Genetics of Myeloproliferative Neoplasms

Jakub Szybinski, PhD[a], Sara C. Meyer, MD, PhD[a,b,*]

KEYWORDS

• Myeloproliferative neoplasms • Genetics • Genomics • JAK2 • Calreticulin • MPL
• Genetic alterations

KEY POINTS

- Phenotypic driver mutations in JAK2, calreticulin, and MPL are present in 85% to 90% of myeloproliferative neoplasms and induce constitutive activation of JAK2-STAT signaling.
- Modern sequencing efforts have revealed large parts of the genomic landscape of myeloproliferative neoplasms with additional genetic alterations mainly in epigenetic modifiers and splicing factors.
- Genetic alterations in myeloproliferative neoplasms are subject to clonal evolution as myeloproliferative neoplasms progress, with high molecular risk mutations impacting dynamics and outcome.
- Because JAK2 V617F is not specific to myeloproliferative neoplasms and is seen in other myeloid malignancies, it is important to note JAK2 V617F is recurrently detected in clonal hematopoiesis of indeterminate potential.
- The expanding insight into the genetic basis has facilitated diagnosis and prognostication of myeloproliferative neoplasms and poses novel candidates for targeted therapeutic intervention.

BACKGROUND

Myeloproliferative neoplasms (MPN) are hematopoietic stem cell disorders with dysregulated production of mature myeloid blood cells including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). They were first grouped together as an entity in 1951 by William Dameshek, suggesting that they are driven by a so far undiscovered stimulus.[1] Over the last 15 years, constitutive

Conflicts of interest: S.C. Meyer has consulted for and received honoraria from Celgene/BMS.
Funded by: Swiss National Science Foundation and others.
[a] Department of Biomedicine, University Hospital Basel and University of Basel, Hebelstrasse 20, Basel CH-4031, Switzerland; [b] Division of Hematology, University Hospital Basel, Petersgraben 4, Basel CH-4031, Switzerland
* Corresponding author. Division of Hematology, University Hospital Basel, Petersgraben 4, Basel CH-4031, Switzerland
E-mail address: sara.meyer@unibas.ch

Hematol Oncol Clin N Am 35 (2021) 217–236
https://doi.org/10.1016/j.hoc.2020.12.002 **hemonc.theclinics.com**
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activation of JAK2-STAT signaling has been revealed as a common characteristic of MPN owing to somatic mutations in the tyrosine kinase JAK2, the chaperone protein calreticulin (CALR) or the thrombopoietin receptor MPL in the majority of patients[2] (Fig. 1). In addition, the advent of modern sequencing technologies has enabled detailed investigation of the genomic landscape of MPN. A set of additional mutations frequently seen also in other myeloid malignancies, often co-occurs contributing to the clinical phenotype, disease dynamics, and overall outcome[3] (Fig. 2). In this review, we provide an overview of the genetics in MPN, which by now provides us with helpful diagnostic biomarkers[4] and contributes to refined prognostication of several MPN subsets.[5,6] Importantly, the extensive characterization of the genetic basis has revealed several promising candidates, which could serve as targets for novel, mechanism-based therapeutic approaches, which represents a current need of patients with MPN.

SOMATIC DRIVER MUTATIONS MEDIATING CONSTITUTIVE JAK2-STAT ACTIVATION

The discovery of the JAK2 V617F mutation in 2005 by several groups using different methodologies was a breakthrough for the field and initiated the era of genetic characterization of MPN, which has progressed at a rapid pace.[7–10] A somatic JAK2 V617F

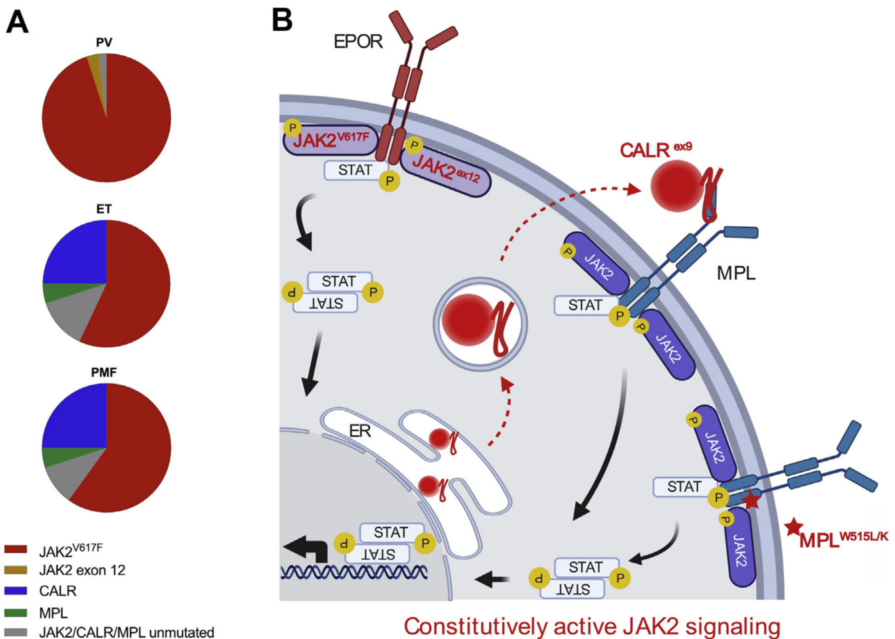

Fig. 1. Somatic driver mutations in MPN activating JAK2-STAT signaling. (*A*) Approximate frequencies of JAK2 V617F, JAK2 exon 12, CALR and MPL mutations in PV, ET, and PMF. JAK2/CALR/MPL unmutated cases are referred to as triple negative MPN. (*B*) JAK2 V617F mutations occur in association with EPOR and MPL in all MPN subtypes including PV, ET, and PMF. JAK2 exon 12 mutations exclusively occur in association with EPOR in PV. CALR mutations locate to exon 9 and occur in ET and PMF. MPL mutations are in exon 10 with missense mutations affecting mostly residue W515 and occur in ET and PMF. Somatic driver mutations in JAK2, CALR, and MPL converge on constitutively activated JAK2-STAT signaling. EPOR, erythropoietin receptor; ER, endoplasmic reticulum; MPL, thrombopoietin receptor.

