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Germline genetic factors in the pathogenesis of myeloproliferative neoplasms

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Abstract

Myeloproliferative neoplasms (MPN) are clonal hematological malignancies that lead to overproduction of mature myeloid cells. They are due to acquired mutations in genes encoding for *JAK2*, *MPL* and *CALR* and that result in the activation of the cytokine receptor/JAK2 signaling pathway. In addition, it exists germline variants that can favor the initiation of the disease or may affect its phenotype. First, they can be common risk alleles, which correspond to frequent single nucleotide variants present in control population and that contribute to the development of either sporadic or familial MPN. Second, those that predispose to the onset of MPN with a higher penetrance and lead to familial clustering of MPN. Finally, some extremely rare genetic variants can induce MPN-like hereditary disease. We will review these different subtypes of germline genetic variants and discuss how they impact the initiation and/or development of the MPN disease.

Keywords: sporadic and familial MPN, germline variants, risk allele, SNV, CNV.

Introduction

Myeloproliferative neoplasms (MPN) are clonal hematological malignancies that lead to overproduction of mature myeloid cells. They appear with an incidence rate varying from 1 to 3.7 per 100,000 persons per year [1-3]. They are due to acquired mutations in genes encoding for the tyrosine kinase *JAK2*, the thrombopoietin (TPO) receptor *MPL* and calreticulin (*CALR*), that arise at the level of hematopoietic stem cell (HSC) and result in the activation of the cytokine receptor/*JAK2* signaling pathway. All these mutations are phenotypic drivers since all the mouse models harboring them recapitulate the disease and its progression. In addition, it exists germline variants that can favor the initiation of the disease or may affect its phenotype (essential thrombocythemia (ET) or polycythemia vera (PV)) or its transformation into leukemia contributing to the heterogeneity of MPN. These germline variants can be classified in three different types. First, they can correspond to common risk alleles, which slightly increase the risk of MPN development. They correspond to frequent single nucleotide variants (SNV) present in control population and that contribute to the development of either sporadic or familial MPN. Second, other germline variants can predispose to the onset of MPN with a higher penetrance and lead to familial clustering of MPN mostly compatible with an autosomal dominant inheritance. Finally, they can be extremely rare genetic variants inducing a non-clonal disease (MPN-like hereditary diseases) without the acquisition of others mutations. We will review these three different subtypes of germline genetic variants and discuss how they impact the initiation and/or development of the MPN disease or MPN-like disease (figure 1).

I-Common germline risk alleles increasing the development of sporadic MPN

Genome-wide association studies (GWAS) performed in several diseases have revealed various risk alleles that correspond to common SNV. One of them, the *JAK2* haplotype namely 46/1 or “GGCC” haplotype, has been reported as an increased risk factor in sporadic MPN. Thereafter, some GWAS were performed using very large cohorts of control

population and MPN that revealed some additional risk alleles particularly on *TERT* and *MECOM* loci.

1) The *JAK2* haplotype

Using a candidate approach in loci related to the *JAK2*/*STAT* signaling including *EPOR*, *MPL*, *G-CSFR* and *JAK2*, the group of Tefferi was the first to identify the association with *JAK2* SNV in 179 MPN patients [4]. Based on this study and on previously identified *JAK2* SNV by GWAS in other diseases, three groups have highlighted by different approaches the *JAK2* haplotype as a predisposition factor to the acquisition of *JAK2* V617F in MPN with an odds ratio (OR) of 3.7.

In order to understand the frequency of multiple acquisition of *JAK2* V617F in one patient, the group of Kralovics developed an allele-specific assay based on the detection of both rs12343867 (C allele) in the intron 14 of the *JAK2* gene and the *JAK2* V617F mutation in the same allele [5]. They not only observed several acquisitions of *JAK2* V617F on both alleles in the same individual, but also a significant bias toward the acquisition of *JAK2* V617F mutation in the C allele. Overall, they showed that 85% of MPN patients (333 cases) carried the *JAK2* V617F mutation in association with the C allele. Moreover, heterozygous and homozygous patients for the C allele were more likely to be *JAK2* V617F positive than other MPN patients with homozygous T allele. Finally, they determined the *JAK2* haplotype based on 4 SNV (rs3780367, rs10974944, rs12343867, rs1159782) associated with the acquisition of *JAK2* V617F mutation.

The group of Cross analyzed 6 different SNV (rs7864782, rs10758669, rs7046736, rs12343867, rs10974944, rs2031904) and found that the *JAK2* V617F mutation occurred on the same haplotype in 77% of the tested cases [6]. They extended the result to 14 SNV and defined that the *JAK2* V617F also arose in the specific *JAK2* haplotype in 354 MPN patients harboring the 3 diseases compared to several cohorts of controls, even if a stronger association was found for PV.

The group of Levine studied SNV that were previously associated with *JAK2* V617F patients by the group of Tefferi who demonstrated the association with rs7046736, rs10815148 and rs12342421 [4, 7]. They found an association with rs10974944 in a cohort of *JAK2* V617F-PV and ET patients (OR=4.3 for PV and 2.1 for ET) and that the *JAK2* V617F mutation occurred in cis with this SNV. Moreover, they evidenced that *JAK2* rs12500918 was more specific to PV than to ET.

Altogether the *JAK2* haplotype was further confirmed by GWAS analyses in *JAK2* V617F-positive MPN by several groups with different type of cohorts [8-10].

From these works, 2 hypotheses, that can be intertwined, may explain the enrichment of *JAK2* V617F mutation with the *JAK2* haplotype: i) the “fertile” hypothesis in which the *JAK2* V617F mutation occurs on any haplotype, but is selected by the *JAK2* haplotype-induced proliferative advantage, ii) the hypermutability hypothesis in which the *JAK2* haplotype is more susceptible to acquisition of mutations. One argument in favor of this latter hypothesis is that one SNV of the *JAK2* haplotype has been found to be a replication timing quantitative trait locus (rtQTL) [11] that could promote the mutability of the *JAK2* gene. Another argument is the identification of a weak association of the *JAK2* haplotype with the *JAK2* exon 12-positive MPN (OR=2.1) [12]. In contrast, the association between *JAK2* haplotype and *MPL*-mutated MPN is against this hypothesis [13]. However this association is controversial [14] as well as the association of *JAK2* haplotype with *CALR*-mutated MPN [10, 15, 16].

2) Others common SNV

As mentioned above, the group of Tefferi was the first to identify the association of MPN with several *JAK2* SNV included or not in the *JAK2* haplotype in 179 MPN patients. Moreover, they also identified one *EPOR* variant (rs318699, A allele) specifically associated with PV and not ET or PMF [4]. Other SNV in genes controlling the *JAK2*/STAT signaling such as *SH2B3* were also studied. *SH2B3* (*LNK*) is an adaptor protein that negatively regulates the *JAK2* signaling mediated by *MPL* and *EPOR*. The *Sh2b3* knock-out (KO) mice

develop an ET-like phenotype with increased erythroid colonies [17, 18]. The rs3184504 (T allele), which corresponds to R262W coding variant of *SH2B3*, was reported to be significantly associated with a cohort of 169 ET patients and 148 PMF patients and more particularly with *JAK2* V617F PMF compared to controls (OR= 1.32) [19]. Another study confirmed these results in 285 MPN patients and reported that rs78894077 (allele T corresponding to R242S) was more frequently associated with ET [20]. The R242 residue is located in the PH domain of LNK that bind the phosphatidylinositol 3-phosphate at the plasma membrane; the variant can cause loss-of-function activity and increase *JAK2* signaling. Another SNV (rs6198) located in the 3'UTR of *NR3C1* (glucocorticoid receptor) was reported to be more frequently associated with PV and MF than with ET and also to transformation into leukemia [21]. However this result was not confirmed in another cohort [22]. Very recently, another loss-of-function SNV (rs4537545) in *IL6R*, another cytokine receptor involved in inflammation, was shown to reduce the risk of *JAK2* V617F mutation acquisition and MPN development after genotyping 107,969 Danes [23].

