




BRIEF REPORT

The Copenhagen founder variant *GP1BA* c.58T>G is the most frequent cause of inherited thrombocytopenia in Denmark

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Abstract

Background: The classic Bernard-Soulier syndrome (BSS) is a rare inherited thrombocytopenia (IT) associated with severe thrombocytopenia, giant platelets, and bleeding tendency caused by homozygous or compound heterozygous variants in *GP1BA*, *GP1BB*, or *GP9*. Monoallelic BSS (mBSS) associated with mild asymptomatic macrothrombocytopenia caused by heterozygous variants in *GP1BA* or *GP1BB* may be a frequent cause of mild IT.

Objective: We aimed to examine the frequency of mBSS in a consecutive cohort of patients with IT and to characterize the geno- and phenotype of mBSS probands and their family members. Additionally, we set out to examine if thrombopoietin (TPO) levels differ in mBSS patients.

Patients/Methods: We screened 106 patients suspected of IT using whole exome- or whole genome sequencing and performed co-segregation analyses of mBSS families. All probands and family members were phenotypically characterized. Founder mutation analysis was carried out by certifying that the probands were unrelated and the region around the variant was shared by all patients. TPO was measured by solid phase sandwich ELISA.

Results: We diagnosed 14 patients (13%) with mBSS associated with heterozygous variants in *GP1BA* and *GP1BB*. Six unrelated probands carried a heterozygous variant in *GP1BA* (c.58T>G, p.Cys20Gly) and shared a 2.0 Mb region on chromosome 17, confirming that it is a founder variant. No discrepancy of TPO levels between mBSS patients and wild-type family members ($P > .05$) were identified.

Conclusion: We conclude that the most frequent form of IT in Denmark is mBSS caused by the Copenhagen founder variant.

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KEYWORDS

Bernard-Soulier syndrome, DNA, pedigree, sequence analysis, thrombocytopenia, thrombopoietin

1 | INTRODUCTION

The classic Bernard-Soulier syndrome (BSS) associated with severe thrombocytopenia, giant platelets, and bleeding tendency is caused by homozygous or compound heterozygous variants in *GP1BA*, *GP1BB*, or *GP9* resulting in absent or very low levels of the von Willebrand factor (VWF) receptor GPIb-IX-V on platelets and megakaryocytes.¹ However, BSS is occasionally inherited as an autosomal dominant trait due to dominant variants in *GP1BA* and *GP1BB*. The molecular mechanism responsible for the monoallelic form of BSS is a conundrum. The first described *GP1BA* variant associated with monoallelic BSS (mBSS) was the Bolzano founder variant (p. Ala156Val) affecting multiple Italian families due to common ancestry.² Subsequently, many singular cases of mBSS associated with at least 18 different heterozygous variants in *GP1BA* have been described.^{3–11} In most cases, the reported variants, including the Bolzano founder variant, are located in the extracellular leucine-rich repeat domains of *GP1BA*. These domains contain the binding site for VWF and are responsible for the induction of hepatic thrombopoietin (TPO) generation possibly via the Ashwell-Morell receptor.¹² Carriers of dominant *GP1BB* variants do not exclusively have reduced platelet GPIIb β , but also impaired expression of GPIIb α and GPIIX because their correct assembly into the GPIb-IX-V complex is affected.¹³ Currently, more than 10 different heterozygous variants in *GP1BB* associated with mBSS have been reported, and so far, no causal variants in *GP5* or *GP9* have been associated with mBSS.

1.1 | Aims

The aim of the study was to examine the frequency of mBSS in a consecutive cohort of patients with inherited thrombocytopenia (IT). Moreover, we repeatedly encountered a specific *GP1BA* variant c.58T>G and set to investigate if this was in fact a Danish founder variant. Because platelet production indirectly depends on the induction of hepatic TPO generation by platelet GPIIb α , we examined the TPO levels from patients with mBSS.

2 | METHODS

One hundred and six patients suspected of IT were included in our study. Thrombocytopenia was defined as a platelet count $<145 \times 10^9/L$. The study was approved by the local ethics committee (H-15011677) and the data registry (30–1470). Bleeding phenotype was evaluated using the ISTH bleeding assessment tool (BAT) with significant bleeding defined as ISTH-BAT >5 for women and

Essentials

- Monoallelic Bernard-Soulier syndrome may be a frequent cause of inherited thrombocytopenia.
- We screened 106 patients suspected for inherited thrombocytopenia by genome sequencing and identified causal heterozygous variants in *GP1BA* and *GP1BB* in 13%.
- Co-segregation analyses, flow cytometry, and immunofluorescence confirmed autosomal dominant inheritance and mild macrothrombocytopenia with reduced GPIb-IX levels.
- The predominant cause of inherited thrombocytopenia in Denmark is the newly identified Copenhagen founder variant: *GP1BA* (c.58T>G, p.Cys20Gly).

