Case Report

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A Trp33Arg Mutation at Exon 1 of the *MYH9* Gene in a Korean Patient with May-Hegglin Anomaly

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In this report, we describe a Korean patient with May-Hegglin anomaly from a mutation of the *MYH9* gene. The proband was a 21-year-old man with thrombocytopenia. He did not have a bleeding tendency. His neutrophil count was normal at 7490/mm^3; however, the neutrophils contained abnormal basophilic inclusions in their cytoplasm. The platelet count was decreased at 15000/mm^3 with giant platelets. Coagulation test results were not remarkable. Direct sequencing of *MYH9* revealed that he was heterozygous for a mutation in exon 1, which was a 97T>A substitution mutation affecting codon 33, substituting tryptophan with arginine (Trp33Arg). Family study showed that both of his parents had normal phenotype and genotypes, indicating a *de novo* occurrence of the mutation in the proband.

Key Words: May-Hegglin anomaly, *MYH9*, thrombocytopenia, Korean

INTRODUCTION

May-Hegglin anomaly (MHA) is a rare autosomal dominant disorder characterized by giant platelets and pale-blue inclusions in leukocytes. The clinical characteristics and the hereditary pattern of MHA were first described in 1909 and 1945, respectively.[1,2] The mutated gene is the gene encoding myosin heavy chain 9 (*MYH9*) located on chromosome band 22q12-13, which is comprised of 40 exons encoding the nonmuscle myosin heavy-chain IIA (NMMHC-IIA), a cytoskeletal contractile protein.[3,4] In this report, we describe a Korean patient with MHA from a missense mutation in exon 1 of *MYH9*.

CASE REPORT

The proband, a 21-year-old Korean soldier, was referred to Bundang CHA Medical Center because of thrombocytopenia. He had been diagnosed as having giant platelet syndrome 8 years previously in a tertiary hospital without further investigation

or follow-up. Recently he was found to have thrombocytopenia on routine health checkup at an army hospital. He had no personal or familial history of a bleeding tendency.

Complete cell counts at the present visit showed a platelet count at 36000/mm^3. Peripheral blood smear revealed giant platelets and basophilic oval-shaped inclusion bodies in the cytoplasm of granulocytes. Based on the history and laboratory findings, he was suspected to have MHA, one of the *MYH9*-related disorders. His parents and younger brother paid a visit for family study and had their blood drawn for cell counts and molecular genetic analyses (Fig. 1A).

To confirm the diagnosis of May-Hegglin anomaly disease, molecular genetic analysis was performed with informed consent provided by the patient and family members. DNA was extracted from leukocytes obtained from peripheral blood using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). All exons and their flanking intronic sequences of *MYH9* were amplified by polymerase chain reaction by pairs of primers designed by the authors (available upon request). Direct sequencing was performed using the same primers. The mutation identified was described according to the guidelines by the Human Gene Variation Society (http://www.hgvs.org/mutnomen/), having the A of the ATG-translation initiation codon as +1 at the nucleotide level and the 1st Met as +1 at the protein level. The direct sequencing of the *MYH9* gene in the patient showed that he was heterozygous for a missense mutation in exon 1 of *MYH9*. The mutation was a transversion variation (c.97T>A) substituting the 33rd residue tryptophan with arginine (p.Trp33Arg) (Fig. 1B).

Wright-Giemsa stain of peripheral blood showed cytoplasmic inclusion in neutrophils (Fig. 2A). Immunofluorescence analysis of neutrophil NMMHC-IIA showed abnormal diffuse NMMHC-IIA localization, with several circular to oval cytoplasmic inclusions (Fig. 2B). The family study showed that both of his parents and his brother had normal phenotype and genotypes, indicating a *de novo* occurrence of the mutation in the proband.

