Naturally Occurring Mutations in Glycoprotein Ibα That Result in Defective Ligand Binding and Synthesis of a Truncated Protein

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The platelet GPIb-V-IX complex is the receptor for the initial binding of von Willebrand factor (vWF) mediating platelet adhesion. The complex is composed of four membrane-spanning glycoproteins (GP): GPIbα, GPIbβ, GPIX, and GPV. Bernard-Soulier syndrome results from a qualitative or quantitative defect in one or more components of the platelet membrane GPIb-V-IX complex. We describe the molecular basis of a novel Bernard-Soulier syndrome variant in two siblings in whom GPIbα was not detected on the platelet surface but that was present in a soluble form in plasma. DNA sequence analysis showed that the affected individuals were compound heterozygotes for two mutations. One, inherited from a maternal allele, a Trp498→Arg mutation in GPIbα containing the Trp498→Arg mutation were transiently transfected into Chinese hamster ovary (CHO) cells stably expressing the GPIb-V-IX complex (CHOpIX), the expression of GPIbα was similar to the wild-type (WT) GPIbα, but did not bind vWF. When plasmids encoding GPIbα containing the Trp498→Arg mutation were transiently transfected into CHOpIX, the surface expression of GPIbα was barely detectable compared with the WT GPIbα. Thus, this newly described compound heterozygous defect produces Bernard-Soulier syndrome by a combination of synthesis of a nonfunctional protein and of a truncated protein that fails to insert into the platelet membrane and is found circulating in plasma.

The expression of the GPIb-V-IX complex is dependent on the coordinated assembly of at least three gene products, the α- and β-subunits of GPIb, and GPIX. The molecular basis of Bernard-Soulier syndrome has been characterized in several published cases to date providing further evidence that each of these subunits has a critical role in the coordinate assembly of the functional complex. Whereas GPV has also been shown to be absent in Bernard-Soulier syndrome, it does not seem to be necessary for the surface expression of the complex.

Given that each polypeptide is encoded by its own gene and that the coordinate assembly of the complex is required, it is surprising that Bernard-Soulier syndrome is so rare. Most of the mutations characterized that result in Bernard-Soulier syndrome are within the GPIbα gene. These mutations have been caused by nonsense mutations producing a truncated GPIbα protein or mutations that have been localized to the leucine-rich motif (LRM) of GPIbα. A single case having a mutation in GPIbα that changed a cysteine residue involved in disulfide bonding has also been described. We and other investigators have recently characterized a mutation within the GPIbα gene.

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transmembrane region of GP Ibα that affected anchoring of the GP Ibα polypeptide in platelets and results in a circulating soluble GP Ibα. A number of mutations have been identified within GPIX, two of which resulted from an amino acid change in the LRM or the region flanking the LRM in GPIX, and two other point mutations that changed a cysteine to a tyrosine in GPIX and another that caused a nonsense codon. There has been a single published report of a mutation within the promoter for GP Ibβ that resulted in Bernard-Soulier syndrome.

This report describes the molecular genetic basis for a novel variant mutation within the gene coding for GP Ibα that is responsible for Bernard-Soulier syndrome.

MATERIALS AND METHODS

Case History

A 5-year-old girl and her 20-year-old brother from eastern Iceland were diagnosed as having Bernard-Soulier syndrome. The boy presented at 15 months of age with easy bruising and a platelet count of 29,000 to 67,000/mm³. He was treated initially with steroids. At 5 years of age, he underwent a splenectomy, as he was believed to have idiopathic thrombocytopenic purpura. At 8 years of age, an episode of epistaxis required hospital admission, and large platelets were noted on a blood smear. Platelet aggregation studies showed normal response to ristocetin. The platelet count was 120,000/mm³, the bone marrow aspirate was normal, and the platelet count has remained at approximately 120,000/mm³ since the splenectomy. The younger sister was first admitted to hospital at 16 months of age because of episodes of bleeding and easy bruising. Her bleeding time was prolonged (>13 minutes) and platelet count was 75,000/mm³. She has had several episodes of bleeding requiring transfusion. There are six other siblings, none of whom has any bleeding symptoms. The mother had several episodes of bleeding requiring transfusion. There are no other siblings, none of whom has any bleeding symptoms. The mother and father are unrelated and unaffected clinically with normal platelet counts and morphology.