>3 for men.¹⁴ Of note, all patients received oral and written information about the high-throughput sequencing (HTS) analysis and signed the informed consent form to allow publication of their data in concordance with the Declaration of Helsinki. For co-segregation analysis of family members, single gene diagnosis was performed. Whole-genome and -exome sequencing (WGS and WES), Sanger sequencing, germline variant calling, and classification algorithms have been previously described in detail.^{8,15,16} Platelet diameters were measured by optical microscopy on May-Grünwald-Giemsa-stained blood smears and by software-assisted image analysis. The maximum diameter of 200 platelets was evaluated in each patient, and platelets belonging to clumps were excluded. Immunofluorescence (IF) was performed as previously described.¹⁷ Confocal laser scanning microscopy was performed on a Leica SP5 confocal laser scanning microscope (Leica). For image acquisition, secondary antibodies were labelled with fluorophores AF488 and AF565. Fluorescence emission was collected between 505 and 5015 nm for AF488 and 566 and 600 nm for AF565. Image processing was performed on Fiji (ImageJ v.1.53c).

Plasma TPO concentrations in probands and their healthy relatives were analyzed by a commercially available solid phase sandwich ELISA test (Sanquin). Normal TPO levels, as determined in a population of 193 healthy individuals, ranged from 4 to 32 AU (2.5th–95.5th percentile).¹⁸ The levels of TPO in patients were compared to healthy relatives using a Mann-Whitney *U*-test. Platelet phenotype and function were evaluated using a standardized and accredited flow cytometry (FC) analysis as previously described.⁸ Data were analyzed by Kaluza flow cytometry software v.2.1 (Beckman Coulter). In-house reference levels (RL) were available. A FC diagnosis of mBSS was made by calculating the relative ratio between patient

GP1b α (CD42b) in percentage of the median RL and patient GPIIb (CD41a) in percentages of the median RL.¹⁹

A founder variant is defined by at least 1.0 Mb shared region of the genome.²⁰ The size of the haplotype associated with the *GP1BA* c.58T>G variant was estimated from WGS variant calling using proband samples from six families. All incompatible variants (i.e., homozygous reference variant in one sample and homozygous alternative variant in another sample) were manually inspected in Integrative Genomics Viewer (IGV)²¹ to confirm the size of the shared haplotype. Identity by descent (IBD) of the genomes was estimated with PLINK 1.9²² after initial pruning of sites in linkage disequilibrium using 50 kb window size, variant count to shift a window of five, and variance inflation factor threshold of two.

3 | RESULTS AND DISCUSSION

Our cohort consists of 106 patients (67% females; median platelet count $98 \times 10^9/L$ and range $[3-143 \times 10^9/L]$; 58% had macrothrombocytopenia [MT]). A causal variant was identified in 50 patients (49%; Table S1 in supporting information). In the cohort, we identified five rare heterozygous variants in *GP1BA* (c.58T>G; c.98G>A; c.247C>T) and *GP1BB* (c.236_244del; c.236_244dup), in 14 Danish probands (13%; Table 1). Co-segregation analyses were carried out in 46 members from the 14 individual families resulting in a total of 31 thrombocytopenic patients emphasizing an autosomal dominant inheritance. Pedigrees are depicted in Figure 1. All identified variants co-segregated with thrombocytopenia in the assessed family members and the median platelet count was $90 \times 10^9/L$ (range 44–131). Peripheral blood smears from all probands demonstrated macrothrombocytes. The mean platelet diameter value in three patients carrying the *GP1BA* p. Cys20Gly variant were 3.7 μm (2–8.77), 3.69 μm (1.61–9.61), and 3.38 μm (1.37–9.9) and the percentage of large platelets were 40%, 35%, and 26% respectively (Figure 2A–F). These results are consistent with the previously reported data on 117 cases of mBSS—103 of them carrying the Bolzano founder variant.²³

A typical finding of enlarged platelets is high mean fluorescence intensity (MFI) values for glycoprotein receptors on the surface (due to a larger surface area). However, in patients with mBSS the relative expression of GPIb α (CD42b) is reduced compared to GPIIb (CD41a). Examinations by FC in 16 patients demonstrated a reduced relative ratio of CD42b to CD41a (median ratio 0.52, range 0.40–0.67). The GPIb-IX levels were reduced by 50% and this was confirmed by IF and confocal laser scanning microscopy (Figure 2G–N). Thus, FC and IF, combined with co-segregation analyses, confirmed a diagnosis of autosomal dominant BSS in the 14 index patients. Consequently, five different heterozygous variants in *GP1BA* and *GP1BB*, identified in 14 index patients, were classified as pathogenic or likely pathogenic.

