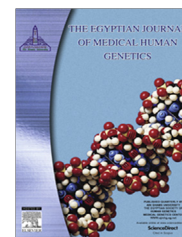
The Egyptian Journal of Medical Human Genetics (2017) 18, 1–7

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



REVIEW

Non-invasive prenatal screening for chromosomal abnormalities using circulating cell-free fetal DNA in maternal plasma: Current applications, limitations and prospects

Cláudia Amorim Costa *

Faculty of Medicine, University of Porto, Porto, Portugal

Received 16 June 2016; accepted 25 July 2016
Available online 27 August 2016

KEYWORDS

Prenatal screening;
Genetic counseling;
Medical genetics;
Genetic testing;
Chromosome abnormality disorders;
Preventive health services

Abstract *Background:* Prenatal screening for chromosomal aneuploidies was initiated in the 1970s, based in maternal age. With the introduction of serum and ultrasound biomarkers, new screening methodologies, with higher detection rates and lower false-positive rates, were implemented. More recently, cell-free fetal DNA testing was presented as a non-invasive test that uses maternal plasma to obtain fetal DNA in order to search for fetal aneuploidies or other chromosomal imbalances.

Methodology: Searches of PubMed were performed, being restricted to English-language publications and to humans. The search period was from January 2010 to July 2016. A total of 3416 citations were examined by title and abstract, 159 were analyzed integrally and a backward search of relevant studies led to the analyses of an additional 67 articles.

Results: When compared to other prenatal screening methods of common aneuploidies, cell-free fetal DNA testing has the best performance. However, its high cost and failure rate prevent at present time its implementation as a universal prenatal aneuploidy screening. Recent inclusion of microdeletions and microduplications in the panel of chromosomal anomalies to be screened by cell-free fetal DNA testing is a matter of concern, because of the low positive predictive value for these changes, and the associated significant cumulative false-positive rate.

Discussion: Cell-free fetal DNA testing represents the best screening method for common aneuploidies, and should its cost decrease, its use may be more widespread. But presently, contingent screening strategies may represent a cost-effective alternative. This review provides a current overview of this relevant theme.

© 2016 Ain Shams University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Abbreviations: aCGH, array comparative genomic hybridization; cffDNA, cell-free fetal DNA; CMA, chromosomal microarray analysis; FISH, fluorescence in situ hybridization; hCG, human chorionic gonadotropin; NIPS, non-invasive prenatal screening; NIPT, non-invasive prenatal testing; PAPP-A, pregnancy-associated plasma protein A; PPV, positive predictive value; QF-PCR, quantitative fluorescent polymerase chain reaction

* Corresponding author at: Rua Doutor António Macedo 309, 3° esquerdo. 4420-430 Valbom GDM, Porto, Portugal.
E-mail addresses: cisascosta@gmail.com, mimed09079@med.up.pt.

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2016.07.004
1110-8630 © 2016 Ain Shams University. Production and hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Contents

1. Introduction . . . 2
2. Methods . . . 2
3. Prenatal diagnosis of chromosomal anomalies . . . 2
4. Prenatal screening evolution . . . 3
5. NIPT vs standard screening . . . 4
6. Limitations of cell-free fetal DNA testing . . . 5
7. Conclusions . . . 5
Conflict of interest . . . 6
References . . . 6

1. Introduction

The presence of fetal cells in maternal plasma was first identified in the 1950s but its isolation had limited success [1]. However, the discovery of cell-free fetal DNA in maternal plasma in 1997 completely altered non-invasive prenatal screening applications [1]. The cell-free DNA present in the plasma normally has approximately 150–180 base pairs in length and its majority originated from apoptotic cells. [2] Particularly, cell-free fetal DNA (cffDNA) has its origin in the placental cytotrophoblastic cells, which are released into maternal bloodstream during pregnancy [2] and usually accounts for approximately 10–20% of the average of cell-free DNA in the maternal plasma in the second trimester of gestation [3]. Despite several reports describing a 1% increase in cffDNA fraction per gestational week, some authors observed stabilization or even decrease in cffDNA fraction along the pregnancy [4]. Some variables are known to affect cffDNA concentration in maternal plasma, for example maternal weight, number of previous gestations and gestational age [3]. However, it is still impossible to predict which patients will present higher or lower levels of cffDNA, which suggests that other factors control the amounts of fetal and maternal DNA circulating in the plasma of each pregnant woman [4]. There are well documented cases of false non-invasive prenatal screening (NIPS) results, which may derive mostly from fetoplacental mosaicism, maternal chromosomal abnormalities, low DNA fetal fraction, vanishing twin and/or errors associated with the procedures [3]. Currently, non-invasive prenatal screening is usually performed at or after 10 weeks of gestational age until the end of the first trimester, but can be done later in the pregnancy [3].

