Webinars

Constitutional thrombocytopenia

EuroBloodNet

Constitutional thrombocytopenia related to GPIb-IX-V complex defects

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Verona – Italy
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for rare or low prevalence complex diseases
Network Hematological Diseases (ERN EuroBloodNet)

Conflicts of interest

No conflicts of interest to declare

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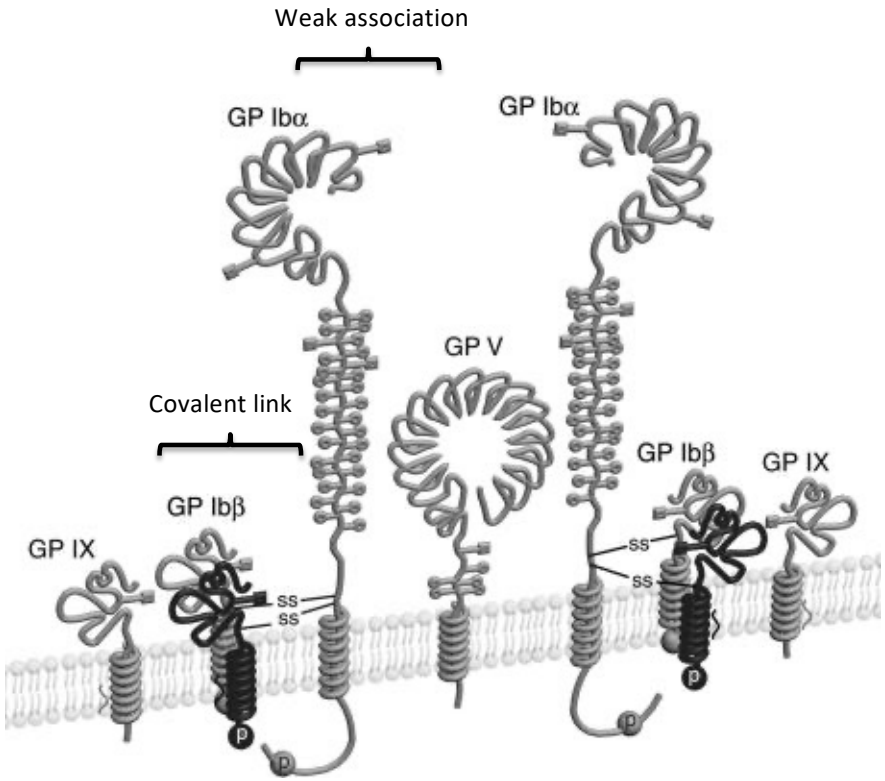
1. Features of the GPIb-IX-V receptor
2. Biallelic Bernard-Soulier syndrome (bBSS)
3. Monoallelic Bernard-Soulier syndrome (mBSS)
4. Diagnostic approach
5. Other disease associated: Platelet-type von Willebrand disease

GPIb-IX complex

- Expressed only on megakaryocytes and platelets
- Second most abundant adhesion receptor on platelets
- Involved in diverse functions
  - Primary haemostasis
  - Thrombopoiesis
  - Platelet clearance
  - Others



Luo et al. Blood 109:603, 2007

Subunits GPIbα, GPIbβ, and GPIX (in a ratio of 1:2:1)

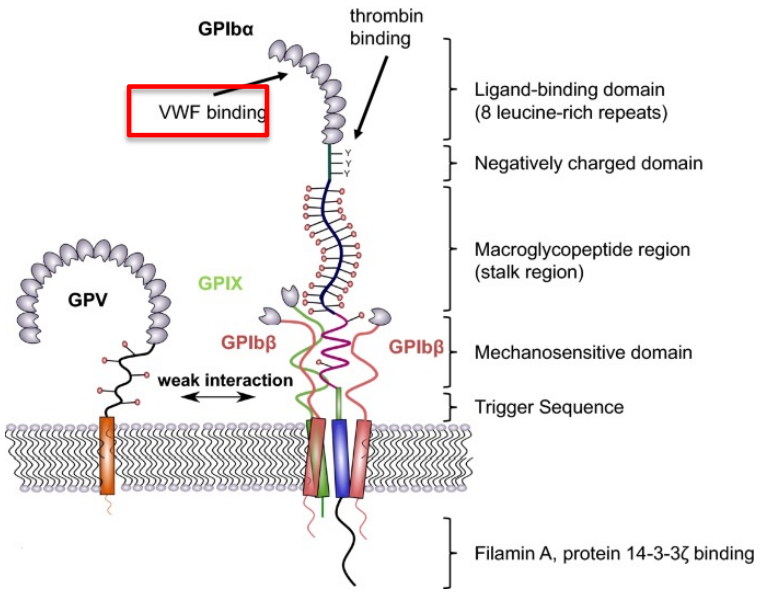
- associate in the endoplasmic reticulum
- mature in the Golgi apparatus
- translocate to the plasma membrane

Expression and correct assembly of all three subunits are necessary for the expression of the complex on cell surface

