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Case report

Severe congenital thrombocytopenia and platelet dysfunction due to novel *WAS* gene mutation: case report

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Abstract

Wiskott-Aldrich syndrome (WAS), X-linked thrombocytopenia, and X-linked congenital neutropenia collectively are designated WAS-related disorders. All are attributable to pathogenic variants of the WAS protein (WASp) and present a broad spectrum of hematopoietic cellular defects that chiefly involve platelets and lymphocytes. Pathogenic mutations in the *WAS* gene (located at Xp11.22-23) are implicated, affecting 12 exons.

Herein, we describe a neonate with congenital thrombocytopenia and platelet dysfunction due to a novel c.1500_1504dup (p.Asp502Gly) variant of the *WAS* gene. This mutation produces a frameshift, with substitution of aspartic acid for glycine at position 502 of the protein, and causes a downstream stop-loss codon. Clinically, the infant displayed severe thrombocytopenia and thrombasthenia, in the absence of other WAS-related traits (i.e., immune deficiency, eczema). Once a multigene panel analysis was complete, conditioning and then successful hematopoietic stem-cell transplantation took place at the age of 8 months. This case highlights the importance of genetic testing in instances where other diagnostics prove inconclusive.

Keywords

Thrombocytopenia, Wiskott-Aldrich syndrome, X-linked thrombocytopenia, mutation, hematopoietic stem-cell transplantation, infant.

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Introduction

Thrombocytopenia is relatively common in the neonatal period, occurring in 1-5% of infants at birth [1]. However, severe thrombocytopenia (platelets < 50 × 10^9/L) is seldom encountered (0.1-0.5% of neonates) [1]. In term babies, severe neonatal thrombocytopenia is usually a consequence of bacterial sepsis, perinatal asphyxia, or neonatal alloimmunity [2]. Prior to diagnosis, clinical evaluations, diagnostic testing, and patient management may be fraught with difficulty.

Wiskott-Aldrich syndrome (WAS), X-linked thrombocytopenia (XLT), and X-linked congenital neutropenia (XLN) collectively are designated WAS-related disorders. All are attributable to pathogenic variants of the WAS protein (WASp) and present a broad spectrum of hematopoietic cellular defects that chiefly involve platelets and lymphocytes. Pathogenic mutations in the *WAS* gene (located at Xp11.22-23) are implicated, affecting 12 exons.

Herein, we describe a male neonate with a novel *WAS* genetic variant. Presenting symptoms were severe thrombocytopenia and platelet dysfunction.

Case report

The patient described herein is a male Caucasian infant born as the second child of non-consanguineous parents. During her first pregnancy, the otherwise healthy mother (no prior signs of bleeding diathesis) had developed mild thrombocytopenia (133 × 10^9/L). This prompted neonatal testing, despite a lack of discernable bleeding or other clinical irregularities; and severe thrombocytopenia (23 × 10^9/L) was discovered on the second day of life. The infant was then transferred to the Neonatal Intensive Care Unit (NICU).

Upon arrival, a platelet transfusion was given, and a 2-day course of empiric intravenous immunoglobulin therapy was administered. The platelet count thereafter rose to 93 × 10^9/L. Platelet levels were monitored daily and remained in the range of 85-71 × 10^9/L during the next 5 days, after which they again began to decline. Neonatal allo- or isoimmune thrombocytopenia had been excluded through testing. Because isolated thrombocytopenia may at times reflect congenital cytomegalovirus (CMV) infection [3], and confirmatory polymerase chain reaction (PCR) testing was not immediately available (for technical reasons), valganciclovir was administered empirically pending urinary PCR testing for CMV. Meanwhile, counts < 20 × 10^9/L warranted platelet transfusion.

Bone marrow aspiration confirmed the presence of megakaryocytes, ruling out congenital amegakaryocytic thrombocytopenia. Platelet aggregation tests showed marginal response to collagen, adenosine diphosphate, or ristocetin; and the von Willebrand factor activity: ristocetin cofactor ratio was increased on 2 occasions (2.16 and 2.45, respectively). Flow cytometry immunophenotyping of peripheral blood lymphocytes also showed increased CD4:CD8 ratios (9.2 and 6.2, respectively) on 2 occasions, due to lower CD8 counts. Brain and abdominal ultrasound studies were normal.

During the NICU stay, no opportunistic infections emerged, and the infant's skin was normal in appearance (no eczema). However, platelet transfusions were required every 7-10 days, in accord with platelet life span.

