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Three New Mutations in a Gene Causing Hermansky–Pudlak Syndrome: Clinical Correlations

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Hermansky–Pudlak syndrome (HPS) consists of oculocutaneous albinism, a platelet storage pool deficiency, and ceroid lipofuscinosis. HPS is common in northwest Puerto Rico, where affected individuals are homozygous for a 16-bp duplication in the gene *HPS*. Two other homozygous frameshift mutations in *HPS* were previously identified among non-Puerto Rican patients. Eighteen non-Puerto Rican HPS families were studied and *HPS* mutations in three of them identified. One mutation, T322insC, has been previously described. However, three additional mutations, E133X, T322delC, and S396delC, have not been reported. Two families exhibited compound heterozygosity for these mutations, although most previously reported HPS patients have been homozygous for a particular mutation. All the newly described mutations were associated with decreased or undetectable levels of *HPS* RNA by Northern blot analysis of fibroblasts, and all had significant pigment dilution. To date, all mutations in *HPS* result in a truncated protein, suggesting that the C-terminal portion of the HPS protein is functionally important. © 1998 Academic Press

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Hermansky–Pudlak syndrome (HPS) is an autosomal recessive, multisystem disorder characterized by oculocutaneous albinism, a bleeding tendency, and lysosomal accumulation of ceroid lipofuscin (1,2). The albinism manifests with congenital nystagmus, iris transillumination, decreased visual acuity, and variable reductions in skin pigmentation (3,4). The bleeding diathesis is due to the absence of dense bodies in platelets; electron microscopic evidence of this provides the *sine qua non* for diagnosing HPS (5). Ceroid lipofuscin consists of an incompletely characterized lipid–protein complex thought to accumulate in cellular lysosomes and believed to cause pulmonary fibrosis (6,7) and granulomatous colitis (8) in some patients. The pulmonary involvement of HPS begins with restrictive disease and progresses inexorably to death, usually in the fourth or fifth decade (2). Although rare in the general population, HPS is quite frequent in northwest Puerto Rico, where it occurs with a prevalence of 1 in 1800 (9) due to a founder effect. A pocket of HPS has also been reported in an isolated village in the Swiss Alps (10,11).

Recently, an HPS-causing gene was mapped by linkage analysis to chromosome 10q2 (12,13). The gene was subsequently identified, its cDNA sequenced (14), and the organization of the gene characterized (15). The HPS gene has 20 exons coding for a 700-amino-acid, 79.3-kD protein of unknown function, with no known homology to other proteins. Only three mutations in the *HPS* gene have been reported to date. A homozygous 16-bp duplication in exon 15 was found in all northwest Puerto Rican HPS patients (14), and appears to be associated with an increased risk for pulmonary fibrosis (16). A homozygous 1-bp insertion known variously as

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T322insC or P324insC (14) was found in six Swiss patients and one Irish patient, and a homozygous 1-bp insertion at codon A441 was reported in a single inbred Japanese patient (14). A recent article noted mutation hotspots in the *HPS* gene (17), with mention of several specific mutations. In addition, two HPS patients from central Puerto Rico completely lacked a mutation in *HPS*, strongly suggesting locus heterogeneity for the disease (18).

We studied 22 non-Puerto Rican HPS patients in 18 families and identified three new mutations in the *HPS* gene, i.e., two frameshifts and a nonsense mutation. All resulted in decreased or undetectable levels of *HPS* RNA by Northern blot analysis. This represents the first report of compound heterozygosity in HPS patients, whose clinical characteristics are also presented.

MATERIALS AND METHODS

Subjects

All patients were enrolled in a protocol approved by the NICHD Institutional Review Board to study the clinical, biochemical, and molecular aspects of HPS. Informed consent was obtained from each patient or the patient's parents. All patients were previously diagnosed by absence of platelet dense bodies on wet-mount electron microscopy by Dr. James G. White of the University of Minnesota. For consistency in future publications, patient numbers correspond to those of a master file of all NICHD patients with HPS.

Polymerase Chain Reaction Detection of the 16-bp Duplication

Genomic DNA was extracted from peripheral leucocytes isolated from acid citrate dextrose-(ACD)-anticoagulated blood using a standard protocol (19). A 269-bp fragment spanning exon 15 of *HPS* was amplified by polymerase chain reaction (PCR) as previously described (14,18). PCR products were electrophoresed in 3% agarose and stained with ethidium bromide.

