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Whole exome sequencing for diagnosis of hereditary thrombocytopenia

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Abstract

Hereditary thrombocytopenia comprises extremely diverse diseases that are difficult to diagnose by phenotypes alone. Definite diagnoses are helpful for patient (Pt) management.

To evaluate the role of whole exome sequencing (WES) in these Pts.

Cases with unexplained long-standing thrombocytopenia and/or suggestive features were enrolled to the observational study. Bleeding scores and blood smear were evaluated. The variant pathogenicity from WES was determined by bioinformatics combined with all other information including platelet aggregometry, flow cytometry, and electron microscopy (EM).

Seven unrelated Pts were recruited. All were female with macrothrombocytopenia. Clinical bleeding was presented in four Pts; extra-hematological features were minimal and family history was negative in every Pt. WES successfully identified all the 11 responsible mutant alleles; of these, four have never been previously reported. Pt 1 with *GNE*-related thrombocytopenia showed reduced lectin binding by flow cytometry, increased glycogen granules by EM and a novel homozygous mutation in *GNE*. Pts 2 and 3 had phenotypic diagnoses of Bernard Soulier syndrome and novel homozygous mutations in *GP1BB* and *GP1BA*, respectively. Pt 4 had impaired microtubule structures, concomitant delta storage pool disease by EM and a novel heterozygous *TUBB1* mutation. Pt 5 had sitosterolemia showing platelets with reduced ristocetin responses and a dilated membrane system on EM with compound heterozygous *ABCG5* mutations. Pts 6 and 7 had MYH9 disorders with heterozygous mutations in *MYH9*.

This study substantiates the benefits of WES in identifying underlying mutations of macrothrombocytopenia, expands mutational spectra of four genes, and provides detailed clinical features for further phenotype-genotype correlations.

Abbreviations: ADP = adenosine diphosphate, BS = bleeding score, EM = electron microscopy, ExAC = Exome Aggregation Consortium, GP = glycoprotein, ITP = immune thrombocytopenia, MFI = mean fluorescence intensity, MPV = mean platelet volume, NGS = next generation sequencing, Pt = patient, WES = whole exome sequencing.

Keywords: hereditary platelet disorders, platelets, whole exome sequencing

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1. Introduction

Hereditary thrombocytopenia is a heterogeneous disorder caused by germline mutations in various essential components in megakaryopoiesis and platelet biogenesis. In many diseases, there are lower numbers of platelets that are larger in sizes, termed macrothrombocytopenia. Before the year 2000, only a few responsible genes were identified, for example, May-Hegglin anomaly (*MYH9* gene), Bernard Soulier syndrome (*GP1BA, GP1BB,* or *GP9* genes). Following the applications of next generation sequencing, pathogenic variants have been identified in approximately 50% to 60%.[1–4] In most cases, the molecular mechanisms underlying macrothrombocytopenia remain unclear. Studying these diseases may not only yield future treatments, but also give deeper insights into the mechanisms of human platelet production by discovering novel molecules in megakaryocyte development. However, next generation sequencing investigations frequently yield uncharacterized variants of unknown clinical significances. Therefore, bioinformatics prediction, clinical correlations, complete Pt phenotyping, and family studies are required for correct diagnosis.[3,4]

The definitive diagnosis of inherited thrombocytopenia gives several clinical benefits. Firstly, unnecessary and potentially toxic treatments for more common diseases causing thrombocytopenia, that is, immune thrombocytopenia (ITP) or myelodysplastic syndromes can be avoided. Screening and monitoring for associated abnormalities may be indicated, such as renal or auditory defects, in MYH9 disorders. Genetic testing is useful as different *MYH9* mutations confer unequal risks of other organ involvements.[5] Furthermore, autosomal dominant thrombocytopenia from *ANKRD26, ETV6,* and *RUNX1* mutations was associated with future hematological malignancies.[1] This information may be helpful for genetic counseling and stem cell donor selection from siblings for leukemia treatment. Finally, amegakaryocytic thrombocytopenia may be resulted from the defects in either *MPL* or thrombopoietin gene that requires different modes of treatments, that is, bone marrow transplantation or a thrombopoietin mimetic, respectively.[6]

In this study, we aimed to evaluate the roles of whole exome sequencing (WES) together with clinical and laboratory data to diagnose seven Pts with hereditary thrombocytopenia. Four cases harbored variants that have never been described. Further investigations were performed to support the pathogenicity of the variants and to potentially find helpful unique features for diagnoses of these diseases in the future.

2. Methods

2.1. Patients and setting

This is a cross-sectional study in a tertiary care hospital. Seven consecutive Pts with suspected hereditary thrombocytopenia were enrolled from 2014 to 2019. The inclusion criteria were thrombocytopenia since the first blood test with no previous history of normal platelet counts with or without bleeding tendency since childhood and/or thrombocytopenia with suggestive features, for example, numerous giant platelets on blood smear, associated abnormalities of *MYH9*-related disorders, or unresponsiveness to ITP therapy. Pts with acquired disorders that could explain thrombocytopenia were excluded.

The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Chulalongkorn University and was conducted in accordance with the international guidelines for human research protection as Declaration of Helsinki, The Belmont Report, The Council for International Organizations of Medical Sciences (CIOMS) guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

2.2. Platelet counts and morphology

Complete blood counts were determined by Sysmex series XN-9203 (Sysmex, Thailand Co., Ltd.). Wright-Giemsa stained blood smears were examined and reviewed by a clinical pathologist (PR).

2.3. Platelet aggregation assay

Platelets were incubated at 37°C for 2 minutes with continuous magnetic stirring at 37°C for 10 minutes. After stimulation by agonists, the changes in light transmission were measured by the Platelet Aggregation Chromogenic Kinetic System-4 (PACKS-4) aggregometer (Helena Laboratories, USA).

