Genomics of Myeloproliferative Neoplasms

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Abstract

Myeloproliferative neoplasms (MPN) are a group of related clonal hematological disorders characterized by excess accumulation of one or more myeloid cell lineages and a tendency to transform to acute myeloid leukemia. Deregulated JAK2 signaling has emerged as the central phenotypic driver of BCR-ABL1-negative MPN and a unifying therapeutic target. In addition, MPN show unexpected layers of genetic complexity, with multiple abnormalities associated with disease progression, interactions between inherited factors and phenotype driver mutations, and effects related to the order in which mutations are acquired. Although morphology and clinical laboratory analysis continues to play an important role in defining these conditions, genomic analysis is providing a platform for better disease definition, more accurate diagnosis, direction of therapy and refined prognostication. There is an emerging consensus with regard to many prognostic factors, but a clear need to synthesize genomic findings into robust, clinically actionable and widely accepted scoring systems, and well as the need to standardize the laboratory methodologies that are employed.
Introduction

The World Health Organization classification\(^1\) recognizes seven subtypes of myeloproliferative neoplasms (MPN). Chronic myeloid leukemia is unique in that is defined by the presence of a specific somatic abnormality, the \(BCR-ABL1\) fusion gene. Of the \(BCR-ABL1\)-negative MPN (the focus of this review), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are often referred to collectively as classic MPN. Chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL) and MPN unclassifiable (MPN-U) are much less common and referred to as atypical or non-classic MPN. During their diagnostic work up, MPN need to be carefully distinguished from other myeloid entities by a combination of morphologic, clinical laboratory, cytogenetic, molecular genetic and, increasingly, genomic analysis.

Classic \(BCR-ABL1\) negative MPN

Deregulation of JAK2/STAT signalling

Direct or indirect dysregulation of JAK2 signalling by somatically acquired mutations has emerged as a central phenotypic driver of classic MPN. JAK2 is a non-receptor tyrosine kinase which plays an essential role in transducing signals from class 1 cytokine receptors critical for normal myelopoiesis, notably the erythropoietin receptor, the thrombopoietin receptor (TPOR; encoded by the \(MPL\) gene) and the granulocyte colony-stimulating factor receptor\(^2,3\).

The principal driver mutations in MPN are indicated on Figure 1. JAK2 is directly activated by acquisition of the \(JAK2\) V617F mutation, seen in the majority of MPN\(^4-7\). \(JAK2\) V617F mutation burdens are usually higher in PV and PMF compared to ET, with many cases demonstrating a homozygous mutant clone\(^8\). Mutations of \(JAK2\) exon 12, typically complex insertion/deletion events, are seen in roughly one third of cases of \(JAK2\) V617F-negative PV\(^9\).
Indirect dysregulation of JAK2 signalling occurs principally by activating mutations in MPL or CALR. MPL mutations are located in exon 10 and most commonly result in amino acid substitutions at W515 of the thrombopoietin receptor (TPOR).\textsuperscript{10,11} W515 is located within a small amphipathic motif of the human receptor at the junction between the transmembrane and cytoplasmic domains that is required for maintaining TPOR in its inactive conformation and prevents its autonomous activation; mutation of this residue abrogates this function thereby constitutively activating JAK2 and downstream signalling.\textsuperscript{10,12}

\textit{CALR} exon 9 mutations are found in about 25-30\% of patients with ET and PMF.\textsuperscript{13,14} All pathogenic \textit{CALR} result in a common frameshift encoding a novel C-terminus with a high positive charge, with 80\% of mutants being type-1 (52bp deletion) or type-2 5bp insertion).\textsuperscript{13,14} Calreticulin is a highly conserved chaperone protein that directs the correct conformation and intracellular trafficking of glycoproteins, as well as in homeostatic control of calcium levels. Remarkably, calreticulin mutants specifically activate TPOR, and thereby JAK2, after binding to its extracellular N-glycosylation residues.\textsuperscript{15-17}

\textit{Triple negative MPN}

ET and PMF negative for standard phenotype driver mutations (\textit{JAK2}, \textit{CALR} and \textit{MPL}) are referred to as ‘triple negative’. High throughput sequencing identified non-canonical activating \textit{JAK2} or \textit{MPL} mutations in a minority of triple negative MPN, some of which were somatic but others were inherited, thus identifying cases with hereditary thrombocythemia misdiagnosed as MPN. Similarly, some cases had no evidence for a clonal disorder suggesting that they had also been misdiagnosed. Other cases, particularly those with a diagnosis of PMF, had clonal disease associated with a range of mutations characteristic of MDS/MPN and other myeloid disorders e.g. in \textit{CBL}, \textit{SH2B3}, \textit{TET2}, \textit{ASXL1} and \textit{SRSF2}.\textsuperscript{14,18,19}

