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CASE REPORT Open Access

Mutational spectrum of the *AP3B1* gene in an Iraqi family affected with Hermansky–Pudlak syndrome type 2

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Abstract

Background Hermansky–Pudlak syndrome type 2 (HPS2) is a rare autosomal recessive inherited disease present with partial oculocutaneous albinism, nystagmus, prolonged bleeding time, and immunodeficiency.

Case presentation We aimed at identifying a genetic mutation in an Iraqi family affected by HPS type 2. Here, we applied whole-exome sequencing to identify mutations in the proband. Moreover, we applied Sanger sequencing to confirm the candidate variant. We found a homozygous novel single nucleotide substitution (c.892A>T) in the exon 8 of the *AP3B1* gene in the proband.

Conclusion This study is the first Iraqi case report of a diagnosis of HPS type 2 caused by *AP3B1* mutation. Our data expand the spectrum of mutations in *AP3B1* gene in HPS type 2 and highlight the importance of molecular prenatal evaluation and relevant genetic counseling.

Background

Hermansky–Pudlak syndrome (HPS), first described in 1959, is a rare autosomal recessive hereditary multisystem disease that comprises a spectrum of different subtypes [1–3]. Patients with HPS typically present with tyrosinase-positive oculocutaneous albinism, nystagmus, bleeding diathesis due to platelet function disorder, and systemic complications resulting from abnormal intracellular vesicles trafficking or formation [4–6].

Currently, 10 subtypes of HPS (HPS1–HPS10) are described in humans, caused by variants in nine unique genes. Patients with HPS type 2 and HPS type 10 present with various symptoms such as neutropenia, immunodeficiency, and neurological abnormalities in addition to the core features of platelet dysfunction and albinism [7–9]. Patients with HPS type 2 absence of the β3A-subunit in the adaptor protein-3 (AP-3) is relevant for sorting of lysosomal [10, 11].

In this study, we performed whole-exome sequencing followed by a Sangar sequencing approach for an Iraqi family with HPS type 2 to identify the underlying genetic defect.

Case presentation

A 9-year-old male born to an Iraqi consanguineous parents (Fig. 1) presented with nystagmus, a tendency to bleed, and platelet dysfunction. The patient also presented with oculocutaneous albinism and recurrent infections of the upper respiratory airways since the first month of life. His parents mentioned that during the first 15 months of life, he had developed normally. We reviewed the entire medical record for the proband. There was evidence of prolonged bleeding. The patient tolerated circumcision with hemorrhagic complications.

