

Case Report

Compound Heterozygous ITGB3 Pathogenic Variants In A Patient With Glanzmann Thrombasthenia

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Abstract

Glanzmann Thrombasthenia is a rare platelet function disorder which is characterized by decreased expression and/or dysfunction of the platelet receptor α IIB β 3 (GPIIb/IIIa). The disease is due to alterations in ITGA2B or ITGB3 the genes encoding for the receptor subunits α Ib (GPIIb) and β 3 (GPIIIa) and mostly inherited autosomal recessively. We report about a one-year old girl presenting with petechiae and hematomas shortly after birth. Platelet light transmission aggregometry was impaired after stimulation with ADP, arachidonic acid and collagen. Stimulation with ristocetin reached normal values, but showed an undulating course. Flow cytometry revealed severely decreased expression of CD41 (α IIB β 3). Molecular genetic analysis of the candidate genes and family genotyping identified two compound heterozygous variants in Exon 10 of ITGB3: c.1552C>T (p.Gln518*) and c.1639T>G (p.Cys547Gly). According to the guidelines of ACMG the variants were classified as pathogenic (Class 5). The nonsense variant c.1552C>T has not been reported before.

Keywords: Inherited platelet disorder; Glanzmann Thrombasthenia; ITGB3; Bleeding symptoms

1. Introduction

Glanzmann Thrombasthenia (GT) is a well characterized platelet disease with mostly autosomal recessive inheritance (OMIM #273800). The key feature of GT is severely impaired or absent platelet aggregation in response to multiple platelet agonists except for ristocetin and a reduction/absence of integrin α IIb β 3 surface expression or dysfunction of the receptor [1]. The integrin α IIb β 3 is the main platelet fibrinogen receptor and consists of 2 subunits: α IIb (*ITGA2B*; 30 exons) and β 3 (*ITGB3*; 15 exons). The encoding genes are less than 200 kb apart on chromosome 17q21 [2-4]. There are three classical types of GT. In type I, α IIb β 3 expression is very low (<5%), whereas in GT type II α IIb β 3 expression is slightly reduced (5-20%) and in variant-type GT α IIb β 3 is dysfunctional and may be due to a defect in genes involved in integrin activation [1, 5, 6]. The subunits of α IIb and β 3 consolidate as a non-covalent heterodimer [7]. The subunit α IIb has four major extracellular domains known as β -propeller, thigh, calf-1 and calf-2. The subunit β 3 comprises of 8 domains: four EGF (epidermal growth factor) domains, the domains bI, hybrid, PSI (plexin/semaphoring/integrin) and the β -tail [8-14]. Variants causing GT can affect biosynthesis as well as structure and function of α IIb β 3 complex [15]. To date the public version of Human Genome Mutation Database (HGMD) comprises 204 pathogenic variants in *ITGA2B* and 149 in *ITGB3* [access 06/2020]. Affected patients are suffering from moderate to severe hemorrhagic syndrome. Symptoms can appear rapidly after birth including gastrointestinal bleeding, petechiae, hematomas and mucocutaneous bleeding (i.e. epistaxis) [16]. There are several other defects in the primary hemostasis which may present with similar symptoms (i.e. other platelet receptor defects). Therefore, comprehensive diagnostics are important to identify the cause of bleeding diathesis.

Here, we report compound heterozygous pathogenic variants in *ITGB3* causing Glanzmann disease, to our knowledge one of them is *novel* and has not been reported before.

2. Patient, Materials and Methods

The index patient is a one-year old Caucasian girl presenting with petechiae and hematomas distributed over the whole body already several hours postpartum. The girl also experienced postnatal cytomegalic virus infection and has two small muscular ventricular septal (VSD) defects, which are not relevant considering hemodynamics. After the baby started crawling even larger hematomas developed. Dentition caused prolonged bleeding for a day and was stopped using tranexamic acid. Sometimes mild bleeding symptoms were observed after scratching. Post-puncture bleeding symptoms occurred for 2 hours after blood sampling. Blood analyses revealed that the platelet count was increased (502 G/l). Normal values were found for red and white blood cell count and blood smear. Global coagulation tests (aPTT, INR) and von Willebrand parameters were normal. The girl is the first child of non-consanguine parents. The mother is easily prone to hematomas after insignificant trauma. During delivery the mother suffered major blood loss due to placenta accreta and received two red blood cell concentrates. There was no bleeding during and after a nose operation. No bleeding diathesis is reported for the father.

2.1 Platelet Functional Analyses

2.1.1 Platelet count and platelet aggregometry: Platelet count was measured using an automated cell counter (Sysmex XN Series, Norderstedt, Germany). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation as described before [17]. Platelet counts in PRP were adjusted to a concentration of 250 G/L with PPP. Platelet aggregometry analyses were performed after stimulation with the platelet agonists: collagen (0,19 mg/mL; Mölab, Germany), adenosine diphosphate (ADP; 20 µmol/L; Mölab, Germany), epinephrine (100 µM Mölab, Germany), and ristocetin (0.6 und 1.5 mg/mL; Mölab, Germany) using APACT 4004 (Labitec, Ahrensburg, Germany).

