

# Bernard-Soulier syndrome in three siblings with a supernumerary small marker chromosome

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## Abstract

*The Bernard-Soulier syndrome (BSS) is a rare congenital bleeding disorder, inherited in an autosomal recessive manner. It is caused by an impaired synthesis or dysfunction of platelet glycoprotein Ib/IX/V complex, which functions as receptor for the von Willebrand factor and thrombin. This paper describes a Polish family with BSS. In three siblings, two symptomatic and one asymptomatic, thrombocytopenia with giant platelets, impaired aggregation to ristocetin and impaired Ib/IX/V complex was diagnosed.*

*Additionally, in each of these three persons, karyotype analysis performed on peripheral blood lymphocytes showed a mosaic presence of supernumerary small marker chromosome (sSMC).*

**Key words:** Bernard-Soulier syndrome, Ib/IX/V glycoprotein complex, supernumerary small marker chromosome.

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## Introduction

The Bernard-Soulier syndrome (BSS) was first described in 1948 [1]. It is a very rare, heterogenous disease in both the clinical picture and the molecular background. BSS is inherited in an autosomal recessive manner but autosomal dominant inheritance was also described [2, 3]. In literature there also appear descriptions of BSS due to somatic mutation [4] and to the appearance of antibodies interfering with von Willebrand factor (vWF) binding [5].

In homozygotes, BSS develops as a severe bleeding disorder while in heterozygotes, the defect is usually mild or asymptomatic. Prolonged bleeding time, mild or severe thrombocytopenia and the presence of giant platelets, comparable to lymphocytes in size, were observed. Platelets in patients showed impaired adhesion to injured vessels, impaired aggregation with ristocetin and throm-

bin and proper aggregation with ADP and collagen [6-9]. The BSS molecular background is the disruption of the synthesis or/and expression of glycoproteins (GP) Ib, IX and V. They form a 2 : 2 : 1 complex on the platelet surface which is a receptor for the vWf and thrombin [10-12]. GPIX and V are single chain proteins, while glycoprotein Ib is composed of two polypeptides:  $\alpha$  (145kD) and  $\beta$  (22kD). The GP Ib  $\alpha$  gene is located on chromosome 17 (13), GP Ib $\beta$  on chromosome 22 (14), GP IX and V on chromosome 3 (15, 16).

The principal function of the GPIb/IX/V complex is participation in the adhesion of blood platelets to the vessel walls in a fast flow at injury site. It takes place in the presence of vWF which provides a bridge between GPIb of platelets and subendothelial collagen [6, 7, 17]. The binding of the GPIb/IX/V complex with vWF can also

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induce the transfer of a signal into the cell and initiate a series of transformations leading to the formation of a haemostatic clot. Although the site of the vWF binding is at the N-end of the Iba subunit, all four polypeptides as well as their mutual configuration, are responsible for the proper adhesion of blood platelets. GPIb $\alpha$  expression depends on the presence of both GPIb $\beta$  and GPIX and, to a lesser extent, GPV [18, 19, 20]. All four glycoproteins contain a leucine-rich fragment [21] which plays a crucial part in the formation of the complex.

The paper describes the Bernard-Soulier syndrome in three siblings with a supernumerary small marker chromosome. The frequency of small supernumerary marker chromosomes in human cells has been estimated at approximately 0.45 per 1000 newborns. They are usually seen as additional single marker chromosome, too small to be identified by banding cytogenetics alone. In minority of cases, a sSMC appear in a mosaic state form [22]. Only about one third of the sSMC cases have been correlated with a specific clinical picture, i.e. the i(18p), der(22), i(12p) (Pallister Killian syndrome) and inv dup(22) (cat-eye) syndromes; most of the remaining sSMC have not yet been correlated with clinical syndromes [29].

## Patients and methods

### Patients

Investigations involved two sisters aged 19 (P-19) and 24 (P-24) hospitalized in the Institute of Haematology and Blood Transfusion for haemorrhagic diathesis of varying intensity as well as three asymptomatic family members: mother (aged 45, P-45), sister (aged 22, P-22) and brother (aged 14, P-14). Patient P-24 had a several months history of bleeding from the digestive tract in the course of an ulcerative inflammation of the large intestine. Her platelet count was  $119 \times 10^9/l$ . Patient P-19 had a several year history of frequent and spontaneous epistaxis, gingival bleeding on teeth-washing and microbleedings on the depilation of lower limb hair. Her platelets count ranged from 50 to  $70 \times 10^9/l$ . Both sisters were prone to bruising. Haemorrhagic diathesis did not develop in P-45, P-22 and P-14. The father, who is healthy, did not report for investigations. Case history did not reveal any blood relation between the patients' parents.

