



# Biological, clinical features and modelling of heterozygous variants of glycoprotein Ib platelet subunit alpha (GP1BA) and glycoprotein Ib platelet subunit beta (GP1BB) genes responsible for constitutional thrombocytopenia

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**Abstract:** Constitutional thrombocytopenias are rare disorders, often difficult to discriminate from acquired thrombocytopenias. More than 80 genes have been described as being at the origin of these diseases. Among them, several variants of the *GP1BA* and *GP1BB* genes, coding for the GpIb-IX-V glycoprotein complex, have been reported in the literature. The study reported here aimed at describing newly identified monoallelic anomalies affecting the *GP1BA* and *GP1BB* genes on a clinical, biological and molecular level. In a cohort of 9 patients with macrothrombocytopenia, eight heterozygous variants of the *GP1BA* or *GP1BB* genes were identified. Five of them had never been described in the heterozygous state. Computer modelling disclosed structure/function relationships of these five variants.

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**Introduction**

Constitutional thrombocytopenias (CT) are rare diseases difficult to distinguish from acquired thrombocytopenias, in particular immune thrombocytopenia (ITP). A CT diagnosis must be evoked in the presence of a number of characteristics: i)thrombocytopenia known since childhood, ii)family history, iii)poor response to ITP treatment and iv)analysis of platelets and megakaryocytes morphology and platelet functions. CT confirmation then relies on molecular analysis. Advances in sequencing techniques have made possible to better characterize the genetic abnormalities at the origin of these pathologies, as they often affect genes involved in megakaryopoiesis and platelet formation.

More than 80 genes have thus been described as being potentially responsible for CT. Among them, some are coding for molecules of the membrane glycoprotein complex Ib-IX-V (GPIb-IX-V). However, publications concerning these mutations are rare and, to date, less than 30 such variants have been reported in the literature.

The GPIb-IX-V glycoprotein complex, the platelet receptor for Willebrand factor (VWF), is essential for primary hemostasis since it allows platelets to bind subendothelial collagen molecules, which become accessible in case of vascular injury. It is composed of 4 subunits, respectively GPIb $\alpha$ , Ib $\beta$ , IX and V, encoded respectively by the *GP1BA*, *GP1BB*, *GP9* and *GP5* genes. Homozygous or double heterozygous deleterious sequence variants of *GP1BA*, *GP1BB* and *GP9* are at the origin of Bernard-Soulier syndrome (BSS), characterized by thrombocytopenia with increased platelet size and the presence of giant platelets, as well as severe platelet function disorders related to quantitative or qualitative deficiency of the GPIb-V-IX complex (1-3). However, some variants are responsible for an attenuated disease phenotype when present in the heterozygous state. The latter are called monoallelic BSS with an autosomal dominant transmission (4).

To date, 13 *GP1BA* variants (4-16) and 14 *GP1BB* variants (15-22), considered to be responsible for monoallelic BSS, have been described in the literature (Table 1). No variant responsible for monoallelic BSS has been identified so far in the *GP9* gene.

From a molecular point of view, all described heterozygous *GP1BA* variants show clustered substitutions within the leucine-rich repeat (LRR) domains of GPIb $\alpha$ , located in the ligand-

binding site (LBD) (residue His1-Thr266) which contains the VWF-binding site (5-7). There also are variants of GPIIb $\beta$  similarly involving the LRR domain of the protein.

Clinically, patients present with moderate mucocutaneous bleeding, such as epistaxis, ecchymosis, menorrhagia and sometimes prolonged post-surgical bleeding (4-23). Platelet counts show moderate thrombocytopenia with an increase in mean platelet volume. Morphologic analysis discloses giant platelets in variable proportions. Surface expression of the GPIIb-IX-V complex, assessed by flow cytometry, shows decreased or normal levels, depending on the type of antibody used and the degree of damage to the complex (11). Platelet functional studies often show decreased aggregation in the presence of ristocetin and, in most cases, normal aggregation in the presence of ADP and collagen (6,8,9,12,14,20-22).

From a pathophysiological point of view, the origin of MTP is still not completely elucidated. The GPIIb-IX-V complex is closely linked to the cytoskeleton, required to sustain platelet shape (2) and also involved in megakaryopoiesis since proplatelet formation is mediated by actin filaments remodelling (24). Moreover, published data indicate that macrothrombocytopenia (MTP) may be due to abnormal interactions between the intracytoplasmic portion of GPIIb $\alpha$  and actin-binding proteins. Indeed, the latter monitor MK maturation and production of the demarcation membrane system, thus controlling platelet size (25,26). This suggests that a GPIIb-IX-V complex deficiency would have a direct impact on platelet production and would be the cause of MTP.

At the molecular level, variants that cause monoallelic BSS appear to have a dominant negative effect (11,21). Indeed, in the majority of heterozygous carriers, including relatives of patients with classical BSS, a normal copy of the GP1BA or GP1BB genes is found without thrombocytopenia nor giant platelets (24). Thus, only specific variants cause MTP in monoallelic BSS, inducing a significant deficit - qualitative or quantitative - of the GPIIb-IX-V complex, despite the presence of a normal GP1BA or GP1BB allele (27). The putative effect of these dominant-negative variants is the presence of an abnormal GPIIb subunit that compromises the delivery of the GPIIb-IX-V complex to the surface, resulting in a quantitative and qualitative GPIIb-IX-V deficit (28). Indeed, most variants affect residues that are embedded in the protein structure and disrupt proper folding of the LRR region, thus impairing the formation of the GPIIb-V-IX complex (4,5,7-9,11,15,20,21,28-30)

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100 The aim of the study reported here was therefore to describe clinically, biologically and  
101 molecularly new monoallelic variants affecting the *GP1BA* and *GP1BB* genes. Moreover, the  
102 structure/function relationship of each variant was analysed in depth by molecular modelling  
103 of the mutated protein.

