Unraveling the germline inheritance of the *JAK2* F556V gene mutation in familial thrombocythemia: a comprehensive analysis of 11 family members and potential implications for surveillance

Familial thrombocythemia refers to an ultra-rare inherited syndrome that is characterized by elevated platelet counts (>450x10^9/L).[1] Most commonly, thrombocytosis is clonal, driven by acquired somatic mutations in genes regulating thrombopoiesis, and often associated with myeloproliferative neoplasms (MPN) or other hematologic malignancies.[2-5] Somatic mutations are common in MPN, most notably in *JAK2* V617F, which is found in 95% of polycythemia vera and in 50-60% of essential thrombocythemia and primary myelofibrosis (PMF).[2-4] *JAK2* V617F leads to a constitutive activation of Janus kinase 2 (JAK2) involved in intracellular signal transduction, resulting in increased proliferation, differentiation and cytokine release. While the *JAK2* V617F mutation has been extensively studied in the context of sporadic MPN, understanding of germline *JAK2* mutations is less advanced.

Several families with germline *JAK2* mutations have been described, such as *JAK2* V617I, R564Q or H608N.[6-9] Here, we present *JAK2* F556V, another germline *JAK2* mutation, in a large family. This mutation has been described before only in one individual in an investigation of triple-negative MPN patients: in this comprehensive analysis of whole-exome sequencing data in 8.8% of cases a non-exon 14 *JAK2* mutation was identified. *JAK2* G571S, G335D, and V625F were classified as germline mutations, while the germline status of *JAK2* F556V remained unclear. Of note, *JAK2* F556V is a gain-of-function mutation, activating JAK-STAT5 signaling.[10]

Our case report now identifies *JAK2* F556V as a germline mutation in a family with elevated platelet counts. This adds a significant new aspect to the existing literature by elucidating the hereditary nature of *JAK2* mutations within familial thrombocythemia.

A 32-year-old woman presented to the hematology specialist practice because of elevated platelet (PLT) counts. Over several months, her PLT count consistently remained slightly above 430x10^9/L. The primary suspicion was a myeloproliferative disorder, specifically ET. Morphological evaluation was assessed in peripheral blood and subsequently bone marrow smears. The normocellular peripheral blood (white blood cell count [WBC]: 8,800/μL; hemoglobin: 13.3 g/dL; PLT: 437x10^9/L) showed mild eosinophilia (but below 1,500/μL), and no definite blasts. Thrombocytosis with anisocytosis was seen. The bone marrow was age-related normocellular and showed a slight increase in megakaryocytes. Granulopoiesis and erythropoiesis were normal and the percentage of blasts in the bone marrow was below 5%. Based on morphology, an MPN could not be confirmed, but also not be ruled out as observations could be based on reactive changes. Molecular genetic diagnostics were conducted to confirm or refute this suspicion. All patients gave their informed consent for genetic analyses and the use of laboratory results for research purposes. The study adhered to the tenets of the Declaration of Helsinki and was approved by the laboratory´s institutional review board. The following MPN driver genes were investigated in peripheral blood using polymerase chain reaction (PCR) and amplicon-based next-generation sequencing: *BCR*::*ABL1*, *JAK2* (exons 12 and 14), *CALR* (exon 9), and *MPL* (exon 10). Surprisingly, no alterations were detected in any of these typical driver genes. When blood samples were collected from other family members mild thrombocytosis was seen in some of them. Therefore, a family history of thrombocytosis was suspected. Further diagnostic steps were initiated in the bone marrow aspirate: chromosome banding analysis showed a normal female karyotype 46,XX in 20 investigated metaphases. A gene panel was analysed using capture-based enrichment library prep (Illumina DNA Prep; Illumina, San Diego, CA, USA) followed by the IDT Enrichment Workflow (IDT Integrated DNA Technologies; Coralville, IA, USA). The targeted genes included: *ASXL1*, *BCOR*, *BCORL*, *DNMT3A*, *EZH2*, *IDH1*, *IDH2*, *JAK2* (complete coding region), *MPL* (complete coding region), *SF3B1*, *SRSF2*, and *TET2*. In exon 13 of the *JAK2* gene, a variant was detected: *JAK2* chr9:5072516T>G; c.1666T>G; p.F556V, with a variant allele frequency of 46%. Classification based on public databases categorized this variant as of uncertain significance (VUS), although predicting a strong pathogenicity based on functional protein predictors (VarSome).[11] The identified variant occurs in the European (non-Finnish) population at an extremely low frequency of 0.0032% according to data from gnomAD v.2.1.1.[12] Functional studies, as reported in Wu *et al.*,[10,13] demonstrated that this variant exhibits a gain-of-function, a constitutive activation of the JAK2 kinase, similar to what is observed with the well-known V617F mutation. JAK2 contributes to thrombocytosis by promoting megakaryocyte proliferation, altering

