Brief Report

In Patients with JAK2 Unmutated CMN, Are CalR and MPL Gene Mutations Predictive of Diagnosis or Clinical Course?

Tarık Onur Tiryaki *, Aynur Dağlar-Aday, Meliha Nalçacı, Akif Selim Yavuz

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In Patients with JAK2 Unmutated CMN, Are CalR and MPL Gene Mutations Predictive of Diagnosis or Clinical Course?

Tarık Onur Tiryaki [1,*], Aynur Dağlar Aday [2], Meliha Nalçacı [1] and Akif Selim Yavuz [1]

[1] Faculty of Medicine, Department of Internal Medicine, Division of Hematology, Istanbul University, Istanbul, Turkey

[2] Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics, Istanbul University, Istanbul, Turkey

* Correspondence: tonurtiryaki@gmail.com

Abstract: Philadelphia (Ph) negative myeloproliferative neoplasms (MPNs) are disorders caused by abnormal proliferation of myeloid cells in the peripheral blood. Mutations that are responsible for the majority of these cases are those affecting Janus kinase 2 (JAK2), Calreticulin (CalR), and myeloproliferative leukemia virus oncogene (MPL). In this study, we aimed to assess the frequency of CalR and MPL gene mutations and the clinical effects of these mutations in JAK2 gene unmutated MPN patients who were followed up. Despite the lack of statistical significance in 46 patients, it was notable that CalR mutations were more common in patients with esansiyel thrombocytosis (ET), while MPL mutations were only found in patients with primary myelofibrosis (PMF). We found no correlation between thrombosis, leukemic transformation, and driver mutations. The triple negative group had a lower survival rate, but this difference was not statistically significant.

Keywords: myeloproliferative disorders; primary myelofibrosis; essential thrombocythemia

1. Introduction

Philadelphia (Ph) negative myeloproliferative neoplasms are disorders caused by abnormal proliferation of terminal myeloid cells in the peripheral blood. This disease group may exhibit hemostasis and thrombosis anomalies but may progress to acute leukemia [1-3]. The three classic types of Ph negative myeloproliferative neoplasms include polycythemia vera (PV), esansiyel thrombocytosis (ET), and primary myelofibrosis (PMF). The WHO classification also includes an unclassifiable MPN, chronic neutrophilic leukemia (CNL), and chronic eosinophilic leukemia (CEL). A combination of gene polymorphisms, driver mutations, and clinical parameters may indicate prognosis and clinical course (overt myelofibrosis or transformation to acute leukemia, thrombosis, and significant hemorrhage).

Different gene mutations and activating signal pathway mutations play important roles in pathogenesis, and some gene mutations also affect diagnosis. The most common mutation in Bcr-Abl negative chronic myeloproliferative diseases is the JAK2 gene mutation (accounting for ~ 95% of PV and 60% of ET and PMF). Approximately 20% of ET patients and 10–15% of PMF patients may have no mutation in the driver gene and are currently referred to as "triple-negative" (TN) patients [3]. In 2016 hematopoietic and lymphoid tissue tumor classification, 2016 World Health Organization (WHO) emphasized the importance of CalR receptor and MPL gene mutation in PMF and ET diagnosis. It contributed to the revision of diagnostic criteria [3].

The CalR gene is involved in cell growth and division, movement, cell interconnection, and control. It also plays an important role in intracellular calcium homeostasis and protein folding in the endoplasmic reticulum. CalR gene mutations have been identified in approximately %20-25 of patients with ET and PMF. All mutations are seen on exon 9 of chromosome 19 as insertions or

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deletions. The most common mutations are in the Type 1 and Type 2 CalR genes, but more than 50 different mutations have been identified. Mutant CALR is the result of frameshift mutations caused by exon 9 deletions or insertions, type-1 52-bp deletion, and type-2 5-bp insertion.

The MPL gene, which is located on the short arm of the 1st chromosome, encodes thrombopoietin receptors. In experiments, it has been demonstrated that MPL gene mutation leads to the proliferation of hematopoietic cells without growth factors and activates signal transduction pathways such as JAK/STAT, MAPK, and PI3K/AKT. Therefore, they cause uncontrolled proliferation. It is a significant risk factor for microvessel disturbances, suggesting platelet hyperreactivity; arterial thromboses are increased. MPL exon 10 mutations (mainly involving codon W515) occur in 5–10 % of patients with JAK2 V617F-negative ET or PMF. The most common MPL mutations are W515L (tryptophan-to-leucine substitution) and W515K (tryptophan-to-lysine substitution). Other MPL mutations (MPL W515S, W5151A, and MPL S505N) have also been reported in cases of hereditary thrombocytosis.

