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Review of Genetic Diagnostic Approaches for Glanzmann Thrombasthenia in Korea

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Inherited platelet function disorders (IPFDs) are a disease group of heterogeneous bleeding disorders associated with congenital defects of platelet functions. Normal platelets essential role for primary hemostasis by adhesion, activation, secretion of granules, aggregation, and procoagulant activity of platelets. The accurate diagnosis of IPFDs is challenging due to unavailability of important testing methods, including light transmission aggregometry and flow cytometry, in several medical centers in Korea. Among several IPFDs, Glanzmann thrombasthenia (GT) is a most representative IPFD and is relatively frequently found compare to the other types of rarer IPFDs. GT is an autosomal recessive disorder caused by mutations of *ITGA2B* or *ITGB3*. There are quantitative or qualitative defects of the GPIIb/IIIa complex in platelet, which is the binding receptor for fibrinogen, von Willbrand factor, and fibronectin in GT patients. Therefore, patients with GT have normal platelet count and normal platelet morphology, but they have severely decreased platelet aggregation. Thus, GT patients have a very severe hemorrhagic phenotypes that begins at a very early age and persists throughout life. In this article, the general contents about platelet functions and respective IPFDs, the overall contents of GT, and the current status of genetic diagnosis of GT in Korea will be reviewed.

Key words: Blood platelet disorders, Thrombasthenia, Platelet function tests, High-throughput nucleotide sequencing, Whole exome sequencing, Whole genome sequencing

REVIEW ARTICLE

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INTRODUCTION

Platelets are small fragmented blood cells without nucleus, which are originated from megakaryocytes of bone marrow [1]. Normal platelets play most important roles in primary hemostasis in case of vascular damage, and they are also involved in immune responses, inflammatory reactions, and wound healing [1]. The process of primary hemostasis occurs though several steps with many essential molecules [2]. After vascular damage and exposure of subendothelial collagen, circulating platelets of plasma adhere to the collagen of exposed subendothelium [2]. This step 'adhesion' is mediated by the interaction of platelet surface receptors and adhesive proteins; 1) binding of Glycoprotein (GP) Ia/IIa (integrin α2β1) and collagen at low shear rates and 2) binding of GPIb/V/IX complex and von Willebrand factor (vWF) at high shear rates [3]. After adhesion, platelet 'activation' occurs by adenosine diphosphate (ADP) and thromboxane A2 (TXA2) through signal transduction of G-protein coupled receptors, tyrosine kinase, or GPIIb/IIIa (integrin αIIbβ3) [3]. Activated platelets make their shape irregular with pseudopodia and centralization of granules [1]. The α-granules contain vWF, fibrinogen, coagulation factors, mitogenic factors, and angiogenic factors [4]. The dense granules (δ-granules) contain ionized calcium, ADP, ATP, serotonin and epinephrine [4]. The 'secretion' of these platelet granules promotes the recruitment of circulating platelets into the initial plug [5]. And the cross-linking between the receptor GPIIb/IIIa of platelet and ligands (vWF and fibrinogen) results platelet

'aggregation' which make platelets firmly connected [3]. Further, coagulation factors bind to the phosphatidylserine of platelet phospholipid bilayer membrane, and then generate thrombin in secondary hemostasis; 'procoagulant activity' of platelets [1]. Thrombin formation on the platelet surface make more stable hemostatic plug [1].

If there is a congenital problem with platelets causing defects during these various processes, life-long hemostatic disorders occur, and they are called as 'inherited platelet function disorders (IPDSs)'. According to the function of platelets, IPFDs are classified as follows [6,7]; 1) Defect of platelet adhesion (Bernard–Soulier syndrome and pseudo-von Willebrand disease), 2) Defect of platelet activation (ADP receptor $P2Y_{12}$ defect and TXA2 receptor defect), 3) Defect of secretion of granules (Gray platelet syndrome, Paris–Trousseau/Jacobsen syndrome, Chediak–Higashi syndrome, and Hermansky–Pudlak syndrome), 4) Defect of platelet aggregation (Glanzmann thrombasthenia [GT]), 5) Defect of procoagulant activity (Scott syndrome). The function of platelets, involved genes, inheritance pattern, and defects of platelets of respective IPFDs are summarized in Table 1.

