

ONLINE MUTATION REPORT

P gene mutations in patients with oculocutaneous albinism and findings suggestive of Hermansky-Pudlak syndrome

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Oculocutaneous albinism (OCA) is a genetically heterogeneous disorder characterised by abnormally low amounts of melanin in the eyes, skin, and hair. In addition to hypopigmentation of the skin and eyes, OCA patients have ocular manifestations including nystagmus, foveal hypoplasia with reduced visual acuity, and strabismus.¹ Several subtypes of OCA exist. OCA2, the most common subtype, results from mutations in the *P* gene.^{2,3} OCA2 patients have a broad range of phenotypes, with minimal to moderate pigmentation of the hair, skin, and iris that may darken with age.¹ OCA1 is the second most common type and is caused by mutations in the tyrosinase gene, *TYR*.^{4,5} The lack of functional tyrosinase results in the complete absence of pigmentation in hair and skin. Rarer forms of OCA include OCA3, also known as "rufous/red albinism" and associated with mutations in the *TYRP1* gene,⁶ and OCA4, associated with mutations in the *MATP* gene.⁷ Finally, some genetic defects in intracellular vesicle formation and trafficking have OCA as a major vesical component.^{8,9} For example, Chediak-Higashi syndrome (CHS) is characterised by giant intracellular granules, an often fatal diathesis to infection, and variable degrees of hypopigmentation.¹⁰ Hermansky-Pudlak syndrome (HPS) involves OCA as part of a constellation of findings that include platelet storage pool deficiency and, in some patients, accumulation of ceroid pigment, pulmonary fibrosis, and/or granulomatous colitis.¹¹ The sine qua non of HPS is absence of platelet dense bodies on whole mount electron microscopy.¹² CHS and HPS can have overlapping phenotypes. For example, HPS-2, associated with mutations in the β 3A subunit of adaptor complex-3,¹³ manifests with neutropenia and childhood infections reminiscent of CHS.^{14,15}

Because of our interest in hypopigmentation and disorders of intracellular vesicles, we investigated all patients having OCA plus a history of bleeding, frequent infections, or some other HPS-related symptoms. We screened eight such individuals with clinical manifestations suggestive of HPS. Five of the eight had definitive molecular evidence for OCA2. We now describe those patients and their *P* gene mutations.

METHODS

Patients

All patients had OCA plus some suggestion of HPS or an HPS-related syndrome. Each was enrolled in a protocol approved by an NIH institutional review board and written informed consent was obtained from each patient or their parents.

Electron microscopy

Platelets were examined for dense bodies by whole mount electron microscopy as previously described.¹²

Mutation analyses

DNA was extracted from whole blood using standard techniques. HPS-causing genes were screened using several different methods. *HPS1* was investigated using Northern

Key points

- Eight patients with oculocutaneous albinism (OCA) and other features suggestive of Hermansky-Pudlak syndrome were evaluated for mutations in HPS-associated genes and the OCA2-associated *P* gene. Since the clinical presentations were suggestive of Hermansky-Pudlak syndrome (HPS), we initially screened for mutations in HPS-causing genes. No *HPS1*, *AP3B1A*, or *HPS3* mutations were detected. Next, we screened for mutations in the *P* gene associated with OCA2, the most common form of OCA.
- Three of the eight patients exhibited previously described mutations in the *P* gene in the homozygous or compound heterozygous states, making the diagnosis of OCA2. In two other patients, a single mutant allele of the *P* gene was detected. The identification of *P* gene mutations in HPS-like patients with manifestations suggesting HPS indicates that OCA2, and perhaps other types of OCA, should be considered in such individuals.
- Differentiating among the various types of albinism remains a difficult task, since many patients carrying the diagnosis of OCA (or even ocular albinism) actually have HPS, while patients thought to have HPS or an HPS-like syndrome may have another basis for their hypopigmentation, demonstrating the importance of molecular diagnosis.

blotting and direct sequencing, *AP3B1A* using Western blot analysis with an antibody to the β 3A subunit of adaptor complex-3, and *HPS3*¹⁶ by Northern blotting. For *P* gene mutation screening, primers covering all coding sequences and intron-exon boundaries were used to amplify the patients' genomic DNA, as described elsewhere.¹⁷ PCR products were purified by the QIAquick Purification Kit and sequenced using an ABI 377 sequencer. Screening of the *P* gene 2.7 kb deletion was performed as previously described.³ The sequences were analysed with Sequencher 3.1.1 software. When necessary, clonal analysis was applied to determine heterozygosity using a TA cloning Kit (Invitrogen).

RESULTS

Molecular findings

Initially, we used the criterion of absence or presence of dense bodies in platelets to categorise all our patients with clinical manifestations suggestive of HPS. Eight patients had dense bodies in their platelets and were subjected to further

Abbreviations: CHS, Chediak-Higashi syndrome; HPS, Hermansky-Pudlak syndrome; OCA, oculocutaneous albinism

molecular analyses. No *HPS1*, *AP3B1A*, or *HPS3* mutations were identified in any of the eight patients in the initial molecular analyses using direct sequencing, Northern blotting, or Western blotting. Next, since *OCA2* is the most common type of albinism, we screened for mutations in the *OCA2* associated gene, the *P* gene. Five of the eight patients had previously reported *P* gene mutations.^{3 18–20} Both *P* allele mutations were identified in three patients, while only one mutation was found in the other two patients (table 1).

