SHORT REPORT

De novo missense variants in the RAP1B gene identified in two patients with syndromic thrombocytopenia

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Abstract
We present two independent cases of syndromic thrombocytopenia with multiple malformations, microcephaly, learning difficulties, dysmorphism and other features. Exome sequencing identified two novel de novo heterozygous variants in these patients, c.35G>T p.(Gly12Val) and c.178G>C p.(Gly60Arg), in the RAP1B gene (NM_001010942.2). These variants have not been described previously as germline variants, however functional studies in literature strongly suggest a clinical implication of these two activating hot spot positions. We hypothesize that pathogenic missense variants in the RAP1B gene cause congenital syndromic thrombocytopenia with a spectrum of associated malformations and dysmorphism, possibly through a gain of function mechanism.

KEYWORDS
Kabuki syndrome, learning difficulties, malformations, microcephaly, pancytopenia, RAP1B, thrombocytopenia

1 | INTRODUCTION

Hereditary thrombocytopenia (HT) is a heterogeneous group of genetic disorders characterized by a low platelet count causing impaired hemostasis. Until today, about 40 genes have been associated with HT.¹

Patients with Kabuki syndrome (KS, MIM#147920/MIM#300867) present with intellectual disability, postnatal growth deficiency, organ malformations, skeletal anomalies and other features.² Typical facial dysmorphism include long palpebral fissures with eversion of the
lateral third of the lower eyelid, large ears and persistent fetal fingertip pads. Hematological symptoms, mainly idiopathic thrombocytopenic purpura (ITP), occur in up to 20% of all KS cases. Recently, pathogenic variants in the RAP1A and RAP1B genes have been identified to cause KS with a less pronounced phenotype, designated Kabuki-like syndrome.

2 | MATERIALS AND METHODS

Whole exome sequencing (WES) was performed as trio analysis in case 1 and as singleton analysis in case 2 (see Supplemental Methods). The two cases in this study were matched using GeneMatcher.

3 | CLINICAL DESCRIPTION

Case 1 is a 36-year-old female patient of non-consanguineous parents presenting with unclear pancytopenia, multiple congenital malformations, mild intellectual disability, endocrine disorders, dysmorphism and other features (Figure 1A-D). The family history was negative for comparable diseases. Her parents and two brothers were unaffected.

The patient was born at term by caesarian section due to transverse presentation. Thrombocytopenia with 30 to 50 × 1000/μL platelets, with normal erythropoiesis and granulopoiesis was documented for the first time at the age of 14 months. Later, leucopenia with lymphopenia and anemia as well as splenomegaly developed. She showed easy skin bruising without muscle or joint bleeding.

The patient presented with feeding difficulties and failure to thrive in infancy. She developed postnatal microcephaly, developmental delay (sitting up at 18 months, crawling at 2 years, walking at 2 years 9 months), a reduced muscle tone and learning difficulties. Dysmorphism (Figure 1A) included a preauricular tag, upslanting palpebral fissures, a flat mid face, scarce eyebrows, low-set posteriorly rotated ears, hypoplastic primary and permanent teeth, brachydactyly and lack of pubic and axillary hair. Her skin was thin and dry with multiple nevi and hematomas, and she had broken nails due to nail biting. She showed short stature with growth hormone deficiency and obesity (BMI 34.2 kg/m² at age 36 years). Pubarche and menarche did not set in while thelarche did occur.

Congenital malformations included congenital hip dysplasia, unilateral cystic renal hypoplasia, an obstructive hydroureter and a complex structural abnormality of the brain affecting the supratentorial ventricle system including aplasia of anterior parts of the septum.

FIGURE 1 Case 1 at A, 6 months; B, 5 years; C, 14 years and D, 36 years of age. Mild dysmorphism with upslanting palpebral fissures, flat midface, scarce eyebrows and low-set posteriorly rotated ears. A, Arrow marks a preauricular tag. D, Note the dysplastic teeth, a bruise on the chest wall and an overall progeroid appearance. Case 2 at 4 years, E, F, and 10 years, G, H. E, Note bruises on the face. E, G. Mild dysmorphism with hypertelorism, anteverted nostrils, long philtrum and thin upper-lip. F, H. Low-set and posteriorly-rotated ears.
pellucidum and formation of a partial cyclop ventricle, in addition to hypoplasia of hypothalamic structures, hypoplastic pituitary gland, abnormal sphenoid sinus with hyperintense structures (cranial MRI scan at the age of 20 years; no image records available). Susceptibility to infection presented as recurrent middle ear infections in childhood and non-healing leg ulcers in adulthood.

