

# JAK 2V617F Mutation: Frequency and Relation to Clinical and Laboratory Features of BCR-ABL Negative Myeloproliferative Diseases

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## ABSTRACT

In this study, we planned to investigate frequency of the JAK2 V617F mutation and its relation with clinical and laboratory findings of BCR-ABL negative myeloproliferative diseases (MPD) which consist of polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). Totally 65 patients were included in the study which composed of 28 (43.1 %) PV, 29 (44 %) ET and 8 (12.3 %) IMF patients. Forty one (63%) patients were female and 24 (37%) were male. Mean age of patients was 64.02±12.42. Frequency of the JAK2V617F mutation was found in 25 (89.3%) of PV, 18 (62.1%) of ET and 2 (25%) of IMF patients. We found no difference in gender and frequency of thrombosis, constitutional symptoms, pruritus, hemorrhage, splenomegaly, bone marrow fibrosis, cytoreductive treatment requirement, arterial/venous thrombosis between JAK2 V617F mutated and unmutated PV and ET and IMF patients. When compared homozygous and heterozygous JAK2 V617F mutated PV and ET patients according to these variables, there was no significant difference. There were no statistically significant difference in Hemoglobin (Hb), hematocrit (HCT), leukocyte, lactate dehydrogenase (LDH), EPO and ferritin levels between JAK2 V617F mutated and unmutated PV and ET patients. HCT levels and leukocyte counts were significantly higher in JAK2 V617F mutated than unmutated IMF patients ( $p=0.046$ ,  $p=0.046$ ). Other variables were not found different. Comprehensive prospective studies are necessary for determining the relationship of the JAK2 V617F mutation with clinical and laboratory findings .

**Keywords:** JAK2 V617F mutation, Polycythemia vera, Essential thrombocythemia, Idiopathic myelofibrosis

## ÖZET

**JAK 2V617F Mutasyonu: BCR-ABL Negatif Myeloproliferatif Hastalıklardaki Sıklığı, Klinik ve Laboratuvar**

### Bulgularla İlişkisi

Bu çalışmada, polisitemia vera (PV), esansiyel trombositemi (ET) ve idiopatik myelofibrozisten (IMF) oluşan BCR-ABL negatif myeloproliferatif hastalıklarda JAK2 V617F mutasyonunun sıklığı, klinik ve laboratuvar bulgularıyla ilişkisini araştırmayı planladık. Çalışmaya 28 (%43.1) PV, 29 (%44) ET, 8 (%12.3) IMF hastası olmak üzere toplam 65 hasta alındı. Hastaların 41'i (%63) kadın, 24'ü (%37) erkekti. Ortalama yaş 64.02±12.42 idi. Bu mutasyonun sıklığı, PV hastalarının 25'inde (%89.3), ET hastalarının 18'inde (% 62.1) ve IMF hastalarının 2'sinde (%25) olarak bulundu. JAK2 V617F mutasyonu olan ve olmayan PV ve ET ve IMF hastaları arasında cinsiyet, tromboz sıklığı, konstitüsyonel semptomlar, pruritus, hemoraji, splenomegali, kemik iliği fibrozisi, sitoredüktif tedavi ihtiyacı, arteriyel/venöz tromboz gelişimleri açısından bir fark bulamadık. Heterozigot ve homozigot JAK2 V617F mutasyonu olan PV ve ET hastaları, bu parametreler açısından karşılaştırıldığında, anlamlı bir fark yoktu ( $p>0.05$ ).

**Anahtar Kelimeler:** JAK V617F Mutasyonu, Polistemia vera, Esansiyel trombositemi, İdiopatik myelofibrozis

JAK2 V617F mutasyonu olan ve olmayan PV ve ET hastaları arasında hemoglobin (Hb), hematokrit (HCT), lökosit, laktik dehidrojenaz (LDH), EPO ve ferritin düzeyleri açısından istatistiksel bir fark yoktu ( $p>0.05$ ). Hematokrit düzeyleri ve lökosit sayıları JAK2 V617F mutasyonu olan IMF hastalarında mutasyon olmayanlara göre belirgin olarak daha yüksekti ( $p=0.03$   $p=0.01$ ). Diğer parametreler farklı bulunmadı. Klinik ve laboratuvar bulgularla JAK2 V617F mutasyonu arasındaki ilişkiyi tanımlamak için daha geniş kapsamlı prospektif çalışmalar gereklidir.

