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Case Report

Identification of a novel *MYH9* mutation (p. V782Y) in a Chinese patient with thrombocytopenia: a case report

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ABSTRACT

MYH9-related diseases (*MYH9*-RD) are a group of autosomal dominant diseases caused by mutations in the *MYH9* gene, which are featured by thrombocytopenia, giant platelets and granulocyte cytoplasmic inclusion bodies. *MYH9*-RD patients generally suffer from bleeding syndromes, progressive kidney disease, deafness, or cataracts. Here, we reported on a case of *MYH9*-RD. A novel heterozygous mutation of *MYH9* (c.2344-2345delGTinsTA, p.T782Y) was discovered by targeted sequencing technology. Immunofluorescence analysis of neutrophils confirmed abnormal aggregation of MYH9 protein. The results of this study should expand the *MYH9* gene mutation spectrum and provide reference for subsequent researchers and genetic counseling.

Keywords: *MYH9*-related diseases (*MYH9*-RD), thrombocytopenia, p.V782Y mutation

INTRODUCTION

MYH9 (OMIM*160 775) is a large gene localized on chromosome 22q12.3, spanning more than 106 kbp and composed of 41 exons[1−2]. It encodes non-muscle myosin heavy chain Ⅱ A (NMMHC-Ⅱ A), a cytoskeletal contractile protein, which plays an important role in cytokinesis[3−4], cell migration[4−5], and signal transduction[6−8]. Mutations in *MYH9* often lead to a rare, autosomal dominant disorder known as *MYH9*-related disease (*MYH9*-RD). *MYH9*-related diseases were initially described as four syndromes: May-Hegglin anomaly (MHA), Fechtner syndrome (FS), Sebastian syndrome (SBS), and Epstein syndrome (EPS)[9−10]. In recent years, it has been discovered that another autosomal dominant disease, Deafness, Autosomal Dominant 17 (DFNA17; OMIM# 603622), is also caused by mutations in the *MYH9* gene[11−14]. It is recognized that all of these disorders actually represent different clinical presentations of the same

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disease, presently known as *MYH9*-RD.

Regarding the prevalence of *MYH9*-RD, it's estimated more than 1 ∶ 500 000, with about one-third of patients being sporadic cases[1]. Due to the low prevalence and varying clinical manifestations, some patients only present with mild skin petechiae and ecchymosis which can be easily misdiagnosed as idiopathic thrombocytopenic purpura (ITP), and therefore receive incorrect clinical treatment. The correct diagnosis of hereditary chronic thrombocytopenia is crucial for planning appropriate treatment.

Here, we reported on a case of *MYH9*-RD which had been misdiagnosed as ITP. The results of this case study may deepen the understanding of *MYH9*-RD for subsequent researchers and genetic counseling.

CASE REPORT

A 6-year-old girl was hospitalized in the Children's Hospital of Soochow University in October 2019 due to "intermittent epistaxis and abnormal blood tests for more than 1 year". Analysis of the proband's family medical history showed that the child and her immediate family members had no other diseases related to vision, kidney function or hearing development (***Table 1***). All participating individuals provided informed consent in accordance with the Declaration of Helsinki. This study was approved by the Medical Ethics Committee of the Children's Hospital of Soochow University, China.

Table 1 **Clinical phenotypes in *MYH9*-RD and the p. V782Y mutation patient**

Phenotypes	Syndromes					
	MHA	FS	EPS	SBS	DFNA17	The case
Thrombocytopenia	+	+	+	+	−	+
Inclusion bodies	+	+	−	+	−	+
Hearing loss	−	+	+	/	+	*
Nephritis	−	+	+	−	−	−
Cataract	−	+	−	/	−	*

"+" means there is a corresponding phenotype; "−" represents the opposite; "/" means the phenotype is late-onset; "*" means that it is unknown whether the phenotype is delayed.

In this study, the proband was examined at the local hospital for "epistaxis" at the age of four. Her blood test indicated that platelets were 30×10^9/L, and other examinations showed no obvious abnormalities. In September 2019, after ITP treatment, her peripheral blood test showed EB virus IgG (+), CMV IgG (+) and IgM (−), and she was given gamma globulin therapy. Bone marrow aspiration revealed 8 megakaryocytes and few platelets. Her platelet content rose to 80×10^9/L, and she was discharged from hospital. Soon after being discharged from the hospital, the blood test showed that the platelets had reduced again to $30–50\times10^9$/L, and 6 capsules of prednisone acetate day were given per day. The local outpatient clinic gradually reduced the dose (about 1 capsule per week) and gave acetic acid. Prednisone was taken orally at 3 capsules/day, but her platelets and hemoglobin still gradually decreased.

