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As a Rare Disease Bernard–Soulier Syndrome in Differential Diagnosis of Immune Thrombocytopenic Purpura: A Case Report

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Abstract **Introduction:** Bernard–Soulier syndrome is a rare autosomal recessive disease that causes a deficiency of glycoprotein Ib, the receptor for von Willebrand factor, which is important for clot formation. It is estimated to occur in fewer than 1 per 1 million persons. The differential diagnosis includes von Willebrand disease, immune thrombocytopenic purpura, May–Hegglin anomaly, thrombocytopenia-absent radius syndrome, grey platelet syndrome, and other inherited giant platelet disorders. **Case Presentation:** A 30-year-old Turkish woman was admitted to the Department of Heamatology for evaluation of thrombocytopenia. Because she had repeated epistaxis during admission, she was assessed to evaluate for haemorrhagic diathesis. She had been diagnosed with immune thrombocytopenic purpura and given steroid therapy at different times. Peripheral blood smear was characterized by neutrophils 75%, lymphocytes 20%, monocytes 5%, giant platelets and platelets forming three to four clusters, a normal red blood cell morphology. A decreased ristocetin-induced platelet aggregation was detected in low and high concentration. On the basis of these findings, Bernard–Soulier syndrome was screened with flow cytometry and genetic mutation. CD41a 86.2%, CD42a 92.9%, CD42b 92.5%, and CD61 87.8% were detected in flow cytometry. Normal platelet GPIb/IX levels by flow cytometry turned down the suspected diagnosis. genetic analysis, we have applied to our patient and her parents. We detected same mutation in patient and her mother. Laboratuar parameters and flow cytometry of mother did not support diagnosis of Bernard–Soulier syndrome. **Conclusion:** Bernard–Soulier syndrome should be considered before a young patient is diagnosed with immune thrombocytopenia purpura and peripheral blood smear should be examined for giant platelets.

Keywords: *thrombocytopenia, Bernard–Soulier syndrome, immune thrombocytopenic purpura*

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1. Introduction

Bernard–Soulier syndrome, also called haemorrhagiparous thrombocytic dystrophyis is an inherited platelet disorder transmitted in an autosomal recessive manner and estimated to occur in fewer than 1 per 1 million persons. Bernard–Soulier syndrome usually presents in the newborn period, infancy, or early childhood. It affects both males and females. It is associated with quantitative or qualitative defects of the platelet glycoprotein complex GPIb/IX/V and characterized by prolonged bleeding time, thrombocytopenia, increased megakaryocytes, and decreased platelet survival [1]. There are four types of Bernard–Soulier syndrome: *GP1BA*, *GP1BB*, *GP IX*, and *GPV*. The genetic lesion may be localized to 1) the *GPIBA* gene (chromosome 17pter-p12), 2) the *GPIBB* gene (chromosome 22q11.2), 3) the *GPIX* gene (chromosome 3q21), or possibly 4) the *GPV* gene (chromosome 3q29) [2,3].

Immune thrombocytopenic purpura is an autoimmune disease characterized by increased platelet destruction due to antibodies against circulating platelets. Immune thrombocytopenic purpura is a diagnosis of exclusion. First, one must show that there are no blood abnormalities other than a low platelet count and that no physical signs are present other than bleeding [4].

We herein present a case of a patient who was under follow-up with immune thrombocytopenic purpura for 2 years. She was considered to have Bernard–Soulier syndrome but afterward was diagnosed with immune thrombocytopenic purpura again.