**Anahtar Kelimeler:** JAK2 V617F mutasyonu, Polisitemia vera, Esansiyel trombositemi, İdiopatik myelofibrozis

## INTRODUCTION

Myeloproliferative diseases (MPD) are heterogenous, clonal group of disease which occur in response to cytokines independently. In MPD, hematopoietic cells are hypersensitive to cytokines and they perform uncontrolled proliferation. Since 2008, World Health Organisation (WHO) has named this group as myeloproliferative neoplasms (MPN). MPN are chronic myelogenous leukemia (CML), BCR-ABL positive, chronic neutrophilic leukemia, polycythemia vera (PV), idiopathic myelofibrosis (IMF), essential thrombocythemia (ET), chronic eosinophilic leukemia, not otherwise specified, mastocytosis and myeloproliferative neoplasms, unclassifiable.<sup>1</sup>

Janus Kinase 2 (JAK2) is a non receptor tyrosine kinase molecule which plays an important role in lifecycles of hematopoietic progenitor cells. JAK2 contributes to synthesis of erythropoetin (EPO), thrombopoetin, GM-CSF receptors and serves as tyrosine kinase for them. After an acquired point mutation of JAK2, resulting in valine to phenylalanine substitution at the 617 amino acid, JAK2 escapes from otoregulator system and becomes active without physiological signal. JAK2 gene is on 9th chromosome and this point mutation was identified in BCR-ABL negative MPD in 2005.<sup>2-6</sup> According to the studies, JAK2 V617F mutation is seen 97% of PV, 57% of ET and 50% of IMF.<sup>3</sup> In addition, JAK2 V617F mutation has been identified in atypical MPD, myelodysplastic syndrome, and erytroleukemia. For this reason, this mutation is not a specific genetic marker like BCR-ABL translocation used in CML diagnosis. But recently, it has been started to use in diagnostic algorithm especially for PV. Four different mutation were identified in exon 12 area of JAK2 gene in patients with JAK2 V617F negative PV but not in patients with ET. Patients with exon 12 mutation generally have low EPO levels and more dominant erytroid lineage than other

series in bone marrow. In a study, exon 12 mutation was identified in 4 of 20 patients with PV and idiopathic erythrocytosis and there were no difference in age, gender, leukocyte count between two groups.<sup>5</sup> But patients with exon 12 mutation had higher Hb and lower platelet count than patients without the mutation. There were no difference in thrombosis development and myelofibrotic transformation between two groups.<sup>5</sup> Another mutation identified in MPD is MPL mutation (MPLW515L). MPL is described in juxtamembran region of thrombopoetin receptor in IMF and rarely ET but not in PV.<sup>7</sup> This mutation was seen 5% in IMF patients and these patients had reported to have lower Hb levels and higher transfusion requirement. MPL mutation was seen 1% of ET patients and it's clinical importance is not clear. Patients with JAK2 mutation was reported to have higher Hb, leukocyte counts and more cellular bone marrow.<sup>8</sup> While some studies showed increase risk of thrombosis in JAK2 positive ET patients<sup>4,9,10,11</sup>, some studies showed no relationship between JAK2 mutation and thrombosis in ET patients.<sup>12,13,14</sup> Thrombosis, an important morbidity and mortality cause in MPD, was found related to neutrophile activation on platelet-neutrophile complexes.<sup>10,15-19</sup> JAK 2 mutation was reported to provide a convenient situation for formation of platelet-neutrophile aggregates<sup>10</sup> and this can explain why hydroxyurea is more effective than anagrelide in thrombosis prevention.<sup>15,19</sup> Another study with 397 PV patients reported that thrombosis risk was not related to mutation prophile (wild type, heterozygous or homozygous).<sup>20</sup> Primignani and coworkers showed that JAK2 V617F mutation was found in 35% of 73 patients with portal vein thrombosis and in 40% of 20 patients with Budd Chiari syndrome.<sup>19</sup> Boissinot and coworkers found JAK2V617F mutation in 58% of patients with idiopathic Budd Chiari syndrome.<sup>21</sup> JAK2 V617F was seen in 38-43% of splanchnic vein thrombosis with normal or

