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Clinical risk features and genetic findings in essential thrombocythemia and polycythemia vera of childhood and adolescence: prospective insights from the German MPN Childhood Registry

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The authors declare no relevant conflict of interest.

Author Contributions

YLB designed the research study, performed the research, analyzed the data, and wrote the paper; ThRe performed the research and analyzed the data; FF, WN, and NK analyzed the data; JB, VR, AG, ZW, and MP performed the research; MB, NDD, MH, BS, CMN, ME, AY, WS, HC, MM, and TiRi, analyzed the data and wrote the paper; AK designed the research study, performed the research, analyzed the data, and wrote the paper.

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Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

Abstract

Our understanding of essential thrombocythemia (ET) and polycythemia vera (PV) in children and adolescents is limited to retrospective analyses partly including young adults. Here, we present the largest set of prospectively acquired clinical and genetic data of pediatric patients up to the age of 18 years at diagnosis of ET and PV from the German myeloproliferative neoplasms (MPN) Childhood Registry. Forty-five patients were diagnosed with ET (n=39; 87%) or PV (n=6; 13%). Thirty-one cases (69%) had at least one established clinical adverse prognostic marker at diagnosis represented by symptoms of hyperviscosity/microcirculatory disturbance and/or splenomegaly in 22 patients (49%) each.

Twenty patients (44%) carried *JAK2*V617F (ET n=14; PV n=6), one patient (2%) showed a non-V617F *JAK2* variant of uncertain significance, eight (18%) had a pathogenic *CALR* variant (Type I/II), and 14 (31%) did not show a known somatic (likely) pathogenic driver variant in the classical gene regions of *JAK2*, *CALR*, or *MPL*. No additional cooperating non-driver variants commonly observed in adult patients with MPN were detected using targeted sequencing. However, we identified likely pathogenic germline variants in five patients without a known somatic driver variant: *JAK2*:c.2624C>A p.(Thr875Asn), *JAK2*:c.2047A>G p.(Arg683Gly), *THPO*:c.13+1G>A r.spl p.?, *FLT3*:c.2525A>G p.(Tyr842Cys), and *SAMHD1*:c.500A>C p.(His167Pro).

Neither the high prevalence of clinical risk features nor the observed enrichment of germline variants identified through targeted sequencing have been previously described in the context of pediatric MPN.

This study confirms that pediatric MPN carries a substantial early disease burden and identified potential predisposing variants with possible implications for cancer surveillance and therapy.

Keywords

Myeloproliferative neoplasms (MPN); essential thrombocythemia (ET), polycythemia vera (PV), pediatric MPN, clinical symptoms, variant profile, predisposition

Introduction

Essential thrombocythemia (ET) and polycythemia vera (PV) are chronic myeloproliferative neoplasms (MPNs) driven by clonal hematopoiesis and overproduction of platelets and erythrocytes, respectively ^{1,2}. These disease entities are almost exclusively diagnosed and therefore well defined in adult patients. Their occurrence in children and adolescents, however, is rare and remains insufficiently characterized. With very isolated exceptions ³, our current knowledge of MPN in pediatric patients is still merely based on retrospective or even historical data analyses, which in part included also young adult patients ⁴⁻⁶. As a consequence, current diagnostic and therapeutic approaches in pediatric patients are largely extrapolated from adult data despite growing evidence suggesting biological differences ⁷.

Diagnosis of ET and PV in children and adolescents can be particularly challenging. Their clinical presentation has been reported to be often subtle or incidental ^{5,8}. In addition, pediatric patients appear to exhibit a distinct molecular (likely) pathogenic variant profile, with a significant proportion of triple negative cases lacking driver variants in *JAK2* (encoding for the JAK2 kinase), *CALR* (encoding for Calreticulin R), and *MPL* (encoding for the thrombopoietin-receptor, CD110), representing the molecular hallmark of adult MPNs leading to constitutive activation of the JAK–STAT pathway ^{9,10}. At the same time, reactive thrombocytosis and secondary erythrocytosis occur much more frequently in the pediatric population, aggravating their distinction from true MPN without known genetic markers. Current World Health Organization (WHO) diagnosis criteria for MPN, developed primarily for adults, may therefore not adequately reflect pediatric disease biology ^{1,2,11}.

Notably, both conditions, ET and PV alike, are associated with significant risk of thrombotic and hemorrhagic complications also when diagnosed in childhood and adolescence ⁶. In addition, the long-term risks of progression to primary myelofibrosis or acute myeloid leukemia remain uncertain due to the scarcity of longitudinal pediatric data ^{5,6,9}. Given the long life expectancy of affected individuals, even low event rates may translate into a substantial cumulative disease burden. Current prognostic risk scores used in adult MPN to guide therapeutic decisions proved not applicable in the pediatric population, and, in young patients, novel risk factors such as hyperviscosity symptoms and splenomegaly have been established ⁶. However, further specific clinical or genetic prognostic markers, as well as a genetic predisposition underlying the comparably early onset of MPN in childhood and adolescence, remain unknown.

To meet this clear need for prospective data on pediatric patients with MPN to refine diagnostic criteria and inform age- and risk-adapted management strategies, we established the German national MPN Childhood registry.

Here, we present prospectively acquired clinical and genetic diagnostic data based on centralized review, including reference cytomorphology, histology, and genetics from this registry.

Methods

Patient population and study design

Children and adolescents aged 0-18 years diagnosed with MPN based on the current WHO/ICC classification ^{1,2} and prospectively enrolled in the German national MPN Childhood Registry between January 2023 and January 2026 were included in this study.

The German national MPN Childhood Registry is conducted in accordance with the Declaration of Helsinki, approved by the ethics board of the medical faculty Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Germany (EK_22-365-B), and registered at ClinicalTrials.gov (Identifier: NCT07410247). The infrastructure of the registry comprises centralized clinical, hematologic, morphologic, histologic, and genetic reference review.

Informed consent was obtained from the patients' legal representatives and, where applicable, from the patients, after providing age-appropriate oral and written information.

Clinical definitions

Splenomegaly was defined as palpable splenomegaly on physical examination. Acquired von Willebrand disease was diagnosed based on a reduced VWF antigen ratio and/or loss of high-molecular-weight multimers. Hyperviscosity/microcirculatory symptoms comprised headache, stomachache, dizziness, and erythromelalgia. Thrombosis was defined as imaging-confirmed arterial or venous thrombotic events, including Budd-Chiari syndrome. Pseudotumor cerebri was diagnosed based on papilledema on ophthalmoscopy in the presence of a normal cerebral magnetic resonance imaging (MRI).

Laboratory methods

All routine diagnostic analyses were performed in certified laboratories, and each diagnosis was confirmed by the German national MPN Childhood Registry.

Fluorescence R-banding (karyotyping) and fluorescence *in situ* hybridization (FISH) were conducted on cultured bone marrow aspirates or cultured peripheral blood samples, following established protocols^{12,13}. Whenever feasible, 15–25 metaphases were analyzed, and karyotypes were assigned according to the current International System for Human Chromosome Nomenclature ISCN 2024¹⁴. To exclude *BCR::ABL1*-positive malignancies, FISH analyses on interphase nuclei were conducted using a dual color fusion probe for *BCR::ABL1* (Vysis LSI *BCR::ABL1* Dual Color; Abbott, Wiesbaden, Germany). Whenever possible, 200 interphases were analyzed. The cut-off level for the *BCR::ABL1* probe was determined by analyzing 1000 interphase nuclei from 10 healthy donors (cut-off level: 2%; data not shown). (Molecular-)Cytogenetic analysis was performed in all but 11 cases (*JAK2* n=3, *CALR* n=4, triple negative n=4) (Table 1).

As part of routine diagnostics, deep targeted DNA sequencing with a custom panel was performed on DNA samples isolated from patients' bone marrow aspirates or peripheral blood according to the manufacturer's instructions (Integrated DNA Technologies, IA, USA and Illumina, San Diego, USA). The custom panel includes 142 leukemia-associated genes/gene regions (Table S1). In routine diagnostics, depending on the referring physician's request, only *JAK2* (exon 12-14), *CALR* (exon 9), and *MPL* (exon 10 and 12) were analyzed, or a broader panel (MPN panel) was evaluated. Within the present study, all 142 leukemia-associated genes/gene regions were evaluated in all patients.

The gold standard for assessing germline variants is DNA analysis of fibroblasts. As fibroblast material was unavailable, non-clonal T cells were isolated by fluorescence-activated cell sorting (FACS) after staining of bone marrow mononuclear cells with anti-human CD3 (SK7) PerCP-Cy 5.5 (BD Biosciences; Milpitas, California 95035 USA) as an alternative method. Cells were first gated based on the lymphocyte population and then sorted into CD3⁺ and CD3⁻ fractions with a purity of CD3⁺ cells exceeding 98%. DNA was isolated and germline origin of *THPO*, *FLT3*, and *SAMHD1* was confirmed by sequencing using standard protocols.

Bioinformatics and filtering strategy

The human reference genome hg38 (UCSC Genome Browser build hg38) was used for the alignment of all detected reads. The sequence data were processed using the Dragen (Illumina) and megSAP analysis pipeline (<https://github.com/imgag/megSAP>). For subsequent analyses, GSvar and Integrative Genomics Viewer (IGV) were used (<https://github.com/imgag/ngs-bits> and <http://software.broadinstitute.org/software/igv/>). Variants were classified according to the standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP)¹⁵. If available, published ClinGen Variant Curation Expert Panel specifications were applied. The present analysis focused on variants classified as likely pathogenic (class 4) or pathogenic (class 5), with one exception (variant of uncertain significance (VUS) in the *JAK2* gene).

Statistical analysis

Categorical variables are reported as n/N (%), with the denominator N reflecting the number of patients with available data for the respective parameter. Patients with missing data for a given variable are explicitly excluded from that denominator and listed in Table S2. Continuous variables are summarized as median (range). Associations between genetic subgroup and clinical features were assessed using Fisher's exact test given the small subgroup sizes, with two-sided p-values reported. A p-value <0.05 was considered statistically significant. All analyses were performed using GraphPad Prism (version 11).

