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Novel mutation in two brothers with Hermansky Pudlak syndrome type 3

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ABSTRACT

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder causing oculocutaneous albinism, bleeding disorder and ceroid lipofuscinosis. Platelets from HPS patients are characterized by impaired secretion of dense (δ)-bodies (CD63). Meanwhile, there are ten known human *HPS* genes, each leading to a particular clinical HPS subtype (HPS1-HPS10).

We report on two Turkish brothers showing typical HPS phenotype comprising oculocutaneous albinism and bleeding symptoms. Pathological bleeding time as well as platelet aggregometry analyses revealed impaired platelet function. The brothers demonstrated absence of platelet δ-granule secretion as measured by flow cytometry. Using molecular genetic analyses, a novel homozygous 1 bp-deletion in the *HPS3* gene was identified in both brothers. In addition, the younger brother with HPS3 demonstrated psychomotoric retardation and cranial gliosis (magnetic resonance imaging, MRI). Array-CGH analysis revealed a de novo 0.482 Mb deletion on chromosome 17 which is not present in his brother and parents.

In this study, we identified a novel 1 bp-deletion in the *HPS3* gene causing HPS3 phenotype in two brothers. In patients with oculocutaneous albinism and increased bleeding symptoms platelet function should be analyzed. The identification of the molecular genetic defect allows the classification to a particular HPS subtype and is important for therapy and prognosis.

1. Introduction

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder which was described in 1959 for the first time [1]. Since then, HPS has been recognized as an increasing group of genetically distinct diseases resulting from abnormal formation or trafficking of intracellular vesicles. There are 10 genetic loci (*HPS1-HPS10*) associated with HPS in humans. Additional human HPS genes may be discovered in future, corresponding to the 15 known distinct genes which were cloned in mouse strains [2,3].

The specific organelles affected in HPS are the lysosomes and lysosome-related organelles (LROs), such as the melanosomes and the platelet dense bodies, which all share certain integral membrane proteins [4,5]. HPS is characterized by oculocutaneous albinism, increased bleeding symptoms and lysosomal accumulation of ceroid lipofuscin. Oculocutaneous albinism due to impaired melanosome formation manifests with congenital nystagmus, iris transillumination, decreased visual acuity and reduced skin pigmentation [6,7]. Bleeding diathesis is due to the absence of dense (δ)-bodies in platelets resulting in the lack of a secondary platelet aggregation response [8,9]. The clinical presentation varies from mild to major bleeding symptoms including easy bruising, petechiae, epistaxis, and prolonged bleeding after surgery or trauma requiring transfusions in some cases [9,10]. Ceroid lipofuscin, an incompletely characterized lipid-protein complex, is thought to accumulate in cellular lysosomes and may cause pulmonary fibrosis [11,12], granulomatous colitis [13,14] and cardiomyopathy [15] in some patients. Neutropenia and susceptibility to recurrent infections were observed in HPS2 and HPS10 patients so far [16–18].

All HPS proteins are associated in multi-protein complexes essential for biogenesis and intracellular trafficking of lysosomal organelles [19]. However, certain function of the particular subunits of these multi-protein complexes is not yet determined. The biogenesis of lysosome-related organelle complex (BLOC)-1 consists of the subunits HPS7

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(dysbindin), HPS8 (BLOS3), HPS9 (pallidin), and the mouse gene products of muted, cappuccino, snapin, BLOS1 and BLOS2. BLOC-2 contains HPS3, HPS5 and HPS6. HPS1 and HPS4 subunits are associated within BLOC-3. The adapter protein complex AP-3 comprises the HPS2 (β3A)-, HPS10 (δ), μ3- and σ3-subunits. Mutations within particular HPS genes can lead to dysfunction of the corresponding protein complex and thus to defective maturation of melanosomes and platelet dense bodies. The human HPS subtypes (HPS1-HPS10) have in part different clinical features [20]. Patients with those defects of HPS proteins that belong to the same multi-protein complex (e.g. BLOC-3) show a similar clinical HPS phenotype (e.g. HPS1, HPS4).

HPS3 gene spans approximately 4.4 kb on chromosome 3q24 and comprises 17 exons. The major *HPS3* transcript is 4.7 kb in size [21]. The 1004-amino acid polypeptide has a predicted molecular weight of 113.7 kDa. The mRNA transcript is 4.4 kb and appears to be ubiquitous, expressed in heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. Together with HPS5 and HPS6, HPS3 protein is part of the cytosolic BLOC-2 which is involved in vesicle trafficking. Lack of endogenous HPS3 can therefore affect membrane trafficking. Meanwhile, eight disease causing mutations have been reported in *HPS3* [21–23] (HPSD, Hermansky-Pudlak Syndrome Database, http://liweilab.genetics.ac.cn/HPSD/).

This study reports on two brothers diagnosed with HPS3 due to a novel one base deletion in exon 15 of *HPS3* leading to a reading frameshift and premature stop codon. In addition, the younger brother presented with severe psychomotoric retardation. Magnetic resonance imaging (MRI) scan revealed cranial gliosis. In this patient, array-CGH analysis revealed an additional 0.482 Mb deletion on chromosome 17 which had not been identified in the older brother.

