Non-invasive prenatal diagnosis using Massively Parallel Sequencing of cell-free DNA in maternal blood

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Introduction

The risk of miscarriage of invasive prenatal tests, like amniocentesis or chorionic villus sampling, has led to the development of non-invasive prenatal tests. These tests are only screening tests but they assess the risk of carrying a fetus with an anomaly, reducing the number of invasive tests that have to be performed. In 1997, the presence of fetal cell-free DNA (cfDNA) in maternal plasma during pregnancy was demonstrated (approx. 10% of total cfDNA in maternal plasma) and, since then, a variety of methods have been used to analyse it as a prenatal test. The most used method nowadays is Massively Parallel Sequencing (MPS).

OBJECTIVE: to do a bibliographic research to describe the MPS technique and its applications in prenatal diagnosis, the kind of anomalies that can be detected, the advantages and limitations of this method and the future expectations.

What is Massively Parallel Sequencing?

MPS: DNA sequencing technique that allows a large-scale production of genomic sequences (reads) rapidly and simultaneously in a single run.

MPS used for the study of cfDNA in maternal plasma:

The approach is based in comparing the number of reads mapping to a chromosome of interest with the number of reads mapping to normal chromosomes of reference (known as counting). There is the need to determine a threshold to stabilise the quantity of reads at which one chromosome is considered over- or under-represented.

What anomalies can be detected?

QUANTITATIVE ANOMALIES

- Sex
- Aneuploidies
- Trisomy 21 (+ Robertsonian translocations)
- Trisomy 18
- Trisomy 13
- Sex chromosomes aneuploidies

What are the advantages and limitations?

ADVANTAGES

- There is no need to differentiate between the fetal and the maternal DNA
- Can detect all the common aneuploidies in just one test
- Can be performed with small fractions of fetal cfDNA in maternal plasma
- Can be done after 10 weeks of gestation (1r trimester)
- Applicable in all pregnancies and independent of the gender of the fetus
- Has a high sensitivity (true positive rate ≥90%) and specificity (true negative rate ≥99%)
- Has a very low false positive rate (1-3%) and false negative rate (<1%) → however, they have to be considered in every case

LIMITATIONS

- High cost and time consuming technique
- There is a bias due to the GC content of every chromosome (chromosomes are amplified faster or more slowly depending on the GC content) → can be corrected using bioinformatics algorithms
- Cannot detect polyploidies or single gene disorders (qualitative anomalies)
- Difficulties to detect mosaicism cases (placental, fetal or maternal mosaicisms)
- Some difficulties with multiple pregnancies
- This techniques is only a SCREENING TEST → positive results have to be confirmed by invasive procedures

What can be expected in the future?

Targeted sequencing

- Sequencing only the chromosomes of interest.
  - Lower cost
  - Only detects anomalies in the chromosomes studied and has lower sensitivity and specificity

Subchromosomal abnormalities

- Detection of abnormalities that only affect a part of the chromosome.
  - Detection at a higher resolution than traditional karyotyping
  - Requires high fractions of fetal cfDNA

Molecular karyotype

- Determination of the ploidy of every chromosomes region.
  - Study of deletions or duplications among all the genome
  - Requires a higher throughput increasing the cost

Whole-genome sequencing

- Sequencing all the fetal genome.
  - Detection of both quantitative and qualitative anomalies.
  - Requires the genetic maps of the mother and the father and has a high cost

Conclusions

- MPS is a very effective technique for the analysis of quantitative abnormalities in the fetus.
- It is indicated in pregnant women with an increased risk of carrying a fetus with an aneuploidy, but not in average risk mothers.
- Allows a significant reduction in high risk women who will have to undergo invasive tests.
- It implies some ethical concerns among a part of the population.
- Improvements in the technique are expected to turn it into a DIAGNOSTIC TEST.

References