

Non-invasive prenatal diagnosis using cell-free fetal nucleic acids in maternal plasma: Progress overview beyond predictive and personalized diagnosis

Georgia Tounta · Aggeliki Kolialexi ·
Nikolas Papantoniou · George Th. Tsangaris ·
Emmanuel Kanavakis · Ariadni Mavrou

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Abstract The discovery of circulating cell-free fetal DNA (cffDNA) in maternal plasma allowed for the development of alternative methodologies that may facilitate safe non-invasive prenatal diagnosis (NIPD). The low concentration of cffDNA in maternal plasma, however, and the coexistence of maternal DNA limit its clinical application to the detection or exclusion of fetal targets that are not present in the mother, such as Y chromosome sequences, the *RHD* gene in a RhD-negative woman and genetic conditions inherited from the father. Strategies for NIPD of monogenic disorders and fetal chromosomal aneuploidies have also been achieved using next-generation sequencing and could be introduced to the clinics as soon as cost-effective and high throughput protocols are developed.

Keywords Non-invasive prenatal diagnosis · Cell-free fetal nucleic acids · Monogenic disorders · Chromosomal aneuploidies · Fetal gender · Fetal RhD status

Introduction

Prenatal diagnosis is now part of established obstetric practice in many countries. To perform it, fetal genetic material is conventionally obtained by invasive techniques such as amniocentesis and chorionic villus sampling. Since procedural related miscarriage rate of about 1% has been reported, invasive prenatal diagnosis is reserved for pregnancies at risk for certain fetal genetic conditions. These include fetal chromosomal aneuploidies and monogenic disorders with relatively high prevalence in the relevant populations. For chromosomal aneuploidies, mainly Down Syndrome (DS), risk calculation is based on maternal age, ultrasonographic findings and maternal serum biochemical markers. For certain monogenic disorders, such as thalassaemia and cystic fibrosis, identification of a positive family history and confirmation of the carrier status of the parents are among the strategies used in order to identify high risk pregnancies that may be referred for invasive prenatal diagnosis.

Non-invasive testing, using maternal peripheral blood as a source of fetal genetic material, has long been the goal for early prenatal diagnosis avoiding the risk of miscarriage. In contrast to popular belief that placenta forms an impermeable barrier between mother and fetus, there is proof for bidirectional traffic between the fetus and the mother during pregnancy [1]. Multiple studies indicate that both intact fetal cells and cell-free fetal nucleic acids (cffNA) cross the placenta and can be found in maternal circulation. Intact-fetal cells present an attractive target for non-invasive prenatal diagnosis (NIPD) of fetal chromosomal abnormalities. Isolation and analysis of fetal cells from maternal circulation have been extensively investigated and several methods for fetal cell enrichment have been developed [2–

G. Tounta · A. Kolialexi (✉) · E. Kanavakis · A. Mavrou
Department of Medical Genetics,
Athens University School of Medicine,
Athens, Greece
e-mail: akolial@med.uoa.gr

N. Papantoniou
1st Department of Obstetrics & Gynecology,
Athens University School of Medicine,
Athens, Greece

G. T. Tsangaris
Proteomics Research Unit,
Biomedical Research Foundation of the Academy of Athens,
Athens, Greece

4]. For the time being, results have been disappointing due to the scarcity of intact fetal cells in maternal circulation (1 cell per 1 ml of maternal blood) and the low efficiency of enrichment methods. In addition, chromosome analysis by Fluorescent In Situ Hybridization (FISH) is difficult due to the abnormally dense apoptotic nuclei of fetal cells [5, 6].

In 1997, Lo et al. reported the presence of cell-free fetal DNA (cffDNA) in maternal plasma and serum in amounts significantly increased, as compared to fetal DNA extracted from the cellular fraction of maternal blood [7, 8]. Following this discovery, a new area of research was developed.

This review summarizes the current status of NIPD using cffDNA in maternal plasma and emphasizes recent developments that may allow in the future routine application of NIPD for assessment of chromosomal abnormalities and monogenic disorders.