104  
105 **Materials and methods**

106 This non-interventional, retrospective and prospective bicentric cohort study was submitted  
107 and approved by the GNEDS (Groupe Nantais d'Ethique dans le Domaine de la Santé). All  
108 patients were informed of the study and none refused to participate.

109 The cohort consists of 9 patients with MTP, recruited from the Clinical Haemostasis centres of  
110 Nantes and Rennes University Hospital. For all of them, a heterozygous variant of the *GP1BA*  
111 or *GP1BB* genes had been identified in the context of thrombocytopenia exploration.

112 Platelet counts were assessed by impedancemetry and fluorocytometry, together with mean  
113 platelet volume (MPV) and immature platelet fraction (IPF) assessment, using an XN9000  
114 instrument (Sysmex, Kobe, Japan). Platelet aggregation assays were performed on citrated  
115 platelet-rich plasma with the following agonists: adenosine diphosphate (ADP) (5 µM),  
116 epinephrine (5 µM), arachidonic acid (1 mM), collagen (1.25 µg/mL) and ristocetin (1.5 mg/mL,  
117 1.2 mg/mL and 0.7 mg/mL). GPIb-IX-V expression was measured by flow cytometry using a  
118 quantification kit (PLT GP/receptors, Biocytex, Marseille France) on a Navios (Beckman  
119 Coulter, Miami, FL) instrument.

120 The search for sequence variants was performed by high throughput sequencing on a MiSeq  
121 sequencer (Illumina, San Diego, CA) targeting coding sequences and the intron-exon junctions  
122 of the *GP1BA*, *GP1BB* and *GP9* genes with an average depth of 300 reads. Mutated and normal  
123 reads were assessed with an automated algorithm (SOPHiA Genetics, Lausanne, Switzerland)  
124 detecting the homozygous or heterozygous status of each of the variants. The presence of  
125 each variant and its status were also systematically confirmed by Sanger sequencing. Only the  
126 probands were available, and it was not possible to perform familial triad analyses as reported  
127 by Sivapalaratnam et al.(20).

The possible pathogenicity of the variants was assessed using recommendations from the American College of Medical Genetics and Genomics and Clinvar (Supplemental table 1).

Structural visualization of proteins of interest was performed from genomic characteristics, in order to visualize the localization and potential structural impact of variants on the GPIb-IX-V complex. Protein sequences were retrieved from UniProt (<https://www.uniprot.org/>): UniProtKB - P07359 (GP1BA\_HUMAN), UniProtKB - P13224 (GP1BB\_HUMAN) and residues numbering used on the figures refers to these sequences. Protein structures were obtained from the Protein Data Bank (PDB, <https://www.rcsb.org/>). For GPIb $\alpha$ , the following PDB codes were used: 1GWB (GPIb $\alpha$  wild type, residues 18 to 283), 1SQ0 (GPIb $\alpha$  carrying N37D and N175D mutations, in complex with VWF) and 1P8V (GPIb $\alpha$  carrying the N37D mutation, in complex with  $\alpha$ -trombin). For GPIb $\beta$ , the PDB code 3RFE, corresponding to wild-type GPIb $\beta$  subunit (residues 27 to 143) was used.

Finally, the structural amino-acid residues of interest were identified and visualized with the BIOVIA Discovery Studio Visualizer (Dassault Systèmes, San Diego, CA).

## **Results**

Eight pathogenic variants were found for the 9 patients included in the study, respectively 3 in the *GP1BA* gene and 5 in the *GP1BB* gene (according to the ACMG criteria).

Among these, 3 have not been previously described in MTP (respectively *GP1BA* [p.Leu155Pro], *GP1BB* [p.Leu168dup] and gene deletion) but 2 (*GP1BB* [p.Cys141\*] and [p.Leu137Pro] had already been found in the homozygous state in a BSS context. Moreover, *GP1BA* [p.Leu73Phe] and [p.Asn150Ser] as well as *GP1BB* [p.Leu16Pro] had already been described in a heterozygous state.

### ***GP1BA* gene**

Patients with *GP1BA* variants (Table 2) displayed a very discreet haemorrhagic symptomatology, mainly affecting mucosal sites when present. Platelet counts showed moderate thrombocytopenia (49 to 79x10<sup>9</sup>/L), variable MPV (11.2 to 14.2 fL), and, in the 2 patients tested, an increase in IPF. GPIb $\alpha$  expression was low in one patient. Abnormalities in platelet function were observed in the presence of ADP, collagen and arachidonic acid. The platelet count was too low to perform aggregometry in one case.

The location in the GPIb $\alpha$  subunit of the 3 mutated residues is depicted on Figure 1.

*p.(Leu73Phe)*

This variant had already been described by Miller *et al.* in 1992 in a patient with MTP (7). The leucine residue at position 73 is located within the second leucine rich repeat (LRR) domain of the GPIb $\alpha$  subunit. Its substitution with a phenylalanine, which is a larger amino acid, may create steric hindrance and disrupt the structure. Furthermore, adjacent residues are involved in VWF binding, making it likely that the *p.(Leu73Phe)* variant could affect this molecular interaction (Figure 2).

*p.(Asn150Ser)*

Sequencing disclosed a c.449A>G substitution predicting a *p.(Asn150Ser)* missense. This was previously described by Ghalloussi *et al.* in 2017 (9). This team suggested that this variant would affect megakaryopoiesis by decreasing the number of mature megakaryocytes (9). Structurally, residue 150 is positioned at the end of the 5th LRR domain (Figure 1). Asn150 is buried but its lateral chain participates in the maintenance of the structure via hydrogen bonds. Its substitution to a serine, which is a smaller amino acid, may have an impact on the conformation of GPIb $\alpha$  (Figure 3).

