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IDENTIFICAZIONE DELLA MUTAZIONE V617F
del gene della Janus Kinase-2

AMPLI JAK2 Cat. N. 1.427

The chronic myeloproliferative Philadelphia-negative diseases (CMPD) are a group of stem hemapoieteic disorders including the polycythemia vera (PV), the essential thrmobocytosis (TE) and the idiopatich myelofibrosis (CIMF).
In 2005 the presence of a somatic mutation (V617F) of gene Janus Kinase 2 (JAK2), coding for an important protein for signal transduction inducted by hemapoieteic growth factors, in patients CMPD Ph negative has been shown; it phosphorylates different cytoplasmic molecules, particularly the STAT (Signal Transducers and Activators of Transcription). The protein JAK2 belongs to the Janus kinases family; these proteins are composed by seven regions: JH1-JH7. Particularly, JH1 is the kinase action region. JH2 is pseudo-kinase domain, important for JH1 catalytic activity and involved in the inhibitory regulation of that activity.
The mutation V617F, is caused by the nucleotidic substitution G>T at nucleotide 1849 in the eson 14, inducing the substitution of the valine amino acid with phenylalanine at codon 617 (GTC>TTC). This mutation involves a portion of the pseudo-kinase JH2 of JAK2, important in controlling JH1 inhibitory activity. This is an acquired somatic mutation, that can be found only in myeloid cells (erythroid line, granulocyte-macrophage line, megakaryocyte line) in the heterozygous and homozygous state. The mutation is called "gain-of-function mutation" because it determines a consituent activation of the JAK-STAT pathway, able to give a proliferative advantage and cytokine-independent growth of the hemapoieteic cells.
The mutation V617F has been often noticed in patients with:
- polycythemia vera (65-97%), 20% more than other homozygous patients.
- essential thrmobocytosis (23-57%), generally present in heterozygous form.
- myelofibrosis (35-95%)

Less frequently V617F has been found in other diseases such as chronic myelomonocytic leukemia, the myelodysplastic diseases (MDS), systemic mastocytosis (SM), the chronic neutrophilic leukemia, the hypereosinophilic syndrome and the atypical chronic myeloproliferative diseases. The mutation JAK2-V617F has never been found in healthy subjects. The mutation's study can explain the disease and can be used as a diagnostic and prognostic tool.
The AMPLI-JAK2 kit allows to identify, thanks allele-specific PCR (ARMS-PCR, Amplification Refractory Mutation System - Polymerase Chain Reaction), the wild-type allele (normal) e mutated; in particular, a 229 bp amplified for the normal allele and 279 bp amplified for the mutated allele. The same mix PCR provides a PCR internal control (quality control/DNA quantity) which is a primer pair not allele specific that runs along the eson 14 of JAK2 gene and amplifies a 463 bp fragment.
The allele-specific PCR, rather than other methods (sequencing, RFLP, etc) allows to identify the mutation even when there is a small amount of cell (sensibility 1-2% of mutated cells; specificity 99%).

Principle of method: A) extraction of genomic DNA B) amplification C) detection on agarose gel.
Applicability: On extracted and purified DNA from whole blood.
Tests: 45
Stability: over 12 months if correctly stored.

REAGENTS AND STORAGE

AMPLIFICATION	
Mix PCR JAK2	-20°C
H_2O RNase/DNase-free	-20°C
Taq Polymerase (5U/µl)	-20°C
DNA-V617F control	-20°C

References:
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· Baxter EJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365:1054-1061.
· Kralovics R, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779-1790.
· Levine RL, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7:387-397.
· Steensma DP.JAK2 V617F in Myeloid Disorders: Molecular Diagnostic Techniques and Their Clinical Utility. J Mol Diagn 2006, 8:397–411.
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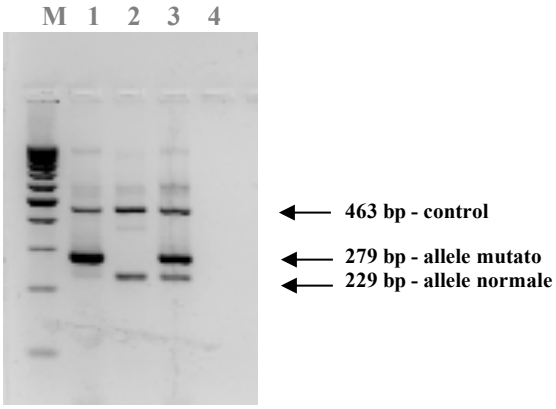
ANALYSIS OF RESULTS

The Mix PCR JAK2 allows to detect simultaneously the amplification of the normal and mutated allele. In particular, an amplified of 229 bp for the normal allele and one of 279 bp have been obtained. Furthermore the same mix PCR contains an internal control (quality/quantity control of DNA) that amplifies a fragment of 463 bp.



Agarose gel containing:
M)Marker 100 bp ladder
1) DNA with V617F mutation
2) DNA with no mutation
3) DNA with V617F mutation
4) negative (DNA)