HJOG
An Obstetrics and Gynecology International Journal

Review

# Non - invasive prenatal diagnosis and cell free DNA analysis

**Kalinderi Kallirhoe, Fidani Liana**
Department of Medical Biology and Genetics, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

**Correspondence**
Fidani Liana, Department of Medical Biology and Genetics, Medical School, Aristotle University of Thessaloniki, GR 54124, Thessaloniki, Greece
**E - mail:** lfidani@med.auth.gr

## Abstract

Prenatal diagnosis is a very important part in the obstetric care of a pregnant woman. The use of invasive methods of prenatal screening provides great accuracy in the prenatal diagnosis of genetic and chromosomal abnormalities of the fetus; however it carries a small risk of fetal miscarriage. For this reason, the development of non-invasive methods of prenatal diagnosis is an area of intense research in which recently great progress has been made.

**Keywords:** prenatal diagnosis; cell free DNA

The term prenatal diagnosis refers to all the diagnostic tests and methods that achieve early intrauterine diagnosis of fetal anomalies or genetic disorders, providing the opportunity to the parents to terminate a pregnancy in case of a congenital abnormality. The main indications for prenatal diagnosis are increased maternal age (> 35 years), a previous pregnancy with fetal chromosomal abnormality, the presence of a balanced translocation or aneuploidy in a parent, increased risk of monogenic disease due to a positive family history, reproductive problems such as repeated pregnancy losses and exposure to potentially teratogenic factors. Prenatal diagnostic procedures can be invasive and non - invasive. The main invasive prenatal methods currently used are amniocentesis, carried out at 16 - 18 weeks of gestation and chorionic villus sampling, carried out at 8 - 11 weeks of gestation[1]. The small but finite risk of miscarriage after invasive prenatal procedures led to intensive efforts to improve methods of non - invasive prenatal diagnosis. In this review, the main methods of prenatal diagnosis are described, focusing on new advances in the field of non - invasive prenatal diagnosis.

## Ultrasonography and maternal serum screening

Ultrasound scanning is one of the main methods of non - invasive prenatal diagnosis, as it is a safe and painless method for both the fetus and the mother. Ultrasound examination is performed during the first trimester of pregnancy, at 6 - 14 weeks of gestation and aims to confirm the existence of a pregnancy, to exclude the probability of an ectopic or molar pregnancy and to detect fetal viability. In the 11 - 14 week, fetal ultrasound scanning is used to measure nuchal translucency (NT). NT is the thickness of the fluid accumulated in the cervical area, between the fetal spine and skin. It normally has a size of 1 - 2 mm, while in cases of chromosomal abnormalities mainly trisomy 21 (Down syndrome) or severe

heart disease, its size is > 3 mm. Specifically in Down syndrome, the NT measurement in combination with the maternal age and maternal serum levels of beta chorionic gonadotropin (β - hCG) and PAPP - A protein may lead to the detection of up to 92 % of pregnancies with Down syndrome[2]. This percentage increases with the addition of other ultrasound markers, foremost of which is the absence / hypoplasia of fetal nasal bone[3].

During the second trimester, between 20 - 24 weeks of gestation, another ultrasound (level II) scan is performed by a specialized obstetrician - gynecologist. With this, a detailed examination of all the organs of the fetus is done. If anomalies incompatible with life are diagnosed and the fetus has not exceeded the viability limit of 24 weeks, the possibility of interrupting the pregnancy is discussed. Some additional ultrasound markers, such as short femur and short humerus, the increased nuchal fold, dilatation of the renal pelvis, the echogenic bowel, echogenic cardiac foci and cysts of choroid plexus are used to further evaluate the possibility of chromosomal abnormalities. During the second trimester of pregnancy, between 16 - 20 weeks of gestation, the triple test (alfa test) can also be performed in order to check for chromosomal abnormalities, mainly for Down syndrome. Provided the precise determination of the gestational age, by measuring the biparietal diameter of the fetus, measurements of maternal serum levels of beta - hCG (β - HCG), alpha - fetoprotein (AFP) and estriol (E3) are made[3]. These measurements are recorded in a computer program and the probability of a chromosomal abnormality or an open lesion of the central nervous system is determined. With the triple test up to 67% of pregnancies with Down syndrome and a significant proportion of trisomy 18 and 13 can be diagnosed[4]. In case of a lesion of the central nervous system, the sensitivity of the method is 90%. The addition of inhibin - A measurement can improve the diagnostic sensitivity by approximately 7%. After the 24th week of gestation, uterine artery Doppler is also performed by measuring blood flow velocity. In case of increased resistance in both uterine arteries there is an increased risk of preeclampsia, placental abruption or intrauterine growth restriction (IUGR)[3].

