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Compound Heterozygosity for a Novel Nine-Nucleotide Deletion and the Asn45Ser Missense Mutation in the Glycoprotein IX Gene in a Patient With Bernard-Soulier Syndrome

Jeanne Drouin,[1] Nancy L. Carson,[3] and Odette Laneuville[2]*

[1] Department of Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
[2] Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
[3] Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada

Bernard-Soulier syndrome (BSS) is a rare inherited bleeding disorder due to quantitative or qualitative abnormalities in the platelet glycoprotein (GP) Ib/IX/V complex, the major von Willebrand factor receptor. The complex comprises four subunits, each encoded by a separate gene. Several mutations have been described for each of the subunits, except for GPV, as a cause of BSS. We describe here the genetic basis of the disorder in a child with BSS. Flow-cytometric analysis of the patient's platelets showed a markedly reduced surface expression of all three glycoproteins of the GPIb/IX/V complex. DNA sequencing analysis showed the patient to be a compound heterozygote for two mutations in the *GPIX* gene, a novel nine-nucleotide deletion starting at position 1952 of the gene that changes asparagine 86 for alanine and eliminates amino acids 87, 88, and 89 (arginine, threonine, and proline) and a previously reported point mutation that changes the codon asparagine (AAC) for serine (AGC) at residue 45. Her mother was heterozygous for the Asn45Ser mutation, and her father, for the nine-nucleotide deletion. Our findings suggest that the additive effects of both mutations in the *GPIX* gene are responsible for the BSS phenotype of the patient. Am. J. Hematol. 78:41–48, 2005. © 2004 Wiley-Liss, Inc.

Key words: Bernard-Soulier syndrome; glycoprotein IX; deletion mutation; compound heterozygote; flow cytometry

INTRODUCTION

Bernard-Soulier syndrome (BSS) is a rare hereditary bleeding disorder which is usually inherited in an autosomal recessive manner. The disease is characterized by variable thrombocytopenia, giant platelets, a prolonged bleeding time, and absence of ristocetin-induced platelet aggregation in the presence of von Willebrand factor (vWF). The syndrome is due to a quantitative or qualitative deficiency of the glycoprotein (GP) Ib/IX/V complex, the major receptor for vWF on the platelet surface [1]. An optimal interaction between the complex and vWF is critical for platelet adhesion to damaged vascular subendothelium [2].

The GPIb/IX/V complex comprises four distinct transmembrane polypeptide subunits, GPIbα (143 kDa), GPIbβ (25 kDa), GPIX (22 kDa), and GPV (82 kDa), with a stoichiometry of 2:2:2:1 [1]. A disulfide bridge links GPIbα and GPIbβ to form GPIb, which in turn associates non-covalently with GPIX and GPV. The binding site for vWF is located in the N-terminal portion of the GPIbα subunit. All four GPs have a

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*Correspondence to: Dr. Odette Laneuville, Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Ontario, Canada K1H 8M5. E-mail: olaneuvi@uottawa.ca

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similar gene structure and are members of the leucine-rich family of glycoproteins, containing motifs of conserved leucine-rich amino acid sequences (LRM) at the N-terminus [3].

The four GP genes are distinct and are located on different chromosomes [1]. They are simple in structure and intron-free except that for *GPIbβ*, which contains a short intron in the coding region. To date, most of the mutations identified in BSS patients have been found in the *GPIbα* gene, but some are also found in the *GPIbβ* and *GPIX* genes [4]. None have been found in the *GPV* gene. Most defects involve missense or nonsense mutations, and deletions are rare.

The current study describes the molecular defects present in a patient with BSS. Platelet glycoprotein analysis using flow cytometry demonstrated markedly decreased expression of GPIbα GPIX, and GPV on the platelet surface. DNA analysis revealed normal coding sequences in the *GPIbα* and *GPIbβ* genes and both a missense and a novel deletion mutation in the *GPIX* gene, making the patient a compound heterozygote. The patient's mother was heterozygous for the missense mutation, and father, for the deletion mutation.

MATERIALS AND METHODS

Patient Profile

The patient, a girl born in 1989, was first investigated at 6 years of age when found to have an iron-deficiency anemia following repeated epistaxes. She had experienced prolonged bleeding from cuts, severe epistaxes, and spontaneous bruising since early childhood. Laboratory tests showed a hemoglobin level of 75 g/L, a microcytic blood picture, and low serum ferritin. The platelet count was moderately decreased ((60–80) $\times$ 10^9/L), and numerous giant platelets were noted in her blood film. There were no inclusions in her leukocytes. Her platelets aggregated normally with ADP, collagen, and epinephrine, but their response to ristocetin was abnormal (no response at 1.0 mg/mL and decreased response at 1.5 mg/mL). FVIIIC was 2.54 U/L (normal range, 0.50–2.00 U/L), von Willebrand factor antigen was 1.56 U/L (normal range, 0.40–1.50 U/L), and von Willebrand factor activity (RiCof) was 1.80 U/L (normal range, 0.50–1.50 U/L). The patient's parents and her half-sister were asymptomatic. Hemostatic and platelet aggregation studies in both parents were normal. There was no history of consanguinity. The patient's mother is of French-Canadian and Irish descent, and her father is an American Indian of the Ojibway tribe. He was adopted as a child and does not know his biological parents.

