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Idtenification Of 5 New Mutations In 9 Glanzmann's Thrombasthenia Patients

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BACKGROUND

Glanzmann Thrombasthenia (GT) is a rare autosomal recessively inherited bleeding syndrome characterized by a lack of platelet aggregation due to a defect within the fibrinogen receptor GPIIb-IIIa. Patients with Glanzmann Thrombasthenia often suffer from severe bleeding complications in case of injury or surgery. Prolonged bleeding time is caused by the diminished, lacking or dysfunctional glycoprotein (GP) IIb-IIIa, which mediates platelet aggregation via fibrinogen..

Concerning this investigation, we performed a molecular and functional characterization of 9 GT patients belonging to 4 unrelated families. In the case of patient GE we were able to perform analysis in first degree relatives as well.

METHODS

Platelet function was analyzed using agonist induced platelet aggregation (ADP, collagen, ristocetin, epinephrin, arachidonic acid). Platelet integrin expression and agonist induced PAC1-expression (resp. fibrinogen binding) on the platelets' surface was investigated using monoclonal fluorescence-labeled antibodies (anti-CD42a, anti-CD42b, anti-CD41/CD61, anti-CD36 and PAC1) and flow cytometry.

Molecular analysis of genomic DNA was performed by polymerase chain reaction and cycle sequencing technique (ABI 310, Applied Biosystems). We analyzed the coding regions of GPIIb (30 exons) and GPIIIa (14 exons) and the flanking splicing site regions. Sequences were compared to wild type sequences applying the DNASIS Max V2.0 Software (Hitachi).

