—Case Reports—

Renal Biopsy-induced Hematoma and Infection in a Patient with Asymptomatic May-Hegglin Anomaly

Tae Matsumoto[1], Takeshi Yanagihara[2], Kaoru Yoshizaki[2], Masami Tsuchiya[1], Mika Terasaki[3], Kiyotaka Nagahama[3*], Akira Shimizu[3], Shinji Kunishima[4**] and Miho Maeda[1]

[1]Department of Pediatrics, Nippon Medical School Tama Nagayama Hospital, Tokyo, Japan
[2]Department of Pediatrics, Nippon Medical School Musashikosugi Hospital, Kanagawa, Japan
[3]Department of Analytic Human Pathology, Graduate School of Medicne, Nippon Medical School, Tokyo, Japan
[4]Department of Advanced Diagnosis, Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan

The May-Hegglin anomaly is characterized by inherited thrombocytopenia, giant platelets, and leukocyte cytoplasmic inclusion bodies. The Fechtner, Sebastian, and Epstein syndromes are associated with mutations of the *MYH9*-coding nonmuscle myosin heavy chain IIA, similar to the May-Hegglin anomaly, and are together classified as *MYH9* disorders. *MYH9* disorders may include symptoms of Alport syndrome, including nephritis and auditory and ocular disorders. A 6-year-old boy was diagnosed with an *MYH9* disorder after incidental discovery of hematuria and proteinuria. Focal segmental glomerulosclerosis was detected on renal biopsy. However, despite no prior bleeding diatheses, he developed a large post-biopsy hematoma despite a preprocedural platelet transfusion calculated to increase the platelet count from 54,000/μL to >150,000/μL. Idiopathic thrombocytopenic purpura is a major cause of pediatric thrombocytopenia following acute infection or vaccination, and patients with *MYH9* disorders may be misdiagnosed with idiopathic thrombocytopenic purpura and inappropriately treated with corticosteroids. Careful differential diagnosis is important in thrombocytopenic patients with hematuria and proteinuria for the early detection of thrombocytopenia. Patients with *MYH9* disorders require close follow-up and treatment with angiotensin II receptor blockers to prevent the onset of progressive nephritis, which may necessitate hemodialysis or renal transplantation. The need for renal biopsy in patients with *MYH9* disorders should be carefully considered because there could be adverse outcomes even after platelet transfusion. (J Nippon Med Sch 2021; 88: 579–584)

Key words: *MYH9* related disease, May-Hegglin anomaly, focal segmental glomerulosclerosis, puncture biopsy, thrombocytopenia

Introduction

The May-Hegglin anomaly, characterized by a triad of thrombocytopenia, macrothrombocytopenia, and leukocyte cytoplasmic inclusions (e.g., Döhle bodies), is a rare inherited thrombocytopenic macrothrombocytopenia that results from an *MYH9* gene anomaly, which generates a nonmuscle myosin heavy chain IIA (NMMHC-IIA) mutant[1,2]. It has an incidence of 1 in 20,000[3] and occurs across a broad age range, although predominantly in young adults[4,5]. *MYH9* disorders are difficult to diagnose because of nonspecific symptoms or unclear investigational findings.

We report an *MYH9* disorder in a 6-year-old boy who underwent renal biopsy with hematuria and proteinuria

* Present address: Department of Pathology, Kyorin University School of Medicine, Tokyo, Japan
**Present address: Department of Medical Technology Gifu University of Medical Science, Gifu, Japan
Correspondence to Tae Matsumoto, Department of Pediatrics, Nippon Medical School Tama Nagayama Hospital, 1–7–1 Nagayama, Tama-city, Tokyo 206–8512, Japan
E-mail: tae@nms.ac.jp
https://doi.org/10.1272/jnms.JNMS.2021_88-609
Journal Website (https://www.nms.ac.jp/sh/jnms/)