*p.(Leu155Pro)*

From a structural point of view, residue 155 is positioned at the beginning of the 6th LRR domain (Figure 1). Because the leucine at position 155 is buried, its replacement to a proline may strongly disrupt the structure of the hydrophobic core (Figure 4). Moreover, the 2 residues upstream of Leu155 are also proline residues (Pro156, Pro157), and the *p.(Leu155Pro)* variant will result in 3 consecutive prolines, likely to form a polyproline helix that could induce the formation of a twist and would thus distort the initial structure of GPIb $\alpha$ . This could impair interactions with  $\alpha$ -thrombin (Figure 4).

*GP1BB* gene

The haemorrhagic symptomatology of patients with *GP1BB* gene variants (Table 3) is variable, depending on the variant, and mostly affects mucosal sites when present. Platelet counts



showed very moderate thrombocytopenia (from 94 to  $147 \times 10^9/L$ ), a variable but most often clearly increased MPV (from 11 to 14.3 fL) and increased IPF in all the patients tested. Low GPIb $\alpha$  expression was disclosed in 3/4 patients. Abnormalities of platelet functions were observed in the presence of low doses of ristocetin in 4 of the 5 patients tested.

From a molecular point of view, because of the location of the variants, prediction of their impact was possible for 2 out of 5 (Figure 5).

*p.(Leu137Pro)*

The heterozygous c.410T>C variant results in the p.(Leu137Pro) substitution, already described in the homozygous state in BSS (31). This residue is buried and located at the C-terminal domain of the GPIb $\beta$  subunit, more precisely in the  $\alpha 2$  helix. Its replacement by a proline is likely to strongly disrupt the helix structure, which would impact subunits assembly of the GPIb-IX-V complex, and would explain the quantitative deficit observed in this patient (Figure 5).

*p.(Cys141\*)*

A heterozygous c.423C>A substitution was disclosed in the *GP1BB* gene, resulting in a stop codon that predicts the nonsense variant p.(Cys141\*). Cysteine 141 is positioned at the end of the C-terminal domain of GPIb $\beta$ , and is involved in a disulphide bridge with cysteine 95 (Figure 5). The p.(Cys141\*) variant would lead to a soluble form of GPIb $\beta$  with the added loss of the disulphide bridge with Cysteine 95, that could impact the C-terminal domain conformation. This soluble form could either interact with other members of the GPIb-IX-V complex or compete with membrane-bound GPIb $\beta$  if both forms are present. This variant has been reported in the homozygous state in BSS (22) (Figure 5).

*p.(Leu16Pro)*

This variant, previously described (20,21), was found in 2 siblings. In these two patients, sequencing disclosed a heterozygous c.47T>C variant on the *GP1BB* gene, predicting a p.(Leu16Pro) missense. This residue is located at the level of the signal peptide, therefore not visible on the available structures of GPIb $\beta$ . This mutation might lead to abnormal addressing or cleavage of the mature protein, thus leading to a quantitative GPIb $\beta$  deficiency.

*p.(Leu168dup)*

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214 Sequencing identified a heterozygous variant c.502\_504dup that predicts a duplication of the  
215 Leu168 residue. The latter is located at the transmembrane domain of the GPIIb $\beta$  subunit and  
216 not present in GPIIb $\beta$  available structures. The variant is likely to disrupt the helix structure of  
217 this domain and its association with the other subunits of the complex.

218 *Exon deletion*

219 Sequencing found a deletion of *GP1BB* gene exon 2 in the heterozygous state in one patient.  
220 This translated in decreased expression of the GPIIb-GPV-GPIX complex as shown by GPIIb $\alpha$   
221 assessment in flow cytometry (table 2).

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223 **Discussion**

224 This molecular study of patients with constitutional thrombocytopenia allowed for the  
225 discovery of five variants that had never been described before in MTP. One is located on the  
226 *GP1BA* gene and four on the *GP1BB* gene. Of note, p.(Leu137Pro) and p.(Cys141\*), on the  
227 *GP1BB* gene, had previously been described as homozygous pathogenic variants in BSS cases  
228 (31,32). Here, although only the probands (and not their parents or siblings) were tested,  
229 heterozygosity was confirmed by NGS and Sanger, demonstrating the presence of normal and  
230 variant sequences.

231 An attempt to predict and explain the impact of these variants based on the location of the  
232 residues involved on the available structures of the extracellular domain of GPIIb $\alpha$  and GPIIb $\beta$   
233 was possible for 5 of the 8 variants observed. Of note, these data are somehow speculative  
234 since it was not intended in this study to perform functional studies of the variant proteins.  
235 Indeed, two are located either at the level of the signal peptide or in the transmembrane  
236 region and one results in a total loss of the coding region. The data obtained confirm and  
237 complete available literature data.

238 The p.(Leu16Pro) substitution, found at the level of the signal peptide, is likely to cause  
239 abnormalities in GPIIb $\beta$  addressing and cleavage, resulting in a quantitative deficiency.  
240 Deletion of the *GP1BB* gene causes a complete absence of GPIIb $\beta$  expression.

241 Concerning the clinico-biological phenotype of the patients, several results are interesting to  
242 note. Their hemorrhagic symptomatology is discrete or non-existent and mainly affects

243 mucosal sites. This is compatible with the previous reports (3-23). Moreover, as expected, von  
244 Willebrand levels of the patients were normal, without discrepancy between activity and  
245 antigen assays.