Reverse Transcription (RT) PCR and Subcloning

Prior to publication of the intron/exon boundaries of the *HPS* gene (15), we performed mutation analysis on cDNA fragments. Total RNA was extracted from cultured fibroblasts using Trizol reagent, and cDNA was synthesized by reverse transcription using 5 μg of total RNA, according to the manufacturer's protocol (Gibco-BRL, Grand Island, NY). The 2.1-kb coding region of the *HPS* cDNA was amplified using seven sets of primers as previously described (18). PCR amplification was performed using 2 μl of the first-strand cDNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dGTP, 200 μM dATP, 200 μM dTTP, 200 μM dCTP, 0.2 μM primers, and 2.5 U *Taq* DNA polymerase in a final volume of 50 μl. After an initial denaturation step at 94°C for 3 min, PCR amplification was conducted for 30 cycles with a denaturation step at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 2 min, and a final elongation step at 72°C for 10 min. For patient 31, the primer sequences 5′-GCTTGCCAAGATGAAGTGCGTC-3′/5′-GAACAGTGGCAAGCAAGGGTG-3′, corresponding to nucleotides neg9-12 and 2166–2146 (18), were employed. The PCR products were subcloned into the vector pCR 2.1 (Invitrogen, Carlsbad, CA), and clones were selected and sequenced using primers designed for fragments HP-2 and HP-3 (18).

Single-Strand Conformational Polymorphism Analysis

For some patients, single-strand conformational polymorphism (SSCP) analysis was performed on cDNA fragments, obtained as described above. After the *HPS* gene organization was reported (15), each exon was amplified from genomic DNA (300 ng) in a 25-μl reaction using a radioactive PCR mixture consisting of 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dGTP, 200 μM dATP, 200 μM dTTP, 200 μM dCTP, 1 μCi [α-^{32}P]dCTP, 0.2 μM primers, and 2.5 U *Taq* DNA polymerase. The primers were derived from intronic sequences and employed under conditions previously described (15). The PCR products were denatured with 10 μl of formamide and heating at 94°C for 2 min, and were fractionated at room temperature and/or at 4°C by electrophoresis for 14–16 h on a 0.5× MDE gel (AT Biochemical, Malvern, PA). The gel was prepared in 0.6× TBE, with or without 5% glycerol, and subjected to autoradiography.

Direct DNA Sequencing

Sequencing was performed using a Plasmid Sequencing Kit or a PCR Product Sequencing Kit from Amersham Life Science Inc. (Cleveland, OH). Electrophoresis was performed using a 5.5% Long Ranger precast gel (Stratagene, La Jolla, CA). All PCR-generated products were sequenced in both the forward and reverse directions. For patient 33, PCR amplification and sequencing of exon 13 were also performed with

a second pair of primers, i.e., 5′-TGATGATGCTTA-GGGTTGGC-3′/5′-TGGGGAGGACTGGAACTTG-3′. The 5′ primer, contained within intron 12, overlapped the previously published primer (15) by 12 bp, but the 3′ intron 13 primers did not overlap each other.

Northern and Southern Blots

Total RNA was isolated from cultured fibroblasts using Trizol reagent (Life Technologies, Grand Island, NY), and 20 μg was electrophoresed on a 1.2% agarose/3% formaldehyde gel and blotted onto a Nytran nylon membrane (Schleicher & Schuell, Keene, NH) in the presence of 20× SSC. The blot was prehybridized and then hybridized with ExpressHyb solution (Clontech, Palo Alto, CA) at 68°C, and probed using full-length human *HPS* cDNA random primer labeled with [α-^{32}P]dCTP (duPont/NEN, Wilmington, DE). After hybridization, the blot was washed several times at room temperature with 2× SSC/0.05% SDS and once with 0.1× SSC/0.1% SDS at 50°C and exposed to Kodak XAR film with an intensifying screen for 24 h at −70°C.

For Southern blots, genomic DNA (10 μg) was digested overnight with 50 U of *Eco*RI, *Alu*I, or *Xba*I (Gibco BRL), and the products were electrophoresed at 20 V in 1× TAE buffer overnight on a 0.7% agarose gel. The DNA was denatured and blotted onto a Nytran nylon membrane according to the manufacturer's "neutral" protocol (Schleicher & Schuell, Keene, NH). Hybridization was performed as for the Northern blots, but with a 48-h exposure.

Genotype Analysis

For genotype analysis of patient 33 and her parents, 13 microsatellite markers spanning chromosome 10, as well as 10 other markers representing chromosomes 6, 7, 8, 9, 12, 13, and 14, were obtained from Bioserve Biotechnologies, Laurel, Maryland, or Research Genetics, Huntsville, Alabama. PCR products were labeled using random priming and [α-^{32}P]-dCTP, denatured, and analyzed on 6% denaturing gels.