### Methods

The bleeding time was determined by the template method with the use of a Precisette Set (Knoll Feimechanik, Germany). The platelet survival time was determined by isotope method with the use of allogenic platelets labeled with an isotope of radioactive chrome  $-\text{Na}_2^{51} \text{CrO}_4$  [24]. The platelet count was determined in a haematological analyser Sysmex K 4500 (Sysmex Corp., Japan) and concurrently by the Nygaard method [25]. The megakaryocyte count in bone marrow was determined in a bone marrow

smear and by of a histological evaluation of the marrow. Platelet aggregation in response to ADP, collagen and ristocetin (Sigma, St. Louis, USA) was examined in platelet-rich plasma with the use of a lumiaggregometer produced by Chrono-Log (Haverton, USA). Anti-platelet antibodies to GPIb, IIb/IIIa and Ia/IIa membrane glycoprotein were determined by immunoenzymatic test (MAIPA), Kiefel and al. method [26]. Glycoprotein expression examination was performed by flow cytometry [27] with the use of antibodies targeted at GPIb $\alpha$  (clone SZ2), GPIb/IX (clone SZ1, Immunotech, Marseilles, France) and GPIb/IX/V (clone Gi 10, Biochemicals, San Diego, USA) on FACS Calibur Becton Dickinson apparatus (California, USA). Cytogenic investigations were performed GTW and by fluorescent hybridization in situ (FISH) on peripheral blood lymphocytes. Karyotypes were described according to ISCN 2005 [28]. Abnormalities found in G-banded metaphases were subsequently studied using FISH with adequate DNA Probes. Studies on supernumerary marker were performed using painting DNA Probes for chromosomes 3, 17, 21, and 22 (ONCOR, Gaithersburg, USA) and centromeric probes for chromosomes 13, 14, 21 and 22 (QBiogene, Kreatach, Amsterdam, The Netherlands). Hybridization signals were analysed with Nikon Fluorescence microscope (NICON, Tokyo, Japan) and documented with Lucia Imaging System (Laboratory Imaging, Praha, Czech Republic).

## Results

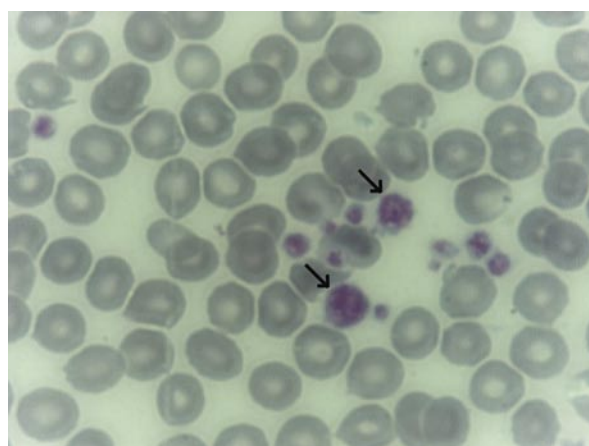
In two patients, P-19 and P-24, the bleeding time was prolonged and in the remaining family members it was normal. The platelet count was reduced to  $<100 \times 10^9/l$  in patient P-19 and her asymptomatic sister, P-22. The survival time of  $^{51} \text{Cr}$ -allogenic platelets in patients with haemorrhagic diathesis was normal. In three patients, including two with haemorrhagic diathesis (P-19 and P-24) and one asymptomatic (P-22), a peripheral blood smear revealed the presence of the giant platelets of over 30 fl (table 1, figure 1). Anti-platelet antibodies were not detected in any of the patients studied (table 1).

Aggregometric investigations revealed impaired aggregation of blood platelets to ristocetin in P-19, P-24, P-22 and normal aggregation to ADP and collagen in all the patients studied. Cytometric studies show a diminished expression of GPIb $\alpha$ , GPIb/IX and GPIb/IX/V in P-19, P-24, P-22 (table 2). The example of blood platelet histogram with a diminished expression of GPIb/IX complex illustrated figure 2. The blood platelet cytograms in patients with a diminished GPIb/IX complex confirmed the presence of giant platelets. In all the women studied, investigations revealed female karyotype with presence of a clone of cells with a supernumerary marker chromosome (sSMC), morphologically similar in each case. A mosaic condition was observed: metaphases with the marker accounted for 1/17 in P-19, 2/14 in