2. Case Presentation

A 30-year-old Turkish woman was admitted to the Department of Heamatology for evaluation of thrombocytopenia. Because she had repeated epistaxis during admission, she was assessed to evaluate for haemorrhagic diathesis. She had been diagnosed with immune thrombocytopenic purpura and given steroid therapy at different times. There was no family history for any disease. Her body temperature was 36°C, pulse rate was 92 bpm, and respiratory rate was 15/min. Her skin was pale and there were no petechiae or purpura. No abnormally enlarged lymph nodes were palpable in any part of her body. Her abdomen was not distended, and her spleen and liver were not palpable. Laboratory values were as follows on Table 1. Peripheral blood smear was characterized by neutrophils 75%, lymphocytes 20%, monocytes 5%, giantplatelets (Figure 1) and platelets forming three to four clusters, a normal red blood cell morphology. There were no signs of concurrent or antecedent infections. Serologic examinations for Human Immunodeficiency Virus and hepatitis B and C were all negative. Thrombocytopenia was revealed in addition to giant platelets in the peripheral blood; thus, Bernard–Soulier syndrome was considered, and aggregation tests were applied two times. A decreased ristocetin-induced platelet aggregation was detected in low and high concentration (Figure 2), whereas other tests (platelet aggregation collagen, platelet aggregation epinephrine, platelet aggregation ADP, level of von Willebrand Factor+Cofactor, von Willebrand Factor, von Willebrand Factor antigen) were normal. On the basis of these findings, Bernard–Soulier syndrome was screened with flow cytometry and genetic mutation. CD41a 86.2%, CD42a 92.9%, CD42b 92.5%, and CD61 87.8% were detected in flow cytometry (Table 2). Normal platelet GPIb/IX levels by flow cytometry turned down the suspected diagnosis. Using direct Sanger sequencing (ABI 3130 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA),with previously reported primers [9] we have characterized a heterozygote missense variant resulting from a GTG>GCG (c.92T>C, p.V31A, rs201827537, NCBI refSeq: NG_008767.2) replacement at codon 31 of the GP1BA sequence (Figure 3 and Figure 4 shown from F and R sequences, respectively), corresponding to a Val→Ala amino acid substitution (technical information is available upon request) in our patient.