On the platelet surface, GPIb-IX weakly interacts with GPV in a ratio of 2:1

Features and structure of GPIb-IX-V subunits

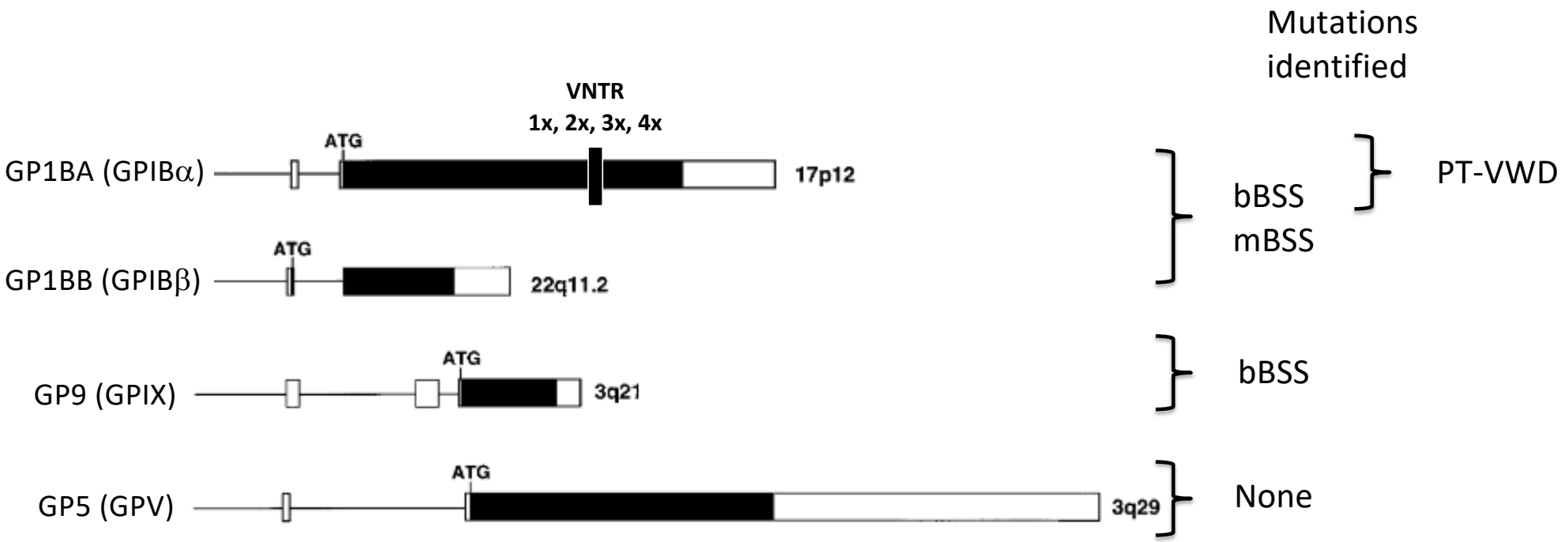
Subunit/Gene	GPIbα GP1BA (17p12)	GPIbβ GP1BB (22q11.2	GPIX GP9 (3q21)	GPV GP9 (3q21)
	Leucine-rich repeated (LRR) proteins			
Amino acids (kDa)	652 (3 VNTR) (135 kDa)	206 (25 kDa)	177 (17 kDa)	560 (83 kDa)
Signal peptide	16	26	16	16
Extracellular glycosylated domain	515 (17-531) 8 LRR	121 (27-147) 1 LRR	131 (17-147) 1 LRR	507 (17-523) 15 LRR
Transmembrane domain	21 (532-552)	25 (148-172)	21 (148-168)	21 (524-544)
Cytoplasmic tail	100 (553-652)	34 (173-206)	9 (169-177)	16 (545-560)
Interactions among subunits	Transmembrane non-covalent interactions			Weakly associated with GPIbα by interactions in the transmembrane domain
	Disulphide bonds between GPIbα and each subunit of GPIbβ			



Bendas & Schlesinger. Exp Hematol Oncol 11:19, 2022

Ristocetin: an antibiotic that triggers binding of VWF to GPIbα inducing platelet agglutination (RIPA test), used for diagnosis

Genes encoding the GPIb-IX-V subunits

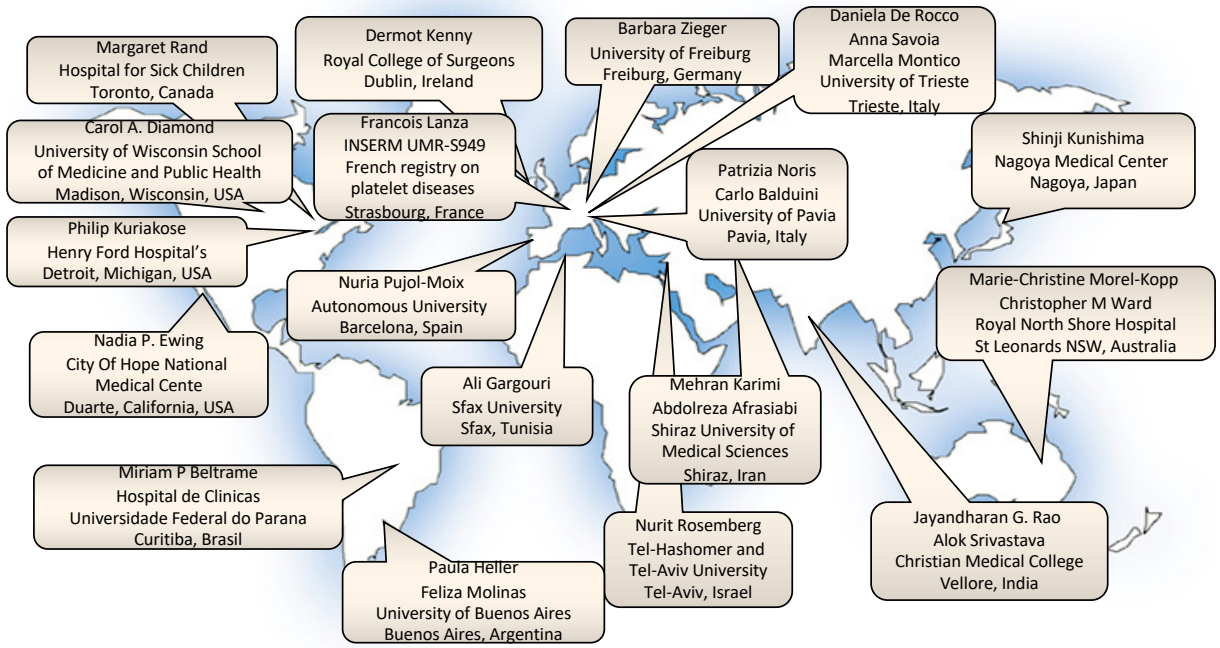


Adapted from Lopez et al. Blood 91:4397, 1998

Bernard-Soulier syndrome

Rare disease: 1 per 1 million live births

International Bernard-Soulier Consortium (2014)



