EXPERT REVIEWS

Understanding the genetic basis of Glanzmann thrombasthenia: implications for treatment

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Glanzmann thrombasthenia (GT) is characterized by mucocutaneous bleeding due to platelets that fail to aggregate in response to physiologic stimuli. GT, a rare inherited disease, is caused by quantitative or qualitative deficiencies of αIIbβ3, an integrin receptor for adhesive proteins. Coded by the *ITGA2B* and *ITGB3* genes, αIIbβ3 mediates platelet-to-platelet attachment, aggregation and clot retraction. Despite widespread mutation analysis, the reason for the extensive variation in both the severity and intensity of bleeding among affected individuals remains poorly understood. Although genetic defects of *ITGB3* affect other tissues where β3 is present as αvβ3 (the vitronectin receptor), the bleeding phenotype continues to dominate. The authors now examine the relationship between genotype and phenotype in classic and variant forms of GT, and reassess if the nature of the gene mutation influences bleeding and treatment aimed at restoring hemostasis.

Keywords: αIIbβ3 integrin • Glanzmann thrombasthenia • inherited bleeding syndrome • platelet aggregation • treatment

Glanzmann thrombasthenia (GT) is the most common inherited disease of platelet function [1–3]. Patients are born with a bleeding syndrome that can be variable in both frequency and intensity, which on rare occasions is life threatening. The hemorrhagic tendency is characterized by episodes of spontaneous mucocutaneous bleeding and easy bruising. Severe trauma-related bleeding can be a major complication. In GT, platelets fail to assume their normal hemostatic function at sites of vessel injury. While attachment to collagen and other subendothelial components occurs normally, thrombasthenic platelets show decreased spreading on collagen [4]. Most importantly, thrombus formation fails as GT platelets lack or have nonfunctional αIIbβ3 integrin (formerly known as the GPIIb-IIIa complex). In normal platelets, αIIbβ3 mediates the final step of platelet aggregation in response to exposure to physiological agonists of platelet activation (i.e., ADP, epinephrine, collagen, thrombin and so on). Prior to platelet stimulation, αIIbβ3 exists in a nonactive bent conformation. When platelets are stimulated, the integrin changes conformation and straightens, which in turn causes the regions of the molecule essential for the binding of fibrinogen (Fg) and other soluble adhesive proteins to be exposed [5,6]. The latter assure aggregation by cross-linking adjacent platelets in a Ca^{2+}-dependent manner. Platelet αIIbβ3 also transmits the forces generated by intracellular cytoskeletal proteins during clot contraction and ensures the transport of Fg to the α-granules through receptor-mediated endocytosis where it constitutes a storage pool. These processes also fail in patients who lack αIIbβ3, however clot retraction and fibrinogen storage pools have been reported in patients with residual integrin expression (see section on GT classification) [1,3,5].

Although the GT phenotype is well defined, bleeding severity differs considerably among affected individuals [1–3,7,8]. In this review, the authors discuss the current situation with regard to mutation analysis, present variant types (where platelet surfaces express 50–100% of normal levels of dysfunctional integrin) and discuss possible additional effects associated with β3 deletion for while αIIb is largely restricted to the megakaryocyte (MK) lineage, β3 is widespread

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in its cellular distribution, with it being present in tissues as αvβ3, the vitronectin receptor [5,6]. The authors will look for correlations between genotype and phenotype, closely examine treatment in GT and assess how knowing the mutation responsible for the loss of αIIbβ3 or its dysfunction can influence therapy.

Bleeding manifestations

Bleeding in GT is primarily mucocutaneous in nature, and epistaxis, gingival, urinary and gastrointestinal (GI) bleeding are common symptoms (Table 1). Purpura, petechiae and easy bruising are frequent and often present at birth [1,2,9]. Although some patients have only minimal bruising, others have frequent, even fatal, hemorrhages. Surgery (including dentistry) has an increased bleeding risk, while accidents and trauma may trigger severe hemorrhage. However, hemarthrosis (bleeding into joints) that marks certain coagulation disorders is rare. Prevention of untoward health problems includes vaccination for hepatitis B, good dental hygiene, avoiding anti-inflammatory drugs, maintaining blood iron levels and refraining from physical contact sports [1,9]. For women, pregnancy and childbirth (including postpartum bleeding) require preventative measures to control bleeding but with adequate precautions delivery is likely to be successful [8]. Young female GT patients may be prescribed oral contraceptives to control prolonged and life-threatening blood loss. High doses of estrogen followed by estrogen–progestin may limit excessive bleeding during menarche, while menorrhagia in later life can be controlled by oral contraceptives. Nevertheless, generally in GT, the incidence of bleeding decreases with age [2]. Dissection of platelet and myeloid cell defects by conditional targeting of the β3-integrin subunit in mice has confirmed that platelet deletion of αIIbβ3 alone accounts for the perturbed hemostasis [10]. However, the reasons why some patients bleed more frequently than others still remain elusive. The primary treatment is transfusion of preferably HLA-compatible platelets to avoid immune response and platelet rejection.

Causes of αIIbβ3 deficiency in Glanzmann thrombasthenia

GT has autosomal-recessive inheritance and mutations affect either the *ITGA2B* or *ITGB3* genes that code for αIIb and β3, respectively [3]. Defects of genes for chaperone proteins necessary for αIIb or β3 processing can potentially occur but have yet to be proven for the rare patients where *ITGA2B* or *ITGB3* gene screening has proven negative [11]. *ITGA2B* or *ITGB3* are closely located at chromosome 17q21.31–32, but while their genomic organization has been established, surprisingly little is known of the factors controlling their expression [12–14]. αIIbβ3 is extremely abundant in normal platelets with up to 100,000 copies on the surface, an expression that varies by at least twofold between individuals [14]. Furthermore, a substantial internal pool is present with recycling as a continuous process, although much of this pool becomes surface exposed after secretion [15]. Such findings imply that measures of αIIbβ3 copy number alone cannot guarantee detection of obligate heterozygotes in GT; carriers with one normal and one mutated allele do not have a bleeding phenotype.

A regularly updated database lists the mutations for 318 patients [201]. Large gene deletions are rare in GT despite an early report revealing a deletion of 4.5 kb extending from intron 1 to intron 9 of *ITGA2B* that resulted in the loss of exons 2–9 and no detectable αIIb in platelets [16]. Nonsense mutations are abundant, as are out-of-frame and in-frame small deletions and insertions;

Table 1. Common treatments for Glanzmann thrombasthenia.

Bleeding syndrome	Local procedure	Transfusion	Others
Superficial injury, gum bleeding	Compression, fibrin glue Use of autologous platelet clots	Rarely platelet transfusion	Tranexamic acid DDAVP
Nose bleeding	Compression 10 min, gauze soak, nose packing, fibrin sealants, cauterization	Platelet transfusion for intense bleeding rFVIIa (restricted use)†	Tranexamic acid
Menorrhagia	–	Platelet transfusion for intense bleeding Red blood cell transfusion during acute phase‡	Oral contraceptives, tranexamic acid
Gastrointestinal bleeding, angiodysplasia	Argon plasma coagulation	Platelet transfusion, red blood cell transfusion during acute phase	Long-action octreotide with or without hormone therapy
Surgery	Fibrin glue applied to surgical site	Repeated platelet transfusions.	Tranexamic acid
Surgery in case of isoimmunization or refractoriness to platelet transfusion	Fibrin glue to surgical site	rFVIIa (90 µg/kg every 2 h) associated or not with HLA-matched platelet transfusions	Tranexamic acid DDAVP
Severe trauma-related bleeding	Fibrin glue applied to site	Repeated HLA-matched platelet transfusions and/or associated rFVIIa	Tranexamic acid DDAVP

†Use is generally restricted to patients with inhibitors whose presence renders platelet transfusion ineffective.
‡Red blood cells are given when bleeding is severe (for all situations).
DDAVP: 1-desamino-8-D-arginine vasopressin or desmopressin; rFVIIa: Recombinant factor VIIa.

