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A novel nonsense mutation in a patient with Hermansky-Pudlak syndrome type 4

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1. Introduction

Hermansky-Pudlak syndrome (HPS; MIM#203300) is a rare multisystemic disorder which was first described in 1959 [1]. During the subsequent decades the different subtypes of HPS have been described to present with divergent clinical manifestations including oculocutaneous albinism, bleeding diathesis (platelet storage pool deficiency) and other organ involvement due to lysosomal accumulation of ceroid lipofuscin [2,3]. The key pathological feature of HPS is the disrupted biogenesis and/or function of specialized lysosomes which are termed lysosome-related organelles (LRO), including melanosomes, platelet δ-granules and secretory lysosomes [3,4,5]. Oculocutaneous albinism is a consequence of impaired melanosome formation which manifests with congenital nystagmus, iris transillumination, decreased visual acuity and reduction in skin pigmentation [6,7]. Bleeding diathesis is due to the absence of platelet δ-granules [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 red blood cell transfusions in some cases [10,11]. Ceroid lipofuscin, an incompletely characterized lipid-protein complex, is thought to accumulate in cellular lysosomes and may cause pulmonary fibrosis [12–16], granulomatous colitis [17,18] and renal dysfunction in some patients [6]. Especially, patients with HPS1, HPS2, or HPS4 are predisposed to interstitial lung disease. Pathogenic variants in 10 genetic loci (*HPS1–HPS10*) are known to cause HPS in humans. The proteins encoded by these *HPS* genes are associated in multi-protein complexes, *i.e.* BLOC-3 consists of the subunits HPS1 and HPS4. Patients with BLOC-3 deficiencies seem to show a more severe phenotype of hypopigmentation, frequently develop granulomatous colitis, and suffer from adult-onset pulmonary fibrosis [3,19]. *HPS4* gene maps to human chromosome 22q12.1 and the major *HPS4* transcript (GenBank NM_022081) contains a 2127-bp open reading frame (14 exons) that codes for a 708-amino acid polypeptide with a predicted molecular weight of 76.9 kDa. This study reports on a Turkish boy with a novel nonsense mutation in exon 11 of *HPS4* leading to a premature termination of HPS4 protein.

2. Patients, materials and methods

We report on a 1 year-old boy (IV.1) who presented with hematoma, epistaxis, oculocutaneous albinism, and nystagmus. The patient is the first child of consanguineous parents (III.5 and III.6, cousins) of Turkish descendants (Fig. 1). When circumcision had been performed in Turkey moderately increased bleeding tendency and slightly impaired wound healing were reported. Platelet count and size were normal. Surprisingly, the *in-vivo* bleeding time (Ivy) was within normal limits (5 min 30 s, normal < 6 min) at the day of investigation. Von Willebrand factor (VWF) antigen, aPTT and prothrombin time were normal. At the age of 15 months an injury of the upper lip had led to uncontrolled bleeding so that surgical treatment and application of tranexamic acid

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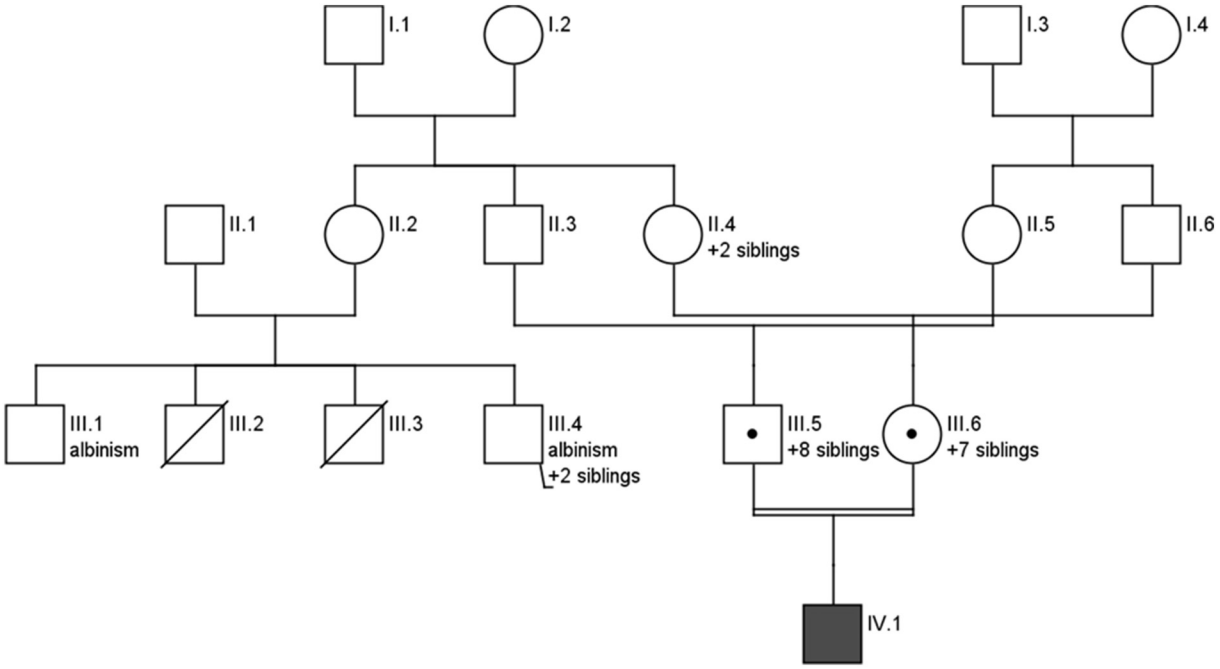

