

Supplementary Material

Table S1. Bleeding questionnaire and bleeding scores for the patients II-1, II-2, and III-1 with BSS according to [17].

Symptom	-1	0	1	2	3	4	Score II-1	Score II-2	Score III-1
Epistaxis	-	No or trivial (<5)	>5 OR more than 10'	Consultation only	Packing or cauterization or antifibrinolytics	Blood transfusion or replacement therapy	3	1	1
Cutaneous	-	No or trivial (<1cm)	>1cm AND no trauma	Consultation only			N/A	N/A	N/A
Bleeding from minor wounds	-	No or trivial (<5)	> 5 OR more than 5'	Consultation only or sterile strips	Surgical hemostasis or antifibrinolytics	Blood transfusion or replacement therapy	1	1	1
Oral Cavity	-	No	Reported at least one	Consultation only	Surgical hemostasis or antifibrinolytics	Blood transfusion or Replacement therapy	1	1	N/A
GI Bleeding	-	No	Identified cause	Consultation or Spontaneous	Surgical hemostasis, antifibrinolytics, blood transfusion	Replacement therapy	N/A	N/A	N/A
Tooth extraction	No bleeding in at least 2 extractions	None done, No bleeding in 1	Reported, no consultation	Consultation only	Resuturing, repacking or antifibrinolytics	Blood transfusion or Replacement therapy	1	1	1
Surgery	No bleeding in 2 surgeries	None done, No bleeding in 1	Reported, no consultation	Consultation only	Surgical hemostasis or antifibrinolytics	Blood transfusion or Replacement therapy	N/A	N/A	N/A
Menorrhagia	-	No	Reported, or consultation only	Antifibrinolytics or Pill use	D&C, Iron therapy	Blood transfusion or Replacement therapy or Hysterectomy	N/A	1	N/A
Post-partum hemorrhage	No bleeding in at least 2 deliveries	No deliveries, No bleeding in 1 delivery	Reported, or consultation only	D&C, Iron therapy, Antifibrinolytics	Blood transfusion or replacement therapy	Hysterectomy	N/A	N/A	N/A
Muscle hematomas	-	Never	Post trauma no therapy	Spontaneous no therapy	Spontaneous or traumatic, requiring replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion	N/A	N/A	N/A
Hemarthrosis	-	Never	Post trauma no therapy	Spontaneous no therapy	Spontaneous or traumatic, requiring replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion	N/A	N/A	N/A
CNS Bleeding	-	Never	-	-	Subdural, any intervention	Intracerebral, any intervention	N/A	N/A	N/A
-	-	-	-	-	-	Bleeding score:	6	5	3

Not Available (N/A)

Table S2. Heterozygous gene variants from WES analysis filtered for the macrothrombocytopenia phenotype. Reference genome: GRCh38; variants and indels were filtered for coding regions and splice sites; minimal coverage 15×. These gene variants were identified in both patients (II-1, II-2), which were completely absent in healthy individuals (III-2, III-3).