low blood counts and in 18-37% of portal vein thrombosis.<sup>22</sup> In another study, JAK2 mutation was found in 74% of 19 idiopathic portal or hepatic vein thrombosis and JAK2 mutation was reported as a valuable genetic marker in latent MPD diagnosis.<sup>23</sup> In a study, ET patients with JAK2 V617F mutation were found to have higher Hb, neutrophile levels than patients without the mutation.<sup>24</sup> In addition, ET patients with JAK2 mutation were reported to have increased bone marrow erythropoiesis and granulopoiesis, more venous thrombosis, lower erythropoietin and ferritin levels and more frequent polycytemic transformation than patients without the mutation.<sup>15</sup> ET patients with JAK2 V617F mutation were reported to have lower platelet counts and lower hydroxyurea dose requirement for controlling of platelet level. Campbell et al thought that JAK2 mutation positive ET was phenotypical similar with PV and these diseases could be accepted as continuation of each other.<sup>5</sup> Another study reported that homozygous JAK2 V617F mutated PV patients had higher hemoglobin and neutrophile counts, more tendency to pruritus and bone marrow fibrosis than unmutated or heterozygous mutated PV patients.<sup>24</sup> But thrombosis or hemorrhage frequency, cytogenetic abnormalities, transformation to myelofibrosis and acute leukemia were similar in JAK2 unmutated, heterozygous or homozygous mutated PV patients.<sup>24</sup> One of the recent studies reported that increase in mutation degree resulted in increase in the frequency of thrombosis, leukocytosis and splenomegaly.<sup>24</sup> Generalised pruritus was found more frequent in homozygous JAK2 mutated PV patients than unmutated or heterozygous mutated PV patients.<sup>24,25</sup>

There are few data about relation of JAK2 mutation to leukemic transformation. Kraloyics et al were found no relationship for JAK2 mutation and leukemic transformation in 244 MPD patients.<sup>4</sup> Jelinek and friends showed low JAK2 mutation incidence of secondary AML in IMF and PV patients.<sup>25</sup>

In a retrospective study with 63 PV patients performed by Tefferi et al, there was found no clinical difference in JAK2 mutated and unmutated PV patients but some differences in homozygous and heterozygous mutated patients. Homozygous PV patients had higher Hb levels and more frequent bone marrow fibrosis.<sup>23</sup>

JAK2 V617F mutation in IMF was found in relationship with high leukocyte count, poor prognosis and aggressive disease.<sup>26</sup>

## PATIENTS AND METHODS

This study was approved by Baskent University Institutional Review Board and Ethics Committee. After getting informed consent from 28 PV, 29 ET and 8 IMF patients, genomic DNA isolation was performed by using High Pure Isolation Kit (Roche Molecular Biochemicals, Mannheim, Germany) and spin column method. In order to identify JAK2 V617F point mutation, DNA samples were added into mixture of PCR which prepared with primary probe set diluted specific to reaction and "LightCycler Fast Start DNA Master Hybridization Probe" (Roche Molecular Biochemicals, Mannheim, Germany) kit content.

Analysis were performed with Light Cycler 2.0 analysis program. Genotype was identified according to "Melting Curves". Melting curves were made after determining  $t_m$  degrees 53°C for mutant series and 62°C for wild type (naturel type). A peak seen only at 62°C, only at 53°C and at both  $t_m$  accepted as wild type, homozygous mutant and heterozygous mutant respectively.

