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Regular Article

Novel Bernard-Soulier syndrome variants caused by compound heterozygous mutations (case I) or a cytoplasmic tail truncation (case II) of GPIbα

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ABSTRACT

A defective platelet glycoprotein (GP) Ib/IX/V complex [von Willebrand factor (VWF) receptor] results in Bernard-Soulier syndrome (BSS), which is characterized by macrothrombocytopenia and impaired ristocetin- and thrombin-induced platelet aggregation. We found 2 independent BSS-variant families: Case I [compound heterozygous mutations, p.Glu331X and a frame shift by a deletion at c.1444delA of GPIbα (GP1BA) terminating at a premature stop codon (p.Thr452ProfsX58)], and case II [homozygous nonsense mutation at c.1723C>T, p.Gln545X]. Case I platelets expressed no GPIbα, resulting in absence of ristocetin-induced platelet aggregation (RIPA) and 50% reduction in thrombin-induced aggregation with no shape change. The mother's platelets had 50% the expression level of A-type GPIbα (4-repeated VNTR: variable number of tandem repeats, p.[Thr145Met; Ser399_Pro411[4]]); the father's platelets had the same expression level of C-type GPIbα (2-repeated VNTR, p.Ser399_Pro411dup) as the mother's platelets. The mother's RIPA was significantly higher than the father's. Thrombin-induced aggregation was normal in both parents. Case II platelets expressed a GPIbα with an abnormal cytoplasmic tail, p.Gln545X-truncated GPIbα, which complexed with GPIX and GPV on the cell surface; its expression level of the complex was normal. Case II platelets had reversible RIPA, with no ATP release, and weak thrombin-induced aggregation without shape change. These results suggest that a signaling process through the GPIbα cytoplasmic tail required for full platelet activation is defective in BSS variant case II and a length polymorphism of GPIbα is associated with a modified level of RIPA heterozygous BSS case I.

Introduction

Bernard-Soulier syndrome (BSS), a bleeding disorder exhibiting macrothrombocytopenia, is caused by defects in the platelet GPIb/IX/V complex, a receptor for von Willebrand factor (VWF) and thrombin. GPIb, a covalently linked heterodimer of GPIbα and GPIbβ subunits, is non-covalently associated with GPIX and GPV in resting platelets. VWF and thrombin binding sites in GPIbα are restricted to the N-terminal region including both leucine-rich repeats and a sulfated tyrosine sequence. Conformational changes in the leucine-rich repeats and anionic charge in the sulfated tyrosine are important for VWF and α-thrombin binding. Thus, mutations in the genes encoding GPIbα [1–9], GPIbβ [8,10–18], and GPIX [2,19–31] cause BSS [32].

The GPIbα gene (GP1BA) contains 3 major polymorphic loci that exhibit the HPA-2 alloantigen due to a substitution of c.524C>T, a variable number of repeats (VNTR) encoded from S399 to T411 [33], and a C>T substitution (c.1324insTCAGAGCCCGCCCCCAGCCCGACTACCCCGGAGCC CACC) in the second repeat of the VNTR designated as C'-type [34]. Four haplotypes of length polymorphism of GPIbα exist due to repeat numbers of 1 – 4 in the VNTR: types A – D, respectively [35], which are associated with linkage disequilibrium of HPA-2 and VNTR, where

Abbreviations: BSS, Bernard-Soulier syndrome; RIPA, Ristocetin-induced platelet aggregation; GP, Glycoprotein; VNTR, Variable number of tandem repeats; VWF, von Willebrand factor.

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T145(ACG) is linked with the gene encoding types D or C, while M145(ATG) is linked with the gene encoding types C, B, and A [36]. Whether this polymorphism is linked to coronary artery disease (CAD) [37] or not [38,39] is controversial. This study investigated platelet functions in two Japanese families with new variant types of BSS, which are novel mutations in GPIbα associated with delay of platelet activation, in which length polymorphism VNTR of GPIbα (case I) and cytoplasmic truncation of GPIbα (case II) are involved. Our determination of the molecular level changes in GPIb found in these two BSS variants and their associated defects in platelet activation may provide further insight into the GPIbα-dependent activation pathways in human platelets.

Methods and Methods

Materials

Human α-thrombin and ADP (Sigma-Aldrich, St. Louis, MO), ristocetin (Lundbeck, Copenhagen, Denmark), and collagen (Hormon Chemie, Munich, Germany) were obtained commercially.

Antibodies

The following colleagues kindly provided antibodies: GI27 against cytoplasmic domain of GPIbβ [40], Dr. Sentot Santoso (Netherlands Red Cross, Netherlands); KMP9 (anti-GPIX antibody), Dr. Shosaku Nomura (Kansai Medical University, Okasa, Japan); polyclonal anti-GPIb antibody, Dr. Makoto Handa (Keio University, Tokyo, Japan); SW16 (anti-GPV antibody), Dr. Piet Modderman (Netherlands Red Cross,Netherland). The following antibodies were purchased from the indicated sources: anti-GPIbα antibodies TM60 (MBL, Nagoya, Japan), PL52-4 and WGA-3 (Takara Bio, Otsu, Japan), and SZ2 (Immunotech, Marseille, France); anti-GPIX antibodies FMC25 (ICN Biomedicals, Irvine, California, USA) and SZ1 (Immunotech, Marseille, France); anti-GPIIIa antibody T-74, anti-P-selectin antibody 2T60, and anti-CD36 antibody GS95 (MBL, Nagoya, Japan). GS70 (anti-GPIbα) and GS64 (anti-GPIX) were developed in our laboratory.