The hematological workup included normal chromosome studies in bone marrow, and no evidence of congenital erythropoietic porphyria, paroxysmal nocturnal hemoglobinuria, anti-thrombocyte antibodies, dyskeratosis congenita, leukemia, lymphoma, myelodysplastic syndrome and myeloproliferative syndrome. Thrombocyte survival time was remarkably reduced. Methylation sensitive MLPA analysis for del (15)(q11q13) was unremarkable for Prader-Willi syndrome.

Case 2 is a 13-year-old male patient of non-consanguineous parents presenting with thrombocytopenia, multiple congenital anomalies and learning difficulties (Figure 1E-H). The family history was negative for comparable diseases. Both parents and his sister were unaffected.

Unilateral kidney agenesis was diagnosed during pregnancy. He was born at term with normal growth parameters: weight 3790 g, height 50 cm, OFC 34 cm. He presented with congenital central thrombocytopenia below 20 × 1000/μL platelets, right kidney agenesis, ventricular septal defect and bilateral inguinal hernia. Mild facial dysmorphism was observed with hypertelorism, anteverted nostrils, long philtrum and thin upper lip, low-set and posteriorly rotated ears (Figure 1E-H). He had normal developmental milestones, walk was achieved at 14 months and there was no speech delay. He attended mainstream school with auxiliary help because of learning difficulties with graphism, syntactic comprehension, logical reasoning and attention deficit. At age 13 years, severe central thrombocytopenia (around 10 × 1000/μL) with low megakaryocytes count persists and is associated with a mild leukopenia (3.8 × 1000/μL) and lymphopenia (1.3 × 1000/μL) with no anemia. There is no immunodeficiency. Growth parameters are normal (weight on the mean, height +1 SD) except postnatal microcephaly with OFC ~2.5 SD. Brain MRI performed at 13 years old revealed two nodular heterotopias of the right ventricle and hypoplasia of the cerebellar vermis (Figure 2). Ophthalmological examination showed hypermetropia and astigmatism.

The thrombocytopenia was responsible for minor to mild hemorrhage with a Buchanan score around 1 to 2.7 The patient usually presents with petechiae and bruises without mucosal or internal bleeding. Flow cytometry revealed no deficit in platelet glycoproteins (GPIIb/IIIa, GPIb).

Standard karyotype, array-CGH, sequencing of genes involved in known RASopathies and telomere length study were normal.

A comparison of the patients’ features is given in Table S1. A list of the patients’ features in HPO terms is given in Table S2.

4 | RESULTS

WES was performed and variants obtained. In case 1, trio filtering exclusively identified a de novo heterozygous missense variant in the RAP1B gene, c.35G>T p.(Gly12Val) (NM_001010942.2) in 33 of 71 reads. This variant was validated by Sanger sequencing as de novo in the patient (Figure S1A). In case 2, prioritization using the deep phenotyping tool PhenoTips8 (see Table S2 for HPO terms used, and Table S3 containing variant list after filtering) identified a heterozygous missense variant in the RAP1B gene, c.178G>C p.(Gly60Arg) (NM_001010942.2) in 81 of 188 reads. Sanger sequencing confirmed the de novo occurrence of this variant (Figure S1B). Importantly, no CDC42 variant was detected in both our patients although their
phenotype showed similarities to two patients with CDC42-associated syndromic thrombocytopenia.9

Both \textit{RAP1B} variants have not been described as germline variants in public databases. The amino acid positions 12 and 60 both involve a glycine residue and are highly conserved among species (phyloP: 9.48 for p.Gly12 and 9.53 for p.Gly60, Figure S2). Both variants are predicted to be disease causing and in silico structural predictions argue for a deleterious effect on the basis of conformational changes of the GTPase domain (Figure S3). Furthermore, since both variants occur in domains sharing significant sequence homology in RAS-related genes (codons 12-13 and 59-61) and frequently involved in the malignant activation of RAS oncogenes,10 we hypothesize that these variants contribute to the presented phenotypes through a gain of function mechanism.