Approval for the study was obtained from the Ottawa Hospital Research Ethics Board. Following informed consent, blood samples were obtained from the patient, her parents, and healthy control subjects. Venous blood was collected either in vacuum tubes containing EDTA or was mixed with a 3.8% solution of sodium citrate corresponding to 1/10 of the collected blood volume.

Analysis of GPIb/IX/V Expression by Flow Cytometry

The expression of the GPIb/IX/V complex was determined by two independent methods: (1) staining of the platelets in PRP by direct immunofluorescence using FITC-conjugated monoclonal antibodies; and (2) staining of the platelets in whole blood by indirect immunofluorescence using the Platelet GPIb/IX/V quantification kit from BioCytex (obtained from Alexis Corporation, San Diego, CA).

For the direct immunofluorescence study, PRP was prepared from blood collected in EDTA, and samples were processed as previously described [5]. For the indirect immunofluorescence study, a commercial kit developed by BioCytex was used (Alexis Corporation), and samples of citrated whole blood were processed according to the manufacturer's protocol. A calibration curve was constructed by incubating with the secondary antibody and analyzing, in parallel, calibration beads coated with defined, increasing quantities of mouse IgG immunoglobulin molecules. The calibration curve was used to convert the mean fluorescence intensity of samples into an absolute number of molecules of the GPIb/IX/V subunits expressed per platelet.

For both methods, flow-cytometric analysis of platelet GPIb/IX/V expression was done using the Epics Profile II analyzer (Coulter Electronics, Inc., Hialeah, FL).

Antibodies

For the direct immunofluorescence flow-cytometric study of PRP, the following FITC-conjugated monoclonal antibodies were used: a mouse IgG negative control, Beb-1 (anti-GPIX) and RUU-PL 7F12 (anti-GPIIIa) obtained from Becton-Dickinson (San Jose, CA), and SZ2 (anti-GPIbα) obtained from AMAC (Westbrook, ME). For the platelet GP quantification study using indirect immunofluorescence and flow cytometry, a kit was obtained from BioCytex (Alexis Corporation), containing the following antibodies: negative control mouse IgG, SZ2 (anti-GPIbα), SZ1 (anti-GPIX), and SW16 (anti-GPV).

DNA Extraction, Amplification, Subcloning, and Sequencing

Genomic DNA was isolated from citrated whole blood using the QIAamp DNA Blood Mini Kit (Qiagen,

TABLE I. Oligonucleotide Primers Used for PCR Amplification of Genomic DNA*

Gene	Primer pair	Sense (primer position)	Antisense (primer position)	Expected size (bp)
GPIbα	Iba1	AGGTCTTTCTGCCTGCCTGT (2812–2831)	TAGCCAGACTGAGCTTCTCC (3566–3585)	773
	Iba2	AAGGCAATGAGCTGAAGACC (3511–3530)	CTTGTGTTGGATGCAAGGAG (4088–4107)	596
	Iba3	TCCACTGCTTCTCTAGACAG (4056–4075)	GGCTGATCAAGTTCAGGGAT (4473–4492)	436
	Iba4	CACAAGCCTGATCACTCCAA (4394–4413)	TTCTCTCAAGGTCCCCAAAC (4959–4978)	584
GPIbβ	Ibb1	GCCTTATCGCTCGGCTCT (12614–12631)	GTTGTGTCGACAGGGAAGG (13110–13128)	514
	Ibb2	AGCTTACTGCTCCTGCTGCT (12983–13002)	AGGGTCCTGTCGAGTTTGC (13630–13648)	665
GPIX	IX1	ATGTCAGGCTCCGCTACATC (1546–1565)	GGTGGAGTCTGGGGACCT (2230–2247)	701
	IX2	CTCTCTCTGCAGCCAGCC (1624–1641)	CCCAGCTGGTAGCCTGTC (2028–2045)	421

*Primer sequences correspond to the published sequences of GPIbα (GenBank M22403), GPIbβ (GenBank AF006988), and GPIX (GenBank M80478). The nucleotide position of each primer is indicated in brackets. The sizes of the PCR products obtained for each primer pair (sense and antisense) are indicated as base pairs (bp).

