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**REVIEW ARTICLE**

# Noninvasive prenatal diagnosis using Cell free fetal DNA in maternal blood: Current efforts and future perspectives

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**ABSTRACT**

Cell-free fetal DNA (cffDNA) is fetal DNA circulating freely in the maternal blood stream. cffDNA can first be observed as early as seven weeks gestation, and the amount of cffDNA increases as the pregnancy progresses. cffDNA originates from the trophoblast making up the placenta. Analysis of cffDNA provides a method of non-invasive prenatal diagnosis. Maternal blood sample is collected after 10 weeks of pregnancy but can be detected as early as the fifth week. The test measures the relative amount of free fetal DNA in the mother's blood. A variety of methods have been used for mutation detection in fetal DNA. Millions of small fragments of cffDNA from maternal plasma are amplified and sequenced. After the fragments are mapped to the human genome and analyzed for frequency/density along each chromosome, fetal Down syndrome and a variety of other chromosomal abnormalities can be detected with a high degree of accuracy for families at high risk for inherited genetic disorders. In industrialized countries, cffDNA tests are now routinely being offered to RhD-negative patients at increased risk of isoimmunization. cffDNA concentration can also be used as a screening tool for preeclampsia. The high degree of accuracy of cffDNA as a method of noninvasive prenatal testing during pregnancy carries a number of socio-ethical implications when it is used for early detection fetal sex for suspected X-linked diseases. It is important for women to receive detailed pretest counseling that explains the benefits and limitations of the test.

**Keywords:** Aneuploidy, cell free fetal DNA, Down syndrome, maternal blood, prenatal testing, screening.
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Prenatal testing for fetal chromosomal aneuploidies includes both screening and invasive diagnostic methods. Screening methods encompasses analysis of serum markers and/ or ultrasound markers of fetal Nuchal translucency (NT) or Nuchal fold thickness (NFT), but has suboptimal sensitivity and specificity [1, 2]. Prenatal diagnostic procedures such as chorionic villus sampling (CVS) or amniocentesis are highly accurate but they are invasive and carry significant risks to the fetus and mother, including loss of the fetus [3]. Numerous efforts

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have been made for several decades to develop a maternal blood test with improved accuracy for the detection of major fetal aneuploidies. Such blood tests could improve current screening practices for fetal aneuploidy. Initial efforts targeting isolation and analysis of circulating fetal cells in the maternal bloodstream have not proven successful, because of the challenges in detecting sufficient fetal cell numbers in circulation [4-6]. By contrast, analysis of cell-free fetal DNA (cffDNA) in maternal circulation has shown promise for evaluation of fetal aneuploidy.

cffDNA is fetal DNA circulating freely in the maternal blood stream. It can be sampled by venipuncture on the mother. Analysis of cffDNA provides a method of non-invasive prenatal diagnosis. cffDNA originates from the trophoblast making up the placenta [7, 8]. It is estimated that 2-6% of the DNA in the maternal blood is of fetal origin [9]. The fetal DNA is fragmented and makes its way into the maternal bloodstream via shedding of the placental microparticles into the maternal bloodstream [10].

cffDNA can first be observed as early as 7 weeks gestation, and the amount of cffDNA increases as the pregnancy progresses [11]. cffDNA diminishes quickly after the birth of the baby, so that it is no longer detectable in the maternal blood approximately 2 hours after birth [12]. cffDNA is significantly smaller than the maternal DNA in the bloodstream [13].

Studies have looked at, and some even optimized, protocols for testing non-compatible RhD factors, sex determination for X-linked genetic disorders and testing for single gene disorders [14]. Current studies are now looking at determining aneuploidies in the developing fetus [15, 16]. These protocols can be done earlier than the current prenatal testing methods, and have no risk of spontaneous abortion, unlike current prenatal testing methods [17]. Non-invasive prenatal testing (NIPT) has been implemented in the United Kingdom (UK) and parts of the United States (US); as technology continues to advance it is almost certain that we will see a shift from the current methods of chorionic villus sample (CVS) and amnio-centesis to NIPT [18], which have risks of miscarriage about 1 in 100 pregnancies and 1 in 200 pregnancies, respectively [19, 20].