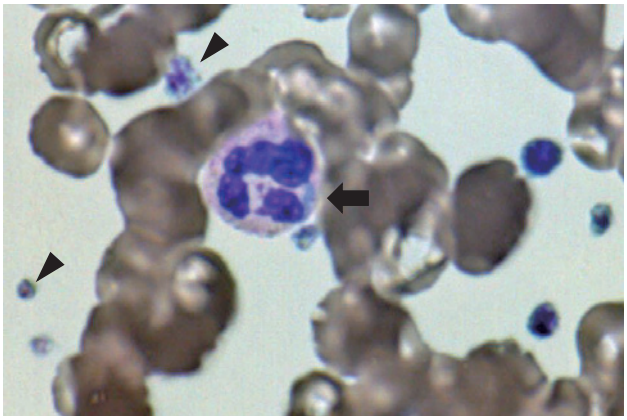

Fig. 1 Hematological analysis of a peripheral blood sample
Giant platelets (arrowhead) and Döhle body (arrow; agglomeration of modified myosin protein) stained blue in granulocytic cytoplasm (hematoxylin-eosin staining, ×400).

and consequently sustained a large post-biopsy hematoma despite preprocedural platelet transfusions. We focus on the need for invasive investigations for differential diagnosis and associated adverse outcomes.

Case Report

A 6-year-old boy was referred to our hospital in Tokyo, Japan for the evaluation of hematuria and proteinuria that were incidentally detected during a routine school health checkup. The patient was asymptomatic and had no relevant medical or family history of kidney disease. His urinary protein-to-creatinine ratio was high at 3.09 g/gCre (normal <0.15 g/gCre). He had thrombocytopenia (platelet count 54,000/μL) but no purpura, hematoma, or bleeding diathesis. Hematology revealed several giant platelets (**Fig. 1**, arrowheads) and leukocytes with Döhle bodies (**Fig. 1**, arrow), which indicated an *MYH9* disorder, characterized by thrombocytopenia (**Table**). He had no cataracts or hearing loss, which are associated with some *MYH9* disorders. A renal biopsy was planned to determine the extent of glomerulonephritis for further clinical workup and treatment planning. Considering his young age, we aimed to prevent a future necessity for hemodialysis or renal transplantation. Pre-procedurally, 10 units of platelets were transfused to increase his platelet count to approximately 150,000/μL. Under intravenous sedation and anesthesia, renal biopsy was performed uneventfully with minimal blood loss. Three hours post-biopsy, he complained of abdominal pain and nausea. Ultrasonography revealed a hypoechoic region, indicative of a large hematoma, extending along two-thirds of the kidney from the superior pole to the lateral convex border, and an echo-free space in the Pouch of Douglas. He became febrile 7 days after renal biopsy and was managed with complete bed rest, analgesics, icepack application, and empirical antibiotic therapy for a suspected hematoma infection. Absorption fever secondary to increased cytokine levels and activity during hematoma absorption was considered.

Table Results of the patient's initial investigations

Parameters	Values
White blood cell (mm^3/μL)	7,100
Hemoglobin (g/dL)	13.5
Platelet (mm^3)	5.4
Döhle bodies	Positive
Megathrombocytes	Positive
Bleeding time (m)	6
International normalized ratio of prothrombin time	1.09
Activated partial thromboplastin time (s)	27.7
Fibrinogen (mg/dL)	274
Hepaplastin test (%)	93
antithrombin III (%)	131.6
Fibrin/fibrinogen degradation products (μg/mL)	<2.5
D-dimer (μg/mL)	0.73
Aspartate aminotransferase (IU)	26
Alanine aminotransferase (IU)	15
Lactate dehydrogenase (IU)	300
Total protein (g/dL)	6.1
Albumin (g/dL)	3.8
Blood urea nitrogen (mg/dL)	13.3
Creatinine (mg/dL)	0.33
Sodium (mEq/L)	142
Potassium (mEq/L)	4.4
Chloride (mEq/L)	106
Calcium (mEq/L)	9.0
Phosphorus (mEq/L)	4.8
Magnesium (mEq/L)	1.9
Cystatin C (mg/L)	0.87
Platelet-associated immunoglobulin G (ng/10^7 cells)	109
Antiplatelet antibody	Negative