Results

Patient characteristics at diagnosis of ET/PV

Between January 2023 and January 2026, 45 pediatric patients diagnosed with either ET (n=39; 87%) or PV (n=6; 13%) based on the current WHO/ICC classification (1, 2) were prospectively enrolled in the German national MPN Childhood Registry. Median age at diagnosis was 14 years (range 0-18 years). Thirty patients (67%) were female, and 15 (33%) were male. Demographic patient data are listed in Table 1, Table S2 and Figure 1A.

All triple-negative cases presented with significant, otherwise unexplained thrombocytosis. Reference bone marrow morphology analyses were performed in 41 out of the 45 cases (91%), including all but one case with triple-negative ET and a bone marrow trephine was centrally reviewed in 37 patients (82%). In all cases, morphological and histological findings were consistent with the diagnostic criteria of the current WHO/ICC classification^{1,2}. In six patients (13%), a mild myelofibrosis (MF-1) was described at diagnosis. All of these individuals were diagnosed with ET, three were positive for *JAK2V617F*, two for *CALR* and one was triple-negative.

Overall, twenty-two individuals (49%) showed at least one disease-associated clinical symptom (headache, pseudo-tumor cerebri, stomachache, erythromelalgia, dizziness, thrombosis, Budd-Chiari syndrome, and bleeding) at the time of diagnosis, comprising 19 of the 39 cases with ET and three of the six patients with PV with pronounced headache being most prominent in 17 individuals (38%; ET (n=15), PV (n=2)) (Table 1 and S2, Figure 1C). Three of these patients presented with papilledema on ophthalmoscopy, while magnetic resonance imaging showed no cerebral abnormalities. All these three patients with pseudo-tumor cerebri (#13, #17, #22), presenting at ages 9, 11, and 13 years, were female, diagnosed with ET, and *JAK2V617F*-positive. Two of the three also had splenomegaly, and two additionally presented with a concurrent thrombotic event. In two of the three cases, antiproliferative treatment with interferon was initiated. Four patients (9%) had abdominal pain (ET (n=3), PV (n=1)). Thrombotic events were reported in six cases (13%), including two with Budd-Chiari syndrome (4%). Among these patients, five were female, ET was diagnosed in 5 cases and PV in 1, and all patients were positive for *JAK2V617F*. In every case, the thrombotic event represented the initial clinical manifestation. An acquired von Willebrand disease was reported in 16 individuals (36%), 14 cases with ET and two cases with PV. Overall, palpable splenomegaly was present in 22 cases (49%), including 17 of the 39 patients with ET and five of the six patients with PV. Of 20 *JAK2V617F*-positive cases, 15 (75%) had splenomegaly, whereas 2 of 3 (67%) *JAK2* other cases, 3 of 8 (38%) *CALR*-positive cases and 2 of 14 (14%) triple-negative cases had splenomegaly (Table 1 and S2, Figure 1B and 2). Median platelet count in patients with ET was 1082 G/L and median leukocyte count in this group was 9.9 G/L. In patients with PV, the median hemoglobin level was 17.0 g/dl, the median platelet count 821 G/L, and the median leukocyte count 11.3 G/L (Table 1 and Figure 2). *JAK2V617F*-positive MPN patients exhibited a significantly higher prevalence of splenomegaly compared with triple-negative MPN patients (15/20 vs. 2/14; p = 0.0069). In contrast, acquired von Willebrand disease

(aVWD) was significantly more frequent among triple-negative MPN patients than among *JAK2*V617F-positive patients (7/14 vs. 5/20; $p = 0.0161$).

In 23 cases (51%), treatment was initiated after diagnosis. Interferon was applied in ten of these cases, combined with acetylsalicylic acid (ASA) in three individuals. Hydroxyurea was given to three patients, in one case combined with ASA. Ruxolitinib and anagrelide were used in one patient each. ASA alone without antiproliferative treatment was started in eight cases (Table 2 and Figure 3).

Genetic variant profile at diagnosis

Deep targeted DNA sequencing comprising 142 leukemia-associated genes/gene regions was performed in all 45 patients of this cohort. Centralized analyses for somatic variants in the classical driver gene regions (*JAK2* (exon 12-14), *CALR* (exon 9), and *MPL* (exon 10)) showed that of the 39 individuals with ET, 14 (36%) were *JAK2*V617F positive and one patient carried a *JAK2* other variant, classified as VUS with an allele fraction (VAF) of 32% (c.2155G>T p.(Val719Leu)). In addition, eight ET patients (18%) had a pathogenic variant in the *CALR* gene ($n=6$ with a type I and $n=2$ with a type II *CALR* variant). Fourteen patients (31%), all with ET, did not show a (likely) pathogenic variant in the aforementioned gene regions. All six patients with PV carried the *JAK2*V617F variant. No (likely) pathogenic variant in the *MPL* gene was detected in our cohort of pediatric MPN patients (Figure 3).

Analysis of the entire panel of 142 leukemia-associated genes/gene regions for additional somatic variants revealed only one pathogenic variant in the *FANCL* gene in a *JAK2* V617F-positive patient with PV (#11), with a low variant allele fraction (VAF) of 4%.

Five of the 45 patients (11%), all of whom had an ET phenotype and were negative for known (likely) pathogenic somatic variants in the classical driver gene regions, showed additional likely pathogenic variants, all subsequently confirmed to be of germline origin. These comprised *JAK2*:c.2624C>A p.(Thr875Asn), *JAK2*:c.2047A>G p.(Arg683Gly), *THPO*:c.13+1G>A r.spl p.?, *FLT3*:c.2525A>G p.(Tyr842Cys), and *SAMHD1*:c.500A>C p.(His167Pro). For both patients (#7, #23) with a likely pathogenic *JAK2* variant, a germline origin of the variant was confirmed by segregation and/or confirmation in finger nails and CD8+ cells. For patients #26, #27, and #32 with a likely pathogenic variant in *THPO*, *FLT3*, and *SAMHD1*, respectively, germline origin was confirmed by resequencing of DNA from sorted non-clonal T cells. These findings were in line with a VAF between 46 and 48% and previous descriptions in the literature¹⁶⁻¹⁸ (Table 3 and Figure 3).

The first of these patients (#7), a 16-year-old female, presented with persistent thrombocytosis. Her mother had developed Budd-Chiari syndrome at the age of 18 years. The sister underwent a liver transplant at the age of 5 years due to suspected Budd-Chiari syndrome. We detected the likely pathogenic variant in NM_004972.4(*JAK2*):c.2624C>A p.(Thr875Asn) with a VAF of 50%. The same variant was detected in the mother and the sister.

A male patient (#23), 4 years of age, also had an elevated platelet count. His grandmother had been diagnosed with MPN in older adulthood and his cousin, like the patient, in early childhood. Sequencing identified the likely pathogenic variant NM_004972.4(*JAK2*):c.2047A>G p.(Arg683Gly) with a VAF of 46%. This variant was also detected in the DNA of proband's #23 finger nails and CD8+ cells, confirming the germline origin of this variant in the enrolled patient.

Another patient (#26) presented at the age of only two years with marked thrombocytosis. The boy was asymptomatic and had an unremarkable family history. Central reference bone marrow diagnostics confirmed morphologic and histological criteria consistent with MPN according to WHO/ICC classification, initially distinguishing this case from non-clonal hereditary thrombocytosis. The likely pathogenic variant NM_000460.4(*THPO*):c.13+1G>A r.spl p.? associated with hereditary thrombocytosis was identified¹⁶.

In a 16-year-old girl (#27) initially showing headache and bleeding signs with a platelet count of 3083 G/L, deep targeted DNA panel sequencing uncovered the likely pathogenic variant NM_004119.3(*FLT3*):c.2525A>G p.(Tyr842Cys). The patient's family history was unremarkable regarding MPN.

In a further female patient (#32) aged 17 years and diagnosed with ET after having presented with persistent thrombocytosis and von Willebrand disease without further clinical abnormalities, the likely pathogenic variant NM_015474.4(*SAMHD1*):c.500A>C p.(His167Pro) was detected. No other MPN was reported in the family history.

The proportional distribution of pathogenic somatic driver variants in the genes *JAK2* and *CALR*, triple-negative patients, as well as additional pathogenic somatic and likely pathogenic germline variants, including their allele fractions, is illustrated in Figure 3 and listed in Table 3.

Classical (molecular-)cytogenetic analyses were performed in 34 of the 45 patients (76%). In one patient, a most likely constitutional inversion 10, inv(10)(p13q22), was detected in all metaphases which is not associated with MPN. All other determinations yielded unremarkable results.

Discussion

This analysis describes initial clinical and genetic characteristics in a pediatric cohort comprising 45 patients below the age of 18 years who were diagnosed with ET or PV and consecutively enrolled in the German MPN Childhood Registry. It is the first report based on prospectively acquired data through central reference diagnostics. Two key observations of our study should be highlighted. The first affects the substantial early disease burden of pediatric MPN reflected by the high proportion of patients presenting with symptoms of hyperviscosity/microcirculatory disturbance and/or splenomegaly. The second represents the identification of likely pathogenic germline variants in various genes including *FLT3* and *SAMHD1*, which had not previously been reported in association with pediatric MPN. These aspects are discussed below within the overall context.

Nearly half of the patients were symptomatic at the time of diagnosis and over forty percent presented with symptoms of hyperviscosity or microcirculation disturbance, respectively, mostly reflected by severe headache. We found an association between the diagnosis of MPN and pseudotumor cerebri in at least three cases. This phenomenon has been described previously in pediatric patients with chronic myeloid leukemia¹⁹. The clustering of highly symptomatic cases with pseudotumor cerebri within the *JAK2*V617F-positive ET subgroup in this cohort supports a proposed underlying pathogenic mechanism of increased cerebrospinal fluid outflow resistance secondary to hyperviscosity/microcirculatory disturbance. In the context of MPNs, to our knowledge, this association has not been reported before. Severe thrombotic events, including Budd-Chiari syndrome, had led to the diagnosis of MPN in six patients (13%). A remarkably high proportion of 49% of individuals in this pediatric cohort had splenomegaly. Hyperviscosity or microcirculation disturbance have been identified as significant risk factors for the development of thrombosis and splenomegaly as a risk factor for disease progression and bleeding in young patients with MPN below the age of 25 years by Sobas et al.⁶. As a central novel finding of our study, it can thus be concluded that a majority of 31 of the 45 pediatric patients (69%) in our cohort had at least one established adverse prognostic marker already at the time of diagnosis.

