Nonsense mutation in the glycoprotein Ib α coding sequence associated with Bernard–Soulier syndrome

(platelet receptor/thrombosis/bleeding disorder/platelet adhesion/thrombocytopenia)

JERRY WARE, SUSAN R. RUSSELL, VICENTE VICENTE, RÜDIGER E. SCHARF, AARON TOMER, ROBERT McMILLAN, AND ZAVERIO M. RUGGERI*

Roon Research Center for Cardiovascular Disease and Thrombosis, Division of Experimental Hemostasis and Thrombosis, Department of Molecular and Experimental Medicine and Committee on Vascular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Communicated by Ernest Beutler, December 28, 1989

ABSTRACT Three distinct gene products, the α and β chains of glycoprotein (GP) Ib and GP IX, constitute the platelet membrane GP Ib–IX complex, a receptor for von Willebrand factor and thrombin involved in platelet adhesion and aggregation. Defective function of the GP Ib–IX complex is the hallmark of a rare congenital bleeding disorder of still undefined pathogenesis, the Bernard–Soulier syndrome. We have analyzed the molecular basis of this disease in one patient in whom immunoblotting of solubilized platelets demonstrated absence of normal GP Ib α but presence of a smaller immunoreactive species. The truncated polypeptide was also present, along with normal protein, in platelets from the patient's mother and two of his four children. Genetic characterization identified a nucleotide transition changing the Trp-343 codon (TGG) to a nonsense codon (TGA). Such a mutation explains the origin of the smaller GP Ib α , which by lacking half of the sequence on the carboxyl-terminal side, including the transmembrane domain, cannot be properly inserted in the platelet membrane. Both normal and mutant codons were found in the patient, suggesting that he is a compound heterozygote with a still unidentified defect in the other GP Ib α allele. Nonsense mutation and truncated GP Ib α polypeptide were found to cosegregate in four individuals through three generations and were associated with either Bernard–Soulier syndrome or carrier state phenotype. The molecular abnormality demonstrated in this family provides evidence that defective synthesis of GP Ib α alters the membrane expression of the GP Ib–IX complex and may be responsible for Bernard–Soulier syndrome.

Blood platelets play a pivotal role in normal hemostasis and in pathological vascular occlusion. Paramount to their function are membrane glycoproteins (GPs) that specify the critical ligand interactions involved in platelet adhesion and aggregation. Among these, the GP Ib–IX complex functions as a binding site for von Willebrand factor (vWF) (1–3), resulting in an interaction that mediates platelet attachment to components of exposed subendothelium (4), and as a high-affinity thrombin receptor necessary for the rapid activation of platelets at low concentrations of thrombin (5, 6). An absent or dysfunctional GP Ib–IX complex is the cause of a rare congenital bleeding disorder, the Bernard–Soulier syndrome (7–9).

The GP Ib–IX complex is formed by the noncovalent stoichiometric association of GP Ib and GP IX (10). GP Ib is a disulfide-linked heterodimer that consists of an α (143 kDa) and a β (22 kDa) subunit (11). GP IX (17 kDa) is a single-chain polypeptide (10). The cDNAs for all three components of the complex have been cloned and characterized (12–14), and the

primary structure of GP Ib α has also been determined, in part, by direct protein sequencing (15). Each of these proteins is encoded by a separate gene, suggesting that association into a complex would require the correct independent expression of each individual component. Immunological studies have established that all components of the GP Ib–IX complex are decreased or missing on platelets from Bernard–Soulier patients, with a considerable degree of heterogeneity among different pedigrees (16–24). It is assumed that the etiology of the syndrome is related to genetic defects in any of the various components of the GP Ib–IX complex, but no experimental data are presently available in this regard.

