Glanzmann's thrombasthenia: modulation of clinical phenotype by α2C807T gene polymorphism

GIOVANNA D'ANDREA, MAURIZIO MARGAGLIONE ON BEHALF OF THE GLANZMANN'S THROMBASTHENIA ITALIAN TEAM (GLATIT)

Background and Objectives. The expression of Glanzmann's thromboasthenia (GT) varies, even among patients carrying the same mutation. It is conceivable that other gene loci may influence the clinical expression of GT and lead to specific phenotypes.

Design and Methods. To investigate GT clinical heterogeneity we screened 25 GT patients with a known $\alpha_{IIb}\beta_3$ molecular defect for thrombophilic mutations (FV Leiden, FII A20210) and the platelet glycoprotein (GP) α_2 C807T gene polymorphism.

Results. The FV Leiden mutation was found in 1 patient, the FII A20210 mutation in none. Three GT patients were homozygous for the T807 allele and showed a mild clinical expression of GT whereas none of the patients presenting with a moderate or severe GT phenotype carried the α_2 TT genotype (p=0.037, two-sided exact test). In patients carrying the same mutation, the clinical GT phenotype was milder in those with the TT807 genotype.

Interpretation and Conclusions. Since the platelet α_2 C807T gene polymorphism is associated with $\alpha_2\beta_1$ receptor density on the platelet surface, our findings suggest that the level of $\alpha_2\beta_1$ on platelets may be an additional factor affecting GT clinical expression.

Key words: Glanzmann's thrombasthenia, polymorphisms, genes, clinical heterogeneity, integrins.

Haematologica 2003; 88:1378-1382
http://www.haematologica.org/2003_12/1378.htm

From the Unità di Aterosclerosi e Trombosi, I.R.C.C.S. "Casa Sollievo della Sofferenza", S. Giovanni Rotondo (MM); Genetica Medica, Università di Foggia, Italy (Gd'A).

Correspondence: Maurizio Margaglione, MD, Unità di Aterosclerosi e Trombosi IRCCS "Casa Sollievo della Sofferenza", viale Cappuccini, San Giovanni Rotondo, Foggia. E-mail: ate.tro@operapadrepio.it

Glanzmann's thromboasthenia (GT) is an autosomal recessively inherited bleeding diathesis marked by prolonged bleeding time, normal platelet count, and absence of platelet aggregation in response to platelet agonists such as ADP, collagen, arachidonic acid, and thrombin.[1] Quantitative or qualitative abnormalities of the platelet $\alpha_{IIb}\beta_3$ integrin (also known as the glycoprotein complex IIb-IIIa) have been shown to be responsible for this disorder. Mutations within the genes that code for $\alpha_{IIb}\beta_3$ subunits have been described in GT patients. Like other integrins, α_{IIb} and β_3 subunits are prominent integral components of the platelet membrane and form heterodimers containing specific sites for platelet-to platelet cohesion.[2] The $\alpha_{IIb}\beta_3$ integrin serves as a platelet receptor for fibrinogen, fibronectin, vitronectin, and von Willebrand factor.[3] In addition, the $\alpha_{IIb}\beta_3$ integrin modulates, to some extent, calcium influx, cytoplasmic alkalinization, tyrosine kinase phosphorylation, and clot retraction.[4] The heterogeneity of GT, on the basis of platelet function testing[5] or using crossed immunoelectrophoresis, Western blotting, flow cytometry and fibrinogen binding, has been stressed.[6] However, it is not known whether these classifications correspond to the molecular defects assigned to different patients.

It has been demonstrated that the clinical phenotype of various diseases inherited in a classic Mendelian fashion can be modulated by a series of factors, inherited as well as acquired.[7] Platelets play a pivotal role in procoagulant activity and thrombus formation. There is increasing evidence suggesting that platelet glycoprotein polymorphisms may have a role as genetic risk factors for arterial thrombosis.[8] Although in healthy individuals the role of these variants may be negligible, they can become important in subjects who are prone to bleeding, such as GT patients, in modulating the clinical phenotype.

