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LETTER

Chronic myeloproliferative neoplasms

A typical acute lymphoblastic leukemia *JAK2* variant, R683G, causes an aggressive form of familial thrombocytosis when germline

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To the Editor:

There has been considerable interest in the study of inherited predisposition to myeloproliferative neoplasm (MPN) over the last two decades [1]. Despite this, distinguishing sporadic MPN from familial MPN and hereditary erythrocytosis/thrombocytosis remains a diagnostic challenge [1]. Thrombopoietin variants were the first described in cases of familial thrombocytosis [1], however variants in the thrombopoietin receptor (TPOR, also known as MPL) and Janus kinase 2 (JAK2), two of the MPN driver mutated genes, have since been identified in several families suffering from hereditary thrombocytosis [1]. Most of these patients presented with isolated thrombocytosis and an increased risk of thrombosis, with only a few cases having splenomegaly.

Here, we describe a family with a germline *JAK2* R683G variant and an aggressive form of familial thrombocytosis. Five individuals in this family presented with an MPN-like syndrome characterized by thrombocytosis and massive splenomegaly that required splenectomy in adulthood. In addition, four out of five identified patients suffered severe thrombotic events with one of the patients dying in the aftermath. Bone marrow only presented megakaryocytic hyperplasia. Figure 1 summarizes clinical and laboratory data from the family and Supplementary Fig. 1 includes bone marrow and spleen histology images of two of the patients along with description of the findings.

We performed targeted next-generation sequencing (NGS) with a panel of genes implicated in myeloid neoplasms, as previously described [2]. All affected members had the *JAK2* R683G variant with a variant allele frequency (VAF) of around 50% (Fig. 1). We also found a *TET2* Q1084P variant, but it was not present in all patients and an unaffected family member also had this variant (Fig. 1). Additionally, *TET2* Q1084P has a minor allele frequency (MAF) of 0.0026 in the ExAC database so we considered this variant to be a low frequency polymorphism.

To test if the R683G variant was germline, we performed NGS and Sanger sequencing (Supplementary Fig. 2) on peripheral blood and saliva of three of the patients, all showing the variant in both samples and similar VAFs (Fig. 1). We also performed whole exome sequencing to discard the possibility that other variants that could be responsible of the phenotype but no alternative potentially causal variants were found (Supplementary Table 1 includes all found non-synonymous and splicing variants with a

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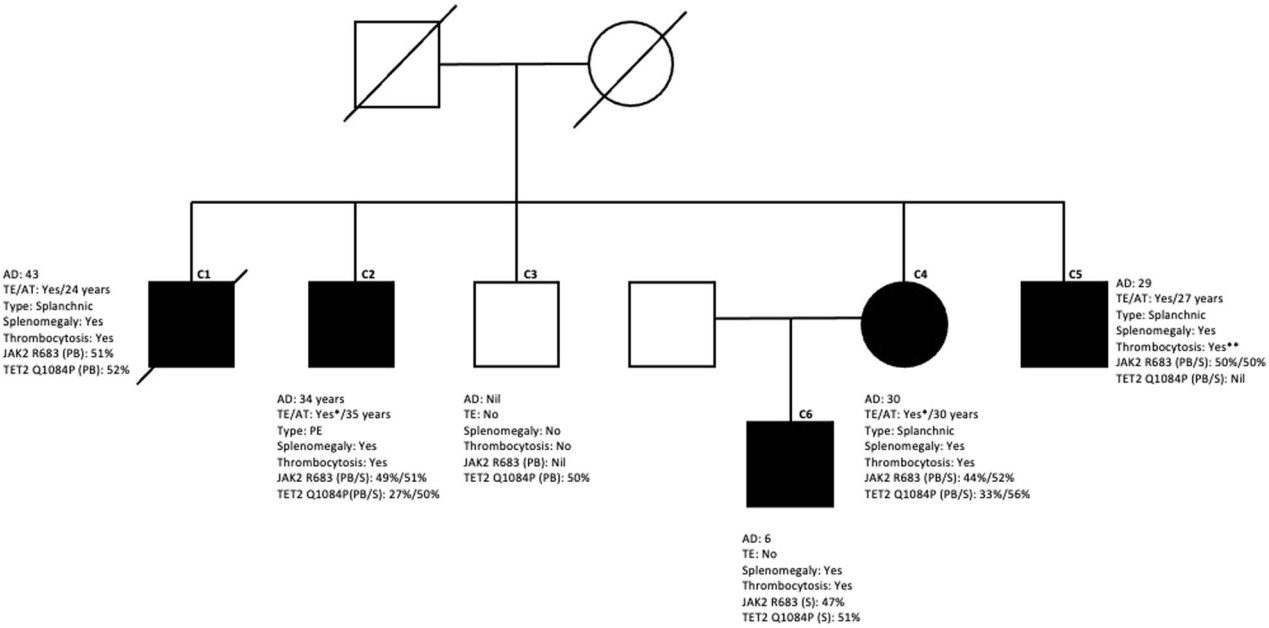

Fig. 1 Family pedigree with clinical and sequencing information of each of the family members. AD age at diagnosis of the myeloproliferative-like syndrome, TE thrombotic event, AT age at thrombosis, PE pulmonary embolism, PB peripheral blood sample, S saliva sample. % represents the variant allele frequency (VAF) of each of the samples. *Thrombosis after splenectomy. ** Thrombocytosis after splenectomy.

minor allele frequency (MAF) of <0.01 present in all affected members).

