Journal of Applied Biological Sciences
E-ISSN: 2146-0108 15(3): 330-341, 2021
Research Article

DISTRIBUTION OF JAK2V617F MUTATION IN CHRONIC MYELOPROLIFERATIVE DISEASES

Akın Yigin [1*], Hatice Korkmaz Guvenmez [2], Bulent Antmen [3]

[1] *Harran University, Veterinary Faculty, Genetics Department, Şanlıurfa, Turkey*
[2] *Çukurova University, Art and Science Faculty, Department of Biology, Balcalı, Adana, Turkey*
[3] *Department of Pediatric Hematology and Oncology, Acıbadem Hospital, Adana, Turkey*

**Corresponding Author:*
E-mail: akinyigin@yahoo.com

(Received 18th May 2021; accepted 30th June 2021)

ABSTRACT. Recent studies have linked mutations in JAK2 to polycythemia vera (PV), essential thrombocythemia (ES), primary myelofibrosis (PMF), as well as other myeloproliferative disorders. In this study, it was aimed to determine the prevalence of JAK2V617F mutation in a broad study group diagnosed with pediatric AML and ALL and adult hematological malignancies diagnosed with PV, ET, CML, PMF and to associate with clinical findings. Patients diagnosed with acute leukemia in the pediatrics group (164 with ALL, 52 with AML), as well as 176 adult patients with PV, ET, CML and PMF, were included in the study. In parallel with cytomorphological, immunohisto-chemical and immunohistometric studies, DNA isolation from whole blood was performed in patients and mutations of the JAK2V617F gene were detected by Realtime PCR and sequence analysis. A significant association was found between JAK2V617F mutation and thrombocyte values due to thrombocytosis, which is the main pathogenomic sign of the disease in ET. In adult groups of patients with ET and PV, a statistically significant difference was found between genotype and allele distribution (p=0.000), and it was found that the patient with T allele may pose a risk of disease in terms of allele distribution (p=0.000). The JAK2V617F mutation was found to be insignificant in hematologic malignancies for childhood leukemias compared to adults. As a result, the distribution of JAK2 mutations in patients diagnosed with pediatric and adults hematological malignancies diagnosed was found to be similar to the results reported in the literature. Especially in the differential diagnosis of PV and ET in hematological malignancies and in the follow-up of the response to treatment, the detection of JAK2V617F mutation by Realtime PCR is useful as a screening test and the importance of the JAK2V617F mutation detected in MPDs patients.

Keywords: *JAK2V617F mutation, AML, ALL, ET, PV*

INTRODUCTION

Chronic myeloproliferative diseases (MPDs) were first identified by Dameshek in 1951. MPDs are clonal hematologic malignancies caused by the proliferation of advanced and undeveloped cells in the bone marrow and peripheral blood of one or more myeloid cell generations of clonal hematopoietic stem cell irregularities [1, 2].

Mutations that can occur in all genes involved in the control of the cell cycle (protooncogene and/or tumor suppressor), chimeric genes formed by chromosomal translocations, and disruption of coordination between the proliferation and differentiation of hematopoietic precursor cells lead to the development of MPDs.

Somatic mutation profiles are now used in identifying subtypes of various myeloid neoplasms, risk classification, and prognostics, including acute myeloid leukemia

(AML), myelodysplastic syndrome, and myeloproliferative neoplasms. In particular, the genetic differences and associated metabolic pathway activities identified by NGS (New Generation Sequencing) Technology in these patients provided important information about mutations and clonal evolution in myeloid leukemia and led to drug development [3].

Seven different chronic MPDs are defined according to the World Health Organization classification; Polisitemia-Vera (PV), Essential Thrombocythemia (ET), Idiopathic Myelofibrosis (IMF) and Chronic Myeloid Leukemia (CML), rarely sub-types of chronic neutrophilic leukemia, hypereosinophilic syndrome and eosinophilic leukemia. Such disorders in gene control are known to cause leukemias. The term" leukemia " means white blood, that is, blood rich in white blood cells. Leukemia is a cancer that forms in leukocytes, and these cells fight infections in the body. There are two main types of white blood cells: lymphoid and myeloid. ALL (Acute Lymphoid Leukemia) affects lymphoid cells, whereas AML (Acute Myeloid Leukemia) affects myeloid cells. The most common form, AML, has eight subtypes (M0-7) and ALL has three subtypes (L1-3). Leukemia cells are abnormal cells, unable to perform their functions like normal cells [4, 5, 6].