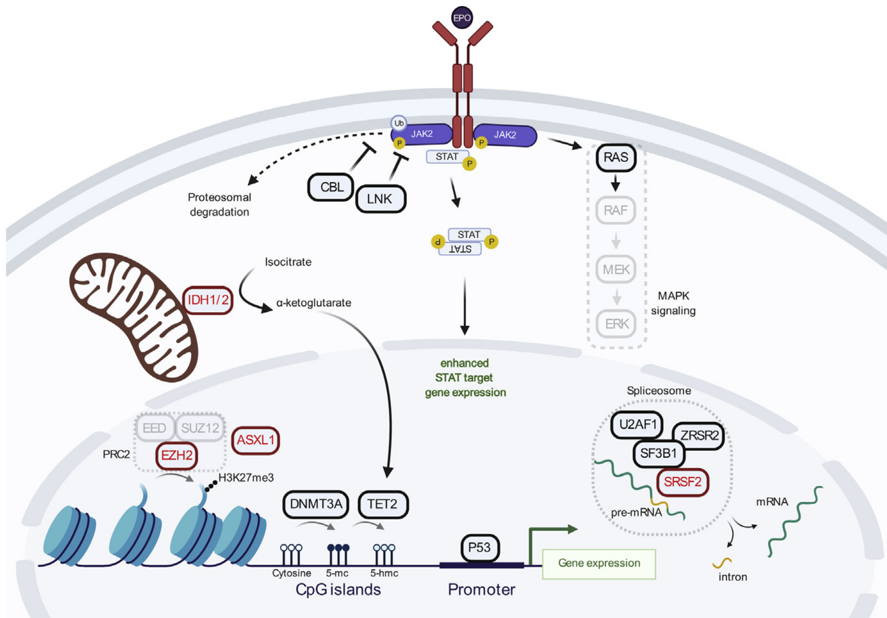

Fig. 2. Somatic mutations in genes broadly affected in myeloid malignancies occurring in MPN. Somatic mutations in myeloid cancer genes are often co-mutated in MPN, particularly in PMF. Epigenetic regulators involved in DNA methylation (DNMT3A), demethylation (TET2), and in histone modification relating to the PRC2 complex (EZH2, ASXL1), as well as factors involved in messenger RNA splicing (SRSF2, U2AF1, SF3B1, ZRSR2) are most frequently affected. IDH1 and IDH2 mutations lead to accumulation of the oncometabolite 2-hydroxyglutarate instead of physiologic α-ketoglutarate, which interferes with TET2 function. Factors activating (RAS) or regulating (CBL, LNK/SH2B3) signaling as well as factors involved in transcriptional regulation/DNA repair (TP53) are also found mutated. Mutations in ASXL1, EZH2, IDH1/2, and SRSF2 (highlighted in *red*) are considered high molecular risk (HMR) mutations, because they confer adverse prognosis, whereas mutations in TP53, IDH1/2, and SRSF2 are enriched in blast-phase MPN. 5-hmc, 5-hydoxymethylcytosine; 5-mc, 5-methylcytosine; EPO erythropoietin; PRC2, polycomb repressive complex 2.

mutation is present in the majority of patients with MPN, including 95% of PV and 50% to 60% of ET and PMF (see **Fig. 1**, **Table 1**). The JAK2 non–receptor tyrosine kinase essential for hematopoietic cytokine signaling via erythropoietin, thrombopoietin and granulocyte colony stimulating factor receptors is constitutively activated by a G to T transition in exon 14 mediating a valine to phenylalanine substitution at position 617 of the protein. Functionally, the inhibitory effect of the JAK2 pseudokinase domain on the kinase domain is abrogated.[11] It results in the constitutive activation of JAK2-driven signaling pathways, including STAT1, STAT3, and STAT5 transcription factors, as well as the PI3K/AKT and the MAPK signaling pathway promoting proliferation, differentiation, and survival of myeloid progenitor cells.[12] The JAK2 V617F mutation acquired at the level of hematopoietic stem cells is in line with the occurrence of erythrocytosis, thrombocytosis, and/or leukocytosis in the peripheral blood.[13] However, the question how JAK2 V617F may induce the differential phenotypes of PV with predominant erythrocytosis, ET with isolated thrombocytosis, and PMF with increased megakaryocytes, bone marrow fibrosis, and progressing cytopenias in patients displaying the same V617F missense mutation in JAK2 is incompletely understood. It has been shown that gene dosage and mutant allele frequency impact on the clinical

Table 1
Approximate frequencies of somatic driver mutations in MPN

Mutation		Frequency (%)			
Gene	**Location**	**PV**	**ET**	**PMF**	**Molecular Function**
JAK2	V617F exon 14	95	50–60	50–60	Non–receptor tyrosine kinase mediating hematopoietic cytokine signaling
JAK2	exon 12	2–3	-	-	
CALR	exon 9	<1	26	18–32	ER chaperone protein interacting with thrombopoietin receptor MPL
MPL	exon 10	<1	4	5–9	Thrombopoietin receptor

presentation, because a low allele burden of JAK2 V617F rather presents as ET versus higher mutational burden presenting rather as PV.[14] Of note, it has been shown that the MPN clone is often homozygous for JAK2 V617F in PV owing to a loss of heterozygosity at chromosome 9p by uniparental disomy, whereas JAK2 V617F is mostly heterozygous in ET.[15] The concomitant presence of additional mutations as discussed elsewhere in this article, as well as the order in which the mutations are occurring during clonal evolution, may also impact the clinical picture.[3,16]