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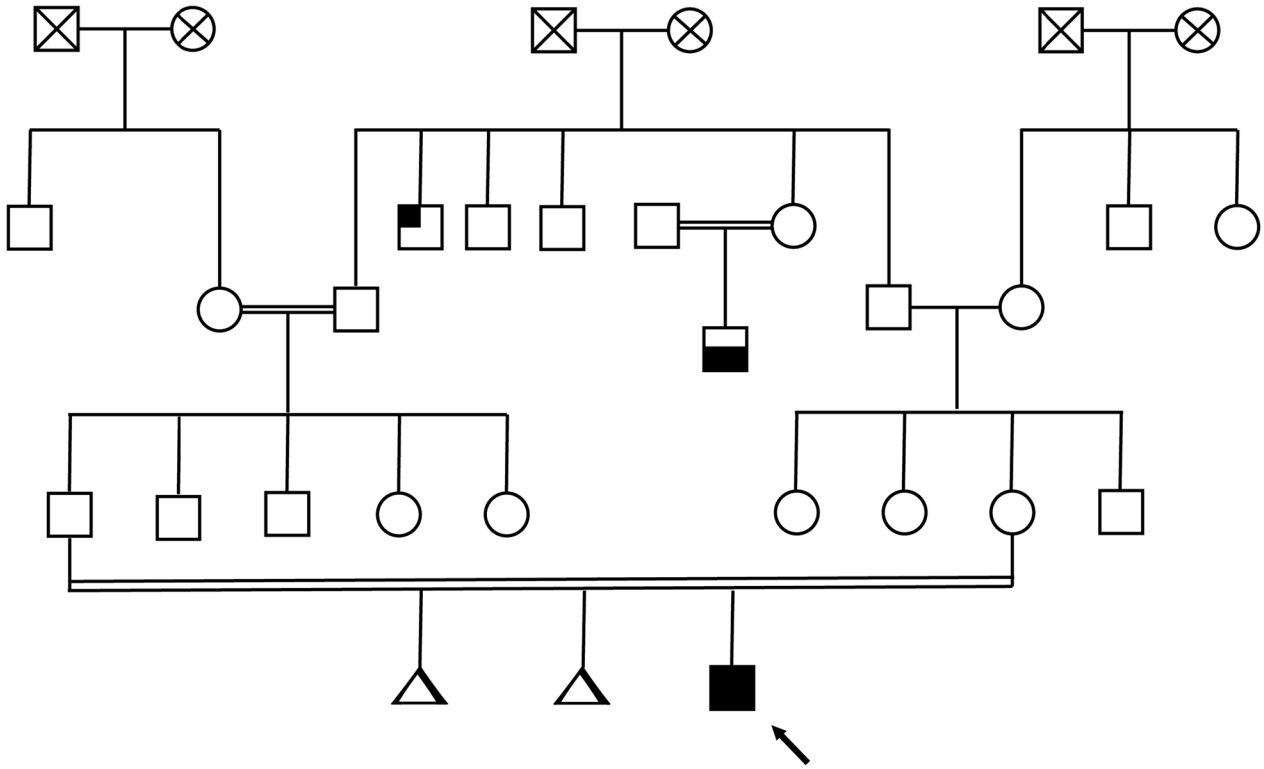

Fig. 1 The family pedigree studied. Squares represent male individuals, circles represent female individuals, black square represents the patient, triangle represent spontaneous abortion, slashes represent deceased individuals, and arrow represents the proband

At the age of 18 months, a tonsillectomy was performed. About 14 days after the tonsillectomy, severe bleeding from the wounds occurred so the boy needed resuscitation. The proband suffered from severe hypoxia and developed severe mental and statomotoric retardation. Measurements of prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet counts were normal, but he presented with neutropenia (mean peripheral blood polymorphonuclear granulocytes count 0.82 ± 0.45 G/L). Finally, laboratory tests showed neutropenia and prolonged bleeding time. Platelet count was $403 \times 10^3/\mu L$, but bleeding time (Duke method) was extended to 7.5 min (the usual time is about 2–5); The proband was clinically suspected to have HPS type 2. Thus, we applied whole-exome sequencing, which may allow confirmation of a diagnosis of HPS in this family. The studied family had a previous history of two spontaneous abortions.

Whole-exome sequencing test with a focus on HPS genes was performed for the proband. Various known filtering procedures were applied, such as coverage of more than six reads, a minimum quality score of 10, an allele frequency between 75 and 100%, a minor allele frequency (MAF) of $\leq 0.1\%$ in the 1000 Genomes database (https://www.internationalgenome.org/), exome aggregation consortium (ExAC) and the exome variant server (EVS) for the NHLBI exome sequencing project (ESP). Following that, to verify the true positive of whole-exome sequencing identified variant, direct Sanger sequencing using an ABI 3730XL sequencer (Applied Biosystems Inc., Foster City, CA, USA) was performed in the patient and family members.

A novel homozygous potentially pathogenic c.892A > T; p.Arg298Ter mutation in *AP3B1* gene (NM_003664.5) associated with HPS type 2 (OMIM#: 608233) was detected. In terms of inheritance, the parents of the patient were both heterozygous carriers for the *AP3B1* gene mutation (Fig. 2). This nonsense mutation (c.892A > T; p.Arg298Ter) has not been reported in other patients with HPS type 2, but it is a severe loss-of-function mutation. This mutation describes a substitution mutation at the codon 298 (Arg), which leads to premature termination of the *AP3B1* protein (AGA > TGA) (Fig. 2D). Reported mutations in *AP3B1* gene are summarized in Table 1 based on Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php). Interestingly, no pathogenic mutations were detected in the other genes in the proband.

