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Clinical And Molecular Insights into Glanzmann's Thrombasthenia in China

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Abstract

Glanzmann's thrombasthenia (GT) is a rare bleeding disorder characterized by spontaneous mucocutaneous bleeding. The disorder is caused by quantitative or qualitative defects in integrin $\alpha\text{IIb}\beta 3$ (encoded by *ITGA2B* and *ITGB3*) on the platelet and is more common in consanguineous populations. However, the prevalence rate and clinical characteristics of GT in nonconsanguineous populations have been unclear. We analyzed 97 patients from 93 families with GT in the Han population in China. This analysis showed lower consanguinity (18.3%) in Han patients than other ethnic populations in GT-prone countries. Compared with other ethnic populations, there was no significant difference in the distribution of GT types. Han females suffered more severe bleeding and had a poorer prognosis. We identified a total of 43 different *ITGA2B* and *ITGB3* variants,

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Conflict of Interest

All authors declare that they have no conflict of interest.

Authorship:

1. Lu Zhou contributed to study design, data acquisition and interpretation and manuscript preparation.
2. Jiang Miao, Hua Wang, and Zhaoyue Wang contributed to manuscript preparation.
3. Hongjie Shen, Zhenni Ma, Zhanli Xie, Lan Dai and Fei Yang contributed to gene sequencing.
4. Ziqiang Yu, Zhaoyue Wang, Jing Lin, Jie Yin, Lijuan Cao, Jian Su, and Huiping Shi contributed to collecting patients' clinical data.
5. Changgeng Ruan and Lijun Xia contributed to study design, data analysis and interpretation and manuscript preparation.

including 25 previously unidentified, in 45 patients. These variants included 14 missense, four nonsense, four frameshift, and three splicing site variants. Patients with the same genotype generally manifested the same GT type but presented with different bleeding severities. This suggests that GT clinical phenotype does not solely depend on genotype. Our study provides an initial, yet important, clinical and molecular characterization of GT heterogeneity in China.

Graphical Abstract

1. Lower consanguinity in Chinese Han patients than other ethnic groups.
2. Female patients had more severe bleeding and a poorer prognosis.
3. Twenty-five novel mutations including four recurrent ones were identified.

Keywords

Glanzmann's thrombasthenia; α IIB β 3; variant; ethnic; platelet

Introduction

Glanzmann's thrombasthenia (GT) is a rare autosomal recessive genetic bleeding syndrome characterized by defects in platelet aggregometry. Its incidence is approximately one in one million (1). Integrin α IIB β 3, composed of α IIB and β 3 subunits, plays a vital role in platelet aggregation by serving as a specific receptor for fibrinogen. The gene *ITGA2B*, located on chromosome 17q21.31, codes for the platelet GP α IIB, while the gene *ITGB3*, coding for the glycoprotein subunit β 3, is located on chromosome 17q21.32. Pathogenic variants in either subunit can lead to a quantitative or qualitative defect in α IIB β 3 to cause GT. While we have made some progress in understanding GT, many questions remain unanswered.

The clinical phenotype of patients with GT is variable (2, 3). Some suffer from severe bleeding, while others have only mild bleeding. It could present as petechiae, purpura, mild bruising, recurrent epistaxis, gingival bleeding and menorrhagia, gastrointestinal bleeding, intracranial hemorrhage or hematuria. Spontaneous remission with age can also occur in most patients, however some patients' conditions worsen after adulthood (2, 4). Some studies found bleeding severity in GT was not only influenced by the extent of α IIB β 3 deficiency, but also the abundance and functioning of other platelet receptors involved in aggregation and adhesion (5–7). However, the clinical phenotypic heterogeneity of GT remains poorly understood. Currently, based on the expression and functionality of residual subunits, GT is classified into three types: <5% residual α IIB β 3 signifies type I, 5%–20% residual of α IIB β 3 comprises type II and >20% of residual α IIB β 3 constitutes variant-type GT. Due to the clinical phenotypic heterogeneity of GT, a diagnosis of GT is often

