Bernard-Soulier syndrome: first human case due to a homozygous deletion of GP9 gene

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Keywords

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Bernard–Soulier Syndrome (BSS) is a rare (1:1 million) hereditary bleeding disorder caused by defects in the platelet glycoprotein (GP)-Ib/IX/V complex, a receptor for von Willebrand factor (VWF) and thrombin (Lanza, 2006; Berndt & Andrews, 2011). Patients typically present with epistaxis, petechial or gingival bleeding with onset already in infancy. They present with macrothrombocytopenia and their platelets do not agglutinate in response to ristocetin, while maintaining a normal aggregation in response to a variety of aggregating agents. GPIb/IX/V complex consists of two GPIbα and four GPIbβ subunits stabilized by disulphide bonds (Luo *et al.*, 2007). This heterodimer is non-covalently associated with two GPIX and one GPV subunits. The N-

terminal residues of GPIbα form seven leucine-rich repeats (LRRs) and include the binding sites for VWF and thrombin. BSS is due to biallelic loss-of-function pathogen variants (deletions, insertions and nonsense mutations) in *GPIBA*, *GPIBB* or *GP9* genes encoding GPIb/IX/V complex (Savoia *et al.*, 2014). However, so far, no mutation in *GP5* causing BSS has been reported yet. Most of the mutations prevent the formation of the complex or trafficking it through the endoplasmic reticulum and Golgi apparatus and alter receptor expression (Salles *et al.*, 2008; Savoia *et al.*, 2011; Nurden *et al.*, 2012).

The International Consortium for the study of BSS described 60 gene variations in *GP1BA* (28%), 59 in *GPIBB* (28%) and 92 in *GP9* (44%) (Savoia *et al.*, 2014). Most of variations (85%) were homozygous and most cases were products of consanguineous marriages (more than 50% of the families, mostly first cousins). Family members with only one mutant allele (BSS carriers) are usually asymptomatic with normal platelet counts; however, they may sometimes show slightly enlarged platelets and decreased GPIb/V/IX complex expression, as well as a moderately reduced response to ristocetin (Noris *et al.*, 2012).

Here, we describe the first human case of BSS due to deletion of the entire *GP9* gene.

A male newborn developed a severe form of congenital nephrotic syndrome of the Finnish type (CNS) with proteinuria, hypoalbuminaemia and oedemas. He is the first baby of a consanguineous couple. A homozygous mutation in the *NPHS1* gene was previously identified in two other family members and confirmed in our patient (Fig 1A).

