



Review

Diagnosis- and Prognosis-Related Gene Alterations in *BCR::ABL1*-Negative Myeloproliferative Neoplasms

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Abstract: *BCR::ABL1*-negative myeloproliferative neoplasms (MPNs) are a group of hematopoietic malignancies in which somatic mutations are acquired in hematopoietic stem/progenitor cells, resulting in an abnormal increase in blood cells in peripheral blood and fibrosis in bone marrow. Mutations in *JAK2*, *MPL*, and *CALR* are frequently found in *BCR::ABL1*-negative MPNs, and detecting typical mutations in these three genes has become essential for the diagnosis of *BCR::ABL1*-negative MPNs. Furthermore, comprehensive gene mutation and expression analyses performed using massively parallel sequencing have identified gene mutations associated with the prognosis of *BCR::ABL1*-negative MPNs such as *ASXL1*, *EZH2*, *IDH1/2*, *SRSF2*, and *U2AF1*. Furthermore, single-cell analyses have partially elucidated the effect of the order of mutation acquisition on the phenotype of *BCR::ABL1*-negative MPNs and the mechanism of the pathogenesis of *BCR::ABL1*-negative MPNs. Recently, specific *CREB3L1* overexpression has been identified in megakaryocytes and platelets in *BCR::ABL1*-negative MPNs, which may be promising for the development of diagnostic applications. In this review, we describe the genetic mutations found in *BCR::ABL1*-negative MPNs, including the results of analyses conducted by our group.

Keywords: *BCR::ABL1*-negative myeloproliferative neoplasms; gene mutations; diagnostic marker; prognosis; genetic background



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1. Introduction

Myeloproliferative neoplasms (MPNs) are characterized as a clonal proliferation of hematopoietic stem/progenitor cells, which cause an increase in one or more mature myeloid lineage cells. MPNs consist of multiple subgroups: chronic myeloid leukemia (CML); polycythemia vera (PV); essential thrombocythemia (ET); prefibrotic primary myelofibrosis (PMF); overt PMF; chronic neutrophilic leukemia (CNL); chronic eosinophilic leukemia (CEL); and unclassifiable MPN, not otherwise specified (Figure 1) [1,2]. In general, CML involves a typical driver gene alteration, the *BCR::ABL1* fusion gene, and more rare diseases, namely CNL, CEL, and unclassifiable MPN, not otherwise specified, have been treated as independent diseases. In contrast, patients with *BCR::ABL1*-negative MPNs (PV, ET, prefibrotic PMF, and overt PMF) transform to other subgroups with a 10–15% frequency and share common gene mutations, namely *JAK2* mutations (V617F and exon 12), *MPLW515L/K*, and *CALR* exon 9 frameshift mutations, in a mutually exclusive manner [3–9]. The frequencies of the three gene mutations in our cohort are shown in Figure 2A. Since these mutations exhibit oncogenic properties, they are defined as driver mutations of *BCR::ABL1*-negative MPNs (Figure 2B). Ahead of the *MPL* and *CALR* mutations, *JAK2* mutations were listed as one of the major criteria in the 2008 WHO classification [10]. After identifying *MPL* and *CALR* mutations, the three driver genes are now available for a revised 2022 WHO classification, which highlights the importance of genetic testing

in *BCR::ABL1*-negative MPN, and have become essential for the definitive diagnosis of *BCR::ABL1*-negative MPNs [1,2]. However, a portion of patients with ET and PMFs (approximately 10–15%) exhibit negativity for all these driver mutations, which are considered to be triple-negative (TN). As for the diagnosis of TN, an exclusion of the possibility of nonneoplastic blood cell mass elevation should be carefully conducted in addition to a bone marrow (BM) biopsy which is mandatory to confirm that the histopathological characteristics of the BM morphology match the diagnostic criteria of *BCR::ABL1*-negative MPNs regardless of the presence of driver gene mutations, because no diagnostic markers have been identified [11]. To find novel gene alterations, genome-wide approaches targeting TN cases have been performed, and although several novel driver mutations in *BCR::ABL1*-negative MPNs have been found, the functional role of these mutations in the pathogenesis of *BCR::ABL1*-negative MPNs remains unclear [12]. Recently, *CREB3L1* overexpression in RNA from the platelets of MPN patients was identified, which may be a comprehensive diagnostic marker for *BCR::ABL1*-negative MPNs [13]. In addition, several mutations on the genes functioning as epigenetic modifiers and splicing factors were identified not only in leukemias/myelodysplastic syndromes but also in *BCR::ABL1*-negative MPNs, and the association of these mutations with the prognosis of *BCR::ABL1*-negative MPNs has been analyzed (Figure 2C) [14–17]. Furthermore, the influence of genetic background on the predisposition to *BCR::ABL1*-negative MPNs has been suggested in studies with large cohorts and familial *BCR::ABL1*-negative MPN pedigrees [18,19]. This article describes genetic abnormalities identified in *BCR::ABL1*-negative MPNs and their associations with the development or prognosis of *BCR::ABL1*-negative MPNs.

