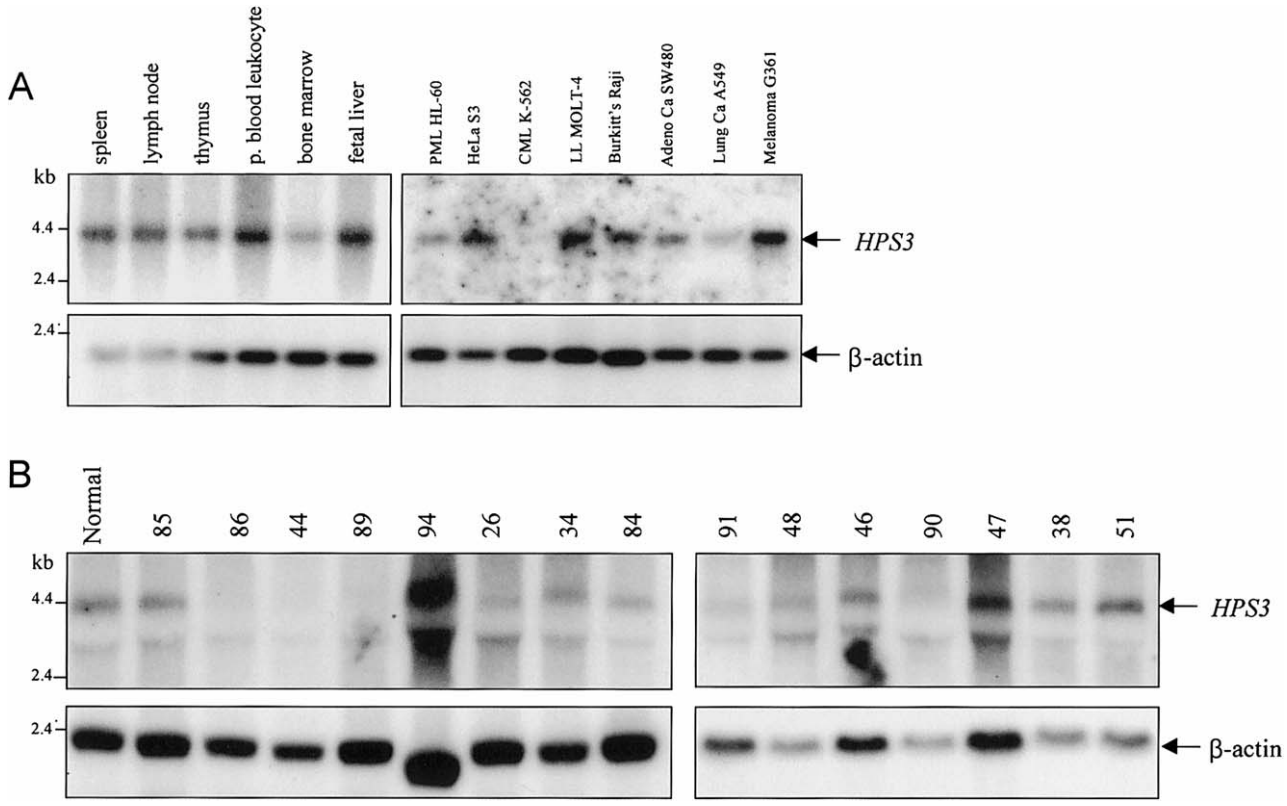

Figure 2 Northern blots demonstrating *HPS3* expression. *A*, Multiple-tissue northern blots hybridized with an *HPS3* cDNA (*upper panel*) and β-actin (*lower panel*) probe. The tissue/cell source of RNA is indicated above each lane. p = peripheral; PML = promyelocytic leukemia; CML = chronic myelogenous leukemia; LL = lymphoblastic leukemia; Adeno Ca = colorectal adenocarcinoma. *B*, Northern blots of mRNA isolated from fibroblasts cultured from patients with HPS who did not have HPS-1 or HPS-2 disease. Patient numbers correspond to a master list of NICHD patients with HPS.

fication of exon 6, followed by restriction enzyme digestion with *Dra*III (New England Biolabs) for 3 h at 37°C. The insertion in patient 34 was demonstrated by cDNA amplification, using forward primer 5′-CCTCGGCTGTTGATTCAACAG-3′ (nt 2277–2297) and reverse primer 5′-TCCTCTACTTGTACTTTGGCA-3′ (nt 3233–3213).

