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Identification and clinical characterization of Hermansky-Pudlak syndrome alleles in the Pakistani population

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TO THE EDITOR

Hermansky-Pudlak Syndrome (HPS) is a genetically heterogeneous, recessive disorder that results in defects of multiple cytoplasmic organelles, including melanosomes, platelet-dense granules and lysosomes (Tomita and Suzuki, 2004; Witkop et al., 1990). Salient clinical features of HPS include Oculocutaneous albinism (OCA), bleeding, lysosomal ceroid storage, nystagmus, strabismus, iris trans-illumination, foveal hypoplasia, bruising, and a significant reduction in visual acuity (Gahl et al., 1998; Witkop et al., 1990). In approximately half of HPS1 and HPS4 cases, pulmonary fibrosis also has been documented, with onset often in the early twenties to early forties (Gahl et al., 1998). To date, mutant alleles in nine genes have been linked to HPS (Wei and Li, 2013). The currently known HPS proteins have been categorized into three biogenesis of lysosome-related organelle (LRO) complexes, BLOC-1, -2 and -3 (Dell'Angelica, 2004; Huizing et al., 2008; Wei and Li, 2013; Wei, 2006). HPS2 also constitute a subunit of adaptor complex-3, which has critical functions in pigment-producing cells (Dell'Angelica et al., 1999). Together with other proteins, BLOC complexes participate in endolysosomal trafficking to facilitate LRO biogenesis (Wei et al., 2013).

According to the HPSD, OMIM and HGMD databases, as of October 2015, only one mutation in *HPS8* (*BLOC1S3*) was previously reported in the Pakistani population. Here, using whole exome sequencing (WES) we report seven pathogenic variants (Table S1) in five *HPS* genes segregating with the phenotype in seven large consanguineous Pakistani families (Figure 1; see also Supplementary Data online). In first family PKAB83,

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sequencing revealed a transition mutation (c.1342T>C, p.(Trp448Arg), Figure S1). Molecular modeling data revealed that the p.Trp448 residue is located in the β-strand of the secondary structure and replacing it with smaller, positively charged, less hydrophobic arginine residue will slightly destabilize the local conformation (Figure S2). We performed detailed hematological examinations (Table 1, Table S2) and chest computerized tomography (CT) scans of the affected individuals. All hematological parameters were within the normal range (Table S2). However, we observed reduced platelet aggregation among the affected individuals (Table 1). CT scans of affected individuals V:6 and V:7 revealed no evidence of diffuse interstitial lung disease, bronchiectasis, pulmonary hypertension, intra pulmonary/mediastinal mass or lymphadenopathy was found. As stated earlier, the lung involvement in HPS1 patients usually begins in adulthood and an absence of pulmonary fibrosis in these affected individuals might be due to their relative young age. Indeed, the CT images of the affected individual V:7 (30 yrs old) revealed small subsegmental fibrotic areas in the apico-posterior segment of the left upper lobe and in the apical segment of the right upper lobe. These clinical findings suggest milder form of HPS phenotype among the affected individual of family PKAB83.

In family PKAB126, a new nonsense mutation (p.(Gln686*)) in the *HPS1* gene was segregating (Figure 1 and S1) with the HPS phenotype (Table 1 and Table S2). The affected individual had reduced vision and platelet aggregation abilities besides OCA (Table 1). On the other hand, the affected individuals of family PKAB114 were homozygous for a 121 bp genomic deletion (Table S3) that results in the partial loss of intron and a splice acceptor site for second coding exon of *HPS1* (Figure 1 and S3). Human Splicing Finder program predicted cryptic splicing and premature truncation of the HPS1 protein (p.(Pro41Aspfs*12)) due to this deletion mutation.

In contrast, the affected individuals of family PKAB139 were homozygous for a transition mutation (c.1509G>A) in the *HPS3* (Figure 1, Table 1), which is predicted to replace an evolutionarily conserved methionine (p.(Met503Ile); Figure S4). Molecular modeling of central region of HPS3 revealed that p.Met503 residue is located in an μ-helix and replacing it with isoleucine that does not prefer μ-helices as secondary structure might lead to loss of interactions (Figure S2). Although limited clinical information was available for family PKAB139, the sixteen-year-old affected individual had no history of gastrointestinal problems, bleeding diathesis and his bleeding time and clotting time were normal (Table 1).