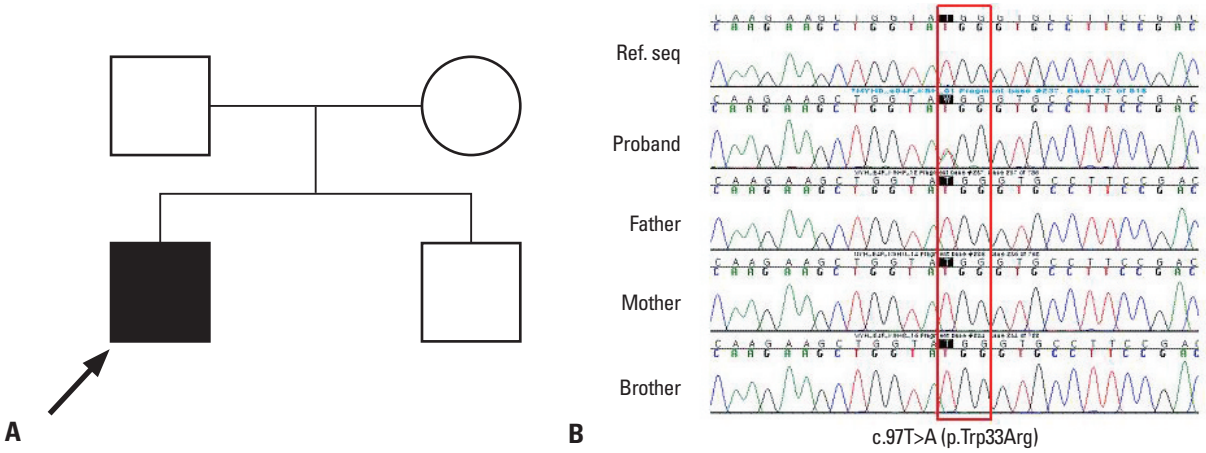

Fig. 1. (A) The pedigree of the patient (arrow). (B) The results of direct sequencing of *MYH9* in the proband and family members. The proband was heterozygous for a missense mutation, c.97T>A, p.Trp33Arg. His parents did not have the mutation, indicating a *de novo* occurrence of the mutation in the proband. His brother was also homozygous for the wild-type sequence, as were his parents.

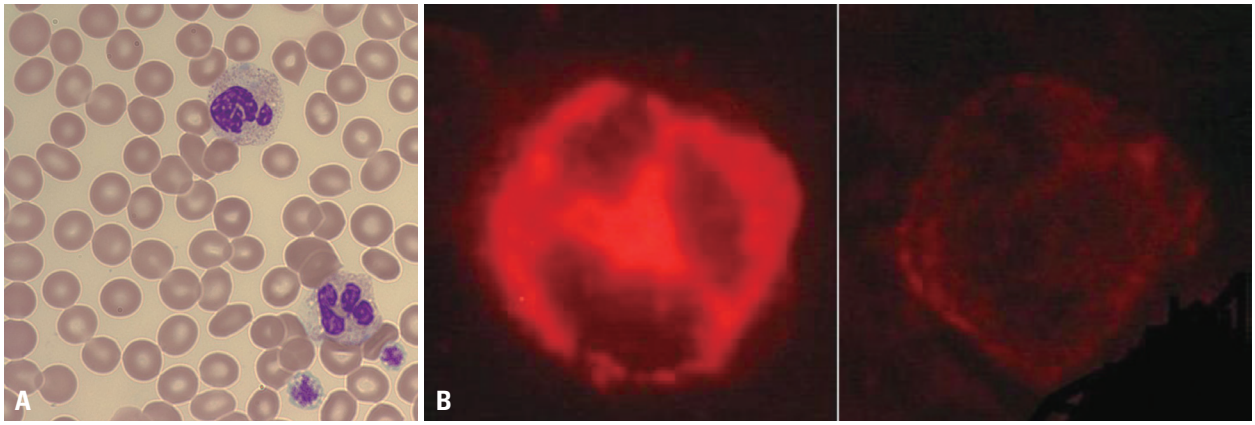

Fig. 2. (A) Cytoplasmic inclusion in neutrophils (Wright-Giemsa ×1000). (B) Diffuse granular pattern of fluorescence signals from anti-NMMHC-A antibody in neutrophils.

