Abstract

Since the discovery of cell-free fetal DNA in peripheral blood of pregnant women, cell-free nucleic acids in maternal plasma are explored in relation to non-invasive prenatal diagnosis of various fetal conditions and pregnancy complications. Non-invasive prenatal diagnosis of monogenic diseases represented by *TSC1*-linked tuberous sclerosis could be achieved by detection of paternally-inherited mutant allele in the pool of maternal alleles in plasma. Reliability of detection of mutant allele could be improved by simultaneous mutation haplotype analysis or detection of universal fetal marker. None of the 3 methods (allele-specific real-time PCR, SNaPshot minisequencing and quantitative fluorescent PCR) evaluated using artificial mixtures and maternal plasma samples reliably and accurately detected low-frequency allele distinguished by point mutation, SNP or microsatellite in *TSC1* gene or in its close proximity. We developed a strategy for prediction of proportion of informative couples for panel of SNPs of interest that can be applied to any monogenic disease. Exploiting differential methylation of promoters of genes *RASSF1A*, *HLCS* and *OLIG2* in maternal and fetal genome, we failed to establish functional fetal marker. MicroRNAs of placental origin released into plasma could serve as biomarkers of placental insufficiency. We demonstrated that although levels of tested microRNAs did not differ between physiological and pathological placentas and between plasma samples of pregnant women at onset of preeclampsia and/or IUGR and of healthy controls with uncomplicated pregnancies, levels of miR-520a*, miR-520h, miR-525 and miR-526a were significantly higher in early pregnancy samples from women that later developed placental insufficiency. Chromosome 21-derived microRNAs were previously reported to be overexpressed in tissues of patients with Down syndrome and we demonstrated that relative quantification of 4 of them (miR-99a, let-7c, miR-125b and miR-155) allowed the detection of trisomic samples up to 1% content in artificial mixtures.

Key words: non-invasive prenatal diagnosis, cell-free nucleic acids, maternal plasma, microRNAs, tuberous sclerosis, placental insufficiency, Down syndrome