In a family of Sindh origin, LUAB13, WES revealed a putative +5 splice site mutation (c.276+5G>A) in intron 4 of *HPS4* segregating with the phenotype (Figure 1). HPS4 together with HPS1 constitute BLOC-3, a Rab guanine nucleotide exchange factor (GEF) that is essential for the biogenesis of LRO (Gerondopoulos et al., 2012). *In silico* prediction suggests that the skipping of exon 4 due to c.276+5G>A change results in a reading frameshift and premature truncation. However, as the mutation is not present at the canonical splice donor site, an *in vivo* c.276+5G>A change may result in a leaky splicing error. Hematological analyses of the three affected individuals ranging from 34 to 55 yrs old revealed milder phenotype with no platelet abnormalities and history of bleeding diathesis (Table 1).

Multiple isoforms are transcribed at the *HPS4* locus (Anderson et al., 2003). The full-length *HPS4* isoform encodes a 708 amino acid polypeptide (Figure S5). The Genome Browser annotation of the human *HPS4* locus (NCBI build GRCh37) also contains a smaller cDNA clone (DB104830), isolated from the human thymus and transcribed by the first two coding exons of *HPS4* and is predicted to encode a polypeptide of 89 amino acids (Figure S5). The open reading of the shorter isoform is predicted to encounter a stop codon at the beginning of intron 4 (Figure S5) and encode only two additional evolutionarily conserved amino acids beyond exon 4 (Figure S4). The mutation segregating in LUAB13 is predicted to replace the serine present at the end of this shorter transcript with an asparagine (Figure S4). However, the differential activities of the various isoforms of *HPS4* are currently not well understood.

To date, nine mutations have been reported in the *HPS6* gene that are responsible for the *HPS* phenotype in humans (Huizing et al., 2009). In family PKAB167, our sequencing revealed a novel homozygous missense mutation (p.(Pro275Ser)) in the *HPS6* (Figure 1 and S4). The p.(Pro275Ser) change was predicted disease causing by at least two prediction programs with relatively moderate CADD scores (Table S1). Our molecular modeling data suggest that the p.Pro275 residue is part of interpro domain termed as Bloc-2 complex, Hps6 subunit (IPR017218). Prolines are known to be very rigid and therefore induce a special backbone conformation and replacing it with a smaller, less hydrophobic residue serine is predicted to disturb this special conformation (Figure S2). Clinical evaluation of the available affected individuals revealed no history of bleeding disorders and her bleeding and clotting times were within the normal range (Table 1).

Finally, our genetic analysis of the LUAB11 family revealed a nonsense mutation (p.(Gln78*); Figure 1 and S4) in the *PLDN* (*HPS9*). The same mutation was previously identified in two individuals, including a 9-month-old Indian male and a 17-year-old Italian female (Badolato et al., 2012; Cullinane et al., 2011). Both affected individuals had OCA and nystagmus but no hemorrhagic problems (Badolato et al., 2012; Cullinane et al., 2011). However, the Italian female had a history of recurrent cutaneous infections (Badolato et al., 2012). In family LUAB11, the 4-year-old female had OCA, photophobia, nystagmus, prolonged bleeding and clotting times (Table 1), which indicate platelet dysfunction.

