DNA-Alignment Driven *JAK2* Exon 12 Mutation Analysis Defines 2 Broad Genetic Mutant-"Clusters" and Simplifies Genetic Testing by PCR-SSP

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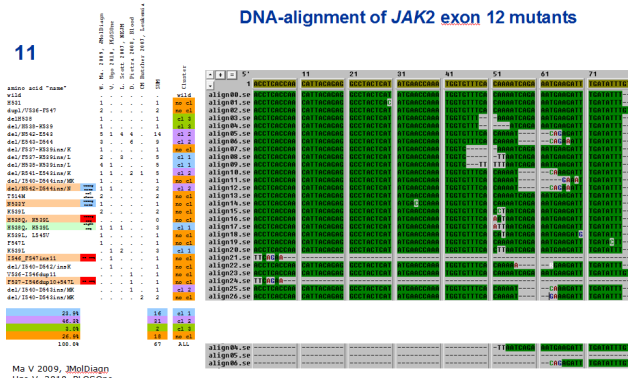
Introduction and Purpose

Somatic V617F mutation located in exon 14 of the JAK2 gene, is known to be present in approximately 90-95% of Polycythemia Vera patients. In V617F negative patients a variety of different mutations in JAK2 exon 12 has been identified with comparable gain of function effects of the Janus Kinase as reported for V617F. We have developed a method, that simplifies the detection of the different mutations in exon 12.

Materials and Methods

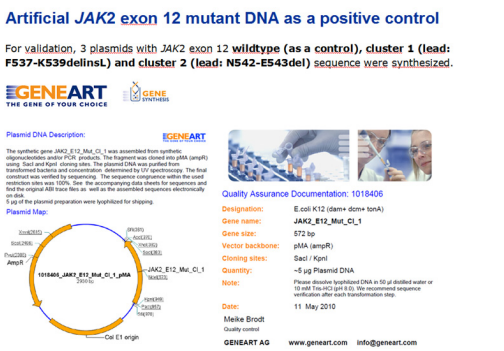
In order to design a practicable genotyping approach, 25 different JAK2 exon 12 mutant alleles known so far, were analysed using a DNA alignment software tool [references 1-5, Figure 1]. Following this analysis, artificial plasmid DNA for the wildtype and the two clusters was used as positive control DNA (Figure 2). Applying PCR Using Sequence Specific Priming (PCR-SSP), the two major allelic groups (clusters) of JAK2 exon 12 mutant alleles were made detectable (Figure 3 and 4).

Figure 1



JAK2 exon 12 cluster 1 and cluster 2 detect 23.9% and 46.3% (Σ = 70.2%) of all observed mutant alleles.

Figure 2



Results

Cluster 1 alleles were characterized by a A1616T, A1617T substitution, (n= 4 alleles, 16 observations) and cluster 2 by a 6 bp deletion, which may be positioned after T1619 for all of them (n= 5 alleles, 31 observations). Representative alleles are F537-K539delinsL and N542-E543del for cluster 1 and 2, respectively. Using artificial DNAs, PCR-SSPs were proven to work correctly and within a wide range of emulated DNA concentrations. The method should be capable of detecting 23.9% (cluster 1) and 46,3% (cluster 2), or 70.2% in total of all previously observed JAK2 exon 12 mutant alleles (total n= 67). Together with our in-house adapted V617F PCR-SSP, most of the pathogenic JAK2 mutations are assessed "all-in-one" using genomic DNA.

Figure 3

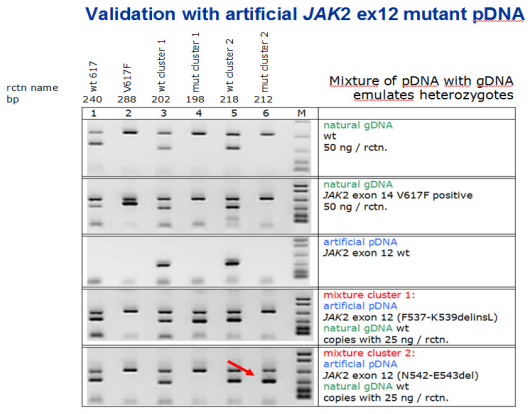
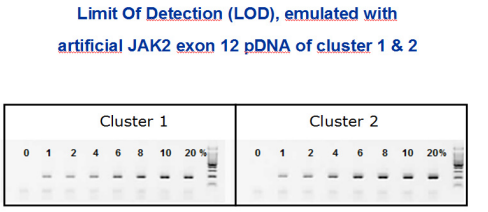


Figure 4



100% correspond to 50 ng gDNA per PCR-SSP (=13'800 copies / 10 µL rctn).

1% of mutated *JAK2* exon 12 pDNA (cluster 1 and 2) is reliably detected (=150 copies / 10 µL rctn).

Conclusion

JAK2 genotyping is an important criterion for the diagnosis of PV and other myeloproliferative disorders according to WHO. Using the PCR-SSP method presented, JAK2 exon 12 / exon 14 mutation detection is simplified drastically and provides a pragmatic approach to assess V617F negative cases, which normally present with isolated polyglobulia. Identified positive samples for exon 12 mutations may be DNA sequenced for final allele definition.

References

Ma V 2009, JMolDiagn, Ugo V, 2010, PLOSOne, Scott LM, 2007, NEJM, Pietra D, 2008, Blood, Butcher CM, 2008, Leukemia

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