In this study, we aimed to investigate the frequency of CalR and MPL gene mutations and complications in patients with JAK2 unmutated bcr-abl negative chronic myeloproliferative disease in our center.

2. Materials and Methods

2.1. Patients

This study was approved by the Institutional Ethics Committee (İstanbul University, Faculty of Medicine Ethics Committee, İstanbul, Turkey). The procedures followed were those of the Helsinki Declaration of 1975, as revised in 2000. Samples were obtained after patients provided written informed consent. The patients and data were selected retrospectively at the İstanbul Faculty of Medicine, Department of Hematology. The primary objective of the study was to evaluate the demographic features, treatments, survival, and status of CalR and MPL gene mutation in patients with JAK2 unmutated CMPNs in our center. Forty-six patients with ET and PMF were enrolled (28 females and 18 males with a mean age of 53,5 years, range 23-93 years); they had been diagnosed at the Department of Hematology between March 2004 and January 2013 according to the WHO criteria. According to WHO criteria, patients≥18 years old with a diagnosis of PV, ET, or PMF were enrolled in the study. Patients were excluded if the JAK2 gene mutated, did not fulfill WHO criteria for PV, ET, or PMF, and did not attend follow-ups regularly.

2.2. JAK2, CalR and MPL Gene Analysis

For each patient, genomic DNA was isolated from peripheral blood. The concentration of the isolated DNA samples was determined with a Nanodrop 2000c spectrophotometer (Thermo Scientific, USA). A tetra-primer PCR assay assessed the JAK2 V617F mutation in all patients. Patients with non-mutated JAK2 PMF or ET were further evaluated for CalR and MPL gene status. CalR (exon 9) and MPL W515 L / K (exon 10) mutations were analyzed on the LightCycler 480II Real-Time PCR device (Roche Diagnostics). CalR mutation analysis was performed by high-resolution melting (HRM) analysis, and MPL W515L / K mutation analysis was performed by HRM and allele-specific PCR analysis. Regarding the reliability of the study, each sample was studied twice. The samples were divided into groups by comparing the differences in the melting curves obtained due to the analysis. Samples that differ in the melting curves due to the analysis were considered potential mutation carriers. If a CalR mutation is detected, TaqMan-based analysis is used for verification.

2.3. Statistical Analysis

All statistical analyses were performed using SPSS software (version 25.0, SPSS, Chicago, IL, USA). Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used to evaluate the data. The Kolmogorov-Smirnov and Shapiro-Wilk

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tests tested the suitability of quantitative data for normal distribution. The Mann-Whitney U test compared two groups of non-normally distributed data. Wilcoxon Signed Ranks test was used for intra-group comparisons of non-normally distributed parameters. Fisher's Exact test was used to compare qualitative data. Overall survival (OS) was defined as the period between diagnosis and death because of any reason or last contact. OS evaluation was performed by using the Kaplan-Meier method. Significance was at least $p < .05$.

3. Results

Forty-six patients who were diagnosed with JAK2 V617F unmutated chronic myeloproliferative Bcr-Abl negative disease between March 2004 and January 2013 were included in the study; 73.9%(n:34) of all patients had ET, and 26.1%(n:12) had PMF. All PV patients were carrying the JAK2 gene mutation, so they were not included. The ages of the cases ranged between 23 and 93 (28 females and 18 males, with a mean age of 53,5 years). Table 1 summarizes the baseline characteristics of all patients.

Table 1. Main demographic and hematological features of 46 patients with JAK2 unmutated bcr-abl negative chronic myeloproliferative disease.

Variable	Total CMN (n:46)	PMF (n:12)	ET(n:34)	*p*
Male/female, % male	18/28 (39.1)	2/10 (16.6)	16/18 (47.0)	*.06*
Age (onset), y	53.5 (23-93)	55.1 (33-85)	53 (23-93)	*.97*
Leucocytes (×10⁹/L)	8.9 (3.6-24.5)	9.4 (3.6-23.4)	8,8 (4.9-24.5)	*.80*
Hemoglobin (g/dL)	11.6 (5.8-15.9)	9,7 (5.8-11.1)	12,3 (7.9-15.9)	*.001*
Platelets (×10⁹/L)	857 (147-2631)	533 (147-921)	993 (326-2631)	*.001*

CalR Type 1 gene mutation in 26.1% (n = 12) of cases, CalR Type 2 gene mutation in 13.0% (n = 6), MPL-L gene mutation in 2.2% (n = 1) and MPL-K gene mutation in 6.5% (n = 3) are detected Table 2. Twenty-eight (60.8%) patients were triple negative for CalR and MPL gene mutations.