5 | DISCUSSION

We identified \textit{de novo} variants in the \textit{RAP1B} gene (c.35G>T p. (Gly12Val) and c.178G>C p.(Gly60Arg)) in two unrelated patients with thrombocytopenia, microcephaly, learning difficulties, renal malformations, structural anomalies of the brain and other features.

\textit{RAP1B} is a member of the RAS superfamily of small GTPases, which are involved in many cellular processes. Murine skeletal development,11 rat diabetic nephropathy,12 rat neuronal development,13 and murine neutrophil migration14 are regulated by \textit{RAP1B}-dependent pathways. Studies have shown a link between \textit{RAP1B} activation and platelet function in human and mice.15-17 Thrombocytopenia due to defective thrombocyte production has been described in Rap1a/b-double knockout mice.17 Interestingly, a somatic p.Gly12Arg variant has been described in myelodysplastic syndrome indicating that \textit{RAP1B} contributes to the pathophysiology of myeloid disorders.18 Furthermore, \textit{RAP1B} was identified to act as a key modulator of lymphocyte recruitment during immune reaction.19 These reports indicate a possible causative link between pathogenic variants in \textit{RAP1B} and thrombocytopenia and pancytopenia, respectively, bleeding diathesis and recurrent infections. Future investigations may determine whether bleeding diathesis including easy bruising is merely caused by a reduced thrombocyte count or additionally by defective platelet function.

There is no major overlap between the symptoms of our patients and the international consensus diagnostic criteria for KS.2 Therefore, we propose a \textit{RAP1B}-associated phenotype distinct from KS. Notably, the previously described variants p.Arg163Thr in the \textit{RAP1A} gene and p.Leu151Glu in the \textit{RAP1B} gene in patients with Kabuki and Kabuki-like syndromes both resulted in a loss of function and decreased downstream \textit{BRAF} activation/MEK/ERK signaling.5 Moreover, no hematological findings were reported in these patients by the authors.

There is strong evidence that the p.Gly12Val and p.Gly60Arg variants in the \textit{RAP1B} gene lead into a dysregulation of the downstream pathway. Both substitutions have been described previously as dominant constitutively active in RAS-related proteins.10,20-22 The variant p.(Gly12Val) is located within the phosphate binding loop (L1) of the catalytic \textit{RAP1B} domain.23 Almost every amino acid change at codon 12 of RAS-related proteins has been shown to alter the protein function.20 Especially a valine at this position seems to have a strong dominant activating effect in transfected cells.20 It has been shown that this activation occurs through impacted GTPase activity, which in turn leads to a relative surplus of active GTP.22 The variant p.(Gly60Arg) is located in the GTPase domain. In the active form of the protein, this residue is linked by a hydrogen bond to the gamma-phosphate of the nucleoside triphosphate.24 The same substitution in \textit{let-60 ras} gene causes hallmarks of gain-of-function RAS mutations in \textit{Caenorhabditis elegans}.20,21 Missense variants involving flanking residue 61 in RAS-related proteins are also responsible for dominant activation of the pathway, by altered GTPase activity.25 Therefore, we conclude that the \textit{RAP1B} variants identified contribute to the presented phenotypes through dysregulation of the MEK/ERK pathway.

In summary, we hypothesize that germline gain-of-function variants in the \textit{RAP1B} gene are causative for congenital syndromic thrombocytopenia. Further functional studies and identification of more affected patients may elucidate the clinical variability of \textit{RAP1B}-associated syndromes.

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ETHICS APPROVAL

The patients and their families gave informed consent for diagnostics testing and research studies including WES. The study was approved by the hospitals' ethics committees (Hannover Medical School, ethic vote number 4121; Institutional Review Board of the University Institute of Hematology, Saint-Louis Hospital, Paris). The patients gave consent for publication of the data including identifiable pictures.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data of this study are not publicly available due to privacy or ethical restrictions.