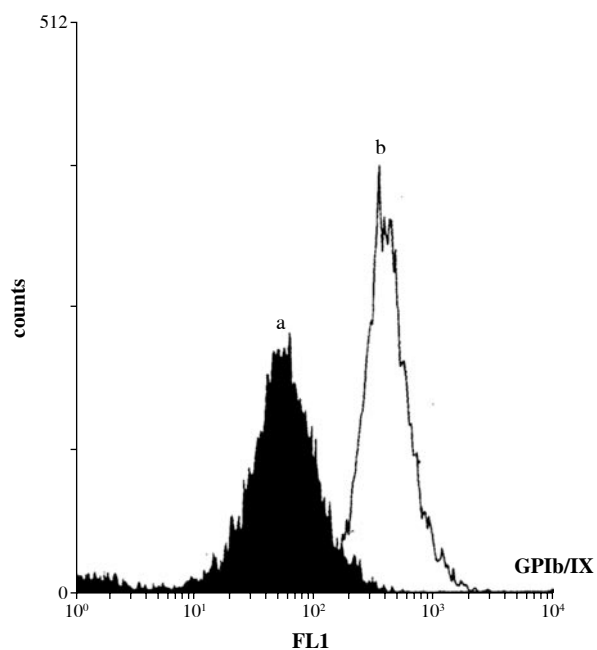
**Table 1.** Results of morphological, cytogenetic and immunological investigations

Methods of investigation	Patients studied/age				
	P-19	P-24	P-22	P-14	P-45
bleeding time	16'40''	15'30''	9'40''	6'10''	6'40''
platelets number	65×10 <sup>9</sup> /l	119×10 <sup>9</sup> /l	80×10 <sup>9</sup> /l	230×10 <sup>9</sup> /l	175×10 <sup>9</sup> /l
number of megakariocytes in bone marrow	N	N	n.s.	n.s.	n.s.
platelet survival time (days)	7	9	n.s.	n.s.	n.s.
presence of giant platelets	+	+	+	-	-
anti-platelet antibodies	-	-	-	n.s.	n.s.
presence of sSMC	+	+	+	n.s.	n.s.

N – within normal range; n.s. – not studied; (+) – present; (-) – not present.



**Fig. 1.** Picture of peripheral blood smear taken with an inverted phase microscope (May-Grunwald-Giemsa stain, enlargement ×1000). Large platelets with stained granules visible in the centre of the field (indicate by arrows)



**Fig. 2.** Histograms illustrating a decline of GPIIb/IX/V expression determined by cytometry (FL – fluorescence intensity) in patient P-24 with BSS (a) and healthy donor (b)

**Table 2.** Results of aggregometric and cytometric investigations in individual family members

Methods of investigation	Patients studied/age					
	P-19	P-24	P-22	P-14	P-45	Donor
<b>aggregometric investigations</b>						
aggregation of blood platelets (% of normal value) in the presence of:						
ADP	N	N	N	N	N	69-88
collagen	N	N	N	N	N	70-90
ristocetin	10	15	14	N	N	70-90
<b>cytometric investigations</b>						
percentage of platelets with normal expression of:						
GPIIb/IX	10.3	32.5	23.4	88.7	77.2	87-97
GPIIb/IX/V	12.5	10.4	13.5	78.1	79.0	77-98
GPIIb $\alpha$	25.5	29.4	33.9	59.5	45.9	40-61

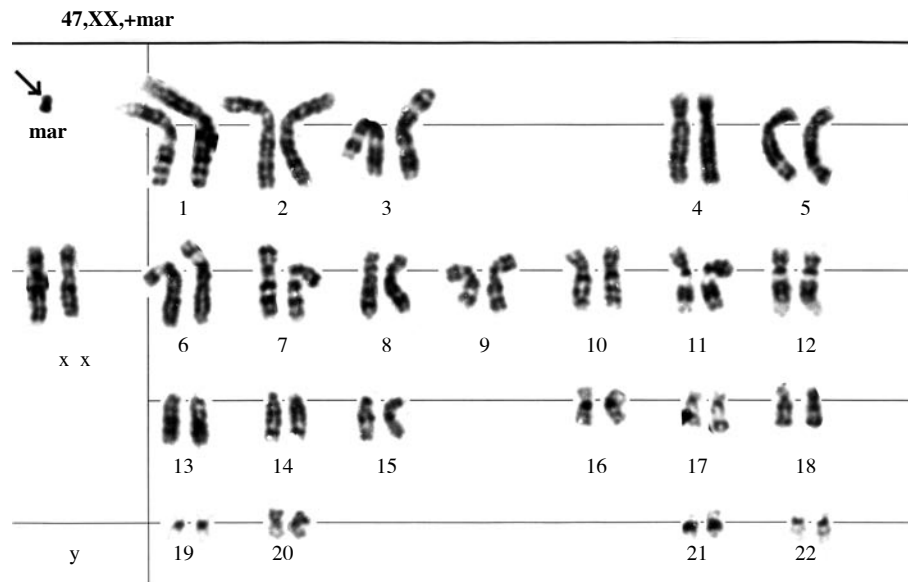
P-24 and 3/18 in P-22 (figure 3). Additional FISH tests with some (3, 17, 21 and 22) whole chromosome painting probes could not define the origin and structure of the sSMC. The example of FISH analysis illustrated figure 4.