Apart from these studies based on candidate genes, three GWAS were performed to find out more risk alleles. Oddsson *et al.*, have searched for germline risk alleles in 2230 Icelanders by GWAS based on whole genome sequencing. They subsequently tested the association between the identified SNV and MPN using 237 patients (PV, ET and MF). They found a significant association between the risk allele *TERT* rs2736100 (allele C) and any MPN subtypes independently of *JAK2* V617F [9]. This SNV might be more frequently associated with *CALR*-mutated cases [24]. It is localized in the second intron of *TERT* gene, which encodes for the reverse transcriptase involved in the telomere maintenance and it increases the size of telomeres. It has been associated with other types of cancers (glioma, lung cancers). Even if the mechanism of increased risk of MPN (OR=2.09) is unclear, it did not appear to impact on the acquisition of *JAK2* V617F. However, the authors correlate the presence of this variant in the Icelandic population with higher red blood cell, platelet and monocyte/granulocyte counts, suggesting that it induces the proliferation of the myeloid lineage.

Tapper *et al.* performed a three-stage GWAS meta-analysis with 10,083 controls and 3,437 MPN cases and identified additional SNV increasing the risk of MPN [10]. Thanks to the high number of cases, they were able to focus on the different types of MPN harboring the different mutations. In *JAK2* V617F-negative MPN cases including *CALR* and *MPL*-mutated cases, they replicated the association with the *JAK2* haplotype (rs12339666). They also identified a novel SNV (rs2201862) with significant effect, which is located downstream of *MECOM* (*MDS1-EVII* locus) and three others with more moderate effects, rs9376092 located between the *HBS1L* and the *MYB* genes, rs4858647 upstream of the *THRB* and *RARB* genes and the *TERT* rs2736100. The *JAK2* haplotype and the rs9376092 *HBS1L-MYB* had lower association with triple-negative MPN than with *CALR/MPL*-positive MPN. In *JAK2* V617F-positive MPN cases, they confirmed the *JAK2* haplotype and *TERT* rs2736100 displayed a stronger effect than in *JAK2* V617F-negative MPN, while the *MECOM* rs2201862 and the *HBS1L-MYB* rs9376092 harbor similar effects whatever the driver mutation. Moreover, when they compared the SNV with subtypes of diseases, they found association between the *JAK2* haplotype and *JAK2* V617F-positive PV and between *HBS1L-MYB* rs9376092 and *JAK2* V617F-positive ET. Interestingly, they could correlate this variant with a decreased *MYB* expression in BFU-E and CFU-GM in ET. These data also correlate with the fact that *MYB* knockdown induces an ET-like phenotype [25]. More recently, a replication study in another cohort including 939 MPN and 483 controls led to similar results [26]. They found an association of *MECOM* with all MPN except for ET and *THRB-RARB* was only slightly associated with PMF. Altogether, these data show that several risk alleles involving different loci may contribute to the risk of developing sporadic MPN.

In another study using a cohort of 252,637 controls, Hinds *et al* identified 0.2% of *JAK2* V617F asymptomatic carriers and performed a GWAS between controls and the *JAK2* V617F carriers and MPN patients (ET, PV, PMF) (497 *JAK2* V617F carriers and 726 MPN) [8]. They also observed a strong association with the *JAK2* haplotype (rs59384377, rs10974900), *TERT* locus (rs7705526, rs2853677), *SH2B3* locus (rs7310615) and a variant rs1548483 located upstream *TET2*. This study has also expanded the number of weaker common risk

alleles in MPN in the following loci *CHEK2* (rs555607708), *ATM* (rs1800056), *PINT* (rs58270997) and *GFI1B* (rs621940).

Altogether, *JAK2*, *TERT* and *MECOM* SNV were uncovered as master ones even if other weak SNV were also identified to aggregate sporadic MPN. Moreover, the different SNV are involved in JAK2/STAT signaling (*JAK2*, *SH2B3*, *EPOR*, *IL6R*), the control of transcription factors that regulate erythropoiesis and megakaryopoiesis (*GFI1B*, *MECOM*, *HBS1L-MYB*), epigenetic factor (*TET2*), effectors or downstream targets of P53 involved in cell cycle/DNA repair (*TERT*, *CHEK2*, *ATM*, *PINT*). Finally, they can be involved in the risk of development of one or several MPN (*JAK2* haplotype, *TERT*, *MECOM*) or are more restricted to certain type of diseases or genotypes (*JAK2* SNV, *HBS1L-MYB*) or are implicated in disease progression (*NR3C1*) (Table 1).

II- Rare germline variants that favor the clustering of MPN cases in families

Rare germline factors can predispose more strongly to the development of MPN leading to clustering of MPN cases in families that are generally transmitted through autosomal dominance with incomplete penetrance. These families are very important to study since these predisposition factors or pathways could also be involved in the initiation of sporadic cases or other hematological malignancies.

1) Epidemiology, clinical and molecular profiles of familial MPN

One very informative study was done by Langren *et al.* who performed a population-based study including 11,000 MPN patients including ET, PV, MF, 25 000 first-degree relatives and 99,000 controls. They established that first-degree relatives have an increased risk by 5-7 fold to develop a MPN of any type [27]. Moreover, they display a mild increasing risk of developing chronic myeloid leukemia (CML) and none of developing other myeloid malignancies such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and overlap entities. Moreover, it was also observed that there was an increased frequency of

other malignancies such as chronic lymphoid leukemia (CLL) and non-Hodgkin lymphoma, or solid cancers (brain and melanoma) [28]. Another study indicating that germline factors contribute to MPN development is the one performed in 158 twin pairs, in which it was identified in 4 pairs both twins with MPN [29].

Generally, MPN families are reported to account for less than 5% of the cases, but one study based on 458 cases has estimated a prevalence of 7.6%, suggesting that they might be underestimated to reduced penetrance or infraclinical presentation [30]. In term of disease(s), these families can be homogenous or heterogeneous. About half families harbor only one disease (ET or PV or MF), while the others present mixed phenotypes generally ET and PV [31]. In rare cases, other MPN such as CML or any types of hematological myeloid malignancies could co-occur [31-34]. One study of 93 pedigree reported that familial cases of MPN are completely indistinguishable from sporadic MPN in terms of clinical presentation, age of onset, prognosis, complications (hemorrhage and thrombosis), and disease progression (MF or leukemia) [34]. They were also shown to be completely comparable to sporadic cases in term of molecular landscape with similar acquired mutations and recurrence. Indeed, *JAK2* V617F, *CALR* and *MPL* mutations were found to be somatically acquired when comparing tumoral to non-tumoral samples in such families [35, 36] and with similar frequency in all families including big families with mixed phenotypes of ET/PV and MF [32, 36]. Very interestingly, 10-15% of MPN cases in families remain triple-negative, which is around the same frequency as in sporadic cases. Those familial cases are of importance to find new acquired molecular drivers. Nevertheless, some cases can present a mutation level at the limit of detection and one can imagine that the predisposing allele cooperates with very low allele burden of an acquired mutation to give a full-blown disease.

Familial cases present also additional mutations that seem similar to the sporadic cases. Particularly *TET2* mutations have been detected in ET, PV that progress into MF or leukemia [37]. Others mutations were also reported in epigenetic molecules such as *ASXL1* or *IDH1/2* [32].

The disease anticipation is still unclear even if studies strongly suggest a lower age of diagnosis in the second-generation patients (median of 38 years) than in the first-generation (median of 64 years) [38]. Of note, in some families the age of onset can be also lower than in sporadic cases around 40 years due to higher penetrance and severity of the predisposition factor [32]. In addition, identifying a familial history of MPN could induce a bias in the MPN clinical diagnosis. In these families, a systematic follow-up of blood counts of asymptomatic cases should be of benefit in the most highly penetrant families, accompanied with early molecular detection of acquired mutations. In some cases, the detection of the drivers was known before the onset of the full-blown disease [39], suggesting that it could be planned to treat patient very early or to search for bone marrow donor outside the family in the case of very penetrant predisposing factors that favor the transformation into leukemia.

Taking into account the low incidence of MPN disorders, the fact that several cases aggregate in families suggest that germline variants predispose strongly to the initiation and/or development of the disease. However, until now, low-penetrance risk alleles have been associated with some families and very rare predisposition germline variants have been identified. Among them, only one is associated with a high penetrance (*TCL1A/GSKIP/ATG2B* locus).

2) Germline low-penetrance risk alleles predisposing to familial MPN

Even if the germline low-penetrance risk alleles predispose to sporadic MPN, some of them have been identified to explain the clustering of MPN cases in families.