\textit{MPN genomes: cytogenetics, arrays and exome sequencing}
Karyotypic abnormalities detected by conventional cytogenetic analysis are seen in a minority of PV and ET (<10%), but about a third of PMF. Gain of chromosome 9 is seen recurrently in PV and associated with JAK2 V617F (JAK2 is located at 9p24). Interstitial deletions of 13q and 20q are seen in all MPN subtypes suggesting the presence of one or more tumour suppressor genes in these regions. Of these, 20q deletion is the most frequent and may target the imprinted genes L3MBTL1 and SGK2. Other recurrent abnormalities in MPN for which the molecular basis is unknown include +8 and der(6)t(1;6).

Single nucleotide polymorphism (SNP) array analysis has confirmed that PV and ET genomes are relatively simple with very few somatic copy number changes. By contrast, copy number changes are more common in PMF and MF evolving from PV or ET. SNP arrays detect frequent regions of acquired uniparental isodisomy (aUPD) in MPN. Such regions arise from mitotic recombination and enable genetic variants to progress from a heterozygous to a homozygous state without change in DNA copy number. Chromosome 9p is most commonly affected and is almost always associated with homozygosity for JAK2 V617F. Other chromosomes are affected less frequently, e.g. aUPD at 4q, 7q and 11q are associated with mutations in TET2, EZH2 and CBL, respectively, whereas aUPD14q is associated with an imprinting defect.

Exome sequencing identified a median of 6.5 total somatic mutations in ET and PV, increasing to 13 in MF, consistent with the latter being a more advanced stage of disease. Most of these mutations are passengers that almost certainly play no role in the disease process. RNAseq has not thus far identified any new in-frame oncogenic fusion genes, although this technique may detect loss of function events. Whole genome sequencing for MPN has not been reported, but may, potentially reveal new somatic changes in non-coding regions of the genome. Overall, however, it is anticipated that the MPN genome is largely understood, at least in terms of the spectrum and frequency of somatic mutations. By contrast, the epigenome remains poorly understood.

Initiating mutations and phenotype modifying mutations
JAK2 V617F can be detected in the general population at a prevalence of 0.1-0.2%, roughly 10x the prevalence of diagnosed MPN.\textsuperscript{31-35} Although some of these mutated individuals turn out to have a previously undiagnosed MPN, many have blood counts within the normal range and thus represent examples of what is now termed age related clonal hematopoiesis (ARCH) or clonal hematopoiesis of indeterminate potential (CHIP). Some of these individuals subsequently develop an MPN but many maintain normal blood counts. A JAK2 V617F mutation burden of >2% has been proposed as suggestive of an MPN,\textsuperscript{36} but this is not a clean cut off and some individuals with ARCH/CHIP have higher JAK2 mutation burdens.

Broad population-based mutations screens indicate that ARCH/CHIP is associated with mutations in several different genes with most affected individuals having only one discernible mutation.\textsuperscript{37-39} Up to 50% of JAK2 V617F mutated PV and ET cases also have no additional pathogenic mutations\textsuperscript{40,41} and thus whilst it appears that JAK2 V617F is sufficient to drive clonal hematopoiesis and an MPN phenotype in many cases, additional factors may play a role in promoting the likelihood of an MPN phenotype developing.

About 50% of JAK2 V617F mutated ET and PV cases have pathogenic mutations in additional genes, most commonly DNMT3A and TET2. The great majority of JAK2 V617F positive PMF cases have additional mutations, particularly those encoding chromatin modifiers and splicing factors, suggesting that these abnormalities promote an MF phenotype.\textsuperscript{40,41}

