# Fetal Nucleic Acids in Maternal Plasma

## Toward the Development of Noninvasive Prenatal Diagnosis of Fetal Chromosomal Aneuploidies

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**The discovery of cell-free fetal nucleic acids in maternal plasma has opened up new possibilities for noninvasive prenatal diagnosis. Over the last few years, a number of approaches have been demonstrated to allow such circulating fetal nucleic acids to be used for the prenatal detection of chromosomal aneuploidies. One such approach involves the enrichment of fetal DNA, such as by size fractionation or by the controversial formaldehyde treatment technique. A second approach involves the targeting of fetal-specific nucleic acid molecules, including fetal-specific epigenetic markers and placenta-specific mRNA markers. A third approach involves the development of highly discriminatory quantitative methods for chromosome dosage analysis using digital polymerase chain reaction technology. It is likely that these and other methods yet to be developed would allow noninvasive prenatal diagnosis of chromosomal aneuploidies by maternal plasma nucleic acids to be realized in the near future.**

*Key words:* **noninvasive prenatal diagnosis; plasma DNA; plasma RNA; epigenetics; Down syndrome**

## Introduction

Prenatal diagnosis is an established part of modern obstetrics service. However, invasive techniques, such as amniocentesis, are commonly used for the definitive diagnosis of fetal chromosomal aneuploidies, including trisomy 21. Because of the potential risks associated with such invasive methods, a long-sought goal in prenatal diagnosis research is to develop noninvasive methods that do not carry such a risk. The discovery of circulating cell-free fetal DNA in maternal plasma in 1997 has opened up new possibilities for noninvasive prenatal diagnosis.[1,2] As circulating fetal DNA only represents a mean of ~3% of the DNA that is present in maternal plasma,[3] the use of maternal plasma DNA for the noninvasive prenatal diagnosis of fetal chromosomal aneuploidies has presented considerable technical challenge. However, remarkable advances have occurred in this area recently. This review summarizes some of these developments.

## Enrichment of Fractional Fetal DNA Concentrations

One approach involves the enrichment of the fractional fetal DNA concentrations from the above-mentioned mean of ~3% to a level that is amenable to commonly available analytical strategies. One variant of this approach has been proposed by Dhallan *et al.*, who attempted to enhance the fractional fetal

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DNA concentration by suppressing the maternal DNA background in maternal plasma through the stabilization of maternal blood cells using formaldehyde.[4] They then applied this approach to the prenatal detection of Down syndrome.[5] The main problem with the formaldehyde approach is that this method can only be reproduced by some,[6] but not other investigators.[7,8]

An alternative approach is based on the discovery that circulating fetal DNA is shorter than maternal DNA in maternal plasma.[9] Thus, through the preferential isolation of short DNA from maternal plasma, it has been demonstrated that the fractional concentration of fetal DNA can be enhanced.[10] The main disadvantage of this approach is that currently an electrophoretic method is used for the size fractionation of plasma DNA, a procedure that is relatively labor-intensive and potentially prone to contamination. It is also unknown whether this approach would provide the degree of fetal DNA enrichment necessary for the noninvasive prenatal diagnosis of fetal chromosomal aneuploidies.

