Report of a Young Girl With *MYH9* Mutation and Review of the Literature

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Summary: *MYH9* mutations cause the inherited macrothrombocytopenic syndromes of May-Hegglin anomaly, Fechtner syndrome, Sebastian syndrome, and Epstein syndrome, collectively referred to as *MYH9*-related disease. We present the case of a girl with *MYH9*-related disease whose diagnosis was facilitated by platelet electron microscopy and *MYH9* sequencing. We discuss our patient's clinical presentation, now with 12 years of follow-up. We also discuss management and her possible prognosis given her specific *MYH9* mutation.

Key Words: macrothrombocytopenia, *MYH9*, immune thrombocytopenic purpura, May-Hegglin anomaly, Epstein syndrome

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Inherited thrombocytopenias are a heterogenous group of diseases characterized by a reduced number of platelets and classified according to platelet size.[1,2] Several congenital thrombocytopenias are associated with large platelets, including heterozygous and homozygous forms of Bernard-Soulier syndrome, gray platelet syndrome, and *MYH9*-related disease (*MYH9*-RD).[3] *MYH9*-RD represents a clinical spectrum of disease caused by mutations within the gene coding for nonmuscle myosin heavy chain IIA (NMMHC-IIA).[3]

We present a case of a young girl with a macrothrombocytopenia and *MYH9* gene mutation.

MATERIALS AND METHODS

Medical Records

We reviewed 12 years of medical records of a female patient, heterozygous for a *MYH9* mutation, who was initially diagnosed with acute immune thrombocytopenic purpura (ITP).

Peripheral Blood Smear and Bone Marrow Aspirate

The patient's peripheral blood smear and bone marrow aspirate were reviewed under light microscopy.

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Platelet Electron Microscopy (EM)

Platelet EM was performed at the Duke Electron Microscopy Laboratory. Whole blood was collected without vacuum from the patient and anticoagulated with acid citrate dextrose in a 4 parts blood to 1 part anticoagulant ratio. The sample was centrifuged at 500*g* for 15 minutes to prepare a buffy coat. The upper plasma layer was removed, and the buffy coat was fixed in situ with 3% glutaraldehyde in White's saline [a 10% solution of a 1:1 mixture of: (1) 2.4 mmol/L NaCl, 0.1 mmol/L KCH, 46 mmol/L $MgSO_4$, and 64 mmol/L $Ca(NO_3)_2 \cdot 4H_2O$ and (2) 0.136 mol/L $NaHCO_3$, 8.4 mmol/L NaH_2PO, and 0.1 g/L of phenol red, pH 7.4][4] for 1 hour. The buffy coat was then further fixed in 1% osmium tetroxide at 4°C for 1 hour, then dehydrated in a graded alcohol series and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined on a Phillips 400 electron microscope (FEI, Hillsboro, OR).

DNA Analysis

Consent was obtained for DNA analysis. Molecular sequencing of the *MYH9* gene was performed at the Duke Molecular Diagnostics Laboratory. This assay uses polymerase chain reaction (PCR) amplification followed by Sanger DNA sequencing to detect mutations in the *MYH9* gene causative for *MYH9*-RD. This assay targets exons 1, 10, 16, 21, 24, 25, 26, 30, 31, 37, 38, 39, and 40 of the *MYH9* gene. These specific coding sequences and flanking intronic sequences (minimum of 20 base pairs) are amplified from purified genomic DNA by PCR. The primers used for PCR contain M13 universal primer "tails" at their 5' ends, and have 3' ends that are homologous to their genomic target sequence. PCR products are treated with an exonuclease/phosphatase mixture (ExoSAP-IT) and sequenced using universal M13 forward and reverse primers (M13 Forward/-20 and M13 Reverse/-27) with the Big Dye Terminator v3.1 Cycle Sequencing Kit. These products were purified with the Big Dye XTerminator Purification Kit and resolved using the ABI 3130xl Genetic Analyzer. Data were analyzed by the ABI Data Collection software v3.0, Sequencing Analysis software 5.2 and SeqScape software v2.6.

RESULTS

A $2\frac{3}{4}$-year-old white female was evaluated for spontaneous ecchymoses since she started walking at 13 months of age. She had been diagnosed with ITP based on platelet counts varying from 5 to 12×10^9/L with normal white blood cell counts and hemoglobin concentrations. Bone marrow aspirate was remarkable for increased megakaryocytes with nuclear hypolobation with early cytoplasmic maturation (Fig. 1A). She was not treated before our evaluation.

