

## LETTER TO THE EDITOR

### Low frequency of *JAK2* exon 12 mutations in classic and atypical CMPDs

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The *BCR-ABL* negative chronic myeloproliferative disorders (CMPDs) are a group of stem cell clonal haematological malignancies characterised by abnormal proliferation and survival of one or more myeloid lineage cells. It has been proposed that they are caused by abnormalities in some signal transduction pathways mainly due to acquired somatic mutations, some of them in tyrosine kinase genes. One of the most prevalent mutations is V617F in the pseudokinase domain (JH2) coded by *JAK2* exon 14. This mutation, reported in 2005, has been associated with nearly 95% of patients diagnosed of polycythaemia vera (PV) and near a half of the patients with essential thrombocythaemia (ET) and primary myelofibrosis (MF). However, the frequency of this mutation is below 20% for the remaining chronic myeloproliferative disorders and is absent in lymphoid neoplasms. *JAK2*V617F has been an important milestone in the knowledge of the molecular mechanisms leading to classic myeloproliferative disorders. However, there are still some patients with PV, ET and MF lacking V617F whose molecular defect is unknown. The disease in these cases could be due to other abnormalities in *JAK2* or other related genes. In fact, in a few cases new point mutations have been reported, affecting also the JH2 domain, and one mutation affecting the JH1 domain [1] and reviewed in [2], some of them in other haematological malignancies. In addition, 10% of patients diagnosed of MF and some with ET show somatic activating mutations in the thrombopoietin receptor gene (*MPL*) inducing constitutive cytokine-independent activation of the JAK-STAT pathway.

Recently, different groups have described novel mutations in *JAK2* exon 12 in V617F-negative CMPD patients [2], [3], [4], [5] and [6]. The mutations reported affect amino-acid residues between F537-E544 and lie in a highly conserved region proximal to the JH2 domain of this gene. These findings support the idea that *JAK2* is one of the main candidates to study the pathogenesis of these disorders. In addition, the patients with *JAK2* exon 12 mutations seem to show distinctive clinical features different from V617F-positive patients such erythrocytosis,

and also a distinctive bone marrow morphology [5] and [7]. These findings could define a new myeloproliferative variant associated with erythrocytosis but this is still unclear [4].

We have studied the coding region of *JAK2* (25 coding exons) in 39 patients diagnosed of classic (PV n=19, TE n=7, MF n=2) and atypical CMPD (n=11) by denaturing high performance liquid chromatography (dHPLC) with the WAVE<sup>®</sup> 4500 System (Transgenomic Ltd, Omaha, NE) and direct sequencing of the abnormal elution profiles. Sequencing results were analyzed with Mutation Surveyor v2.51 software (SoftGenetics, LLC, State College, PA), and abnormal profiles were checked manually. All the patients were negative for *JAK2*V617F analyzed by amplification refractory mutation system PCR (ARMS-PCR) and confirmed by dHPLC. Twenty-one normal samples were also included as control group in order to detect potential differences in the frequencies of the polymorphisms observed in the analyses.

Two of the 19 PV patients carried silent variants in exons 19 and 25, 96506 C>T (D820D) and 141607 C>T (exonic non-coding region) respectively, not previously described as polymorphisms in the NCBI SNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). Both of these mutations were in heterozygous state and were not detected in the control group. If either both changes are rare polymorphisms or silent mutations affecting *jak2* function (by means, for example, of increased mRNA stability) remains to be elucidated.

Another two of the 19 PV patients harbored *JAK2* exon 12 heterozygous mutations (Fig. 1). One male patient had a 6 bp in-frame deletion, between positions 1627 to 1632 of the cDNA sequence (Fig. 1A and B). This mutation results in the loss of two aminoacids (E543-D544del) and it has been described previously in six PV patients (five female and one male) [5] and one female patient with idiopathic erythrocytosis (IE) [6]. The other patient, also male, had another 6 bp in-frame deletion, located between positions 1624 to 1629, (Fig. 1A and C) resulting also in the loss of two aminoacids (N542-E543del). This mutation has been described in eleven PV patients (five female and six male) [3], [5], [7] and [8] and five IE patients (three female and two male) [6]. Both of them are the most frequent recurrent non-V617F mutation in *JAK2* and lie in the same “hot” region where other indel mutations have been described. No other mutation was identified in the 25 *JAK2* coding exons in the remaining 35 V617F-negative CMPDs patients, despite the fact that dHPLC is a very sensitive method.

In our series, twenty CMPDs samples other than PV or IE have been included. No *JAK2* mutations were found in these samples, confirming that *JAK2* exon 12 mutations are specific for PV and IE phenotype as reported by other groups [5] and [7].