Non-invasive prenatal screening is usually based on massive parallel sequencing or on single nucleotide polymorphism pattern analysis of cell-free fetal DNA in maternal plasma [2,5–10]. The quantity of cffDNA present in the maternal plasma determines the test accuracy, the lowest accepted being approximately 4% [4]. Non-invasive prenatal screening applications are multiple and their value was first demonstrated in the determination of fetal sex, Rhesus D status and monogenic disorders [1].

In the last five years, it was found that detection of fetal aneuploidies was also possible through the study of circulating fetal cell-free DNA in the maternal plasma, with a very high sensitivity and specificity for the detection of trisomy 21, and slightly lower performance for trisomy 18, trisomy 13 and sex chromosome aneuploidies (SCAs: 45, X; 47, XXX; 47, XXY; 47, XYY) [2].

More recently, companies started promoting non-invasive prenatal screening also for microdeletions [2] and microduplications [11].

Since the demonstration of the feasibility of non-invasive analysis of fetal DNA to screen for chromosomal anomalies, non-invasive prenatal screening has gained a growing role in prenatal testing and it is essential to review its applications, major limitations and likely developments in the future.

2. Methods

Searches of PubMed were performed using the following search terms: "non invasive DNA prenatal screening", "non invasive prenatal test accuracy", "cell-free DNA analysis trisomy", "NIPS for fetal abnormalities", "noninvasive prenatal diagnosis and standard screening", "Prenatal screening review" and "massive parallel sequencing". These were restricted to English-language publications and to humans. The search period was from January 2010 to February 2016. Then, a total of 3416 citations were examined by title and abstract in order to identify all relevant articles. A sum of 159 were analyzed integrally, including a backward search of relevant studies, which led to the analyses of an additional 67 articles.

3. Prenatal diagnosis of chromosomal anomalies

Since, in 1966, it was demonstrated that fetal cells obtained through amniocentesis could be cultured in vitro to obtain a fetal karyotype, the era of prenatal diagnosis started.

A few years later, other prenatal invasive procedures, such as chorionic villus sampling and cordocentesis, became available and were used initially for the study of fetal chromosomes, originally for the detection of aneuploidies and, after banding techniques were discovered, also for the diagnosis of balanced and unbalanced structural abnormalities [12,13].

Prenatal diagnosis of chromosomal anomalies remained based on fetal karyotyping for several decades, which in turn required that at-risk women would be subjected to an invasive procedure, either chorionic villous biopsy (usually performed between the 10th and the 13th gestational week), amniocentesis (usually carried out at 16 plus weeks) or, rarely, cordocentesis (later in pregnancy), each of these procedures having a risk of fetal loss that ranges from 0.5% to 1% for amniocente-

sis and chorionic villous biopsy, and 1–2% for cordocentesis [14].

In view of the above-referred procedure-related risks of fetal loss, prenatal diagnosis was reserved for pregnant women at an increased risk of carrying a chromosomally abnormal fetus, initially assessed by maternal age and previous obstetric history, and later by a number of screening methods described in the next section.

After invasive procedures, multiple analysis can be performed in order to establish the presence of a chromosomal abnormality, with the karyotype being considered the gold-standard until very recently.

Indeed, karyotyping is a reliable technique in the identification of most chromosomal anomalies, namely polyploidy, aneuploidy, mosaicism, and structural rearrangements, including balanced translocations and inversions.

However, karyotyping (also referred to as conventional cytogenetics) has some important limitations, namely the fact that it is labor-intensive, requires cell culture (which may fail) and has a low resolution. If the need for cell culture implies a delay of 10–14 days before a diagnosis can be reached (which is understandably a very stressful time for parents), its low resolution can lead to false negative results [15–17]. In fact, karyotyping can only detect unbalanced anomalies of at least 5–20 Mb in size [11].