Families I–VI carried the same heterozygous variant in *GP1BA* (c.58T>G, p. Cys200Gly) not previously reported in the gnomAD database. To determine if the variant is a founder variant, we certified (1) that patient genomes were unrelated and (2) that the region around

the variant of interest is shared by all genomes. The patients had no cryptic relatedness across the genomes, and they shared a 2.0 Mb region (chr17:3,745,359–5,699,382) around the *GP1BA* c.58T>G variant (1.1 Mb upstream from the variant and 0.9 Mb downstream; Figure S1 in supporting information). Therefore, the genetic mapping confirmed that *GP1BA* c.58T>G is indeed a Danish founder variant. The c.58T>G variant likely breaks the disulfide bond between Cys20 and Cys33, thereby disrupting the local stability of the protein secondary structure (Figure S2 in supporting information). The altered protein folding in the N-terminal ligand-binding-domain (LBD) of GPIb α could potentially lead to a decreased binding to the VWF receptor and hereby reduce the pro-platelet formation by the megakaryocytes.²⁴ We identified the *GP1BA* variant c.98G>A, which was previously described as a likely pathogenic cause of mBSS,⁴ in three members of family VII. Three probands representing family VIII, IX, and X, carried the *GP1BA* c.247C>T variant, which co-segregated with MT; it was subsequently classified as pathogenic. The founder variant c.58T>G and the c.98G>A variant are located in the leucine rich repeat N-terminus of GPIb α required for platelet-mediated hepatic TPO production.

In the probands from family XI, XII, and XIII we found the same previously described deletion in *GP1BB* (c.236_244del, p. Pro79_Leu81del) located in the leucine-rich repeat domain.¹⁹ Interestingly, the proband from family XIV carried a duplication in the same locus of the *GP1BB* gene (c.236_244dup, p. Pro79_Leu81dup), also co-segregating with thrombocytopenia. Thus, we suspect that the leucine-rich repeat domain of *GP1BB* is less stable and perhaps prone to pathogenic variants. The crystal structure of the GPIIb β protein with the affected domains and locations of the identified variants are shown in Figure S2. Heterozygous variants in *GP1BB*, causal of inhibited trafficking of the GPIb-IX-V complex to the platelet surface or GPIb-IX-V dysfunction, may cause mBSS. Variants that simply impair the platelet expression of GPIIb β have not been shown to cause MT.²⁵ Consequently, the identified *GP1BB* variants in our cohort may disrupt the function or reduce trafficking of the GPIb-IX-V complex.

Four patients were previously misdiagnosed with immune thrombocytopenia (ITP) of which two patients were treated with immunosuppressants during pregnancy. In total, 6 patients had given 13 births and no bleeding complications occurred. Only 2 of the 14 index patients had significant ISTH-BAT scores. We did not identify any other significant variants in bleeding-associated genes in the two probands with significant ISTH-BAT scores. Additional variants associated with MTP are listed in Table S2 in supporting information. Following the American College of Medical Genetics guidelines, results of flow cytometry, and the absence of inclusion bodies regarding the *MYH9* variants, we concluded that none of the additional variants were implicated in MTP phenotype. Nor did we identify any pathogenic genetic variants in 23 additional *TIER1* and *TIER2* genes associated with MT. Taken together, mBSS constituted 13% (14/106) of the IT diagnoses and was thus found to be the most frequent cause of IT in the Öresund region. This result was due to the *GP1BA* c.58T>G founder variant, identified in six unrelated

TABLE 1 Clinical characteristics of patients with inherited thrombocytopenia from 14 Danish families with monoallelic Bernard-Soulier syndrome

