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CASE REPORT

R1933X mutation in the *MYH9* gene in May-Hegglin anomaly mimicking idiopathic thrombocytopenic purpura

Chih-Chien Sung [a], **Shih-Hua Lin** [a], **Tai-Kuang Chao** [b], **Yeu-Chin Chen** [c,*]

[a] *Division of Nephrology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[b] *Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[c] *Division of Hematology/Oncology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*

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May-Hegglin anomaly (MHA) is a rare autosomal dominant disorder characterized by the triad of thrombocytopenia, giant platelets, and inclusion bodies in leukocytes. Recent evidence links MHA to mutations in the *MYH9* gene. MHA has not been reported in Taiwan before. We report a 25-year-old Taiwanese man who presented with prolonged bleeding after dental extraction. Examination of peripheral blood smear revealed thrombocytopenia (platelet = 35,000/μL), giant platelets, and Döhle-like cytoplasmic inclusions in neutrophils. A strong family history of thrombocytopenia favored hereditary macrothrombocytopenia over idiopathic thrombocytopenic purpura (ITP). Electron microscopy revealed a spindle shape and parallel order of filaments in the inclusions, consistent with the diagnosis of MHA. We performed mutational analysis using polymerase chain reaction followed by direct sequence of the *MYH9* gene for the patient, his maternal uncle and cousin, and all showed the same heterozygous R1933X mutation in exon 40. MHA should be considered when a young patient has thrombocytopenia, frequently misdiagnosed as ITP. Morphological examination of peripheral blood smear, family history tracing and genetic studies are required to make an accurate diagnosis and avoid unnecessary and even harmful therapies such as corticosteroids and splenectomy.

The authors have no conflicts of interest relevant to this article.

* Corresponding author. Division of Hematology/Oncology, Department of Internal Medicine, Tri-Service General Hospital, Number 325, Section 2, Cheng-Kung Road, Neihu 114, Taipei, Taiwan.
E-mail address: yeu-chin@yahoo.com.tw (Y.-C. Chen).

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Introduction

Hereditary macrothrombocytopenias with leukocyte inclusion bodies, *MYH9* disorders, are a group of rare autosomal dominant disorders characterized by thrombocytopenia, giant platelets, and Döhle-like inclusions in granulocytes. *MYH9* disorders, including May-Hegglin anomaly (MHA), Sebastian syndrome, Fechtner syndrome and Epstein syndrome, all have largely overlapping phenotypes and result from mutations in the *MYH9* gene on chromosome 22, which encodes the nonmuscle myosin heavy chain-IIA (NMMHC-IIA) protein.[1] To date, at least 33 mutations of the *MYH9* gene have been identified.[2,3]

MHA was first described by May, a German physician, in 1909, and was subsequently described by Hegglin, a Swiss physician, in 1945.[4] Thrombocytopenia may occur in 50% of the patients with this anomaly, but severe bleeding is unusual.[5] Most patients are asymptomatic, discovered incidentally, and require no specific treatment. MHA is frequently misdiagnosed as idiopathic thrombocytopenic purpura (ITP) without careful inspection of blood smears and a thorough family history. The most serious impacts of this disease are iatrogenic managements due to misdiagnosis. Herein, we report a young man presenting with prolonged bleeding after dental extraction. To the best of our knowledge, this is the first case report of MHA caused by R1933X mutation from Taiwan.

Case report

A 25-year-old man visited our hematology clinic due to prolonged bleeding after dental extraction. He had experienced several episodes of prolonged nasal bleeding and conjunctival hemorrhage since childhood. On physical examination, there was no ecchymosis, petechia or palpable hepatosplenomegaly, confirmed by abdominal ultrasonography. The patient displayed normal hearing function and no cataracts.

