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# *JAK2* EXON 12 VARIANTS IN VIETNAMESE PATIENTS WITH *JAK2* V617F-NEGATIVE PRIMARY MYELOFIBROSIS

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SUMMARY

Primary myelofibrosis is the severe form of myeloproliferative neoplasms that causes scar tissue in the bone marrow, leading to low production of blood cells and thus, life span shortening. Besides the most common variant *JAK2* V617F, the association between these disorders with other variants in the *JAK2* gene, especially the exon 12 variants have been poorly studied. In our research, the *JAK2* exon 12 variants were detected by amplification and sequencing from genomic samples of five cases with V617F-negative primary myelofibrosis. Results showed that among 5/14 primary myelofibrosis patients with V617F-negative profile, only two patients carried exon 12 variants (*JAK2* c.1592A>G p.H531R, and c.1613A>C p.H538P). *In silico* analysis indicated that the variant c.1613A>C p.H538P was novel and potentially pathogenic. The positioning demonstration by Missense3D tools indicated that this variant localized in the proximity to the pathogenic variant V617F, suggesting a potential effect on the enzymatic activity of Janus kinase 2. This initial data can be used as a genetic diagnostic criterion for myeloproliferative neoplasms. Nonetheless, the effect of p.H538P needs to be verified by further investigations.

**Keywords:** *Janus kinase 2*, exon 12 variants, Primary myelofibrosis, Myeloproliferative neoplasms

## INTRODUCTION

Primary myelofibrosis (PMF) is the most aggressive form of myeloproliferative neoplasms (MPN) disorder that involved the abnormal proliferation of hematopoietic stem cells and scar formation in the bone marrow tissue. The deficiency of blood cell production can cause many signs and symptoms such as: enlarged spleen and liver, bone pain, fever, easy bruising, easy internal bleeding, due to deficiency of platelet production, and increased susceptibility to infectious factors (Tefferi 2005). The annual

incidence rate of PMF is approximately one case per 100 000 people in the European population (Moulard *et al.*, 2014).

The median life expectancy of a primary myelofibrosis patient is from 4 to 5.5 years, depending on the age, gender of the patient, and status at diagnosis (Cervantes *et al.*, 2008). The mortality rate tends to increase in old PMF patients (more than 65 years old), or patients with a low level of hemoglobin concentration (less than 10 g/dL) and a leukocyte count more than $25 \times 10^9$/L (Passamonti *et al.*, 2010).

The specific etiology of PMF still remains unknown but the risk factor can include some genetic abnormalities, exposure to certain chemicals and drugs, viral infection or weakened immune systems, or an autoimmune disease. Among those, the genetic factor has been proven to play a crucial in controlling the clinical phenotype of myeloproliferative neoplasms. The driver variants of PMF disorder are indicated to be located within the *JAK2*, *CALR*, or *MPL* genes. Among those, the variant *JAK2* V617F is the most common variant since this one was present in nearly 50% of the PMF patients (Vainchenker, Kralovics 2017). *JAK2* encoded for the Janus kinase 2 enzyme, which plays a key role in the signal transduction and activation of the JAK-STAT transcription pathway, a complex of signaling interactions that is strongly associated with hematological malignancies (Vainchenker, Constantinescu 2013). The protein structure of Janus kinase 2 was composed of four domains: FERM, SH2L, pseudokinase JH2 domain, and tyrosine kinase JH1 domain (Scott 2011). The V617F variant has been indicated to have a crucial effect on structural perturbations of the JH2 pseudokinase domain, especially with Src homology 2-JH2 linker, αC-helix side of JH2, and ATP binding site (Hammaren *et al.*, 2019). Besides V617F, the *JAK2* exon 12 variants, which are located in a loop close to the kinase domain, were also detected occasionally in patients with hematologic oncology (Maddali *et al.*, 2020, Makani *et al.*, 2017, Scott *et al.*, 2007). Although JAK2 V617F is the most common genetic variant observed among PMF patients, there is a subset of PMF cases and other MPN-type patients who carry different variants occurring within exon 12 of the JAK2 gene (Martinez-Aviles *et al.*, 2007, Scott *et al.*, 2007). Therefore, in this study, we investigated the prevalence of exon 12 variants among Vietnamese PMF patients with V617F-negative results.

## MATERIALS AND METHODS

### Patients

Peripheral blood samples were taken from 14 primary myelofibrosis patients at the Vietnam Military Medical University. All of the patients had been well informed about the purpose and possible risks of this research and signed in the consent forms. The ethical review of this study was approved by the Ethical Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology (No 4-2021/NCHG-HDDD). The information on the age, gender, and ethnicity of these patients was described in our previous study (Ngoc *et al.*, 2022).