overlooked, as a normal platelet count on a routine blood smear does not exclude a diagnosis of GT. In addition to a complete medical history, a GT diagnosis requires a comprehensive laboratory workup, including platelet aggregation analysis, and a confirmation of integrin $\alpha\text{IIb}\beta 3$ deficiency by flow cytometry or western blotting with monoclonal antibodies that recognize either αIIb or $\beta 3$ subunits or the $\alpha\text{IIb}\beta 3$ complex. The best diagnostic method for GT is through genetic testing of the *ITGA2B* and *ITGB3* genes, which allows a definitive diagnosis.

To date, 256 variants in *ITGA2B* and 164 variants in *ITGB3* have been identified (<http://sinaicentral.mssm.edu/intranet/research/glanzmann/search>). However, most were individual case reports (8–14). Large patient cohort studies were concentrated in countries with high intermarriage rates, such as Iraqi Jews (15), Indians (16, 17), Iranians (4, 18) and French (2, 19, 20). To date, scant clinical data and GT prevalence rates have been reported from nonconsanguineous populations. The Han ethnic group in China is one of the largest ethnic groups in the world. They constitute approximately 92% of the population in China. However, there has been no systemic analysis of GT in the Han Chinese. It is also unknown whether clinical phenotypic differences exist among GT patients in the Han Chinese compared to other ethnic populations. We sought to reveal the clinical and genotypic characteristics of GT patients in the Han population, explore the potential correlation between clinical manifestation and genotype, and compare clinical and genetic differences among different ethnic populations. To this end, we analyzed the clinical features of 97 GT patients from the Han population in China. Our study provided an initial yet important investigation of GT in the Han ethnic group.

Materials and Methods

Patients

97 GT patients from 93 families from the Han population in China were enrolled. GT diagnosis was based on clinical manifestations, platelet aggregation analysis and flow cytometry analysis of $\alpha\text{IIb}\beta 3$ expression. Aggregation defects associated with other disorders, such as acute lymphoblastic, myeloblastic leukemia, acquired post-transfusion or post-pregnancy anti- $\alpha\text{IIb}\beta 3$ antibodies that block platelet function, were excluded. Demographic data including the patient's age, gender, consanguinity, family history and clinical history of bleeding were collected. Bleeding degree was evaluated according to the WHO bleeding score: Grade 0, no bleeding; 1, mild petechial or minimal bleeding; 2, occasional significant bleeding requiring transfusion or recombinant FVIIa; 3, repeated episodes of bleeding requiring multiple transfusions or other treatments; and 4, major or life-threatening bleeding and hospitalizations (21). The study was approved by the ethics committee of the Soochow University, Suzhou, China. Informed consent was obtained from individuals in accordance with the institutional ethics guidelines.

Platelet aggregation analysis

Peripheral blood samples were obtained from 97 patients, and platelet-rich plasma (PRP) was prepared. Platelet aggregations were performed in the presence of collagen 1.2 $\mu\text{g/mL}$,

adenosine diphosphate (ADP) 2 μ M and 1.5 mg/ml ristocetin as agonist. Platelet aggregometry was performed using a CHRONO-LOG MODEL 700 aggregometer.

Flow cytometry

Expression of glycoproteins on the surface of washed platelets was performed by flow cytometry with a Cytomics TM FC500 flow cytometer using fluorescein isothiocyanate conjugated anti-CD41, anti-CD61 and anti-CD42 monoclonal antibodies (MoAbs). For PAC-1 binding, washed platelets were preincubated with 0.25 U/ml thrombin for 30 min in 37°C. 25 μ g/ml FITC-labeled PAC-1 was then added.