Monoclonal antibodies and reagents. The anti-GP Ibα antibody AP-1 blocks vWF binding to GP Ibα. MBC 142.2, 142.6, and 142.11 are monoclonal antibodies (MoAbs) raised against purified GP Ibα that do not inhibit the binding of vWF to GP Ibα. An anti-GPI MoAb (FMC 25) was purchased from Harlan Bioproducts (Indianapolis, IN). AP2 is a MoAb against the GPIIb-IIIa complex. An affinity-purified platelet GPIIb-specific rabbit polyclonal antibody was a generous gift of Dr Sander S. Shapiro (Cardence Foundation for Hematologic Research, Philadelphia, PA).

Purified glycopcalcin was obtained from outdated platelets as described and provided by Dr P.A. Kroner (Blood Center of Southeastern Wisconsin). Bortocetin was purified according to the method of Laemmli. The separated proteins were electroblotted onto a polyvinylidene difluoride (PVDF) membrane (Novex, San Diego, CA) as described by Towbin et al.

Immunoprecipitation. GP Ibα MoAb 142.2 was coupled to cyanogen bromide-activated Sepharose 4B beads (Sigma). PPP was pre-cleared by incubating with uncoupled Sepharose CL-4B beads for 1 hour at room temperature. The beads were centrifuged at 1,000 g and the plasma added to the antibody-coupled beads and incubated overnight at 4°C. The beads were washed and the immunoprecipitated complexes from plasma eluted in 2% SDS. All samples were boiled at 100°C for 3 minutes. The samples were then analyzed by SDS-PAGE on an 8% to 16% exponential gradient in the presence of 20 mmol dithiothreitol. Immunoblotting was performed in the same manner as described above, using the antibody MBC 142.11.

Flowerrysm of analysis of platelets in whole blood. The platelet GPIb-IX complex and GP IbβIla were analyzed by flow cytometry, using a two-color, two-antibody technique. A total of 100 µL of whole blood was diluted 1:10 in PBS and divided into six 50-µL aliquots. Samples were then either stained, incubated with 2 µg/mL of mouse IgG as a negative control, 2 µg/mL of fluorescein isothiocyanate (FITC)-conjugated MoAb AP1, 2 µg/mL of FITC-conjugated AP2, a combination of a biotin-conjugated AP2 plus FITC-AP1, or a combination of a biotin-conjugated AP1 plus FITC-conjugated AP2. The samples were incubated in the dark for 20 minutes at room temperature.

Further experiments were performed to evaluate the surface expression of GP Ibα and of GP Ib on platelets using the anti-GP Ibα antibodies 142.2 and 142.11 and the anti-GP Ibα antibody FMC-25. In these experiments, platelets were identified and gated, both by their PE-AP1 fluorescence intensity and by their physical properties on a forward versus side-scatter plot. Data for these platelets were collected through this gate and were analyzed for fluorescence with the antibodies 142.2 and 142.11 and FMC-25.

PCR amplification of genomic DNA. Genomic DNA was isolated from peripheral blood lymphocytes (PBLs) as described. DNA was amplified by the polymerase chain reaction (PCR), using primer pairs based on the published genomic sequence of GP Ibα. For DNA sequence analysis, the full-length coding region for mature GP Ibα was amplified with primers 162-181 (GCCCCTGCAATTCTCCTCACC) and 2653-2634 (AAGCTCCCCAGTGCTGCATGGG). The target se

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quences were amplified in a 50-µL reaction volume containing 500 to 1,000 ng of genomic DNA, 30 pmol of each primer, and 0.2 mmol of each dNTP in a reaction buffer consisting of 60 mmol/Tris-HCl pH 9.0, 15 mmol (NH₄)₂SO₄, 2 mmol/L MgCl₂, and 1 U of Taq polymerase (Perkin Elmer, Foster City, CA) and 4% (vol/vol) DMSO. PCR amplification was performed in a programmable thermal cycler (model 9600, Perkin Elmer) for 35 cycles of 45 seconds denaturation at 96°C, annealing for 1 minute at 60°C, and extension for 1 minute at 72°C. PCR products containing the entire coding region for GPIbα were cloned into the pCRII.1 cloning vector using the TA cloning kit (Invitrogen, San Diego, CA).

DNA sequencing. Direct sequence analysis of the entire coding region of PCR-amplified GPIbα from each subject and a minimum of three clones each from the two patients, the mother, and father was performed using the Prism Ready Reaction DyeDeoxy terminator cycle sequencing kit and an Applied Biosystems (Foster City, CA) model 373A DNA Sequencer. Sequencing primers were synthesized on an Applied Biosystems model 394/4DNA synthesizer.