## Sample Collection method and diagnosis

Maternal blood sample is collected after 10 weeks of pregnancy but can be detected as early as the fifth week [21]. The test measures the relative amount of free fetal DNA in the mother's blood which consists of approximately 2-6% of the total [9].

Limitations include the concentration of all cell-free DNA in maternal blood is low, the total amount of cell-free DNA varies between individuals, cell free fetal DNA molecules are out-numbered by maternal cell-free DNA molecules, the fetus inherits half the genome from the mother. However, there are ways around these limitations [22].

## Cell-free DNA purification

Procedures for isolation of plasma from maternal blood include centrifugation, followed by isolation and purification of cell-free DNA [23]. Addition of formaldehyde to maternal blood samples increases the percentage of free fetal DNA. The purpose of formaldehyde is to stabilize intact cells, and inhibit further release of maternal DNA. The mean percentage of free fetal DNA in maternal blood ranges from 0.32% to 40%, with a mean percentage of 7.7 without formaldehyde-treatment [24]. The mean percentage of free fetal DNA with formaldehyde treatment increases to 20%. However, Benachi et al. and Chinnapapagari et al. have highlighted that the range of results varies from 5% to 96% [25, 26]. Another way to increase the fetal DNA is based on physical length of DNA fragments. Fetal DNA is smaller in size, a standardized size fractionation can comprise up to 70% of total cell-free DNA [27].

## Specification of fetal DNA and mutation detection

Different methods have been used for mutation detection in fetal DNA. To detect fetal DNA, the majority of studies focus on detecting paternally inherited sequences [28, 29]. For example, primers can be designed to target the Y chromosome of male fetuses for polymerase chain reaction (PCR).

### Real-time quantitative PCR (RT-qPCR)

Fluorescent probes are used to monitor the accumulation of amplicons produced throughout the PCR process. Thus, increase in reporter fluorescent signal is proportional to the number of amplicons generated. The appropriate real-time PCR protocol is designed according to the mutation or genotype to be detected. Point mutations are analyzed with qualitative real-time PCR with the use of allele-specific probes. Insertions and deletions are analyzed by dosage measurements using quantitative real-time PCR. Real-time PCR assays for single cell analysis have been developed for a Y-chromosome marker [30], a common Tay-Sachs disease mutation [31], the most common cystic fibrosis mutation [32], and a wide range of thalassemia mutations and HbS [33].

### Nested PCR

Yatama et al. evaluated the use of nested PCR to detect the Y chromosome in fetal DNA of maternal plasma. Nested PCR showed 96% sensitivity and 88% specificity [29].

### Digital PCR

Absolute quantification can be achieved rather than relative quantification compared to RT-PCR. Thus, point mutations, copy number variations, loss of heterozygosity or aneuploidy can be detected [16, 34]. Digital PCR can differentiate between maternal blood plasma and fetal DNA in a multiplex fashion [35].

### Shotgun sequencing

High-throughput shotgun sequencing technology from plasma of pregnant women, obtaining about 5 million sequence tags per patient sample can be done. Using this method Fan et al. were able to identify aneuploid pregnancies; trisomy detected at gestational ages as early as 14th week. Shot-gun sequencing can be done with a Solexa/Illumina platform. Whole fetal genome mapping by parental haplotype analysis using sequencing of cell free fetal DNA was done in 2010. [20] Chiu et al. studied 753 pregnant women, using a 2-plex massively parallel maternal plasma DNA sequencing and trisomy was diagnosed with z-score greater than 3.The test had 100% sensitivity, 97.9% specificity, positive predictive value of 96.6%, and negative predictive value of 100% [36].