Subsequent sequencing efforts in JAK2 V617F–negative MPN revealed several small insertions and deletions or missense mutations in exon 12 of JAK2 in 2007, which occur exclusively in PV, accounting for 2% to 3% of patients, but not in ET and PMF.[17] Interestingly, JAK2 exon 12 mutated patients with PV preferentially present with pronounced erythrocytosis in the absence of concomitant thrombocytosis or leukocytosis, whereas JAK2 V617F rather is associated with an older age at diagnosis and erythrocytosis, often accompanied by neutrophilia and/or thrombocytosis.[18] The association of these specific JAK2 genotypes with differential PV phenotypes has been recapitulated in preclinical models as well.[19]

Although JAK2 V617F or exon 12 mutations provide a genetic basis for the constitutive activation of JAK-STAT signaling in the vast majority of patients with PV, a large proportion of patients with ET and patients with myelofibrosis are negative for genetic alterations in JAK2.[12] In 2006, missense mutations in the thrombopoietin receptor MPL, which signals through JAK2, were identified at position 515 in 4% of patients with ET and 5% to 9% of patients with PMF, but not in patients with PV.[20] Although W515L represents the most frequent alteration in the MPL gene, W515K and rarely others have also been reported and similarly induce constitutive activation of JAK2 signaling.[21]

After these exciting discoveries in 2005 to 2007, the genetic basis of JAK2- and MPL-unmutated ET and PMF remained elusive for several years, leaving 30% to 40% of patients without a known driver mutation. The adaptor protein LNK (SH2B3) and the Casitas B-lineage lymphoma proto-oncogene CBL, negative regulators of JAK2, were found mutated in a relatively small proportion of patients, also leading to JAK-STAT activation.[22,23] Although LNK, an inhibitor of erythropoietin and thrombopoietin signaling, is mutated in 0% to 9% of patients with MPN, inactivating CBL mutations interfere with ubiquitin ligase activity and induce prolonged activation of JAK2 signaling in up to 6% of PMF or secondary acute myeloid leukemia (AML). It was only in 2013 that 2 groups reported another breakthrough discovery identifying a putative driver mutation in 67% to 88% of the JAK2 and MPL wild-type MPN patients. By applying whole exome sequencing to individuals with wild-type genotypes for JAK2 and MPL, both groups independently identified somatic mutations in CALR, an endoplasmic reticulum (ER) chaperone protein that has not been previously implicated in

cancer development.[24,25] Under physiologic conditions, CALR is involved in the appropriate folding of glycoproteins in the lumen of the ER containing a C-terminal ER retention signal with a characteristic KDEL sequence.

CALR is also implicated in calcium homeostasis given its negatively charged C-terminus facilitating calcium binding. Of the more than 35 CALR mutations identified, all localize to C-terminal exon 9 and result in a 1 bp frameshift inducing a novel C-terminal sequence. The 2 most frequent mutations accounting for 85% of the alterations include a 52 bp deletion ($CALR^{del52}$, type 1) with 44% to 53% of patients and a 5 bp insertion ($CALR^{ins5}$, type 2) in 32% to 42% of patients. Alternative insertions or deletions with lower frequencies account for the remainder (type 1 like or type 2 like). It rapidly became clear that the observed CALR mutations all interfere with the distal ER retention signal via the altered C-terminal sequence, with the loss of the typical negative charge and that activation of JAK2-STAT signaling as a common feature of MPN was preserved also in CALR mutant MPN. Intense research efforts over the last years have delineated how JAK2-STAT activation from mutated CALR may occur and how the CALR chaperone may function as an oncogene in MPN. Several elegant studies have shown that ER-located mutant CALR associates with MPL, inducing aberrant activation of MPL-JAK2 signaling, whereas mutant CALR would also leave the ER and associate with MPL at the cell surface.[26–28] These findings provide a plausible explanation as to why CALR mutant MPN phenocopy MPL mutant MPN to large extents. Differential aspects relating to CALR as a driver include the findings of more pronounced thrombocytosis, slightly lower hemoglobin levels, presentation at a younger age, and a lesser incidence of thromboembolic events in CALR versus JAK2 V617F mutant ET, which has implications for prognostication and clinical management.[29] In PMF, patients with a CALR mutant show a more favorable prognosis as compared with patients with JAK2 V617F and MPL mutations, which primarily relates to type 1 CALR mutations, with a significantly prolonged survival, making them a relevant parameter for modern, molecularly based prognostication schemes in myelofibrosis.[5,6,30,31]

Although JAK2, CALR, and MPL mutations are mostly mutually exclusive in MPN given their redundant effects with constitutive activation of JAK-STAT signaling, "double hits" with 2 concomitant JAK2 mutations as, for example, JAK2 V617F and a JAK2 exon 12 mutation or JAK2 V617F with a concomitant JAK2 R1063H mutation, have been reported, albeit rarely.[3,32,33] Alternatively, concomitant CALR or MPL mutations may also rarely occur, mostly in the setting of low allele burden JAK2 V617F. The clonal architecture of such cases is not entirely clarified.