Family	Patient	Age and gender	Pit Count $\times 10^9/L$	MPV (Ref.: 8–13 fL)	Family variant	Frequency (gnomAD) (%)	ACMG Class	GPIIb α (CD42b) Ref.: 45–69 MFI*	GPIIb (CD41a) Ref.: 20–33 MFI*	RR CD42b/CD41a**	ISTH-BAT score	Multiplate ristocetin Ref.: 65–116 U
I	Proband	39F	68	Failed	GPIBA c.58T>G, p. Cys20Gly	N/A	5	42	36	0.53	2	22
II	Proband	44F	107	15	GPIBA c.58T>G, p. Cys20Gly	N/A	5	39	40	0.43	1	15
	Mother	76F	94	Failed				36	41	0.40	0	ND
	Brother	40M	96	Failed				ND	ND	ND	0	16
III	Proband	29F	73	Failed	GPIBA c.58T>G, p. Cys20Gly	N/A	5	40	40	0.47	7	ND
	Father	71M	103	Failed				51	48	0.54	6	60
IV	Proband	43F	94	14	GPIBA c.58T>G, p. Cys20Gly	N/A	5	41	43	0.40	2	ND
	Father	70M	54	Failed				ND	ND	ND	ND	ND
	Son	19M	85	Failed				ND	ND	ND	ND	ND
	Half-brother	35M	91	16				ND	ND	ND	ND	ND
V	Proband	43F	128	12	GPIBA c.58T>G, p. Cys20Gly	N/A	5	ND	ND	ND	3	ND
	Daughter	14F	59	Failed				ND	ND	ND	1	ND
VI	Proband	35F	114	14,6	GPIBA c.58T>G, p. Cys20Gly	N/A	5	ND	ND	ND	0	ND
	Daughter	7F	131	13,8				ND	ND	ND	0	ND
VII	Proband	61M	81	Failed	GPIBA c.98G>A, p. Cys33Tyr	N/A	4	ND	ND	ND	0	18
	Son	31M	90	Failed				40	37	0.55	0	30
	Son	28M	89	Failed				32	26	0.55	0	ND
VIII	Proband	54F	94	Failed	GPIBA c.247C>T, p. Leu83Phe	0.00083	5	49	36	0.62	3	44
	Cousin	49M	84	Failed				ND	ND	ND	ND	ND
	Son	25M	83	ND				ND	ND	ND	ND	ND
IX	Proband	25F	44	Failed	GPIBA c.247C>T, p. Leu83Phe	0.00083	5	50	36	0.63	6	ND
	Father	49F	78	12				49	40	0.55	0	75
X	Proband	29M	64	ND	GPIBA c.247C>T, p. Leu83Phe	0.00083	5	ND	ND	ND	3	ND

(Continues)

TABLE 1 (Continued)

Family	Patient	Age and gender	Plt Count $\times 10^9/L$	MPV (Ref.: 8–13 fL)	Family variant	Frequency (gnomAD) (%)	ACMG Class	GPIIb α (CD42b) Ref.: 45–69 MFI*	GPIIb (CD41a) Ref.: 20–33 MFI*	RR CD42b/CD41a**	ISTH-BAT score	Multiplate ristocetin Ref.: 65–116 U
XI	Proband	43F	112	Failed	GPIIb c.236_244del, p. Pro79_Leu81del	N/A	5	33	32	0.47	2	40
	Father	77M	87	Failed				ND	ND	ND	ND	ND
XII	Proband	63M	107	14	GPIIb c.236_244del, p. Pro79_Leu81del	N/A	5	ND	ND	ND	0	37
XIII	Proband	33F	105	Failed	GPIIb c.236_244del, p. Pro79_Leu81del	N/A	5	47	32	0.67	3	ND
	Father	71M	107	Failed		N/A		52	48	0.48	6	60
	Brother	31M	131	12		N/A		ND	ND	ND	0	44
XIV	Proband	37M	85	Failed	GPIIb c.236_244dup, p. Pro79_Leu81dup	N/A	4	43	58	0.47	1	51
	Daughter	7F	123	14				ND	ND	ND	ND	ND

Notes: All patients had macrothrombocytes in peripheral blood smear.

*In-house reference level. **The relative ratio of CD42b and CD41a was calculated as: patient CD42b median reference level/patient CD41a in percentage of CD41a median reference level; CD42b median reference level = 56.1MFI and CD41a median reference level = 25.4MFI.

Abbreviations: ACMG, American College of Medical Genetics; gnomAD, the genome aggregation database; F, female; ISTH-BAT, International Society on Thrombosis and Haemostasis Bleeding Assessment Tool; M, male; MFI, mean fluorescence intensity; MPV, mean platelet volume; ND, not done; Plt, platelet; RR, relative ratio.