### Mass spectrometry (MALDI-TOF)

Matrix-assisted laser desorption ionization/time of flight mass spectrometry (MALDI-TOF MS) combined with base extension after PCR allows cell free fetal DNA detection with single base specificity and single DNA molecule sensitivity. DNA is first amplified by PCR, then linear amplification with base extension reaction (with a third primer) is designed to anneal to the region upstream of the mutation site. Either 1-2 bases are added to the extension primer to produce two extension products from wild-type DNA and mutant DNA. Since single base specificity is achieved, it is better than hybridization-based techniques with Taqman probes. Ding et al. found no false negatives and no false positives when looking for fetal DNA across 16 individuals he studied [37]. Akolekar et al. found MALDI-TOF mass spectrometry had 99.1% accuracy with 98.9% sensitivity and 99.2% specificity [38].

### Epigenetic modifications

Differences in gene activation between maternal DNA and fetal DNA can be exploited by epigenetic modifications. Most current research is focused on using epigenetic modifications to detect cffDNA [39]. Hypermethylated RASSF1A promoters have been reported as universal fetal marker to confirm the presence of cffDNA [40].

### Detection of mRNA derived from genes

mRNA transcripts from genes expressed in the placenta is detectable in maternal plasma [41]. The mixture of plasma is centrifuged and aqueous layer transferred, RNA is extracted. RT-PCR is set up for selected RNA expression. Specifically, hPL and beta-hCG mRNA is stable in maternal blood. This can help to confirm the presence of fetal DNA in the maternal plasma [22].

## Current uses of cffDNA

### Early detection of fetal sex

X-linked diseases occur 5 per 10,000 live births [42]. The most common sex-linked diseases includes Duchene muscular dystrophy and hemophilia. Ultrasonography is unreliable during the first trimester of pregnancy as the genitalia are not fully developed. Other methods

for sex determination before the use of cffDNA are invasive and performed at an advanced gestation. There is a small risk of miscarriage [43]. The main method is to target the SRY gene on the Y chromosome and DYS14 sequence [44].

Most X-linked diseases are evident in males because they lack the second X-chromosome that can compensate for the diseases allele. Some X-linked diseases include fragile-X syndrome, Duchene muscular dystrophy and Hemophilia. In the case of X-linked diseases, if cffDNA can determine gender, invasive testing can be eliminated [44]. In Europe, tests for sex determination are available as early as 7 weeks of gestation [45].

Lack of the Y chromosome in the maternal plasma suggests that the fetus is female, but could also indicate failure to detect cffDNA in the maternal plasma. Thus, paternal polymorphisms or sex independent markers are further used to detect cffDNA. High heterozygosity of these markers must be detected for them to be application [46].

**Genetic studies for families at high risk for inherited genetic disorders**

Severe monogenic diseases for which prenatal diagnosis is more commonly applied to include cystic fibrosis, beta-thalassemia, sickle cell anemia, spinal muscular atrophy, myotonic dystrophy, fragile-X syndrome, Duchene muscular dystrophy and Hemophilia [28]. Both autosomal dominant and recessive disorders have been detected noninvasively by analyzing paternally inherited DNA [44].

**Routine prenatal screening for Rhesus factor**

Mixing of fetal cells carrying paternal RhD antigens into maternal circulation may result in the sensitization of an RhD-negative mother. Rhesus blood group (D antigen) is used to determine the risk of hemolytic disease in the fetus. In hemolytic disease, the maternal antibodies destroy RhD-positive fetal red blood cells. This leads to lethality for the fetus. A significant amount of blood can be exchanged between mother and infant during birth, CVS, amniocentesis and accidents [22]. In UK and other industrialized countries, cffDNA tests are now routinely being offered to RhD-negative patients at increased risk of isoimmunization.

In US, prophylactic treatment is recommended for all RhD-negative pregnant women to prevent isoimmunization in case of RhD incompatibility. Amniocentesis still serves as the gold standard diagnostic tool for those women who require antenatal fetal blood genotyping in US, but it has been suggested that technology using cell-free fetal DNA may ultimately replace this invasive procedure [47].

