



HAL
open science

A new heterozygous mutation in GP1BA gene responsible for macrothrombocytopenia

Dorsaf Ghalloussi, Noémie Saut, Denis Bernot, Xavier Pillois, Philippe Rameau, Gérard Sébahoun, Marie-Christine Alessi, Hana Raslova, Veronique Baccini

► **To cite this version:**

Dorsaf Ghalloussi, Noémie Saut, Denis Bernot, Xavier Pillois, Philippe Rameau, et al.. A new heterozygous mutation in GP1BA gene responsible for macrothrombocytopenia. *British Journal of Haematology*, In press, 10.1111/bjh.14986 . hal-01744130

HAL Id: hal-01744130

<https://hal.science/hal-01744130>

Submitted on 11 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

A new heterozygous mutation in GPIBA gene responsible for macrothrombocytopenia

Ghalloussi, Dorsaf; Aix-Marseille Université, Medicine Faculty
Saut, Noémie; APHM Hôpital La Timone, Laboratoire d'Hématologie
Bernot, Denis; APHM, Hôpital la Timone, Laboratoire d'Hématologie
Pillois, Xavier; CHU Bordeaux, Laboratoire d'Hématologie; Inserm 1034,
Adaptation Cardiovasculaire à l'Ischémie
Rameau, Philippe; PFIC, UMS AMMICA (UMS 3655 CNRS/US 23 INSERM)
Gustave Roussy Cancer Campus
Sebahoun, Gérard; APHM Hôpital Nord, Laboratoire d'Hématologie
Alessi, Marie-Christine; NORT Laboratory, UMR 1062 INSERM, Aix Marseille
Université, Medicine Faculty; APHM Hôpital La Timone, Laboratoire
d'Hématologie
Raslova, Hana; INSERM, U1170
Baccini, Véronique; UMR1062 INSERM, Aix Marseille Université, Medicine
Faculty; APHM Hôpital Nord, Laboratoire d'Hématologie

Key Words:

MEGAKARYOCYTOPOIESIS, PLATELET DISORDERS, PLATELET GENETIC
DISEASES, PLATELET MEMBRANE, PLATELETS

Congenital macrothrombocytopenias are a heterogeneous group of rare inherited disorders characterized by decreased platelets count with enlarged platelet size. However, patients with isolated macrothrombocytopenia are often misdiagnosed with idiopathic thrombocytopenic purpura (ITP) and wrongly treated with immunoglobulin injection, steroid administration and splenectomy.

Bernard-Soulier Syndrome (BSS) is a bleeding disorder caused by defects in the platelet GPIb/IX/V complex, a receptor for von Willebrand factor (vWF) and thrombin. Patients show a 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 GPIb α and four GPIb β subunits stabilized by disulfide bonds (Luo *et al.*, 2007). This heterodimer is non-covalently associated with two GP1X and one GPV subunits. The N-terminal residues of GPIb α form seven leucine-rich repeats (LRRs) and include the binding sites for vWF and thrombin. The BSS disease is due to homozygous mutations in *GPIBA*, *GPIBB* or *GP9* genes encoding GPIb/IX/V complex (Savoia *et al.*, 2014). However, some families with hereditary macrothrombocytopenia and mild or no bleeding diathesis were described with heterozygous mutations in both *GPIBA* (Savoia *et al.*, 2001; Vettore *et al.*, 2008) or *GPIBB* gene (Savoia *et al.*, 2014; Kunishima *et al.*, 2001). These patients show mild thrombocytopenia with slightly increased mean platelet volume (MPV), a variable percentage of giant platelets, a modest decrease of cell-surface expression of the GPIb/IX/V complex, sometimes leading to a reduced aggregation in response to ristocetin. However, due to the absence or paucity of biological signs, the use of gene sequencing is mandatory to pinpoint the GPIb complex involvement. 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. The most common reported variation is a *GP1BA* missense mutation, leading to an amino acid substitution (Ala172Val), known as the Bolzano variant located in the sixth LRR (Savoia *et al.*, 2001). Here, we describe a French family with a new mutation in *GPIBA* gene located in the fifth LRR.