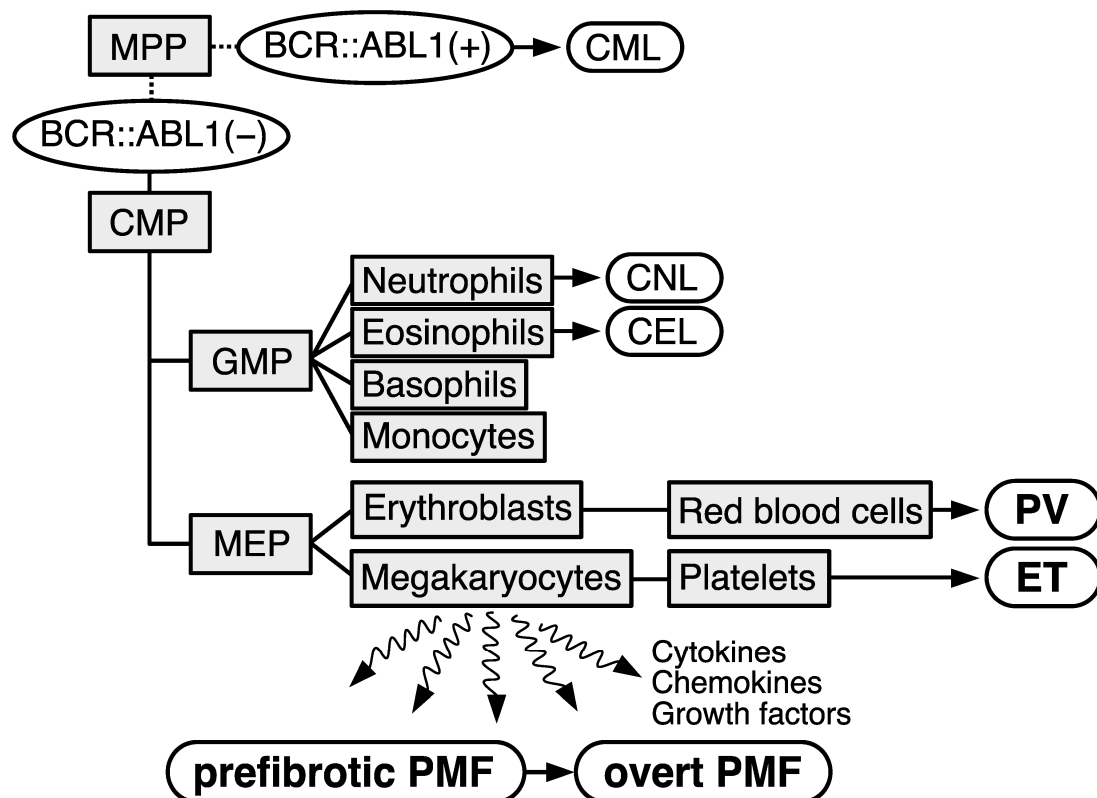


Figure 1. Schematic illustration describing the subtypes of myeloproliferative neoplasms (MPNs). Cell types are depicted as light gray squares. MPP: multipotent progenitor; CMP: common myeloid progenitor; GMP: granulocyte-monocyte progenitor; MEP: megakaryocyte–erythrocyte progenitor. Subtypes of MPNs are depicted as white rounded rectangles. Chronic myeloid leukemia (CML) exhibits *BCR::ABL1* gene (*BCR::ABL1*(+)). Other subtypes are stratified as *BCR::ABL1*-negative (–) MPNs. CNL: chronic neutrophilic leukemia; CEL: chronic eosinophilic leukemia; PV: polycythemia vera; ET: essential thrombocythemia; PMF: primary myelofibrosis.