**Routine prenatal screening for aneuploidy**

Increased quantities of cffDNA are detected in maternal plasma for fetal trisomy 13 and trisomy 21 [48], but not in fetal trisomy 18 [49]. With complete trisomy, the mRNA allele in maternal plasma isn't the normal 1:1 ratio, but is in fact 2:1. Allelic ratios determined by epigenetic markers can also be used to detect the complete trisomies. Massive parallel sequencing and digital PCR for fetal aneuploidy detection can be used without restriction to fetal-specific nucleic acid molecules. Several cell-free fetal DNA and RNA technologies are under development to test a pregnancy for aneuploidy, mostly focusing on Down syndrome testing. Sampling of cffDNA from maternal blood for analysis by massively parallel sequencing (MPSS) is estimated to have a sensitivity of between 96 and 100%, and specificity between 94 and 100% for detecting Down syndrome. It can be performed at 10 weeks of gestational age [50].

**Identification of Preeclampsia**

cffDNA concentration can be used as a screening tool for preeclampsia. Lo et al. studied quantitative variations of cffDNA in maternal circulations for preeclampsia [51]. cffDNA was measured by using PCR to the SRY gene. The levels were fivefold more in preeclampsia pregnancies than normal. These findings were consistent with subsequent studies [52]. The largest study compared 120 preeclampsia pregnant women with similar number of gestational age matched normal women. The cffDNA concentrations were 176 vs 75 genome equivalents/mL at 29 weeks of gestations.

**Future perspectives**

Cell-free fetal DNA can be used for whole genome sequencing. Thus, it can determine the complete DNA sequence of every gene of the baby [53]. This will be more clinically useful in the future, as large numbers of scientific studies continue to be published detailing clear associations between specific genetic variants and disease [54].

**Issues for counseling**

Given the demonstrated value of non-invasive prenatal testing in high risk pregnancies, this testing should be an option available to pregnant women found to be at increased risk of fetal Down syndrome, trisomy 18, and Trisomy 13 on the basis of currently available screening tests or ultrasound findings. It is important for these women to receive detailed pretest counseling that explains the benefits and limitations of the test [55]. Counseling should include discussion of following points with the clients:

Non-invasive cffDNA testing should not be considered equivalent to conventional cytogenetic analysis of CVS or amniocytes.

Cytogenetic testing of CVS or amniocytes detects 100% of cases of Down syndrome, trisomy 18, and trisomy 13, whereas the test done on cffDNA may miss some cases.

The currently available tests screen only for Down syndrome, trisomy 18, and trisomy 13. Other trisomies, triploidy, and structural chromosomal abnormalities would not be detected by the commercially available cffDNA test.

cffDNA testing has a higher rate of false-positive results than current diagnostic tests based on cytogenetic analysis of amniocytes or chorionic villi.

Some women will have a cffDNA positive result and not carry a fetus with Down syndrome, trisomy 18, or trisomy 13 (false positive). No irrevocable obstetrical decision should be made in pregnancies with a positive cffDNA test for Down syndrome without confirmatory invasive diagnostic testing. cffDNA testing fails to provide a result in a small percentage of women.

**Ethical issues**

Gaining reassurance in a pregnancy will become much safer for many women by removing the risks associated with invasive diagnostic tests. In addition, testing for information/preparation i.e. when termination of pregnancy would not be chosen whatever the result will be easier and perhaps a more common choice. However, by removing the need to discuss the miscarriage risk, maternal plasma DNA testing may provide an apparent ease of access that could compromise informed decision making by increasing the likelihood that the remaining implications of test outcomes would not be considered adequately [56]. Excellent communication between women and health professionals is essential to understand the implications of an apparently simple and safe blood test offered to detect Down syndrome or other conditions [57]. Consideration will need to be given to the potential commercialization of this technology and whether and how regulation would be implemented.

An important issue is related to fetal sex determination. The high degree of accuracy of NIPT as early as 7 weeks of pregnancy carries a number of socio-ethical implications, such as the selective termination of fetuses according to sex [58]. Other issues subsequent to the removal of miscarriage risk include the possibility that women may seek prenatal diagnosis for an increasing number of conditions or for paternity testing [59].

