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High prevalence of the natural Asn89Asp mutation in the *GP1BB* gene associated with Bernard–Soulier syndrome in French patients from the genetic isolate of Reunion island

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Keywords

Bernard-Soulier syndrome; Reunion island; platelet GPIbβ; Leucin-rich repeat domain; GP1BB

Congenital defects in the platelet GPIb-IX-V complex which result in quantitative deficiencies cause the Bernard-Soulier syndrome (BSS) bleeding disorder (Nurden and Nurden 2011). BSS is an autosomal recessive disease characterized by moderate to severe thrombocytopenia, giant platelets and mucocutaneous bleeding (Lanza 2006).

Mutations in any one of the three genes *GP1BA*, *GP1BB* or *GP9* may lead to impaired expression of the GPIb-IX-V complex. The prevalence of BSS has been estimated at less than one in a million live births, but higher frequencies have been observed in particular regions.

The island of Reunion is a French overseas territory where isolation from mainland resulted in founder effects observed for different inherited diseases (Richard*, et al* 1995, Rodius*, et al* 1994). Occurrence of BSS has been known for several years in a number of patients

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Addendum

H. Randrianaivo-Ranjatoelina, C. De Thoré, M. Dreyfus, C. Lavenu-Bombled, and M. Fiore followed patients and collected data; ML Jacquemont collected genealogic information and established the genealogic trees; M.J. Baas and A. Dupuis performed sequencing; R. Li performed 3-D modelling; C. Gachet, critically reviewed the manuscript; F. Lanza and M. Fiore designed the study, analyzed the results and wrote the manuscript.

referring to the main hospitals of Reunion island. Then, these patients were analyzed for genetic defects in the *GP1BA*, *GP1BB*, and *GP9* genes. Sequencing of the *GP1BB* coding region revealed a similar homozygous c.265A>G transition in the 13 affected individuals. This mutation predicts a p.Asn89Asp amino acid change in GPIbβ. Analysis of 7 relatives from 5 families showed that they were all heterozygous for the same mutation (Supplemental Fig. 1 and Supplemental Table I).

The Asn to Asp change falls within the C-terminal region flanking the single LRR motif of GPIbβ. Alignment of corresponding sequences from other species revealed that the Asn mutated in the patient is highly conserved (Supplemental Fig. 2A). As shown in the 3D-model, the side chain of the Asn forms 6 close-knit hydrogen bonds with polar backbone atoms (Supplemental Fig. 2B) (McEwan*, et al* 2011). The mutation will replace the carboxamide group with a carboxylate, and although the change is relatively small, it will likely perturb the intricate hydrogen bond network around this residue. Therefore, the mutation is expected to be quite destabilizing. In 2003, Strassel et al. reported a homozygous p.Asn89Thr substitution in a BSS patient and showed that this single amino acid substitution in the extracellular domain of GPIbβ affects the expression of both GPIbα and GPIX in transfected CHO cell lines (Strassel*, et al* 2003). As illustrated in WB analysis of one patient (BSS_1), GPIbα, GPIbβ and GPIX subunits, which are probably not correctly associated, were easily degraded leaving only trace amounts or absence of them in platelet lysates (Supp. Fig. 3).

Characteristics of the population study is described in Supplemental Table I. In total, 13 homozygous patients and 11 heterozygous carriers were included. In the group of BSS patients, median age at diagnosis was 8 years (range 0–53 years) and seven patients (54%) had a late diagnosis after the age of 7 (Fig. 1A). In thrombocytopenic heterozygous patients (6/11), the diagnosis of inherited thrombocytopenia was realized at a very late stage (median +/− SD = 42.5 +/− 13 y). Among reasons for diagnostic delay, patients with inherited thrombocytopenia may be mistaken for or misdiagnosed as immune thrombocytopenia (ITP). Indeed, 38% of BSS patients were misdiagnosed as ITP and received treatment for this reason. Furthermore, three patients were splenectomized without receiving any benefit. The study of Savoia et al. also showed that 4 out of 13 BSS patients (31%) had an erroneous diagnosis of autoimmune thrombocytopenia, treated with intravenous immunoglobulins, steroids and/or splenectomy (Savoia*, et al* 2011). Among heterozygous carrier, at least one patient was misdiagnosed as ITP, but did not receive any treatment.