Transient expression. A Bsg I and BsaGI restriction fragment (nucleotides 218–847) of the subcloned GPIbα PCR product amplified from genomic DNA containing the T → C substitution at nucleotide 777 in GPIbα (Cys⁶⁵ → Arg) was subcloned into pGEM-7 containing the WT GPIbα cDNA, from which a BsI and BsaGI restriction fragment had been excised. An XhoI/MluI restriction fragment, containing the entire coding region for GPIbα, was then excised from this pGEM-7GPIbαC⁶⁵ → R and inserted into the mammalian expression vector pCI-NEO (Promega, Madison, WI), which carries the human cytomegalo-virus (CMV) promoter and the SV40 origin of replication. This expression vector (pCI-NEOGPIbαC⁶⁵ → R) now contained the entire coding region for GPIbα with the point mutation at codon 65. In a similar manner, a Pst I restriction fragment (nucleotides 1246–2206 and containing the G → A substitution at nucleotide 2078 resulting in W⁴⁹⁸ → stop) of the subcloned GPIbα PCR product was subcloned into pGEM-7 containing the WT GPIbα cDNA, from which a Pst I restriction fragment had been excised. Again, an XhoI/MluI restriction fragment, containing the entire coding region for GPIbα, was excised from pGEM-7GPIbαW⁴⁹⁸ → stop and inserted into the expression vector pCI-NEO. This expression vector (pCI-NEOGPIbαW⁴⁹⁸ → stop) now contained the entire coding region for GPIbα with the point mutation at codon 498. Constructs containing both the WT and mutant GPIbα were sequenced to ensure that no additional mutations had been introduced.

For transient expression studies, CHO BIX cells (kindly provided by Dr José A. López, Baylor College of Medicine, Houston, TX) were used. CHO BIX cells are CHO cells that stably surface express human GPIbα and GPIbβ and GP IX at high levels. 42 These cells were additionally transiently transfected with either the WT GPIbα, the construct containing the pCI-NEOGPIbαC⁶⁵ → R, the construct containing pCI-NEOGPIbαW⁴⁹⁸ → stop or mock-transfected with the plasmid pCI-NEO alone. Expression plasmids were introduced into CHO BIX cells in the presence of lipofectamine (GIBCO-BRL), following the protocol of Felgner et al. 43 In brief, 1.5 × 10⁶ cells were plated in 100-mm dishes and grown overnight. 6.6 mL of OPTI-MEM–reduced serum media (GIBCO-BRL) containing 72 µg of lipofectamine and 6 µg of the appropriate plasmid DNA was added, and the cells were incubated for 5 hours. The transfection media was removed and 8 mL of culture media was added; incubation was continued at 37°C for 48 hours.

Transfected cells were detached from tissue culture plates with 3 mmol EDTA, centrifuged at 250g and resuspended in HBSS with 1% bovine serum albumin (BSA) and 1% normal donkey serum. 3 × 10⁵ cells were transferred to each well of a 96-well V-bottom plate (Dynatech, Chantilly, VA) and incubated for 30 minutes simultaneously with 5 µg/mL of vWF, 1 µg/mL of botrocetin the anti-GPIbα antibody MBC 142.2, and a rabbit polyclonal antibody to vWF (5 µg/mL and 3 µg/mL, respectively). A concentration of 1 µg/mL of botrocetin was used, as previous investigations from our laboratory have shown no significant difference in vWF binding with 1 µg/mL of botrocetin as compared with 5 µg/mL. 44 The cells were then washed twice and incubated for an additional 30 minutes in a darkened room with a 1:100 dilution of PE-conjugated affinity-purified Fab(‘)₂ donkey anti-mouse IgG and a 1:320 dilution DTAF-conjugated affinity-purified Fab(‘)₂ donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) to detect vWF binding in cells expressing GPIbα. The cells were then washed twice, resuspended in 2% paraformaldehyde, and analyzed in a Becton Dickinson FACScan flow cytometer.