Platelet Size

Platelet-size histograms of 10,000 platelets were obtained from the Coulter Multisizer II (Beckman Coulter, Fullerton, CA); platelet size was obtained from the impedance signal accumulated in 256 channels.

Reference of GP1BA

Genbank accession number J02940.1 for GP1BA was referred to those from BSS cases in this study. The initial Met of the signal peptide is numbered as −16 [34]. The GP1BA sequence obtained from the BSS families has been registered in the Genbank database (Table 1).

Case Reports

All blood samples were collected from the subjects and analyzed after obtaining their written informed consent in accordance with the Ethical Committee of the Tokyo Metropolitan Institute of Medical Science and the Declaration of Helsinki.

Case I

The case I patient, a 34-year-old Japanese female whose case report was described elsewhere [41], had a lifelong history of recurrent epistaxis and menorrhagia. Her parents were not consanguineously related and exhibited no abnormal bleeding. Her platelets showed a giant platelet phenotype and did not express GPIbα, leading to the diagnosis of BSS.

Case II

The case II patient was a 23-year-old female exhibiting recurrent epistaxis and menorrhagia. She was diagnosed with BSS at age 2, as she had a severe bleeding disorder, giant platelets, and complete absence of RIPA, with the following parameters: platelet count, 11.0×10^4/μl; bleeding time, >10 minutes; clotting time, 7 minutes; prothrombin time (PT), 10.6 seconds; and partial thrombin time (PTT), 52.5 seconds; fibrinogen level, 294.3 mg/dl; levels of other coagulation factors were within the normal range [42]. Except for the current RIPA level, which was discrepant from the total absence of RIPA obtained at age 2, the other laboratory parameters were the same as those obtained at age 2. The patient's family showed no consanguinity. Her platelets were ~3.9 μm in diameter, 1.7-fold larger than normal control platelet size (mean size: 2.3 μm), confirming the typical giant-shape phenotype previously diagnosed morphologically at age 2. Her father's platelets were only slightly larger than normal (approximately 2.5 μm).

Allele-specific PCR to Observe the Linkage of Single Adenine Deletion to VNTR

The DNA region encoding the VNTR region (1275 – 1323) (Genbank accession no. J02940.1) and 7-polyadenine (1436 – 1442) was amplified by PWO SuperYield DNA polymerase (Roche Applied Science) with the following primer pairs: P3F (1000 – 1020, 5'-ACAACCCCCTGGGGT CTATTC-3') and P7RDEL (1437 – 1457, 5'-AGTTCAGGGATGGTTTTTTG-3'), designed to anneal to the mutant allele, and P3F and P6RNOR (1437 – 1457, 5'-AGTTCAGGGATGGTTTTTTTG-3'), designed to anneal to the normal allele. Optimal PCR conditions for maximal yield of the VNTR region were used: 35 cycles of denaturation at 96 °C, 1 minute; annealing at 62 °C, 2 minutes; and elongation at 72 °C, 3 minutes. Amplified DNA fragments were analyzed on 1.5% agarose gel.

Ristocetin-induced Platelet Aggregation (RIPA) and ATP-release

RIPA levels were examined in the same plasma and at the same platelet counts by replacing autologous plasma in platelet-rich plasma (PRP) with normal pooled plasma: the PRP sample (pH adjusted to 6.5 by adding citric acid) was centrifuged to pellet the platelets and the plasma removed completely; then the platelets were resuspended in normal plasma to 3.0×10^5 platelets/μl. Platelet aggregation was measured using a Hematracer (Niko Biosicence, Tokyo, Japan). To observe agglutination without signal transduction through GPIb/IX/V, RIPA was determined in the presence of PGE_1 (1 μg/ml), while PGE_1 was excluded to

Table 1
GP1BA mutations in case I and case II BSS describing by different nomenclatures.

BSS	Allele	Ref ID	Accession No. J02940 (*)		VNTR
Case I	Paternal	AB576652.1	c.[1081G>T;1324insTCAGAGCCCGCCCCCAGCCCGACTACCCCGGAGCCCACC]	p.Glu331X	C'
	Maternal	AB576654.1	c.[1324insTCAGAGCCCGCCCCCAGCCCGACTACCCCGGAGCCCACC;1444delA]	p.[Ser399_Pro411dup;Thr452ProfsX58]	C'
	Father	AB576655.1	c.1324insTCAGAGCCCGCCCCCAGCCCGACTACCCCGGAGCCCACC	p.Ser399_Pro411dup	C'
	Mother	AB576651.1	c.[524C>T;1285_1323[4]]	p.[Thr145Met;Ser399_Pro411[4]]	A
Case II	Patient	AB576653.1	c.[1285_1323dup;1723C>T]	p.[Ser399_Pro411dup;Gln545X]	C

(*) Numbering of A of ATG as +43 and Met as −16 (GenBank: J02940.1) are used.