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## RAPID AND NON INVASIVE PRENATAL DIAGNOSIS

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### INTRODUCTION

Prenatal screening and diagnosis are routinely offered at antenatal care clinic visits, and are important in decision making about the continuation of pregnancies affected by genetic conditions for which there are no cures, and prevention through therapeutic abortion is a reasonable option. Prenatal screening is offered to all pregnant women and include fetal ultrasonography and maternal serum biochemistry to select the pregnancies at-risk for chromosomal abnormalities. However, these methods have limited sensitivities (60.0-75.0%) and specificities (false positive rate of 5.0%). Even when used in combination and taking into account maternal age, the identification rate of affected fetuses does not exceed 90.0% [one]. Prenatal diagnosis is usually preformed for detection of chromo-

somal aneuploidies or monogenic diseases in “high risk” pregnancies. Diagnostic testing currently requires a sample of fetal cells obtained either by chorionic villus sampling (CVS) between 10 and 14 weeks gestation or by amniocentesis after 15 weeks of gestation. However, these invasive procedures carry a risk of miscarriage of around 1.0% [2].

**Prenatal Diagnosis of Chromosomal Abnormalities.** Chromosomal abnormalities (numerical or structural) occur in 1 of 160 live births, with extra copies of chromosomes 21, 18, and 13 accounting for the majority of numerical alterations that are not related to sex chromosomes. The prevalence of trisomies is highest in the first trimester because of subsequent miscarriage and demise of aneuploid conceptuses during pregnancy [3]. Conventional cytogenetic techniques (karyotyping) are usually used to detect aneuploidies and large (5-10 Mb) rearrangements in fetal cells (amniocytes, trophoblasts), however, these are time-consuming (2-3 weeks), subjective (small rearrangements) and expensive. The development of molecular methods for the rapid, targeted detection of aneuploidies of chromosomes 13, 18, 21 and the sex chromosomes by quantitative fluorescent polymerase chain reaction (QF-PCR) [4,5] using fetal DNA, do not provide a genome-wide screen for unexpected imbalances, but are rapid (24-48 hours), accurate and inexpensive. Multiplex ligation probe amplification (MLPA) is a recent technique for relative quantita-

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tion of up to 40 to 45 nucleic acid targets. Several MLPA commercial kits are used for prenatal detection of common aneuploidies (chromosomes 13, 18, 21, X and Y), common microdeletion syndromes and subtelomeric copy-number changes, identification of marker chromosomes, and detection of familial copy-number changes in single genes [6-8]. The most powerful technique for genome wide screening is array comparative genomic hybridization (aCGH), which has the potential to combine the speed of DNA analysis with a large capacity to scan for subtle genomic abnormalities (approximately additional 10.0% of karyotyping) respective to the resolution of the used arrays [9-11], but is expensive, time-consuming and requires a high degree of expertise.

**Non Invasive Prenatal Diagnosis.** The discovery of cell-free fetal DNA (cffDNA) in maternal plasma in 1997 opened up new avenues for prenatal diagnosis [12,13]. Fractional concentration of fetal DNA is ~10.0%, coexists with a background of maternal DNA and is present in maternal plasma from approximately the 6th gestational week [14]. Techniques, such as real-time PCR (ReTi-PCR) and digital PCR, provide sufficient sensitivity for reliable non invasive assessment of this cffDNA pool for paternally inherited traits such as sex and RHD status, offering possibilities for non invasive prenatal diagnosis of X-linked disorders (such as Duchenne/Becker muscular dystrophy, Hemophilia A, Hemophilia B, *etc.*) and RhD incompatibility, respectively [15,16]. By detecting the presence of fetal-specific paternally inherited mutant alleles in maternal plasma, diagnosis of autosomal dominant diseases transmitted by the father could be made non invasively, whereas the absence of such alleles could be used to exclude fetal inheritance of autosomal recessive diseases [14,17-20]. Quantification of cffDNA, specific fetal and maternal DNA and mRNA single nucleotide polymorphism allelic ratios have been used to detect fetal aneuploidies, however, the limitations of these techniques affect the accuracy of the diagnosis [21-23]. Improvements were made after the discovery of the unmethylated *SEPINB5* gene that turned out to be the first sex- and polymorphism-independent fetal DNA marker found in maternal plasma [24-27]. The differential methylation of placenta and maternal blood provides a rich source of markers for non invasive prenatal diagnosis, however, further

research is needed to render the techniques widely applicable. Implementing the new and robust next generation sequencing techniques in detection of the fetal aneuploidy made the detection for Down's syndrome to have 98.6-100.0% sensitivity and 96.8-97.9% specificity [28,29].