Taken together, these results underline that MPNs occurring in childhood and adolescence represent serious diseases and warrant close clinical surveillance. Antiproliferative therapy (interferon, hydroxyurea, ruxolitinib, or anagrelide) and/or aspirin were initiated after diagnosis in 23 of 45 patients (51%) guided primarily by the presence of disease-associated risk features (symptoms of hyperviscosity/microcirculatory disturbance, marked splenomegaly, or thrombosis), consistent with the absence of validated pediatric risk-stratification tools. Whether

earlier or more consistent therapeutic intervention would improve outcomes will require prospective follow-up data.

Cytogenetic analyses performed in 34 patients (76%) revealed no disease-associated abnormalities at diagnosis, suggesting a subordinate role of cytogenetic aberrations in MPN of children and adolescents. However, larger case series and longitudinal data are required for final conclusions.

Analyses concerning pathogenic somatic driver variants revealed that with 20 patients (44%), the largest subgroup including all patients with PV in this cohort carried the *JAK2*V617F variant, and eight individuals (18%) had a pathogenic variant in the *CALR* gene (six type I and two type II variants), all with an ET phenotype. No patient in this cohort carried a (likely) pathogenic variant in the *MPL* gene. This is in accordance with earlier studies showing a considerably lower proportion also of pathogenetic somatic *MPL* variants in young compared to older patients with MPN^{6,9,10}.

Fourteen cases (31%) were triple-negative for (likely) pathogenic variants in the classical driver genes in *JAK2*, *CALR*, and *MPL*, all of which were classified as ET. This latter subgroup of patients appears to be smaller than previously reported for other pediatric cohorts, with up to more than 70% of triple-negative cases^{8,9,20}. This could at least in part be due to the full spectrum of centralized reference diagnostics provided by the registry stringently excluding secondary reactive cases and emphasizing the significance of bone marrow diagnostics, particularly in triple-negative MPN. In this pediatric cohort, no correlation between age and pathogenic variant group was observed. The *JAK2*V617F alteration was associated with splenomegaly as an adverse prognostic factor in 15 out of the 20 cases (75%), whereas two out of the 14 triple-negative individuals (14%) had a splenomegaly ($p=0.0069$). Thrombotic events occurred only in patients carrying the somatic *JAK2*V617F variant, supporting previous observations that this variant represents a major risk factor for thrombosis also in young patients⁵.

In line with preceding studies, deep targeted DNA panel sequencing of 142 leukemia-associated gene regions identified a low number of additional (likely) pathogenic somatic variants^{10,20}, and only one *JAK2*V617F-positive patient carried a small clone with a pathogenic somatic variant in *FANCL*. In addition, we identified the somatic *JAK2*:c.2155G>T p.(Val719Leu) with a VAF of 32% in another case negative for *JAK2*V617F and *CALR*, respectively. This alteration, however, was classified as a VUS. None of the pediatric patients in this cohort exhibited clonal driver mutations in *TET2* or *DNMT3A*, nor mutations in *ASXL1*, *EZH2*, *IDH1/2*, *SRSF2*, *SF3B1*, *U2AF1*, *NRAS*, *KRAS*, or *TP53* as described in adult patients diagnosed with MPN^{21,22}. This observation again indicates a distinct underlying genetic landscape of MPN in pediatric compared to adult patients.

Deep targeted sequencing in this study identified additional likely pathogenic variants in five patients (11%) of the cohort, all of whom with an ET phenotype and negative for the classical somatic driver variant *JAK2*V617F or a *CALR* variant. These variants were located in *JAK2* (NM_004972.4(*JAK2*):c.2624C>A p.(Thr875Asn) and NM_004972.4(*JAK2*):c.2047A>G p.(Arg683Gly)) in two cases and in *THPO* (NM_000460.4(*THPO*):c.13+1G>A p(?)), *FLT3* (NM_004119.3(*FLT3*):c.2525A>G p.(Tyr842Cys)), and *SAMHD1* (NM_015474.4(*SAMHD1*):c.500A>C p.(His167Pro)) in one case each (Table 2). A germline origin was confirmed for all five variants in *JAK2*, *THPO*, *FLT3*, and *SAMHD1* through resequencing of DNA from finger nails or highly purified non-clonal T cells or through segregation analysis, consistent with their VAF between 46 and 50% and previous reports in the literature¹⁶⁻¹⁸. These latter genetic findings represent the second key aspect of our study including likely pathogenic germline variants in *FLT3* and *SAMHD1* in pediatric MPN, expanding the known genetic landscape beyond the previously described germline *JAK2* and *THPO* variants. These findings further emphasize the importance of extended molecular analyses in pediatric MPN.

Previously described additional pathogenic variants in young patients with MPN were predominantly of germline origin and affecting distinct loci of the *JAK2* gene²³⁻²⁹. In this pediatric cohort, two patients with a family history for MPN carried a likely pathogenic germline *JAK2* resulting in the amino acids changes p.(Thr875Asn) and p.(Arg683Gly), respectively. The *JAK2* p.(Thr875Asn) had been linked earlier to essential thrombocythemia³⁰. Pathogenic germline variants in young patients with MPN have also been found in the genes *THPO*, *VHL*, *EPOR*, *EPAS1*, and *EGLN1*³¹⁻³³. One patient in the present analysis also harbored a likely pathogenic germline variant in *THPO* as an indication of an underlying hereditary thrombocythemia¹⁶. Data on somatic as well as germline genetic variants outside of the aforementioned genes, their classification, and their functional/prognostic role are sparse for young patients with MPN^{20,34-36}. Therefore, it appears of particular interest, that another individual in this cohort carried a likely pathogenic *FLT3* variant. Lately, this alteration has been identified as a novel germline driver in acute myeloid leukemia and was thus associated with leukemia predisposition¹⁷. The close association of *FLT3* with different signaling pathways involved in proliferation and/or inhibition of apoptosis renders this gene an attractive therapeutic candidate, and like pathogenic somatic *FLT3* variants, such *FLT3* germline alterations could also become the potential target of approved therapy with tyrosine kinase inhibitors (Figure 4). Additionally, one patient showed a likely pathogenic *SAMHD1* variant. This gene has just recently come into focus in cancer research and is meanwhile considered a tumor suppressor gene¹⁸. This alteration could also be of particular interest, as it has been found that depletion and/or dysfunction of *SAMHD1* impairs DNA damage response, increasing susceptibility to the targeted inhibition of poly-(adenosine 5'-diphosphate ribose) polymerase (PARP)³⁷ and chemotherapy, enhancing antitumor responses³⁸ (Figure 4). These findings, however, may warrant future functional investigations. The identification and future characterization of potential underlying hereditary tumor predisposition syndromes in pediatric MPN might be of major relevance for patients and families concerning possible cancer surveillance and therapeutic options. Notably, no pathogenic variants in *SH2B3* were detected in our analysis, suggesting at least a low proportion of *SH2B3* disorders underlying the MPN phenotypes as described here³⁹. Figure 4 illustrates the interconnectivity of novel variants identified in this study within the JAK-STAT pathway.

The additional genetic alterations described here have been identified using a targeted leukemia gene panel only, and for example, pathogenic variants in DNA repair genes, including *BRCA1/2*, which have been observed in familial MPN⁴⁰, were not included. One might therefore speculate that more comprehensive sequencing approaches could detect additional pathogenic somatic as well as germline variants in a significantly higher proportion of pediatric MPN.

Together, our findings show that pediatric MPN presents with a clinically and genetically distinct, symptom-rich phenotype at diagnosis that warrants dedicated pediatric risk-stratification and follow-up strategies.

As a limitation of this study, it needs to be emphasized that it focused on clinical and genetic characteristics and resulting therapeutic interventions at the time of diagnosis, and, given the chronic trajectory of these diseases and the limited maximum 3-year follow-up available since the establishment of the registry, could not yet consider longitudinal outcomes, which will be subject to separate future analyses.

The results of this study underline the given clinical and scientific need for national and joint international efforts to further characterize MPN in children and adolescents.