Toronto, Canada). Polymerase chain reaction (PCR) amplification of the *GPIbα*, *GPIbβ*, and *GPIX* genes was done using a set of primers (Table I) synthesized by the University of Calgary Core DNA Services (University of Calgary, Calgary, AB, Canada), the *Taq* DNA polymerase kit (Gibco BRL, Toronto, Canada) and a thermocycler (Perkin Elmer Cetus DNA Model 480). For each pair of oligonucleotides, the conditions were optimized to ensure maximum yield and specificity. Each PCR reaction contained between 50 and 150 ng of genomic DNA, 40 ng of each primer, each dNTP at a final concentration of 100 μM, 1.25 U *Taq* DNA polymerase, 2.5 μL of PCR buffer, 4% dimethyl sulfoxide, and varying concentrations of Mg^{2+}. The volume of each reaction was brought up to 25 μL with water. Magnesium ion concentrations were as follows: 0.75 mM for Iba1, Iba2, Iba3, Iba4, and Ibb2; 1.0 mM for Ibb2, IX1, and IX2. DNA amplification was performed as follows: denaturing at 94°C for 3 min followed by 30 cycles of 1 min at 94°C, 1 min at 58°C (for Iba2, Iba3, Iba4, and Ibb2) or at 62°C (for Iba1, Ibb1, IX1, and IX2), and 2 min at 72°C. After 30 cycles, the reactions were incubated at 72°C for 10 min to increase the yield of amplification. PCR products were analyzed on a 1% agarose gel stained with ethidium bromide.

Both strands of each PCR fragment were sequenced using the original PCR primers. Sequencing was performed using an ABI PRISM dye terminator cycle sequencing-ready reaction kit on an Applied Biosystems Model 373 Stretch DNA sequencer (Applied Biosystems, Foster City, CA). Sequencing reactions were performed on 120 ng of gel-purified DNA prepared with the QIAquick Gel Extraction kit (Qiagen) and 20 ng of primer. Sequences were confirmed with sense and antisense primers and analyzed with the BLAST program against published sequences. In order to sequence the patient's alleles separately, PCR fragments obtained with the GPIX primers were subcloned in the pGEM-T vector (Promega, Madison, WI) using T4 DNA ligase. Plasmid DNA was introduced into the JM-109 bacterial strain and grown overnight. Plasmid DNA was isolated, digested with *Apa*I and *Pst*I, and resolved on a 1% agarose gel to confirm the integrity and size of the DNA insert. Sequencing was then performed using SP6 and T7 primers.

RESULTS

GPIb/IX/V Expression Determined by Flow Cytometry

The levels of GPIX, GPIbα, and GPIIb/IIIa on the surface of platelets from the BSS patient and her parents were assessed by staining the PRP samples with FITC-conjugated monoclonal antibodies. The immunofluorescence readings as expressed by the mean fluorescence intensity (MFI) showed a markedly decreased expression of the GPIX and GPIbα polypeptides on the patient's platelets as compared to that seen in both her parents and in a healthy individual (Fig. 1). On the other hand, an increased expression of GPIIb/IIIa on the BSS platelets was measured. This finding is consistent with other reports [6–8] and is likely due to the platelets' large size. A small peak of positive events with lower fluorescence was also obtained with the anti-GPIIIa