A somatic mutation at position 617 (JAK2-V617F) acquired in the JAK2 gene leads to the encoding of phenylalanine instead of valine. JAK mutations that result in this amino acid exchange are often found in patients with leukemia and MPD. Heterezygous and homozygous V617F mutations in the JH2 region of JAK2 have been observed in the vast majority of classic myeloproliferative patients, such as PV, ET, idiopathic. Miscarriage has also been common in other myeloproliferative diseases. As a result of these mutations, JAK2 activation increases and its regulation changes. It has also been shown by recent animal experiments to increase the high sensitivity of the cytokine receptor [6]. The JAK2V617F point mutation is located within the JH2 domain. The JAK2 protein plays a key role in intracellular signaling production at the cytokine receptor. Erythroprotein receptor EPO-R and thromboprotein receptor TPO-R are required for hematopoiesis. As recently shown, the point mutation at position V617F in the gene encoded for the JAK2 protein is also effective in tyrosine kinase activation [7].

In our study, we aimed to determine the prevalence of JAK2V617F mutation in a large study group diagnosed with pediatric AML and ALL and adult hematological malignancies diagnosed with PV, ET, CML, PMF and to associate it with clinical findings.

MATERIALS AND METHODS

Patients

Patients who applied to the Department of Pediatric Hematology at Çukurova University Faculty of Medicine, Department of Pediatrics between 2009-2012 and diagnosed as a result of cytomorphological, immunohistochemical and immune flowcytometric studies were included in the study. Of the 216 patients diagnosed with Acute Leukemia, 164 were pediatric patients with ALL and 52 with AML. This study also included 176 adult patients diagnosed with MPD in the Internal Medicine Hematology department, and a total of 392 patients were examined.

During the diagnosis, 3 mL of blood was taken from the patients into tubes with K3EDTA and stored at +4 C. Morphological subgroups were made according to the FAB classification [8]. At the same time, surface markers were examined by means of

monoclonal antibodies with flow cytometric study for immunophenotyping. 2 ml of bone marrow aspiration samples taken from the patients were taken into tubes containing EDTA and the samples were passed through flow cytometry (BD FACS Calibur / USA) device to determine the percentage ratios in the blastic cell population. Blasts were immunophenotyped with CD2, CD3, CD19, CD7, CD10, CD13, CD22, CD33, CD34, HLA DR, intrastoplasmic MPO, CD117 and Anti TdT. In immunophenotyping studies, those over 30% of CD surface marker antigens were evaluated as positive. Patients diagnosed with ALL and AML were divided into subgroups according to FAB classification and immune phenotyping. ALL are immunophenotypically divided into CALLA (+) Pre B-ALL, CALLA (+) early pre-B-ALL, Mature B-ALL, and T-ALL subgroups.

The patients were followed up and treated in the Pediatric Hematology Unit of Çukurova University Faculty of Medicine. BFM TR-ALL 2000 chemotherapy protocols were applied according to risk groups in patients with ALL diagnosis. AML-BFM 2000 protocol was applied to patients with AML diagnosis. Laboratory and clinical findings required for the diagnosis of PV, ET, CML and PMF in adult MPDs patients of internal medicine were evaluated according to WHO criteria.

DNA extraction

DNA isolation from 10 ml blood taken from patients was performed manually using the isolation kit (Roche, 11796828001) and stored at -80ºC.

Analysis of the JAK2 V617F mutation

Commercial JAK2 V617F genomic primer probe design kit was used to identify mutations. For the detection of JAK2 V617F mutations; Reaction mixture ddH2O 7.4 µL, Mg + 2 (25mM) 1.6, master-mix 4.0 µL, LC probe mix 2.0 µL and RealTime PCR (Light Cycler Roche Diagnostic, GmBh, Germany) were genotyped using the device. 10 minutes at 95 °C, 10 seconds at 95 °C, 10 seconds at 53 °C, 20 seconds at 72 °C for melting curve, 20 seconds at 95 °C and 85 °C at 40 ^{0}C for 30 seconds. Melting curves are 53ºC for mutant JAK2 617F / F, 62ºC for wild type JAK2 617 V / V, and 53 hC and 62ºC for heterozygous alleles JAK2 617 V / F.

Sequence Identification Method

Reaction composition (total 50µL); 50 ng DNA (prepared in 1x ABI buffer) 1.5 mmol / L MgCl2, 125 nmol / L dNTP (from each dNTP), 0.8 µmol / L forward and reverse primer, 5U AmpliTaqGold (enzyme). Amplification was implemented in ABI9700 equipment as follows. (10 minutes of denaturation / activation at 95°C), 20 cycles, performed at 3 varying temperatures: 40 seconds at 95°C, 30 seconds at 67-47°C, 30 seconds at 72°C. In addition, 18 cycles were completed at 47°C (bonding temperature) and 72°C (elongation temperature) in 10 minutes. The amplicons obtained were purified by purification with the High Pure PCR Product Purification Kit (Roche 11732676001) kit (with 50 µL AE buffer). 5 µL of the cleared amplicon was taken, 10 µL of 10 µmol / L forward primer and 5 µL of BigDye v.3.1 ready reaction mix was added. The excess dye was precipitated by adding an equal volume of 2.2% SDS (sodium dodecyl sulfate) and heating at 98 ° C for 5 minutes. The cleaned amplicon was passed through the Centricep Colon (Princeton Seperations, Adelphia, NJ). The sample was dried with

SpeedVac (Savant, Farmingdale, NY) and dissolved in formamide and sequence analysis was performed with ABI3100 DNA sequence equipment [9].