The *HPS3* exon 5 skipping, observed in patients of Ashkenazi Jewish descent, was shown by PCR amplification of cDNA, using forward primer 5′-TTGTGCATTTCCTGTTGCCCTG-3′ (nt 549–570) and reverse primer 5′-CTTGGGTGACTGTCTTCTCTCT-3′ (nt 1532–1511). The presence of the 1303+1G→A mutation, in the homozygous or heterozygous state, was confirmed by amplification of exon 5 genomic DNA followed by restriction enzyme digestion with *Rsa*I (New England Biolabs) for 3 h at 37°C.

For screening of this mutation in Ashkenazi Jewish populations, 185 anonymous samples were obtained from the National Laboratory for the Genetics of Israeli Populations, through L. Brody of the National Human Genome Research Institute, and 50 anonymous samples were obtained from patients with Gaucher disease, through E. Sidransky of the National Institute of Mental Health.

Results

Among our patients with HPS, 31 lacked the 16-bp duplication in exon 15 of *HPS1,* as well as any other mutation in the coding region of *HPS1*. In addition, these 31 patients expressed a normal contingent of the β3A subunit of AP-3, as determined by western blotting of fibroblast extracts. Thus, diagnoses of HPS-1 disease and HPS-2 disease were essentially eliminated.

An initial screening for *HPS3* mutations involved northern blot analyses, using RNA obtained from patients' cultured fibroblasts. It was previously determined that heart, brain, placenta, lung, liver, kidney, skeletal muscle, pancreas, and fibroblasts express *HPS3* (Anikster et al. 2001), and we further demonstrated that all other human tissues or cell lines tested, with the exception of CML line K562, also produce *HPS3* mRNA (fig. 2*A*). The transcript size was ~4.4 kb, and no alternatively spliced product was detected on the

Table 1

Characteristics of Patients with Mutations in *HPS3*

Patient Number	Age (years), Sex	Ancestry[a]	*HPS3* Mutation 1	*HPS3* Mutation 2	Visual Acuity[b] (OD, OS)	Bleeding[c]	FVC (%)[d]	GI[e]	Comments[f]
26	3, F	AJ/Iri-Ger	1303+1G→A	2621−2A→G	60, 60	E, Br	NA	−	11q24 Del
91	7, M	Ger/Swi	2729+1G→C	C1329T	160, 200	E, Br	75	−	…
90	12, F	PR/Ita	Del3904bp	Unknown	63, 80	Br	86	−	Central PR
34	15, M	Iri/Eng	3027Ins89bp	Unknown	100, 125	E, Br	90	−	PDA
86	25, F	AJ/AJ	1303+1G→A	1831+2T→G	160, 100	E, Br, M	96	−	OA
44	30, F	AJ/AJ	1303+1G→A	1303+1G→A	125, 100	E, Br, M	84	−	Transfusion
100	44, F	AJ/AJ	1303+1G→A	1303+1G→A	100, 160	E, Br	92	−	OA
89	52, F	AJ/AJ	1303+1G→A	1303+1G→A	80, 100	Br, M	93	−	…

[a] AJ = Ashkenazi Jewish; Iri = Irish; Ger = German; Swi = Swiss; PR = Puerto Rican; Ita = Italian; Eng = English.

[b] Visual acuity expressed in terms of vision at 20 feet (e.g., 160 indicates 20/160 acuity). OD = right eye; OS = left eye. Patient 26 had an acuity of 10/30 when tested by use of Allen cards.

[c] E = epistaxis; Br = bruising; M = menorrhagia.

[d] FVC = forced vital capacity (expressed as percent of predicted value). Patient 91 may have been too young to provide optimal effort.

[e] GI = gastrointestinal symptoms of colitis; − = absent.

[f] Patient 26 has Jacobsen's syndrome, with an 11q24-11qter deletion. The father of patient 90 is from the central Puerto Rican area where the 3,904-bp deletion in *HPS3* was found. PDA = patent ductus arteriosus. OA = ocular albinism. Patient 44 required a transfusion for bleeding after wisdom teeth removal.

multiple-tissue northern blots. The expression of *HPS3* mRNA in fibroblasts encouraged us to employ northern blots of fibroblast RNA to screen each of our 31 candidate patients for abnormalities in *HPS3* expression. Representative examples illustrate the different amounts or sizes of *HPS3* mRNA produced by the patients' cultured fibroblasts (fig. 2*B*). In addition to the expected 4.4-kb *HPS3* transcript, a second mRNA band of ~3 kb hybridized to the *HPS3* cDNA probe. This band could represent an alternatively spliced form of *HPS3*, or nonspecific hybridization to the probe. In any event, this screening procedure pointed to certain patients (26, 34, 44, 86, 89, 90, 91, and 100) later shown to have mutations in *HPS3* (see table 1).

