

Hematologic and genetic characterization of an *MYH9*-related disorder in a Chinese family

We describe a Chinese family with an *MYH9*-related disorder in which a novel mutation V1516L at exon 31 of the *MYH9* gene was identified. To the best of our knowledge, this is the first reported Chinese family with *MYH9* mutation and supports the pan-ethnic nature of the disorder.

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Due to a common genetic basis, the term *MYH9*-related disorder is currently used to denote a spectrum of autosomal dominant inherited conditions characterized by various combinations of macrothrombocytopenia, neutrophil cytoplasmic inclusions, nephritis, deafness and cataract. The spectrum includes May-Hegglin anomaly (MHA), Sebastian syndrome, Fechtner syndrome and Epstein syndrome.¹ The gene defect is mapped to chromosome 22q12-13 and involves the *MYH9* gene^{2,3} that encodes non-muscle myosin heavy chain-A (NMMHC-A) protein, an integral part of myosin II. We characterized the hematologic and genetic features of a Chinese family with an *MYH9*-related disorder.

A 25-year old Chinese woman, who suffered from bilateral juvenile glaucoma of gradual onset since the age of 16 and a painful blind left eye enucleated at the age of 22 due to absolute glaucoma, was admitted to hospital for right eye surgery to maintain intra-ocular pressure under control. She volunteered a history of thrombocytopenia since childhood that did not require treatment. There was no personal history of a bleeding tendency and all previous eye operations had been uneventful. Laboratory investigations showed isolated thrombocytopenia (Table 1). A blood film showed giant platelets and Dohle-body like inclusions in the neutrophils, appearing as faint bluish blocks located at the periphery of the cytoplasm (Figures 1 A-B). A morphological diagnosis of MHA was made. Direct questioning revealed a history of thrombocytopenia in her mother who had had a previous bone marrow aspiration performed for suspected immune thrombocytopenia purpura (ITP) that had showed no detectable morphological abnormality. Her mother had cataracts but no glaucoma. The elder brother of the proband's mother suffered from renal failure, was undergoing dialysis and had hearing problems. A family study (Table 1) revealed hematologic abnormalities in four out of seven family members. The age of the individual may have an effect on the non-hematologic phenotypes since renal damage, deafness and cataracts can be acquired rather than congenital, thus explaining their occurrence in the two eldest affected family members.

Electron microscopy ultrastructural examination⁴ of neutrophils from the index patient showed round cytoplasmic inclusions about 0.5-1µm in diameter without a membrane. These inclusions comprised clusters of ribosomes and segments of rough endoplasmic reticulum, without longitudinal filaments (Figure 1C). There was another form of elongated inclusions, about 2 µm long and 0.8 µm across with ribosomes distributed as cross-striated bands in the matrix (Figure 1D). These were similar to the Fechtner/Sebastian inclusions described by

Table 1. Hematologic and clinical features of the index patient and her family members.

Subject	Sex (years)	Age	Hb (g/dL)	WBC ($\times 10^9/L$)	Plt ($\times 10^9/L$)	Clinical features
Index patient	F	25	13.4	8.52	58	Bilateral juvenile glaucoma; 1+ proteinuria; audiogram not done
Mother	F	51	14.5	6.76	128	Cataracts; microscopic hematuria; audiogram not done
Father	M	52	14.3	12.7	243	Normal
*Maternal uncle	M	62	12.2	6.31	85	Renal failure on dialysis, deafness
*Cousin 1	F	34	11.6	5.11	48	Asymptomatic; urinalysis and audiogram not done
Son of cousin 1	M	6	13.8	7.83	51	Asymptomatic; urinalysis and audiogram not done
*Cousin 2	F	32	13.5	6.91	214	Normal

Hb: hemoglobin level; WBC: white blood cell count; Plt: platelet count performed on an Abbott Cell-Dyn 3200 automated hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA); *elder brother of the index patient's mother; *daughters of the maternal uncle.

