Detecting Microdeletion Syndromes: Next Step of Non-Invasive Prenatal Testing (NIPT)

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INTRODUCTION

Prenatal screening and diagnostic tests are well-established procedures for fetal monitoring during the pregnancy.

- Non-Invasive techniques:
  - Ultrasound and maternal serum screening
  - Assessment of pregnant women at risk of having a fetus with chromosomal abnormalities
  - Limited sensitivity and high false-positive rate.

- Invasive procedures:
  - Amniocentesis and chorionic villus sampling (CVS)
  - Provide fetal tissue for genetic diagnosis
  - Significant risk of fetal loss (1%)

Many researchers have focused on finding alternative sources of fetal genetic material. The discovery, in 1997, of circulating cell-free fetal DNA (cffDNA) in maternal plasma opened up the field of non-invasive prenatal testing (NIPT).

NIPT: TECHNIQUES AND APPLICATIONS

Chromosomal Anomalies

First strategies to quantify chromosomal dosage:

- Epigenetic allelic ratio (EAR) approach: quantification of the maspin gene promoter, which is located in chromosome 18 and has differential methylation pattern between placentas and maternal blood cells.
- Polyphthalic allelic ratio analysis on FPG in blood, a placental-specific miRNA transcribed from a chromosome 22 gene.
- Digital relative chromosome dosage (RDY) analysis. A polymorphism-independent approach to compare chromosome 21 dosage from trisomy 21 fetuses with a reference sample.
- Several limitations, such as the lack of reproducibility, complex experimental and data analysis procedures and suboptimal diagnostic results.
- MPS technology for NIPT of chromosomal Anomalies.
- Large-scale clinical trials reported a sensitivity >99.6% and specificity >97.9%.
- NIPT for chromosomal aneuploidy is clinically available from 2011.
- Professionals societies recommend it for screening of fetal aneuploidy in high-risk pregnant women.
- Patients with positive NIPT results are recommended to undergo an invasive diagnostic procedure for an confirmatory diagnosis.

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Fetal Sex Determination

- Ultrasound can be helpful to confirm fetal sex in the second trimester, except when there is a suspicion of fetal ambiguity.
- Clinically indicated to those pregnant women at risk of having a fetus with a X-linked genetic disorder.
- NIPT of fetal sex determination was rapidly adopted into clinical practice. It is reliable from 7th week of gestation.
- Technique: Real-time PCR to detect Y chromosome sequence (SPY or DYS4 in maternal plasma).
- Sensitivity and specificity are 95.4% and 98.6%, respectively.
- A positive result of this test suggests that the pregnancy involves a male fetus, who is at risk of a sex abnormality.
- Clinical practice. It is a reliable and widely used approach for fetal sex determination.

RHD Genotyping

- Indicated in pregnancies of RHD negative women whose partners are heterozygous for the RHD gene. In cases of RHD positive, there is a high risk of mother sensitization and attacks fetal blood, causing an hemolytic disease.
- Nowadays, many countries are performing NIH for RHD genotyping to assess the real need of anti-Rh immunoglobulin as a prophylactic treatment.
- Technique: multiplex real-time PCR targeting multiple RHD exon and SRY gene sequence.
- Sensitivity of 99.4%

Single-gene disorders

The implementation of NIPT has not been as easy and fast as in the previous applications.

First approaches:

- In-paternal inherited autosomal dominant diseases and autosomal recessive diseases:
  - PCR assay to detect mutant paternally inherited alleles.
  - When both mother and father carry the same mutant allele:
    - Determination of the paternally inherited wild-type allele by detecting paternal specific polymorphisms in disease locus.
- In-maternally inherited autosomal dominant diseases:
  - Digital relative mutation dosage (RDY) approach: Detecting the ratio between mutant alleles and wild-type alleles in maternal plasma.

Development of MPS technologies:

- At the present, the genome-wide profile of the fetus can be deduced from the maternal plasma.
- It is possible to screen all exons and gene-related targeted genes can be analyzed in the same sample.

NEXT STEP: Microdeletion Syndromes

Clinically significant microdeletions and duplications occur in 1-2% of all structurally normal pregnancies and they are independent of maternal age.

Deep sequencing approaches:

- Detection of 4–5 Mb deletions on chromosome 21 in maternal plasma samples obtained at the 15th week of gestation (Peters et al. 2013).
- Detection of significant decrease in the representation of 3 Mb, corresponding to 22q11.2 region (Di George syndrome). (Jensen et al. 2012).
- Detection of seven cases of microdeletions, duplications, translocations and trisomy 20 in a 100 kb resolution. (Srinivasan et al. 2013).
- Low coverage MPS assay with ESPS bioinformatics method.
- Detecting large-scale deletions/duplications in maternal plasma of >10 Mb in fetal genome with a 100% sensitivity and 99.92% of specificity. (Chen et al. 2012).
- Targeted SNP-based approach. (Opper et al. 2013).
- Detection of 5 the microdeletions syndromes that have more severe phenotype: 22q11.2 (Di George syndrome), 1p36, Cri-du-chat, Prader-Willi, and Angelman deletions.
- Technique: targeted multiplex PCR, sequencing and NATUS algorithm.

- High coverage of sequence reads, clinically validate because of the high costs.
- Selection of specific genes and pathways.
- Single nucleotide variants (SNVs), insertion-deletions (Indels), and copy number variations

CONCLUSIONS

- Since the discovery of the cffDNA in maternal plasma in 1997, the field of noninvasive prenatal testing has suffered a very fast development. At the present, NIPT for fetal sex determination and fetal RHD genotyping, based on a real-time PCR assay, are clinically well established. As mentioned before, the extremely fast development of the MPS technology brought to clinical practice the application of NIPT for some single-gene disorders and fetal aneuploidies. Furthermore, in the near future, the detection of fetal subchromosomal abnormalities by NIPT would also be translated into clinical practice after large-scale clinical trial validation.

REFERENCES


Figure 1. Overview of the key developments in the field of NIPT. Adapted from Romo et al. (2016)