**Supplemental Material :**

**About Method**

We used a Roche Nimblegen design to establish an in-home probe panel to capture 1086 regions including exons and flanking regions of 59 genes associated with Inherited Platelet Disorders (IPD) in patients referred to the CRRP (Centre de Référence des Pathologies Plaquettaire) of Trousseau Hospital. All patients or their legal tutors gave written informed consent and the analysis was performed on DNA extracted from patients whole blood according to standard procedures.

Sequence data generated by high throughput sequencing on MiSeq System (Illumina Corp.) was mapped to the Reference Human Genome (hg19) with MiSeq integrated computer software (MiSeq Reporter, Illumina), which uses a Burrows-Wheeler-Aligner (BWA) and Genome Analysis Tool Kit (GATK) for variant-calling of single nucleotide variants (SNVs) and short insertions/deletions (InDels).

Secondary data analysis, sequence alignment and variant detection was performed with Annovar and Integrative Genomics Viewer (IGV) (Broad Institute, Cambridge, MA, USA) software. The coverage per base was ≥ 98 %. The first step consisted of a quality filter based on a Phred score >20, Quality >20 and Read coverage >30 at each position within the reads, to indicate high sequence quality. Data was then filtered according to the severity of the consequence, in order to prioritize variants leading to an amino acid change in the protein sequence (missense, nonsense, frameshift) and those in the splice site.

Apart from exceptional cases, synonymous and intronic variants were disregarded. Minor allelic frequencies (MAFs) were consulted in the Exome Variant Server, 1000 Genomes Browser and exome aggregation consortium (ExAC) databases; variants with a MAF of <0.02 were selected for further analysis. The other variants were searched for across sources, such as the dbSNP152, the Catalog of Somatic Mutations in Cancer (COSMIC), the National Center for Biotechnology Information (NCBI) ClinVar, the HGMD professional database, Varsome Genomics Community, PubMed, Online Mendelian Inheritance in Man (OMIM), and locus-specific mutation databases in an attempt to identify variants known to cause IPDs

Several *in silico* tools, Polymorphism Phenotyping v2 (PolyPhen-2), Sorting Intolerant From Tolerant (SIFT), Mutation Taster, MutationAssessor and Functional Analysis Through Hidden Markov Models (fathmm), were used to predict the functional effects and pathogenicity of the novel variants.

We followed the guidelines of the American College of Medical Genetics and Genomics and Association for Molecular Pathology, to qualify each identified variant as a “pathogenic variant” (PV), “likely pathogenic variant” (LPV) or “variant of uncertain significance”(VUS).

Sanger sequencing was used for validation and segregation analysis following standard protocols.

**About SNV and segregation :**

**The SNVs**

The propositus (I-1) harbored four SNV in the genes RUNX1 (NM\_001754), ETV6 (NM\_001987) and FLI-1 (NM\_002017).

RUNX1 carries two SNVs : c.167 T>C, p.Leu56Ser and c.335T>C p. Leu112Pro .

The first, p.L56S is a known polymorphysm ([rs111527738](https://varsome.com/variant/hg19/rs111527738)) whose frequency varied from 0.017 in European to 0.00013 in East Asian population. It is considered « likely benign according » to ACMG rules because of its frequency in healthy population and its high tolerant score (1.17) in the protein (as in Metadome Analysis , [Hum Mutat.](https://www.ncbi.nlm.nih.gov/pubmed/31116477) 2019 Aug;40(8):1030-1038.).

The latter, p.L112P is a private variant, observed in some hematological malignancy but we don’t know whether the mutation iss acquired by leukemia or congenital in origin. The ACMG scores (PM1, PM2, PP2 and PP3 in accordance with the revised ACMG version, 2017) categorize. p.L112P as a PV. Indeed, the variant p. L112P falls on the RUNT domain in a hot-spot of length 61 base-paire where 14 non-VUS coding variants have been localized (12 pathogenic and 2 benign), pathogenicity = 85.7%, proximity score 6.328 which is more than threshold 2.472). Moreover, the computational verdict is clearly « pathogenic » because of 11 pathogenic predictions from DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI, REVEL and SIFT vs no benign predictions. No publications are so far available making the proof of a role of p.L112P in the FPD/AML (Familial Platelet Disorder with Associated Acute Myeloid Malignancies, MIM n° 601399), although one should keep in mind this issue in the follow up of the patient.

The gene ETV6 carries the c.602T>C, p.Leu201Pro SNV, initially described in association with the familial thrombocytopenia THC5 (Thrombocytopenia 5 with increased risk of malignancy, MIM n° 616216). Its role in the thrombocytopenia is now conflicting by reason of its frequency in healthy population (minor allele frequency of 0.008 in the european population). According to ACMG scores (BS2, BP6, PP2, PP3) the p.L101P is also considered as « likely benign » (high frequency, ClinVar report of the variant and a rather conflicting verdict on its potential pathogenicity based on computational analysis).