RESULTS

Of 22 non-Puerto Rican patients representing 18 independent families, none exhibited the 16-bp duplication common among Puerto Rican patients (14,18). However, 4 of these patients had pathological mutations in the *HPS* gene, suggested by SSCP analysis and verified by sequencing. Their clinical characteristics are described below.

Case Reports

Patient 31 was a 6-year-old girl of Italian, German, and Ukranian ancestry. Born with pale skin, she was noted to have nystagmus at 2 months of age and pale retinas at 3 months of age, when oculocutaneous albinism was diagnosed. Bruising began at 7–8 months of age, and HPS was diagnosed at 18 months based on abnormal platelet aggregation studies. Epistaxis occurred in the winter months, and prolonged bleeding accompanied a cut lip and placement of myringotomy tubes. Asthma was diagnosed at 1 year of age. There were no signs of colitis or pulmonary fibrosis.

Height was 119 cm (75%), weight 21.8 kg (75%). The patient's hair was straw-colored and her skin was fair (Fig. 1A). Ophthalmic examination showed a best-corrected visual acuity of 20/100 in each eye with a moderate hyperopic astigmatic correction. Subtle horizontal nystagmus was observed in primary position and became more evident on lateral gaze. This girl also had a 30 prism diopter exotropia. In each eye, the iris had moderate pigmentation but the fundus showed remarkable hypopigmentation and foveal hypoplasia (Fig. 1A). The macula was moderately transparent. The remainder of the physical examination was normal. Blood and urine chemistry values were normal. The platelet count was 311,000/mm^3.

Patient 33 was a 9-year-old girl of Italian, English, Irish, French, and Norwegian ancestry diagnosed with oculocutaneous albinism at 2 weeks of age. Bruising at age 1 year led to the diagnosis of HPS at 18 months of age. Severe bleeding granulomatous colitis required numerous and repeated platelet and whole blood transfusions between ages 2.7 and 6.7 years. This patient has been described by others as patient 2 of a report on granulomatous colitis in HPS (20). Current therapy includes hydrocortisone, 6-mercaptopurine, and cyclosporine. Other complications of her disease or its treatment include osteoporosis and compression fractures, *Candida* esophagitis, repeated urinary tract infections, supraventricular tachycardia, hypotensive episodes, mild renal tubular Fanconi syndrome, and occasional epistaxis, which resolved on administration of 1-desamino-8-D-arginine vasopressin (21).

Height was 122.4 cm (3 cm below the 5th centile), weight 31.9 kg (50th centile). The patient was mildly

