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CASE REPORT

A NOVEL LIKELY PATHOGENIC VARIANT IN THE *RUNX1* GENE AS THE CAUSE OF CONGENITAL THROMBOCYTOPENIA

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ABSTRACT

Introduction: Heterozygous pathogenic and likely pathogenic sequence variants in the *RUNX1* (Runt-related Transcription Factor 1) gene are a common genetic cause of decreased platelet count and/or platelet dysfunction and an increased risk of developing myelodysplasia and acute myeloid leukemia. The majority of causative variants are substitutions, which rarely occur de novo. The aim of this case report is to present a patient with congenital thrombocytopenia caused by a deletion variant in exon 9 in the *RUNX1* gene.

Case report: A one-month-old male infant was admitted to the Clinical Hospital Center Rijeka because of anemia and thrombocytopenia verified in the course of an acute viral infection. During follow-up, he occasionally had petechiae and ecchymoses on the lower extremities after mild trauma, with no other symptoms. The patient had persistent slightly decreased values of platelets with normal morphology, but with pathological aggregation with adrenaline and adenosine diphosphate. Due to the unclear etiology of persistent mild thrombocytopenia, he was referred for genetic testing at the age of five. Genomic DNA was isolated from the patient's peripheral blood and whole-exome sequencing was performed using the next-generation sequencing method. A heterozygous frameshift variant, c.1160delG (NM_001754.4), was identified in exon 9. The variant is classified as likely pathogenic.

Conclusion: To the best of our knowledge, the heterozygous variant c.1160delG in the *RUNX1* gene was first described in our patient. Although pathogenic variants in the *RUNX1* genes are very rare, persistently low platelet counts of unclear etiology should raise suspicion of an underlying genetic disorder.

Keywords: Blood Platelet Disorders, Genetic Testing, Thrombocytopenia

INTRODUCTION

Thrombocytopenia is a condition characterized by decreased platelet counts in peripheral blood below 150 x 10^9/L, associated with an increased risk of bleeding predominantly into the skin and mucous membranes. It is classified as congenital and acquired. Causal factors for the development of congenital thrombocytopenia are sequence variants in genes encoding transcription factors important for megakaryocyte differentiation, platelet formation, or release [1].

According to the Human Phenotype Ontology database, 328 genes associated with thrombocytopenia have been described until now, among which the most common are *RUNX1* (Runt-related Transcription Factor 1), *ETV6* (ETS Variant Transcription Factor 6), and *ANKRD26* (Ankyrin Repeat Domain 26). Pathogenic sequence variants in these genes can cause specific syndromes such as TAR (Thrombocytopenia-absent radius) syndrome, Wiskott-Aldrich syndrome, Bernard-Soulier syndrome, Gray platelet syndrome, and Alport syndrome.

The first family with chronic thrombocytopenia and thrombocytopathy in childhood was described in 1978. Three of the ten siblings in this family died of myeloproliferative neoplasms [2]. This is considered the first described case of RUNX1 familial platelet disorder with associated myeloid malignancies (RUNX1-FPDMM).

The *RUNX1* gene encodes a protein of the same name, which is a transcription factor responsible for controlling hematopoiesis. Heterozygous pathogens and likely

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pathogenic sequence variants in the *RUNX1* gene are often present in patients with decreased platelet count and/or dysfunction and are an indicator of the increased risk of developing myelodysplasia and acute myeloid leukemia. The most common causal variants are point mutations that lead to missense, nonsense, or frameshifts changes in the RUNX1 protein. Also, several large deletions and gene duplications have been described [3]. To date, 16 likely pathogenic and 27 pathogenic deletions of the *RUNX1* gene have been described in the ClinVar database. Only two variants of unclear significance were described in exon 9, one substitution and one deletion.

The aim of this paper is to present a patient with congenital thrombocytopenia caused by a rare variant in the *RUNX1* gene, a heterozygous frameshift deletion, which is likely a de novo variant.

CASE REPORT

A one-month-old male infant was admitted to the Department of Pediatrics, Clinical Hospital Center Rijeka, due to anemia and thrombocytopenia verified during an acute viral infection. The father was treated for acute lymphoblastic leukemia in childhood, and at the age of 30 for thyroid cancer. Father and sister suffer from atopic diseases. In the first hospitalization, the patient received a red blood cell transfusion, followed by erythropoietin beta for six weeks. During follow-up, he occasionally had petechiae and ecchymoses on the lower extremities after mild trauma, with no other symptoms. Bone malformations have not been identified. He had normal values of erythrocytes and hemoglobin, occasionally mild neutropenia, persistently slightly decreased values of platelets that were normal morphology, but impaired functions - pathological aggregation with adrenaline and adenosine diphosphate (ADP). Glanzmann's thrombasthenia and Bernard Soulier's syndrome were ruled out. Specific platelet antibodies were negative several times, and on one occasion indirect antineutrophil antibodies were positive. Bone marrow aspiration showed impaired hematopoiesis, especially erythropoiesis and some hypolobulated megakaryocytes.

At the age of 18 months, the boy had the first episode of typical febrile convulsions. At 20 months he was hospitalized for recurrent febrile convulsions and bilateral pleuropneumonia complicated by sepsis and hemolytic-uremic syndrome. He was treated with the combination of parenteral antimicrobial therapy and irradiated red blood cell and platelet transfusions. At the age of two, he was hospitalized for Candida pelliculosa sepsis and treated with parenteral antifungals, intravenous immunoglobulins due to transient hypogammaglobulinemia, and granulocyte colony-stimulating factor due to severe neutropenia. Comprehensive immunological testing did not reveal an immune disorder. Repeated bone marrow aspiration was normal. At the same age, he underwent allergy skin testing because of atopic dermatitis, but the causative allergen was not found.