The proband shows a mild thrombocytopenia ($89 \times 10^9/L$) with large platelets (MPV: 13.1 fL; Advia[®], Siemens) without bleeding diathesis. Platelets aggregated normally with ADP and collagen but agglutination response to 1.25 mg/mL ristocetin as the GPIb/IX/V complex

platelet surface expression were modestly decreased (Table 1).

The member 2 (his sister) exhibits more pronounced bleeding symptoms with menorrhagia, epistaxis, postoperative bleeding that did not require transfusion (Table 1). Platelet volume in the two patients was slightly elevated (11.5-13.1 fL) with 17% of large platelets, but without giant platelets on the peripheral blood smear (Table 1 and Figure 1A). Pedigree analysis reveals an autosomal dominant inheritance pattern since several members of different generations exhibit macrothrombocytopenia (Supplementary data).

The morphology of the propositus bone marrow cells revealed small megakaryocytes with reduced and vacuolated cytoplasm (Figure 1B). *In vitro* study of MKs derived from peripheral blood CD34⁺ cells in presence of TPO and SCF revealed a reduction in the percentage of mature CD41⁺CD42⁺ in the propositus as compared to control whereas the ploidy level was not affected (Figure 1C).

Sanger sequencing of the patient's *GPIBA*, *GPIBB*, and *GPIX* gene revealed a novel single nucleotide mutation located in *GPIBA* (Figure 1D). The substituted residue was highly conserved in *GPIBA* orthologs from ten distantly related species. This variation was called deleterious by diverse prediction algorithms (Polyphen-2 score 1, SIFT score 0) and was not reported in the ExAC and 1000G databases (Supplementary data).

The first reported thrombocytopenic patients with large platelets, slightly reduced aggregation to ristocetin and GPIb/IX/V platelet surface expression carried monoallelic Ala172Val substitution in GPIb α (Savoia *et al.*, 2001) known as the Bolzano variant. Other monoallelic mutations in the *GPIBA* gene have since been identified (Savoia *et al.*, 2001; Vettore *et al.*, 2008) and some were also discovered in the *GPIBB* gene associated with macrocytosis though not always with thrombocytopenia (Savoia *et al.*, 2014; Kunishima *et al.*, 2001). Macrothrombocytopenia due to haploinsufficiency of the *GPIBB* gene is also a finding frequently observed in patients with the diGeorge syndrome (*del22q11*) (Liang *et al.*, 2007) but these patients are mainly diagnosed according to extra-hematological symptoms. In our laboratory, we sequenced *GPIBA*, *GPIBB* and *GPIX* genes in all patients with mild macrothrombocytopenia and we found only one family carrying the substitution of asparagine by a serine at position 150 that was not yet listed by the International Consortium for the study of BSS. Thus, in contrast to the Bolzano variant, our results do not support a mutational founder effect. Like the Bolzano variant, we have observed a variable phenotype among the mutation carriers despite similar platelet phenotype. A combination of genetic, environmental and lifestyle factors may explain the discrepancies such as gender. Indeed, the individual with the highest bleeding score predominantly suffered from gynecological and obstetrical bleeding.