Cell-free fetal nucleic acids in maternal plasma

Cell-free fetal DNA represents extracellular DNA which can be detected in maternal peripheral blood as early as 18 days following embryo transfer in *in vitro* fertilization pregnancies [9]. In contrast to fetal cells, it is cleared from maternal circulation shortly after delivery, with a mean half-life of 16 min [10]. The proportion of cffDNA, however, is only 3–6% of the total amount of cell-free DNA (cfDNA), the remaining portion of DNA being contributed by the mother, mainly from maternal blood cells. Later studies using digital PCR showed that the actual amount of cffDNA is somewhat higher (~19%) but still represents a minor fraction of the total amount of cfDNA in maternal plasma [11]. The concentration increases with gestational age, from the equivalent of 16 fetal genomes per millilitre of maternal blood in the first trimester to 80 in the third trimester, with a sharp peak during the last 8 weeks of pregnancy [8, 12].

It is generally accepted that cffDNA in maternal plasma is derived from syncytiotrophoblasts undergoing apoptosis and is, therefore, fragmented [13, 14]. Studies regarding the size distribution of cffDNA have confirmed that it is on the average 300 bp or smaller, in contrast to the maternal cfDNA fragments, which are considerably larger [15, 16].

In addition to cffDNA, cell-free fetal mRNA (cffmRNA) sequences of placental origin also exist in maternal plasma. The proof of principle of cffmRNA in maternal plasma was first demonstrated in pregnant women carrying a male child, with the detection of Y chromosome-specific zinc finger protein mRNA [17]. This finding was further confirmed a few years later, through the analysis of placental-specific mRNA, human placental lactogen (hPL) and the b subunit of human placental chorionic gonadotrophin [18].

Recent data have also demonstrated that placental miRNAs are also released from the placenta and are present

in maternal plasma in detectable quantities. Quantification of placental miRNAs in maternal plasma may offer a non-invasive tool for monitoring gene regulation in the placenta. Placental miRNAs exhibit exceptional stability which is probably due to their association with subcellular particles such as syncytiotrophoblast microparticles [18–21].

Technical aspects

The scarcity of cffDNA in maternal blood and its co-existence with maternal DNA represent the two major limitations for the use of cffDNA for diagnosis. Both maternal plasma and serum contain cffDNA, however, plasma is the material of choice for prenatal diagnosis since it contains less maternal background DNA. Usually, isolation of plasma cfDNA is performed manually with commercially available kits, although automation of the process has also been reported [22, 23]. It is important to note that special care should be taken to avoid external contamination during isolation and amplification.

Various methods have been used to overcome the presence of maternal background cfDNA, including methods based on the size difference of maternal fragments [15, 24]. Efforts to increase the relative proportion of fetal DNA compared to the larger maternal fraction have also included the use of formaldehyde, as a fixative, to prevent lysis of maternal cells during the isolation of the maternal plasma [25]. The formaldehyde enrichment technique, however, has not been reproducible by other laboratories.

Different methodologies applied for the detection of cffDNA include conventional PCR, restriction analysis, quantitative fluorescence real-time PCR (QF-PCR) and automated sequencing [11, 26–31].

Since QF-PCR is more sensitive compared to conventional PCR, enabling the detection of very low copy numbers of DNA, it represents the optimal method for reliable NIPD. The main advantage of QF-PCR is that it is quantitative and collects data in the exponential growth phase of the reaction, which is the most specific and precise one. The technique is less time consuming and offers an extra level of protection against contamination. A wide range of Ct values in each QF-PCR and poor repeatability of some replicates is reported, partly due to the variability of target copy number in maternal plasma. It is, therefore, recommended to perform several replicates from each maternal sample in order to increase the probability of fetal DNA detection and to avoid false-negative results [32].

Recently, new sophisticated molecular techniques such as mass spectrometry, massively parallel genomic sequencing (MPS) and digital PCR have emerged and are applied in the field of NIPD [33, 34]. They have higher sensitivity, but the

expensive and complicated handling processes required by these techniques make them less useful for clinical practice.