**Discussion**

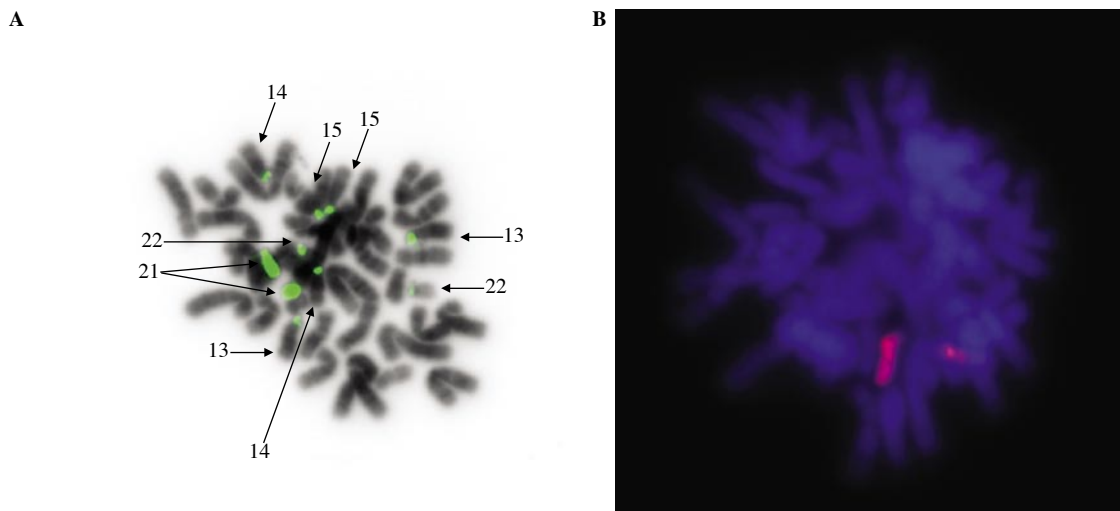
The peripheral blood of three sisters was studied (two with haemorrhagic diathesis and prolonged bleeding time and one asymptomatic). In our patients abnormal karyotype

developed simultaneously with the BSS. The development of BSS seems to be confirmed by thrombocytopenia, presence of giant platelets (P-19, P-24, P-22) and reduced GPIb/IX/V complex expression.

In all the women studied, investigations revealed female karyotype with the presence of a clone of cells with a supernumerary marker chromosome (sSMC), morphologically similar in each case. A small supernumerary marker chromosome (sSMC) is defined as a structurally abnormal chromosome that cannot be identified by classical cytogenetics



**Fig. 3.** Karyotype (P-24) with the presence of an additional marker in the left top corner. (GTG stain)



**Fig. 4.** FISH on P-24 metaphases. (A) Two copies of chromosomes 13, 14, 15, 21 and 22 indicated by arrows (green signals of centromeric and painting probes respectively). (B) Two normal copies of chromosome 17 (red signals of whole painting probes)

alone, and is equal in size or smaller than chromosome 20 of the same metaphase spread [22]. The sSMCs chromosomes can be generated from different chromosomes, in most cases (69%) from acrocentrics [29]. Most of the sSMCs are too small to be characterized for their chromosomal origin by traditional banding techniques, but require molecular cytogenetic techniques for their identification. Patients with such a small marker sometimes demonstrated low degrees of mosaicism for sSMC. The hypothesis was that the presence of sSMC as other genetic and non-genetic factors, including modifier genes at separate loci, mosaicism, unstable mutations, allelic variations at the haploid locus, chance and environmental interaction and can be involved in variable clinical expression, even in the same family.

In the studied family, hyperdiploid cells with sSMC comprised between 6% and 22% of the analyzed metaphases. Using DNA painting Probes, we have shown that sSMCs do not originate from chromosomes 17, 22 and 3 on which GPIb $\alpha$ , GPIb $\beta$ , GPIX and V genes are located and do not contain centromeres of acrocentric chromosomes 13, 14, 15, 21 and 22. We could not to explain the the origin and clinical importance of a small, additional chromosomal marker in P-19, P-24 and P-22.

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