***JAK2* haplotype.** It was initially found to be present in 2 cases of PV in one family [6]. Then, the study of Olcaydu *et al.*, showed in 88 familial cases that *JAK2* haplotype is more frequent in familial MPN compared to controls. However, it was not more prevalent in familial MPN compared to sporadic cases indicating that it cannot be responsible for familial clustering [28] even if there was significant trend enrichment in *JAK2* V617F-positive cases [40]. In another study, two pedigrees with both *JAK2* V617F and *CALR*-mutated patients were carriers of the *JAK2* haplotype [16]. However, the low amount of *CALR*-mutated cases

did not allow concluding whether the *JAK2* haplotype predisposes to familial *CALR*-mutated cases in general. Overall, the *JAK2* haplotype does not seem to be involved in familial MPN.

TERT rs2736100 was found significantly enriched in familial compared to sporadic MPN including ET, PV and MF [40]. However, this variant only explains few families. Interestingly, the association of *TERT* rs2736100 and *JAK2* haplotype presents an additive risk while independently predisposing to familial cases, suggesting that their predisposition mechanisms might be different and cooperate together.

Germline *SH2B3* mutation such as E208Q was reported in one family with two MPN cases segregating with a *JAK2* V617F-ET and a *CALR* type 1-mutated PMF [41]. In the meantime, it was also found as germline in two families with two MPN cases (PV and ET) but only segregated with the *JAK2* V617F-positive PV and not with the *JAK2* V617F-positive ET cases. Thus, this last study strongly suggests that this germline SNV does not explain the familial clustering [42], but could rather be involved in PV phenotype. The involvement of *SH2B3* variants as germline MPN predisposition factors remains a topic of debate.

TET2 mutations have been described to predispose to sporadic myeloid and lymphoid malignancies such as MDS, chronic myelomonocytic leukemia (CMML) and MPN[43]. Very interestingly, a familial germline *TET2* *D1858fs* mutation resulting in the truncation of the catalytic C-terminal part of the protein was found in a PV suggesting that *TET2* mutant can predispose to the development of MPN, but with a reduced penetrance as the relative with the *TET2* mutation was asymptomatic [44]. However, in two other families, 2 germline *TET2* mutations (A241V and R1440Q) were identified but without segregation in all the affected cases.

3) Germline highly penetrant variants predisposing to familial MPN

RBBP6 is a RING finger ubiquitin ligase located in the nucleus. It was initially found as binding to RB and then to P53. It favors the MDM2/P53 interaction and the degradation of P53. The *RBBP6* R1569H variant was found in an Australian pedigree with 5 affected members presenting almost only PMF [45]. It is located near the P53 interacting site. The *RBBP6* R1569H was detected in three MPN family members with *JAK2* V617F, *CALR del52* or *MPL* W515L and eight asymptomatic family members suggesting a low-penetrance of the mutation. The *JAK2* haplotype and *TERT* rs2736100 seem to have additive effect with *RBBP6* variant since all affected cases were associated with one of these risk factors. Two other variants E1654G and R1451T were also identified in a single case after testing 66 MPN families [45].

Three novel potential candidate variants for familial PV were identified in a four-generation Finnish family. They include *ZXDC* (P644R), *ATN1* (R234Q) and *LRRC3* (R234Q) [46]. *ZXDC* seems to be involved in the transcription of both MHC class I and II by interacting with CIITA (class II transactivator) [47]. *ATN1* is a nuclear transcriptional corepressor expressed in the hematopoietic system shown to be involved in neurodegenerative diseases. [48]. *LRRC3* is the leucine-rich repeat whose function is not known.

Identification of structural variants affecting the *TCL1A/GSKIP/ATG2B* locus. This germline structural variant with autosomal dominant inheritance was identified in six large families originated from the French West Indies with more than fifty members presenting myeloid disorders [32]. They develop not only classical MPN in 2/3 of the cases, but also CMML, CML, MDS and AML. Moreover, the MPN patients diagnosed with ET were actively progressing to MF and AML of any types, but with very poor prognosis. The spectrum and frequency of molecular abnormalities in signaling molecules in affected members from these families were identical to those found in sporadic MPN. The germline alteration consists in a 700 kb copy-number variation (CNV) duplicated in tandem located on

chromosome 14q32.13-q32.2 that segregates with all affected members of the families. The penetrance reaches 90%. No clear pathogenic mutation was found in this region, which contains 6 genes (*TCL1A*, *BDKRB1*, *BDKRB2*, *ATG2B*, *GSKIP*, *AK7*). Three genes are expressed in the hematopoiesis (*TCL1A*, *ATG2B* and *GSKIP*). However, only *ATG2B* and *GSKIP* are expressed in myeloid hematopoiesis and are overexpressed in primary hematopoietic cells from patients compared with donor cells including CD34⁺ progenitors. Functional studies demonstrated that *ATG2B* and *GSKIP* stimulate hematopoietic progenitors and cooperate with acquired signaling mutations such as *JAK2* V617F or with epigenetic mutations like *TET2* to modify the response of hematopoietic progenitors to cytokines. Thus, this study highlighted a novel predisposition locus including two genes *ATG2B* and *GSKIP* responsible for the high induction of MPN and myeloid malignancies of poor prognosis.

Little is known about *ATG2B* and *GSKIP* functions. *ATG2A/B* encodes important players in autophagy particularly in autophagosome formation. Autophagy is a process by which cellular material is degraded by lysosomes or vacuoles and recycled. This mechanism is important in cancer, but also in many others process including normal hematopoiesis. Indeed, deficiency in *Atg7*, an autophagy-related gene, leads to severe anemia, thrombocytopenia but also to an AML-like phenotype in mice [49-51]. Deregulated autophagy could affect HSC self-renewal capabilities in aging subjects [52]. Somatic loss-of-function mutations in *ATG2B* have been identified in breast, gastric and colorectal cancers [53]. The *GSKIP* gene encodes a negative regulator of GSK3 β , and its mutation may thus mimic activation of the Wnt/ β -catenin signaling pathway involved in HSC homeostasis and normal megakaryopoiesis, as well as in the transformation in CML and the development of leukemia stem cells in AML [54-57]. Conditional *Gskip* KO mice have been generated and harbor developmental defects leading to a cleft palate and perinatal lethality caused by respiratory distress [58].

Very recently, two other families presenting hematological diseases were identified harboring a germline tandem duplication of 1.8 Mb and 700 kb respectively on chromosome 14q32 overlapping our previously published region and encompassing only the *TCL1A* gene, suggesting that this gene or the deregulation of this locus must be involved in the

predisposition mechanism [59, 60]. *TCL1A* has numerous roles, particularly, it interferes with AKT and ATM signaling, but also regulate the transcription in cooperation with AP1 transcription factor [61]. *TCL1A* expression is restricted to the lymphoid lineage. It plays an essential role in lymphomagenesis, but has not been implicated in myeloid malignancies. While Hahn reported a four-generation family with 7 cases presenting PMF, *de novo* AML and MDS (RARS-T and RCMD-RS), the study of Babushok identified a five-generation pedigree with 4 affected cases of lymphoma, *de novo* and secondary AML, MDS (RS-MLD-RS) and PMF. The ET/PMF phenotype was more prevalent in the 6 French West Indies families without any hematological malignancies related to ringed sideroblasts and the presence of a *SF3B1* acquired mutation that is highly frequent in the two others families. ET/PMF phenotype of the West Indies families is probably related to the role of *ATG2B/GSKIP* in megakaryopoiesis. Thus, it is possible that *TCL1A* may play a role in the predisposition to hematological malignancies and that *ATG2B* and *GSKIP* might induce a bias toward MPN. It is also interesting to note that uniparental disomy (UPD) of 14q32.12 region was also found in 1.6% of myeloid malignancies (particularly ET and PMF), as well as trisomy of chromosome 14 in 1.4 % of the cases [62].

Altogether in most familial MPN, the predisposing allele has not been identified and data linkage analyses suggest a large genetic heterogeneity, each locus being linked to a few families. Among the genes that have been discovered, some are more related to particular phenotypes (*RBBP6* in PMF, *ZXDC/ATN1/LRRC3* in PV) and some genes are more specifically associated with various hematological malignancies with enriched ET/PMF (*TCL1A/ATG2B/GSKIP*). Several seem to play a role in signaling such as the duplicated locus *TCL1/ATG2B/GSKIP* and *SH2B3* variant, some may favor genetic instability such as *TERT* variant and *RBBP6* mutation through the regulation of telomere shortening or increased mutagenesis through P53 downregulation (Table 1).