Screening for the Central Puerto Rican Founder Deletion in HPS3

Genomic DNA from the 31 patients was analyzed for the presence of the 3,904-bp central Puerto Rican deletion in *HPS3*, using a multiplex PCR assay (Anikster et al. 2001). No non–Puerto Rican patient exhibited the deletion. Only one individual (patient 90) was found to be heterozygous for the deletion. Her father, who was from central Puerto Rico, also carried the deletion, but her Italian mother did not (fig. 3). We screened the patient's cDNA to identify a mutation in the maternal allele, but the coding region had an entirely normal sequence. As expected, northern blot analysis showed a reduced amount of *HPS3* mRNA (fig. 2*B*).

Other HPS3 *Mutations*

HPS3 cDNA of all 31 patients was PCR amplified in three overlapping fragments, and each fragment was analyzed by agarose gel electrophoresis before sequencing. Fragment 1 was of normal size for all patients, but cDNA from three Ashkenazi Jews (patients 44, 89, and 100) was not amplified, because—as we later recognized—one of the primers was located in a skipped exon (see *HPS3* Mutations in Patients of Ashkenazi Jewish Descent section below). Fragment 2 was smaller for one patient (patient 86); subsequent sequencing showed skipping of exon 9, changing the reading frame. The alteration was confirmed by genomic DNA analysis, which revealed a heterozygous 1831+2T→G splice-site mutation.

Fragment 3 displayed an abnormal size when cDNA was amplified from three patients (91, 26, and 34). Patients 26 and 91 had a smaller-than-normal fragment 3, and sequencing showed skipping of exon 14. Genomic

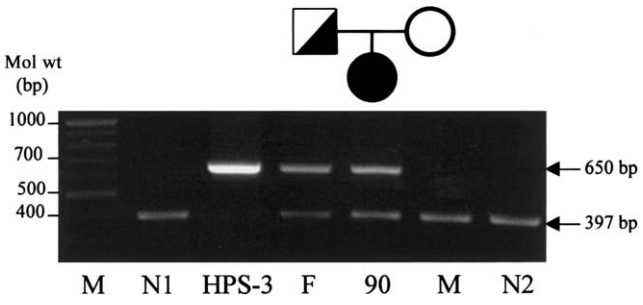

Figure 3 Multiplex PCR amplification identifying the 3,904-bp founder deletion of central Puerto Rico. Patient 90 and her father (F) have the deletion in the heterozygous state, but the mother (M) lacks the deletion. M = 100-bp DNA ladder; N1 and N2 = normal control DNA; HPS-3 = DNA from a central Puerto Rican patient with HPS-3 who was homozygous for the deletion.

DNA analysis of the exon 14 splice sites revealed a heterozygous 2621−2A→G mutation in patient 26, and patient 91 carried a 2729+1G→C mutation. PCR analysis of the family of patient 91 (fig. 4*A*) indicated that the mutation was inherited from the mother and transmitted to the proband's two full sisters (one affected and one unaffected). The proband's unaffected half sister did not carry the mutation. Sequence analysis of the second allele of patient 91 revealed a C1329T missense mutation in exon 6, resulting in an R396W amino acid change and creation of a recognition site for the restriction enzyme *Dra*III. Incubation of PCR-amplified exon 6 fragments of all family members with *Dra*III (fig. 4*B*) indicated that the father is heterozygous for the C1329T mutation, that both of his affected children inherited the mutant allele, and that his unaffected daughter inherited his normal allele. Northern blot analysis (fig. 2*B*) revealed a decreased amount of *HPS3* mRNA in the cultured fibroblasts of patient 91.