Genomic coordinate	Gene	RefSeq	cDNA	Protein	II-1 VAF [%]	II-2 VAF [%]
62728902	<i>KANK4</i>	NM_001320269	c.517T>C	p.Y173H	52.94	40.00
145608556	<i>POLR3C</i>	NM_001303456	c.290G>A	p.R97Q	39.02	35.29
148004763	<i>NBPF26</i>	NM_001351372	c.3796G>A	p.E1266K	41.07	46.15
153012650	<i>SPRR2D</i>	NM_006945	c.173C>T	p.T58I	41.03	55.56
153430347	<i>S100A7</i>	NM_002963	c.241G>T	p.D81Y	58.62	58.33
156106994	<i>LMNA</i>	NM_001282625	c.1579C>T	p.R527C	93.40	55.38
161332307	<i>SDHC</i>	NM_001035511	c.430G>C	p.E144Q	37.93	48.84
201179738	<i>IGFN1</i>	NM_001164586	c.5717C>A	p.A1906E	46.67	30.77
43768308	<i>THADA</i>	NM_001345925	c.3254C>T	p.T1085M	50.00	42.50
54120827	<i>PSME4</i>	NM_014614	c.4022G>A	p.R1341H	62.86	37.50
54155315	<i>PSME4</i>	NM_014614	c.1442T>C	p.M481T	43.82	50.00
152404198	<i>NEB</i>	NM_001164507	c.20212G>A	p.D6738N	63.04	55.88
152404248	<i>NEB</i>	NM_001164507	c.20162T>C	p.L6721P	56.76	57.14
152530992	<i>NEB</i>	NM_001164507	c.3986A>C	p.D1329A	50.00	43.55
160634452	<i>CD302</i>	NM_001198759	c.5416T>C	p.S1806P	31.25	56.25
186653566	<i>FSIP2</i>	NM_173651	c.1703A>T	p.Y568F	51.14	61.54
186665996	<i>FSIP2</i>	NM_173651	c.11963C>T	p.S3988F	43.24	52.73
13600704	<i>BOD1L1</i>	NM_148894	c.7820A>T	p.N2607I	50.91	36.00
47574926	<i>ATP10D</i>	NM_020453	c.3278A>G	p.K1093R	47.06	30.43
62812764	<i>ADGRL3</i>	NM_001322402	c.2552C>T	p.T851M	54.43	43.75
88084724	<i>KLHL8</i>	NM_001292003	c.1807_1810del	p.T603fs	46.30	51.61
108868556	<i>CYP2U1</i>	NM_183075	c.1151G>T	p.R384I	51.46	44.44
155533337	<i>FGG</i>	NM_000509	c.140C>T	p.T47I	39.02	60.71
54786796	<i>PLPP1</i>	NM_176895	c.205A>G	p.I69V	77.78	46.88
74412411	<i>ANKRD31</i>	NM_001164443	c.3964T>C	p.S1322P	54.10	43.33
79616698	<i>SPZ1</i>	NM_032567	c.664G>A	p.E222K	45.83	50.00
151180378	<i>G3BP1</i>	NM_198395	c.1142G>A	p.G381D	37.84	46.81
42858231	<i>C6orf226</i>	NM_001008739	c.296T>G	p.V99G	39.13	30.77
42931129	<i>GNMT</i>	NM_018960	c.658A>T	p.T220S	47.73	39.29
76023429	<i>FILIP1</i>	NM_001289987	c.2127_2128insGG	p.R710fs	55.21	52.27
17379805	<i>AHR</i>	NM_001621	c.2356A>G	p.M786V	47.37	52.17
141813647	<i>PTK2</i>	NM_005607	c.1036C>T	p.P346S	47.22	41.46
144810414	<i>FAM83H</i>	NM_198488	c.1217C>A	p.A406D	43.75	39.13
134351730	<i>PRRC2B</i>	NM_013318	c.4214T>G	p.L1405R	40.00	69.23
139265345	<i>CARD9</i>	NM_052814	c.575A>G	p.Q192R	59.09	50.00
96162517	<i>TBC1D12</i>	NM_015188	c.147_164del	p.49_55del	46.15	30.77
3141665	<i>OSBPL5</i>	NM_020896	c.592G>A	p.V198I	60.47	42.86
7712604	<i>OVCH2</i>	NM_198185	c.1535C>G	p.T512S	51.85	53.85
10862595	<i>YBX3</i>	NM_003651	c.692A>C	p.Q231P	60.71	40.00
15777273	<i>EPS8</i>	NM_004447	c.2113G>A	p.A705T	45.45	57.14

26217934	<i>RASSF8</i>	NM_001164747	c.607G>C	p.E203Q	51.43	48.89
96379884	<i>HAL</i>	NM_001258334	c.1106G>A	p.R369Q	53.85	50.00
123057763	<i>KNTC1</i>	NM_014708	c.2214G>C	p.E738D	52.54	51.72
4835997	<i>GP1BA</i>	NM_000173	c.98G>A	p.C33Y	55.00	60.00
74003318	<i>EVPL</i>	NM_001320747	c.6034C>T	p.R2012W	64.71	40.74
78306001	<i>RNF213</i>	NM_001256071	c.3713G>A	p.R1238Q	47.27	68.29
7049171	<i>LAMA1</i>	NM_005559	c.674G>A	p.R225H	47.06	39.66
8825397	<i>MTCL1</i>	NM_015210	c.3889T>C	p.S1297P	41.38	50.00
8825398	<i>MTCL1</i>	NM_015210	c.3890C>T	p.S1297F	39.29	51.35
54423856	<i>WDR7</i>	NM_015285	c.2032G>T	p.A678S	34.21	50.00
17212814	<i>MYO9B</i>	NM_001130065	c.287A>G	p.Q96R	42.22	53.85
18980866	<i>GDF1</i>	NM_001492	c.251G>A	p.G84E	50.00	50.00
19033544	<i>DDX49</i>	NM_019070	c.767A>G	p.N256S	41.38	60.00
43990796	<i>PHLDB3</i>	NM_198850	c.1393G>A	p.A465T	39.39	54.84
53116748	<i>ZNF83</i>	NM_001348019	c.1070A>G	p.N357S	50.91	53.33
53912045	<i>ZNF765</i>	NM_001040185	c.1237T>G	p.C413G	34.25	39.47
42315606	<i>MYBL2</i>	NM_002466	c.394G>C	p.E132Q	48.75	60.00
153220494	<i>HCFC1</i>	NM_005334	c.3270_3356del	p.1090_1119del	48.65	56.25

VAf – variant allele frequency

Table S3. Mean fluorescence intensity of antigens expression on platelets in patients II-1, II-2 and one healthy individual III-3.