Current clinical applications of non-invasive prenatal diagnosis using cfDNA

Fetal sex determination

Fetal sexing, the first clinical application of NIPD, is based on the detection of Y chromosome specific targets in maternal plasma [7]. Positive detection suggests that pregnancy involves a male fetus, while absence is interpreted as a female bearing pregnancy. The majority of the studies for non-invasive sex determination, uses QF-PCR to detect *SRY* and/or *DYS14* sequences in maternal plasma [35–37]. Accuracy of fetal sex assessment has been confirmed by many research group to be better than 95%, so that its use has been adopted in a clinical setting [37, 38].

The most important clinical indication for fetal sex determination is an X-linked genetic disorder and it has been estimated that their cumulative incidence is around 5 in 10 000 live births [39]. Early determination of fetal sex non-invasively could limit the number of invasive diagnostic procedures required for each specific disease to male fetuses only, sparing most female bearing pregnancies from unnecessary invasive diagnostic testing.

Sex determination is also important in cases where development of external genitalia is ambiguous, in families with a history of conditions associated with ambiguous development of the external genitalia, some fetal ultrasound findings and occasionally, discrepancy between genetic sex and the appearance of the external genitalia on fetal ultrasound.

Non-invasive prenatal fetal sex detection has also been suggested for the management of some endocrine disorders, such as congenital adrenal hyperplasia, where masculinization of the female fetus can be suppressed by maternal administration of dexamethasone from the 6th week of gestation [35, 40]. This approach can allow either for slightly delayed administration of maternal steroids to women reluctant to expose fetuses unnecessarily to the potential side effects of dexamethasone, or for prompt cessation of treatment if the fetus is confirmed to be male.

Fetal RHD determination

Rhesus D (RhD) system incompatibility between a RhD-negative pregnant woman and her fetus can result in maternal alloimmunization and haemolytic disease of the fetus (HDFN) in subsequent pregnancies when the fetus is RhD-positive [41]. Routine postnatal injection of immunoglobulin anti-D to all RhD-negative pregnant women,

significantly prevents the occurrence of HDFN and has been successfully introduced in developed countries.

Prenatal determination of the fetal RhD status can be achieved by PCR amplification of *RHD* sequences in amniotic fluid or chorionic villus samples [42, 43]. These invasive procedures, however, carry not only the risk of miscarriage but most importantly, testing RhD-negative pregnant women may lead to immunization due to fetomaternal haemorrhage [44, 45].

The advent of NIDP using cfDNA to determine fetal RhD status offers significant potential for a change in the way RhD-negative pregnant women are managed. Since the *RHD* gene is usually completely absent from the genome of RhD-negative mothers, the detection of *RHD* sequences in maternal blood implies that the fetus must be RhD-positive.

There are multiple reports of high degrees of accuracy for the non-invasive prenatal determination of the fetal RhD status, but to date, clinical application has been confined to women known to be at high risk for HDFN [32, 46–48]. The benefits of mass testing antenatally for fetal RhD status by analysis of cfDNA in maternal plasma of RhD-negative mothers could reduce the use of anti-D and the number of anti-D donors exposed to blood products for hyperimmunization. Women carrying a RhD-negative fetus (approximately 40%) would be spared unnecessary exposure to anti-D with its associated discomfort and risk from viral (hepatitis C) or prion (variant Creutzfeldt-Jacob disease) contamination.

QF-PCR technology is considered the optimal method for the reliable detection of *RHD* sequences using cfDNA [49, 50]. Efforts have also been made for non-invasive prenatal diagnosis of fetal RhD status by mass spectrometry (MS). Grill et al. used an automated system for the extraction of cell-free DNA from maternal plasma and detected the presence of fetal *RHD* exon 7 by MS technology provided by Sequenom to detect SNPs (Sequenom, Inc., San Diego, CA) [51]. Validation of the assay showed the presence of 2.5% RhD-positive genomic DNA in a background of RhD-negative genomic DNA. Five out of 178 samples examined were incorrectly diagnosed as RhD-negative. Grill's report is the only one in the literature which applies MS for NIPD RhD testing. The main advantage of this approach over QF-PCR is that it has the potential of multiplex analysis of several different loci in a single assay but further studies are necessary in order to determine the clinical utility of the technique.