2.4. Flow cytometry

Platelet membrane glycoproteins (GPs) were detected using fluorescein-conjugated antibodies and flow cytometry. Citrated whole blood was centrifuged at 150 g for 15 minutes without brake at room temperature. Subsequently, platelets rich plasma was stained with the fluorescein isothiocyanate-conjugated (FITC) anti-human CD41a (GPIIb), phycoerythrin-conjugated (PE) anti-human CD42b (GPIbα, both from BioLegend) or allophycocyanin-conjugated anti-human CD36 (BD Biosciences) for 20 minutes. To analyze lectin expression, the fluorescein isothiocyanate-labelled sialic acid-binding lectin, *Sambucus nigra* (SNA) lectin, and the PE-streptavidin labeled sialic acid-binding lectin, *Maackia amurensis* lectin II (MAL-II) (both obtained from Vector Laboratories, California), for 30 minutes at room temperature. The BD FACSAria II (Becton Dickinson, Franklin Lakes, NJ) was used for analysis. To correct for the increased fluorescence intensity in giant platelets, the mean fluorescence intensities (MFIs) of lectins were divided by MFIs of the CD41a for comparison with those of normal platelets.

2.5. Electron microscopy analysis

Platelets were isolated from whole blood by centrifugation at 150 g for 15 minutes followed by 400 g for 15 minutes at room temperature. Subsequently, 3% glutaraldehyde was added to fix platelets for 60 minutes and postfixed with 2% osmium tetroxide in phosphate buffer for 45 minutes. Platelets were then washed twice with phosphate buffer for 2 minutes and dehydrated in ethanol. The sample was embedded in 100% resin at 70°C for 24 hours to allow complete resin infiltration. Thin section was performed by an ultramicrotome, placed on mesh copper grids and stained with uranyl acetate and lead citrate. All images were examined by electron microscopy (EM) (JEOL 1210, JEOL, Japan).

2.6. Whole exome sequencing

Genomic DNA was isolated from peripheral blood leukocytes after informed consent. The DNA sample was prepared as an Illumina sequencing library followed by the exome capture step. The sequencing libraries were enriched by SureSelect Human All

Table 1

Characteristics, phenotypes, and genotypes of the patients.

Pt	Age (yr)	BS	BAT	Platelet Count (/μL)	Phenotype	Variant	Diagnosis
1	24	5	6	17,000	-Giant platelets -EM: Increased glycogen granules	*GNE* c.G1417A* (p.G473S) (Homozygous)	*GNE*-related Thrombocytopenia
2	14	10	11	38,000	-Giant platelets -No GpIb expression	*GP1BB* c.406G>T* (p.Glu136Ter) (Homozygous)	Bernard Soulier syndrome
3	37	5	5	56,000	-Giant platelets -No GpIb expression	*GP1BA* c.663C>A* (p.N221K) (Homozygous)	Bernard Soulier syndrome
4	24	7	11	109,000	-Large platelets -Impaired ADP- and epinephrine-induced aggregation -EM: Unclear microtubular coils Reduced dense bodies	*TUBB1* c.151_153del* (p.Tyr51del) (Heterozygous)	*TUBB1* variant and Delta storage pool disease
5	34	−1	0	93,000	-Large platelets -Reduced GpIIb and GpIb expression -Reduced RIPA -EM: Dilated DMS	*ABCG5* c.1217G>A (p.R406Q) *ABCG5*c.751C>T (p.Gln251Ter) (Compound heterozygous)	Sitosterolemia
6	27	0	0	56,000	-Giant platelets -Döhle bodies -Cataract in the young	*MYH9* c.3494G>T (p.R1165L) (Heterozygous)	MYH9 disorder
7	48	−2	0	9,000	-Giant platelets -Döhle bodies	*MYH9* c.5797C>T (p.Arg1933Ter) (Heterozygous)	MYH9 disorder

Age = Age of testing, BAT = ISTH-SSC Bleeding Assessment Tool, BS = MCMDM-1VWD Bleeding score, DMS = demarcation membrane system, EM = electron microscopy, GNE = UDP-N-acetylglucosamine 2-epimerase, GpIb = glycoprotein Ib-IX-V, Pt = patient, RIPA = ristocetin-induced platelet aggregation, Yr = years.
* Novel variants.

Exon V5 Kit. The captured libraries were sequenced using Illumina HiSeq 4000. Singleton-WES analysis was made and all SNVs and Indels were filtered by the following filtering criteria;

(1) located in exons or flanking introns of the listed genes,
(2) not synonymous,
(3) rare with 1000G minor allele frequency of less than 1%,
(4) less than 0.1% in the Genome Aggregation Database (GnomAD),
(5) less than 10 alleles in 2166 Thai exome controls,
(6) (if the variant is a missense) predicted to be damaging by SIFT and Polyphen, and/or
(7) related to the phenotypes of the Pts.

Only hematopoietic system variants could be associated with the current features of Pts. All process was performed and analyzed by Excellence Center for Genomics and Precision Medicine, King Chulalongkorn Memorial Hospital, the Thai Red Cross Society.

3. Results

3.1. Clinical information

All data are summarized and displayed in Table 1. The ages of consultation ranged from 14 to 48 years old and all of them were female. Pt bleeding symptoms were evaluated by both the MCMDM-1VWD Bleeding score (BS)[7] and ISTH-SSC bleeding assessment tool.[8] These 2 scores were well-correlated. Pt 1 to Pt 4 had bleeding tendency since childhood, while Pt 5 to Pt 7 had only thrombocytopenia without bleeding. Pt 1 and Pt 6 had been treated as ITP including steroids and immunosuppressive agents with no response. The nonhematological abnormality was found only in Pt 6 who developed cataract at the age of 10. She had not received corticosteroids at that time. The platelet counts ranged from 9,000 to 109,000/microliter. Blood smear showed large platelets in all Pts.

After complete investigations, the geneticists, laboratory scientists, and hematologists were discussed on all available data to give final diagnoses. The information was subsequently reported back to the referring hematologists to aid genetic counseling and disease management.

