Cell-Free DNA

Screening for Single-Gene Disorders and Determination of Fetal Rhesus D Genotype

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KEYWORDS

• Prenatal diagnosis • cfDNA • Noninvasive prenatal testing • Single-gene disorders • Prenatal screening • Rhesus D genotype

KEY POINTS

- The use of cell-free DNA (cfDNA) for diagnosis of single-gene disorders is an evolving technology, and its application is limited at this time.
- The limitations of cfDNA technology are most notable in clinical settings involving X-linked and autosomal recessive conditions, in part because background maternal mutant alleles greatly outnumber those of fetal origin.
- Examples of single-gene disorders where cfDNA has been used include rhesus D genotyping, skeletal dysplasias, congenital adrenal hyperplasia, and β-thalassemia.
- Patients undergoing prenatal diagnosis for evaluation for single-gene testing should undergo invasive testing.
- Determination of fetal rhesus D genotype with cfDNA is highly accurate with sensitivities above 99% and very low false-negative rates.

INTRODUCTION

The introduction of cell-free DNA (cfDNA) into the prenatal arena in 2011 has revolutionized prenatal screening for fetal aneuploidy. The use of cfDNA is also being investigated in the use of screening for single-gene disorders. The phenotypes of conditions caused by single-gene disorders are highly variable and depend on the specific gene location as well as the amount of genetic material that is duplicated or deleted. The use of expanded carrier screening panels will result in an increased number of couples identified at risk for having an offspring affected with a single-gene disorder. Family history may influence an individual's predisposition to single-gene disorders. Advanced paternal age is associated with an increased risk of de novo dominant

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single-gene mutations, including achondroplasia, neurofibromatosis, Marfan syndrome, osteogenesis imperfect, and Apert syndrome. X-linked disorders associated with advanced paternal age in the maternal grandfather include fragile X, hemophilia B, and Duchenne muscular dystrophy. Experts advise that patients should undergo invasive testing for prenatal diagnosis of single-gene disorders. This article provides an overview of the status of cfDNA screening for single-gene disorders as well as an overview of the use of cell-free DNA in rhesus D (RhD) genotyping.

CELL-FREE DNA

During pregnancy, placental tissue undergoes continuous turnover of the villous trophoblast, thereby releasing apoptotic debris and cfDNA into the maternal circulation. Although cfDNA is often referred to as fetal, the genetic material derives from the placenta and circulates in the maternal plasma as short random genomic DNA fragments of 150 to 200 base pairs.[1–4] The fetal fraction or percentage of circulating DNA that is contributed by the fetus, is 3% to 13% of total cfDNA in the maternal circulation.[5,6] The fetal fraction increases with gestational age and is undetectable within hours after delivery.[7]

Identification of cfDNA in the maternal serum prompted a robust scientific investigation into its origin and potential clinical applications.[8–12] Noninvasive prenatal testing or cfDNA screening subsequently evolved as a powerful screening tool for the common autosomal and sex chromosome aneuploidies.

Screening Technology and Reporting

Various methodologies for analysis of cfDNA screening include massively parallel shotgun sequencing, targeted massively parallel sequencing, and single-nucleotide polymorphism–based approaches.[13–16] The technology uses cfDNA to screen for fetal aneuploidies. Overall, the detection rate for cfDNA screening for aneuploidy is 99.4% with a false-positive rate of 0.16%.[17] All modalities carry a high sensitivity and specificity rate for trisomy 21 and 13. A recent meta-analysis reported pooled detection rates for trisomy 21 at 99.7% with a false-positive rate of 0.04%.[18] Similarly, pooled detection rates for trisomy 13 were cited at 99.0% with a false-positive rate of 0.04%. For trisomy 18, however, these rates were slightly lower with a pooled detection rate of 97.9% with a false-positive rate of 0.04%.

Reporting of results can vary depending on laboratory, with some reporting risk as positive or negative, whereas others cite specific risk of aneuploidy using a numeric value. Positive and negative predictive values, however, are apt to be more meaningful from a clinical standpoint and depend on the prevalence of the condition in the screened population; higher disease prevalence results in higher positive predictive value. When applied to aneuploidy screening, positive predictive values are higher among older women given the increasing prevalence of the trisomic aneuploidies with maternal age. On this basis, the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine recommend that all cfDNA screening results include report of positive predictive and residual risk values.[6]

Occasionally, test results are indeterminate. Such no call results may occur in the setting of a low fetal fraction, aneuploidy, maternal obesity, treatment with low-molecular-weight heparin, or states of high cell turnover, or consanguinity.[19–23] Although low fetal fraction may be attributed to early gestational age, it has also been associated with pregnancies affected by aneuploidy; rates of aneuploidy among patients with indeterminate results have been reported as high as 23%.[24] Thus, a no