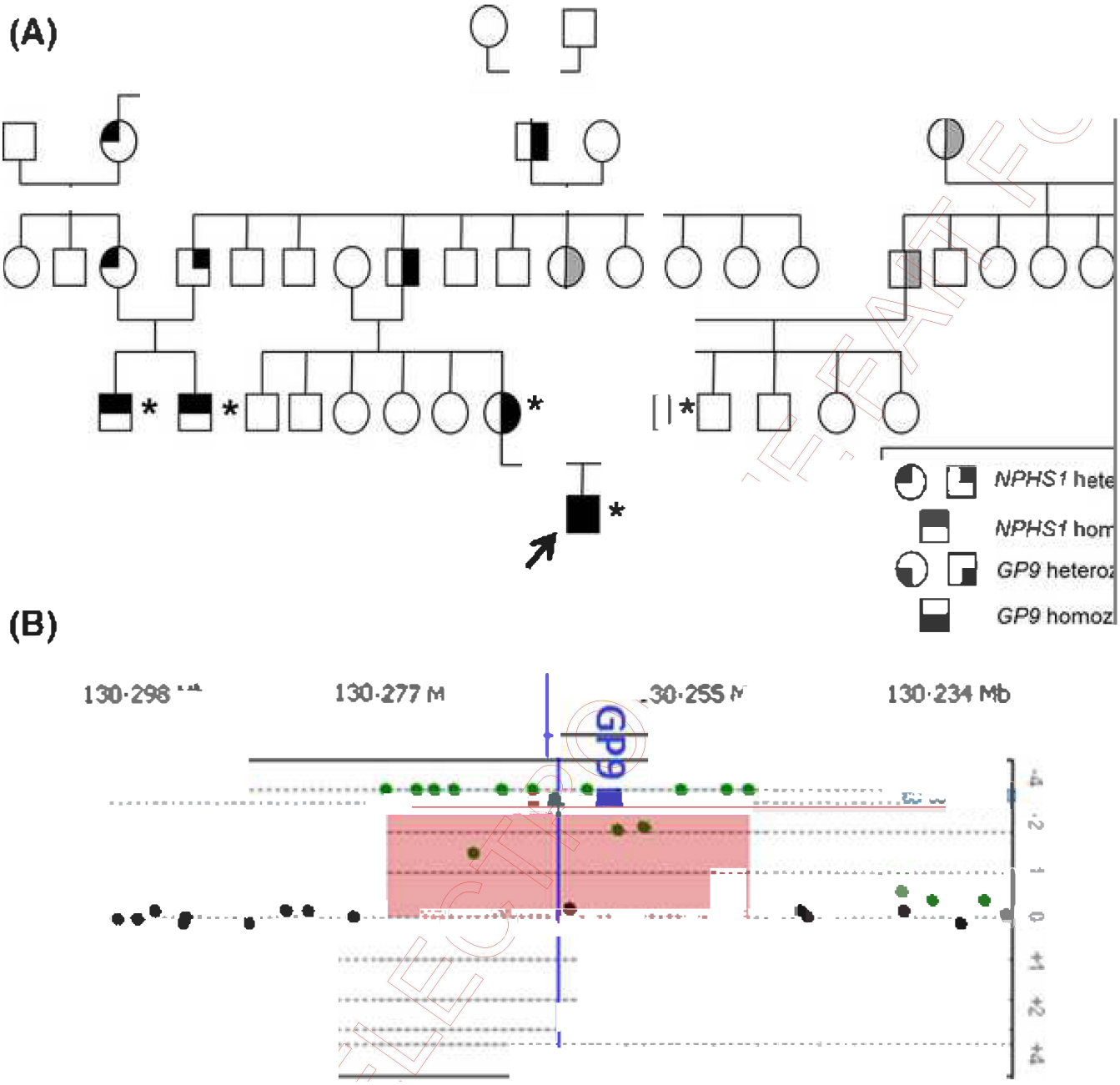

Fig. 1 (A) Genealogic tree; * – genotype persons; grey – possible genotypes. (B) Screen shot from array-CGH analysis showing the homozygous deletion of *GP9* gene.

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The prognosis for CNS is poor as the majority of cases die within six months of life. However, intravenous albumin supplementation, nutritional management, treatment of complications, dialysis and renal transplantation have been shown to improve the growth and development of affected children (Holmberg *et al.*, 1995).

The *NPHS1* gene has a size of 26 Kb and 29 exons. It codes a transmembrane protein named 'nephrin'. The detection rate for this gene mutation varies among different ethnic groups. It approaches 98% in Finnish children with CNS (Kestila *et al.*, 1998).

During hospitalization, laboratory studies disclosed a profound thrombocytopenia (9 g/l).
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Antibodies to HPA5b were present in the maternal serum while the father's platelets were HPA5b-positive. So, the selected diagnosis was a maternal alloimmunization.
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Treatment for the CNS was initiated with daily intravenous albumin supplementation, thyroxine, antithrombin and intravenous immunoglobulin. Angiotensin-converting enzyme inhibitors and non-steroidal anti-inflammatories drugs were added. Nephrectomy and renal transplantation were programmed.

However, two months later, the thrombocytopenia was persistent with high transfusion frequencies (one per week). The morphology of the patient's bone marrow cells revealed the presence of some small megakaryocytes (MKs) with reduced cytoplasm and sometimes vacuolated (Fig 2A) and blood smear examination revealed giant platelets (Fig 2B).