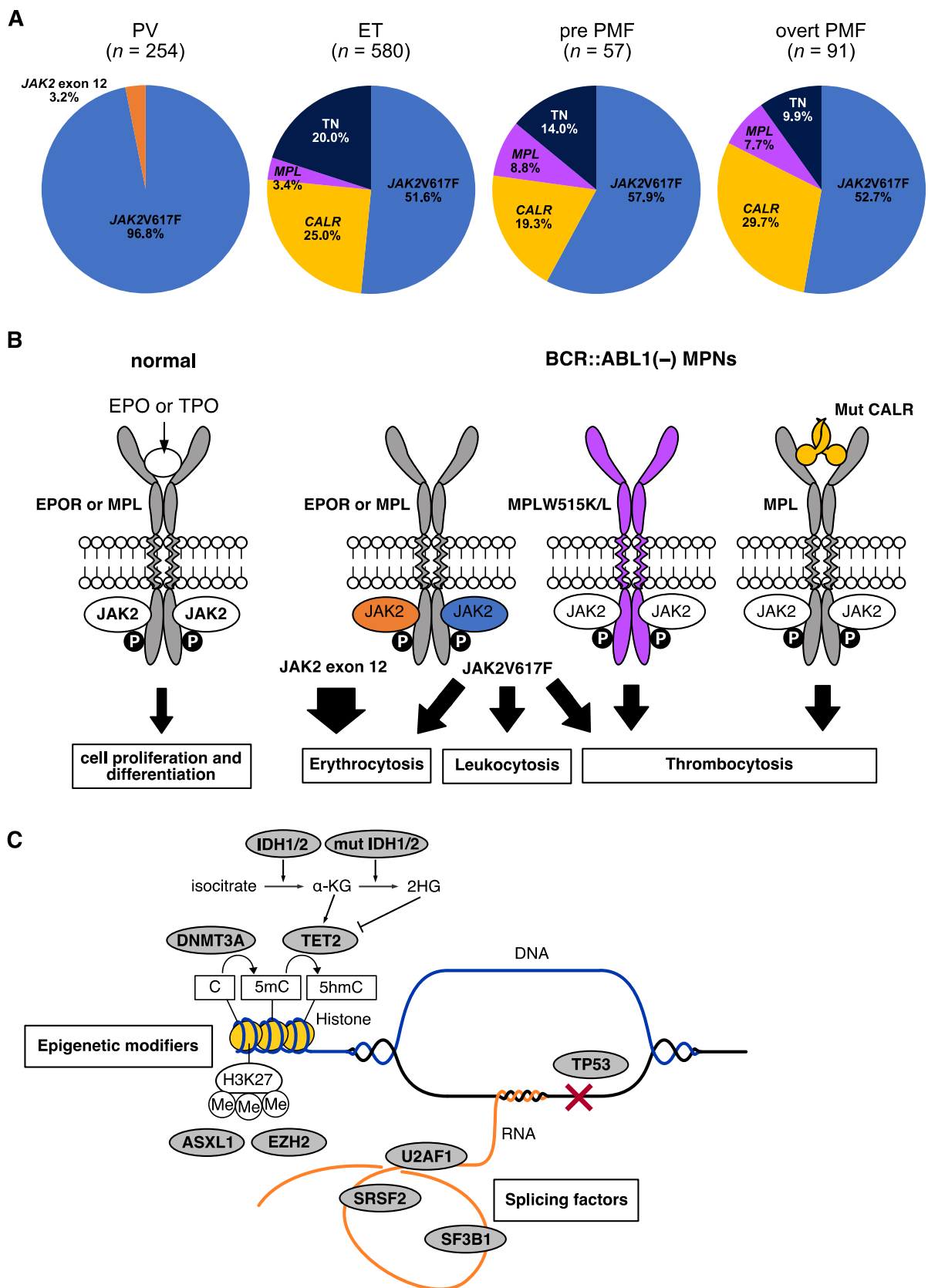


Figure 2. (A): Distribution of driver gene mutations for *BCR::ABL1*-negative MPNs in our Japanese cohort. Triple-negative: TN. (B): Driver gene mutations in *BCR::ABL1*-negative MPNs. Cell proliferation signal is regulated through the binding of cytokines (erythropoietin: EPO or thrombopoietin: TPO) to the receptors (erythropoietin receptor: EPOR or thrombopoietin receptor: MPL, left panel). However,

in the driver gene-mutated *BCR::ABL1*-negative MPNs, downstream signal cascades constitutively activate owing to the mutant proteins without the binding of cytokines. *JAK2* exon 12 mutations activate strong signals, especially for erythrocytosis, whereas *JAK2V617F* activates trilineage signals (erythrocytosis, leukocytosis, and thrombocytosis). *MPLW515L/K* and mutant *CALR* activate *MPL* signaling and trigger thrombocytosis (right panels). (C): Typical nondriver gene transcripts identified in *BCR::ABL1*-negative MPNs. Mutations that occur in these genes disrupt the gene regulations and may affect the prognosis of *BCR::ABL1*-negative MPNs. *IDH1/2* generates α -ketoglutaric acids (α -KG) from isocitrate. Mutant *IDH1/2* generates 2-hydroxyglutaric acid (2HG) from α -KG, resulting in the suppression of *TET2*. *DNMT3A* and *TET2* act as DNA methylation and demethylation enzymes by methylating cytosine to 5-methylcytosine (5mC) and oxidizing 5mC to 5-hydroxymethylcytosine (5hmC), respectively. *ASXL1* and *EZH2* regulate the transcription through the trimethylation of histone 3 lysine 27. *SF3B1*, *SRSF2*, and *U2AF1* function as splicing factors. *TP53* is a well-known guardian gene of carcinogenesis.

2. *JAK2* Mutations

The *JAK2* mutations considered as driver mutations of *BCR::ABL1*-negative MPNs are *V617F* substitution and complex mutations, including missense and in-frame deletions/insertions at exon 12 [3–6]. These mutations concentrate around the JH2 domain, which suppresses the kinase activity of the JH1 domain in *JAK2* under a static state. *JAK2* mutations decrease the suppression of kinase activity by the JH2 domain, resulting in the constitutive activation of *JAK2*. The *JAK2V617F* mutation is a single nucleotide alteration from guanine to thymine at nucleotide position 1849, which causes an amino acid change from V (valine, GTC) to F (phenylalanine, TTC) at codon 617. In addition, the *JAK2V617F* mutation was initially identified from the three driver gene mutations of *BCR::ABL1*-negative MPNs [3–5] and has been most frequently identified among the patients with *BCR::ABL1*-negative MPNs, and the positivity is approximately 97% in PV and approximately 50% in ET and PMF. In contrast, *JAK2* exon 12 mutations are specific for PV, with 3% positivity (Figure 1A), and a variety of mutations have been identified at *JAK2* exon 12 (Supplementary Table S1, according to the Catalogue Of Somatic Mutations In Cancer (COSMIC) database, as of June 2023) [20,21]. Clinically, patients with PV harboring the *JAK2V617F* mutation exhibit pancytosis, including leukocytosis, thrombocytosis, and erythrocytosis, whereas those harboring the *JAK2* exon 12 mutation show only an aggressive increase in red cell mass. As for the relationship between the prognosis and *JAK2* mutations, no significant differences between *JAK2V617F*-mutated and *JAK2* exon 12-mutated patients with PV have been observed [22]; nevertheless, *JAK2V617F* is a well-known risk factor of thrombosis [23,24]. Thrombosis promoted through the increased neutrophil extracellular trap formation was observed in the *JAK2V617F*-mutated murine model [25]. *JAK2V617F* is also useful for monitoring the efficacy of treatments or predicting the outcome of patients. Pegylated interferon- α , for example, is one of the recently developed drugs against MPNs and decreases the *JAK2V617F* allele burden in patients [26–28]. To date, pegylated interferon- α is the only agent that specifically affects the hematopoietic stem/progenitor cells of *BCR::ABL1*-negative MPNs, indicating the need for methodologies that quantify the mutant burden to monitor the efficacy of such agents. Therefore, a unique quantitative technique for assessing the *JAK2V617F* allele burden, alternately named binding-probe competitive PCR (ABC-PCR), has been developed [29]. The *JAK2V617F* mutant allele load (allele burden) in Japanese patients with *BCR::ABL1*-negative MPNs quantified by utilizing ABC-PCR showed the tendency of distribution of *JAK2V617F* allele burden between the subtypes of *BCR::ABL1*-negative MPNs (Figure 3A). Furthermore, ABC-PCR enables precise quantification of the *JAK2V617F* allele burden by correlating it with fluorescence intensity and may be as effective as massively parallel sequencing (MPS, Figure 3B). By utilizing ABC-PCR, the relationship between the allele burden and clinical significance was clarified; the increase in mutant burden during the follow-up is associated with the transformation to secondary myelofibrosis [30]. Furthermore, more studies have shown