Statistical analysis

IBM SPSS 20.0 package program, which is a modern analysis program, was used for statistical analysis of the data obtained from patient groups. To determine whether the groups were distributed normally, One Sample Kolmogorov-Smirnov test, comparison of patient groups, One Way ANOVA and Mann-Whitney U tests, and genotype distribution was evaluated with Chi-square (X2) test. As a result of the evaluation, results with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

In this study, Real-time PCR method was used to detect single nucleotide polymorphism (SNP) of mutations, and sequencing was performed with sanger sequences to verify the results. A total of 392 patients were followed in this study, including 216 (AML, ALL) children and 176 (PV, ET, CML, PMF) adults. The child group consists of 164 individuals diagnosed with ALL and 52 diagnosed with AML.

The age range of patients with ALL is 1-17 and their mean age is 7.1 ± 4.5 years. Sixty-eight (59.6%) of ALL patients are boys, 46 (40.4%) of them are girls and the ratio of girls to boys is 1.4: 1. Patients with AML are between the ages of 1-15 and their average age is 10 16 of the patients with AML were male (55.2%) and 13 (44.8%) were female, and the female: male ratio was calculated as 1.2: 1. In the adult group, the mean age of 22 patients with CML, whose ages are 29-78, is 56 ± 2, and 10 of the patients with CML are male (50%), 12 (50%) are female, and the female: male ratio is 1. 79 patients with PV were between the ages of 17-83, the mean age was 53.6 ± 2.3 and 47 of these patients were male (57%), 32 (43%) were female and the female: male ratio was 1.3: 1. distributed. The ages of 51 patients with ET are in the range of 25-85, their average age is 48.5 ± 3.5. 21 of the patients were male (40%), 30 (60%) were female, and the female: male ratio was 1: 1.6.

According to the FAB classification, the subtype distribution of ALL is ALL-L1 (85%), ALL-L2 (14%) and ALL-L3 (1%). In this study, the subtype distribution of ALL according to the FAB classification (n = 164), 114 (69.5%) of the patients were in the L1 subgroup, 46 (28.0%) were L2 and 4 (2.5%) It is in the L3 subgroup.) B-ALL and 41 (25%) were classified as T-ALL. According to the FAB classification, 22 (42.3%) of the patients with AML (n = 52) were in the M1 subgroup, 18 (34.6%) in the M2 subgroup, and 3 (5.8%) in the M3 subgroup. 6 (11.5%) were in the M4 subgroup, 3 (5.8%) were in the M5 subgroup, and there were no patients in the M0, M6, and M7 subgroups.

Table 1. *Comparison of some data of ALL and AML groups*

	ALL n=164 (mean±SD)	***AMLn=52 (mean±SD)***	**P**
Age (year)	7.59±4.24	9.25±4.87	p>0.05
Hb g/dl	7.71± 2.09	7.41±1.86	p>0.05
WBC (/mm^3)	86323 ± 89555/mm^3	60252 ± 71079/mm^3	p>0.05
PLT(/mm^3)	53617 ± 52539/mm^3	56431 ± 44410/mm^3	p>0.05
Survival (months)	7.6±6.5	9.1±8.2	p>0.05
Event (relaps)	24 (%14.6)	12 (%23)	
Survival (eksitus)	23 (%14)	14 (%26.9)	
JAK2V617F Mutations	**0 (%0)**	**1 (%1.92)**	**p=0.759**

When patients with ALL were compared in terms of FAB classification, immunophenotype, event-free life, mortality and survival according to risk groups, no statistically significant relationship was found (p> 0.05). When the patients with AML were compared in terms of event-free life, mortality and survival according to the FAB classification, no statistically significant relationship was found (p> 0.05).

Table 2. *Comparison of some data of PV, ET, KML and PMF groups*

	PV n=79 (mean ± SD)	***ET n=51 (mean ± SD)***	***KML n=22 (mean ± SD)***	***PMF*** *n=24* ***(mean ± SD)***	***P***
Age (year)	51.73 ± 14.26	48.0 ± 15.91	50.09 ± 16.97	47.12 ± 17.29	
HCT (%)	50.36 ± 4.85	35.37 ± 7.27	33.46 ± 9.71	29.3 ± 9.49	p=0.000
WBC (/mm^3)	11154 ± 5489/mm3	11811 ± 5812/mm3	17342 ± 12251/mm3	9796 ± 55632/mm3	p=0.001
PLT (/mm^3)	519810 ± 312570/mm3	1096700 ± 3.43/mm3	489.96 ± 4.44/mm3	260200 ± 1.31/mm3	p=0.000
RBC	3686.7± 3020.9	3003.78 ± 2347	3172.9 ± 2254	4153.1 ± 1750.32	p=0.253
JAK2V617F Mutation	71(%89.8)	22(%43.1)	1(%4.5)	15(%62.5)	

A statistically significant difference was found for leukocytes when comparing all patient groups in adults (p = 0.001). The mean HCT values at diagnosis of patients with PV diagnosis are 50.4 4.85 g / dL for all patients, the mean value for patients with

JAK2V617F mutation is 50.2 3.8 g / dL, and the mean PLT values at diagnosis of patients with ET are all patient averages For the patients with JAK2V617F mutation, the mean value was determined as $1076x10^3$µL.

