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LETTER TO THE EDITOR

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Autosomal dominant macrothrombocytopenia caused by a rare *GPIBB* variant: The importance of DNA sequencing

Dear editors,

We have read with great interest the article by Hayward et al[1] on diagnostic testing for platelet function disorders (PFD). Concerning molecular genetic testing, classified as third-step laboratory tests in the most recent International Society on Thrombosis and Haemostasis (ISTH) guideline,[2] a survey among 123 diagnostic laboratories revealed that only 12%-16% of participants perform these kind of tests. In this light, it was stated that DNA sequencing techniques have an overall low diagnostic yield, unless a specific disorder is suspected from the clinical picture.[1] Screening for rare disorders in all patients with suspected PFD would pose an important financial burden on patients and healthcare authorities. We fully agree with the authors on this point; however, we would like to emphasize the added value genetic testing may have in specific cases by presenting two patients in which DNA sequencing was paramount in assessing a correct diagnosis of PFD and identifying the underlying cause of macrothrombocytopenia associated with variable bleeding tendency. All testing described in this report was performed for diagnostic purposes.

Two unrelated patients were both referred to a hematologist for diagnostic elaboration of mild macrothrombocytopenia, persistently found in routine peripheral blood analyses. The first patient was a 32-year-old woman. Her medical history revealed multiple bleeding symptoms, such as frequent ecchymoses, gingival bleeding, metrorrhagia, and bright red blood loss per rectum. She presented with an ISTH-bleeding assessment tool (BAT) score of 7 at the time of first consultation.[3] The second patient, a 22-year-old man, had no documented bleeding diathesis. Both patients did not report to take any drugs potentially interfering with platelet function. Family history revealed that the mother of the first patient and the father of the second patient also had macrothrombocytopenia, without apparent bleeding symptoms.

Complete blood counts, determined on Sysmex hematology analyzers, showed moderate thrombocytopenia, which already persisted for several years in both patients (patient one, 95-141 x 10^9 platelets/L; patient two, 88-103 x 10^9 platelets/L), along with increased platelet volume (patient one, mean platelet volume 12.9-14.0 fL) or platelet anisocytosis (patient two, multiple large platelets on blood smear) and elevated immature platelet fractions (patient one, 15.4%-17.0%; patient two, 23.4%-29.5%). Of note, routine coagulation assays (PT, aPTT, and fibrinogen) were normal for both patients. Factor VIII coagulant activity and von Willebrand factor (VWF) screening tests (antigen and ristocetin cofactor activity) were determined for the female patient only and were normal as well. The presence of large platelets, mildly reduced platelet counts, positive family history, and a bleeding diathesis, at least in one patient, led to the suspicion of the presence of a congenital platelet disorder, such as Bernard-Soulier syndrome (BSS). As no syndromic features and no structural abnormalities in platelets (other than increased volume and anisocytosis), erythrocytes, or leukocytes were noticed, in vitro platelet aggregation and expression of the glycoprotein (GP) IB-IX-V receptor on platelets were evaluated, according to ISTH recommendations.[2]

LTA was performed according to the guidelines[4] on a Chrono-Log® 700 lumiaggregometer (Chrono-log corporation), yielding normal results with all tested agonists for both patients. Maximal aggregation with 1.5 mg/mL ristocetin, being an important diagnostic test in BSS, was 93%-99%. In addition to LTA, a lumiaggregometry-based ATP-release assay using thrombin (1 U/mL) as agonist was performed on the Chrono-Log® 700, yielding a clearly reduced result for patient one (ATP-release 0.3 nmol, local cutoff 0.5 nmol) and absent ATP-release for patient two. The latter result could not be confirmed, as no second sample was available. To exclude δ-storage pool disease, platelet electron microscopy was subsequently performed on a Zeis EM900 transmission electron microscope (Carl Zeis). Normal platelet dense granule content was found for patient one, while an inconclusive result was obtained for patient two due to low platelet numbers in fixed platelet preparations. Consequently, presence of a storage pool disorder could not be fully excluded for the latter patient. Evaluation of the expression of the GP Ib-IX-V receptor complex on platelet surface was performed by flow cytometric detection of CD42b, being the α-subunit of GPIb, using a FACSCanto™ II flow cytometer (BD). Slightly reduced GPIb expression was found for patient two only (CD42b mean fluorescent intensity (MFI) 67%, expressed as % to control), while it was normal in patient one (CD42b MFI 90%).

As the above mentioned analyses could not provide a clear explanation for the existing macrothrombocytopenia and variable bleeding tendency, high-throughput DNA sequencing for inherited platelet disorders was performed using the ThromboGenomics diagnostic platform.[5] DNA sequencing revealed the presence of a heterozygous c.47T > C variant (p.Leu16Pro) in *GP1BB*, the gene encoding the GPIbβ-subunit of the GP Ib-IX-V receptor. This missense mutation has recently been associated with autosomal dominant macrothrombocytopenia and monoallelic BSS,[6,7] as discussed below.