In the third trimester, with an ultrasound after 28 weeks of gestation, an estimation of fetal development is done based on measurements such as biparietal diameter, head and abdominal circumferences and fetal femur length[3]. If symmetrical IUGR is assessed, congenital or chromosomal abnormalities or intrauterine infections are suspected. In asymmetrical IUGR, there is increased probability of placental dysfunction and preeclampsia. In the third trimester of pregnancy the fetal biophysical profile is monitored by the movements of the fetal body and limbs, the fetal breathing, the fetal heart rate as well as the amount of amniotic fluid. Towards the end of pregnancy, ultrasound scanning is useful to check the position of the fetus and of the placenta and to measure the amount of amniotic fluid.

Apart from ultrasound and biochemical markers, non - invasive methods of prenatal diagnosis include the use of magnetic resonance imaging and analysis of fetal cells in maternal circulation, however these methods have serious drawbacks and its use is very limited, thus they will not be discussed in this review. The development of proteomics and the search for new biomarkers especially for aneuploidies such as trisomy 21 could help a lot in the field of non - invasive prenatal diagnosis, however so far no clear and valid results have come out[5].

One of the most important recent achievements in the field of noninvasive prenatal diagnosis is the discovery of cell free fetal DNA in the maternal circulation[6]. The existence of cell free DNA in the maternal circulation makes it possible that in the near future genetic tests for prenatal diagnosis could be done with a blood test in the pregnant woman.

## Cell - free DNA analysis

According to the discovery of Lo et al in 1997, about 10% of the DNA in the maternal circulation is of fetal origin, while the remaining 90% is maternal DNA[6-8]. Fetal DNA consists of small fragments of <313bp which can be detected in the maternal circulation

from the 5th week[9] and disappear two hours after birth[10]. The main source of cell free fetal DNA appears to be the apoptosis of trophoblast cells. Although the presence of a large amount of maternal DNA complicates the identification and analysis of fetal DNA, nevertheless today the analysis of cell free fetal DNA has already started to be used in clinical practice in the context of non - invasive prenatal diagnosis for the determination of sex, Rhesus genotype and recently for Down syndrome. The diagnosis of monogenic diseases and other aneuploidies may also be performed in clinical practice very soon.

## Fetal sex determination

The first application of cell free fetal DNA was to determine fetal sex. More specifically, the detection of DNA sequences of the Y chromosome (SRY, DYS14 genes) in the maternal plasma indicates that the pregnant woman carries a male fetus. This knowledge can be used to prevent sex - linked diseases such as hemophilia and muscular dystrophy[11]. Conversely, in pregnancies where there are no detectable DNA sequences of the Y chromosome it is assumed that the fetus is female and in such a case the pregnant women does not need to undertake an invasive prenatal testing. The accuracy of the determination of fetal sex by analysis of cell free fetal DNA has been found to be > 95%, that is why its use in clinical practice was the first to be implemented and has led to a 50% reduction of invasive prenatal diagnostic methods in pregnancies with a high risk of sex-linked diseases[12,13]. Although, the free fetal DNA, can be detected almost by the fifth week of gestation, the validity of the results is 100% from the seventh week of gestation and afterwards[14].

Timely and accurate knowledge of the sex of the unborn fetus is particularly useful in cases of congenital hyperplasia of the adrenal cortex as well. In this condition there is a shortage of the 21 hydroxylase, enzyme resulting in hyperandrogenism which in females results to the masculinization of the external genital organs[15]. To prevent this, corticosteroids can be administered[16].Thus, the detection of fetal sex at 7 weeks of gestation, much earlier than by ultrasound scanning, is particularly useful in these circumstances.