Among several IPFDs, GT is a most representative IPFD with phenotypes of severe repetitive bleeding episodes throughout the patient's life. The accurate diagnosis and differential diagnosis of IPFDs is challenging owing to the unavailability of essential testing methods, including light transmission aggregometry and flow cytometry, in several medical centers in Korea. Although each IPFD is very rare, but among them, GT is a relatively frequently found disease. Thus, in this review, I review the current status of genetic diagnosis of GT in Korea along with the overall contents of GT.

GT

GT (OMIM #273800) was firstly presented as 'hereditary hemorrhagic thrombasthenia without reduction of platelet numbers' by Eduard Glanzmann in 1918 [8]. It is an autosomal recessive disorder caused by mutations of *ITGA2B* or *ITGB3* [9]. It is a very rare autosomal recessive IPFD which occurs with a prevalence of 1 in 1,000,000 individuals [10]. However, Glanzmann thrombasthenia often show higher prevalence up to 1 in 200,000 individuals in some populations due to consanguineous marriages [8]. In patients with Glanzmann thrombasthenia, there are quantitative or qualitative defects in the GPIIb/IIIa complex of platelet, which is the binding receptor for von Willbrand factor, fibrinogen, and fibronectin [11,12]. Thus, patients with Glanzmann thrombasthenia have normal platelet count and normal platelet morphology. But they have severely decreased platelet aggregation in response to ADP, epinephrine, serotonin, thrombin, or collagen. Thus, GT patients have life-long, severe hemorrhagic phenotypes [9].

By the large scale study about the clinical manifestations of patients with Glanzmann thrombasthenia, most patients had typical bleeding symptoms during the first year of life [13]. The median age at diagnosis of Glanzmann thrombasthenia was about 7 years old [13]. Severe persistent epistaxis and easy bruising were the most common symptom in patients with Glanzmann thrombasthenia [11,13]. And menorrhagia was severe during the menstruation in the female patients with Glanzmann thrombasthenia [11]. Although intracranial hemorrhage was rare, approximately 1% of the patients with Glanzmann thrombasthenia had hemorrhage of central nervus system [11,13]. In total, over 80% of patients with Glanzmann

Table 1. Classification of IPFDs according to the platelet functions

Disease	Function of platelet	Defect	Genes	Inheritance
Bernard–Soulier syndrome	Adhesion	GPIb/V/IX	*GPIBA, GPIBB, GP9*	AR (rarely AD)
Pseudo-von Willebrand disease	Adhesion	GPIbα	*GPIBA*	AD
ADP receptor $P2Y_{12}$ defect	Activation	ADP receptor	*P2RY12*	AR
TXA2 receptor defect	Activation	TXA2 receptor	*TBXA2R*	AD
Gray platelet syndrome	Secretion	α-granule	*NBEAL2*	AR (rarely AD)
Paris–Trousseau/Jacobsen syndrome	Secretion	α-granule	*FLI1*	AD
Chediak–Higashi syndrome	Secretion	Dense granule	*LYST*	AR
Hermansky–Pudlak syndrome	Secretion	Dense granule	*HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, BLOC1S6*	AR
Glanzmann thrombasthenia	Aggregation	GPIIb/IIIa	*ITGA2B, ITGB3*	AR
Scott syndrome	Procoagulant activity	PS expression	*ANO6*	AR

AR, autosomal recessive; AD, autosomal dominant; IPFDs, inherited platelet function disorders; GP, glycoprotein; ADP, adenosine diphosphate; TXA2, thromboxane A2; PS, phosphatidylserine.

thrombasthenia needed red blood cell transfusion [11].

Because of the platelet GPIIb/IIIa defect, the platelets of Glanzmann thrombasthenia exhibit normal count and normal morphology, but platelet function analyser-100 measurements is significantly abnormal with prolonged closure times in both ADP/collagen and adrenaline/collagen cartridges [14]. In platelet aggregation test by light transmission aggregometry, only platelet agglutination in ristocetin (binding of GPIb/IX and von Willebrand factor) is intact, but the platelet aggregation is severely diminished in response to ADP, epinephrine, and collagen [13,14]. Flow cytometry using antibodies to GPIIb (CD41) and GPIIIa (CD61) is useful for diagnosis of Glanzmann thrombasthenia [14]. The genetic test for 2 genes for Glanzmann thrombasthenia, *ITGB2A* and *ITGB3*, are diagnostic. Glanzmann thrombasthenia typically develops as loss-of-function mutations in *ITGB2A* or *ITGB3* genes, encoding GPIIb or GPIIIa of platelets, respectively [9]. However, very rarely, gain-of-function mutations in *ITGB3* had also been reported, which results enhanced fibrinogen binding, thus severe hemorrhagic phenotype [15].