Detailed bleeding history for each patient is also reported in Supplemental Table 1. Bleeding diathesis was variable and severity ranged from 5 to 22 (median +/− SD = 11 +/− 4.6) in BSS patients according to the ISTH-BAT (Fig. 1B). All menstruated women (5/8 patients) had menorrhagia that required, in 60% of them, estroprogestinic treatment associated or not with antifibrinolytics. One spontaneous intracerebral haemorrhage was reported at the age of 9 months (BSS_{10}). Nine patients (75%) received platelet transfusions to arrest bleeding episodes or in preparation to surgery. Recombinant activated factor VII was administered twice in one patient following head trauma and gingivorrhagia. It is interesting to note that thrombopoietin analog was used off-label, as a therapeutic alternative, in one patient (BSS_{11}) for which we observed a significant improvement of daily rectorrhagia with

complete disappearance of bleeding. Finally, while patients from Reunion island bear the same mutation in GPIbβ, our results demonstrated that there was some heterogeneity in the severity of bleeding history.

Reasons for the varying clinical phenotype despite a single homozygous mutation could be explained by the existence of others relevant haemostatic traits, such as von Willebrand factor, or by the personal history of each patient (trauma-related bleedings).

ISTH-BAT of heterozygous carriers ranged from 0 to 7 (median +/− SD = 1.0 +/− 2.1) (Fig. 1B). Only one patient had abnormal bleeding scores associated to diverse bleeding symptoms. None of the heterozygous patients received hemostatic treatment. Similar results were found in the study of Savoia et al. where 7 heterozygous carriers were asymptomatic (Savoia, *et al* 2011), whereas the study of Bragadottir et al. reported statistically significant bleeding in the heterozygous group (Bragadottir, *et al* 2015).

BSS patients had severe to minor macrothrombocytopenia, ranging from 26 to 112 $\times 10^9$ L^{-1} (Median+/−SD: 45 +/− 27.2 $\times 10^9$ L^{-1}) (Fig. 2A). No correlation was observed between platelet count and ISTH bleeding score (p=0.37). Among heterozygous carriers, six (55%) had minor thrombocytopenia (< 150 $\times 10^9$ L^{-1}), whereas the remaining patients had normal platelet counts. When available, MPVs were normal or only slightly increased **in this group**. In BSS patients, GPIbα and GPIX expression, evaluated by flow cytometry on platelet membrane, was markedly reduced, whereas the level of the GPIIb-IIIa complex was twice normal, due to the increased platelet size (Fig. 2B). In heterozygous carriers, GPIb-IX complex was less, but also significantly reduced compared to controls (Fig. 2B). Finally, on a biological level, heterozygous patients had a high inter-individual variability regarding their platelet counts, whereas low GPIb-IX expression showed less variation, representing a good biological screening marker for these patients.