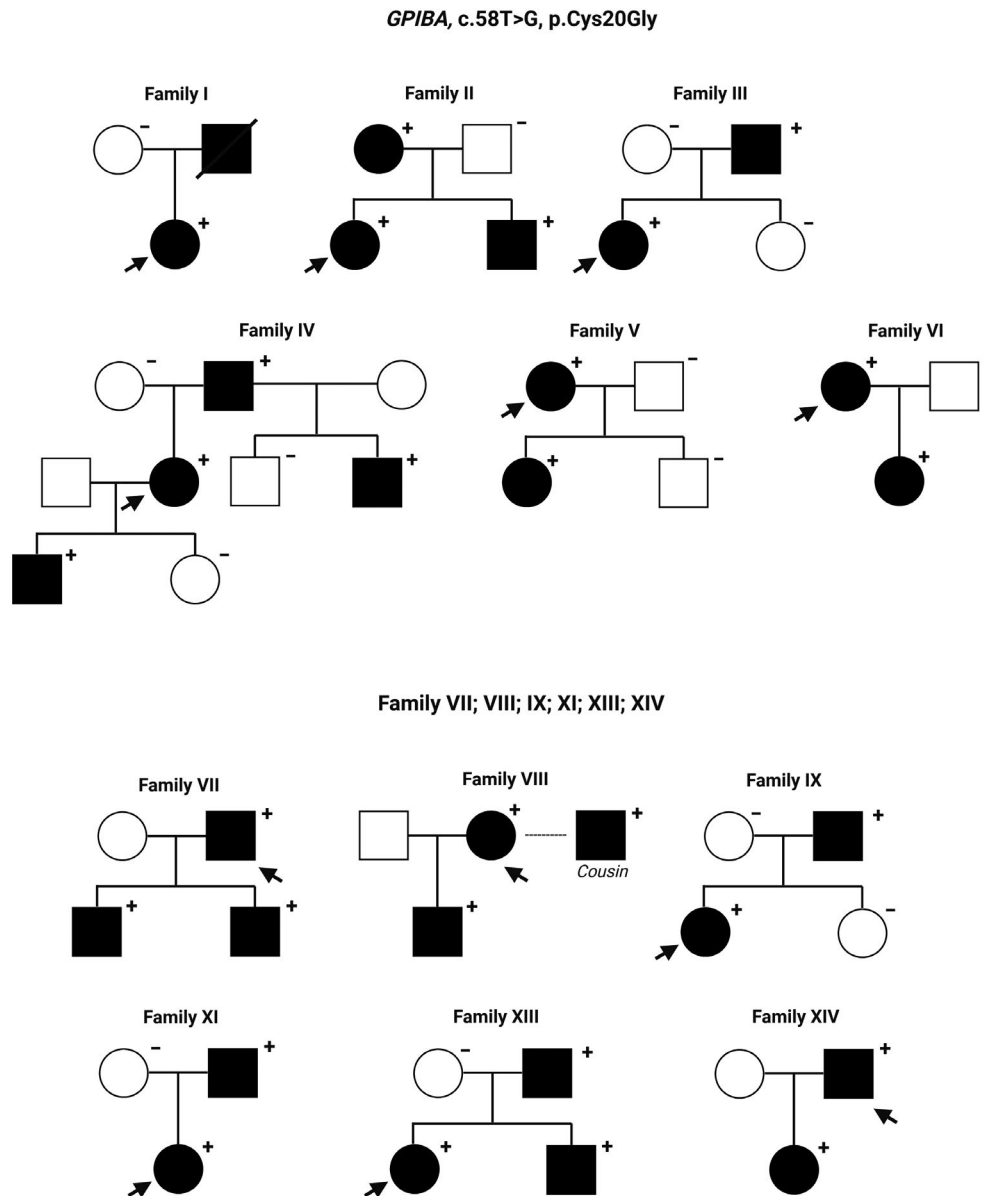


FIGURE 1 Pedigrees of 14 families with monoallelic Bernard-Soulier syndrome. A, Six families with the *GP1BA* c.58T>G, p. Cys20Gly founder variant. B, Family VII carries a *GP1BA* c.98G>A, p. Cys33Tyr variant. Families VIII, IX, and X has a *GP1BA* c.247C>T, p. Leu83Phe variant. Families XI, XII, and XIII share a *GP1BB* c.236_244del, p. Pro79_Leu81del variant and family XIV harbors a *GP1BB* c.236_244dup, p. Pro79_Leu81dup variant. Arrow indicates index patient. "+" or "-" indicate carrier status of the family variant. Filled family member illustrates thrombocytopenia and all depicted variants co-segregate with thrombocytopenia

probands. Hence, *GP1BA* c.58T>G was named the Copenhagen variant. Compared to the Italian population, in which 20% of IT are caused by the Bolzano founder variant,²⁶ the frequency of mBSS in our cohort was lower.

We measured TPO in 18 patients with mBSS and 10 healthy relatives and found a median plasma TPO in patients of 16.5 AU/ml (range 6–79 AU/ml), which did not differ significantly from the median TPO in healthy relatives (17 AU/ml; range 7–28 AU/ml; $P > .05$; Table 2). Our results indicate a relative deficiency of TPO production and may suggest that patients with mild mBSS could benefit from TPO-receptor agonist (TPO-RA) treatment prior to major surgery or childbirth. In contrast, Noris et al. measured levels of TPO in 46

patients with the monoallelic Ala156Val Bolzano variant and found increased levels compared to healthy controls.² We do not have a plausible explanation for the discrepancy between TPO levels in our studies. The first phase II trial administering the TPO-RA eltrombopag in different types of IT was published in 2020 and included two patients with mBSS. They both had a major response with the lowest treatment dose of 50 mg/day.²⁷

Because the GPIb-IX-V receptor membrane complex plays a pivotal role in thrombosis, a reduction in the expression of GPIb-IX-V in mBSS patients may protect against arterial thrombosis and thereby promote a survival advantage. To the best of our knowledge, no data has been published on the risk of thrombosis in mBSS. Yin et al.