## Molecular Targeting of Fetal-Specific Nucleic Acid Populations

An alternative approach is the targeting of specific nucleic acid populations in maternal plasma which are virtually completely fetal-specific. One such population is fetal-specific DNA methylation markers.[11–14] One of these markers is the *SERPINB5* gene, which is hypomethylated in the placenta and hypermethylated in maternal blood cells.[12] Hypomethylated *SERPINB5* can therefore be regarded as a fetal-specific marker in maternal plasma, as most of the background maternal DNA in maternal plasma is probably derived from hematopoietic cells.[15] As this gene is located on chromosomal 18, it has been demonstrated that allelic ratio analysis of a single nucleotide polymorphism (SNP) in the hypomethylated form of *SERPINB5* can be used as a marker of trisomy 18 in maternal plasma.[16] This approach is called the epigenetic allelic ratio approach (EAR).[16] The main disadvantage of this approach is that it is dependent on bisulfite conversion, a method that may destroy a large proportion of the input DNA.[17] In addition, the allelic ratio approach can only be used if the fetus is heterozygous for the SNP concerned. Furthermore, it would be necessary to search for similar epigenetic changes on the other chromosomes involved in common aneuploidies, such as chromosome 21.[18] In addition, because of the limitation in the number of circulating fetal DNA molecules, if the volume of plasma used is insufficient, the accuracy of the method would diminish on account of statistical sampling errors.[16] As placenta-specific epigenetic markers have also been found on chromosomes not normally involved in fetal chromosomal aneuploidies (e.g., the *RASSF1A* gene on chromosome 3),[13] it is also potentially possible to determine the fetal chromosome 21 status by measuring the relative concentrations of fetal-specific epigenetic markers on chromosome 21 to those on one or more reference chromosomes.

An alternative approach to target fetal-specific nucleic acid species is to use placental-specific mRNA species.[19] Through the use of allelic ratio measurement on placenta-specific mRNA species transcribed from a chromosome involved in an aneuploidy (e.g., the *PLAC4* gene on chromosome 21 for Down syndrome), the aneuploidy status can be ascertained.[20] This approach is called the RNA–SNP allelic ratio approach, and appears to have a high sensitivity of 90% and a high specificity of 96.5%.[20] Compared with the epigenetic approach, the advantages of the mRNA approach include (1) the fact that multiple copies of mRNA are transcribed from a gene active in the placenta; and (2) mRNA markers can be readily detected by reverse transcriptase polymerase chain reaction (PCR) or other amplification strategies, without having to involve a more complicated procedure like bisulfite conversion. Similar to

the EAR approach, the main limitation of the RNA–SNP approach is that it would only work if the fetus is heterozygous for the analyzed SNP. To cover a large proportion, multiple placental mRNA markers and multiple SNPs would be needed.

## High-Precision Analytical Approach

Another approach towards the detection of fetal chromosomal aneuploidies is the development of highly discriminatory quantitative methods which would allow the detection of a small excess of nucleic acid sequences from a particular locus, even when applied to a sample in which fetal nucleic acid molecules only represent a minor population. Recently, Lo *et al.* have demonstrated that digital PCR would allow this goal to be achieved.[21] For example, it has been shown that for a DNA sample in which 25% is derived from the placenta and 75% is derived from maternal blood cells, digital PCR would allow the correct classification of the chromosome 21 aneuploidy status 97% of the time, as long as at least 7680 DNA molecules are available for analysis.[21] Thus, the work by Lo *et al.* provides a set of benchmark parameters documenting the statistical relationship between the fractional concentration of the fetal nucleic acid target, the number of nucleic acid molecules, and the frequencies of correct classification of the aneuploidy status.[21] For investigators working on fetal DNA enrichment techniques,[4,10] these parameters would be useful to guide the degree of fetal DNA enrichment needed. For workers studying the use of epigenetic[16,18] or mRNA markers,[20] the data from Lo *et al.* would indicate the number of fetal-specific epigenetic or mRNA marker molecules needed for robust diagnosis. With the development of rapid methods for conducting digital PCR analysis, such as microfluidics PCR[22] and emulsion PCR,[23] it is expected that this powerful technology will have an impact on the clinical application of noninvasive prenatal diagnosis in the near future.

## Conclusions

After a decade of development, the use of cell-free fetal nucleic acids in maternal plasma for the noninvasive prenatal detection of chromosomal aneuploidies has been realized in principle. A number of alternative approaches have been shown to be of promise. It is hoped that these and further approaches yet to be devised will deliver a new generation of tests for noninvasive prenatal diagnosis over the next few years, making prenatal investigations safer for pregnant women and their fetuses.

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### Conflicts of Interest

The author holds patents and has filed patent applications on aspects of the use of fetal nucleic acids in maternal plasma for noninvasive prenatal diagnosis. Some of these technologies have been licensed to Sequenom, to which the author is a consultant.