The rate of CalR gene mutation in patients with PMF who do not have JAK2 gene mutation was 41.6%. On the other hand, the rate of MPL gene mutation in this population was 33.3%. In the ET group who have unmutated JAK2, the CalR mutation rate was 35.2%, and MPL mutation was not detected. CalR Type 1 and MPL-L gene mutations were detected together in one patient with PMF. CalR Type 1 and CalR Type 2 gene mutations were detected in one patient with ET. Twenty-six (%56,5) patients were negative for CalR Type 1, Type 2, and MPL L/K gene mutations. There was no statistically significant difference between the rates of incidence of CalR Type 1, CalR Type 2, and MPL-L gene mutations in the two disease groups ($p > .05$), but there was a statistically significant difference between the incidence rates of MPL-K gene mutation in those groups ($p = .003$; $p < .05$). The incidence of the MPL-K gene mutation was higher in patients with diagnosed PMF than in those with ET. At the same time, it was noteworthy that none of the patients with ET had MPL-L and MPL-K mutations.

Table 2. CalR and MPL gen mutation status in patients with PMF and ET.

Gene Mutation	PMF (n=12,%)	ET (n=34,%)	CMN(N=46,%)	*P*
CalR-Type 1	4 (33.3)	8 (23.5)	12 (26.1)	*.51*
CalR- Type 2	1 (8.3)	5 (14.7)	6 (13.0)	*.57*
MPL-L	1 (8.3)	0 (0)	1 (2.2)	*.09*
MPL-K	3 (25.0)	0 (0)	3 (6.5)	*.003*

The patients were evaluated about their mutational status and ages; there was no statistical significance between the ages and CalR Type 1 and 2 gene mutations in patients with essential thrombocythemia ($p = .62$, $p = .81$, respectively). None of the patients with ET had MPL-L and MPL-

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K mutations. Similarly, no significant difference was found between the gene mutations and age in patients with PMF (CalR Type 1 gene mutation p= .71, MPL-K gene mutation p= .30). The mutational status for the CalR Type 2 and MPL-L were not statistically significant.

The mean follow-up period was 99 months in patients with PMF and 120 months in patients with ET (p= .27). No effect of mutation status on survival was observed in our subgroup analyses. In this study, we also did not observe significant differences in overall survival between patients in the triple-negative group and others (110.3 months vs. 121.4 months, respectively, p= .53). In this period, leukemic transformation was observed in 3 of 46 patients (n:2 (%5.9) ET, n:1 (%8.3) PMF patients). CalR gene mutation was present in one ET patient with leukemic transformation, while the other two patients were triple negative. Major thrombotic events were observed in 6 patients; all were ET patients, 3 had CalR mutation, two had CalR type 1, and one had CalR type 2. The mortality ratio was higher in patients with PMF, regardless of mutational status (%58.3 and %14.7, p= .006).

4. Discussion

In this study, we researched the frequency and clinical effects of CalR and MPL gene mutation in JAK2 gene mutation negative chronic myeloproliferative disease. Because of the small sample size, we were unable to detect statistical significance, but the results were clinically valuable. We know that JAK2 V617F gene mutation has a pivotal role in the diagnosis of chronic myeloproliferative neoplasms; it only occurs in 50-60% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF). MPL and CalR gene mutations are also frequently used in clinics besides being included in WHO diagnostic criteria. There is an increase in the number of driver mutations with increasing age and disease progression. Driver mutations and germline polymorphisms predict whether patients have essential thrombocythemia, polycythemia vera, or myelofibrosis. Genomic data can be integrated with clinical parameters to predict patient outcomes. MPN patients will benefit from this improvement in management [4,15].

In ET patients, JAK2 V617F driver mutation can be found in approximately 60%, CalR and MPL mutations in approximately 20% and 3%, respectively [5,6]. The CalR gene mutation is found in 20-80% of patients with ET without JAK2 and MPL gene mutations. In our study, the rate of CalR gene mutation in ET with unmutated JAK2 is similar to the rates in the literature (%35.2).

MPN who do not have these three genetic mutations (JAK2, CalR, and MPL) are called triple-negative (triple-negative ET accounts for 10-20% of ET). They are at greater risk of leukemic transformation and have worse outcomes [6,8]. It is well known that patients diagnosed with ET who carry MPL mutations have the worst prognosis; however, among our ET patients, no patient carried an MPL mutation [7].