Contrary to expectations, the hematoma persisted after his fever subsided, and bedside ultrasonography on alternate days showed a hypoechoic region. The hematoma gradually pooled at the superior renal pole. Computed tomography revealed a persistent hematoma 7 days post-biopsy, indicating continuous low-volume bleeding/oozing from the kidney puncture site. We transfused 10 units of platelets over 3 days, and his platelet count increased to 228,000/μL. Thereafter, the hematoma disappeared, and he was discharged 18 days after the biopsy. Genetic testing revealed that a mutation of exon 24 on *MYH9* caused his hematuria and proteinuria[6], and an an-

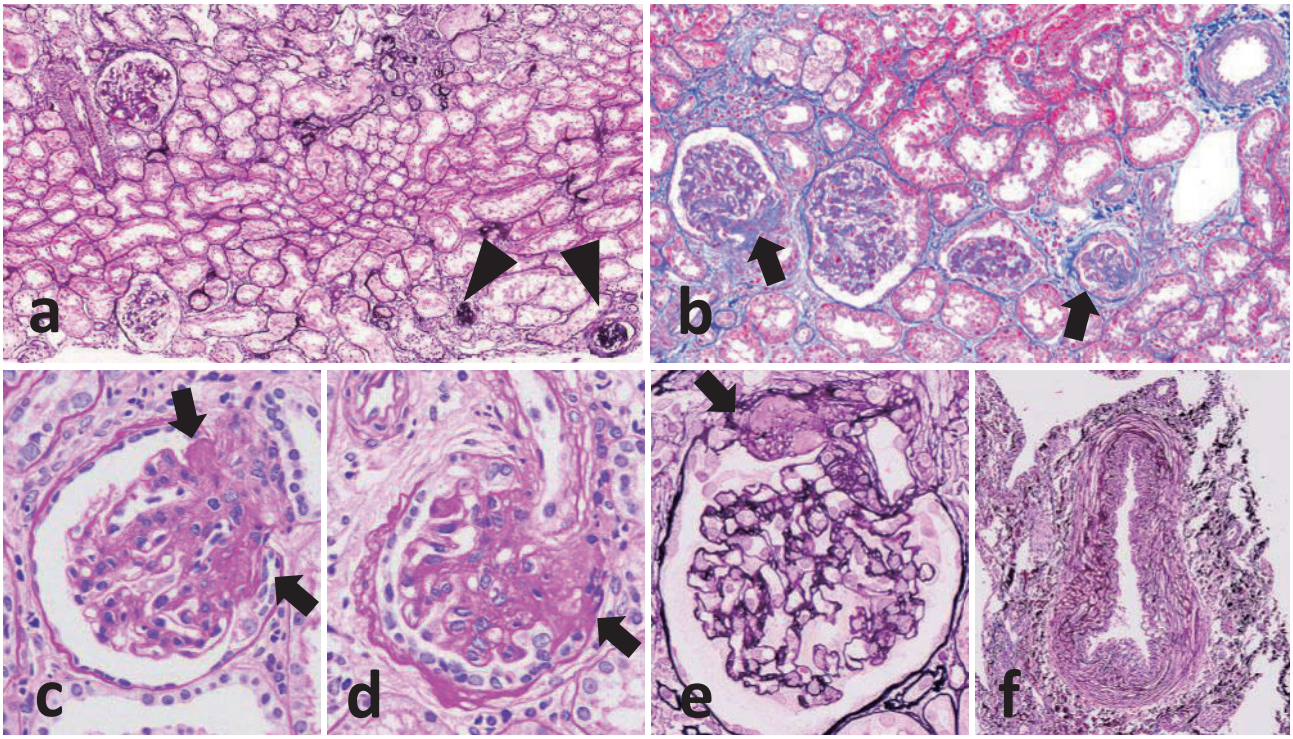

Fig. 2 Light microscopy findings in a renal biopsy specimen
Among 34 glomeruli (a: Periodic acid silver-methenamine (PAM) stain, ×150; b: Masson stain, ×200), two are globally sclerotic (a: arrowhead). The glomeruli have minor abnormalities, and some had segmental glomerulosclerosis (b: arrow). Three glomeruli show segmental glomerulosclerosis with exudative lesions in the perihilar region (c–e: arrow) (c, d: Periodic acid-Schiff stain, ×600; e: PAM stain, ×600), indicative of perihilar focal segmental glomerulosclerosis. An arcuate artery is present in the renal corticomedullary area (f: PAM stain, ×200).