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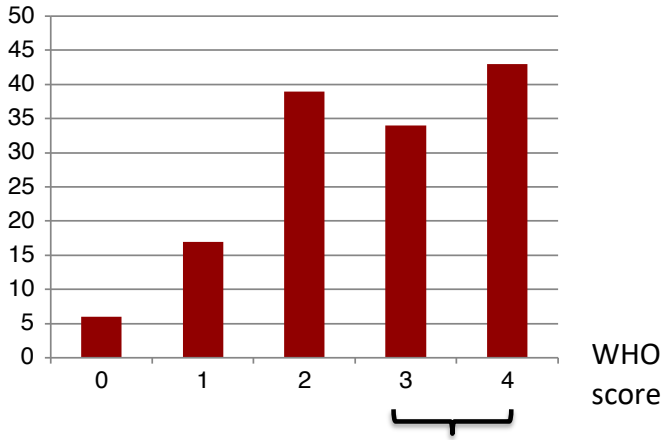
Savoia et al. Hum Mut 35:1033, 2014

Features of patients with biallelic BSS (bBSS)

(161 patients from 121 unrelated families)

- Age at diagnosis: mean **16 years** (range birth - 75 ys)
- Moderate/severe thrombocytopenia
  Cell counters: mean **51x10^9/L**; range 5 - 175x10^9/L
- Platelet macrocytosis
  - MPV: mean **14.8 fL**; range from 9.3 - 27 fL
  - Diameter: mean **4.8 µm;** range 2.9 to 7.5
  (*normal values: 2.4 µm, range 1.9 to 3.4*)
- Absent (80% of pts) expression of GPIb-IX
- Severe platelet function: absent or markedly reduced **RIPA**
- Variable (moderate to severe) bleeding tendency since the first years of life (independent of platelet count)
- **Misdiagnosis ITP (50%)**



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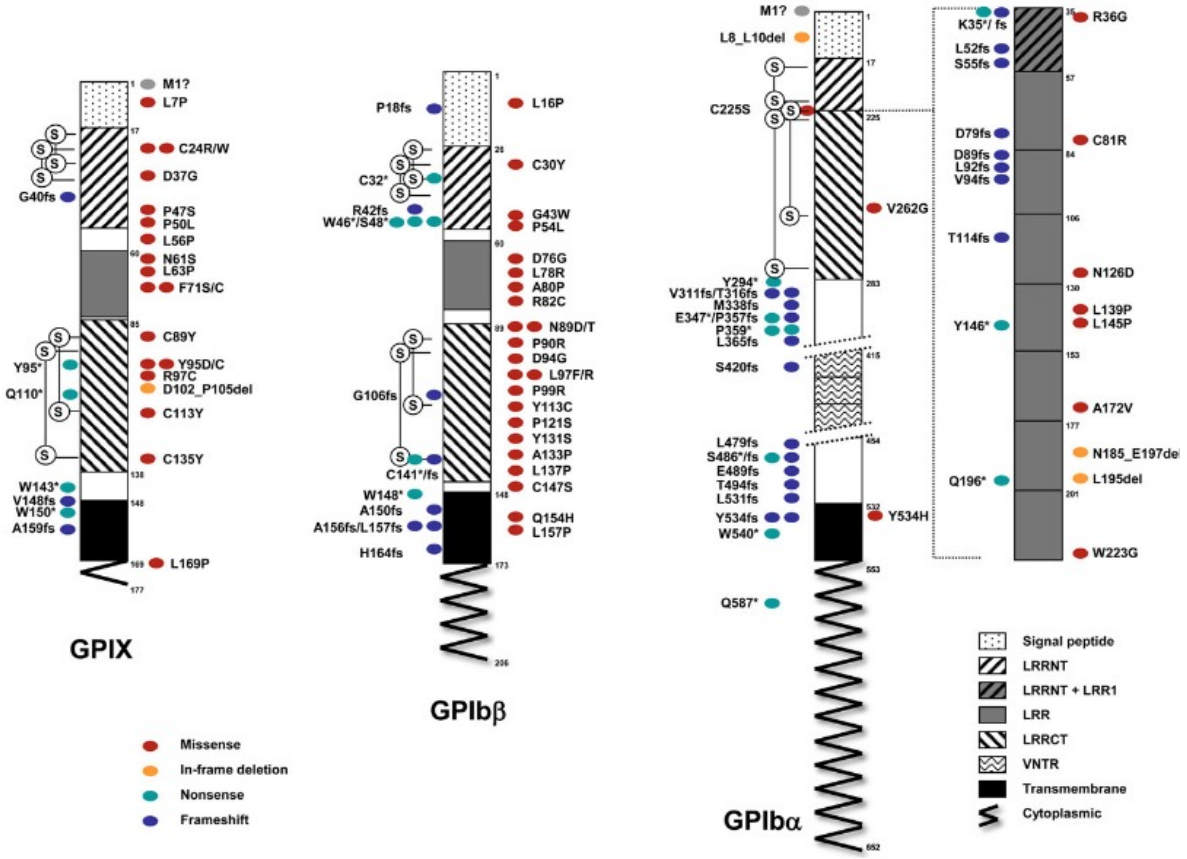
Wide spectrum of point mutations of GP1BA, GP1BB, and GP9 genes

Point mutations (N=112)
Coding regions
- Missense
- Nonsense
- Frameshift

Promoter (N=1)
No splicing

Homozygous (85%)
Compound heterozygous (13%)
Some founder effect

} Rare disease



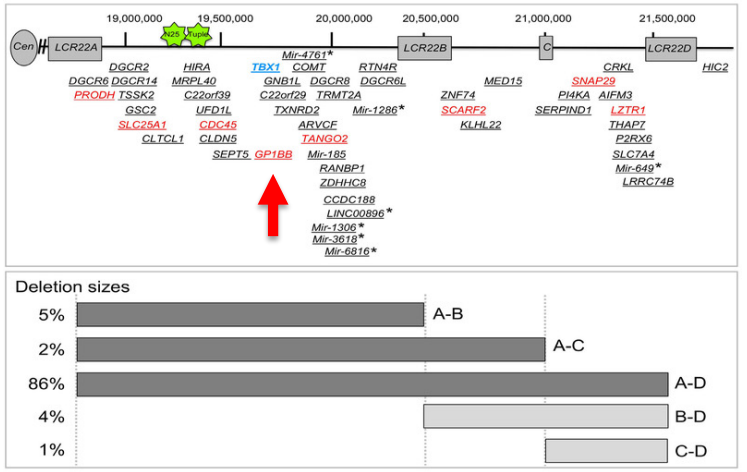
Savoia et al. Hum Mut 35:1033, 2014

bBSS: hemizygosity of GP1BB

GP1BB point mutation (allele 1) and microdeletion on 22q11.2 (allele 2)
Combined clinical features of BSS and of 22q11.2 deletion syndrome