After learning about the patient's medical history, etc., we conducted a detailed evaluation of the patient. Physical examination revealed no abnormality. Bone marrow biopsy showed that hematopoietic tissue was rare. Routine blood examination: white blood cells, 9.26×10^9/L; red blood cells, 2.9×10^{12}/L; lymphocytes, 71.2%; hemoglobin, 104 g/L; total number of platelets, 22×10^9/L; neutrophils, 22.7%. In view of the patient's conditions, carbazochrome sodium sulfonate was given to prevent bleeding; vitamin C to improve vascular circulation; compound glycyrrhizin to protect the liver; prednisone acetate and ganciclovir to resist the virus. Thrombopoietin was given subcutaneously to the patient, but the platelet increase was not significant. Through high-depth targeted sequencing to detect genes related to the blood system, we found one predictive damaging mutation site (NM_002473, c. 2344-2345delGTinsTA, P. T782Y) related to the proband's phenotypes (***Fig. 1***). The evidences for the mutation site were PS2, PS3, PM2, and PP3. According to ACMG guidelines, this variant is determined to be pathogenic.

The motor domain of NMMHC-ⅡA is located at the N-terminus, which is encoded by exons 2–19. The N-terminus contains the actin binding site and the ATP hydrolysis domain. Exons 19 and 20 encode the binding region of the myosin light chain, which (sometimes called the "neck") pivots to convert the force generated by the motor domain into motion[1–2]. The mutation (p. T782Y) was located in the key structural domain of the neck encoded by exon 19 (***Fig. 1B***). This may affect the normal rotation of this region and cause abnormal protein function.

To verify our diagnosis, three members of the entire family underwent blood smear examinations and immunofluorescence staining of the NMMHC-ⅡA protein. The blood smear result of the proband was normal and there were no giant platelets (***Fig. 2B***), which was consistent with our previous blood routine examinations. In our previous inspections, there was suggestion that the platelet volume had a tendency to increase. The MPV of the patient in the last result was 13.6, which was a little higher than the normal value. However, "Döhlelike" inclusion bodies could be seen in neutrophils (***Fig. 2C***).

