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.
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. 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. Subsequently, many singular cases of mBSS associated with at least 18 different heterozygous variants in GP1BA have been described. 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. Carriers of dominant GP1BB variants do not exclusively have reduced platelet GPIbIX, but also impaired expression of GPIba and GPIX because their correct assembly into the GPIb-IX-V complex is affected. 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 GPIbα, 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 × 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 >3 for men. Of note, all patients received oral and written information about the high-throughput sequencing (HTS) analysis and signed th 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, 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).

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). 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. 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
GPIbα (CD42b) in percentage of the median RL and patient GPIbβ (CD41a) in percentages of the median RL. A founder variant is defined by at least 1.0 Mb shared region of the genome. 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., homoygous reference variant in one sample and homozygous alternative variant in another sample) were manually inspected in Integrative Genomics Viewer (IGV) to confirm the size of the shared haplotype. Identity by descent (IBD) of the genomes was estimated with PLINK 1.9 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 x10^9/L and range [3-143 x 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 x 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. Cys200Gly variant were 3.7 µm (2.87, 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 mBSS—103 of them carrying the Bolzano founder variant. The 117 cases of mBSS—103 of them carrying the Bolzano founder variant were manually inspected in Integrative Genomics Viewer (IGV) to confirm the size of the shared haplotype. Identity by descent (IBD) of the genomes was estimated with PLINK 1.9 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.

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 GPIbα (CD42b) is reduced compared to GPIbβ (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 GPIbα could potentially lead to a decreased binding to the VWF receptor and hereby reduce the pro-platelet formation by the megakaryocytes. We identified the GP1BA variant c.98G>A, which was previously described as a likely pathogenic cause of mBSS, 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 GPIbα 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. 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 GPIbβ 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 GPIbβ have not been shown to cause MT. 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
| Family | Patient | Age and gender | Platelet Count ×10^9/L | MPV (Ref.: 8–13 fL) | Family variant | Frequency (gnomAD) (%) | ACMG Class | GPLIbα (CD42b) Ref.: 45–69 MFI* | GPLIIb (CD41a) Ref.: 20–33 MFI* | RR CD42b/CD41a** | ISTH-BAT score | Multiplate ristocetin Ref.: 65–116 U |
|--------|---------|---------------|------------------------|---------------------|----------------|------------------------|------------|-------------------------------|-------------------------------|----------------|----------------|-------------------------------|
| I      | Proband | 39F 68        | Failed                 | GP1BA c.58T>G, p. Cys20Gly | N/A            | 5                      | 42         | 36                            | 0.53                         | 2              | 22                          |                               |
| II     | Proband | 44F 107       | 15                     | GP1BA c.58T>G, p. Cys20Gly | N/A            | 5                      | 39         | 40                            | 0.43                         | 1              | 15                          |                               |
|        | Mother  | 76F 94        | Failed                 |                         |                | 36                     | 41         | ND                            | ND                           | 0.40            | ND                          | 0                             |
|        | Brother | 40M 96        | Failed                 |                         |                | ND                     | ND         | ND                            | ND                           | 0              | 16                          |                               |
| III    | Proband | 29F 73        | Failed                 | GP1BA 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                     | GP1BA c.58T>G, p. Cys20Gly | N/A            | 5                      | 41         | 43                            | 0.40                         | 2              | ND                          |                               |
|        | Father  | 70M 54        | Failed                 |                         |                | ND                     | ND         | ND                            | ND                           | ND             | ND                          | ND                            |
|        | Son     | 19M 85        | Failed                 |                         |                | ND                     | ND         | ND                            | ND                           | ND             | ND                          | ND                            |
|        | Half-brother | 35M 91     | 16                     |                         |                | ND                     | ND         | ND                            | ND                           | ND             | ND                          | ND                            |
| V      | Proband | 43F 128       | 12                     | GP1BA c.58T>G, p. Cys20Gly | N/A            | 5                      | ND         | ND                            | ND                           | 3              | ND                          |                               |
|        | Daughter | 14F 59        | Failed                 |                         |                | ND                     | ND         | ND                            | ND                           | 1              | ND                          |                               |
| VI     | Proband | 35F 114       | 14,6                   | GP1BA c.58T>G, p. Cys20Gly | N/A            | 5                      | ND         | ND                            | ND                           | 0              | ND                          |                               |
|        | Daughter | 7F 131        | 13,8                   |                         |                | ND                     | ND         | ND                            | ND                           | 0              | ND                          |                               |
| VII    | Proband | 61M 81        | Failed                 | GP1BA 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                 | GP1BA c.247C>T, p. Leu83Phe | 0.00083        | 5                      | 49         | 36                            | 0.62                         | 3              | 44                          |                               |
|        | Cousin  | 49M 84        | Failed                 |                         |                | ND                     | ND         | ND                            | ND                           | ND             | ND                          | ND                            |
|        | Son     | 25M 83        | ND                     |                         |                | ND                     | ND         | ND                            | ND                           | ND             | ND                          | ND                            |
| IX     | Proband | 25F 44        | Failed                 | GP1BA 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                     | GP1BA c.247C>T, p. Leu83Phe | 0.00083        | 5                      | ND         | ND                            | ND                           | 3              | ND                          |                               |

(Continues)
**TABLE 1** (Continued)

| Family | Patient | Age and gender | Platelet Count x10⁹/L | MPV (Ref.: 8–13 fL) | Family variant | Frequency (gnomAD) (%) | ACMG Class | GPIbα (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                | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | 5                      | 33                       | 32                  | 0.47             | 2               | 40               |
|        | Father  | 77M 87         | Failed                | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | ND                     | ND                      | ND                  | ND               | ND              | ND               |
| XII    | Proband | 63M 107        | 14                    | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | 5                      | ND                      | ND                  | 0                | 37              |
| XIII   | Proband | 33F 105        | Failed                | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | 5                      | 47                       | 32                  | 0.67             | 3               | ND               |
|        | Father  | 71M 107        | Failed                | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | 52                     | 48                       | 48                  | 0.48             | 6               | 60               |
|        | Brother | 31M 131        | 12                    | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | ND                     | ND                      | ND                  | ND               | 0               | 44               |
| XIV    | Proband | 37M 85         | Failed                | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | 4                      | 43                       | 58                  | 0.47             | 1               | 51               |
|        | Daughter| 7F 123         | 14                    | GP1BB c.236_244del, p. Pro79_Leu81del | N/A             | ND                     | 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 in percentage of CD42b median reference level/patient CD41a in percentage of CD41a median reference level; CD42b median reference level = 56.1 MFI and CD41a median reference level = 25.4 MFI.

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.
proband. 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, 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.
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. These findings suggest that targeting GPIb-IX-V could be a possible prospect for managing endotoxemia and sepsis 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. 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
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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| TABLE 2 | Thrombopoietin (TPO) in patients with monoallelic Bernard-Soulier syndrome and their healthy relatives |
|-----------------|-----------------|-----------------|
| Family variant  | Median TPO Ref. | gp/med 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) | |
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SUPPORTING INFORMATION

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

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