In conclusion, this study identified a private mutation confined to a cluster of families from Reunion island. At a molecular level, this work further stresses the importance of the LRCT domain of the GPIbβ subunit for its proper folding and assembly with other subunits of the GPIb-IX complex. Moreover, clinical and biological data provided by this study may help improving the diagnosis and management of this dense BSS population from Reunion island.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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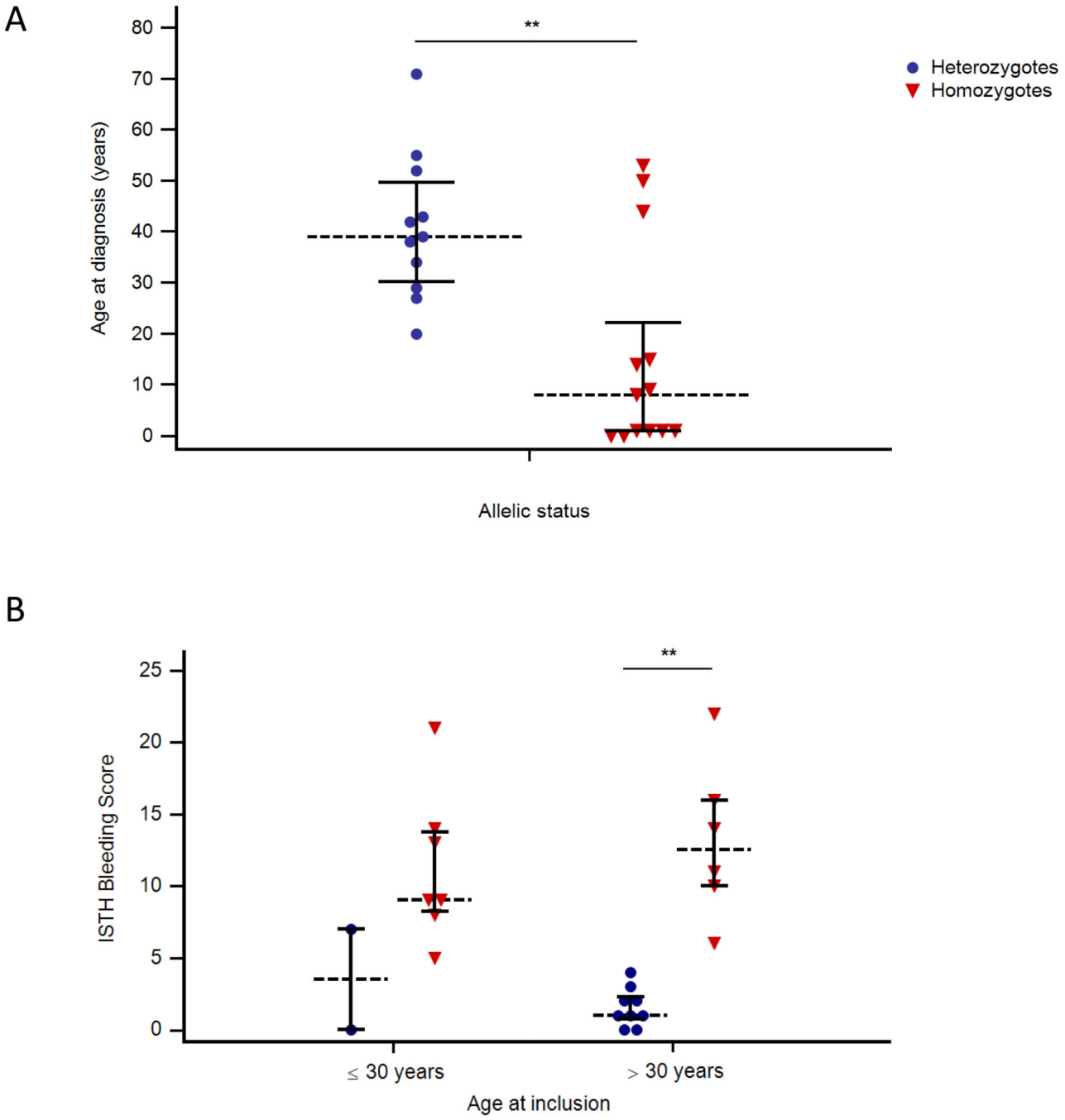

Figure 1. Clinical characteristics of the population study according to allelic status.

(A) Age at diagnosis; (B) ISTH Bleeding Scores according to age at inclusion.Horizontal bars represent medians and interquartiles. ** p< .01.