This nonsense mutation (c.892A > T; p.Arg298Ter) has not been reported in other patients with HPS type 2, but

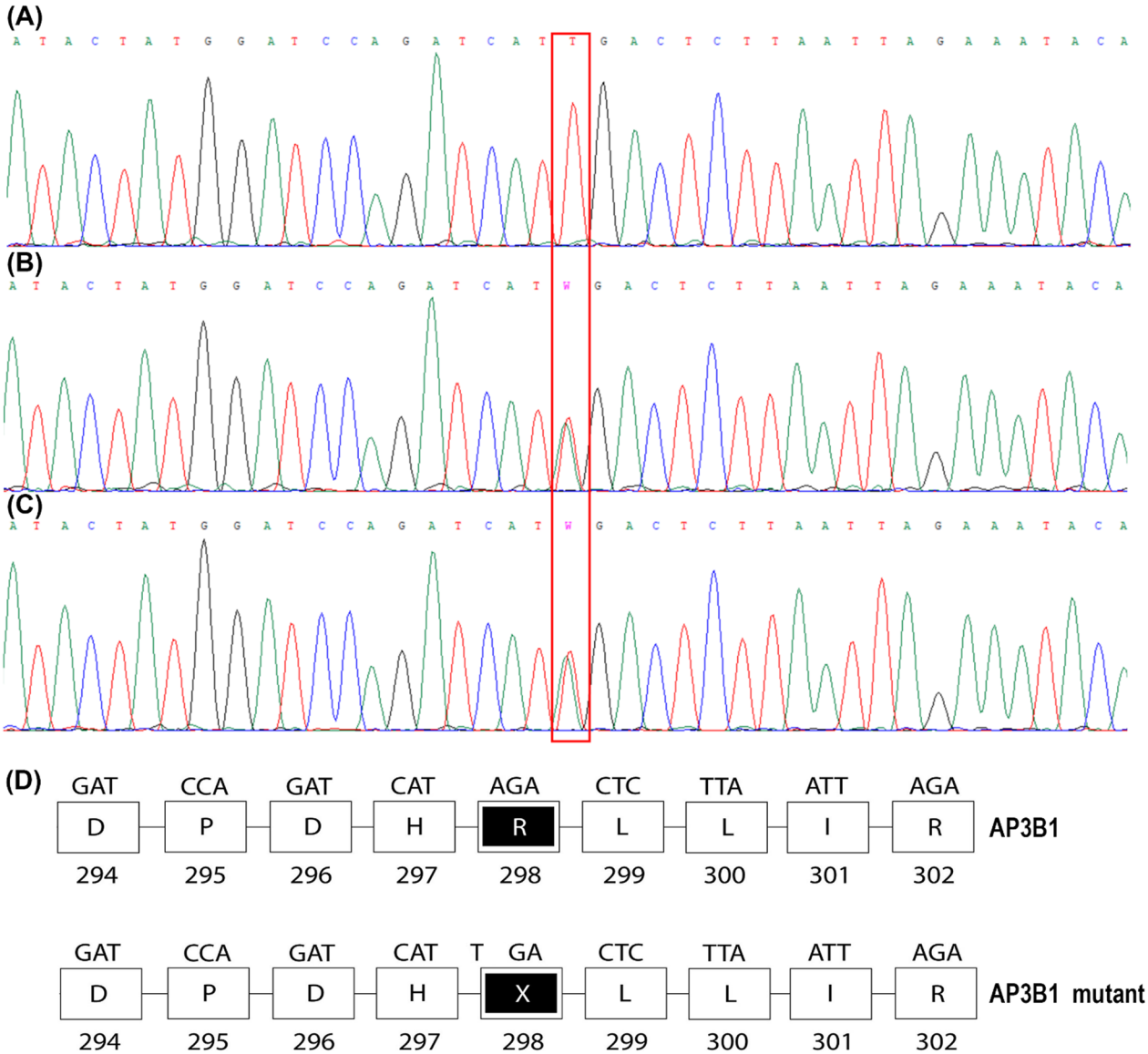

Fig. 2 Sequence chromatograms of the affected son (**A**) and his healthy parents (**B**, **C**). **D** Zoomed-in view of the region containing *AP3B1* Arg298Ter variant, including the amino acid sequences of protein-coding isoform and the mutated sequence caused by the variant

it is a severe loss-of-function mutation. This mutation describes a substitution mutation at the codon 298 (Arg), which leads to premature termination of the *AP3B1* protein (AGA > TGA) (Fig. 2D).