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premature terminations with or without frameshifts frequently accompany splice defects and give rise to truncated proteins and/or mRNA instability [3]. Figures 1 & 2 show this diversity and highlight an extensive repertoire of missense mutations located in GT. These are mapped in Figures 1 & 2 in relation both to the cDNA location of the nucleotide changes and to the position of the affected amino acid within the known structural domains of the mature protein. Although it is mostly concluded that missense mutations primarily affect early protein processing events, recent evidence suggests that mutations affecting short exonic splicing regulators may also promote splicing failure. As a specific example, a missense mutation in αIIb has been suggested to promote exon skipping by altering the exonic splicing regulator sequence recognized by SC35 [17].

Mutations occur across both genes with a higher percentage in *ITGA2B*, possibly because, although it is a smaller gene, it has 30 compared with 15 exons in *ITGB3* (with an accompanying increase in the number of splice sites). Biogenesis of αIIbβ3 starts in the hematopoietic stem cell and continues throughout MK maturation; it is one of the earliest platelet surface receptors to be expressed [18]. The αIIb subunit is synthesized with a signal peptide (pro-αIIb). N-linked glycans are attached to pro-αIIb and to β3 in the endoplasmic reticulum, where pro-αIIbβ3 complexes form and the signal peptide is removed. More carbohydrate modifications accompany the separation of pro-αIIb into mature heavy and light chains in the Golgi apparatus [19]. Mutations in GT can block the production of either subunit, cause RNA instability, interfere with complex formation or prevent (or slow down) trafficking [19–23]. Particular importance is given to the αIIb outer β-propeller domain, where a panorama of mutations that result in protein misfolding and/or impaired calcium binding severely interfere with αIIbβ3 biogenesis (Figures 1 & 3) [23]. If complex formation fails (αIIb with β3; or β3 with αIIb or αv), residual αIIb or β3 undergo proteosomal degradation.

Most families have their own private mutation although apparent mutational hotspots lead to the reappearance of some mutations in unrelated families (Figures 1 & 2) . Consanguinity is often linked to homozygous mutations and contributes to a higher concentration of GT (and of specific mutations) in some ethnic groups (e.g., Iraqi Jews, Palestinian Arabs, French Gypsies), and this precludes an accurate worldwide estimation of disease frequency [3]. The pioneering studies by Peter Newman and his coworkers identified a common 11-bp out-of-frame deletion (c.2031–2041del, exon 13) in *ITGB3* in the Iraqi–Jewish population of Israel that results in a frameshift and premature termination before the transmembrane domain [24]. A 13-bp deletion encompassing the splice acceptor site of exon 4 of *ITGA2B* (IVS3[-3]-418del) is common to Arab kindreds in Israel, Saudi Arabia and Jordan, and through alternative splicing leads to a 6-amino acid deletion (A [106]-Q [111]del) in mature αIIb [24]. Haplotype analysis confirmed a common ancestry for this mutation with a frequency 1:114 in the general Iraqi–Jewish population and suggests that the founder mutation occurred some 300–600 years ago [25,26]. The French gypsy mutation is a IVS15(+1)G->A substitution that induces abnormal splicing and a 8-bp deletion at the 3′-end of exon 15 [27]. A reading frame shift and a stop codon results in a severely truncated form of αIIb. Exclusive to families of Manouche origin, haplotype analysis suggested that this founder mutation originated some 300–400 years ago, almost certainly after the Manouche tribe had migrated from India through Germany before settling in the Alsace region of France [28].

Geographical distribution

GT, although a rare disease, has a worldwide distribution. Estimating frequency remains difficult due to its high concentration in certain ethnic groups. For example, in France an unpublished census by the authors' center suggests around 300 cases of which half are of gypsy origin. A relatively high abundance in France is underlined by the report of Jallu *et al.* who studied 24 cases (none of gypsy origin) and identified 29 mutations of which 17 were new allelic variants [29]. D'Andrea *et al.* studied 30 Italian patients and found 19 new mutations mostly in *ITGA2B* [30]. Here, wide differences in bleeding severity were shown for patients with stop codons predicting truncated proteins and for those with missense mutations. Epidemiological screening in India has been the subject of several reports. These include the finding of 23 new mutations (13 in *ITGA2B* and ten in *ITGB3*) located in 40 families from southern India and then 22 novel mutations, again with a higher proportion in *ITGA2B*, in 45 more Indian patients [11,31]. Correlations between bleeding severity and genotype or indeed αIIbβ3 expression were not made in these Indian studies. In many of the epidemiologic reports, expression studies in transformed cell lines have not been completed so definitive proof that the mutations actually cause the GT phenotype is sometimes lacking. Phenotyping alone has suggested that GT is a relatively common disease in Iran [32]. However, there is as yet little information on the prevalence of GT in many parts of the world, probably due to inadequate screening although initial reports each describing several patients have shown that it is indeed present in China, Africa and South America [33–35].

Diagnosis

Diagnosis of GT starts with questioning on the nature and family history of the mucocutaneous bleeding; aggregation defects associated with acute lymphoblastic or myeloblastic leukemia (or a related disorder), or the presence of acquired post-transfusion or postpregnancy anti-αIIbβ3 antibodies that block platelet function, need to be excluded [1–3]. The possible presence of consanguinity should be ascertained. Bleeding symptoms are mostly present from birth, and in the vast majority of patients, there is a normal platelet count and normal platelet volume. Determining a bleeding score such as that used in von Willebrand disease (VWD) is recommended [36]. Laboratory investigations at the time of diagnosis are listed in Table 2. A prolonged bleeding time (or a prolonged closure time with the Platelet Function Analyzer-100® [Siemens Healthcare Diagnostics Inc., NY, USA]) is accompanied by an absent platelet aggregation with a range of physiologic agonists (ADP, arachidonic acid, collagen, thrombin receptor activating peptide) in platelet-rich plasma. von Willebrand factor