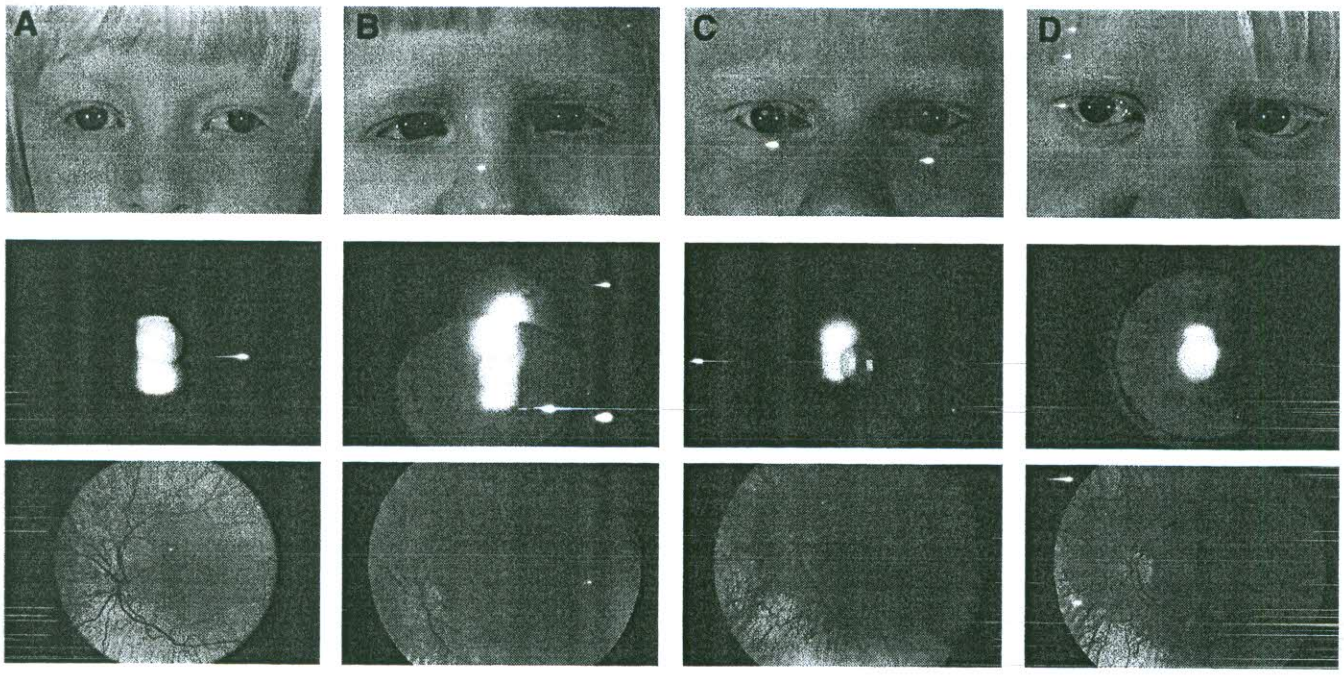

FIG. 1. Pigmentation in patients with defined mutations. Top panel shows skin and hair, middle panel shows iris transillumination, and bottom panel shows the fundus. (A) Patient 31 has white hair and skin. Moderate iris pigmentation prevents complete transillumination, but the fundal hypopigmentation is remarkable. (B) Patient 33 has blond hair and light skin, with marked iris transillumination and fundal hypopigmentation peripherally. (C, D) Patients 39 and 41 have white hair, light skin, marked transillumination of the iris with some residual pigmentation, and peripheral hypopigmentation of the fundus.

Cushingoid in appearance. Skin was pale and hair was tan (Fig. 1B). Ophthalmic examination revealed best-corrected visual acuities of 20/200 and 20/160, with moderate hyperopic astigmatism. Horizontal and rotatory nystagmus and an alternating esotropia were observed. Iris transillumination was marked in both eyes (Fig. 1B), with no pigmentation identified on slit-lamp biomicroscopy. On fundus examination, there was marked peripheral hypopigmentation with foveal hypoplasia. The macula was not transparent. The remainder of the physical examination was normal. The creatinine clearance was 88 ml/min/1.73 m^2. Pulmonary function tests were normal. Platelet count was 464,000/mm^3. Functional platelet von Willebrand factor was 37% (normal, 44–150%). All other blood and urine tests were normal.

Patient 39 was a 17-year-old man of Dutch and German ancestry with nystagmus from birth. He was diagnosed with HPS at approximately 1 year of age. The patient bruised easily as a child and sufferred from episodes of prolonged epistaxis at ages 6, 13, and 14. He had no other major bleeds despite playing fullback for his high school football team. There were no pulmonary or gastrointestinal symptoms.

Height was 181 cm; weight 88 kg. Hair and skin pigmentation were light (Fig. 1C). Best-corrected visual acuity was 20/200 in each eye with a hyperopic astigmatic correction. Horizontal and rotatory nystagmus and exotropia were observed. Transillumination of the iris was marked with some pigmentation (Fig. 1C). The fundus was hypopigmented in the periphery, with foveal hypoplasia. The macula was not transparent. The remainder of the physical examination was normal. All urine and blood tests were normal, including a platelet count of 199,000/mm^3.

Patient 41, the sister of patient 39, was a 21-year-old woman with stable nystagmus since birth. The diagnosis of albinism was made at 6 months of age, and HPS was diagnosed at 4 years of age. Bruising occurred at the time of ambulation. An episode of prolonged epistaxis took place at age 12, but menarche, at age 13, was uneventful. There were no other major bleeding episodes. Gastrointestinal and pulmonary symptoms were lacking. The patient was a successful college student.

