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# A New Variant of Hermansky-Pudlak Syndrome due to Mutations in a Gene Responsible for Vesicle Formation

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Hermansky-Pudlak syndrome is a recessive type of oculocutaneous albinism that is prevalent in northwest Puerto Rico due to a founder effect (1–3). In this syndrome, bleeding and bruising occur because of the absence of platelet dense bodies, which normally release serotonin, calcium, and adenosine diphosphate to trigger a secondary aggregation response (4). In addition, the accumulation of a lipid-protein complex called ceroid lipofuscin (5,6) is thought to cause the pulmonary fibrosis (7) and granulomatous colitis (8) seen in this disease.

One gene causing Hermansky-Pudlak syndrome, *HPS-1*, encodes a 700 amino acid protein of unknown function (9–11). Northwest Puerto Rican patients are homozygous for a 16 base pair (bp) duplication in *HPS-1*, but most non-Puerto Rican patients have no mutations in this gene (12,13). Consequently, it has become accepted that several different genes, when mutated, can cause Hermansky-Pudlak syndrome (12–14). This phenomenon, called locus heterogeneity, is also found in mice: 14 different mouse strains manifest a type of Hermansky-Pudlak syndrome (pigment dilution and platelet storage pool deficiency), each due to a different gene (15). To date, three of these genes have been cloned. *Pale ear* is the murine analogue of patients with *HPS-1* mutations (16,17), and *pearl* (18) and *mocha* (19) have defects in adaptor complex-3 (AP-3). One protein subunit of adaptor complex-3, called β3A, is mutated in the *pearl* mouse, while another protein subunit, called δ, is mutated in the *mocha* mouse.

Adaptor complex-3 is an aggregate of four different peptides and serves as a "coat" protein that concentrates in a donor membrane and recruits other membrane components to become part of a newly formed vesicle. These vesicles, such as lysosomes and peroxisomes, are functional compartments that provide an optimal environment for specialized biochemical processes. Adaptor complex-3 is thought to be responsible for the formation of pigment-forming vesicles (melanosomes) and platelet storage vesicles (dense bodies) (20,21).

We describe two brothers with Hermansky-Pudlak syndrome with mutations in the β3A subunit of adaptor complex-3 (22,23).

## CASE REPORTS

We admitted 49 Hermansky-Pudlak syndrome patients to the NIH Clinical Center under an Institutional Review Board-approved protocol (24). Patient 40, aged 20 years, and patient 42, aged 25 years, were brothers who had normal gestations, deliveries, and birth weights. Bilateral congenital hip dislocations, due to dysplastic acetabulae, required closed reduction in patient 40 and splinting in patient 42. The family was of Dutch origin with no known consanguinity or miscarriages. The brothers' healthy parents had two normal, unaffected sons.

Nystagmus was observed in the newborn period. Skin color was light, and the brothers' white hair gradually turned blond. Bruising occurred in early childhood, with recurrent epistaxis decreasing in frequency by adolescence. In patient 42, extraction of several teeth was performed with minimal bleeding. Neither brother experienced hemoptysis, hematemesis, hematochezia, or melena. Hermansky-Pudlak syndrome was suspected when the patients were 8 and 13 years old, and absence of platelet dense bodies was documented by Dr. James White of the University of Minnesota.

From infancy to adolescence, the brothers had recurrent upper respiratory tract infections and episodes of otitis media that responded to antibiotic treatment. Neutropenia was consistently noted. The patients' unaffected siblings did not have recurrent upper respiratory tract infections.

Developmentally, the brothers achieved major milestones on time, but complained of poor balance causing stumbling and falling. They completed 11th grade and work on a family farm and nearby factory. Patient 42 smoked one half to 1 pack of cigarettes daily since age 13 years; patient 40 never smoked. Patient 40 had a physiologic cardiac murmur noted since age 3 years, with normal echocardiograms.

Patient 40 had a height of 172.3 cm, weight of 86.5 kg, and head circumference of 56.8 cm. Patient 42 had a height of 173.1 cm, weight of 67.9 kg, and head circumference of 56.5 cm. The brothers' hair was dark blond.