246 MTP is almost always observed in these patients, whether the variant involves the *GP1BA* or  
247 *GP1BB* gene.

248 Several authors have indeed pointed out that the GPIb $\alpha$  variants associated to MTP were  
249 especially located at the LRR domains (5-7,19,23). Indeed, here p.(Leu73Phe), p.(Asn150Ser)  
250 and p.(Leu155Pro) mutations are positioned within the LRR2, LRR5 and LRR6 domains  
251 respectively. All three could generate conformational changes in the GPIb $\alpha$  subunit, with  
252 increasing functional impact when the modification is located near the VWF binding site.

253 Concerning GPIb $\beta$ , it has previously been hypothesized (19,22) that MTP-associated variants  
254 result in a decreased expression of the molecule, which impacts all subunits of the GPIb-IX-V  
255 complex. This hypothesis is supported by our observations. Both p.(Leu137Pro) and  
256 p.(Leu168dup) alterations could result in structural changes of GPIb $\beta$  which might interfere  
257 with a correct assembly of the GPIb-IX-V complex. The p.(Cys141\*) variant results in a  
258 truncated protein which could be responsible for the production of a soluble form of GPIb $\beta$ .

259 The origin of MTP remains debated. It has been proposed that MTP would not be due to the  
260 absence of an allele but to the presence of an abnormal GPIb-V-IX complex leading to a  
261 dominant negative effect (20). Nevertheless, the majority of first-degree relatives of patients  
262 with BSS, who therefore have a GP1BB allele with a deleterious sequence variation and a  
263 healthy allele, do not have MTP. In addition, only 25% of the patients with a 22q11 deletion  
264 syndrome, who have a complete deletion of a *GP1BB* allele, present MTP (28). In our series,  
265 similarly to these cases of 22q11 deletion, the patient presenting a complete deletion of  
266 *GP1BB* was diagnosed with MTP. The mechanism by which only some mutations of GP1BA or  
267 GP1BB with hemizyosity for these genes present with MTP still remains unclear although  
268 interactions between GPIb-V-IX complex subunits are probably essential (16, 32-34). One  
269 hypothesis would be that the concentration of the GPIb-V-IX complex on the platelet surface  
270 is involved in the terminal phases of megakaryopoiesis. This could be confirmed by studying  
271 the concentration of the GPIb-V-IX complex in patients with a monoallelic abnormality of the  
272 GP1BA or GP1BB gene without MTP. Alternately, in vitro models of megakaryopoiesis from

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3 273 stem cells issued from such patients could shed some light on this phenomenon. Another  
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5 274 hypothesis could be that cryptic anomalies of the second allele are present in patients with  
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7 275 MTP, that might be evidenced in whole genome analyses.  
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9 276 The expression of GPIb $\alpha$  detected by flow cytometry, which reflects the whole GPIb-V-IX  
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11 277 complex, was moderately decreased in most of cases, whatever the variant gene. This  
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13 278 suggests that the proteins resulting from abnormal alleles do not allow the complex to be  
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15 279 properly expressed at the membrane surface.  
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17 280 From a functional point of view, the study of platelet aggregation unexpectedly revealed  
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19 281 anomalies with ADP, arachidonic acid or collagen in association with GPIb $\alpha$  mutations. There  
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21 282 is no clear explanation for this but, paralleling observations in homozygous BSS, this could be  
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23 283 the consequence of an abnormal expression of the CD9 tetraspanin. CD9 and GPIIbIIIa are  
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25 284 stored in the same intracytoplasmic platelet structures and migrate to similar membrane  
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27 285 areas, which is consistent with a possible role of CD9 in platelet aggregation, modulating  
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29 286 GPIIbIIIa and consistent with the abnormalities of platelet aggregation in the presence of other  
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31 287 activators than ristocetin (14, 35, 36). More intriguingly, ristocetin aggregation was normal for  
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33 288 these patients, although the VWF receptor, located in the LRR part of GPIb $\alpha$ , is encoded by  
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35 289 the *GP1BA* gene. It could be that variants in the *GP1BA* gene do not directly impact the binding  
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37 290 site of the VWF A1 domain (residues 60-128) nor the thumb region of the ligand binding  
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39 291 domain, which is important for the interaction between GPIb and VWF (37,38). Moreover,  
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41 292 again surprisingly, 4/5 patients with GPIb $\beta$  variants tested displayed abnormalities affecting  
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43 293 platelet aggregation with intermediate concentrations of ristocetin. This suggests that the  
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45 294 GPIb $\beta$  protein is essential to ensure the proper conformation of GPIb allowing for VWF binding  
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47 295 (39-41). In addition, several studies indicate that the binding of GPIb $\beta$  to GPIb $\alpha$  protects the  
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49 296 latter from lysosomal degradation in (42,43). It is therefore possible that the binding defect  
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51 297 between the two subunits leads to excessive degradation of GPIb $\alpha$ , resulting in an abnormal  
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53 298 functional response to Willebrand factor  
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55 299 This work also demonstrates once again the interest of assessing the IPF, which corresponds  
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57 300 to the fraction of circulating immature platelets, in order to differentiate constitutional from  
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59 301 acquired thrombocytopenias. Indeed, in this cohort, the IPF value was significantly increased  
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302 in all patients whatever the *GP1BA* or *GP1BB* variant. This is in agreement with previous

studies that have attempted to define IPF thresholds to distinguish central from peripheral thrombocytopenias (44,45). It has been shown that, in MTP, the IPF is increased (3 to 5 times higher than in ITP) (46,47). Nevertheless, there are no validated normal values for IPF levels to date, although several studies have measured IPF in cohorts of healthy adults, yielding ranges of 0.2 to 7.7% (44-47). Finally, a recent study found that an IPF level greater than  $11 \times 10^9/L$  is a strong argument in favour of constitutional thrombocytopenia (47), which is confirmed here.