Sequencing analysis and variants identification

Among 97 GT patients, peripheral blood samples of 45 patients were available for genetic analysis. After obtaining patient consent, genomic DNA was prepared from peripheral blood leukocytes isolated from whole blood of 45 GT patients. All exons from *ITGA2B* and *ITGB3* genes were amplified by PCR using intronic primers. DNA sequencing was determined by Sanger sequencing. Sequencing results were aligned with the sequence for *ITGA2B* (NCBI: NG_008331.1) and *ITGB3* (NCBI: NG_008332.2) using the Basic Local Alignment Search Tool (BLAST) software through Pubmed. The pathogenicity of the variants was defined based on the standards and guidelines of ACMG (22). Missense variants were predicted by searching the Pathogenic Variant Assessor (<http://pathogenicvariantassessor.org/r3/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) and Sift (<http://sift.bii.a-star.edu.sg/>). Novel pathogenic variants were further confirmed by a literature survey and consulting the Glanzmann Thrombasthenia Database (<http://sinaicentral.mssm.edu/intranet/research/glanzmann/menu>), Ensembl Database (<http://asia.ensembl.org/index.html>), EXAC Database (<http://exac.broadinstitute.org/terms>) and the Human Gene Pathogenic Variant Database (<http://www.hgmd.cf.ac.uk/ac/index.php>). Variant nomenclature followed the role of Human Genome Variation Society (HGVS).

Results

Clinical features

We examined the clinical features of 97 GT patients from 93 families, including 53 females and 44 males (Supplementary table 1). Patient ages ranged from 1 to 53 years old. Most patients suffered from mucocutaneous petechia (77.3%), epistaxis (69.1%), gingival bleeding (36.1%), post traumatic hemorrhage (19.6%) and gastrointestinal bleeding (12.4%). They also experienced rare GT manifestations, such as hematuria, intracranial hemorrhage and hemarthrosis (5.2%). 90.6% of females had menorrhagia after menarche. 12.5% of females had a ruptured corpus luteum, and 6.25% suffered from infertility (Table 1).

The average bleeding score in female and male patients was 2.96 and 2.61, respectively. Female patients had higher bleeding scores, and a more significant clinical presentation than male patients. No male patient died from GT. However, one female patient died of intracranial hemorrhage, and six female relatives of the probands died of hemorrhage. Symptoms lessened with age in 25.2% (26/97+6) of patients, including 31.8% (14/44) of

males and 20.3% (12/53+6) of females. These results suggest that females suffer more severe bleeding and have a poorer prognosis compared to males (Figure 1).

Comparison of clinical manifestations among different ethnic populations

Given these gender differences, we also compared the clinical manifestations of GT among different ethnic populations. We found that consanguinity based on 3rd and 4th degree inbreeding was present in 17 families. The consanguinity rate was 18.3% (17/93), much lower than Indian 73.3% and 63% (19, 22), Iranian 93.7% and 86.6% (4, 18) and French 39% (2). These patients suffered from recurrent bleeding, 72.1% (70/97) with a bleeding score 3. However, other studies did not show such high bleeding scores (Table 2). We suggest this discrepancy occurred because we evaluated the most severe bleeding status in a patient's disease history. Unfortunately, we did find higher cases of lethality than other studies. 74.2% (72/97) of patients presented with the classical GT type (type I), characterized by an absence or extremely reduced levels (<5%) of α IIB β 3. 23.7% (23/97) of patients were classified as type II, since their α IIB β 3 levels varied between 10 and 20%. 2.1% (2/97) of patients were diagnosed as type III (variant type), because they exhibited a normal or near normal amount of α IIB β 3, which corresponded to approximately 50–100% of control levels (Table 2). All ethnic groups had no significant differences in the distribution of GT type. Type I and type II are the most common, and type III is the rarest.