We used mean value  $\pm$  standart deviation for numeric data and percent/ratio for categorical data of demographic and disease related features. We investigated relation of JAK2 V617F mutation to Hb, HCT, platelet, leukocyte, ferritin, LDH and erythropoietin levels, gender, splenomegaly, constitutional symptoms, pruritus, bone marrow fibrosis, bone marrow cellularity, thrombosis, hemorrhage, cyto-reductive therapy requirement by using univariate analysis. Univariate analysis performed by Ki-square and Mann Whitney U tests SPSS 10 for Windows (Statistical Package for the Social Sciences 10) (SPSS Inc. Chicago, A.B.D).

## RESULTS

Sixty five patients were included in the study. Mean age of the patients was 64.02 $\pm$ 12.42. Forty one patients (63%) were female, 24 patients (37%) were male. Twenty eight patients (43%) were PV, 29 (44.6%) were ET and 8 (12.3%) were IMF. JAK2

**Table 1.** Frequency of JAK2 V617F mutation in myeloproliferative diseases

	JAK2 MUTATION		
	Positive (n,%)	Negative (n,%)	Total (n,%)
PV	25 (89.3%)	3 (10.7%)	28 (100%)
ET	18 (62.1%)	11 (37.9%)	29 (100%)
IMF	2 (25%)	6 (75%)	8 (100%)

V617F is positive in 25 (89.3%) of PV, 18 (62.1%) of ET and 2 (25%) of IMF patients (Table 1).

We compared JAK2 V617F mutated and unmutated 28 PV patients in terms of gender, frequency of thrombosis, constitutional symptoms (weakness, fever, night sweat, weight loss), pruritus, hemorrhage, splenomegaly, bone marrow fibrosis, bone marrow cellularity, requirement of cytoreductive treatment and we found no difference between two groups ( $p > 0.05$ ). All these variables are not statistically different between homozygous and heterozygous JAK2 V617F mutated PV patients. There was no difference in Hb, HCT, leukocyte, LDH, EPO, uric acid, ferritin levels between JAK2 V617F mutated and unmutated PV patients ( $p > 0.05$ ). There were 11 arterial and 2 venous thrombosis in PV patients and found no difference in frequency of thrombosis between JAK2 V617F mutated and unmutated PV patients ( $p > 0.05$ ).

In 29 ET patients, there were no difference in terms of gender, thrombosis frequency, constitutional symptoms, pruritus, hemorrhage, splenomegaly,

bone marrow fibrosis, bone marrow cellularity and requirement of cytoreductive treatment between JAK2 mutated and unmutated patients ( $p > 0.05$ ). All these variables were similar between homozygous and heterozygous JAK2 V617F mutated ET patients. Two groups were compared in terms of Hb, HCT, leukocyte, LDH, EPO, uric acid, ferritin levels and found no difference ( $p > 0.05$ ). There were 8 arterial and 1 venous thrombosis in ET patients. Frequency of arterial and venous thrombosis were similar between JAK2 V617F mutated and unmutated ET patients.

JAK2 mutated and unmutated 8 IMF patients compared in points of gender, frequency of thrombosis, constitutional symptoms, pruritus, hemorrhage, splenomegaly, bone marrow fibrosis, cytoreductive treatment requirement and bone marrow cellularity and found no difference ( $p > 0.05$ ). JAK2 mutated and unmutated IMF patients were compared according to Hb, HCT, leukocyte, LDH, EPO, uric acid, ferritin levels. HCT and leukocyte levels were found higher in JAK2 V617F mutated IMF patients

**Table 2.** Relationship between JAK2V617F mutation and laboratory variables in IMF patients.

	JAK2 MUTATION		
	Positive (median)	Negative (median)	P value
Hemoglobin (g/dl)	12.5	9,04	0.09
Hematocrit (%)	38.7	26.05	0.046
Leukocyte (/mm <sup>3</sup> )	30550	7235	0,046
platelet (/mm <sup>3</sup> )	305500	191000	0.505
LDH (IU/ml)	274.5	303	0.182
Uric acid (mg/dl)	6.4	7.3	1

than unmutated patients ( $p=0.046$ ) (Table-2). Other variables were similar in two groups ( $p>0.05$ ).