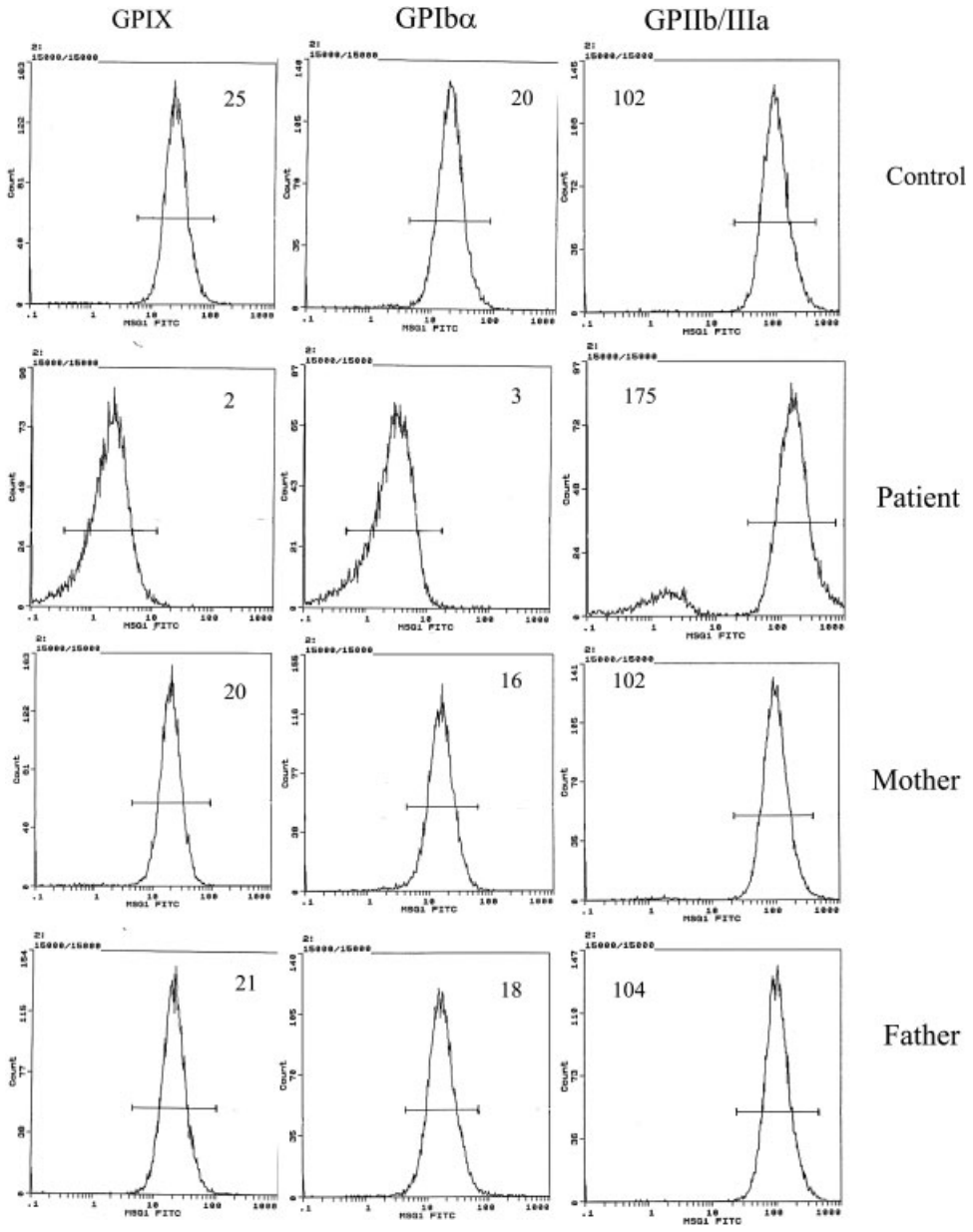

Fig. 1. Flow-cytometric analysis of surface glycoprotein expression on platelets from the patient, her parents, and a healthy control. Histograms represent the platelet number on the *y* axis (linear scale) and, on the *x* axis (log scale), the fluorescence intensities of 15,000 platelets after incubation with anti-GPIX, anti-GPIbα, and anti-GPIIIa monoclonal antibodies. The inset of each panel indicates the mean fluorescence intensity (MFI). The patient's platelets show reduced surface expression of GPIX and GPIbα as evidenced by markedly decreased MFI values compared to platelets from the control and from both her parents. BSS platelets show an increased expression of GPIIb/IIIa, likely because of their abnormally large size.

antibody, possibly representing a small subpopulation of BSS platelets or microparticles. Using a quantitative flow-cytometry method and citrated whole blood, platelets from the proposita were further analyzed for their membrane expression of GPIbα, GPIX, and GPV. Patient platelets showed a markedly reduced absolute number of molecules of all three GPs compared to platelets from healthy donors (Table II). The reduced expression of the GPIb/IX/V complex on the patient's platelets is consistent with the clinical diagnosis of BSS.

DNA Sequencing Analysis

The PCR-amplified DNA coding sequences obtained for the *GPIbα*, *GPIbβ* and *GPIX* genes

TABLE II. Flow-Cytometric Quantitative Analysis of GPIbα, GPIX, and GPV Expression on Patient and Control Platelets Using the Biocytex Kit*

Subject	GPIbα (sites/platelet)	GPIX (sites/platelet)	GPV (sites/platelet)
Proposita	4,800	2,300	3,000
Healthy subject #1	30,500	26,500	9,000
Healthy subject #2	40,000	31,000	14,500
Healthy subject #3	44,000	30,000	18,000
Healthy subject #4	35,000	25,000	12,500
Healthy subject #5	32,000	27,000	14,000
Expected values	34,000 ± 9,000	26,000 ± 6,000	12,000 ± 4,000

*Citrated whole blood was diluted and incubated in the presence of mAbs specific for GPIbα, GPIX, and GPV. The samples and a calibrator were labeled by adding a FITC-conjugated anti-mouse IgG, and fluorescence intensity was measured by flow cytometry (Epics Profile II Analyzer). Results are expressed as the number of antibody-binding sites per platelet.

