Prenatal screening for common aneuploidies using cell-free DNA

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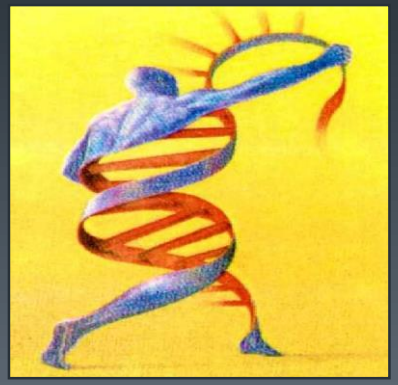
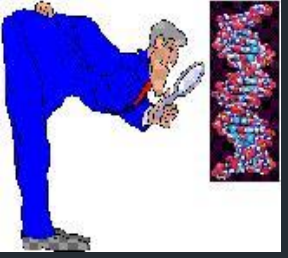


Introduction

- ✓Prenatal screening for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome), and selected sex chromosome aneuploidies can be performed using next-generation sequencing of cell-free DNA (cfDNA) in the maternal circulation.
- ✓Circulating cfDNA is derived from both the mother and the fetal-placental unit and cleared from the maternal circulation soon after delivery

Introduction

- ✓Although this approach is often called "noninvasive prenatal screening" (NIPS) or "noninvasive prenatal testing" (NIPT), these terms are nonspeci!c, as conventional serum screening tests, such as the second-trimester quadruple test or the first-trimester combined test, are also noninvasive.



Cell-free DNA

- ✓The cfDNA test provides excellent performance for women with successful testing (at least 99 percent of Down syndrome pregnancies are detected with a screen-positive rate less than 1 per 1000, <0.1 percent). However, it is still considered a **screening** test due to infrequent false-positive and false- negative results. An invasive procedure (eg, amniocentesis or chorionic villus sampling) and subsequent karyotyping or microarray analysis are considered the gold standard **diagnostic** tests and should be o"ered to women who are screen positive by cfDNA testing.

Cell-free DNA

- ✓Both the mother and the fetal-placental unit produce cfDNA. The primary source of so-called "fetal" cfDNA in the maternal circulation is thought to be apoptosis of placental cells (syncytiotrophoblast), while maternal hematopoietic cells are the source of most maternal cfDNA [1-3]. A lesser source is apoptosis of fetal erythroblasts generating cfDNA in the fetal circulation, and these fragments can cross the placenta and enter the maternal circulation [1,5,6]

Fetal fraction

- ✓ Fetal-placental cfDNA can be detected in maternal blood as early as !ve weeks of gestation and almost always by nine weeks of gestation.
- ✓The relative concentration of fetal cfDNA increases modestly (0.1 percent per week) with gestational age from 10 to approximately 20 weeks, and then increases rapidly (1 percent per week) until term

A low fetal fraction may be due to:

- ✓**Early gestational age**
- ✓**Suboptimal sample collection**
- ✓**Obesity**
- ✓**Fetal karyotype**
- ✓**Other less common factors**
  - ❑In addition, a low fetal fraction has been attributed to maternal
  - ❑use of low molecular weight heparin before 20 weeks of gestation and pregnancies achieved by in vitro fertilization
  - ❑The per fetus fetal fraction is also lower in twin gestation.

SCREENING PERFORMANCE

- ✓Trisomy 21 – DR 99.5 percent,
- ✓Trisomy 18 – DR 97.7 percent,
- ✓Trisomy 13 – DR 96.1 percent,

Sex chromosome aneuploidies

- ✓For sex chromosome aneuploidies, cfDNA detection rates are lower and FPR rates are higher than for the common autosomal trisomies. In the largest meta-analysis that evaluated cfDNA test performance for sex chromosome aneuploidies, the DR and FPR for monosomy X (177 cases and 9079 controls) were 90.3 and 0.23 percent, respectively.
- ✓For the sex chromosome trisomies 47,XXX; 47,XXY; and 47,XYY (56 cases and 6699 controls), the DR and FPR were 93.0 and 0.14 percent, respectively.

Rates and reasons for cfDNA test failures

- ✓The most common reasons for test failure include less than a speci!ed absolute amount of total and/or fetal-placental DNA, fetal fraction below an acceptable level (eg, <3.5 or <4.0 percent), and insuffcient numbers of fragments sequenced and/or aligned.
- ✓Low fetal fraction may be responsible for up to 50 percent of all failures, depending on methodology. Increasing data show that the fetal fraction is lower and the test failure rate is approximately two or three times higher for in vitro fertilization

Reasons for false-positive and false-negative results

a) **Confined placental mosaicism**
b) **Demised twin**
c) Maternal mosaicism
d) Maternal cancer
e) Maternal copy number variants
f) Chance
g) Technical issues
h) Transplant recipient
i) Recent blood transfusion

False negative cfDNA test results

- ✓Confined placental mosaicism
- ✓Borderline low fetal fraction
- ✓Maternal copy number variants
- ✓Technical issues

CLINICAL USE

- ✓Primary screening
- ✓Secondary screening

POST TEST FOLLOW UP

- Screen positive
  - Amniocentises

  or
  - cvs
- Screen negative
- No call or No result
  - Repeat the cfDNA test as soon as possible, if allowed by the laboratory (some failures, like large regions of homozygosity, will always cause the test to fail, and repeat testing is not an option). Repeat testing, when allowed, is successful in approximately 60 to 80 percent of cases .
  - ● Standard serum marker/ultrasound screening.
  - Invasive tests