The insight into the genetic basis of MPN in the last 15 years now provides us with information on somatic driver mutations in a majority of patients, because only approximately 2% of PV and approximately 10% of ET and PMF are unmutated for JAK2, CALR, or MPL. These "triple-negative" MPN require a particularly diligent diagnostic workup, because reactive causes for a phenotype suggestive of MPN as well as alternative myeloid malignancies need to be carefully excluded. It has been reported that triple-negative MPN tested positive for typical somatic driver mutations at low mutant allele burden when resequenced using methodologies with greater sensitivity.[3,32,33] In addition, noncanonical somatic mutations in JAK2 and MPL have also been identified, as well as germline variants, implying a familial basis of thrombocytosis or erythrocytosis of nonclonal origin.[32–34] Truly triple-negative ET have typically been found in young, female patients with a benign prospect.[3] In contrast, triple-negative PMF associates with significantly poorer outcome as compared with CALR, JAK2, or MPL mutant PMF, which show a more favorable prognosis in decreasing order. An adverse prognosis of triple-negative PMF implies increased risk of progression as well as

shortened overall survival and should be considered for decisions on clinical management.[30] With sequencing technologies rapidly moving forward, somatic driver mutations in the triple-negative MPN might also be revealed.

Although the identification of the somatic driver mutations in JAK2, CALR, and MPL has provided us with a set of biomarkers greatly facilitating the diagnosis of MPN, the finding of constitutively activated JAK-STAT signaling resulting from each of these driver mutations has posed a relevant target for therapy.[2] Consequently, JAK2 inhibitors have been developed and 2 compounds, ruxolitinib and fedratinib, have entered clinical use.[35–38] Because these currently available JAK2 inhibitors, which bind to the ATP pocket of the JAK2 kinase domain, are not selective for the JAK2 V617F mutant form of the kinase, they are able to interfere with activated JAK2-STAT signaling not just in JAK2 mutant, but also in CALR or MPL mutant patients, as well as in triple-negative MPN. Of note, the recent findings on mutant CALR being exposed at the cell surface could provide a therapeutic target specific to the MPN clone, which may be addressable in the future.[28]

SOMATIC MUTATIONS IN GENES BROADLY AFFECTED IN MYELOID MALIGNANCIES

Several genes commonly mutated across myeloid malignancies were found to be affected by somatic mutations also in MPN[25,39] (see **Fig. 2**, **Table 2**). Overall, more than one-half of individuals suffering from MPN carry accompanying mutations in these myeloid cancer genes with increasing frequency at more advanced ages and most prominently in PMF. Epigenetic modifiers and factors involved in messenger RNA splicing are predominantly affected, whereas blast phase MPN shows characteristic additional mutations.[3]

Epigenetic Modifiers

The DNA methylation status of CpG islands modulating gene expression results from a complex interplay of methylating and demethylating events. DNMT3A represents a de novo methyltransferase prevalently mutated in AML, most commonly with the hotspot mutation R882H. DNMT3A mutations were also frequently found in MPN with 3% to 15% in patients with PMF and up to 9% in patients with PV and patients with ET.[40] A loss of DNMT3A function has been shown to occur early in MPN disease evolution, typically before the acquisition of JAK2 V617F and to induce a clonal advantage with overall expansion of the hematopoietic stem cell pool.[39,41] The dynamics of clonal evolution seem to relate to specific MPN phenotypes with PMF developing rather from JAK2 V617F mutant clones with preexisting DNMT3A mutations, whereas JAK2 V617F followed by late acquisition of genetic alterations in DNMT3A would favor the occurrence of PV or ET.[42]

DNA demethylation occurs via the generation of 5-hydroxymethylcytosine converted from 5-methylcytosine by TET2 found to be mutated in solid tumors and myeloid malignancies. Also in MPN, TET2 mutations are prevalent and found in 7% to 22% of patients without a clear prognostic effect.[43] Genetic alterations in TET2 mediate expansion of the hematopoietic stem cell pool.[44] Similarly to DNMT3A, mutational order impacts clinical phenotype with patients acquiring JAK2 V617F before a TET2 mutation presenting rather with PV or ET as compared to patients acquiring JAK2 V617F afterwards.[16] The observation that TET2 mutations are mutually exclusive with mutations in the 2 isoforms of isocitrate dehydrogenase IDH1 and IDH2 with largely overlapping DNA methylation and gene expression patterns, has revealed their redundant functional effects. IDH1/2 mutations, initially described in glioblastoma and AML, induce the accumulation of the oncometabolite

Table 2
Approximate frequencies of additional somatic mutations in MPN

Class	Mutated Gene	Frequency (%) PV	ET	PMF	Molecular Function
Epigenetic regulation	DNMT3A	2–7	0–9	3–15	De novo DNA methylase
	TET2	19–22	5–16	10–18	DNA demethylase
	IDH1/2[a]	2	1	0–6	Isocitrate dehydrogenase generating 2-HG
	ASXL1[a]	3–12	1–11	13–37	Chromatin remodeling as Polycomb group protein
	EZH2[a]	0–3	1–3	1–9	PRC2 complex H3K27me3 methyltransferase
	SUZ12	2–3	<1	2	PRC2 complex component
Messenger RNA splicing	SRSF2[a]	3	2	8–18	Serine/arginine-rich splicing factor
	U2AF1	<1	1	6–16	Spliceosome component
	SF3B1	3	5	6–10	Splicing factor 3B protein complex subunit 1
	ZRSR2	5	3	4–10	Spliceosome component
Signaling	N/KRAS	0–1	<1	3–4	Small GTPase activating MAPK pathway signaling
	CBL	1	1	4–7	E3 ubiquitin-protein ligase regulating JAK2
	SH2B3 (LNK)	9	3	3–6	Adaptor regulating hematopoietic signaling incl. JAK2
	PTPN11	<1	0–2	0–2	Protein tyrosine phosphatase dephosphorylating RAS
Transcriptional regulation	RUNX1	0–2	0–2	3–4	Transcription factor involved in differentiation of hematopoietic stem cells
	NFE2	2–3	<1	0–3	Transcription factor involved in myelopoiesis
DNA repair	TP53	1	2–6	1	Transcription factor, cell cycle regulator
	PPM1D	1	2	1	Regulatory inhibitor of TP53

[a] High molecular risk (HMR) mutations.