RESULTS

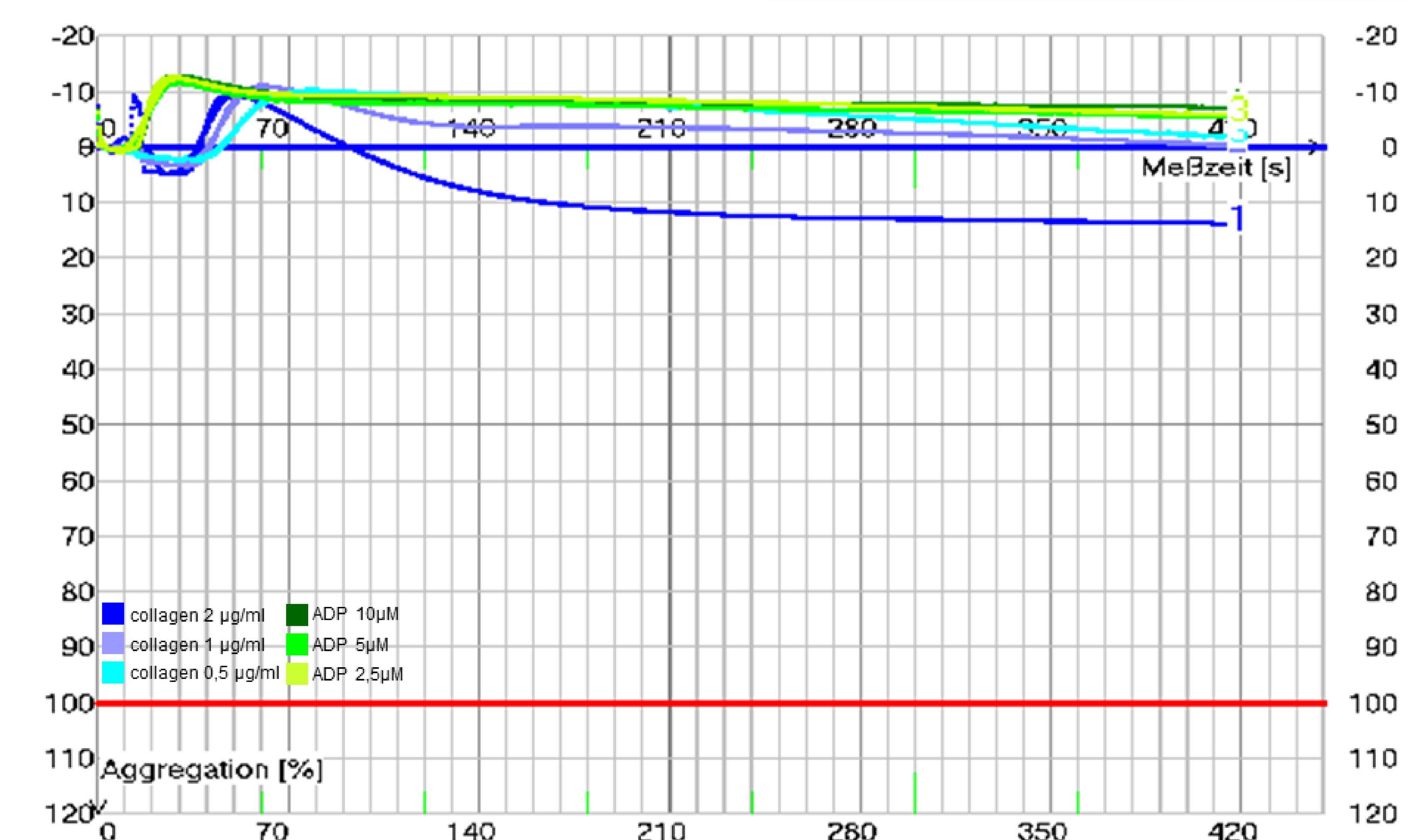

Fig. 1: Aggregation of patient G.E. in response to ADP and collagen. In accordance with a residual receptor expression of less than 1%, the aggregation after stimulation with ADP and collagen was absent. The data concerning the epinephrine, arachidonic acid and ristocetin induced aggregations are not shown, but were performed. Ristocetin-induced aggregation showed a normal pattern.

Patient	Mutation	Subunit	Genotype	Amount of expressed receptor GPIIb/IIIa	Affected domain
GE	missense mutation p. 71 Pro>Arg	GPIIb	compound heterozygous	< 1%	β-Propeller
	missense mutation p.684 Leu>Arg	GPIIb			Calf-1
CM*	missense mutation p.684 Leu>Arg	GPIIb	heterozygous	58%	β-Propeller
CR*	missense mutation p. 71 Pro>Arg	GPIIb	heterozygous	80%	Calf-1
CW+	missense mutation p. 71 Pro>Arg	GPIIb	heterozygous	77%	β-Propeller
CS°	missense mutation p. 71 Pro>Arg	GPIIb	heterozygous	84%	β-Propeller
BM	missense mutation p.176 Pro>Ala	GPIIb	compound heterozygous	N.A.	β-Propeller
	splicing site mutation c. 3092 C>T				intracellular
BJ °	missense mutation p.176 Pro>Ala	GPIIb	compound heterozygous	N.A.	β-Propeller
	splicing site mutation c. 3092 C>T				intracellular
BD	missense mutation p.42 Cys>Trp	GPIIIa	homozygous	< 1%	PSI-domain
WJ	missense mutation p.314 Asp>Ala	GPIIIa	homozygous	< 1%	βA-Domain

Key-explanation: *:parents; °:sibling; +:uncle.