Height was 171 cm; weight 82.7 kg. Hair and skin pigmentation were very light (Fig. 1D). On ophthalmic examination, the best-corrected visual acuity

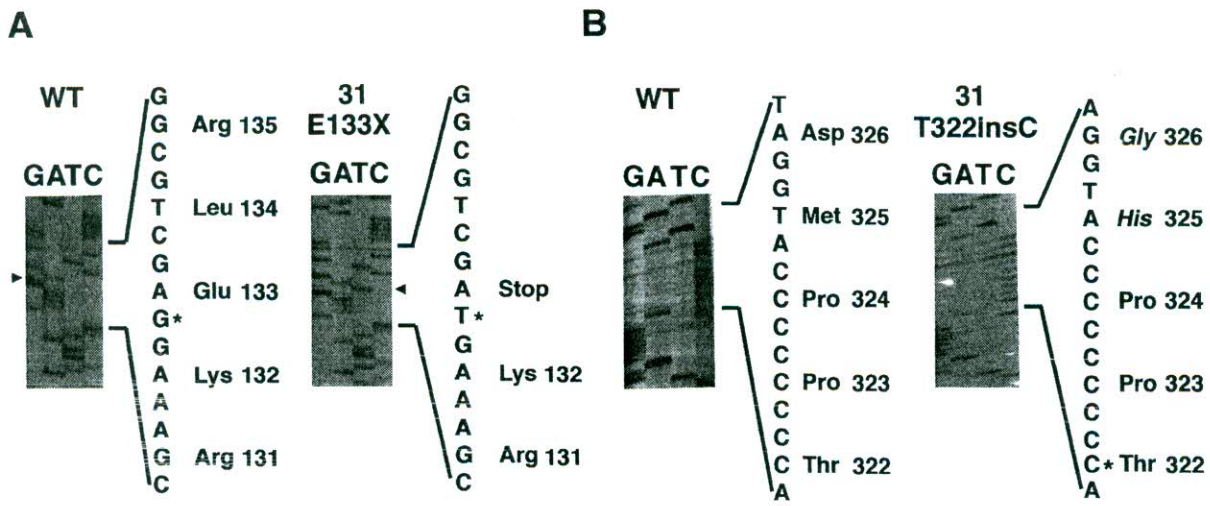

FIG. 2. Cloned cDNA sequencing results for both alleles of patient 31. (A) Normal or wild-type (WT) sequence includes a G at nucleotide 397 (arrowhead). In one allele of patient 31, a T replaces the G (asterisks), yielding the terminator codon TAG. (B) Single-base-pair insertion at codon 322 in the second allele of patient 31. Normal (WT) sequence has a string of eight cytidine residues, whereas patient 31 has nine cytidine residues (asterisk). This frameshift alters downstream amino acids. Sequencing was performed on clones in the 5′ → 3′ direction and verified in the reverse direction.

was 20/160 in each eye, with a hyperopic astigmatic correction. Horizontal and rotatory nystagmus was noted but no strabismus was found. Transillumination of iris was marked with some pigmentation (Fig. 1D). Fundus examination revealed hypopigmentation in the periphery and foveal hypoplasia. The macula was not transparent. The remainder of the physical examination was normal. The platelet count was 201,000/mm^3; all other blood and urine tests were also normal. Chest radiograms and pulmonary function tests were normal.

Mutation Analysis

Direct genomic sequencing of DNA from patient 31 revealed a nonsense mutation at codon E133 of exon 5 and a single-base insertion at codon T322 of exon 11 (Fig. 2). Reverse transcription (RT)-PCR amplification, followed by subcloning and sequencing of cDNA, revealed that the two mutations were located on separate alleles, represented by two separate clones. Therefore, patient 31 represents a compound for the two mutations E133X and T322insC.

Sequencing of exon 13 of patient 33 revealed only S396delC (Fig. 3). Her mother is a carrier of the S396delC mutation, but no mutations were apparent in the father (Fig. 3). Microsatellite analysis, performed for 23 markers on eight different chromosomes, demonstrated that the patient received one allele from each of her parents (data not shown). None of the data were consistent with nonpaternity or maternal uniparental isodisomy. Specifically, all 13 chromosome 10 markers gave results consistent with biparental inheritance, including markers D10S2437 and D10S2436, located 80 kb centromeric and 130 kb telomeric, respectively, to the *HPS* gene. Intron 12 of the patient and her father contained entirely normal sequence, and sequencing of exon 13, using two different 3′ primers, confirmed that the patient had only S396delC while the father showed no abnormalities. Southern blots were performed in an attempt to detect small deletions in the father's DNA. *Eco*RI, *Alu*I, and *Xba*I digests, probed with *HPS* cDNA, showed normal restriction patterns and normal band intensities for patient 33, her mother, and her father (data not shown).

Patients 39 and 41 exhibited compound heterozygosity for T322delC in exon 11 and S396delC in exon 13 (Fig. 4). Sequencing analysis of their parents revealed that the father carries the T322delC mutation and the mother is heterozygous for S396delC (data not shown).