Discussion

This report describes the characteristics of a patient with HPS type 2 due to a novel disease-causing *AP3B1* mutation. This study proved that the nonsense *AP3B1* variant in the exon 8 (c.892A > T or p.Arg298X) leads to an early termination in amino acid production, which would be expected to affect the *AP3B1* protein's function.

It was suspected that the child had HPS because of the combination of oculocutaneous albinism and increased bleeding tendency. In contrast to other subtypes of HPS, HPS type 2 caused by mutations in the β3A-subunit of the adaptor protein complex AP-3 is characterized by immunodeficiency causing increased susceptibility to infections. All reported HPS type 2 patients had severe neutropenia [11, 12]. The case described here also presented with neutropenia.

Wenham et al. in their publication reported two unrelated individuals with HPS type 2, which were homozygous for different deletions *AP3B1* gene mutations. One

Table 1 Reported mutations in *AP3B1* gene

Pathogenic variant	Protein effect	Location	Type of mutation
c.305T > C	p.Leu102Pro	Exon 4	Missense
c.716G > A	p.Trp239Ter	Exon 7	Nonsense
c.904A > T	p.Arg302Ter	Exon 8	Nonsense
c.1525C > T	p.Arg509Ter	Exon 15	Nonsense
c.1739T > G	p.Leu580Arg	Exon 16	Missense
c.1975G > T	p.Glu659Ter	Exon 18	Nonsense
c.2041G > T	p.Glu681Ter	Exon 18	Nonsense
IVS10 ds + 5 G > A	–	Intron 10	Splicing
IVS11 as − 1 G > C	–	Intron 11	Splicing
IVS14 ds + 6T > C	–	Intron 14	Splicing
IVS23 ds − 108 C > G	–	Intron 23	Splicing
c.54_57del	p.Glu19TrpfsTer22	Exon 1	Deletion
c.143_147del	p.Lys48AsnfsTer10	Exon 2	Deletion
c.167_171del	p.Asp56GlyfsTer2	Exon 2	Deletion
c.1744_1749del	p.Val582_Pro583del	Exon 16	Deletion
c.2760_2766del	p.Glu921AsnfsTer4	Exon 23	Deletion
c.3212_3216del	p.Ile1071LysfsTer3	Exon 27	Deletion

patient with a homozygous frameshift *AP3B1* mutation in the exon 2 (c.153_156del) manifests a severe clinical phenotype with albinism, recurrent respiratory infections, failure to thrive, facial dysplasia, pulmonary fibrosis, and developmental delay. No increased bleeding was seen in the patient; however, it is unclear whether the patient has been challenged by surgery in a mucocutaneous area. In addition, the patient also developed transient hepatosplenomegaly, hypertriglyceridemia, and thrombocytopenia strongly suggestive of hemophagocytic syndrome [13].

Previously, Enders et al. also introduced a homozygous nonsense *AP3B1* mutation in exon 8 causing a stop codon at codon 302 (Arg) in a patient with HPS type 2 who showed a severe clinical phenotype similar to the case reported by Wenham et al. [13, 14]. Besides dysplastic characteristics and developmental retardation, he presented with hepatosplenomegaly and recurrent infections and finally developed a lethal hemophagocytic syndrome. First, an increased bleeding tendency had not been recognized; however, he developed severe mucosal bleeding, after tooth extraction. More assessment showed that the two cases described by Wenham et al. and Enders et al. represent the only HPS type 2 patients developing symptoms indicative bleeding symptoms [7, 8, 10–12, 15]. However, there was no insufficient evidence of hemophagocytosis. Interestingly, the case reported in this study revealed a nonsense *AP3B1* mutation leading to a β3A protein that represents an altered amino acid sequence, which can be a highlighted new candidate mutation Arg298X in exon 8 for HPS type 2. So far, this variant has not been reported before. In this study, our patient did not develop any signs or symptoms of hemophagocytosis.