Appendix

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References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
2. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *Am J Hematol*. 2020;95(12):1599-1613.
3. Cario H, Schwarz K, Herter JM, et al. Clinical and molecular characterisation of a prospectively collected cohort of children and adolescents with polycythemia vera. *Br J Haematol*. 2008;142(4):622-626.
4. Giona F, Teofili L, Moleti ML, et al. Thrombocythemia and polycythemia in patients younger than 20 years at diagnosis: clinical and biologic features, treatment, and long-term outcome. *Blood*. 2012;119(10):2219-2227.
5. Ianotto JC, Curto-Garcia N, Lauermanova M, Radia D, Kiladjian JJ, Harrison CN. Characteristics and outcomes of patients with essential thrombocythemia or polycythemia vera diagnosed before 20 years of age: a systematic review. *Haematologica*. 2019;104(8):1580-1588.
6. Sobas M, Kiladjian JJ, Beauverd Y, et al. Real-world study of children and young adults with myeloproliferative neoplasms: identifying risks and unmet needs. *Blood Adv*. 2022;6(17):5171-5183.
7. Kucine N. Myeloproliferative neoplasms in children, adolescents, and young adults. *Curr Hematol Malig Rep*. 2020;15(2):141-148.
8. Kucine N, Chastain KM, Mahler MB, Bussel JB. Primary thrombocytosis in children. *Haematologica*. 2014;99(4):620-628.
9. Randi ML, Geranio G, Bertozzi I, et al. Are all cases of paediatric essential thrombocythaemia really myeloproliferative neoplasms? Analysis of a large cohort. *Br J Haematol*. 2015;169(4):584-589.
10. Lee JW, Jo S, Yoo JW, Kim S, Chung NG, Cho B. Absence of canonical mutations in pediatric essential thrombocytosis: a case series. *Blood Res*. 2024;59(1):32.
11. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia*. 2022;36(7):1703-1719.
12. Schlegelberger B, Zwingers T, Harder L, et al. Clinicopathogenetic significance of chromosomal abnormalities in patients with blastic peripheral B-cell lymphoma. Kiel-Wien-Lymphoma Study Group. *Blood*. 1999;94(9):3114-3120.
13. Schlegelberger BMS, Harder S, Zühlke-Jenisch R, Zhang Y, Siebert R. Classical and molecular cytogenetics of tumor cells. In: Wegner R-D, ed. *Diagnostic Cytogenetics*. Berlin (DE): Springer Berlin Heidelberg; 1999. pp. 151-185.
14. Hastings RJ, Moore S, Chia N. *ISCN 2024: An International System for Human Cytogenomic Nomenclature*. Basel (SW): S.Karger AG; 2024.
15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
16. Kimura H, Onozawa M, Matsukawa T, Goto H, Kondo T, Teshima T. Relative impact of THPO mutation causing hereditary thrombocythemia. *Exp Hematol*. 2024;134:104208.
17. Swaroop A, Johnson NL, Tophkhane MC, et al. FLT3 as a germ line driver in AML: expanding the landscape of leukemia predisposition. *Blood Neoplasia*. 2026;3(1):100197.
18. Schott K, Majer C, Bulashevskaya A, et al. SAMHD1 in cancer: curse or cure? *J Mol Med (Berl)*. 2022;100(3):351-372.
19. Rasmussen AH, Nguyen NT, Molander LD, Rathe M. Secondary pseudotumor cerebri syndrome in a child with chronic myeloid leukemia: report of a case and review of previous cases. *Pediatr Blood Cancer*. 2023;70(2):e29913.
20. Karow A, Nienhold R, Lundberg P, et al. Mutational profile of childhood myeloproliferative neoplasms. *Leukemia*. 2015;29(12):2407-2409.
21. Lundberg P, Karow A, Nienhold R, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood*. 2014;123(14):2220-2228.
22. Luque Paz D, Kralovics R, Skoda RC. Genetic basis and molecular profiling in myeloproliferative

neoplasms. *Blood*. 2023;141(16):1909-1921.

23. Mead AJ, Rugless MJ, Jacobsen SE, Schuh A. Germline JAK2 mutation in a family with hereditary thrombocytosis. *N Engl J Med*. 2012;366(10):967-969.

24. Etheridge SL, Cosgrove ME, Sangkhae V, et al. A novel activating, germline JAK2 mutation, JAK2R564Q, causes familial essential thrombocytosis. *Blood*. 2014;123(7):1059-1068.

25. Kapralova K, Horvathova M, Pecquet C, et al. Cooperation of germ line JAK2 mutations E846D and R1063H in hereditary erythrocytosis with megakaryocytic atypia. *Blood*. 2016;128(10):1418-1423.

26. Aral B, Courtois M, Ragot S, et al. Germline JAK2 L611S mutation in a child with thrombocytosis. *Haematologica*. 2018;103(8):e372-e373.

27. Dvoracek L, Markova J, Holoubek A, et al. A novel germline hyperactivating JAK2 mutation L604F. *Ann Hematol*. 2023;102(10):2725-2734.

28. Song J, Lanikova L, Kim SJ, et al. Novel germline JAK2(R715T) mutation causing PV-like erythrocytosis in 3 generations. Amelioration by Ropog-Interferon. *Am J Hematol*. 2024;99(7):1220-1229.

29. Zimolova V, Burocchiova M, Berkova L, et al. Germline Jak2-R1063H mutation interferes with normal hematopoietic development and increases risk of thrombosis and leukemic transformation. *Leukemia*. 2025;39(11):2745-2757.

30. Yoshimitsu M, Hachiman M, Uchida Y, et al. Essential thrombocytosis attributed to JAK2-T875N germline mutation. *Int J Hematol*. 2019;110(5):584-590.

31. Jones AV, Cross NC. Inherited predisposition to myeloproliferative neoplasms. *Ther Adv Hematol*. 2013;4(4):237-253.

32. Rumi E, Cazzola M. Advances in understanding the pathogenesis of familial myeloproliferative neoplasms. *Br J Haematol*. 2017;178(5):689-698.

33. McMullin MF. Diagnostic workflow for hereditary erythrocytosis and thrombocytosis. *Hematology Am Soc Hematol Educ Program*. 2019;2019(1):391-396.

34. Fu R, Liu D, Cao Z, et al. Distinct molecular abnormalities underlie unique clinical features of essential thrombocythemia in children. *Leukemia*. 2016;30(3):746-749.

35. England JT, Szuber N, Sirhan S, et al. Clinical features and long-term outcomes of a pan-canadian cohort of adolescents and young adults with myeloproliferative neoplasms: a Canadian MPN Group Study. *Leukemia*. 2024;38(3):570-578.

36. Fu R, Dong H, Gu W, et al. Childhood and adolescent essential thrombocythemia and prefibrotic primary myelofibrosis: insights into diagnosis, outcomes, and treatment from a large Chinese cohort. *Leukemia*. 2025;39(1):155-165.

37. Rodriguez-Sanchez A, Quijada-Alamo M, Perez-Carretero C, et al. SAMHD1 dysfunction impairs DNA damage response and increases sensitivity to PARP inhibition in chronic lymphocytic leukemia. *Sci Rep*. 2025;15(1):10446.

38. Sun J, Zheng W, Zhang ZG, et al. Selective depletion of tumor-associated SAMHD1 enhances chemotherapeutic efficacy and antitumor immune responses. *Signal Transduct Target Ther*. 2025;10(1):406.

39. Attardi E, Wlodarski MW. LNKing genotype to phenotype: the expanding clinical spectrum of SH2B3 disorders. *Eur J Hum Genet*. 2025 Sep 23. doi:10.1038/s41431-025-01942-6 [Epub ahead of Print]

40. Elbracht M, Meyer R, Kricheldorf K, et al. Germline variants in DNA repair genes, including BRCA1/2, may cause familial myeloproliferative neoplasms. *Blood Adv*. 2021;5(17):3373-3376.

Table 1 Patient characteristics Demographic, clinical and genetic characteristics of 45 pediatric patients presenting with ET and PV.

	all	Genetic subtype				Diagnosis	
		JAK2 V617F	JAK2 other	CALR	triple- negative	ET	PV
Number of patients	45	20	3	8	14	39	6
Male/female	15/30	7/13	1/2	3/5	4/10	12/27	3/3
Age at diagnosis - median (range)	14 (0-18)	14 (4-17)	4 (0-16)	14 (7-17)	14 (1-18)	14 (0-18)	12 (4-16)
Symptoms/Clinical findings at diagnosis n/total							
└ Splenomegaly	22	15	2	3	2	17	5
└ Headache	17	9	/	4	4	15	2
└ Stomachache	4	1	/	2	1	3	1
└ Erythromelalgia	1	/	/	1	/	1	/
└ Dizziness	1	/	/	1	/	1	/
└ Thrombosis	4	4	/	/	/	3	1
└ Budd-Chiari-syndrome	2	2	/	/	/	2	/
└ Bleeding	1	/	/	/	1	1	/
└ Acquired VWD	16	5	/	4	7	14	2
└ Myelofibrosis	6	3	/	2	1	6	/
(Molecular-)Cytogenetics							
└ no cytogenetic aberrations*	34	18	2	4	10	28	6
└ unknown	11	2	1	4	4	11	/
Blood count at diagnosis#							
└ leukocytes (median, G/l)	9.9	8.58	12.08	7.7	10.2	9.9	11.3
└ platelets (median, G/l)	1067	756	823	1184	1247	1082	821
└ hemoglobin (median, g/dl)	14	16	14	13	12	13	17
└ hematocrit (median, %)	42	46	39	41	38	41	52

Abbreviations: VWD = von Willebrand disease

* with one exception (patient #16): inv(10)?c was detected

four patients (#5, #7, #15, #21) were excluded: blood counts from time point at diagnosis were not available

Table 2 Initial treatment approaches (Interferon (IFN), Hydroxyurea (HU), Anagrelide (ANG), Ruxolitinib (RUXO), Acetylsalicylic acid (ASA); no therapy n=21; unknown n=1)

	No therapy	IFN only	IFN + ASA	HU only	HU + ASA	ANG only	RUXO only	ASA only	Not recorded	N
JAK2V617	5	6	2	1	/	1	1	3	1	20
JAK2 other	2	/	/	/	/	/	/	1	/	3
CALR	4	/	1	/	/	/	/	3	/	8
triple-negative	10	1	/	1	1	/	/	1	/	14
ET	20	6	1	2	1	1	/	7	1	39
PV	1	1	2	/	/	/	1	1	/	6
<u>Total</u>	21	7	3	2	1	1	1	8	1	45

Table 3 Detailed information on the patients with confirmed germline variants.