MFI	II-1		II-2		III-3	
FSC	4566		7118		4480	
CD41	14431	99.3%	13253	99.3%	12656	99.6%
CD61	4886	99.3%	7468	99.3%	3406	99.6%
CD42a (GPIX)	1814	98.9%	3005	98.9%	3803	99.6%
CD42b (GPIbα)	1113	98.5%	4287	98.9%	4622	99.4%
CD9	1236	50.4%	1082	54.1%	5560	99.6%

Mean fluorescence intensity (MFI), forward scatter (FSC); reference limits for percentage values (in-house reference level): CD41- 80-99%, CD61- 75-99%, CD42a- 66-99%, CD42b- 70-98%, cut-of limit for MFI >100.

Table S4. Virtual panel of inherited thrombocytopenia related genes based on the literature review [27].

ABCA12	BLOC1S2	FHOD1	ITGA2	MYO3A	PPP1CB	RASGRP2	STXBP4
ABCB4	BLOC1S3	FLI1	ITGA2B	MYO5A	PPP1CC	RBM8A	STXBP5L
ABCC4	BLOC1S4	FLII	ITGA5	MYO5B	PPP1R12A	RGS10	STXBP6
ABCG5	BLOC1S5	FLNA	ITGB1	NAPA	PPP1R12C	RGS18	SUZ12
ABCG8	BLOC1S6	FMNL1	ITGB3	NAPG	PPP1R14A	RGS19	SYK
ACSL4	BMP4	FMNL3	ITPR1	NBEA	PPP1R2	RGS20	SYTL3
ACTN1	BTBD9	FRMPD1	JAK2	NBEAL2	PRKACA	RGS9	SYTL4
ACVRL1	BTK	FYB	JMJD1C	NFE2	PRKACB	RHOA	TAL1
ADAMTS13	C14orf133	FYN	KIAA1109	NIPSNAP3A	PRKACG	RHOC	TAOK1
ADCY3	C19orf55	GATA1	KIAA2018	NOTCH1	PRKAR1A	RHOF	TBXA2R
ADCY6	C20orf42	GDI2	LAIR1	NOX1	PRKAR2A	ROCK1	TEC
ADCY7	C6orf25	GFI1	LAT	NRG3	PRKCA	ROCK2	TGFBR3
ADORA2B	CD226	GFI1B	LCP2	NSF	PRKCB	RUNX1	THPO
ADRA2A	CD36	GNAI2	LPAR1	NXF1	PRKCD	SCAMP2	TLN1
ADRA2B	CLEC1B	GNAI3	LTBP1	ORAI1	PRKCQ	SCAMP5	TLR2
ADRBK1	CLEC4F	GNAI1	LY6G6F	P2RX1	PRKD1	SCFD1	TMCC2
AK3	CNO	GNAI2	LYN	P2RY1	PRKG1	SELP	TPM1
AKT1	CSK	GNAQ	LYST	P2RY12	PRKG2	SERPINE2	TPM4
AKT2	CTTN	GNAZ	MAP2K2	P2RY13	PTEN	SH2B3	TRAF4
ALOX12	CYCS	GNB2	MAP2K4	PADI2	PTGIR	SIRPA	TREML1
ANKRD12	DAAM1	GNB3	MAP3K9	PDE2A	PTGS1	SLC35D3	TRPM7
ANKRD18A	DIAPH1	GNE	MAPK1	PDE3A	PTK2	SLC9A3R1	TTC37
ANKRD18B	DIAPH2	GNG11	MAPK13	PDE4D	PTPN1	SLC9A3R2	TTF2
ANKRD26	DIAPH3	GNG12	MAPK14	PDE5A	PTPN11	SLFN14	TUBA3C
ANKRD33	DNAH11	GNG13	MAPK8	PDPK1	PTPN12	SMAD1	TUBB1
AP3B1	DNM1L	GNG5	MDS1	PDZD3	PTPN18	SMAD6	UNC13A
AP3D1	DNM2	GP1BA	MECOM	PDZK1	PTPN2	SNAP23	UNC13B
AP3M1	DNM3	GP1BB	MKL1	PEAR1	PTPN6	SNAP25	VAMP2
AP3S1	DTNBP1	GP5	MLK1	PECAM1	PTPN7	SNAP29	VAMP3
APC	EFNB1	GP6	MLPH	PGM3	PTPN9	SNAPIN	VAMP7
ARHGAP1	EPHA4	GP9	MMP17	PHOX2A	PTPRA	SNX1	VAMP8
ARHGAP17	EPHB1	GRAP2	MNX1	PIK3CA	PTPRC	SRA1	VAV1
ARHGAP32	ERG	GRB2	MPL	PIK3CB	PTPRJ	SRC	VAV2
ARHGAP6	ETS1	GRK5	MRPS34	PIK3CD	RAB27A	SRF	VAV3
ARHGDIA	ETV6	GRK6	MUC16	PIK3CG	RAB27B	STIM1	VPS11
ARHGDIB	EXOC1	GUCY1A3	MUC2	PIK3R1	RAB38	STOM	VPS16

