Clinical Case Reports

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CASE REPORT

A point mutation in Phe71Ser in glycoprotein IX as a genetic cause of Bernard–Soulier syndrome: case report

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Key Clinical Message

We need to be aware of rare causes of persistent thrombocytopenia as Bernard–Soulier syndrome (BSS). When BSS is suspected based on family history and giant platelets, genetic test for mutations of GPIbIXV is necessary. Management varies once you recognize the cause. Platelets transfusion and antifibrinolytics are the mainstay of therapy.

Keywords

Platelet, disorder, bleeding, thrombocytopenia, Bernard–Soulier syndrome, glycoprotein XI.

Introduction

Bernard–Soulier syndrome (BSS) is an inherited bleeding disorder transmitted in a rare autosomal recessive manner. It is caused by mutations of the genes encoding for the subunits of the glycoprotein (GP) *Ib-IX-V* complex. Normally, group of linked proteins called *(GP) GP1BA (GPIb), GP1BB (GPIb), GP9 (GPIX), and GP5 (GPV)* are found on the surface of the platelets that activated with vessels wall injury. *(GP) Ib/IX/V* complex acts as a platelet receptor and works as binding site for coagulation factors to help with platelet adhesion to the site of injury [1].

In patients with BSS, defect in the glycoproteins complex leads to an abnormal platelet adherence and increases bleeding tendency [2].

The frequency of BSS based on case reports from Europe, North America, and Japan has been estimated to be one in one million [2]. Clinical presentation of BSS includes purpura, epistaxis, menorrhagia, and mucocutaneous bleed. Laboratory diagnosis is based on prolonged bleeding time, moderate-to-severe thrombocytopenia, giant platelets on peripheral blood smear, and defect in platelet aggregation [3]. Molecular investigations of BSS patients showed that only three genes are responsible for the disease: *GPIba, GPIbb, and GPIX* [3].

The gene of *GPIX* is located on chromosome 3q21.3 and organized in three exons with coding for 177 amino acid sequences of *GPIX* in exon 3. In patients with *GPIX* mutation, eleven substitutions of amino acid have been reported [4].

In this case report, we present a young boy with mutations in *GPIX*.

Case Report

Eight year old Saudi boy presented with history of gum bleeding on 19/12/2016. Other systemic review was unremarkable. He was not on any medications. On physical examination, he was conscious, not in distress. He had purpuric rash and ecchymosis lesions in lower limb. No lymphadenopathy is noted. His abdomen was not distended, and his spleen and liver were not palpable. Other systemic examinations were unremarkable. Laboratory findings were: complete blood count showed hemoglobin of 11.7 g/dL (12.0–14.0 g/dL), white blood cell 6.3×10^9/L ($4.0–12.0 \times 10^9$/L), absolute neutrophilic count 2.5, and platelets were only 20×10^9/L ($150–450 \times 10^9$/L). The remaining of hematological result was within normal limits. Blood smear showed decreased number as well as large and giant platelets as showed in Figure 1. The patient was managed as a case of Idiopathic Thrombocytopenia Purpura (ITP) and received Intravenous immunoglobulin (IVIG) with no response. He responded to platelet transfusion as the platelet count increased to 56×10^9/L ($150–450 \times 10^9$/L). As this finding was highly suggestive of BSS, we arranged a follow-up appointment and sent for genes analysis of BSS.

The result of molecular genetic analysis of the genes *GP1BA, GP1BB, and GPIX* showed a presence of a homozygous nucleotide substitution (TTT-TCT) leading to the change in Phe71Ser in *GPIX*.

Finally, he was diagnosed with BSS and treated with platelet transfusion with improvement.

Methodology

Genomic DNA extraction and capture of the gene region of *GP1BA, GP1BB,* and *GPIX u*sing a DNA extraction kit (Trusight one kit, Illumina) and high throughput sequencing on NextSeq sequencer.

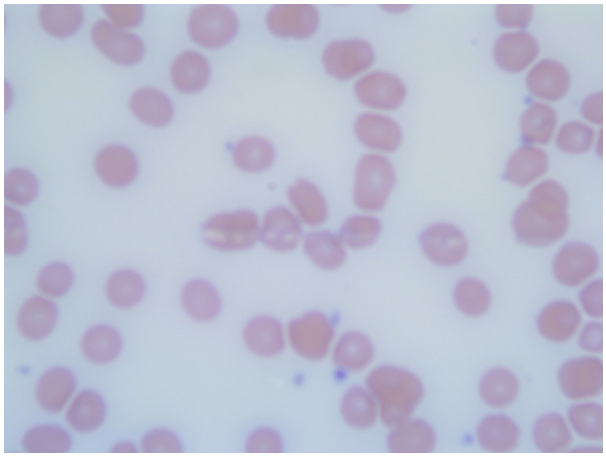

Figure 1. Peripheral blood smear platelets appeared large and giant.