The present studies have been prompted by the observation of a patient with typical phenotypic manifestations of Bernard–Soulier syndrome, including lifelong bleeding tendency, thrombocytopenia with giant platelets, and absent vWF–GP Ib interaction. Structural analysis demonstrated the existence of a genetic defect within one GP Ib α allele, leading to the synthesis of a truncated polypeptide that cannot be inserted into the platelet membrane. These findings provide evidence that the Bernard–Soulier phenotype may be the consequence of a GP Ib α gene lesion. Moreover, the characterization of additional family members demonstrated that the inheritance of Bernard–Soulier syndrome in this family is associated with defective GP Ib α alleles.

MATERIALS AND METHODS

Case Presentation. The proband is a 36-year-old male with a lifelong bleeding disorder that frequently results in spontaneous epistaxis and mucosal hemorrhage requiring transfusion. Blood smears revealing giant platelets with large prominent granulations, a thrombocytopenia of 32,000 per μ l, and a normal number of megakaryocytes in a bone marrow biopsy suggested a diagnosis of Bernard–Soulier syndrome. Blood samples were collected from the patient, his available family members, and normal volunteers of the General Clinic Research Center facility at the Research Institute of Scripps Clinic. All individuals gave their informed consent before blood drawing.

Binding Studies. Antibody-binding studies were performed on platelet-rich plasma prepared from blood collected into one-tenth final volume of 0.11 M trisodium citrate/50 mM disodium ethylenediaminetetraacetate (EDTA), and aprotinin at 200 units (kallikrein inhibitor units)/ml (Sigma). Platelet-rich plasma was prepared with two successive low-speed centrifugations ($70 \times g$, 6 min) to prevent excessive sedimentation of large platelets present in the patient's blood. Binding of vWF was evaluated by using platelets isolated

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mAb, monoclonal antibody; vWF, von Willebrand factor; PCR, polymerase chain reaction; 125 I-LJ-Ib10 and 125 I-vWF, 125 I-labeled LJ-Ib10 and vWF, respectively.

*To whom reprint requests should be addressed.

from blood drawn into one-sixth final volume of acid/citrate/dextrose (pH 4.5) and washed free of plasma constituents by the albumin density-gradient technique of Walsh *et al.* (25) with minor modifications (26). The vWF used in the binding assay was purified from cryoprecipitate and characterized as described (27). The anti-GP Ib α monoclonal antibodies (mAbs), LJ-Ib1 and LJ-Ib10, have been described (28). Protein iodination was performed by the procedure of Fraker and Speck (29) with ^{125}I (Amersham) and Iodo-Gen (Pierce). Platelets were incubated (25°C) with increased concentrations of ^{125}I -labeled LJ-Ib10 (^{125}I -LJ-Ib10; 1.56–50 $\mu\text{g}/\text{ml}$) and ^{125}I -labeled vWF (^{125}I -vWF; 1.56–50 $\mu\text{g}/\text{ml}$), as described (1, 20). Binding parameters were calculated with the computer-assisted program LIGAND (30), assuming $M_r = 170,000$ and $M_r = 275,000$ for mAb LJ-Ib10 and vWF, respectively.

Flow Cytometric Analysis. Platelet-rich plasma was prepared as described above and incubated with saturating concentrations of the anti-GP Ib α mAb, LJ-Ib1. After washing with Tyrode buffer (pH 7.35) to remove excess antibody, the samples were fluorescently tagged by incubating 15 min (25°C) with phycoerythrin-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL). Each sample was also immunolabeled to evaluate concurrently the expression of the GP IIb–IIIa complex. An anti-GP IIb–IIIa mAb, LJ-P4 (obtained and characterized as the anti-GP IIb–IIIa antibodies LJ-P5 and LJ-P9 from ref. 31) was prepared as a fluoresceinated conjugate. The levels of each receptor complex were evaluated by the red (GP Ib α) and green (GP IIb–IIIa) emission spectra. The labeled platelet-rich plasma was analyzed in a FACStar flow cytometer (Becton Dickinson) (32).