Remarkably, the order in which mutations are acquired is not fixed. In some patients JAK2 V617F is acquired early in the disease and then additional mutations appear as subsequent subclonal events. In other patients, clinically covert clonal hematopoiesis is believed to be established by mutations in DNMT3A, TET2 or other abnormalities such as del(20q) or aUPD14q, and JAK2 V617F is acquired as a later event, triggering the onset of an MPN. The order in which mutations are acquired influences the disease phenotype: patients who acquired JAK2 V617F before TET2, DNMT3A or aUPD14q were more likely to develop PV than ET compared to cases where JAK2
V617F was acquired late.\textsuperscript{26,42,43} In addition, detailed analysis of \textit{TET2/JAK2} co-mutated patients indicated that the order in which mutations are acquired influences tumor biology as well as the behaviour of stem and progenitor cells.\textsuperscript{43}

In contrast to \textit{JAK2} V617F, \textit{CALR} mutations show a high degree of clonal dominance throughout hematopoietic development and are believed to be the initiating event in most cases. Additional mutations are thus secondary events associated with disease evolution.\textsuperscript{13,14,40,44} The principal genetic abnormalities and evolution of MPN are shown on Figure 2.

\textit{Factors determining disease phenotype}

Although MPN phenotype driver mutations all induce the activation of JAK2, they do not lead to the same disorder. \textit{JAK2} V617F is associated with PV, ET and PMF while but \textit{MPL} and \textit{CALR} mutations are associated with ET and PMF. \textit{JAK2} V617F mutant ET patients tend to present many of the phenotypic features of PV and an increased risk of thrombosis compared to ET negative for \textit{JAK2} V617F.\textsuperscript{45,46} Cases with \textit{MPL} mutations are on average older and have higher platelet counts, lower hemoglobin levels and reduced bone marrow cellularity at diagnosis than patients with \textit{JAK2} V617F, although, no significant differences in clinical outcome are apparent.\textsuperscript{47}

\textit{CALR} mutations in ET are associated with higher platelet counts, lower haemoglobin levels, lower leukocyte counts and younger age at presentation, while the incidence of thrombotic events is lower than \textit{JAK2} V617F positive ET.\textsuperscript{48-50} Although no differences in survival have been demonstrated for the different driver mutation subgroups,\textsuperscript{50} ET patients with type-1 \textit{CALR} mutations progress to MF much more frequently than those with type-2 mutations.\textsuperscript{51}

In PMF the nature of the MPN driver mutation has a stronger prognostic significance. Compared to \textit{JAK2} V617F mutated cases, patients with \textit{CALR} mutations (most commonly type-1 in PMF) present at a younger age, with higher platelet counts and lower leukocyte counts, but with reduced anemia and transfusion dependency, while they have a better prognosis regarding overall and leukemia-free survival.
Importantly, triple-negative patients for both PMF and post-MPN MF have the worst prognosis and a more severe anemia.\textsuperscript{48,52,53} Compared to PV and ET, the JAK2 V617F and CALR mutant allele burden is significantly higher in MF evolving from either ET or PV, indicating a role for the accumulation of mutated alleles in the process of transformation.\textsuperscript{48}

The reason why some JAK2 V617F positive patients present with PV whereas others present with ET or other myeloid disorders is likely to be multifactorial. As discussed above, the JAK2 mutant allele burden is higher in PV compared to ET and the order in which mutations are acquired can influence the disease phenotype. Constitutional genetics also influences the disease phenotype, with inherited variation in the \textit{HBS1L-MYB} region influencing whether JAK2 V617F mutant cases develop ET or PV.\textsuperscript{24} Phenotypic determinants in MPN may also include factors other than acquired mutations and constitutional genetic modifiers. PV and PMF are more common in males and ET is predominant in females,\textsuperscript{54} whilst CALR-mutated ET is more prevalent in men and JAK2 V617F is more common in women,\textsuperscript{55} suggesting that gender-specific factors may influence MPN phenotype. Hormones could be an explanation for these differences, as oestrogens can inhibit the JAK2 V617F mutated stem cells,\textsuperscript{56} an observation that may explain the observation that JAK2 V617F allele burdens are lower in women compared to men.\textsuperscript{57} Iron status, especially in premenopausal women, is another factor relevant to MPN phenotype.\textsuperscript{54}

\textit{Disease progression and prognostic significance of disease modifying mutations}

Considerable effort has been put into the identification of genetic predictors of transformation from MPN to AML. Robust molecular prognostic markers are still lacking for patients with PV and ET, and risk stratification for these patients is still based mainly on clinical criteria and the presence of phenotypic driver mutations. The mutational burden is certainly higher on transformation of MPN to AML with the appearance of variants in a range of genes that are not unique to MPN but are also seen in other myeloid malignancies (Table 1). Gene expression profiling with adjustment for gender-specific effects has been suggested to provide prognostic information in PV,\textsuperscript{58} but this approach remains unvalidated. Overall, whilst no strong
predictors of transformation have been identified, some clinically useful prognostic factors have emerged, particularly for the management of patients with MF.