No difference was observed between the PMF and CML groups of patients with the JAK2V617F mutation in genotype (p = 0.500 and p = 0.545, respectively) and allele distribution (p = 0.273 and p = 0.545, respectively) between genders. On the other hand, a statistically significant difference was found in genotype and allele distribution between PMF and CML groups. In terms of allele distribution, it was seen that the T allele could pose a risk for the disease (p = 0.000) OR = 0.042, 95% CI = (0.005-0.336).

There was no difference in genotype (p = 0.445) and allele distribution (p = 0.197 and p = 0.221) between the ALL and AML groups among the patients with the JAK2V617F mutation and there was no statistically significant difference between the ALL and AML groups in terms of genotype and allele distribution. not found (p = 0.759), (p = 0.781) OR = 0.994 95% CI = (0.986-1.002).

JAK2V617F mutation was detected in 1 (0.46%) of a total of 216 patients with acute leukemia. When examined in general, no statistically significant difference was found in patients with mutations in terms of surveillance, remission-relapse, and survival time compared to other patients. This mutation was detected in only 1 (1.92%) of 52 patients in the AML group. This mutation rate was not found statistically when compared with the ALL group (p> 0.05). No statistically significant difference was found between patients with JAK2V617F mutation and the remaining AML patients in terms of mortality, survival time and remission-relapse (p> 0.05).

In adult patient groups (CML, PV, ET, PMF), no statistically significant difference was observed in terms of gender and leukocyte values (p> 0.05), but a significant correlation was found between the presence of JAK2V617F mutation and platelet values in the same patient groups (p = 0.000).

Patients diagnosed with acute leukemia in the pediatrics group (164 with ALL, 52 with AML), as well as 176 adult patients 79 with PV, 51 with ET, 22 with CML and 24 with PMF, were included in the study.

When DNA samples isolated from patient blood were amplified by PCR, 20 samples (1 sample from pediatric patients, 19 samples from adult patients) were taken for sequence analysis in order to check the accuracy of the samples with positive results according to Tm values.

MPD's show clonal proliferation in pluripotent hematopoietic stem cells. The JAK2V617F mutation plays an important role in the majority of MPDs patients. In chronic myeloproliferative diseases, the JAK2V617F mutation has been studied by many researchers in recent years, especially in adults. In the study conducted by Passamonti et al in 2010, RealTime PCR analysis showed that the presence of JAK2V617F mutation and hemoglobin concentration, leukocyte count, spleen size (p = 0.001) and worn and reorganized bone marrow cellularity (p = 0.002) in 320/338 (94.7%) patients with PV found that it was directly related to allele load, as well as inversely related to thrombocyte count (p <0.001). In this study, while there was no significant association between Hct values and JAK2V617F mutation in adult groups of patients with KML, ET, PMF, this study showed a significant association between JAK2V617F mutation and erythrocyte values and leukocyte values in PV patients (p=0.000) (p=0.001). Again, in the study of Jones et al, in 2005, a total of 129/480 (27%) ARMS were studied in patients with MPDs. They confirmed the same results by Sequence, detecting a 17/99 (17%) JAK2V617F mutation in patients with CML at an average age of 62 (17-77). In this study, the

frequency of KML mutation was 1/22 (4.5%) and earlier, that is, in the 50s (29-78). JAK2V617F mutation was detected in none of 164 patients with ALL (0%), 1 of 52 patients with AML (1.92%), 71 of 79 patients with PV (89.8%), 22 of 51 patients with ET (43.1%), 1 of 22 patients with CML (4.5%) and 15 of 24 patients with PMF (62.5%), and parallel results were obtained in other studies [10, 11].

Malak et al, (2012) studied 227 MPDs patients and found a JAK2V617F mutation in 79/97 (81%) patients with PV, 66/105 (62%) patients with ET, 7/14 (50%) patients with PMF, 0/4 (0%) patients with CML, and reported that the average age in PV patients was 58 (20-86). They determined mean values of leukocytes (12 $x10^9$/l), thrombocytes (529 $x10^9$/L) and Hct (56%). In patients with ET, they reported an average age of 51 (8-86), mean values of leukocytes ($9x10^9$/l), thrombocytes (824 $x10^9$/L) and Hct (43%). The average age in patients with PMF is 61 (27-78), and the average blood values are as follows; leukocytes ($17x10^9$/l), thrombocytes ($285x10^9$/L) and Hct (39%) respectively. Mean age was 48 (13-68) and blood values were leukocytes ($113x10^9$/l), thrombocytes ($340x10^9$/L) and Hct (39%) in patients with CML. In our study, in parallel with Malak et al., the average HCT values in the diagnosis of PV patients were 50,4±4.85 g/dL for all patient averages, the average value for patients with JAK2V617F mutation was 50,2±3.8 g/dL, the average PLT values in the diagnosis of ET patients were $1096x10^3$μL for all patient averages, and the average value for patients with JAK2V617F mutation was $1076x10^3$μL [12].