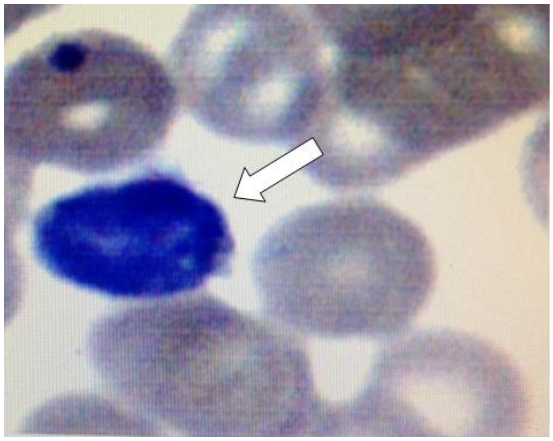

Figure 1. One of the giant platelets

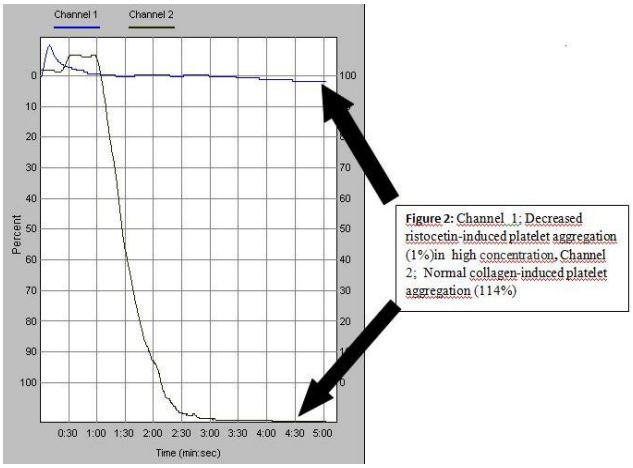

Figure 2. Decreased ristocetin-induced platelet aggregation

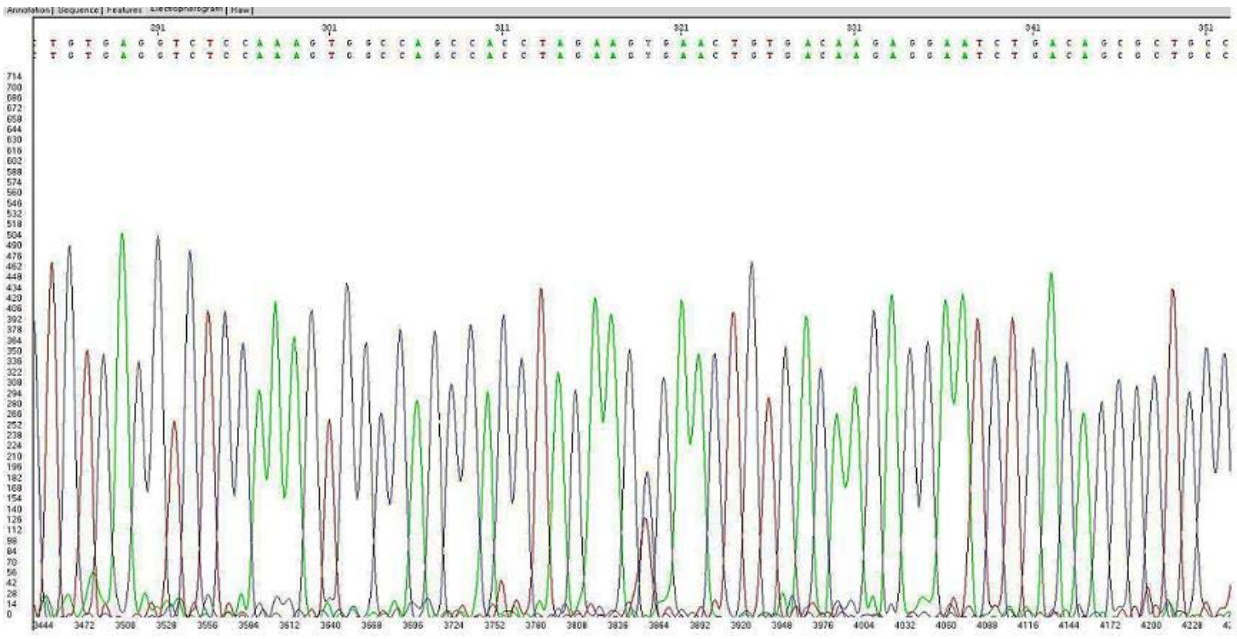

Figure 3. Forward sequence, showing the missense variant, c.92T>C

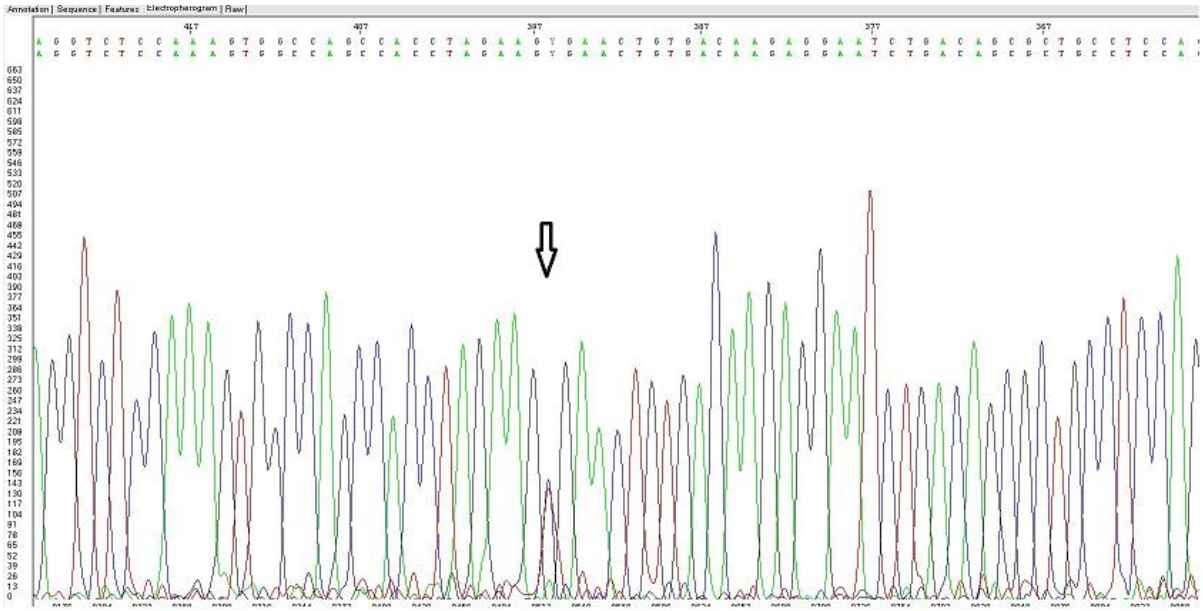

Figure 4. Reverse sequence, showing the missense variant, c.92T>C

Table 1. Laboratuar parameters of patient

Parameter	Value	Normal Range
Leukocyte count	9,100/µL	4.000-10.000/µL
Haemoglobin	13,6 g/dL	13-17 g/dL
Haematocrit	39.5%	39.5-50.3%
Mean corpuscular volume (MVC)	85,5 fL	80.7-90.5fL
Mean corpuscular haemoglobin (MCH)	29.6 pg	27.2-33.5 pg
Mean platelet volume (MPV)	14,1fL	6,6-10,6 fL
Mean corpuscular haemoglobin concentration (MCHC)	34,6 gm/dL,	32.7-35.7 gm/dL,
Red cell distribution width	13,5%	11.8-14.3%
Platelet count	41.000/µL (in EDTA tubes)	150.000-450.000 /µL
Platelet count	48.000/µL (in citrate tubes)	150.000-450.000 /µL
Prothrombin time	12 sec	9-13.5 sec
Partial thromboplastin time	30 sec	18-35 sec
Bleeding Time	9 minute	4-10 minute
Lactate dehydrogenase	184 U/L	110–240 U/L
Total bilirubin	0.2 mg/dL	0.2-1.0 mg/dL
Immunoglobulin G direct Coombs test	negative	