### JAK2 V617F genotyping and exon 12 sequencing

The E.Z.N.A.® Blood DNA Mini Kit (OMEGA Bio-Tek) was used to extract genomic DNA from the collected blood samples. The sequence of the *JAK2* exon 12 region was determined by sequencing by

ABI Big Dye Terminator v3.1 Sequencing Standard Kit with forward (5'-TCAAAGTTCAATGAGTTGACCCC-3') and reverse (5'-CATCTAACACAAGGTTGGCAT-3') primer.

### *In-silico* prediction

The position of altered amino acid in the structure of protein was demonstrated by the Missense3D (Ittisoponpisan *et al.*, 2019). The potential effect of an amino acid substitution on the structure and function of the coding protein was analyzed *in silico* by several tools (PhD-SNP, SIFT, Polyphen-2). Finally, a multiple alignment to detect the conserved region of amino acid sequence was conducted using the UCSC Genome Browser on Human version GRCh38/hg38 (https://genome.ucsc.edu/).

## RESULTS AND DISSCUSSION

Result showed that 2 of 5 V617F-negative PMF patients had genetic variants in the exon 12, including *JAK2* c.1592A>G (p.H531R), and c.1613A>C (p.H538P) (Transcription ID: ENST00000381652.4). The *in-silico* prediction by PhD-SNP, PolyPhen-2, and SIFT indicated that the effect of *JAK2* p.H531R was neutral, but *JAK2* p.H538P could be a deleterious variant (Figure 1).

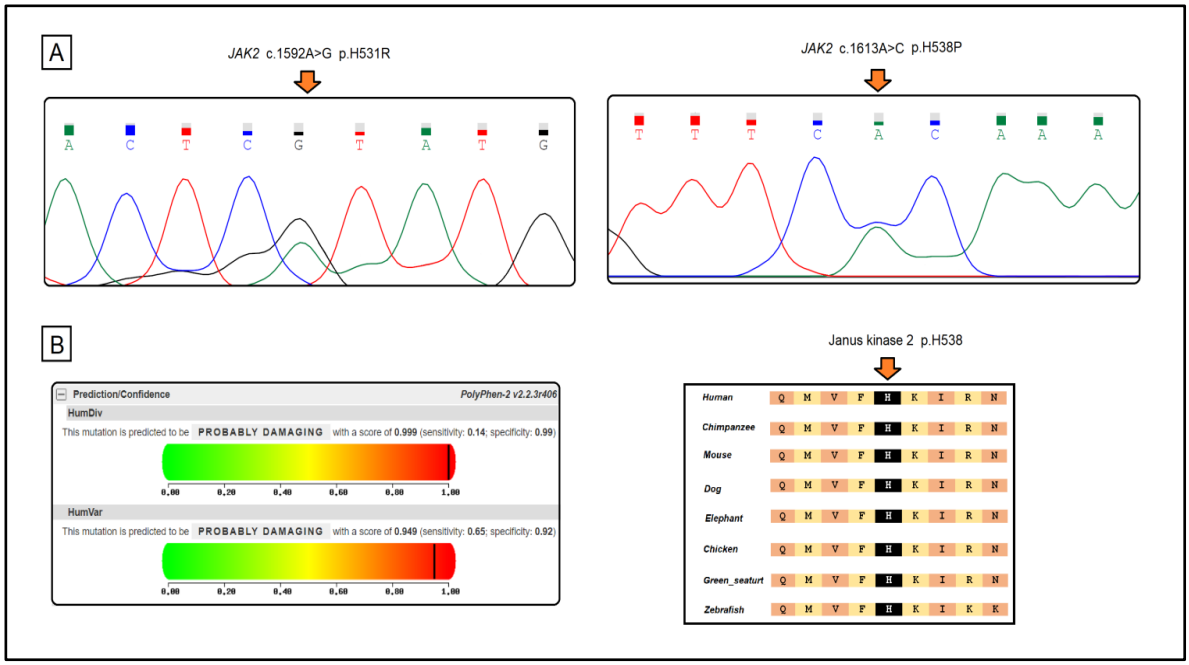


**Figure 1.** (A) Detection of the two novel variants JAK2 c.1592A>G (p.H531R) and c.1613A>C (p.H538P) by Sanger sequencing. (B) The in-silico prediction of p.H538P effect by PolyPhen-2 (Left) and Conservation analysis (Right).