3.2. Patient 1: GNE-related thrombocytopenia

The Pt had easy bruising and gastrointestinal bleeding since 4 years old. She later developed hypermenorrhea since menarche. There was no detectable myopathy. Both of her parents had normal platelet counts and did not have any bleeding tendency. Her platelets were very large on blood smear (Fig. 1A). Due to giant platelet sizes, her platelet count by automate complete blood count was probably falsely lower than the actual count and her mean platelet volume (MPV) was spuriously normal (9.5 fl). Platelet aggregation was not performed due to platelet counts below 50,000/microliter. Flow cytometry showed normal expression of GPIIb and GPIbα on platelet membrane (Fig. 2A) excluding the diagnosis of Glanzmann thrombasthenia and Bernard Soulier syndrome, respectively. Under EM, platelets displayed markedly increased glycogen granules as compared with normal platelets (Fig. 1B and C).

WES revealed a novel homozygous variant in the *GNE* (UDP-N-acetylglucosamine 2-epimerase) gene, c.G1417A (p.G473S), substituted an amino acid glycine with serine. This variant has not been identified in the Exome Aggregation Consortium (ExAC) database but was found in 2/154 individuals in the in-house Thai Exome database.

To verify the reduced sialic acid expression of Pt 1 by flow cytometry, the mean fluorescent intensity (MFI) ratios between *SNA*/GPIIb and MALII/GPIIb were 0.164 and 0.252, which were

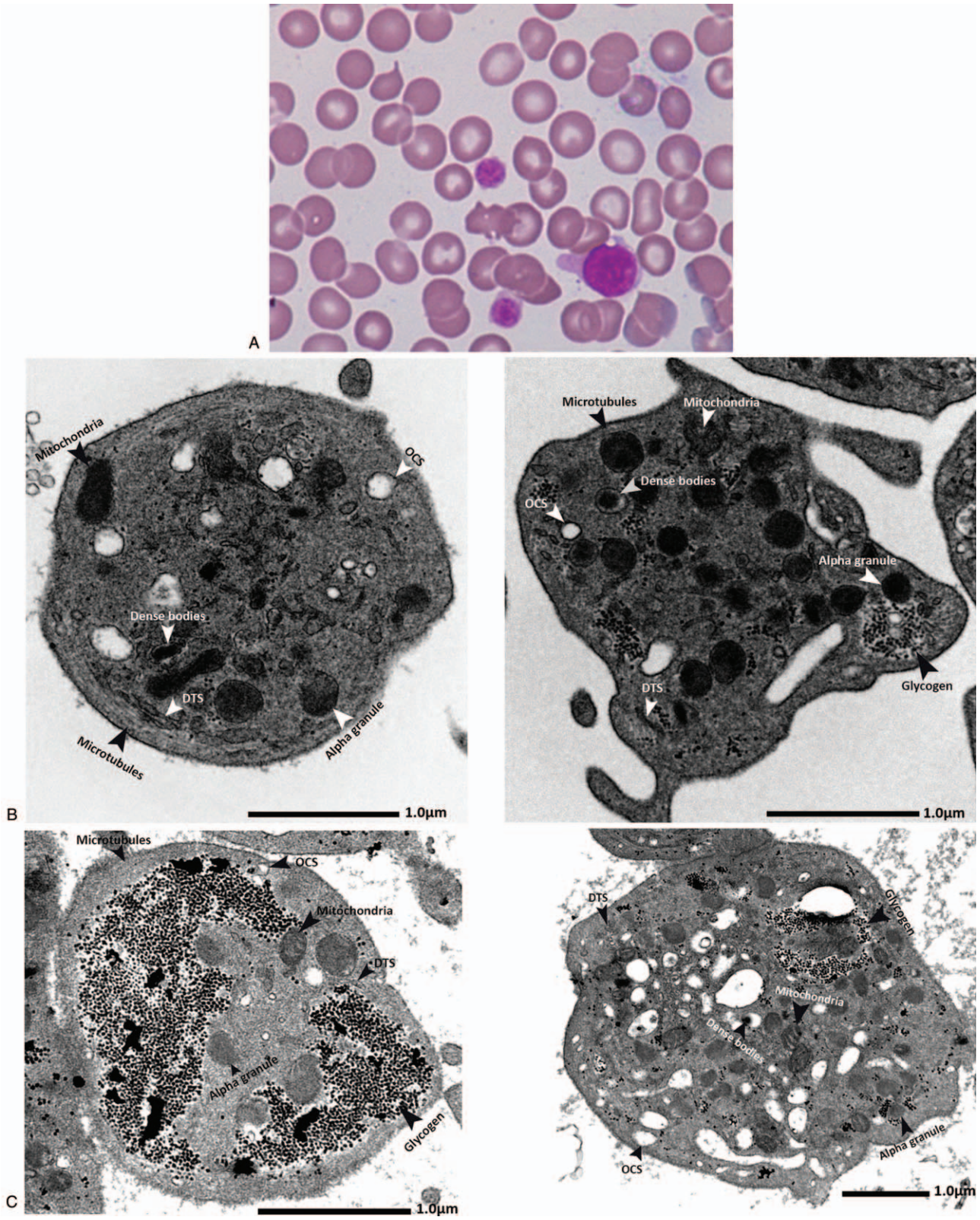

Figure 1. Patient 1 with *GNE*-related thrombocytopenia. A. The blood smear revealed very large and round platelets. B. Electron microscopy of normal platelets exhibited circumferential microtubules. Cytoplasm contains organelles, such as alpha granules, dense bodies, mitochondria, open canalicular system, and dense tubular system. C. The ultrastructure of patient platelets showed increased glycogen granules and unclear microtubule coils.