22q11.2 deletion syndrome
(Velocardiofacial syndrome - DiGeorge syndrome)

Variable clinical phenotype
(penetrance not complete)
Congenital heart defects
Palatal anomalies
Developmental delay
Facial dysmorphisms



LCR: Low Copy Repeat

Morrow et al. Am J Med Genet A 176: 2070, 2018

Macrothrombocytopenia and bleeding tendency could be the only clinical features

↓

microdeletions on 22q11.2 should be evaluated

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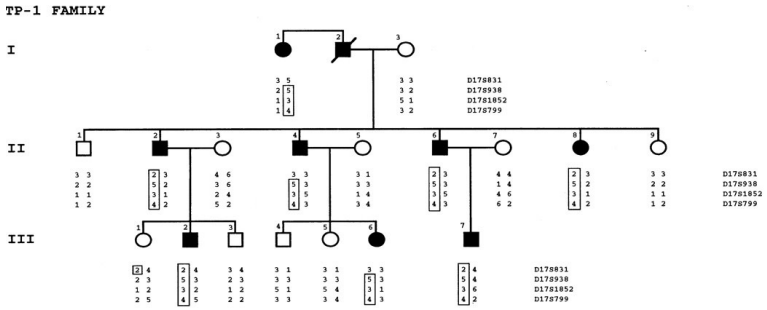
Monoallelic form of BSS (mBSS)

- BSS is classically described as a recessive disorder
- Heterozygous subjects are expected to be asymptomatic with normal platelet count and function
- Heterozygous individuals with either
  - reduced platelet count
  - increased MPV
  - defect of GPIb-IX complex
  - reduced RIPA
  - any combination of these
- In some patients these abnormalities are severe enough to elicit a bleeding diathesis

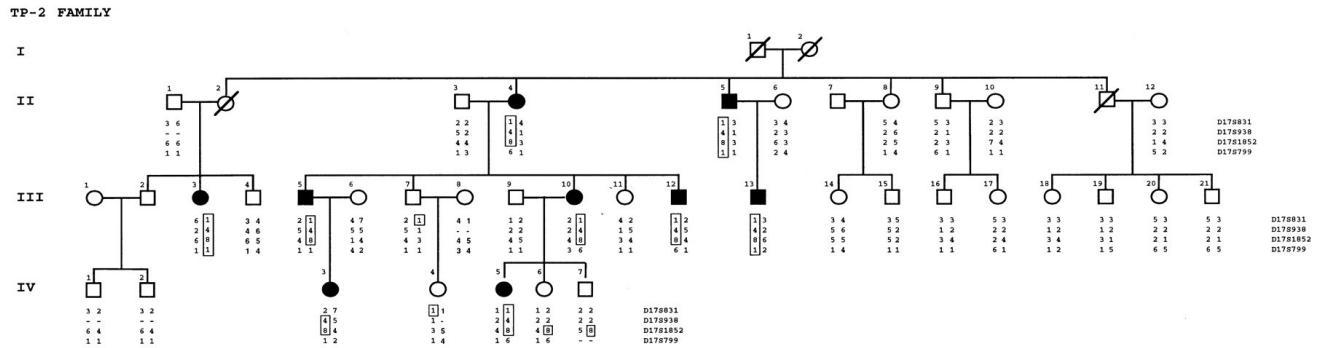
Autosomal dominant macrothrombocytopenia in Italy is most frequently a type of heterozygous Bernard-Soulier syndrome

Anna Savoia, Carlo L. Balduini, Maria Savino, Patrizia Noris, Maria Del Vecchio, Silverio Perrotta, Simona Belletti, Vincenzo Poggi, and Achille Iolascon

Blood 97: 1330, 2001



Identification of heterozygous Ala172Val (also known as Bolzano) of GP1BA
The most common variant in mBSS



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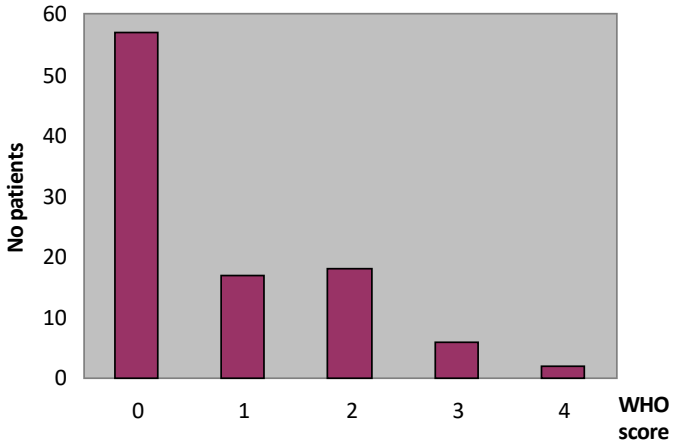
Monoallelic BSS: Ala172Val of GP1BA (103 patients from 42 unrelated families)

- Age at diagnosis: mean **33** years (range 1 - 93 ys)
- Mild bleeding tendency
- Mild thrombocytopenia
  Cell counter: mean 89 x 10^9/L; range 21 – 147 x 10^9/L
  Optical plt count: mean **103** x 10^9/L; range 21 – 162 x 10^9/L
- Platelet macrocytosis
  Diameter: mean **3.5** µm; range 2.3 to 5.1
  (normal values: 2.4 µm, range 1.9 to 3.4)
- Founder effect (common haplotype T-T-3)



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Noris et al. Haematologica 97:82:2012