These limitations led to the use of other techniques, in the early 1990's, like fluorescence in situ hybridization (FISH) analysis, which can be applied in uncultured cells (thereby allowing a rapid diagnosis of aneuploidies), and can be used to detect submicroscopic rearrangements (microdeletions and duplications), thus overcoming partially the limited resolution of conventional cytogenetics [11].

However, FISH provides an analysis limited to targeted genomic regions of interest and, hence, cannot be used to screen the whole genome for imbalances.

Likewise, quantitative fluorescent polymerase chain reaction (QF-PCR) can be used as a rapid diagnostic tool for detection of most common aneuploidies (trisomy 21, 18 and 13 and SCA) with high sensitivity and specificity [15,18–26].

Indeed, rapid QF-PCR is able to detect numerical chromosome abnormalities in a few hours (detecting 95% of chromosome abnormalities with potential risk for the fetus within 24 h from sampling), which may even help in reducing the need for conventional cytogenetic analysis in prenatal diagnosis [15,18,19,21,23,27–37].

Moreover, the possibility of automation of QF-PCR allows that a high number of samples can be processed at a low cost, which is not possible with FISH [15,24,27,33].

However, as was the case with FISH, QF-PCR also represents a targeted approach, that is, it does not provide a genome-wide screening.

Recently, another technique was developed that overcomes the shortcomings of both conventional cytogenetics and FISH or QF-PCR, allowing a high resolution genome-wide screening of genomic imbalances – the array comparative genomic hybridization (aCGH), also known as chromosomal microarray analysis (CMA).

The efficacy of aCGH in the postnatal setting, where it shows a considerably higher diagnostic yield (15–20%) than G-banded karyotype (~3%) in the study of patients with mental retardation and/or autism-related disorders, encouraged the gradual introduction of this technique in prenatal diagnosis [38–40].

In fact, CMA provides a sort of "molecular karyotyping", that not only identifies chromosomal aneuploidies, but also detects gains and losses of DNA that are too small to be detected by conventional karyotyping (unbalanced genomic rearrangements as small as 10–100 kb in size can be detected by CMA) [11], without being focused exclusively in targeted areas as is the case with FISH.

Despite the significant advances in prenatal diagnosis, enabling smaller and smaller changes to be detected, the truth is that all methods described in this section rely on the analysis of fetal material, which is traditionally obtained through invasive procedures, with the inevitable associated risks. In other words, the higher diagnostic yield of the more recent techniques has diminished the number of false negative diagnoses, but not the need for invasive procedures.

The bottom line is, therefore, that a special emphasis must be placed in an accurate identification of risk, in order to limit the number of unwarranted invasive procedures.

4. Prenatal screening evolution

Prenatal screening for chromosomal anomalies (mostly, trisomy 21 and other common aneuploidies like trisomy 13 and 18) started in the 1970s and was based initially on maternal age alone [41,42]. Accordingly, pregnant women of 35 years or older were considered at high risk for such aneuploidies and, therefore, with indications of amniocentesis [41].

However, since at that time most pregnancies occurred before the age of 35, more than 75% of the affected pregnancies remained undetected, since no diagnostic test was offered to "low risk" ages [43].

In the 80's, it was found that the levels of maternal serum α-fetoprotein in pregnancies of trisomy 21 fetuses were lower on average than in the case of euploid fetuses [44]. This led to the development of a screening process that combined woman's maternal serum α-fetoprotein level and her age, making it possible to identify an additional 20% of all pregnancies affected by trisomy 21 in women under age 35 [41]. A few years later, the measurement of human chorionic gonadotropin (whose levels were found to be higher in trisomy 21 pregnancies) was added to serum α-fetoprotein and maternal age, which was dubbed the double test, carried out between the 14th and 22nd weeks of gestation, thereby ensuring a higher detection rate for trisomy 21 [44].

An improvement in the assessment of risk was later introduced, by adding unconjugated estriol levels (lower in trisomy 21 pregnancies) to the previously referred second trimester markers, giving rise to the so called the triple test, which allowed the detection of about 77% of affected pregnancies for a false-positive rate of 5% [43].