that thrombotic risks increase in patients bearing high allele burden [31] and more frequent cooccurrence of chronic kidney disease and tendency for disease progression [32], implicating the relationship between the clinical relevance and mutant allele burdens. The series of results obtained by groups studying Western patients and our group studying Japanese patients demonstrate that the quantification of the *JAK2V617F* allele burden may be used as an indicator of drug efficacy or to predict the adverse prognosis of *BCR::ABL1*-negative MPNs. Moreover, the highly sensitive detection of *JAK2V617F* is desired for the early diagnosis of MPNs. Melting curve analysis after T allele enrichment (MelCaTle) detects the *JAK2V617F* allele at a single-copy level by eliminating the *JAK2* wild-type allele using a peptide nucleic acid probe and *BstXI* restriction enzyme [33]. The combination of ABC-PCR and MelCaTle enables precise detection of the *JAK2V617F* mutation at a single-molecule level and accurately quantify the *JAK2V617F* burden in patients with MPNs.

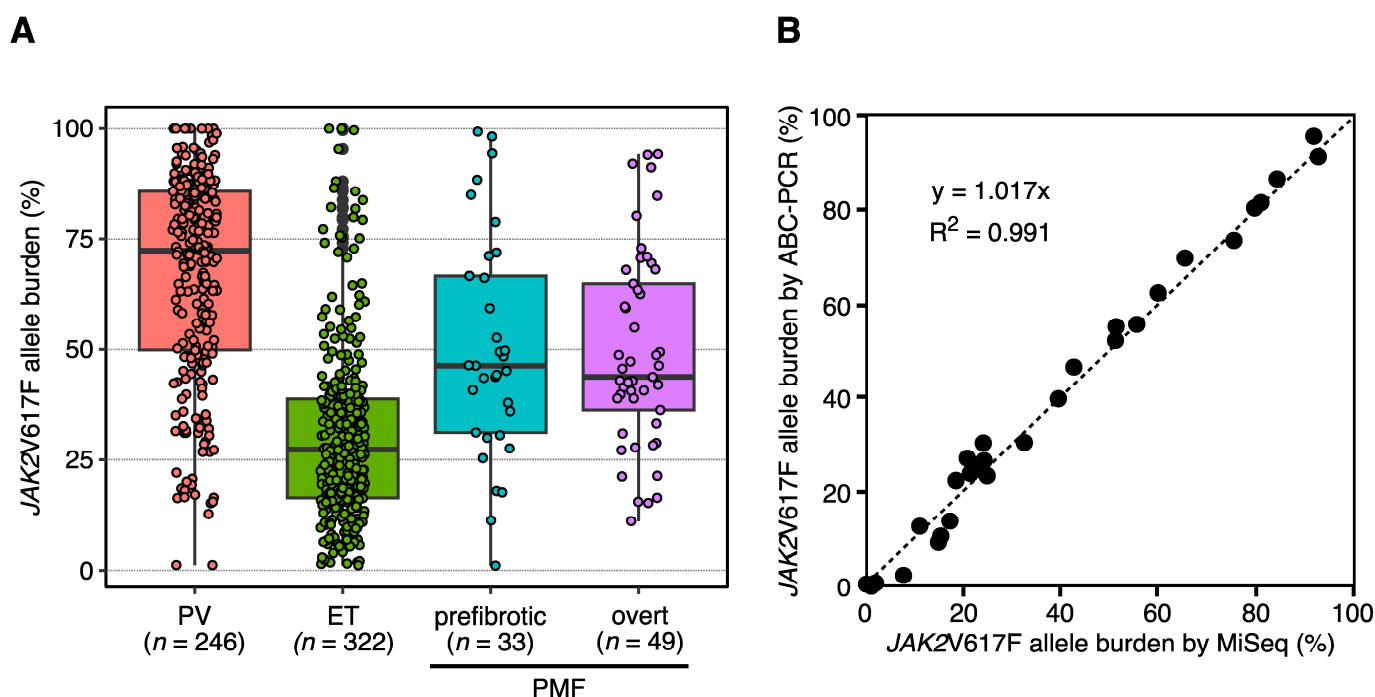


Figure 3. (A): Scatter plot showing the relationship between *JAK2V617F* allele burden measured using massively parallel sequencing (MiSeq, x-axis) and those measured using ABC-PCR (y-axis). The correlation coefficient of R^2 was calculated as 0.991; (B): box plot showing the *JAK2V617F* allele burden among patients with PV (red), ET (green), prefibrotic PMF (blue), and overt PMF (purple) harboring the *JAK2V617F* mutation (>1.0%). The median/mean *JAK2V617F* allele burden is 72.3/66.8% in PV, 27.3/30.2% in ET, 46.3/50.2% in prefibrotic PMF, and 43.7/49.3% in overt PMF.