We have recently identified a large spectrum of mutations within the α_{IIb} and β_3 genes in 30 patients with GT. The wide spectrum of clinical disease expression, ranging from mild to severe even among patients carrying the same mutation, provided evidence for a role of different loci or circumstantial factors in the modulation of the clinical phenotype.[9] We, therefore, evaluated inherited prothrombotic risk factors (FV Leiden, FII A20210) and the platelet glycoprotein (GP) α_2 C807T gene polymorphism in GT patients with a clearly characterized molecular defect in order to assess whether these gene variants play a major role in modulating the clinical expression of the GT.

Table 1. Clinical and genetic characteristics of GT patients.

Patient (sex)	*Bleeding severity*		*GP α_{IIb}/β_3 gene mutation*	*GP α_2 C807T*	*FV G1691A*	*FII G20210A*
PF (M)	moderate	α_{IIb}	Homozygous A108V	CC	GG	GG
LF (F)	moderate	α_{IIb}	Homozygous premature stop	CT	GG	GG
DRP (M)	mild	α_{IIb}	G236E/premature stop	CC	GG	GG
FF (M)	moderate	α_{IIb}	Premature stop/?	CT	GG	GG
MR (M)	moderate	α_{IIb}	P145A/I374T	CT	GG	GG
GM (F)	mild	α_{IIb}	G349D/L721V-R724P	CT	GG	GG
PL (M)	moderate	α_{IIb}	A550D/?	CT	GG	GG
MR (M)	moderate	β_3	Homozygous premature stop	CT	AG	GG
FN (M)	mild	α_{IIb}	Homozygous alternative splicing	CC	GG	GG
BM (M)	mild	α_{IIb}	C674L/?	TT	GG	GG
GI (F)	mild	β_3	Homozygous D217V	CT	GG	GG
SM (F)	mild	β_3	R93W/C575R	TT	GG	GG
LPR (F)	moderate	β_3	R93W/C575R	CC	GG	GG
DOM (M)	mild	β_3	C575R/?	CC	GG	GG
CG(F)°	severe	β_3	Homozygous C575R	CT	GG	GG
CI(F)*°	moderate	β_3	Homozygous C575R	CT	GG	GG
CD(M)*°	moderate	β_3	Homozygous C575R	CT	GG	GG
VA(F)	moderate	α_{IIb}	Homozygous premature stop	CC	GG	GG
ME(F)	severe	α_{IIb}	Alternative splicing/R946X	CC	GG	GG
LRI (F)	moderate	α_{IIb}	Homozygous C674L	CT	GG	GG
CT (F)	mild	α_{IIb}	Homozygous R335X	TT	GG	GG
ER (M)	moderate	α_{IIb}	Premature stop/?	CT	GG	GG
CE(F)	moderate	α_{IIb}	Homozygous C674L	CT	GG	GG
ZM(M)	mild	α_{IIb}	Homozygous premature stop	CT	GG	GG
FV (F)	severe	α_{IIb}	G349D/L721V-R724P	CC	GG	GG

*M: male; F: female; *twin; °sibs. ?: mutation not identified.*

Design and Methods

Patients

The characteristics of the GT patients analyzed here have been previously reported.[9] The gene mutation could not be identified in 5 of 30 patients and these 5 patients are not included in the present study. Thus, 25 GT patients were analyzed: their clinical and genetic characteristics are summarized in Table 1. Bleeding symptoms were evaluated by examining available hospital records. Mild bleeders were defined as those who bled only after trauma or surgery or had minor symptoms, such as epistaxis. Moderate bleeders were defined as those with a history of spontaneous or life-threatening hemorrhages, such as gastrointestinal bleeding, and severe bleeders as those who had repeated episodes requiring platelet transfusions. Informed

consent to the study was obtained from patients after the local Human Ethics Committees had approved the protocol. The studies were carried out according to the Principles of the Declaration of Helsinki.

Genotyping

Polymerase chain reaction (PCR) analyses were carried out using standard procedures.[9] The G1691A polymorphism in the FV gene and the G20210A variant in the prothrombin gene were screened for as previously reported.[10] The platelet α_2 C807T gene polymorphism was investigated as described by Di Paola *et al.*[11]

Flow cytometric analysis of CD41 expression on platelets

Data obtained from GT patients using monoclonal antibodies against CD42a and Cd42b, as previously reported,[9] were adjusted using the algorithm proposed by Sharp *et al.*[12] and then expressed as percent of the mean fluorescence intensity recorded in a control subject investigated at the same time.

