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Mini Review

Genetic Testing for Inherited Platelet Disorders

Constance P Chen[1] and Dong Chen[2*]

[1]University of Minnesota Medical School, Minneapolis, MN 55455

[2]Division of Hematopathology, Mayo Clinic, Rochester, MN 55905

***Corresponding author:** Dong Chen MD PhD, Division of Hematopathology, Mayo Clinic, Rochester, Minnesota, United States 55905.

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Abstract

Inherited platelet disorders (IPDs) are rare bleeding diatheses due to quantitative or qualitative platelet defects. Due to the lack of highly specific platelet function tests, diagnosing the underlying cause of IPDs remains challenging. Therefore, genetic testing has an important role in investigating patients with IPDs. A major hurdle of genetic testing arises with interpreting the genetic information to identify disease-causing mutations definitively. Thorough phenotype and genotype correlation studies of the patients and their family members and collaboration of the clinical and research communities are essential.

Keywords: Inherited Platelet Disorder, Thrombocytopenia

Introduction

Platelets are derived from megakaryocytes in bone marrow and have crucial roles in primary hemostasis [1]. The platelets' proper shape, size, and biological function are critical for their adhesion and aggregation [2]. Adhesion of platelets to the site of vessel injury involves their direct binding to subendothelial collagen through receptors GPVI and GPIa/IIa. VWF serves as a bridge between platelets and collagen via GPIb-V-IX receptors. The initial adhesion activates platelets that undergo shape change, forming filopodia with an increased density of surface receptors that aid in the adhesion and secretion of alpha and dense granules. Released ADP and thromboxane A2 help platelet-to-platelet interaction and lead to platelet GPIIb/IIIa activation and subsequent platelet aggregation via GPIIb/IIIa binding to fibrinogen.

The Prevalence of Inherited Platelet Disorders

Inherited platelet disorders (IPD), caused by defects in platelet synthesis or function, have unclear prevalence [3,4]. A recent population genetic study on the frequency of naturally occurring loss-of-function variants in genes associated with platelet disorders from a large genome aggregation database showed that 0.329% of subjects in the general population had a clinically meaningful loss-of-function variant in a platelet-associated gene [5]. Therefore, the total prevalence of IPDs could be close to that of symptomatic von Willebrand disease (VWD), at about 0.1-1% [6].

General Steps to Investigate Potential IPDs

Diagnosis of an IPD requires both clinical and laboratory investigations. Clinical assessment involves a comprehensive review of a patient's medical history, such as bleeding assessments, cardiovascular diseases, hematopoiesis status, malignancy history, neuromuscular diseases, etc. A thorough family history is also important to establish an assumptive diagnosis of IPDs and to facilitate future family clustering studies to confirm the pathogenicity of a genetic variant. To reliably assess bleeding phenotypes, the International Society of Thrombosis and Hemostasis (ISTH) bleeding assessment tools (BAT) can be used to assess bleeding severity [7,8].

Once an IPD is suspected via clinical assessment, laboratory studies are conducted. The standard laboratory tests include complete blood count (CBC) and platelet indices, peripheral blood smear, and platelet function tests such as platelet aggregation and granule release (serotonin or ATP release) [9]. Ancillary tests such as flow cytometry and transmission electron microscopy are helpful in further documenting platelet phenotypic defects. These laboratory results can confirm the clinical suspicions of IPDs and provide laboratory phenotypic data for future genetic data interpretation.

The Benefits of Genetic Testing

With the advent of next-generation sequencing (NGS), genetic testing for IPDs has become a useful tool for confirming clinical and laboratory phenotypes, allowing for more accurate diagnosis and subclassification of IPDs. For example, of the 11 subtypes of gene mutations causing Hermansky-Pudlak syndrome (HPS), only patients with HPS-1, -2, and -4 mutations develop potentially fatal pulmonary fibrosis [10]. Genetic testing can diagnose rare IPDs when the results of phenotypic laboratory tests are inconclusive or unavailable. Many IPDs may have non-specific platelet aggregation testing results, making a definitive diagnosis difficult. In addition, platelet function tests may not be available in some medical institutions [11]. Genetic testing can also assess the individual's prognosis and risk of developing hematologic neoplasms for certain IPDs (e.g., RUNX1 mutation-associated thrombocytopenia) [12,13]. Finally, genetic testing can provide invaluable insight into platelet biology and IPD mechanisms.