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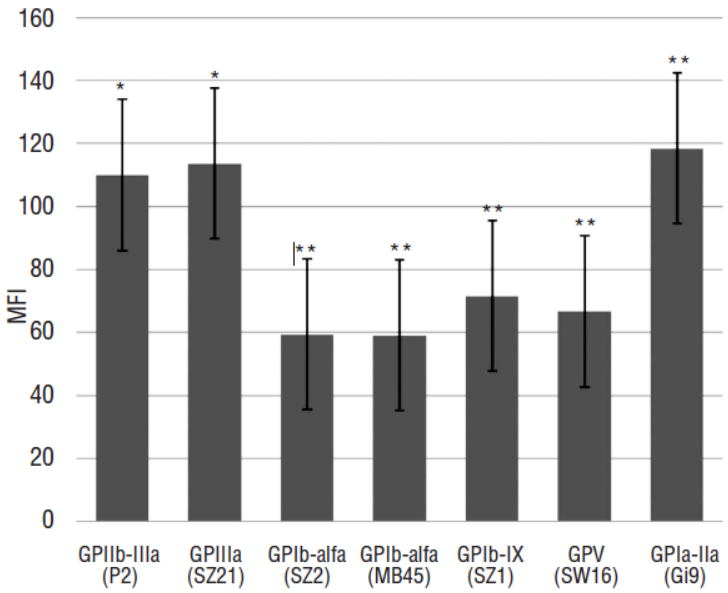
Expression of GPIb-IX-V subunits in Ala172Val patients (N. 39)

Significant reduction (40%)



Noris et al. Haematologica 97:82, 2012

In vitro platelet aggregation

- **ADP, collagen**
  Normal in all patients
- **RIPA (3.0 mg/mL)**
  Normal in all patients
- **RIPA (1.5 mg/mL)**
  Normal in 18 patients
  Decreased in 5 patients

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Variants of GP1BB in mBSS

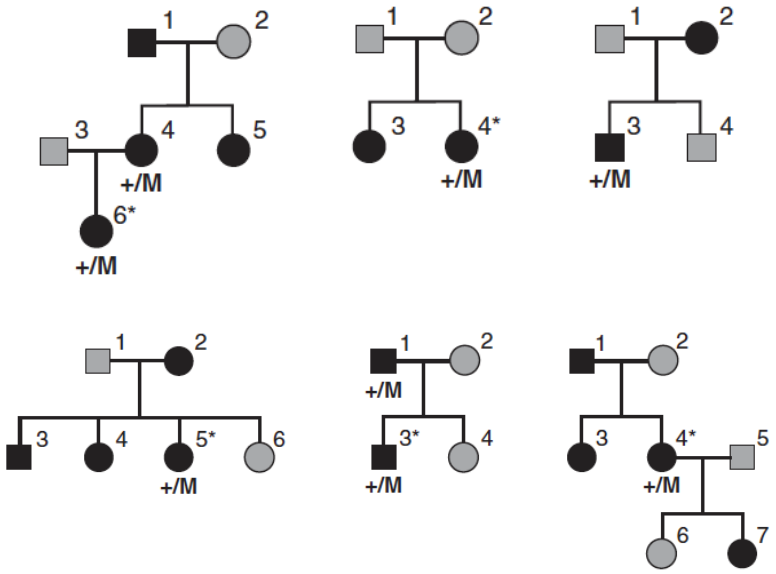
Eight variants *in 18 families*
Sivapalaratnam et al. Blood 129:520, 2017

c.DNA	Protein	N. of families
c.3G>C	p.Met1?	1
c.47T>C	p.Leu16Pro	2
c.127G>T	p.Gly43Trp	2
c.137G>A	p.Trp46*	2
c.203C>T	p.Thr68Met	1
c.236_244del	p.Pro79_Leu81del	1
c.338A>G	p.Tyr113Cys	6
c.395T>A	p.Leu132Gln	1
c.448delG	p.Ala150Argfs*43	2

Same phenotype as in Bolzano patients

Platelet count: mean 107.9x10^9/L (range, 47–172x10^9/L)
MPV: mean 12.74 fL (range, 10.7–14.3 fL)

c.179C>T (Tyr113Cys)
frequent cause in Japan



Sivapalaratnam et al, Blood 129:520, 2017

GP1BB in mBSS: c.179C>T (Leu60Pro) frequent cause after Ala172Val in Italy

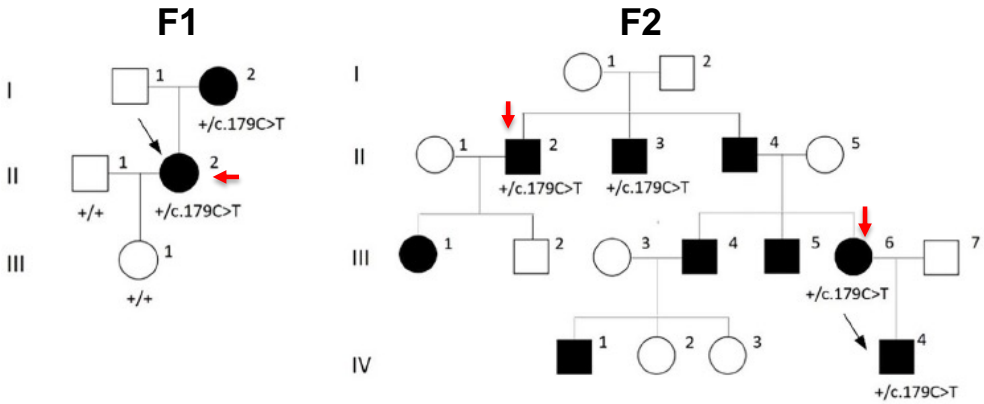
Mild thrombocytopenia

- Mean platelet count 92×10^9/L, range 42–142

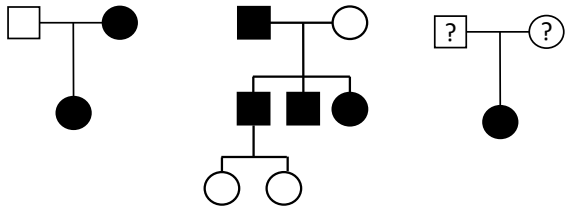
Platelet macrocytosis

- Mean platelet diameter of 3.5 µm; range 3.1–4.6, **Reduction (about 50%) of GPIb-IX complex**