The molecular analysis identified homozygous mutation in the *GPIX* gene. This is a rare variant in well-conserved region of the protein which causes the substitution of a nonpolar and aromatic phenylalanine with a polar and aliphatic serine. This mutation previously classified as pathogenic in ClinVar and HGMD (Human Gene Mutation Database). This mutation may lead to production of an altered *GPIX* subunit that is broken down early or that cannot get to the platelet surface. Lack of this subunit on the surface of platelets prevents formation of the *GPIb-IX-V* complex and leads to the related bleeding disorder. Therefore, this mutation is likely the cause of the symptoms of the patient (Table 1).

Discussion

BSS is a rare inherited platelet disorder that was first reported in 1948 by Jean Bernard and Jean-Pierre Soulier as a case of a young male patient with prolonged bleeding time and thrombocytopenia with large giant platelets that is caused by abnormal function of the platelet *Gp Ib-IX-V* receptor complex [5].

More than 100 cases of BSS cases have been reported in the literature with 1:1 ratio of female-to-male affection [6].

Until now, 47 genetic defects have been associated with BSS and the largest number of mutations in *GPIBA* (20 mutations) followed by 16 mutations in *GPIBB* and 11 mutations in *GPIX*. These genetic defects could be divided into three categories: a missense mutation that can result into a decrease expression of single abnormal receptor or most likely to an unstable complex, nonsense mutation that leads to smaller subunits that lack the transmembrane region, frameshift insertions or deletions resulting in a new polypeptide [7].

In our patient, the gene analysis revealed a point mutation in the codon 71 of the *GPIX* gene which leads to the substitution of a phenylalanine to a serine that was observed in all clones with no other mutations detected in the *GPIba* and *GPIbb* genes. These results suggest that the mutation reported here can be the genetic cause that is responsible for the BSS phenotype.

All the genetic mutations of *GPIX* that have been previously reported are arranged in (Table 2).

In this case, we identified a homozygous mutation within the *GPIX* gene. A substitution of phenylalanine to serine at position 71 leads to the production of abnormal *GPIX* subunit. The absence of this subunit expression on the surface of the platelet prevents the formation of *GPIb-IX-V* complex that is necessary for binding of Von

Table 1. Genomic DNA extraction with homozygous mutation in the GPIX gene

Gene	Reference sequence	cDNA	Protein	dbSNP	Class
GPIX	NM_000174.3	c.[212T>C] c.[212T>C]	Phe71Ser Phe71Ser	rs12191803	Pathogenic
	(dbSNP) Single-Nucleotide Polymorphism database				

Willebrand Factor to platelets resulting in bleeding tendency that is observed clinically.

Conclusions

Bernard–Soulier syndrome is a rare inherited platelet-bleeding disorder that is characterized by episodes of recurrent bleeding. Laboratory features in BSS show prolonged bleeding time and thrombocytopenia with large giant platelets. This disease is transmitted in autosomal recessive manner. Up to date, eleven mutations in form of amino acid substitution of *GPIX* in patients with BSS have been reported. We identified a homozygosity point mutation in the *GPIX* gene of patient with BSS that caused a substitution (TTT-TCT) of a phenylalanine 71 to serine. This nucleotide substitution caused an absence of *GPIX* expression on the platelet's membrane. In our case, the dysfunction of *GPIb–IX* complex is caused by this mutation resulting in clear diagnosis of this patient with BBS.

We need to be aware of rare causes of persistent thrombocytopenia as BSS. When BSS is suspected based on family history and giant platelets on peripheral smear, genetic test for mutations of GPIbIXV is necessary. Management varies once you recognize the cause. Platelets transfusion and antifibrinolytics are the mainstay of therapy in our patient.

Table 2. Genetic mutations of GPIX

		GPIX mutation			
Age	Gender	Nucleotide substitutions	The change in Amino acid	Genotype	Reference
59	Female	GAC-GGC	Asp 21 Gly	Heterozygous	[8]
59	Female	AAC-AGC	Asn 45 Ser	Heterozygous	[8]
23	Male			Homozygous	[9]
6	Female			Heterozygous	[20]
7	Male	CTG-CCG	Leu 7 Pro	Homozygous	[10]
2.5	Male	TGT-CGT	Cys 8 Arg	Homozygous	[11]
49	Male	CTT-CCT	Leu 40 Pro	Homozygous	[12]
75	Male	TTT-TCT	Phe 55 Ser	Homozygous	[13]
39	Female			Homozygous	[14]
46	Female	TGT-TAT	Cys 73 Tyr	Homozygous	[15]
31	Male				
46	Male	TGT-TAT	Cys 97 Tyr	Homozygous	[16]
44	Female	TGG-TGA	Trp 127 Stop	Homozygous	[17]
39	Female				
30	Female				
–	–	GCC-ACC	Ala 156 Thr	Homozygous	[18]
–	–	GCC-ACC	Ala 140 Thr	Homozygous	[19]

Authorship

SI, AG, DS, and BB: wrote the manuscript and reviewed it with the literature review. HI: Main author, supervised, corrected, and reviewed the manuscript and provided direct care to the patient. All authors: read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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