The most widespread approach used in prenatal *RHD* diagnosis is an assay that detects at least two different exons of the *RHD* gene. Many laboratories prefer to include amplification of exon 7, because it contains the most sequence differences to the highly homologous *RHCE* gene, thus improving specificity for *RHD* and allowing

for the detection of fetal *RHD* even in the 7th week of gestation, without giving false-positive results [47, 52].

Fetal monogenic autosomal disorders

Many human diseases are caused by mutations in a single gene and it has been estimated that their combined occurrence is around 3.6 per 1000 live births [39]. Prenatal diagnosis of single gene disorders using invasive techniques is an accepted part of clinical practice and is performed when there is a positive family history for a particular disease.

NIPD for autosomal dominant disorders using maternal plasma has been reported, mainly based on the detection of paternally inherited DNA sequences in maternal circulation. Such an approach can avoid conventional prenatal diagnosis in some cases. It is also important to note that detection of large-scale mutations, caused by expansion, insertion or duplication, is restricted to sequences less than 300 base-pairs in length, due to the fragmented nature of cfDNA [53]. NIPD of maternally transmitted autosomal dominant diseases is not as straightforward. The large maternal DNA background in maternal plasma renders it technically challenging to determine whether a fetus has inherited a mutation from its mother.

To date, use of cfDNA for NIPD has been reported for the following dominant single gene disorders in at least one pregnancy:

- Huntington's disease – detection of a paternally inherited expansion of 37 repeats [54]
- Achondroplasia – identification of a specific point mutation which accounts for more than 98% of cases [26, 27, 55]
- Myotonic dystrophy – a paternally inherited expansion of 70 repeats has been detected using cfDNA [56]

In autosomal recessive disorders, cfDNA can only be used to determine the carrier status of the fetus through detection of the paternally inherited disease allele, in cases where the maternal and paternal ones differ. This information could be used to reduce the number of invasive procedures required, either by increasing the risk for an affected fetus (from one in four to one in two), or by determining that the fetus has not inherited the paternal disease allele and therefore cannot be affected. To date, fetal carrier status has been performed using cfDNA in Cystic fibrosis and Congenital adrenal hyperplasia [57, 58]. For haemoglobinopathies, cfDNA has been used to detect paternally inherited mutations that cause β -thalassaemia, in carriers of a different β -thalassaemia or sickle cell mutation (leading to sickle β -thalassaemia disease) and Hb Lepore. Sickle cell anaemia, however, the most common haemoglobinopathy, is not yet prenatally diagnosed using cfDNA,

since it is caused by two identical copies of a single point mutation [29, 59, 60].

In order to extend NIPD to cases where the father and mother shared the same mutation Lum et al. developed a digital PCR based approach, called relative mutation dosage (RMD) [61]. RMD analysis can be used for diagnosis in cases of pregnant women heterozygous for a known mutation through determination of the dosages of the mutant and wild-type alleles of the disease-causing gene. For a woman carrying a fetus homozygous for the mutation there should be proportionally more mutant sequences than the non-mutant ones in maternal plasma, while in cases with an heterozygous fetus, equal amounts of mutant and non-mutant sequences should be detected in maternal plasma. Similarly, when the fetus is normal homozygous, there should be proportionally more non-mutant sequences than mutant sequences in maternal plasma.