Table 1. Mutations and Clinical Features in MYH9-Related Thrombocytopenia[5-7,16,19-24]

Exon	Mutation	Number of affected families	Thrombocytopenia	Hearing loss	Renal impairment	Cataract
1	W33R	3	+	-	-	-
1	W33C	2	+	+	-	-
1	P35A	1	+	-	-	-
1	L46F	2	+	+	NA	NA
1	N93D	1	+	+	NA	NA
1	N93K	2	+	-	-	-
1	A95T	1	+	-	-	-
1	A95V	3	+	-	-	-
1	S96L	4	+	+	+	-
10	K371N	1	+	-	-	-
16	R702C	7	+	+	+	+
16	R702H	4	+	+	+	+
16	R705H	1	-	+	NA	NA
16	Q706E	1	+	NA	NA	NA
16	R718W	2	+	+	+	+
20	K850E	2	+	+	-	-
21	E894K	2	+	+	-	-
24	E1066-A1072del	1	+	-	-	+
25	V1092-R1162del	1	+	-	+	-
25	D1114P	1	+	-	+	-
26	T1155A	1	+	+	+	+
26	T1155I	5	+	-	-	-
26	R1165C	5	+	+	+	+
26	R1165L	2	+	+	+	+
26	L1205-Q1207del	1	+	-	-	-
30	R1400W	2	+	-	+	-
30	D1424Y	2	+	+	+	+
30	D1424N	13	+	+	+	+
30	D1424H	3	+	+	+	+
30	D1424G	1	+	+	-	-
30	A1436T	1	+	-	-	-
30	D1447V	1	+	NA	NA	NA
31	E1475K	1	+	-	-	-
31	V1516L	1	+	NA	+	+
31	V1560G	1	+	-	-	-
32	Q1563K	2	+	-	-	-
37	I1816V	1	+	+	+	-
38	E1841K	21	+	-	+	+
40	G1924fs	1	+	NA	NA	NA
40	D1925fs	1	+	-	-	-
40	P1927fs	1	+	-	-	-
40	R1933fs	1	+	-	-	-
40	R1933X	14	+	+	+	-
40	G1940R	1	+	NA	NA	NA
40	E1945	2	+	-	+	-

NA, no data available; fs, frameshift; del, deletion.

DISCUSSION

Mutations in the *MYH9* gene are responsible for a group of related thrombocytopenias: May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome, based on the morphologic aspects of Döhle-like bodies and the combination of different clinical findings at the time of diagnosis. These disorders are characterized by mild to severe thrombocytopenia with large-sized platelets. The typical clinical manifestation is macrothrombocytopenia; however, the bleeding tendency is usually moderate. Thrombocytopenia ranges from mild to severe and remains stable in an individual throughout their lifetime. Other clinical features may include high tone deafness, cataracts, leukocyte inclusions, and kidney disease leading in some cases to renal failure.

The *MYH9* gene encodes the nonmuscle myosin heavy chain, class IIA. To date, 43 different mutations of the *MYH9* gene in more than 200 families have been identified.[5-7] Most causative *MYH9* mutations reported to date have been missense, although in-frame deletions of a few amino acids, as well as nonsense and frameshift mutations have also been reported.[8,9] Some of the reported mutations have occurred *de novo* as with our case. The proportion of cases caused by *de novo* mutations is 20-38%.[10] In Korea, until recently, seven families of MHA have been identified.[11-18] Of them, mutation analysis was performed in only two families and revealed previously reported mutations.[17,18]

In this report, we described a patient with MHA due to a missense mutation in the *MYH9* gene. The mutation was a T-to-A substitution in exon 1 of *MYH9* (c.97T>A), replacing tryptophan of the codon 33 with arginine (p.Trp33Arg) on the head domain of NMMHC-IIA. Two cases of Trp33Arg have also recently been reported in patients with MHA, as abstracts,[5,6] and this is the third report in the world of this mutation and the first in Asia, to the best of our knowledge. Until now, 43 mutations in 14 different exons have been described.[5-7,16,19-24] Exons 1-19 encode for the head and neck domains of NMMHC-IIA , while exons 20-40 encode the tail portion of the protein.[7] Exon 1, 16, 26, 30 and 40 are relatively frequent sites of mutations. The mutation in our patient occurred on the actin binding site of the head domain of NMMHC-IIA at exon 1.

Previous studies reported that patients with a mutation in exon 1 of *MYH9* had a high frequency of hearing or renal impairment (Table 1).[5-7,17,19-24] The patient in the present report showed no evidence of hearing or renal impairment. However, further monitoring is needed for the possible development of these complications.

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