Molecular genetic analysis

Among the 45 GT patients with genomic DNA samples, sequencing results were compared with the sequence for *ITGA2B* (NCBI: NG_008331.1) and *ITGB3* (NCBI: NG_008332.2) using the Basic Local Alignment Search Tool (BLAST) software through Pubmed. We detected 43 different variants (*ITGA2B*: n = 28, *ITGB3*: n = 15), which reveals the genetic diversity of GT in the Han population from China (Supplementary table 2). These variants included 51% (22/43) missense, 23% (10/43) nonsense, 12% (5/43) splice site, 12% (5/43) frameshift, and 2% (1/43) deletion (Figure 2). Fifteen patients were homozygotes, twenty-one patients were compound heterozygotes, while seven patients were identified only one heterozygous variant and two patients harbored no pathogenic variant on *ITGA2B* or *ITGB3* (Figure 2). These results stand in consensus with other reports that did not identify pathogenic variants in some patients (Table 2) (16, 17, 23). Here, our 25 novel pathogenic variants included 14 missense (*ITGA2B*: n = 9, *ITGB3*: n = 5), 4 nonsense (*ITGA2B*: n = 2, *ITGB3*: n = 2), 3 splicing (*ITGA2B*), and 4 frameshift (*ITGA2B*: n = 1, *ITGB3*: n = 3). Among them, 11 were “pathogenic variant”, and 14 were “likely pathogenic variants” (Supplementary table 2). We uncovered 9 amino acid substitutions in α IIB located in the β -propeller domain (c.812C>G (p.A271G), c.659A>G (p.Y220C), c.1022C>T (p.A341V), c.962G>T (p.G321V)) and the calf domain (c.2330C>A (p.A777D), c.2159T>C (p.L720P), c.2570G>T (p.C857F), c.2029G>A (p.E677K), c.2172G>A (p.M724I)), whereas the 5 β 3 missense pathogenic variants occurred in the β I domain (c.412G>A (p.V138M), c.842A>C (p.H281P)), IEGF domain (c.1495T>C (p.C499R), c.1646G>C (p.C549S)) and the β tail domain (c.2147G>T (p.G716V)) (Figure 3).

Four recurrent mutations suggest a possible founder effect

Notably, we identified c.1750C>T (p.R584X), c.2671C>T (p.Q891X), c.2333A > C (p.Q778P) in *ITGA2B* and c.1199G>A (p.C400Y) in *ITGB3* in 7, 4, 7, and 2 unrelated families, respectively. We first reported p.R584X in two type I GT siblings in 1993 (10). They were both heterozygous. Indeed, we found more patients harboring this mutation here. This p.R584X mutation involves a CpG hotspot, which may alter its reproducibility. p.Q891X is another mutation previously identified in Chinese type I GT (24). Functional studies show that impaired transport of the pro- α IIb β 3 complex from the endoplasmic reticulum to the Golgi underlies thrombasthenia (24). p.C400Y was previously identified in the Chinese population (25). Transient transfection in CHO cells showed that this mutation causes an 85% to 90% reduction in GPIIb/IIIa surface expression, even though these cells retain the ability to adhere to immobilized fibrinogen. These results suggest that these variants are pathogenic and reveal hotspot mutations in China, which may reflect a founder effect. Further, p.Q778P has been reported in seven Japanese patients (26–28) and two Korean patients (29), suggesting that p.Q778P may be another hot spot pathogenic variant in the Asian population.

Clinical phenotypic variation in a compound heterozygous patient

Twenty-one compound heterozygotes were identified in this study. Among them, GT39 was unique. He was 25 years old and the only son of unrelated Chinese parents. He never manifested any abnormal bleeding episode until he had a head injury at the age of 25. CT scan showed extradural hemorrhage. The number and size of platelets were within the normal range. Aggregation analysis showed no reactivity to ADP (2 μ M). Gene analysis revealed a compound heterozygous variant c.2570G>T (p.C857F) and c.1769G>A (p.R590Q) in *ITGA2B*. Surface expression of CD41 and CD61 on the patient's platelets were 65.1% and 77.5%, respectively. These results raised the possibility of an additional qualitative abnormality. So, we analyzed expression of PAC-1 binding by flow cytometry in resting state and with stimulation by thrombin (0.25 U/ml). We found that expression of PAC-1 binding accounted for 43.3% (MFI: 2.67/6.17) of the patient's platelets compared to controls at resting state. When stimulated by thrombin, the patient's platelets responded poorly compared to controls. Our results demonstrated a functional defect in this patient's platelets, which further verified that this patient has a variant form of GT (Figure 4).

