MYH9 disorder: diagnosis using immunofluorescence and genetic testing in Thai children and adolescents with macrothrombocytopenia

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Abstract

MYH9 disorder is characterized by macrothrombocytopenia with or without granulocyte DÖhle body-like inclusion bodies. Diagnosis is made by immunofluorescence analysis and genetic study of the *MYH9* gene. Our collaborative study between Thailand and Japan began with 67 Thai patients with macrothrombocytopenia. Of these, 11 patients(16.4%), aged 4 months-22 years with platelet counts ranging from 2,000-99,000/uL were diagnosed with *MYH9* disorder. *MYH9* gene mutations occurred in exons 1,16,30,38,40. One novel mutation was identified (c.4338T>C, p.F1446A). The results indicate that patients with macrothrombocytopenia should be tested for *MYH9* disorder in order to avoid misdiagnosis to the other diseases, such as chronic immune thrombocytopenia.

Patients with mutations in the Head domain of the *MYH9*gene

Patients 1-6 presented at the age of [?]1 year. They were diagnosed with alloimmune thrombocytopenia (patients 3, 5 and 6), or cITP (patients 1 and 2). Their platelet count range was 2,000-65,000/uL. Patients 1-3 had mucocutaneous bleeding. Patients 1 and 6 were treated with prednisolone while patients 3 and 5 received intravenous immunoglobulin. Patient 4 was initially suspected of *MYH9* disorder because granulocyte IB were observed by a hematologist from TMSG. IFA demonstrated small diffuse precipitation in all patients except patient 6, who had small-to-medium diffuse precipitation. Five out of six patients had mutations in exon 1, of which p.S96L is the most common (Table 1 and Figure 1).

Non-HMs were identified in patients 1, 3 and patient 6. The hearing test of patient 6, at 5 years old, showed mixed hearing loss (Right ear: hearing frequency 1 kHz at 22 dB for air and 30 dB for bone, Left ear: hearing frequency 4 kHz at 40 dB). This patient had a high urine protein/creatinine ratio of 0.49 and had been treated with an angiotensin inhibitor after diagnosis.

Patients with mutations in the Tail domain of the *MYH9*gene

Patients 7-11 presented from birth to 20 years of age. Their platelet count range was 3,000-99,000/μL. All were diagnosed with cITP and treated with prednisolone, except patient 10. Mild bleeding symptoms were reported in patients 7 and 8. Granulocyte IB were found in all patients except patient 7. IFA revealed medium diffuse precipitation in patients 7-9, and large localized precipitation in patients 10 and 11. Genetic testing revealed mutations in exon 30, 38 and 40. Mutations in exon 30 were the most common, including a novel p.F1446A mutation (Table 1 and Figure 1).

Non-HMs were demonstrated in patients 8 and 9, cousins who carried a similar mutation. Patient 8 had cataracts in both eyes. Patient 9, the older cousin, had microscopic hematuria, cataracts in both eyes, and mild sensorineural hearing loss in both ears (hearing frequency 4 kHz: Right ear at 23 dB, Left ear at 30 dB).

Family history was demonstrated in four patients: patients 8 and 9 (cousins described above), and patients 5 and 11, who each had a parent with thrombocytopenia and similar *MYH9* mutations.

DISCUSSION

Our investigation found that it is important to observe granulocyte IB carefully and to test for *MYH9* disorder in patients with unknown causes of macrothrombocytopenia, since 17% of our macrothrombocytopenia patients were ultimately diagnosed with *MYH9* disorder. A previous study had findings that support this: 12.4% of patients with thrombocytopenia, tested by targeted next generation sequencing of 27 common genes, were found to have *MYH9*disorder[11]. Testing for *MYH9* disorder takes on even greater importance when we consider that many patients are believed to be undiagnosed due to mild bleeding symptoms.[1,12]This is supported by our findings, since 6 out of 11 patients with*MYH9* disorder had no bleeding symptoms at all, and the others had only mild symptoms.

Different methods can be used to identify patients with *MYH9*disorder, though their reliability varies. Specifically, MPV, a parameter on most automated blood cell counters, can indicate platelet size, but only half of our patients whose MPV was measured had MPV that would indicate giant platelets (i.e., [?]11 fL). In addition, relying on family history to identify patients who might have *MYH9* disorder is unreliable since 30% of patients have previously been reported as sporadic cases[5] and our report demonstrated that 7 out of 11 (64.0%) patients had no family history of thrombocytopenia.

Therefore, to ensure diagnosis of *MYH9* disorder, according to the results of our study, observation of platelet size and granulocyte IB on PBS were the most useful screening tests, despite their drawbacks: all patients had large or giant platelets and 9 out of 11 patients had granulocyte IB. Definitive diagnosis can then come from IFA of the NMMHC-IIA since IFA is sensitive and specific in detecting *MYH9*disorder. Mutation analysis is also important to determine the prognosis of patients. IFA can locate mutations in the *MYH9* gene according to the patterns of localization: type I, large oval-spindle precipitation usually associates with mutations in the tail domain (exons 1-16) and types II and III, small to medium-sized circle-oval precipitation, usually associate with mutations in the head domain (exons 17-40).[12] Since the *MYH9* gene contains 40 exons, Sanger sequencing of all exons is quite time consuming and increases the cost of investigation. The present report demonstrated patterns of IF localizations that mostly correlated with the site of mutations. Specifically, 6 patients with small diffuse to medium localization patterns had common mutations in the head domain, and 5 patients with medium to large localization patterns had mutations in the tail domain.

Our findings regarding the age at which non-HMs occur are in line with previous research. Specifically, 3 younger patients (ages 2, 3 and 11) with non-HMs had mutations in the head domain (exons 1 and 16), similar to previous reports where mutations in the head domain were associated with non-HMs, especially p.R702C, which occurs in younger age groups,[13-17] and p.S96L and p.W33R.[18-23] Likewise, 2 older patients, aged 15 and 19, had non-HMs in the tail domain (exon 30), in agreement with previous studies that found tail domain mutations were associated with non-HMs in older age groups.[2,14,18] This cohort demonstrated a novel mutation in the tail domain, p.F1446A that associated with non-HMs in adolescent age group.

4. CONCLUSION

Increased diagnosis of *MYH9* disorder was made possible by applying using appropriate laboratory techniques. The simplest screening approach is the observation of PBS. IFA, meanwhile, is the gold standard for diagnosing *MYH9* disorder, with the added benefit of guiding the location of *MYH9* gene mutations. Following this protocol will greatly increase the likelihood of diagnosing *MYH9* disorder.

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CONFLICT OF INTEREST

The authors state no conflicts of interest.

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