3. MPL Mutations

The majority of MPL mutations involve the substitution of W (tryptophane, c.1542-1544TGG) at codon 515 with other nucleic acids, causing W515L/K/A/R (leucine/lysine/alanine/arginine) mutations [7,34]. In addition to these W515 mutations, the substitution of S (serine) at codon 505 with N/C (asparagine/cysteine) has been identified [35,36]. These mutations are located at the membrane-spanning segment of MPL and are considered to involve conformation changes that trigger constitutive activation of the downstream molecules. Although the frequency of MPL mutations in *BCR::ABL1*-negative MPNs is low (5% at most in ET, <10% in PMF), considering that mutant CALR binds to MPL and activates downstream signals [37], signal activation through MPL may play a key role in the pathogenesis of *BCR::ABL1*-negative MPNs [38]. Patients harboring *MPL* mutations are also relatively rare, but a meta-analysis unifying seven studies clarified that patients with ET harboring an *MPL* mutation showed higher risks for thrombosis than those harboring a *JAK2V617F* mutation [39]. In line with this study, Japanese patients with ET harboring

MPL mutations demonstrated a higher risk for thrombosis [40]. Therefore, *MPL* mutations may be one of the adverse factors of thrombosis.

4. *CALR* Exon 9 Frameshift Mutations

The *CALR* mutation is the most recently discovered among the driver gene mutations in *BCR::ABL1*-negative MPNs and is found in approximately 20–30% of patients with ET and PMF [8,9]. This mutation is characterized by the presence of a deletion or insertion at the end of exon 9, the final exon of the *CALR* gene. Over 100 variations have been found (Supplementary Tables S2 and S3, according to the COSMIC database, as of January 2023), all of which cause the same frameshift and produce a common amino acid sequence at the C-terminus when translated into protein. Among them, deletions of 52 bases (type 1, p.L367fs*46) and insertions of 5 bases (type 2, p.K385fs*47) are the major mutations, accounting for approximately 85% of the observed variations (Figure 4). Mutant *CALR* activates downstream signaling by forming homomultimeric complexes through a new amino acid sequence generated by the mutation, changing the structure of the *CALR* protein and allowing it to bind with *MPL* [37,41,42]. Patients with overt PMF harboring a *CALR* mutation have a better prognosis than those with other driver gene mutations [43]. Regarding the *CALR* mutations in overt PMF, *CALR* type 1 mutations are dominant, and patients harboring the type 2 mutation exhibit a poorer prognosis than those with the type 1 mutation [44,45].

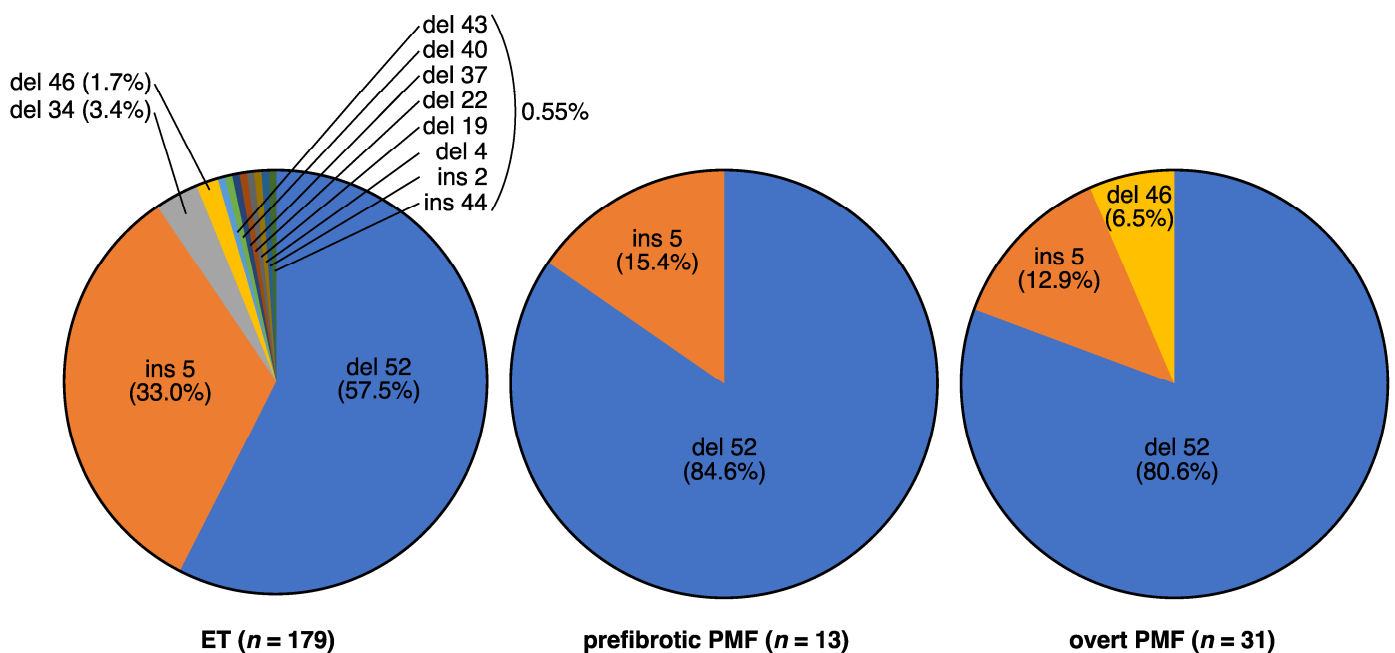


Figure 4. Distribution of *CALR* frameshift mutations analyzed in our data. ET (left, $n = 179$), prefibrotic PMF (middle, $n = 13$), and overt PMF (right, $n = 31$).