Patient	Gender	Age at diagnosis	Phenotype	Variant	VAF (%)	Classification (ACMG)	Clinical symptoms	Family history
#7	female	16 years	ET	NM_004972.4(JAK2): c.2624C>A p.(Thr875Asn)	50*	likely pathogenic (PS4_mod, PM1, PM2_sup, PP2, PP3)	persistent thrombocytosis, splenomegaly	mother with Budd-Chiari syndrome (18 years), sister with suspected Budd-Chiari syndrome (5 years) - received a liver transplant
#23	male	4 years	ET	NM_004972.4(JAK2): c.2047A>G p.(Arg683Gly)	46*	likely pathogenic (PS4_mod, PM1, PM2_sup, PP3_mod)	persistent thrombocytosis, splenomegaly	grandmother with MPN (older adulthood), cousin with MPN (early childhood)
#26	male	2 years	ET	NM_000460.4(THPO): c.13+1G>A r.spl p.?	46*	likely pathogenic (PVS1, PM2_sup)	persistent thrombocytosis	/
#27	female	16 years	ET	NM_004119.3(FLT3): c.2525A>G p.(Tyr842Cys)	48*	likely pathogenic (PS4_mod, PM1, PM2_sup, PP3_mod)	headache, bleeding signs, persistent thrombocytosis	/
#32	female	17 years	ET	NM_015474.4(SAMHD1):c.500A>C p.(His167Pro)	47*	likely pathogenic (PM1, PM2_sup, PP3_str)	persistent thrombocytosis, von Willebrand disease	/