<i>ARHGEF12</i>	<i>F2R</i>	<i>GUCY1B3</i>	<i>MUTED</i>	<i>PIK3R3</i>	<i>RAB4A</i>	<i>STX11</i>	<i>VPS18</i>
<i>ARHGEF3</i>	<i>F2RL3</i>	<i>HBB</i>	<i>MYB</i>	<i>PIK3R5</i>	<i>RABGGTA</i>	<i>STX12</i>	<i>VPS33A</i>
<i>ARRB1</i>	<i>FARP2</i>	<i>HOOK3</i>	<i>MYH10</i>	<i>PLA2G4A</i>	<i>RAC1</i>	<i>STX2</i>	<i>VPS33B</i>
<i>ASPN</i>	<i>FCER1G</i>	<i>HOXA11</i>	<i>MYH13</i>	<i>PLA2G4C</i>	<i>RAF1</i>	<i>STX4</i>	<i>VPS39</i>
<i>BAK1</i>	<i>FCGR2A</i>	<i>HPS1</i>	<i>MYH9</i>	<i>PLCB2</i>	<i>RAI1</i>	<i>STX6</i>	<i>VPS41</i>
<i>BCL2L1</i>	<i>FERMT1</i>	<i>HPS4</i>	<i>MYL9</i>	<i>PLCB3</i>	<i>RAP1B</i>	<i>STX7</i>	<i>VPS4B</i>
<i>BCOR</i>	<i>FERMT3</i>	<i>HTR2A</i>	<i>MYLK</i>	<i>PLCG2</i>	<i>RAP1GAP</i>	<i>STXBP1</i>	<i>VPS52</i>
<i>BET1L</i>	<i>FGD3</i>	<i>CHD3</i>	<i>MYLK2</i>	<i>PLDN</i>	<i>RAP1GAP2</i>	<i>STXBP2</i>	<i>VPS8</i>
<i>BLOC1S1</i>	<i>FGR</i>	<i>INPP5D</i>	<i>MYO18B</i>	<i>PPP1CA</i>	<i>RAP1GDS1</i>	<i>STXBP3</i>	<i>VWF</i>
					<i>ZFPMI</i>	<i>WDR66</i>	<i>WAS</i>

Table S5. The reference limits and maximum aggregation results in the patients II-1 and II-2 (2017).

Name of agonists	Reference limits	Patient II-1	Patient II-2
Platelet count in PRP (* 10 ⁹ /L)	150-300	58	102
Platelet count in PPP (* 10 ⁹ /L)	0-20	0	1
Aggregation - collagen			
Collagen 2 - Amax (%)	74.5 - 87.3	23.7	71.5
Collagen 5 - Amax (%)	74.7 - 88.9	45.3	75.8
Aggregation - ADP			
ADP 5 - Amax (%)	57.0 - 86.2	28	48.9
ADP 5 - disaggregation (%)	0 - 10.0		
ADP 10 - Amax (%)	66.6 - 90.7	31.3	69.1
ADP 10 - disaggregation (%)	0 - 10.0		
Aggregation - ristocetin			
Ristocetin - Amax (%)	77.8 - 97.1	20.2	20.3
Ristocetin correction - Amax(%)	N.A.	39.5	36.4
Low ristocetin - Amax (%)	0.2 - 4.7	1.4	2.5
Aggregation - arachidonic acid			
Arachidonic acid - Amax (%)	73.2 - 89.6	6.7	71.5
Spontaneous aggregation	less than 5	N.D.	N.D.

The reference limits were determined based on the results of the 2.5 - 97.5 percentile. Reference aggregation limits were obtained from 50 peripheral blood donors. Not applicable N.A., not done N.D.