2.1.2 Flow cytometry: Flow cytometry analysis (Navios, Beckman Coulter, Krefeld, Germany) of CD41, CD42b, CD62P (Beckman Coulter, Krefeld, Germany) and platelet-bound fibrinogen (Dako, Carpinteria, CA) was performed according to standard protocol of LADR GmbH medical care unit (Bremen, Germany).

2.2 Molecular genetic analysis

Written Informed consent was given for all family members. Genomic DNA from EDTA blood was isolated using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). For the index patient all exons of the genes *ITGA2B* and *ITGB3* were amplified by PCR with intronic primers. The purification products were sequenced directly. Analysis was performed by sequence assembly and alignment software Codon Code® Aligner and variant analyzing software Alamut® visual. Variants were classified according to the guidelines of the ACMG (American College of Medical Genetics and Genomics). Family genotyping was done for the two pathogenic variants in *ITGB3*.

3. Results

Platelet aggregometry analysis revealed severely impaired aggregation after stimulation with ADP, adrenaline, collagen and arachidonic acid. Agglutination after stimulation with ristocetin reached normal values (Table 1). Flow cytometry analysis showed reduced basal expression of CD41 (GPIIb/IIIa) compared to healthy control (Figure 1) whereas expression of CD42 (GPIb) was normal. Also CD62 expression was normal after activation with TRAP and ADP. Molecular genetic analysis of the index patient identified two heterozygous variants in exon 10 of *ITGB3* (Table 2). One variant (c.1552C>T) is resulting in a premature Stop Codon at amino acid 518 (p.Gln518*; position without signal peptide: p.Gln492*). The variant is neither listed in population data bases (dbSNP, gnomAD, ESP) nor in disease databases (HGMD public version, Sinai Central, ClinVar).

The second heterozygous variant c.1639T>G leads to an exchange of the highly conserved (up to *C. elegans*) cysteine at position 547 by the physiochemical different glycine (p.Cys547Gly; position without signal peptide: Cys521Gly). This non-synonymous coding variant is found in dbSNP as a not validated entry (rs902952044) and in gnomAD with low allele frequency of 0.0032% (amount of counted allele frequencies: 1/ amount of all alleles: 30984). The variant is listed in HGMD public version. *In silico* prediction is concordant pathogenic in PolyPhen2,

Mutation Taster and SIFT. Family genotyping of Exon 10 identified heterozygous c.1639T>G (p.Cys547Gly) as paternal variant, whereas, c.1552C>T (p.Gln518*) was found maternally. Family genotyping confirmed compound heterozygosity in index patient (Figure 2).

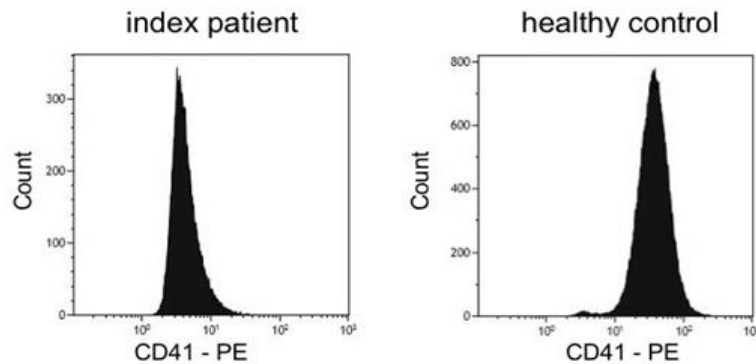


Figure 1: Flow cytometric quantification of platelet surface α IIb β 3 (CD41). Reduced expression of CD41 (Arbitrary unit: 340) in patient's platelets compared to healthy control (Arbitrary unit: 780).