Statistical analysis

All the analyses were performed using the Statistical Package for Social Sciences (SPSS 10.0 for PC).

Results

The FV A1691 (FV Leiden) gene mutation was found in only one GT patient, a moderate bleeder, with a homozygous single nucleotide deletion (224Gdel) within exon 3 of the β_3 integrin subunit. None of the GT patients carried the FII A20210 gene mutation. Thrombophilic gene mutations have been suggested to affect the clinical expression of hemorrhagic diseases such as hemophilia A. In the present setting, the FV Leiden and the FII A20210 gene mutation do not appear to play a major role in modulating the clinical phenotype of GT patients. A larger group of GT patients, including a sufficient number of carriers of thrombophilic mutations, are needed to address this issue adequately.

Among the 25 GT patients analyzed, 8 (32.0%) were homozygous for the C allele of the platelet glycoprotein α_2 C807T gene polymorphism, 3 (12.0%) were homozygous for the T allele, whereas the remaining 14 (56.0%) were heterozygotes. Allele frequencies observed were not different from those predicted by the Hardy-Weinberg equilibrium. Moreover, the frequencies in this series of patients were similar to those reported in different groups of non-hemorrhagic individuals.[11,13]

The platelet α_2 C807T gene polymorphism is associated with statistically different expressions of the $\alpha_2\beta_1$ receptor on the platelet surface: subjects with the TT genotype show a high $\alpha_2\beta_1$ density.[11] The $\alpha_2\beta_1$ receptor is the primary receptor that mediates platelet adhesion to collagen and the lack of the α_2 glycoprotein impairs platelet response to collagen.[14] Both homozygous and heterozygous carriers of the C807 allele showed a variable clinical GT phenotype, ranging from mild to severe forms (Table 1). On the other hand, all 3 GT patients who were homozygous for the T807 allele showed a mild clinical expression of the GT disease (Table 2). Taken together, none of the patients with a moderate or severe GT phenotype carried the α_2 TT genotype whereas 3 out of 9 patients with the mild phenotype had the TT genotype (p=0.037, two-tailed exact test). Of the patients carrying the α_2 TT genotype, 2 (BM, SM), had no platelet surface $\alpha_{IIb}\beta_3$ receptor, as detectable by flow cytometry.[9] Although flow cytometry data were not available in the third patient (CT), the homozygous stop mutation identified (R335X) apparently predicts the complete absence of the platelet receptor on the membrane surface. These findings support the hypothesis that carriership of the TT genotype may moderate the bleeding tendency of GT patients. When the clinical GT phenotype in subjects carrying the same mutation was analyzed (Table 3), it was found that of the 2 subjects with compound heterozygosity β_3 R93W/C575R, the one (SM) who carried the TT807 genotype showed a mild clinical GT phenotype while the other patient (LPR), who had the CC807 genotype, suffered from a moderate form of GT. Three GT patients carried the α_{IIb} C674L gene mutation: only the subject (BM) with the TT807 genotype had a mild GT clinical phenotype. Moreover, in patients with null alleles the only subject carrying the TT807 genotype showed a mild clinical expression of GT whereas the only one with a severe form carried the CC807 genotype. Finally, in GT patients carrying the TT807 genotype, levels of $\alpha_2\beta_2$ on platelets were compared to those found in patients carrying the CC807 genotype, using flow cytometric data obtained employing different

Table 2. Distribution of α_2C807T genotypes according to Glanzmann's thrombastenia phenotype.

		Glanzmann's thrombasthenia phenotype		
		mild	*moderate*	*severe*
α_2 C807T	CC	3	3	2
Polymorphism	CT	3	10	1
Genotypes	TT	3		

p=0.064 (two-tailed exact test).

Table 3. Clinical and genetic characteristics of patients carrying the same GT mutation or null alleles.