III- Extremely rare germline Mendelian SNV that cause hereditary MPN-like diseases

Germline Mendelian variants can also be responsible for the development of MPN-like diseases transmitted through a complete penetrance at a young age (Table 1). These families correspond to hereditary diseases that are extremely rare and that affect only one myeloid lineage leading to hereditary thrombocytosis, erythrocytosis or neutrophilia and present a polyclonal hematopoiesis contrary to familial MPN. They are generally of good prognosis and not associated with myeloid transformation. These entities are very important to decipher since causative factors affect the overproduction of red blood cells, platelets and granulocytes and some of these genetic factors are already known to be involved in the development of sporadic MPN.

1) Hereditary thrombocytosis

Hereditary thrombocytosis (HT) is characterized by a megakaryocytic hyperplasia and an increased platelet count (more than 450,000 G/L). Platelets are generated through the megakaryopoiesis that is mainly driven by the TPO/MPL axis. Many germline factors contributing to the development of HT are members of this pathway.

THPO is the gene encoding for the TPO that is a hormone essentially produced by the liver [63]. It has been identified several heterozygous mutations in the *THPO* gene resulting in HT. They correspond to gain-of-function mutations located in the 5'UTR or affecting the consensus donor splice site of exons 2 and 3 of *THPO* leading to an increased translation of mRNA and thereby of TPO protein level [64-68].

MPL is the gene encoding for the TPO receptor. Gain-of-function as well as loss-of-function mutations may lead to HT because MPL has a double function: signaling and TPO clearance. The effects on the platelets level results from the equilibrium between these two functions.

In HT families, *MPL S505N* (gain-of-function mutation) has been identified first in one Japanese family and then in 8 Italian pedigrees with a founder effect. This mutation leads to an amino acid (AA) change in the transmembrane part of the receptor. Thus, it induces MPL

homodimerization and activation leading to a constitutive activation of the receptor and downstream signaling pathway as well as to an independence/hypersensitivity to TPO [69, 70]. **MPL W515R** was also been found in another HT family. This mutation leads to an AA change in the amphipathic juxtamembrane domain of MPL and induces a gain-of-function activity in Ba/F3-MPL cells lines [71].

Others *MPL* mutations have been found in HT leading to AA changes located in the extracellular part of MPL more precisely in the first cytokine receptor motif of the receptor. It concerns the *MPL* K39N, P106L and R102P mutants. In these cases, patients exhibited increased level of TPO in their serum. TPO is produced by the liver and its plasma level is mainly regulated *via* MPL internalization and thus its level is dependent of the platelet mass. In case of a defective MPL surface expression, TPO is not cleared enough by the platelets and increases in the serum mimicking the effect of *THPO* mutations. Functional studies of these mutations have highlighted different molecular mechanisms resulting to HT.

The Baltimore polymorphism **MPL K39N** is a frequent African-American variant (7%). It has not been associated with autosomal dominant form of HT *per se* but was associated with a higher risk of thrombocytosis. The severity of HT is dependent on the genotype reaching more than 800,000 G/L platelets at homozygous state and 500,000 G/L at heterozygous state. The mutant expression was found decreased at the cell surface in an incomplete glycosylated immature form suggesting a loss-of-function variant [72].

MPL P106L (Gain or loss-of-function) was originally found in one Arab HT family and then in one Kuwaiti, one Saudian and one Qatari HT families [73-75]. The patients with homozygous P106L mutations present high thrombocytosis with a huge amount of TPO in the serum suggesting an autosomal recessive transmission of the HT and a loss-of-function mutant. Indeed, it was shown that this mutant is incorrectly addressed to the plasma membrane and presents a defective trafficking in megakaryocytes. It was also suggested that this mutant is activated in the endoplasmic reticulum by cytoplasmic TPO [75]. Another more likely study showed that the low level of this mutant at the cell surface responds to TPO [74]. The latter study suggested that *MPL* P106L is insufficient to clear the TPO entirely through

the platelet mass leading to increased TPO level that is sufficient to induce the growth of megakaryocyte precursors that express higher level of MPL P106L on their surface than megakaryocytes. Thus, in *MPL* P106L patients there is a paradoxical thrombocytosis through a defective TPO clearance whose mechanism is close to what has been described with *MPL* or *JAK2* deletion specifically in megakaryocytes [76, 77].

MPL R102P was identified at heterozygous state in a HT with several family members harboring mild abnormal platelets levels around 600,000 G/L but again with high TPO levels in the serum. This mutation was previously demonstrated to be a loss-of-function mutant since it is generally found in congenital amegakaryocytopenia (CAMT) at homozygous state and results in a strong thrombocytopenia and aplasia. This disease is lethal unless the patients are allografted. Studies have shown that the MPL R102P is blocked in the endoplasmic reticulum (ER) and not expressed at all at the plasma membrane [78, 79]. Thus, again in such case of HT, it could be hypothesized that at heterozygous state, the MPL R102P mutant is blocked in ER and only the normal MPL is addressed to the surface mimicking the effect of MPL P106L mutant: i.e decreased clearance by platelets, increased TPO levels sufficient to allow the proliferation of megakaryocyte precursors and to lead to a paradoxical thrombocytosis [80].

Germline mutations of *JAK2* have been identified in HT. Many heterozygous gain-of-functions mutations have been described in the coding sequence of AA V617 as in sporadic cases, but with a different AA change such as V617I, or at different sites located in different domains: the FERM domain (*JAK2* T108A, *JAK2* G335D, *JAK2* L393V), the pseudokinase domain (*JAK2* R564Q, *JAK2* G571S, *JAK2* H608N, *JAK2* L611S, *JAK2* V625F, *JAK2* S755R), the kinase domain (*JAK2* R867Q, R938Q) of the protein. *JAK2* L611S was identified as *de novo* germline mutation in a young patient with thrombocytosis [81] (Figure 2). *JAK2* H608N was found in one family associated with thrombocytosis and no myelofibrosis. All *JAK2* H608N cases were associated with the *JAK2* haplotype suggesting a potential cooperation between both of them. Using Ba/F3 cell models, the team showed that it

exhibits a low gain-of-function activity on the phosphorylation of STAT5 [82]. *JAK2* R564Q was found in one family of HT with 3 members. They were not clonal or associated with others mutations as expected. The authors showed that it activates the MPL phosphorylation and the *JAK2*/STAT pathway but at a lower level than *JAK2* V617F using both Ba/F3 cell lines expressing MPL and patient platelets. They also show that it gives a proliferative advantage, a hypersensitivity to TPO and a better anti-apoptotic response compared to controls. Very interestingly, they showed that this mutant is more sensitive to SOCS3 and to ruxolitinib compared to *JAK2* V617F [83]. *JAK2* V617I was also found in HT with mild thrombocytosis (600,000 G/L). No spontaneous progenitor colony growth was observed with the patients contrary to *JAK2* V617F patients, but a total increase in CFU-G/GM and CFU-GM were observed with the patients, contrary to *JAK2* V617F patients. Nevertheless, the granulocytic progenitors were hypersensitive to G-CSF. Moreover, modeling *JAK2* V617I in Ba/F3 cell lines expressing MPL but not EPOR showed a constitutive phosphorylation of STAT1/3/5 but lower than with *JAK2* V617F [84, 85]. A transplantation mouse model was performed after retroviral transduction of *JAK2* V617I construct [86]. It showed no effect on blood parameters but when bone marrow and spleen were analyzed, they exhibited myeloid progenitor and megakaryocyte hyperplasia. Altogether, these studies showed a low gain-of-function activity of *JAK2* V617I compared to *JAK2* V617F that is specific of MPL and, at a lower extent, of G-CSFR.

As the other mutations found in HT, those affecting the pseudokinase (*JAK2* S755R) or the kinase domain (*JAK2* R867Q and R938Q) of the protein displayed very similar activity. Of note a double mutation (*JAK2* S755R/R938Q) was found in the same allele. *JAK2* R867Q and *JAK2* S755R/R938Q were very specific of MPL, since they induce a spontaneous proliferation of Ba/F3-MPL but not of Ba/F3-EPOR associated with a constitutive activation of signaling pathways. However, these effects were less pronounced than with *JAK2* V617F. Megakaryocytic hyperplasia (increased CFU-MK colonies) was observed in patients compared to controls. Moreover, these mutants were more stable compared to *JAK2* V617F and bound more tightly to HSP90 chaperone. Finally, they were less sensitive to ruxolitinib

contrary to JAK2 R564Q [87]. It is important to note that a pedigree with *JAK2* R867Q was identified in Japan with patients harboring thrombocytosis alone or progressing to PV [88].