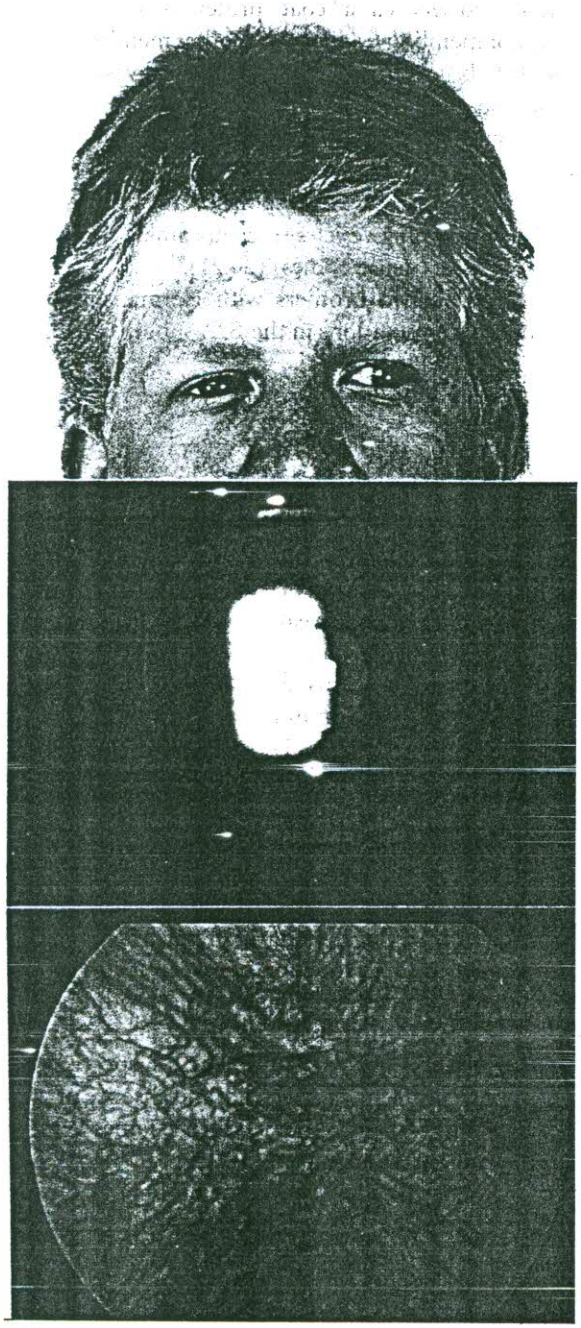

**Figure 1.** The hair of patient 40 is tan-blond, and the skin is somewhat light (top panel). Marked transillumination of the iris is apparent (middle panel). The fundus has areas of hypopigmentation peripherally (bottom). Similar findings were seen in patient 42 (see Figure 2B of reference 24).

Dark adaptation was normal, but iris transillumination and fundal hypopigmentation were marked (Figure 1). Both brothers had horizontal nystagmus, radial opacities of both lenses, and decreased visual acuity (Table 1). Visual evoked potentials showed an asymmetric pattern typical for albinism. The skin was hypopigmented, with acanthosis nigricans, actinic keratoses, hypertrichosis, darkly pigmented irregular nevi, and brown-pink macules. The tympanic membranes and oropharynx were clear. No cervical lymphadenopathy was observed. Neurologic examination revealed dysmetria and poor tandem gait, worse in patient 42, who also had a postural and intention tremor and decreased vibratory sense in his feet. The neurological examinations were otherwise normal. Magnetic resonance imaging studies of the head revealed no cerebellar abnormalities; patient 40 had incidental mastoid air cell opacification.

Laboratory results, compared with other Hermansky-Pudlak syndrome patients, are shown in Table 1. Absolute neutrophil counts of patient 40 from 7 months to 20 years of age were 275, 310, 570, 720, and 832 cells per $\mu$L; those of patient 42 from 11 to 25 years of age were 320, 480, 550, 725, 750, 790, and 1160 cells per $\mu$L. The neutrophils had normal morphology. On electron microscopy, dense bodies were absent from the platelets of the affected brothers, but present in all other family members (Figure 2). Bleeding times exceeded 20 minutes but the prothrombin time, partial thromboplastin time, and von Willebrand factor levels were normal. Platelet aggregation tests were abnormal upon adenosine diphosphate and collagen stimulation, but normal with epinephrine and ristocetin. Bone marrow aspiration showed mild granulocytic hypoplasia. Pulmonary function tests were slightly reduced (Table 1). High-resolution thin-section tomography of the chest revealed bilateral scarring in the anterior upper lobes in patient 40 and mild interstitial fibrosis in patient 42. Immunologic studies performed at ages 6 months (patient 40) and 5 years (patient 42) revealed normal T cell rosettes, nitroblue tetrazolium tests, neutrophil functions, and neutrophil morphology on electron microscopy. Karyotypes were normal.