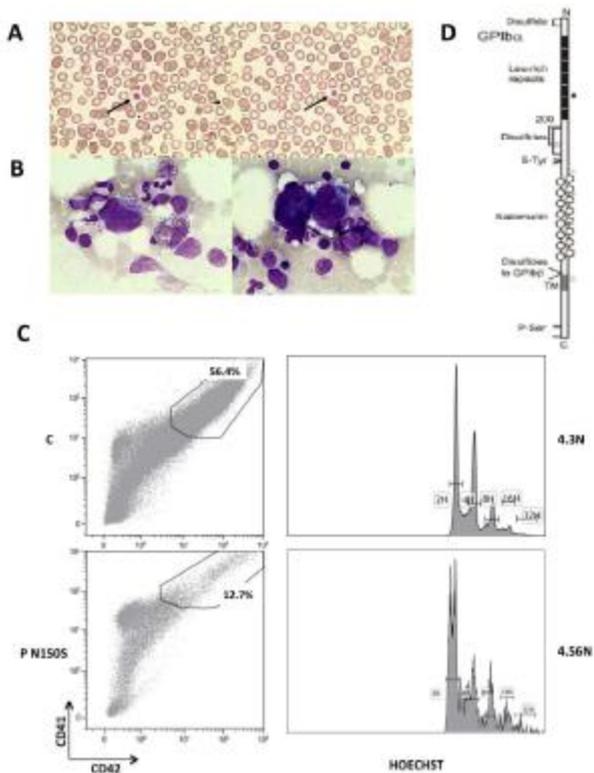
Balduini *et al.* (Balduini *et al.*, 2009) showed that megakaryocyte maturation was not affected in subjects with monoallelic Bolzano mutation while proplatelet formation was severely reduced *in vitro*. In this study, the maturation was evaluated according to the morphology of CD34⁺ derived megakaryocytes at day 12. We observed fewer megakaryocytes (CD41⁺CD42⁺ cells) at day 13 of culture without reduction of modal ploidy. Those results are consistent with those described in a murine model of BSS (Ware *et al.*, 2000).

We reported here a new monoallelic mutation in the *GPIBA* gene that slows-down megakaryocyte differentiation without reducing polyploidization.

Diagnosis of monoallelic BSS has to be suspected in case of thrombocytopenia, rather moderate with a dominant transmission pattern. The important element is the presence of macroplatelets (> 10%) but only few even no giant platelets (< 5%). These two criteria associated with discrete quantitative and qualitative alterations of the GPIb/IX/V complex may help to orient the diagnosis towards a defect in GPIb/IX/V complex.

References

- Balduini, A., Malara, A., Pecci, A., Badalucco, S., Bozzi, V., Pallotta, I. Noris, P., Torti, M. & Balduini, C.L. (2009) Proplatelet formation in heterozygous Bernard-Soulier syndrome type Bolzano. *Journal of Thrombosis and Haemostasis*, **7**, 478-484.
- Kunishima, S., Naoe, T., Kamiya, T. & Saito, H. (2001) Novel heterozygous missense mutation in the platelet glycoprotein Ib beta gene associated with isolated giant platelet disorder. *American Journal of Hematology*, **68**, 249-255.
- Liang, H.P., Morel-Kopp, M.C., Curtin, J., Wilson, M., Hewson, J., Chen, W. & Ward, C. (2007) Heterozygous loss of platelet glycoprotein (GP) Ib-V-IX variably affects platelet function in velocardiocardiac syndrome (VCFS) patients. *Thrombosis and Haemostasis*, **98**, 1298-1308.
- Luo, S.Z., Mo, X., Afshar-Kharghan, V., Srinivasan, S., Lopez, J.A. & Li, R. (2007) Glycoprotein Ibalpha forms disulfide bonds with 2 glycoprotein Ibbeta subunits in the resting platelet. *Blood*, **109**, 603-609.
- Savoia, A., Balduini, C.L., Savino, M., Noris, P., Del Vecchio, M., Perrotta, S., Belletti, S., Poggi, & Iolascon, A. (2001) Autosomal dominant macrothrombocytopenia in Italy is most frequently a type of heterozygous Bernard-Soulier syndrome. *Blood*, **97**, 1330-1335.
- Savoia, A., Kunishima, S., De Rocco, D., Zieger, B., Rand, M.L., Pujol-Moix, N., Caliskan, U., Tokgoz, H., Pecci, A., Noris, P., Srivastava, A., Ward, C., Morel-Kopp, M.C., Alessi, M.C., Bellucci, S., Beurrier, P., de Maistre, E., Favier, R., Hezard, N., Hurtaud-Roux, M.F., Latger-Cannard, V., Lavenu-Bombled, C., Proulle, V., Meunier, S., Negrier, C., Nurden, A., Randrianaivo, H., Fabris, F., Platokouki, H., Rosenberg, N., HadjKacem, B., Heller, P.G., Karimi, M., Balduini, C.L., Pastore, A. & Lanza, F. (2014) Spectrum of the mutations in Bernard-Soulier syndrome. *Human Mutation*, **35**, 1033-1045.
- Vettore, S., Scandellari, R., Moro, S., Lombardi, A.M., Scapin, M., Randi, M.L., & Fabris, F. (2008) Novel point mutation in a leucine-rich repeat of the GPIIb/IIIa chain of the platelet von Willebrand factor receptor, GPIIb/IIIa, resulting in an inherited dominant form of Bernard-Soulier syndrome affecting two unrelated families: the N41H variant. *Haematologica*, **93**, 1743-1747.
- Ware, J., Russell, S. & Ruggeri, Z.M. (2000) Generation and rescue of a murine model of platelet dysfunction: the Bernard-Soulier syndrome. *Proceedings of the National Academy of Sciences*, **97**, 2803-2808.

