Review

CellPress

*Special Issue: Nurturing the Next Generation*

# Noninvasive fetal genomic, methylomic, and transcriptomic analyses using maternal plasma and clinical implications

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**The discovery of cell-free fetal DNA in maternal plasma opened up new possibilities for noninvasive prenatal testing (NIPT). Conceptual advances in single-molecule counting have resulted in robust methods for the NIPT of fetal chromosomal aneuploidies and subchromosomal aberrations. Such methods are employed worldwide and are among the most rapidly adopted genomic tests. Furthermore, approaches for fetal whole-genome sequencing from maternal plasma, as well as for targeted detection of many single-gene disorders, have been reported. Recently, fetal methylome and transcriptome sequencing from maternal plasma have also been achieved, potentially allowing fetal physiological and pathological processes to be monitored noninvasively using maternal blood. These advances herald exciting future applications in prenatal medicine.**

## Discovery of cell-free fetal DNA in maternal plasma and analytical challenges

In 1997 the presence of cell-free fetal DNA in maternal blood was demonstrated by using PCR to amplify a chromosome Y-specific sequence from the plasma and serum of pregnant women carrying a male fetus [1]. This discovery was the beginning of a new area of research, with exciting implications for noninvasive prenatal testing.

Since then many aspects of the biology of cell-free fetal DNA have been elucidated. Cell-free fetal DNA has been found to be fragmented (smaller than 200 bp) [2,3], cleared shortly after delivery [4], and to exhibit highly-variable inter-individual concentrations that generally increase with gestational age. Fetus-derived DNA has been identified by a variety of fetus- specific markers, such as chromosome Y-specific sequences [1], epigenetic markers [5,6], and SNPs [7]. One major challenge in the noninvasive analysis of cell-free fetal DNA in maternal plasma is that it would generally need to be performed in the presence of a high background of maternal DNA. However, even with this latter difficulty, the analysis of cell-free fetal DNA has quickly made its way into clinical applications. With the advent of single-molecule counting, several investigators have described the noninvasive prenatal testing of aneuploidies [8–10], subchromosomal aberrations [11], and monogenic diseases [12,13]. These developments have rapidly impacted upon prenatal medical practice. Furthermore, recent developments in the field have allowed noninvasive prenatal determination of fetal methylomic [14] and transcriptomic profiles [15], thus further opening up exciting research and clinical possibilities. Figure 1 summarizes the timeline of several developments in the field.

## Fetal genomic analysis

### *Chromosomal aneuploidy detection*

Aneuploidy refers to conditions where an extra or a missing copy of a chromosome is found in the genome. The presence

### Glossary

***De novo* mutation:** germline genomic variants present in the fetus but not present in either parent.
**Digital PCR:** a PCR technology that involves the dilution of a DNA sample or the compartmentalization of the sample into multiple compartments, and subjecting each diluted aliquot or compartment to a PCR, with multiple PCRs being carried out simultaneously. The degree of dilution or the number of compartments is such that each PCR would characteristically only contain one or zero copies of the target molecule. Counting of the number of positive PCRs then allows the starting number of target DNA molecules in the sample to be calculated.
**Haplotype:** the combination of a group of alleles at polymorphic loci on a chromosome arm.
**Massively parallel sequencing (MPS):** a high-throughput DNA sequencing method in which multiple parallel sequencing reactions (typically on the order of millions, or even billions) are conducted within a small area, such as on a glass slide or in an array of reaction wells.
**Molecular karyotyping:** the genome-wide scanning of molecular aberrations within the human genome, such that information analogous to that conventional cytogenetic analysis can be obtained.
**Noninvasive prenatal testing (NIPT):** typically refers to the new generation of prenatal tests that are based on the analysis of cell-free fetal DNA in maternal plasma.
**SNP:** the most common type of genetic variation in the human genome. SNPs represent a difference in the DNA sequence at a single nucleotide position.

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*Keywords:* noninvasive prenatal diagnosis; fetal epigenome; fetal transcriptome; massively parallel sequencing; cell-free DNA.