Fetal markers

Significant effort has been made to detect fetal identifiers in maternal plasma samples. For fetal chromosome Y or *RHD* detection assays, failure to detect the targeted cfDNA sequences could be a result of failed or degraded fetal DNA. It is prudent therefore to confirm the presence of fetal material in maternal plasma before reporting a negative result. Detection of Y-chromosome specific sequences, such as *SRY* or *DYS14*, is used in order to confirm the presence of cfDNA in the sample tested [49]. This, however, can only be applied in pregnancies bearing a male fetus. An alternative approach involves detection of paternally inherited polymorphisms that are unique to the fetus, but insertion/deletion polymorphisms or single nucleotide polymorphisms (SNPs) are only useful as internal positive controls if they are absent in the maternal genome and the paternal-unique allele has been inherited by the fetus [62, 63]. Depending on the parental genotype and fetal inheritance, a particular polymorphism may not be applicable to all pregnancies. Consequently, a panel of polymorphisms is needed to ensure that at least one member of the panel is appropriate for any given pregnancy.

A major area of current research aims at finding universal fetal-specific markers, independent of sex or paternally inherited polymorphisms, that could be used either as diagnostic tests or to confirm the presence and quantify cfDNA. Recent approaches have targeted fetal DNA sequences in maternal plasma that are epigenetically different from maternal ones [64]. CpG methylation in the promoter regions of genes is involved in the regulation of gene expression. As tissues in the body have different gene expression profiles, the methylation status of certain genes also exhibits tissue-specific patterns. Chim et al. [64] studied the methylation profile of the promoter of *serpin*

peptidase inhibitor, *SERPINB5*, and showed that it is hypomethylated in placental tissues but hypermethylated in maternal blood cells. Using methylation specific PCR, the placental-derived hypomethylated *SERPINB5* could be detected and distinguished from maternally derived hypermethylated molecules in maternal plasma. The hypomethylated *SERPINB5* sequences were shown to be pregnancy-associated, as they disappeared from maternal plasma within 24 h after delivery. *SERPINB5* was the first universal circulating cffDNA marker that could be applied in all pregnancies regardless of fetal gender and genotype. However, methylation-specific PCR requires use of bisulphite conversion, which alters unmethylated cytosines to uracil nucleotides and thereby results in differences in the genetic sequence of methylated and unmethylated DNA molecules. Yet, bisulphite conversion degrades up to 95% of the DNA molecules in a sample [65]. This would substantially reduce the amount of fetal DNA in a maternal plasma sample and may result in false-negative results, particularly in early pregnancy when fetal DNA concentrations are very low.

For this reason, researchers looked for fetal epigenetic markers that could be detected in maternal plasma without bisulphite conversion. Hypomethylated *RASSF1A* sequences derived from maternal blood cells can be removed from maternal plasma using methylation-sensitive restriction enzyme digestion, revealing only the fetal hypomethylated target (Fig. 1) [66]. Several studies demonstrated the value of using digestion resistant *RASSF1A* DNA sequences as a positive control for NIPD of fetal RhD status [66–68]. False-negative diagnosis can be avoided in samples that are negative for both *RHD* and *RASSF1A* sequences, as failure to detect hypermethylated *RASSF1A* sequences signifies the lack of fetal DNA in maternal plasma sample. These developments improve the reliability of the applications of cffDNA analysis when used in clinical setting.

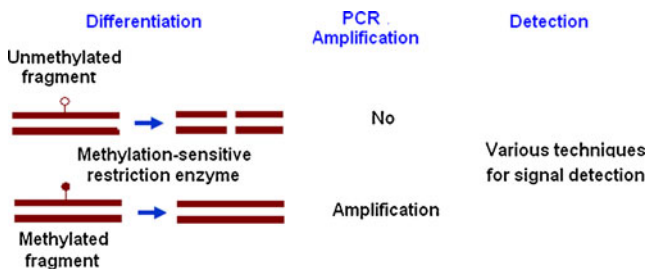


Fig. 1 General schema of DNA methylation analysis using methylation-sensitive restriction enzymes that selectively digest unmethylated DNA, so that only methylated fragments remain available for detection