Patient (sex)	*Bleeding severity*	*GP α_{IIb}/β_3 gene mutation*	*GPα_2 C807T*	*FV G1691A*	*FII G20210A*
SM (F)	mild	R93W/ C575R	TT	GG	GG
LPR (F)	moderate	R93W/ C575R	CC	GG	GG
LRI (F)	moderate	Homozygous C674L	CT	GG	GG
CE(F)	moderate	Homozygous C674L	CT	GG	GG
BM (M)	mild	C674L/?	TT	GG	GG
Null alleles					
LF (F)	moderate	Homozygous premature stop	CT	GG	GG
DRP (M)	mild	G236E/ premature stop	CC	GG	GG
FF (M)	moderate	Premature stop/?	CT	GG	GG
MR (M)	moderate	Homozygous premature stop	CT	AG	GG
FN (M)	mild	Homozygous alternative splicing	CC	GG	GG
VA(F)	moderate	Homozygous premature stop	CC	GG	GG
ME(F)	severe	Alternative splicing/R946X	CC	GG	GG
CT (F)	mild	Homozygous R335X	TT	GG	GG
ER (M)	moderate	Premature stop/?	CT	GG	GG
ZM(M)	mild	Homozygous premature stop	CT	GG	GG

?: mutation not identified.

monoclonal antibodies. Flow cytometric data were available in 5 GT patients with the CC807 genotype and in 2 GT patients carrying the TT807 genotype. Corrected mean fluorescence intensity in the two GT patients with the TT807 genotype (CD42a: 105.3+14.5%; CD42b: 102.7+1.1%) was higher than that observed in GT patients with the CC807 genotype (CD42a: 88.5+8.4%, Mann-Whitney U-test p = n.s.; CD42b: 96.0+2.1%, Mann-Whitney U-test p = 0.026).

Discussion

Various improvements in the molecular characterization, diagnosis, and treatment of GT have been made in the last years. However, it is unclear why differences in severity of bleeding develop in GT patients with similar levels of the $\alpha_{IIb}\beta_3$ receptor on their platelet surface or with the same mutation. An association has been found between the α_2 C807T polymorphism and bleeding risk in patients with type I von Willebrand's disease, suggesting that this polymorphism may account for the phenotypic variability in the presence of similar levels of von Willebrand factor antigen.[11]

To the best of our knowledge, this is the first report that has investigated the molecular mechanisms outside the α_{IIb} and the β_3 genes leading to different clinical expressions of GT. Present data suggest an association between the platelet α_2 C807T gene polymorphism and the clinical phenotype of GT. Since the platelet α_2 C807T gene polymorphism is associated with $\alpha_2\beta_1$ receptor density on the platelet surface,[13] our findings suggest that the level of $\alpha_2\beta_1$ on platelets may be one of the additional factors that affects severity of bleeding in this particular disease. Employing monoclonal antibodies against CD42a and CD42b, it was found that GT patients carrying the TT807 genotype showed higher corrected mean fluorescence intensity than patients with the CC807 genotype. These findings further support the hypothesis that platelet α_2 C807T gene polymorphisms may play a role in the clinical expression of GT. Although the present study is on a small-scale, given the low prevalence of GT (about 1/1,000,000) our results suggest a mitigating effect of the TT807 genotype on the clinical severity of GT. In fact, we found that co-inheritance of different α_2 C807T genotypes in subjects sharing the same molecular defect is associated with different clinical GT phenotypes. The difficulty in establishing a phenotype-genotype correlation in various diseases with Mendelian inheritance indicates that, although the monogenic model is useful for identifying the primary genetic cause of familial disorders, it may fail to make accurate genotype-based phenotypic predictions. The identification of modifying loci, which act together to lead to a specific phenotype, is a pivotal step to a better understanding of the variability in the clinical expression of certain diseases, such as GT. The platelet glycoprotein α_2 C807T gene polymorphism seems to interact with an impair-

ment of the platelet $\alpha_{IIb}\beta_3$ integrin expression to produce different GT clinical phenotypes. Whether the identification of α_2 C807T genotypes would be helpful in the clinical management of GT, needs to be further investigated in different series of GT patients.