2. Patients, materials, and methods

2.1. Informed consent

Informed consent for the performed studies was obtained from the patients' family in accordance with the guidelines of the local ethics committee. The study protocol was approved by the ethics committee of the University of Freiburg (Germany).

2.2. Patients

2.2.1. Patient 1

The boy born in 1997 is the second child of consanguine (cousins) parents of Turkish origin. His older sister (*1994) and his parents are healthy. Oculocutaneous albinism with typical hypopigmentation, nystagmus, and pronounced visual impairment (20% vision) were detected very early in life. At the age of four years, the boy showed a prolonged bleeding (4 weeks) during a circumcision and increased bleeding diathesis (e.g. epistaxis and hematomas). At the age of eight years, he came to the Department of Pediatrics and Adolescence Medicine, Medical Center – University of Freiburg, where Hermansky-Pudlak syndrome was diagnosed. Because of recurrent epistaxis, laser treatment of Kiesselbach's plexus was performed at the age of 12 years. No further HPS complications (pulmonary fibrosis, cardiomyopathy, granulomatous colitis or increased susceptibility to infection) have been observed so far. The boy shows a normal psychomotor development and has just graduated from secondary school.

Platelet count and size were normal, and the blood smear revealed no abnormalities. Bleeding time was prolonged (>15 min, normal <6 min). Von Willebrand factor (VWF) antigen, collagen binding capacity, multimeric analysis, aPTT and prothrombine time were normal.

2.2.2. Patient 2

The brother was born in 2002 in the 38th week of gestation with a birth weight of 3280 g. As his brother, he showed oculocutaneous albinism, pronounced visual impairment, nystagmus and an increased bleeding tendency (prolonged bleeding after tonsillectomy, severe epistaxis requiring hospitalisation). In contrast to his older brother, he already presented with delayed achievement of gross motor development milestones in the first two years of life. He slowly learned walking independently at the age of two years, however, now most of the time he is still walking on toes with a wide base until the current age of 14 years despite several therapeutic attempts with botulinum toxin and operative correction. Besides this delayed motoric development he presented an erethic and autistic like behavior already as an infant and he also showed a distinctive disorder of speech development with an actual vocabulary below 100 words. Over the years he developed an increasing obesity. A symptomatic epilepsy with rare tonic and gelastic seizures has been suspected but could not be confirmed in long-term video-EEG. An anti-epileptic treatment was not necessary. Repeated brain MRI (magnetic resonance imaging) scan revealed multiple T2-hyperintense lesions in the peripheral white matter and being predominantly located in the frontal and parietal lobes (Fig. 1). These lesions were hypointense on T1-weighted imaging, did not enhance upon contrast administration and displayed neither diffusion restriction nor pathologic signal loss on susceptibility weighed imaging. No other abnormalities could be discovered and repeat MRI scans showed no progression of the lesions described above.

As his brother, he presented with prolonged bleeding time (>15 min, normal <6 min) and normal values for platelet count and platelet size. Von Willebrand factor (VWF) antigen, collagen binding capacity, multimeric analysis, aPTT and prothrombine time were normal.

So far, he did not develop any signs of pulmonary fibrosis, cardiomyopathy or granulomatous colitis.

2.3. Platelet studies

2.3.1. Isolation of platelets and platelet poor plasma

Citrated-anticoagulated blood samples were obtained after informed consent from the controls, patient and the parents, respectively. For platelet function analyses platelet rich plasma (PRP) and platelet poor plasma (PPP) was prepared from citrated blood by centrifugation as described [24].

2.3.2. Platelet aggregation assay

Platelet numbers in PRP were adjusted to a concentration of 250 G/l with PPP. Platelet agglutination/aggregation was analyzed on platelet aggregometer APACT 4 (Labor Fibrintimer, BRD) using following agonists: adenosine diphosphate (ADP; 2.0 and 4.0 μmol/l; MP Biomedicals, USA), arachidonic acid (0.3 mg/ml; möLab, UK), collagen (2.0 μg/ml; Nycomed, Austria), epinephrine (8 μmol/l; Aventis, BRD), and ristocetin (1.2 mg/ml; American Biochemical and Pharmaceutical LTd, UK).

2.4. Flow cytometry analysis

2.4.1. Platelet surface GPIIb/IIIa and GPIb/V/IX ex vivo

Aliquots of diluted PRP (5×10^7 platelets/ml) were fixed as described [24]. Fixed platelets were stained with fluorescein labelled monoclonal antibody (mab) against CD41, CD42a and CD42b and analyzed as described before [24].

2.4.2. Von Willebrand factor (VWF) and fibrinogen binding to platelets

Diluted PRP (5×10^7 platelets/ml) was incubated with anti-VWF-FITC or anti-fibrinogen-FITC as described, respectively [24]. Stimulation took place with different concentrations of ristocetin (0.2–1.0 mg/ml), ADP (0.25–2.0 μmol/l) and thrombin (0.025–0.5 U/ml) for 3 min at room temperature, respectively. Platelet activation by thrombin was performed in the presence of 1.25 mM of the peptide Gly-Pro-Arg-Pro to prevent fibrin-polymerisation. Platelets were fixed and analyzed by flow cytometry as described [24].

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