**Conclusion**

Non-invasive prenatal testing using massive parallel sequencing of cell-free fetal DNA to test for trisomies 21, 18, and 13 is an option available at some centres to high-risk pregnant women alternative to invasive amniocentesis. Pretest counseling of these women should include a discussion of the limitations of non-invasive prenatal testing. Before these methods can replace current prenatal screening options, validation studies using them in average-risk pregnancies need to be done. As the cost of the technology is high compared with current screening methods, cost-effectiveness studies are also needed.

**References**

1. ACOG Practice Bulletin No. 77. Screening for chromosomal aneuploidies. Obstet Gynecol. 2007;

109: 217–227.

2. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011; 31(1): 7–15.

3. Caughey AB, Hopkins LM, Norton ME. Chorionic villus sampling compared with amniocentesis and the difference in the rate of pregnancy loss. Obstet Gynecol. 2006; 108(3): 612–616.

4. Bianchi DW, Simpson JL, Jackson LG, et al. Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY I data. National Institute of Child Health and Development Fetal Cell Isolation Study. Prenat Diagn. 2002; 22(7): 609–615.

5. Guetta E, Simchen MJ, Mammon-Daviko K, et al. Analysis of fetal blood cells in the maternal circulation: challenges, ongoing efforts, and potential solutions. Stem Cells Dev. 2004; 13(1): 93–99.

6. Lo YM, Chiu RW. Prenatal diagnosis: progress through plasma nucleic acids. Nat Rev Genet. 2007; 8: 71–77.

7. Alberry M, et al. "Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast". Prenatal Diagnosis. 2007; 27 (5): 415-418.

8. Gupta AK, et al. "Detection of fetal DNA and RNA in placenta-derived syncytiotrophoblast microparticles generated in vitro". Clinical Chemistry. 2004; 11: 2187-90.

9. Lo ES, Lo YM, Hjelm NM, Thilaganathan B. "Transfer of nucleated maternal cells into fetal circulation during the second trimester of pregnancy". British Journal of Haematology. 1998; 100 (3): 605-606.

10. Smets EM, Visser A, Go AT, van Vugt JM, Oudejans CB. "Novel biomarkers in preeclampsia". Clinica Chimica Acta: International Journal of Clinical Chemistry. 2006; 364: 22-32.

11. Lo YM, et al. "Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis". American Journal of Human Genetics. 1998; 62 (4): 768-775.

12. Lo YM, et al. "Rapid clearance of fetal DNA from maternal plasma". American Journal of Human Genetics. 1999; 64 (1): 218-224.

13. Chan KC, et al. "Size distributions of maternal and fetal DNA in maternal plasma". Clinical Chemistry. 2004; 50 (1): 88-92.

14. Hahn S, Chitty LS. "Noninvasive prenatal diagnosis: current practice and future perspectives". Current Opinion in Obstetrics & Gynecology. 2008; 20 (2): 146-151.

15. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. "Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood". Proceedings of the National Academy of Sciences of the United States of America. 2008; 105 (42): 16266-16271.

16. Lo YM, et al. "Digital PCR for the molecular detection of fetal chromosomal aneuploidy". Proceedings of the National Academy of Sciences of the United States of America. 2007; 104 (32): 13116-13121.

17. Lo YM, et al. "Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma". The New England Journal of Medicine. 1998; 339 (24): 1734-1738.

18. Allyse M, Sayres LC, King JS, Norton ME, Cho MK. "Cell-free fetal DNA testing for fetal aneuploidy and beyond: clinical integration challenges in the US context". Human Reproduction. 2012; 27 (11): 3123-3131.

19. Mujezinovic F, Alfirevic Z. "Procedure-related complications of amniocentesis and chorionic villous sampling: a systematic review". Obstetrics and Gynecology. 2007; 110 (3): 687-694.

20. Lo YM. "Fetal nucleic acids in maternal plasma". Annals of the New York Academy of Sciences. 2008; 1137: 140-143.

21. Guibert J, et al. "Kinetics of SRY gene appearance in maternal serum: detection by real time PCR in early pregnancy after assisted reproductive technique". Human Reproduction. 2003; 18 (8): 1733-1736.

