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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