The Limitations of Genetic Testing

Despite the benefits of genetic testing, it has several limitations. Genetic testing may have limited yield if the patients and family members have a low pre-test probability of an IPD. Therefore, a thorough clinical and laboratory investigation is critical. Genetic testing may not find mutations if they are not in the region of the genome that is being tested. Or if the mutations are large duplications or deletions or they are in a region of the genome that is highly homologous. Inadequate gene variant curation can lead to the misclassification of variants, which may result in an IPD misdiagnosis. Finally, genetic tests can be expensive and may have reimbursement issues.

The Importance of Patient Consent

Genetic testing holds the potential to unveil hereditary predispositions to malignancy or other significant health insights, necessitating the careful consideration of several ethical principles. These include autonomy, wherein patients retain the right to consent to genetic testing. Confidentiality stands paramount, demanding the safeguarding of genetic information to preserve patient privacy. Non-maleficence underscores the acknowledgment of potential risks associated with genetic testing, such as psychological distress, discrimination, and stigmatization, prompting healthcare providers to mitigate these risks diligently. Additionally, beneficence underscores the potential benefits of genetic testing in facilitating informed healthcare decisions, though its recommendation should hinge upon a careful assessment of the risk-benefit balance. Lastly, justice calls for efforts from healthcare providers and policymakers to ensure equitable access to genetic testing for all eligible patients [14].

Types of Inherited Platelet Disorders

IPD-related genes can be grouped based on different major phenotypes: congenital thrombocytopenia and platelet functional defects. These IPDs have been thoroughly described in the literature. Some recent updates are summarized as follows.

The prevalence of congenital thrombocytopenia is unknown due to its diagnostic challenges. Many young patients are often misdiagnosed with immune thrombocytopenia (ITP) and receive inadequate clinical treatment [15]. Based on a recent high-throughput NGS study of 2396 patients, common genetic alterations of macrothrombocytopaenia are alpha-actinin 1 (*ACTN1*), glycoprotein 1B-alpha/beta (*GP1BA/B*), cytoskeleton proteins b tubulin (*TUBB1*), MYH9 [16] and diaphanous-related formin 1 (*DIAPH1*) [17]. Thrombocytopenias with normal-sized platelets are generally caused by gene mutations that affect hematopoiesis. The World Health Organization (WHO) 5th classification enlisted three autosomal-dominant, inherited thrombocytopenias with associated increased risk of secondary hematologic neoplasms: *RUNX1, ETV6*, and *ANKRD26* [18]. The affected patients usually have a family history of congenital thrombocytopenia and later onset of hematologic neoplasms. A subgroup of normocytic thrombocytopenias is caused by congenital amegakaryocytic thrombocytopenia. Non-syndromic congenital amegakaryocytic thrombocytopenia is a recessive disorder due to an *MPL* mutation. Patients usually have severe thrombocytopenia and absent megakaryocytes in the bone marrow [19]. Amegakaryocytic thrombocytopenia with radioulnar synostosis is caused by autosomal-dominant *HOXA11* mutations [20] or autosomal-recessive mutations in *MECOM* [21]. Besides bleeding diathesis, patients with these gene mutations also have an increased risk of bone marrow failure and/or myelodysplastic syndrome [22]. The most common microcytic thrombocytopenias are Wiskott-Aldrich syndrome, an X-linked disorder characterized by severe immunodeficiency, small platelets, and eczema. Recently, two new autosomal-recessive disorders with small platelets have been described: *FYB* and *ARPC1B* mutation-associated microthrombocytopenias. The former shows increased basal expression of P-selectin and PAC-1 [23], and the latter is associated with chronic inflammation and eosinophilia [24].

