Abstract

The analysis of cell-free fetal DNA (cffDNA) in maternal plasma became an important component of non-invasive prenatal diagnostics in recent years. Detection of Y chromosome sequences in free circulating DNA (cfDNA) indicates the presence of a male fetus; the absence of the Y-chromosomal signal confirms the female gender. The aim of this work is to confirm the utility of insertion-deletion polymorphisms (INDEL) for determination of the female sex of the fetus using the analysis of cffDNA by Digital Droplet PCR (ddPCR).

In the thesis, X chromosomal INDEL polymorphisms with a suitable allelic frequencies were selected from databases to allow the determination of the presence of the paternal X chromosome in cffDNA and to lead to the confirmation of the female sex of the fetus by a positive amplification signal and to rapid determination of the fetal fraction size of the circulating DNA.

Molecular genetic examination of these polymorphisms was established using ddPCR method. A population study was carried out to verify the utility of the proposed polymorphisms with regard to non-invasive prenatal diagnosis. We examined X chromosomal INDEL polymorphisms: rs2307932, rs16397, rs16637, rs3048996, rs16680 using the ddPCR methodology. In order to obtain population data, we performed tests from the buccal swabs of 50 unrelated women. For all INDEL polymorphisms, we tested the performance of ddPCR in 20%, 10%, 5% and 2.5% mixtures of homozygotes for deletion and insertion forms. We investigated the plasma of 13 pregnant women with the absence of Y chromosomal signal in cffDNA. Testing resulted in a small fraction (representing the paternal X-linked allele on maternal background) in all artificial mixtures. We confirmed the presence of the paternal X chromosome in 12 of 13 pregnant women (92.31% sensitivity) by a positive amplification signal.

Our established approach allows to confirm female fetus by a positive amplification signal in non-invasive prenatal diagnosis based on cfDNA analysis of pregnant women.

Key words: INDEL polymorphisms, cell-free fetal DNA, sex determination , Digital Droplet PCR