## References

1. Lo, Y.M.D. *et al*. 1997. Presence of fetal DNA in maternal plasma and serum. *Lancet* **350:** 485–487.
2. Lo, Y.M.D. & R.W.K. Chiu. 2007. Prenatal diagnosis: progress through plasma nucleic acids. *Nat. Rev. Genet.* **8:** 71–77.
3. Lo, Y.M.D. *et al*. 1998. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am. J. Hum. Genet.* **62:** 768–775.
4. Dhallan, R. *et al*. 2004. Methods to increase the percentage of free fetal DNA recovered from the maternal circulation. *JAMA* **291:** 1114–1119.
5. Dhallan, R. *et al*. 2007. A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. *Lancet* **369:** 474–481.

6. Benachi, A. *et al*. 2005. Impact of formaldehyde on the in vitro proportion of fetal DNA in maternal plasma and serum. *Clin. Chem.* **51:** 242–244.
7. Chung, G.T. *et al*. 2005. Lack of dramatic enrichment of fetal DNA in maternal plasma by formaldehyde treatment. *Clin. Chem.* **51:** 655–658.
8. Chinnapapagari, S.K. *et al*. 2005. Treatment of maternal blood samples with formaldehyde does not alter the proportion of circulatory fetal nucleic acids (DNA and mRNA) in maternal plasma. *Clin. Chem.* **51:** 652–655.
9. Chan, K.C. *et al*. 2004. Size distributions of maternal and fetal DNA in maternal plasma. *Clin. Chem.* **50:** 88–92.
10. Li, Y. *et al*. 2005. Detection of paternally inherited fetal point mutations for beta-thalassemia using size-fractionated cell-free DNA in maternal plasma. *JAMA* **293:** 843–849.
11. Poon, L.L. *et al*. 2002. Differential DNA methylation between fetus and mother as a strategy for detecting fetal DNA in maternal plasma. *Clin. Chem.* **48:** 35–41.
12. Chim, S.S.C. *et al*. 2005. Detection of the placental epigenetic signature of the maspin gene in maternal plasma. *Proc. Natl. Acad. Sci. USA* **102:** 14753–14758.
13. Chiu, R.W. *et al*. 2007. Hypermethylation of RASSF1A in human and rhesus placentas. *Am. J. Pathol.* **170:** 941–950.
14. Chan, K.C. *et al*. 2006. Hypermethylated RASSF1A in maternal plasma: a universal fetal DNA marker that improves the reliability of noninvasive prenatal diagnosis. *Clin. Chem.* **52:** 2211–2218.
15. Lui, Y.Y. *et al*. 2002. Predominant hematopoietic origin of cell-free DNA in plasma and serum after sex-mismatched bone marrow transplantation. *Clin. Chem.* **48:** 421–427.
16. Tong, Y.K. *et al*. 2006. Noninvasive prenatal detection of fetal trisomy 18 by epigenetic allelic ratio analysis in maternal plasma: theoretical and empirical considerations. *Clin. Chem.* **52:** 2194–2202.
17. Grunau, C., S.J. Clark & A. Rosenthal. 2001. Bisulfite genomic sequencing: systematic investigation of critical experimental parameters. *Nucleic Acids Res.* **29:** E65–65.
18. Old, R.W. *et al*. 2007. Candidate epigenetic biomarkers for non-invasive prenatal diagnosis of Down syndrome. *Reprod. Biomed. Online* **15:** 227–235.
19. Ng, E.K.O. *et al*. 2003. mRNA of placental origin is readily detectable in maternal plasma. *Proc. Natl. Acad. Sci. USA* **100:** 4748–4753.
20. Lo, Y.M.D. *et al*. 2007. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. *Nat. Med.* **13:** 218–223.
21. Lo, Y.M.D. *et al*. 2007. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. *Proc. Natl. Acad. Sci. USA* **104:** 13116–13121.
22. Warren, L. *et al*. 2006. Transcription factor profiling in individual hematopoietic progenitors by digital RT-PCR. *Proc. Natl. Acad. Sci. USA* **103:** 17807–17812.
23. Diehl, F. *et al*. 2005. Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proc. Natl. Acad. Sci. USA* **102:** 16368–16373.