In their study in (2013), Barosi et al examined the phenotypic variations of the JAK2V617F mutation in 304 primary myelofibrosis (PMF) patients and major risks in (PMF) patients with qualitative and quantitative analysis with allele specific PCR and Real Time PCR. In these patients, 193/304 (63.4%) JAK2V617F mutation was detected with mean age values of 63 (52-71, 5) and leukocyte: 9.3 $x10^9$/l, trombocyte: 246 $x10^9$/L. In this study, the average age of patients diagnosed with PMF was 47 (14-77), leukocyte values were 9.5 $x10^9$/l, trombocyte values were 238 $x10^9$/L and 15/24 (62.5%) JAK2V617F mutations were detected [13].

When adults and children are compared, the JAK2V617F mutation in childhood leukemia, hematologic malignancies are, as reported in the literature by Sulong et al 2005, Frohling, et al, (2006), Levine et al (2005), in ALL patients as 0%, and in patients with CML was found in low percentages such as 1.92%. In the Pediatric Group, the relationship was not significant when survival was statistically examined only between patients carrying the JAK2V617F mutation in ALL and AML ($p>0.05$) Sulong et al, (2005) performed JAK2V617F mutation analysis on a total of 126, 42 of which were relapses and, Levine et al. (2005) in 222 pediatric patients with ALL. Fröhling et al., (2006), studied the same mutation in a total of 152 AML patients, including 85 AML (M5), 53 AML (M6) and 14 AML (M7) patients. Jelinek et al. (2005) identified a JAK2V617F mutation in a total of 39 patients with 20 AML (M0) and (M5), 8 AML (M6) and 11 AML (M7) subgroups [14, 15,16, 17].

Föhling et al, (2006) studied the JAK2V617F mutation in subgroups (M5), (M6) and (M7) in AML patients. When the patients were analyzed, the JAK2V617F mutation was found to be important in a significant percentage of patients, especially in MPDs in secondary AML. In contrast, the JAK2V617F mutation was detected at very low rates in de novo AML patients. But the frequency of JAK2V617F is also limited in specific subtypes of de novo AMLs. Heterozygous JAK2V617F mutation was determined as 0/85 (0%) AML M5, 1/53 (1.88%) AML M6 and 0/14 (0%) AML M7 of patients. These findings were also supported by the study of Jelinek et al. in (2005), JAK2V617F

mutation 0/20 (0%), AML in subtypes between M0 to M5 0/8 (0%) AML M6, 2/11 (18%) AML and in subtypes M7 is specified. 1/52 (1.92%) AML (M6) JAK2617F mutation was detected in this study. 7 of our patients were observed to have MPDs followed by secondary AML patients. Of the 5 patients carrying the JAK2V617F mutation, 3 had polistemia vera, 1 had antecedent ET, and 1 had AML from idiopathic myelofibrosis. As reported in both studies, it was concluded that allele patients carrying the JAK2V617F mutation returned from precursor MPDs to secondary AML (Fröling et al., 2006). But no conversion from precursor MPDs was observed in patients in this study [15,17].

In their study in (2007), Larsen et al examined the JAK2V617F tyrosine kinase mutation in PV, ET and PMF patients. In this study, the presence of chronic myeloproliferative disease in all three different diseases shows three different phenotypes, although they carry the same JAK2V617F positive mutation. However, physiological and genetic modifications are defined according to the allele charge in the phenotype. They studied 165 patients with Ph (-), and the JAK2V617 mutation was detected in 94/95 (99%) of patients with PV, 21/40 (53%) of patients with ET, and 13/30 (43%) of patients with PMF. They did a quantitative analysis with Realtime PCR. They found a very high degree of compatibility between different JAK2V617F allele loads and disease ($p=0.001$ between ET and PV and $p < 0.00001$ between PV and PMF). While the analysis was limited only to newly diagnosed patients, this significant difference was still found ($p=0.02$ between ET and PV and $P < 0.0008$ between PV and PMF). In this study, a statistically significant difference was found between the ET and PV groups in terms of genotype and allele distribution ($p=0.000$) and it was observed that the T allele may pose a risk for disease in terms of allele distribution ($p=0.000$, or=4.834 95% CI = (2.778-8.411). However, a statistically significant difference was found between the PMF and PV groups in terms of genotype and allele distribution ($p=0.002$) and it was found that the T allele may pose a risk for disease in terms of allele distribution ($p=0.001$) OR=2.900 95% CI = (1.480-5.682) [18].