Platelet function anomalies may arise from various sources, including receptor anomalies, storage pool deficiencies, and signal transduction pathway defects. Scott syndrome, a rare autosomal recessive disorder, manifests with compromised platelet aggregation, thrombocytopenia, and prolonged bleeding time. This condition stems from mutations in the *ANO6* gene, responsible for encoding phospholipid scramblase, and TMEM16F, which facilitates phosphatidylserine exposure-an essential process for binding coagulation factors to the cell membrane [25]. Bernard-Soulier syndrome (BSS) results from defects in the platelet GPIb-V-IX complex, responsible for facilitating the binding of von Willebrand factor (VWF) to platelets, essential for platelet adhesion to the subendothelium. Diagnosis of BSS typically involves assessing decreased platelet surface GPIb-V-IX levels through flow cytometry. A recent study highlighted that platelet disorders associated with Filamin A (FLNA) mutations exhibit compromised GPIb-V-IX function, leading to laboratory phenotypes similar to BSS [26]. Glanzmann thrombasthenia (GT) is a rare autosomal-recessive disorder characterized by a defect in the platelet-fibrinogen surface receptor, GPIIb/GPIIIa complex. Patients with GT usually have abnormal platelet aggregation responses to all agonists except for ristocetin [27].

Platelets contain dense and alpha granules. The most well-characterized platelet dense granule deficiency is Hermansky-Pudlak syndrome (HPS), an autosomal recessive disorder caused by mutations in 11 genes, with HPS1 being the most common [28]. In addition to platelet defects, HPS patients may have oculocutaneous albinism, nystagmus and decreased visual acuity, granulomatous colitis, pulmonary fibrosis, neutropenia, and immunodeficiency [29]. Platelet alpha granule deficiencies are also known as gray platelet syndromes (GPS) since the abnormal platelets look "gray" due to the lack of alpha granule coloration on a Wright-Giemsa stained peripheral blood smear under light microscopy. The inheritance patterns include autosomal-recessive, autosomal-dominant, and X-linked patterns. The *NBEAL2* gene mutations cause classical GPS (autosomal recessive). [30] *GFI1b* mutations cause the autosomal-dominant GPS [31], while *GATA1* mutations cause the X-linked GPS [32].

Platelet signal transduction defects are caused by mutations of the key signal transduction genes. They include *RASGRP2*, thromboxane A2 synthesis genes (*TBXA2R, TBXAS1, and PTGS1*), and phospholipase A2 (*PLA2G4A*) [33].

Gene Variant Interpretation and the Challenges

In 2015, the American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP) published a guideline providing a consensus approach for sequence variant interpretation [34]. This guideline specified 28 criteria, and each assigned a direction (benign or pathogenic) and level of strength (stand-alone, very strong, strong, moderate, or supporting). Rules for combining these criteria were established to assign a pathogenicity assertion for each sequence variant into one of the following five categories: benign, likely benign, variant of uncertain significance (VUS), likely pathogenic, and pathogenic. However, because of the rarity of genetic studies of IPDs, some of the pathogenic criteria do not apply, such as PS4 (the prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls), PM1 (Located in a mutational hotspot and/or critical and well-established functional domain without benign variation), PP2 (Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease) and PP5 (Reputable source recently reports variant as pathogenic) as recently recommended by ClinGen [35].

Interpretation of VUS is difficult due to insufficient evidence to assign them to the benign or pathogenic category based on limited information. Conflicting data of previously further compound this difficulty reported variants on the gene product and their validity. ACMG recommended 3 criteria (PP1, PS3, and PS4) to characterize VUS variants further [36]. The PP1 criterion is a cosegregation with a disease in multiple family members in a gene definitively known to cause an IPD. The PS3 criterion is to experimentally validate the deleterious effects of a variant on protein function, or expression can be employed to identify its role in the molecular pathogenesis of the disease. However, this method is often limited by the availability of cellular/animal models. Finally, the PS4 criterion requires the prevalence of the affected individuals to be significantly increased compared with the prevalence in controls. Unfortunately, due to the rarity of IPDs, this method is usually not feasible.

Conclusion

Genetic testing for IPDs will become increasingly important for improving diagnosis and providing personalized management and therapy. Continued collaborations in clinical and research communities will be the key to improving gene variant curation and accuracy of diagnosis of IPDs. These advances will enable more accurate diagnosis, personalized management and therapy, and help to improve the quality of life for patients with IPDs.

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None

Conflict of Interest

None

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