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Hermansky-Pudlak Syndrome in Two African-American Brothers

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Abstract

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding disorder, and, in some patients, granulomatous colitis and/or a fatal pulmonary fibrosis. There are eight different subtypes of HPS, each due to mutations in one of eight different genes, whose functions are thought to involve intracellular vesicle formation and trafficking. HPS has been identified in patients of nearly all ethnic groups, though it has primarily been associated with patients of Puerto Rican, Northern European, Japanese and Israeli descent. We report on the diagnosis of HPS type 1 in two African-American patients. Both brothers carried compound heterozygous mutations in *HPS1*: previously reported p.M325WfsX6 (c.972delC) and a novel silent mutation p.E169E (c.507G>A), which resulted in a splice defect. HPS may be underdiagnosed in African-American patients and other ethnic groups. A history of easy bruising or evidence of a bleeding disorder, combined with some degree of hypopigmentation, should prompt investigation into the diagnosis of HPS.

Keywords

albinism; Hermansky-Pudlak syndrome; platelet dense bodies; pulmonary fibrosis; splice site mutation; silent mutation

INTRODUCTION

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder first reported in 1959 by Czech physicians in 2 unrelated adults [Hermansky and Pudlak, 1959]. The disorder is characterized by oculocutaneous albinism, a bleeding disorder due to absent platelet dense granules and, in some patients, lysosomal accumulation of ceroid lipofuscin [Gahl et al., 1998]. A subset of patients develops fatal pulmonary fibrosis [Garay et al., 1979] and some patients have granulomatous colitis [Gahl et al., 1998, Hussain et al, 2006].

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HPS displays locus heterogeneity; eight human genes are now associated with HPS [Wei, 2006; Huizing et al., 2008], and there are at least fifteen mouse models with an HPS phenotype [Li et al., 2004]. Both the locus heterogeneity and the clinical manifestations of HPS can be explained by the inability to form certain intracellular vesicles or direct them to their proper destination. This shortcoming affects melanosomes in melanocytes, dense bodies in platelets, and lysosomes in some other cell types [Huizing et al., 2008]. Of the eight HPS subtypes, pulmonary fibrosis is only associated with HPS subtypes 1 and 4 and recurrent infections are only associated with HPS subtype 2 [Wei, 2006; Huizing et al., 2008]. HPS subtypes 3, 5 and 6 are not associated with pulmonary fibrosis or recurrent infections. HPS subtypes 7 and 8 have been described in single family case reports [Gahl, 2007].

HPS has been identified primarily in patients of Puerto Rican, Northern European, Japanese and Israeli descent, although individuals with HPS have been ascertained in other ethnic groups [Witkop et al., 1990; Schallreuter et al., 1993; Oh et al., 1996; Huizing et al., 2001; Anderson et al., 2003; Huizing et al., 2004; Ito et al., 2005; Schreyer-Shafir et al., 2006; Wei, 2006]. Here we report the diagnosis of HPS in African-American patients.

MATERIALS AND METHODS

Patients and cells

The patients were enrolled in a protocol approved by the National Human Genome Research Institute (NHGRI) institutional review board to evaluate the clinical and molecular aspects of HPS. Patient numbers correspond to a master file of all NHGRI patients with HPS. Informed assent was obtained from the patients and informed consent was obtained from the parents. The diagnosis of HPS was based on the presence of albinism and the absence of platelet dense bodies on whole-mount electron microscopy [Gahl, 2007]. Skin fibroblast primary cultures, which were obtained from 4-mm punch biopsies, were grown in Dulbecco modified Eagle's 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 was prepared from fresh citrated blood, placed on copper grids and treated as previously described [Witkop et al., 1987; Hazelwood et al., 1997]. A Philips model 301 electron microscope was used to examine the air-dried grids.

Mutation analysis

Genomic DNA was extracted from whole blood using the Gentra Puregene Blood Kit (Qiagen, Valencia, CA). Patients were screened for mutations in known candidate genes *HPS1*, *HPS3*, *HPS4*, *HPS5*, and *HPS6* using intron-based, exon specific primers for PCR amplification and sequencing by standard methods [Sambrook et al., 1989]. Primer sequences are available upon request.