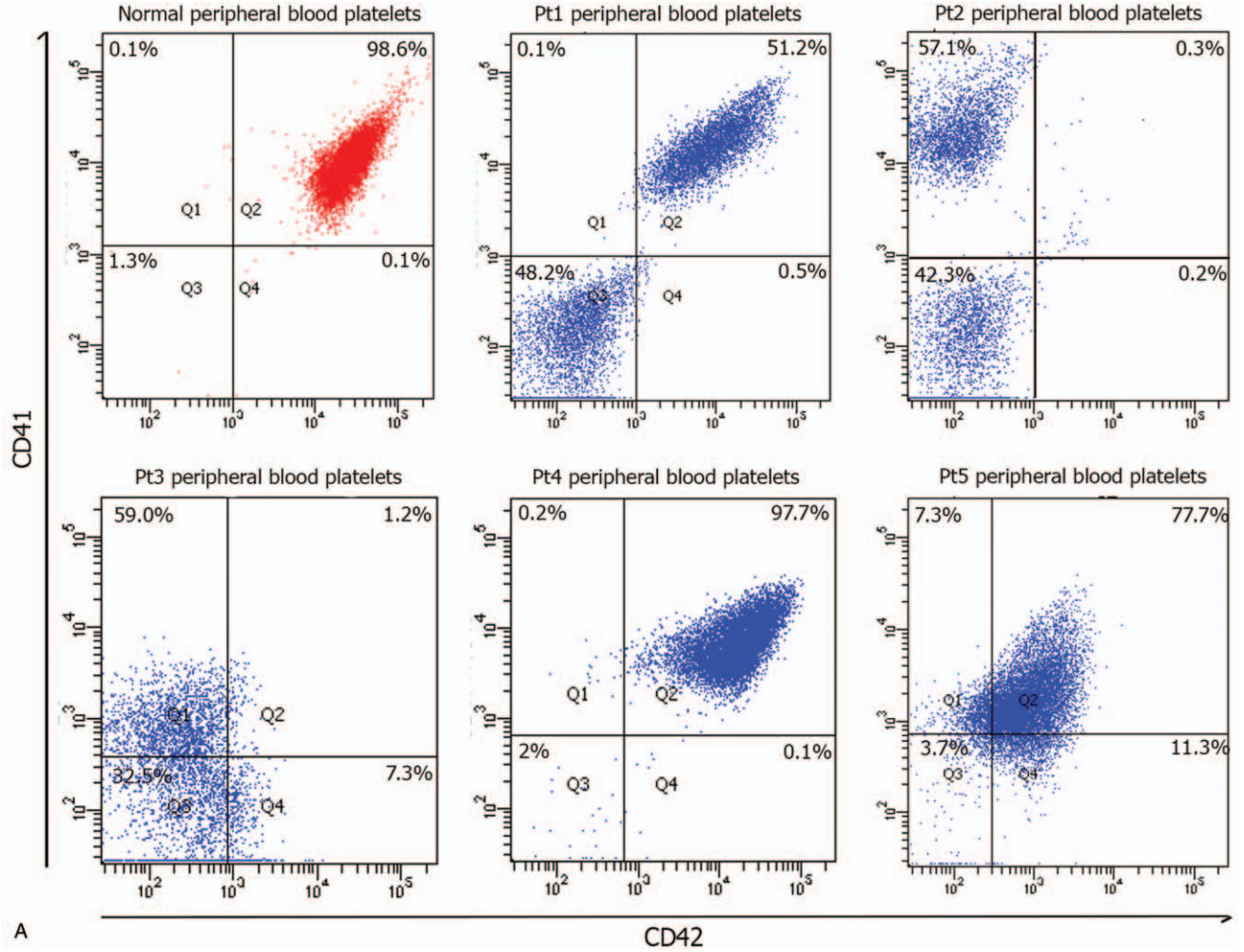

Figure 2. Flow cytometry studies. A. Flow cytometry showed normal expression of glycoprotein (Gp) Ibα (CD42) in Patient 1 (Pt1), no expression of GpIbα in Pt2 and Pt3 with Bernard Soulier syndrome, and reduced expression of GpIIb (CD41) and GpIbα in Pt5 with sitosterolemia. B. Flow cytometry of Pt 1 with GNE thrombocytopenia showed reduced the ratios of mean fluorescence intensities of sialic acid-binding lending lectins (*Sambucus nigra* lectin and MALII) and the MFI of GpIIb (CD41) compared with those of normal controls.

lower than those of normal platelet controls, which were 0.566 and 0.728, respectively (Fig. 2B).

3.3. Patient 2 and Patient 3: Bernard Soulier syndrome

Both of them had macrothrombocytopenia associated with mucosal bleeding since childhood. Flow cytometry exhibited the marked deficiencies of GPIbα with normal expression of GPIIb (Fig. 2A). Ristocetin-induced platelet aggregation was impaired.

WES discovered the novel homozygous variant, c.406G>T (p.Glu136Ter) in the *GP1BB* gene in Pt 2 and a new homozygous mutation, c.663C>A (p.Asn221Lys) in the *GP1BA* gene in Pt 3 confirming the diagnoses of Bernard Soulier syndrome.

3.4. Patient 4: TUBB1-related thrombocytopenia and delta storage pool disease

The Pt had a history of easy bruising, epistaxis, gastrointestinal bleeding, hypermenorrhea, and recurrent postprocedural bleeding since childhood. Her father and sister did not have bleeding problem (BS 0). Her mother reported epistaxis and bleeding postsurgery, but the MCMDM-1VWD BS (3) and ISTH-SSC bleeding assessment tool score (3) were within normal limits. She had mild thrombocytopenia with mildly enlarged platelets with peripheral clear zones devoid of pink granules (Fig. 3A). Flow cytometry showed normal expression on platelet membrane GP IIb and GPIbα (Fig. 2A).

Light transmission aggregometer revealed a lack of secondary wave after adenosine diphosphate (ADP) stimulation and no primary wave after epinephrine. The responses to ristocetin, collagen, and arachidonate were normal. EM exhibited large platelets with the dilated and hypertrophic open canalicular system and the microtubule coils were not seen. The number of alpha granules was normal but the number of dense granules was markedly decreased (Fig. 3B). Despite the larger sizes, the number of dense granules of Pt 4 was 0.32 per platelets compared with 0.69 per normal platelets.