The quadruple test was proposed in 1996 and included, in addition to the markers used in the triple test, the measurement of inhibin A levels (higher in trisomy 21 pregnancies), thereby increasing the detection rate of trisomy 21 to over 80% for the same false-positive rate [42].

In the late 1990's, biochemical first trimester screening of trisomy 21 was found to be possible through the measurement of the serum concentrations of the free beta subunit of human chorionic gonadotropin and of pregnancy-associated plasma

protein A (PAPP-A), between the 8th and 13th week of pregnancy (usually carried out between 10 and 13), with a performance that is similar to that achieved with the second trimester double test [44].

The incorporation of the measurement of nuchal translucency associated with the two first-trimester serum markers (free beta subunit of human chorionic gonadotropin and PAPP-A), which was termed the combined test, made possible an early screening for trisomy 21, with a detection rate of approximately 85% for a false-positive rate of 5% [44].

In 1999, integrated screening was proposed, involving the calculation of the risk of trisomy 21 based on the use of multiple markers in both the first (between 10 and 13 weeks of gestation) and second trimesters of pregnancy [42,44]. This test achieved higher detection rates (around 94% for a 5% false-positive rate) compared to screenings carried out in either trimester alone [44]. The integrated test involved, in its most complete form, the use of six markers: ultrasound measurement of nuchal translucency and maternal serum PAPP-A in the first trimester and serum α-fetoprotein, unconjugated estriol, human chorionic gonadotropin (hCG) and inhibin A in the second trimester, with the risk of trisomy 21 being calculated when levels of all markers are determined in the second trimester [45].

It is now part of current obstetrical care to offer pregnant women prenatal screening for trisomy 21, with any of the tests mentioned above, the combined test being increasingly popular, in view of its high detection rate and early result.

Indeed, one of the most important aims of prenatal screening of chromosomal aneuploidies is to achieve a reliable detection of these abnormalities (translated in a high detection rate and low false-positive rate), preferably at the earliest possible time in the pregnancy, in order to reassure patients at low risk and allow patients with a screen positive result to undergo an invasive test to confirm or exclude a chromosomal change [46].

Traditional screening tests, despite having high detection rates, lead to unnecessary invasive procedures in approximately 5% of patients (false-positives), which entail a small (but not negligible) risk of fetal loss (estimated as 0.5–1%).

Table 1 Detection rates at a 5% false-positive rate of standard prenatal screening [44].

	Detection rate (%)
Integrated test	94
Combined test	85
Quadruple test	76
Triple test	69
Double test	59

In Table 1 a summary of the performance of traditional prenatal screening tests is presented, with the comparison of their detection rates of trisomy 21 for a fixed false-positive rate of 5% [44].

5. NIPT vs standard screening

In order to analyze cffDNA testing performance, the weighted pooled detection rates and false-positive rates, as determined by a meta-analysis of published papers up to January 2015, are presented in Table 2 [47].

More recently, a study compared the performance of cffDNA testing and standard screening in the first trimester of pregnancy (combined test) in the general population [46]. Regarding the detection rate, cffDNA testing identified correctly all 38 pregnancies with trisomy 21, which means a detection rate of 100% (CI: 90.7–100%) [46]. Standard screening had a detection rate of 78.9% (CI: 62.7–90.4%; $P = 0.008$), which means it identified 30 of 38 cases of trisomy 21 as positive [46]. For cffDNA testing, there were 9 false positives among 15,803 women (false-positive rate: 0.06% [CI: 0.03–0.11%]) [46]. With standard screening a false-positive rate of 5.4% (CI: 5.1–5.8%; $P < 0.001$) was observed [46]. cffDNA testing and standard screening had a positive predictive value (PPV) of 80.9% (CI: 66.7–90.9%) and 3.4% (CI: 2.3–4.8%), respectively ($P < 0.001$) [46]. cffDNA correctly identified all 19 pregnancies with trisomy 21, 6 of which among 11,994 women with maternal age under 35 years [46]. For this group, PPV was 76.0% [CI: 54.9–90.6%) [46]. Standard screening showed a mid-trimester risk of trisomy 21 less than 1 in 270 in 14,957 women and in this group cffDNA testing identified 8 of 8 women with trisomy 21, with 8 false positive results [46]. In this last group, PPV was 50.0% (CI: 24.7–75.3%) [46].

