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Study of the p.V617F and Exon 12 Mutations in *JAK2* Gene Among Iranian Chronic Myeloproliferative Patients

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Abstract

Background: Chronic myeloproliferative disorders (CMPD) occur due toclonal proliferation of the single hematopoietic stem cells and result in an increased number of mature and immature cells in the peripheral blood. The mutations in *JAK2* gene are identified in large numbers of CMPD patients.
Objectives: The aim of this study was to investigate thep.V617F (c.1849G > T) mutation as well as exon 12 mutations in *JAK2* gene in the CMPD patients.
Methods: Philadelphia chromosome negative CMPD patients were recruited for this study. In order to study p.V617F and JAK2 exon 12 mutations in *JAK2* gene, FRET probe real-time PCR, allele specific PCR and PCR-direct sequencing were utilized.
Results: JAK2 p.V617F mutation was found in polycythemia vera, Essential thrombocytosis and idiopathic myelofibrosis (67%, 52% and 50% respectively) but not in idiopathic erythrocytosis patients. Also no mutation was found in JAK2 exon 12 of these patients.
Conclusions: Our data regarding p.V617F was in concordance with the previous studies. The absence of any mutation in exon 12 of our patients may be due to extracting DNA from whole blood cells instead of granulocytes, that may impact the detection rate of cycle sequencing method.

Keywords: Myeloprolifrative Disorders, Polycythemia Vera, Essential Thrombocytosis, Idiopathic Myelofibrosis, Idiopathic Erythrocytosis, JAk2

1. Background

The myeloproliferative disorders are clonal hematologic malignancies with the feature of abnormal proliferation of myeloid lineages. This group of disorders is divided into two classes. Philadelphia (Ph) chromosome positive (Ph+) patients constitute CML patients and Ph- patients comprise polycythemia vera (PV), essential thrombocytosis (ET) and idiopathic myelofibrosis (IMF) (1-3).

The Janus kinase (JAK) family (including JAK1, JAK2, JAK3, and TYK2 members) is a group of non receptor-tyrosine kinase proteins that have critical role in JAK/STAT signal transduction pathway (2, 4).

Asignificant number of non-CML CMPNs have a somatic gain of function mutation (p.V617F) in the exon 14 of *JAK2* gene which is located on chromosome 9p24 (1, 5-8). This single nucleotide mutation happens in the pseudokinase domain of JAK2 (6).

Alsonon-synonymous substitutions, deletions and duplications are identified in JAK2 exon 12 which affect a region adjacent to the start of the pseudo-kinase domain (9).

2. Objectives

In this study we evaluated the prevalence of p.V617F and the mutations of exon 12 of *JAK2* gene in non-CML CMPD patients.

3. Methods

One hundred and forty Ph-CMPN patients (76 men (54%) and 64 women (46%)) who were referred by hematologist-oncologists to Tehran Medical Genetics Laboratory were recruited to this study. Philadelphia negative (Ph-) chromosome status was already established by either karyotyping the patients' bone marrow or analyzing mRNA from their peripheral blood for BCR-ABL

translocation. These patients were subdivided into 63 PV, 63 ET and 14 IMF cases. Seventy nine another patients who could not be categorized to either of the aforementioned subgroups were grouped together as idiopathic erythrocytosis (IE) and were subjected to investigation too. Informed written consent was obtained from all patients and their parents for carrying out research on their specimens. The study was approved by the local Ethics Committee.

3.1. DNA Extraction

DNA was extracted from whole blood using salting out method.

3.2. Melting Curve Analysis for Diagnosis of JAK2 p.V617F Mutation

The genotyping assay using fluorescence resonance energy transfer (FRET) probes and melting curve analysis was carried out using the primers and probes designed by Murugesan et al. (1). An amplicon of 177 bp in length was generated using a PCR forward primer, 5'-TTCCTTAGTCTTTCTTTGAAGCA-3', and a reverse primer, 5'-GTGATCCTGAAACTGAATTTTCT-3'. A sensor probe, 5'-ATGGAGTATGTGTCTGTGG-fuorecein-3', and an anchor probe, 5'-LCR640-ACGAGAGTAAGTAAAACTACAGGCT-phosphate-3', were used to perform melting curve analysis (1).

PCR was carried out in Corbett Life Science, Rotor-gene 6000, in a total volume of 20 μL containing 50 ng of genomic DNA. The final reaction contained 200 μmol/L of dNTPs, 4 mmol/L of $MgCl_2$, 0.1 μmol/L of forward primer, 0.5 μmol/L of reverse primer, and 0.2 μmol/L of each of the sensor and anchor probes. The following PCR program was used: initial denaturation at 95°C for 10 minutes; 45 amplification cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; Melting analysis was performed as follows: 95°C for 15 seconds, 75°C for 15 seconds and 95°C for 30 seconds.

