Two novel ITGA2B mutations in a Glanzmann thrombasthaenia family associated with different platelet phenotypic expression

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Dear Sir,

Glanzmann thrombasthaenia (GT) is a rare inherited platelet function disorder caused by mutations in the *ITGA2B* and *ITGB3* genes coding for the α_{IIb} and β_3 platelet surface proteins, respectively. After release from the ER, α_{IIb} and β_3 assemble to form the large heterodimeric transmembrane $\alpha_{IIb}\beta_3$ complex, the most abundant receptor on the platelet surface that mediates platelet plug formation *via* interaction with fibrinogen, von Willebrand factor (VWF), vitronectin and fibronectin[1]. Hundreds of mutations in the *ITGA2B* and *ITGB3* genes (including missense and nonsense mutations, insertions, deletions, and splicing site variations) have been reported to damage the synthesis, structure and/or function of the $\alpha_{IIb}\beta_3$ complex, leading to GT[2]. Patients with GT suffer from lifelong moderate-to-severe haemorrhagic symptoms, which can become manifest immediately after birth with epistaxis, easy bruising, petechiae, menorrhagia and gastrointestinal bleeding[1]. Consistent with GT recessive inheritance, heterozygous subjects are usually asymptomatic, and carrier status can only be ascertained by direct gene sequencing or flow cytometry, though the latter approach is less sensitive[3].

Here we report two new *ITGA2B* mutations, (c.512delC, c.1686delG), responsible for GT when they occur in compound heterozygosity. The proband is a 35-day old female infant born from a twin delivery. Immediately after delivery, she developed a large parietal and cervical cephalohaematoma, severe anaemia, petechiae and ecchymosis; these complications were attributed to the vacuum-assisted delivery. After one month, the patient again showed widespread petechiae and ecchymoses after minimal pressure. She was, therefore, referred to our centre for assessment for rare coagulation disorders. Blood drawing led to abnormal bleeding at the venipuncture site. Basic haemostatic analyses were conducted, revealing a prolonged closure time at PFA-100®, absent ADP-, adrenaline- and collagen-induced platelet aggregation, and ristocetin-induced platelet aggregation (RIPA) at the lower limit of normal (Table I). All the other haemostatic parameters investigated were normal (Table I). Flow cytometry of platelet surface antigens revealed the absence of the $\alpha_{IIb}\beta_3$ complex, leading to the diagnosis of GT. Expression of the other main platelet antigens, in particular GPIbα and GPIX, was normal.

Sequencing of *ITGA2B* and *ITGB3* genes showed that the proband is compound heterozygous for two new *ITGA2B* mutations: c.512delC and c.1686delG, mapping in exon 4 and exon 17 of *ITGA2B*, respectively. Both deletions cause a frameshift with the consequent generation of premature stop codons resulting in an α_{IIb} protein 181 amino acids long, truncated at the β-propeller domain as a consequence of the c.512delC mutation (p.Cys161Alafs*22), and in an α_{IIb} protein 563 amino acids long, truncated at the thigh domain as a consequence of the c.1686delG mutation (p.Val552Cysfs*13). Neither of the variations was found in the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org) or the 1000 Genomes (1000GP; http://www.1000genomes.org) databases. A C/T transition involving cysteine 512 reportedly caused GT in a Chinese patient[4], but so far there have been no reports of deletions of this nucleotide. The proband's mother and father carry heterozygous c.512delC and c.1686delG mutations, respectively. Results of haemostatic screening were normal in the proband's mother (except for a mild reduction in collagen-induced platelet aggregation), consistent with the absence of any haemorrhagic diathesis; her bleeding score (BS), measured with the International Society on Thrombosis and Haemostasis (ISTH) Bleeding Assessment Tool (BAT), was 0 (normal value in females 0-5). The proband's father, on the other hand, revealed defective ADP-, collagen- and adrenaline-induced platelet aggregation (Table I), suggesting a pathological effect of the c.1686delG mutation even in heterozygosity. The laboratory test results were confirmed on two different occasions. These findings were unexpected because GT is a recessive inherited disease and, to our knowledge, the rare GT-related heterozygous mutations causing defective platelet function are gain-of-function, not frameshift mutations[5]. It is worth noting, however, that the defective platelet aggregation did not seem to produce a bleeding tendency, since the proband's

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Table I - Main haemostatic findings in the proband and her parents.

Subjects	ABO/age	PTT (sec)	PFA100# (sec)	Platelet aggregation				VWF:Ag (U/dL)	VWF:CB (U/dL)	VWF:RCo (U/dL)	FVIII:C (U/dL)	Bleeding score°	*ITGA2B* mutations
				ADP (%)	*Collagen (%)*	*Adrenalin (%)*	*Ristocetin* (%)*						
Proband	O/35 days	32.9	> 215	0	0	0	42.9	87.5	100.1	98.8	112.3	10	c.512delC/ c.1686delG
Father	O/37 years	29	136	23.7	23	7.5	58.1	41	44.3	49.2	61.1	0	c.1686delG
Mother	A/ 31years	28	132	56.25	17.5	71.9	72.5	74	72.55	126	100.1	0	c.512delC
Normal range		22-32	94-193	42-70	40-72	49-71	60-84	60-160	65-150	60-130	60-160	0-3/0-5 (M/F)	

#Assayed with the collagen/EPI cartridge. *Ristocetin concentration 1.2 mg/mL. °Calculated with the International Society on Thrombosis and Haemostasis Bleeding Assessment Tool. M/F: male/female; PTT: partial thromboplastin time ; PFA: platelet function analyser; ADP: adenosine diphosphate; VWF:Ag: von Willebrand factor antigen; VWF:CB: von Willebrand factor collagen binding; VWF:RCo: von Willebrand factor ristocetin co-factor; FVIII:C: factor VIII activity.

father has a BS of 0 (normal value in males 0-3), though he suffered from frequent epistaxis in childhood. He also showed a mild decrease in plasma VWF antigen (VWF:Ag), VWF collagen binding (VWF:CB), and VWF ristocetin co-factor (VWF:RCo): 41 U/dL, 44.3 U/dL and 49.2 U/dL, respectively. This prompted us to analyse the VWF gene to check for any co-existence of a mild form of VWD with GT. Direct sequencing of all coding exons, intron-exon boundaries, 5' and 3' untranslated regions of the VWF gene revealed no mutations, so we conclude that the proband's father belongs to the class of normal O-blood group individuals whose circulating VWF lies at the lower limit of normal. This result is consistent with the finding that intraplatelet VWF content was normal (98.8 U/dL; normal value 70-140 U/dL), confirming the absence of a defect in VWF synthesis.

Our observations suggest that certain *ITGA2B* mutations have some phenotypic expression on platelet function, even in heterozygosity, but do not significantly influence bleeding symptoms.

The Authors declare no conflicts of interest.

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