Table 2. Results of Platelet Aggregation and Coagulation Assays

Test	Result	Unit	Normal Range
PlateletAggregationRistocetin (Final concentration of 0,5-0,7 and 1,2-1,5 mg/mL)	1	%	>%60
PlateletAggregationCollagen (Final concentration of 2-5 µM)	114	%	>%60
PlateletAggregation ADP (Final concentration of 5-10 µM)	89	%	>%60
PlateletAggregationEpinephrine (Final concentration of 0,5-10 µM)	112	%	>%60
vWF:RCof	62	%	>%40
International normalizedratio (INR)	1.0	Second	0,7-1,2
Activatedpartialthromboplastin time (APTT)	25	Second	22-35
CD41a	86.2	%	>20
CD42a	92.9	%	>20
CD42b	92.5	%	>20
CD61	87.8	%	>20
vWF:Ag	82	%	>60

3. Discussion

As with other congenital platelet function defects, it often presents as a bleeding disorder evidenced by perioperative and postoperative bleeding, bleeding gums, easy bruising, heavy menstrual periods, epistaxis, or abnormally prolonged bleeding from small injuries [1]. The differential diagnosis includes other inherited giant platelet disorders, von Willebrand disease, and immune thrombocytopenic purpura [3]. The diagnosis can be confirmed by platelet aggregation studies. In Bernard-Soulier syndrome platelets do not aggregate to ristocetin, and this defect is not corrected by the addition of normal plasma, distinguishing it from von Willebrand disease. The platelet responses to physiologic agonists are normal, with the exception of low concentrations of thrombin (1). The patient in our case was diagnosed with immune thrombocytopenia purpura by an outside centre 2 years ago and was administered various treatments, including steroids. The giant platelets seen in peripheral blood are the hallmark of the disease.

Howard et al. and Caen and Levy-Toledano found that platelets in Bernard–Soulier syndrome failed to aggregate to ristocetin, a peptide antibiotic known to aggregate normal platelets but not the platelets affected by von Willebrand disease [5,6]. *In vitro* platelet aggregation was studied in citrated platelet-rich plasma as described [7]. To minimize the loss of denser platelets, the platelet-rich plasma was obtained by sedimentation of blood for 20–30 min. Platelet-rich plasma's platelet count was 220,000/µL. The platelet-agonist ristocetin was applied in two doses. Application of low dose (0.6 mg/mL) ristocetin produced no platelet aggregation, whereas high dose (1.25 mg/mL) ristocetin reduced platelet aggregation (both from Sigma Chemical Co, St. Louis, MO, USA). The diagnosis of von Willebrand disease was excluded in our patient because she had normal levels of von Willebrand factor and her platelets did not aggregate with ristocetin, a defect that was not corrected by the addition of normal plasma.

Flow cytometry has been widely used as a tool in the diagnosis of leukaemias, lymphomas, and many other immune haematological diseases like Bernard–Soulier syndrome. The expression of platelet membrane glycoproteins was investigated in platelet-rich plasma by flow cytometry with an Epics XL flow cytometer (Beckman Coulter, USA) as previously reported [7]. The following monoclonal antibodies were used: AP2, which recognizes GPIIb/IIIa (CD41a/61); MB45 and SZ2 against GPIbα (CD42b); SZ1, which recognizes GPIX (CD42a) andwas used as the negative control. Fluorescein isothiocyanate-conjugated goat anti-mouse antibodies (GAM-FITC) were also purchased from Coulter. Ten thousand platelet events were collected, and the value of mean fluorescence, expressed in arbitrary units, was recorded. A sample from a healthy donor (the same since the beginning of this study) was run with the patient's samples as a control. The patient and the mother had normal CD41a, CD42a, CD42b, and CD61 levels, which is an unusual finding in Bernard–Soulier syndrome.