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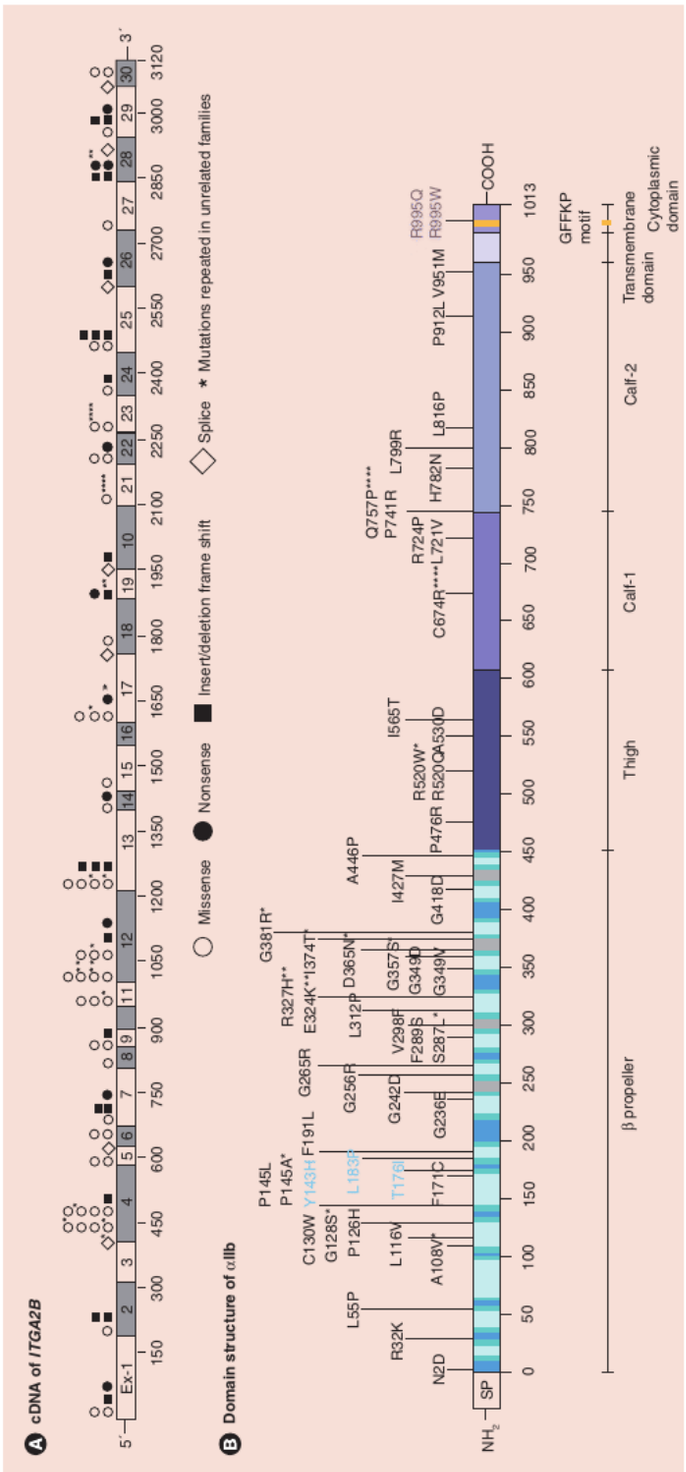

Figure 1. Schematic representation of the *ITGA2B* gene illustrating a wide spectrum of mutations that give rise to Glanzmann thrombasthenia. Data were obtained from a survey of the literature and from consulting the Glanzmann thrombasthenia database. In **(A)**, the defects are designated a symbol according to their type. Mutations are found all along the gene. In **(B)**, missense mutations are identified by a single letter amino acid code and are aligned with respect to the structural domains of each subunit. Those that primarily prevent αIIbβ3 expression are in black; mutations in blue are variant forms with expression of nonfunctional αIIbβ3; in mauve, mutations are associated with macrothrombocytopenia. Asterisks indicate the number of times the defect has been reported in apparently unrelated families. Data taken from [201].

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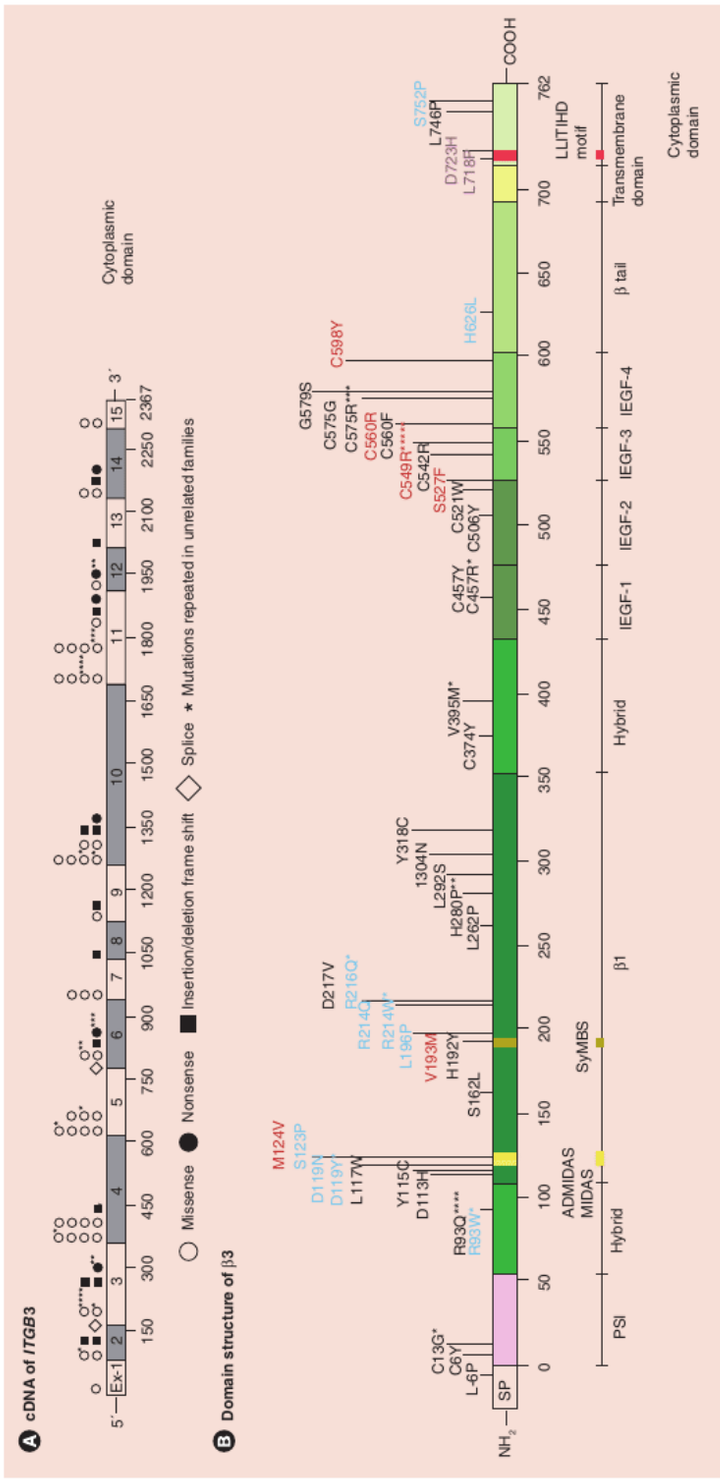

Figure 2. Schematic representation of the *ITGB3* gene illustrating a wide spectrum of mutations that give rise to Glanzmann thrombasthenia. Data were obtained from a survey of the literature and from consulting the Glanzmann thrombasthenia database. In **(A)**, the defects are designated a symbol according to their type. Mutations are again found all along the gene. In **(B)**, missense mutations are identified by a single letter amino acid code and are aligned with respect to the structural domains of each subunit. Those that primarily prevent αIIbβ3 expression are in black; mutations in blue are variant forms with expression of nonfunctional αIIbβ3; in red, the substitutions give activated integrin; while in mauve, mutations are associated with macrothrombocytopenia. Asterisks indicate the number of times the defect has been reported in apparently unrelated families. Missense mutations giving rise to Glanzmann thrombasthenia are widely distributed across both genes. In contrast, variant forms are more likely to have *ITGB3* gene defects.
Data taken from [201].

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(VWF)- and GPIb-dependent ristocetin-induced platelet agglutination normally occurs, although the light transmission patterns in a platelet aggregometer often show cycles of association and dissociation – a finding that has yet to be explained [37]. Clot retraction is defective in many patients but may occur if there is residual functional αIIbβ3 – even if amounts are too low to support aggregation.

