DNA-ALIGNMENT DRIVEN JAK2 EXON 12 MUTATION ANALYSIS DEFINES 2 BROAD GENETIC MUTANT-"CLUSTER"-VARIANTS AND SIMPLIFIES GENETIC TESTING APPLYING PCR-SSP

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Introduction: V617F mutation located in exon 14 of the JAK2 gene, is known to be present in approximately 90-95% of Polycythemia Vera (PV) 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.
Methods: In order to design a practicable JAK2 exon 12 genotyping approach, 24 different JAK2 exon 12 mutant alleles published so far (Scott L, 2007, Butcher CM, 2007, Pietra D, 2008, Ma W, 2009, Ugo V 2010) were analysed using a DNA alignment software tool (www.generunner.com). Following this analysis, artificial DNA (Geneart, Germany) was used as positive control DNA for the development of the test. Applying PCR Using Sequence Specific Priming (PCR-SSP), two major allele-groups ("cluster") of JAK2 exon 12 mutant alleles were made detectable.
Results: Interpreting a DNA alignment of 24 different JAK2 exon 12 mutant alleles showed pronounced similarity for some of them, and led to the definition of 2 major allele-groups, "cluster 1 and 2". Cluster 1 alleles were characterized to show a combined A1616T, A1617T substitution, leading to a K539L amino acid exchange (n = 4 alleles, 16 observations). Cluster 2 alleles were characterized 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. Synthetic wild-type and mutant DNA for both clusters, each with the sequence of the representative alleles given above, were used to develop specific PCR-SSPs. Using these artificial DNAs, PCR-SSPs were proven to work correctly and within a wide range of emulated DNA concentrations. In theory, the developed method should be capable of detecting 23.9% (cluster 1) and 46,3% (cluster 2), or 70.2% in total of all previously reported JAK2 exon 12 mutant allele observations (total = 67). Together with our in-house adapted V617F PCR-SSP, we present an easy approach to assess most of the pathogenic JAK2 mutations "all-in-one".
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 may be simplified drastically. Positive samples for exon 12 mutations may then be DNA sequenced for final allele definition.