Impaired platelet function in Hermansky-Pudlak syndrome associated with novel mutations in *HPS3*, *HPS6* and *HPS8* genes

Lisa Pieri, Silvia Linari, Francesca Salvianti, Monica Attanasio, Giancarlo Castaman

Center for Bleeding Disorders and Coagulation, Department of Oncology, Careggi University Hospital, Florence, Italy

ABSTRACT

We report three unrelated cases of Hermansky-Pudlak syndrome (HPS) characterized by novel mutations in *HPS3* (compound heterozygosity for c.1457G>A and c.1813G>T), *HPS6* (homozygous c.210_211insGGGCC), and *BLOC1S3* (homozygous c.299dupC) genes. No spontaneous bleeding tendency was observed despite the presence of several platelet function abnormalities. Screening test with PFA-100 failed to detect the abnormality. This report confirms the pattern of low bleeding risk in patients with rare HPS and the need for detailed platelet function studies despite normal hemostatic screening tests.

Introduction

Hermansky-Pudlak syndrome (HPS) is a heterogeneous autosomal recessive multisystemic disorder characterized by oculocutaneous albinism, variable bleeding tendency, and, in some individuals, pulmonary fibrosis, granulomatous colitis, or immunodeficiency.[1] The disorder was first described by two Czechoslovakian physicians in two unrelated patients with oculocutaneous albinism, bleeding tendency, and pigmented macrophages in bone marrow.[1] The estimated worldwide prevalence of HPS is 1-9 per 1,000,000 individuals, but it can differ per subtype due to founder variants. The HPS type 1 subtype is the most common with a prevalence in northwestern Puerto Rico of 1:1,800.[2]

Currently, 11 subtypes have been molecularly characterized in humans (*HPS-1* to *HPS-11*). The common feature is a defect in multiple tissue-specific lysosome-related organelles (LROs), that include melanosomes of eyes and skin, dense granules in platelets, lytic granules in cytotoxic T cells and natural killer cells and lamellar bodies in lung epithelial cells and keratinocytes. The different clinical manifestations of HPS are associated with the dysfunctional LRO type involved.[2] Molecular studies show that all the known 11 mutated genes in *HPS* encode for subunits of four ubiquitously expressed multi-subunits complexes: BLOC-1 (biogenesis of lysosome-related organelles complex-1), BLOC-2, BLOC-3 and AP-3 (adaptor protein-3).[2] The HPS phenotype is related to the complex affected: patients with mutations in BLOC-1 genes (*HPS*-7, HPS-8, HPS-9, *HPS*-11) and BLOC-2 genes (*HPS*-3, *HPS*-5, *HPS*-6) have generally a mild phenotype with hypopigmentation and bleeding diathesis only. Individuals with mutations in genes involved in BLOC-3 (*HPS*-1, *HPS*-4) and AP-3 complex (*HPS*-2, *HPS*-10) are mostly associated with severe oculocutaneous albinism, pulmonary fibrosis and granulomatosis colitis.[2]

Bleeding diathesis includes easy bruising, epistaxis, gingival bleeding, menorrhagia, postpartum haemorrhage and prolonged bleeding after surgery, and it is attributed to an impaired platelet function.[2-4] The platelet function disorder (PFD) in HPS is characterized by an almost complete absence of dense granules, that contain calcium, serotonin, ATP and ADP, pyrophosphates and polyphosphates.[4]

The diagnosis of PFD in HPS is made through functional tests as platelet aggregation test and/or measuring the release and content of dense granules by lumi-aggregometry or flow cytometry,[3,4] according to the international guidance of the ISTH on inherited platelet function disorders [5]. Platelet function analyser (PFA), which is more commonly available in hemosta-

Correspondence: Giancarlo Castaman, Center for Bleeding Disorders and Coagulation, Department of Oncology, Careggi University Hospital, Florence, Italy.
E-mail: giancarlo.castaman@unifi.it

Key words: congenital platelet function disorders; platelet genetics; platelet function tests.