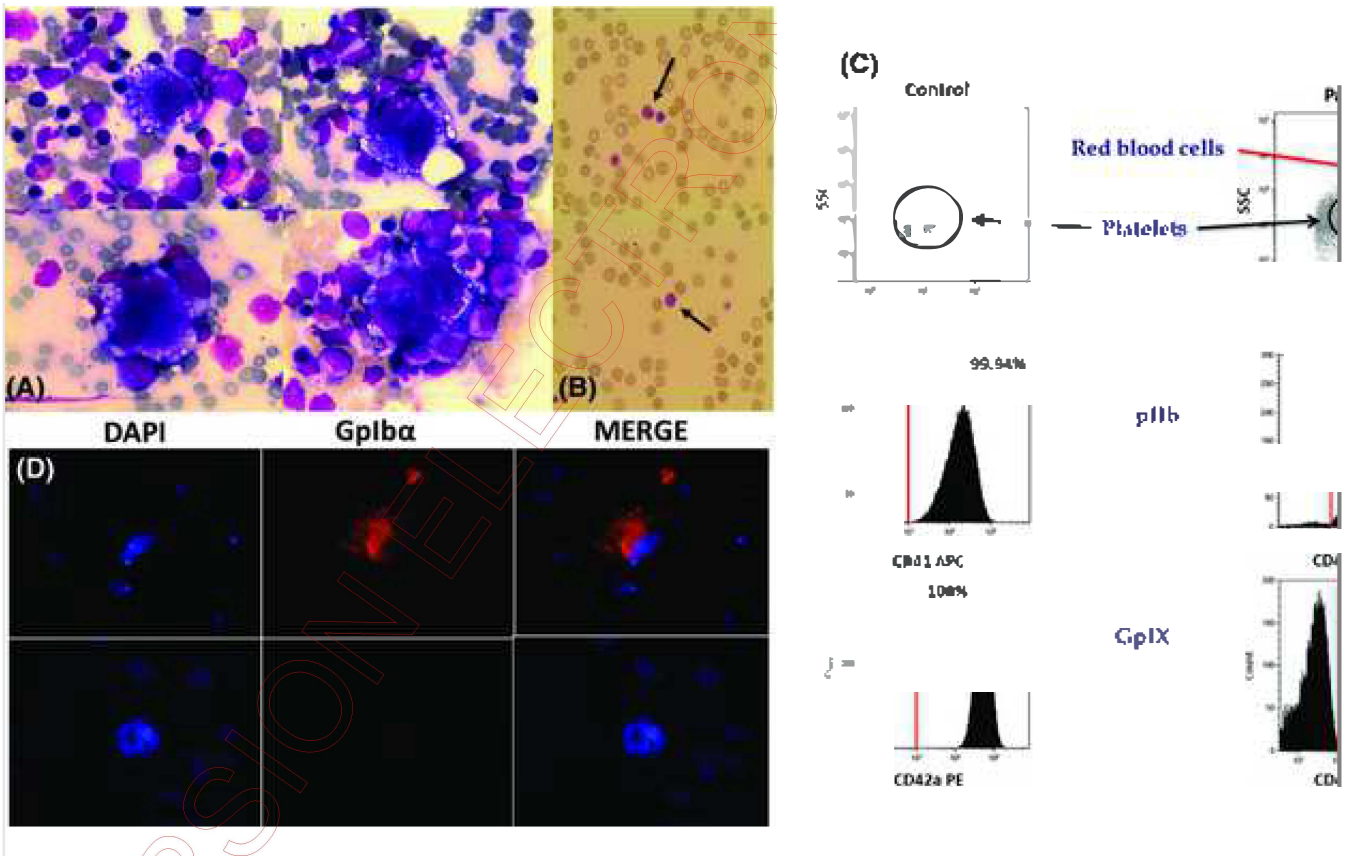

Fig. 2 Bone marrow smear (A) revealed the presence of some small megakaryocytes with reduced cytoplasm and sometimes vacuolated and blood smear (B) showed giant platelets (arrow). Flow cytometry labelling was realized on platelet-rich plasma obtained after centrifugation for the control and after whole-blood sedimentation for the patient because of the platelets' size. Platelets' patient were negative for GpIX (C, right) like immunolabelling of GpIb/IX/V complex of patients' megakaryocytes (D, lower) whereas control platelets (C, left) and megakaryocytes (D, upper) were positive.

In vitro study of patient's MKs derived from bone marrow $CD34^+$ cells in the presence of thrombopoietin (TPO) and stem cell factor (SCF) **(**Bluteau *et al.*, 2014**)** revealed no differences in percentage of $CD41^+$ cells and in the ploidy level **(**Lordier *et al.*, 2012**)** between the patient and an healthy donor (Figures S1 and S2).

Platelet glycoprotein surface expression was explored by flow cytometry and showed an absence of GPIX expression (Fig 2C). Likewise, immunolabelling of megakaryocytes on a bone marrow smear was negative for GPIbα showing the absence of GPIb/IX/V complex expression (Fig 2D).

Sanger sequencing of the patient's *GPIBA* and *GPIBB* did not reveal damaging variation but the *GP9* gene was not amplifiable. So, we analyzed the DNA extracted from peripheral whole blood by array-comparative genomic hybridization (array-CGH) by using the commercial Agilent 1M SurePrint G3 Human CGH Microarray (Agilent Technologies, Santa Clara, CA, USA) with an overall median and average probe spacing of 2·6 and 3 Kb, respectively. The data were extracted using the Feature Extraction v.10.7.1.1 software (Agilent Technologies), analyzed with DNA Analytics v.4.0.85 software (Agilent Technologies) using the ADM-2 algorithm with a threshold of 5, and compared with the human genome reference sequence hg19. The array-CGH analysis revealed a homozygous deletion on the long arm of chromosome 3 (3q21.3) between the genomic positions 128 770 580 and 128 796 076 (first and last deleted probe, hg19), so a deletion in the range 26–31 Kb containing only the *GP9* gene (Fig 1B).

Four deletions encompassing the *GP9* gene are reported in the ClinVar database (Landrum *et al.*, 2014). Three of these are very large deletions identified in individuals with developmental disabilities or congenital anomalies. The fourth deletion, identified in a clinical setting, is described as a gross deletion encompassing the *GP9* gene with unknown boundaries. However, the zygosity and clinical data are lacking.

Our report is the first human case of homozygous deletion encompassing only the *GP9* gene in a patient carrying another genetic abnormality in a context of strong consanguinity.

Since diagnosis, he was bi-nephrectomized and kidney-transplanted for the CNS. Platelets are no longer transfused and bleeding complications are treated by tranexamic acid without systematic platelet transfusion.

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Supplementary Material

Fig S1. Megakaryocyte differentiation was deduced from control (A) or patient (B) peripheral blood $CD34^+$ cells and analyzed at day 9 of culture.
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Fig S2. Megakaryocyte differentiation was deduced from control (A) or patient (B) peripheral blood $CD34^+$ cells and analyzed at day 9 of culture.

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