The Prevalence of JAK2 Exon 12 Mutations in Vietnamese Patients with JAK2 V617F-Negative Polycythemia Vera: Frequent or Rare?

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Keywords
Janus kinase 2 · JAK2 V617F · Genetic mutation · Myeloproliferative disorders · Polycythemia vera

Abstract

Purpose: Polycythemia vera is a hematological malignancy characterized by the overproduction of red blood cells in the bone marrow. Pathogenesis of polycythemia vera was thought to be caused by genetic mutations of the Janus kinase 2 (JAK2) gene, especially the JAK2 V617F and exon 12 mutations, since those mutations were found frequently in the patients. The prevalence of JAK2 exon 12 mutations among polycythemia vera patients in Vietnam has not been studied yet. Objectives: The overall study objective was to investigate the frequency of JAK2 exon 12 mutations among V617F-negative polycythemia vera patients in Vietnam. Methods: In this study, the occurrence of these mutations was investigated in a clinical population of 76 Vietnamese polycythemia vera patients by polymerase chain reaction-restriction fragment length polymorphism and Sanger sequencing. Results: The result showed that 53 of the patients were V617F-positive, and in 23 V617F-negative patients, only four individuals carried two JAK2 exon 12 mutations. Analysis by different in silico tools predicted that all the two exon 12 mutations detected in this study (JAK2 c.1592A>G; p.H531R and c.1616A>G p.K539R) were benign. Conclusion: These results suggested that the causative mutations in this V617F-negative subgroup might be located in another genetic region, and mutations in exon 12 might not be as common among the V617F-negative polycythemia vera patients as thought.

Introduction

Polycythemia vera (PV) is one type of blood cancer that belongs to the Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) group. The reported annual incidence of PV was about 44 cases per 100,000 of the population [1] and was more frequent in the elderly population than in the younger ones. The median survival time of PV patients was about 2 years without any treatment and approximately 13.5 years with treatments [2]. This disorder was caused by an abnormal condition in the bone marrow, leading to the excessive production of erythrocytes. According to the World...
Health Organization (WHO) criteria, a patient was diagnosed with PV if the hemoglobin concentration in blood exceeded 16.5 g/dL in males and 16.0 g/dL in females or if the hematocrit exceeded 49% in males and 48% in females [3].

The causes of PV are similar to the other clonal hematopoiesis disorders resulting from the malignant transformation of hematopoietic stem cells, caused by an increase in the number of myeloid blood cells. Many studies have demonstrated that genetic mutations play a decisive role in these overproductions. A large number of genes lead to the change of protein activities in different receptors and downstream effectors. Genes have been elucidated such as the Janus kinase 2 gene (JAK2), the calreticulin (CALR), myeloproliferative leukemia virus (MPL), ASXL1 transcriptional regulator 1 (ASXL1), Tet methylcytosine dioxygenase 2 (TET2) [4–6]. Among those, a genetic mutation in the JAK2 gene was considered the most common in PV since about 90% of PV cases carried this mutation: JAK2 V617F [7]. Besides V617F, mutations in the JAK2 exon 12 region were also detected in a large proportion of PV patients with JAK2 V617F-negative background [8, 9], thus, these variations were considered potential causatives of polycythemia vera.

In this study, a population of Vietnamese PV patients was determined to have the JAK2 V617F genotype by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. With the group of PV patients with V617F-negative, we sequenced the JAK2 exon 12 to identify the prevalence of mutations in this region.

Materials and Methods

Patients

A population of 76 patients was recruited at the Vietnam Military University for this study. All of them were diagnosed with PV based on their clinical history and hemoglobin blood parameters.

JAK2 V617F Genotyping

The total genomic DNA samples were extracted from PV patients’ peripheral blood using the QIAamp® DNA Mini Blood Kit (QIAGEN). JAK2 V617F genotype was identified by the PCR-RFLP method. The DNA fragment containing V617F was amplified in a 40 μL PCR reaction with 50 ng of genomic DNA, 1X PCR buffer, 2.5 mM dNTPs, 1 μM of each primer, and 5 U of Taq DNA polymerase. Sequences of the oligo primers were: (FP) 5′-AGTTCAATGAGTTGACCCCCT-3′; (RP) 5′-ACATCTAACA-CAAGGTTGGA-3′. Thermocycling was at 95°C for 5 min, 40 cycles of denaturation at 95°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 40 s, with one final extension at 72°C for 10 min. PCR products were purified by the GeneJET PCR Purification Kit (Thermo Scientific) and sequenced by the ABI 3500 Genetic Analyzer Sequencer (Applied Biosystems) using the ABI Big Dye Terminator v3.1 Sequencing Standard Kit.

In silico Analysis

The effect of detected mutations was predicted in silico using different software such as PROVEAN [10], PredictsSNP2 software [11], the Predictor of human Deleterious Single Nucleotide Polymorphisms (PhD-SNP) tool [12], Sorting Intolerant From Tolerant (SIFT) software [13], and the Polyphen-2 tool [14]. The novelty of detected mutations was checked by the dbSNP version 151 and the 1000 Genome Project databank.

Results

The result of JAK2 V617F genotyping showed that in 76 PV patients, 53 carried the mutant T allele (classified as JAK2 V617F-positive). Thus, JAK2 V617F mutation was detected in 69.7% of the PV cases. In detail, 29 of the patients had the homozygous TT genotype and 24 patients had the heterozygous GT genotype. To avoid false results, 10% of the samples were genotyped again by Sanger sequencing. All the samples showed similar results between the two methods, Sanger sequencing and PCR-RFLP. The remaining 23 PV patients with the homozygous wild-type GG genotype (classified as JAK2 V617F-negative) were proceeded to JAK2 exon 12 analysis.