Immunoblotting. Platelets for immunoblotting analysis were washed twice by differential centrifugation in modified calcium-free Tyrode buffer, pH 7.35, containing leupeptin at 200 $\mu\text{g}/\text{ml}$, 10 mM EDTA, and aprotinin at 20 units/ml. Solubilization of membrane proteins was obtained by resuspending washed platelets in a buffer composed of 20 mM Tris (pH 7.35), 150 mM NaCl, 10 mM EDTA, 5 mM *N*-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, and 2% NaDodSO $_4$.

Genetic Characterizations. Genomic DNA was isolated from whole blood as described (33). Oligonucleotides for the polymerase chain reaction (PCR) (34) were synthesized based on the published genomic DNA sequence of GP Ib α (35). Oligonucleotides used were as follows: Ib α 10, 5'-AATTCAGACAAGTTTCCCGTCTACA-3'; Ib α 7, 5'-TTC-TCTCAAGGTCCCAAACCTCC-3'; Ib α 6, 5'-CCGAAT-TCTGAGGGCGATAAGGTGCGTGCC-3'; and Ib α 11, 5'-ATGGAAGCTTGGAGTTTGGAGAATGTGATGG-3'. These oligonucleotides correspond to the following nucleotide numbers of ref. 35: 1342–1357, 2422–2446, 1435–1457, and 1642–1665, with some additional nucleotides added to Ib α 6 and Ib α 11 (italics) to incorporate restriction sites within the 5' ends of the amplified molecules. PCR reactions were performed with a DNA thermal cycler (Perkin-Elmer/Cetus) and *Thermus aquaticus* (Taq) polymerase (Perkin-Elmer/Cetus). The reactions were done in a 100- μl mixture containing 1–2 μg of genomic DNA, 1 μg of each synthetic oligonucleotide primer, and buffer consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl $_2$, 0.1% gelatin, and 200 mM of each dNTP. PCR conditions were 35 cycles of 30 sec at 94°C, 30 sec at 52°C, and 1 min at 72°C. Amplified fragments were purified by electrophoresis through a 2% agarose gel before cloning and DNA sequence analysis (36). Single-stranded DNA was sequenced in a dideoxy chain-termination reaction using T7 DNA polymerase (Pharmacia) and [α - ^{35}S]dATP (37, 38). *Ava* II restriction digests were performed according to the manufacturer's recommended conditions (New England Biolabs).

RESULTS

Analysis of Platelet Membrane Receptors. The anti-GP Ib α mAb, LJ-Ib10, recognizes an epitope located within residues Ala-238–Arg-293 of GP Ib α . The binding of ^{125}I -LJ-Ib10 to platelets from the propositus, the propositus' family members (Fig. 1), and normal individuals was determined, and the results are summarized in Table 1. Platelets from the propositus (II-1) had no detectable binding of ^{125}I -LJ-Ib10. Two children of the patient (III-3, III-4) and his mother (I-2) had $\approx 50\%$ normal amounts of GP Ib α antigen. Two other children (III-1 and III-2) displayed values closer to normal. Corresponding results were obtained with a second anti-GP Ib α mAb, LJ-Ib1, which recognizes a conformational epitope present in native GP Ib α . In agreement with the lack of GP Ib α epitopes, the ristocetin-induced binding of vWF to the patient's platelets was also undetectable (Table 1). In contrast, the mother and all four children of the patient were clinically normal and had moderately reduced-to-normal levels of vWF binding.

Flow cytometric analysis of the patient's platelets corroborated the results obtained with the binding studies. By using the mAb LJ-Ib1 and indirect labeling with phycoerythrin-conjugated goat anti-mouse IgG, the patient's platelets had undetectable GP Ib α antigen (Fig. 2A). Labeled platelets from a child of the propositus (III-4) displayed $\approx 50\%$ binding of the anti-GP Ib α antibody (Fig. 2B) when compared to normal platelets (Fig. 2C). Platelet membrane expression of GP IIb–IIIa was simultaneously examined with a fluorescein-conjugated antibody, LJ-P4. This antibody demonstrated an increased membrane immunofluorescence (increase of $\approx 70\%$) on the patient's platelets as compared with the platelets from a healthy individual (Fig. 2A and C). This result indicates an increased number of GP IIb–IIIa complexes on the abnormally large platelets. Overall, these results are consistent with a diagnosis of typical Bernard-Soulier syndrome.