It has also been described a compound heterozygous patient harboring two missense mutations (E846D and R1063H) located in the kinase domain and presenting an erythrocytosis with megakaryocyte hyperplasia [89]. The three family members (proband and both parents) revealed hypersensitivity to EPO of the erythroid progenitors and using Ba/F3 cell lines expressing EPOR. Both mutants induced a milder constitutive activation of signaling pathways than *JAK2* V617F with a stronger effect of E846D over R1063H.

Similarly, two other *JAK2* heterozygous germline mutations (*JAK2* T108A and L393V) presented both thrombocytosis and erythrocytosis and were shown to display mild gain-of-function activity on STAT5 activation in Ba/F3-EPOR cell lines [90].

Altogether all the germline *JAK2* mutants are mild gain-of-function compared to *JAK2* V617F and act in the majority of cases specifically *via* MPL, giving HT. However, some mutants can also interact with G-CSFR or EPOR giving mixed phenotypes of erythrocytosis or myeloid hyperplasia associated or not with higher platelet counts. For now, it is not elucidated why some mutants interfere more with MPL and some others more with EPOR or G-CSFR, but it is possible that mild *JAK2* mutants could activate more easily MPL like in the case of low amount of *JAK2* V617F (mostly heterozygous cells) in ET.

Hereditary erythrocytosis (also named primary erythrocytosis or primary familial and congenital polycythemia (PFCP) or ECYT1) is characterized by an increased hemoglobin, hematocrit and red cell mass with low EPO level or with high EPO level (secondary polycythemia or ECYT2/3/4). While in both cases there is an isolated erythrocytosis, PFCP are characterized by an EPO hypersensitivity of the erythroid progenitors and low serum EPO level, which is absent in secondary erythrocytosis. The main axis controlling the erythropoiesis is the EPO/EPOR axis, by analogy with the TPO/MPL axis in HT. Germline mutations have been identified notably in *SH2B3*, *EPOR* or *EPO* genes or in genes that control EPO production.

First germline *SH2B3* mutations such as E208Q or E400K have been identified in erythrocytosis [91, 92]. While the E208Q mutation induces an hypersensitivity to TPO in Ba/F3-MPL [93], the E400K does not affect the sensitivity to EPO in Ba/F3-EPOR [91].

Around 20 different *EPOR* mutations in the exon 8 have been identified in PFCP all leading to a truncation of the receptor in C-terminal. All these mutations led to an EPO hypersensitivity in terms of proliferation and signaling activation. EPOR is generally pre-associated as a dimer at the cell-surface. EPO binding induces the formation of a 120° angle between the D1 domains of the two EPOR monomers and the reorientation of the continuous rigid alpha helix formed by the transmembrane domain and the cytosolic juxtamembrane region that contains a rigid hydrophobic motif composed of residues L278, I282 and W283. This is essential for the dimerization of JAK2 molecules and activation of the receptor. Moreover, the regulation of the receptor is fine-tuned; first by negative regulators such as phosphatases SHP-1 and suppressors of cytokine signaling such as SOCS3 and CIS; second by the internalization and degradation of the EPO-EPOR complex. In PFCP, the mechanism generally admitted to explain EPO hypersensitivity is the loss of negative regulatory sites in the C-terminal part in the mutant [94-100]. More recently, our group has identified a new *EPOR* mutation p.Gln434Profs*11 leading to a truncation of 64 AA in the C-terminal part, but generating a new C-terminal tail due to the frameshift mutation. The patient exhibited an important EPO hypersensitivity of the erythroid progenitors nearly at the same level as *JAK2* V617F MPN patients. This was shown not to be due to loss of C-terminal part but rather to a new C-terminal tail (particularly the motif MDTVP) that increases the dimerization of the receptor and primes the EPOR to EPO. The same phenotype could also be observed with other frameshift mutants containing the MDTVP motif already identified in PFCP. Thus, this work highlighted a more complex mechanism for the EPOR mutants than the simple absence of negative regulatory sites [101].

***EPO* mutations or *EPO*-regulated gene expression.**

Very recently, *EPO* mutations have been identified in erythrocytosis in a Norwegian pedigree with autosomal dominant isolated erythrocytosis with high levels of EPO in serum [102] and in another small pedigree [103]. By linkage analysis, the authors determined a region in the chromosome 7 and the targeted sequencing of the loci allowed the finding of heterozygous *EPO* mutations located in the exon 2 (c.32delG and c.19delC). The group of Skoda studied these variants functionally. These mutations were predicted to be loss-of-function, but surprisingly allows the use of a second promoter in the intron 1 leading to the generation of EPO mRNA transcript with a shorter signal peptide. The EPO is in the great majority produced by the kidney, but with the use of the second promoter, the new *EPO* mRNA isoform is mainly produced by the liver. This was demonstrated by the editing of *EPO* gene in a hepatocyte cell line with increased EPO production and was confirmed in Ba/F3-EPOR cell lines, which were able to proliferate after transduction of the mutated cDNA.

Since EPO is regulated by the oxygen level mainly through the transcriptional factor Hypoxia-inducing factor 2 (HIF2 α), all the molecules controlling these factors could potentially be involved in changes in the EPO levels. The erythropoiesis is generally regulated through the production of EPO *via* the oxygen sensing pathway. Under normoxic conditions, HIF2 α encoded by the *EPAS1* gene is hydroxylated by the dioxygenase PHD (prolyl hydroxylase domain also called EGLN). The resulting hydroxylated HIF2 α is then recognized by the E3 ubiquitin ligase VHL for its degradation. Under hypoxia, HIF2 α remains non-hydroxylated, is stabilized and accumulates in the nucleus where it binds to HIF-response element upstream the *EPO* gene. Thus, loss-of-function mutations in *VHL* (ECYT 2) or in *EGLN1/PHD2* (ECYT3) or gain-of-function mutations in *EPAS1/ HIF2A* (ECYT4) have been identified in secondary polycythemia.

VHL mutations have been identified as germline homozygous or heterozygous compound, suggesting an autosomal recessive transmission of the disease. The first one (R200W) was found in Chuvash Russian family. The Chuvash mutation was modeled in a knock-in

mouse reproducing polycythemia [104]. This mutation may be particular because it has been shown that it modifies the affinity of the interaction of VHL with SOCS1 and thus induces a decrease in the degradation of phosphorylated JAK2 [105]. Later, more than 15 other mutations have been identified in non-Chuvasch cases including heterozygous mutations with autosomal dominant trait [106]. More recently, a *VHL* mutation in cryptic exon 1 (E1') found in 7 families as homozygous or heterozygous compound led to cryptic exon retention and downregulation of VHL expression [107]. These cases only presented with erythrocytosis. Moreover, a synonymous mutation in exon 2 of *VHL* was found in 2 families and led to an altered splicing of *VHL* [107].

***EGLN1* or *PHD2* P317R** heterozygous mutation was first identified by the group of Percy *et al.* in one family leading to a marked decreased activity [108]. Later, using a knock-in mouse model, they prove that this mutant is responsible of the disease since the mice developed an erythrocytosis that was reversible in an *HIF2a*^{-/+} but not *HIF1a*^{-/+} deficient context [109]. Then more than 25 mutations in *PHD2* were reported including missense, frameshift or nonsense and leading to erythrocytosis [110].

EPAS1* or *HIF2A heterozygous mutations have been identified in congenital erythrocytosis with autosomal dominant trait. These mutations lead to AA changes near the hydroxylation site of EPAS1 thereby reducing the hydroxylation itself or its binding to VHL. Altogether, this results in aberrant stabilization of this transcriptional factor and increased *EPO* gene expression. In addition to erythrocytosis some *HIF2A* or *PHD2* mutations can also give rise to others tumors indicating that germline mutations can give rise to various spectra of diseases depending on the type of mutations.

Other mutations can also be responsible for congenital erythropoiesis such as hemoglobin (***Hbb***) variants leading to increased O₂ sensing and O₂ affinity or mutations in biphosphoglycerate mutase (***BPGM* gene**). The BPGM is an enzyme that generates the 2,3 BPG which bind to Hb and decreases affinity for O₂. Defective BPGM mutants lead to increased red blood cell production due to reduced availability of O₂ [111].

