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Case Report
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Identification of a *JAK2* FERM Domain Variant Associated With Hereditary Thrombocytosis

Jan Müller[1], Naomi Azur Porret[2], Axel Rüfer[1]

Correspondence: Jan Müller (jan.mueller.1@luks.ch).

Hereditary thrombocytosis is a rare congenital hematologic disorder that is caused by single gene defects that affect only the megakaryocytic lineage, show polyclonal hematopoiesis and exhibit either an autosomal dominant or autosomal recessive Mendelian inheritance pattern.[1,2] A growing number of causative variants within the thrombopoietin (*THPO*) gene and the MPL proto-oncogene, thrombopoietin receptor (*MPL*) gene with different clinical characteristics have been described throughout the last decades and were reviewed by Teofili and Larocca.[2] More recently several variants within the *JAK2* gene have been described in families with hereditary thrombocytosis which are germline and located in exons encoding either the kinase or pseudo-kinase domain of *JAK2*.[3–6] Here we report a single germline variant in the N-terminal FERM domain of *JAK2* associated with hereditary thrombocytosis in a Swiss family.

The index patient, a 45-year-old woman, was evaluated for long-standing thrombocytosis with a platelet count of approximately 500 × 10^9 per liter (normal values 130-330 × 10^9 per liter). Her medical history revealed a spontaneous internal carotid artery dissection (ICAD) 2 years prior to the evaluation leading to a mild but persistent motoric aphasia. Otherwise history and physical examination were unremarkable and therefore not suggestive of any secondary cause of the thrombocytosis. On abdominal ultrasound there was no hepatosplenomegaly.

Thus, a work up with regard to a suspected myeloproliferative neoplasm (MPN) was carried out. The molecular genetic analysis in the peripheral blood was negative for *JAK2* V617F, calreticulin (*CALR*), *MPL* W515L/K driver mutations, and the *BCR-ABL1* translocation. The bone marrow examination showed a slightly enhanced but qualitatively normal megakaryopoiesis and a quantitatively and qualitatively normal myelo- and erythropoiesis. Based on those results, a MPN, in particular an essential thrombocythemia, was excluded.

A detailed family history revealed a mild thrombocytosis in both healthy daughters of the index patient and therefore the possibility of hereditary thrombocytosis was considered. After confirmation of a mild thrombocytosis in both daughters (platelet count 360 × 10^9 and 490 × 10^9 per liter, respectively), a next-generation sequencing analysis of the *JAK2*, *THPO*, and *MPL* genes was performed in the index patient. A heterozygous *JAK2* c.668T>C, p.(Ile223Thr) variant (Figure 1A), located in the N-terminal FERM domain of the *JAK2* gene, was detected (transcript: NM_004972.3, rs562010686).

This variant was subsequently confirmed with Sanger sequencing in both daughters. Based on those findings, a diagnostic work up of family members was initiated. All participants gave written informed consent. There was no history of vascular events in affected family members, except for the ICAD in the index patient. In addition to the index patient and her daughters, mild thrombocytosis was found in the mother and in all tested siblings (no data in 1 sister). Furthermore, the only child of 1 sister had a platelet count within the upper limit of the normal range (301 × 10^9 per liter). The subsequent Sanger sequencing revealed the same heterozygous *JAK2* c.668T>C, p.(Ile223Thr) variant in all tested family members (Figure 2), showing an autosomal dominant Mendelian inheritance pattern with nearly complete phenotypical penetrance.

In summary, a diagnosis of hereditary thrombocytosis associated with a heterozygous *JAK2* FERM domain variant, *JAK2* c.668T>C, p.(Ile223Thr), was made in this family.