WES revealed potential pathogenic mutations in 2 genes, *CD36* and *TUBB1*. The trio-analysis revealed double heterozygous *CD36* gene variants inherited from her mother. The first is a heterozygous splicing c.429+3dupG variant that was found in 9/121150 individuals in the ExAC database and 4/1084

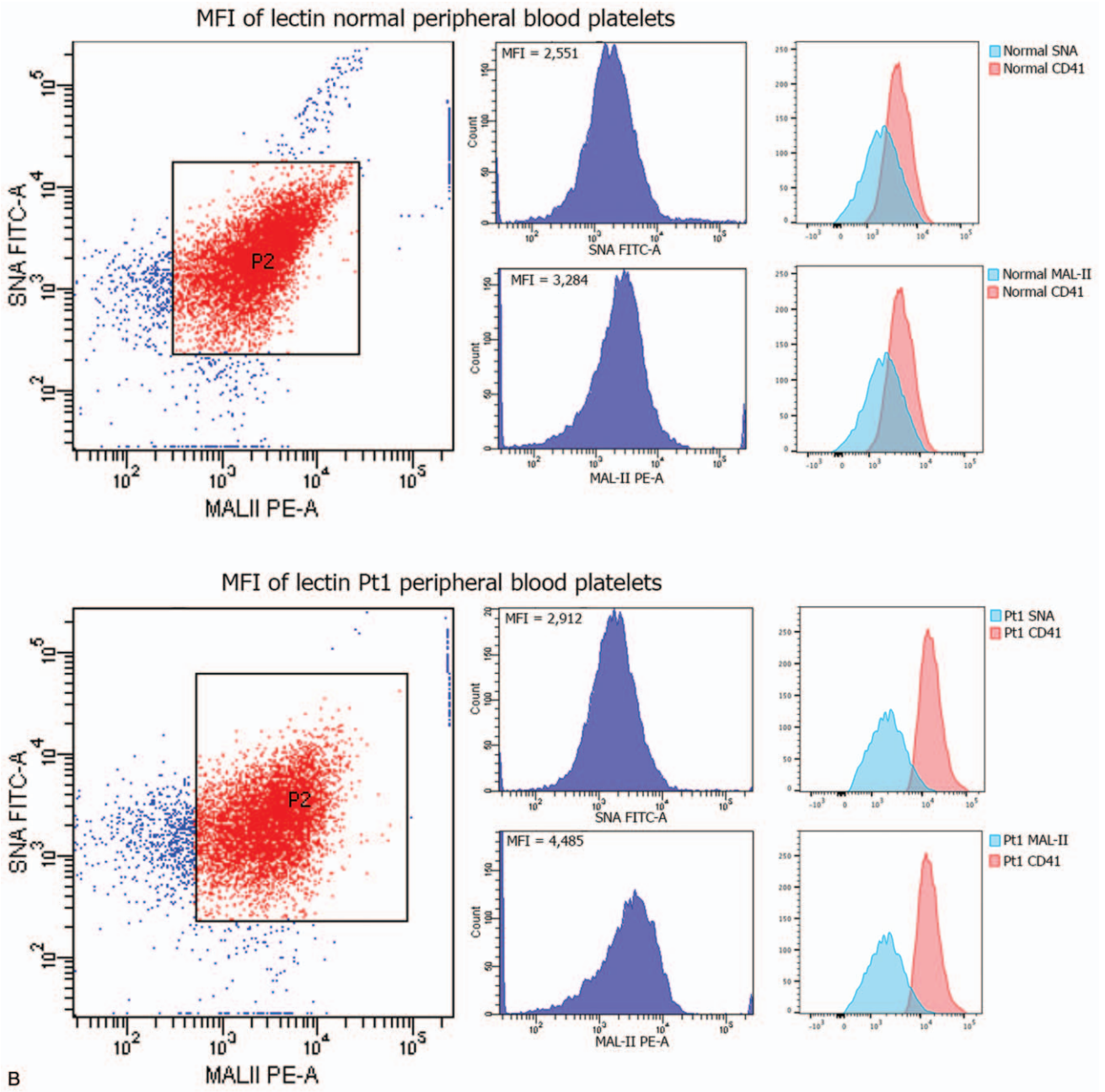

Figure 2. (Continued).

individuals in the in-house Thai Exome database. The second is a heterozygous missense variant, c.1027C>A (p.Leu343Ile) that has not been identified in the ExAC database but was found in 1/1084 individuals in the in-house Thai Exome database. The SIFT/Polyphen/MCAP programs predict this variant to be probably pathogenic. However, flow cytometry showed normal expression of CD36 on platelets.

Moreover, WES reported a novel probably pathogenic heterozygous in-frame deletion variant, c.151_153del (p.Tyr51del) in the *TUBB1* gene, which was known to be related to an autosomal dominant macrothrombocytopenia. The WES analyses of her parents revealed that this mutation was inherited from her father. Both parents had normal platelet counts but the blood smear of her father occasionally showed giant platelets (Fig. 3C).

3.5. Patient 5: Sitosterolemia

The Pt presented with asymptomatic macrothrombocytopenia. She had hypercholesteremia (low density lipoprotein cholesterol 185 mg/dL) which was normalized by simvastatin 10 mg per day (low density lipoprotein cholesterol 112 mg/dL). There was no xanthoma and no clinical evidence of atherosclerosis. Her platelets were mildly enlarged with the MPV of 12.9 fL (9.4–12.3 fL). There was no anemia or reticulocytosis but occasional stomatocytes were seen (Fig. 4A).

Flow cytometry showed lower expression of GPIIb and GPIbα on platelet membrane (Fig. 2A). The Pt platelets showed normal aggregation to ADP and collagen. However, the platelets failed to aggregate using ristocetin at the concentrations of 1.0, 1.2, and