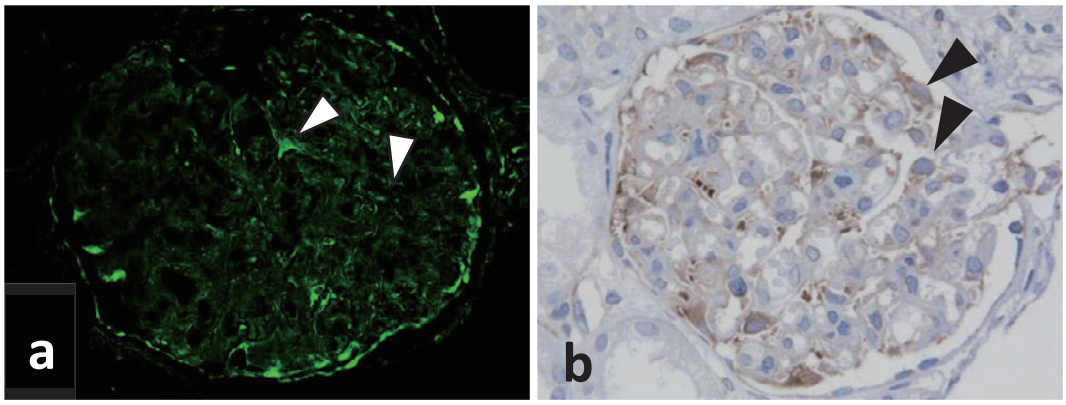

Fig. 3 Myosin expression in the patient's podocytes
Intraglomerular weak and irregular myosin expression shown using immunofluorescence (a: ×600) and immunohistochemistry (b: ×600).

giotensin II receptor blocker was prescribed to maintain his renal function[7].

Histology revealed that the biopsy specimen had 34 glomeruli: two glomeruli were globally sclerotic (**Fig. 2a**), and three exhibited segmental glomerulosclerosis with exudative changes and perihilar adherence to Bowman's capsule (**Fig. 2b-e**). The remaining glomeruli had minor abnormalities; no arteriolar changes were noted (**Fig. 2f**). These findings indicated perihilar focal segmental glomerulosclerosis (FSGS). Focal tubular atrophy, interstitial fibrosis, and mild mononuclear cell infiltration around the atrophic tubules were apparent. Immunofluorescence staining showed nonspecific complements.

Renal NMMHC-IIA expression was evaluated with immunofluorescence (**Fig. 3a**) and immunohistochemistry (**Fig. 3b**) using the rabbit polyclonal human NMMHC-

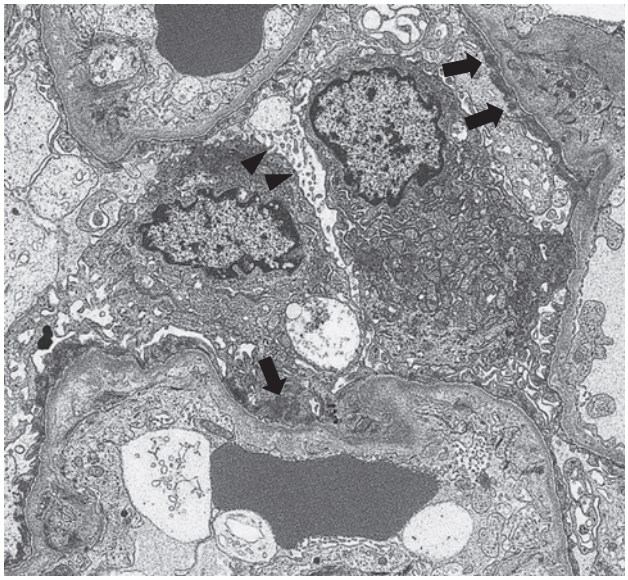

Fig. 4 Electron microscopy of podocytes
The foot processes of podocytes, containing microvilli (arrowheads) and increased mitochondria, show irregular distribution and diffuse effacement in almost all surfaces with the aggregation of actin filaments (arrows).