2) Hereditary neutrophilia

In 2009, our group studied a three-generation pedigree with 12 patients suffering from neutrophilia (WBC median 21,350 cells/mm³ with >70% segmented neutrophils or band cells and <10% immature granulocytes). A *CSF3R* T640N mutation was identified to segregate with all cases. *In silico* modeling showed that this mutation led to a higher homodimerization of G-CSFR by forming interhelical hydrogen bonds between monomers. This mutation induced a spontaneous growth of granulocytic cells including CFU-G as well as a constitutive signaling in progenitors from patients. Moreover, the phenotype of patients was recapitulated in two different mouse models. First, in bone marrow transplantation after retroviral transfer, the mice presented a splenomegaly as well as high levels of GR-1 precursors in bone marrow, blood and particularly in spleen. Second, in a patient-derived xenograft model, the mice showed a myeloid bias at the expense of lymphoid B cells as well as a mobilization of progenitors in the blood. Altogether, this mutation is a gain-of-function mutation that induces a hyperplasia of the myeloid lineage, but contrary to other hereditary diseases can predispose to MDS since one patient from the family developed such hematological malignancies [112].

Conclusion and Future considerations

As described in this review, germline factors contributing to the development and/or evolution of sporadic and familial MPN are classified into three categories according to their allelic frequency in the general population and their penetrance (Figure 1). First, there are the common germline risk alleles, which increase weakly the risk of development of both sporadic and familial MPN. Second, the low-frequency germline variants that favor the clustering of MPN cases in families and third, the very rare germline Mendelian alleles that cause MPN-like hereditary diseases with a high penetrance. Altogether, these variants involve 3 major pathways (Figure 3). The *JAK2* pathway seems to be the major one, with different germline variants associated with variable functional effects in *JAK2* gene itself and in *EPO/EPOR*, *CSF3R*, *THPO/MPL* and *SH2B3* genes. This pathway is also the driver of acquired MPN with mutations in *JAK2*, *MPL* and *CALR* genes, suggesting that there is no

major difference in the pathogenesis of true and hereditary MPN-like disorders. The main difference is that the first one is clonal, implying that to obtain a clonal dominance many epigenetic modifications have occurred, which may explain the increased risk in thrombosis and of leukemia. The second are polyclonal and in most cases does not seem to predispose to leukemia and to slightly increase the risk of thrombosis. This could be interesting to better understand the mechanism of thrombosis in MPN because in hereditary disorders the variants are present outside the hematopoietic cells including endothelial cells. However, they do not develop disorders such as Budd Chiari, which has been suggested to be the consequence of *JAK2 V617F* in endothelial cells. Thus, they could be interesting models to understand the mechanism of thrombosis in MPN. In addition, this pathway may be also involved in true predisposition, maybe because germline and acquired mutations cooperate in intensifying the signaling increasing the probability of the acquired mutations to give rise to a MPN. The control of cell cycle/DNA repair is also a germline pathway recurrently affected (*TERT*, *RBBP6*, *CHEK2*, *ATM*, and *PINT* genes). Finally, transcriptional factors such as *MECOM* and *MYB* regulating myeloid gene expression are involved and probably impact the proliferation/differentiation processes. Accordingly, these pathways fit with the two predisposition hypotheses: “fertile ground” and “hypermutability”. Alongside these three pathways, one structural variant with three potential candidates (*ATG2B*, *GSKIP*, *TCL1A*) has been shown to be a very highly penetrant predisposing factor in familial MPN but the underlying molecular mechanism is still unclear. Deciphering the latter will pave the way for the identification of new molecules involved in this predisposition pathway.

One should note, that apart from *TERT*, none of the identified predisposition genes involved in familial MDS or/and AML have been implicated in familial MPN [113, 114]. However, they can impact some common main pathways such as transcription (the RNA helicase *DDX41* and the *GATA2*, *RUNX1*, *CEBPA* transcriptional factors), DNA repair/stress response (*ATM*, *P53*, *BRCA1/2*, *FANC*, *XPC* genes); and signaling (*ANKRD26* or *PTPN11* genes). The penetrance and the age of onset could vary between the different predisposing variants. They can be associated or not with preexisting defects or organ dysfunction. Alternatively, they can

be associated with only hematological defects such as thrombocytopenia, neutropenia or pancytopenia.

Overall, some of the MPN germline factors have been also involved not only in hematological malignancies but also in others cancers and the spectrum of diseases could be very different depending on the type of mutation. *VHL* mutations are also involved in pheochromocytoma, renal cancers and hemangioblastoma, *TERT* variants predisposes to many types of cancers (breast, thyroid, gliomas, lung...). Even some *SH2B3* variants could be associated with non-malignant diseases such as type 1 diabete or coronary heart disease. Thus, different variants in the same gene be associated with different phenotypes either because they contribute directly to the pathophysiology of different organs or because their effects are compensated in certain organs by other related genes.

The initiation of MPN is still very poorly understood and the discovery of some molecules or some pathways involved in familial clustering could be very important to dissect the initiation of sporadic MPN. However, this task is very challenging since many groups including ours have performed WES with no significant results. Several explanations may be proposed: (i) most of candidate variants are private germline variants restricted to one or two pedigrees and often associated with a reduced penetrance and consequently they do not perfectly segregate with the disease within families. Therefore, the demonstration of their implication in the disease require complex functional studies (ii) Complex structural variants or non-coding variants located either in deep-intronic regions or in regulatory regions missed by WES that focuses on coding variants may be involved. Their detection will be facilitated by whole-genome sequencing; (iii) oligogenic inheritance based on a combination of several low-penetrance variants may be responsible of familial clustering. These variants are currently difficult to identify.

Practice Points

. The majority of germline risk factors remains unknown and the disease initiation is difficult to predict

- . Following carefully patients at risk (familial cases) is of importance using sensitive detection methods of acquired mutations to diagnose and eventually treat as soon as the MPN develops.
- . Germline factors causing hereditary diseases are generally of good prognosis

Research agenda

- . Understand the function of already described germline variants
- . Understand the mechanism of predisposition to acquired and familial MPN
- . Development of global and combined approaches (RNA-seq, WGS) to find out more germline predisposing variants

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Figure legends

Figure 1: Disease predisposition according to allele frequency and penetrance of germline variants.

Figure 2: Localization of germinal variants within JAK2. The structure of the JAK2 is shown with functional domains, protein structural domains (JH7-JH1) and position of amino acid changes.

JH: JAK Homology domains; FERM: four-point-one ezrin, radixin, moesin; SH2: src homology2; A: ATP Nucleotide binding site; L, Kinase loop.

Figure 3: Pathways involved in predisposition to MPN and MPN-like diseases.

JAK2/STAT pathway: germline variants in *JAK2*, *EPO*, *EPOR*, *CSF3R*, *THPO*, *MPL*, *SH2B3* genes. Cell cycle/DNA repair pathway: germline variants in *TERT*, *RBBP6*, *CHEK2*, *ATM*, *PINT* genes. Myeloid gene expression pathway: germline variants *MECOM*, *MYB*, *GFI1B*, *TET2*. 14q CNV rearrangements with unknown function. Colored in blue: common germline risk variants increasing weakly the development of sporadic and familial MPN; in green: rare germline intermediate and low-penetrance variants; in orange: extremely rare germline Mendelian SNV that cause MPN-like hereditary diseases.