5. Triple-Negative *BCR::ABL1*-Negative MPNs

A portion of patients with *BCR::ABL1*-negative MPNs (none or rare in PV, 10–15% in ET, and ~10% in PMF) have none of the driver mutations, referred to as TN cases. Noncanonical somatic mutations at driver genes of *BCR::ABL1*-negative MPNs (e.g., *JAK2G571S* and *MPLS204F/P*) have been identified in TN cases; however, it should be considered that these mutations do not account for all the remaining cases (Supplementary Table S4) [12,46] and no evidence of cytokine-independent cell growth has been reported for these mutations. Whole exome sequencing and analysis of TN-ET have shown that approximately half of the patients exhibited polyclonal cell differentiation, which implies that some cases of thrombocytosis in TN-ET may be caused by nonneoplastic diseases [12,47]. In addition, several cases of nonneoplastic erythrocytosis (NNE) showing low EPO levels (<4.2 IU/mL,

low EPO NNE) were identified in our analysis. BM biopsy samples derived from low EPO NNE patients were diagnosed by histopathologists, resulting in the denial of PV in all cases [48]. Careful histopathological diagnosis of promising diagnostic markers for *BCR::ABL1*-negative MPNs is mandatory for such puzzling cases.

6. *CREB3L1* as a Novel Diagnostic Marker of *BCR::ABL1*-Negative MPNs

To diagnose TN cases in practice, a histopathological diagnosis of the BM biopsy is required. However, the discrimination of TN from reactive cases is challenging because the pathological diagnosis of *BCR::ABL1*-negative MPNs is not always reproducible, even for expert hematopathologists. By focusing on the fact that typical clinical presentations (i.e., thrombocytosis) of ET are similar regardless of the presence and type of driver gene mutations, and that the downstream RNA expression may be common and different from that in reactive cases, differential expression analysis utilizing RNA from platelet-rich plasma (PRP) obtained from ET and reactive thrombocytosis patients was conducted. As a result, *CREB3L1* was found to be specifically overexpressed among ET patients [13]. *CREB3L1* is a transcription factor that localizes in the endoplasmic reticulum (ER), migrates into the nucleus in response to ER stress, and induces the expression of various genes [49]. Although the role of *CREB3L1* in the pathogenesis of *BCR::ABL1*-negative MPNs remains unclear, the IRE1a/XBP1 pathway, which is an ER stress-responsible pathway other than *CREB3L1*, is activated by the CALR type 1 mutation and drives *BCR::ABL1*-negative MPNs [50]. In breast and bladder cancer, *CREB3L1* is highly methylated, and *CREB3L1* expression is inversely correlated with tumor grade, indicating that it acts in a tumor-suppressive manner [51,52].

Expansion of the testing of *CREB3L1* overexpression for other subtypes of *BCR::ABL1*-negative MPNs harboring one of the driver gene mutations in the validation analysis by employing quantitative PCR revealed that *CREB3L1* was overexpressed widely among *BCR::ABL1*-negative MPNs compared with those of reactive cases and healthy volunteers (Figure 5). The area under the ROC curve showed that the sensitivity and specificity were both 1.0000, indicating that the *CREB3L1* overexpression in PRP discriminates driver gene-mutated *BCR::ABL1*-negative MPNs from reactive cases [13]. Further investigations are required to determine whether *CREB3L1* expression is a diagnostic marker for *BCR::ABL1*-negative MPNs, including TN cases. In our cohort, 20 cases without any driver mutations were definitively diagnosed with TN-ET according to the pathological characteristics. Among them, eight cases did not express *CREB3L1* mRNA. Based on these findings, TN-ET cases were stratified into two groups (*CREB3L1*-positive TN-ET and *CREB3L1*-negative TN-ET) and the clinical parameters of the two groups were monitored. As a result, the platelet counts of two patients with *CREB3L1*-negative TN-ET decreased to normal levels during observation. In both cases, the BM biopsies at the time of initial diagnosis were consistent with ET, but the BM examinations performed at the time of spontaneous regression were negative for ET [13]. Therefore, aggressive treatments, such as anticancer drugs, should be avoided, and careful follow-up observation is recommended in cases with *CREB3L1*-negative TN-ET.

