Noninvasive prenatal detection of genetic defects

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Chapter 8

Summary

Samenvatting

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Current prenatal diagnostics is mainly based on obtaining fetal DNA through invasive procedures which are associated with a small, but significant risk of fetal loss. The discovery of the presence of cell-free fetal DNA (cffDNA) in maternal plasma opened possibilities for less or noninvasive alternative procedures.

CffDNA is comprised of small fragments of fetal extracellular DNA derived from placental cells that go into apoptosis. It circulates in maternal plasma and can be detected already very early in gestation. However, the majority of total cell-free DNA (cfDNA) in maternal plasma is of maternal origin and the fetal contribution is relatively small in the first trimester. Despite this high maternal background, the use of cffDNA in noninvasive prenatal diagnostics (NIPD) and noninvasive prenatal testing (NIPT) has been described successfully. In this thesis, we describe novel applications and approaches for the use of cffDNA in both NIPD and NIPT.

For all these novel applications, either NIPT or NIPD, it is important to know the fetal fraction in the maternal background. Independent confirmation of the presence of fetal DNA in the sample is therefore required. Most often male specific markers (e.g. *SRY* and *DYS14*) are used to confirm the presence of cffDNA or to determine the fetal fraction in a sample. However, using these markers a positive result can only be obtained in case of a male fetus. Therefore we have developed a novel approach to detect and confirm the presence of cffDNA in maternal plasma in a sex-independent way. **Chapter 2** describes a novel assay for the detection of fetal specific methylated *RASSF1A* (*mRASSF1A*) using a combination of bisulfite conversion and pyrophosphorolysis-activated polymerization. *RASSF1A* is differentially methylated between mother and fetus. These differences in methylation were used to specifically detect and thus confirm the presence of fetal *mRASSF1A* sequences. We have shown that this qualitative approach to determine cffDNA in maternal plasma can be used successfully with high sensitivity and specificity and can be applied early in gestation. In the future, digitalization and thus quantification of this assay could be beneficial in the determination of the fetal fraction.

NIPT for fetal aneuploidy screening is perhaps one of the best studied applications for the use of cffDNA and is frequently performed on PCR based sequencing platforms. These platforms are known for GC content related bias in sequencing data. **Chapter 3** describes the use of Helicos Single Molecule Sequencing (SMS) as an alternative non-PCR based sequencing platform for NIPT of fetal trisomy 21 (T21). We have directly compared SMS to the PCR based Illumina platform and show that when using SMS, the mean number of sequencing reads is not influenced by GC content, thereby showing that SMS sequencing data is not GC biased. While, using the Illumina platform, the mean number of sequencing reads generated increases when GC content of the chromosome increases. Moreover, we show that when using SMS, all fetuses with T21 can be correctly identified and distinguished more clearly from euploid fetuses. In **chapter 4**, we additionally describe the use of SMS for other fetal trisomies. We show that SMS is also successful for trisomy 18 (T18) screening. However, for trisomy 13 (T13) screening SMS turns out to be less successful. To elucidate the cause of this poor performance further testing is needed.

In contrast to NIPT, NIPD for monogenic disorders currently represents only a small part of the field of noninvasive prenatal genetic testing. NIPD can be used for the detection of paternally inherited sequences. However, not all NIPD can currently be addressed by a single or universal approach, such as for instance NGS. **Chapter 5** describes the use of High-Resolution Melting Curve Analysis (HR-MCA) as a non-NGS PCR mediated alternative for the detection of paternally inherited point mutations in maternal plasma. We show that by blocking the

maternal background with locked nucleic acid (LNA) blocking probes, the detection of the paternally inherited allele of the fetus can be enhanced. We show this proof of principle for both an autosomal dominant and an autosomal recessive disorder.

Chapter 6 additionally shows the use of another PCR based approach for the detection of fetal paternally inherited repeats in Huntington disease (HD). HD is caused by the expansion of a trinucleotide (CAG) repeat in the Huntingtin (*HTT*) gene. The fetal paternally inherited repeat can detected by use of a PCR based approach with primers flanking the repeat. We describe a validation study for NIPD for HD and showed that we could detect a fetal repeat of up to 70 CAG repeats in maternal plasma. In this validation study, all paternally inherited repeats for all fetuses at risk for HD could be detected. We additionally show that in cases where the fetus has inherited the normal paternal allele, the detection strongly depends on the parental genotypes.

In the future, the number of applications for NIPT and particularly NIPD is expected to increase. Even though the use of fragmented cffDNA from maternal plasma still has its challenges (e.g. relative low amounts, short in size), we show that by careful consideration of the most efficient approach for each application, these challenges may be overcome. This makes cffDNA a very potential source for use in NIPT and NIPD. Based on the ongoing improvements of these tests, both NIPT and NIPD will soon become available to more women, although a universal approach for all applications is not feasible yet. Nevertheless, it is to be expected that in the near future, the majority of prenatal diagnostics will be replaced by either NIPD or NIPT.