IIA antibody (PRB-440P; BioLegend, CA, USA). There was intraglomerular weak, irregular myosin expression in patient's specimen podocytes, respectively, indicating reduced myosin expression. Electron microscopy revealed irregular distribution and diffuse effacement of podocyte foot processes on the glomerular basement membrane (**Fig. 4**), consistent with FSGS and aggregation of actin filaments in podocytes in effaced areas of foot processes with microvilli formation.

Written informed consent was obtained from the patient's mother for publication of this case report and any accompanying images.

Discussion

Genetic testing revealed an *MYH9* disorder with a rare mutation on exon 24 (EXON24p.E1066_A1072del) that destroys the double-stranded structure of NMMHC-IIA, which matched the pathological findings[6]. NMMHC-IIA are distributed in various cells with various functions, including stress fibers in neutrophils for cell structure and mitosis. The abnormal NMMHC-IIA structure in individuals with *MYH9* mutations results in the formation of leukocytes with Döhle bodies. NMMHC-IIA is essential for proplatelet differentiation from blood megakaryocytes, which explains thrombocytopenia in *MYH9* disorders. NMMHC-IIA is distributed in podocytes opposite to the glomerular basement membranes to inhibit protein filtration. Podocytes provide an essential filtration layer with the network of foot processes, and pathological changes cause proteinuria.

Most patients with *MYH9* disorders caused by exon 24 mutations develop signs, such as hematuria, proteinuria, cataracts, and macrothrombocytopenia, in adulthood and remain asymptomatic until they develop chronic kidney disease, with illness onset depending on the genotype. Patients with incidentally discovered asymptomatic *MYH9* disorders tend to be misdiagnosed with idiopathic thrombocytopenia[8]. The first opportunity for a diagnosis in this asymptomatic patient occurred with the detection of hematuria and proteinuria on a routine school health checkup. Since the 1970s, all students younger than 18 years in Japan have undergone mandatory annual checkups for urinary protein, hematuria, and glucosuria to screen for chronic kidney disease with an aim to prevent future hemodialysis or kidney transplantation[9]. This patient came to our hospital for an evaluation of proteinuria and hematuria. Blood investigations revealed the triad of the May-Hegglin anomaly: thrombocytopenia, giant platelets, and cytoplasmic inclusion bodies, which led to the diagnosis of an *MYH9* disorder. Before the incidental detection of urinary abnormalities, the patient was healthy and had not previously undergone blood investigations or experienced bleeding problems; therefore, this was the first identification of thrombocytopenia. Some patients with isolated hematuria are monitored with urinalysis for years because the hematuria has no pathological effect. Patients with proteinuria tend to have severe kidney disease, and thus, hematuria is sometimes overlooked. In our patient, hematological investigations facilitated an accurate diagnosis. Hematological investigation of students with urinary abnormalities can help detect occult *MYH9* disorders despite the absence of symptoms or proteinuria. Approximately 10% of patients who are initially diagnosed with idiopathic thrombocytopenia are subsequently diagnosed with secondary thrombocytopenia[10]. In our patient, prophylactic platelet transfusion was undertaken to increase the plasma platelet count to more than 150,000/μL to avoid a post-renal biopsy hematoma, although a platelet count higher than 100,000/μL generally suffices for invasive hematological treatment. Hemorrhage as a complication of renal biopsy has been reported in 1.2% of patients[11]. Among the hemorrhagic complications after a pediatric kidney biopsy, hematoma occurs in 11-18% of cases, and transfusion is needed in 0.9% of cases despite a normal platelet count[12]. Syndromes with macrothrombocytopenia may be associated with abnormal hemostatic function; however, we did not

ascertain the patient's platelet function before the biopsy. Flow cytometry can effectively evaluate platelet function[13], thereby facilitating the detection of abnormal hemostatic function. Thus, macrothrombocytopenia could possibly have resulted in the large hematoma[14].

MYH9 disorders have previously been reported to strongly influence the prognosis of FSGS[15]. The NMMHC-IIA, coded by the *MYH9* gene, is a motor protein that modulates the movement of the actin filament in muscles. The NMMHC-IIA is distributed throughout several types of cells and may polymerize or depolymerize as relevant to perform a variety of roles in each cell. NMMHC-IIA contributes to the movement of the foot processes in podocytes, which function as the filtration layer and maintain capillary wall motion. Animal experiments have demonstrated that functional and structural changes in podocytes caused by NMMHC-IIA protein anomalies coded by *MYH9* mutations can cause permselective membrane failure[16]. This demonstrates the importance of NMMHC-II in glomerular filtration[17,18].

