Rare variants in \textit{GP1BB} are responsible for autosomal dominant macrothrombocytopenia

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Key Points

- Variants in \textit{GP1BB} can cause autosomal dominant macrothrombocytopenia.

The von Willebrand receptor complex, which is composed of the glycoproteins Ibα, Ibβ, GPV, and GPIX, plays an essential role in the earliest steps in hemostasis. During the last 4 decades, it has become apparent that loss of function of any 1 of 3 of the genes encoding these glycoproteins (namely, \textit{GP1BA}, \textit{GP1BB}, and \textit{GP9}) leads to autosomal recessive macrothrombocytopenia complicated by bleeding. A small number of variants in \textit{GP1BA} have been reported to cause a milder and dominant form of macrothrombocytopenia, but only 2 tentative reports exist of such a variant in \textit{GP1BB}. By analyzing data from a collection of more than 1000 genome-sequenced patients with a rare bleeding and/or platelet disorder, we have identified a significant association between rare monoallelic variants in \textit{GP1BB} and macrothrombocytopenia. To strengthen our findings, we sought further cases in 2 additional collections in the United Kingdom and Japan. Across 18 families exhibiting phenotypes consistent with autosomal dominant inheritance of macrothrombocytopenia, we report on 27 affected cases carrying 1 of 9 rare variants in \textit{GP1BB}. (\textit{Blood}. 2017;129(4):520-524)

Introduction

The earliest step in hemostasis is the tethering of platelets to the damaged endothelium through their cell surface receptor for von Willebrand factor (VWF). The glycoproteins (GPs) Ibα and Ibβ, in association with GPV and GPIX, form the transmembrane receptor complex on platelets for VWF,1,2 The leucine-rich repeat (LRR) domain of GPIbα contains the binding site for VWF, whereas the \(\beta\) chain contributes to the surface expression of the complex, which, by phosphorylating its intracellular domain, also participates in downstream signaling. GPIbβ is synthesized from a 1.5-kb mRNA transcribed from \textit{GP1BB} and is highly expressed in megakaryocytes, but absent from other blood cell progenitors.3 The mRNA encodes a 206-amino-acid-long transmembrane protein with a 22-kD molecular mass and an extracellular LRR domain.4,6

Bernard and Soulier were the first to describe a patient with a rare autosomal recessive syndrome (Bernard-Soulier syndrome; BSS) characterized by giant platelets with concomitant thrombocytopenia complicated by severe bleeding.7 Platelets from patients with this syndrome do not agglutinate in response to ristocetin and show a subtly reduced aggregation response to thrombin. Over the course of 4 decades, 45, 39, and 28 variants have been identified in \textit{GP1BA}, \textit{GP1BB}, and \textit{GP9}, respectively, that, if present in compound heterozygous or homozygous form, cause the VWF receptor to be...
absent or reduced in function. Only 4 variants in GP1BA with dominant effects have been reported, one being the “Bolzano variant,” and a further 3 observed in 3 isolated pedigrees. Only 2 variants in GP1BB have been reported as being possibly responsible for dominant macrothrombocytopenia. One encoding Y113C was identified in 2 Japanese families and led to initial speculation that variants in the subunits of the GPIb/IX complex could span a spectrum of platelet and bleeding phenotypes. Another, encoding R42C, was observed in only 1 Japanese patient, and data from family or functional studies supporting an association with a dominant encoding R42C, was observed in only 1 Japanese patient, and data from family or functional studies supporting an association with a dominant phenotype were lacking. There are no reports of variants in GP9 having a dominant effect, and BSS cases resulting from variants in GP5 do not exist because GPV is not required for receptor expression.

We found a significant statistical association between rare monoallelic nonsynonymous variants in GP1BB and macrothrombocytopenia. Family history and cosegregation data from 18 pedigrees, in which 27 affected cases carry 1 of 9 rare variants in GP1BB, are consistent with autosomal dominant inheritance.

**Study design**

**Study population**

The study population comprises a discovery collection and 2 validation collections. The discovery collection consists of cases with an assumed inherited bleeding or platelet disorder (BPD) of unknown molecular etiology or their relatives enrolled in the National Institute for Health Research (NIHR) Biobank Resource after providing informed written consent. The Biobank cohort was enrolled during a 4-year period and is composed of 1542 patients with a BPD or their close relatives, with a further 5422 patients with other rare inherited disorders or their close relatives. The validation collections contain data from 75 patients with thrombocytopenia assessed with the ThromboGenomics diagnostic platform, and 301 Japanese probands referred during an 8-year period for a suspected diagnosis of inherited macrothrombocytopenia and their relatives. Institutional review board approval was obtained for this international multicenter study.