Identification of a Variant GP Ib α . To evaluate whether normal GP Ib α antigen was synthesized but not transported to the membrane surface, platelet lysates were subjected to immunoblotting analysis. Platelets from the propositus, his family members, and normal controls were solubilized, and platelet extracts were immunoblotted with LJ-Ib10 after NaDodSO $_4$ /PAGE. In the patient, normal GP Ib α polypeptide was absent, but a smaller immunoreactive species, with molecular mass equal to ≈ 40 kDa, could be detected, albeit in greatly reduced concentration (Fig. 3). Three family members, the mother and two children of the propositus (III-3 and III-4), also exhibited the abnormal GP Ib α peptide, but, in

FIG. 1. Family pedigree. Two individuals were not studied in this analysis, the deceased father of the propositus (I-1) and the wife of the propositus, a normal individual with no history of bleeding (II-2). Genetic characterization identified the nonsense mutation that is illustrated as a black allele and segregates through three generations. A second, presumably abnormal, GP Ib α allele is illustrated as a cross-hatched allele in the propositus and two of his children (III-1, III-2).

Table 1. Binding of an anti-GP Ib α mAb (125 I-LJ-Ib10) and 125 I-vWF to intact platelets

	Binding, %	
	125 I-LJ-Ib10	125 I-vWF
II-1 (propositus)	0	0
I-2 (mother of propositus)	46	63
III-1 (child of propositus)	75	95
III-2 (child of propositus)	80	80
III-3 (child of propositus)	55	76
III-4 (child of propositus)	48	65
Normal	100	100

Platelets were incubated (25°C) with increased concentrations of 125 I-LJ-Ib10 (1.56–50 μ g/ml) and 125 I-vWF (1.56–50 μ g/ml) as described (1, 20). Values are listed as a percent of normal values [125 I-LJ-Ib10 = 25,342 $\pm$ 5,371 (SD), n = 4; 125 I-vWF = 41,829 $\pm$ 5,296 (SD), n = 4].

addition, they also displayed the normal-sized GP Ib α species; the two remaining children (III-1 and III-2) had only the normal-sized GP Ib α antigen (Fig. 3). Immunoblotting analysis with an anti-GP IX mAb, LJ-RA8, demonstrated undetectable quantities of GP IX in patient platelets (Fig. 4). In support of the results from the binding assays, it was apparent that the propositus could only synthesize an abnormal GP Ib α molecule, whereas his mother and two of the children synthesized both normal and abnormal species. The normal-sized protein could be detected on the platelet surface, although in reduced amount, and was assembled in a functionally normal GP Ib–IX complex.

Genetic Basis of the Variant GP Ib α . The inheritance of an abnormally small GP Ib α antigen through three generations (I-2, II-1, III-3, III-4) suggested an underlying genetic defect in this family. To investigate the molecular basis for the observed variant molecule, we used a strategy based on use of the PCR. As previously reported, the coding sequence for GP Ib α is contained within a single exon (36). This gene arrangement was exploited by designing oligonucleotides to amplify GP Ib α sequence from genomic DNA (Fig. 5A). An initial PCR (oligonucleotides Ib α 10 and Ib α 7) was performed to amplify a fragment containing the coding sequence for amino acid residues 250 through the 3' untranslated sequence (Fig. 5A). Amplified fragments were cloned and subjected to

DNA sequence analysis. Initially, six different clones from the propositus were characterized. Three of the six clones contained a guanine-to-adenine transition at nucleotide 1613 (numbering according to ref. 35) in the GP Ib α coding strand. This mutation alters the Trp-343 codon (TGG) to a nonsense codon (TGA). This change predicts the synthesis of a truncated protein that would only contain the first 342 of the 610 amino acids of mature GP Ib α . The nonsense mutation is consistent with the immunoblotting result as an explanation for the observed truncated peptide seen in solubilized platelets.