Complete blood counts were normal except for thrombocytopenia (platelet = 35,000/μL). Other laboratory data, including electrolytes, creatinine, blood urea nitrogen, liver profiles, von Willebrand factor antigen, prothrombin time, and activated partial thromboplastin time, were all within normal limits. Urinalysis was unremarkable. A flow cytometric study, using dual markers of CD 62P and PAC-1, revealed normal platelet function. The activated platelets proportion was 1.36% before ADP (10 μM, final concentration) stimulation, which rose to 63.6% (normal range = 57.12 ± 17.59%) after ADP stimulation. Bone marrow examination revealed increased megakaryocytes without dysplasia. Initially, a provisional diagnosis of ITP was made, based on clinical and laboratory findings.

However, further review of the family history disclosed some relatives, including his mother, one uncle, and one cousin all have thrombocytopenia as well (Fig. 1). All affected family members did not have nephritis, deafness and cataract. Therefore, hereditary thrombocytopenia was considered. A review of the patient's peripheral blood smear revealed a few giant platelets and Döhle-like cytoplasmic inclusions in neutrophils, appearing as conspicuous

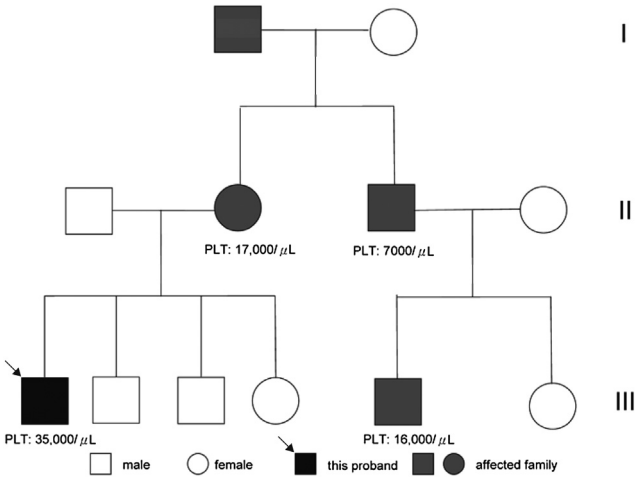

Figure 1 Pedigree of this patient and some affected family members. PLT = platelets.

bright blue oval to spindle shapes, as illustrated in Fig. 2A. A bone marrow smear disclosed the same inclusions in myeloid cells (see Fig. 2B). Electron microscopy revealed giant platelets of about 32 fL in size with adequate alpha granules and dense particles (Fig. 3A) and leukocyte inclusions composed of spindle-shaped and parallel ordered filaments (Fig. 3B), which are consistent with the diagnosis of MHA. We performed mutational analysis using a polymerase chain reaction by amplification of exons 1, 16, 26, 30, 38 and 40, followed by direct DNA sequencing of the *MYH9* gene in the patient, his maternal uncle and cousin. A heterozygous thymine to cytosine single-base substitution at nucleotide 5797 (CGA to TGA) was found in exon 40, resulting in a recurrent mutation from arginine to terminal at codon 1933 (R1933X), confirming the diagnosis of MHA.

Discussion

We report this case and his family with MHA due to a heterozygous R1933X mutation in the *MYH9* gene in Taiwan. The patient sought medical attention because of prolonged bleeding after dental extraction. The morphological features of peripheral blood smear and a family history of thrombocytopenia pinpointed a hereditary macrothrombocytopenia. *MYH9*-related disorder was diagnosed based on leukocyte inclusions in the peripheral blood smear. Fechtner and Epstein syndromes were not likely, due to the absence of Alport manifestations, including nephritis, deafness, and cataract.[6] Electron microscopy clearly demonstrated features of typical MHA, with spindle-shaped and parallel ordered filaments in leukocyte inclusions. Mutational analysis showed a heterozygous R1933X mutation, which has been reported as a causative mutation for *MYH9*-related disorder.[2]

Clinical presentations of MHA, as well as *MYH9*-related disorders are, usually, mild bleeding tendency, easy bruising, epistaxis, menorrhagia in woman, and postoperative hemorrhage, which depend on the severity of the thrombocytopenia.[7,8] Some patients can remain asymptomatic.[9] *MYH9*-related disorders are sometimes