The mainstay of treatment for the patients with Glanzmann thrombasthenia is platelet transfusion [8]. In patients with IPFDs, platelet transfusion is standard modality to control bleeding episodes and is also helpful for perioperative care for prophylaxis [6]. However, adverse events such as transfusion-transmitted infections, allergic reactions, or development of antibodies to platelet surface proteins or HLA antigens should be considered. Thus, transfusion of leukocyte-depleted platelet from the HLA-matched single donor is the most appropriate method to decrease alloimmunization [16]. And recombinant activated factor VII (rFVIIa, Novoseven™) had been approved for use in patients with GT in case of hemorrhagic episodes or prophylaxis before invasive procedures in 2004 [8]. The rFVIIa is recommended by the European Medicines Agency recommend for the patients with GT who have platelet antibodies or refractory hemorrhages despite of platelet transfusions [8]. The rFVIIa, alone or combination with platelet transfusion and/or anti-fibrinolytic agents, is efficient and safe treatment for all patients with GT [17]. In Korea, the use of rFVIIa at 90 μg/kg/dose every 2 hours was approved for the treatment of bleeding or prophylactic manage before invasive procedure in patients with GT with platelet antibodies or platelet refractoriness. In addition, adjuvant anti-fibrinolytic agents also can be used. Anti-fibrinolytic agents (tranexamic acid or aminocaproic acid) are effective for mucosal bleeding and minor surgical procedures [18]. They also can be used in adjunctive therapy for major bleeding episodes with other modalities [18]. And there have been successful hematopoietic stem cell transplantations in patients with GT [16,18]. In patients with IPFDs associated with severe repetitive hemorrhagic problems or high potential for marrow aplasia or malignant transformation, hematopoietic stem cell transplantation can be considered as a curative treatment [14].

There are some rarer types of IPFDs with similar phenotypes compare to Glanzmann thrombasthenia; Bernard–Soulier syndrome, leukocyte adhesion deficiency type III, or *RASGRP2*-related platelet dysfunction [19]. Light transmission aggregometry and flow cytometry are the first-line screening tests recommended by the International Society of Thrombosis and Haemostasis for differential diagnosis of IPFDs [20]. Light transmission aggregometry is known as the gold standard method for diagnosing IPFDs with high sensitivity and specificity [20]. It requires a large sample volume for diagnosis [20]. Further, it is expensive and time-consuming with poor reproducibility [20]. On the contrary, flow cytometry can be conducted with a small sample volume with high sensitivity and specificity [20]. However, it is also expensive and laborious with high inter-laboratory variability [20].

There is a big problem that these two essential tests are only available for research purposes in a few Korean medical centers due to the nature of the Korean national health insurance system. Thus, it is very difficult to accurately diagnose and identify each type of IPFD cases in Korea. The prevalence Korean IPFDs and the distributions of their genetic abnormalities remain also unknown. Therefore, the Benign Hematology Committee of the Korean Pediatric Hematology Oncology Group (KPHOG) conducted a multicenter study for diagnosing IPFDs by next-generation sequencing (NGS) [21], and the results will be discussed in the next chapter.

KOREAN STUDY FOR GT

Although light transmission aggregometry and flow cytometry are recommended first-line tests by ISTH for differential diagnosis of IPFDs [20], only very few Korean centers can fully conduct these essential tests. In other words, it is very difficult to accurately diagnose the patients with IPFD in Korea. Only anecdotal cases of GT had been genetically confirmed and reported in Korea in the past [7,22]. Therefore, the Benign Hematology Committee of the Korean Pediatric Hematology Oncology Group (KPHOG) conducted the multicenter study for Korean IPFDs using NGS from March 2017 to December 2020

Table 2. Baseline clinical information and genetic variants of Korean patients with Glanzmann thrombasthenia by KPHOG study

Clinical information	N
Male : Female	6 : 4
Age of symptom onset (months, range)	1 (0–48)
Bleeding symptoms	
Easy bruising	7
Gum bleeding	6
Whole body petechiae after birth	5
Persistent epistaxis	3
Delayed wound healing	2
Hematoma after vaccination	1
Bleeding after procedure	1
Melena	1
Anal bleeding	1
Hematemesis	1
Muscle hematoma	1
Genetic variants	
ITGB3	
c.1913+5G>T	9 (45%)
c.1451G>T (p.Gly484Val)	1 (5%)
c.917A>C (p.His306Pro)	1 (5%)
c.1595G>T (p.Cys532Phe)	1 (5%)
ITGA2B	
c.2333A>C (p.Gln778Pro)	3 (15%)
c.2975del (p.Glu992Glyfs*)	1 (5%)
c.257T>C (p.Leu86Pro)	1 (5%)
c.1750C>T (p.Arg584*)	1 (5%)
c.1184G>T (p.Gly395Val)	1 (5%)
c.2390del (p.Gly797Valfs*29)	1 (5%)

KPHOG, Korean Pediatric Hematology Oncology Group.