Northern blots revealed absence of *HPS* RNA in fibroblasts of patients 33, 39, and 41. Patient 31 had a decreased amount of *HPS* RNA (Fig. 5).

Patients Lacking HPS Mutations

Eighteen patients, representing 15 independent families, were not found to have mutations in the

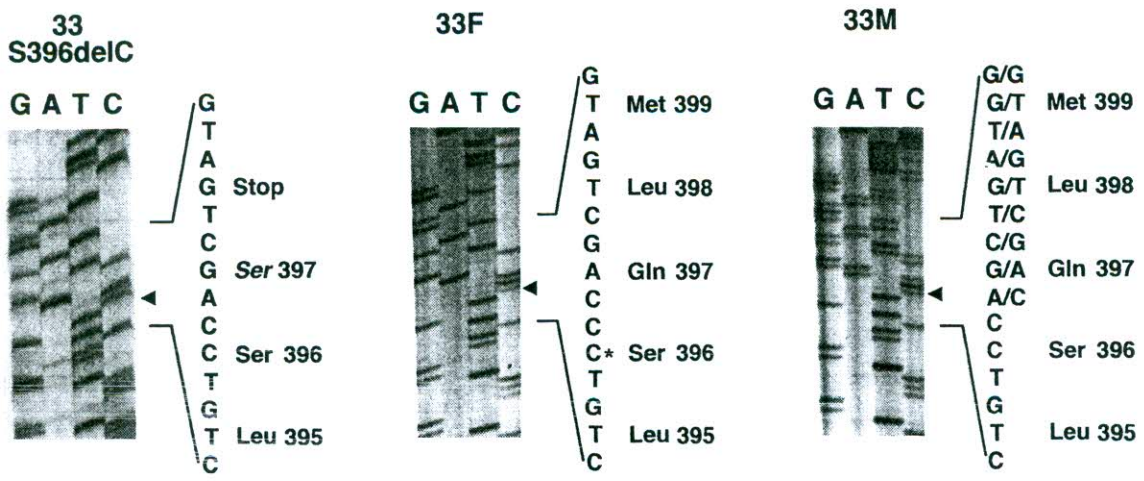

FIG. 3. Mutation analysis of patient 33 and her parents. Direct sequencing was performed on genomic DNA in the 5′ → 3′ direction and verified in the reverse direction. Patient 33 has two cytidine residues at codon 396 (arrowhead), where her father (33F) and all normal individuals have three cytidines. The patient's C deletion results in a termination signal at codon 398. The patient's mother (33M) shows heterozygosity, i.e., doublets after the single base deletion.

HPS gene. Sixteen of these 18 had a normal amount and size of *HPS* mRNA (data not shown); RNA was not available from one patient, and another patient had a reduced amount of normally sized *HPS* RNA. Nevertheless, none of the 18 patients showed any evidence of a mutation in *HPS*. For six patients, genomic DNA was subjected to SSCP analysis of each exon, and all aberrant SSCP patterns were pursued by direct exon sequencing. No pathological mutations were discovered. For the remaining 12 patients, SSCP analysis was performed on cDNA segments of the *HPS* gene, as previously described (18). Again, abnormal patterns were followed up by sequencing, and no pathological changes were found.

Sequencing of exons showing abnormal SSCP patterns did reveal a variety of polymorphisms. Some, including G283W, IVS14, +7, +8, IVS16, −15, and R603Q, were previously reported (15). Others are