Patient 34 exhibited a larger-than-normal fragment 3 after cDNA amplification (fig. 5*A*). Sequencing revealed an 89-bp insertion in cDNA between nt 3027 and nt 3028, that is, at the splice site between exons 16 and 17 (not shown). The 89-bp insertion appeared to be part of intron 16 (nt 44102–44191 on *HPS3* genomic DNA). Sequencing of intron 16 revealed a heterozygous G→A mutation at nt 44101 (fig. 5*B*). This mutation introduces a new splice site, resulting in additional exonic material between exons 16 and 17 (fig. 5*C*). The 89-bp insertion in patient 34 results in a change after amino acid 962, such that 23 new amino acids are translated before a premature stop is reached at codon 986. This mutation is present in the heterozygous state in patient 34. In searching for the second mutation, all 17 exons were sequenced, but no other mutations were found. No normal-sized bands of *HPS3* mRNA or cDNA were produced by this patient's fibroblasts.

HPS3 *Mutations in Patients of Ashkenazi Jewish Descent*

Of our 31 candidate patients, 6 were of Ashkenazi Jewish descent. Five of these (patients 26, 44, 86, 89, and 100) showed skipping of exon 5 when the appropriate portion of their cDNA was amplified (fig. 6*A*). Sequencing the intron/exon boundaries of these patients' genomic DNA revealed a 1303+1G→A splice-site mutation, present in the homozygous state in patients 44, 100, and 89 and in the heterozygous state in patients 26 and 86. Splice-site mutations present on the second alleles of patients 26 and 86 had already been identified (table 1). Amplification of a 908-bp *HPS3* cDNA region revealed a very faint fragment of normal length for the homozygous patients, and a nearly normal amount of a normal-sized band in the heterozygous patients, with an extra, "skipped" band of lesser intensity (fig. 6*A*). The fibroblasts of homozygously affected patients expressed negligible *HPS3* mRNA, whereas those of the heterozygous patients showed decreased expression on northern blots (fig. 2*B*). The 1303+1G→A splice-site mutation was not found in any patients with HPS who were not of Ashkenazi Jewish descent.

The splice-site mutation deletes an *Rsa*I restriction site (fig. 6*B*), and we took advantage of this to estimate the gene frequency among Ashkenazi Jews. We screened DNA samples from a total of 235 Ashkenazi Jews (drawn from a commercial database of 185 unrelated individuals and a set of 50 patients with Gaucher dis-

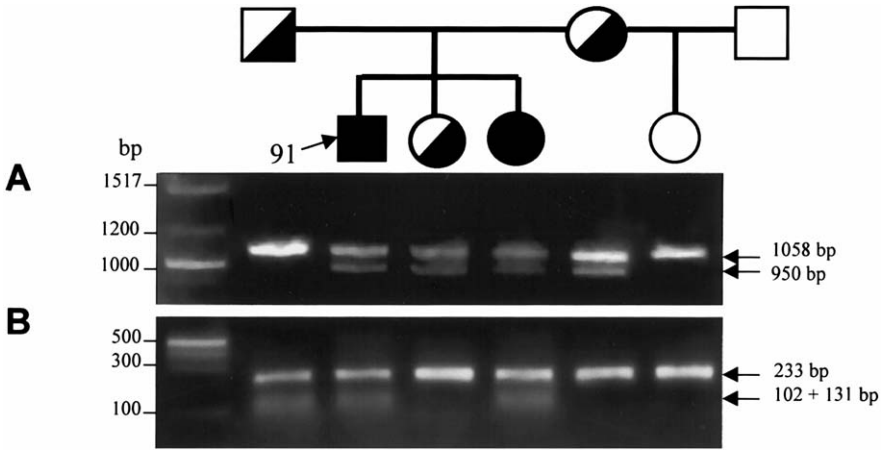

Figure 4 Mutation analysis in patient 91 and his family. *A,* PCR amplification of a 1,058-bp *HPS3* cDNA fragment showing heterozygous skipping of exon 14 (with an additional 950-bp band) in patient 91, his two full sisters, and his mother. *B,* PCR amplification of exon 6 *HPS3* genomic DNA (233-bp), followed by restriction enzyme digestion with *Dra*III. The paternal C1329T mutation introduces a *Dra*III recognition site, resulting in 102-bp and 131-bp bands after digestion. Patient 91, his father, and his affected sister carry the C1329T mutation.