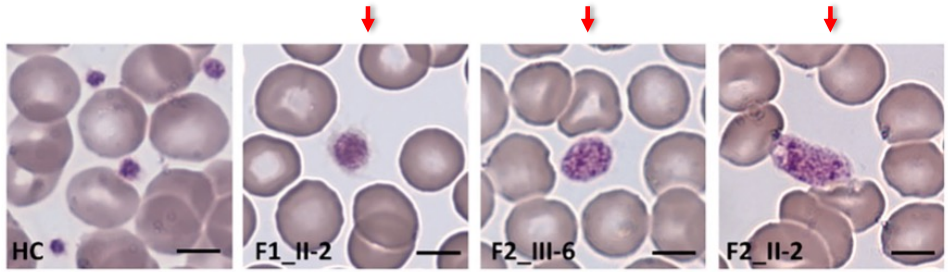
No or very mild bleeding tendency



Mutation reported in other Italian patients



Ferrari et al. Br J Haematol 184:855, 2019



Barozzi et al. Annals of Hematology 102:677, 2023

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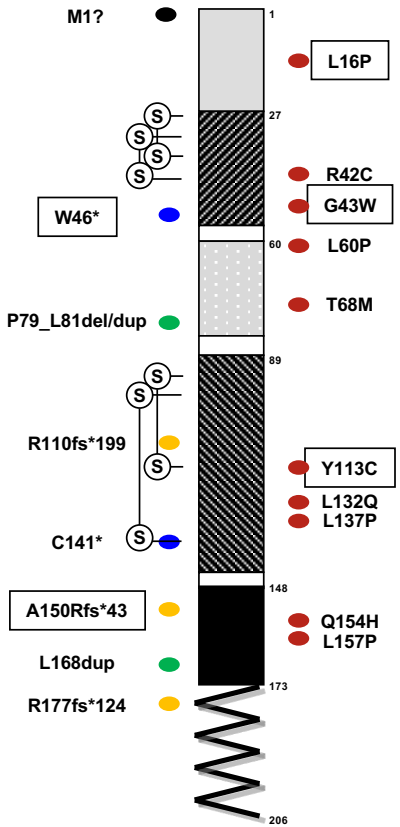
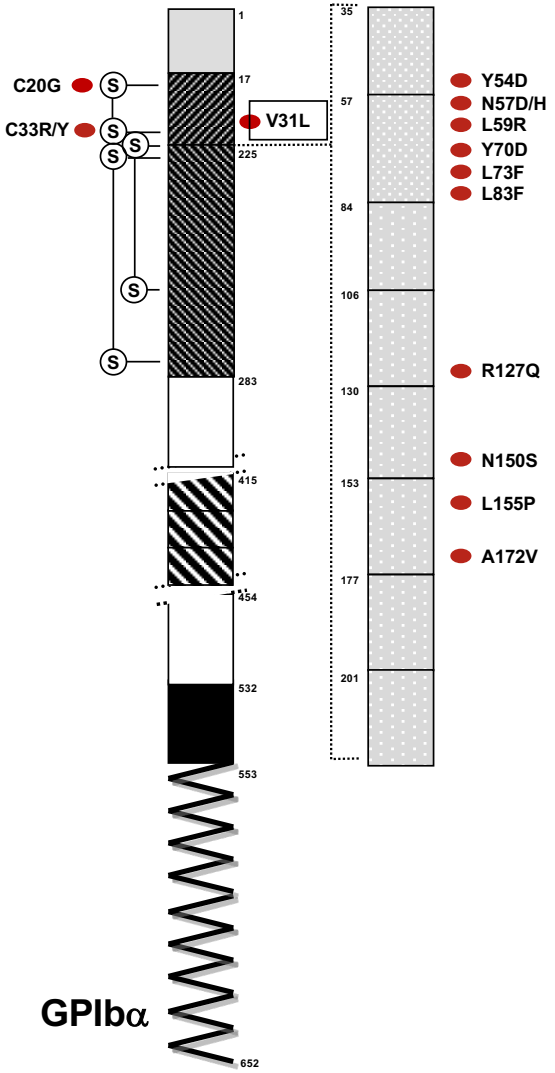
Known variants in mBSS

- Missense
- In-frame del/dup
- Nonsense
- Frameshift

Some of these variants also identified in bBSS



GPIbβ



GPIbα

- Signal peptide
- LRRNT
- LRR
- LRRCT
- VNTR
- Transmembrane
- Cytoplasmic

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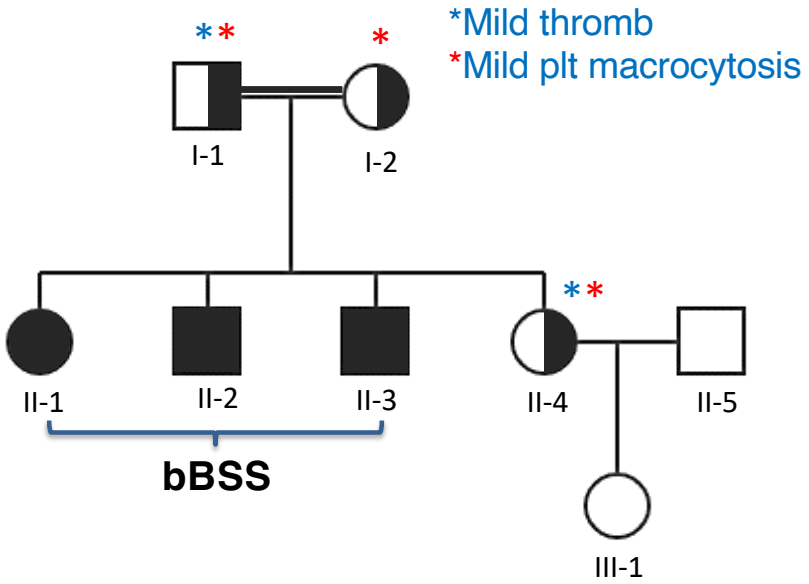
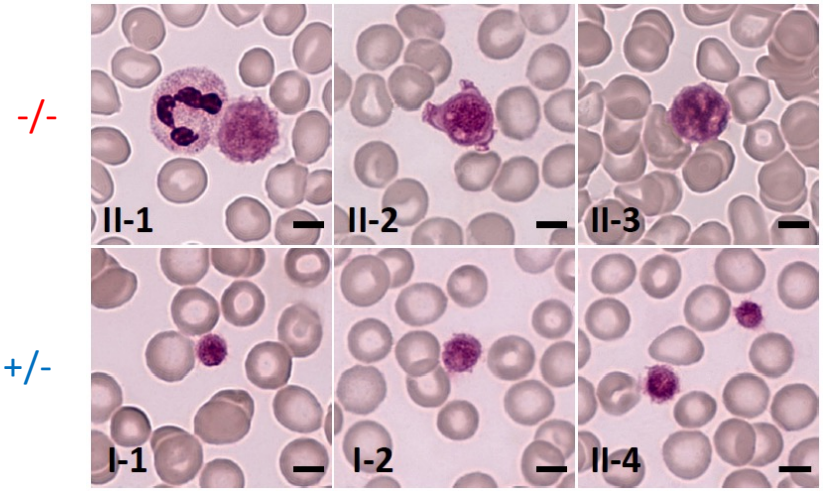
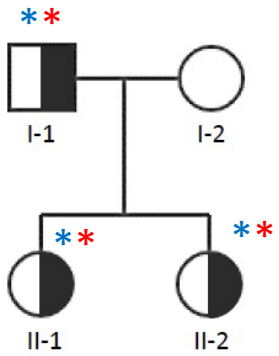
bBSS/mBSS associated with the same mutation of GP1BB



