Acquired somatic mutation in essential thrombocythemia

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Abstract. A total of 104 patients with ET evaluated at a single centre, were genotyped for the presence of the mutant JAK2 allele. The V617F mutation was detected in genomic DNA of peripheral blood samples of 104 ET patients by tetraprimer PCR technique. Mutation JAK2V617F was detected in 56 patients (53.8%), JAK2V617F heterozygous was found in 52 patients (50%) and homozygous in 4 patients (3.8%). The V617F positive group had higher haematocrit and leucocyte levels (p<0.05).

Key Words: Myeloproliferative disorders, essential thrombocythemia, JAK2V617F.

Introduction

Essential thrombocythemia is a clonal myeloproliferative disease involving a hematopoietic stem cell and manifesting predominantly as thrombocytosis and it is associated with thrombohemorrhagic complications and myeloid transformation to diseases such as myelofibrosis and acute myeloid leukaemia. The molecular pathogenesis of the MPDs has been poorly understood, except for chronic myeloid leukaemia where the BCR-ABL fusion protein exhibits constitutive kinase activity. A similar mechanism was thought to be likely responsible for the MPDs (Tefferi et al. 2005, Mesa 2007). In 2005 a unique acquired clonal mutation in JAK2 was found by five different research teams. A unique valine to phenylalanine substitution at position 617 (V617F) in the pseudokinase autoinhibitory JAK2 domain causes the constitutive activation of the JAK - STAT signalling pathway and leads to autonomous cell growth in a cytokine-independent manner. This mutation was observed in the majority of PV patients and in about half of ET and IMF patients. The traditional diagnostic criteria of the Polycythemia Vera Study Group was based mainly on a sustained increase in platelet count (higher than 600 x 109/l) and the exclusion of reactive thrombocytosis and other MPDs or myelodysplastic disorders. JAK2 V617F has been adopted in the new WHO diagnostic criteria for ET, PV and IMF (Tefferi & Vardiman 2008). In this study we evaluated 104 patients with ET from a single institution and studied the prevalence of JAK2 V617F mutation and the clinical correlations.

Materials and methods

This study included 104 patients with ET at Oncology Institute, Haematology Department, Cluj-Napoca. Descriptive and statistically analyzed data were obtained from the entire cohort of diagnosed patients. All these patients were considered in our estimate of the prevalence of the JAK2 V617F. JAK2 mutation analysis was done at Medical Genetics Department by tetraprimer PCR and PCR-RFLP techniques (Jones et al. 2005 with some modifications). DNA for mutation screening was derived from 300µL peripheral blood using Wizard Genomic DNA Purification Kit (Promega, Ma, USA) and amplified by polymerase chain reaction (PCR) with 4 primers: 2 specific for Jak2 gene, amplifying a fragment of 463bp, and 2 specific for the normal V617 allele and F617 mutant allele. The mutant allele specific primer together with the reverse primer specific for Jak2 gene form a fragment of 279bp and the specific primer for the normal allele with the forward primer specific for Jak2 gene a fragment of 229bp. So, the normal allele and the abnormal one are studied in the same reaction having as control the amplification of a larger fragment of Jak2 gene. The presence of both 279 and 229bp fragments signifies the positivity of Jak2, the intensity of mutant allele signal depending on the mutant clone expansion. PCR amplifications were performed in an Eppendorf thermocycler in a reaction volume of 25μL consisting of: 12.5μL 2xPCR Master Mix containing Taq-DNA polymerase 0.05U/μL recombinant, MgCl2 4mM, dNTPs mix 0.4mM each (Fermentas MBI, Vilnius, Lithuania), 10 pm of Jak2 gene specific primers, 8 pm of normal allele specific primers and the mutant one, 75ng genomic DNA and nuclease free water up to 25μL. The conditions of amplification were: one cycle of 7 min at
94°C, 33 cycles supposing denaturation for 35 s at 94°C, annealing 40 s at 57°C and elongation 45 s at 72°C. The results were processed using SPSS program.

Results and discussion

The study cohort consisted of 104 patients with ET. Our study showed a predominance of females patients-68 (65.4%), males-36 (34.6%), similar to other studies (Finazzi & Harrison 2005). The median age at diagnosis was 53.79 years (range 17-81 years), comparable with the age from the literature. 25 (24.03%) patients were diagnosed when they were less than 40 years old, mostly women (78.5%) (In literature 20% of the subjects have less than 40 years) (Finazzi & Harrison 2005, Passamonti et al. 2004, Cortellazo et al. 1990, Jensen et al. 2000). The clinical and laboratory characteristics of these patients are presented in Table 1, which also displays the information according to JAK2 V617F mutational status. Mutation screening was performed on genomic DNA of peripheral blood from all 104 patients with ET and JAK2 V617F was detected in 56 patients (53.8%). JAK2 V617F heterozygous was found in 52 patients (92.8%) and homozygous in 4 patients (7.1%). Mutational frequency in ET (53.8%) from the current study is similar to those cited for ET in previous reports (23-57%) (Baxter et al. 2005, James 2008, Kralovics et al. 2005, Levine & Gilliland 2008, Zhao et al. 2005). A minority of patients with ET (3%) were found to be homozygous for the JAK2 mutation (mutant JAK2 in more than 75%), similar to our study (3.8%). This phenomenon is detected in a substantial proportion of patients with PV (30%) and IMF, as a result of mitotic recombination affecting chromosome 9p.

Those patients with JAK2 V617F positive had higher haematocrit and leucocytes levels (p<0.05) similar to other study (Antonioli et al. 2005).

Conclusions

The discovery of specific molecular abnormalities had greatly facilitated the diagnostic approach in patients with MPD and justifies the preoccupation of the high number of researchers groups to understand the biological mechanisms in the light of Jak2 V617F mutation discovery.

Table 1. Clinical and laboratory features at diagnosis for 104 patients with ET.

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<tr>
<th>Patients (n=104)</th>
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<tr>
<td>Age (years) median and range</td>
<td>53.79 (17-81)</td>
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<tr>
<td>Sex F/M</td>
<td>68/36 (65.4/34.6%)</td>
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<tr>
<td>Haemoglobin g/dl, median and range</td>
<td>14 (5-13.4)</td>
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<tr>
<td>Haematocrit %, median and range</td>
<td>41 (21-54)</td>
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<td>Leucocytes x 10^9/l</td>
<td>12.172 (4.500-75.00)</td>
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<td>Platelet counts 10^9/l</td>
<td>1085.150 (451.000-6000.000)</td>
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<td>JAK2 V617F: heterozygous/homozygous</td>
<td>52/4 (50/3.8 %)</td>
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References


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