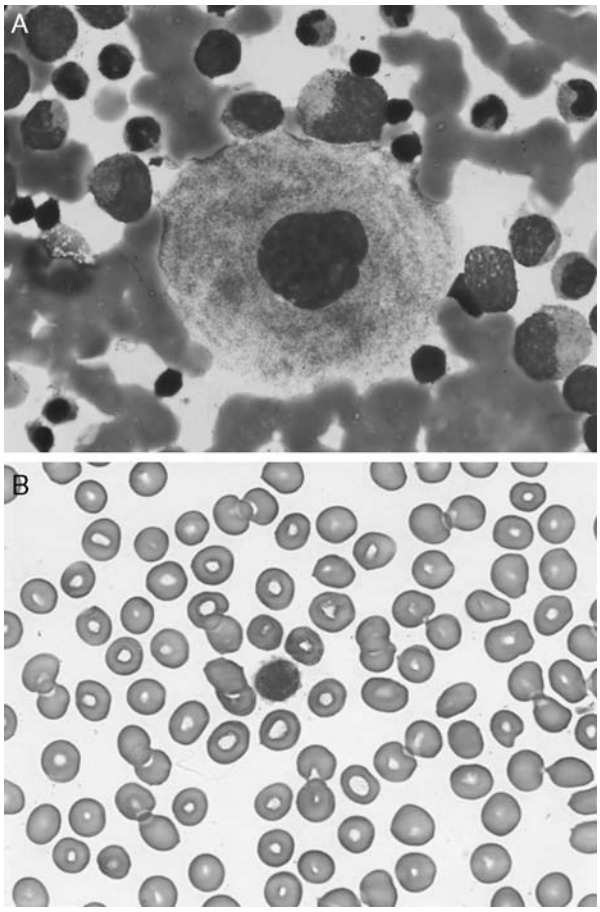

FIGURE 1. A, Bone marrow aspirate, ×100. Megakaryocyte with nuclear hypolobulation and early cytoplasmic maturation. B, Peripheral blood smear, ×100. Thrombocytopenia with giant platelets. Normal red cell and white blood cell morphology. No abnormal inclusions in the white blood cells.

Upon evaluation at our institution, she continued to have isolated thrombocytopenia. Peripheral blood smear was notable for macrothrombocytes, roughly the size of red blood cells (Fig. 1B). There were no Döhle bodies within the leukocytes seen on light microscopy. Flow cytometry for GP1b to evaluate for Bernard-Soulier syndrome could not be done because of the degree of thrombocytopenia. There was no family history of thrombocytopenia, deafness, nephritis, or presenile cataracts.

She was initially treated for presumed ITP, but she did not respond to anti-D intravenous immunoglobulin or corticosteroids. On the basis of persistent macrothrombocytopenia and lack of response to ITP therapy, she was diagnosed with a giant platelet syndrome of unknown etiology. Five years after her initial presentation, platelet EM was done at Duke University Medical Center which showed platelets that were enlarged and spherical, without the usual discoid shape of control platelets. EM did not detect abnormal inclusions within her leukocytes. Her microtubule network was noted to be somewhat disorganized, and 20% to 40% of her platelets had prominent dense tubular systems (Fig. 2). She was noted to have normal α and dense granules and unremarkable platelet organelles.

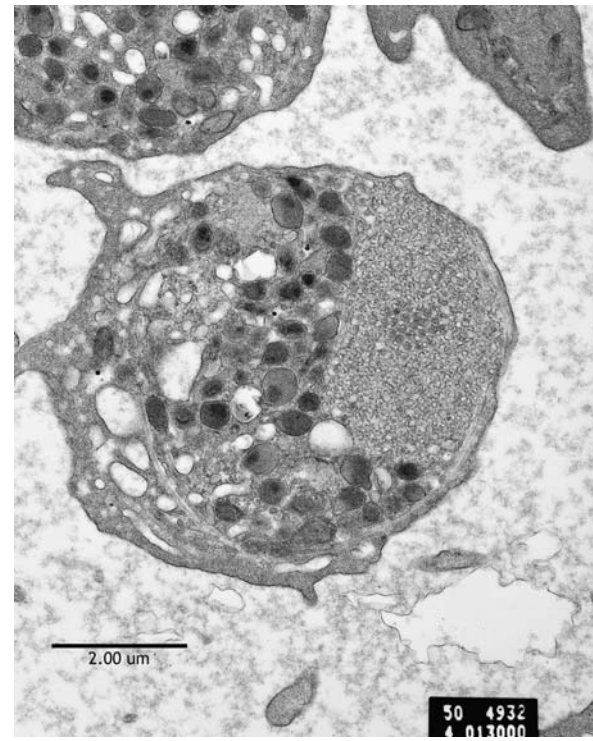

FIGURE 2. Platelet electron microscopy, ×13,000. Macrothrombocyte with a prominent dense tubular system. Ultrastructure is otherwise unremarkable; no abnormal leukocyte inclusions were observed.

Subsequent sequencing of the *MYH9* gene showed a heterozygous nucleotide substitution at cDNA nucleotide 283 (c.283G > A; exon 1; Fig. 3); the normal allele was also present. This change alters the alanine codon at position 95 to a threonine codon (p.Ala95Thr).