Lastly, the gene FLI-1 bears a c.203G>T, p.Arg68Leu, a very rare variant with a estimated frequency in caucasian population of 0,000295. According to the ACMG classification this variant should be considered as VUS, with incertain clinical significance ; its position in the protein is moderately conserved and the the tolerance score calculate with Metadome is 1, which is quite neutral. Nevertheless, the p.R68L falls on the short NLS1 (nuclear localisation signal) sequence which could be important for a correct nuclear localization of the protein.

FLI1 gene is involved with different types of thrombocytopenia, depending on the kind of alterations. Typically, point mutations are identified in BDPT-21 (Bleedind Desorder Platelet Type 21, OMIM n°617443) and « classically » located in the ETS domain. Loss of one allele of the gene following the microdeletion of 11q24.3 chromosomal region is, on the other hand, associated with syndromic thrombocytopenias, e.g. Paris-Trousseau Syndrome or in Jacobsen Syndrome.

**Segregation analysis :**

FLI1 (exon2) c.203G>T: p.R68L

ETV6 (exon5) c.602T>C: p.L201P

**RUNX1 (exon4) c.335T>C: p.L112P**;

RUNX1 (exon4) c.167T>C: p.L56S

FLI1 (exon2) c.203G>T: p.R68L

ETV6 (exon5) c.602T>C: p.L201P

RUNX1 (exon4) c.167T>C: p.L56S

Mother

Patient

Father

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As indicated in the family plot, the propositus share three out four SNVs with her parents.

Both parents have a normal blood count, are not thrombocytopenic and don’t carry particular physical features.

Therefore, the mode of transmission and the aforementioned epidemiological and computational data, seem to exclude the involvement of ETV6 p.L201P and RUNX1 p.L56S variants transmitted by the mother. Deep functional an morphological analysis (agregrometric tests, ATPmetry, cytofluorimetry , MGG staining and electron microscopy) of mother platelets don’t revealed any significant abnormalities , suggesting a benign significance of both variants.

The FLI p.R69L variant is clearly transmitted by the father. The fathers platelet count was normal and he did not present any feature compatible with a Paris Trousseau or Jacobsen Syndrome ;

thus making the role of FLI1 in the thrombocytopenia unlikely. Again, we searched for morphological and functional alterations in order to explore a possible qualitative rather than quantitative disorder

Detailed functional an morphological analysis (agregrometric tests, ATPmetry, cytofluorimetry , MGG staining and electron microscopy) of father platelets don’t revealed significant abnormalities. Likewise he did not present any feature compatible with a Paris Trousseau or Jacobsen Syndrome.

Thus the new p.L112 RUNX1 variant, appears as the most likely candidate, associated to the platelet disorder of the patient. However, it is not clear what role RUNX1 mutation may have in the facial dysmorphism, mental retardation and ventricular septal defect.

In this regard, we did an extensive genetic analysis by High Density CGH array and WES (Whole Exome Analysis) to search for additional alteration, in particular a deletion of 11q243 region.

**Three causal mutations in one patient**

Part of the answer comes from what was said above. It is clear that the patient carries three rare variants of genes ( four with the frequent RUNX p.L59S variant) involved with platelets disorders but the database information and the familial distribution of three of them strongly suggest that those variants are not causal.

We focus on the role of a de novo mutation of RUNX1 (p. L112P) because of epidemiologic and predictive arguments. Moreover a detailed morphological analysis confirmed the presence of an alpha granules quantitative abnormality as reported in FDP/AML.

We cannot conclude on the specific role of the rare FLI1 variant in the pathology because of lack of public data and because of incomplete analysis of FLI1 locus in the patient.

Nevertheless we known that RUNX1 interacts with FLI1 by direct protein-protein interactions to synergistically activate the transcription of the MPL promoter (Ref : MOLECULAR AND CELLULAR BIOLOGY, Aug. 2009, p. 4103–4115) or synergystically downregulate MYH10 trascription (Anthony et al. Blood 2012 ; 120 (13) : 2719-2722).

The patient's clinical history indicates the existence of very deep and symptomatic thrombocytopenia, which is not very frequent in the FDP / AML patients.

In this case, the additional FLI 1 mutation could play a role and explain the clinical presentation. The endogenous TPO plasma level should be measured and MPL expression and binding evaluated.

Thus, this case illustrates how subtle and complex is to establish a causal genetic relationship with the clinical phenotype.