Discussion

By comparing clinical differences between the Han population and other ethnic groups, we revealed key distinguishing characteristics. First, consanguinity contributes to a much lower frequency in GT patients from the Han population than other ethnic populations, as most GT patients in the Han population were compound heterozygotes (46.7%). We found seven patients (GT2, GT3, GT12, GT22, GT27, GT34, GT36) who has only one heterozygous variant in *ITGA2B* or *ITGB3* gene. It is possible that the other allele may be deleted or the second pathogenic variant may be located in other gene, which we are not able to find due to the limitation of our current detection method. This heterozygous genetic pattern prevents elimination of these pathogenic variants, suggesting asymptomatic, heterozygous subjects compose a relatively high frequency in the Han population. This result underscores the

necessity for gene screening. These results can update the pathogenic variant database to improve early diagnosis of acataleptia patients. In addition, it can facilitate prenatal diagnosis and carrier detection, which is important to control the disorder among family members. Second, the mortality rate in China is relatively high. We suggest the mortality rate is related to the low consanguinity rate, since doctors may ignore the possibility of a recessive genetic disorder in a non-consanguineous family. Proper care and appropriate treatment in emergencies can largely reduce the mortality risk of GT. Therefore, it is vital to improve our understanding of this rare disease to improve the early diagnosis rate for disease management.

The reasons for the heterogeneity of bleeding symptoms in GT have been unclear. Evaluating the phenotype of patients with the same genotype will elucidate the relationship between genotype and phenotype in GT. GT4, GT17b, and GT18 were all Q891X homozygotes and type I GT, but the severity of the bleeding varied significantly. This suggests that genotype is not the only determinant of bleeding heterogeneity and further suggests that heterogeneity is a multifactorial symptom. However, genotype may regulate α IIb β 3 expression, since patients with the same genotype generally had the same GT type (Table 3). We also examined whether different domains correlate with bleeding heterogeneity, however we did not find a significant correlation (data not shown).

Investigating GT variants could elucidate integrin function, providing insight into disease phenotypes and stimulating drug development with α IIb β 3 inhibitors for novel treatment strategies (30). GT39 had only one bleeding episode in the patient's lifespan and harbored a compound heterozygous pathogenic GT variants as c.1769G>A (p.R590Q) and c.2570G>T (p.C857F) located on thigh and calf-2 domain of *ITGA2B*, respectively. C857 is a highly conservative and buried residue. It forms a disulfide bond with C911 (31) (Figure 4). F857 breaks the disulfide bond to influence integrin stability or conformation, which affects ligand binding. R590 is a highly conservative and exposed residue (Figure 4). Q590 changed the polarity of amino acids in the site, such that the positively charged arginine was converted into an uncharged glutamine. This change could affect α IIb β 3 conformation. So far, several pathogenic variants have been identified that underlie the loss-of-function variant forms (32, 33). Proportionally, the highest concern is *ITGB3*, and lowest concern is *ITGA2B* (32, 33). Moreover, most are located on the headpiece of the α IIb β 3 complex (32, 33). Only a few defects on other parts have been identified, specifically β 3 H626L, β 3 S752P and α IIb V951M. This case implied an interesting structure–function relationship between the different α IIb β 3 domains and highlighted the importance of α IIb for proper function α IIb β 3, especially other domains except β -propeller. We acknowledge this case was unique compared to the majority of GT cases. He did not have any abnormal bleeding before extradural hemorrhage, although he had bumps, small traumas or needle injections prior to that event. It is possible that other factors affect platelet aggregation besides *ITGA2B* and *ITGB3* genes.