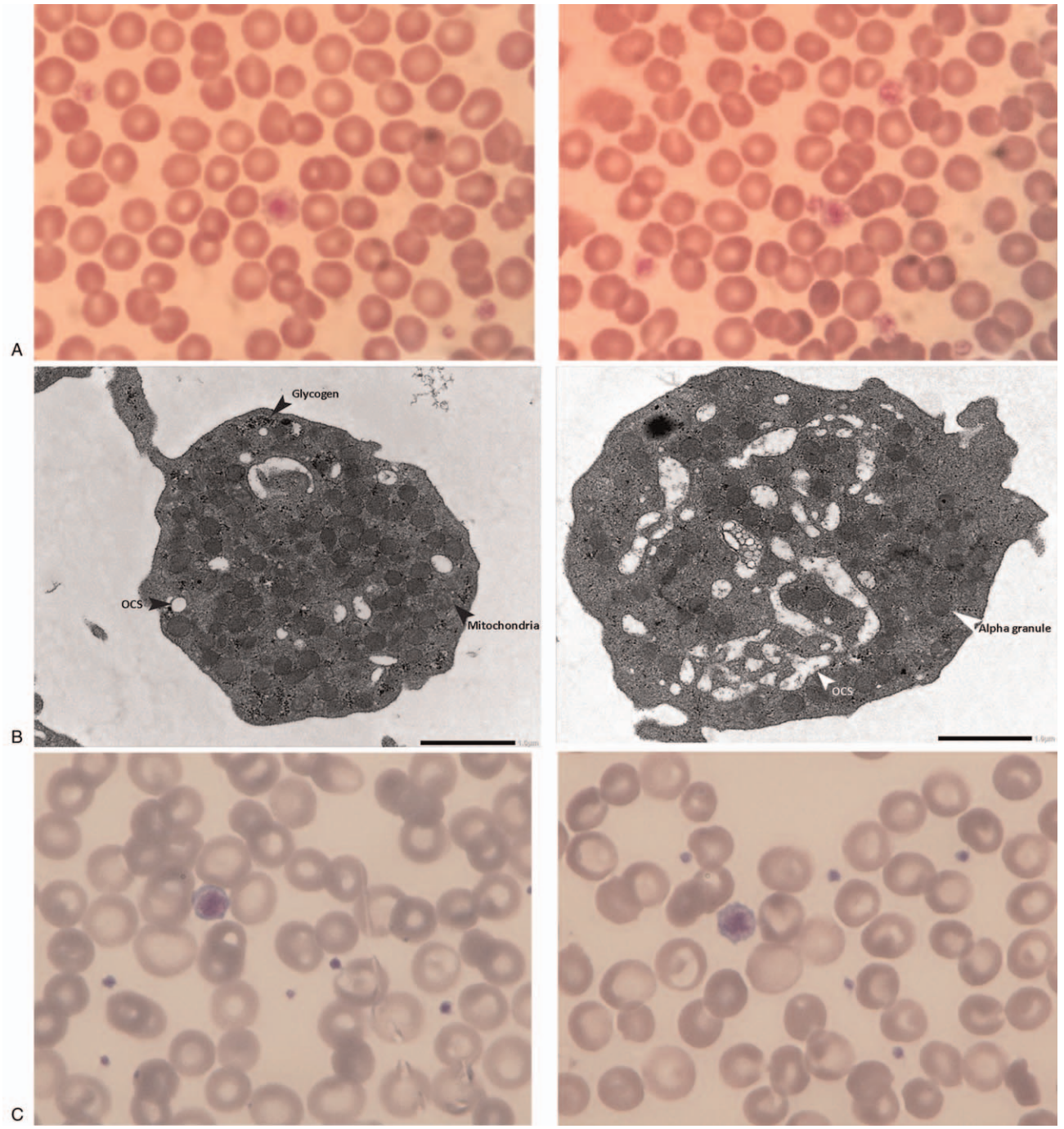

Figure 3. Patient 4 with *TUBB1* thrombocytopenia and delta storage pool disease. A. The blood smear of the patient showed enlarged platelets with peripheral zones lacking of granules. B. The ultrastructure of the patient platelets showed dilated intracytoplasmic membrane complexes, unclear microtubule coils, and reduced dense granules. C. The blood smear of the patient's father revealed occasional giant platelets with peripheral clear zones.

1.4 mg/ml but responded to 1.6 mg/ml ristocetin. Interestingly, EM showed large platelets containing dilated and hypertrophic intra-cytoplasmic membrane complexes (Fig. 4B).

WES revealed 2 known compound heterozygous variants in the *ABCG5* gene. The first one is missense variant, c.1217G>A (p.R406Q) that was previously described[9] and the second one is a truncated mutation, c.751C>T (p.Gln251Ter) that was found previously in sitosterolemia.[10] PCR-Sanger sequencing also confirmed the presence of these mutations.

3.6. Patient 6 and Patient 7: MYH9 disorder

These 2 Pts had no bleeding problem but Pt 6 presented with cataracts at a young age. Blood smear revealed Döhle bodies in neutrophil cytoplasm (Fig. 5). The renal and liver functions, as well as hearing, were normal.

WES revealed heterozygous *MYH9* gene variants, the c.3494G>T (p.R1165L) in Pt 6 and the c.5797C>T (p.Arg1933Ter) in Pt 7.