Figure 1

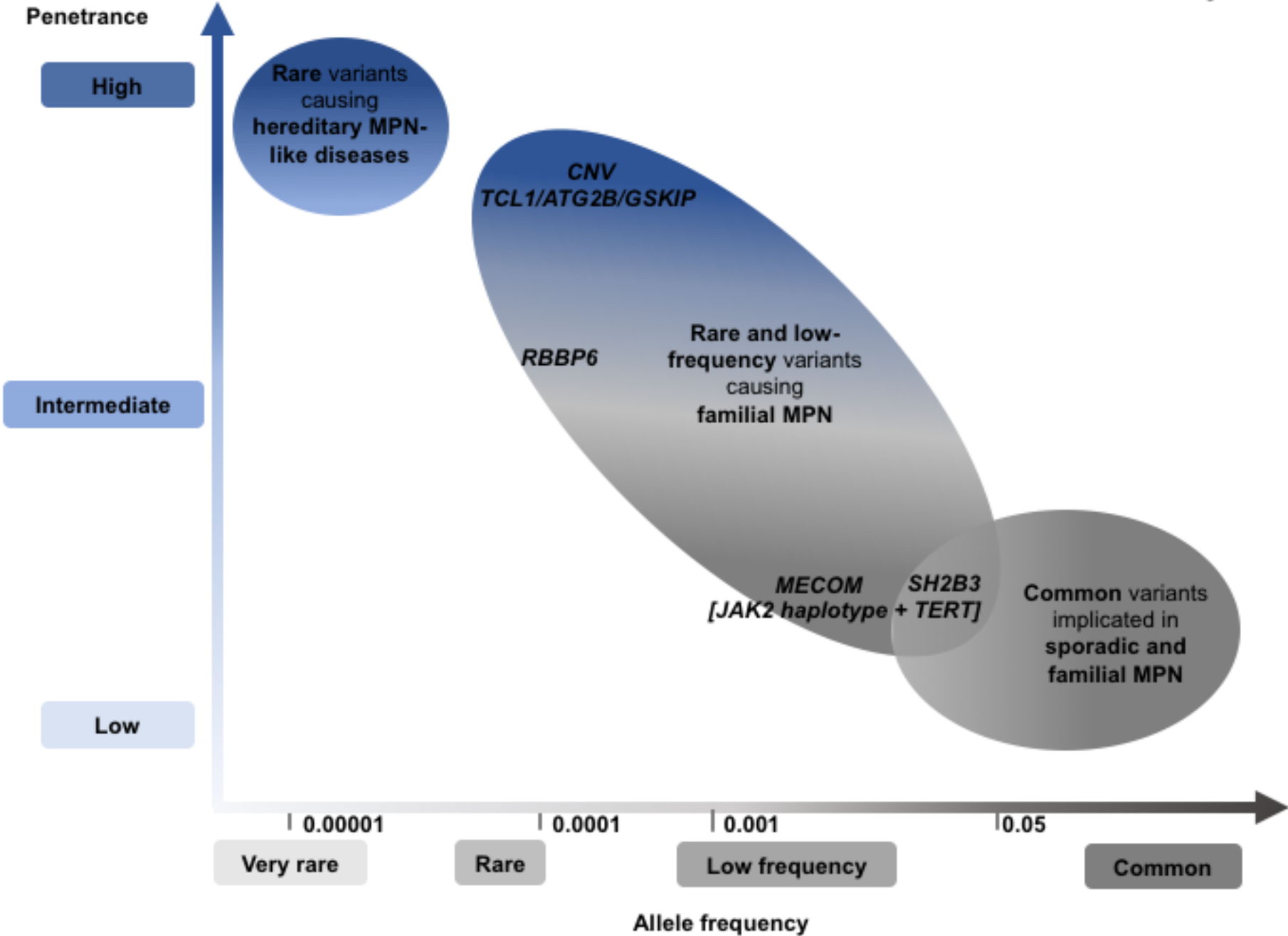


Figure 2

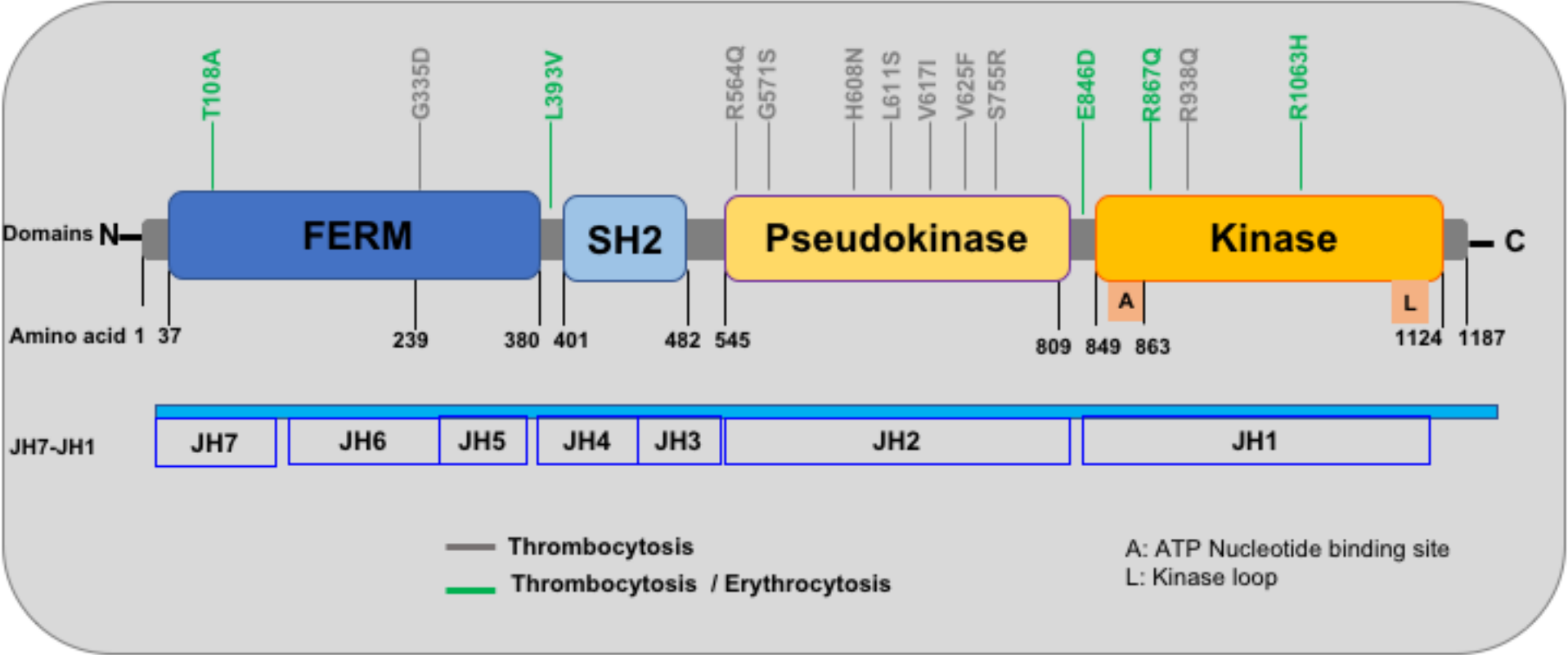


Figure 3

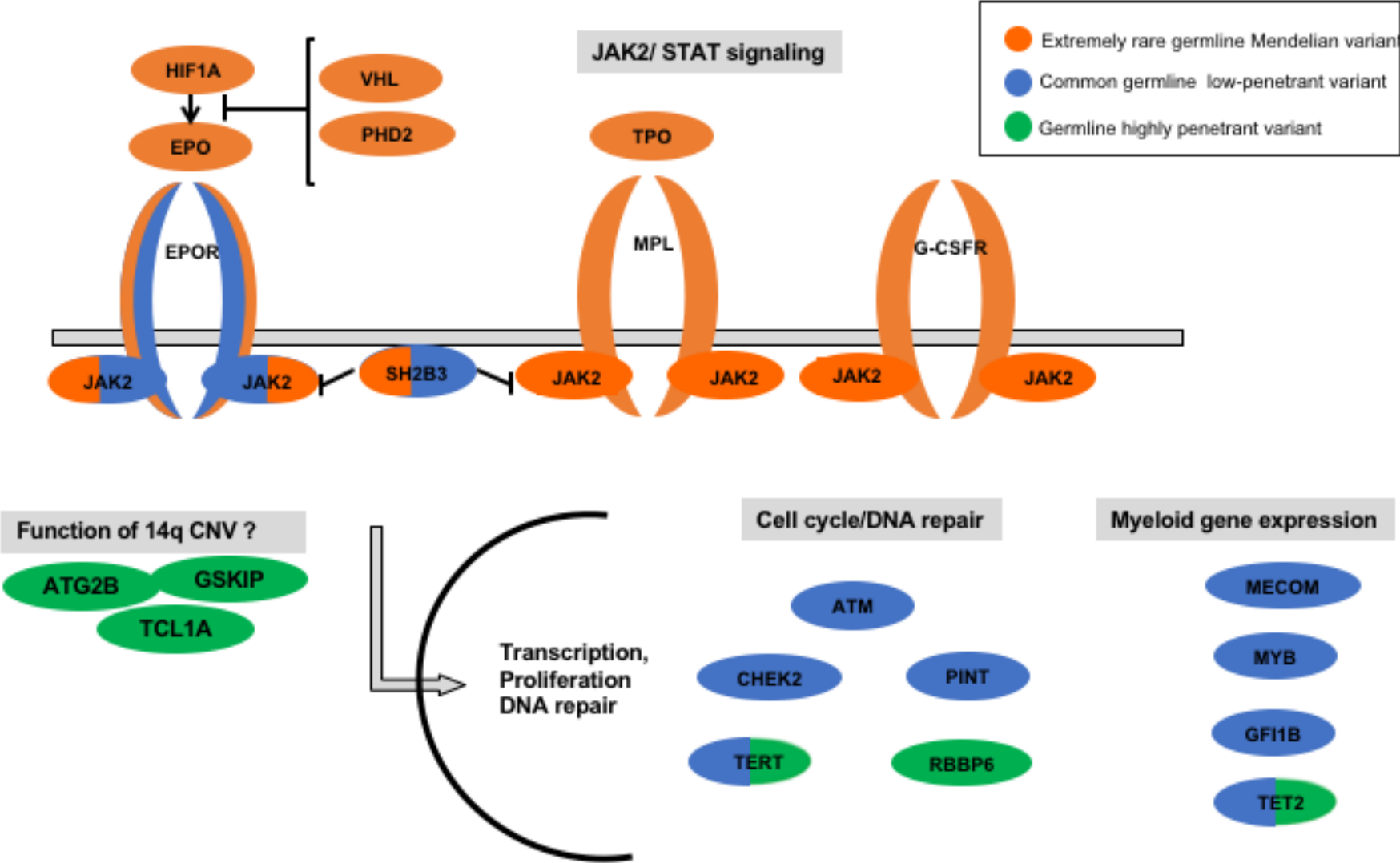


Table 1: Germline factors in MPN

High frequency common variant, low risk	Variants	Type of disease(s)	References
<i>ATM</i>	rs1800056	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN	[8]
<i>CHEK2</i>	rs555607708	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN	[8]
<i>EPOR</i>	rs318699	<i>JAK2</i> V617F-PV	[4]
<i>GFI1B</i>	rs621940	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN	[8]
<i>HBS1L</i> and <i>MYB</i> genes	rs9376092	All MPN (<i>JAK2</i> V617F-ET; <i>CALR/MPL</i> -positive MPN)	[10, 26]
<i>JAK2</i>	rs10758669, rs3808850, rs10974947	<i>JAK2</i> V617F-PV	[4]
	rs12343867, rs3780367, rs10974944	<i>JAK2</i> V617F-MPN	[5]
	rs10974944	<i>JAK2</i> V617F and <i>CALR</i> mutated -MPN	[24]
	rs7864782, rs10758669, rs7046736, rs12343867, rs10974944, rs2031904	<i>JAK2</i> V617F-MPN	[6]
	rs10974944, rs12500918	<i>JAK2</i> V617F-MPN	[7]
	rs10974944, rs1034072	MPN	[9]
	rs12339666	<i>JAK2</i> V617F-MPN	[10]
	rs12343867	<i>JAK2</i> exon 12 mutated - positive PV	[12]
	rs59384377, rs10974900	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN,	[8]
	rs12339666, rs10974944	Controversial on <i>CALR/MPL</i> mutated MPN	[10, 13, 15, 16]
	rs10974944	Familial MPN	[28]
	rs10974944	Familial <i>JAK2</i> V617F MPN	[40]
	rs10974944	<i>JAK2</i> V617F but not <i>CALR</i> mutated sporadic MPN cases <i>JAK2</i> V617F and <i>CALR</i> mutated familial cases	[16]
<i>NR3C1</i>	rs6198	MPN leukemic transformation	[21]
<i>IL6R</i>	rs4537545	<i>JAK2</i> V617F sporadic MPN cases	[23]
<i>MECOM (MDS1-EVII)</i>	rs2201862	All MPN All MPN except ET	[10] [26]
<i>SH2B3 (LNK)</i>	rs3184504 - p.R262W	ET, PMF	[19, 20]
	rs78894077 - p.R242S	ET	[20]
	rs7310615	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN	[8]
	p.E208Q	<i>JAK2</i> V617F-ET and <i>CALR</i> type 1 mutated PMF familial cases	[41]
	p.E208Q	Two families, only segregated with two <i>JAK2</i> V617F-positive PV, not <i>JAK2</i> V617F-positive ET cases	[42]
<i>TERT</i>	rs2736100	MPN	[9, 10, 24]
	rs2736100	Sporadic and familial MPN	[40]
	rs7705526, rs2853677	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN	[8]
<i>TET2</i>	rs1548483	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN	[8]
	p.D1858fs	PV	[44]
<i>THRB</i> and <i>RARB</i>	rs4858647	MPN stronger effect in <i>JAK2</i> V617F-negative MPN (<i>CALR</i> , <i>MPL</i>) PMF only	[10] [26]

<i>PINT</i>	rs58270997	<i>JAK2</i> V617F asymptomatic carriers, <i>JAK2</i> V617F-MPN	[8]