*Mild thromb
*Mild plt macrocytosis





	Subject	Gender/ Age (Years)	Automated Platelet Count, $\times 10^9$/L [1]	Microscopic Platelet Count, $\times 10^9$/L [2]	MPV, Fl [3]	Mean Platelet Diameter, µm [4]	Giant Platelets [5]	ISTH BAT Score [6]	Bleeding Symptoms
	I-1	M/59	107	129	14.1	3.07	No	0	None
	I-2	F/55	175	197	14.5	2.95	No	0	None
-/-	II-1	F/34	31	56	20.4	4.05	Yes	1	Mild menorrhagia
-/-	II-2	M/33	22	45	18.9	4.45	Yes	0	None
-/-	II-3	M/31	17	55	20.2	4.61	Yes	0	None
	II-4	F/30	103	110	14.5	3.01	No	0	None

Macrothrombocytopenia as expected in bBSS but without bleeding

Barozzi et al. Int J Mol Sci 22:10190, 2021

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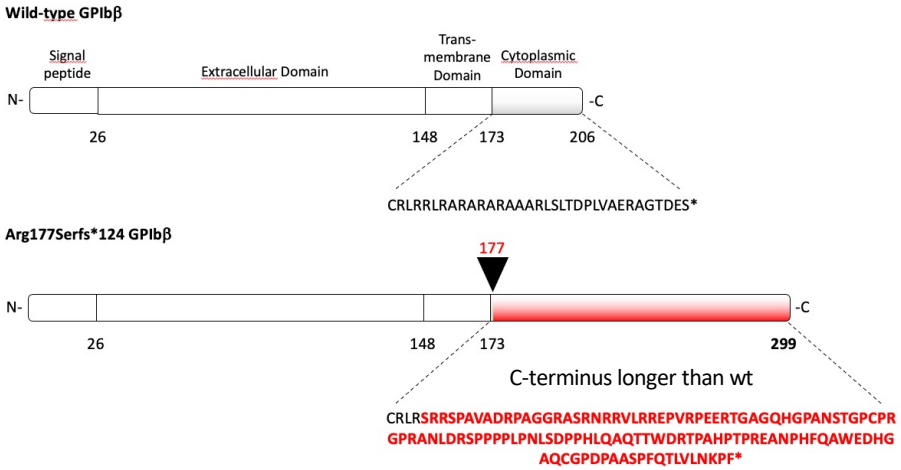
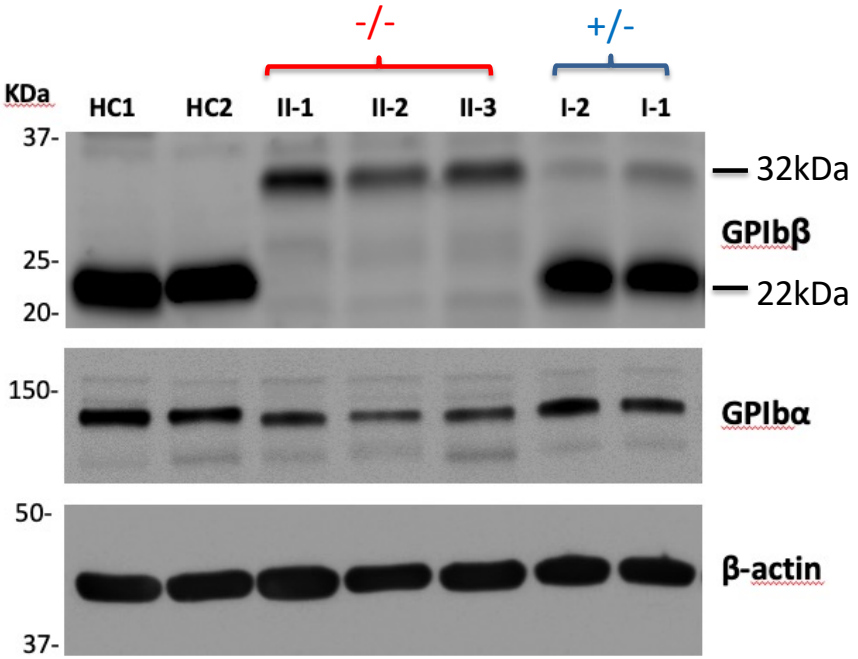
c.528_550del (p.Arg177Serfs*172) of GP1BB: partial degradation

c.528_550del (p.Arg177Serfs*172)





Likely degradation of mutant protein

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Barozzi et al. Int J Mol Sci 22:10190, 2021

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Expression of GPIb-IX subunits: 30% of control, consistent with WB data

	Subject	GPIbα (SZ2), % of Controls	GPIbα (MB45), % of Controls	GPIb-IX (SZ1), % of Controls	GPIIb (P2) % of Controls	GPIIIa (VIPL2), % of Controls
-/- ≈30%	II-1	36.7 ± 2.1	31.1 ± 2.2	31.5 ± 2.9	231.8 ± 3.1	205.2 ± 4.3
	II-2	37.2 ± 1.1	29.3 ± 1.4	28.9 ± 1.3	252.2 ± 14.4	215.3 ± 10.2
	II-3	29.7 ± 3.2	31.4 ± 1.5	28.5 ± 1.8	267.3 ± 8.7	199.5 ± 12
+/- ≈70%	I-1	62.8 ± 2.9	87.3 ± 3.9	60.2 ± 4.3	155.6 ± 10.4	161.7 ± 14.2
	I-2	63.7 ± 2.5	69.7 ± 5.4	67.7 ± 5.6	139.0 ± 8.5	145.3 ± 7.3
	II-4	70.1 ± 6.9	65.3 ± 4.3	67.3 ± 3.7	145.5 ± 3.8	155.3 ± 6.9

Note: Expression of the glycoproteins was calculated as the percentages of the mean fluorescence intensity with respect to healthy individuals processed in parallel (controls) and represent the means ± SD of two separate experiments.