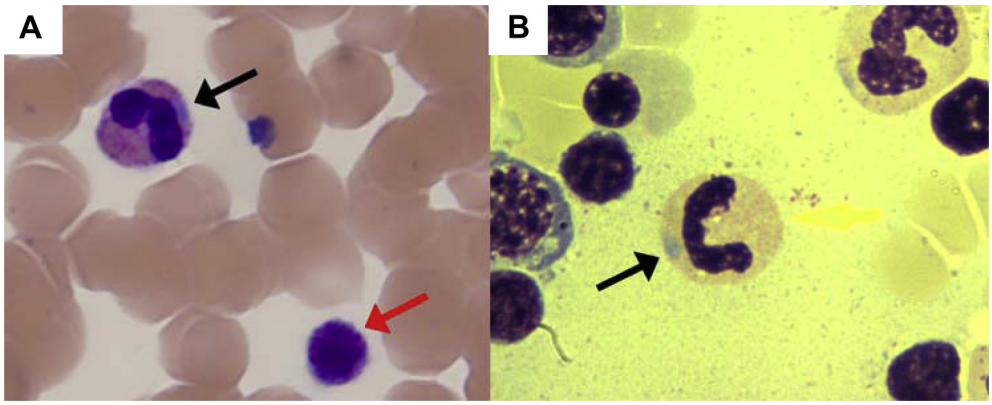

Figure 2 (A) Giant platelet (red arrow) and Döhle-like cytoplasmic inclusion in neutrophil, appearing as conspicuous bright blue oval to spindle shapes (black arrow) on peripheral blood smear; (B) Döhle-like cytoplasmic inclusion in myeloid cells (black arrow) on bone marrow smear (2A ×1000, 2B ×1000; all Wright's stain).

accidentally discovered during routine blood tests in asymptomatic individuals. *MYH9*-related disorders cannot be distinguished from ITP by clinical symptoms and platelet count. The patient's family history and careful examination of the patient's peripheral blood smear are very important for distinguishing two diseases (Table 1).

The diagnosis of *MYH9*-related disorders has been conventionally made on morphological examination revealing a triad of giant platelets, thrombocytopenia, and inclusions in the cytoplasm of leukocytes on May-Grunwald-Giemsa or Wright's stained blood smear, where 2—4 μm oval or spindle-shaped, sky-blue inclusions are present in the peripheral cytoplasm. Epstein syndrome does not have leukocyte inclusions. An audiogram, ophthalmologic screening and renal function assessment (creatinine clearance and proteinuria) should be evaluated for Alport manifestations, including nephritis, deafness, and cataract. Fechtner and Epstein syndromes have Alport manifestations. The MHA is distinguished from Sebastian syndrome by ultrastructural differences in their leukocyte inclusion bodies.[6] Ultrastructurally, MHA lacks limiting membrane and contains clusters of ribosomes oriented along the axis of thin parallel filaments 7—10 nm in diameter. Sebastian syndrome also contains ribosomes, but lacks parallel filaments depolymerized ribosomes.[9]

Mutation analysis is helpful for the diagnosis of *MYH9* disorders, and a full molecular assessment requires screening of 40 exons. Genetic testing has been postulated to help assess the risk of the development of high-tone hearing loss, cataracts, or renal impairment, although there is debate about the extent of mutation-phenotype correlation in *MYH9* disorders.[2] By the reported clinical features of *MYH9* disorders caused by a heterozygous R1933X mutation, most patients had hearing impairment and rare renal impairment.[2] However, in this patient and his family, they all did not have renal and hearing function impairment.