The observed nucleotide change abrogates the recognition sequence for the restriction enzyme *Ava* II [GG(A/T)CC] (Fig. 5A). Taking advantage of this, we obtained confirming evidence that the identified mutation existed in the genomic DNA of the patient and was not the consequence of misincorporation by *Taq* polymerase. First, PCR was performed, as described above (oligonucleotides Ib α 10 and Ib α 7). An aliquot of this product was then amplified with a second round of PCR by using internal oligonucleotides (Ib α 6 and Ib α 11) (Fig. 5A). The two-step method of PCR to generate the 246-base-pair (bp) fragment yielded a single fragment visible in an agarose gel, whereas a single PCR using oligonucleotides (Ib α 6 and Ib α 11) synthesized the 246-bp fragment and several other nonspecific fragments. The second PCR predictably amplified a single 246-bp fragment containing two *Ava* II recognition sites from normal GP Ib α sequence, as schematically shown in Fig. 5A, and demonstrated with the visualization of 95-, 90-, and 61-bp fragments in Fig. 5B. The described mutation destroys the second *Ava* II site, resulting in a 151-bp band. As predicted from the characterization of the recombinant clones, the propositus exhibited a composite restriction pattern compatible with one allele containing the identified nonsense mutation and another allele without the same defect. Restriction fragments of 151, 95, 90, and 61 bp are demonstrated in Fig. 5B from amplified propositus DNA. Although the 61-bp fragment appears faint in the agarose gel, a critical examination of the greater fluorescing intensity of the 95/90-bp fragments as compared with the 151-bp fragment was indicative of a non-separating doublet. These results confirm that the patient has a defective GP Ib α allele that would synthesize a truncated protein. Absence of any detectable normal protein suggests that the second allele lacking the nonsense mutation carries a

FIG. 2. Flow cytometric analysis of immunofluorescent platelets. Platelet-rich plasma was analyzed using a two-color method. Platelet-rich plasma was prelabeled at saturating concentrations with LJ-Ib1 (anti-GP Ib α mAb) and detected with a phycoerythrin-conjugated (red) goat anti-mouse IgG. The samples were also labeled with a fluorescein-conjugated (green) anti-GP IIb–IIIa (LJ-P4) antibody. Combination of the two colors allowed identification and quantitation of platelets expressing GP Ib–IX and GP IIb–IIIa complexes recorded on a logarithmic scale. The platelet membrane immunofluorescence is displayed for the propositus (A), a daughter of the propositus (III-4) (B), and a normal individual (C). The red immunofluorescence indicates the absence of GP Ib α expression in the patient and reduced levels of expression from the child when compared with a healthy individual. In contrast, green immunofluorescence is higher in the patient as compared to the child and a normal individual, indicating an increased number of GP IIb–IIIa receptor complexes on the patient's giant platelets. Contour lines represent numbers of platelets with the innermost contours corresponding to the highest numbers of platelets.

FIG. 3. Immunoblotting of GP Ib α antigen. Membrane glycoproteins were solubilized, reduced with 20 mM dithiothreitol, and electrophoresed in a NaDodSO₄/7.5% polyacrylamide gel. After electrophoresis, the samples were transferred onto nitrocellulose paper for immunoblot analysis with mAb LJ-Ib10. Positive bands were visualized by autoradiography after reactivity with an ¹²⁵I-rabbit anti-mouse IgG. (Left) Samples from a normal blood donor (N) and the Bernard-Soulier patient (II-1). (Right) Similar analysis with a normal blood donor (N) and several family members identified by their pedigree designation in Fig. 1.

distinct abnormality, thus making this Bernard-Soulier patient a compound heterozygote.