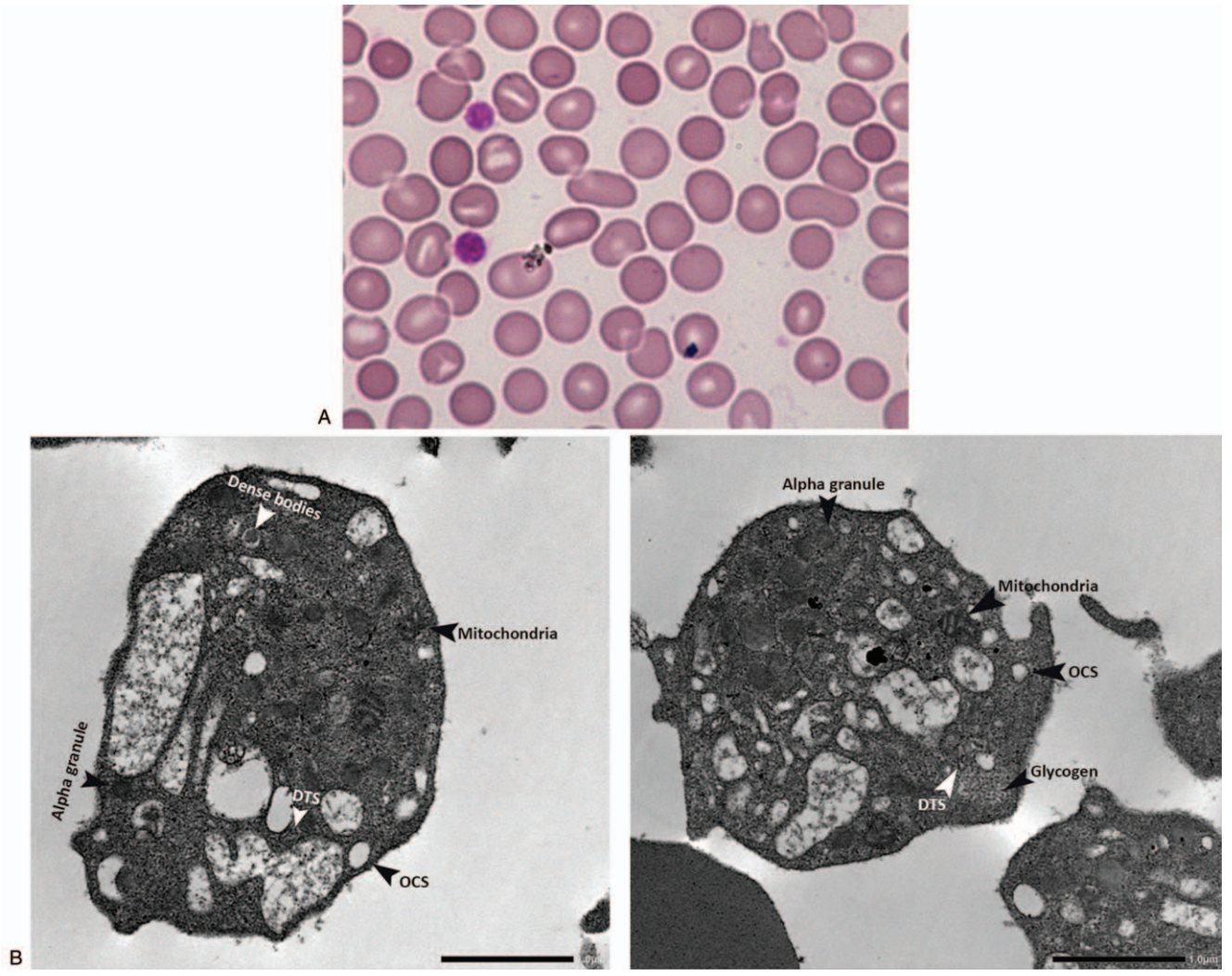

Figure 4. Patient 5 with sitosterolemia. A. The blood smear revealed large platelets with occasional stomatocytes. B. The ultrastructure of platelets showed the large open canalicular system and the unclear circumferential bands of microtubules.

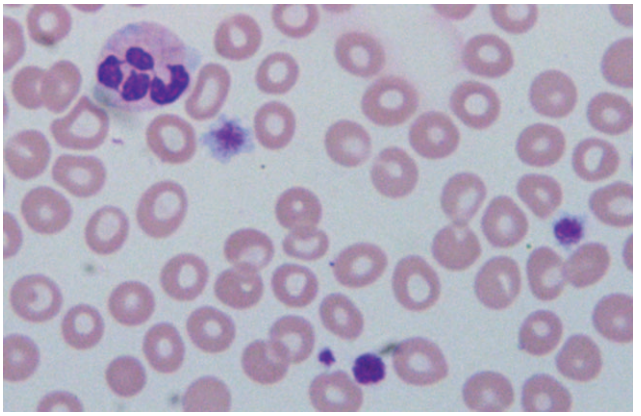

Figure 5. The blood smear of Patient 6 with MYH9 disorder showed large platelets and Döhle bodies in a neutrophil.

4. Discussion

In our study, the WES showed potentials to determine genetic variants in Pts with hereditary thrombocytopenia. Four of 7 Pts revealed the novel variants by using the exome sequence data explored in the ExAC database and the in-house Thai Exome database. Interestingly, extra-hematological manifestations were absent in most of our Pts. Based solely on clinical information, candidate genes were unknown, therefore, prohibiting the use of Sanger sequencing. The applications of the WES technology to find the candidate genes in conjunction with clinical data, blood smear, flow cytometry, EM, and platelet aggregometry significantly contribute to the precise diagnosis.

In our study, Pt 1 had a novel missense mutation in the *GNE* (UDP-N-acetylglucosamine 2-epimerase) gene which controls biosynthesis of N-acetylglucosamine, a sialic acid precursor.[10] The diagnosis was supported by decreased lectin binding under flow cytometry. Most of platelet surface GP contain N-linked and O-linked glycans, which are covered by sialic acids. The desialylation exposed surface β-galactose residues which were

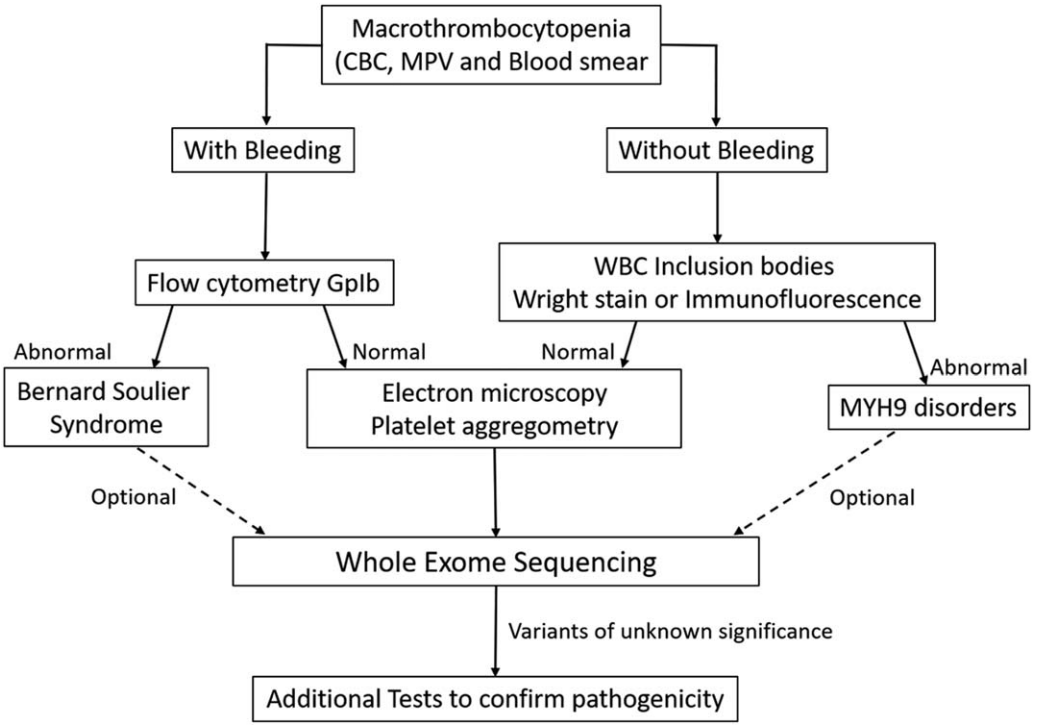

Figure 6. The proposed diagnostic scheme for hereditary macrothrombocytopenia (BS: Bleeding score).