## Rhesus genotyping

It is well known that Rhesus D (RhD) (+)) fetuses carried by a RhD (-) mother are at risk of hemolytic disease after prior sensitization. Therefore anti - RhD globulin is prophylactically administered in RhD (-) pregnant women, blocking the process of sensitization. In the last 40 years, the administration of anti - RhD globulin has reduced the rates of death from hemolytic disease from 1/2,200 to 1/21,000 live births. Nowadays, prophylactic anti - RhD globulin is recommended in all RhD (-) pregnant[17]. Following this strategy, 38% of RhD (-) pregnant women that carry a RhD (-) fetus receive anti - RhD globulin without having any need of it[18]. Therefore it is of particular clinical importance to know the fetal RhD genotype before birth. The presence of RhD sequences in the plasma of an RhD negative pregnant woman indicates that the unborn fetus is RhD (+)[12, 19 - 22]. By using cell free fetal DNA the accuracy of the detection of the fetal Rh genotype is 94.8%[23]. With the aid of cell free fetal DNA and the early identification of fetal Rh genotype, RhD (-) pregnant women with a RhD (-) fetus can avoid unnecessary administration of anti - RhD globulin. However, the benefit compared to the cost of cell free DNA prenatal diagnosis of fetal RhD genotype remains a matter of debate[24,25].

## Single gene disorders

Prenatal diagnosis is particularly useful for common monogenic diseases such as the thalassemias and cystic fibrosis, but also for rarer monogenic diseases in families with a positive family history. The non - invasive prenatal diagnosis using cell free fetal DNA initially focused on identifying autosomal dominant diseases of paternal origin[26]. In such cases, the detection or not of paternal mutations in the maternal plasma, can lead to the confirmation or exclusion of the inheritance by the fetus of an autosomal dominant disease transmitted from the father. In the case of an autosomal recessive disease or an autoso

mal dominant disease of maternal origin, the large amount of maternal DNA in the plasma (maternal DNA background), makes it difficult to determine the possibility of inheritance of a maternal mutation to the fetus. If the couple carries different mutations for an autosomal recessive disease and the fetus has inherited a normal allele from the father it will not express the disease, regardless of whether it has inherited a mutation from the mother. Greater difficulties are faced if both the father and mother carry the same mutation for an autosomal recessive disease. In such cases new methods have begun to be implemented such as the relative mutation dosage (RMD) method using digital PCR[27-29]. In this method, the amounts of the mutant and normal alleles at a particular locus are compared in the maternal plasma. This methodology is used mainly in cases where the mother is heterozygous for a known mutation. For a pregnant woman heterozygous for a mutation who carries a fetus heterozygous for this particular mutation, equal amounts of mutant and normal sequences are expected in the maternal plasma. If the fetus is homozygous for the mutation, more mutant compared to non - mutant sequences are expected to be detected in the maternal plasma, whereas if the fetus is homozygous for the normal sequences a larger proportion of non - mutated relative to mutated sequences are expected to be found[30]. In conclusion, the RMD method can diagnose autosomal dominant diseases inherited from the mother, whereas when this method is combined with techniques for the detection of mutations of paternal origin, autosomal recessive diseases can also be found, enabling the non invasive prenatal detection of all monogenic diseases simply by a DNA analysis test in the maternal plasma[30].

**Aneuploidies**

Chromosomal aneuploidies, such as trisomy 21, are the most common reasons why couples proceed to prenatal diagnosis. The use of non - invasive prenatal diagnostic techniques in such cases would be very useful eliminating the small but finite risk of miscarriage. However, the use of cell free fetal DNA in the diagnosis of chromosomal aneuploidies presents certain difficulties such as the existence of a high rate of maternal DNA in the maternal plasma, which complicates the analysis of fetal DNA, as well as the fact that the fetal DNA is free and not within cells.