There are four types of Bernard–Soulier syndrome [2]. The *GP1BA*, *GP1BB,* and *GPIX* genes were screened for mutations using genomic DNA samples from the proband and parents. The missense variant we have defined—resulting from a GTG>GCG (c.92T>C, p.V31A, rs201827537, NCBI refSeq: NG_008767.2) replacement at codon 31 of the GP1BA sequence, corresponding to a Val→Ala amino acid substitution—is not listed in the Human Gene Mutation Database (HGMD) as a disease causing mutation, whereas it is settled as a clinically unknown variant [8]. We could not study *GPV* gene mutation because of technical incompetence. Parental screening (Both of mother and father had normal haematological parameters, Table 3) revealed maternal inheritance of the mutation.

Table 3. Laboratuar parameters of mother and father

Name of Tests	Mother	Father	Unit
Leukocyte count	7,600	6,300	/µL
Hemoglobin	12,4	14,3	g/dL
Platelet	189.000	213.000	/µL
PlateletAggregationtests	Normal	Normal	>%60
Peripheralbloodsmear	Normal	Normal	

Giant platelets in peripheral smear as well as a low ristocetin aggregation made us consider the diagnosis of Bernard–Soulier syndrome. The absence of a decrease in the levels of CD41a, CD42a, CD42b, and CD61 in flow cytometry and the detection of a previously undefined mutation in genetic testing prompted us to make a family screening for Bernard–Soulier syndrome.

Normal CD41a, CD42a, CD42b, and CD61 levels as well as a normal thrombocyte count and aggregation in our patient's mother moved us away from the possibility that this mutation was due to Bernard–Soulier syndrome, and thus our patient was diagnosed with immune thrombocytopenic purpura.

4. Conclusion

Although giant platelets and decreased ristocetin-induced platelet aggregation in our patient made us consider Bernard-Soulier syndrome, flow cytometry and gene analysis did not support the diagnosis of Bernard-Soulier syndrome. We could not explain decreased ristocetin-induced platelet aggregation. As a conclusion, Bernard–Soulier syndrome should be considered before a young patient is diagnosed with immune thrombocytopenia and peripheral blood smear should be examined for giant platelets.

5. Consent

Written informed consent was obtained from the patient's next of kin for publication of this manuscript and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

This report reflects the opinion of the authors and does not represent the official position of any institution or sponsor. IB was responsible for reviewing previous research, journal hand searching, and drafting the report. MAE and KY were responsible for provision of published trial bibliographies, and preparing photographs. IN, MK contributed to the final draft of the manuscript and analysis of relevant data. EK and IK were responsible for project coordination. All authors read and approved the final manuscript.

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Ethical Approval

This case report was approved by the Institutional Ethics Committee of the Inonu University Medical School.

References

[1] Kostopanagiotou G., Siafaka I., Sikiotis C., Smyrniotis V., "Anesthetic and perioperative management of a patient with Bernard-Soulier syndrome," J ClinAnesth, 16 (6). 458-460. Sep. 2004.

[2] López JA., Andrews RK., Afshar-Kharghan V., Berndt MC.,"Bernard-Soulier syndrome," Blood, 91 (12). 4397-4418. Jun. 1998.

[3] Lanza F., "Bernard-Soulier syndrome (hemorrhagiparous thrombocytic dystrophy)," Orphanet J Rare Dis, 16.1.46. Nov. 2006.

[4] Neunert C., Lim W., Crowther M., Cohen A., Solberg L Jr., Crowther MA., "The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia," Blood, 117 (16). 4190-4207. Apr. 2011.

[5] Howard MA., Hutton RA., Hardisty RM., "Hereditary giant platelet syndrome: a disorder of a new aspect of platelet function," Br Med J, 2 (5866):586-588. June. 1973.

[6] Caen JP., Levy-Toledano S., "Interaction between platelets and von Willebrand factor provides a new scheme for primary haemostasis," Nat New Biol, 244 (135): 159-160. Aug. 1973.

[7] Savoia A., Balduini CL., Savino M., Noris P., Del Vecchio M., Perrotta S., Belletti S., Poggi., Iolascon A., "Autosomal dominant macro thrombocytopenia in Italy is most frequently a type of heterozygous Bernard-Soulier syndrome, "Blood, 97 (5): 1330-1335. Mar. 2001.

[8] http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=201827537

[9] Kunishima S., Lopez JA., Kobayashi S., Imai N., Kamiya T., Saito H., Naoe T., "Missense Mutations of the Glycoprotein (GP) Ibβ Gene Impairing the GPIb α/β Disulfide Linkage in a Family With Giant Platelet Disorder," Blood, 89. 2404-2412. Apr. 1997.