were all of the predicted size in all subjects (Table I). No mutations were found in the coding regions of the *GPIbα* and *GPIbβ* genes. In the patient, we found two mutations in the *GPIX* gene: a previously reported single-base substitution [6,9–12] and a novel nine-nucleotide deletion. The substitution changed asparagine (AAC) to serine (AGC) at residue 45 (Asn45Ser) (Fig. 2A,C). The proposita (Fig. 2A) and her mother (Fig. 2C) were heterozygous for the Asn45Ser missense mutation, while her father did not have it (Fig. 2B).

In order to confirm the length of the deletion, the PCR product generated with the IX2 primers was subcloned in the pGEMt vector, and six colonies were sequenced. Examples of antisense sequences from clones carrying the deletion mutation or without it are presented in Fig. 3. Starting from the second nucleotide in codon 86, the mutation deleted nine nucleotides, thus changing amino acid 86 to Ala (Asp86Ala), and deleting amino acids 87, 88, and 89 (Arg, Thr, Pro). The proposita and her father were heterozygous for the deletion, while the mother did not have this defect (Fig. 3).

DISCUSSION

We report a novel genetic abnormality in a BSS patient with a classical disease phenotype, that of compound heterozygosity for both a missense and a deletion mutation in the *GPIX* gene. No mutation was detected in the *GPIbα* and *GPIbβ* genes. This is the first report of a deletion mutation in the *GPIX* gene, a defect that leads to the loss of nine nucleotides. It is found here on one of the patient's alleles, while an already reported point mutation, Asn45Ser, is found on the other allele. Parents of the proposita are not related and are asymptomatic. Each parent is a carrier for one mutation, indicating that the patient has inherited a defective gene from both of them. Analysis of patient platelets using flow cytometry shows a markedly decreased surface expression of all three glycoproteins of the GPIb/IX/V complex components, GPIX being the most severely affected.

Several mutations affecting the GPIX peptide have been described in BSS patients. All represent single-nucleotide mutations within the coding sequence of the *GPIX* gene, the most common resulting in an Asn45Ser point mutation within the leucine-rich extracellular domain of the polypeptide [6,9–12]. The nine-nucleotide deletion we report is the first

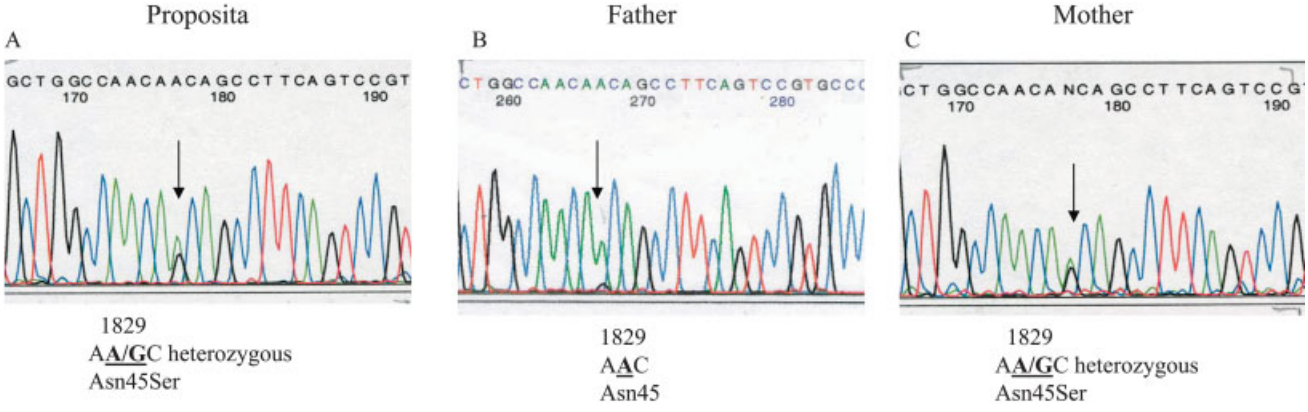

Fig. 2. DNA sequence analysis of the *GPIX* gene from the proposita (A), her father (B), and her mother (C). The 701 nucleotides PCR product generated with the IX1 pair of primers was sequenced as described under Materials and Methods. Sequencing of the antisense strand of the PCR product shows the simultaneous presence of A and G at position 1829 (according to NCBI database accession number M80478) in the patient and her mother, which causes a heterozygous Asn45Ser substitution. The substitution is not present in the father. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