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The platelet transmembrane GP Ib-IX-V receptor complex plays an essential role during the initial steps of primary hemostasis by adhering platelets to damaged subendothelium via VWF. A variety of mutations in *GP1BA*, *GP1BB*, *and GP9*, the genes encoding the GPIbα, GPIbβ, and GPIX subunits, respectively, have been identified to cause BSS when present in homozygous or compound heterozygous condition. In its classical from, BSS is inherited in an autosomal recessive way (biallelic BSS) and in vitro ristocetin-induced platelet aggregation and GP Ib-IX-V expression are absent or strongly reduced, enabling diagnosis based on LTA and flow cytometry in most cases. Rare monoallelic mutations in *GP1BA* and *GP1BB* may cause an autosomal dominant form of BSS, usually characterized by mild macrothrombocytopenia and less severe or absent bleeding tendency.[6,8,9]

The c.47T >C variant in the GP1BB gene results in a missense variant p.Leu16Pro. This variant is absent from the population database gnomAD.[10] Leu16Pro is located in the signal peptide and is predicted to interfere with SEC61-mediated secretion and/or signal peptide removal, but secretion studies for this variant were not performed.[8] Pathogenicity scoring for the c.47T >C variant supports a deleterious defect (SIFT 0.01 and CADD 22.50). According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology variant classification guidelines,[11] this variant can be classified as a likely pathogenic variant (PM2, PS4, and PP4). The c.47T >C variant has previously been seen in three unrelated index cases with mild macrothrombocytopenia with cosegregation data in the affected parent that also carries the variant.[6,7] Sivapalaratnam et al described three cases originating from two families presenting with normal or slightly reduced platelet counts and increased platelet volume. Abnormal platelet agglutination to ristocetin was found in 2/3 patients. Mildly reduced GPIb expression was reported for the two related patients, while data for the third patient were not available. The authors mentioned bleeding in both cases without further details.[6] Babuty et al described two related patients (father and son) carrying the same mutation. They both had moderate macrothrombocytopenia and high immature platelet fraction. Reduced, albeit mild, GPIb expression in platelets was found, together with impaired ristocetin-induced platelet aggregation. The father showed various bleeding symptoms, including frequent bruising, epistaxis, gingival bleeding, and postoperative hematoma. No increased bleeding tendency was noticed in his son.[7]

Interestingly, while mild macrothrombocytopenia was a constant finding in nearly all cases carrying the monoallelic c.47T >C mutation in *GPIBB*, heterogeneity in in vitro platelet aggregation response to ristocetin and platelet GPIb expression seems to exist. In contrast to the previously described cases, our patients showed normal platelet aggregation to ristocetin and slightly reduced expression of GPIb on platelets was found in one patient only. In addition to the variable bleeding tendency, this may illustrate the variable biological phenotype of patients carrying this mutation. The cohort study presented by Sivapalaratnam *et al* showed that clinical and laboratory findings for dominant forms of macrothrombocytopenia are indeed highly variable.[6] Cosegregation studies would support the genotype-phenotype correlation for our cases; however, we did not have access to samples from pedigrees. Concerning the possibility of other factors underlying the macrothrombocytopenia, it should be noted that all genes currently known for macrothrombocytopenia were evaluated in our patients using the ThromboGenomics gene panel test[5] and no other variants were found that could modify the phenotype. However, noncoding variants and/or variants in novel other genes cannot be excluded as modifiers. The variable phenotype may pose a challenge to diagnostic algorithms solely based on LTA and flow cytometry. If mutation analysis would not have been included in the diagnostic work-up, our patients would not have received an adequate diagnosis. This would have put them at risk of being misdiagnosed with immune thrombocytopenia. In this light, it could be interesting to evaluate in further research how many cases with macrothrombocytopenia would need to be sequenced to find a pathogenic variant. Downes et al[5] found that approximately 50% of thrombocytopenia patients remained without a genetic diagnosis using the ThromboGenomics gene panel test; however, this cohort was not separated into thrombocytopenia versus macrothrombocytopenia.

In conclusion, the presented cases highlight the value of mutation analysis when congenital macrothrombocytopenia is suspected, as platelet aggregation assays and flow cytometric detection of GP Ib-IX-V expression might not be sufficient to detect monoallelic BSS subtypes, in contrast to classical biallelic BSS.

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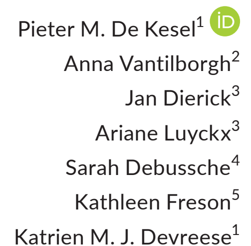
CONFLICT OF INTERESTS

The authors have no competing interests.



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