Several questions however remain pending regarding these observations. First, patients with these forms of MTP have very variable clinical features, both in the literature and in this cohort. The spectrum of clinical manifestations ranges from the absence of haemorrhagic signs (4 patients) to severe hematoma and minor haemorrhagic signs. It was also noted that, in some cases, patients carrying the same variant (within the same family) presented hemorrhagic signs of different severity. It is therefore likely that any given mutation is one element, among others, involved in the bleeding phenotype.

In an attempt to explain the heterogeneity of the clinical-biological phenotype between the different patients of this series, we can refer to the recent studies of polygenic scores for FBC traits (46) which seem to be especially relevant in the context of MTP. This model predicts that associated gene variants can explain a large part of the heritability of traits through the transregulation of major genes. For example, a network involving two genes encoding the GPIIb-V-IX receptor, GP1BB and GP9, and 8 other highly coexpressed genes can be mentioned. These genes are all trans-regulated by the ARHGEF3 gene, which is one of the main genes regulating megakaryopoiesis and could therefore influence the severity of thrombocytopenia as well as platelet size (48, 49).

The pathophysiological mechanisms of the impact of these mutations on megakaryopoiesis remain to be elucidated. For this, it would be necessary to analyze larger series of patients and to carry out directed mutagenesis studies. It would also be interesting to compare the proteomics of these variants with that of normal individuals, as performed by immunoblot for *GP1BA* p.Cys33Tyr by Skalníková et al. (13).

Finally, the results of this study suggest that variants affecting the *GP1BA* and *GP1BB* genes are a fairly frequent cause of MTP. It is nonetheless probably underestimated, especially since

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3 333 patients display moderate thrombocytopenia with an often mild hemorrhagic phenotype. It  
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5 334 may be proposed that a better etiological investigation of thrombocytopenias should be  
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7 335 performed in order to avoid inappropriate diagnoses that could be at the origin of useless or  
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9 336 even deleterious therapies.  
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338 Authorship statement

339 DF performed the research and analysed the data and wrote the paper, QA performed the  
340 research and contributed essential tools, BS saw the patients and performed the research, BP  
341 performed the research and contributed essential tools, BA saw the patients and performed  
342 the research, TM saw the patients and performed the research, SM saw the patients and  
343 performed the research, TC saw the patients and performed the research, DN saw the patients  
344 and performed the research, EM performed the research and contributed essential tools, GB  
345 saw the patients and performed the research, BMC analysed the data and wrote the paper,  
346 MF saw the patients, designed the research study, analysed the data and wrote the paper.

347 Conflicts of interest

348 The authors report no conflicts of interest.

349 Data availability.

350 All data generated or analysed during this study are included in this article. Further enquiries  
351 can be directed to the corresponding author

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## Tables

Table 1: Compilation of the literature about GP1BA and GP1BB heterozygous mutations in patients with thrombocytopenia including the mutations reported here.

Table 2: Clinical and biological features of the three patients with a *GP1BA* gene mutation. Normal values: platelet count: 150-450x10<sup>9</sup>/L; MPV (mean platelet volume): 7.4-11.7 fL; IPF (immature platelet fraction): 1.1-6.1%; GPIb $\alpha$  surface expression: 27000-49000 sites/platelet. AA: arachidonic acid; Col: collagen, ADP: adenosine diphosphate.

Table 3: Clinical and biological features of the six patients with a *GP1BB* gene mutation. Normal values: platelet count: 150-450x10<sup>9</sup>/L; MPV (mean platelet volume): 7.4-11.7 fL; IPF (immature platelet fraction): 1.1-6.1%; GPIb $\alpha$  surface expression: 27000-49000 sites/platelet. ND : not done.

## Figure legends

Figure 1: Ribbon drawing showing the crystal structure of the GPIb $\alpha$  N-terminal domain (PDB code 1GWB). The location of the residues related to the 3 mutations detected: p.(Leu73Phe) (L73), p. (Asn150Ser) (N150) and p.(Leu155Pro) (L155) are depicted in yellow. These 3 residues are buried and may participate in the maintenance of the structure of GPIb $\alpha$  especially via hydrogen bonds. Their mutation into phenylalanine, serine and proline respectively, may have a significant impact on the conformation of the protein and therefore on binding to its partners such as VWF or  $\alpha$ -thrombin.

Figure 2: (A) Ribbon diagram of the structure of the GPIb $\alpha$  unit (grey and pink shading) in complex with the VWF A1 domain (green) (PDB code 1SQ0). The Leu73 residue (L73) is showed in yellow in the vicinity of the  $\beta$ -finger residues (E21, V25, A26, D34), LRR1 (H53 and S55) and LRR2 (D79) of GpIb $\alpha$ , involved in interactions with VWF (dotted lines). The substitution of this residue with a phenylalanine, which is a larger residue, may impact the structure of GPIb $\alpha$  and its binding to VWF. (B) Zoom around residue Leu73 showing the interactions between VWF and GPIb $\alpha$  (pink dotted lines: hydrophobic, green dotted lines: hydrogen bond, orange dotted lines: electrostatic).