Fig. 1. Pedigree of the Turkish family.
The patient's parents (III.5 and III.6) are first cousins. Two cousins of the patient's father have been recorded to show albinism (III.1 and III.4). Two brothers of III.1 and III.4 deceased as newborn (III.2) and due to an accident (III.3), respectively. The dark symbol shows the affected (phenotype and homozygous genotype) index patient, symbols with a dot inside show heterozygous mutation carrier (phenotype unknown), and white symbols show other family members (phenotype and genotype unknown). Additional siblings in generation II and III of whom we did not receive any information were not displayed in the pedigree. Double line indicates consanguine bond.

was necessary. At the age of 4 years, prolonged epistaxis had been treated with coagulation because treatment with tranexamic acid was initially inefficient. Interestingly, then the *in-vivo* bleeding time (Ivy) was prolonged bleeding (9 min 30 s). A tongue bite at the age of almost 6 years was treated surgically and further bleeding could be stopped with tranexamic acid (local and systemic). So far, no other HPS complications, *e.g.* pulmonary fibrosis, cardiomyopathy, kidney failure or granulomatous colitis have been observed. Within the patient's family in the paternal line two cases of albinism were documented (Fig. 1:III.1 and III.4). Informed consent for the performed studies was obtained from the patient's parents in accordance with the guidelines of the local ethics committee.

2.1. Platelet lumiaggregometry and platelet aggregometry

ATP secretion in whole blood was determined according to Ingerman-Wojenski and Silver with firefly luciferase on a Whole Blood Lumi-Aggregometer (Chrono-Log) [20]. In brief to 0.9 ml diluted whole blood (1:2 in saline) 100 µl of Chrono-Lume firefly luciferase reagent was added, and the reaction was started with 1) 2 nmol of ATP Standard (Chrono-Lume, Chrono-Log, Haverton PA, USA); 2) 1 U/ml Thrombin (Siemens Healthcare, Marburg, Germany), and 3) 2 µg/ml Collagen Horm® (Takeda, Linz, Austria). Platelet aggregometry was performed as described before [21].

2.2. Cell concentration and flow cytometric analyses

Platelet count was measured using an automated cell counter (Sysmex N21, Hamburg, Germany). Flow cytometry analysis (FACS Calibur, BD, Heidelberg, Germany) of CD41a, CD42b and CD62P (Miltenyi Biotec, Bergisch Gladbach, Germany) was done according to standard institutional protocol.

2.3. Molecular genetic analyses

Genomic DNA was isolated from EDTA-blood samples (Blood and Cell Culture DNA Kit, Qiagen GmbH, Hilden, Germany). For the index patient all exons and splice site regions of the first seven HPS associated genes except *HPS2* (*HPS1*, *HPS3–7*) were Sanger sequenced as described previously [22]. Variants were named according to HGVS nomenclature and analyzed using supporting software ALAMUT® VISUAL. Family genotyping and mutation confirmation was performed using two independent PCR preparations.

3. Results

3.1. Platelet function analyses

Platelet aggregation/agglutination after stimulation with collagen, adenosine diphosphate, epinephrine, arachidonic acid, and ristocetin was impaired (Table 1). Especially treatment with collagen and arachidonic acid was not effective to stimulate platelet aggregation. In addition, the patient's platelets demonstrated severely reduced ATP

Table 1
Laboratory investigation of the patient's platelets.

Lumiaggregometry		
Stimulation	Max. aggregation/agglutination	Normal control
Collagen	2%	70–95%
ADP	65%	75–90%
Epinephrine	25%	70–90%
Arachidonic acid	0%	75–95%
Ristocetin	74%	85–95%

Lumiaggregometry			
Stimulation	Parameter	Value	Normal control
Thrombin (1 E/ml)	ATP	3.3 nmol/10^9	13–40 nmol/10^9
Collagen (2 µg/ml)	ATP	< 0.5 nmol/10^9	> 7 nmol/10^9