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REFERENCES
1. Almazni I, Stapley R, Morgan NV. Inherited thrombocytopenia: update on genes and genetic variants which may be associated with bleeding. Front Cardiovasc Med. 2019;6:80.
2. Adam MP, Banka S, Bjornsson HT, et al. Kabuki syndrome: international consensus diagnostic criteria. J Med Genet. 2019;56(2):89-95. https://doi.org/10.1136/jmedgenet-2018-105625.
3. Kawame H, Hannibal MC, Hudgins L, Pagon RA. Phenotypic spectrum and management issues in kabuki syndrome. J Pediatr. 1999;134(4):480-485.
4. Cantoni S, Fattizzo B. Clinical course and management of adult-onset immune-mediated cytopenia associated with kabuki syndrome. Eur J Intern Med. 2019;69:e3-e5.
5. Bogershausen N, Tsai IC, Pohl E, et al. RAP1-mediated MEK/ERK pathway defects in kabuki syndrome. J Clin Invest. 2015;125(9):3585-3599.
6. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36(10):e3-e5.
7. Buchanan GR, Adix L. Grading of hemorrhage in children with idiopathic thrombocytopenic purpura. J Pediatr. 2002;141(5):683-688.
8. Girdea M, Dumitriu S, Fiume M, et al. PhenoTips: patient phenotyping software for clinical and research use. Hum Mutat. 2013;34(8):1057-1065.
9. Takenouchi T, Okamoto N, Ida S, Uehara T, Kosaki K. Further evidence of a mutation in CDC42 as a cause of a recognizable syndromic form of thrombocytopenia. Am J Med Genet A. 2016;170A(4):852-855.
10. Barbacid M. Ras genes. Annu Rev Biochem. 1987;56:779-827.
11. Maruyama T, Jiang M, Abbott A, et al. Rap1b is an effector of Axin2 regulating crosstalk of signaling pathways during skeletal development. J Bone Miner Res. 2017;32(9):1816-1828.
12. Xiao L, Zhu X, Yang S, et al. Rap1 ameliorates renal tubular injury in diabetic nephropathy. Diabetes. 2014;63(4):1366-1380.
13. Schwamborn JC, Puschel AW. The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. Nat Neurosci. 2004;7(9):923-929.
14. Kumar S, Xu J, Kumar RS, et al. The small GTPase Rap1b negatively regulates neutrophil chemotaxis and transcellular diapedesis by inhibiting akt activation. J Exp Med. 2014;211(9):1741-1758.
15. Franke B, Akkerman JW, Bos JL. Rapid Ca2+−mediated activation of Rap1 in human platelets. EMBO J. 1997;16(2):252-259.
16. Stefanini L, Bergmeier W. RAP1-GTPase signaling and platelet function. J Mol Med (Berl). 2016;94(1):13-19.
17. Stefanini L, Lee RH, Paul DS, et al. Functional redundancy between RAP1 isoforms in murine platelet production and function. Blood. 2018;132:1951-1962.
18. Gyan E, Frew M, Bowen D, et al. Mutation in RAP1 is a rare event in myelodysplastic syndromes. Leukemia. 2005;19(9):1678-1680.
19. Reedquist KA, Ross E, Koop EA, et al. The small GTPase, Rap1, mediates CD31-induced integrin adhesion. J Cell Biol. 2000;148(6):1151-1158.
20. Seeburg PH, Colby WW, Capon DJ, Goeddel DV, Levinson AD. Biological properties of human c-ha-ras1 genes mutated at codon 12. Nature. 1984;312(5989):71-75.
21. Schutzman JL, Borland CZ, Newman JC, Robinson MK, Kokel M, Stern MJ. The Caenorhabditis elegans EGL-15 signaling pathway implicates a DOS-like multisubstrate adaptor protein in fibroblast growth factor signal transduction. Mol Cell Biol. 2001;21(23):8104-8116.
22. Colby WW, Hayflick JS, Clark SG, Levinson AD. Biochemical characterization of polypeptides encoded by mutated human ha-ras1 genes. Mol Cell Biol. 1986;6(2):730-734.
23. Noguchi H, Ikegami T, Nagadoi A, et al. The structure and conformational switching of Rap1B. Biochem Phys Res Commun. 2015;462(1):46-51.
24. Vetter IR, Wittinghofer A. The guanine nucleotide-binding switch in three dimensions. Science. 2001;294(5545):1299-1304.
25. Der CJ, Finkel T, Cooper GM. Biological and biochemical properties of human rasH genes mutated at codon 61. Cell. 1986;44(1):167-176.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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