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Review

Cell-free DNA screening for fetal aneuploidy as a clinical service ☆

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ABSTRACT

Non-invasive prenatal testing (NIPT) through the analysis of cell free (cf)DNA is revolutionizing prenatal screening for fetal aneuploidy. Current methods used in clinical practice include shotgun massively parallel sequencing (s-MPS); targeted (t-MPS); and an approach that takes advantage of single nucleotide polymorphism (SNP) differences between mother and fetus. Efficacy of cfDNA testing for the common autosomal trisomies far exceeds that of conventional screening. Depending on the methodology used, reasons for discordancy between cfDNA results and fetal karyotype can include true fetal mosaicism, confined placental mosaicism, presence of a maternal karyotype abnormality, insufficient counting due to low fetal fraction, and a vanishing twin. Among the possible cfDNA strategies a Primary test has the highest performance but is expensive, while a Contingent cfDNA test can achieve high performance at a relatively low cost. Practicalities to be considered in the provision of testing include pretest counseling about the scope and accuracy of the testing, the interpretation of results when there is a low fetal fraction and follow-up studies for positive test results. The role of first trimester nuchal translucency measurement and conventional biochemical testing needs to be reassessed in the context of the use of cfDNA.

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Introduction

Testing maternal plasma for cell free (cf)DNA represents the latest progression in a series of technologies developed and implemented since the 1980s for use in fetal aneuploidy screening. Although the latest technology has considerably superior performance to earlier methods, the aim is the same, namely, the identification of pregnancies at sufficiently high risk of aneuploidy to warrant invasive prenatal diagnosis. This involves either second trimester amniocentesis or first trimester chorionic villus sampling (CVS). Both procedures are associated with fetal loss and screening strategies have been designed to maximize aneuploidy detection while minimizing the proportion of women offered invasive testing.

Until recently, most developed countries had either adopted the first trimester Combined test as the preferred strategy or were in the process of doing so. Some had also introduced sequential protocols such as the Contingent test, which build on Combined test markers and offer second trimester serum markers or more detailed first trimester ultrasound to a large subgroup of women with borderline Combined test risks. Others had extended the Combined test by incorporating concurrent additional serum and ultrasound markers.

cfDNA testing is also widely referred to as non-invasive prenatal testing (NIPT). Improved screening strategies using this test alone or in combination with existing screening tests are rapidly replacing older protocols and revolutionizing prenatal screening for aneuploidy. However, these developments involve a number of practical issues. In this paper these issues are discussed and information for those planning or delivering such a service is provided.

cfDNA testing methods

There are currently three broad cfDNA testing methods available: shotgun (genome-wide) massively parallel sequencing (s-MPS); targeted (t-)MPS that focuses on specific chromosomes of interest; and an approach that takes advantage of single nucleotide polymorphism (SNP) differences between mother and fetus. Laboratories differ in the bioinformatics that are used to classify results as 'positive' or 'high risk'.

s-MPS relies on identification and counting of large numbers of DNA fragments in plasma specimens. MPS is used to simultaneously sequence millions of genome-wide fetal and maternal fragments and informative sequences are mapped to discrete loci on all chromosomes [1,2]. If fetal trisomy is present, there will be a relative excess of counts for a given chromosome and with a monosomy deficit. Large numbers of counts are necessary since in most cases the fetal fraction (FF) of cfDNA is low and the excess or deficit in the assigned DNA fragments is small. The observed distribution of counts between chromosomes is compared with the expected distribution for euploid cases. Variant bioinformatics include a z-score; a likelihood ratio; [3] and adjustment for guanine–cytosine base content of the sequences, as well as the use of moving averages to smooth data [4,5].

t-MPS is similar to s-MPS insofar as it uses sequencing but also selectively enriches for chromosomal regions of interest (e.g. 21, 18, 13, X and Y) and counts this subset [6]. A patient-specific risk score can be generated by adjusting for the FF and then combines the results with maternal and gestational ages [6].

The SNP approach takes advantage of DNA polymorphic differences between the mother and fetus by comparing buffy coat (maternal), and maternal plasma (maternal and fetal) [7]. Inclusion of a sample (blood or saliva) from the father is helpful but not essential. A multiplex PCR amplification of nearly 20,000 SNP sequences is carried out in a single reaction followed by sequencing. Each product is evaluated based on the hypothesis that the fetus has trisomy, monosomy or is euploid. After considering the positions of the SNPs on the chromosomes and the possibility that there may have been recombination, a maximum likelihood is calculated for each option. Results are presented as risk scores.

The various cfDNA tests available should not be expected to be equivalent; [8] for example, counting methods (s-MPS and t-MPS) that involve greater depth of sequencing should have greater efficacy when FF is low or when testing for small copy number variations. Of these two counting methods, t-MPS could potentially involve greater depth of sequencing for the chromosomes of interest while requiring considerably less total sequencing and thereby potentially lower cost.

The SNP method has several advantages. It can exclude imbalances that are maternal as well as identify additional haplotypes that may be indicative of undetected multiple pregnancies. It can provide information about parent of origin of aneuploidy and, in theory, recombination. It can also detect diandric triploidy, non-paternity, consanguinity, and uniparental disomy. In ART pregnancies using an unrelated egg donor, account would need to be taken of extra fetal alleles that are not present in the surrogate mother.

Performance for common autosomal trisomies

The performance of an aneuploidy screening test can be judged by three parameters: the detection rate (DR), the proportion of affected pregnancies with 'positive' screening results; the false-positive rate (FPR), the proportion of euploid pregnancies with positive results; and, the positive predictive value (PPV), the risk of

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