Having excluded the most common causes of neonatal thrombocytopenia, a Reference Laboratory (Barcelona, Spain) was tasked with examining the following genes: *ADAMTS13*, *ANKRD26*, *CYCS*, *GATA1*, *GP1BA*, *GP1BB*, *GP9*, *ITGA2B*, *ITGB3*, *JAK2*, *MASTL*, *MPL*, *MYH9*, *RUNX1*, *SRC*, and *WAS*. Capture and enrichment of exonic and flanking intronic regions of genes in the REFLAB MedExome (Roche, Basel, Switzerland) sequencing panel were facilitated by Roche NimbleGen SeqCap EZ HyperCap Library technology, relying on NextSeq (Illumina, San Diego, CA, USA) for massive sequencing. Variants of interest were identified with regard to a reference genome (hg19), once filtered according to specific quality criteria. Annotation of detected variants was enabled through various bioinformatics applications: Alamut Visual (Interactive Biosoftware [SOPHiA, Lausanne, Switzerland]), Variant Interpreter (Illumina), and Ingenuity Variant Analysis (QIAGEN, Venlo, the Netherlands). Reference sources of record included the following: Single Nucleotide Polymorphism Database (dbSNP), 1000 Genomes Project (1KGP), Exome Aggregation Consortium (ExAC), Genome Aggregation Database (gnomAD), Human Gene Mutation Database (HGMD v2018.4), ClinVar, Leiden Open Variation Database (LOVD), disease-specific repositories, and the testing facility's own data. Other open-source software tools

(MutationTaster, SIFT, and PolyPhen-2) served to assess the potential impact of variant calls on protein structure and functionality. The average depth of readings obtained was 165 (4-fold), being > 20-fold in 98.8% of regions analyzed.

Above testing revealed a novel c.1500_1504dup (p.Asp502Gly) variant of the *WAS* gene. This mutation produces a frameshift, with substitution of aspartic acid for glycine at position 502 of the protein, causing a stop-loss codon downstream. The parents themselves declined to be tested.

After discharge and during the first 6 months of life, the infant continued to receive platelet transfusions every 7-10 days. There were no serious infections during this period, and typical features of WAS (immune deficiency or eczema) were absent. Once the multigene analysis was complete, preliminary conditioning and successful hematopoietic stem-cell transplantation (HSCT) were undertaken at 8 months of age.

Discussion

WAS-related disorders, including WAS, XLT, and XLN, are characterized by a broad spectrum of hematopoietic cellular defects, largely confined to platelets and lymphocytes [4]. Such diseases are caused by pathogenic mutations in the *WAS* gene (located at Xp11.22-23), affecting 12 exons. This gene is encoded for WASp, a cytoplasmic 502-amino acid protein involved in signal transduction from cell surface receptors to actin cytoskeleton. It is chiefly expressed in non-erythroid hematopoietic cells, playing a pivotal role in their functional integrity [5].

Although WAS-related disorders often overlap in symptomology, WAS is usually associated with microthrombocytopenia, eczema, and immunodeficiency; whereas microthrombocytopenia and severe neutropenia are sole features of XLT and XLN, respectively [4]. XLT typically results from pathogenic missense *WAS* variants, allowing most afflicted male patients to still produce WASp and display only mild thrombocytopenia. Nonsense, frameshift, or splice-site variants may well trigger severe disease, but consistent correlations between specific pathogenic variants and clinical outcomes have yet to be established [4].

Within the realm of neonatal thrombocytopenia, WAS-related disorders must be considered in male infants with persistent thrombocytopenia [6]. Rarely observed mutations of the *WAS* gene perhaps elicit the broadest range of potential clinical manifestations, from recurrent mild thrombocytopenia to the classic WAS phenotype, where thrombocytopenia coexists with eczema, immunodeficiency, and a greater likelihood of developing tumors or autoimmune manifestations later in life [7].

The infant reported herein seemed to qualify as XLT, showing a novel *WAS* gene mutation and the expected phenotype (i.e., thrombocytopenia only). However, the platelet deficiency was severe (counts as low as 5×10^9/L); and mean platelet volume was normal, not diminished as in WAS or XLT [8]. This particular scenario, although rare, has been documented before [9, 10]. Remarkably, no serious bleeding ensued under our care, likely due to rigorous surveillance and platelet support.

After a poor response to standard thrombocytopenic treatment, successful HSCT at 8 months of age brought full recovery in this instance, confirming what others have found. Ultimately, HSCT appears curative in the setting of WAS or in other immunodeficient states accompanied by congenital thrombocytopenia [11].

Conclusion

WAS-related disorders clearly pose therapeutic challenges, especially if thrombocytopenia is severe at birth. Our delineation of this novel *WAS* mutation and its corresponding patient phenotype may help convey a better understanding of the pathophysiology entailed and improve treatment.

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Ethical approval

Informed parental consent was obtained before genetic testing took place. This report was approved by the local hospital ethics authority.

Declaration of interest

The Authors have no conflicts of interest to declare. Funding: No specific funding was sought or acquired from any public, commercial, or not-for-profit agency.

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