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3 521  
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5 522 Figure 3: Ribbon drawing of the GPIb $\alpha$  unit (PDB code 1GWB) in the area of residue Asn150  
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7 523 (N150, in yellow) showing its participation in the hydrogen bonding network (green dotted  
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9 524 lines). This residue is located at the end of the 5<sup>th</sup> LRR domain. Its substitution with a serine  
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11 525 which is a smaller residue may disrupt this network, weakening the native conformation.  
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14 527 Figure 4: (A) Ribbon diagram of the GPIb $\alpha$  unit complex structure (in grey and pink shading)  
15  
16 528 with  $\alpha$ -thrombin (orange) (PDB code 1P8V), showing residue Leu155 (L155, yellow) at the  
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18 529 beginning of LRR6 and the two neighbouring prolines (P156 and P157) close to  $\alpha$ -thrombin.  
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20 530 The substitution of Leu155 with a proline will result in 3 consecutive prolines which may  
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22 531 strongly constrain this area involved in the binding to  $\alpha$ -thrombin. (B) Zoom around residue  
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24 532 Leu155.  
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27 534 Figure 5: (A) Structure of the GPIb $\beta$  unit (PDB code 3RFE) showing the location of residues  
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29 535 Leu137 (L137) and Cys141 (C141) (yellow) in the C-terminal capping region. The C-terminal  
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31 536 capping region of GPIb $\beta$  is involved in the formation of the GPb-IX-V complex. Leu137 is  
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33 537 located more precisely in the  $\alpha$ 2 helix. Its substitution with a proline is likely to strongly disrupt  
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35 538 the helix structure which would certainly impact subunits assembly of the GPb-IX-V complex.  
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37 539 Cysteine 141 is positioned at the end of the C-terminal domain of GPIb $\beta$  and is engaged in a  
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39 540 disulphide bridge with cysteine 95. The p.(Cys141\*) variant would lead to a soluble form of  
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41 541 GPIb $\beta$  without the disulphide bridge with cysteine 95. The appearance of this soluble form  
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43 542 could disturb the correct formation of the GPb-IX-V complex. (B) Zoom around residues  
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45 543 Leu137 and Cys141.  
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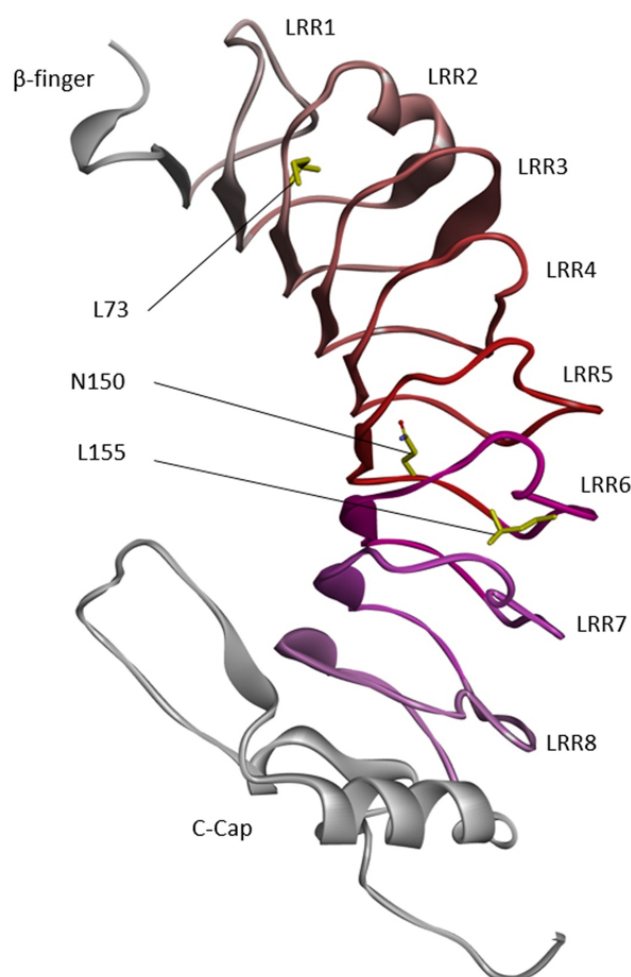


Figure 1: Ribbon drawing showing the crystal structure of the GPIIb N-terminal domain (PDB code 1GWB). The location of the residues related to the 3 mutations detected: p.(Leu73Phe) (L73), p. (Asn150Ser) (N150) and p.(Leu155Pro) (L155) are depicted in yellow. These 3 residues are buried and may participate in the maintenance of the structure of GPIIb especially via hydrogen bonds. Their mutation into phenylalanine, serine and proline respectively, may have a significant impact on the conformation of the protein and therefore on binding to its partners such as VWF or  $\alpha$ -thrombin.

159x172mm (150 x 150 DPI)

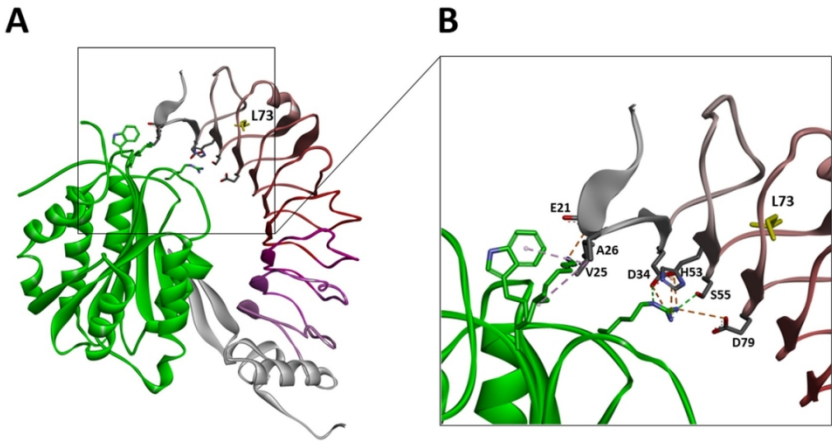


Figure 2: (A) Ribbon diagram of the structure of the GPIIb unit (grey and pink shading) in complex with the VWF A1 domain (green) (PDB code 1SQ0). The Leu73 residue (L73) is showed in yellow in the vicinity of the  $\alpha$ -finger residues (E21, V25, A26, D34), LRR1 (H53 and S55) and LRR2 (D79) of GpIb, involved in interactions with VWF (dotted lines). The substitution of this residue with a phenylalanine, which is a larger residue, may impact the structure of GPIb and its binding to VWF. (B) Zoom around residue Leu73 showing the interactions between VWF and GPIb (pink dotted lines: hydrophobic, green dotted lines: hydrogen bond, orange dotted lines: electrostatic).