Flow cytometry analyzing the binding to platelets of monoclonal antibodies (MoAbs) specific for αIIbβ3 allows the detection and quantification of the integrin deficiency [38–40]. If this facility is not available, αIIbβ3 deficiency can be shown by immunofluorescence microscopy using fluorochrome-labeled MoAbs to αIIbβ3 or by antibody-dependent cytochemical staining on a blood film. For variant GT, the presence of normal or near normal amounts of nonfunctional αIIbβ3 is confirmed by flow cytometry, and the absence of binding of the activation-dependent MoAb, PAC-1 or fluorochrome-labeled Fg, after stimulation of platelets with ADP [39,40]. Whenever possible, diagnosis should be confirmed by genetic analysis. While mutations can be detected by sequencing platelet RNA, the poor expression and limited stability of RNA in platelets limits this approach for diagnosis, while some mutations themselves affect RNA stability [21]. Despite these reservations, studies on mRNA are useful for defining the correct mechanism of gene inactivation. In early analyses of genomic DNA, preliminary PCR-based screening procedures of exons and splice sites such as single strand conformation polymorphism analysis were used to detect potential mutations [38,41]. While successful for many patients, such methods have only around a 70% detection rate and so most laboratories now perform direct sequencing of the 45 exons and their splice sites that compose the *ITGA2B* and *ITGB3* genes [11,22,29]. Once detected, mutations should be confirmed on a second DNA sample; their penetration within families or ethnic groups can be determined using restriction enzyme-based assays or by high-resolution melting-point analyses [42,43]. It should be emphasized, however, that a negative result does not rule out a second mutation in ethnic groups [25]. Other investigations that continue after diagnosis are given in Table 2.

Integrin αvβ3 in thrombasthenia

Compared with the high density of αIIbβ3, normal platelets only express 50–100 copies of αvβ3, making its quantification difficult [3]. Nonetheless, studies using an αvβ3-specific radiolabeled MoAb showed that platelets of Israeli–Arabs with the founder *ITGA2B* mutation (c.IVS3[-3]-418del + frameshift) preventing αIIbβ3 expression had a twofold increase in αvβ3: in contrast, platelets of Iraqi–Jewish patients with the founder *ITGB3* mutation (c.2031–2041del/premature termination) lacked both integrins [44]. Nevertheless, clinically, the Iraqi–Jewish and Arab patients are indistinguishable [24,44]. While stop codons and mutations leading to a loss of expression of β3 will affect αvβ3 and αIIbβ3 equally, this is not necessarily the case for missense mutations with residual β3 expression. For example, β3 Leu196Pro and β3 His280Pro, a mutation prevalent in Japan, differentially affect αIIbβ3 and αvβ3 function [45,46]. Thus, αIIbβ3Pro196 supported CHO cell spreading on Fg, but αvβ3Pro196 did not and the difference extended to clot retraction. As αvβ3 is expressed in endothelial cells, osteoclasts and smooth muscle cells among others, such results imply that the effect of β3 amino acid substitutions on these cells must be assessed individually. Likewise, missense mutations affecting αIIb may differentially affect αvβ3 and αIIbβ3 expression and/or function [22].

Table 2. Laboratory testing for Glanzmann thrombasthenia.

Investigation	Results	Remarks
Laboratory investigations at diagnosis		
Bleeding time	Prolonged	No longer recommended
PFA-100 closure time	Absence of cartridge occlusion	Replacement for bleeding time
Aggregation (citrated PRP)	Null with ADP-Collagen-AA-TRAP	Absent αIIbβ3 function
Ristocetin agglutination	Normal but can be reversible (or cyclic)	Normal GPIb function
Clot retraction	Null–partial (or even normal)	Can occur if residual αIIbβ3 is present
Flow cytometry	<5% (type I), 5-20% (type II), >20% variants showing no activation after stimulation	Rare patients with activating mutations
Molecular biology *ITGA2B* and *ITGB3*	Mutation: homozygote or compound heterozygote	Heterozygotes nonsymptomatic
Others: platelet count and size	Usually normal	Platelet anisocytosis has been reported
Laboratory investigations after diagnosis		
Blood cell counts/levels of Hb	Normal or anemia	Low if bleeding
Ferritin	Normal or decreased	To be supplemented
Anti-HLA antibodies	Positive or not	Seen after transfusion
αIIbβ3 isoantibodies	Positive or not	Seen after transfusion or pregnancy

Hb: Hemoglobin; PFA: Platelet function analyzer; PRP: Platelet-rich plasma.

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Classification of Glanzmann thrombasthenia

In the older nomenclature, patients with <5% residual αIIbβ3 were classified as type I GT, those with 5–20% αIIbβ3 as type II GT; while rare patients with >20% αIIbβ3 but with a qualitative defect preventing function were called variant-type disease [1]. It was assumed that residual αIIbβ3 in type II GT could permit clot retraction and the uptake of Fg into α-granules. Thus, a type II French patient with a β3 Cys598Tyr mutation and approximately 10% αIIbβ3 expression had >60% of the normal content of α-granule Fg and platelets that supported clot retraction [1,47]. Similarly, a patient with a β3 Ser162Leu mutation and 30% αIIbβ3 reacted with fibrin while a *ITGB3* Ser752Pro substitution in a GT variant allowed Fg trafficking to α-granules and normal clot retraction (see section on 'variant GT') while failing to support activation-dependent soluble Fg binding [48,49]. Nevertheless, the type II nomenclature should be used sparingly, for it is now clear that the functional response of the platelets depends not only on the residual expression of αIIbβ3 but, in the case of missense mutations, also on the modifying effect of the amino acid substitution. Patients have been reported with 10–30% αIIbβ3 that fail to support clot retraction while some variants with normal or near normal αIIbβ3 and mutations in β3 are also unable to support aggregation, clot retraction or Fg uptake [50–52].

Variant-type Glanzmann thrombasthenia

In variant-type GT, patients express αIIbβ3 at levels that would normally allow platelet aggregation, but amino acid substitutions change the integrin structure so that it is unable to support a normal hemostatic function. As stated in the section on 'diagnosis', variants are characterized by the inability of their platelets to bind soluble Fg or antibodies that recognize activation-dependent determinants on αIIbβ3. The nature and position of the mutation defines the residual functional response [3]. Most variant forms concern *ITGB3*. Significantly, β3 also contains a series of amino acid substitutions that fail to affect αIIbβ3 expression or function but in normal platelets constitute alloantigen systems responsible for the production of alloantibodies, giving rise to neonatal alloimmune or post-transfusion thrombocytopenia; fewer alloantigen systems are carried by αIIb [53,54].

Mutations affecting extracellular domains of β3

These have provided critical information on the active sites of αIIbβ3 [3]. The first to be described, a homozygous Asp119Tyr substitution within the β3 I-like domain abrogated αIIbβ3 function by inducing the loss of a divalent cation (Mg^{2+}) structured Fg-binding site within the metal ion-dependent adhesion site (MIDAS) [52]. As illustrated in Figure 3, homozygous substitutions of Arg214 (Arg214Gln or Trp) in the ADMIDAS (adjacent to MIDAS) Ca^{2+}-binding domain of β3 make αIIbβ3 unstable, with the result that the integrin becomes sensitive to divalent-cation chelation and is unable to bind Fg [3]. Three-dimensional structures of the unactivated and activated (Fg-binding) forms of αIIbβ3 have defined key residues essential for Fg binding located on both the β3 MIDAS and ADMIDAS domains [3–6]. The fact that SNPs giving rise to alloantigen systems but without modifying function often affect the β-tail domain of β3 with a membrane proximal location implies an interesting structure–function relationship between the different domains of β3 [53,54].