Additionally, 9 of 10 pregnancies with trisomy 18 were correctly identified by cffDNA whereas standard screening identified only 8 of 10 [46]. Regarding trisomy 18, cffDNA testing had 1 false positive result, for a false-positive rate of 0.01 (CI: 0–0.04%) and a PPV of 90.0% (CI: 55.5–99.7%), whereas the combined test had 49 false positive results, corresponding to a false-positive rate of 0.31% (CI: 0.23–0.41%) and a PPV of 14% (CI: 6.3–25.8%) ($P < 0.001$ for both comparisons) [46].

A total of 11,185 women underwent both cffDNA testing and standard screening for trisomy 13 and there were 2 confirmed cases [46]. Both cases were identified by cffDNA testing whereas standard screening only identified 1 [46]. With cffDNA testing there was 1 false positive result, while standard screening had 28 false positive results, which corresponds to a

Table 2 Pooled weighted detection rates and false-positive rates of cffDNA testing [47].

	Detection rate [95% Confidence Interval (CI)]	False-positive rate [95% CI]
Trisomy 21		
Singleton pregnancies	99.2% [98.5–99.6%]	0.09% [0.05–0.14%]
Twin pregnancies	93.7% [83.6–99.2%]	0.23% [0.00–0.92%]
Trisomy 18	96.3% [94.3–97.9%]	0.13% [0.07–0.2%]
Trisomy 13	91.0% [85.0–95.6%]	0.13% [0.05–0.26%]
Monosomy X	90.3% [85.7–94.2%]	0.23% [0.14–0.34%]
SCA (other than monosomy X)	93.0% [85.8–97.8%]	0.14% [0.06–0.24%]

false-positive rate of 0.02% (CI: 0–0.06%) and 0.25% (CI: 0.17–0.36%), respectively (P < 0.001) [46].

This last report shows a higher sensitivity and specificity of cffDNA testing than standard screening in the detection of trisomy 21 in general pregnant population and a false-positive rate nearly 100 times lower than standard screening, which is also supported by the other reports mentioned [46]. Regardless of maternal age, cffDNA testing was found to be more sensitive and with lower false-positive rates than the combined test [46].

It should be noted that a limitation of this study is that it only compared cffDNA testing with the combined test, when other conventional screening methods (like the integrated screening) have higher sensitivity and specificity than first trimester screening [46]. However, in view of the known performance of the integrated screening (see Table 1, above), there is no doubt that cffDNA testing would be far superior.

6. Limitations of cell-free fetal DNA testing

There is presently a debate regarding the place of cffDNA testing in current prenatal care, either as a first line universal screening tool to all pregnant patients, or as a second tier test whose application would be reserved for high risk first-trimester screening results (this being the first-line screening test) [36,41]. The major present limitations described for cffDNA testing being implemented as a method of universal screening are its failure rate (percentage of inconclusive or no results) and its high cost [42,47].

In some cases, cffDNA fails to provide a result mainly because of sample quality associated with low fetal DNA fraction or when the result is too close to a determined cut-off [47]. In the large multicentric study conducted by Norton et al., no results on cffDNA testing were obtained in approximately 3% of patients, either due to a fetal fraction below 4%, fetal fraction that could not be measured, or high assay variance or an assay failure [46].

Regarding cffDNA fraction, it should be noted that it is inversely associated with maternal weight, as observed by Norton et al., who observed a median maternal weight of 93.7 kg in women with a low fetal fraction and 65.8 kg in women with a successful result on cffDNA testing (P < 0.001) [46].

Prevalence of aneuploidy in the group with no results on cffDNA testing (1 in 38, 2.7%) was higher than the prevalence in the overall cohort (1 in 236, 0.4%) (P < 0.001) [46]. In the study of Norton et al., in the 192 women with less than 4% fetal fraction, 9 (4.7%) had an aneuploid pregnancy [46]. Standard screening did yield a high risk result (screen positive) in 6 of these 9, with risks ranging between 1 in 26 and 1 in 2 [46].