The data presented here support the idea that other somatic aberrations remain to be identified, and also that some PV patients have mutations other than V617F in *JAK2* or in other genes. In

addition, the frequency of *JAK2* exon 12 mutations in PV V617F-negative patients (2/19 or 10.5%) was significantly lower in our series than the frequency observed by other groups (30-100%) [3], [4], [5] and [7], maybe due to the small size of our series. In addition, both mutations in exon 12 were found in male patients in contrast with the slightly female predominance described by other groups [3] and [6]. However, despite the discrepancy in the frequencies observed, the mutations reported here reside in the same *JAK2* mutational “hot spot region”. This region must play an important role in *JAK2* activity that needs to be investigated in further analyses.

The data presented here reinforce the importance of searching mutations by screening methods in *JAK2* exon 12, at least for some CMPDs, in addition to the highly prevalent V617F. This will lead to a better diagnosis of some patients, who might also become potential candidates for future anti-jak2 therapies.

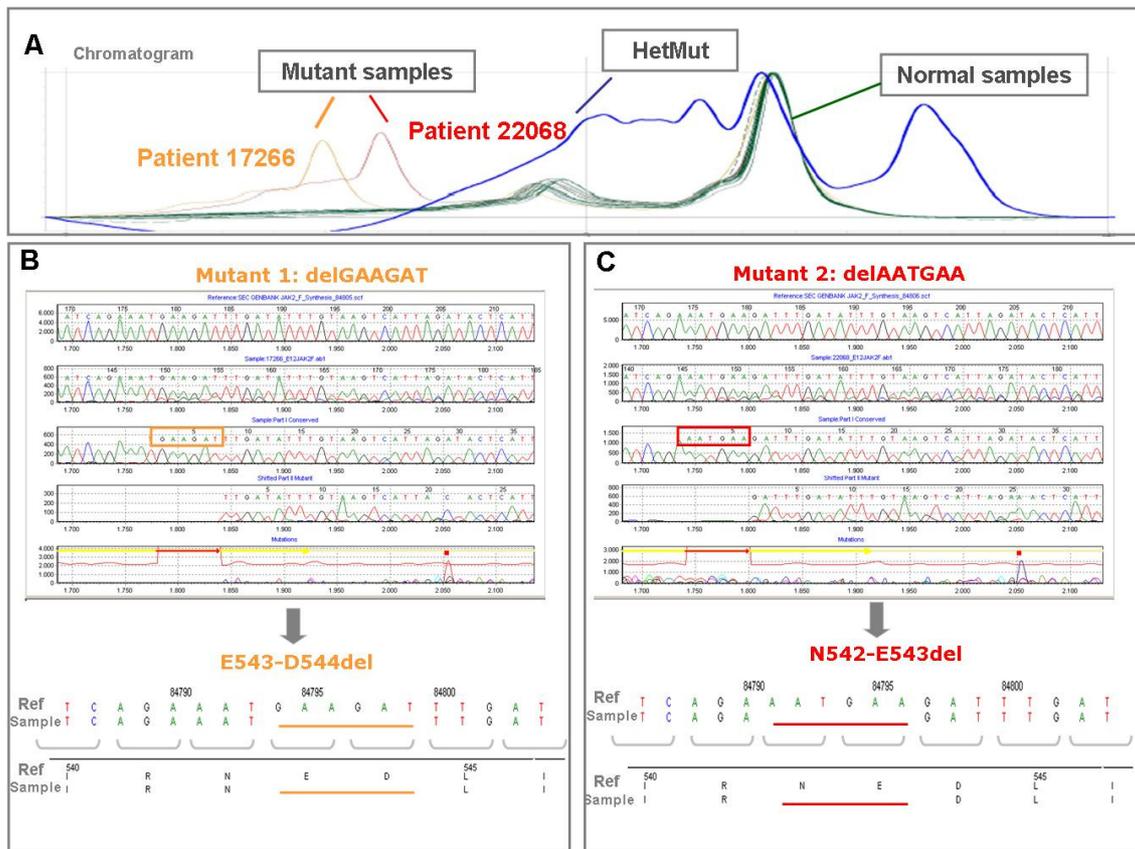
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### **Keywords**

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**Figure 1.**

**Panel A.** Mutational analysis by denaturing high performance liquid chromatography (dHPLC) of *JAK2* exon 12 in 39 samples from CMPDs patients. There are different elution profiles: green profile from patients samples lacking mutations, blue profile from heteroduplex mutant-normal (mutant created by mutagenesis and used as reference for the analyses), orange and red profiles from patient samples with mutations in *JAK2* exon 12.

**Panel B.** *JAK2* exon 12 sequencing results from the 17266 patient sample with orange profile. The sample sequence has been compared with the genomic reference sequence obtained from Ensembl (<http://www.ensembl.org>) showing a 6-bp in-frame deletion (**GAAGAT**). This mutation results in the loss of two aminoacids (E543-D544del).

**Panel C** *JAK2* exon 12 sequencing results from the 22068 patient sample with red profile. Comparing this sequence with the genomic reference sequence, a 6-bp deletion (**AAGATT**) can be observed, resulting in the loss of two aminoacids (N542-E543del)

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