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Invited critical review

Glanzmann thrombasthenia: An update

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ABSTRACT

Glanzmann thrombasthenia (GT) is a rare autosomal recessive disorder characterized by qualitative or quantitative abnormalities of the platelet membrane glycoprotein (GP) IIb/IIIa. Physiologically, this platelet receptor normally binds several adhesive plasma proteins, and this facilitates attachment and aggregation of platelets to ensure thrombus formation at sites of vascular injury. The lack of resultant platelet aggregation in GT leads to mucocutaneous bleeding whose manifestation may be clinically variable, ranging from easy bruising to severe and potentially life-threatening hemorrhages. In this review we discuss the main characteristics of GT, focusing on molecular defects, diagnostic evaluation and treatment strategies.

Contents

1. Introduction

Glanzmann thrombasthenia (GT) is a rare autosomal recessive disorder characterized by a deficiency or functional defect of platelet glycoprotein (GP) IIb/IIIa, which mediates aggregation of activated platelets by binding the adhesive proteins, fibrinogen, von Willebrand factor (VWF) and fibronectin [1–4]. First described in 1918 by Glanzmann as "hereditary hemorrhagic thrombasthenia" [5], this syndrome is characterized by mucocutaneous bleeding with a variable clinical presentation ranging from mild bruising to severe and potentially fatal hemorrhages [6].

In this article, we review the current knowledge and discuss the more recent advances in the diagnosis and management of GT.

2. Molecular biology

The two genes, encoding GPIIb (*ITGA2B*) and GPIIIa (*ITGB3*) are closely associated at chromosome 17q21. The GPIIIa subunit is initially present as a propeptide that associates with GPIIb early in the biosynthetic pathway, and is translationally cleaved into heavy and light chains during passage through the Golgi apparatus where the two peptides remain linked through a disulfide bridge. Both subunits are intrinsic membrane glycoproteins with large, glycosylated, extracellular domains and biologically active cytoplasmic domains. The membrane glycoprotein GPIIb consists of four major domains, β-propeller, thigh, calf-1 and calf-2 domains [7]. The main contact site with GPIIb is located in the β-propeller, while calf-1 and calf-2 domains contribute minor interfaces. When the complex formation between GPIIb and GPIIIa fails, neither subunit traffics to the membrane systems that regulate platelet synthesis, thus affecting surface expression of both subunits [8]. In type I disease, from no to little GPIIIa/GPIIb is produced or detectable on the platelet surface, while in type II GT their number is measurable but low.

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Although the overall number of families affected by severe inherited platelet disorders is typically considered to be low, GT represents an exception of sorts, because of a significant number of worldwide cases due to a broad worldwide distribution and comparative commonality in certain ethnic groups (e.g., India, Arab Countries and Israel) [8]. Accordingly, the GT-associated mutations that have been identified at the molecular level has substantially increased in recent years, enabling the development of an Internet database (Glanzmann Thrombasthenia Database), that is regularly updated and includes clinical, biochemical, and mutation information on reported patients (http://med.mssm.edu/glanzmanndb). This database includes 82 mutations to date in the *ITGA2B* gene (Table 1) and 51 mutations in the *ITGB3* gene (Table 2), a difference attributable to the larger size of the *ITGB3* gene (65 kbp–15 exons) as compared with the *ITGA2B* gene (17 kbp–30 exons). The GT mutations located within the GPIIb ß propeller comprehends those within and surrounding the calcium-binding domains, within and around the third blade of the propeller, and within the fourth to seventh blades of the propeller [10]. The latter mutations compromise the transport of the GPIIb/IIIa complex to the platelet surface. Another group of mutations is located within the vicinity of the third blade of the ß propeller, containing a predicted ß-turn structure that has been involved in ligand-binding of GPIIb/IIIa and other integrin receptors. Some missense mutations might also result in functionally defective receptors. The GT mutations in the *GPIIIa* gene comprehend those located within the cation-binding sphere, within the conserved DXSXS amino acid motif, close to the putative coordinating sites, and within the sphere of the MIDAS domain. Additional mutations might result in severe abnormalities of GPIIb/IIIa function but do not affect surface expression, whereas others result in the intracellular retention of misfolded receptor complexes [9].