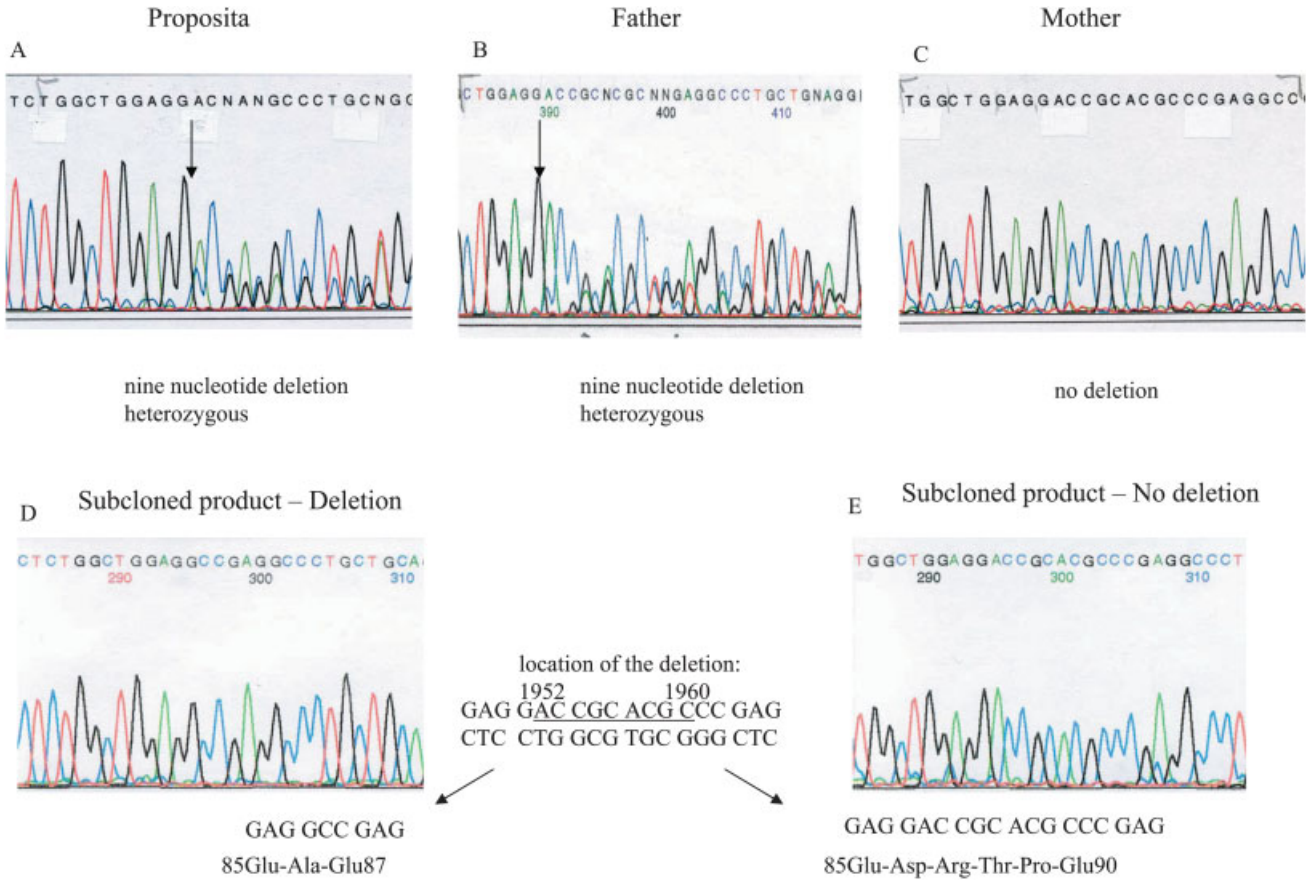

Fig. 3. DNA sequence analysis of the *GPIX* gene from the proposita (A), her father (B), and her mother (C). Arrows in A and B show the nine-nucleotide deletion in the patient and her father. The 421-bp PCR product generated with the IX2 pair of primers from the patient's genomic DNA was subcloned in the pGEM*t* vector and sequenced. Sequences of the antisense strands from clones containing the deletion (D) and without it (E) are presented. Nucleotides 1952–1960 (according to NCBI database accession number M80478) inclusively are deleted as shown. The mutant protein has an Ala residue in position 86 and lacks the Arg, Thr, and Pro residues in positions 87, 88, and 89, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

case of a deletion mutation in that gene. It affects four codons, changing Asp to Ala at position 86 and eliminating the Arg, Thr, and Pro residues at positions 87, 88, and 89 in the GPIX polypeptide. The altered sequence is found within the extracytoplasmic domain of the protein, lying between a leucine-rich motif (LRM) at the N-terminal region and the transmembrane domain. Three point mutations in BSS patients have been mapped to this region: Cys73Tyr, Cys97Tyr, and Trp126STOP [13–15]. As is the case in our study, these mutations all lead to decreased surface expression of the GPIb/IX/V complex on platelets, GPIX being the most severely affected. That the deletion mutation seen in our patient affects four amino acids of the GPIX polypeptide without abolishing the expression of the complex may be due to the fact that she is heterozygous for two mutations affecting protein segments that are not in close proximity.