A strategy used to overcome these difficulties is to analyze nucleic acids specific to the fetus, such as mRNA expressed exclusively in the placenta or placental epigenetic markers specific for the studied chromosome. A considerable number of such markers have been used, including the placenta - specific 4 (PLAC4) and chromosome 21 open reading frame 105 (c21orf105) mRNA for the chromosome 21[31,32], serpin peptidase inhibitor, clade B (ovalbumin), member 2 (SERPINB2) mRNA for chromosome 18 and hypermethylated holocarboxylase synthetase (HLCS) for chromosome 21[33,34]. These markers are specific for the fetus, as they contain fetal - specific single nucleotide polymorphisms (SNP) alleles, whereas they do not contain maternal - specific SNP alleles. In order to extract information about the chromosome dosage of free nucleic acid molecules specific for the fetus, two methods are used. The first method is the allelic ratio approach such as the RNA - SNP approach that determines the amount of heterozygous alleles in specific nucleic acid molecules. Therefore, if the RNA - SNP method for PLAC4 mRNA is applied in the maternal plasma for an euploid fetus, the amount of the PLAC4 mRNA alleles will be almost equal, while if the fetus has a trisomy 21 one of the alleles will be overrepresented in the maternal plasma[31,32]. Similar studies after analysis of SERPINB2 mRNA have been done for trisomy 18. The methodology of allelic ratio is applied to fetal epigenetic markers as well (epigenetic allelic ratio approach, EAR). More specifically, in the EAR method placental - specific epigenetic markers are used such as hypomethylated SERPINB2 on chromosome 18 and mRNA expressed in the placenta such as PLAC4 mRNA, and the ratio for specific alleles on the target gene can be determined. In general, the ratio of 1:1 for both alleles is expected in a euploid fetus, while ratios of 1:2 or 2:1 are expect-

ed for a trisom ic fetus[33]. The main drawback of this method is that it can only be applied to heterozygous fetuses, thus multiple markers are needed to be tested in order to enhance the population coverage of the method.

The second method used is the EGG method (epigenetic - genetic approach)[34]. In this method, a fetal - specific epigenetic marker from the potentially aneuploid chromosome, e.g. HLCS for chromosome 21, is used as well as a fetal - specific genetic marker from a non - aneuploid chromosome, e.g. the Y chromosome for male fetuses or a SNP - allele of paternal origin[34]. HLCS gene located on chromosome 21 is hypermethylated in the placental tissue, from which the cell free fetal DNA comes from, while it is hypomethylated in maternal blood cells from where the majority of the maternal DNA originates. By using specific PCR which can identify the hypermethylated HLCS, fetal DNA is amplified and then is compared to a fetal - specific genetic marker. In cases of trisomy 21, the epigenetic/genetic ratio will be increased due to the extra chromosomal dosage derived from fetus[35]. The advantage of this method is that it does not require the placental epigenetic marker to contain a polymorphism.

Besides quantifying fetal - specific DNA sequences in the maternal plasma, an alternative approach is to directly detect fetal aneuploidy by determining the total (maternal + fetal) amount of the studied aneuploid chromosome in the maternal plasma and comparing it with other chromosomes in the maternal plasma[36]. For this purpose, specific quantitative methods such as digital and RMD PCR methodology are used. According to this strategy, if a pregnant woman carries a fetus with trisomy 21, an additional dose of chromosome 21 derived from the fetus will be present which means a 50% increase of the copies of the fetal chromosome 21. For example, in a sample from the plasma of a pregnant woman who carries a fetus with trisomy 21 and in which the fetal DNA is 12%, a 6% (half of 12%) increase is expected in the total number of DNA copies of chromosome 21 in this sample[35]. Thus, non - invasive prenatal diagnosis of trisomy 21 could be done by comparing the number of copies of a genetic region on chromosome 21 with the number of copies of another reference chromosome. For an euploid fetus, the dose of chromosome 21 is expected to be equal to the reference chromosome and for a fetus with trisomy 21 a 50% increase of fetal DNA is expected[35].