β3 cytoplasmic domain mutations

These have proved crucial in defining how inside-out signaling activates αIIbβ3. A heterozygous Ser752Pro substitution in the β3 cytoplasmic tail combined with a nonexpressed allele resulted in 50% αIIbβ3 and a loss of platelet aggregation. Platelets stimulated by physiologic agonists failed to bind Fg, although they did following direct surface-based activation of the integrin [49]. Significantly, kindlin-3, a cytoplasmic protein required for integrin activation (for more information on kindlin-3, see section on 'leukocyte adhesion deficiency-III') failed to interact with the β3 Pro752 cytoplasmic domain [55]. The ability of this patient's platelets to support clot retraction, possess a normal α-granule Fg pool and to secrete Fg attached to αIIbβ3 [56,57] suggests that these are kindlin-3 unrelated events. Possibly the latter are mediated by low affinity Fg binding and lead to a mild bleeding syndrome. Significantly, β3 Pro752 also blocks 'outside-in signaling' and cell spreading after integrin engagement on surface-bound adhesive proteins [3]. In comparison, an African–American child, who was a compound heterozygote for a nonexpressed allele and a novel truncating cytoplasmic domain mutation, possessed platelets with intermediate amounts of αIIbβ3 and β3 but with only eight membrane proximal amino acids of the 47 that compose the cytoplasmic tail [58]. Here, the β3 cytoplasmic tail lacked elements responsible for both talin and kindlin binding; furthermore, studies on this patient permitted the identification of FcγRII as a partner for αIIbβ3 in outside-in signaling [59,60]. CHO cells expressing αIIbβ3 Arg724Ter failed to spread on Fg confirming defective outside-in integrin signaling and showing absent FAK phosphorylation [58]. Significantly, this patient had a more severe bleeding history than the patient with the more specific Ser752Pro mutation.

Mutations affecting αIIb

Proportionally fewer mutations of *ITGA2B* give rise to variant GT and intriguingly also to alloantigen systems [3,54]. A heterozygous Cys674Arg substitution in αIIb that disrupts the Cys674–Cys687 disulfide gave platelets with 30% αIIbβ3 when associated with a null allele [61]. Association and trafficking of the subunits was retarded due to an abnormal interaction of αIIbArg674 with the BiP chaperone. A Japanese variant with a homozygous 6-bp insertion causing the addition of Arg-Thr within the Cys146–Cys167 loop of αIIb had platelets fully expressing αIIbβ3 unable to bind Fg or PAC-1 when activated [62]. Similarly, a heterozygous Tyr143His substitution in αIIb, combined with the null expression of the second allele, gave about 40% levels of αIIbβ3 that failed to bind PAC-1 or Fg when activated [63]. As shown in Figure 3, the Tyr143His substitution interferes with a hydrogen bond. It should be emphasized that the GT phenotype depends on both the position of the

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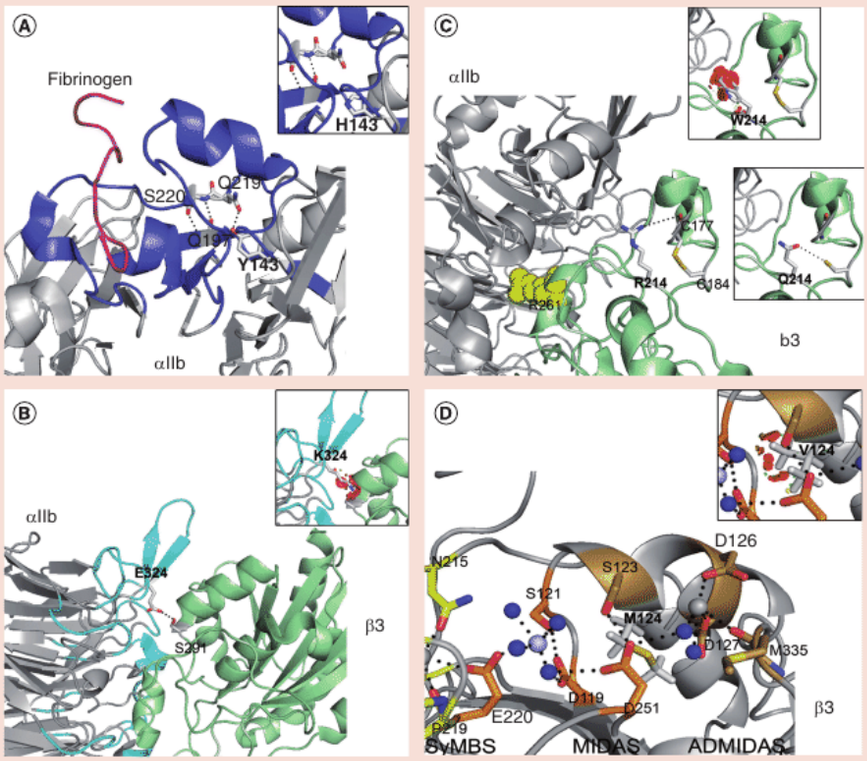

Figure 3. Structural modeling of selected missense mutations affecting αIIb or β3. Amino acid substitutions within the αIIb subunit are shown in **(A)** and **(B)**, while those in β3 are presented in **(C)** and **(D)**. The position and structural importance of the mutated amino acid is shown in the inserts at the right hand side of each panel. **(A)** αIIb Tyr143His (Y143H). Illustrated is a computer-drawn ribbon diagram of the β-propeller structure of the αIIb headpiece. Highlighted in blue are the α-helices that form part of the propeller structure interacting with fibrinogen. The left- and right-hand side helices form the sides of the domain receiving fibrinogen, while the middle helix constitutes the base of this domain. Q197 with S220 and Y143 with Q219 form hydrogen bonds (dashed lines) important in stabilizing this structure. The insert shows the mutation change with H143 causing the loss of the hydrogen bond with Q219 and destabilization. **(B)** αIIb Glu324Lys (E324K). Shown is a ribbon diagram of αIIb (gray) and β3 (green) headpieces. In light blue, are parts of the β-propeller at the interface with β3. E324 of αIIb localizes to this interface and forms a hydrogen bond with S291 of the β3 headpiece. The K324 substitution (insert) is shown by a graphical white stick representation; graphical 'bumps' (red discs) represent steric interactions caused by the presence of the K. Taken together, the loss of the hydrogen bond with additional steric repulsive forces may cause a destabilization of the heterodimer and altered trafficking. For clarity of representation this image has been turned 180°. **(C)** β3 Arg214Gln or Trp (R214Q or W). Shown is a ribbon diagram of αIIb (gray) and β3 (green) headpieces. R261 (yellow spheres) in β3, penetrating into the β-propeller structure is shown in a space-filling format. β3 R214 forms a hydrogen bond with the oxygen atom of the main chain of C177 that forms a disulfide with C184. This structure is in close proximity with the β-propeller of the αIIb headpiece. When R214 is mutated, we observed either a loss of the hydrogen bond with the appearance of steric repulsive forces as represented by graphical 'bumps' (red discs) (mutation W214, upper insert) or a break of the disulfide (mutation Q214, right window). These altered structures contribute to a decrease in the stability of the interaction between the β3 and αIIb headpieces. **(D)** β3 Met124Val (M124V). Shown are metal coordination sites in the SyMBS (yellow), MIDAS (orange), and ADMIDAS (brown) domains of the β1 domain of β3 in the bent (resting) conformation; N atoms are blue sticks and O atoms red sticks; Ca^{2+} (gray spheres), Mg^{2+} (pale blue sphere), and water (blue small spheres). Metal coordination and hydrogen bonds are shown as dashed lines; E220 contributes to both SyMBS and MIDAS, although colored orange. M124 (white stick), although not involved in metal coordination or hydrogen bonds, extends deep into the β1 structure. V124 (insert) leads to steric overcrowding that could result in increased spacing of the MIDAS and ADMIDAS domains, with M335 taking a position as in the active open conformation. Models were obtained using the PyMol Molecular Graphics System Version 1.3 (Schrödinger LLC; [202]) and 2vdo (for **A, B** and **C**) and 3fcs (for **D**) pdb files for crystal structure of αIIb and/or β3 subunits. Amino acid changes are rotamer incorporated from the Dunbrack Backbone library with the maximum probability.
ADMIDAS: Adjacent to MIDAS; MIDAS: Metal ion-dependent adhesion site.