Mutations in genes involved in transcription or DNA damage response are clearly associated with leukemic transformation, particularly RUNX1 and TP53 but also IKZF1 and CUX1. Mutations in TP53 may persist in a heterozygous state for an extended period of time during chronic phase MPN without clonal expansion. However, after loss of the wild-type allele by either chromosomal deletion or aUPD, the hemizygous or homozygous TP53 clone rapidly expands, leading to leukemic transformation. Mutations in RUNX1 and other transcription factors are usually acquired at the time transformation, thus limiting their use as predictive factors.

Impairment of polycomb repressive complex 2 (PRC2) function also appears to be an important pathogenic mechanism that promotes disease progression. Both EZH2 and ASXL1 mutations are associated with an increased risk of leukemic transformation and inferior survival. In PMF, ASXL1 is an important prognostic indicator, with CALR wild type, ASXL1 mutated cases having the worst prognosis.

Mutations in genes encoding chromatin modifiers are not consistently associated with prognosis. Whereas DNMT3A mutations are not considered to be prognostically significant, there are conflicting data regarding the association between TET2 mutations and the risk of leukemic transformation. Rather than promoting disease progression, mutations in these genes may be considered as landscaping events that create a ‘fertile ground’, i.e. an environment more conducive to the development of a myeloid neoplasm. Early studies indicated, unexpectedly, that JAK2 V617F mutated MPN may evolve to JAK2 unmutated AML. Most likely this is due to the presence of a pre-existing, clonal phase driven by DNMT3A or TET2 mutations with this clone independently acquiring JAK2 V617F (leading to an MPN) or other mutations (leading to AML). Alternatively, it is possible that some individuals have an inherited tendency to develop myeloid malignancies and thus develop MPN and AML as truly independent clones.
In PMF the presence of mutations in any one of ASXL1, SRSF2, IDH1/2 or EZH2 has been shown to be associated with reduced overall survival and increased risk for leukemic transformation. Specifically, the number of these “high-molecular-risk” (HMR) mutations was inversely correlated with median survival in PMF independently of standard risk scores. Notably, HMR mutations are enriched in triple negative PMF, while spliceosome mutations (SRSF2, SF3B1, U2AF1) are infrequent in CALR-mutant patients compared with the JAK2/MPL-mutant and triple-negative patients. Mutational analysis in PMF patients who received ruxolitinib revealed that patients with ≥3 mutations of any type had significantly lower odds of a spleen response and a shorter time to treatment discontinuation. Patients with ≥1 mutations in ASXL1, EZH2, IDH1 or IDH2 had shorter survival and time to treatment discontinuation, and were significantly less likely to have a spleen response. Additional mutations may correlate with other relevant clinical factors, for example in PMF the presence of U2AF1 mutations is strongly associated with severe anemia and thrombocytopenia. Overall, these data suggest that broad mutational analysis, for PMF patients at least, should be integrated into routine management.

Genetic predisposition
Familial predisposition to MPN may occur in up to 5-8% of apparently sporadic cases. MPN phenotype driver mutations are never inherited but are frequently acquired somatically in familial cases, as they are in sporadic cases. As noted above, inherited, relatively weakly activating JAK2 and MPL mutations are associated with hereditary erythrocytosis or thrombocytosis, disorders that are non-clonal but which may occasionally be mistaken for true MPN.

The causes of familial predisposition to MPN are largely unknown. The inheritance patterns are heterogeneous and attempts to aggregate families for linkage analysis have largely failed, suggesting there are probably a variety of different germline mutations of variable penetrance driving the effect. Thus far, only one clear explanation for high penetrance germline predisposition has emerged: analysis of four large families identified a 14q duplication including ATG2P and GSKIP that segregated with the MPN phenotype (ET/AML) and provided a ‘fertile ground’ for
Large epidemiological studies have suggested the presence of weakly penetrant, common risk factors in the general population. Several of these factors have been identified, but just two (JAK2 46/1 and TERT) account for the major part of the population attributable risk for development of an MPN, although the underlying reasons for this effect remain obscure. JAK2 46/1 (also known as GGCC) strongly predisposes to JAK2-mutated MPN but also, to a much lesser degree, to MPN with other mutations. TERT variants strongly predispose to all MPN. Although these effects are strong in genetic epidemiological terms, their penetrance is very low and consequently they cannot be used to predict whether any individual is likely to develop an MPN. Nevertheless, aggregation of these factors may contribute to apparent familial clustering.