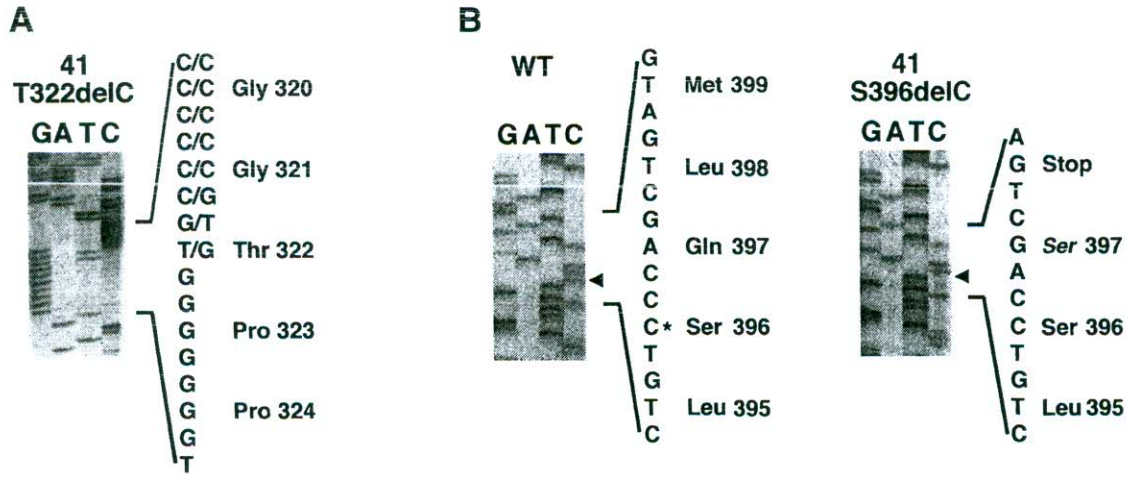

FIG. 4. Mutation analysis of patient 41. (A) Direct genomic DNA sequencing showing the C deletion at codon 322, which appears as a deleted G in a run of eight guanine residues on this 3′ → 5′ sequencing gel. Doublets above the deletion signify heterozygosity for this mutation. (B) Sequencing was also performed on cDNA clones of patient 41 in the 5′ → 3′ direction. The series of three cytidines in the normal (WT) sequence has been altered in patient 41 by a C deletion (arrowhead) at codon 396, resulting in a downstream termination signal. All sequencing was verified in both directions. A brother, patient 39, also displayed the T322delC and S396delC mutations.

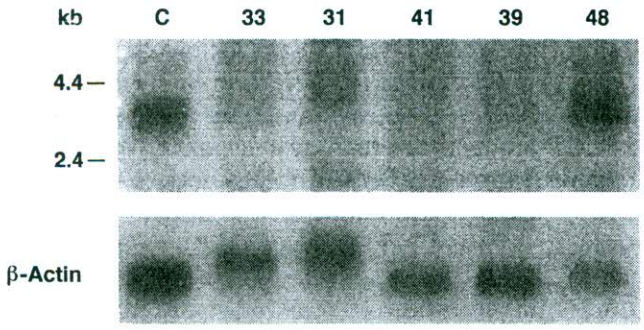

FIG. 5. Representative Northern blot of patient RNA, probed with the *HPS* gene cDNA. The *HPS* RNA band runs at approximately 3.0 kb. Control (C, lane 1) and a typical non-Puerto Rican HPS patient (patient 48, lane 6) have a normal contingent of *HPS* RNA. Patients 33 (lane 2), 41 (lane 4), and 39 (lane 5) lack *HPS* RNA. Patient 31 has a reduced amount of *HPS* RNA. β-Actin message was used to control for the amount of RNA loaded on the gel. Different migration patterns appear to be related to variable degrees of RNA degradation.

newly described, and consist of the silent mutations L212L (CTC → CTT), F538F (TTC → TTT), and I545I (ATC → ATA).

DISCUSSION

Each of the three previously reported, disease-causing mutations in the *HPS* gene occurred in the homozygous state, generally within an inbred population arising outside the U.S. mainland (14). The Puerto Rican 16-bp duplication is the most common, but the Swiss and Irish share the T322insC mutation, and the A441insA mutation was found in a consanguineous Japanese family. All these mutations involve insertional frameshifts predicted to yield no normal, intact HPS protein.

Studying an American population of more diverse and integrated ethnicities, we present new information regarding *HPS* gene mutations. Three patients (31, 39, and 41) exhibited compound heterozygosity for *HPS* (Table 1). Patient 31, although of Italian, German, and Ukrainian heritage, carried the Swiss/Irish T322insC mutation; the additional cytidine in a run of eight results in termination of translation at codon 452. On her other allele, this girl exhibited a nonsense mutation in the *HPS* gene, E133X. Patients 39 and 41 are also compound heterozygotes, having received an S396delC mutation from their German/Dutch mother and a T322delC mutation from their German father. The S396 deletion of cytidine in a row of three results in termination at codon 398; the C deletion at codon T322 in a run of eight causes termination at codon 330. The T322delC mutation, as well as the T322insC mutation, occurs at a mutation "hotspot" reported by Oh *et al.* (17).