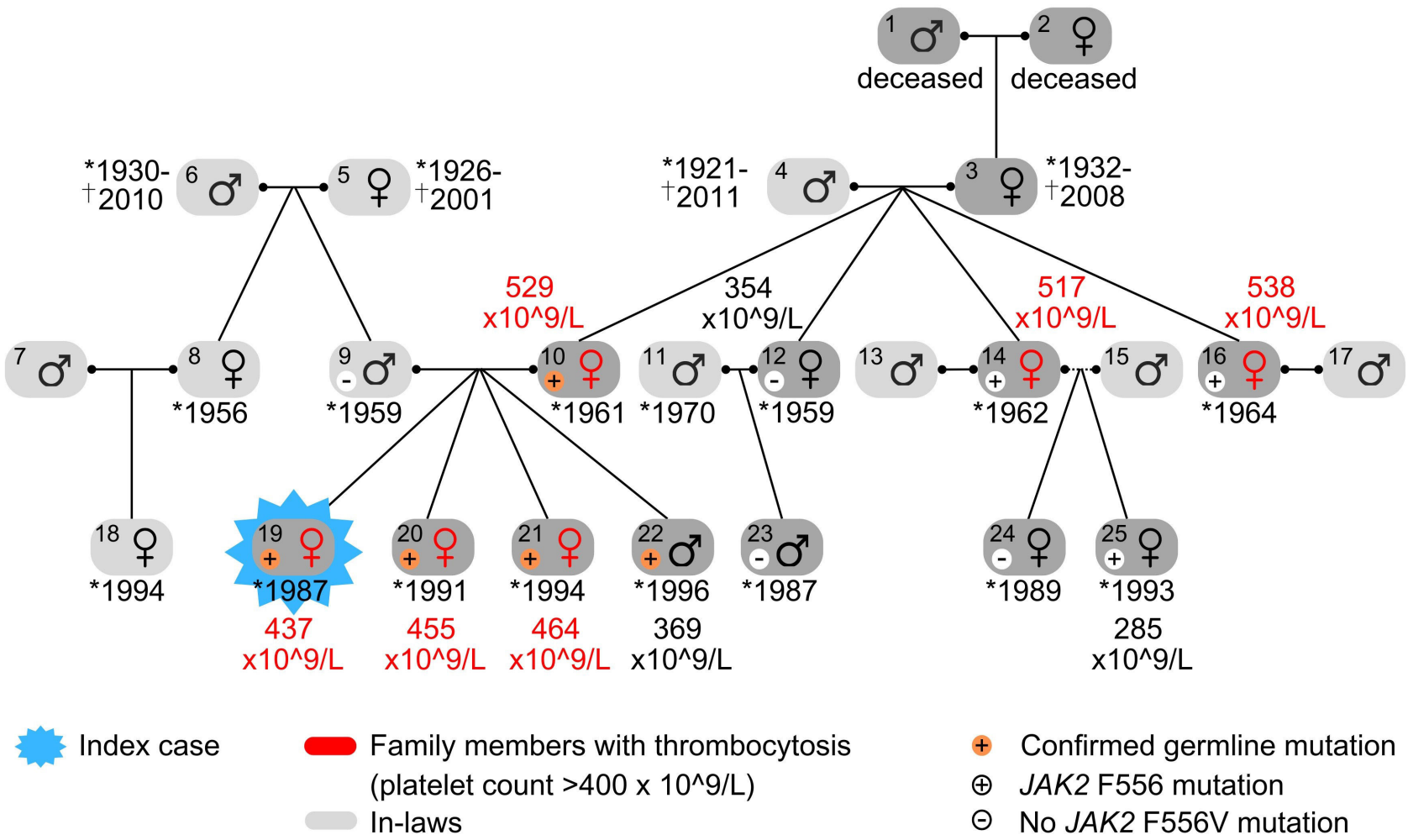

Figure 1. Pedigree of the initial patient. Initial patient marked in light blue. Red sex symbols represent patients with thrombocytosis. Thrombocytosis was based on platelet counts exceeding 400x10^9/L following standard diagnostics.[16] Platelet counts are given. +/- indicate the *JAK2* F556V alteration tested with either detection (+) or no detection (-). The orange background of *JAK2* mutation status (+/-) shows the germline confirmation by detection in buccal swap and nail clippings. Light gray boxes show married-in family members. The year of birth of the patient is indicated where known.

bone marrow dynamics, and driving platelet production through cytokine signalling and genetic mutations.[10,14] Although *JAK2* F556V has been previously identified in one MPN triple-negative patient,[10] its precise classification as either a somatically acquired mutation or a germline alteration can only be solved by testing of normal tissue/germline material that was not available in the previously mentioned study. To further investigate the possibility of germline involvement, we then performed in our family specific amplicon sequencing on samples obtained from our patient's buccal swap and nail clippings. The presence of the variant was confirmed in both tissues with approximately 50% variant allele frequency (VAF), respectively. With a potential familial explanation for the thrombocytosis now identified, we extended our investigation to include the patient's parents and three siblings. The father did not exhibit any alterations, while the mother and all three siblings showed heterozygosity for the *JAK2* F556V variant (~50% VAF) in their blood, respectively. Both the mother and siblings further confirmed the germline nature of the variant through analysis of buccal swaps and nail clippings. Subsequently, we expanded our study to other maternal family members. Among the three sisters of the mother, two of them displayed mild thrombocytosis and carried the variant in their peripheral blood. Also one of the sisters transmitted the variant to one of her two children. Overall, we detected the