DNA sequencing showed no mutations in the *HPS-1* gene in either brother. Sequencing of the $\beta$3A subunit of adaptor complex-3 (22) revealed that the brothers had different mutations in each of their two alleles. Specifically, a 63-bp deletion, 1166-1228del ($\Delta$390-410), was inherited from the patients' father, and a 1739T->G substitution (L580R) was inherited from their mother (22). The two unaffected brothers are carriers for 1166-1228del.

## DISCUSSION

We describe two brothers with oculocutaneous albinism, a bleeding tendency, mild pulmonary fibrosis, recurrent upper respiratory tract infections in childhood with persistent neutropenia, congenital hip dislocations, a mild balance defect and radial opacities of the ocular lens. Absence of platelet dense bodies supports the diagnosis of Hermansky-Pudlak syndrome (4), and the dermatologic,

**Table 1.** Characteristics of Hermansky-Pudlak Syndrome Patients with Mutations in Adaptor Complex-3 Compared with Other Patients with the Syndrome

| Characteristic | Patient 40 | Patient 42 | Patients with 16-bp Duplication in *HPS-1*† (n = 25) | Patients without 16-bp Duplication in *HPS-1*† (n = 24) |
|---|---|---|---|---|
| | Value(s)* | | Mean ± SD, Range | |
| Age (years) | 20 | 25 | 24.3 ± 15.2 | 18.7 ± 13.3 |
| Hemoglobin (g/L) | 130, 142 | 141, 148 | 138 ± 14 | 135 ± 14 |
| White blood cells ($\times 10^3/\mu L$) | 2.3, 2.4 | 2.6, 2.8 | 7.0 ± 2.1 | 6.3 ± 2.9 |
| Neutrophils ($\times 10^3/\mu L$) | 0.57, 0.72 | 0.79, 1.16 | 3.8 ± 1.2 | 3.4 ± 2.0 |
| Platelets ($\times 10^3/\mu L$) | 149, 173 | 162, 170 | 271 ± 67 | 270 ± 93 |
| Prothrombin time(s) | 12.1 | 11.8 | 12.1 ± 0.4 | 12.3 ± 0.5 |
| Partial thromboplastin time(s) | 26.0 | 23.4 | 30.1 ± 3.2 | 30.9 ± 4.0 |
| Corrected visual acuity | | | | |
| –OD | 20/200 | 20/200 | 20/80–20/250 | 20/50–20/200 |
| –OS | 20/160 | 20/160 | 20/80–20/250 | 20/50–20/200 |
| Pulmonary function (% of predicted) | | | | |
| Forced vital capacity | 90 | 90 | 88 ± 21 | 97 ± 19 |
| Forced expiratory volume in 1 sec | 82 | 87 | 89 ± 19 | 94 ± 22 |
| Total lung capacity | 91 | 87 | 88 ± 21 | 96 ± 16 |
| Diffusing capacity of carbon monoxide | 104 | 105 | 80 ± 22 | 91 ± 25 |

* Some measurements were made at two different times.

† From reference 24. All 25 duplication patients were Puerto Rican; the 24 nonduplication patients include 4 with different mutations in *HPS-1* and 20 with mutations at other, undefined loci.

bp = base pair; HPS = Hermansky-Pudlak syndrome.

ocular, and pulmonary findings are reminiscent of a defect in *HPS-1* (Table 2) (24). However, frequent infections and neutropenia are not part of Hermansky-Pudlak syndrome (24–26).

Could these brothers have another type of oculocutaneous albinism, such as Chediak-Higashi syndrome (27) or Griscelli syndrome (28)? In these autosomal recessive disorders, hypopigmentation is associated with immunologic deficiency, neutropenia, and an accelerated histiocytic phase resembling lymphoma (29,30). Patients with Chediak-Higashi syndrome also manifest a bleeding tendency due to deficient platelet dense bodies (31). However, our patients did not exhibit giant lysosomes in their granulocytes, the pathognomonic abnormality of Chediak-Higashi syndrome (27). Moreover, our patients had granulocytic hypoplasia as a cause of their neutropenia, whereas Chediak-Higashi syndrome patients have increased granulocytic precursors, with impaired mobilization from the marrow (32). Finally, our patients, already adults, have no evidence of an accelerated histiocytic

**Table 2.** Comparison of Adaptor Complex-3 Deficient Patients with Patients Having *HPS-1* Mutations, Chediak-Higashi Syndrome, or Griscelli Syndrome

| | HPS-2 (Adaptor Complex-3 Deficiency) | HPS-1 (*HPS-1* Mutations) | Chediak-Higashi Syndrome | Griscelli Syndrome |
|---|---|---|---|---|
| Oculocutaneous albinism | + | + | + | + |
| Absent platelet dense bodies | + | + | + | – |
| Pulmonary fibrosis | + | + | – | – |
| Neutropenia | + | – | + | + |
| Giant intracellular granules | – | – | + | – |
| Dysfunction of neutrophils, lymphocytes, and NK cells | – | – | + | + |
| Accelerated histiocytic phase | – | – | + | + |
| Death in childhood | – | – | + | + |

HPS = Hermansky-Pudlak syndrome; NK = natural killer.