In order to overcome these two major limitations of cffDNA testing (high cost and failure rate in a small, albeit not negligible, percentage of patients), different strategies can be considered as an alternative to universal cffDNA testing: the use of cffDNA as a second-line test limited to patients who are considered screen positive according to a predefined risk cut-off, as assessed by first trimester screening, thereby representing an alternative to invasive procedures; contingent testing, using a two-stage approach, in which all pregnant women are offered conventional first-trimester screening, but with two cut-off risks, one high risk and the other low risk, with invasive procedures and non-invasive prenatal testing (NIPT) being offered to the high risk and intermediate risk groups, respectively [42].

Recently, Wald and Bestwick proposed a reflex DNA testing protocol, in which all patients would be offered a first trimester screening test with a low-risk cut-off (say, 1 in 800), and all cases with a risk at or above the adopted cut-off would be offered NIPT [48]. This strategy would ensure a high detection rate at an extremely low false-positive rate [48].

Some authors fear that if NIPT is implemented as a primary universal screening, it may be considered that ultrasound is no longer needed as part of the first-trimester screening [42,47]. However, it cannot be overemphasized that ultrasound is the most accurate method of determining the gestational age in the first-trimester with crown-rump length measurement, assessing chorionicity in multiple pregnancies, detecting major fetal malformations, screening at a very early stage for major cardiac abnormalities (particularly if Doppler examination is included), as well as evaluating several parameters (namely, mean arterial pressure and uterine artery Doppler pulsatility index), which all represent important prognostic indicators of the ongoing pregnancy [42].

Recently, some commercial companies have begun to offer testing for known clinically important microdeletion syndromes (namely, 1p36 deletion; cri-du-chat – 5p14-15 deletion; Prader Willi/Angelman syndromes – 15q11.2 deletion; and DiGeorge syndrome – 22q11.2 deletion) [49]. It is only likely that the spectrum of subchromosomal rearrangements that can be detected by this technology will expand dramatically in the near future possibly with whole genome coverage, much like aCGH [50].

However, it should be noted that even if as high sensitivities and specificities are achieved for these microdeletions by NIPT as it happens for trisomy 21, the very low frequency of some of the microdeletions for which NIPT is currently available, makes PPV for these disorders very low, with most of the subsequent diagnostic tests not confirming the suspected alteration.

Moreover, by adding different conditions (like SCA and microdeletions) for NIPT in addition to common aneuploidies (trisomies 21, 13 and 18), each with an inherent small false-positive rate, will result in a significant cumulative false-positive value.

7. Conclusions

Screening for fetal aneuploidies by NIPT is more reliable than what is achieved by standard screening [42]. It presents higher detection rates, higher specificity, and higher positive predictive values [46,47]. Despite its superior performance, non-invasive prenatal screening is currently more expensive than standard screening which still prevents its universal implementation for aneuploidy screening [46,47].

Until the costs of NIPT drop significantly and make universal prenatal screening with this method cost-effective, contingent or reflex DNA strategies can provide a good and affordable compromise between high detection rate for common aneuploidies and extremely low false-positive rate, thereby reducing dramatically unnecessary invasive procedures.

It should be noted, however, that NIPT still cannot completely replace invasive testing, because no definite diagnosis

of chromosomal anomaly can be made by cffDNA testing alone, all abnormal results needing to be confirmed by chromosomal analysis (conventional karyotyping or other methods) after chorionic villus biopsy or amniocentesis [46].

Nowadays, cffDNA testing is presented as a safe test with reliable results in prenatal diagnosis which attracts companies' investment, increasing market competition [42]. Media attention and, consequently advertisement, may influence couples' decision about being submitted to non-invasive prenatal screening [42,51]. It is a matter of concern that most informative leaflets about cffDNA testing provided by non-invasive prenatal screening distributors do not include all the crucial information recommended by professionals [51].

In the clinical practice, the risks, limitations and benefits associated with every test should be discussed with the patient, informing what alternatives they have in terms of prenatal screening [51].

Conflict of interest

There is no conflict of interest.

References

[1] Porreco RP et al. Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA. Am J Obstet Gynecol 2014;211(4):365.e1–365.e12.

[2] Neufeld-Kaiser WA, Cheng EY, Liu YJ. Positive predictive value of non-invasive prenatal screening for fetal chromosome disorders using cell-free DNA in maternal serum: independent clinical experience of a tertiary referral center. BMC Med 2015;13:129.