According to our current knowledge, CalR gene mutation is associated with younger age and has a higher platelet count [4]. In ET patients, CALR gene mutation appears to be associated with a decreased risk of thrombosis. In contrast, mutation of JAK2 is associated with an increased risk of thrombosis and a lower risk of myelofibrotic transformation [7,9-11]. There were 1.9% fatal and non-fatal thrombotic events per patient/year [12]. Half of our six patients with thrombosis (all in the ET group) during follow-up had a mutation in the CalR gene (2 had CalR type 1 mutation and 1 had CalR type 2).

There have been a range of reports regarding the progression to the accelerated phase at the age of 10 years, ranging from 0.7 to 1.9% [6,10]. CalR gene-mutated ET more frequently progressed to the blast or accelerated phases than JAK2-mutated ET [2]. Leukemic transformation was observed in 1 patient carrying the CalR gene mutation in our groups.

Type 2 CalR gene mutation causes more fibrosis than Type 1 CalR gene mutation. CalR type 2 mutations in ET patients are also associated with a higher platelet count than CalR type 1 mutations [4]. There is a tendency for CalR type 2 mutations to be associated with ET, whereas type 1 mutations are associated with PMF [3]. The CalR type 1 gene mutation was seen more frequently in PMF cases in our group as well.

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PMF harbors 52-67% mutations of JAK2 V617F, %4-12 CalR gene mutation, and %4-6 MPL mutation [14]. In the PMF group, CalRmutations were associated with lower leukocyte counts, lower bone marrow cellularity, and more megakaryocytes. On the other hand, a study in patients with PMF showed that the mean survival time was significantly longer in patients with CalR gene mutation than without CalR mutation. This correlation was not detected in our study; this may be related to the size of the population concerned. It is noteworthy that our PMF group did not experience any thrombosis or leukemic transformation.

MPLW515 L/K patients presented reduced total and erythroid bone marrow cellularity, whereas the numbers of megakaryocytes, megakaryocytic clusters, and small-sized megakaryocytes significantly increased. MPL gene mutation has been reported in %5-10 of patients with JAK2-negative ET or PMF [15]. The incidence of MPL gene mutation was statistically higher in our patients with PMF. The difference between ET and profibrotic MF might be challenging in some cases. The presence of atypical megakaryocytes, granulocytic proliferation, and clinical symptoms such as increased LDH or splenomegaly support a diagnosis of prefibrotic PMF. It is distinguished from ET by abnormally large and dense megakaryocyte clusters. MPL gene mutation may be more valuable in patients with suspicious diagnosis, especially post-ET myelofibrosis patients. In our patients, it was noteworthy that no MPL mutation was observed in any patient with ET. It is possible that, in rare instances, the presence of MPL mutation could be a beneficial indicator of PMF.

MPN is a genetically heterogeneous disease consisting of germinal and somatic mutations that contribute to disease. This heterogeneity can be demonstrated using next-generation sequencing techniques. Studies have shown that atypical JAK2, CALR, and MPL variants are essential for diagnosing these diseases [16]. It has been shown that there is a high rate of poor prognostic mutations, such as SH2B3 and ASXL1, in the presence of driver mutations containing these atypical variants [16,17]. Due to this situation, sequencing techniques for new generations have become more critical.

In conclusion, the results of these studies suggest that MPL gene mutations may make PMF diagnoseable earlier and that ET patients with CalR gene mutations are more likely to develop leukemic transformation and thrombosis. Further research is anticipated to contribute to the diagnosis, treatment, and prediction of the course of MPN.

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Informed Consent Statement: Patients had provided written informed consent.

Data Availability Statement: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Baumeister J,; Chatain N,; Sofias AM,; Lammers T,; Koschmieder S. Progression of Myeloproliferative Neoplasms (MPN): Diagnostic and Therapeutic Perspectives. Cells. 2021;10(12). http://doi: 10.3390/cells10123551.
2. Kim SY,; Im K,; Park SN,; Kwon J,; Kim JA,; Lee DS. CALR, JAK2, and MPL mutation profiles in patients with four different subtypes of myeloproliferative neoplasms: primary myelofibrosis, essential thrombocythemia, polycythemia vera, and myeloproliferative neoplasm, unclassifiable. Am J Clin Pathol. 2015;143(5):635-44. 9 http:// doi: 10.1309/AJCPUAAC16LIWZMM.