1471-4914/
 http://dx.doi.org/10.1016/j.molmed.2014.12.006

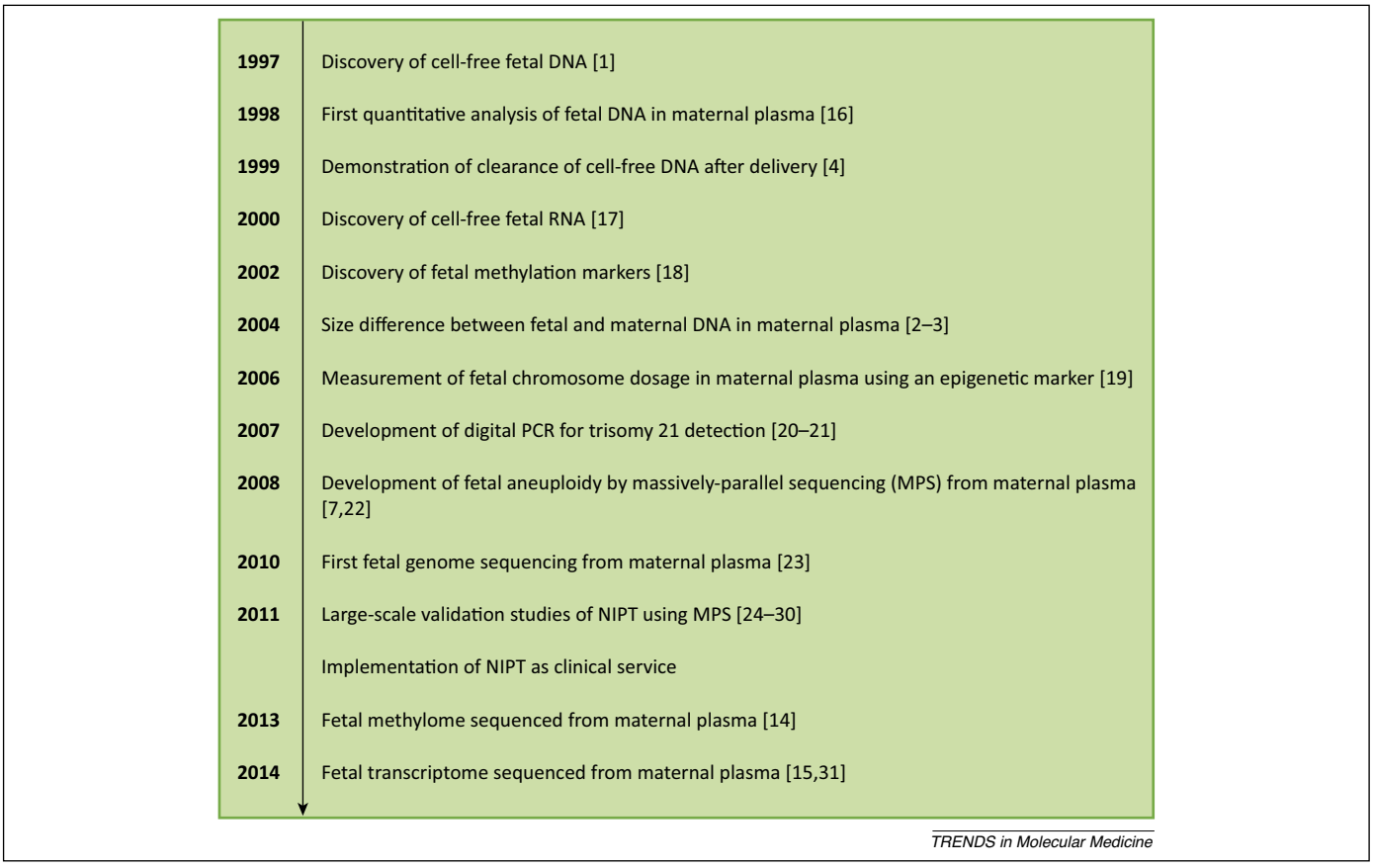


**Figure 1**. Timeline of key developments of nucleic acid based noninvasive prenatal testing. Abbreviations: MPS, massively parallel sequencing; NIPT, noninvasive prenatal testing.

of an extra chromosome is termed trisomy, whereas the lack of a single chromosome copy is known as monosomy. Trisomy 21 is the most common chromosomal aneuploidy, occurring in 1 of 750 newborn babies. Trisomic babies who are born can have lifelong medical problems. Hence, one of the major reasons for undergoing prenatal diagnosis is for assessment of fetal trisomy 21 status. Conventional prenatal diagnostic tests require invasive sampling of fetal tissues using amniocentesis or chorionic villus sampling. These procedures, however, carry a miscarriage risk of 0.5–1% [16]. Therefore, a noninvasive method to detect trisomy 21 would prevent unnecessary risk of physical harm to the fetus. Noninvasive screening procedures typically involve ultrasonography combined with the measurement of proteins and hormones in maternal blood. However, many screening protocols using these parameters have false-positive rates as high as 3–5% [17]. Such false-positive results would have required many pregnant women to undergo further unnecessary invasive prenatal diagnostic tests.

Cell-free fetal DNA in maternal blood has opened up a new possibility for noninvasive prenatal testing (NIPT; see Glossary) because it allows access to fetal genetic material that can be obtained safely. A trisomy 21 fetus with three copies of chromosome 21, rather than two copies, releases proportionally more chromosome 21 DNA into maternal plasma than does a euploid fetus. In 2007, two reports detailed an approach that can be used for detecting the small increase in chromosome 21-derived genetic material in maternal plasma in trisomy 21 pregnancies using digital PCR [18,19]. The investigators reasoned that the degree of increase would be dependent on the fractional concentration of fetal DNA in maternal plasma. They also deduced that, as the fractional concentration of fetal DNA decreases, more DNA molecules would need to be counted [18,19]. However, because digital PCR only amplifies DNA molecules in maternal plasma that have binding sites for the predetermined PCR primers, most plasma DNA molecules, which do not have the primer binding sites, are not analyzed and thus the diagnostic information contained in them is wasted.

In 2008, two further reports outlined the use of random massively-parallel sequencing (MPS) of maternal plasma DNA, in which the diagnostic information contained in any plasma DNA molecule with sequences that can be mapped back to the human genome can be used [8,20]. Instead of isolating fetus-specific reads or looking for fetus-specific markers, they counted all the plasma DNA reads (i.e., maternal and fetal) for sequencing, and with alignment methods the chromosomal location of each DNA molecule can be ascertained. The genomic representation (GR) of chromosome 21 is the proportion of plasma sequencing reads aligned to chromosome 21 over the total number of aligned reads. Comparing this GR with a group of normal controls, the chromosome 21 GR will be increased if the fetus has trisomy 21 (Figure 2A).

Other than this random MPS approach, targeted sequencing approaches based on SNPs have also been developed [10,21,22]. One variant involves the use of hybridization-based capture of the genomic regions of interest, followed