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amino acid that is mutated and the nature of the amino acid that is substituted. This was first shown by Milet-Marsal *et al.* who studied a Glu324Lys substitution in αIIb in a patient with classic type-I GT [64]. Expression studies in which αIIb mutated at amino acid 324 was transiently expressed with wild-type β3 in COS-7 cells clearly showed that whereas αIIb Lys324 led to a block in synthesis, substitution by other amino acids led to variable expression of the integrin. Glu324 is a highly conserved amino acid in the third β-strand of blade 5 of the αIIb β-propeller. As illustrated in Figure 3, the Glu324Lys substitution results in loss of a hydrogen bond and severe steric hindrance within the heterodimer structure.

Activating mutations in β3

Most activating mutations involve breakage of disulfide bonds within the β3 EGF domains where the cysteine-rich core confers a structural constraint on the receptor [65,66]. Mostly, they severely limit αIIbβ3 expression; this was the case for a Cys549Arg mutation in the EGF-3 domain of β3 in six Jordanian families with severe GT that disrupted a conserved disulfide between Cys549 and Cys588, and also for Cys542Arg and Cys457Tyr mutations in families studied by the authors [66–68]. At the same time, mutating these cysteine residues resulted in variable constitutive activation (PAC-1 binding) when αIIbβ3 was expressed in baby hamster kidney cells [66]. For these patients, the very reduced αIIbβ3 level in their platelets is probably sufficient to account for the bleeding phenotype. Mutations that allow a greater expression of spontaneously active αIIbβ3 on platelets include Cys560Arg (or Phe) and Cys598Tyr [39,47]. The gain-of-function Cys560Arg mutation was detected in a French man with mild bleeding and moderate thrombocytopenia. It allows approximately 20% surface αIIbβ3 expression. Platelet aggregation and clot retraction are much reduced but not absent; platelet Fg content is normal. His platelets spontaneously bound antibodies to activation-dependent epitopes on αIIbβ3 and, significantly, Fg was detected on freshly isolated platelets *ex vivo*. This phenotype resembles platelet-type VWD where spontaneous VWF binding to GPIbα results in a bleeding rather than a thrombotic syndrome [69]. Interestingly, a regulatory role was suggested for free Cys560 [66]. To gain insight into the *in vivo* effects of this activating mutation, a conditional 'knock-in' mouse model with platelets possessing human αIIbArg560β3 was generated using lentivirus-based technology [70]. The mice possessed platelets whose characteristics closely mimicked those of the patient and significantly, a notable bleeding tendency led to a greatly increased mortality.

Mutations outside the cysteine repeat domains can also give rise to activating mutations. In one such patient, a heterozygous β3 Met124Val substitution one residue downstream from the MIDAS core occurred in combination with a second heterozygous inactivating β3 (Asp119Tyr) mutation [71]. The patient's platelets failed to bind Fg or PAC-1 for β3 Val124 strongly interfered with αIIbβ3 biosynthesis and the β3 Tyr119 form predominated. As shown in Figure 3, β3 Met124 although not involved in metal coordination or hydrogen bonds, extends deep within the β1 structure; its substitution by Val leads to steric overcrowding and increased spacing of the MIDAS and ADMIDAS domains and an active open conformation. This may explain why integrin with β3 Val124 showed spontaneous activation when expressed in CHO cells, although receptor clustering was hypothesized at the time [71]. Another interesting mutation is the novel Ser527Phe mutation in β3 that induced a high affinity αIIbβ3 receptor by hindering adoption of the bent conformation [72].

Macrothrombocytopenia in patients with *ITGA2B* & *ITGB3* mutations

While a characteristic of GT patients with mutations in *ITGA2B* and *ITGB3* is a normal platelet size and count (Table 2), there are exceptions to this rule. For example, an Italian boy with mild bleeding whose platelets aggregated minimally to ADP but which give a slow, irreversible aggregation to thrombin; also showed moderate thrombocytopenia and platelet anisocytosis with some enlarged forms [41,73]. The surface expression of αIIbβ3 was about 18%, although curiously the internal pool was maintained, suggesting a recycling defect. A heterozygous Arg995Gln substitution within the GFFKR sequence of the cytoplasmic domain of αIIb was shown to be associated with a null allele. The latter is now known to be linked to a maternally inherited heterozygous 13bp intronic deletion (c1440-13_1440-1del) within the splice acceptor for exon 15 [43]. Transfection studies suggested that the Arg995Gln substitution led to a partially activated integrin. The more recent observation that a heterozygous *ITGA2B* Arg995Trp mutation in five patients from three Japanese families gives a similar phenotype when associated with a normal allele has confirmed that substitution of Arg995 accounts for the macrothrombocytopenia [74]. In the Japanese families, the normal second allele meant that surface αIIbβ3 levels were 50–70% of controls. Platelets from these patients spontaneously bound PAC-1 yet the platelet aggregation response was reduced (although not absent). Cytoplasmic domain residues αIIb Arg995 and β3 Asp723 form a salt bridge and breaking this clasp is a key step in integrin activation [75]. Strikingly, macrothrombocytopenia without a significant platelet aggregation defect and no bleeding characterized 5 members of a family with a heterozygous Asp723His substitution in β3 [76]. Interestingly, this mutation (which was also partially activating) resulted in RhoA downregulation when transfected in CHO cells; these responded with a unique microtubule-dependent 'proplatelet-like' formation when plated on Fg [77]. The fact that abnormal proplatelet formation also occurred in MKs cultured in vitro from the propositus's $CD34^+$ stem cells suggested that increasing the αIIbβ3 affinity state had a major effect on platelet production.

Moderate-to-severe mucocutaneous bleeding, mild thrombocytopenia and large platelets with defective aggregation were inherited in two Italian families as an autosomal dominant trait linked to a heterozygous in-frame deletion, causing loss of amino acids 647–686 from the transmembrane domain of β3 [78]. The result was a low surface expression of αIIbβ3 with a reduced activation on stimulation, and abnormal platelet spreading on Fg but normal clot retraction. A heterozygous Leu718Pro mutation in the membrane-proximal region of the β3 cytoplasmic domain of

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a Spanish woman also led to severe bleeding, thrombocytopenia and platelet anisocytosis [79]. Platelet aggregation was defective and this time there was also a secretion defect with little surface expression of P-selectin and CD63. The surface expression of αIIbβ3 was low and platelets showed impaired spreading and defective lamellipodia formation on Fg as well as a decreased binding of PAC-1 when stimulated. Interestingly, a differential effect on the processing of β3 integrins was also noted in transiently transfected cells for the β3Leu718Pro mutation [79].

In conclusion, upregulated αIIbβ3 reactivity and an altered MK maturation and/or timing of proplatelet formation can result in macrothrombocytopenia; this is a new phenotype given by specific mutations in either *ITGA2B* or *ITGB3*. Whether such patients should be included in the definition of GT is an open debate.