Erken in 2008, reported that they conducted a leukocytosis risk assessment for ET patients, and the study results support the knowledge that leukocytosis is a risk factor for the development of thrombosis. In this study, the relationship between JAK2V617F allele load and leukocytosis was also examined, but it was noted that significance was not shown. But in the study conducted by Erken, PV and ET cases observed a relationship that could be significant when examined it in terms of JAK2 mutation and the presence of leukocytosis at the time of diagnosis ($p=0.06$). In our patients, a statistically significant difference was found between the ET and PV groups in terms of genotype and allele distribution in parallel with Erken's study ($p=0.000$) [19].

In 2010 Denys et al, the JAK2V617F mutation was studied by different methods in cases without PV, ET, PMF and MPN patients. Results were obtained as follows: 11/13 AS-PCR, 11/13 Larsen-PCR, 11/13 LNA-PCR, 9/12 sequence in PV patients; 14/20 AS-PCR, 14/40 Larsen-PCR, 14/20 LNA-PCR, 11/16 sequence in ET patients; 8/11 AS-PCR, 9/11 Larsen-PCR, 8/11 LNA-PCR, 8/9 sequence in PMF patients; 4/69 Larsen-PCR, 0/69 LNA-PCR, 0/37 sequence in non-MPN 0/69 AS-PCR patients. In our study, all patients were analyzed with melting curve analysis in realtime PCR. Some of the cases that were positive were confirmed by sequence analysis. From the point of view of this study, it is important to use current diagnostic methods and the results are parallel [20].

Poopak et al, 2013 investigated the frequency of JAK2V617F mutation in Iranian patients with MPDs and its association with the clinic and laboratory. 615 patients were found to be positive for mutations in 175 (28.4%) of the examined patients. The positive

group reported 79 (45.1%), ET and 62 (35.4%), PV and 27 (15.4%) PMF, 7 (4%) non-classified PMFs [21].

Speletas et al., (2007) examined the relationship between allele-specific PCR analysis and clinical and laboratory findings of JAK2V617F mutation in 166 patients with MPDs. According to the results, the JAK2V617F mutation was increased depending on age in positive patients (p = 0.02). In MPDs cases, it was observed that the presence of leukocytosis at the time of diagnosis increased three-fold and the frequency of thrombosis doubled. However, this association between the presence of leukocytosis and a history of thrombosis at the time of diagnosis and the JAK2V617F mutation was not statistically significant. Vannucchi et al showed a significant association between JAK2V617F mutation and thrombotic event development in ET patients, and did not determine the same relationship for PV cases where the mutation was positive. The results of Larsen et al, (2007) are of the same parallelism. In our study, the relationship between the presence of leukocytosis and the history of thrombosis in the adult group and the JAK2V617F mutation was statistically significant as (p=0.001) and (p=0.000) respectively [18, 22].

In their study in (2007), Shide et al. studied the JAK2617F mutation by performing PCR and direct DNA sequence analysis in 9/15 (60%) ET patients. Although they found a single allele in 8 patients, they detected a homologous JAK2 mutation in 1 (11%) patient. In our study, it was observed that 22/51 (43.1%) ET patients were statistically important in terms of allele distribution and that T (24.8%) allele may pose a risk for the disease and was confirmed by sequence analysis [23].

Murugesan et al, (2006) developed and validated the detection of the JAK2V617F (G→T) mutation from peripheral blood granulocytes and bone samples embedded in paraffin block in chronic MPDs patients with the realtime PCR fluorescence hybridization probe. They 47/114 (41.2%) identified JAK2617 mutation. Mutations detected in patients were as follows: PV patients 22/23 (96%), ET patients 9/15 (60%), AML patients 0/3 (0%), CML patients 0/19 (0%), CCML patients 0/2 (0%), MDS patients 5/70 (7%). The importance of clinical genotyping of the JAK2V617 mutation was also emphasized in this study [24].

Baxter et al, (2005) studied JAK2 mutations of tyrosine kinase in MPDs in humans. Valine 617 PHE single point mutation was identified in 71 (97%) of 73 PV patients, 29 (57%) of 51 ET patients, and 8 (50%) of 16 Idioaptic myelofibrosis patients. These mutations were found to be at varying rates in granulocytes [25].

In the study of Döhner et al, (2006) the positive relationship between JAK2V617F mutation and genetic lesion t(8;21) in AML patients was studied. They observed the presence of active JAK2V617F mutation in a single region in patients with t(8;21) (N=64) and inv16/t(16;16)-positive AML (N=99). As a result, it is known that the JAK2617F mutation is not a very common finding in the pathogenesis of patients with AML and occurs predominantly before MPDs. But still, according to the analysis of the researchers, the mutation was identified in a t(8;21) positive AML patient. It has been noted that this finding may be a result of auxiliary gene mutations model. It was found that the frequency of JAK2 mutations (1.92%) of our 52 patients with AML was similar and was thought to be a finding that supports this idea [26].