α-ketoglutarate interfering with proper TET2 function.[45] Of note, IDH1/2 mutations, which are detected in 1% to 6% of patients with MPN, significantly impact prognosis in PMF, with a higher risk for secondary AML, earlier transformation, and lower overall survival. In addition, IDH1/2 mutations are enriched in blast phase MPN occurring in 19% to 31% of patients.[46]

The factors involved in histone methylation via polycomb repressive complex 2 are also mutated in MPN, as for example, EZH2 and ASXL1, which mediate adverse prognostic effects. Genetic alterations in EZH2, the enzymatic component of polycomb repressive complex 2 mediating methylation at H3K27, are detected particularly in patients with PMF at 1% to 9%.[47] Several studies demonstrated aggravating effects on bone marrow fibrosis and observed EZH2 mutations to associate with adverse prognosis and decreased overall survival.[48,49] ASXL1, which is involved in mediating polycomb repressive complex 2 function, is mutated in 13% to 37% of patients with PMF and 1% to 12% of patients with PV and patients with ET.[50] Analogous to its adverse prognostic effect in other myeloid malignancies, ASXL1 mutations are also unfavorable in MPN and associate with a poor outcome.[51]

In addition to somatic mutations in epigenetic regulators modifying the epigenetic landscape in MPN, JAK2 V617F itself has also been reported to mediate epigenetic functions. JAK2 V617F phosphorylates the protein arginine methyltransferase PRMT5, which is increasingly studied in different malignancies, including MPN.[52,53] Additional reports have described the potential of JAK2 V617F to localize to the nucleus impacting on histone H3 phosphorylation.[54]

Splicing Factors

Mutations in genes involved in messenger RNA splicing are frequent in myeloid malignancies, particularly in myelodysplastic syndromes (MDS). Splicing factors are also recurrently mutated in MPN with up to approximately 20% of patients with PMF harboring genetic alterations in SRSF2, U2AF1, SF3B1 or ZRSR2 leading to missplicing.[55] Although SF3B1 is typically seen in MDS/MPN with ringed sideroblasts and thrombocytosis and rather associates with a favorable prognosis in this setting,[56] SRSF2 confers an increased risk for leukemic transformation and shortened overall survival in patients with PMF.[57]

Signaling Molecules

Beyond the somatic driver mutations in JAK2, CALR, and MPL, additional signaling molecules may also be subject to mutational events implicated in MPN pathogenesis. RAS isoforms, including KRAS and NRAS, which are central drivers of MAPK pathway signaling, represent well-established oncogenes not only in solid tumors, but also in myeloid malignancies.[58] Although AML particularly and certain MDS/MPN overlap syndromes (eg, chronic myelomonocytic leukemia) recurrently show somatic RAS mutations, N/KRAS have also been found mutated in MPN,[3,25,39] for which a relevance of MAPK signaling has been established.[59] Recent studies evaluating the significance of RAS activation in myelofibrosis have shown somatic N/KRAS mutations in 6% of patients.[3] They were typically subclonal relative to other, clonal genetic alterations and associated with progressive disease and additional, high molecular risk mutations. N/KRAS mutations predicted increased risk for leukemic transformation and significantly shorter overall survival, which was improved in patients treated with the JAK2 inhibitor ruxolitinib.[60]

High Molecular Risk Mutations and Blast Phase Myeloproliferative Neoplasms

Concomitant mutations in myeloid cancer genes as discussed elsewhere in this article play significant roles in determining MPN phenotypes, progression, and outcome.[3] Although the sequence of mutation acquisition may determine the presentation of PMF versus PV/ET as shown for TET2 and DNMT3A, several studies have demonstrated adverse prognostic effects mediated by a greater overall number of mutations, which often increase upon progression.[39] Unfavorable outcomes have directly been related with mutations in ASXL1, IDH1/2, EZH2, and SRSF2, which are therefore designated as high molecular risk mutations and are increasingly implemented in modern, molecularly-based prognostication schemes.[55] In blast phase MPN, mutations in IDH1/2 and SRSF2 are enriched and occur at an increased frequency.[46,61] In contrast, JAK2 V617F is frequently lost upon transformation to blast phase MPN, highlighting a clonal evolution from early subclones preceding JAK2 V617F or from independent JAK2 V617F negative clones.[62,63] Of note, genetic alterations in the tumor suppressor TP53 are seen in up to 35% of patients upon transformation. TP53 mutations often herald blast phase when acquired in advanced MPN and mostly affect both alleles via independent emergence of mutations or uniparental disomy at chromosome 17p.[61]