Finally, a new method for the quantitative detection of DNA sequences from an aneuploid chromosome in maternal plasma is the massively parallel sequencing (MPS)[37]. With this method, sequencing of many small nucleic acid molecules in a sample is done and thereafter the chromosomal location of these molecules is determined. For example, a pregnant woman who carries a fetus with trisomy 21 will increase the percentage of specific gene fragments (sequences) of chromosome 21 in comparison to the data obtained from pregnancies carrying euploid fetuses. The use of MPS has been considered for the non - invasive prenatal diagnosis of trisomy 21 and is expected to be applied to other aneuploidies as well[38,39]. Currently, the major limiting factor for MPS is the high cost of this methodology. Nevertheless, considering that the cost of sequencing is expected to be lowered in the near future, the MPS methodology could become more affordable and could be widely applied in routine clinical practice in the context of non invasive prenatal diagnosis.

## Conclusion

The development of non - invasive methods of prenatal diagnosis is a field of intense research in which great progress has already been achieved. The discovery of cell free fetal DNA in the maternal blood 17 years ago, has brought substantial changes and new opportunities in the context of antenatal screening. Currently, the analysis of cell free fetal DNA is used for determining fetal sex, Rhesus genotype and for identifying mutations of paternal origin. Sex determination has resulted in 50% reduction of invasive prenatal screening methods, while the identification of Rhesus genotype has limited the use of anti - D globulin to Rhesus D (-) pregnant women who carry Rhesus D (+) fetuses. Although the detection of aneuploidies and of all monogenic diseases by analy-

sis of cell free fetal DNA has been described, a lot of research is carried out to find the best technical approach and methodology, in order to put these applications in routine clinical practice. Advances in technology as well as in molecular techniques are expected to lead to the implementation of new reliable and affordable methods of non - invasive prenatal diagnosis with the view that in the near future, prenatal testing could be performed with just a blood draw and a low cost DNA test.

## Conflict of interest

All authors declare no conflict of interest.

## References


1. Stembalska A, Slezak R, Pesz K, Gil J, Sasiadek M. Prenatal diagnosis - principles of diagnostic procedures and genetic counseling. Folia Histochem Cytobiol 2007;45 Suppl 1:S11-6.
2. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first - trimester screening for trisomy 21 in 75,821 pregnancies: results and estimation of the potential impact of individual risk - orientated two - stage first - trimester screening. Ultrasound Obstet Gynecol 2005;25:221-6.
3. Collins SL, Impey L. Prenatal diagnosis: types and techniques. Early Hum Dev 2012;88:3-8.
4. Reynolds T. The triple test as a screening technique for Down syndrome: reliability and relevance. Int J Womens Health 2010;2:83-8.
5. Cho CK, Diamandis EP. Application of proteomics to prenatal screening and diagnosis for aneuploidies. Clin Chem Lab Med 2011;49:33-41.
6. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7.
7. Lo YM, Tein MS, Lau TK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768-75.
8. Lun FM, Chiu RW, Chan KC, Leung TY, Lau TK, Lo YM. Microfluidics digital PCR reveals a higher than expected fraction of fetal DNA in maternal plasma. Clin Chem 2008;54:1664-72.
9. Galbiati S, Smid M, Gambini D, et al. Fetal DNA detection in maternal plasma throughout gestation. Hum Genet 2005;117:243- 8.
10. Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet 1999;64:218-24.
11. Costa JM, Benachi A, Gautier E. New strategy for prenatal diagnosis of X - linked disorders. N Engl J Med 2002;346:1502.
12. Wright CF, Burton H. The use of cell - free fetal nucleic acids in maternal blood for non - invasive prenatal diagnosis. Hum Reprod Update 2009;15:139-51.
13. Scheffer PG, van der Schoot CE, Page - Christiaens GC, Bossers B, van Erp F, de Haas M. Reliability of fetal sex determination using maternal plasma. Obstet Gynecol 2010;115:117-26.
14. Bustamante - Aragonés A, Rodríguez de Alba M, Perlado S, et al. Non - invasive prenatal diagnosis of single - gene disorders from maternal blood. Gene 2012;504:144-9.
15. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. Lancet 2005;365:2125-36.
16. Rijnders RJ, van der Schoot CE, Bossers B, de Vroede MA, Christiaens GC. Fetal sex determination from maternal plasma in pregnancies at risk for congenital adrenal hyperplasia. Obstet Gynecol 2001;98:374-8.
17. National Institute for Clinical Excellence (NICE). Technology Appraisal Guidance 41. Guidance on the Use of Routine Antenatal Anti - D Prophylaxis for RhD - negative Women. London 2002.
18. Daniels G. Human Blood Groups. 2nd ed. Oxford: Blackwell Science; 2002, p. 195- 74.
19. Lo YM, Hjelm NM, Fidler C, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med 1998;339:1734-8.
20. Faas BH, Beuling EA, Christiaens GC, von dem Borne AE, van der Schoot CE. Detection of fetal RHD-specific sequences in maternal plasma. Lancet 1998;352:1196.
21. Finning K, Martin P, Summers J, Massey E, Poole G, Daniels G. Effect of high throughput RHD typing of fetal DNA in maternal plasma on use of anti - RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study. BMJ 2008;336:816-8.