The utility of using a molecular biology approach in the diagnosis GT is still widely debated. There are several hurdles, including the large number of mutations widely distributed over the entire genes (exons and splice sites), and the fact that although most families carry a single distinct mutation, the same mutation can occasionally occur in unrelated families from widely different geographic locations [10]. To some extent, several other limitations to a genetic testing approach for the diagnosis of GT would mimic those of other haemostasis defects, such as that for von Willebrands disorder [11] and hemophilia [12]. Nevertheless, some specialized laboratories are already providing this service, which may be facilitated by using a prescreening step with single-strand conformation polymorphism (SSCP) analysis. A polymerase chain reaction (PCR) amplification of individual exons is performed using oligonucleotides designed to cover ~300-bp fragments of the required gene. Afterwards, the PCR products are separated by electrophoresis under stringent conditions and detection is enabled by silver staining on minigels [13]. Unfortunately, PCR-SSCP cannot be guaranteed to detect every single mutation, and up to 30% of mutations might go undetected. An alternative but more challenging approach involves the sequencing of mRNA isolated from the platelets of the patient [14], which also suffers from several disadvantages including the poor stability and quantity of mRNA in the anucleated platelet and the inability to detect splice-site mutations. The direct sequencing of amplified fragments of genomic DNA, including exons and splice sites, provides an attractive alternative option [10].

3. Laboratory diagnosis

The approach undertaken to confirm a laboratory diagnosis of GT may depend on whether the patient presents with a non-specified disorder of primary haemostasis versus that as an undiagnosed relative of a known GT-affected family (Fig. 1). In the case of the former, the process will typically follow a serial investigation, whereas in the latter, a more direct approach is warranted. Several algorithms

Table 1
Glycoprotein IIb gene (*ITGA2B*) mutations causing Glanzmann thrombasthenia.

Exon	Mutation	Phenotype	RNA splicing	Aminoacid substitution
1	IVS1-9del4.5kb	Del: out of frame	Alternative	Premature termination
1	48GA	Nonsense		W16X
1	188GA	Ins: out of frame	Alternative	R63K(R32K)
2	IVS2(+1)GA		Alternative	
2	243insG	Ins: out of frame		71X
2	258TC	Missense		L86P(L55P)
2	288delC	Del: out of frame		Premature termination
4	IVS3(-3)418del	Del: out of frame		Premature termination
4	399CG	Missense		L147V(L116V)
4	416CT	Missense		A139V(A108V)
4	470CA	Missense		P157H(P126H)
4	G475A	Missense		G159S(G128S)
4	480CG	Del: in frame	Alternative	S(129)-S(161)del
4	520TC	Missense		Y174H(Y143H)
4	527CT	Missense		P176L(P145L)
5	IVS5(+2)CA	Ins: out of frame	Alternative	Premature termination
7	IVS7(-2)AG		Alternative	
5	575–580ins	Ins: in frame		R192T193 (R161T162)
5	605TG	Missense		F202C(F171C)
5	620CT	Missense		T207I(T176I)
6	641TC	Missense		L214P(L183P)
7	683CT	Nonsense		Q228X(Q197X)
7	693Tins	Ins: out of frame		Premature termination
7	726delC	Del: out of frame		
8	800GA	Missense		G267E(G236E)
8	818GA	Missense		G273D(G242D)
9	855Cins	Ins: out of frame		Premature termination
9	859GC	Missense		G287R(G256R)
11	C953T	Missense		S318L(S287L)
11	959TC	Missense		F320S(F289S)
11	985GT	Missense		V329F(V298F)
12	1063GA	Missense		E355K(E324K)
12	1073GA	Missense		R358H(R327H)
12	1073delGTGT	Del: out of frame		
12	1096CT	Nonsense		R366X(R335X)
12	1139GA	Missense		G380D(G349D)
12	1139GT	Missense		G380V(G349V)
12	1162GA	Missense		G388S(G357S)
12	1186GA	Missense		D396N(D365N)
13	1214TC	Missense		I405T(I374T)
13	1230delC	Del: out of frame		
13	1233Ains	Ins: out of frame		Y411X(Y380X)
13	1214TC	Missense		I405T(I374T)
13	1346GA	Missense		G449D(G418D)
13	1366–1371del	Del: in frame		V(425)D(426)del
13	1374CG	Missense		I458M(I427M)
14	1413CG	Nonsense		Y471X(Y440X)
15	IVS15(+1)GA	Del: out of frame	Alternative	Premature termination
15	1520CG	Missense		P507R(P476R)
17	1651CT	Missense		R551W(R520W)
17	1652GA	Missense		R551Q(R520Q)
17	1742CA	Missense		A581D(A550D)
17	1750CT	Nonsense		Arg553Stop
18	1787TC	Missense		I596T(I565T)
18	IVS17(-1)GA	Del: in frame	Alternative	D(585)-Q(626)del
18	1787TC	Missense		I596T(I565T)
19	1882CT	Nonsense		R628X(R597X)
19	1912Gins	Ins: out of frame		Premature termination
20	IVS19(-2)AG	Del: out of frame	Alternative	Premature termination
20	2013delG	Del: out of frame		663X
21	2113TC	Missense		C705R(C674R)
23	2333AC	Missense		Q778P(Q747P)
22	2236GT	Nonsense		E746X(E715X)