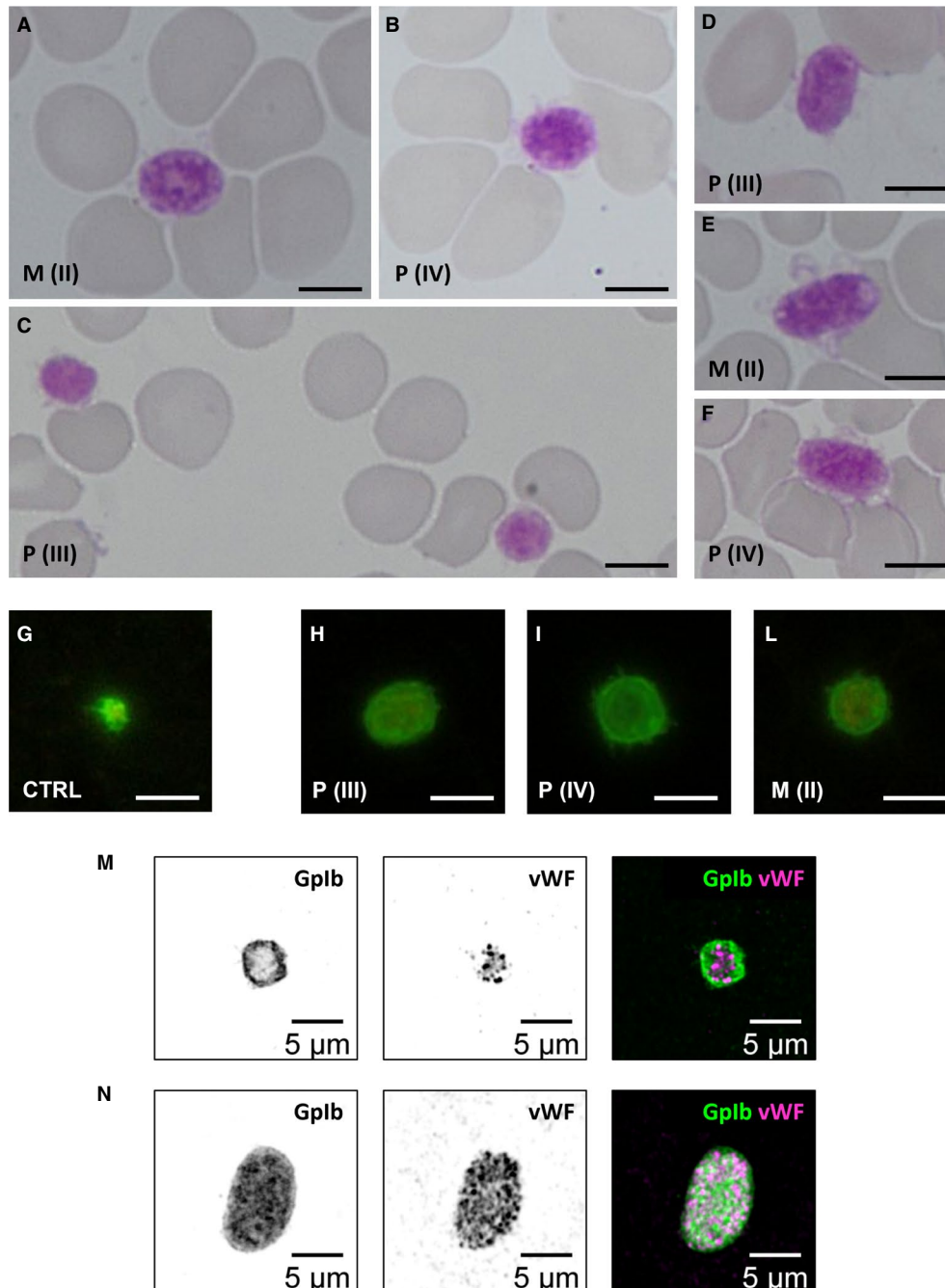


FIGURE 2 Light- and immunofluorescence microscopy. Representative light microscopy platelet images are shown from three patients with the *GP1BA* p. Cys20Gly variant (A–F). A heterogeneous platelet population with large platelets (platelets with mean platelet diameter (MPD) $>3.9 \mu\text{m}$)²³ and peculiar elongated platelets (D–F) was found in the investigated subjects. By standard immunofluorescence microscopy (G–L), a partial reduction of the GpIb staining in the larger platelets of the patients was observed with respect to control. Confocal laser scanning microscopy immunofluorescence image panels (CTRL, control; M, control and N, patient; P, proband; M, mother). The roman letters indicate the corresponding pedigrees. Scale bars correspond to $5 \mu\text{m}$

selectively inhibited the VWF-binding function of GPIb-IX by a peptide inhibitor and discovered that the process of lipopolysaccharide-induced thrombocytopenia in sepsis was attenuated, while the sepsis mortality of mice expressing a functionally deficient mutant of GPIb-IX was significantly decreased.²⁸ These findings suggest that targeting GPIb-IX-V could be a possible prospect for managing

endotoxemia and sepsis²⁹ and with relevance to the present study, inherited variants in *GP1BA* or *GP1BB* may protect against death from sepsis and thus provide a survival advantage.²⁸ In conclusion, genetic screening of patients suspected for IT has improved the diagnostic outcome in our clinics and resulted in the identification of a Danish founder variant causing mBSS. All identified variants in