[21]; this study aimed for differential diagnosis and finding causative genetic variants of Korean IPFDs. Targeted exome sequencing, followed by whole genome sequencing was performed. Clinical manifestations were also collected. As a result, unrelated 10 families of GT were found and the causative genetic variants were identified. The median age of symptom onset was very early (1 month after birth), and the most common hemorrhagic symptoms are easy bruising, gum bleeding and whole body petechiae after birth. These clinical manifestations are described in Table 2.

Identified variants of Korean GT patients by KPHOG study are also shown in Table 2. Among the identified variants, c.1913+5G>T of *ITGB3* was the most common (9/20, 45%) [21]. This variant was found as homozygotes in three unrelated patients, and heterozygotes in other three unrelated patients [21]. Park *et al.* [22] also previously had reported that homozygote of c.1913+5G>T in *ITGB3* was found repeatedly among four unrelated Korean GT patients. The variant c.1913+5G>T in *ITGB3* had also been described as g.29107G>T by Tanaka *et al.* [23]; which causes aberrant splicing, thus resulting in a premature stop codon [23]. Because the variant c.1913+5G>T in *ITGB3* is most commonly found and is also frequently found as a homozygote in KPHOG study [21], it is thought to be the founder mutation of Korean GT [22]. Next, c.2333A>C (p.Gln778Pro) of *ITGB2B* was second common (3/20, 15%) and was identified as heterozygote in three unrelated GT patients. This variant had been reported in both Korean patients [22] and Japanese patients with GT [24]. Considering its relatively high minor allele frequency (0.012% in East Asia by ExAC database), it is suggested as Asian founder mutation of GT [21].

Other known variants of GT from Japanese patients, c.917A>C (p.His306Pro) of *ITGB3* [25,26] and c.257T>C (p.Leu86Pro) of *ITGA2B* [27] were found in this Korean study [21]. And c.1750C>T (p.Arg584*) of *ITGA2B* is found in both Korea [22] and Japan [28]. The variant c.2975del (p.Glu992Glyfs*) of *ITGA2B* had been previously reported in Korean subject [22]. In addition, four novel variants - c.1451G>T (p.Gly484Val) and c.1595G>T (p.Cys532Phe) of *ITGB3* and c.1184G>T (p.Gly395Val) and c.2390del (p.Gly797Valfs*29) of *ITGA2B* - were identified in four unrelate subjects with GT [21]. Based on the results of this study, KPHOG is planning to establish a Korean registry of IPFDs. In addition, KPHOG is seeking ways to expand the application of NGS for accurate diagnosis of IPFDs.

CONCLUSION

Platelets have important functions in primary hemostasis with wound healing and formation of immune barrier [1]. Various genetic variants are associated with platelet function and respective IPFDs are rare and hemorrhagic symptoms are similar [6,7]. Further, the modalities for accurate diagnosis and differential diagnosis of each IPFDs are difficult for access in Korea [21]. Over the past few decades, many researchers worldwide have elucidated the etiology of different types of IPFDs [29,30]. Various IPFD-associated genes have been identified and molecular characterization of these disorders is ongoing using NGS [29,30]. With this information, we can better understand the IPFDs.

The prevalence of genetically confirmed IPFDs in Korea has not yet been reported. This may be because the prevalence of IPFDs in Koreans is very low, and the accessibility to the diagnostic tests is also low. These problems indicate the need to establish a network among the Korean physicians for the accurate diagnosis and comprehensive management of the patients

with IPFDs. A domestic IPFD study by KPHOG showed that the genetic confirmation of IPFDs was possible using NGS. Therefore, the application of NGS for IPFDs is thought to be useful for accurate and differential diagnosis of each IPFDs. Although this study is only the beginning, it is expected to be useful for future large-scale research and establishment of the Korean IPFD registry.

CONFLICTS OF INTEREST

There are no potential conflicts of interest relevant to this article.

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