Previous studies revealed that HPS type 2 could be a consequence of the *AP3B1* gene mutations. In this regard, Jung et al. [15], in their publication, showed causative homozygous genomic exon deletion of the *AP3B1* gene in two patients with HPS type 2. Furthermore, Wenham M, et al., evaluating pathogenic genomic defects in two patients, novel mutations in *AP3B1* mutation (c.2078_2165del; p. Glu693fsX13 and c.153_156del; p. Glu52fsX11) and concluded that mutations in *AP3B1* gene accounted for HPS type 2 [13]. Subsequently, Nishikawa et al. [16] reported a Japanese patient with a novel compound heterozygous pathogenic mutation (c.188T > A; p.Met63Lys [exon 2] and c.2546T > A; p. Leu849Ter [exon 22]) in *AP3B1* gene related with HPS type 2. In this study, we also investigated the genetic defect of HPS type 2 in an Iraqi family using whole-exome sequencing and presented a novel homozygous *AP3B1* c.892A > T mutation that resulted in a premature stop codon.

Conclusion

We have detected a novel nonsense *AP3B1* mutation causing HPS type 2 in an Iraqi family. The present study revealed that whole-exome sequencing may be used as an efficient and cost-effective molecular diagnostic strategy for detecting HPS type 2 patients. Moreover, detection of HPS type 2 causing *AP3B1* gene mutation may be helpful for surveillance and management in at-risk relatives.

Abbreviations

aPTT	Activated partial thromboplastin time
AP-3	Adaptor protein-3
ESP	Exome sequencing project
ExAC	Exome aggregation consortium
EVS	Exome variant server
HPS	Hermansky–Pudlak syndrome
MAF	Minor allele frequency
PT	Prothrombin time

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Author contributions

MN and AIA analyzed and interpreted the data. MN wrote the manuscript. AIA helped to review the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compare ethical strand.

Consent for publication

Written informed consent was obtained from the parents of the patient for this publication.

Competing interests

The authors declared there is no conflict of interest.