*germline origin confirmed

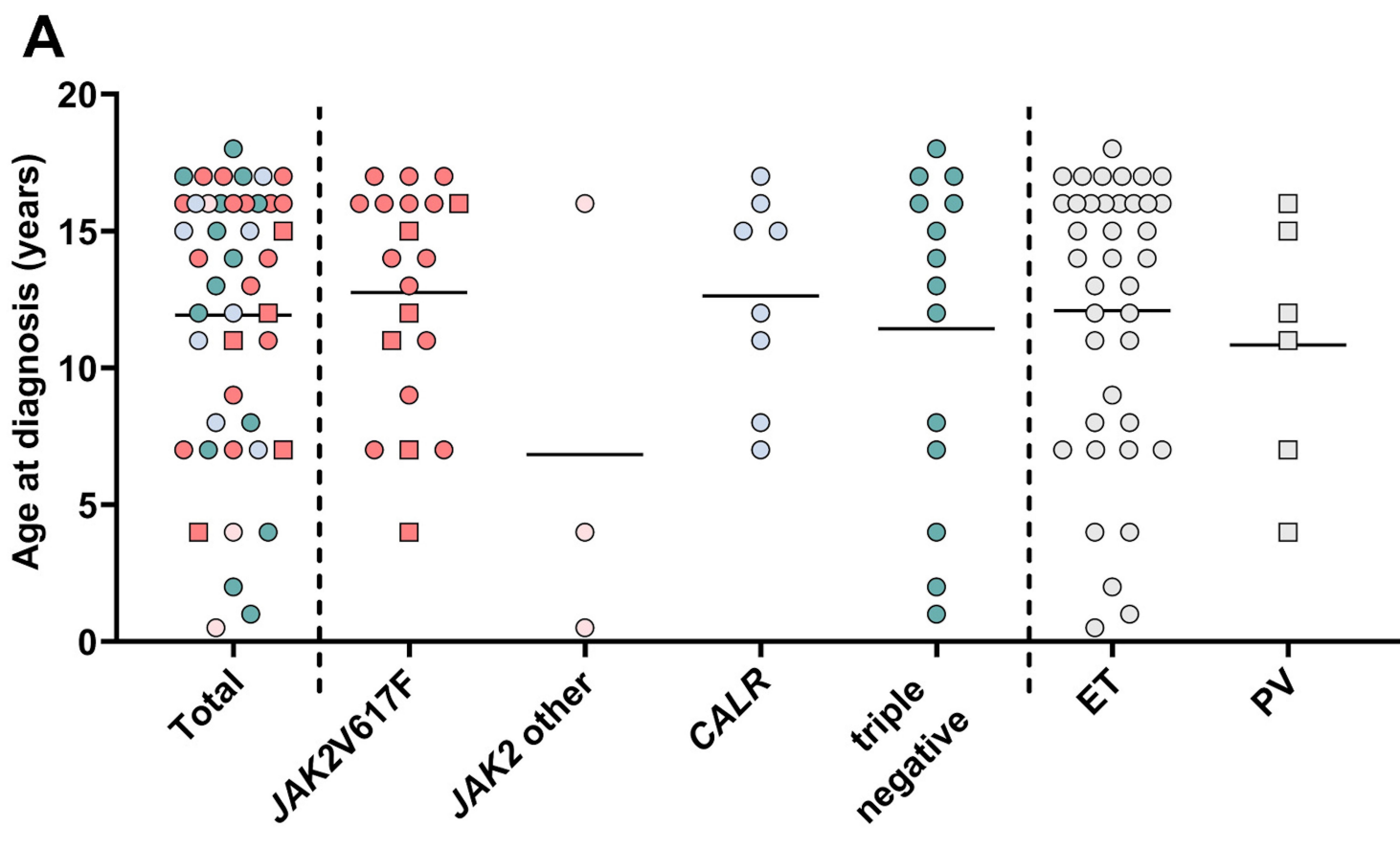
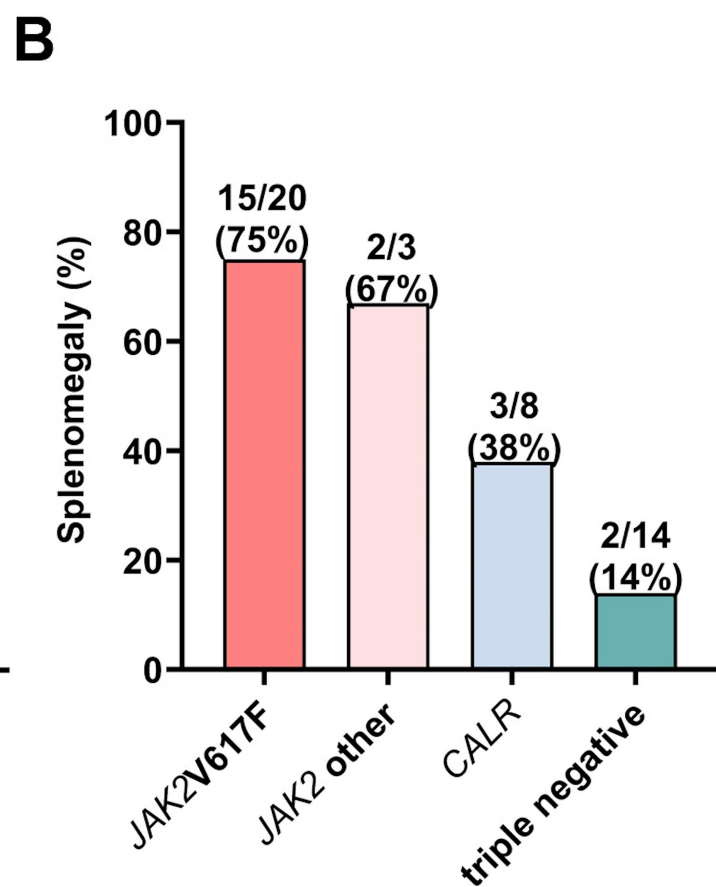
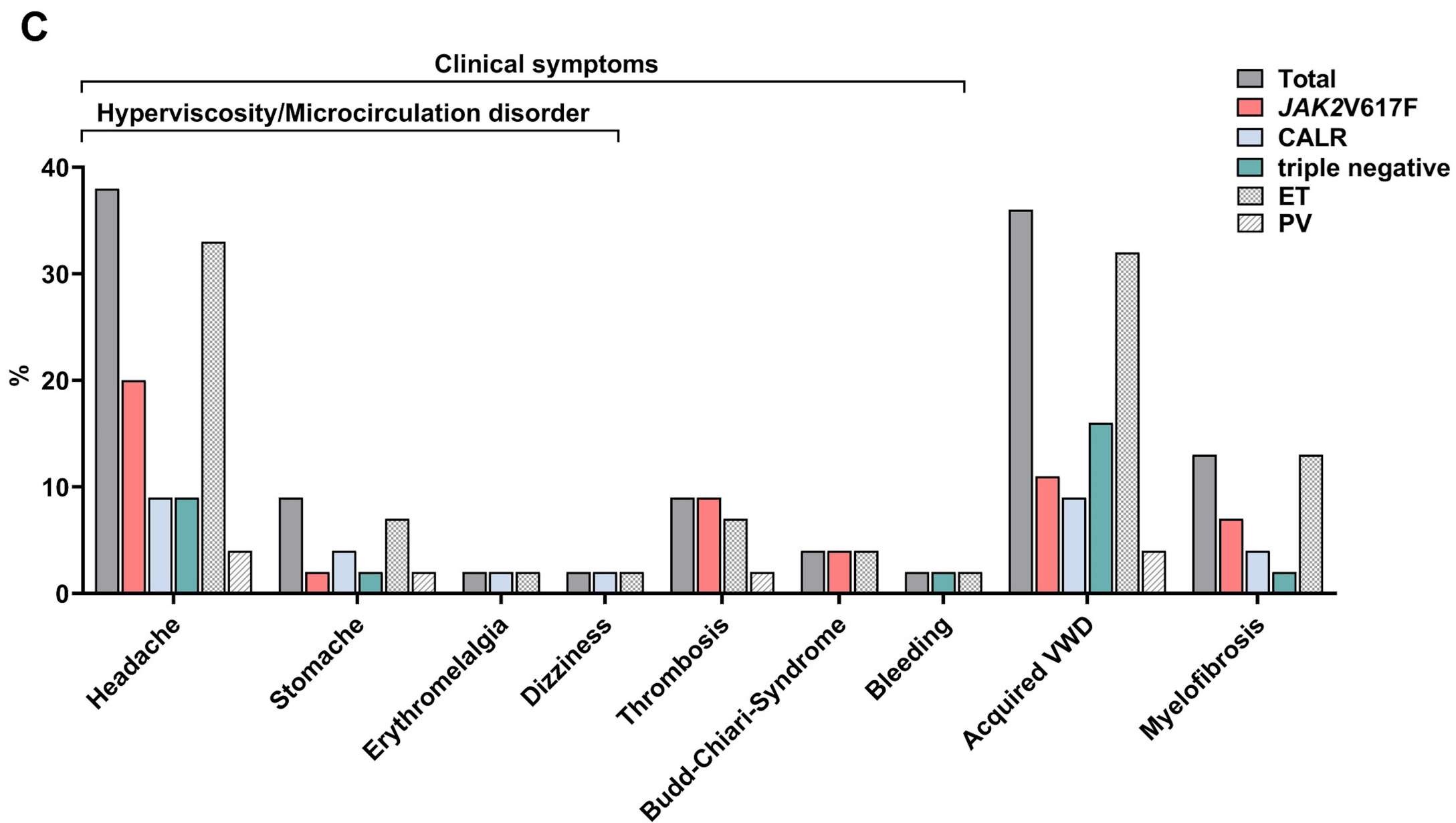
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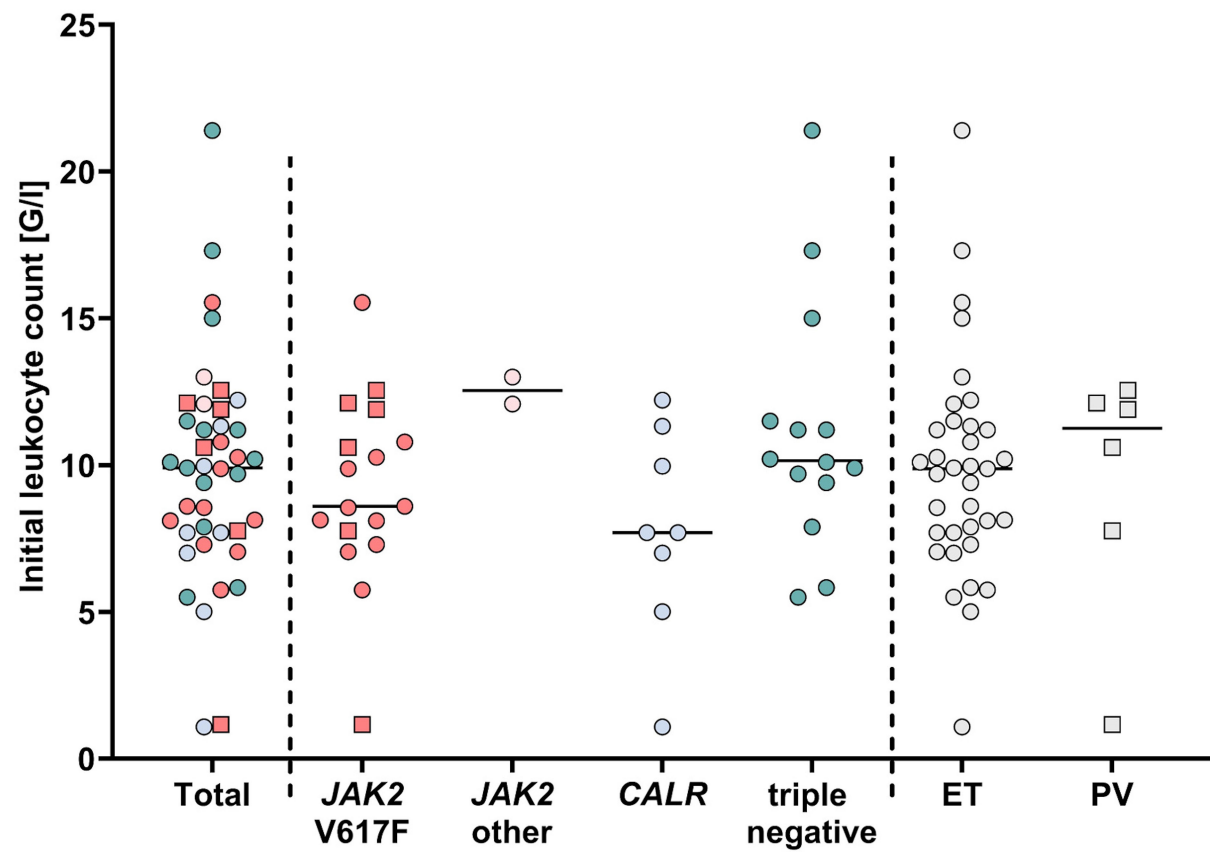
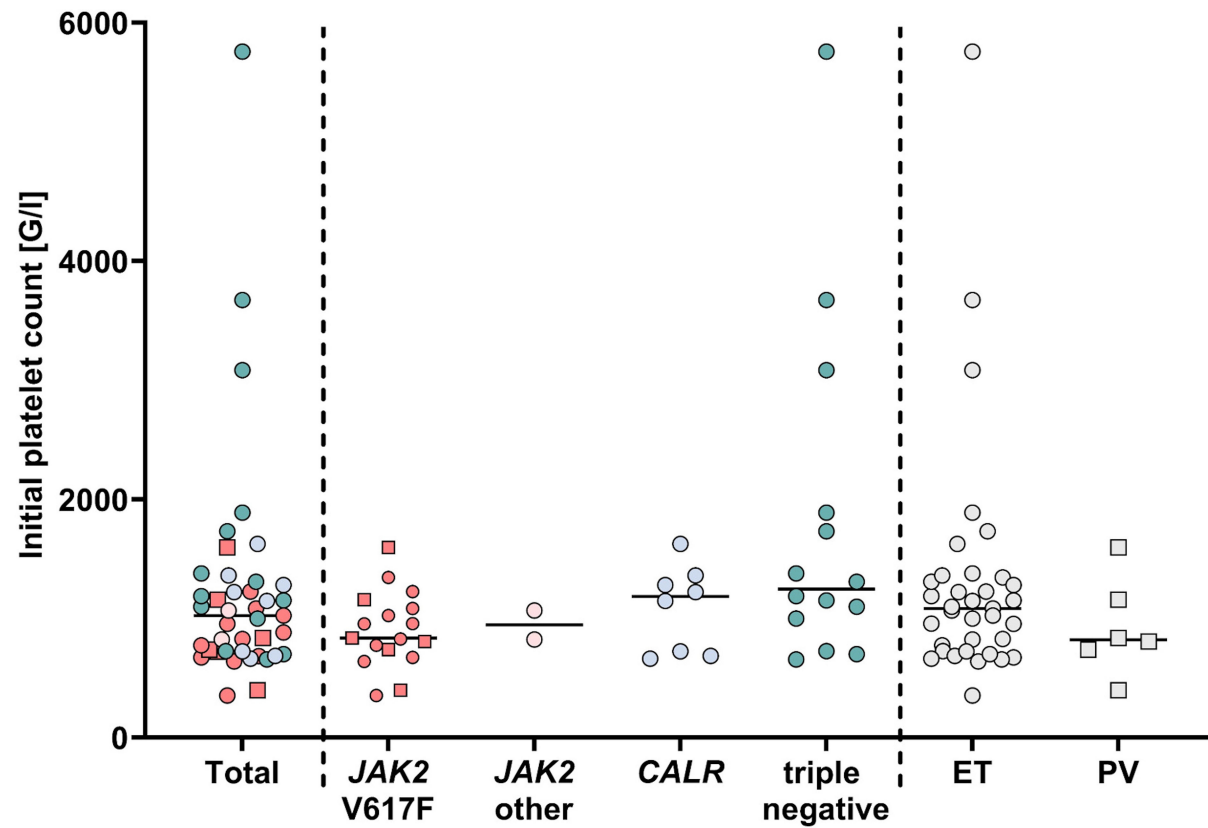
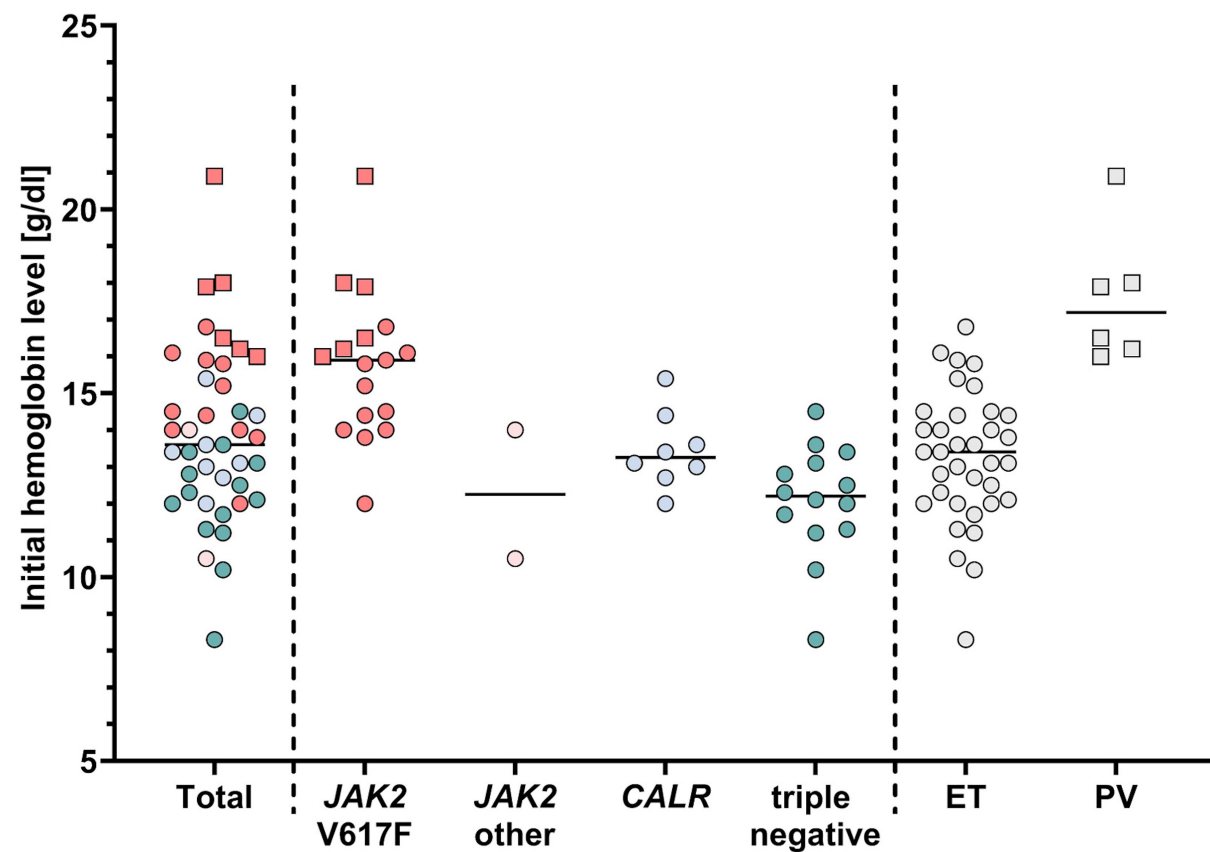
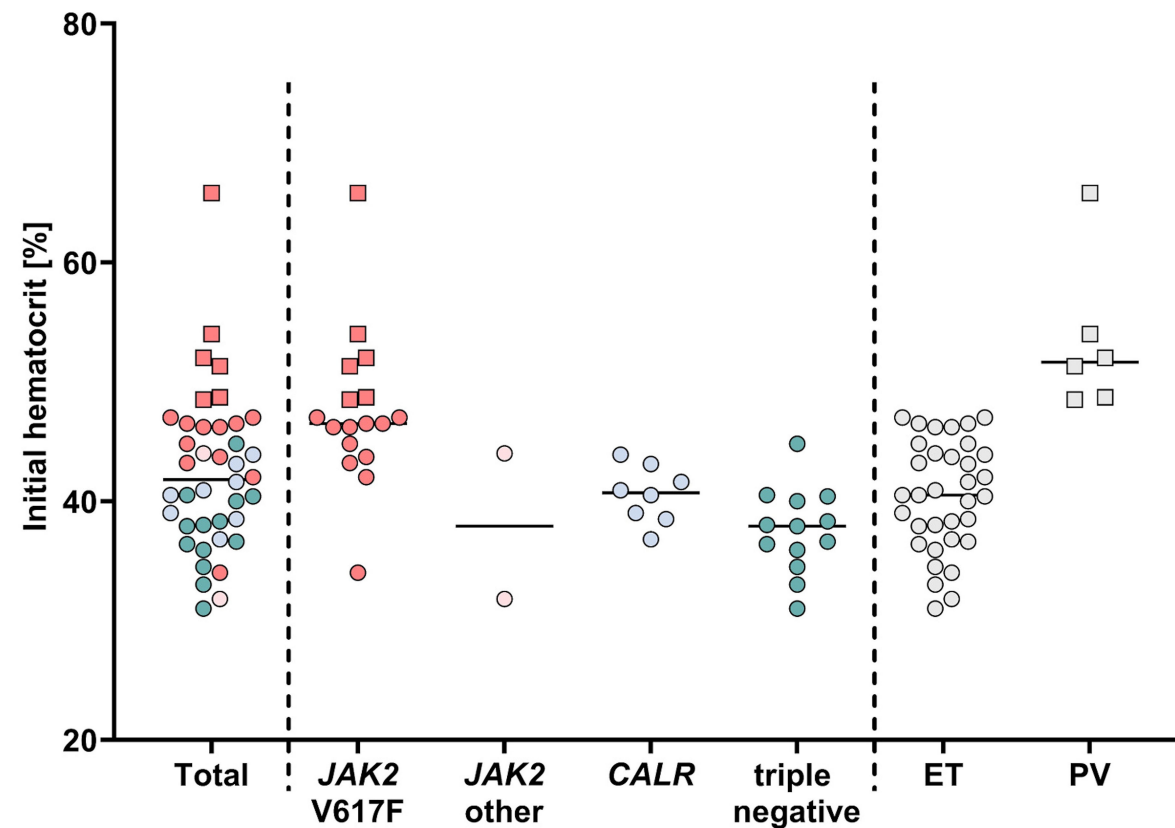
Figure 1 Demographic and clinical cohort characteristics. (A) Age distribution, (B) proportion of patients with splenomegaly according to known somatic driver variant status, (C) clinical presentation at the time of diagnosis overall, according to genotypes, and according to phenotypes.