## DISCUSSION

We aimed to investigate frequency of JAK2 V617F mutation and relation to clinical and laboratory findings of patients with PV, ET and IMF. The limitation of our study is smaller patient (especially in IMF patients) population than literature.

Frequency of MPD increases with age and median age is 60.<sup>3</sup> Similarly, mean age of patients in our study was  $64.02 \pm 12.42$  (19-87). JAK2 V617F mutation was identified 97% in PV, 57% in ET and 50% in IMF patients (4). In our study, we found the mutation 89.3% in PV, 62.1% in ET and 25% in IMF patients.

We didn't find any difference in frequency of arterial and venous thrombosis in PV patients ( $p>0.05$ ). There are insufficient data about this in studies investigating arterial and venous thrombosis in PV patients.<sup>11,16,27,28</sup>

Frequency of homozygous JAK2 V617F mutation in PV is higher than in ET patients. In PV patients frequency of homozygous JAK2 V617F mutation is about 30% and the mutation seems to be related to polycytemic phenotype.<sup>29</sup> In a study with 63 PV patients, Hb, leukocyte, platelet count, pruritus, myelofibrosis and acute leukemic transformation, hemorrhage/thrombosis frequency were compared in homozygous and heterozygous JAK2 V617F mutated patients. Hb level, pruritus and frequency of myelofibrosis transformation were higher in homozygous mutated patients than heterozygous mutated patients. Thrombosis and hemorrhage frequencies were similar in homozygous mutated and heterozygous mutated patients.<sup>23</sup> In our study, frequency of homozygous JAK2 V617F mutation was 9.1% and it was lower than expected. Homozygous and heterozygous patients were not different in point of thrombosis, constitutional symptoms, pruritus, hemorrhage, splenomegaly, frequency of bone marrow fibrosis, bone marrow cellularity and requirement of cytoreductive treatment.

In a study with 150 ET patients, JAK2V617F mutated patients were reported to have higher median age, leukocyte count, platelet count and frequency

of transformation to PV than unmutated patients. There were no difference according to splenomegaly, cytogenetic abnormalities, vasomotor symptoms, frequency of thrombosis and hemorrhage, requirement of cytoreductive treatment and median survival between two group.<sup>30</sup> In a prospective study conducted with 806 ET patients, JAK2 V617F mutated patients had higher Hb, leukocyte counts, more erythropoiesis and granulopoiesis in bone marrow, more frequent venous thrombosis and polycytemic transformation, less EPO and ferritin levels than unmutated patients. In addition, this study reported that JAK2 V617F mutated patients have more benefit from hydroxyurea than anagrelid.<sup>5</sup> Our study showed no difference in terms of frequency of thrombosis, constitutional symptoms, pruritus, hemorrhage, splenomegaly, bone marrow fibrosis, bone marrow cellularity and cytoreductive treatment, between JAK2 mutated and unmutated ET patients. In addition, Hb, HCT, leukocyte, LDH, EPO, uric acid and ferritin levels were similar in two groups.

In a study conducted with ET patients, JAK2V617F mutation was reported to be in relationship with venous thrombosis rather than arterial thrombosis.<sup>15</sup> In a recent study, 867 patients with ET wild-type, ET V617F, and 415 patients with PV showed a rate of thrombosis of 1.4%, 2.1%, and 2.7%/patients/year. Actuarial probability of arterial and venous thrombosis in the first 5 years of diagnosis was roughly similar in the three groups. The curves of mutated ET patients diverged from wild-type, and after 10 to 15 years the ET-mutated arm approached PV.<sup>31</sup> In our study, frequency of arterial and venous thrombosis were found similar in JAK2 V617F mutated and unmutated ET patients.