GERMLINE GENETIC FACTORS INVOLVED IN MYELOPROLIFERATIVE NEOPLASMS

Familial clustering of MPN with 5% of patients with MPN having an affected family member has suggested germline predisposition alleles, which increase the susceptibility to acquire somatic MPN driver mutations.[64] In 2009, several groups identified a so-called 46/1 or GGCC haplotype still representing the strongest germline predisposing factor for MPN today with 3- to 4-fold increased risk to develop sporadic or familial MPN.[65] The haplotype marked by several single nucleotide variants (as eg, rs10974944) encompasses the JAK2 gene itself and the somatic JAK2 V617F has typically been found in cis with the predisposition allele.[66] In addition, GWAS studies have identified germline susceptibility alleles also in TERT, MECOM, TET2, and SH2B3 (LNK) genes.[67] Although more prevalent risk alleles may promote the occurrence of sporadic or familial MPN, rarer germline factors would more strictly associate with familial cases as, for example, variants in RBBP6 involved in p53 function.[68] Importantly, analyses of multiple pedigrees demonstrated analogous clinical manifestations, genetic landscape, prognosis, and dynamics of progression for familial MPN as compared with sporadic cases.[69] In contrast, very rare germline variants with a high penetrance, which are involved in erythropoietin and thrombopoietin signaling, have been shown to mediate nonclonal, “MPN-like” diseases affecting only 1 myeloid lineage with a benign prognosis and no prospect of transformation. Such germline mutations in THPO, MPL, and JAK2 genes have been reported in hereditary thrombocytosis, whereas variants in EPOR, EPO, SH2B3, VHL, EGLN1, and EPAS1 (HIF2A) are known in hereditary erythrocytosis and germline mutations in CSF3R in hereditary neutrophilia. These hereditary MPN-like disorders have been reviewed in detail elsewhere.[68]

SOMATIC DRIVER MUTATIONS OF MYELOPROLIFERATIVE NEOPLASMS IN CLONAL HEMATOPOIESIS

Somatic driver mutations of MPN, including JAK2 V617F, are not restricted to MPN, but may occur in MDS, MDS/MPN overlap syndromes, and AML at lower frequencies.[70,71] Of note, the JAK2 V617F mutation has also been identified in clonal hematopoiesis of indeterminate potential (CHIP). This condition has gained increasing interest in recent years and delineates a hematopoietic clone arising from a hematopoietic stem cell with a somatic myeloid cancer gene mutation in a healthy individual with normal peripheral blood counts.[72] Although first insights came from X-chromosome inactivation studies showing an age-dependent skewing of hematopoiesis, modern sequencing methodologies have revealed clonal hematopoiesis characterized by somatic mutations in a greater than expected proportion of healthy persons. CHIP turns out to be prevalent and represents an age-dependent process, with 0.6% of individuals younger than 60 years, but up to 19.5% of individuals greater than 90 years of age presenting with clonal hematopoiesis. The most frequent are mutations in DNMT3A, TET2, ASXL1, SRSF2, and SF3B1 followed by JAK2 V617F.[73–76] Differential patterns have been described with DNMT3A and JAK2 V617F CHIP observed already in young adults with an increasing prevalence at older ages, whereas CHIP, characterized by splicing factor mutations as, for example, in SRSF2, rather present after the age of 70 years with a high risk for evolution to a myeloid malignancy.[77]

Why would JAK2 V617F CHIP matter? On the one hand, clonal hematopoiesis has been associated with an increased risk for vascular events, including coronary heart disease and ischemic stroke. This risk for vascular complications is particularly high for JAK2 V617F mutant CHIP and has been shown to increase with CHIP clone

size.[78] It is likely that JAK2 V617F CHIP cooperates with classic vascular risk factors as hypertension, hyperlipidemia, diabetes, and smoking and may further promote vascular complications. Thus, JAK2 V617F CHIP may promote morbidity and mortality in so far healthy individuals in the absence of overt MPN. On the other hand, similar to CHIP with other myeloid cancer gene mutations, JAK2 V617F CHIP represents a premalignant stage with an increased risk of developing MPN. A study in more than 4000 healthy individuals reported higher platelet counts in JAK2 V617F versus wild-type CHIP, but still in the normal range, highlighting a propensity toward MPN development.[77] In a complementary approach, patients with JAK2 V617F mutant MPN with an availability of blood samples several years before MPN diagnosis were studied, which demonstrated JAK2 V617F CHIP in a majority of them.[79] Of note, the dynamics of clonal evolution were very variable between individuals ranging from an 0.36% to 6.20% annual increase of the JAK2 V617F allele burden, also among patients without co-mutations in common myeloid cancer genes. The 46/1 haplotype promoted the expansion of JAK2 V617F CHIP clones, but additional, as yet unknown factors are likely to contribute to this process. Given the prevalence and clinical consequences of JAK2 V617F CHIP, investigations into the biological processes driving clonal evolution and progression to overt MPN as well as vascular complications in pre-MPN and MPN phases are warranted.

CLINICAL SIGNIFICANCE OF GENETIC MARKERS IN MYELOPROLIFERATIVE NEOPLASMS

In the last 15 years, the insight into the genetics of MPN has been substantially extended and has started to immerse clinical practice at several levels including diagnosis, prognostication and therapeutic management.