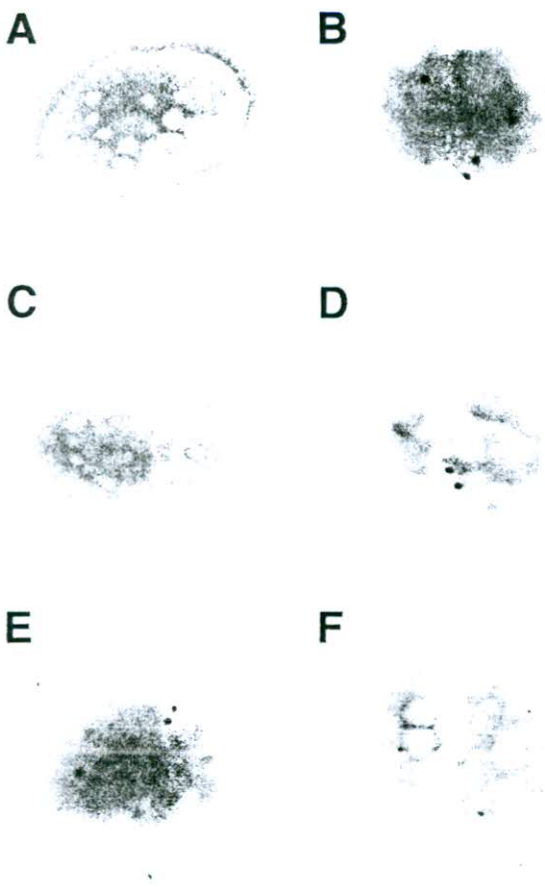


**Figure 2.** Electron micrographs of platelets show absence of dense bodies in patient 40 (**A**) and patient 42 (**C**) but presence of dense bodies in a normal control (**B**), an unaffected brother of the patients (**D**), the mother (**E**), and the father (**F**).

phase, which generally leads to death from infection before 10 years of age in Chediak-Higashi syndrome (29) and Griscelli syndrome (30).

Hence, the clinical features of our patients most closely resemble those of Hermansky-Pudlak syndrome (Table 2). We propose to name this disorder "HPS-2", recognizing that it is part of a spectrum of disease between Hermansky-Pudlak syndrome and Chediak-Higashi syndrome. More HPS-2 patients are required to determine if mild balance defects, radial opacities of ocular lenses, and acetabular dysplasia are integral components of this disorder.

The molecular basis of HPS-2 in our patients is compound heterozygosity for two mutations in the β3A subunit of adaptor complex-3 (22). Adaptor complex-3, composed of 4 subunits (β3A, δ, μ3, and σ3) (20,21,33), functions in the formation of new vesicles and in the transport of cargo proteins to lysosomes (34). In addition, melanosomes, which are specialized lysosomes (35), and platelet dense bodies are thought to form via the action of adaptor complex-3. When the δ subunit of adaptor complex-3 is mutated, the result is the *garnet* mutant of *Drosophila*, with its characteristic eye color (36), or the *mocha* mouse, with its balance problems (19). When the β3A subunit is defective, the result is the *pearl* mouse or the HPS-2 human. Our patients resemble their murine counterparts in manifesting pigment dilution, nystagmus, and a platelet-storage pool deficiency. They differ from the mice in having neutropenia, balance problems, and normal dark adaptation.

The role of adaptor complex-3 mutations in Hermansky-Pudlak syndrome suggests that other genes involved in vesicle formation and protein trafficking may also be defective in the Hermansky-Pudlak syndrome and in the Chediak-Higashi syndrome. Aberrant tyrosinase has been demonstrated in melanocytes from patients with Hermansky-Pudlak syndrome (37) and Chediak-Higashi syndrome (38).

The elucidation of an adaptor complex-3 subunit defect represents an important step toward determining the genetic causes of other types of Hermansky-Pudlak syndrome and providing a better understanding of vesicle formation and trafficking (39).

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