Pregnancy complications

Various problems associated with placental growth and development result in altered levels of cffDNA in maternal plasma. Elevated concentrations of cffDNA have been detected in pregnancy-related disorders associated with abnormal placentation such as preeclampsia, the leading cause of prematurity [69–71]. Preeclampsia complicates around 5–10% of pregnancies, and if it is not treated on time it can become life threatening for both mother and child. Although preeclampsia stems from a defective placenta, the underlying cause is unknown and the only available treatment is delivery of the fetus. Numerous studies have shown that the level of cffDNA (usually measuring Y chromosome DNA in pregnancies with a male fetus) is elevated by 2–3-fold before the onset of preeclampsia and 2–14-fold during preeclampsia [8, 71]. Elevations in cffDNA have also been reported in pregnancies with preterm contractions that did not respond to colytic treatment and resulted in premature deliveries, in contrast to those that responded to such treatment [72]. A significant number of additional pregnancy-related disorders have been linked to increased concentrations of cffDNA. These include hyperemesis gravidarum (severe morning sickness), invasive placentation (in which the placenta contacts the maternal bloodstream), intrauterine growth restriction, fetomaternal haemorrhage and polyhydramnios [73]. Hence, quantitative cffDNA analysis may assist in predicting pregnancy related complications. It should be noted, however, that the absolute level of circulating cffDNA fluctuates over short periods throughout pregnancy and varies with both ethnicity and maternal weight, raising important questions about the diagnostic utility of adding cffDNA concentration to the current panel of biomarkers [74–76].

Non-invasive prenatal diagnosis of fetal chromosomal aneuploidies

Since fetal and maternal alleles are by nature indistinguishable, detection of extra fetal chromosomes in maternal plasma poses a substantial challenge. Initially published work in the field revealed increased levels of circulating DNA in pregnancies known to carry DS or trisomy 13 fetuses as compared to chromosomally normal ones [77, 78]. It was therefore suggested that this increase of cffDNA levels in maternal plasma could be used as a marker for NIPD of fetal aneuploidies. Discordant results, however, among different research groups were reported given that the levels of cffDNA vary widely and, as mentioned previously, are also elevated in a number of pregnancy related complications [75]. More successful and specific diagnostic strategies are required, therefore, in order to identify non-invasively fetal aneuploidies.

An alternative more specific diagnostic strategy for NIPD of fetal aneuploidies focuses on the analysis of classes of nucleic acids in maternal plasma that are fetal-specific, because maternal blood cells do not express the target mRNA or have a different methylation profile for an epigenetic fetal marker. These fetal nucleic acids include as already mentioned, *SERPINB5* and placenta-specific 4 (*PLAC4*) mRNA [79].

SERPINB5, one of the proposed universal fetal markers, has been studied in order to diagnose trisomy 18 as it is located on the specific chromosome [79]. Bisulphite modification was followed by methylation-specific PCR and primer extension to assess the allelic ratios. This generated a sensitivity of 100% and a false-positive rate of 9.7%. Multiple differentially methylated genes have also been found on chromosome 21 [20]. This approach however requires several manipulations, thus making it less feasible for clinical practice.

Selective targeting by differentially methylated markers in placenta and maternal blood cells has recently been combined with microfluidics digital PCR for non-invasive detection of fetal trisomy 21 [80]. Chromosome dosage analysis was performed by comparing the dosage of an epigenetic chromosome 21 marker (*HLCS*, a hypermethylated fetal-DNA marker) with that of reference chromosomes, *RASSF1A* on chromosome 3 and *ZFY* on the Y chromosome. The ratio of *HLCS* to *RASSF1A* showed great overlap between euploid and trisomy 21 samples. The comparison between *HLCS* and *ZFY* can discriminate aneuploid fetuses, but its use is limited to women carrying male fetuses.

Lo et al. reported quantification of *PLAC4* mRNA deriving from chromosome 21, for the NIPD of fetal DS [81]. Euploid cases have equal ratios of each allele (1 : 1 ratio), whereas if an aneuploidy is present, the ratio is 2 : 1 (Fig. 2). This approach appears to be quite promising, as the authors were able to detect fetal DS with a sensitivity of 90% and a specificity of 96%. The technique, however, is not applicable to all pregnancies, as it requires that the fetus has inherited two different SNP alleles in the region analyzed.