After the second year of life, the boy did not have any serious infections and was regularly monitored on an outpatient basis. Physical and mental development was normal.

Due to the unclear etiology of persistent mild thrombocytopenia, he was referred for genetic testing at the age of five. Genomic DNA was isolated from the patient's peripheral blood and whole-exome sequencing was performed using next-generation sequencing, which included the analysis of 34 genes associated with the clinical presentation. Sequencing was performed using Illumina NovaSeq 6000. The analysis included sequencing of coding regions of genes defined based on prominent clinical presentation, as well as regions on the intron-exon boundary that may result in changes/defects in splicing.

A heterozygous variant was identified in the *RUNX1* gene (c.1160delG deletion of one guanine, NM_001754.4). The variant leads to a frameshift at the 387th position of the amino acid sequence encoded by *RUNX1*. The variant is located in exon 9 and was not described in the genome of 138,000 controls in the gnomAD project or the ClinVar database of clinically relevant variants. By ACMG/AMP standards and guidelines for the interpretation of sequence variants (Richards et al. 2015), modified by ACGS recommendations, the variant was classified as likely pathogenic (evidence categories PVS1_STR, PM2).

DISCUSSION

In our patient, whole-exome sequencing revealed the presence of a heterozygous deletion of one guanine in exon 9 (c.1160delG), which was classified as a likely pathogenic variant leading to a frameshift in the RUNX1 protein.

Pathogenic heterozygous variants in the *RUNX1* gene represent an established cause of mild to moderate thrombocytopenia, functional and ultrastructural platelet defects, and predisposition to myelodysplastic syndrome, acute myeloid leukemia, and less frequently acute T-cell lymphoblastic leukemia. Pathogenic heterozygous variants in the *RUNX1* gene show high penetrance with variable expressivity and anticipation [4]. The clinical presentation is highly variable, ranging from the mild and moderate bleeding tendency to symptoms suggestive of the proliferation of immature hematopoietic cells such as fatigue, shortness of breath, fever, infections, and bleeding. The lifelong risk for malignant hematological diseases is 44% and the average age of occurrence is 33 years [4].

The association of the c.1160delG variant with thrombocytopenia has not been described to date, but there is evidence to suggest its pathogenicity. Although the variant presumably does not lead to nonsense-mediated decay, at least 94 amino acids of the RUNX1 protein are expected to be lost with changes in the transactivational domain / inhibitory domain / VWRPY motif, and protein extension by an additional 113 amino acids. So far, other C-terminal variants with a shift in the reading frame have been described, some of which lead to a loss of transactivational ability and a dominant-negative effect [5,6,7]. Furthermore, the variant is not present in the control population of the gnomAD project and is compatible with the referral diagnosis.

Consistent genotype-phenotype correlations have not been identified in the *RUNX1* gene [8]. Due to the identified rare type of sequence variant, we performed a genotype-phenotype association analysis for all deletions in the *RUNX1* gene recorded in the ClinVar database. The analysis showed that there is no genotype and phenotype correlation among the described deletions in the ClinVar database. The remaining two recorded changes in exon 9 of the *RUNX1* gene clinically presented as RUNX1-FPDMM, which matches with the clinical picture of our patient.

The variant was classified as likely pathogenic (evidence categories PVS1 STR, PM2) according to ACMG/AMP standards and guidelines for the evaluation of sequence variants (Richards et al. 2015), modified by ACGS recommendations.

CONCLUSION

To the best of our knowledge, the heterozygous frameshift c.1160delG variant in the *RUNX1* gene was first described in our patient. This sequence variant is the third described pathogenic or likely pathogenic variant in exon 9. Although pathogenic variants in the *RUNX1* genes are very rare, persistently low platelet counts of unclear etiology should raise suspicion of an underlying genetic disorder.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article

REFERENCES

1. Almazni I, Stapley R, Morgan N, Inherited Thrombocytopenia: Update on Genes and Genetic Variants Which may be Associated With Bleeding, Front Cardiovasc Med. 2019; 6: 80.
2. Luddy RE, Champion LA, Schwartz AD. A fatal myeloproliferative syndrome in a family with thrombocytopenia and platelet dysfunction. Cancer. 1978; 41: 1959-63.
3. Galera P, Dulau-Florea A, Calvo KR. Inherited thrombocytopenia and platelet disorders with germline predisposition to myeloid neoplasia. Int J Lab Hematol. 2019; 41(Suppl. 1): 131– 141.
4. Luo X, Feurstein S, Mohan S, Porter CC, Jackson SA, Keel S, et al. ClinGen Myeloid Malignancy Variant Curation Expert Panel recommendations for germline RUNX1 variants. Blood Adv. 2019 Oct 22; 3(20): 2962-2979.
5. Tsai SC, Shih LY, Liang ST, Huang YJ, Kuo MC, Huang CF, et al. Biological Activities of RUNX1 Mutants Predict Secondary Acute Leukemia Transformation from Chronic Myelomonocytic Leukemia and Myelodysplastic Syndromes. Clin Cancer Res. 2015 Aug 1; 21(15): 3541-51.
6. Harada H, Harada Y, Niimi H, Kyo T, Kimura A, Inaba T. High incidence of somatic mutations in the AML1/RUNX1 gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. Blood. 2004 Mar 15; 103(6): 2316-24.
7. Tang JL, Hou HA, Chen CY, Liu CY, Chou WC, Tseng MH, et al. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood. 2009 Dec 17; 114(26): 5352-61.
8. Deuitch N, Broadbridge E, Cunningham L, et al. RUNX1 Familial Platelet Disorder with Associated Myeloid Malignancies. 2021 Mar 4 [Updated 2021 May 6]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022.