RESULTS

GPIbα Is Undetectable on the Patient’s Platelet Surface

FACS analysis of whole blood showed that the MoAb AP1 failed to recognize GPIbα on the platelet surface of these two patients (Fig 1). Although there was no detectable binding of AP1 to platelets from either of the affected siblings, the binding of AP1 was similar in both parents, as compared with three normal controls. By contrast, binding of AP2 was normal in both patients and their parents (data not shown). Further analysis of patients' platelets from the eldest patient showed that neither of the anti-GPIbα antibodies, 142.2 and 142.11, bound to platelets (Fig 2). By contrast, the expression of GPIbX was normal in the patients' platelets. Immunoblot analysis of platelet
lysate with the monoclonal antibodies MBC 142.6 confirmed the absence of GPIbα in either of the patient’s platelets (Fig 3). Immunoblot analysis of platelet lysate under reduced conditions with the anti-GPIbβ polyclonal antibodies demonstrated that GPIbβ was present in the patients platelets, albeit in markedly reduced amounts (Fig 4). Soluble GPIbα can be immunoprecipitated from the patient’s plasma. Immunoprecipitation of plasma with MBC 142.2 and immunoblotting with MBC 142.6 demonstrated the presence of a protein of approximately 130-kD in both siblings and their parents. This band had a mobility similar to that of purified glycocalcin. However, this was significantly reduced in both patients compared to their parents (Fig 5). Thus, it seems unlikely that the absence of detectable GPIbα on the patient’s platelets was caused by cleavage of glycocalcin from the platelet surface, as this would result in a greater amount of circulating glycocalcin.

**Patients have compound heterozygous mutations within the GPIbα gene.** To determine the molecular basis for the absence of GPIbα, we amplified the entire coding region of GPIbα from both patients’ genomic DNA. The PCR-amplified DNA of both patients were of the predicted size according to the published
sequence. Direct sequence analysis of PCR-amplified DNA showed a heterozygous $T \rightarrow C$ substitution at nucleotide 777 and a heterozygous $G \rightarrow A$ substitution at nucleotide 2078 (Fig 6). The $T \rightarrow C$ substitution changes a free cysteine within the second leucine-rich repeat of GPIba to an arginine. The $G \rightarrow A$ substitution results in a premature stop codon within the transmembrane region of GPIba.

In addition to these mutations, a number of other differences were noted from the published sequence. Within the intron, there was an $A \rightarrow T$ polymorphism at nucleotide 497 and a $T \rightarrow C$ polymorphism at nucleotide 532 within the 5' untranslated region of GPIba. We followed the segregation of these mutations within the affected family by amplification and direct sequencing of the entire coding region of both parents. In addition, the entire coding region was also cloned and sequenced in both parents. An allele from the mother contained the $T \rightarrow C$ substitution at nucleotide 777; this allele also contained two of the variable number of tandem repeats in the macroglycopeptide region. The mother was homozygous for an A in the A497T polymorphism within the intron, and her WT allele contained only one of the tandem repeat polymorphisms. The allele from the father contained the $G \rightarrow A$ transversion at nucleotide 2078. The father was homozygous for the $T$ polymorphism within the intron and the $T \rightarrow C$ polymorphism at...
nucleotide 532 within the 5' untranslated region of GPIbα. Both fathers' alleles contained two of the variable number of tandem repeats within the macroglycopeptide region. Consequently, the affected children had inherited the T777 → C mutation from the mother and the G2078 → A mutation from the father.

The mutations are either nonfunctional or not expressed in a mammalian expression system. Transfected alone, GPIbα is not expressed on the cell surface of CHO cells in appreciable quantities.\(^{1,6}\) Studies examining the role of GPIbβ and GPIX have shown that all three subunits are required for efficient expression of the ligand-binding subunit on the surface of transfected cells.\(^{1,6}\) Therefore, to investigate the effect of this mutation on surface expression, both the WT GPIbα and the constructs containing the mutations were transiently transfected individually into CHO cells that stably express both GPIbβ and IX.\(^{1,6}\) These cells were then incubated with vWF, botrocetin, and antibodies against GPIX and vWF, and then analyzed by flow cytometry. Botrocetin was used in these investigations, as the patients' platelets failed to aggregate in the presence of ristocetin. As shown in Fig 7, a significant fraction (9.77%) of the cells transfected with the WT GPIbα construct exhibited an appreciable increase in surface fluorescence when reacted with the anti-GPIbα antibody 142.11. Furthermore, a significant number of the cells expressing GPIbα bound vWF (2.94%). Surprisingly, there was an increase in surface fluorescence of cells transfected with the C65 → R construct when reacted with the anti-GPIbα antibody 142.11, which was similar to the WT transfections, however none of these cells bound vWF. By contrast, surface expression of the construct containing the W498stop construct was barely detectable, and none of these cells bound vWF.