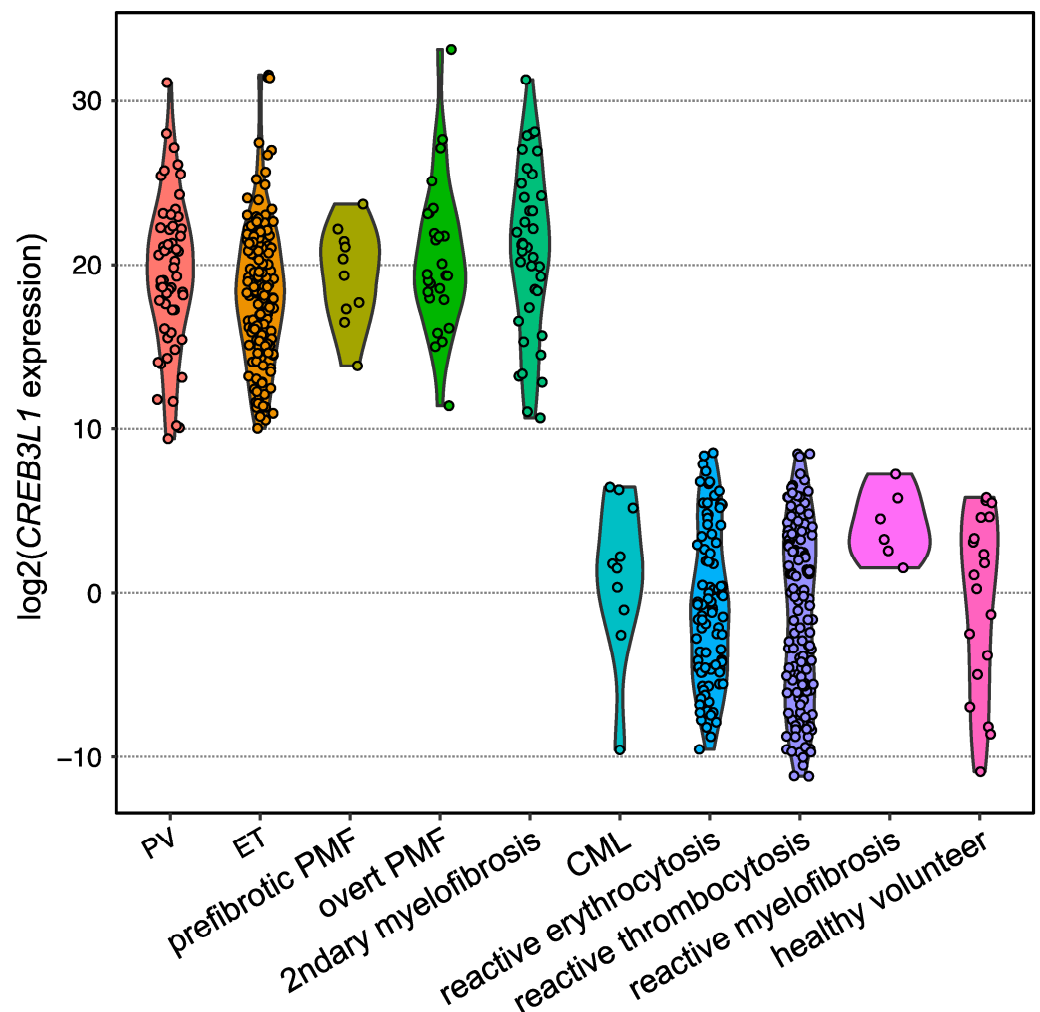


Figure 5. Violin plot showing the expression levels of *CREB3L1* measured using reverse transcription quantitative PCR. Dots represent the *CREB3L1* levels of the individuals, which are expressed as the value relative to the mean expression levels among healthy volunteers. *B2M* was used as an internal control.

7. Nondriver Mutations and Their Association with the Prognosis of *BCR::ABL1*-Negative MPNs

Comprehensive genome analyses, such as MPS, have identified some mutations relating to epigenetic modification and RNA-splicing among patients with *BCR::ABL1*-negative MPNs at low frequency [14,15]. MPS-based comprehensive target resequencing methodology focusing on these nondriver mutations also revealed that the mutations highly accumulated in patients with *BCR::ABL1*-negative MPNs were correlated with a poor prognosis. Based on these findings, mutation-enhanced prognostic scoring systems based on the positivity of nondriver mutations have been proposed and are widely used to estimate the prognostic risks of patients with *BCR::ABL1*-negative MPNs. For example, the mutation-enhanced international prognostic scoring system (MIPSS) 70+ v2.0, which was originally designed for patients with PMF aged 70 years or younger and eligible for transplantation, uses the *CALR*, *ASXL1*, *EZH2*, *IDH1/2*, *SRSF2*, and *U2AF1Q157* mutations [53]. MIPSS-ET and -PV are available for classifying the prognostic risks of patients with ET and PV, using the *SF3B1*, *SRSF2*, *TP53*, and *U2AF1* mutations for ET and the *SRSF2* mutation for PV [54].

Similar to the results obtained by other groups, patients with PMF harboring *ASXL1*, *EZH2*, and/or *SRSF2* mutations exhibited significantly shorter 5-year overall survival, and these gene mutations are also the poor prognostic factors of PMF that were demonstrated in the Japanese cohort [16]. Furthermore, regarding ET and PV, the frequencies of *ASXL1*

and *EZH2* mutations increase as the diseases progresses from ET or PV to prefibrotic PMF and overt PMF, whereas the frequencies of *DNMT3A* and *TET2* mutations are unrelated to disease type. This implies that *ASXL1* and *EZH2* mutations are related to disease progression, whereas *DNMT3A* and *TET2* mutations may trigger the disease. Logistic regression analysis showed that *ASXL1* mutation-positive ET/PV patients had a high rate of progression to leukemia and myelofibrosis [17]. Nonetheless, the prognosis of *BCR::ABL1*-negative MPNs may be affected by the timing and order of the acquisition of these mutations. Although the effect of timing remains unclarified, the effect of acquisition order on the phenotype of *BCR::ABL1*-negative MPNs has been studied. Ortmann and colleagues cultured mononuclear cells isolated from the peripheral blood of *BCR::ABL1*-negative MPN patients who were positive for both *JAK2V617F* and *TET2* mutations in methylcellulose medium and examined the positivity of *JAK2* and *TET2* mutations in the BFU-E colonies. As a result, patients with *JAK2V617F*-first colonies had stronger clinical symptoms and were at higher risk of developing thrombosis and PV than those with the *TET2*-first colonies. The authors proposed a model in which the respective clinical symptoms differ according to the acquisition order of *JAK2* and *TET2* mutations [55]. Moreover, a colony assay targeting a patient with myelodysplastic syndrome (MDS)/MPN-RS-T harboring both *JAK2* exon 12 (p.H538_K539delinsL) and *SF3B1E622D* mutations demonstrated that *SF3B1*-mutated clones existed in isolation but all clones with *JAK2* exon 12 mutations had accompanying *SF3B1* mutations. This indicates that the *SF3B1* mutation triggers pancytopenia in the cells and then the *JAK2* mutation is acquired as a second-hit mutation, causing thrombocytosis in the patient to rescue the pancytopenia to some extent [56]. The above two studies show that the first-hit gene mutations characterize the basal phenotype of the disease, and the second-hit mutations contribute additional clinical presentations of the disease; therefore, the order in which the gene mutations are acquired may explain the development and progression of the disease. More recently, comprehensive RNA expression analysis with single-cell resolution has been employed to estimate cell profiling in patients. Tong and colleagues found prominent megakaryocyte lineage priming and elevated interferon signaling in hematopoietic stem cells (HSCs) in *JAK2V617F*-mutated ET patients, and the pathogenesis and therapeutic responses were dependent on the *JAK2V617F* heterogeneity of HSCs [57]. A more precise association between the disease development and traits, including single nucleotide polymorphisms (SNPs) or the differentiation and proliferation of neoplastic cells in *BCR::ABL1*-negative MPNs, may be revealed by novel approaches such as the network genome-wide association studies (networkGWAS) and RNA velocity-based algorithms (e.g., CellRank) [58–60].