The *JAK2* R683G variant is overwhelmingly associated with B-cell malignancies, being present in ~20% of pediatric B acute lymphoblastic leukemia (B-ALL) cases with [3] and 10% of cases without [4] Down syndrome. The R683 residue is located in the pseudokinase domain of JAK2 at the hinge between the two lobes of the pseudokinase domain. This domain, where the pathogenic *JAK2* V617F variant is also located, regulates the activation of the JAK2 kinase domain. Models of JAK2 kinase and pseudokinase domains based on the X-ray crystallographic structure of the related Janus kinase, TYK2, suggest that the R683G variant impairs the inhibitory interaction between the pseudokinase and the kinase domain, resulting in constitutive activation of JAK2 and pathological cell proliferation [5].

We decided to further characterize the functional consequence of the variant, in particular to address whether cytokine receptors associated with myelopoiesis enhance the pathological activity of this JAK2 variant, as previously established with the V617F variant [6], as well as its sensitivity to JAK1/2 inhibitor ruxolitinib. For this, we performed a dual luciferase assay in HEK293T cells [7] to examine the STAT5 transcriptional activity of *JAK2* R683G mutants in the presence of either of the three homodimeric cytokine receptors required for JAK2 V617F pathogenic activity: the receptors for thrombopoietin, erythropoietin (EPOR) and granulocyte colony stimulating factor (GCSFR). As shown in Fig. 2, JAK2 R683G constitutively

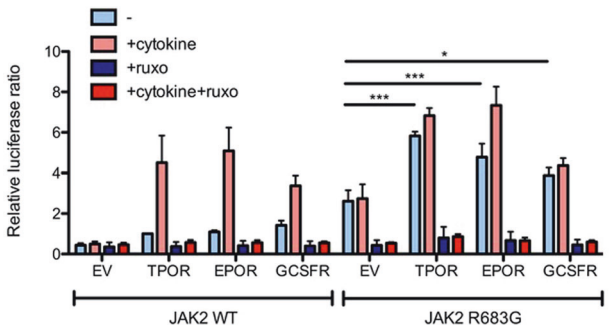

Fig. 2 Dual luciferase assay for STAT5 transcription activity of R683G mutants and its sensitivity to ruxolitinib. HEK 293T cells were transiently transfected with plasmids for the expression of JAK2 WT or R683G, together with pMX-IRES-GFP empty vector (EV) or HA-tagged human TPOR, EPOR or GCSFR, and treated or not with a cocktail of cytokines (TPO, EPREX, GCSF) and ruxolitinib. Mean ± SD is shown for $n = 3$ independent experiments performed in triplicate, normalized to relative luciferase activity with overexpression of TPOR WT and JAK2 WT in the absence of cytokine stimulation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by Student's t test.

activated STAT5 in the absence of additional cytokine receptors, but this activity increased in the presence of homodimeric cytokine receptors, reminiscent of JAK2 V617F. The largest effect was seen with co-expression of TPOR. Importantly, this was also sensitive to ruxolitinib (as described previously by others) [8]. Thus, biochemically, JAK2 R683G supports signaling by cytokine receptors other than only those expressed in the B-lineage. Further studies are needed to explain why activator mutations in the

same gene and domain of the protein are so differently distributed between different entities.

Our findings help improve our understanding of the biology of both lymphoid and myeloid neoplasms. Although initially exclusively associated with B-ALL, *JAK2* R683G has recently been described in a single case of sporadic ET, and in one case of Down syndrome-related myeloid leukemia [7, 9]. Of interest, like JAK2 V617F, JAK2 R683G requires the presence of F595 in the helix C of the pseudokinase domain in order to induce constitutive activation both in luciferase and long term proliferation assays of cell lines expressing EPOR [10]. As with JAK2 V617F, a substitution at A498 to Phe inhibits constitutive activation by *JAK2* R683G [11]. Finally, expression of *JAK2* R683G induces also basal constitutive activity via the interferon gamma receptor [11]. Taken together with the fact that none of our patients presented with ALL nor lymphoid malignancy this indicates that the variant has oncogenic potential in a range of hematopoietic lineages, in agreement with a potential to activate signaling via many receptors. The cell of origin, and which cytokine receptors are expressed, may ultimately determine the type of neoplasm for acquired variants and the type of amplified lineage for hereditary conditions, rather than a very high level of specificity of JAK mutants for one particular receptor.

The clinical implications of familial thrombocytosis are poorly understood and there is no consensus about anti-aggregation or cytoreductive therapy [1]. Our results suggest that the magnitude of JAK-STAT activation has consequences on the phenotype of the disease. For example, patients with JAK2 V617I [12], which does not induce full cytokine-independence or with R564Q [13], which has a smaller effect on cellular growth than V617F, do not present with splenomegaly. On the other hand, the germline *MPL* S505N variant, which results in cytokine-independence, does produce splenomegaly [14]. While a *JAK2* V617F variant was recently claimed to be germline and confer a predisposition to familial MPN [15], the reported allele frequency of *JAK2* V617F in peripheral blood of 15% does not support such a conclusion, and other results reported in this manuscript are instead suggestive of a *JAK2* 46/1 haplotype, which is associated with the acquisition of *JAK2* V617F and susceptibility to MPN [1]. As a consequence, our finding of a germline *JAK2* R683G, which gives cytokine-independence in vitro and results in familial thrombocytosis, is the sole example of an inherited *JAK2* variant that strongly activates signaling to drive an aggressive familial MPN.

The broad application of NGS gene panels in routine diagnosis undoubtedly continues to help us identify novel variants, but these variants need to be characterized in order to understand the molecular mechanics of MPN-like disorders and give a more nuanced approach to patient treatment. Our work provides evidence that germline *JAK2* variants present in acquired B-cell malignancies can cause familial thrombocytosis and suggests receptor preference of the *JAK2* variant may influence the disease phenotype and outcomes.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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