mutation in eight of 11 blood relatives investigated. Figure 1 depicts the family pedigree of the initial patient, illustrating the inheritance pattern of the *JAK2* F556V variant. The majority of the individuals within the affected family exhibit slightly increased thrombocyte concentrations. However, solely the index case (case 19) experienced a thromboembolic event, specifically an infarction of the middle cerebral artery. It is important to note that a direct causal linkage has not been established in this instance, and additional risk factors could potentially have influenced this outcome.

Interestingly, proband 3, the mother of three *JAK2* F556V-positive individuals had died from blast crisis (B-ALL) of a Philadelphia chromosome-positive chronic myeloid leukemia (CML). *BCR*::*ABL1* translocation can occur as a secondary event after *JAK2* V617F mutation in patients with essential thrombocythemia who develop chronic myeloid leukemia.[15] Whether this indicates an increased risk of transformation remains unclear but close follow up of the family members seems to be reasonable.

In summary, the present study shows that the *JAK2* F556V mutation can occur as a germline variant and can be passed

on to the offspring. In the present family, this heterozygous germline mutation led to thrombocytosis. Surveillance of family members because of a potential increased risk for any other blood cancer might be discussed.

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Disclosures

TH discloses part ownership of Munich Leukemia Laboratory (MLL). MM is employed by the MLL. MKB and PEP have non conflicts of interest to disclose.

Contributions

MM performed molecular genetics and data analyses and wrote the manuscript. TH was responsible for cytomorphology assessment and was the principle investigator of the study. MKB interpreted the data and wrote the manuscript. PEP provided samples and was the principle investigator of the study.

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Data-sharing statement

Original data can be provided upon request to the corresponding author.

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