230x144mm (150 x 150 DPI)



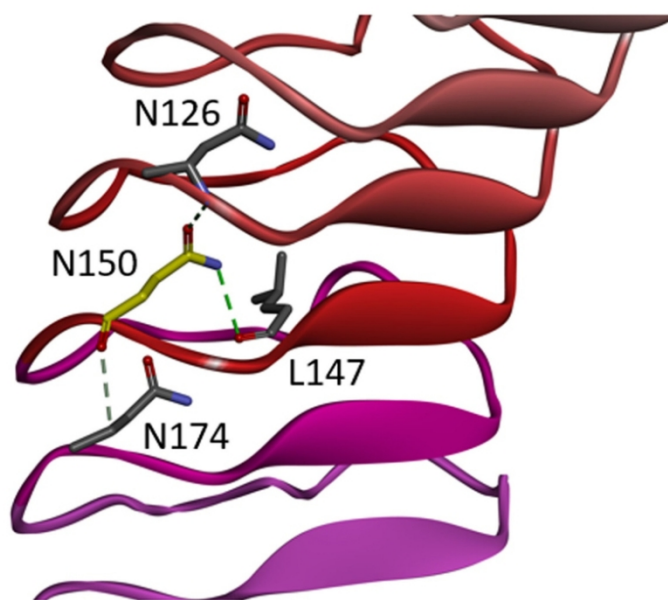


Figure 3: Ribbon drawing of the GPIIb unit (PDB code 1GWB) in the area of residue Asn150 (N150, in yellow) showing its participation in the hydrogen bonding network (green dotted lines). This residue is located at the end of the 5th LRR domain. Its substitution with a serine which is a smaller residue may disrupt this network, weakening the native conformation.

197x168mm (150 x 150 DPI)

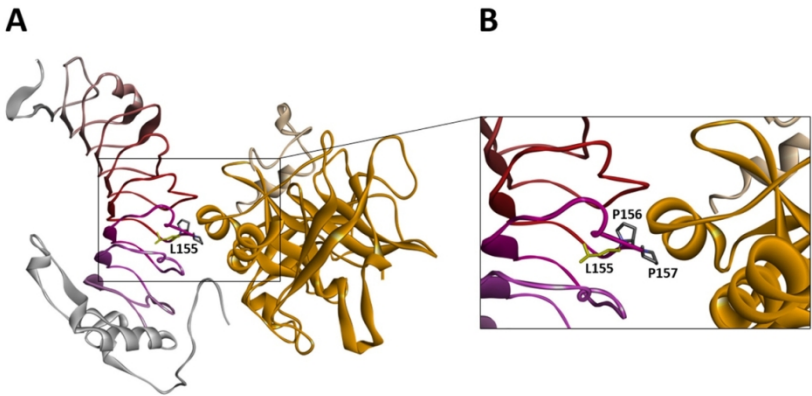


Figure 4: (A) Ribbon diagram of the GPIIb/IIIa unit complex structure (in grey and pink shading) with  $\alpha$ -thrombin (orange) (PDB code 1P8V), showing residue Leu155 (L155, yellow) at the beginning of LRR6 and the two neighbouring prolines (P156 and P157) close to  $\alpha$ -thrombin. The substitution of Leu155 with a proline will result in 3 consecutive prolines which may strongly constrain this area involved in the binding to  $\alpha$ -thrombin. (B) Zoom around residue Leu155.

240x149mm (150 x 150 DPI)

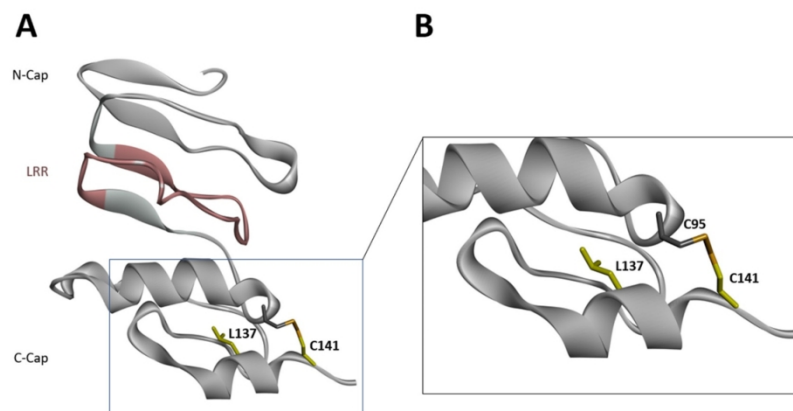


Figure 5: (A) Structure of the GPIIb/IIIa unit (PDB code 3RFE) showing the location of residues Leu137 (L137) and Cys141 (C141) (yellow) in the C-terminal capping region. The C-terminal capping region of GPIIb/IIIa is involved in the formation of the GPIIb/IIIa-IX-V complex. Leu137 is located more precisely in the  $\alpha 2$  helix. Its substitution with a proline is likely to strongly disrupt the helix structure which would certainly impact subunits assembly of the GPIIb/IIIa-IX-V complex. Cysteine 141 is positioned at the end of the C-terminal domain of GPIIb/IIIa and is engaged in a disulphide bridge with cysteine 95. The p.(Cys141\*) variant would lead to a soluble form of GPIIb/IIIa without the disulphide bridge with cysteine 95. The appearance of this soluble form could disturb the correct formation of the GPIIb/IIIa-IX-V complex. (B) Zoom around residues Leu137 and Cys141.

242x148mm (150 x 150 DPI)

Table 1.