We report a heterozygous *JAK2* c.668T>C, p.(Ile223Thr) variant associated with hereditary thrombocytosis in a Swiss family. This very rare variant has previously only been described in population studies. The minor allele frequency, as listed in the following databases, is gnomAD 0.0008%, 1000Genomes 0.02%, ExAC 0.0017%; the highest frequency is found in South Asian populations: 0.1% in 1000Genomes. To our knowledge, no clinical phenotype has been related to this variant so far. Nevertheless, a functional analysis using different bioinformatic prediction tools tends toward a damaging effect of the variant. *JAK2* c.668T is a highly conserved nucleotide (phyloP 4.81). GVGD (v2007) shows class C35, SIFT (v6.2.0): deleterious (score: 0.04), MutationTaster (v2013): disease causing (prob: 1) PolyPhen2 (v2.2.2r398): benign.

So far different single gene variants in the *THPO*, *MPL*, and *JAK2* gene loci have been described as causes of hereditary thrombocytosis.[1,2] Depending on the location, the clinical relevance in respect to thrombocytosis-associated symptoms and the risk of thromboembolic events in particular differs widely among these variants. Several high risk variants as

[1]Centre for Hematology, Department of Medicine, Cantonal Hospital Lucerne, Switzerland
[2]Hematologic Molecular Diagnostics, University Hospital for Hematology and Hematologic Central Laboratory, University Hospital Bern, Switzerland
NAP and AR contributed equally to the article and both should be considered as last authors.

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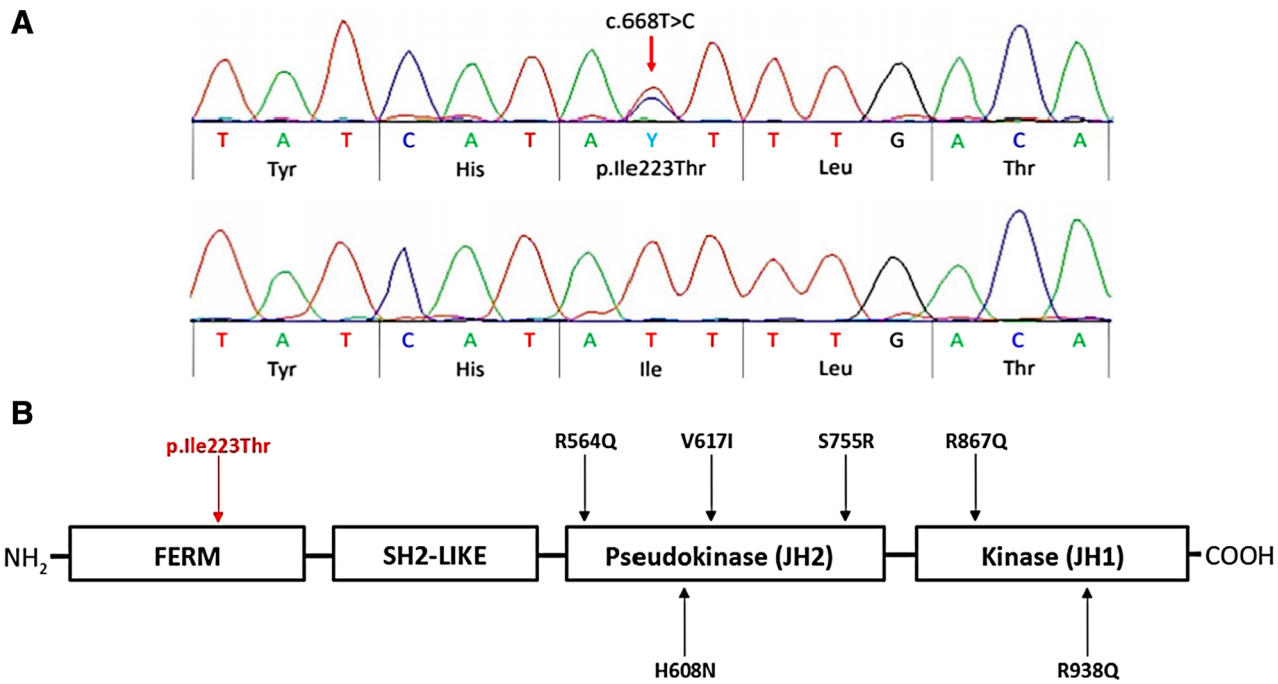

Figure 1. Sequence and location of the variant. (A) Electropherogram trace of the detected *JAK2* c.668T>C, p.(Ile223Thr) missense variant (top) in comparison to the wild-type control (bottom). (B) Locations of germline *JAK2* variants associated with hereditary thrombocytosis: black text and arrows indicating previously described variants within the kinase- and pseudokinase domain; red text and arrow indicating the novel FERM domain variant. FERM = 4.1 protein ezrin radixin moesin; SH2 = Src homology-2.