[3] Song Y et al. Non-invasive prenatal testing for fetal aneuploidies in the first trimester of pregnancy. Ultrasound Obstet Gynecol 2015;45(1):55–60.

[4] Shi X et al. Feasibility of noninvasive prenatal testing for common fetal aneuploidies in an early gestational window. Clin Chim Acta 2015;439:24–8.

[5] Chan KC. Clinical applications of the latest molecular diagnostics in noninvasive prenatal diagnosis. Top Curr Chem 2014;336:47–65.

[6] Langlois S et al. Current status in non-invasive prenatal detection of Down syndrome, trisomy 18, and trisomy 13 using cell-free DNA in maternal plasma. J Obstet Gynaecol Can 2013;35(2):177–83.

[7] Bayindir B et al. Noninvasive prenatal testing using a novel analysis pipeline to screen for all autosomal fetal aneuploidies improves pregnancy management. Eur J Hum Genet 2015;23(10):1286–93.

[8] Benachi A et al. Cell-free DNA analysis in maternal plasma in cases of fetal abnormalities detected on ultrasound examination. Obstet Gynecol 2015;125(6):1330–7.

[9] Shaw SW et al. Noninvasive prenatal testing for whole fetal chromosomal aneuploidies: a multicenter prospective cohort trial in Taiwan. Fetal Diagn Ther 2014;35(1):13–7.

[10] Sun K, Jiang P, Chan KC. The impact of digital DNA counting technologies on noninvasive prenatal testing. Expert Rev Mol Diagn 2015;15(10):1261–8.

[11] Peters DG et al. Recent advances of genomic testing in perinatal medicine. Semin Perinatol 2015;39(1):44–54.

[12] Lichtenbelt KD, Knoers NV, Schuring-Blom GH. From karyotyping to array-CGH in prenatal diagnosis. Cytogenet Genome Res 2011;135(3–4):241–50.

[13] Simpson JL. Invasive procedures for prenatal diagnosis: any future left? Best Pract Res Clin Obstet Gynaecol 2012;26(5):625–38.

[14] Bustamante-Aragones A et al. Non-invasive prenatal diagnosis of single-gene disorders from maternal blood. Gene 2012;504(1):144–9.

[15] Cirigliano V et al. Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR, results of 9 years of clinical experience. Prenat Diagn 2009;29(1):40–9.

[16] Sjogren B, Uddenberg N. Prenatal diagnosis for psychological reasons: comparison with other indications, advanced maternal age and known genetic risk. Prenat Diagn 1990;10(2):111–20.

[17] Marteau TM et al. The psychological effects of false-positive results in prenatal screening for fetal abnormality: a prospective study. Prenat Diagn 1992;12(3):205–14.

[18] Pertl B et al. Rapid detection of chromosome aneuploidies by quantitative fluorescence PCR: first application on 247 chorionic villus samples. J Med Genet 1999;36(4):300–3.

[19] Pertl B et al. Rapid prenatal diagnosis of aneuploidy by quantitative fluorescent PCR on fetal samples from mothers at high risk for chromosome disorders. Mol Hum Reprod 1999;5(12):1176–9.

[20] Schmidt W et al. Detection of aneuploidy in chromosomes X, Y, 13, 18 and 21 by QF-PCR in 662 selected pregnancies at risk. Mol Hum Reprod 2000;6(9):855–60.

[21] Adinolfi M, Sherlock J. Prenatal detection of chromosome disorders by QF-PCR. Lancet 2001;358(9287):1030–1.

[22] Cirigliano V et al. Clinical application of multiplex quantitative fluorescent polymerase chain reaction (QF-PCR) for the rapid prenatal detection of common chromosome aneuploidies. Mol Hum Reprod 2001;7(10):1001–6.

[23] Cirigliano V et al. Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR. Assessment on 18,000 consecutive clinical samples. Mol Hum Reprod 2004;10(11):839–46.

[24] Cirigliano V et al. Rapid prenatal diagnosis by QF-PCR: evaluation of 30,000 consecutive clinical samples and future applications. Ann N Y Acad Sci 2006;1075:288–98.