Reduced in vitro platelet aggregation

	Subject	RIPA, % of controls	
		1.5 mg/mL	3.0 mg/mL
-/-	II-1	18	81
	II-2	15	75
	II-3	21	83
+/-	I-1	77	nd
	I-2	85	nd
	II-4	85	nd

Hypomorphic mutation?

- Expression of GPIb-IX
- Platelet aggregation
- Bleeding tendency

Barozzi et al. Int J Mol Sci 22:10190, 2021

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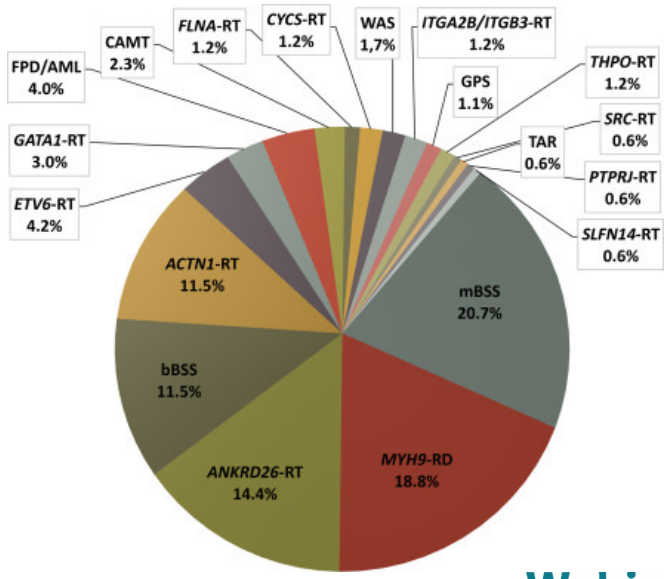
Bernard-Soulier syndrome

	bBSS	mBSS
Genetics	Autosomal recessive	Autosomal dominant
Bleeding	Moderate/severe	Absent/mild
Thrombocytopenia	Variable degree	Mild
Platelet size	Very large (giant)	Large
Platelet function (RIPA)	Defective	Normal

Pavia series
335 consecutive Italian families
with molecular diagnosis
Pecci and Balduini. Blood Reviews 48:100784, 2021



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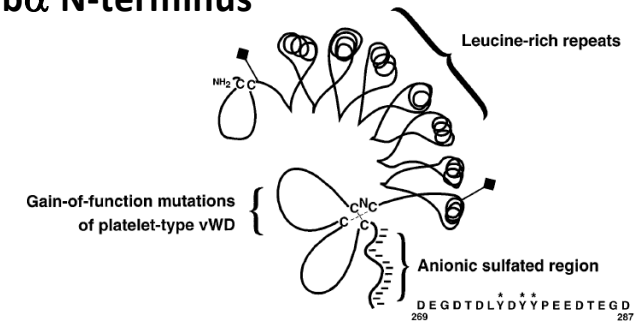
Diagnostic approach

- ✓ Thrombocytopenia known since childhood
- ✓ Family history (mBSS is AD with almost complete penetrance)
- ✓ Peripheral blood smear examination, as electronic counters do not estimate correctly platelet count and volume
- ✓ RIPA: differential diagnosis of bBSS with other thrombocytopenias characterized by giant platelets (MYH9-related disease)
- ✓ Flow cytometry of the GPIb-IX subunits
- ✓ Mutational screening: important for mBSS as no specific assay recognizes this form

Platelet-type von Willebrand disease (PT-VWD)

- Rare autosomal dominant disease
- Mild thrombocytopenia
- Mild/moderate bleeding tendency
- Increased ristocetin-induced platelet agglutination (RIPA)
- Mutations (GoF) of GPIbα enhancing affinity for VWF
- Similarities with type 2B VWD (cases with PT-VWD misdiagnosed as type 2B VWD)
- Differential diagnosis using **RIPA-mixing tests** (patient's platelets mixed with control plasma and vice versa) and/or **sequencing** of the VWF and GP1BA genes

GPIbα N-terminus



Lopez et al. Blood 91:4397, 1998

Arg127Gln Leu194Phe	LRR5 and LRR7
Trp246Leu **Gly249Val** **Gly249Ser** **Asp251Tyr** **Met255Val**	**C-terminal disulphide loop**
Pro462_Ser470del	macroglycopeptide

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Montario et al. Br J Haematol 203:673, 2023
Bury et al. Blood Adv 6:2236, 2022

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Take home messages

1. bBSS and mBSS are rare forms of inherited thrombocytopenia
2. Characterized by variable expressivity
3. Difficulties in diagnosis leading to possible underestimation and/or misdiagnosis
4. In presence of giant platelets, RIPA recognizes bBSS
5. Molecular genetic testing, at least for mBSS (inconclusive diagnosis for presence of VUS)

Open questions

1. **Why thrombocytopenia?** GPIb-IX complex is closely linked to the cytoskeleton, which is involved in proplatelet formation and sustains platelet shape
2. **Effect of mutations in mBSS?** Haploinsufficiency or dominant negative effect of specific mutations, leading to abnormal GP subunits impairing formation and/or function of the GPIb-IX complex
3. **Clinical heterogeneity explanation?** Hypomorphic mutations and/or association of pathogenic variants with adverse/favorable polygenic scores (PGS)

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