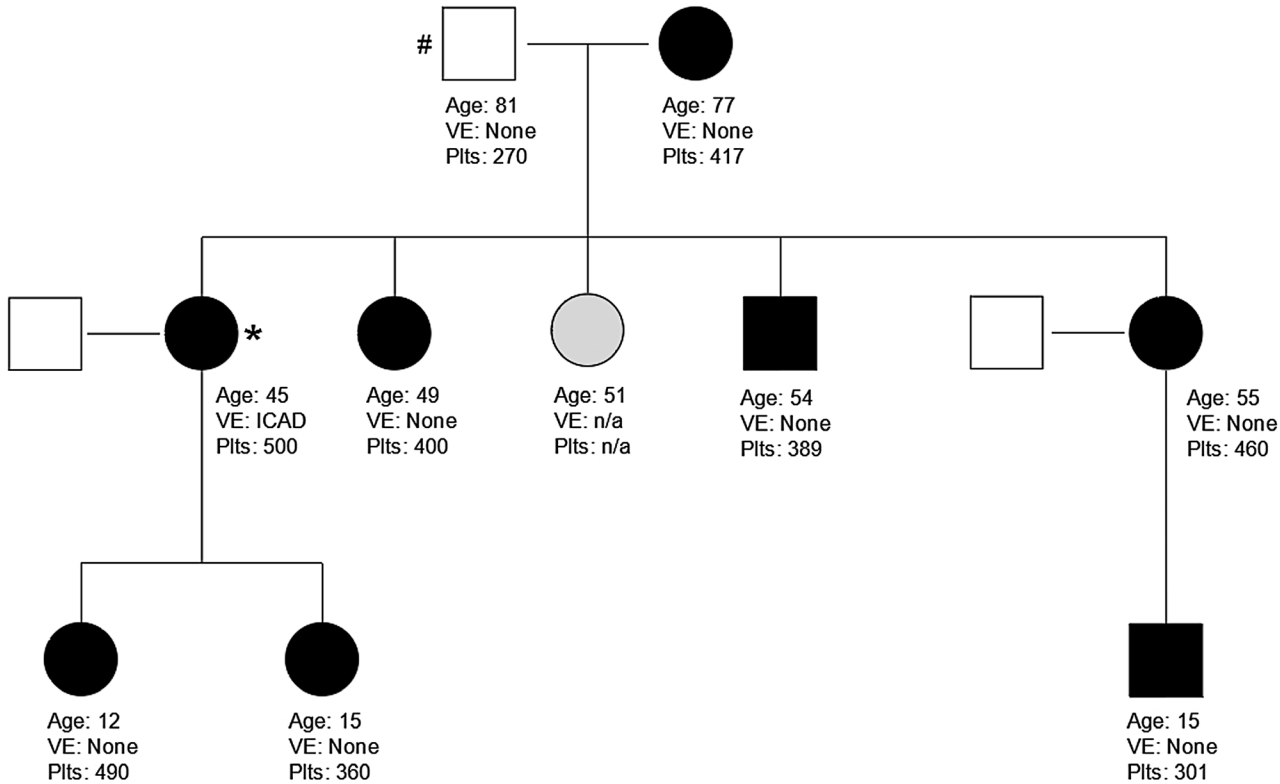

Figure 2. The JAK2 c.668T>C, p.(Ile223Thr) pedigree. Squares indicate male family members; circles indicate female family members. *Index patient. #Served as wild-type control. Black shading: family members who are *JAK2* c.668T>C, p.(Ile223Thr) positive; grey shading: family member without data. ICAD = internal carotid artery dissection; n/a = not available; Plts = platelet count ($\times 10^9$ per liter]; VE = history of vascular events.

well as clinically silent variants have been described in the *THPO*, *MPL*, and *JAK2* gene loci.[3–9] It underlines that the location of the variant within the gene locus is decisive for the clinical course and emphasizes the importance of identifying possible new causative variants in correlation to their clinical phenotype.