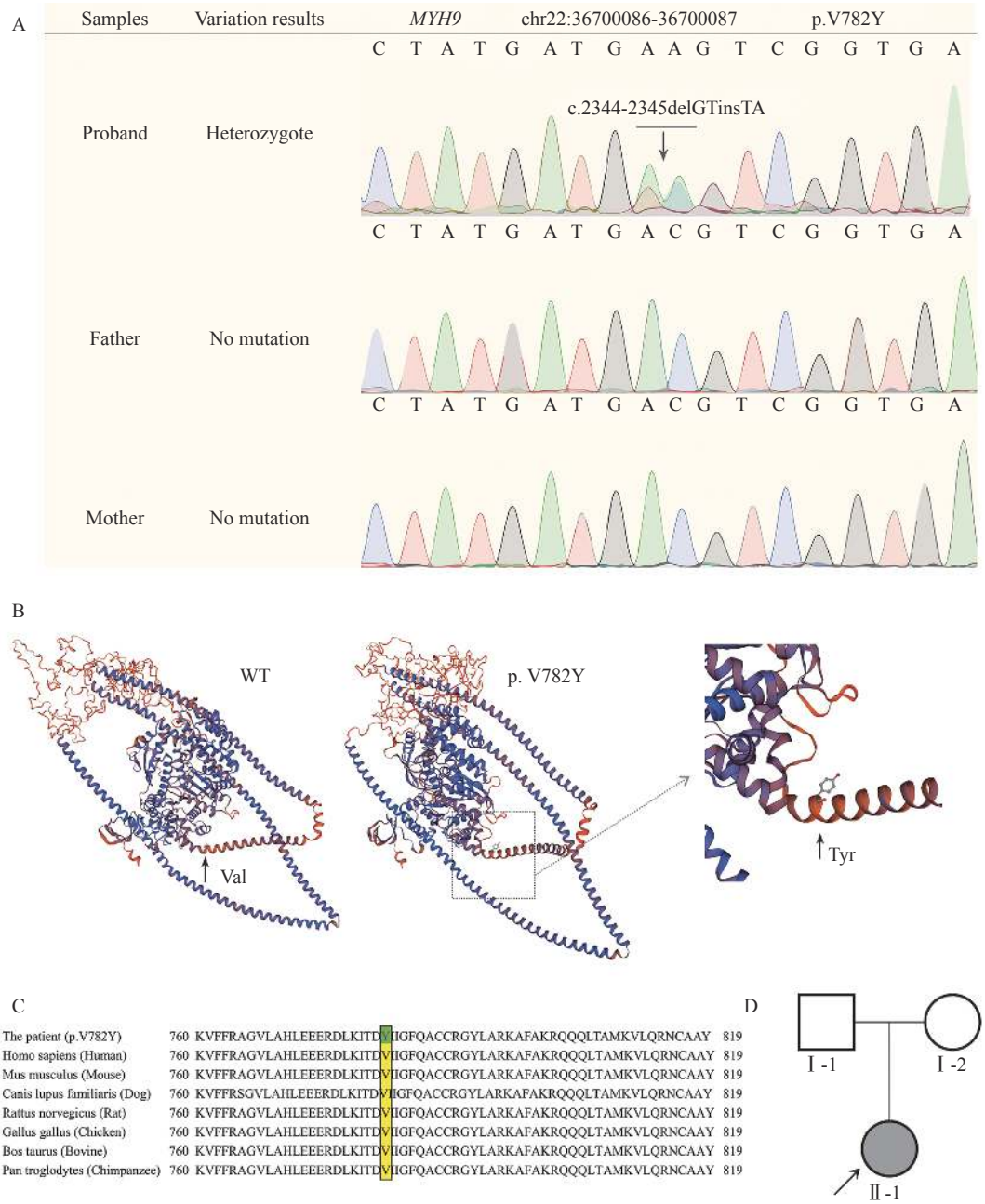

***Fig. 1* Co-separation of p.T782Y site and protein structure prediction.** A: The mutation at the corresponding sites in the family. B: The predicted maps of the protein structure after mutations based on the SWISS-MODEL website (https://swissmodel.expasy.org/). C: The conservation of this site in different species. D: "↗" pointing to the proband.

Although not all *MYH9*-RDs have inclusion bodies, the presence of these *MYH9* related inclusion bodies helps distinguish *MYH9* abnormalities from other hematological abnormalities with high specificity and sensitivity[15−16]. The detection of abnormal NMMHC-ⅡA localization by immunofluorescence staining is considered by many experts as the gold standard for the diagnosis of *MYH9*-RD[17−19]. Therefore, immunofluorescence analysis was performed to confirm the diagnosis of *MYH*9-RD. As can be seen from ***Fig. 2D*** and ***Fig. 2E***, compared with normal people (parents' result), the proband with p.T782Y mutation had abnormal aggregation of NMMHC-ⅡA protein in neutrophils.

DISCUSSION

MYH9-RD is the most prevalent form of inherited thrombocytopenia worldwide[20]. Patients may present with thrombocytopenia and granulocyte inclusions, with or without nephritis or sensorineural hearing loss. Patients with *MYH9*-RD present with compli-