The JAK2V6147F mutation has been reported at very low rates, especially in denovo AML patients. It shows that the JAK2/STAT5 signal transmission pathway is structurally activated from the megakaryocytic leukemic cell line. JAK2V617F was studied in 85 AML M5 patients, 53 AML M6 patients and 14 AML M7 patients, and heterezygous mutations were identified in only one AML M6 patient [15].

In contrast, another study conducted in AML patients in parallel with our findings showed a JAK2 1849 G>T mutation in only 2 M7 subgroup patients out of 20 AML M0 and M5, 8 AML M6 and 11 AML M7 patients. Although the precursor MPD was present in 7 patients, secondary AML patients and JAK2 1849 G>T mutation was detected in 5 of these patients. 3 of these 5 patients reported the presence of pre-existing polistemia vera, essential thrombocytemia in one patient, and idiopathic myelofibrosis in another patient [17].

CONCLUSION

Since the first identification of the JAK2V617F mutation, researchers have been trying to determine many hematological malignancies and their frequency and importance in non-hematological cancers. As of today, studies have shown that the JAK2V617F mutation is important to influence the clinical treatment strategy of ET, PV, PMF, myeloproliferative diseases. In these diseases, it has been ascertained that JAK2V617F mutation is one of the most fundamental points of the pathophysiological mechanism. After showing these results, drugs with monoclonal antibody properties were made that target the uncontrolled JAK pathway and play a role in the targeted treatment that will briefly act as an inhibitor of JAK2. This whole process was realized by understanding the relevant pathophysiological mechanism in the enzyme tyrosine kinase, in which the JAK2V617F mutation is involved.

As a result of this research, the identification of the JAK2V617F mutation and its clinical significance in diseases were revealed. Research findings showed the prevalence of the JAK2V617F mutation in a large study group diagnosed with pediatric AML and ALL and adult hematological malignancies diagnosed with PV, ET, CML, PMF and were associated with clinical findings. Accordingly, it has been thought that in some types of leukemia, especially in the adult period, JAK2V617F may play a role as an operator gene mutation that aids diagnosis.

Acknowledgement. Approval of the Ethics Committee for this study was obtained from the Ethics Committee of the Çukurova University Faculty of Medicine with the Ethics Committee number: 17 and decision number: 12. It was also supported by Çukurova University Scientific Research Projects as Project No: FEF2008D13.

REFERENCES

[1] Topak, A. (2015): Investigatıon of GSTM1, GSTT1, GSTP1 Gene Polymorphisms In JAK2 V617F Mutatıon Positive Chronic Myeloproliferative Disorder. Uludağ University 1-65.

[2] Theocharides, A. P. (2018): The genetic dissection of myeloproliferative neoplasms. University of Zurich. 1-18.

[3] Branford, S., Kim, D. D. H., Apperley, J. F., Eide, C. A., Mustjoki, S., Ong, S. T., Hughes, T. P. (2019): Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia *33*(8), 1835-1850.

[4] Hsu, H. (2007): Pathogenetic Role of JAK2 V617F Mutation in Chronic Myeloproliferative Disorders. R.O.C. Chin Med Assoc, 70(3): 89-93.

[5] Kaushansky, K. (2007): The chronic myeloproliferative disorders and mutation of JAK2: Dameshek's 54 year old speculation comes of age. Best Practice & Research Clinical