Contributions: all the authors contributed equally, read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Informed consent: written informed consent was obtained from legally authorized representatives for anonymized patients' information to be published in this article.

Availability of data and materials: all data underlying the findings are fully available.

Received: 23 July 2024.
Accepted: 18 October 2024.

Bleeding, Thrombosis and Vascular Biology 2024; 3:150
doi:10.4081/btvb.2024.150

sis laboratories, has unfortunately generally a low sensibility to screen patients with PFD due to dense granules defect.

Case Report

We report three novel cases of HPS in three unrelated children. All were Caucasian, with no family history of HPS, and had their first access due to nystagmus. Parents were not consanguineous (Table 1). Written informed consent was obtained from the patients' parents prior to testing.

The Pediatric Bleeding Score Questionnaire (PBQ) was calculated according to Bowman *at al*.[6] PBQ is considered positive in children if bleeding score is ≥2. PFA-100 was performed using both ADP/collagen and ADP/epinephrine cartridges; results were recorded as a closure time (CT) and expressed in seconds. Light Transmission Aggregometry (LTA) was performed on platelet rich plasma (PRP) according to the Born method. The maximum percentage of variation of light transmission from the base level (MAX%) using platelet poor plasma as a reference was recorded. Lumi-aggregometry was performed on PRP and measured the change in fluorescence emission given by the release of ATP simultaneously with platelet aggregation. The agonists used were ADP (2 and 10 µmol/L), collagen (2µg/mL), arachidonic acid (1 mmol/L), ristocetin (1.5 mg/mL, only for LTA) and epinephrine (5 µmol/L) (Table 2). A measure of dense granule content was not performed. LTA was used as a screening test of platelet function, while Lumi-aggregometry was performed both for confirmation of LTA results and for the study of dense granule content release. Results of platelet aggregation were consistent with both methods.

The first patient was born in 2019. At 3 months of age, he developed nystagmus with photophobia, no bleeding symptoms were reported (PBQ=0). Two novel heterozygous variants were identified in *HPS3* gene (c.1457G>A and c.1813G>T). Both variants, according with VarSome engine, lead to aminoacidic substitution, p.(Trp486Ter) and p.(Gly605Ter), respectively. Both are considered pathogenic. *HPS3* gene codes for a protein of the complex BLOC-2 responsible for lysosome-associated organelle biosynthesis. Platelet count and PFA-100 were in normal range. LTA revealed a reduced aggregation only after stimulation with epinephrine. ATP release from platelets was absent after stimulation with ADP 2 and 10 µmoles/L and epinephrine.

The second patient was born in 2017 and soon after birth had nystagmus with foveal hypoplasia resulting in oculo-cutaneous albinism. No bleeding symptoms were reported (PBQ=0). Compound heterozygosity for two variants were identified, both in the *HPS6* gene, which also codes for a protein of the complex BLOC-2 responsible for lysosome-associated organelle biosynthesis. The first variant (c.1A>G) was identified in the asymptomatic father. The c.1A>G changes the ATG (Met) codon with a codon coding for Val, therefore the consequence should be the loss of the start codon. Concordantly, VarSome classified this variant as likely pathogenic; however, in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) it is reported as variant of uncertain significance (VUS).

Table 1. Patients' genetic and clinical features.

Case nr	HPS type	Age (years)	Gender	Ethnicity	Gene	Mutations	Clinical features	PBQ score
1	HSP-3	4	M	Caucasian	*HSP3*	c.1457G>A c.1813G>T	Nystagmus, photophobia	0
2	HSP-6	5	F	Caucasian	*HSP6*	c.1A>G c.210_211insGGGCC	Nystagmus, oculo-cutaneous albinism	0
3	HSP-8	11	M	Caucasian	*BLOC1S3*	Homozygous c299dupC	Nystagmus, oculo-cutaneous albinism	0

Table 2. Platelet function analysis. Abnormal results are in bold.