[25] Mann K et al. Development and implementation of a new rapid aneuploidy diagnostic service within the UK National Health Service and implications for the future of prenatal diagnosis. Lancet 2001;358(9287):1057–61.

[26] Mann K et al. Strategies for the rapid prenatal diagnosis of chromosome aneuploidy. Eur J Hum Genet 2004;12(11):907–15.

[27] Cirigliano V, Voglino G, Adinolfi M. Non-invasive screening and rapid QF-PCR assay can greatly reduce the need for conventional cytogenetic analyses in prenatal diagnosis. Reprod Biomed Online 2005;11(6):671–3.

[28] Mansfield ES. Diagnosis of Down syndrome and other aneuploidies using quantitative polymerase chain reaction and small tandem repeat polymorphisms. Hum Mol Genet 1993;2(1):43–50.

[29] Pertl B et al. Rapid molecular method for prenatal detection of Down's syndrome. Lancet 1994;343(8907):1197–8.

[30] Pertl B et al. Rapid detection of trisomies 21 and 18 and sexing by quantitative fluorescent multiplex PCR. Hum Genet 1996;98(1):55–9.

[31] Pertl B et al. Quantitative fluorescence polymerase chain reaction for the rapid prenatal detection of common aneuploidies and fetal sex. Am J Obstet Gynecol 1997;177(4):899–906.

[32] Adinolfi M, Sherlock J, Pertl B. Rapid detection of selected aneuploidies by quantitative fluorescent PCR. BioEssays 1995;17(7):661–4.

[33] Adinolfi M, Pertl B, Sherlock J. Rapid detection of aneuploidies by microsatellite and the quantitative fluorescent polymerase chain reaction. Prenat Diagn 1997;17(13):1299–311.

[34] Sherlock J et al. Assessment of diagnostic quantitative fluorescent multiplex polymerase chain reaction assays performed on single cells. Ann Hum Genet 1998;62(Pt 1):9–23.

[35] Verma L et al. Rapid and simple prenatal DNA diagnosis of Down's syndrome. Lancet 1998;352(9121):9–12.

[36] Cirigliano V et al. Rapid detection of chromosomes X and Y aneuploidies by quantitative fluorescent PCR. Prenat Diagn 1999;19(12):1099–103.

[37] Cirigliano V et al. Assessment of new markers for the rapid detection of aneuploidies by quantitative fluorescent PCR (QF-PCR). Ann Hum Genet 2001;65(Pt 5):421–7.

[38] Miller DT et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010;86(5):749–64.

[39] Shinawi M, Cheung SW. The array CGH and its clinical applications. Drug Discov Today 2008;13(17–18):760–70.

[40] American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No: 581: the use of chromosomal microarray analysis in prenatal diagnosis. Obstet Gynecol 2013;122(6):1374–7.

[41] Palomaki GE, Haddow JE. Maternal serum alpha-fetoprotein, age, and Down syndrome risk. Am J Obstet Gynecol 1987;156(2):460–3.

[42] Cuckle H, Maymon R. Development of prenatal screening – a historical overview. Semin Perinatol 2016;40(1):12–22.

[43] Wald NJ et al. Maternal serum screening for Down's syndrome in early pregnancy. BMJ 1988;297(6653):883–7.

[44] Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. N Engl J Med 1999;341(7):461–7.

[45] Cuckle HS et al. Contingent screening for Down syndrome – results from the FaSTER trial. Prenat Diagn 2008;28(2):89–94.

[46] Norton ME et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med 2015;372(17):1589–97.

[47] Gil MM et al. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2015;45(3):249–66.

[48] Wald NJ, Bestwick JP. Incorporating DNA sequencing into current prenatal screening practice for Down's syndrome. PLoS One 2013;8(3):e58732.

[49] Cuckle H, Benn P, Pergament E. Cell-free DNA screening for fetal aneuploidy as a clinical service. Clin Biochem 2015;48(15):932–41.

[50] Yatsenko SA et al. Maternal cell-free DNA-based screening for fetal microdeletion and the importance of careful diagnostic follow-up. Genet Med 2015;17(10):836–8.

[51] Deprest J et al. In case you missed it: the Prenatal Diagnosis editors bring you the most significant advances of 2015. Prenat Diagn 2016;36(1):3–9.