22. Wright CF, Burton H. "The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis". Human Reproduction Update. 2009; 15 (1): 139-51.

23. Chiu RW, et al. "Effects of blood-processing protocols on fetal and total DNA quantification in maternal plasma". Clinical Chemistry. 2001; 47 (9): 1607-1613.

24. Dhallan R, et al. "Methods to increase the percentage of free fetal DNA recovered from the maternal circulation". The Journal of the American Medical Association. 2004; 291 (9): 1114-1119.

25. Benachi A, et al. "Impact of formaldehyde on the in vitro proportion of fetal DNA in maternal plasma and serum". Clinical Chemistry. 2005; 51 (1): 242-244.

26. Chinnapapagari SK, Holzgreve W, Lapaire O, Zimmermann B, Hahn S. "Treatment of maternal blood samples with formaldehyde does not alter the proportion of circulatory fetal nucleic acids (DNA and mRNA) in maternal plasma". Clinical Chemistry. 2005; 51 (3): 652-655.

27. Li Y, et al. "Size separation of circulatory DNA in maternal plasma permits ready detection of fetal DNA polymorphisms". Clinical Chemistry. 2004; 50 (6): 1002-1011.

28. Traeger-Synodinos, J. "Real-time PCR for prenatal and preimplantation genetic diagnosis of monogenic diseases". Molecular Aspects of Medicine. 2006; 27: 176-191.

29. Al-Yatama MK, et al. "Detection of Y chromosome-specific DNA in the plasma and urine of pregnant women using nested polymerase chain reaction". Prenatal Diagnosis. 2001; 21 (5): 399-402.

30. Pierce KE, Rice JE, Sanchez JA, Brenner C, Wangh LJ. "Real-time PCR using molecular beacons for accurate detection of the Y chromosome in single human blastomeres". Molecular Human Reproduction. 2000; 6 (12): 1155-1164.

31. Rice JE, Sanchez JA, Pierce KE, Wangh LJ. "Real-time PCR with molecular beacons provides a highly accurate assay for detection of Tay-Sachs alleles in single cells". Prenatal Diagnosis. 2002; 22 (12): 1130-1134.

32. Pierce KE, Rice JE, Sanchez JA, Wangh LJ. "Detection of cystic fibrosis alleles from single cells using molecular beacons and a novel method of asymmetric real-time PCR". Molecular Human Reproduction. 2003; 9 (12): 815-820.

33. Vrettou C, et al. "Real-time PCR for single-cell genotyping in sickle cell and thalassemia syndromes as a rapid, accurate, reliable, and widely applicable protocol for preimplantation genetic diagnosis". Human Mutation. 2004; 23 (5): 513-521.

34. Quake S. "At the interface of physics and biology". Bio Techniques. 2007; 43 (1): 19.

35. Zimmermann BG, et al. Digital PCR: a powerful new tool for noninvasive prenatal diagnosis? Prenatal Diagnosis. 2008; 28 (12): 1087-1093.

36. Chiu RW, Lo YM. "Pregnancy-associated micro RNAs in maternal plasma: a channel for fetal-maternal communication?" Clinical Chemistry. 2010; 56 (11): 1656-1657.

37. Ding C. "Maldi-TOF mass spectrometry for analyzing cell-free fetal DNA in maternal plasma". Methods in Molecular Biology. 2008; 444: 253-67.

38. Akolekar R, Farkas DH, Van Agtmael AL, Bombard AT, Nicolaides KH. "Fetal sex determination using circulating cell-free fetal DNA (ccffDNA) at 11 to 13 weeks of gestation". Prenatal Diagnosis. 2010; 30 (10): 918-23.

39. Papageorgiou EA, et al. "Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21". Nature Medicine. 2011; 17 (4): 510-13.

40. White HE, Dent CL, Hall VJ, Crolla JA, Chitty LS. "Evaluation of a novel assay for detection of the fetal marker RASSF1A: facilitating improved diagnostic reliability of noninvasive prenatal diagnosis". PloS One. 2012; 7 (9): e45073.