NMMHC-IIB or C has a function similar to that of NMMHC-IIA in the kidney, eye, and ear and, therefore, can substitute for abnormal NMMHC-IIA. Cataracts or hearing disabilities often appear later than thrombocytosis or macrothrombocytosis because of the substitutional function of NMMHC-IIB or C. Our patient was screened for cataracts or hearing disability, but glomerulonephritis was the only sign at diagnosis[6,19].

The results of the renal biopsy indicated FSGS[15] and suggested the presence of an *MYH9* disorder. In this case, blood examination suggested an *MYH9* disorder before the definitive diagnosis by genetic testing. As the patient was asymptomatic, it is debatable whether the renal biopsy was indicated at that time. Primary medical treatment without a renal biopsy is initiated in patients with pediatric nephrotic syndrome because most have minimal changes. Therefore, renal biopsy can be deferred until a specific diagnosis.

This patient's genetic testing led to the subsequent genetic testing and diagnosis of his mother who had never had proteinuria, hematuria, bleeding tendency, hearing loss, or cataracts. Gene testing was conducted on three sample types: blood and kidney cells, which develop from the mesoderm, and are collected from the urine; fingernails and hair roots, which develop from the ectoderm; and buccal mucosal cells, which develop from the endoderm. The mother was found to have a mosaic mutation in somatic cells. All germ layers had, to some extent, the *MYH9* gene mutation, which is presumed to occur as a mutation in the early embryonic gastrula stage; however, 0% mutation was found in kidney cells in the patient's mother's urine sample[20]. This was possibly the reason for her asymptomatic state.

In conclusion, this patient was diagnosed with an *MYH9* disorder following the incidental detection of hematuria and proteinuria during a school checkup in the absence of symptoms. However, he sustained a huge hematoma and fever after a renal biopsy, despite a preprocedural platelet transfusion to increase his platelet count to more than 150,000/μL. It is important to consider an *MYH9* anomaly in the differential diagnosis of thrombocytopenia with giant platelets. The need for a renal biopsy should be carefully considered because, in patients with an *MYH9* disorder, the renal pathology can be predictive of FSGS. Clinicians can determine the primary treatment without a renal biopsy based on typical histopathological characteristics indicative of FSGS. Patients with hematuria and proteinuria complicated by thrombocytopenia should be carefully evaluated, and an *MYH9* disorder should be considered in the differential diagnosis. A renal biopsy in a patient with an *MYH9* disorder is associated with a risk of bleeding complications and should be carefully considered for each patient.

Conflict of Interest: The authors declare that they have no conflicts of interest.

References

1. Kunishima S, Saito H. Advances in the understanding of MYH9 disorders. Curr Opin Hematol [Internet]. 2010 Sep; 17(5):405–10. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20601875
2. Balduini CL, Pecci A, Savoia A. Recent advances in the understanding and management of MYH9-related inherited thrombocytopenias. Br J Haematol [Internet]. 2011 Jul;154(2):161–74. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21542825
3. Fernandez-Prado R, Carriazo-Julio SM, Torra R, Ortiz A, Perez-Gomez MV. MYH9-related disease: it does exist, may be more frequent than you think and requires specific therapy. Clin Kidney J [Internet]. 2019 Aug;12(4):488–93. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31384439
4. Seri M, Pecci A, Di Bari F, et al. MYH9-related disease: May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome are not distinct entities but represent a variable expression of a single illness. Medicine (Baltimore) [Internet]. 2003 May;82(3):203–15. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12792306
5. Savoia A, De Rocco D, Panza E, et al. Heavy chain myosin 9-related disease (MYH9-RD): neutrophil inclusions of myosin-9 as a pathognomonic sign of the disorder. Thromb Haemost [Internet]. 2010 Apr;103(4):826–32. Available from: https://www.ncbi.nlm.nih.gov/pubmed/