RNA was extracted from fibroblasts of patient #122 using Trizol reagent (Invitrogen, Carlsbad, CA) and transcribed into cDNA using the SuperScript III system per the manufacturer's instructions (Invitrogen, Carlsbad, CA). cDNA was subjected to standard PCR amplification using HotStart Taq Master Mix (Qiagen, Valencia, CA) at an annealing temperature of 60 °C. For *HPS1* mutation analysis primer sequences, 5'-AAGTTCGGGCAGTCAGAGAA-3' and 5'-AGCTGGCACTGTGGCTAGA-3', were designed to amplify a normal 598-bp fragment that spanned the exon-exon boundaries between exons 3 and 7 of the primary transcript (Genbank Accession NM_000195). Resultant PCR fragments were cloned into the TOPO TA Cloning Kit per the

manufacturer's instructions (Invitrogen, Carlsbad, CA). Clones were purified using the QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA) and subjected to automated sequence analysis on a Beckman CEQ 8000 using the CEQ Dye Terminator Cycle sequencing kit, according to the manufacturer's instructions (Beckman, Fullerton, CA).

RESULTS

Clinical findings

Two African-American brothers, patients #122 (age 13 years) and #132 (age 12 years), were diagnosed with HPS by the finding of absent platelet dense bodies on electron microscopy (Figs. 2B,C). Both boys had been diagnosed with oculocutaneous albinism at birth. They also had excessive bruising throughout childhood that had become more apparent in the two years prior to the diagnosis of HPS. The younger brother had a gastrointestinal bleeding episode due to a ruptured polyp requiring platelet transfusion immediately prior to his diagnosis.

The elder brother (patient #122) had a height of 175 cm (>95th centile), weight of 71 kg (>95th centile), blood pressure of 101/59 and oxygen saturation of 99% on room air. The hair was brown (Fig. 1A) and the face had freckles visible across the nose. Visual acuity was 20/160 bilaterally. Horizontal nystagmus and a right exotropia were noted. The irides showed transillumination (Fig. 1B), and the retinal periphery was pale (Fig. 1C). Pigment was present in the central posterior pole of the retina but the foveal reflex was absent. Routine serum chemistry results were normal. An incidental finding was a low mean corpuscular volume (MCV) of 64.1 cubic micrometers. Subsequent hemoglobin electrophoresis testing was consistent with a presumptive diagnosis of alpha thalassemia trait. The platelet count was approximately normal at 374,000 per mm³ of blood. Computed tomography of the chest (high resolution) and pulmonary function testing demonstrated no evidence of pulmonary fibrosis. Due to a history of blood in stools, this child underwent colonoscopy, which identified three hyperplastic rectal polyps, and polypectomy was recommended.

The younger brother (patient #132) had a height of 154 cm (50–75th centile), weight of 45 kg (50–75th centile), blood pressure of 96/58 and oxygen saturation of 98% on room air. Best-corrected visual acuity was 20/160 in the right eye and 20/100 in the left eye respectively. Horizontal nystagmus was present. Iris transillumination and hypopigmentation of the retinal periphery were noted during the ophthalmic examination. There was pigment present in the macular area but the foveal reflex was absent. Routine serum chemistry results were normal. Hematologic testing revealed a normal hemoglobin and white blood cell count, a slightly low MCV of 77.4 fL and an approximately normal platelet count of 354,000 per mm³ of blood. Chest x-ray was normal except for decreased chest AP diameter. Computed tomography of the chest (high resolution) and pulmonary function testing showed no evidence of pulmonary fibrosis.

Platelet studies

Lack of dense bodies was demonstrated in the platelets of both brothers using whole mount and transmission electron microscopy (Fig. 2).

Molecular analysis

Both brothers were compound heterozygous for mutations in the *HPS1* gene and carried a previously reported mutation p.M325WfsX6 (c.972delC) in exon 11 [Oh et al., 1998] and a novel mutation c.507G>A (p.E169E) in exon 6 (Fig. 3A). Due to the silent nature of the latter mutation and its position in the 5' splice site of exon 6 (Fig. 3B), we predicted an

alteration in the splicing of *HPS1* mRNA. Reverse transcription of mRNA, followed by PCR analysis across four exon-exon boundaries, verified the production of a larger mRNA transcript in the patient's RNA, but not in control RNA (Fig. 3C). Sequencing of the larger transcript revealed activation of a cryptic intronic splice site and insertion of 43 base pairs of intervening intronic sequence (Fig. 3D). This insertion results in an altered reading frame of the mutant transcript, predicting that it will be targeted for nonsense-mediated decay or that it will produce a truncated protein with a premature stop codon 26 amino acids downstream of E169E.

Sequencing of the cloned single strand fragments of cDNA demonstrated that the mutations observed are on different alleles of the *HPS1* gene.