Agonist	Maximum percentage of variation of light transmission ADP 2 µmol/L (nv >42%)	 ADP 10 µmol/L (nv >57%)	 Collagen 2 µg/mL (nv >58%)	 Arachidonic acid 1 mmol/L (nv >60%)	 Ristocetin 1.5 mg/mL (nv >68%)	 Epinephrine 5 umol/L (nv > 4%)
Case #1, *Hsp-3*	**44%***	65%*	63%	70%	100%	**17%**
Case #2, *Hsp-6*	**31%***	**50%***	**50%**	68%	68%	**10%**
Case #3, *Hsp-8*	**44%***	71%*	**23%**	62%	85%	**14%**
Agonist	**nM ATP release measured by lumi-aggregometry ADP 2 µmol/L (nr 0.96-4 nM)**	** ADP 10 µmol/L (nr 0.84-5.7 nM)**	** Collagen 2 µg/mL (nr 0.54-1.95 nM)**	** Arachidonic acid 1 mmol/L (nr 0.68-4.2 nM)**	** Ristocetin 1.5 mg/mL**	** Epinephrine 5 mmol/L (nr 0.68-2.44 nM)**
Case #1, *Hsp-3*	No release	No release	n.a.	n.a.	n.a.	No release
Case #2, *Hsp-6*	No release	0.15	0.19	0.24	n.a.	No release
Case #3, *Hsp-8*	No release	No release	No release	n.a.	n.a.	No release

nv, normal values; nr, normal range in 20 individuals; n.a., not assessed; *monophasic reversible platelet LTA curves; all experiments were performed in duplicates.

The second variant (c.210_211insGGGCC) was found in the asymptomatic mother, leads to the frameshift variation p.(Trp71Glyfs*158) and is classified as likely pathogenetic by VarSome software. Platelet count and PFA-100 were in normal range. No release from platelet dense granules was detected in the patient after stimuli with ADP 2 µmol/L and epinephrine, while minimal ATP release was obtained after stimulation with ADP 10 µmol/L, arachidonic acid and collagen. LTA showed slightly impaired aggregation after stimulation with ADP 2 µM/L and epinephrine.

The third patient was born on 2012 and presented nystagmus with oculo-cutaneous albinism, without bleeding symptoms (PBQ=0). He was diagnosed with HPS type 8 with the novel homozygous mutation c.299dupC in *BLOC1S3*, that introduces the frameshift p.(Ala101Glyfs*44) and a subsequent stop codon. Platelet count and PFA-100 were in normal range. LTA revealed reduced aggregation after stimulation with collagen and epinephrine. ATP release from platelets was absent after stimulation with ADP 2 and 10 µmol/L, collagen and epinephrine.

HPS is a very heterogeneous disorder, although clinical oculocutaneous albinism is present in almost all patients. We described three cases of HPS with newly identified mutations involving BLOC-2 and BLOC1S3 proteins without, at least at last follow up, bleeding symptoms. However, no one of these patients required invasive procedure nor had any severe trauma, so it cannot be excluded that they may have an increased bleeding risk after more challenging stimuli other than activity of daily living.

As expected, dense granules release was almost absent and LTA showed only mild aggregation reduction to few agonists, mainly epinephrine. However, platelet delta granules content, which characterizes the HPS platelet phenotype, was not measured in our patients and thus we cannot link the reduced release to a reduction of delta granules or of their content in patients' platelets. PFA was normal in all cases, confirming its poor sensitivity when screening for PFD with storage pool deficiency.[3,4]

Conclusions

These data also confirm that *HPS3*, *HPS6* and *HPS8* variants are usually associated with no or mild bleeding tendency, although low or absent platelet delta granules.[4] An open issue is if these patients can have an increased bleeding risk in case of surgery, and what kind of prophylaxis could be necessary and useful. The use of tranexamic acid, desmopressin and activated recombinant factor VII was described in one case of menorrhagia.[7] Response to desmopressin was variable in a HSP patient series from Puerto Rico, with a significant correlation with poor response in patients with *HSP1* mutations.[8]

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