In summary, our data suggest a genotype-phenotype correlation in which presumably the missense alleles identified are hypomorphic and associated with a milder phenotype, whereas truncating alleles result in classical severe HPS. The results provided here will improve our knowledge of the molecular epidemiology of HPS, diagnoses and genetic counseling. We expect that further analyses of the missense alleles identified here in the *HPS* genes are expected to increase our understanding of mutation-structure and structure-function relationships, and therefore the pathogenesis of HPS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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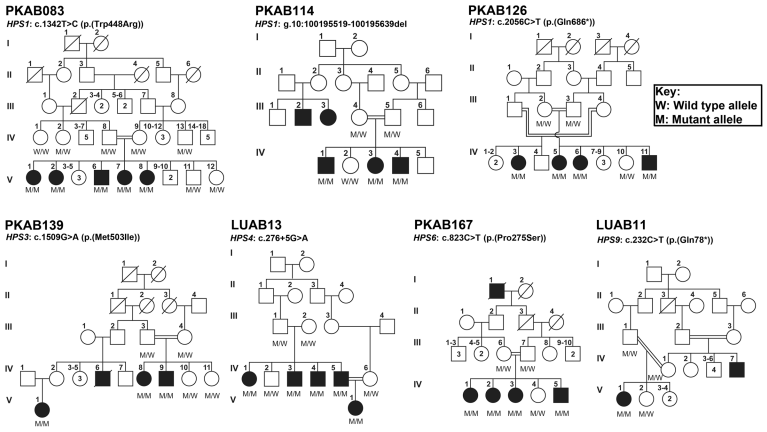

Figure 1. HPS phenotypes in seven Pakistani families co-segregate with mutations in known *HPS* genes

The filled and empty symbols represent the affected and unaffected individuals, respectively. A double line uniting two individuals represents a consanguineous marriage. Given also are the *HPS* genes mutations and two point LOD scores for each family, along with the genotypes for the mutated loci.