Since diagnosis, the patient's platelet count has consistently ranged from 1 to 21×10^9/L, as counted by an automated cell counter and confirmed by review of the peripheral blood smear. She bruises easily and occasionally has gingival bleeding, but she has never developed joint, gastrointestinal or other frank or abnormal bleeding despite several childhood accidental traumas. She is premenarchal and has no evidence of renal disease, hearing loss, or cataracts. She was given aminocaproic acid around the time of extraction of baby teeth and did well without bleeding complication.

DISCUSSION

We describe a young girl with inherited macrothrombocytopenia who was initially diagnosed with ITP. Family history of thrombocytopenia or other associated symptoms can be clues to the diagnosis of an inherited thrombocytopenia. However, in this case, there was no family history of thrombocytopenia. Correct diagnosis was facilitated by careful review of the peripheral blood smear along with platelet EM and *MYH9* gene sequencing.

Platelet EM has been a useful tool for evaluation of platelet structure, function, and pathology.[5] Whereas platelets in *MYH9*-RD were initially thought to be morphologically normal apart from their size, platelet EM has shown the microtubule networks are generally disorganized. Furthermore, while granulocyte inclusions in

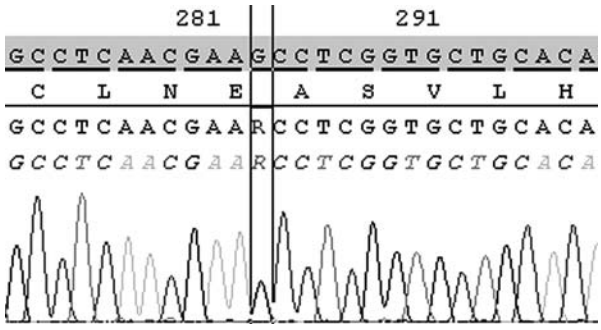

FIGURE 3. Electropherogram showing heterozygous *MYH9* mutation. A heterozygous c.283G>A mutation was detected in exon 1 by a targeted sequencing approach. The reference sequence with coding nucleotide numbers is provided above, with single letter amino acid abbreviations given below.

May-Hegglin anomaly were traditionally described as being different than those of Sebastian syndrome and Fetchner syndrome, EM of multiple patients with *MYH9*-RD showed the relationship between the type of inclusion and clinical syndrome was not consistent.

In 2000, the *MHY9* gene was linked to inherited macrothrombocytopenias,[6,7] and genetic testing and improved EM techniques led to better characterization as these diseases as single clinical spectrum. Genetic testing is used to confirm the diagnosis of a *MYH9*-RD and might provide prognostic information. To date, over 40 mutations, mainly missense nucleotide substitutions, have been identified within the *MYH9* gene.[3] Both inherited and de novo mutations have been described,[8] and the clinical phenotype of individuals with *MYH9*-RD is quite variable.[9] The phenotype can vary even among family members with the same mutation.[10]

The patient reported here is heterozygous for the A95T mutation, which affects the motor domain region of the NMMHC-IIA protein. This mutation was reported in 2001 by Kunishima et al[11] in a Korean family diagnosed with MHA, who presented with only macrothrombocytopenia and the characteristic leukocyte inclusions. In general, individuals with mutations affecting the motor domain of NMMHC-IIA are more likely to develop extrahematologic manifestations over time and often have significant bleeding.[9] They also tend to have lower platelet counts. Our patient has severe thrombocytopenia but had no leukocyte inclusions observed on EM. To date, she has had mild bleeding symptoms and no extrahematologic manifestations. Nonetheless, on the basis of the prior reports and her degree of thrombocytopenia, we continue to educate our patient about prevention and treatment of bleeding and remain vigilant for development of extrahematologic manifestations.

Patients with *MYH9*-RD should avoid activities with risk of trauma or injury and should receive regular dental care to prevent gingival bleeding and reduce the need for invasive dental procedures. Patients should avoid medications with antiplatelet effects. Treatment for bleeding episodes may include the antifibrinoltyics aminocaproic acid or tranexamic acid. In the event of life-threatening bleeding, platelet transfusion may be necessary. We recommend HLA-matched leuko-reduced platelets to reduce the risk of alloimmunization.[12] Alternative therapies include desmopressin acetate and recombinant factor VIIa. Also, the use of eltrombopag, an orally available thrombopoietin receptor agonist, has recently been described for patients with *MYH9* mutations and could be used as preventive management of ongoing bleeding symptoms.[13] Menorrhagia is common in women with *MYH9*-RDs and generally responds to treatment with oral contraceptives.[12] Although women with *MYH9*-RD do not seem to be at higher risk of bleeding during pregnancy or postpartum,[14] these women should be managed in consultation with a hematologist.

In summary, light microscopy, platelet EM, and *MYH9* sequencing were keys to the diagnosis of *MYH9*-RD in our patient. The clinical course of our patient varies from previously reported cases with the same or similar mutations. Given the unpredictability of the disease course she will benefit from ongoing follow-up and management of hematologic and extrahematologic manifestations cataracts, hearing loss, and nephritis.

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