TABLE 2 Thrombopoietin (TPO) in patients with monoallelic Bernard-Soulier syndrome and their healthy relatives

Family variant	Median TPO Ref.: 4–32 AU/ml
GP1BA c.58T>G, p. Cys20Gly	13 (range 6–79)
GP1BA c.247C>T, p. Leu83Phe	16.5 (range 14–19)
GP1BB c.236_244del, p. Pro79_Leu81del/dup	23 (range 16–35)
Healthy relatives	17 (range 7–28)

GP1BA and GP1BB have been uploaded to the ISTH Gold variant database to aid the global interpretation of genetic variants associated with IT.³⁰

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

EL and MR designed the research study, conducted the data analyses, and wrote the manuscript. NB, EZ, and SR conducted the data collection. AØR conducted the mapping of the reported variants. MG conducted the founder variant analysis. SRO conducted the flow cytometry analyses. CZ performed light- and immunofluorescence microscopy. RP performed confocal laser scanning microscopy analysis. NB, EZ, AØR, MG, SR, SRO, RP, and CZ contributed to the writing and critical revision of the manuscript.

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REFERENCES

- Diz-Küçükkaya R, López JA. Inherited disorders of platelets: membrane glycoprotein disorders. *Hematol Oncol Clin North Am*. 2013;27:613–627. <https://doi.org/10.1016/j.hoc.2013.03.005>
- Noris P, Perrotta S, Bottega R, et al. Clinical and laboratory features of 103 patients from 42 Italian families with inherited thrombocytopenia derived from the monoallelic Ala156Val mutation of GPIIb (Bolzano mutation). *Haematologica*. 2012;97:82–88. <https://doi.org/10.3324/haematol.2011.050682>
- Bastida JM, Lozano ML, Benito R, et al. Introducing high-throughput sequencing into mainstream genetic diagnosis practice in inherited platelet disorders. *Haematologica*. 2018;103:148–162. <https://doi.org/10.3324/haematol.2017.171132>
- Downes K, Megy K, Duarte D, et al. Diagnostic high-throughput sequencing of 2396 patients with bleeding, thrombotic, and platelet disorders. *Blood*. 2019;134:2082–2091. <https://doi.org/10.1182/blood.2018891192>
- Ghalloussi D, Saut N, Bernot D, et al. A new heterozygous mutation in GP1BA gene responsible for macrothrombocytopenia. *Br J Haematol*. 2018;183:503–506. <https://doi.org/10.1111/bjh.14986>
- Guéguen P, Dupuis A, Py JY, et al. Pathogenic and likely pathogenic variants in at least five genes account for approximately 3% of mild isolated nonsyndromic thrombocytopenia. *Transfusion*. 2020;60:2419–2431. <https://doi.org/10.1111/trf.15992>
- Kunishima S, Imai T, Hamaguchi M, Saito H. Novel heterozygous missense mutation in the second leucine rich repeat of GPIIb/IX/V affects GPIIb/IX/V expression and results in macrothrombocytopenia in a patient initially misdiagnosed with idiopathic thrombocytopenic purpura. *Eur J Haematol*. 2006;76:348–355. <https://doi.org/10.1111/j.1600-0609.2005.00612.x>
- Leinøe E, Gabriellite M, Østrup O, et al. Outcome of an enhanced diagnostic pipeline for patients suspected of inherited thrombocytopenia. *Br J Haematol*. 2019;186:373–376. <https://doi.org/10.1111/bjh.15886>
- Miller JL, Lyle VA, Cunningham D. Mutation of leucine-57 to phenylalanine in a platelet glycoprotein IIb alpha leucine tandem repeat occurring in patients with an autosomal dominant variant of Bernard-Soulier disease. *Blood*. 1992;79:439–446.
- Trizuljak J, Kozubík KS, Radová L, et al. A novel germline mutation in GP1BA gene N-terminal domain in monoallelic Bernard-Soulier syndrome. *Platelets*. 2018;29:827–833. <https://doi.org/10.1080/09537104.2018.1529300>
- Vettore S, Scandellari R, Moro S, et al. Novel point mutation in a leucine-rich repeat of the GPIIb/IX/V chain of the platelet von Willebrand factor receptor, GPIIb/IX/V, resulting in an inherited dominant form of Bernard-Soulier syndrome affecting two unrelated families: the N41H variant. *Haematologica*. 2008;93:1743–1747. <https://doi.org/10.3324/haematol.12830>
- Grozovsky R, Begonja AJ, Liu K, et al. The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling. *Nat Med*. 2015;21:47–54. <https://doi.org/10.1038/nm.3770>
- Hadjkacem B, Elleuch H, Gargouri J, Gargouri A. Bernard-Soulier syndrome: novel nonsense mutation in GPIIb gene affecting GPIIb-IX complex expression. *Ann Hematol*. 2009;88:465–472. <https://doi.org/10.1007/s00277-008-0611-8>
- Elbatarny M, Mollah S, Grabell J, et al. Normal range of bleeding scores for the ISTH-BAT: adult and pediatric data from the merging project. *Haemophilia*. 2014;20:831–835. <https://doi.org/10.1111/hae.12503>
- Dandanell M, Friis-Hansen L, Sunde L, Nielsen FC, Hansen TV. Identification of 3 novel VHL germ-line mutations in Danish VHL patients. *BMC Med Genet*. 2012;13:54. <https://doi.org/10.1186/1471-2350-13-54>
- Leinøe E, Zetterberg E, Kinalis S, et al. Application of whole-exome sequencing to direct the specific functional testing and diagnosis of rare inherited bleeding disorders in patients from the Oresund Region, Scandinavia. *Br J Haematol*. 2017;179:308–322. <https://doi.org/10.1111/bjh.14863>
- Zaninetti C, Greinacher A. Diagnosis of inherited platelet disorders on a blood smear. *J Clin Med*. 2020;9:539. <https://doi.org/10.3390/jcm9020539>
- Folman CC, von dem Borne AE, Rensink IH, et al. Sensitive measurement of thrombopoietin by a monoclonal antibody based sandwich enzyme-linked immunosorbent assay. *Thromb Haemost*. 1997;78:1262–1267.
- Sivapalaratnam S, Collins J, Gomez K. Diagnosis of inherited bleeding disorders in the genomic era. *Br J Haematol*. 2017;179:363–376. <https://doi.org/10.1111/bjh.14796>
- Perić S, Glumac JN, Töpf A, et al. A novel recessive TTN founder variant is a common cause of distal myopathy in the Serbian population. *Eur J Hum Genet*. 2017;25:572–581. <https://doi.org/10.1038/ejhg.2017.16>
- Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform*. 2013;14:178–192. <https://doi.org/10.1093/bib/bbs017>
- Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*. 2015;4:7. <https://doi.org/10.1186/s13742-015-0047-8>