These variants have not been reported on the three DNA variant databases 1000 Genome, dbSNPs, and gnomAD (v2.1.1). The variant *JAK2* c.1592A>G (p.H531R) was detected in our recent publication among Vietnamese V617F-Negative Polycythemia Vera (PV) patients (Nguyen *et al.*, 2022), while the c.1613A>C (p.H538P) has not been published before as our knowledge, thus considered to be novel. This variant has

not been detected among the V617F-positive PMF patients as well as the controls. A recent study detected variants in the same position in 3 *JAK2* V617F-negative MPN patients (1 PV patient carried the p.H538_K539delinsL variant and 2 PMF patients carried the p.H538_K539delinsL and p.H538_K539delinsQL variants) (Maddali *et al.*, 2020). Since genetic variants located in the *JAK2* exon 12 region had been indicated to be infrequent among the MPN patients with V617F-negative profile (Tavakoli, Naing 2017), the potential mechanism of those variants contributing to the pathogenesis of Myeloproliferative Disorder is poorly understood. The incidence of exon 12 variants among MPN patients was about 3% of the cases (Scott 2011), among those the most frequent variant were N542-E543del, E543-D544del, F537-K539delinsL, K539L (10%), and R541-E543delinsK variants. In mice, N542-E543del variant carriers suffered from idiopathic erythrocytosis and their longevities were reduced dramatically (died prematurely) with enlarged spleens. These transgenic mice showed a low expression level of hepcidin and high expression of transferrin receptor-1 and erythroferrone, the key regulators that enhance iron resorption, causing overproduction of erythrocytes (Grisouard *et al.*, 2016).

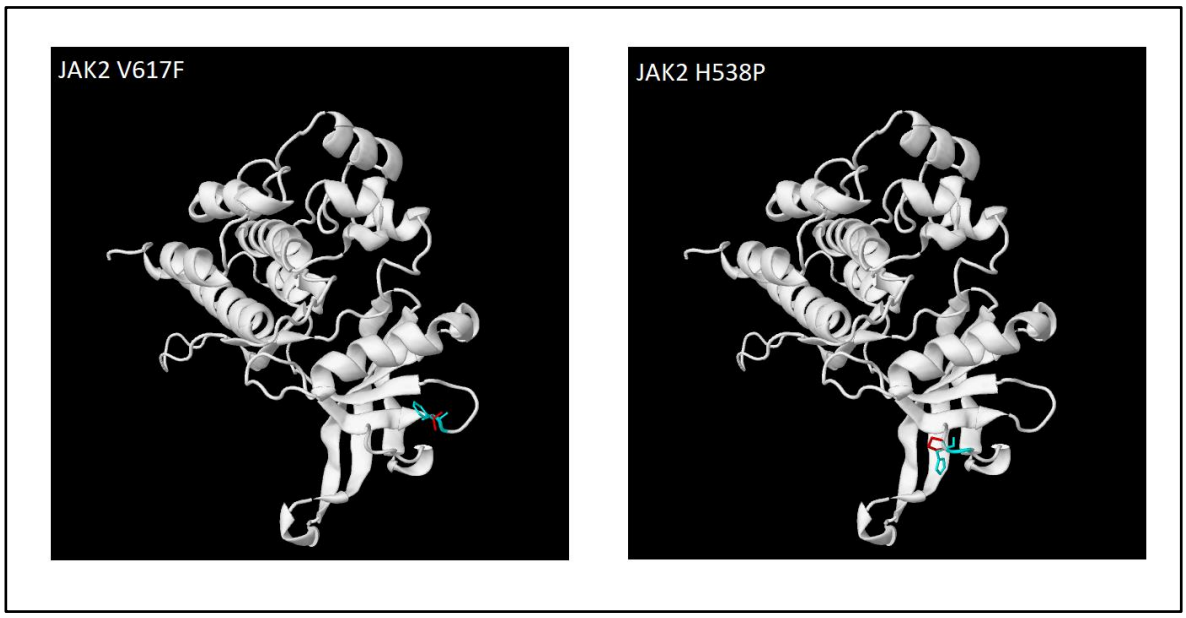


**Figure 2.** Residue V617F (Left) and H538P (Right) corresponding to the detected variant on the structure of pseudokinase domain of Janus kinase 2. The wild type residue was shown by pale blue and the mutant residue was shown by red color.

On the structure of Janus kinase 2, the V617F variant which was indicated to change the interactions between the JH1 tyrosine kinase domain and the JH2 pseudokinase domain through steric interference (Scott 2011), and this variant was demonstrated to be present in the majority of MPN patients (Vainchenker, Kralovics 2017). Located in the same JH2 domain, the variant H538P was close to the V617F variant (Figure 2),

suggesting that this variant might have a significant effect. However, this hypothesis must be confirmed by further functional studies.

## CONCLUSION

In this study, we observed that 2/5 (40%) of our V617F-negative PMF patients carried the *JAK2* exon 12 variants, which were located on the JH2 pseudokinase domain of Janus kinase 2 protein (c.1592A>G p.H531R, and c.1613A>C p.H538P). Among the two variants, the novel variant *JAK2* c.1613A>C p.H538P was predicted to be potentially pathogenic by several *in-silico* analyses. The 3D structural analysis also revealed that p.H538P located closely to the pathogenic variant *JAK2* V617F, a variant frequently detected in MPN patients, demonstrating that this substitution of an amino acid residue might have a significant effect on the structure of Janus kinase 2 and thus, on the pathology of primary myelofibrosis.

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