To confirm that the truncated peptide is the product of the allele that contains the nonsense mutation, we applied the same PCR strategy and restriction fragment polymorphism analysis to amplified genomic DNA from the available members of the propositus' family. The mother (I-2) and two children (III-3 and III-4) of the propositus, in whom synthesis of the smaller GP Ib α antigen had been demonstrated by immunoblotting analysis, also exhibited a restriction pattern with *Ava* II that by agarose gel analysis was identical to that of the propositus. The representative pattern seen in the mother is shown in Fig. 5B. The two children (III-1, III-2) in whom only normal GP Ib α was present exhibited a pattern identical to that seen in amplified normal DNA. These results verify that the nonsense mutation has a pattern of inheritance that follows the truncated GP Ib α antigen seen in lysed platelet material.

DISCUSSION

The biosynthetic processes leading to membrane expression of the GP Ib-IX complex in megakaryocytes, and conse-

FIG. 4. Immunoblotting of GP IX antigen. Platelet lysates as described in Fig. 3 were transferred onto nitrocellulose and treated with the anti-GP IX mAb, LJ-RA8. Lysate from a normal (N) donor reveals a single polypeptide of $\approx$ 17 kDa. Lysate from the patient's platelets (II-1) shows absence of detectable GP IX protein.

FIG. 5. Primary structure of the GP Ib α protein, PCR strategy and genetic characterization. (A) The GP Ib α precursor consists of a 16-residue signal peptide (SP) that is removed from the 610-residue mature GP Ib α molecule. Several identified protein domains include a series of leucine-rich repeats (LRR) (12), a transmembrane domain (TM), a vWF-binding domain (3), and the epitope of mAb LJ-Ib10 contained within the diagrammed regions. The location of Trp-343 within the linear sequence is shown by the corresponding single-letter abbreviation W. The two coding regions that were targeted by the PCR are aligned under the appropriate linear sequence of GP Ib α . A diagrammatic illustration of the *Ava* II restriction pattern for the amplified 246-bp target fragment from a normal and mutant sequence is schematically represented. (B) Amplified 246-bp GP Ib α fragments were digested with the restriction enzyme *Ava* II and subsequently analyzed by electrophoresis through a 2% agarose gel. The gel contains an undigested sample (left lanes) and one digested with *Ava* II (right lanes) for the DNA from a normal individual (N), the propositus (II-1), and the mother of the propositus (I-2).

quently in platelets, are not presently understood. It has been assumed, but not demonstrated, that defective synthesis of each one of the three distinct components may lead to abnormalities in the number of copies of the complex assembled and present on the cell surface. An analogous pathogenic mechanism has been demonstrated for abnormalities of adhesion receptors of the integrin family, which are composed of two distinct subunits (39). Thus, Bernard-Soulier syndrome might resemble leukocyte adhesion deficiency, which can result from a defective gene for the β subunit of a leukocyte integrin, preventing normal association and surface expression of the complex (40). The situation, however, is additionally complicated by the possible deficiency in Bernard-Soulier platelets of a fourth gene product, GP V, the biosynthesis and membrane expression of which must be related to that of the components of the GP Ib-IX complex, even though no association of the mature product with the latter has been demonstrated (16, 19). Because of this, it has been proposed that Bernard-Soulier syndrome may result from the genetic defect of a single common regulatory element, albeit unknown, responsible for the production of four distinct proteins.

The present studies provide experimental evidence on the pathogenesis of a typical Bernard-Soulier phenotype in one family. A nonsense mutation in the GP Iba coding sequence and the predicted abnormal gene product were found associated with either decreased or absent membrane expression of normal GP Iba. In the proband, this abnormality must have combined with another lesion, yet unrecognized, to create a genetic compound with absence of any detectable levels of normal GP Iba and GP IX. Whether this unknown lesion is in the other GP Iba allele remains open to speculation—even though it is the most probable occurrence. Preliminary Southern blots of genomic DNA from the proband have identified some abnormal restriction fragments associated with either the GP Iba gene or sequences 5' to the gene. Whether these abnormal restriction fragments are related to the dysfunctional allele has not yet been determined. We propose that this allele should be defective; otherwise, the absence of normal α chain will be related to obliteration of another component of the complex or of the putative common regulatory element. Because Bernard-Soulier syndrome is transmitted as an autosomal recessive trait, the two latter possibilities would require the presence of at least two additional abnormal alleles in conjunction with the one carrying the mutation described here.