22. Van der Schoot CE, Hahn S, Chitty LS. Non-invasive prenatal diagnosis and determination of fetal Rh status. Semin Fetal Neonatal Med 2008;13:63-8.
23. Geifman - Holtzman O, Grotegut CA, Gaughan JP. Di agnostic accuracy of noninvasive fetal Rh genotyping from maternal blood - A meta - analysis. Am J Obstet Gynecol 2006;195:1163-73.
24. Goodspeed TA, Allyse M, Sayres LC, Norton ME, Cho MK. Translating cell - free fetal DNA technology: structural lessons from non - invasive RhD blood typing. Trends Biotechnol 2013;31:7-9.
25. Szczepura A, Osipenko L, Freeman K. A new fetal RHD genotyping test: costs and benefits of mass testing to target antenatal anti - D prophylaxis in England and Wales. BMC Pregnancy Childbirth 2011;11:5.
26. Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaihara T. Prenatal DNA diagnosis of a single - gene disorder from maternal plasma. Lancet 2000;356:1170.
27. Lun FM, Tsui NB, Chan KC, et al. Noninvasive prenatal diagnosis of monogenic diseases by digital size selection and relative mutation dosage on DNA in maternal plasma. Proc Natl Acad Sci USA 2008;105:19920-5.
28. Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci USA 1999;96:9236-41.
29. Chiu RW, Cantor CR, Lo YM. Non - invasive prenatal diagnosis by single molecule counting technologies. Trends Genet 2009;25:324-31.
30. Chiu RW, Lo YM. Non - invasive prenatal diagnosis by fetal nucleic acid analysis in maternal plasma: the co ming of age. Semin Fetal Neonatal Med 2011;16:88-93.
31. Lo YM, Tsui NB, Chiu RW, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med 2007;13:218-23.
32. Go AT, Visser A, Betsalel OT, van Vugt JM, Blankenstein MA, Oudejans CB. Measurement of allelic - expression ratios in trisomy 21 placentas by quencher extension of heterozygous samples identified by partially denaturing HPLC. Clin Chem 2008;54:437-40.
33. Tong YK, Ding C, Chiu RW, et al. Noninvasive prenatal detection of fetal trisomy 18 by epigenetic allelic ratio analysis in maternal plasma: theoretical and empirical considerations. Clin Chem 2006;52:2194-202.
34. Tong YK, Jin S, Chiu RW, et al. Noninvasive prenatal detection of trisomy 21 by an epigenetic - genetic chromosome - dosage approach. Clin Chem 2010;56:90-8.
35. Chiu RW, Lo YM. Noninvasive prenatal diagnosis empowered by high - throughput sequencing. Prenat Diagn 2012;32:401-6.
36. Chiu RW, Cantor CR, Lo YM. Non - invasive prenatal diagnosis by single molecule counting technologies. Trends Genet 2009;25:324-31.
37. Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet 2010;11:31-46.
38. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci USA 2008;105:16266-71.
39. Chiu RW, Chan KC, Gao Y, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci USA 2008;105:20458-63.