Haematology 20(1): 5-12.
[6] Noor, S., Rıchard, B., And Ward, A.L. (2010): Shooting the messenger: Targeting signal transduction pathways in leukemia and related disorders. Critical Reviews in Oncology/Hematology 34-44.
[7] Vainchenker, W., Dusa, A., And Constantınescu, S.N. (2008): Constantinescu JAKs in pathology: Role of Januskinases in hematopoietic malignancies and immunodeficiencies. Seminars in Cell &Developmental Biology 19: 385–393.
[8] Neame, P. B., Soamboonsrup, P., Browman, G. P., Meyer, R. M., Benger, A., Wilson, W. E., McBride, J. A. (1986): Classifying acute leukemia by immunophenotyping: a combined FAB-immunologic classification of AML. Blood 1-8.
[9] Lay, M., Mariappan, R., Gotlib, J., Dietz, L., Sebastian, S., Schrijver, I., & Zehnder, J. L. (2006): Detection of the JAK2 V617F mutation by LightCycler PCR and probe dissociation analysis. The Journal of Molecular Diagnostics 8(3): 330-334.
[10] Passamonti, F., Rumi, E., Pietra, D., Elena, C., Boveri, E., Arcaini, L., Cazzola, M. (2010): A prospective study of 338 patients with polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. Leukemia 24(9): 1574-1579.
[11] Jones AV, Kreil S, ZK. (2005): Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 106:2162–2168.
[12] Malak, S., Labopin, M., Saint-Martin, C., Bellanne-Chantelot, C., Najman, A., & French Group of Familial Myeloproliferative Disorders. (2012): Long term follow up of 93 families with myeloproliferative neoplasms: life expectancy and implications of JAK2V617F in the occurrence of complications. Blood Cells, Molecules, and Diseases 49(3-4): 170-176.
[13] Barrio, S., Gallardo, M., Arenas, A., Ayala, R., Rapado, I., Rueda, D., ... & Martinez-Lopez, J. (2013): Inhibition of related JAK/STAT pathways with molecular targeted drugs shows strong synergy with ruxolitinib in chronic myeloproliferative neoplasm. British journal of haematology 161(5): 667-676.
[14] Sulong, S., Case, M., Minto, L., Wilkins, B., Hall, A., & Irving, J. (2005): The V617F mutation in Jak2 is not found in childhood acute lymphoblastic leukaemia British journal of haematology 130(6): 964-965.
[15] Fröhling, S., Lıpka, D.B., Kayser, S., Scholl, C., Schlenk, R.F., Döhner, H., Gıllıland, G., Levıne, R.L., And Döhner, K. (2006): Rare occurrence of the JAK2 V617F mutation in AML subtypes M5, M6, M7 Blood 107(3):1242-1243.
[16] Levine, R.L., Loriaux, M., Huntly, B.J.P., Loh, M.L., Beran, M., Stoffregen, E., Berger, R., Clark, J.J., Willis, S.G., Nguyen, K.T., Flores, N.J., Estey, E., Gattermann, N., Armstrong, S., Look, A.T., Griffin, J.D., Bernard, O.A., Heinrich, M.C., Gilliland, D.G., Druker, B., And Deininger, M.W.N. (2005): The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. Blood 106: 3377-3379.
[17] Jelinek, J., Oki, Y., Gharibyan, V., Bueso-Ramos, C., Prchal, J. T., Verstovsek, S., Issa, J. P. J. (2005): JAK2 mutation 1849G> T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome–negative CML, and megakaryocytic leukemia. blood, 106(10): 3370-3373.
[18] Larsen, T. S., Pallisgaard, N., Møller, M. B., & Hasselbalch, H. C. (2007): The JAK2 V617F allele burden in essential thrombocythemia, polycythemia vera and primary myelofibrosis–impact on disease phenotype. European journal of haematology, 79(6): 508-515.
[19] Erken, E. (2008): Miyeloproliferatif Hastalıklarda Jak2 Mutasyonu ve Klinik Bulgular. Çukurova Üniversitesi Tıp Fakültesi İç Hastalıkları Anabilim Dalı.
[20] Denys, B., El Housni, H., Nollet, F., Verhasselt, B., & Philippé, J. (2010): A real-time polymerase chain reaction assay for rapid, sensitive, and specific quantification of the JAK2V617F mutation using a locked nucleic acid-modified oligonucleotide. The Journal

of Molecular Diagnostics *12*(4): 512-519.

[21] Poopak, B., Hagh, M. F., Saki, N., Elahi, F., Rezvani, H., Khosravipour, G., Falah, P. (2013): JAK2 V617F mutation in Iranian patients with myeloproliferative neoplasms: clinical and laboratory findings. Turkish Journal of Medical Sciences *43*(3): 347-353.

[22] Speletas, M., S., Katodrıtou, E., Daıou, C., Mandala, E., Papadakıs, E., Kıoumı, A., Rıtıs, K., And Korantzıs, I. (2007): Correlations of JAK2–V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leukemia Research 31: 1053–1062.

[23] Shide, K., Shimoda, K., Kamezaki, K., Kakumitsu, H., Kumano, T., Numata, A., Harada, M. (2007): Tyk2 mutation homologous to V617F Jak2 is not found in essential thrombocythaemia, although it induces constitutive signaling and growth factor independence. Leukemia research 31(8): 1077-1084.

[24] Murugesan, G., Aboudola, S., Szpurka, H., Verbic, M.A., Maciejewski, J.P., Tubbs, R.R., And Hsi, E.D. (2006): Identification of the JAK2 V617F Mutation in Chronic Myeloproliferative Disorders Using FRET Probes and Melting Curve Analysis. Am J Clinic Pathology 125: 625-633.

[25] Baxter, E.J., Scott, L.M., Campbell, P.J., East, C., Fourouclas, N., Swanton, S., Vassiliou, G.S., Bench, A.J., Boyd, E.M., Curtin, N., Scott, M.A., Erber, W.N., And Green. A.R. (2005): Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 365: 1054-1061.

[26] Dohner, K., Du, J., Corbacioglu, A., Scholl, C., Schlenk, R. F., & Dohner, H. (2006): JAK2V617F mutations as cooperative genetic lesions in t (8; 21)-positive acute myeloid leukemia. Haematologica 91(11): 1569-1570.