recognized by the lectin asialoglycoprotein receptor on hepatocytes to initiate phagocytosis resulting in decreased platelet survival.[11–15] This causes thrombocytopenia associated with myopathy. Like our case, previous papers[16,17] reported *GNE* mutations causing macrothrombocytopenia with no sign of a muscle disorder. EM displayed the large platelets with normal numbers of alpha granules.[17] The increase in platelet glycogen granules, which has never been described, was first observed in our study. This may be due to the defect in carbohydrate uses in platelets. Whether this finding is helpful to suggest this disorder remains to be confirmed.

In Pt 4, the pathogenicity of the *CD36* variant was excluded by the normal surface expression of CD36 using flow cytometry. Furthermore, previous reports found no bleeding and/or thrombocytopenia in subjects with CD36 deficiency.[18] The *TUBB1* gene mutation was inherited from the father who did not have any bleeding or thrombocytopenia but his blood smear revealed large platelets. Kunishima et al suggested that the mutations of the beta1-tubulin impaired the microtubule assembly in platelets.[19,20] In vitro transfection experiments in HeLa cells using a mutant beta1-tubulin led to microtubule formation defect. The EM displayed round and large platelets with dilated intracytoplasmic membrane complex.[21] In our study, the platelet EM also showed these features supporting the diagnosis of *TUBB1* thrombocytopenia. In addition, the dense granule numbers were decreased. With a lack of secondary wave of ADP-induced platelet aggregation, the coexisting delta storage pool disease was suggested. This may explain the more severe bleeding and thrombocytopenia of Pt 4 compared to her parents. However, the genetic defect of storage pool disease in this Pt could not be identified by WES and the EM studies of her parents were not available. Previously, concomitant Glanzmann thrombasthenia and TUBB1 disorder has been reported.[22] Therefore, defects in more than 1 gene in a Pt with hereditary thrombocytopenia should be always considered.

The Pt 5 had been previously misdiagnosed as Bernard Soulier syndrome due to the reduced response to ristocetin but normal responses to other platelet agonists by platelet aggregometer and GpIbα expression was mildly decreased by flow cytometry. WES result showed the *ABCG5* variants which were previously reported in sitosterolemia, an autosomal recessive disorder caused by mutations in the *ABCG5* or *ABCG8* genes[23] which encode the plant sterol transporter. Pts usually manifest as hypercholesterolemia, xanthoma, premature atherosclerosis, macrothrombocytopenia, and hemolysis.[24] The global apparent prevalence was 1 in 2.6 million[25] but the true prevalence was still unknown because routine lipid tests cannot differentiate between cholesterol and sitosterol and mild thrombocytopenia may be easily missed.[24] In this case, genetic test is the best option to solve this problem. The sitosterol levels cannot be measured in this study, but hypercholesterolemia in this Pt responded well to a low dose statin suggesting that this was cholesterol.[26] The mechanism of macrothrombocytopenia was mediated by platelet hyperactivity with endocytosis of platelet GpIIb and filamin A[27] consistent with lower GpIIb expression by flow cytometry. In addition, GpIbα expression was also low which may explain the reduced ristocetin responses and dilated membrane system by EM in this case similar to Bernard Soulier syndrome platelets.[28]

In this study, WES could confirm the clinical diagnosis of 2 Pts with Bernard Soulier syndrome and 2 Pts with the MYH9 disorder. The *MYH9* mutations were in the coiled-coil domain in Pt 6 and the C-terminal tail in Pt 7 suggesting the intermediate and low risks of extra-hematological manifestations, respectively.[5] Consistently, cataract was diagnosed in Pt 6. The

information may be helpful to determine appropriate frequencies of follow-up for different Pts.

At present, over 50 candidate genes known to affect megakaryopoiesis, platelet formation, and platelet function responsible for inherited platelet disorders were reported.[1,2] However, causative mutations cannot be identified in approximately half of the Pts, especially platelet function disorders that are not involved surface GPs. Some of them might be acquired disorders and, therefore, Pt selection criteria for genetic testing are also critical. In this study, we recruited the Pts who were likely to have congenital defects and, therefore, the probable mutations could be identified in the majority of them. An advantage of WES is the ability to identify the candidate variants. However, gene defects in the noncoding regions or in genes that are not known to be involved in megakaryocytes and platelet could be missed. Furthermore, functional studies are required to confirm the pathogenicity of the candidate defects. We proposed a diagnostic scheme for hereditary macrothrombocytopenia in Figure 6. It should be noted that blood smear examination is required as MPV can be falsely low for very large platelets. Because WES is still relatively costly, flow cytometry for GpIb and staining for Döhle bodies on blood smear (Wright stain or immunofluorescence[29]), as well as bleeding symptoms, are used initially to diagnose the more common Bernard Soulier syndrome and MYH9 disorder, respectively. If these screening tests are nondiagnostic, WES and EM should be performed. Platelet aggregometry may be helpful, but the interpretation is limited in case of severe thrombocytopenia.

In summary, WES is helpful for diagnosis of hereditary thrombocytopenia in the context of multi-modality investigations. Here, we report novel mutations and interesting features including increased platelet glycogen granules in GNE thrombocytopenia and reduced ristocetin response in sitosterolemia platelets. The limitation of this study is a small sample size due to rarity of this condition. Continuing comprehensive phenotyping of these disorders can provide useful information for diagnosis and proper genetic counseling in the future.

Author contributions

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