3.3. Allele Specific PCR

Genotyping results of melting curve analysis were confirmed by Allele specific PCR using the following primers (1): Forward 1: 5'-AGCATTTGGTTTTAAATTATGGAGTATATT-3'; Forward 2: 5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3'; Common reverse: 5'-CTGAATAGTCCTACAGTGTTTTCAGTTTCA-3'

Primer F1 is specific for mutant allele and produces a 203 bp PCR product in the presence of p.V617F mutation. Primer F2 anneals to both mutant and normal alleles and generates a 364 bp PCR product. So it is used as an internal PCR control.

3.4. PCR-Direct Sequencing

PCR and direct sequencing of JAK2 exon 12 of all patients were carried out using the following primers:

F: TGAAATAACTGGCAGGAATACATC

R: GAGAACTTGGGAGTTGCGATA

The reaction contained 1× PCR buffer, 1.3 mM $MgCl_2$, 0.27 mM of each dNTPs, 0.4 Pmol of each primer, 0.2 unit CinnaGen Taq DNA polymerase, 50 - 100 ng template DNA in 30 μL final volume.

PCR program was: 95°C for 5 min (pre-denaturation), followed by 30 cycles including 95°C for 50 sec, 63°C for 50 sec and 72°C for50 sec, and final extension of 72°C for 10 min.

Cycle sequencing was carried out by Macrogen Company (Seoul, Korea) (http://dna.macrogen.com/).

4. Results

One hundred and forty patients including 63 PV, 63 ET and 14 IMF cases as well as 79 individuals with idiopathic erythrocytosis were recruited to this study. Table 1 summarizes the genotyping results for Jak2 p.V617F somatic mutation detected in our patients.

No mutation was found in JAK2 exon 12 in these patients. But a kind of polymorphism (IVS11-90 A > G) was discovered in 33 IE patients as well as4 out of 6 negative controls.

5. Discussion

Due to the overlap in the clinical and laboratory features of the myeloproliferative disorders, the accurate diagnosis forthese patients can be difficult. The JAK2 mutations (such as JAK2 p.V617F and JAK2 exon 12 mutations) are associated with the pathogenesis of the myeloproliferative disorders (2, 10). The JAK2 mutations are reported in more than 95% of patients with polycythemia vera and in 50 to 60% of patients with essential thrombocythosis or idiopathic myelofibrosis (2). The estimated frequency of JAK2 p.V617F mutation is about 65% - 97% in PV, 30% - 57% in ET and 35% - 95% in IMF patients (1, 11-13).

In the present study, real-time PCR and allele specific PCR were applied to assess the JAK2 p.V617F mutation. We detected this mutation in 67% of PV samples, 50% patients with idiopathic Myelofibrosis and in 52.4% with essential thrombocytosis.

PCR-direct sequencing for studying JAK2 exon 12 mutations did not reveal any mutationin these patients. However, IVS11-90 A > G was found in 33 IE patients and 4out of 6 negative controls. This observation suggests the fact

Table 1. Frequency of Different Genotypes of JAK2 p.V617F (c.1849 G > T) Mutation Detected by Real-Time PCR on DNA Extracted From Whole Blood Samples

Diagnosis	Genotyping with Melting Curve Analysis				Percentage of Mutation Negative (GG), %	Percentage of Mutation Positive (GT + TT) , %
	GG	GT	TT	Total		
Polycythemia vera	21	42	-	63	33.3	66.7
Essential thrombocytosis	30	33	-	63	47.6	52.4
Idiopathic myelofibrosis	7	7	-	14	50	50
Idiopathic Erythrocytosis	79	-	-	79	100	0

that the polymorphism is not just seen within the IE community so any relation between the disorder and the polymorphism is rejected.

The differences in the mutation percentages reported in myeloproliferative disorders may be due to the diagnostic criteria (PVSG and WHO), sensitivity of the methods and source of the DNA (1, 2, 6, 10).

According to the previous reports, the homozygous mutants have a low frequency (5% TT). In order to detect the homozygous form of JAK2 mutation, it is required to examine granulocytes extracted from peripheral blood (1, 10). Since the buffy coat was used in this study, all blood cell lineages existed in the samples and it was not possible to detect homozygous mutations. In other words, the heterozygotes we detected may be the combination of both heterozygotes and homozygotes for mutant allele (GT and TT). Further characterization can be performed using the DNA extracted from granulocytes.

Also detection limit of cycle sequencing method is about 10% of mutated allele (14, 15). So isolating DNA from whole blood instead of granulocytes may decrease the sensitivity of this method. This may be the reason we couldn't find any mutation in JAK2 exon 12.

It is essential to evaluate serum erythropoietin (Epo) in patients suspected with polycythemia vera. In these patients the blood erythropoietin is low (2, 10). In the present study, we did not measure Epo levels in patients' blood. Hence, patients who were suspected to suffer from polycythemia vera may be suffering from the other blood disorders.

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Footnotes

Conflict of Interests: It is not declared by the author.

Ethical Approval: The study was approved by the local ethics committee.

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Informed Consent: Informed written consent was obtained from all patients and their parents for carrying out research on their specimens.

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