DISCUSSION

We identified two African American brothers with HPS characterized by hypopigmentation, iris transillumination, pale fundus, decreased visual acuity, nystagmus, and lack of platelet dense bodies. Sequencing of known candidate genes *HPS1*, *HPS3*, *HPS4*, *HPS5*, and *HPS6* revealed that the brothers have HPS type 1, indicated by two compound heterozygous *HPS1* mutations, c.972delC and c.507G>A. c.972delC (exon 11) has been previously described and is considered the most frequent mutation among HPS patients of European descent [Oh et al., 1998]. The c.507G>A (p.E169E, exon 6) mutation has not been previously reported and was not observed in 97 alleles of other HPS patients sequenced in our laboratory. Molecular analysis of c.507G>A revealed the activation of a cryptic splice site at +43 bp within intron 6 of the *HPS1* gene (Fig. 3). This splicing error incorporated 43 additional base pairs of intronic sequence into the final mutant transcript. The cryptic splice site was predicted by Neural Network Splice Site Prediction Tool or NNSPLICE (version 0.9; available online at http://www.fruitfly.org/seq_tools/splice.html) with a high probability score of 0.72 out of 1 [Reese, 1997]. The normal splice site of exon 6 has a score of 0.57, but the c.507G>A change altered this score to below the threshold of 0.40, predicting conversion to an unacceptable splice donor site. These findings indicate that aberrant splicing needs to be considered if mutation analysis reveals missense mutations, whether synonymous or nonsynonymous [Cartegni et al., 2002].

To our knowledge this is the first diagnosis of oculocutaneous albinism in African-American patients due to *HPS1* mutations. It is very likely, however, that African-American HPS patients have been overlooked due to the more prevalent diagnosis of oculocutaneous albinism type 2 (OCA-2), also known as tyrosinase-positive OCA. OCA-2, one of the most common autosomal recessive disorders among southern Africans, results from mutations in the *P*-gene [Stevens et al., 1995; Stevens et al., 1997; Kerr et al., 2000; Garrison et al., 2004]. OCA-2 corresponds to the "pink-eyed dilution" (*p*) gene in the mouse. [Rinchick et al., 1993]. Africans and African Americans with OCA-2 vary phenotypically from having yellow hair and blue-gray or hazel irides to manifesting "brown OCA" with light brown hair and skin color and gray to tan irides [King et al., 2001]. African-Americans with OCA-2 do not develop the granulomatous colitis and fatal pulmonary fibrosis associated with HPS type 1 [Gahl et al., 1998]. These serious complications, and the fact that effective therapeutic approaches exist for them [Gahl et al, 2002; Hussain et al, 2006], make it critical that physicians caring for African-American patients with albinism consider the possibility of HPS prior to assuming a diagnosis of nonsyndromic oculocutaneous albinism. Due to the phenotypic variability in findings of albinism, easy bruising and bleeding history, patients may not come to the attention of a physician until the teenage years. Diagnosed with HPS-1 as young teenagers, our patients were not at an age yet when pulmonary disease typically develops in HPS-1 patients. It is therefore important that they continue to be followed for the development of restrictive lung disease to anticipate therapy [Gahl et al, 2002]. In

summary, clinical evidence of a bleeding disorder, or a history of easy bruising, should be considered and prompt further investigation and testing for platelet dense body deficiency [White, 1969; Witkop et al, 1987; Maurer-Spurej et al, 2002] to diagnose HPS in patients with oculocutaneous albinism in this population and all other ethnic groups.

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Fig. 1. Pigmentation findings in patient #122. **A:** Brown hair. **B:** Iris transillumination, indicating paucity of pigment in the iris. **C:** Pale fundus reflects hypopigmentation of retinal pigment epithelium. **D:** Normal iris transillumination (dotted line outlines the limbus). **E:** Normal fundus.

Fig. 2. Electron microscopy of platelets. **A:** Dense bodies of normal platelets, apparent on whole mount electron microscopy (x2000). **B:** Absent dense bodies in patient #122 (x1600). **C:** Absent dense bodies in patient #132 (x2000). **D:** Transmission electron microscopy of normal platelets, with dense bodies (arrows) and alpha granules (arrowheads) (x5500). **E:** Platelets of patient #122, with normal contingent of alpha granules but no dense bodies (x3300). **F:** Platelets of patient #132, resembling those of his brother (x4200).

Fig. 3. *HPS1* Mutations in two brothers of African American descent. **A:** Genomic DNA sequencing showing compound heterozygous mutations in patient #122. **B:** Schematic representation of the *HPS1* gene and the primers used for PCR analysis to detect the effects of the patient's splice site mutation on his cDNA. **C:** RT-PCR analysis of RNA derived from cultured fibroblasts of a normal individual (lane 1) and patient #122 (lane 2). Patient #122 has an extra, 641-bp band. **D:** Sequence analysis of normal and mutant allele RT-PCR fragments showing the 43-bp of IVS6 inserted into the cDNA of patient #122.