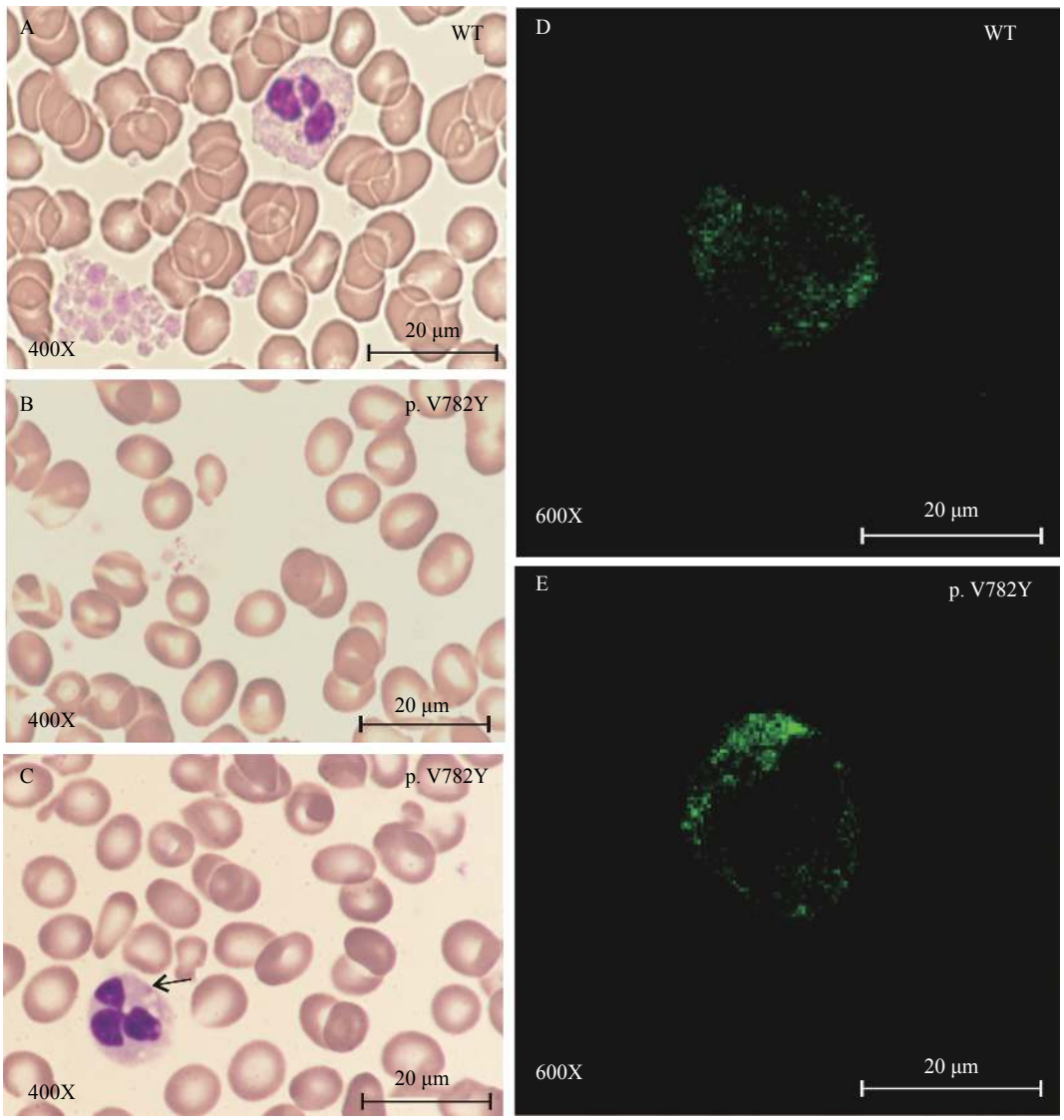

***Fig. 2* Examination of peripheral blood smears and immunofluorescence staining.** A: Control. B: The volume of platelets had not increased. C: After routine blood smear staining, the aggregates of MYH9 protein in the neutrophil granulocyte cytoplasm could be identified as weakly basophilic (sky blue) inclusion bodies, called "Döhle-like" bodies (↗). D: Parental immunofluorescence staining control result. E: Immunofluorescence staining of typical NMHC-ⅡA aggregates in the cytoplasm of granulocytes from the patient with *MYH9*-RD with NMHC-ⅡA antibody.

cations at different ages[21−22]. The main manifestations are thrombocytopenia, giant platelets, and basophilic Döhle-like inclusions in peripheral blood leukocytes[23]. In this case, the proband showed thrombocytopenia, granulocyte inclusions, and long-term epistaxis.

The proband initially received presumptive ITP therapy, but did not respond to intravenous immunoglobulin or corticosteroids. Based on persistent thrombocytopenia and poor response to ITP treatment, she was diagnosed with thrombocytopenia syndrome of unknown etiology. Since the patient had epistaxis at a very young age and conventional immunotherapy was ineffective in the past, combined with the increasing tendency of platelet volume, after excluding other factors, we considered that thrombocytopenia might be caused by genetic factors such as blood system related genes. After high-depth targeted sequencing of key genes related to the blood system, the report concluded a novel mutation in the *MYH9* gene (c. 2344-2345delGTinsTA, p. V782Y). The mutation occurred in the key structural domain of NMMHC-ⅡA, and a variety of prediction software programs showed that the mutation was damaging. An immunofluorescence assay confirmed that this mutation could also cause abnormal aggregation of the protein. According to the location of the mutation, clusters of NMM-ⅡA can be detected as oval-spindle-shaped, oval-shaped, or round inclusion bodies[9].

Here, the patient's protein showed "oval-spindle-shaped" shape, similar to one case reported by Althaus *et al*[9]. The different shapes of NMMHC-ⅡA clusters may have varying degrees of impact on cell function, which requires further studied.

According to the patient's clinical and experimental data, the patient was finally diagnosed as *MYH9*-RD. Although it was proved that the mutation was responsible for the patient's clinical phenotypes, more reports or animal experiments are required to further clarify the correlation between the genotype and phenotype of the mutation and the underlying mechanism.

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