

The co-inheritance of two *ITGB3* variants with additive detrimental effects on platelets leads to variant Glanzmann thrombasthenia

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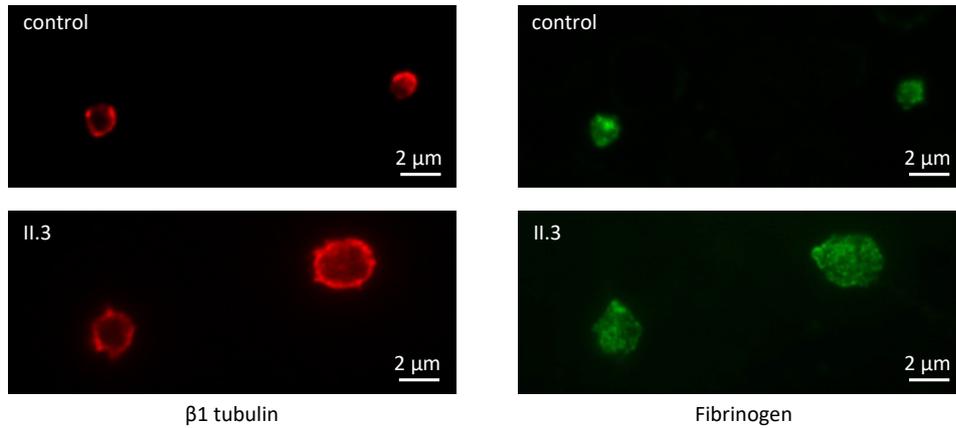
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Supplementary Figure 1

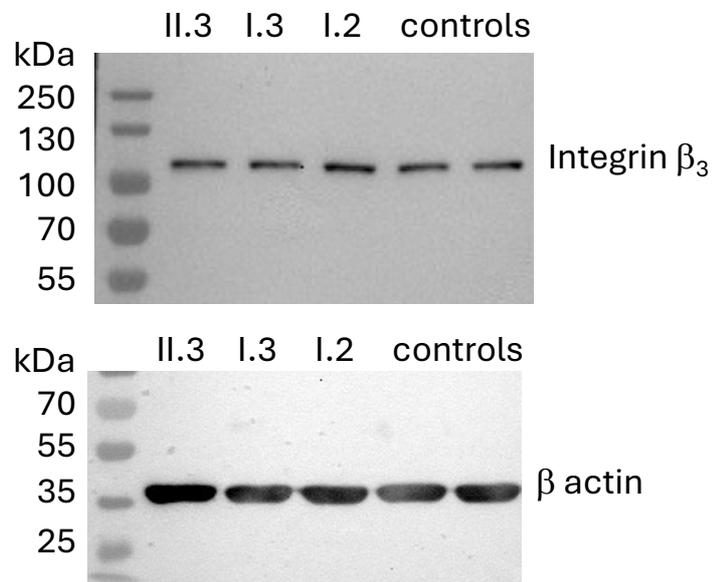
A



B

Median Fluorescence ± SD	CD42b*PE	CD49b*FITC	GPVI*APC
II.3 (proband) (n=3)	51530 ± 8202	1054 ± 783	4506 ± 702
I.1 (aunt) (n=2)	45874 ± 15748	2442 ± 36	6054 ± 982
I.2 (mother) (n=2)	35316 ± 12280	2183 ± 588	4261 ± 340
I.3 (father) (n=2)	32650 ± 721	2249 ± 368	4131 ± 703
II.1 (brother) (n=1)	26928	2501	5119
II.2 (brother) (n=1)	22575	1657	4694
II.4 (cousin) (n=1)	24447	1455	3613
Controls (n=4)	29051 ± 8785	1422 ± 881	3509 ± 437

C



Legend to supplementary Figure 1

A) Fibrinogen content in platelets from the proband (II.3) and a healthy control. β_1 tubulin is stained red to detect platelets (anti- β_1 tubulin from Sigma Aldrich, cat. N. M8064 diluted

1:10000 as primary antibody, goat anti-rabbit Alexa Fluor 568 from Life Technologies as secondary antibody), fibrinogen is stained green (anti-fibrinogen gamma chain from Proteintech, cat. N. 66158 diluted 1:200 as primary antibody, goat anti-mouse Alexa Fluor 488 from Life Technologies as secondary antibody). Immunofluorescence was performed on blood smears fixed with acetone and stored at -20°C.

