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Glanzmann's Thrombasthenia Patients with No Mutations in Both the ITGA2B and ITGB3 Genes as Identified by Conformation Sensitive Gel Electrophoresis (CSGE)

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Abstract

Glanzmann's Thrombasthenia (GT) is an autosomal recessive inherited platelet function disorder that is due to defect platelet aggregation in response to multiple physiologic agonists. The defect may be because of mutations in the genes encoding either ITGA2B or $ITGB_3$ that result in qualitative or quantitative abnormalities of the platelet receptor $aIIbb_3$. A total of 45 unrelated GT patients were analyzed for mutations in all the exons of ITGA2B and ITGB3 genes by a mutation screening technique, Conformation Sensitive Gel Electrophoresis (CSGE). Mutation was identified in 36 out of 45 patients; and in 9 patients no causative gene alterations were identified in both the genes. Only polymorphisms were identified in 5 patients; however, 4 patients did not show any sequence variation in both the genes. Though their mutation status was not identified, their hematological parameters, platelet aggregation, flow cytometry and western blot revealed that they were definite GT (Table 1). Of these 9 patients with no mutations, 5 patients had family history of bleeding; that included death episode, due to prolonged bleeding in all four families and bleeding complication in sibling in one family. The clinical manifestations included epistaxis, Gum bleeding and petechiae in 8 patients and Echymotic spots in 6 patients. The major bleeding complication of gastro- intestinal bleed and eye bleed was seen in 4 patients and 2 patients respectively; one patient had hematuria. Among the 9 patients with no mutation, a blood transfusion was required in 8 patients. Normal values of Prothrombin time, Activated partial thromboplastin time and platelet count in these patients excluded the possibility of other factor

deficiencies and thrombocytopenia. Platelet aggregation was absent with all the four agonists in all these patients. Platelet aIIbb$_3$ analysis by flow cytometry classified 5 patients as type I, one as type II and 3 patients as type III GT. Western blot analysis revealed complete absence of both aIIb and b_3 in 6 patients. In remaining 3 patients, one had trace amount of aIIb and reduced amount of b_3; another had mild amount of b_3 with no trace of aIIb, the third patient had abnormal aIIb protein. GT in these 9 patients may be due to defect in a regulatory element affecting the transcription of these two genes or abnormalities in mechanisms that are responsible for post-translational modifications and trafficking of integrin subunits. Moreover, the strategy to detect mutations in these patients was based on CSGE, whose sensitivity is not 100%. Even though the CSGE technique was carefully set up and tested with known mutations, making it unlikely that nucleotide substitutions were missed in these patients, the literature reports that the sensitivity of the CSGE technique is approximately 80%. The above data shows that these patients need to be considered as definite GT and treated based on Clinical, hematological parameters and flow cytometry, even though they did not show mutations.

						Hematological evaluation									protein analysis			
GT No	**Age Sex**	**CG**	**FH**	**Bld Tfn**	**Ble Syn**	**P/S**	**PC**	**BT (mi**	**CR (%)**	**Platelet aggregation**					**FCM**	**Western blot**		**Polymorphisms**
										ADI	**ADI**	**AA**	**Col**	**Rist**	**aIIb b_3**	**aIIb**	**b_3**	
4	15/M	N	Y	Y	P, Ep, GB, HU	Isolated plt	N	8′	20	Abs	Abs	Red	Abs	Red	20.4%	AN	N	**IIb** c.2188-7C>G
																		IIIa c.1126-30delT
																		IIIa c.1545C>A
																		IIbc.2188-7C>G
5	19/F	Y	Y	Y	P, Ep, GB, Mr	Isolated plt	N	>15′	5	Abs	Abs	Abs	Abs	N	0.13%	Abs	Red	**IIb** c.2187+34_42del 9bp
6	44/M	N	Y	Y	P, ES,	Isolated plt	N	>15′	0	Abs	Abs	Abs	Abs	N	7.72%	Red	Red	Nil

						Hematological evaluation									protein analysis			
GT No	Age Sex	CG	FH	Bld Tfn	Ble Syn	P/S	PC	BT (mi	CR (%)	Platelet aggregation					FCM	Western blot		Polymorphisms
										ADI	ADI	AA	Col	Ris	allb b_3	allb	b_3	
					Ep, GB, GI													
7	12/M	N	Y	Y	P, ES, Ep, GB	Isolated plt	N	>15′	12	Abs	Abs	Abs	Abs	N	1.02%	Abs	Red	Nil
8	46/M	N	Y	Y	P, ES, Ep, GB, GI	Isolated plt	N	>15′	0	Abs	Abs	Abs	Abs	N	0.27%	Abs	Abs	Nil
13	4/M	Y	N	N	P, ES, GB	Isolated plt	N	>15′	0	Abs	Abs	Abs	Abs	N	20%	Abs	Abs	**IIb** c.2188-7C>G
																		IIb c.2188-47C>T
																		IIb c.1600+100delT
																		IIIa c.2040T>C
21	14/M	N	N	Y	ES, Ep, GB, GI, EB	Isolated plt	N	>15′	0	Abs	Abs	Abs	Abs	N	0.2%	Abs	Abs	Nil
22	11m/M	Y	N	Y	P, ES, Ep,	Isolated plt	N	9′	0	Abs	Abs	Abs	Abs	N	21.7%	Abs	Abs	**IIb** c.2188-7C>G

						Hematological evaluation									protein analysis			
GT No	Age Sex	CG	FH	Bld Tfn	Ble Syn	P/S	PC	BT (mi	CR (%)	Platelet aggregation					FCM	Western blot		Polymorphisms
										ADI	ADI	AA	Col	Rist	$\alpha IIb\beta_3$	αIIb	β_3	
					GI, EB													
																		IIb c.3063C>T
33	1/F	Y	N	Y	P, Ep, GB	Isolated	N	>15′	0	Abs	Abs	Abs	Abs	N	3.5%	Abs	Abs	IIb c.2188-7C>G

Table 1: The clinical and hematological parameters along with polymorphisms identified in GT patients with no mutations

Note: *CG: Consanguinity, Bld Tfn: Blood transfusion, P/S: Peripheral smear, PC: Platelet count, BT: Bleeding time, CR: Clot retraction, FCM: Flow cytometry, ADP: Adenosine 5′-diphosphate, ADR: Adrenaline, AA: Arachidonic acid, Collag: Collagen, Risto: Ristocetin sulphate, P: Purpura, Ep: Epistaxis, GB: Gum bleed, HU: Hematuria, Mr: Menorrhagia, ES: Epistaxis, GI: Gastrointestinal bleed, EB: Eye bleed*

Disclosures: No relevant conflicts of interest to declare.

Author notes

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