41. Ng EK, et al. "Presence of filterable and nonfilterable mRNA in the plasma of cancer patients and healthy individuals". Clinical Chemistry. 2002; 48 (8): 1212-1217.

42. Baird PA, Anderson TW, Newcombe HB, Lowry RB. "Genetic disorders in children and young adults: a population study". American Journal of Human Genetics. 1988; 42 (5): 677-93.

43. Scheffer PG, et al. "Reliability of fetal sex determination using maternal plasma". Obstetrics and Gynecology. 2010; 115(1): 117-126.

44. Bustamante-Aragones A, Gonzalez-Gonzalez C, de Alba MR, Ainse E, Ramos C. "Noninvasive prenatal diagnosis using ccffDNA in maternal blood: state of the art". Expert Review of Molecular Diagnostics. 2010; 10 (2): 197-205.

45. Sayres LC, Cho MK. "Cell-free fetal nucleic acid testing: a review of the technology and its applications". Obstetrical & Gynecological Survey. 2011; 66 (7): 431-442.

46. Hill M, Barrett AN, White H, Chitty LS. "Uses of cell free fetal DNA in maternal circulation". Best Practice & Research: Clinical Obstetrics & Gynaecology. 2012; 26 (5): 639-654.

47. Moise KJ. "Fetal anemia due to non-Rhesus-D red-cell alloimmunization". Seminars in Fetal & Neonatal Medicine. 2008; 13 (4): 207-214.

48. Wataganara T, et al. "Maternal serum cell-free fetal DNA levels are increased in cases of trisomy 13 but not trisomy 18". Human Genetics. 2003; 112 (2): 204-208.

49. Chiu RW, Lo YM. "Non-invasive prenatal diagnosis by fetal nucleic acid analysis in maternal plasma: the coming of age". Seminars in Fetal & Neonatal Medicine. 2011; 16 (2): 88-93.

50. Mersy E, Smits JM, Van Winden AP, De Die-Smulders EM, Paulussen DC, MacVille VE, et al. "Noninvasive detection of fetal trisomy 21: Systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012". Human Reproduction Update. 2013; 19 (4): 318

51. Lo YM, et al. "Increased fetal DNA concentrations in the plasma of pregnant women

carrying fetuses with trisomy 21". Clinical Chemistry. 1999; 45 (10): 1747-1751.

52. Leung TN, Zhang J, Lau TK, Chan LY, Lo YM. "Increased maternal plasma fetal DNA concentrations in women who eventually develop preeclampsia". Clinical Chemistry. 2001; 47 (1): 137-39.

53. Yurkiewicz IR, Korf BR, Lehmann LS. "Prenatal whole-genome sequencing--is the quest to know a fetus's future ethical?". N Engl J Med. 2014; 370 (3): 195.

54. Mailman MD, Feolo M, Jin Y, Kimura M, Tryka K, Bagoutdinov R, et al. The NCBI dbGaP database of genotypes and phenotypes". Nat. Genet. 2007; 39 (10): 1181–6.

55. Benn P, Borrell A, Cuckle H, Dugoff L, Gross S, Johnson J, et al. Prenatal detection of Down syndrome using massively parallel sequencing (MPS): a rapid response statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis, 24 October 2011. Prenat Diagn. 2012; 32: 1–2.

56. van den Heuvel A, Chitty L, Hogg J, Marteau T. Will noninvasive prenatal diagnosis using free fetal nucleic acids in maternal blood erode informed choices? Prenat Diagn. 2008; 28 Suppl 1: S16.

57. Bianchi DW. At-home fetal DNA gender testing: caveat emptor. Obstet Gynecol. 2006; 107: 216–8.

58. Marteau TM, Chitty LS, editors. Special Issue: Fetal sexing: Global Perspectives on Practices, Ethics and Policy. Prenat Diagn. 2006; 26: 597–653.

59. Guo X, Bayliss P, Damewood M, Varney J, Ma E, Vallecillo B, et al. A noninvasive test to determine paternity in pregnancy. N Engl J Med. 2012; 366: 1743–5.