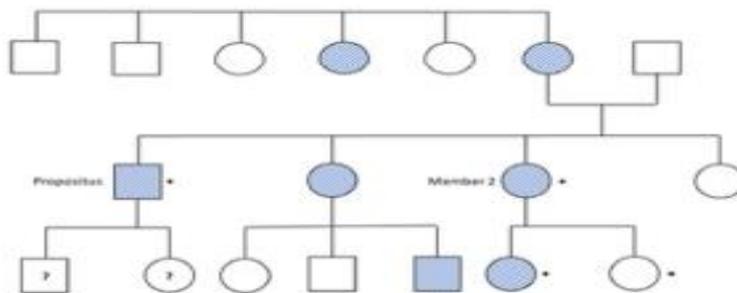


Blood smear (A) shows large platelets (arrow) and bone marrow smear (B) revealed the presence of some small megakaryocytes with reduced cytoplasm and sometimes vacuolated. MK differentiation was induced from control or patient peripheral blood CD34+ cells and analyzed at day 13 of culture (C).

Gates represent mature (CD41+CD42+). The ploidy level (N) was analyzed in the gate of CD41+CD42+ MKs. Mutation location in the protein (asterisk) (D) : amino acid substitutions is located in the fifth LRR. 199x199mm (300 x 300 DPI)

	Propositus	member 2
Age	29	37
Platelet count (10 ⁹ /L)	89	74
MPV (fL)	13.1	11.5
Bleeding score	0	1 to 2
RIPA by 1.25 mg/mL (Intensity, %)	77	62
Normal range : [85-94]		
RIPA by 1.25 mg/mL (Velocity, %)	41	33
Normal range : [85-212]		
GpIb expression (% of control)	69	100
% of large platelets/giant platelets	NP	17/0

Patients characterization : Age, platelet counts and platelet size (MPV) obtained by optical method, bleeding score, platelet aggregation in response to ristocetin (RIPA) 1.25 mg/mL and GpIb expression by flow cytometry (NP : not performed).



Family tree :
Members with macrothrombocytopenias are representing with striped square or circle.
Asterisks highlight patients which DNA was sequenced.
Platelet counts of members with question mark are unknown.

Cristallography studies : Cristallography studies predict that this mutation was probably damaging (by polyphen-2, SIFT and MutationTaster predictive algorithms). It results in the substitution of an asparagin by a serine at position 150. The physicochemical difference between Asn and Ser is very moderate (Grantham dist. : 46 [9-215]).