Rare germline variants high-risk	Variants	Type of disease(s)	References
<i>TCL1A/ATG2B/GSKIP</i>	14q32.13-q32.2 700 kb CNV (g.96,163,103_96,857,129dup)	6 families with ET mainly and other hematological myeloid malignancies (PMF, AML, LMMC, MDS)	[32]
	14q32 700kb CNV encompassing <i>TCL1A</i>	1 family with 7 cases with PMF, <i>de novo</i> AML and MDS (RARS-T and RCMD-RS)	[60]
	14q32 1.8 Mb CNV (chr14:94,387,112_96,219,286dup)	1 family with 4 cases of lymphoma, PMF, <i>de novo</i> and secondary AML and MDS (RS-MLD-RS)	[59]
<i>RBBP6</i>	p.R1569H, p.E1654G, p.R1451T	PMF families, associated with <i>JAK2</i> haplotype and <i>TERT</i> rs2736100	[45]
<i>ZXDC</i> , <i>ATN1</i> and <i>LRRC3</i> (potential candidates)	<i>ZXDC</i> (p.P644R), <i>ATN1</i> (p.R234Q) and <i>LRRC3</i> (p.R234Q)	One PV family	[46]
Extremely rare germline variant causing MPN-like hereditary diseases	Variants	Type of disease(s)	References
<i>EGLN1 (PHD2)</i> (AD)	p.P317R ; More than 25 mutations	Hereditary erythrocytosis	[108, 110]
<i>EPAS1 (HIF2A)</i> (AD)	Around 10 mutations	Hereditary erythrocytosis	[106]
<i>EPO</i> (AD)	p.W11fs and p.P7fs	Hereditary erythrocytosis	[102, 103]
<i>EPOR</i> (AD)	More than 20 truncating mutations	Hereditary erythrocytosis	[94, 95, 97, 98, 101, 106]
<i>SH2B3 (LNK)</i>	p.E208Q, p.E400K	Hereditary erythrocytosis	[91, 92]
<i>VHL (AR)</i>	p.R200W + more than 15 other mutations	Hereditary erythrocytosis	[104, 106]
	Complex splicing defects (new pseudo-exon 1) and in exon 2 (Synonymous variant)	Hereditary erythrocytosis	[107]
<i>JAK2 (AD)</i>	p.T108A, p.L393V	Hereditary erythrocytosis and thrombocytosis	[90]
	p.R564Q	Hereditary thrombocytosis	[83]
	p.H608N	Hereditary thrombocytosis	[82]
	p.L611S	Congenital thrombocytosis	[81]
	p.V617I	Hereditary thrombocytosis	[84, 85]
	p.S755R, p.R867Q, p.R938Q	Hereditary thrombocytosis	[87]
	p.R867Q	Hereditary thrombocytosis with PV progression	[88]
<i>MPL (AD, AR)</i>	p.E846D, p.R1063H	Hereditary erythrocytosis and thrombocytosis	[89]
	Homozygous p.K39N (Baltimore polymorphism)	Hereditary thrombocytosis	[72]
	Heterozygous p.R102P	Hereditary thrombocytosis	[80]
	Homozygous p.P106L	Hereditary thrombocytosis	[73-75]
	Heterozygous p.S505N	Hereditary thrombocytosis	[69, 70]
<i>THPO (AD)</i>	Heterozygous p.W515R	Hereditary thrombocytosis	[71]
<i>THPO (AD)</i>	5'UTR and variants affecting donor splice site of exons 2 and 3	Hereditary thrombocytosis	[64-68]
<i>CSF3R (AD)</i>	p.T640N	Hereditary neutrophilia	[112]

CNV, copy number variation; AD, Autosomal dominant; AR, autosomal recessive