Relationships between genotype & phenotype

Here, the authors look more closely at whether the nature of the gene defect in GT has any effect on the phenotype.

Leukocyte adhesion deficiency-III

Leukocyte adhesion deficiency-III (LAD-III) syndrome is a variant of GT with a readily detectable phenotype, identified not only by severe bleeding but also recurrent infections not seen in classic GT [80]. These patients have a defect common to the inside-out signaling pathways of the β1, β2 and β3 integrins with the result that none function in response to stimulation even though the integrins are intact. Infections range from bacterial pneumonia and early septicemia to fungal disease. Osteopetrosis in some patients confirm abnormal osteoclast function. Mutations in *FERMT3* (encoding kindlin-3) are now known to account for the phenotype [55,81,82]. Kindlin-3 belongs to a family of proteins that cooperate with talin in inside-out integrin activation [75]. Enigmatically, kindlin-3 binding was inhibited by the GT variant β3 Ser752Pro mutation (see section on 'variant-type GT') albeit his mild bleeding phenotype. Stop codons or splicing defects leading to truncation and/or nonexpression of kindlin-3 predominate in LAD-III patients [81,82]. Bialkowska *et al*. have reported that kindlin-3 plays a role in αvβ3-mediated adhesion of endothelial cells [83]. Defective endothelial cell function may therefore contribute to the severe bleeding in this disease.

Influence of SNPs & other factors on the Glanzmann thrombasthenia phenotype

Studies assessing whether certain SNPs favor bleeding or by promoting fibrin formation result in a milder form of the disease in GT are in their infancy. Particular interest surrounds the potential influence of the coexpression of thrombophilic mutations such as Factor (F) V Leiden and the prothrombin A2010 polymorphism. D'Andrea *et al*. screened 25 Italian GT patients, but only one was positive for FV Leiden and none had the prothrombin A20210 mutation [84]. Similarly, we found but a single patient positive for FV Leiden in a series of 14 GT patients [85]. Yet, surprisingly, there are several reports of deep vein thrombosis (DVT) in GT [3]. For example, Ten Cate *et al*. described a 48-year-old man with type I GT with FV Leiden and who, on three occasions, has developed DVT in a lower limb [86]. A more recent report has also described recurrent DVT associated with FV Leiden in a 36-year-old male Brazilian GT patient who was successfully treated with warfarin [87]. DVT has also been diagnosed in a 2-year girl with GT [88]. Quite why DVT is a problem in GT is unclear. Functional studies performed on platelets from French GT patients showed that patients with residual αIIbβ3, although unable to bind Fg, adhered and formed small thrombi when their blood was perfused through collagen-coated capillaries [89]. A thick mesh composed of VWF and fibrin surrounded the thrombi, suggesting residual shear-dependent platelet reactivity. This contrasted with the results for type I GT patients where no thrombi formed. It is important, therefore, that patients who develop DVT are genotyped and their αIIbβ3 deficiency quantified. Perhaps, it is significant that a role for GPIb and VWF has been proposed in venous thrombosis [90].

It is to be expected that the bleeding severity in GT is influenced not only by the extent of the αIIbβ3 deficiency but also the abundance and functioning of other platelet receptors involved in aggregation and adhesion. Indeed, D'Andrea et al proposed that the *ITGA2* C807T SNP associated with a high density of the α2β1 collagen receptor led to a milder form of the disease [84]. A survey of 45 Indian GT patients revealed three with at least one HPA-1b allele on *ITGB3*; a milder form of the disease was suggested when HPA-1b was associated with FV Leiden and a thrombophilic 5,10-methylenetetrahydrofolate reductase SNP [91]. Yet, expression of HPA-1b is in linkage disequilibrium with the French gypsy mutation (on *ITGA2B*) in patients with type I GT and a relatively severe bleeding phenotype [92]. As β3 is not expressed in platelets in type I GT, any effects of HPA-1b must concern αvβ3 and vascular or other blood cells. The frequency of major SNPs affecting GPIbα, αIIb, β3, GPVI or the α2 integrin subunit has not shown major changes in GT patients in another report [92].

The gene encoding αv (*ITGAV*) is found at chromosome 2q32; remarkably, no mutations have been reported to give inherited disease. While this may suggest that αv is embryonically lethal, the absence of β3 is not and so an alternative explanation is that the role of αvβ3 is taken over by other integrins. A major enigma in GT is therefore the absence of a distinctive phenotype when αvβ3 is absent. Despite the relatively small number of patients for whom information is available, a recent survey of other major pathologies associated with GT found no reasons to suggest that brain disorders, postmenopausal bone thickening, bacterial infections, cancer or cardiovascular disease are more or less frequent in GT or that their incidence is changed according to whether *ITGA2B* or *ITGB3* is mutated [3]. Yet, a similar analysis of transgenic mouse models found ample evidence that mice lacking β3 show enhanced angiogenesis and tumor growth, facilitated atherosclerosis when fed a high-lipid diet, increased bone thickening, altered placental function and increased fetal mortality and altered social behavior [3]. Morgan *et al* using Cre/loxP technology in mice showed that conditional depletion of myeloid β3-integrins (including osteoclasts but not from platelets) resulted in osteopetrosis (with significant increases in trabecular bone volume) [10]. Tumor growth

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in these mice was also increased. This strongly underlines how β3-integrins have cell-specific roles. As yet, no such studies have been performed on αIIb-deficient mice. The question remains as to whether additional phenotypes have been overlooked in human GT where the bleeding syndrome predominates for both *ITGA2B* and *ITGB3* defects. An unexpectedly low number of pregnancies have been reported in GT women in Iran [32]. However, those patients have yet to be genotyped.

The coexistence of other coagulation abnormalities in GT patients may increase the bleeding syndrome. For example, type I VWD associated with type I GT gave a severe bleeding syndrome in a child from Toronto, who was a compound heterozygote for mutations in *ITGA2B* [93]. Factor VII deficiency has been reported in the French gypsy population [2]. Whole-exome sequencing and other new-generation sequencing procedures may help define how SNPs and variations in other blood and vascular cell constituents or plasma factors in the coagulation cascade help determine the bleeding tendency in GT as well as other rare diseases [94,95].

In this context, it is quite interesting that variant-type GT can be associated with quite severe bleeding (e.g., those with mutations in the β3 MIDAS and ADMIDAS domains), while an Argentinian male with a β3 Ser752Pro mutation in the cytoplasmic domain that prevented platelet aggregation never experienced a bleeding episode that required treatment [1–3].

Does genotyping influence treatment?

Treatment in GT resembles that used for all inherited platelet disorders with mucocutaneous bleeding [2,9]. The most frequently used strategies are summarized in Table 1. Transfusion of HLA-compatible platelets is the standard response to severe bleeding. Topical measures and nasal packing can deal with less-severe superficial bleeding at accessible sites. DDAVP (desmopressin) that increases plasma levels of VWF and FVIII is an alternative procedure, although relatively little is used in GT. In contrast, recombinant FVIIa (rFVIIa) is now a widely tested alternative to platelet transfusions for patients with antibodies formed after platelet transfusion [7,9,96]. FVIIa is used both to stop bleeding in refractory patients and as cover for invasive procedures or during childbirth; in one report maternal antibodies were said to be present in 73% of pregnancies with postpartum hemorrhage occurring up to 3 weeks after delivery [8]. rFVIIa is thought to enhance platelet deposition in the vicinity of an injured vessel by promoting tissue factor-independent thrombin and fibrin formation. Fibrin binds and incorporates platelets into the clot through its capacity to react with receptors other than αIIbβ3 [97–99]. Adverse events including myocardial infarction, stroke and venous thromboembolism are potential problems with this therapy [9]. The capacity of fibrin to bind GT platelets is the basis for the topical use of fibrin glue; it is also the basis for the use of autologous clots to prevent bleeding and facilitate healing of superficial wounds, a procedure that is particularly useful during tooth extraction or dental implant surgery [100]. Although for most GT patients clot retraction may be compromised, secretion of the pool of biologically active metabolites and growth factors is not. Thus, autologous clots provide an advantage over fibrin sealants. GI bleeding is a major problem in GT, particularly among elderly patients [2]. While rFVIIa is successful in many cases, it can prove refractory; an alternative procedure, particularly for repeated GI bleeding, is electrocoagulation together with prolonged use of octreotide (a somatostatin derivative) with or without estrogen–progesterone combinations (for women) [101,102]. This procedure has been successfully used in three patients in Bordeaux, France [Nurden P, Unpublished Data].