**Data analysis**

Coding of clinical and laboratory phenotypes with Human Phenotype Ontology (HPO) terms and collection of numerical and family history data were performed as described previously. Variants from high-throughput sequencing were called and annotated using Isaic (Illumina, Inc; whole-genome sequencing) or as described previously (capture-based sequencing). We used phenotype similarity regression (SimReg) to identify statistical associations between presence of a variant affecting protein sequence in a gene and similarity to a latent HPO–coded phenotype in the discovery collection, subsequently corroborated using Fisher’s exact test. The variants obtained by high-throughput DNA sequencing of the probands were genotyped by Sanger sequencing in relatives who agreed to participate in this study. These variants were identified in all members of the Japanese collection were obtained by Sanger sequencing. Where possible, complete blood counts were obtained using automated hematology analyzers, VWF receptor expression levels on platelets were determined by cytometry, and platelet function was tested by light transmission aggregometry. Macrothrombocytopenia was deemed present if the platelet count was below 150 × 10^9/L, the mean platelet volume was above 12 fl, or there was clear platelet anisocytosis, with a subset of platelets being abnormally large.

**Results and discussion**

We identified a strong statistical association between the presence of rare nonsynonymous monoallelic DNA variants (population allele frequency less than 1/10 000) in GP1BB and macrothrombocytopenia in the discovery collection (SimReg posterior probability = 0.93 with inferred characteristic phenotype preferentially included the HPO term “Increased mean platelet volume”; Fisher’s P = 2.10 × 10^-9). All 8 probands with macrothrombocytopenia from the discovery collection had a family history suggestive of autosomal dominant inheritance. This mode of transmission was corroborated by results from cosegregation studies (Figure 1; P = 1.95 × 10^-5). Systematic review of rare variants in these 8 cases within the 15 established genes implicated in macrothrombocytopenia, including GP1BA and GP9, did not reveal any alternative variants that could plausibly explain the platelet phenotype. We searched for further cases in the validation collection and identified 4 further probands in the ThromboGenomics data set and 6 further probands in the Japanese collection who carried a variant specific to people of Japanese ancestry (Figure 1).

Altogether, we identified 9 unique variants in GP1BB: 1 resulting in a disruption of the canonical methionine start codon; 1 encoding a premature stop at residue 46; 5 missense variants encoding L16P, G43W, T68M, Y113C, and L132Q; a 3′–5′ acid deletion P79_L81del; and a frameshift in the codon for residue A150 leading to an alternative reading frame with a premature stop after 43 further residues (Figure 2A). Assessment of the potential effect of the missense and in-frame variants on GPIbβ structure, supported by prior work by McEwan et al., is available in supplemental Table 2 and supplemental Figure 1 on the Blood Web site. Only the variant encoding G43W is present in the ExAC database of more than 60 000 exome-sequenced individuals, where it is heterozygous in just 1 individual. It is noteworthy that the variant underlying the premature stop at residue 46, the variant leading to a frameshift at residue 150, and the missense variants encoding L16P, G43W, and Y113C have been previously implicated in BSS. We reason that the effects of these 5 variants in heterozygosity, except for Y113C, went unnoticed because the counts of the parents of these BSS probands were not measured or were not deemed notably low in isolation from other monoallelic cases.

The count and mean volume of platelets of the 18 probands were 107.9 × 10^9/L (range, 47–172 × 10^9/L) and 12.74 fl (range, 10.7–14.3 fl), respectively (Figure 2B; supplemental Table 1). In several cases, May-Grünwald-Giemsa-stained blood smears revealed platelet anisocytosis, with a subset of platelets being abnormally large; however, blood films were not available for all cases (Figure 2C; supplemental Table 1). Electron micrographs of the platelets of B-3 show significantly increased platelet surface area that is intermediate between healthy controls and BSS cases, but includes a small number of giant platelets (Figure 2D). Thus, the mean platelet volumes obtained by automated complete blood count analysis may obscure an increased spread of platelet volumes. Bleeding diathesis, including menorrhagia, epistaxis, spontaneous bleeding, and postpartum bleeding, was reported in 9 of 23 females but in none of the 8 males for whom platelet count and bleeding phenotype information was available, which suggests that the overall propensity to bleeding is, at most, only marginally increased in these patients relative to the general population. There was a reduction of at least 30% of GPIbα on platelets, as measured by flow cytometry in cases from 8 of the 9 families for which measurements were available (supplemental Table 1).

Each VWF receptor complex has 4 GPIbβ molecules, which are covalently bonded with 2 copies of GPIbα through cysteines at residues 147 and 526/527, respectively, and noncovalently paired with 2 molecules each of GPV and GPIIX. This stoichiometry may explain why a single allele encoding a mutated GPIbβ molecule may exert a dominant negative effect on the function of the VWF complex. It could also exacerbate haploinsufficiency in the cases with disruption of the methionine start codon and truncation at residue 46. It is important to note that although the overall association between
monoallelic variants in GP1BB and macrothrombocytopenia is robust, it is possible that some of the 9 variants reported here are individually not causal. Therefore, further work and the continued sharing of genotype and phenotype data from many patients with macrothrombocytopenia will be required to establish the molecular consequences of these variants definitively.
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A complete list of the members of NIHR BioResource appears in the online appendix.

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