P1056 SCREENING OF JAK2 EXON 12 SOMATIC MUTATIONS BY HIGH-RESOLUTION MELTING CURVE ANALYSIS

Topic: 16. Myeloproliferative neoplasms - Clinical

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Background:

JAK2 exon 12 mutations are seen in about 2-5% of JAK2V617F-negative cases of polycythemia vera (PV). Nowadays about 40 different JAK2 exon 12 mutations associated with PV have been identified and classified. To identify all possible variants, it is necessary to use sequencing. However, due to the high cost of sequencing, developing a two-stage algorithm for detecting mutations in JAK2 exon 12 using inexpensive screening is of immediate practical necessity. We have previously proposed a two-stage algorithm for detecting mutations in JAK2 exon 12 using inexpensive screening test by heteroduplex analysis (Subbotina T et al, Haematologica 2017).

Aims:

The aim of this study was to demonstrate the feasibility of HRM analysis using the CFX96 thermocycler and the Precision Melt Analysis software (Bio-Rad, USA) as the preliminary screening test for detection of JAK2 exon 12 mutations.

Methods:

DNA samples of 5 JAK2 exon 12 mutation positive PV patients were included in this study. The identification of the JAK2 exon 12 mutation types and allele burden measurement was carried out by pyrosequencing (Subbotina T et al, Haematologica 2014). All 5 patients have different mutation variant in the 12 exon of the JAK2 and different levels of allelic burden: c.1624_1629delAATGAA – 67%; c.1619_1627TCAGAAATGA→AAA – 14%; c.1623_1628delAAATGA – 15%; c.1622_1627delGAAATG – 33%; c.1612_1616CACAA→TT – 21%. HRM analysis was performed using a Precision Melt Supermix reagent kit in the presence of Eva Green dye (Bio-Rad, USA). PCR with an additional high-resolution melting step was carried out on a CFX96 thermocycler (Bio-Rad, USA) according to the following program: denaturation at 95°C for 2 minutes, then 40 cycles at 95°C for 10 seconds, 58°C in for 30 seconds. The high resolution melting program consisted of denaturation at 95°C for 30 seconds, renaturation at 60°C for 1 minute, and melting at 65°C to 95°C with a 0.2°C gradient in 10 seconds. Each DNA sample was analyzed in duplicate. For the two out five JAK2 exon 12 mutations a threshold determination of the mutant allele presence was analyzed. To analyze the threshold for determining the proportion of the mutant allele, dilution of cloned wild-type and mutated samples was performed to obtain samples with different levels of allelic burden: 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%.

Results:

Figure 1 shows the differential melting plots of the DNA fragments. The analyzed samples were divided into five clusters: first cluster – melting curves of wild type DNA; second cluster – melting curves of DNA from samples with deletion type mutations: c.1624_1629delAATGAA and c.1622_1627delGAAATG; the third cluster – the DNA melting curves from the sample with the combined mutation: c.1612_1616CACAA→TT; the fourth cluster – DNA melting curves from the sample with deletion type mutation: c.1623_1628delAAATGA; fifth cluster – DNA melting curves...
from a sample with the combined mutation: c.1619_1627TCAGAAATG>AAA. The detection thresholds in case of the c.1624_1629delAATGAA and c.1619_1627TCAGAAATg>AAA mutation analysis are 6.25% of the presence of the mutant allele in the samples (data not shown).

Image:

Figure 1. Differential melting plots of the JAK2 gene fragments.

Summary/Conclusion:

Therefore, the HRM analysis that was conducted on the CFX96 allows to screen highly specific for the PV diagnosis mutations in exon 12 of the JAK2 gene. The inclusion of this screening research in the laboratory testing algorithm improves the efficiency and accessibility of molecular genetic technologies in the diagnosis of PV.