This is the second report of BSS showing compound heterozygosity for mutations in the *GPIX* gene. The first report described three siblings with BSS who were heterozygous for single base-pair substitutions affecting codon 45 (Asn45Ser) and codon 21 (Asp21Gly) [9]. This is the first report of the Asn45Ser point mutation being identified in a Canadian family, an A to G change in codon 45 being found in one allele in both the proposita and her mother. This gene defect was first reported in a British patient and later in patients of Austrian, Swedish, Finnish, and Belgian origin [6,9–12]. As suggested by Koskela et al., the Asn45Ser mutation in the *GPIX* gene appears to be an ancient mutation shared by northern and central European populations [12]. In our family, the mother's French and Irish ancestry fits with this hypothesis. However, the origin of the *GPIX* gene deletion mutation found in the father remains to be determined. The father's aboriginal ancestry is well established, but he

was adopted as a child and his biological parents and siblings were not available for study.

The functional role of the GPIX subunit is not well understood. However, this subunit is known to be essential for assembling and anchoring the GPIb/IX/V complex to the platelet surface [16,17]. In addition, studies have shown that the normal surface expression of GPIbα, the subunit responsible for the binding of von Willebrand factor, depends both on the integrity of GPIX and on its interaction with GPIbβ [18,19]. Indeed, a deletion mutation at the N terminus of GPIbβ does not allow surface expression of GPIX, showing that residues 15–32 of GPIbβ are critical for a normal interaction between the latter and GPIX [20]. The mapping of the GPIX residues that are essential for the ordered surface expression of the complex has not been reported yet. However, naturally occurring mutations in BSS patients have been informative. Mutations affecting a leucine-rich domain of GPIX or its amino- and carboxy-terminal flanking regions all give rise to decreased GPIb/IX/V surface expression on platelets, suggesting that these regions are important to ensure the correct assembly and the stability of the complex [21]. The sequences flanking the LRM of GPIbβ, GPIX, and GPIbα are very similar, these regions showing conserved cysteine residues that form disulfide loops within the polypeptide, as observed in GPIbα by crystallography [22]. It has been suggested that *GPIX* gene mutations affecting cysteine residues [13,14,21] prevent the formation of intramolecular disulfide-loop structures that are critical for the interaction of the polypeptide with the GPIbβ subunit. A mutation involving the GPIX signal peptide was recently shown to drastically affect the biosynthesis of both GPIbα and GPIX in affected platelets [23]. While it is not clear how point mutations in the *GPIX* gene not affecting cysteine residues give rise to the BSS phenotype, it has been postulated that these alter the configuration of the GPIX subunit, thus preventing its normal interaction with other constituents of the complex. It is likely that the deletion mutation that we have identified also changes the conformation of the GPIX subunit: the mutation alters one amino acid residue and deletes the subsequent three residues. We speculate that the altered polypeptide cannot interact normally with GPIbβ, thus preventing the normal assembly of the GPIb/IX/V complex and resulting in a decreased platelet surface expression of all its subunits.

In conclusion, we have described the first deletion mutation in the *GPIX* gene in a patient with BSS. The deletion mutation is accompanied by a point mutation in the same gene, resulting in compound heterozygosity. Platelet glycoprotein analysis has shown a markedly decreased surface expression of the GPIb/IX/V complex subunits on BSS platelets. We postulate that the two mutations prevent the interaction of GPIX with GPIbβ and impair the full expression of the GPIb/IX/V complex on the patient's platelet membrane. Our results suggest that a deletion mutation affecting a sequence outside a leucine-rich domain of GPIX or its flanking regions and not involving cysteine residues can also contribute to the BSS phenotype.