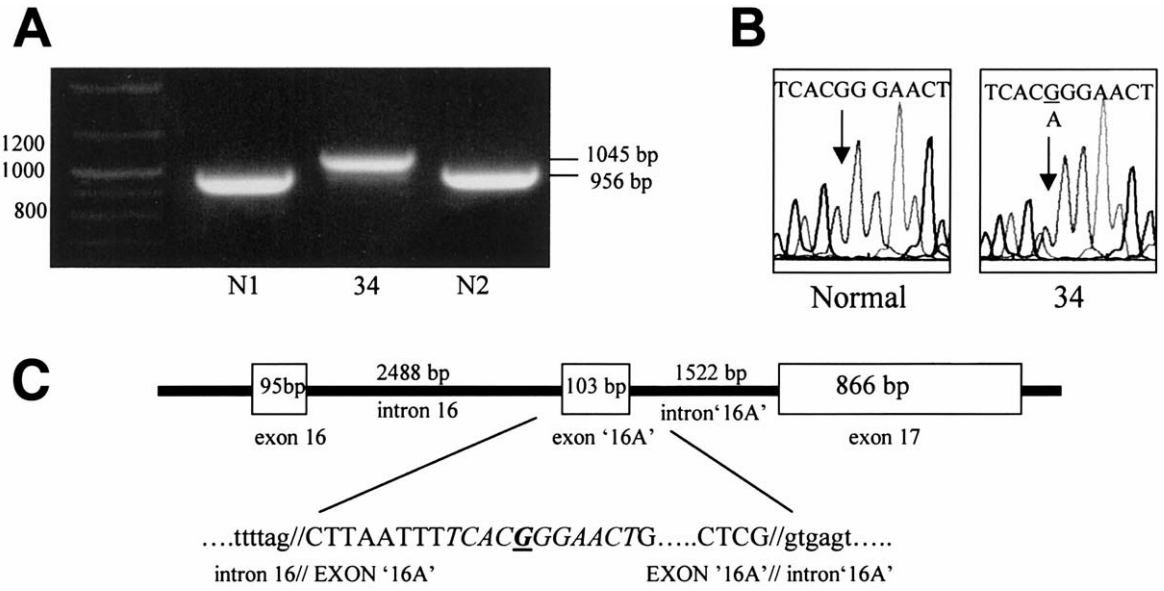

Figure 5 Demonstration of a new splice site in patient 34. *A,* PCR amplification of a 956-bp cDNA fragment in normal cDNA (N1 and N2). Amplification reveals an 89-bp insertion. *B,* Sequencing indicates a heterozygous G44101A mutation in intron 16. *C,* Schematic drawing of the 89-bp insertion the patient's cDNA. Exon 16A is expressed in *HPS3* cDNA in a very rare alternative splice form in normal fibroblasts; however, the G44101C mutation introduces a new consensus splice site that results in insertion of 89-bp of exon 16A in the patient's cDNA.

ease). One of the 235 DNA samples contained a single copy of the mutation.

Clinical Findings

The eight patients with documented *HPS3* mutations were 3–52 years of age. Besides the Ashkenazi Jews from eastern Europe, affected patients had ancestors of Irish, English, German, Swiss, and Italian heritage.

Hair and skin color were mildly hypopigmented, in comparison with other family members, and ranged from light tan to dark brown (fig. 7*A*). Iris transillumination was quite variable and correlated somewhat with the degree of fundus hypopigmentation (fig. 7*B*). Visual acuity ranged from 20/63 to 20/200, and all patients had a history of excessive bruising. Three of the four adult women reported menorrhagia.

Pulmonary function testing revealed forced vital capacity values 75%–96% of predicted (normal, 80%–120%). No patient had a history or any evidence of granulomatous colitis. Two patients, 86 and 100, carried the diagnosis of ocular albinism for 25 and 13 years, respectively. The father of patient 90 was born in the region of central Puerto Rico where the 3,904-bp founder deletion arose (Anikster et al. 2001).

Discussion

The *HPS3* gene, which is responsible for HPS in a genetic isolate in central Puerto Rico (Anikster et al. 2001), also causes a subtype of HPS among non–Puerto Rican patients. The common Puerto Rican deletion in *HPS3*—like the founder duplication in *HPS1* (Oh et al. 1996; Shotelersuk et al. 1998)—has been identified only in members of the founder population, in-