8. Genetic Background Enhancing the Risk of Developing *BCR::ABL1*-Negative MPNs

The accumulation of mutations is highly dependent on age, and elderly individuals sometimes develop clonal hematopoiesis because of acquired mutations [61,62]. Such individuals are diagnosed with clonal hematopoiesis of intermediate potential (CHIP) or age-related clonal hematopoiesis (ARCH) [63,64]. Regarding the development of CHIP/ARCH, it has been experimentally demonstrated using the zebrafish model that mutant clones increase by developing clonal fitness, which is driven by enhanced resistance to inflammatory signals [65]. Notably, *JAK2V617F* has been identified with a frequency of approximately 0.1% in an analysis of 49,488 individuals, and 7 patients harboring *JAK2V617F* (48 individuals were removed for originally having MPNs) were then considered as CHIP/ARCH in the final cohort. Furthermore, the *JAK2V617F* mutant burden in CHIP/ARCH increases by 0.55% per year in the study, implying that the *JAK2V617F*-mutated cells acquire a mild growth advantage, and therefore the development of *BCR::ABL1*-negative MPNs may progress over time [66]. This implication is supported by another investigation, which clarified that *JAK2V617F* mutations occur decades before *BCR::ABL1*-negative MPN diagnosis, increase the fitness of HSCs, and induce a megakaryocyte–erythroid differentiation bias [67]. CHIP/ARCH individuals also have an increased risk of developing hematologic malignancies or cardiovascular diseases in patients with additional mutations [68].

Some studies have investigated the impact of genetic background on the risk of developing *BCR::ABL1*-negative MPNs. For example, the *JAK2* 46/1 haplotype concomitant with *JAK2V617F*, *RBBP6*, *SH2B3*, and *TERT* mutations was shown to increase the risk of developing *BCR::ABL1*-negative MPNs [69–74]. A GWAS analysis of 888,503 individuals, including 2949 patients with *BCR::ABL1*-negative MPNs, identified 17 loci, including *JAK2* and *TERT* [18]. SNPs located at the identified loci, such as rs17879961 (located at *CHEK2* exon 5) and rs534137 (located at the promoter region of *GFI1B*), were considered to induce the instability of HSCs homeostasis and may predispose patients to *BCR::ABL1*-negative MPNs. In addition, a germline frameshift mutation at *Carbohydrate Sulfotransferase 15* (*CHST15*) has been identified among some familial *BCR::ABL1*-negative MPN pedigrees with the same geographical origin [19]. Mutant *CHST15* reduces expression levels of *CHST15* and its target genes, which induces a chronic inflammatory response, and this may also predispose patients to *BCR::ABL1*-negative MPNs. The above mutations may trigger genetic instability in the cells, induce chronic inflammation, and impose cell fitness toward a predisposition to *BCR::ABL1*-negative MPNs.

In addition to SNPs that confer higher susceptibility to develop *BCR::ABL1*-negative MPNs, SNPs that affect the phenotype, prognosis, and response to therapies of *BCR::ABL1*-negative MPNs have been reported and summarized [75]. Although allelic frequencies are not rare (0.114822 for rs6198, 0.279039 for rs1024611, and 0.444187 for rs2431697 by gnomAD, respectively), a poorer prognosis was observed among patients with PMF harboring both *JAK2V617F* and homozygous mutations of rs6198 locating at *NR3C1* than those bearing wild-type *NR3C1* [76], rs1024611 at *CCL2* strongly correlated to the *CCL2* expression and the myelofibrosis grade [77], and homozygous rs2431697 at miR-146a was associated with myelofibrosis progression [78]. Furthermore, *IL28B* rs12979860 homozygous phenotype showed hematologic response in patients with PV treated with interferon- α [79]. These SNPs would be useful to consider as a therapeutic strategy for *BCR::ABL1*-negative MPNs.

9. Conclusions

This review focused on the gene alterations involved in the pathogenesis or development of *BCR::ABL1*-negative MPNs. Notably, the number of mutations associated with *BCR::ABL1*-negative MPNs is smaller than those of acute leukemias and solid tumors, and the genetic mutation analyses play a significant role in confirming diagnosis and prognosis. On top of this, quantitation of mutant burden in the patients can provide important clinical information such as transformation to myelofibrosis and drug responses in the patients with *BCR::ABL1*-negative MPNs.

10. Future Perspective

The function of each mutation found in *BCR::ABL1*-negative MPNs is relatively easy to analyze; therefore, clarifying the pathogenesis of *BCR::ABL1*-negative MPNs may provide a model for other hematopoietic malignancies and solid cancers. The latest comprehensive gene expression technologies and subsequent statistical analyses help to elucidate the cell behavior with single-cell resolution. Moreover, deep machine learning approaches using artificial intelligence may assist in developing novel diagnostic/prognosis markers and help elucidate the pathogenesis of *BCR::ABL1*-negative MPNs, potentially leading to personalized therapies [80].

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