In respect to hereditary thrombocytosis, all causative variants within the *JAK2* gene that have been described so far are gain-of-function variants and located either in the kinase- or pseudo-kinase domain.[1,3–6]

Interestingly, this heterozygous *JAK2* c.668T>C, p.(Ile223Thr) germline variant identified in our pedigree of hereditary thrombocytosis is located in the N-terminal FERM domain of the *JAK2* gene locus and to our knowledge has never been described in the context of hematologic abnormalities before, especially not in hereditary thrombocytosis (Figure 1B).

Published data about the role of *JAK2* FERM domain variants and thrombocytosis are scarce but some in vitro data about the regulating role of the *JAK2* FERM domain in *JAK2* activation exist. Zhao et al[10] demonstrated in vitro that a deletion of the *JAK2* FERM domain results in a *JAK2* hyperactivation state in wild-type *JAK2* as well as in the presence of *JAK2* (*V617F*) mutation, albeit through entirely different mechanisms. In wild-type *JAK2*, they showed an inhibitory effect of the FERM domain, as its deletion resulted in a higher basal *JAK2* activity. In the presence of a *JAK2* (*V617F*) mutation, the deletion of the *JAK2* FERM domain leads to an increased substrate affinity and thus to a functional hyperactivation.[10]

In addition to this in vitro data, Milosevic Feenstra et al[11] described a sporadic germline *JAK2* G335D variant located in the FERM domain in a patient with triple-negative MPN but they were not able to show any functional alteration of *JAK2* activity associated with this variant. The authors speculate that this specific variant is either irrelevant or additional genetic events are necessary to exert a clinical phenotype.[11]

Eder-Azanza et al[12] identified 2 more sporadic *JAK2* FERM domain mutations (p.Y317H and p.N337D) in patients with myelofibrosis and *CALR* mutation and subsequently demonstrated in vitro that p.Y317H but not p.N337D leads to a *JAK2* gain-of-function state. Whether the FERM domain mutation alone or only the combination with the concomitant *CALR* mutation is pathogenic could not be determined in their study.[12]

More recently, Wu et al[13] showed in vitro that a germline *JAK2* F556V variant which was described in triple-negative MPNs increases *JAK2* activity through complex posttranslational mechanisms affecting the protein stability of *JAK2*. They demonstrated that F556V disrupts the structural conformation of the pseudokinase domain and the kinase/pseudokinase domain interactions, finally leading to an overactive *JAK2* protein.[13] At the moment, it remains unknown whether similar posttranslational mechanisms could contribute to functional effects of *JAK2* FERM domain mutations. Here, we report for the first time the association of a single heterozygous variant in the *JAK2* FERM domain with hereditary thrombocytosis. Thus further in vitro investigations regarding possible underlying mechanisms would certainly be worth studying.

So far there is no evidence that this variant is associated with a relevant clinical phenotype other than mild thrombocytosis or a platelet count around the upper limit of the normal range, since no relevant thrombocytosis-associated symptoms, and thromboembolic events in particular, have been reported in our pedigree. There is probably no link between the spontaneous ICAD in the index patient and the hereditary thrombocytosis. This is more likely a coincidence as no causal relationship between these 2 conditions has been published so far. With 1 exception, all affected family members are presently younger than 60 years; therefore, long-term follow-up will deliver more insight regarding the clinical relevance of this germline *JAK2* FERM domain variant associated with hereditary thrombocytosis.

Disclosures

The authors have no conflicts of interest to disclose.

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