Sequencing results in exon 12 of the JAK2 gene detected only 2 mutations in 4/23 individuals (17.4%) (shown in Fig. 1). The most common mutation was JAK2 c.1616A>G p.K539R, detected in 3 individuals. The other mutation (c.1592A>G; p.H531R) was found in only 1 patient (Table 1). All the mutations were found under the heterozygous form. None of the two mutations was novel. In addition, in silico analysis by several tools demonstrated that these mutations had benign effects on the
Fig. 1. Sanger sequencing results of JAK2 V617F and exon 12.
protein structure and function. This result suggested that the two detected mutations JAK2 c.1592A>G; p.H531R and JAK2 c.1616A>G p.K539R in exon 12 might not be the causative reason for polycythemia vera in the JAK2 V617F-negative group.

**Discussion and Conclusion**

The JAK2 gene occupies a key role in the signaling transfer process. Cytokines from the extracellular, such as interferons and interleukins, bind to the JAK and STAT tyrosine transfer cytokine receptor and change their conformation. In the JAK/STATs pathway, the JAK dimers to phosphorylate, causing the phosphorylation of STAT proteins, mainly in STAT3 and STAT5, to form the dimerized STATs. These dimerizations move toward the cell nucleus to act as the transcription factors that play a critical function in cell proliferation, apoptosis, and survival [15]. Any abnormality in the JAK receptor might cause a false signal of the intercellular molecular interaction network, leading to hematopoietic disorders.

Up to date, genetic testing of JAK2 V617F and JAK2 exon 12 mutations has been applied as a major diagnostic criterion for PV following the WHO 2016 guidelines by their popularity in the genome of PV patients around the world. In 2010, research performed on 22 Taiwanese PV patients showed that 17/22 (77%) of the patients were V617F-positive. And interestingly, all other 5/22 (23%) V617F-negative patients carried exon 12 mutations: N542-E543del, F537-K539delinsL, and I540-E543delinsKK. Thus, the proportion of PV patients who had JAK2 V617F or exon 12 mutations was 100% [9]. Another study launched in 90 PV patients in Poland revealed 82/90 (91%) of the patients were V617F-positive. And interestingly, all other 5/22 (23%) V617F-negative patients carried exon 12 mutations: N542-E543del, F537-K539delinsL, and I540-E543delinsKK. Thus, the proportion of PV patients who had JAK2 V617F or exon 12 mutations was 100% [9]. Another study launched in 90 PV patients in Poland revealed 82/90 (91%) of the patients were V617F-positive carriers. Among the 8/90 V617F-negative PV patients, 4 patients had mutations in the exon 12 region, including H538-K539delinsL, E543-D544del, and N542-E543del [8]. In our study, 53/76 (69.7%) of the Vietnamese PV patients were V617F-positive. This part of the result is quite similar to other studies in different populations. However, the result of exon 12 sequencing revealed that only 4/23 (17.4%) of the Vietnamese V617F-negative PV patients had mutations in the JAK2 exon 12. In addition, the two mutations detected JAK2 c.1592A>G; p.H531R and c.1616A>G p.K539R showed no pathogenic effect against tyrosine kinase function by in silico prediction. Of course, a small sample size, which was not sufficient to confirm any potential association between JAK2 exon 12 mutations and PV, was a limitation of our study. However, a recent study published on 1272 MPN patients showed that JAK2 exon 12 mutations were found in only 8/307 V617F-negative MPN cases and in 2–5% of V617F-negative cases of PV [16]. These findings suggested that mutations located in JAK2 exon 12 might not be as popular among V617F-negative PV patients as we thought.

In conclusion, in this research, we found that the incidence of JAK2 V617F-positive in the Vietnamese population was nearly 70% (53/76 patients). Among the JAK2 V617F-negative PV subgroup, only a small proportion carried a JAK2 exon 12 mutation. The result suggested that although the importance of JAK2 in controlling the outcome of PV was well known, this disease might be manipulated by a complex genetic interaction network instead of a single gene disorder. This outcome provided the initial result for further analysis on the molecular mechanism of PV and other MPN disorders.

**Acknowledgments**

We sincerely thank all the PV patients who participated in this study.

**Statement of Ethics**

All of the patients agreed to sign the consent form providing samples for the research. The written informed consent was obtained for participation in this study. This study was ethically approved by the Review Board in Bio-Medical Research of the Institute of Genome Research, the Vietnam Academy of Science and
Technology (No. 4-2021/NCHG-HDDD). Experiments on human subjects were conducted ethically in accordance with the Helsinki Declaration of 1975 as revised in 2008.

Conflict of Interest Statement

All the authors declared no conflict of financial or nonfinancial interests in this study.

Funding Sources

This study was granted by the University of Science and Technology of Hanoi (USTH), the Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam (Grant No. USTH. BIO.01/20–22).

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Author Contributions

Nguyen Thy Ngoc (N.T.N.) designed the study and wrote the manuscript with input from all authors. N.T.N and Nguyen Thuy Linh (N.T.L.) carried out the laboratory work. Nguyen Thi Xuan (N.T.X.) analyzed the data.

Data Availability Statement

All the data generated in our study are available and included in this article. Further inquiries can be sent directly to the corresponding author.