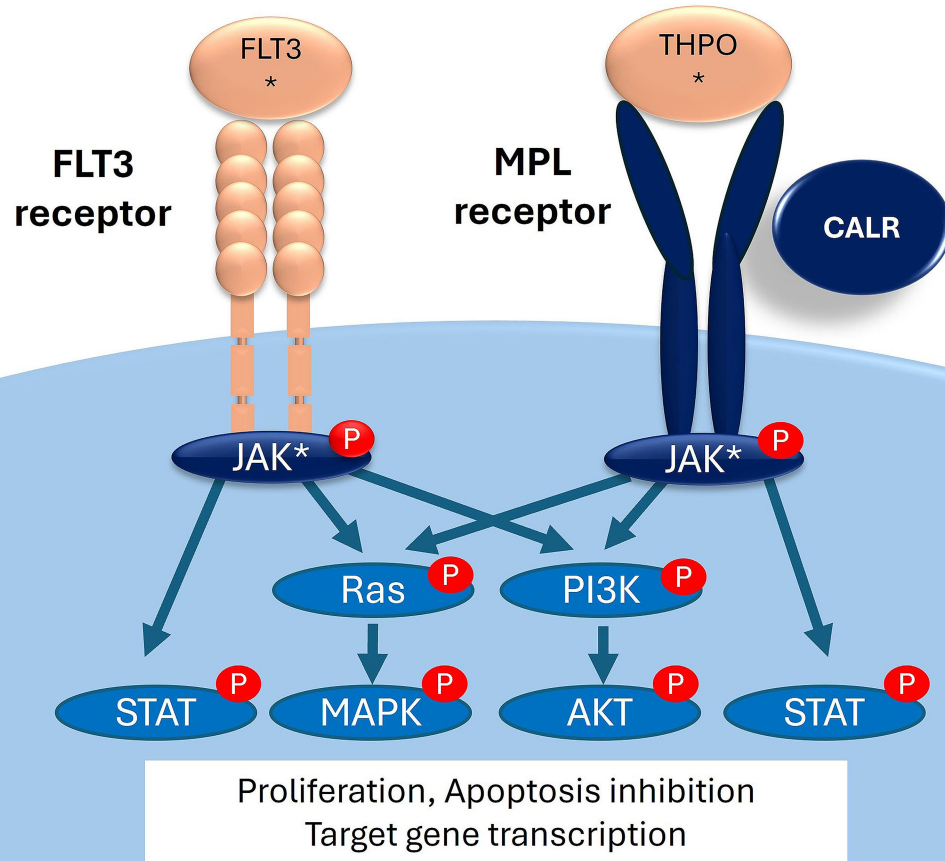
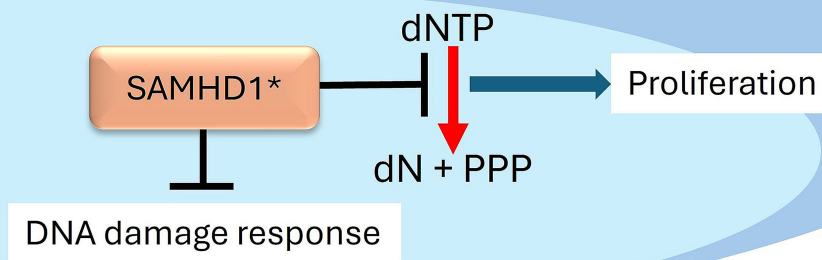
Figure 2 Hematological values at the time of diagnosis overall, according to genotypes, and according to phenotypes (four patients (#5, #7, #15, #21) were excluded: hematological values from the time point at diagnosis were not available).

Figure 3 Distribution patterns of disease phenotype and pathogenic somatic driver variants including variant allele fraction (upper panel), additional somatic variants and likely pathogenic variants with germline origin (central panel) in correlation with initial clinical presentation and resulting treatment regimen (lower panels) in the 45 patients in this cohort at the time of diagnosis.

Figure 4 Synopsis Illustration of the interconnectivity of likely pathogenic germline variants identified in this study within the JAK-STAT pathway.







* Likely pathogenic, confirmed-germline variant of the respective gene/protein

Table S1 Custom panel including 142 leukaemia-associated gene regions

Gene	Exone
<i>ABL1</i>	
<i>ANKRD26</i>	complete with 5'UTR; without 3'UTR
<i>ARID1A</i>	
<i>ASXL1</i>	13, 14
<i>ASXL2</i>	9, 11, 12, 13
<i>ATM</i>	
<i>BAX</i>	
<i>BCL2</i>	
<i>BCOR</i>	
<i>BCORL1</i>	
<i>BIRC3</i>	6-9
<i>BRAF</i>	11 - 16
<i>BTK</i>	14 - 16
<i>B2M</i>	
<i>CALR</i>	
<i>CARD11</i>	4 - 10
<i>CBL</i>	8, 9
<i>CCND1</i>	1
<i>CCND3</i>	5
<i>CD58</i>	1 - 3
<i>CD79A</i>	
<i>CD79B</i>	
<i>CDKN2A</i>	
<i>CDKN2B</i>	
<i>CEBPA</i>	
<i>CHD2</i>	
<i>CREBBP</i>	
<i>CSF3R</i>	
<i>CSNK1A1</i>	
<i>CUX1</i>	
<i>CXCR4</i>	
<i>DDX3X</i>	
<i>DDX41</i>	
<i>DIS3</i>	
<i>DNM2</i>	
<i>DNMT3A</i>	
<i>EGLN1</i>	
<i>EGR2</i>	2
<i>EED</i>	8
<i>EP300</i>	
<i>EPAS1</i>	
<i>EPOR</i>	
<i>ETNK1</i>	
<i>ETV6</i>	
<i>EZH2</i>	
<i>FBXW7</i>	8 - 12
<i>FLT3</i>	
<i>FOXO1</i>	
<i>FOXP1</i>	16 - 18
<i>GATA1</i>	
<i>GATA2</i>	
<i>GNA13</i>	
<i>GNAS</i>	1, 8, 9

GNB1	
H3F3A	2
HRAS	
ID3	
IDH1	
IDH2	
IGLL5	1
IKZF1	
IKZF2	
IKZF3	5
IL2RG	
IL7R	5 + 6
IRF4	
JAK1	14 -17
JAK2	
JAK3	11 - 16, 19, 20, 21
KDM6A	
KIT	1 - 3, 8 - 13, 16 - 18, 20 - 21
KLF2	
KMT2A	
KMT2D	
KRAS	
MAPK1	
MAP2K1	2, 3
MEF2B	
MPL	
MYC	
MYD88	
NF1	
NFE2	
NOTCH1	26 - 34
NOTCH2	26 - 34
NPM1	
NRAS	
NSD2 (WHS)	18, 19, 20, 21
NT5C2	
PAG1	
PAX5	
PHF6	
PIGA	
PLCG2	19, 20, 24
POT1	
PPM1D	
PRDM1	
PRPF8	
PTEN	
PTPN1	complete with 5'UTR
PTPN11	2, 3, 4, 7, 8, 12,13
RAD21	
RB1	
RHOA	
RPS15	
RUNX1	
SAMD9	
SAMD9L	