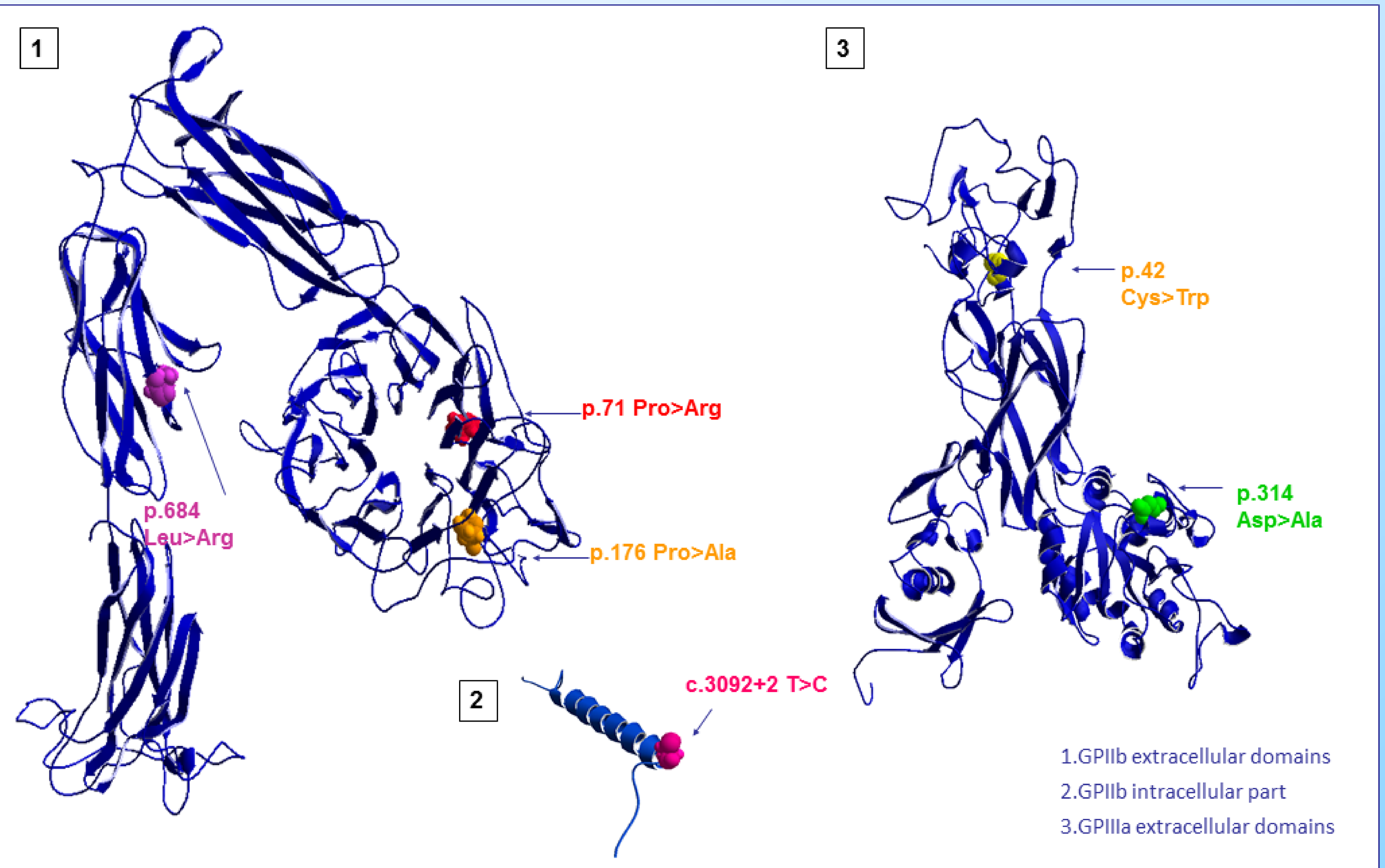

Fig. 2: Schematic illustration of $\alpha_{IIb}\beta_3$ (GPIIb/IIIa). Shown is the distribution of the mutations onto the different domains of α_{IIb} and β_3.

We found 4 different mutations in the α_{IIb} gene and 2 mutations of the β_3 gene in 5 patients. 5 out of 6 mutations are missense mutations. Only one mutation is a splicing site defect.

Overall, 2 patients resulted to be homozygous for a mutation in the β_3 gene. 3 patients are compound heterozygous with both mutations located in the α_{IIb} subunit and 4 patients have a heterozygous genotype with a single mutation in the α_{IIb} subunit.

Tab. 1: This table shows onto which domains, within the two subunits, the various mutations are distributed. Most of the detected mutations are found in the β-propeller of the GPIIb subunit.

The GPIIb subunit consists out of 5 different regions. The GPIIIa subunit consists out of 3 domains and 4 EGF repeating regions. The EGF repeating units are characterized by several disulfide bonds that are inevitable for the complexes' structure. One of the missense mutations, located in the PSI domain of the GPIIIa subunit, leads to an amino acid exchange of cysteine to tryptophan in position 42 and therefore disrupts one disulfide-bond.

Furthermore, the table contains information concerning the genotype of all patients and the residual receptor expression of the fibrinogen receptor GPIIb/IIIa.

CONCLUSION

In the present investigation 5 novel and 1 previously described gene alterations, that is known to cause GT, were identified in 4 unrelated patients and their first-degree relatives. To ascertain the mutations we sequenced the coding regions of α_{IIb} and β_3 subunits including the exon/intron boundaries.

Molecular analysis revealed five point mutations which are all non-conservative substitutions in strictly conserved residues among vertebrates in both genes. One of the mutations in the β_3 subunit leads to the exchange of cysteine in position 42 to tryptophan. The cysteine in position 42 forms a disulfide bond with cysteine 75, which cannot be formed. This could lead to a disruption of the three dimensional structure of the protein and inhibit the congregation with the α_{IIb} subunit. Therefore the protein complex cannot be expressed on the platelet's surface.

In one pair of siblings (BM and BJ), we could again identify the splicing site mutation c.3092+2 C>T. This splicing site defect leads to a complete loss of exon 29, proven by cDNA-sequencing. The mutations are located in the cytoplasmatic domain proximal to the transmembrane region, in the N-terminal area of the GFFKR-motif. Regions proximal to the transmembrane domains of α and β subunits contain highly conserved sequences, such as the GFFKR-motif referring the α_{IIb} subunit, and are found to be important for maintaining the inactive, low affinity, state of the integrin.

In conclusion, our investigation of several patients revealed a number of different mutations within the α_{IIb} and β_3 subunits associated with causing GT. This vast number of different mutations indicates the genetic heterogeneity in the studied group, respectively in GT.

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