23. Noris P, Biino G, Pecci A, et al. Platelet diameters in inherited thrombocytopenias: analysis of 376 patients with all known disorders. *Blood*. 2014;124:e4-e10. <https://doi.org/10.1182/blood-2014-03-564328>
24. Quach ME, Li R. Structure-function of platelet glycoprotein Ib-IX. *J Thromb Haemost*. 2020;18:3131-3141. <https://doi.org/10.1111/jth.15035>
25. Zwifelhofer NMJ, Bercovitz RS, Weik LA, et al. Hemizyosity for the gene encoding glycoprotein Ib β is not responsible for macrothrombocytopenia and bleeding in patients with 22q11 deletion syndrome. *J Thromb Haemost*. 2019;17:295-305. <https://doi.org/10.1111/jth.14357>
26. Pecci A, Balduini CL. Inherited thrombocytopenias: an updated guide for clinicians. *Blood Rev*. 2021;48:100784. <https://doi.org/10.1016/j.blre.2020.100784>
27. Zaninetti C, Gresele P, Bertomoro A, et al. Eltrombopag for the treatment of inherited thrombocytopenias: a phase II clinical trial. *Haematologica*. 2020;105:820-828. <https://doi.org/10.3324/haematol.2019.223966>
28. Yin H, Stojanovic-Terpo A, Xu W, et al. Role for platelet glycoprotein Ib-IX and effects of its inhibition in endotoxemia-induced thrombosis, thrombocytopenia, and mortality. *Arterioscler Thromb Vasc Biol*. 2013;33:2529-2537. <https://doi.org/10.1161/atvbaha.113.302339>
29. Ghimire S, Ravi S, Budhathoki R, et al. Current understanding and future implications of sepsis-induced thrombocytopenia. *Eur J Haematol*. 2021;106:301-305. <https://doi.org/10.1111/ejh.13549>
30. Megy K, Downes K, Simeoni I, et al. Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH. *J Thromb Haemost*. 2019;17:1253-1260. <https://doi.org/10.1111/jth.14479>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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