In GIMEMA MPD Group's study conducted with 639 ET patients, JAK2 V617F mutation was found heterozygous in 57.6%, homozygous in 2.2% and negative 40.2% of patients. This study reported that, JAK2 V617F homozygous mutated patients had increased erythropoiesis and granulopoiesis and lower platelet count than unmutated and heterozygous mutated patients. In addition, homozygous mutated ET patients were found to have higher incidence of splenomegaly and increased requirement of cytoreductive treatment. Homozygous mutated ET patients were reported to have higher

thrombosis incidence than the others. In this study JAK2 V617F mutation was found heterozygous in 67.8% and homozygous in 32.2% in 323 PV patients. Homozygous mutated PV patients were found to have increased erythropoiesis, granulopoiesis, higher incidence of splenomegaly, higher requirement of cytoreductive treatment, lower platelet levels and higher incidence of pruritus than heterozygous mutated PV patients. There were no difference in point of frequency of thrombosis.<sup>32</sup> In another study, JAK2 V617F mutation occurs in a homozygous state in 25% to 30% of patients with PV and 2% to 4% with ET. 118 homozygous patients (104 PV, 14 ET) were older, displayed a higher leukocyte count and hematocrit value at diagnosis and presented larger spleen size. Homozygosity associated with more frequent evolution into secondary myelofibrosis in both PV and ET. Homozygous ET patients displayed a significantly higher risk of cardiovascular events.<sup>24</sup> In our study, frequency of thrombosis, constitutional symptoms, pruritus, frequency of hemorrhage, splenomegaly, bone marrow fibrosis, bone marrow cellularity and requirement of cytoreductive treatment were not significantly different between homozygous and heterozygous mutated patients.

In a study conducted with 152 IMF patients, JAK2 V617F mutated and unmutated patients were not different in terms of age, gender, spleen size, Hb, LDH and platelet levels. But JAK2 V617F mutated patients were found to have higher leukocyte and neutrophil counts than unmutated patients. In addition, JAK2 V617F mutated patients were found to have rarer need of transfusion but less survival than unmutated patients.<sup>33</sup> On the other hand, Tefferi and coworkers reported JAK2 V617F have no effect on survival and leukemic transformation but to have related with thrombosis.<sup>34</sup> In the Barosi and coworkers' study with 304 IMF patients, JAK2 V617F mutation was found in 63.5% of patients. In addition, JAK2 V617F mutated patients were found to have higher Hb, leukocyte and platelet counts and more frequent pruritus than unmutated patients.<sup>35</sup> A recent study showed that a low JAK2V617F allele burden at diagnosis is associated with a myelodepletive rather than myeloproliferative phenotype and associated with shortened survival in PMF patients.<sup>36</sup> In our study, we found JAK2 V617F mutated IMF patients to have higher

HCT and leukocyte levels than unmutated IMF patients. There were no significant difference in point of Hb, leukocyte counts, LDH, EPO, uric acid and ferritin levels between JAK2 V617F mutated and unmutated IMF patients. Frequency of thrombosis, constitutional symptoms, pruritus, hemorrhage, bone marrow cellularity and requirement of cytoreductive treatment were not different between JAK2 V617F mutated and unmutated IMF patients. Difference of these results from literature findings may be related to smaller patient population of IMF. In a study conducted with 6 IMF patients, relation of JAK2 V617F mutation to thrombosis is reported to be unclear.<sup>26</sup>

JAK2 Exon 12 mutation was reported 3-4% in PV patients. In a comprehensive study compared 409 patients with idiopathic erythrocytosis and 719 PV patients, frequency of JAK2 exon 12 mutation was found 3.7%. These patients were younger than JAK2 V617F mutated PV patients and have dominance of female gender. In addition, higher HCT, lower EPO levels, lower platelet and leukocyte counts in JAK2 exon 12 mutated PV patients than JAK2 V617F mutated patients.<sup>6</sup> In our study, JAK2 V617F mutation was negative in 10.7% of PV patients and we couldn't investigate JAK2 Exon 12 mutation in these PV patients.

In this study, we investigated JAK2 V617F mutation frequency and its relation to clinical and laboratory findings of BCR-ABL negative MPD patients. More comprehensive prospective studies are needed to show prognostic value of the mutation and relation to clinical and laboratory variables .

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