Table 1

Clinical findings of Hermansky-Pudlak syndrome in the Pakistani families

Family	ID	Age (yrs)	Gene	Allele	Status	Hair color	Skin tone	Iris color	Visual acuity		Refraction error	Fundus	FVH	PP	NYS	GI problems[#]	BT (2-7 min)	CT (6-7 min)	PT (10-14 sec)	APTT (≤32sec)	P.A.T. (80-100%)
									Right	Left											
PKAB083	IV:13	45	*HPS1*	c.1342T>C	Norm.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	No	2m	4m 40s	NA	NA	NA
	V:1	8			Aff.	Golden	White	Hazel green	6/60	6/60	NA	Albinotic	Yes	Yes	Yes	Yes	2m	3m	NA	NA	52%
	V:2	4			Aff.	Golden	White	Hazel green	6/60	6/60	NA	Albinotic	Yes	Yes	Yes	Yes	3m 15s	3m	NA	NA	15%
	V:6	32			Aff.	Golden	White	Hazel green	6/60	6/60	NA	Albinotic	Yes	Yes	Yes	Yes	2m 15s	6m 20s	14s (12s)	32s	33%
	V:7	30			Aff.	Golden	White	Hazel green	6/60	6/60	NA	Albinotic	Yes	Yes	Yes	Yes	3m 20s	5m 15s	12s (12s)	30s	46%
	V:8	28			Aff.	Golden	White	Hazel green	6/60	6/60	NA	Albinotic	Yes	Yes	Yes	Yes	3m 15s	5m 50s	NA	NA	29%
PKAB114	III:5	63	*HPS1*	genomic deletion	Norm.	NA	NA	NA	NA	NA	NA	NA	NA	NA	No	No	3m 30s	3m 15s	NA	43s	85%
	III:4	58			Norm.	NA	NA	NA	NA	NA	NA	NA	NA	NA	No	No	NA	NA	NA	NA	93%
	IV:3	25			Aff.	Brown	White	Grey	NA	NA	NA	NA	NA	NA	No	Yes	3m 40s	3m 30s	NA	39s	10%
	IV:4	23			Aff.	Brown	White	Green	NA	NA	NA	NA	NA	NA	No	Yes	6m 20s	7m 30s	NA	49s	32%
	IV:1	10			Aff.	Brown	White	Green	NA	NA	NA	NA	NA	NA	No	Yes	NA	NA	NA	NA	22%
PKAB126	III:3	58	*HPS1*	c.2056C>T	Norm.	Black	Wheat	Brown	6/6	6/6	Presbyopic	Normal	No	No	No	No	3m 30s	4m	NA	41s	81%
	III:2	16			Norm.	Black	Wheat	Black	6/6	6/6	Emmetropic	Normal	No	No	No	No	NA	NA	NA	NA	88%
	IV:11	10			Aff.	Yellow brown	Pink white	Hazel green	6/18	6/18	+3.25/−3.50 *22 +2.50/−3.75 *176	Albinotic	Yes	Yes	Yes	No	5m 30s	4m 30s	NA	50s	53%
	IV:6	15			Aff.	Yellow brown	Pink white	Hazel green	6/18	6/18	+5.25/−4.75 *16 +5.25/−2.50 *4	Albinotic	Yes	Yes	Yes	No	5m	4m 30s	NA	44s	35%
	IV:3	12			Aff.	Yellow brown	Pink white	Hazel green	CF	CF	+7.25/−5.25 *24 +6.0/−1.25 *180	Albinotic	Yes	Yes	Yes	No	NA	NA	NA	NA	35%
PKAB139	IV:9	16	*HPS3*	c.1509G>A	Aff.	Dark brown	White	Brown	6/24	6/24	−3.25/−2.25 *168 -4.75/−3.25 *173	Normal	No	Yes	Yes	No	3m (≤5m)	8m (≤11m)	NA	NA	NA
LUAB13	III:1	55	*HPS4*	c.276+5G>A	Norm.	NA	NA	NA	NA	NA	NA	NA	NA	No	No	No	1m 30s (≤3m)	5m (≤11m)	15s	32s	NA
	IV:4	34			Aff.	Brown	Pink white	Brown	NA	NA	NA	NA	NA	Yes	Yes	No	2m (≤3m)	6m (≤11m)	14s	36s	NA
	IV:5	38			Aff.	Brown	Pink white	Brown	NA	NA	NA	NA	NA	Yes	Yes	No	1m 30s (≤3m)	4m 30s (≤11m)	12s	32s	NA
PKAB167	IV:4	18	*HPS6*	c.823C>T	Norm.	Black	Wheat	Brown	6/60	6/24	+10.25/−4.75 *177 +10.0/−3.75 *6	Normal	No	No	No	No	NA	NA	NA	NA	NA
	IV:3	10			Aff.	Red	Pink white	Dark brown	6/60	6/36	+2.0/−1.25 *161 +2.0/−1.0 *149	Albinotic	Yes	Yes	Yes	Yes	2m 45s (≤5m)	4m 50s (≤11m)	NA	NA	NA

Family	ID	Age (yrs)	Gene	Allele	Status	Hair color	Skin tone	Iris color	Visual acuity		Refraction error	Fundus	FVH	PP	NYS	GI problems[#]	BT (2-7 min)	CT (6-7 min)	PT (10-14 sec)	APTT (≤32sec)	P.A.T. (80-100%)
									Right	Left											
	IV:2	14			Aff.	Red	Pink white	Dark brown	6/18	6/18	+0.25/−0.50 [*]63 +0.25/−0.50 [*]65	Albinotic	Yes	Yes	No	Yes	NA	NA	NA	NA	NA
LUAB11	VI:4 [*]	04	*HPS9*	c.232C>T	Aff.	Golden white	Pink white	Light brown	NA	NA	NA	NA	NA	Yes	Yes	Yes	>3m (≤3m)	>11m (≤11m)	>1m	>2m	NA

Normal ranges for tests are given in parenthesis. Norm: Normal; Aff: Affected; NA: Not Available; CF: Count Fingers. FVH.: Foveal hypoplasia; PP: photophobia; NYS: Nystagmus; BT: Bleeding time; CT: Clotting time; PT: Prothrombin time; APTT: Activated Partial Thromboplastin time; P.A.T.: Platelet aggregation test; Y: Yes; N: No.

[#]Gastrointestinal problems including nausea, vomiting, abdominal pain, diarrhea etc.

[*]Had platelet count was 74×10^3/uL (normal range 150 – 400 10^3/uL) at the time of clinical investigation.