SAMHD1
SETBP1
SETD2
SF1
SF3A1
SF3B1 5, 6, 13 - 16, 18, 24
SGK1
SH2B3 2, 3, 4, 5, 6, 7, 8
SMARCA4
SMC1A
SMC3
SOCS1
SRSF2
STAG2
STAT3
STAT5B 15 - 18
STAT6 9 - 14
SUZ12 14 - 16
TCF3 17 - 19
TENT5C (FAM46C)
TET2
THPO
TNFAIP3
TNFRSF14
TP53
TRAF3
U2AF1
U2AF2
UBR5 58
UBTF
VHL
WT1
XPO1 12 - 18
ZRSR2

Table S2 Overview of demographic features, reported somatic and germline variants, hematological and clinical characteristics and therapeutic regimen

#Sample	Age at diagnosis [y]	Gender f/m	Molecular genetic results (variant, VAF in % (depth))	Diagnosis	Initial leukocyte count [G/l]	Initial platelet count [G/l]	Initial hemoglobin level [g/dl]	Initial hematocrit [%]	Headache	Pseudo-tumor cerebri	Stomach-ache	Erythromelalgia	Dizziness	Thrombosis	Budd-Chiari syndrome	Bleeding	Acquired VWD	Myelofibrosis (grade 1-3)/n	Splenomegaly	Therapy initiated
1	16	f	JAK2:c.1849G>T p.(Val617Phe) 21% (929)	ET	8,10	954	14,5	47	y	n	n	n	n	n	n	n	n	n	n	ASS
2	14	m	JAK2:c.1849G>T p.(Val617Phe) 38% (534)	ET	9,88	1082	16,1	46,2	n	n	n	n	n	n	n	n	y	n	y	IFN
3	7	f	JAK2:c.1849G>T p.(Val617Phe) 34% (1258)	ET	15,54	1023	12,0	34	y	n	n	n	n	n	n	n	n	y 1	y	IFN
4	15	m	JAK2:c.1849G>T p.(Val617Phe) 7% (1092)	PV	7,76	396	20,9	65,8	n	n	n	n	n	n	n	n	n	n	y	ASS
5	17	m	JAK2:c.1849G>T p.(Val617Phe) 21% (1009)	ET	1,08	649	9,8	35	n	n	n	n	n	n	y	n	n	y 1	n	HU
6	16	m	JAK2:c.1849G>T p.(Val617Phe) 19% (684)	PV	11,90	836	16,2	48,5	y	n	n	n	n	n	n	n	y	n	y	ASS + IFN
7	16	f	JAK2:c.2624C>A p.Thr875Asn 50% (752)	ET	6,48	531	13,5	39,4	n	n	n	n	n	n	n	n	n	n	y	ASS
8	12	f	JAK2:c.1849G>T p.(Val617Phe) 25% (1404)	PV	12,55	1156	18,0	52	y	n	y	n	n	n	n	n	n	n	y	ASS + IFN
9	16	m	JAK2:c.1849G>T p.(Val617Phe) 13% (1142)	ET	5,75	352	15,9	44,8	n	n	n	n	n	n	n	n	n	n	y	n
10	17	f	JAK2:c.1849G>T p.(Val617Phe) 12% (912)	ET	8,55	826	15,2	46,5	n	n	n	n	n	n	n	n	y	n	y	IFN
11	4	f	JAK2:c.1849G>T p.(Val617Phe) 18% (1020); FANCL:c.654dup p.Pro219ThrfsTer14 4% (1273)	PV	1,16	720	16,5	48,7	n	n	n	n	n	y	n	n	y	n/a	y	n
12	7	f	JAK2:c.1849G>T p.(Val617Phe) 30% (1424)	ET	10,78	683	14	43,2	y	n	n	n	n	n	n	n	y	y 1	y	n
13	9	f	JAK2:c.1849G>T p.(Val617Phe) 28% (1429)	ET	8,13	881	14	46,2	y	y	n	n	n	y	n	n	n	n	y	n
14	16	f	JAK2:c.1849G>T p.(Val617Phe) 15% (1114)	ET	7,04	639	16,8	46,5	n	n	n	n	n	n	n	n	n	n	n	ASS
15	16	f	JAK2:c.1849G>T p.(Val617Phe) 24% (1059)	ET	8,43	191	14,2	42,3	n	n	n	n	n	n	y	n	n	n/a	y	ANG
16	7	m	JAK2:c.1849G>T p.(Val617Phe) 20% (1083)	PV	12,12	1595	16	51,3	n	n	n	n	n	n	n	n	n	n	y	RUXO
17	11	f	JAK2:c.1849G>T p.(Val617Phe) 24% (1532)	ET	8,60	673	15,8	47,0	y	y	n	n	n	n	n	n	n	n/a	y	IFN
18	0	f	JAK2:c.2155G>T p.(Val719Leu) 32% (2052)	ET	12,08	1067	10,5	31,8	n	n	n	n	n	n	n	n	n/a	n/a	n	n
19	11	f	JAK2:c.1849G>T p.(Val617Phe) 30% (1283)	PV	10,60	737	17,9	54	n	n	n	n	n	n	n	n	n	n	n	IFN
20	14	f	JAK2:c.1849G>T p.(Val617Phe) 11% (1448)	ET	7,29	774	14,4	43,7	y	n/a	n	n	n	y	n	n	n/a	n/a	y	n
21	17	m	JAK2:c.1849G>T p.(Val617Phe) 59% (846)	ET	10,45	509	15,3	46,8	n	n	n	n	n	n	n	n	n	n/a	y	n/a
22	13	f	JAK2:c.1849G>T p.(Val617Phe) 14 (1497)	ET	10,26	1224	13,8	42	y	y	n	n	n	y	n	n	n	n	n	IFN
23	4	m	JAK2:c.2047A>G p.(Arg683Gly) 46% (1131)	ET	13,00	823	14	44	n	n/a	n	n	n	n	n	n	n/a	n	y	n
24	15	f	no pathogenic variant	ET	11,50	725	12,5	37,9	n	n	n	n	n	n	n	n	y	n	n	n
25	12	f	no pathogenic variant	ET	9,70	1098	12,0	35,9	y	n	n	n	n	n	n	n	y	n	n	IFN
26	2	m	THPO:c.13+1G>A p.? 46% (750)	ET	10,20	1186	13,1	40,4	n	n	n	n	n	n	n	n	n	n	n	n
27	16	f	FLT3:c.2525A>G p.Tyr842Cys 48% (1668)	ET	10,10	3083	11,2	36,6	y	n	n	n	n	n	n	y	n	n	n	HU
28	14	f	no pathogenic variant	ET	5,83	1377	14,5	44,8	n	n	n	n	n	n	n	n	n	n	n	n
29	4	m	no pathogenic variant	ET	15,00	3672	11,3	33	n	n	n	n	n	n	n	n	y	y 1	y	n
30	8	m	no pathogenic variant	ET	21,40	5758	10,2	31	n	n/a	n	n	n	n	n	n	n/a	n	n	ASS + HU
31	17	f	no pathogenic variant	ET	7,89	1150	8,3	40	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n	n/a	n
32	17	f	SAMHD1:c.500A>C p.His167Pro 47% (1464)	ET	11,20	1887	12,3	38,3	n	n	n	n	n	n	n	n	y	n	n/a	n
33	7	f	no pathogenic variant	ET	17,30	1731	11,7	34,5	y	n	y	n	n	n	n	n	y	n	n	n
34	1	f	no pathogenic variant	ET	11,20	656	12,1	36,4	n	n	n	n	n	n	n	n	n/a	n/a	n/a	n
35	13	f	no pathogenic variant	ET	5,50	699	13,4	n/a	n/a	n	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n
36	16	m	no pathogenic variant	ET	9,40	1307	12,8	38	y	n	n	n	n	n	n	n	y	n/a	y	n
37	18	f	no pathogenic variant	ET	9,90	998	13,6	40,5	n	n	n	n	n	n	n	n	y	n	n	ASS
38	17	m	CALR:c.1154_1155insTTGTC (p.Lys385fs) 29% (1744)	ET	5,01	662	15,4	43,9	y	n	y	n	n	n	n	n	n	n	n	n
39	8	f	CALR:c.1099_1150del p.(Leu367ThrfsTer46) 7% (2133)	ET	7,00	684	12	36,8	n	n	n	n	n	n	n	n	y	n	n	n
40	7	m	CALR:c.1099_1150del p.(Leu367ThrfsTer46) 40% (1039)	ET	1,08	1279	13	38,5	n	n	y	n	n	n	n	n	y	y 1	y	n
41	15	m	CALR:c.1099_1150del p.(Leu367ThrfsTer46) 8% (2484)	ET	9,96	722	14,4	43,1	n	n	n	y	n	n	n	n	n	y 1	n	n
42	12	f	CALR:c.1155_1156insTGTCG p.(Glu386CysfsTer46) 35% (1968)	ET	11,32	1625	13,6	41,6	y	n	n	n	y	n	n	n	y	n/a	y	ASS + IFN
43	15	f	CALR:c.1099_1150del p.(Leu367ThrfsTer46) 5% (915)	ET	12,21	1220	12,7	40,5	n	n	n	n	n	n	n	n	y	n/a	y	ASS
44	11	f	CALR:c.1099_1150del p.(Leu367ThrfsTer46)10% (1266)	ET	7,70	1147	13,1	40,9	y	n	n	n	n	n	n	n	n	n/a	n	ASS
45	16	f	CALR:c.1099_1150del p.(Leu367ThrfsTer46) 25% (1651)	ET	7,70	1360	13,4	39	y	n	n	n	n	n	n	n	n	n/a	n	ASS