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References

1. Hermansky F, Pudlak P (1959) Albinism associated with hemorrhagic diathesis and unusual pigmented reticular cells in the bone marrow: report of two cases with histochemical studies. Blood 14(2):162–169 (**PMID: 13618373**)
2. Velázquez-Díaz P, Nakajima E, Sorkhdini P, Hernandez-Gutierrez A, Eberle A, Yang D, Zhou Y (2021) Hermansky–Pudlak syndrome and lung disease: pathogenesis and therapeutics. Front Pharmacol 12:644671. https://doi.org/10.3389/fphar.2021.644671
3. Hengst M, Naehrlich L, Mahavadi P, Grosse-Onnebrink J, Terheggen-Lagro S, Skanke LHS, Schuch LA, Brasch F, Guenther A, Reu S, Ley-Zaporozhan J, Griese M (2018) Hermansky–Pudlak syndrome type 2 manifests with fibrosing lung disease early in childhood. Orphanet J Rare Dis 13(1):42. https://doi.org/10.1186/s13023-018-0780-z
4. Wei AH, He X, Li W (2013) Hypopigmentation in Hermansky–Pudlak syndrome. J Dermatol 40(5):325–329. https://doi.org/10.1111/1346-8138.12025
5. Loredana Asztalos M, Schafernak KT, Gray J, Berry A, Paller AS, Mancini AJ (2017) Hermansky–Pudlak syndrome: report of two patients with updated genetic classification and management recommendations. Pediatr Dermatol 34(6):638–646. https://doi.org/10.1111/pde.13266
6. Kurnik K, Bartsch I, Maul-Pavicic A, Ehl S, Sandrock-Lang K, Bidlingmaier C, Rombach N, Busse A, Belohradsky BH, Müller-Höcker J, Aslanidis C, Schmitz G, Zieger B (2013) Novel mutation in Hermansky–Pudlak syndrome type 2 with mild immunological phenotype. Platelets 24(7):538–543. https://doi.org/10.3109/09537104.2012.741275
7. Clark RH, Stinchcombe JC, Day A, Blott E, Booth S, Bossi G, Hamblin T, Davies EG, Griffiths GM (2003) Adaptor protein 3-dependent microtubule-mediated movement of lytic granules to the immunological synapse. Nat Immunol 4(11):1111–1120. https://doi.org/10.1038/ni1000
8. Fontana S, Parolini S, Vermi W, Booth S, Gallo F, Donini M, Benassi M, Gentili F, Ferrari D, Notarangelo LD, Cavadini P, Marcenaro E, Dusi S, Cassatella M, Facchetti F, Griffiths GM, Moretta A, Notarangelo LD, Badolato R (2006) Innate immunity defects in Hermansky–Pudlak type 2 syndrome. Blood 107(12):4857–4864. https://doi.org/10.1182/blood-2005-11-4398
9. Ammann S, Schulz A, Krägeloh-Mann I, Dieckmann NM, Niethammer K, Fuchs S, Eckl KM, Plank R, Werner R, Altmüller J, Thiele H, Nürnberg P, Bank J, Strauss A, von Bernuth H, Zur Stadt U, Grieve S, Griffiths GM, Lehmberg K, Hennies HC, Ehl S (2016) Mutations in AP3D1 associated with immunodeficiency and seizures define a new type of Hermansky–Pudlak syndrome. Blood 127(8):997–1006. https://doi.org/10.1182/blood-2015-09-671636
10. Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS (1999) Altered trafficking of lysosomal proteins in Hermansky–Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. Mol Cell 3(1):11–21. https://doi.org/10.1016/s1097-2765(00)80170-7
11. Huizing M, Scher CD, Strovel E, Fitzpatrick DL, Hartnell LM, Anikster Y, Gahl WA (2002) Nonsense mutations in ADTB3A cause complete deficiency of the beta3A subunit of adaptor complex-3 and severe Hermansky–Pudlak syndrome type 2. Pediatr Res 51(2):150–158. https://doi.org/10.1203/00006450-200202000-00006
12. Shotelersuk V, Dell'Angelica EC, Hartnell L, Bonifacino JS, Gahl WA (2000) A new variant of Hermansky–Pudlak syndrome due to mutations in a gene responsible for vesicle formation. Am J Med 108:423–427. https://doi.org/10.1016/S0002-9343(99)00436-2
13. Wenham M, Grieve S, Cummins M, Jones ML, Booth S, Kilner R, Ancliff PJ, Griffiths GM, Mumford AD (2010) Two patients with Hermansky Pudlak syndrome type 2 and novel mutations in AP3B1. Haematologica 95(2):333–337. https://doi.org/10.3324/haematol.2009.012286
14. Enders A, Zieger B, Schwarz K, Yoshimi A, Speckmann C, Knoepfle EM, Kontny U, Müller C, Nurden A, Rohr J, Henschen M, Pannicke U, Niemeyer C, Nurden P, Ehl S (2006) Lethal hemophagocytic lymphohistiocytosis in Hermansky–Pudlak syndrome type II. Blood 108(1):81–87. https://doi.org/10.1182/blood-2005-11-4413
15. Jung J, Bohn G, Allroth A, Boztug K, Brandes G, Sandrock I, Schäffer AA, Rathinam C, Köllner I, Beger C, Schilke R, Welte K, Grimbacher B, Klein C (2006) Identification of a homozygous deletion in the AP3B1 gene causing Hermansky–Pudlak syndrome, type 2. Blood 108(1):362–369. https://doi.org/10.1182/blood-2005-11-4377
16. Nishikawa T, Okamura K, Moriyama M, Watanabe K, Ibusuki A, Sameshima S, Masamoto I, Yamazaki I, Tanita K, Kanekura T, Kanegane H, Suzuki T, Kawano Y (2020) Novel AP3B1 compound heterozygous mutations in a Japanese patient with Hermansky–Pudlak syndrome type 2. J Dermatol 47(2):185–189. https://doi.org/10.1111/1346-8138.15177

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