Clinical and laboratory data

In addition to having dense bodies in their platelets, the five *OCA2* patients also lacked giant inclusions in their leukocytes, eliminating any close resemblance to *CHS*. The patients ranged in age from 1 to 32 years and were of various ethnicities (table 1). The severity of hypopigmentation and ophthalmic findings varied considerably. Skin and hair colour ranged from completely white to brown (fig 1) and visual acuities were between 20/300 and 20/50. No *OCA2* patient had any significant history of bleeding or granulomatous colitis. Only patient no 5 had pulmonary insufficiency; her forced vital capacity was 61% of predicted, total lung capacity 71% of predicted, and diffusing capacity for carbon monoxide 81% of predicted. She complained of dyspnea, but was pregnant at the time and, therefore, a chest CT scan was not performed. All five patients had varying histories of increased infections, including frequent episodes of otitis, sinusitis, pneumonias, and urinary tract infections (table 1).

DISCUSSION

Differentiating among the various types of albinism remains a difficult task. For example, many patients carrying the diagnosis of *OCA* or even ocular albinism^{12–21} actually have *HPS*. Conversely, patients thought to have *HPS* or an *HPS*-like syndrome may have another basis for their hypopigmentation. In fact, here we describe five patients referred to an *HPS* investigational protocol who actually had mutations in the *P* gene, giving them the diagnosis of *OCA2*. In these five cases, the findings suggestive of *HPS*-like disorders included a high frequency of infections, and indications of a bleeding diathesis (bruising, idiopathic thrombocytopenic purpura, history of transfusion) or pulmonary insufficiency. Because *OCA2* is associated solely with hypopigmentation, these additional findings proved to be incidental and not related to the primary cause of albinism in these patients.

Currently, the absence or presence of platelet dense bodies on whole mount electron microscopy makes or excludes the diagnosis of *HPS*. In contrast to the definitive studies of a whole mount examination, however, results of standard transmission electron microscopy of platelets can be ambiguous, since electron-dense organelles such as lysosomes and α -granules can be mistaken for dense bodies. Moreover,



Figure 1 Hair and skin of patients no 1 (A), no 2 (B), no 3 (C), and no 5 (D).

reports indicate that *HPS* patients can have reduced numbers of dense bodies rather than a complete absence of dense bodies,^{22–23} although these studies lacked molecular verification of the diagnosis of *HPS*, and the dense body analysis was performed by transmission electron microscopy. Therefore, even though the eight patients described here had dense bodies in their platelets when examined by electron microscopy, we still subjected these patients to further molecular screening for *HPS* mutations. There is a possibility that Western blotting to detect *AP3B1* gene mutations and Northern blotting to detect *HPS3* gene mutations in this study may have missed some types of mutations. However, the identification of mutations in the *P* gene in these patients suggests that they likely do not carry mutations in *AP3B1* or *HPS3*, since the coincidence of mutations in one of these *HPS*-causing genes and the *P* gene is highly unlikely. Our laboratories continue to search for *HPS* variants who have molecularly verified mutations in *HPS*-causing genes, along with dense bodies which are decreased in number but not completely absent. To date, no such patients have been identified.

Our findings also point to the phenotypic heterogeneity of *OCA2*. Patient #3 is heterozygous for the mutation, 1327G>A (V443I), which is consistent with *P* gene albinism. At the age of 2 months, she was diagnosed with albinism and had nystagmus, white hair, sun sensitivity, and a visual acuity of 20/50. Some of these signs decreased with age, as patient #3 currently has medium brown hair and pigmented skin. We have not discovered this patient's second *P* gene mutation. However, we do not always detect mutations in both alleles; the undetected allele could be located in a promoter region, an intron, or some other area not routinely sequenced.

Table 1 Clinical and molecular findings in *OCA2* patients

Pt/age/sex*	Ethnicity†	Visual acuity	Bleeding	GI‡	Infections**	Pulmonary	Mutations
1/1/M	AAm/Ger-Iri-PR	20/300	None	None	Otitis (7), sinusitis (2), mastitis, impetigo	NA‡	2.7 kb del(ex7)/1327G>A
2/2/F	Fre-Can/Fre-Can	NA	Bruising	Cramp	Otitis (4), urinary tract infection	Asthma	2207C>T/NA
3/22/F	Ger/Eng	20/50	None	None	Otitis frequent, pneumonias (5), sinusitis, yeast	Asthma, normal CT	1327G>A/NA
4/29/F	Ash/Ash	20/100	ITP§	IBS¶	Infectious mononucleosis	None	79C>A/79C>A
5/32/F	AAm/Dut	20/250	Transfusion after surgery	None	Otitis frequent, pneumonias (3), urinary tract infection	Asthma, dyspnea	2.7 kb del(ex7)/819CTGG>GGTC

*Pt, patient number; †AAm, African American; Ger, German; Iri, Irish; PR, Puerto Rican; Fre-Can, French Canadian; Eng, English; Ash, Ashkenazi Jewish; Dut, Dutch; ‡NA, not available; §ITP, idiopathic thrombocytopenic purpura; ††GI, gastrointestinal; ¶IBS, irritable bowel syndrome; **numbers in the parentheses indicate the numbers of infections.

A complete characterisation of the various subtypes of HPS requires examination of individuals who have OCA along with other HPS-related symptoms. Many of these subjects will prove to have common types of albinism such as OCA2, but this should not mitigate against an aggressive pursuit of the entire spectrum of disorders involving hypopigmentation, platelet storage pool deficiency, an infectious diathesis, pulmonary fibrosis, and/or granulomatous colitis.

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