GPIIb Protein mutation	This study	Publications
p.(Cys20Gly)		Leinøe et al, 2021 (15)
p.(Val31Leu)		Ali et al, 2017 (10)
p.(Cys33Arg)		Ma, et al. 2021 (14)
p.(Cys33Tyr)		Skalnikova et al, 2022 (13), Leinøe et al, 2021 (15)
p.(Tyr54Asp)		Kunishima et al, 2006 (6)
p.(Leu57Phe)	✓	Miller et al., 1992 (7)
p.(Asn57 His)		Vettore et al, 2008 (5)
p.(Leu59 Arg)		Trizuljak et al, 2018 (11)
p.(Tyr70Asp)		Kunishima et al., 2006 (6)
p.(Leu83Phe)		Leinøe et al, 2021 (15)
p.(Arg127 Gln)		Bury et al., 2022 (16)
p.(Asn150Ser)	✓	Ghalloussi et al., 2018 (9)
p.(Leu155Pro)	✓	NR
p.(Ala172Val)		Savoia et al., 2001 (4), Noris et al., 2012 (8)

GPIIb Protein mutation	This study	Publications
p.(Met1*)		Sivapalaratnam et al., 2017 (20)
p.(Leu16Pro)	✓	Sivapalaratnam et al., 2017 (20), Babuty et al., 2019 (21)
p.(Pro27Ser)		Busonero et al. 2020 (18)
p.(Arg42Cys)		Kunishima et al., 2001 (19)
p.(Gly43Trp)		Sivapalaratnam et al., 2017 (20)
p.(Trp46*)		Sivapalaratnam et al., 2017 (20)
p.(Leu60Pro)		Ferrari et al., 2019 (23)
p.(Thr68Met)		Sivapalaratnam et al., 2017 (20)
p.(Pro79_Leu81del)		Sivapalaratnam et al., 2017 (20), Leinoe et al, 2021 (15)
p.(Pro79_Leu81dup)		Leinøe et al, 2021 (15)
p.(Arg110fs)		Dunstan-Harrison et al., 2022 (17)
p.(Tyr113Cys)		Sivapalaratnam et al., 2017 (20), Kurokawa et al., 2001 (22)
p.(Leu132Gln)		Savoia et al., 2001 (4)
p.(Leu137Pro)	✓	NR
p.(Cys141*)	✓	NR
p.(Ala150Argfs*43)		Sivapalaratnam et al., 2017 (20)
p.(Leu168dup)	✓	NR
Exon 2 deletion	✓	NR

NR : not previously reported in heterozygous state. Of note, all protein mutations are described according to the HGVS nomenclature

Table 1

<i>GP1BA</i> Mutations	Platelet count (x10 <sup>9</sup> /L)	MPV (fL)	IPF (%)	GPIb-V-IX expression (GPIbα sites/platelet)	Platelet functions (aggregometry)	Clinical features
c.217C>T p.Leu73Phe	49	11.2	67	17 341	Not assessed	Epistaxis
c.449A>G p.Asn150Ser	76	14.2	21.7	30 315	Decreased with AA, Col, ADP	Epistaxis and gum bleeding
c.464T>C p.Leu155Pro	79	12	ND	ND	Decreased with AA, Col, ADP	No bleeding

Table 2

<i>GP1BB</i> Mutations	Platelet count (x10 <sup>9</sup> /L)	MPV (fL)	IPF (%)	GP Ib-V-IX expression (GPIbα sites/platelet)	Platelet functions (aggregometry)	Clinical features
c.410T>C p.(Leu137Pro)	98	11.8	13	25955	Decreased with ristocetin low concentration	Gum bleeding
c.2423C>A p.(Cys141*)	99	12.9	15.7	ND	ND	No bleeding
c.47T>C p.(Leu16Pro)	105	14.1	27.8	37135	Decreased with ristocetin low concentration	Epstaxis, bruising, gum bleeding, hematomas and post-surgical bleeding
c.47T>C p.(Leu16Pro)	108	14.3	22.6	22202	Decreased with ristocetin low concentration	Epistaxis, gum bleeding
c.502_504dup p.(Leu168dup)	147	13.6	21.2	ND	Normal	Bruising, epistaxis, gum bleeding ant menorrhagia
Exon 2 deletion	94	14.3	35	11174	Slightly Decreased with ristocetin low concentration	No bleeding.

## Biological, clinical features and modelization of heterozygous variants of GP1BA and GP1BB genes responsible for constitutional thrombocytopenia

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**Supplemental table 1.**

		ACMG scores			ClinVar
		Varsome	Franklin	InterVar	
<b>GP1BA</b>	p.(Leu73Phe)	4	3.9	4	4/5
	p.(Asn150Ser)	5	3.9	3	4/4
	p.(Leu155Pro)	3.5	3.1	3	fd
<b>GP1BB</b>	p.(Leu137Pro)	4	3.9	4	4
	p.(Cys141*)	4	5	4	4
	p.(Leu16Pro)	4	4.5	3	4/4
	p.(Leu168dup)	3.5	4.5	NC	fd

ACMG scores were calculated according to version 2 (with modulation of the different arguments) based on Richards et al., 2015 and other subsequent publications on the topic. These scores take into account many parameters at the variant locus including population data, literature, protein structure and function, bioinformatic predictions, segregation. ACMG scores are comprised between 1 and 5: 1: benign, 2: likely benign, 3: uncertain significance, 4: likely pathogenic, 5: pathogenic, NC: not calculated.

The three softwares used to perform these calculations can be accessed at

Varsome: <https://varsome.com/>

Franklin: <https://franklin.genoox.com/clinical-db/home>

InterVar: <https://wintervar.wglab.org/>

ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>): this database collates the ACMG scores calculated by the teams of experts over the world who have found the variant under consideration. fd: first description.