Significance of Genetics for Diagnosis of Myeloproliferative Neoplasms

Although the World Health Organization implemented somatic JAK2 V617F, JAK2 exon 12, and MPL mutations promptly into its 2008 revision as a major diagnostic criterion for MPN, CALR mutations were added to the updated version in 2016 (**Table 3**). Thus, the somatic driver mutations, which constitutively activate JAK2-STAT signaling in MPN, serve as helpful biomarkers in approximately 98% of patients with PV and 85% to 90% of patients with ET and patients with PMF, representing a mainstay of MPN diagnosis. The prevalent co-mutations in myeloid cancer genes have also been implemented to provide support in diagnosing the more challenging cases of triple negative PMF. The 2016 World Health Organization guidelines recommend to test for ASXL1, EZH2, TET2, IDH1/2, SRSF2, and SF3B1 in triple-negative PMF to help determining a clonal nature.[4]

The Significance of Genetics for Prognostication in Myeloproliferative Neoplasms

Much has been learned from the sequencing efforts in myeloid malignancies as to differential effects of genetic alterations on the risk of transformation and overall outcome. In MPN, a set of high molecular risk mutations, including ASXL1, EZH2, IDH1/2, and SRSF2,[55] which confer adverse prognosis, as well as mutations enriched in blast phase MPN including IDH1/2 and TP53, have been delineated.[57] In contrast, mutations in CALR, particularly type 1, have been associated with favorable outcome in ET and PMF.[30] Although previous prognostication schemes for PMF, such as the International Prognostic Scoring System (IPSS), the Dynamic IPSS, and the Dynamic IPSS Plus have relied on clinical parameters and blood counts,[80] novel prognostic scoring systems are implementing the genetic make-up of MPN and are increasingly

Table 3
Diagnostic criteria for MPN implementing genetic markers according to the World Health Organization (2016)

	PV	ET	Myelofibrosis: Prefibrotic/Early	Myelofibrosis: Overt/Fibrotic
Major criteria				
Blood (m/f)	Hemoglobin >165 g/L/ 160 g/L or Hematocrit >49%/48% or Red cell mass >25% above normal	Platelet count >450 × 10^9/L	No specific requirement (cytoses or cytopenias possible)	No specific requirement (cytoses or cytopenias possible)
Marrow	Age-adjusted hypercellularity Trilineage growth (panmyelosis) including erythroid, granulocytic and megakaryocytic proliferation Pleomorphic, mature megakaryocytes	Proliferation of megakaryocytic lineage: increased, enlarged, megakaryocytes, hyperlobulated nuclei No increase in granulo/ erythropoiesis Rarely increase in reticulin fibers (grade 1)	Megakaryocytic proliferation/atypia No reticulin fibrosis greater than grade 1 Increased age-adjusted cellularity Granulocytic proliferation, often decreased erythropoiesis	Megakaryocytic proliferation and atypia Reticulin and/or collagen fibrosis of grade 2 or 3
Exclusion	No specific exclusions	Not PV, PMF, other myeloid neoplasm	Not CML, PV, ET, MDS, other myeloid neoplasms	
Genetics[a]	*JAK2 V617F* or exon 12 mutation	*JAK2, CALR*, or MPL mutation	*JAK2, CALR, MPL* mutation or Another clonal marker[b] or absence of reactive BM fibrosis	
Minor criteria				
Additional	Subnormal serum erythropoietin	Presence of a clonal marker or Absence of evidence of reactive cause	Anemia not owing to comorbidity Leukocytosis ≥11 × 10^9/L Palpable splenomegaly LDH above reference	Anemia not owing to comorbidity Leukocytosis ≥11 × 10^9/L Palpable splenomegaly LDH above reference Leukoerythroblastosis

(*continued on next page*)

Table 3
(*continued*)

	PV	ET	Myelofibrosis	
			Prefibrotic/Early	**Overt/Fibrotic**
Required	All 3 major criteria or First 2 major + minor criterion	All 4 major criteria or First 3 major + minor criterion	All 3 major + $\geq$1 minor criterion	All 3 major + $\geq$1 minor criterion

Abbreviations: BM, bone marrow; CML, chronic myeloid leukemia; LDH, lactate dehydrogenase; MDS, myelodysplastic syndromes.

[a] Genetic markers represent an integral part of diagnosis for all MPN subtypes according to the World Health Organization 2016 criteria.

[b] In absence of JAK2, CALR or MPL mutations genotyping of other mutations associated with myeloid malignancies, for example, in ASXL1, EZH2, TET2, IDH1/2, SRSF1, and SF3B1 to determine a clonal nature is recommended.

Table 4
Genetics-based prognostication schemes in myeloproliferative neoplasms

	GIPSS	Patients	MIPSS70	Patients	MYSEC	Patients
Genetic factors						
Driver mutations	*CALR* type 1/like mutation absent	1	*CALR* type 1/like mutation absent	1	*CALR* mutation absent	2
High-risk mutations	Somatic mutation in:	1	Somatic mutation in:	1	—	
	ASXL1	1	ASXL1	1		
	SRSF2	1	EZH2	1		
	U2AF1 Q157		SRSF2	1		
			IDH1/2	2		
			≥2 of the above "high molecular risk" mutations			
Conventional factors						
	Karyotype	2	Hemoglobin <100 g/L	1	Hemoglobin <110 g/L	2
	Very high risk: −7, i(17q), inv(3)/	1	WBC >25 × 10^9/L	2	Platelets <150 × 10^9/l	1
	3q21, 12p-/12p11.2, 11q-/		Platelets <100 × 10^9/l	2	Peripheral blood blasts ≥3%	2
	11q23, +21, other autosomal		Peripheral blood blasts ≥2%	1	Constitutional symptoms	1
	trisomies except +8/+9		Fibrosis ≥ grade 2	1	Age at diagnosis 40–90 y	6–13.5
	Unfavorable: All other if not		Constitutional symptoms	1		
	favorable (including normal,					
	sole 13q-, +9, 20q-, +1, -Y)					
Risk category (points)	Low	0	Low	0–1	Low	0
	Intermediate-1	1	Intermediate	2–4	Intermediate-1	1
	Intermediate-2	2	High	≥5	Intermediate-2	2
	High	≥3			High	≥3

Abbreviations: GIPSS, genetically inspired prognostic scoring system; MIPSS, Mutation-Enhanced International Prognostic Score System; MYSEC, Myelofibrosis Secondary to Polycythemia Vera and Essential Thrombocythemia Prognostic Model; WBC, white blood cell count.