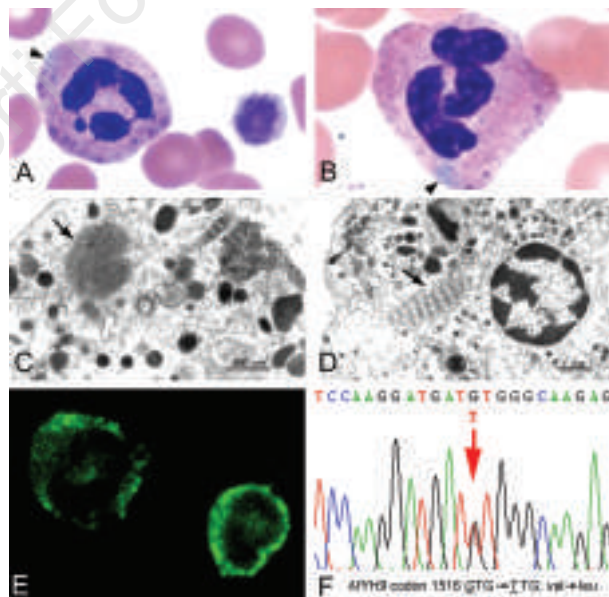


Figure 1. A-B. Cytoplasmic inclusion (arrowhead) in neutrophils (Wright-Giemsa $\times 1000$). (C) Round inclusion comprising a cluster of ribosomes (arrow) (D) Elongated inclusion with cross-striated bands of ribosomes in the matrix (arrow), under electron microscopy. (E) Granular pattern of fluorescence signals from anti-NMMHC-A antibody in neutrophils. (F) *MYH9* mutation detected by automated sequencing.

Pujol-Moix *et al.*⁴ Using polyclonal anti-NMMHC-A antibody (Biomedical Technologies, Stoughton, MA, USA), an immunofluorescence study showed an abnormal granular distribution of NMMHC-A in neutrophils instead of the normal diffuse patterns⁵ (Figure 1E). Direct nucleotide sequencing of all coding sequence of the *MYH9* gene (GenBank Accession number NM 002473) in

the index patient, using a published protocol⁶ that covered 40 exons, showed a missense mutation at codon 1516 (GTG→TTG; val→leu) of exon 31 (Figure 1F). The other four affected family members had the same mutation, whereas two unaffected family members (the father of the index patient and the younger daughter of the maternal uncle) were negative for the mutation.

To the best of our knowledge, this is the first reported Chinese family with an *MYH9* mutation and our findings support the pan-ethnic nature of *MYH9*-related disorders. There are several points of note in the present report. Whether congenital or developmental glaucoma, as seen in the index patient, is associated with MHA is uncertain, and a literature search found no evidence of such an association. Second, it is well known that MHA and other forms of inherited macrothrombocytopenia may mimic idiopathic thrombocytopenic purpura. For a correct diagnosis, a high index of clinical suspicion together with a thorough family history and careful examination of peripheral blood film are needed. Importantly, although the bleeding tendency in *MYH9*-related disorders is generally mild, treatment used for idiopathic thrombocytopenic purpura, such as corticosteroids and splenectomy, is not effective.⁷ Finally, unraveling the molecular defect in these disorders allows rapid diagnostic confirmation through detection of the mutation. The V1516L mutation, located at the coiled-coil domain of the protein and segregating with the disease phenotype in this family, is a novel change. Despite observations that C-terminal mutations or truncation of the tailpiece are associated with hematologic changes only,⁸ other known mutations in the coiled-coil domain can be found in both MHA and Fechtner syndrome.⁹ Recent putative evidence points towards lineage discordant pathogenic mechanisms for the hematologic abnormalities, namely haploinsufficiency of NMMHC-A in the megakaryocytic lineage and a dominant negative effect of the NMMHC-A mutant in granulocytes.¹⁰

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