Patient 33 represents a novel case of either homozygosity or hemizygosity for the S396delC mutation in exon 13. The patient herself shows only one mutation, for which her Irish/English/French/Norwegian mother is heterozygous, while her Italian/English father shows only normal sequence at codon 396. Nonpaternity and maternal uniparental isodisomy of chromosome 10 were extremely unlikely, based on microsatellite analysis. The possibility of a mutation in the primer annealing sites causing no amplification of the allele was excluded by normal sequence of intron 12 in the proband and her father and by the use of two sets of nonoverlapping primers in intron 13. The lack of consanguinity, as well as absence of the mutation in the father, mitigates against the possibility of homozygosity. Other explanations include the unlikely possibility that a new mutation of S396delC occurred on the paternally derived allele at exactly the site of the maternally inherited mutation. Paternal germline mosaicism may also have occurred. The most likely explanation for this situation, however, is that the patient inherited from her father a deletion of a region of *HPS* including exon 13. Southern blot analysis did not demonstrate a deletion in the 33-kb *HPS* gene; reduced intensity or size of some bands might be expected if there were a small deletion in the region. However, it remains possible that a relatively large deletion in the area of exon 13 was passed from father to daughter.

All mutations in the *HPS* gene reported to date (Table 1) have been frameshift or nonsense mutations, meaning that they predict a truncated HPS protein. Consequently, some missense mutations

TABLE 1
Summary of *HPS* Gene Mutations

	Ethnicity	Severity
Previously published (14)		
16-bp dup/16-bp dup	Puerto Rican	+++
T322insC/T322insC[a]	Swiss/Irish	+
A441insA/A441insA	Japanese	+++
This paper		
T332insC/E133X	Italian/German/Ukrainian	++
S396delC/(S396delC)	Italian/English/Irish/ French/Norwegian	++++
T322delC/S396delC	Dutch/German	++

[a] Same as P324 of Ref. (14).

may result in a functional *HPS* gene product, and individuals carrying such mutations may be clinically completely normal. This may also account for the large number of polymorphisms in the *HPS* gene reported here and elsewhere (14,15).

Expression studies in HPS have been reported only for the Puerto Rican 16-bp duplication, in which no *HPS* mRNA was detected (18). We now report that homozygosity (or hemizygosity) for S396delC, as well as compound heterozygosity for T322delC and 396delC, yields no *HPS* mRNA, while compound heterozygosity for T322insC and E133X allows some residual *HPS* RNA synthesis (Fig. 5). This residual expression may have been contributed by either of patient 31's two mutations, and it may have permitted production of the substantial amount of pigmentation in her iris (Fig. 1A, middle panel).

Relatively little genotype/phenotype correlation can be inferred from current findings regarding the *HPS* gene. Patients 31, 33, 39, and 41 all have significant pigment dilution manifest in albinotic skin and hair (Fig. 1). However, pigmentation alone does not define disease severity. Furthermore, the compound heterozygosity of patients 31, 39, and 41 precludes associating a mutation with a specific degree of disease severity in these cases. Patient 33, on the other hand, had only a single *HPS* gene mutation, whether hemizygous or homozygous, and manifested serious granulomatous colitis. A severe phenotype might, then, be attributed to the S396delC mutation, with one caveat. If patient 33 represents a hemizygous individual, then a significant deletion in one of her alleles could cause mutations at loci adjacent to *HPS*, and these may have enhanced the severity of this girl's disease.

In general, the disease severity in our patients is consistent with the hypothesis of Oh *et al.* (14), who suggested that longer HPS peptides, containing both of the protein's putative transmembrane domains, may be more harmful because they allow incorporation of the defective protein into membranes. The two putative transmembrane domains, at amino acids 79–95 and 369–396, would be included in the polypeptide of patient 33 (S396delC) and one of the two *HPS* gene products of patients 39 and 41, as well as the polypeptides produced by the Puerto Rican 16-bp duplication and the A441insA mutations, all of which result in severe disease. However, mutations such as the T322insC and E133X of patient 31 and the T322delC of patients 39 and 41 all occur between the two transmembrane domains, perhaps making incorporation into membranes less likely and possibly explaining their milder phenotypes.

Our data support the previously reported evidence (18) that the disease HPS displays locus heterogeneity. Only 4 of 22 patients (3 of 18 families) exhibited mutations in *HPS* when examined by Northern blot and SSCP analysis followed by sequencing of suspicious gene fragments. These findings are not surprising, in view of the numerous mouse models of HPS mapped to different genetic loci (22,23). Recent evidence indicates that one mouse model, pearl or *pe*, results from mutation of the β3A subunit of the adaptor-3 complex, which appears responsible for proper trafficking of pigment-forming vesicles (24,25). It remains to be determined if pearl or other murine models such as mocha, due to mutation of the delta subunit of the adaptor-3 complex (26), correspond to human HPS subtypes.

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