It appears that the truncated α -chain species identified in this family is the consequence of the described nonsense mutation and not of other unidentified lesions, as demonstrated by the strict correlation between the two abnormal findings in four individuals through three generations. The fact that all the relatives of the proband exhibiting the nonsense mutation had a phenotype consistent with the carrier state for Bernard-Soulier syndrome—namely, 50% membrane expression of GP Iba, supports the contention that the observed genetic abnormality is involved with the pathogenesis of the disease. The proband's allele producing the truncated GP Iba molecule was inherited from his mother (I-2), whereas his other defective allele must have been inherited from his deceased father or results from a *de novo* mutation. Consequently, all four children of the proband must be heterozygotes. Two of them (III-3, III-4) inherited their father's allele containing the nonsense mutation (Fig. 1). The other two (III-1, III-2), whose platelets did not contain the truncated α -chain species, must be heterozygous by inheriting a normal allele from their mother and their father's allele, which is defective for reasons not yet defined but which does not contain the nonsense mutation (Fig. 1). The membrane expression of GP Iba was greater in the latter two individuals than in the other family members who inherited the nonsense mutation; thus, no clear phenotypic consequence of such genotypic composition was detectable.

In summary, these results demonstrate a defective GP Iba allele that causes the synthesis of a truncated form of this component of the GP Ib-IX complex containing only the amino-terminal half of the normal molecule. In the presence of a second abnormal allele that, for unknown reasons, prevents the synthesis of normal GP Iba, a genetic compound is created with phenotypic manifestations typical of Bernard-Soulier syndrome. The molecular pathology demonstrated in this family provides a basis for characterizing other genetic lesions that give rise to this congenital platelet abnormality. This work, in turn, may provide models to increase our understanding of the platelet functions involving the GP Ib-IX receptor complex in hemostasis and thrombosis.

The authors acknowledge the Sam and Rose Stein Trust Fund in the Department of Molecular and Experimental Medicine at the Research Institute of Scripps Clinic. This work was supported, in

part, by Grants HL 31950 and HL 42846 from the National Institutes of Health. R.E.S. is supported by a Heisenberg Stipend (Scha 358/2-1) from the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, F.R.G. Additional support from National Institutes of Health Grant M01RR833 for the General Clinic Research Center facility at the Research Institute of Scripps Clinic is also acknowledged. This is manuscript 6023-MEM/CVB from the Research Institute of Scripps Clinic.