Conclusion

In summary, we investigated the clinical and genetic characteristics of 97 GT patients from 93 families in China. To our knowledge, this is the first large cohort study of GT patients

from the Han population in China. Our results reveal critical insights into GT heterogeneity and demonstrate key differences between phenotypes and genotypes from other consanguineous populations in the world.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Gender	Number	Bleeding score	Death (%)	Relief with age (%)
Male	44	2.61	0	31.8% (14/44)
Female	53(+6)	2.96	11.9% (7/53+6)	20.3% (12/53+6)
Total	97(+6)	2.8	6.8% (7/97+6)	25.2% (26/97+6)

Figure 1.

Han females suffered more severe bleeding and had a poorer prognosis. (A) Comparison of male and female patient demographics: patient number, average bleeding score, the percentage of lethal cases, and the percentage of patients experiencing relief with age. Six female relatives of proband (GT12, GT17a, GT18, GT27, GT43) died of hemorrhage. (B) Column diagram shows percentage of female patients with high bleeding scores greater than male patients. (high: bleeding score ≥ 3 , low: bleeding score < 3)

Figure 2.

Most GT patients in the Han population were compound heterozygous. (A) Circle diagram shows the ratio of all identified mutations in *ITGA2B* and *ITGB3*, including the percentage of missense and nonsense mutations, deletions, frameshift, and splice site mutations. (B) Column diagram shows the number of clinically diagnosed GT patients who harbor different types of *ITGA2B* or *ITGB3* mutations.

Figure 3.

Forty-three different pathogenic variants were identified in 45 GT patients, of which 25 were new and 4 were recurrent pathogenic variants, showing genetic diversity in the Han population. (A) Schematic shows the *ITGA2B* DNA and αIIb domain structure. Variants are designated as a symbol according to their type and grouped to the nearest exon. Solid circle: nonsense variant. Curve: frameshift variant. Solid triangle: deletion variant. Hollow triangle: missense variant. Hollow diamond: splicing variant. Red: recurrent variant. Blue: novel variant. SP: signal peptide. TM: transmembrane domain. CD: cytoplasmic domain. (B) Schematic shows the *ITGB3* DNA and β3 domain structure. Variants are designated as a symbol according to their type and grouped to the nearest exon. Solid circle: nonsense variant. Curve: frameshift variant. Hollow triangle: missense variant. Hollow diamond: splicing variant. Red: recurrent variant. Blue: novel variant. SP: signal peptide. TM: transmembrane domain. CD: cytoplasmic domain.

Figure 4.

Characterization of a unique patient (GT39) with C857F and R590Q variants in *ITGA2B*.

(A) Diagram depicting the C857-C911 disulfide bond within the integrin α IIb β 3 complex.

GPIIb (136.5 kDa) is formed by two subunits, α (GPIIb α , 114 kDa), and β (GPIIb β , 22.5 kDa), joined by a single disulfide bridge between GPIIb β Cys-911 and GPIIb α Cys-857. (B)

Platelet aggregation analysis of GT39 by stimulation with ADP relative to a healthy

individual (control). (C) Glycoprotein expression levels on platelet surfaces analyzed by

flow cytometry. Shadow: control. Black line: GT39. Numbers are mean fluorescence

intensity. (D) PAC-1 binding analysis on platelet surfaces with or without stimulation with

thrombin by flow cytometry. Shadow: control. Black line: GT39. Numbers are mean fluorescence intensity. (E) α IIb R590 and α IIb C857 are highly conserved among species.