Appendix

List of participants in the study: Laura Conti, Vitaliana De Santis, Maria Gabriella Mazzucconi, Giuseppe M. Gandolfo (Dipartimento di Medicina Sperimentale e Patologia, Università "La Sapienza", Roma); Michele Nobile (Divisione di Ematologia, I.R.C.C.S. "Casa Sollievo Della Sofferenza", S. Giovanni Rotondo); Antonio Ciampa (Divisione di Ematologia, Ospedale "G. Moscati", Avellino); Maria Luisa Lupone, Corrado Perricone (Centro per le Emocoagulopatie, Azienda Ospedaliera "Santobono-Pausillipon", Napoli); Maddalena Loredana Zighetti, Marco Cattaneo ("A. Bianchi Bonomi", Emophilia & Thrombosis Center, Università di Milano); Mario Schiavone, Nicola Ciavarella (Servizio di Coagulazione e Sorveglianza Emofilici, Policlinico Universitario, Bari); Maria Luisa Papa (Centro di Emofilia e Trombosi, Ospedale "Nuovo Pellegrini, Napoli); Giovanni Di Minno, Antonio Coppola (Centro di Riferimento Regionale per le Malattie Emocoagulative, Università "Federico II", Napoli); Luigi De Marco (Centro Trasfusionale e Chimica Clinica, C.R.O. Aviano, Pordenone); Rita Santoro, Gaetano Muleo (Servizio di Coagulazione, Unità Socio Sanitaria Locale n. 7, Catanzaro); Aldo Amoriello (Centro Trasfusionale, Azienda Ospedaliera "A. Cardarelli", Napoli); Anna Maria Innoceta (Oncologia-Ematologia, Ospedale Infermi AUSL Rimini).

References

1. Phillips DR, Charo IF, Scarborough RM. GPIIb-IIIa: the responsive integrin. Cell 1991;65:359-62.
2. Caen JP, Castaldi PA, Leclerc JC. Congenital bleeding disorders with long bleeding time and normal platelet count. I. Glanzmann's thromboasthenia (reports on fifteen patients). Am J Med 1966;41:4-26.
3. George JN, Nurden AT, Phillips DR. Molecular defects in interactions of platelets with the vessel wall. N Engl J Med 1984; 311:1084-98.
4. Phillips DR, Charo IF, Parise LV, Fitzgerald LA. The platelet membrane glycoprotein IIb-IIIa complex. Blood 1988;71: 831-43.
5. Caen JP. Glanzmann's thromboasthenia. Clin Haematol 1972; 1:383-92.
6. George JN, Caen JP, Nurden AT. Glanzmann's thromboasthenia: the spectrum of clinical disease. Blood 1990;75: 1383-95
7. Badado JL, Katsanis N. Beyond Mendel: an evolving view of human genetic transmission. Nature Rev Genet 2002;3:779-89.
8. Ardissino D, Mannucci PM, Merlini PA, Duca F, Fetiveau R, Tagliabue L, et al. Prothrombotic genetic risk factors in young survivors of myocardial infarctio. Blood 1999;94:46-51.
9. D'Andrea G, Colaizzo D, Vecchione G, Grandone E, Di Minno G, Margaglione M. Glanzmann's thrombasthenia: identification of 19 new mutations in 30 patients. The Glanzmann's Thrombasthenia Italian Team (GLATIT). Thromb Haemost 2002; 87:1034-42.
10. Margaglione M, Brancaccio V, Giuliani N, D'Andrea G, Cappucci G, Iannaccone L, et al. Increased risk for venous thrombosis in carriers of the prothrombin G→A20210 gene variant. Ann Intern Med 1998;129:89-93.
11. Di Paola J, Federici AB, Mannucci PM, Canciani MT, Kritzik M, Kunicki TJ, et al. Low platelet $\alpha_2\beta_1$ levels in type I von Willebrand disease correlate with impaired platelet function in a high shear stress system. Blood 1999;93:3578-82.
12. Sharp WJ, Khanduri UD, Christie BS. Rapid heterozygote detection in Glanzmann's thrombasthenia. Br J Haematol 1998; 101:66-9.
13. Kunicki TJ, Kritzik M, Annis DS, Nugent D. Hereditary variation in platelet integrin $\alpha_2\beta_1$ density is associated with two silent polymorphisms in the α_2 gene coding sequence. Blood 1997; 89:1939-43.
14. Nieuwenhuis HK, Akkerman JW, Houdijk WP, Sixma JJ. Human blood platelets showing no response to collagen fail to express glycoprotein Ia. Nature 1985;318:470-2.

Pre-publication Report

Contributions

MM and GD'A gave substantial contributions to the conception and design of the study, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and gave final approval of the version to be published. All participants in the GlaTIT Study gave substantial contributions to revising the article critically for important intellectual content and approved the version to be published.

Disclosures

Conflict of interest: none

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by an Associate Editor. The final decision to accept this paper for publication was taken by the Editors. Manuscript received February 11, 2003; accepted October 15, 2003.