3. Barbui T,; Thiele J,; Gisslinger H,; Kvasnicka HM,; Vannucchi AM,; Guglielmelli P,; Orazi A,; Tefferi I A. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood Cancer J. 2018;8(2):15. http:// doi: 10.1038/s41408-018-0054-y.
4. Grinfeld J,; Nangalia J,; Baxter EJ,; Wedge DC,; Angelopoulos N,; Cantrill R,; Godfrey AL,; Papaemmanuil E,; Gundem G,; MacLean C; et al. Classification and Personalized Prognosis in Myeloproliferative Neoplasms. N Engl J Med. 2018;379(15):1416-30. http:// doi: 10.1056/NEJMoa1716614.
5. Tefferi A,; Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. Am J Hematol. 2020;95(12):1599-613. http:// doi: 10.1002/ajh.26008.
6. Tefferi A,; Pardanani A. Essential Thrombocythemia. N Engl J Med. 2019;381(22):2135- 44. http:// doi: 10.1056/NEJMcp1816082.
7. Elala YC,; Lasho TL,; Gangat N,; Finke C,; Barraco D,; Haider M,; Hussein AKA,; Hanson CA,; Ketterling RP,; Pardanani A,; et al. Calreticulin variant stratified driver mutational status and prognosis in essential thrombocythemia. Am J Hematol. 2016;91(5):503-6. http:// doi: 10.1002/ajh.24338.
8. Tharakan S,; Mascarenhas J,; Tremblay D. Understanding triple negative myeloproliferative neoplasms: pathogenesis, clinical features, and management. Leuk Lymphoma. 2024;65(2):158-67. http:// doi: 10.1080/10428194.2023.2277674.
9. Rotunno G,; Mannarelli C,; Guglielmelli P,; Pacilli A,; Pancrazzi A,; Pieri L,; Fanelli T,; Bosi A,; Vannucchi AM. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. Blood. 2014;123(10):1552-5. http:// doi: 10.1182/blood-2013-11-538983.
10. Najim M,; Abu-Tineh M,; Alshurafa A,; Ibrahim MIM,; Ansari S,; Faraj H,; Alateeg S,; Akiki SJ,; Yassin MA. The characteristics of CALR mutations in myeloproliferative neoplasms: a clinical experience from a tertiary care center in Qatar and a literature review. Hematology. 2024;29(1):2360246. http:// doi: 10.1080/16078454.2024.2360246.
11. Guglielmelli P,; Szuber N,; Gangat N,; Capecchi G,; Maccari C,; Harnois M,; Karrar O,; Abdelmagid M,; Balliu M,; Nacca E,; et al. CALR mutation burden in essential thrombocythemia and disease outcome. Blood. 2024;143(13):1310-4. http:// doi: 10.1182/blood.2023023428.
12. Gianelli U,; Thiele J,; Orazi A,; Gangat N,; Vannucchi AM,; Tefferi A,; Kvasnicka HM. International Consensus Classification of myeloid and lymphoid neoplasms: myeloproliferative neoplasms. Virchows Arch. 2023;482(1):53-68. http:// doi: 10.1007/s00428-022-03480-8.
13. Tefferi A,; Wassie EA,; Guglielmelli P,; Gangat N,; Belachew AA,; Lasho TL,; Finke C,; Ketterling RP,; Hanson CA,; Pardanani A,; et al. Type 1 versus Type 2 calreticulin mutations in essential thrombocythemia: a collaborative study of 1027 patients. Am J Hematol. 2014;89(8):E121-4. http:// doi: 10.1002/ajh.23743.
14. Guglielmelli P,; Pacilli A,; Rotunno G,; Rumi E,; Rosti V,; Delaini F,; Maffioli M,; Fanelli T,; Pancrazzi A,; Pietra D,; et al. Presentation and outcome of patients with 2016 WHO diagnosis of prefibrotic and overt primary myelofibrosis. Blood. 2017;129(24):3227- 36. 10 http:// doi: 10.1182/blood-2017-01-761999.
15. Luque Paz D,; Kralovics R,; Skoda RC. Genetic basis and molecular profiling in myeloproliferative neoplasms. Blood. 2023;141(16):1909-21. http:// doi: 10.1182/blood.2022017578.
16. Wang Z,; Tian X,; Ma J,; Zhang Y,; Ta W,; Duan Y,; Li F,; Zhang H,; Chen L,; Yang S,; et al. Clinical laboratory characteristics and gene mutation spectrum of Ph-negative MPN patients with atypical variants of JAK2, MPL, or CALR. Cancer Med. 2024;13(7):e7123. http:// doi: 10.1002/cam4.7123.
17. Zhang S,; Yan J,; He L,; Jiang Z,; Jiang H. STAT5a and SH2B3 novel mutations display malignancy roles in a triple-negative primary myelofibrosis patient. Cancer Gene Ther. 2024;31(3):484-94. http:// doi: 10.1038/s41417-023-00719-7.