- Ruggeri, Z. M., De Marco, L., Gatti, L., Bader, R. & Montgomery, R. R. (1983) *J. Clin. Invest.* **72**, 1–12.
- Stricker, R. B., Wong, D., Saks, S. R., Corash, L. & Shuman, M. A. (1985) *J. Clin. Invest.* **76**, 1274–1278.
- Vicente, V., Kostel, P. J. & Ruggeri, Z. M. (1988) *J. Biol. Chem.* **263**, 18473–18479.
- Weiss, H. J., Turitto, V. T. & Baumgartner, H. R. (1978) *J. Lab. Clin. Med.* **92**, 750–764.
- Jamieson, G. A. & Okumura, T. (1978) *J. Clin. Invest.* **61**, 861–864.
- Harmon, J. T. & Jamieson, G. A. (1986) *J. Biol. Chem.* **261**, 13224–13229.
- Bernard, J. & Soulier, J. P. (1948) *Sem. Hôp. Paris* **24**, 3217–3223.
- Nurden, A. T., Didry, D. & Rosa, J.-P. (1983) *Blood Cells* **9**, 333–358.
- Clemetson, J. J. & Luscher, E. F. (1988) *Biochim. Biophys. Acta* **947**, 53–73.
- Du, X., Beutler, L., Ruan, C., Castaldi, P. A. & Berndt, M. C. (1987) *Blood* **69**, 1524–1527.
- Phillips, D. R. & Agin, P. P. (1977) *J. Biol. Chem.* **252**, 2121–2126.
- Lopez, J. A., Chung, D. W., Fujikawa, K., Hagen, F. S., Papayannopoulou, T. & Roth, G. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5615–5619.
- Lopez, J. A., Chung, D. W., Fujikawa, K., Hagen, F. S., Davie, E. W. & Roth, G. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2135–2139.
- Hickey, M. J., Williams, S. A. & Roth, G. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6773–6777.
- Titani, K., Takio, K., Handa, M. & Ruggeri, Z. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5610–5614.
- Clemetson, K. J., McGregor, J. L., James, E., Dechavanne, M. & Luscher, E. F. (1982) *J. Clin. Invest.* **70**, 304–311.
- Montgomery, R. R., Kunicki, T. J., Taves, C., Pidard, D. & Corcoran, M. (1983) *J. Clin. Invest.* **71**, 385–389.
- Berndt, M. C., Gregory, C., Chong, B. H., Zola, H. & Castaldi, P. A. (1983) *Blood* **62**, 800–807.
- Gruel, Y., Boizard, B., Daffos, F., Forestier, F., Caen, J. P. & Wautier, J. P. (1986) *Blood* **68**, 488–492.
- De Marco, L., Fabris, F., Casonato, A., Fabris, P., Ben, M. G. D., Barbato, A. & Girolami, A. (1986) *Acta Haematol.* **75**, 203–208.
- Nichols, W. L., Kaese, S. E., Gastineau, D. A., Otteman, L. A. & Bowie, E. J. W. (1989) *Mayo Clin. Proc.* **64**, 522–530.
- Greaves, M., Pickering, C., Martin, J., Cartwright, I. & Preston, F. E. (1987) *Br. J. Haematol.* **65**, 429–435.
- Ingerslev, J., Stenbjerg, S. & Taaning, E. (1987) *Eur. J. Haematol.* **39**, 182–184.
- Drouin, J., McGregor, J. L., Parmentier, S., Izaguirre, C. A. & Clemetson, K. J. (1988) *Blood* **72**, 1086–1088.
- Walsh, P. N., Mills, D. C. B. & White, J. G. (1977) *Br. J. Haematol.* **36**, 281–298.
- De Marco, L., Girolami, A., Russell, S. & Ruggeri, Z. M. (1985) *J. Clin. Invest.* **75**, 1198–1203.
- De Marco, L. & Shapiro, S. S. (1981) *J. Clin. Invest.* **68**, 321–328.
- Handa, M., Titani, K., Holland, L. Z., Roberts, J. R. & Ruggeri, Z. M. (1986) *J. Biol. Chem.* **261**, 12579–12585.
- Fraker, D. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80**, 849–857.
- Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* **107**, 220–239.
- Trapani-Lombardo, V., Hodson, E., Roberts, J., Kunicki, T. J., Zimmerman, T. S. & Ruggeri, Z. M. (1985) *J. Clin. Invest.* **76**, 1950–1958.
- Shattil, S. J., Cunningham, M. & Hoxie, J. A. (1987) *Blood* **70**, 307–315.
- Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3**, 2303–2308.
- Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
- Wenger, R. H., Kieffer, N., Wicki, A. N. & Clemetson, K. J. (1988) *Biochem. Biophys. Res. Commun.* **156**, 389–395.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
- Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **71**, 2900–2904.
- O'Toole, T. E., Loftus, J. C., Plow, E. F., Glass, A. A., Harper, J. R. & Ginsberg, M. H. (1989) *Blood* **74**, 14–18.
- Kishimoto, T. K., Hollander, N., Roberts, T. M., Anderson, D. C. & Springer, T. A. (1987) *Cell* **50**, 193–202.