**Non-classic or atypical MPN**

**Chronic neutrophilic leukemia**

Frequent oncogenic mutations of CSF3R are seen in CNL, a rare MPN subtype sharing overlapping features with atypical CML. The initial study found that 50-60% of patients with CNL or atypical CML harboured CSF3R mutations with subsequent reports indicating that such abnormalities were restricted to CNL and essentially absent in aCML and MDS/MPN-unclassified. Co-operative mutations in SETBP1 and ASXL1 appear to be of prognostic significance and correlate with disease progression.

CSF3R encodes the receptor for granulocyte-colony stimulating factor 3, which requires JAK2 to function. Mutations fall into 2 types: nonsense or frameshift mutations leading to premature truncation of the cytoplasmic tail of the receptor and point mutations in the extracellular domain, most commonly T618I, an abnormality that strongly activates the JAK/STAT signalling. CSF3R mutations are
almost always acquired, although a recent report described a child with CNL and an inherited T618I mutation.\textsuperscript{92} Ongoing studies are evaluating whether CNL patients are amenable to treatment with JAK2 or other inhibitors, as suggested by initial reports.\textsuperscript{88,93}

**Chronic eosinophilic leukemia and MPN-unclassified**

CEL (not otherwise specified) is distinguished from idiopathic hypereosinophilic syndrome largely by the finding of a marker of clonality. Most commonly this has been achieved by cytogenetics, although only a very small proportion of suspected cases turn out to have a karyotypic abnormality. Cytogenetics and/or fluorescent in situ hybridization (FISH) is also critical to help identify cases with *FIP1L1-PDGFRα* or other tyrosine kinase fusions such as those involving *PDGFRα, PDGFRβ, FGFR1* or *JAK2*, abnormalities that are amenable to treatment with specific targeted therapies.

A recent study has demonstrated that targeted next-generation sequencing helps to establish clonality in a subset of patients with cytogenetically normal hypereosinophilia that would otherwise have been classified as idiopathic hypereosinophilic syndrome. Importantly, the survival of these cases was significantly shorter than cases without mutations and was indistinguishable from cases with cytogenetically defined CEL, clearly indicating the value of genomic analysis in this disorder.\textsuperscript{94} There are no molecular markers for MPN-U but it is possible that wide mutation screens may help to better define this heterogeneous condition.

**Concluding remarks**

The 2016 revision of WHO classification has incorporated testing for MPN phenotype driver mutations as essential components in the diagnostic workup of MPN. The presence of *BCR-ABL1* defines CML but this fusion needs to be excluded in the diagnosis of classic MPN and other tyrosine kinase fusions need to be excluded in
cases with eosinophilia. It is also suggested that testing for the most frequent disease modifying mutations may assist in the determination of the clonal nature of the disease.\textsuperscript{1} and, as discussed above, many of these abnormalities have prognostic value. Currently, many centers perform a combination of cytogenetics, FISH, reverse transcriptase polymerase chain reaction (RT-PCR), Sanger sequencing and NGS panels in the diagnostic and prognostic work up of MPN. Clearly the time is ripe for genomics-based approaches, for example RNAseq, to supplant these diverse techniques and provide a platform for improved management and understanding of MPN.
References


Table 1. Approximate frequencies of acquired mutations in MPNs

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**Phenotypic/Driver Mutations**

**Initiation/Landscaping mutations**

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**Disease Modifying/Progression Mutations**

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Klampfl et al.25, Delhommeau et al.27, Grand et al.29, Delic et al.30, Lundberg et al.31, Milosevic et al.32, Brecqueville et al.33, Guglielmelli et al.34, Tefferi et al.35, Vannucchi et al.36, Lasho et al.37, Tefferi et al.38, Oh et al.39, Score et al.39.
Figure legends

Figure 1. Phenotypic driver mutations in classic, *BCR-ABL1* negative MPN

Figure 2. Pathways and principal genetic abnormalities in the evolution of *BCR-ABL1* negative MPN