**DISCUSSION**

A critical event in hemostasis is the interaction of vWF with the platelet GPIb-IIIa receptor. Defects in the GPIb-IIIa complex result in the congenital acquired bleeding disorder Bernard-Soulier syndrome. In the present investigation, we have identified, to the best of our knowledge, the first patients with these compound heterozygous mutations within the coding region for the α-subunit of the GPIb-IIIa complex that cause Bernard-Soulier syndrome. Both Western blot and FACS analysis of platelets from both patients failed to demonstrate any GPIbα; however, FACs analysis clearly demonstrated GPIbα on the surface of both parents who are heterozygous carriers, similar to normal volunteers. The clinical diagnosis of Bernard-Soulier syndrome was suggested by giant platelets and an absence of ristocetin-induced platelet aggregation.\(^{34}\) The specific diagnosis was confirmed by detailed platelet membrane glycoprotein analysis and expression studies. In the present investigation, we used botrocetin as an agonist to induce binding of vWF to expressed GPIbα. Previous investigations, using either recombinant soluble GPIbα\(^{25}\) or a chimera containing the vWF binding region of GPIbα,\(^{45}\) have demonstrated no difference in the binding of vWF to GPIbα induced by either ristocetin or botrocetin. Furthermore botrocetin, like ristocetin, fails to induce agglutination of platelets from a Bernard-Soulier syndrome patient.\(^{46}\) Because botrocetin forms a soluble complex with vWF,\(^{47}\) a technical advantage over ristocetin\(^{48}\) when examining the binding of vWF to GPIbα in transfected cells, we used botrocetin as an agonist to study the role of vWF binding to the recombinant expressed GPIbα. Expression studies confirmed that the mutations identified in these affected patients resulted in expression of a nonfunctional protein and the decreased surface expression of GPIbα.

The consensus sequence for the leucine rich repeats for GPIbα and for the entire family of leucine-rich repeats is shown in Fig 8. At the sixth residue within the consensus sequence or binding of vWF is detected in the mock-transfected cells. B: In CHO cells transiently transfected with the wild-type (WT) GPIbα (B), and in CHO cells transfected with any GPIbα construct results in a significant increase in fluorescence when detected with the anti-GPIbα antibody (A); neither GPIbα expression or binding of vWF is detected in the mock-transfected cells. C: Whereas the GPIbα(Cys65 → Arg (C) and GPIbα(Trp498 → stop (D). The x-axis is fluorescence detected with an anti-GPIbα MoAb. The y-axis is fluorescence detected with a polyclonal anti-vWF antibody. A: Neither GPIbα expression or binding of vWF is detected in the mock-transfected cells. B: In cells transfected with the WT GPIbα, GPIbα is readily detectable on the cell surface (9.77% of cells) and binds vWF (2.94% of cells). C: Whereas the GPIbα(Cys65 → Arg construct results in a significant increase in fluorescence when detected with the anti-GPIbα antibody (6.68%) of cells, there is no binding of vWF. D: In the cells transiently transfected with the GPIbα(Trp498 → stop construct, there is no significant increase in surface fluorescence when detected with the anti-GPIbα antibody, and there is no binding of vWF.
receptor that does not bind vWF "in response to the agonist botrocetin" in vitro. Whereas measurement of vWF binding in the presence of ristocetin in vitro may have provided useful confirmatory evidence of the platelet studies in vivo, other investigators have shown that platelets from Bernard-Soulier syndrome patients do not agglutinate in response to botrocetin."

In contrast to the results demonstrated on the patients’ platelet surfaces, the results of the expression studies demonstrate some surface expression, of the C65 — R mutation. This discrepancy has a number of potential explanations. In vivo disruption of the normal trafficking pathways of the complex by causing a conformational change within the protein, as a result of changing a free cysteine could explain the observed Bernard-Soulier syndrome phenotype. The results obtained in vitro, using a eukaryotic expression vector in which transcription is greatly exaggerated may account for the results seen in vitro. Platelets that lack a nucleus do not have the same ability to synthesize proteins; consequently, any protein that may be made in these two patients is below the physiologically relevant range and results in the phenotype described. However, even when expressed in an in vitro system the mutant protein clearly failed to bind vWF.