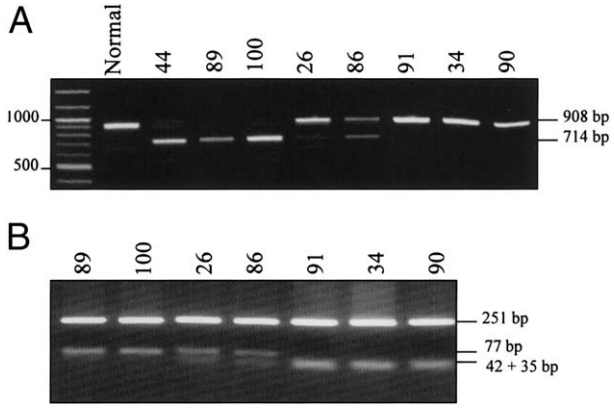

Figure 6 Ashkenazi Jewish mutation in *HPS3*. *A,* PCR amplification of a 908-bp *HPS3* cDNA fragment, showing homozygous and heterozygous skipping of exon 5 (714-bp band). *B,* PCR amplification of exon 5 genomic DNA (328-bp) followed by *Rsa*I restriction enzyme digestion. The 1303+1G→A splice-site mutation deletes an *Rsa*I restriction site. Normal genomic DNA is cut into 251-bp, 42-bp, and 35-bp bands; homozygously mutated genomic DNA is cut into 251-bp and 77-bp bands. Patients 44, 89, and 100 exhibit homozygous 1303+1G→A mutations. Patients 26 and 86 carry the mutation in the heterozygous state. Patients 34, 90, and 91 do not have this mutation.

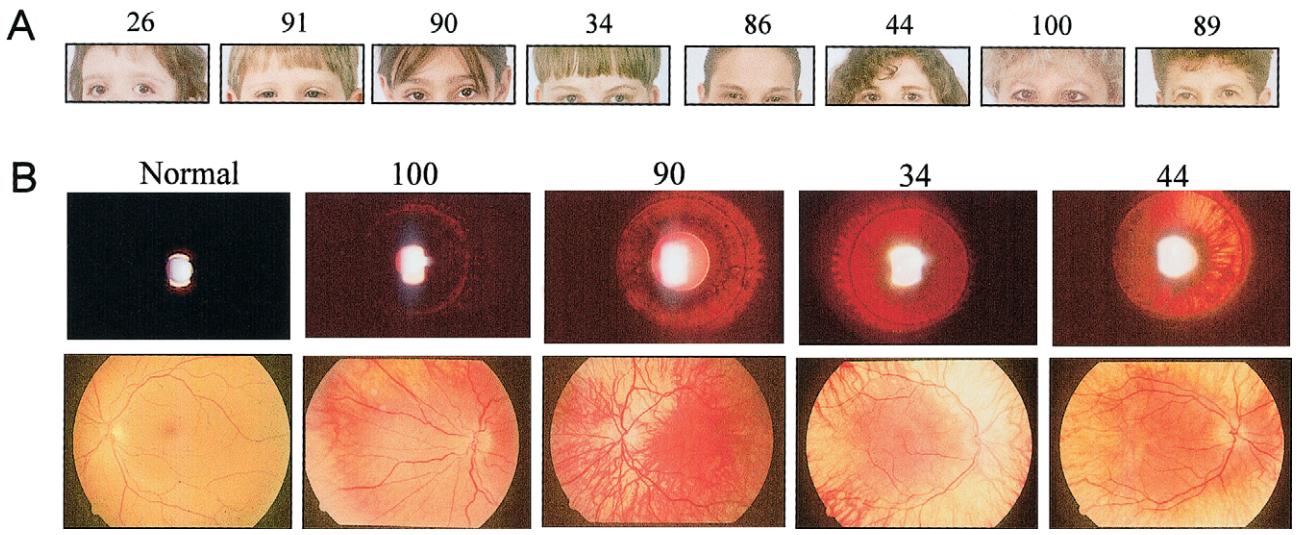

Figure 7 Hypopigmentation of hair, iris, and retina in patients with HPS-3. *A*, Hair pigmentation in patients of increasing age (see table 1). Color ranges from tan (patient 34) to dark brown (patient 90). Patient 100 tints her hair lighter, but her eyebrows are natural in color. *B*, Increasing severity of iris transillumination (*top panel*) and retinal hypopigmentation (*bottom panel*) in four patients with HPS-3. The picture on the left shows the retina of an unaffected control subject. Iris transillumination consists of light transmitted through the iris as a result of reduced pigment in that tissue. The normal iris remains dark when light is shone through the pupil. In the HPS-3 retinas, patchy hypopigmentation results from a reduction of pigment epithelium compared with normal retina (*left*). The degree of fundus hypopigmentation corresponds somewhat with the extent of iris transillumination.

cluding one Puerto Rican/Italian patient (patient 90) who inherited the deletion from her father (fig. 3). Among non–Puerto Rican patients with HPS, however, other *HPS3* mutations give rise to HPS-3 disease.