B) Platelet expression of membrane glycoproteins (GPs) GPIb α (CD42b), GPIa (integrin α 2, CD49b) and GPVI was evaluated by flow cytometry in citrated whole blood diluted 1:10 in Tyrode buffer (PBS) using specific antibodies (all from BD Biosciences, Madrid, Spain). Diluted blood was incubate for 30 minutes at room temperature with, the different antibodies, then samples were diluted with PBS and run in a BD Accuri™ C6 flow cytometer (BD Biosciences, Ann Arbor, MI, USA). The median fluorescence intensity (MFI) was analysed using BD Accuri™ C6 software8.

C) β ₃ expression as assessed by Western blotting in lysates of platelets from the proband (II.3), his father (I.3), his mother (I.2), and two healthy controls (controls). The anti- β ₃ antibody is from Santa Cruz Biotechnology, catalogue number sc-365679 (1:500). The anti- β actin antibody is from Cell Signaling (1:2000).

Supplementary Table 1

Gene	Variant	Variant allele frequency (VAF)						Coverage						Minor Allele Frequency (MAF)
		Proband (II.3)	Father (I.3)	Mother (I.2)	Brother (II.1)	Brother (II.2)	Aunt (I.1)	Proband (II.3)	Father (I.3)	Mother (I.2)	Brother (II.1)	Brother (II.1)	Aunt (I.1)	
ABL1	NM_005157.6 c.3140C>T [p.Ser1047Phe]	0,514	0	0,512	0	0	0,457	177	139	160	133	123	142	-
ACE	ENST00000290866.10 c.503T>C [p.Leu168Pro]	0,436	0	0,342	0	0	0,642	71	61	70	60	58	42	0,000409508
LINC01237	NM_001382368.1 c.287G>C [p.Ter96Serext*46]	0,5	0	0,580	0	0	0,454	106	93	81	76	70	66	0,00039664
MMAB	NM_052845.4 c.569G>A [p.Arg190His]	0,355	0	0,567	0	0	0,258	45	34	37	26	36	31	1,85119E-05
RP1	NM_006269.2 c.4780T>C [p.Tyr1594His]	0,49	0	0,425	0	0	0,491	100	105	101	67	91	57	1,43658E-05
SLC26A5	NM_198999.3 c.1433C>T [p.Ser478Phe]	0,567	0	0,250	0	0	0,571	37	40	40	24	41	28	2,05286E-06
ITGB3	NM_000212.3 c.992A>G [p.Asn331Ser]	0,351	0	0,384	0	0	0,257	54	51	39	42	35	19	-
ITGB3	NM_000212.3 c.59T>G [p.Leu20Arg]	0,491	0,407	0	0	0,573	0	59	54	75	55	75	46	-

Supplementary table 1: Whole exome sequencing carried out on DNA of all family members. Gene variants segregating with thrombocytopenia are shown in bold. These variants were found to be heterozygous in the proband, mother and aunt, and absent in the father and brothers. Variants were considered heterozygous when VAF was 0.25-0.7. Variant coverage and population frequency (MAF<0.01) are also shown. Of these genes, only *ITGB3* has a recognised role in inherited thrombocytopenia. The cousin (II.4) was not included in this analysis.

Supplementary Table 2

	% MKs (CD42+) (mean±SD)	$\alpha_{IIb}\beta_3$ surface expression (CD41 MFI±SD)
II.3 (proband)	14.3±4.4%	73652±4468
I.1 (aunt)	28.6±5.8%	143703±81608
I.2 (mother)	18.7±2.3%	174643±40147
I.3 (father)	28.1±2.7%	198520±118431
II.1 (brother 1)	17±0.87%	231752±39157
II.2 (brother 2)	13.3±3.2%	162548±87403
II.4 (cousin)	5.9±1.6%	156849±82085
Controls	19.87±14%	248522±81633