Recently developed single molecule counting techniques can be used for fetal aneuploidy detection without the restrictions of fetal-specific nucleic acids in maternal plasma:

Determination of chromosome dosage by digital PCR, a highly sensitive technique that uses limiting dilution to isolate single template DNA molecules to be amplified [81, 82]. Digital PCR was tested in a model system for molecular detection of fetal trisomy 21. A nonpolymorphic chromosome 21 locus was compared to one located on a reference chromosome. A change in the ratio of both chromosomes from 2:2 in an euploid fetus to 3:2 in a

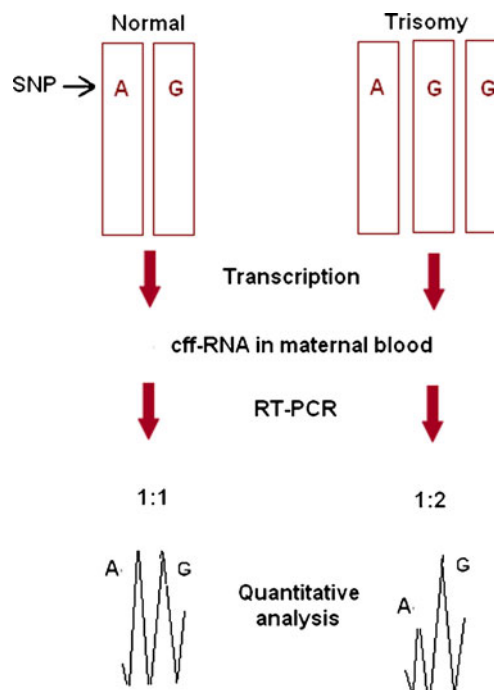


Fig. 2 Calculation of the fetal chromosome dosage using the allelic ratio of a specific heterozygous SNP on cfRNA

trisomic fetus was reported. It is noteworthy that the technique does not specifically distinguish fetal-derived from maternal DNA, thus the degree of increment depends on the concentration of cfDNA. The analytical platform would need to be quantitatively more precise to reliably determine the small expected increment.

Use of massively parallel, or next-generation, sequencing (MPS). MPS can analyse the nucleotide sequences of millions of DNA molecules in each run. The capacity of MPS to differentiate small quantitative alterations in genomic distributions of chromosomes, has allowed detection of higher amounts of chromosome 21 sequences in trisomy 21 pregnancies as compared to euploid pregnancies. Fan et al. tested this technique on cfDNA from plasma of pregnant women with a gestational age of 10–35 weeks [33]. Chiu et al. applied the same technique, but followed a different strategy for data analysis [83]. They used this strategy in order to detect trisomy 21 and the Y and X chromosomes difference between male and female fetuses. They tested an algorithm to calculate the percentage unique sequences for the chromosome of interest in the test sample and compared it with the reference population of that same chromosome. They were able to discriminate trisomy 21 from disomy 21 samples. Both studies demonstrated the feasibility of deep sequencing for NIPD. Measurements of the genomic representations for chromosomes 13 and 18 were less precise [84]. The important advantage of the MPS technique is that it is gender and polymorphism-independent, applicable in all pregnancies and likely to

allow analysis of all frequent forms of aneuploidies in the same test. Currently the technique is technically demanding, the cost per tested sample is high and the throughput per instrument is low (16 samples per week). This prevents its use as a regular test for all pregnant women. Thus, although MPS is certainly one of the most promising approaches, population-based studies, involving prospective studies in low-risk populations are needed to indicate if the technique is robust and can be used for clinical diagnosis.

Conclusions

New advances have been reported regarding NIPD using cfDNA from maternal plasma. Currently fetal sex assessment for X-linked disorders, and RhD incompatibility have been implemented in many diagnostic laboratories as a routine technique. Approaches for the NIPD of monogenic disorders, including both autosomal dominant and recessive, have also been developed. NIPD of fetal chromosomal aneuploidies, the main referral reason for prenatal diagnosis, has also been achieved with the use of next-generation sequencing, but issues related to the cost and throughput of this methodology should be solved before cfDNA can replace fetal genetic material obtained using invasive techniques.

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