20174760
6. Kunishima S, Kitamura K, Matsumoto T, Sekine T, Saito H. Somatic mosaicism in MYH9 disorders: the need to carefully evaluate apparently healthy parents. Br J Haematol [Internet]. 2014 Jun;165(6):885–7. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24611568
7. Pecci A, Granata A, Fiore CE, Balduini CL. Renin-angiotensin system blockade is effective in reducing proteinuria of patients with progressive nephropathy caused by MYH9 mutations (Fechtner-Epstein syndrome). Nephrol Dial Transplant [Internet]. 2008 Aug;23(8):2690–2. Available from: https://www.ncbi.nlm.nih.gov/pubmed/18503011
8. Althaus K, Najm J, Greinacher A. MYH9 related platelet disorders - often unknown and misdiagnosed. Klin Padiatr [Internet]. 2011 May;223(3):120–5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21567368
9. Yamagata K, Iseki K, Nitta K, et al. Chronic kidney disease perspectives in Japan and the importance of urinalysis screening. Clin Exp Nephrol [Internet]. 2008 Feb;12(1):1–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/18175065
10. Lee AC. Isolated thrombocytopenia in childhood: what if it is not immune thrombocytopenia? Singapore Med J [Internet]. 2018 07;59(7):390–3. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30109354
11. Atwell TD, Spanbauer JC, McMenomy BP, et al. The timing and presentation of major hemorrhage after 18,947 image-guided percutaneous biopsies. AJR Am J Roentgenol [Internet]. 2015 Jul;205(1):190–5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26102398
12. Varnell CD, Stone HK, Welge JA. Bleeding complications after pediatric kidney biopsy: A systematic review and meta-analysis. Clin J Am Soc Nephrol [Internet]. 2019 01;14(1):57–65. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30522995
13. Skipper MT, Rubak P, Stentoft J, Hvas AM, Larsen OH. Evaluation of platelet function in thrombocytopenia. Platelets [Internet]. 2018 May;29(3):270–6. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28409645
14. Ishida M, Mori Y, Ota N, Inaba T, Kunishima S. Association of a novel in-frame deletion mutation of the MYH9 gene with end-stage renal failure: case report and review of the literature. Clin Nephrol [Internet]. 2013 Sep;80(3):218–22. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22541678
15. Winkler CA, Nelson G, Oleksyk TK, Nava MB, Kopp JB. Genetics of focal segmental glomerulosclerosis and human immunodeficiency virus-associated collapsing glomerulopathy: the role of MYH9 genetic variation. Semin Nephrol [Internet]. 2010 Mar;30(2):111–25. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20347641
16. Johnstone DB, Zhang J, George B, et al. Podocyte-specific deletion of Myh9 encoding nonmuscle myosin heavy chain 2A predisposes mice to glomerulopathy. Mol Cell Biol [Internet]. 2011 May;31(10):2162–70. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21402784
17. Miura K, Kurihara H, Horita S, et al. Podocyte expression of nonmuscle myosin heavy chain-IIA decreases in idiopathic nephrotic syndrome, especially in focal segmental glomerulosclerosis. Nephrol Dial Transplant [Internet]. 2013 Dec;28(12):2993–3003. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24042022
18. Agrawal S, Agarwal S, Naik S. Genetic contribution and associated pathophysiology in end-stage renal disease. Appl Clin Genet [Internet]. 2010;3:65–84. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23776353
19. Aoki T, Kunishima S, Yamashita Y, Minamitani K, Ota S. Macrothrombocytopenia with congenital bilateral cataracts: A phenotype of MYH9 disorder with exon 24 indel mutations. J Pediatr Hematol Oncol [Internet]. 2018 01;40(1):76–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29200148
20. Kunishima S, Matsushita T, Shiratsuchi M, et al. Detection of unique neutrophil non-muscle myosin heavy chain-A localization by immunofluorescence analysis in MYH9 disorder presented with macrothrombocytopenia without leukocyte inclusions and deafness. Eur J Haematol [Internet]. 2005 Jan;74(1):1–5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15613099

(Received, September 4, 2020)
(Accepted, December 11, 2020)
(J-STAGE Advance Publication, March 9, 2021)