Some subtypes of hereditary macrothrombocytopenias are associated with mutations in the *MYH9* gene on chromosome 22, which encodes 224KD NMMHC-IIA protein. NMMHC-IIA protein is expressed in many cells, including platelets, leukocytes, kidney and cochlea, and is involved

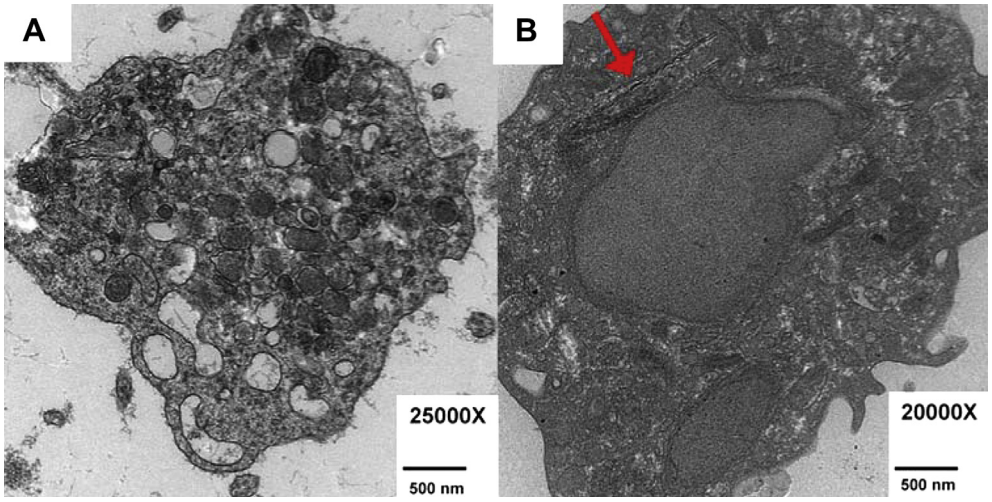

Figure 3 (A) Giant platelet about 32 fL in size with adequate alpha granules and dense particles on electronic microscopy; (B) inclusion consists of ribosomes along parallel microfilaments (red arrow) on electron microscopy (3A ×25,000, 3B ×20,000).

Table 1 Clinical and laboratory differences between MHA and ITP.

	MHA	ITP
Family history	Yes	No
Onset	Life-long (young age)	Recent
Bleeding tendency	Mild	Variable
Previous normal platelet count	No	Yes
Blood smear	Giant platelet, inclusions in neutrophils	Normal or large platelet
Response to platelet transfusion	Good	Poor
Treatment	Observation and supportive treatment	Corticosteroid, intravenous immunoglobulin G, splenectomy

ITP = idiopathic thrombocytopenic purpura; MHA = May-Hegglin anomaly.

in cell motility, cytokinesis, cell polarity, and cell architecture.[4] Mutations of NMMHC-IIA may alter the composition of platelet cytoskeleton and impair cytoskeletal reorganization, which may subsequently cause abnormal platelet formation from megakaryocytes, resulting in thrombocytopenia and giant platelets.[10,11] Anomalies of the podocyte cytoskeleton can damage the glomerular filtration barrier, leading to hematuria and even renal failure.[12] Mechanisms of hearing impairment and cataracts are still obscure, and probably related to an abnormal actin-myosin complex.[13]

There is no known prevention or treatment for the nonhematopoietic consequences of these disorders. Platelet transfusion may be useful for bleeding caused by trauma or surgery. Preoperative use of desmopression (DDAVP) can be considered in patients with May-Hegglin anomaly and other *MYH9*-related disease with thrombocytopenia.[5] Splenectomy, a treatment for refractory idiopathic thrombocytopenic purpura, is contraindicated in all hereditary macrothrombocytopenias, including MHA.[8,14,15]

In conclusion, we describe a male patient with MHA, the first was discovered in Taiwan with R1933X mutation in *MYH9*, who presented with prolonged bleeding after dental extraction. MHA is easily misdiagnosed as idiopathic thrombocytopenic purpura, if careful inspection of blood smear and family history are overlooked. Early recognition of this inherited thrombocytopenia can avoid unnecessary diagnostic studies, such as bone marrow aspiration and biopsy, and even harmful therapies with corticosteroids, immunosuppressive agents and splenectomy.

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