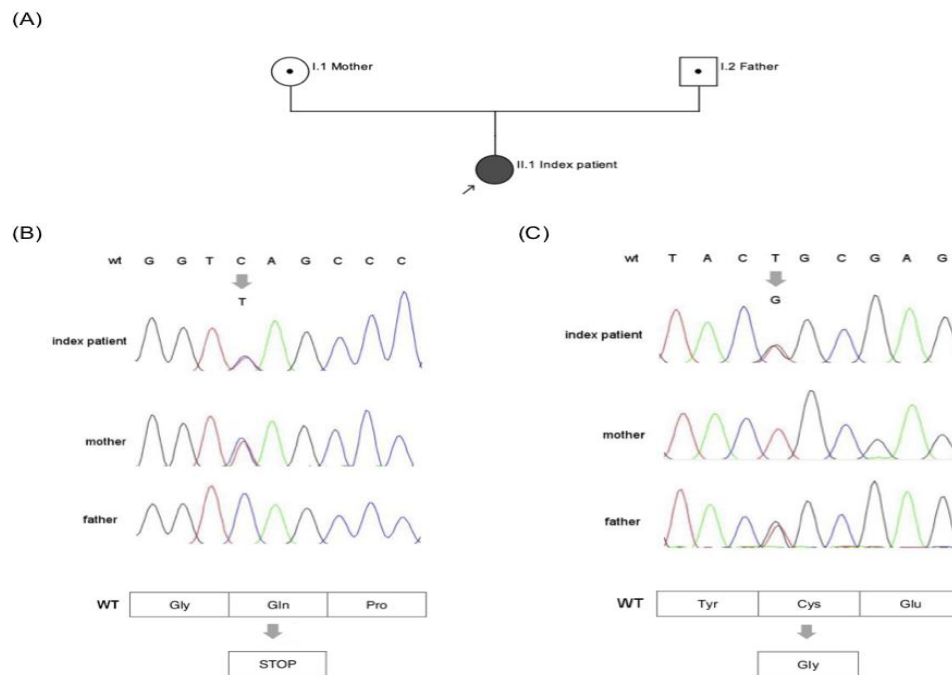


Figure 2: A Pedigree: Parents (I.1 mother and I.2 father) are carrier of heterozygous variants in exon 10; index patient (II.1) is affected compound heterozygous; arrow marks index patient. B Chromatogram (ITGB3, Exon10) shows heterozygous c.1552C>T in index patient and mother, resulting in a premature Stop Codon and wildtype sequence for father (Gln). C Chromatogram (ITGB3, Exon10) shows heterozygous c.1639 T>G in index patient and father and wildtype sequence for mother. Amino acid change is from Cys to Gly.

Inductor	Max. aggregation/agglutination	Normal control
ADP (20µmol)	3%	60-90%
Adrenaline (100 µM).	9%	60-90%
Collagen (0,19 mg/mL)	45%	60-90%
Arachidonic acid (0,5 mg/mL)	3%	60-90%
Ristocetin (0,6 mg/mL)	2%	<20%

Table 1: Platelet aggregometry analysis.

Specification	Variant 1	Variant 2
Nucleotide	c.1552C>T	c.1639T>G
Amino acid	p.Gln518*	p.Cys547Gly
Allele frequency	-	gnomAD: ALL 0.0032%
Variant type	Nonsense	Missense
In silico prediction	-	Pathogenic in PolyPhen2, Mutation Taster, SIFT
Occurrence in HGMD public	-	Yes (Buitrago et al., 2015)
Domain	β3	β3
Class according to ACMG	5 (pathogenic, serious consequences)	5 (pathogenic)
Family genotyping		
Index patient	Heterozygous	Heterozygous
Mother	Heterozygous	WT
Father	WT	Heterozygous

WT, wild type; HGMD access 06/2020

Table 2: Characteristics of the two variants identified in ITGB3 (NM_000212).

4. Discussion

In this study we describe the early onset of Glanzmann Thrombasthenia due to compound heterozygous variants in exon 10 of ITGB3. To our knowledge the nonsense variant has not been reported so far. The nonsense variant c.1552C>T (p.Gln518*) interrupts the reading frame by a premature Stop codon (normal protein length 788 aa). The mRNA produced might be a target for nonsense mediated decay. Due to loss of protein function we classified the variant as pathogenic (Class 5). The second allele carries a heterozygous non-synonymous coding variant c.1639T>G, (p.Cys547Gly). *In silico* prediction was concordant pathogenic in 3 programs. According to UniProt (<https://www.uniprot.org/>) the cysteines at position 547 and 534 form a disulfide bond. The amino acid exchange from cysteine to glycine is likely causing substantial impairment of the tertiary structure due to the loss of the disulfide bond (534-547). The importance of cysteine at position p.547 is supported by the described variant c.1641C>G resulting in exchange from cysteine to Tryptophan (p.Cys547Trp) in a Glanzmann Thrombasthenia

patient [18]. Kannan et al. used molecular modeling and showed that Cys547 is located in the EGF-3 domain of $\beta 3$, which has intense contact with the calf-1 domain of α Ib. They presumed that the loss of Cys547 may cause conformational changes which lead to retention of the aberrant α Ib $\beta 3$ complex [18]. Furthermore, in HEK293 cells expressing the Cys547Gly variant a profound reduction in $\beta 3$ expression was found [19]. Buitrago and colleagues anticipated that Cys547Gly would decrease receptor expression due to the loss of the disulfide bonding. According to these findings we classified the variant as Class 5 (pathogenic).

5. Conclusions

Biochemical and molecular genetic analysis led to early diagnosis for this patient with Glanzmann Thrombasthenia. Early diagnosis helped to treat bleeding symptoms more effectively and improves surgical management. Identifying the disease-causing variants led to a better understanding of molecular genetic mechanisms affecting the α Ib $\beta 3$ integrin receptor. In addition, the results will help regarding the genetic counseling for the parents.

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Conflicts of Interest

We have no conflict of interest to report.

Author Contributions

A.L. and D.B. contributed equally to the study design, performed molecular genetic analyses and data interpretation and wrote the manuscript. M.B. took care of the patient and collected blood samples. K.D. performed platelet aggregometry. J.K. performed flow cytometry analysis. B.Z. contributed to the study design, supervision of the molecular genetic analyses and manuscript editing. All authors critically reviewed the manuscript, with approval of the final draft.

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