For example, patient 91 is heterozygous for a splicing mutation that removes exon 14, consisting of 108 bp. The 36 amino acids eliminated by this in-frame deletion are relatively conserved (81%) between humans and mice (Huizing et al. in press), indicating the importance of the region. The decrease in *HPS3* mRNA (fig. 2*B*) suggests that the truncated message is unstable. The patient's second mutation consists of an R397W missense mutation involving an arginine residue that is conserved from mice to humans but is not part of a specific signaling or binding domain. PCR amplification and restriction-site analysis verified that the patients' two mutations segregate with HPS-3 disease in the family (fig. 4).

Patient 34 exhibited an 89-bp insertion of a portion of intron 16. In attempting to explain how this insertion arose, we noted that FLJ22704, a cDNA clone coding for a protein of unknown function, contains a 103-bp insertion between HPS exons 16 and 17 (called "exon 16A," nt 44089–44191). We speculate that this clone represents an alternatively spliced form of *HPS3* that is normally very weakly expressed. (In fact, we were able to amplify a band from fibroblast cDNA, using a primer located in exon 16A). In patient 34, a G→A mutation at nt 44101, within exon 16A, creates a very strong new splice site, resulting in abundant expression of the 89-bp insertion. The mutated RNA, which contains 23 new amino acids and a premature stop at codon 986, appears stable, because northern blot analysis shows an mRNA signal slightly larger than the normal 4.4-kb *HPS3* mRNA transcript. It is even possible that translation of a truncated protein takes place in this patient.

The second mutation of patient 34, a mutation that is as yet unidentified, is probably severe, because no normal-sized *HPS3* mRNA is expressed by the patient's fibroblasts (fig. 2*B*) and because no normal band appears on PCR amplification of the cDNA region that includes exons 16 and 17 (fig. 5*A*). This suggests a large deletion or a promoter mutation.

Perhaps our most significant finding is the existence of a common mutation in *HPS3* among patients of Ashkenazi Jewish descent. Five of our six Ashkenazi patients with HPS had mutations in *HPS3*, and 8 of their 10 alleles carried the 1303+1G→A splice-site mutation that removes exon 5. This finding, along with the identification of one heterozygous carrier of the mutation among 235 DNA samples from anonymous Ashkenazi Jews, suggests a founder effect among the Ashkenazim. HPS is not recognized as a disorder that affects Jews (Zlotogora et al. 2000), nor has it been listed among the types of albinism in Israel (Gershoni-Baruch et al. 1994). Certainly, the mildness of the patients' hypopigmentation and bleeding contributes to this situation. Nevertheless, our findings show a need for heightened

awareness of the possibility of HPS among Ashkenazi Jews, particularly those with some degree of albinism or platelet storage-pool deficiency. For these individuals, HPS-3 disease and the 1303+1G→A splicing mutation in *HPS3* should be considered first. Further screening of Ashkenazi Jewish populations may help to determine the true frequency of the exon 5 splicing mutation in this population, and haplotype analysis may allow estimation of when the putative founder mutation occurred (Luria and Delbruck 1943; Anikster et al. 2001).

The preponderance of splicing mutations in patients who have HPS-3 along with drastically reduced mRNA production suggests that, in general, missense mutations may not result in a clinically recognizable phenotype. The HPS-3 disease associated with severe *HPS3* mutations is relatively mild, so patients with less-severe mutations may be indistinguishable from individuals without *HPS3* mutations.

The non–Puerto Rican HPS-3 patients we describe here enhance our understanding of HPS in other ways. We know that the *HPS3* founder mutation causes relatively mild disease among the few central Puerto Ricans we have examined (Hazelwood et al. 1997; Anikster et al. 2001). Clearly, the same is true for other *HPS3* mutations affecting non–Puerto Ricans. Hair hypopigmentation (fig. 7*A*), iris transillumination (fig. 7*B*), and visual acuity deficits (table 1) were less severe than those typically seen among patients with HPS-1 (Iwata et al. 2000). In the present study, two individuals (patients 86 and 100) had such mild skin and hair hypopigmentation that they carried the diagnosis of ocular, rather than oculocutaneous, albinism for more than a decade. The patients' histories of bleeding were not impressive; reduction in forced vital capacity was minimal, if present at all; and no history of colitis was elicited. This sample of patients with HPS-3 is small, however, and more and older patients must be examined to determine whether pulmonary and gastrointestinal disease will accompany *HPS3* mutations.