In 1990, George *et al.* reviewed the case histories of 177 GT patients with a more in-depth analysis of the bleeding syndrome in 64 GT patients studied in Paris and concluded that there was no relationship between the frequency or severity of bleeding and the presence or abscence of residual αIIbβ3 in platelets [1]. More than 20 years on, this conclusion still stands for isolated cases; furthermore, there is no indication from the literature that the bleeding syndrome differs with respect to whether the mutation affects *ITGB3* or *ITGA2B*. The intimate structural interactions between αIIb and β3 within the mature integrin probably account for this finding [103,104]. There are, however, certain situations where the bleeding syndrome and/or treatment may be influenced by the nature of the gene lesion. The case of LAD-III has been dealt with earlier in this review (see section on 'leukocyte adhesion deficiency-III'). Another situation concerns the frequent formation of isoantibodies with patients becoming refractory to platelet transfusion (with possible febrile reactions) or, on rare occasions, antibodies causing fetal thrombocytopenia for mothers with GT [105–107]. By binding to αIIbβ3, these antibodies can lead to the elimination of transfused platelets and, in certain cases, block their functional capacity [108,109]. In the case of pregnancy, the transplacental passage of the antibodies may cause fetal thrombocytopenia [105,107]. The authors have recently reassessed platelet antibody formation to αIIbβ3 in a cohort of 24 French patients [110]. Included were 16 patients with the French gypsy mutation with no platelet expression of αIIbβ3 and a severe bleeding syndrome often requiring transfusion. Most of the gypsy patients possessed antibodies to αIIbβ3. For eight patients with other mutations affecting *ITGA2B* or *ITGB3*, only two with premature terminations and platelets lacking surface αIIbβ3 had formed antibodies after transfusion. It was concluded that patients lacking αIIbβ3 are at high risk for isoantibody formation and that rFVIIa should be recommended both to stop bleeding and as a preventative measure to minimize bleeding in the event of surgery.

Parents in families where GT is present, particularly in ethnic groups where consanguinity is common, often ask for antenatal testing and sometimes prenatal testing is requested early in pregnancy. Measuring αIIbβ3 expression on platelets is too imprecise to allow conclusions in view of the twofold variation in the normal population [14]; furthermore, heterozygotes aggregate normally in platelet function testing. Haplotype analysis has been used to identify GT carriers but is very cumbersome to perform [111]. The only sure way to advise prospective parents is to first ascertain the mutations that affect the family and then screen them. This can be done using same-day testing with PCR single strand

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conformation polymorphism (electrophoretic analysis of PCR products), restriction enzyme-based or high-resolution melting point-PCR procedures, or simply by targeted DNA sequencing [42,112]. Taking blood from the cordon is not recommended in prenatal diagnosis as it can lead to fetal loss through bleeding. Chorionic villus sampling or amniocentesis has been performed but there are as yet no reports of non-invasive testing. Even if results are positive, parents should be made aware that bleeding severity can vary considerably among affected members of the same family [2]. It is important to emphasize that other mutations can also give rise to GT in ethnic groups in which a founder mutation dominates, as shown by the detection of three novel mutations in Palestinian Arabs and a 11.2-kb deletion in Iraqi Jews [25,26].

There are now several reports of the use of allogeneic stem cell transplantation to provide a long-term cure of GT in severely affected children [113–115]. The use of HLA-matched marrow transplants from siblings or unrelated donors has proven remarkably successful, despite the inherent risks of these procedures and the possibility that the patients will develop isoantibodies against the functional platelets. Both conventional and reduced conditioning has been tested and stem cells from peripheral blood have also been used as an alternative to marrow [115]. Unfortunately, little is known of either the residual αIIbβ3 content of the recipient's platelets prior to transplantation or of the causal mutation affecting *ITGA2B* or *ITGB3*. Animal models of gene therapy have provided proof of principle that gene therapy will work in GT and a lentivirus-based procedure warrants a clinical trial [116,117]. Obviously, knowledge of the genetic lesion will be essential for gene therapy, and it is likely that patients with stop codons or mutations preventing integrin expression will be the first to be considered, although the potential problems linked to antibody production may be an obstacle. It is also to be recognized that, with the improved care that is now available, prognosis is good in GT.

Expert commentary

GT is the classic inherited disorder of platelet function and provides a clinical picture of the bleeding problems that arise when platelet aggregation, an essential step in thrombus formation at injured sites within the vasculature, is totally absent. Genotyping is now well advanced and a panoply of mutations have been described. While most of these prevent or severely decrease αIIbβ3 synthesis, some abrogate function rather than integrin expression and the identification of the mutated amino acids is helping define active sites on the extracellular and cytoplasmic domains of both subunits, a process helped by the advances in our knowledge of integrin structure obtained from crystallography. Mapping the mutations, and particularly those within ethnic groups, is facilitating diagnosis, improving patient care and treatment and making it possible, for example, to predict which patients are at high risk for inhibitor development after transfusion and, eventually, to identify those most likely to benefit from gene therapy.

Five-year view

The next 5 years will see an increase in epidemiological studies, providing a better knowledge of the incidence of GT worldwide. Mutation screening will become available to most patients who desire it and we predict that the application of new generation whole exome (or genome) and other high-throughput sequencing technologies to rare diseases will identify mutations in *ITGA2B* and especially *ITGB3* that will broaden the GT phenotype and possibly include pathologies affecting tissue cells. It may also become possible to widen the treatment strategies in GT and to predict those most suitable for individual patients taking into account both their phenotype and their genotype. Finally, gene therapy may become a therapeutic option and here the choice of patient will become crucial, although key issues such as safety and possible inhibitor development will need to be overcome. Finally, the development of patient's associations will increase awareness among patients and also add to an improved prognosis, particularly in third world countries.

Information resources

An updated database of mutations giving rise to Glanzmann thrombasthenia can be found online [201].

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Key issues

- Glanzmann thrombasthenia is the most abundant of the inherited disorders of platelet function and studies on the nature of the molecular defect have pioneered the understanding of the pathophysiology of an integrin.
- Knowledge of the bleeding phenotype is important, not only for its control in this rare disease but also for the improved care of patients treated for thrombotic disease with powerful inhibitors of αIIbβ3.
- It is important to better define the factors that influence bleeding in patients whose platelets lack or have nonfunctional αIIbβ3. This will include SNPs and epigenetic factors that both protect and promote the bleeding tendency.
- It is essential to also better understand the nonhemostatic effects potentially associated with the absence of β3 as αvβ3 is present in many tissues. This will include assessing the susceptibility of Glanzmann thrombasthenia patients to major illnesses including cardiovascular disease and cancer.

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