**Prenatal Diagnosis of Monogenic Diseases.** Monogenic diseases are the second most frequent indication for prenatal diagnosis. The incidence of these diseases, depending on the population, is up to 2.0% newborns. Although there are some biochemical tests and ultrasound findings to screen and identify pregnancies at-risk for specific monogenic disorders, still the diagnosis is usually established after the fetus is born in couples with no familial history of the disease. In families at-risk for monogenic disease, prenatal diagnosis is used to determine fetal health and to provide adequate management of the pregnancy and prenatal or perinatal treatment. The new developments in prenatal testing using cffDNA and their translation into clinical practice are going to make a difference in selection of pregnancies at-risk for monogenic disorders that need invasive testing.

**Prenatal Diagnosis at the Research Centre for Genetic Engineering and Biotechnology (RCGEB) "Georgi D. Efremov," Skopje, Republic of Macedonia.** In the last 20 years, the researchers at the RCGEB "Georgi D. Efremov" have performed more than 80 prenatal diagnoses for different monogenic diseases, such as hemoglobinopathies, cystic fibrosis, Duchenne/Becker muscular dystrophy, spinal muscular atrophy, hemophilia A, Lesch Nyhan syndrome, Rett syndrome, phenylketonuria, galactosemia, pseudohypoaldosteronism, *etc.* [30,31]. The prenatal diagnosis was performed on fetal DNA by using standard molecular genetic techniques for direct diagnosis of the disease or by using informative polymorphic DNA markers for indirect diagnosis.

In 2001, the rapid prenatal detection of the most common chromosomal aneuploidies (chromosomes 13, 18, 21, X and Y) by the multiplex QF-PCR (mQF-PCR) method was introduced at the RCGEB "Georgi D. Efremov" [32,33]. We have developed a one-tube mQF-PCR assay for amplification of 22 highly polymorphic short tandem repeat (STR) markers (at least four by analyzed chromosome) (Table 1). Since then, more than 2200 prenatal diagnoses of common aneuploidies in at-risk preg-

**Table 1.** Short tandem repeat markers used in one-tube quantitative fluorescent polymerase chain reaction assay for detection of aneuploidies of chromosomes 13, 18, 21, X and Y.

	Chromosome 13	Chromosome 18	Chromosome 21	Chromosome X	Chromosome Y
STR markers	D13S631	D18S535	D21S1414	AMEL X/Y <sup>a</sup>	AMEL X/Y <sup>a</sup>
	D13S305	D18S391	D21S1446	DXYS218 <sup>a</sup>	DXYS218 <sup>a</sup>
	D13S258	D18S386	D21S1411	TAF9B <sup>b</sup>	MYPT2Y <sup>c</sup>
	D13S1817	D18S390	D21S1435	XHPRT	SRY
				DXS6803	DYS448

<sup>a</sup> Locus on chromosomes X and Y.

<sup>b</sup> Locus on chromosomes X and 3.

<sup>c</sup> Locus on chromosomes Y and 2.

nancies have been performed using the mQF-PCR assay as a stand-alone test [34]. It was also used in the prenatal cases of monogenic diseases to control maternal contamination of the fetal material. The prenatal diagnosis was performed on genomic DNA isolated from fetal cells collected by amniocentesis or CVS. Maternal blood samples were analyzed in all blood contaminated amniotic samples and in most chorionic villi samples. No discordant results were obtained when cytogenetic analysis was performed in addition to QF-PCR. Polymorphic duplications involving STR markers D13S631, D21S1441, D18S978 or D18S535 were detected in seven fetuses; in all fetuses the duplications were inherited from one of the parents. Using this method we were also able to determine the parental origin of the aneuploidy [35,36]. In our experience, the QF-PCR method is an efficient, rapid and reliable method for prenatal diagnosis of the most common chromosome aneuploidies. In addition, it can provide information about the origin of the aneuploidy and maternal contamination of the fetal material.

In some “high risk” pregnancies with normal QF-PCR results, we have used MLPA kits to analyze subtelomeric regions and common microdeletion syndromes. In addition to this, aCGH has been employed in prenatal diagnosis of a few fetuses with specific abnormal ultrasound findings.

We have also evaluated the specificity and sensitivity of the real-time quantitative PCR method for non invasive fetal sex determination using cffDNA from maternal plasma. Our initial results showed that this is a promising approach for fetal gender determination in pregnancies at-risk for a fetus with an X-linked disorder [37]. Our recent study of non invasive determination of fetal RHD status,

using cffDNA from maternal plasma in RhD negative pregnant women, showed 100.0% concordant results with those obtained on fetal DNA from amniocytes or CVS. This is a promising test that can be used in clinical practice for targeted anti-RhD prophylaxis and improvement of management of RHD fetomaternal incompatibility. Using a multi copy marker on Y chromosome (DYS14), we have increased the sensitivity and specificity of the non invasive fetal sex determination using cffDNA. This method will be used in the future for non invasive fetal sex determination in pregnancies at-risk for X-linked disorders. Our further plans include translation of the non invasive tests using cffDNA for diagnosis of monogenic disorders and chromosomal aneuploidies into clinical practice.

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