Strategies for the molecular diagnosis of patients with HPS are now becoming better defined. Obviously, patients of northwest Puerto Rican ancestry should be studied by PCR amplification for the 16-bp duplication in *HPS1* (Oh et al. 1996), and patients from central Puerto Rico should be analyzed by use of multiplex PCR for the 3,904-bp deletion in *HPS3* (Anikster et al. 2001). The few patients with HPS who have histories of childhood infections or neutropenia can be suspected of having HPS-2, with mutations in *ADTB3A* (Dell'Angelica et al. 1999; Shotelersuk et al. 2000). Ashkenazi Jewish individuals, especially those with mild phenotypes, should be examined using restriction enzyme analysis of genomic DNA to detect the 1303+1G→A splice-site mutation. Other mildly affected patients can be investigated by amplifying *HPS3* cDNA in three overlapping fragments or, if RNA is not available, by amplifying and sequencing genomic DNA exon by exon. *HPS1* mutations can be pursued in a similar fashion. As an estimate of the relative likelihood of HPS-1, HPS-2, and HPS-3 disease, we found 7 patients (6 families) with *HPS1* mutations, 3 patients (2 families) with *ADTB3A* mutations, and 8 patients (8 families) with *HPS3* mutations among 43 patients with HPS (38 families) not homozygous for the founder *HPS1* or *HPS3* mutations.

We expect that each HPS-causing gene will provide unique insights into the cell biology of vesicle formation, specifically the creation of melanosomes, dense bodies, and lysosomes from the trans-Golgi network. These organelles are considered to have a common genesis and are known to share certain integral membrane proteins (Shotelersuk et al. 1998; Huizing et al. 2000); they are also abnormal to various degrees in patients with HPS. Patients with HPS-2 provide the most-direct evidence that HPS is a disorder of vesicle formation. In these individuals, *ADTB3A* mutations cause defective production of β3A, a subunit of the coat protein AP-3. This heterotetrameric complex mediates the formation of vesicles, apparently including the melanosome, dense body, and lysosome. HPS-2 fibroblasts display mistrafficking of the lysosomal membrane protein CD63 through the plasma membrane (Dell'Angelica et al. 1999). HPS-2 melanocytes exhibit misrouting of tyrosinase, which does not display a normal melanosomal distribution (Huizing et al. 2001*a*). The association of β3A mutations with HPS and with membrane-trafficking defects suggests that the products of *HPS1* and *HPS3* also function in vesicle formation, although no direct evidence to this effect has been forthcoming. It will be of critical importance to determine whether the *HPS3* gene product interacts with either the *HPS1* or the *ADTB3A* gene product, and these studies are ongoing.

Because the phenotype of HPS can be extremely mild with respect to both pigmentation and bleeding, it may be appropriate to screen for HPS among patients who have some degree of hypopigmentation. Three separate HPS-causing genes are known, so molecular studies could confirm the initial diagnosis. Identification of patients with a bleeding diathesis among those with hypopigmentation would assist in prognosis as well as prophylaxis against serious bleeding episodes or exposure to lung toxins in selected patients. We suggest that HPS screening be performed by whole-mount electron microscopy for identification of platelet dense granules in individuals with oculocutaneous, or even ocular, albinism. A recent study showed that 35% of a German albino population had no mutations in either the tyrosinase or the *P* gene (Passmore et al. 1999); these patients are candidates for having mild HPS.

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Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *HSP1* [accession number U65676], *ADTB3A* [accession number U91931], *HPS3* genomic DNA [accession number AF375663], and *HPS3* cDNA [accession number AY033141])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for OCA1 [MIM 203100], OCA2 [MIM 203200], OCA3 [MIM 203290], Chediak-Higashi syndrome [MIM 214500], Griscelli syndrome [MIM 214450], Hermansky-Pudlak syndrome [MIM 203300], HPS-1 [MIM 604982], HPS-2 [MIM 603401], and HPS-3 [MIM 606118])

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