used. The Molecular IPSS70 adds genetic information on CALR and high molecular risk mutations, as well as on the number of mutations to the classical prognostic factors to assess the urgency of allogeneic hematopoietic stem cell transplantation in patients up to 70 years of age.[5] Rating the prognostic impact of genetics even higher, the genetically inspired IPSS purely relies on genetic information including a subset of high molecular risk mutations and mutations in CALR, as well as cytogenetic abnormalities to predict outcome.[6] The Myelofibrosis Secondary to Polycythemia Vera and Essential Thrombocythemia Prognostic Model specifically assesses prognosis for secondary, post-PV or post-ET myelofibrosis also implementing certain genetic factors[81] (**Table 4**). Promising further developments for prognostication are under way as exemplified by personalized prediction tools based on large-scale genomic data, which will be instrumental to individually assess the prognostic impact of a specific MPN patient's comprehensive genetic profile[3] (https://cancer.sanger.ac.uk/mpn-multistage).

Significance of Genetics for Myeloproliferative Neoplasm Therapy

Most importantly, the genetic characterization of MPN has initiated the era of targeted therapies for these entities, as several genetic lesions are actionable. The identification of JAK2 V617F as well as the observation that also CALR and MPL mutations induce constitutive activation of JAK-STAT signaling has led to the development of JAK2 inhibitors. Although the JAK1/2 inhibitor ruxolitinib represents now a clinical standard of care for the treatment of PMF and PV,[35–37] fedratinib, a JAK2/FLT3 inhibitor, has recently been approved.[38] The fact that current JAK2 inhibitors are not selective for mutant JAK2 turns out to be advantageous in the sense that activated JAK-STAT signaling in CARL and MPL mutant patients is also addressed. However, JAK2 inhibition selective for the MPN clone would be highly desirable to achieve more substantial disease-modifying activity with decreased mutant clone sizes and efforts toward refined JAK2 inhibitors are ongoing.[82] Adaptive changes of JAK2, MPL, or CALR mutant signaling are also extensively explored as co-targets including, for example, MEK-ERK or PI3K-AKT pathways.[59] The increasing insight into the biology of mutant CALR exposed at the cell surface holds potential as a target private to the mutant MPN clone, which could be addressable.[26] The genetic alterations in epigenetic and splicing factors could also provide interesting targets for therapeutic intervention, particularly in patients with JAK2 V617F–deficient founder clones or blast phase MPN with loss of JAK2 V617F. Although hypomethylating agents or histone deacetylase inhibition with beneficial effects in AML are also being studied in MPN,[83,84] PRMT5A or BRD4 inhibition represent innovative approaches to epigenetic targeting with promising preclinical results.[53,85] In addition to single-agent approaches, combination strategies with JAK2 inhibition are particularly explored aiming at increased efficacy of JAK2 inhibitor therapy. Important open questions relate to the significance of specific co-mutational profiles for response to targeted or conventional therapies.[86] Comprehensive genetic characterization of large treatment trials in MPN is desirable to evaluate which patient subgroups would benefit from specific therapeutic approaches.

SUMMARY

Our understanding of the genetics of MPN has expanded at a rapid pace with substantial impact on the way we diagnose, prognosticate and treat MPN. These developments are ongoing and will further refine our understanding of MPN pathogenesis for example, in regard to clonal hematopoiesis as a pre-MPN state, as well as our ability to develop genetically guided, individualized treatment concepts for patients with MPN.

CLINICS CARE POINTS

- JAK2, CALR, and MPL mutations are present in approximately 98% of patients with PV and 85% to 90% of patients with ET and patients with PMF and represent a mainstay for diagnosis of MPN according to World Health Organization criteria.
- In triple-negative MPN, evaluation for ASXL1, EZH2, TET2, IDH1/2, SRSF2, and SF3B1 mutations can assist to determine the clonal nature of the disease.
- Although CALR mutations associate with favorable prognosis in patients with ET and patients with PMF, the presence of high molecular risk mutations, including ASXL1, EZH2, IDH1,/2 and SRSF2, relate to an unfavorable prognosis in MPN.
- Genetic factors are increasingly implemented in modern prognostication schemes, including the genetically inspired IPSS, Molecular IPSS7, and Myelofibrosis Secondary to Polycythemia Vera and Essential Thrombocythemia Prognostic Model scores or novel personalized prediction tools (https://cancer.sanger.ac.uk/mpn-multistage).
- The constitutive activation of JAK-STAT signaling by somatic driver mutations in JAK2, CALR, and MPL provides a rational basis for JAK2 inhibitor therapy in patients with MPN.
- Novel targeted therapies, either as single agents or in combination with JAK2 inhibitors, are currently being developed to provide improved treatment concepts.

ACKNOWLEDGMENTS

The authors acknowledge research support to S.C. Meyer by the Swiss National Science Foundation (PBBEP3-144806, PCEFP3_181357), the Swiss Cancer League/Swiss Cancer Research (KFS-3858-02-2016), the Swiss Bridge Foundation (PSB-4066-06-2016), the Cancer League Basel (KLbB-4784-02-2019), the Swiss Group for Clinical Cancer Research SAKK, the Foundation for the Fight against Cancer, the Nora van Meeuwen-Häfliger Foundation, the Foundation Peter-Anton and Anna-Katharina Miescher, and the Swiss Society of Hematology. Due to space restrictions, 1 or 2 publications may be referenced on behalf of several for certain key discoveries to which several groups have contributed. Figures were created with Biorender software (Biorender.com).

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