The precise mechanism whereby a mutation within the leucine-rich repeat causes Bernard-Soulier syndrome is unclear. Investigations using either overlapping synthetic peptides or site-directed mutagenesis have demonstrated that residues Ser251 — Asp287 are critically important for the binding of vWF to GPIbα induced by the agonists ristocetin and botrocetin. Residues Ser251, Asp287 are located near the N-terminus of the receptor between the leucine-rich repeats and the macroglycopeptide region. Curiously, although there have been several reports of mutations within the leucine-rich region that resulted in Bernard-Soulier syndrome, to date no mutations described within the region bounded by residues 251—287 have resulted in Bernard-Soulier syndrome. Using overlapping synthetic peptides to delineate the vWF binding region of GPIbα, Vincente et al showed that peptides spanning the second and third leucine-rich repeat inhibited ristocetin-dependent binding of platelets to vWF to a similar degree as did synthetic peptides between residues 251—287. By contrast, peptides within the second and third leucine-rich repeat were marginally less effective in inhibiting botrocetin induced binding of vWF compared to peptides within residues 251—287. The corollary of ristocetin or botrocetin-induced binding of vWF to GPIb in vivo is unclear. However, the results of these in vitro experiments in conjunction with the mutations described in vivo would suggest that the structure of the leucine-rich repeats is essential for residues 251—287 to be able to function in the binding of vWF to GPIb. The results of the present investigation suggest that the in vivo mutations that affect C65 within the leucine-rich repeat not only inhibit adequate platelet surface expression but also disrupt function of the receptor when expressed in a mammalian cell line.

The postulated transmembrane region of GPIbα extends from Leu486 to Gly514. The transmembrane region is followed by two charged amino acids that may help anchor the protein within the platelet membrane; after this region is a cytoplasmic domain of approximately 100 amino acids. The second mutation described in this report converts the Trp508 to a stop codon approximately halfway within the transmembrane region. Although the coordinate expression of each of the three subunits is required for efficient surface expression of the complex, in the presence of a truncated transmembrane region it appears that GPIbα is synthesized but fails to anchor within the platelet membrane since GPIbα is found circulating in plasma and GPIX is found on the platelet surface. These results are similar to those described in a recent report by Holberg et al, who identified a patient with the W498stop. However, our expression studies demonstrate for the first time that this mutation results in the lack of surface expression of GPIbα. Although we have not demonstrated that the W498stop mutant is responsible for the soluble GPIbα found circulating in plasma, previous investigations both from our laboratory and from those of other investigators have demonstrated that in patients with Bernard-Soulier syndrome resulting from truncated versions of GPIbα that a soluble form of GPIbα is found circulating in plasma. These "experiments of nature" have been confirmed in expression studies which have demonstrated that truncated GPIbα is readily detectable in culture media. Together, these results strongly suggest that the W498stop mutant explains the soluble GPIbα found circulating in our patients plasma, albeit significantly reduced, as compared with normal. These results are analogous to a case of Bernard-Soulier syndrome previously described by our group, in which a mutation within the transmembrane region caused failure of the protein to anchor within the platelet; however, a soluble form was found in the patient’s plasma.

In the present investigation, GPIbα was not detectable on the platelet surface of the affected patients; however, platelet surface expression of GPIX was similar to that of a normal control. Transfection experiments have shown that at least three of the polypeptides (GPIbα, GPIbβ, and GPIX) are necessary for efficient surface expression of the GPIbα subunit, which is then capable of binding vWF. Therefore, a molecular genetic effect in either of these three subunits could cause Bernard-Soulier syndrome by a reduction in the vWF binding subunit. The interaction of GPIbα with GPIX is less clear. However,
López et al. demonstrated that in cell lines expressing each combination of only two of the three subunits, that two polypeptides would only associate in cells containing GPIbβ. In the present investigation, we demonstrated the presence of GPIbβ in platelet lysate, albeit reduced compared with the normal condition. The results of the present investigation confirm these experiments of López et al., by demonstrating normal GPIX expression with detectable GPIbβ. de la Salle et al.23 also reported similar findings of reduced levels of GPIbβ with normal GPIX in a patient with a mutation in the GPIbα subunit. It appears that GPIX can be expressed normally on the platelet surface with reduced amounts of GPIbβ and absent GPIbα.

In summary, we have identified and characterized a novel BSS resulting from compound heterozygous mutations within the GPIbα gene. One mutation changes a free cysteine within the leucine-rich motif and changes the functional surface expression of GPIbα, the other mutation causes a premature stop codon within the transmembrane region, so that the mutant peptide does not anchor within the plasma membrane and is found circulating in plasma. Each of these mutations, when inherited together with a normal allele in the family reported, does not cause any symptoms. However, compound heterozygotes for these mutation results in the observed phenotype of Bernard-Soulier syndrome.

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