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Table 1

Clinical manifestations of GT patients in Han population

Bleeding symptoms	Female% (n = 53)	Male (n = 44)	Total (n = 97)
Mucocutaneous petechia	79.2% (42/53)	75% (33/44)	77.3% (75/97)
Epistaxis	67.9% (36/53)	70.5% (31/44)	69.1% (67/97)
Gingival bleeding	43.4% (23/53)	22.7% (12/44)	36.1% (35/97)
Gastrointestinal bleeding	9.4% (5/53)	15.9% (7/44)	12.4% (12/97)
Post traumatic hemorrhage	11.3% (6/53)	29.5% (13/44)	19.6% (19/97)
Intracranial hemorrhage	1.9% (1/53)	2.3% (1/44)	2.1% (2/97)
Arthrorrhagia	1.9% (1/53)	0	1% (1/97)
Hematuria	3.8% (2/53)	0	2.1% (2/97)
Symptoms	Female after menarch (n = 32)		
Menorrhagia	90.6% (29/32)		
Rupture of corpus luteum	12.5% (4/32)		
Infertility	6.25% (2/32)		

GT, Glanzmann's thrombasthenia; n, patient number.

Table 2

Comparison of GT patients among Chinese Han with other ethnic populations

Ethnic	N	Consanguinity rate	GT type	Death	No mutation identified	Severe bleeding	Ref
Han	97(+6)	18.3%	type I 74.2%;	7	4.4% (2/45)	72.1%	This study
			type II 23.7%;				
			type III 2.1%				
Indian	15	73.3%	type I 33.3%;	NA	26.7%	33.3%	(23)
			type II 60%;				
			type III 6.7%				
	40	63%	NA	NA	12.5%	NA	(17)
			type I 62.2%;				
			type II 15.6%;				
	45	NA	type III 22.2%	0	20%	33.3%	(16)
			type I 77.9%;				
			type II 13.7%;				
Iranian	95	93.7%	type III 8.4%	0	NA	50.5%	(4)
			NA				
			type I 78.2%;				
French	64	39%	type II 14%;	2	NA	54%	(2)
			type III 7.8%				

GT, Glanzmann's thrombasthenia; NA, not available; N, number of patients; Ref, reference.

Table 3

Recurrent pathogenic variants were identified in some patients

Recurrent variants	Gene	Patient	Hom/Het	GT type	Bleeding score	PAGT		
						ADP	Risto	Collagen
R584X	ITGA2B	GT2	Het	I	2	Absent	Normal	Absent
		GT3	Het	I	3	Absent	Normal	Absent
		GT5	Het	I	4	Absent	Normal	Absent
		GT22	Het	I	3	Absent	Normal	Absent
		GT24	Het	II	3	Absent	Normal	Absent
		GT29	Hom	II	3	Absent	Normal	Absent
		GT40	Het	I	3	Absent	Normal	Absent
		GT4	Hom	I	4	Absent	Normal	Absent
		GT17a	Het	I	3	Small amount	Normal	Absent
		GT17b	Hom	I	3	Absent	Normal	Absent
Q891X	ITGA2B	GT18	Hom	I	2	Absent	Normal	Absent
		GT26	Het	I	2	Absent	Normal	Absent
		GT9	Hom	I	3	Absent	Normal	Absent
		GT12	Het	II	3	Absent	Normal	Absent
		GT14	Hom	I	3	Absent	Normal	Absent
		GT16	Het	I	2	Absent	Normal	Absent
		GT24	Het	II	3	Absent	Normal	Absent
		GT26	Het	I	2	Absent	Normal	Absent
		GT27	Het	I	2	Absent	Normal	Absent
		GT11	Het	I	4	Absent	Normal	Absent
C400Y	ITGB3	GT15a	Het	II	2	Absent	Normal	Small amount
		GT15b	Het	II	2	Small amount	Normal	Absent

Note: GT17a and GT17b are second cousins. GT15a and GT15b are full siblings. PAGT, platelet aggregation test; Hom, homozygous; Het, heterozygous.