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REFERENCES

1. Lopez JA, Andrews RK, Afshar-Kharghan V, Berndt MC. Bernard-Soulier syndrome. Blood 1998;91:4397–4418.
2. Berndt MC, Shen Y, Dopheide SM, Gardiner EE, Andrews RK. The vascular biology of the glycoprotein Ib/IX/V complex. Thromb Haemost 2001;86:178–188.
3. Kobe B, Deisenhofer J. The leucine-rich repeat: a versatile binding motif. Trends Biochem Sci 1994;19:415–421.
4. Kunishima S, Kamiya T, Saito H. Genetic abnormalities of Bernard-Soulier syndrome. Int J Haematol 2002;76:319–327.
5. Drouin J, Sutherland M, McLeish WA, Cantin G, McGregor JL, Jobin F. Bernard-Soulier syndrome in three Canadian families: clinical and laboratory observations. Platelets 1992;3:311–316.
6. Vanhoorelbeke K, Schlammadinger A, Delville JP, et al. Occurrence of the Asn45Ser mutation in the GPIX gene in a Belgian patient with Bernard-Soulier syndrome. Platelets 2001;12:114–120.
7. Ware J, Russell SR, Vicente V, et al. Nonsense mutation in the glycoprotein Ibα coding sequence associated with Bernard-Soulier syndrome. Proc Natl Acad Sci USA 1990;87:2026–2030.
8. Moran N, Morateck PA, Deering A, et al. Surface expression of glycoprotein Ibα is dependent on glycoprotein Ibβ: evidence from a novel mutation causing Bernard-Soulier syndrome. Blood 2000; 96:532–539.
9. Wright SD, Michaelides K, Johnson DJ, West NC, Tuddenham EG. Double heterozygosity for mutations in the platelet glycoprotein IX gene in three siblings with Bernard-Soulier syndrome. Blood 1993;81:2339–2347.
10. Clemetson JM, Kyrle PA, Brenner B, Clemetson KJ. Variant Bernard-Soulier syndrome associated with a homozygous mutation in the leucine-rich domain of glycoprotein IX. Blood 1994; 84:1124–1131.
11. Donner M, Karpman D, Kristoffersson AC, Winqvist I, Holmberg L. Recurrent mutation Asn45→Ser of glycoprotein IX in Bernard-Soulier syndrome. Eur J Haematol 1996;57:178–179.
12. Koskela S, Javela K, Jouppila J, et al. Variant Bernard-Soulier syndrome due to homozygous Asn45Ser mutation in the platelet glycoprotein (GP) IX in seven patients of five unrelated Finnish families. Eur J Haematol 1999;62:256–264.
13. Noda M, Fujimura K, Takafuta T, et al. A point mutation in glycoprotein IX coding sequence (Cys73 (TGT) to Tyr (TAT)) causes impaired surface expression of GPIb/IX/V complex in two families with Bernard-Soulier syndrome. Thromb Haemost 1996;76:874–878.
14. Kunishima S, Tomiyama Y, Honda S, et al. Cys97→Tyr mutation in the glycoprotein IX gene associated with Bernard-Soulier syndrome. Br J Haematol 1999;107:539–545.
15. Noda M, Fujimura K, Takafuta T, et al. Heterogeneous expression of glycoprotein Ib, IX and V in platelets from two patients

with Bernard-Soulier syndrome caused by different genetic abnormalities. Thromb Haemost 1995;74:1411–1415.
16. Lopez JA, Weisman S, Sanan DA, Sih T, Chambers M, Li CQ. Glycoprotein (GP) Ibβ is the critical subunit linking GPIbα and GPIX in the GPIb complex. J Biol Chem 1994;269:23716–23721.
17. Kenny D, Morateck PA, Gill JC, Montgomery RR. The critical interaction of glycoprotein Ibβ with GPIX: a genetic cause of Bernard-Soulier syndrome. Blood 1999;93:2968–2975.
18. Sae-Tung G, Dong JF, Lopez JA. Biosynthetic defect in platelet glycoprotein IX mutants associated with Bernard-Soulier syndrome. Blood 1996;87:1361–1367.
19. Dong J, Gao S, Lopez JA. Synthesis, assembly, and intracellular transport of the platelet glycoprotein Ib-IX-V complex. J Biol Chem 1998;273:31449–31454.
20. Kenny D, Morateck PA, Montgomery RR. The cysteine knot of platelet glycoprotein Ibβ (GPIbβ) is critical for the interaction of GPIbβ with GPIX. Blood 2002;99:4428–4433.
21. Rivera CE, Villagra J, Riordan M, Williams S, Lindstrom KJ, Rick ME. Identification of a new mutation in platelet glycoprotein IX (GPIX) in a patient with Bernard-Soulier syndrome. Br J Haematol 2001;112:105–108.
22. Huizinga EG, Tsuji S, Romijn RAP, et al. Structures of glycoprotein Ibα and its complex with von Willebrand factor A1 domain. Science 2002;297:1176–1179.
23. Lanza F, de la Salle C, Baas MJ, et al. A Leu7Pro mutation in the signal peptide of platelet glycoprotein (GP) IX in a case of Bernard-Soulier syndrome abolishes surface expression of the GPIb-V-IX complex. Br J Haematol 2002;118:260–266.