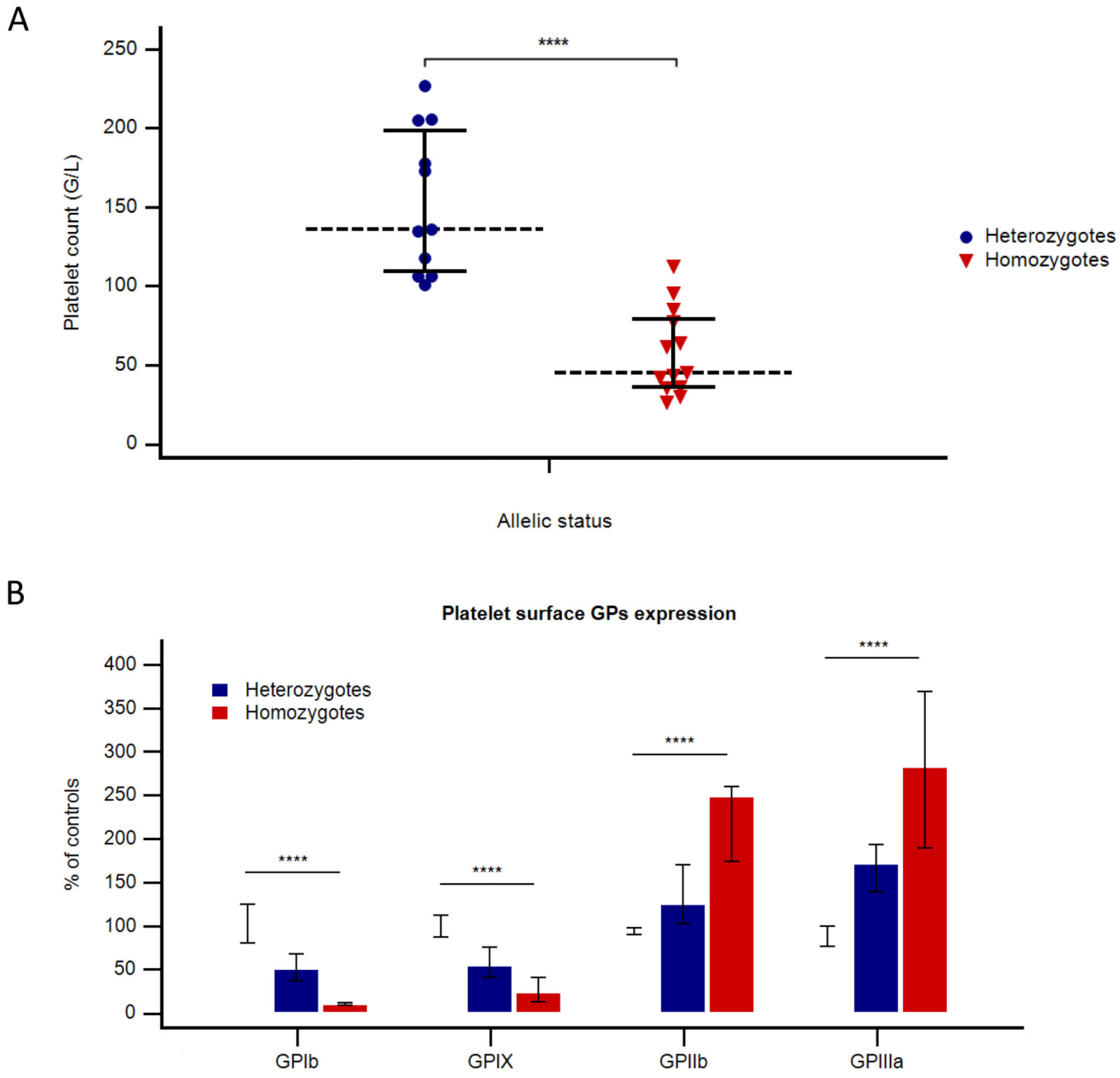

Figure 2. Biological characteristics of the population study according to allelic status.

(A) Platelet counts; (B) Platelet surface GPs expression measured by flow cytometry with different monoclonal antibodies. Horizontal bars represent medians and interquartiles. **** p<.0001.