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

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Introduction

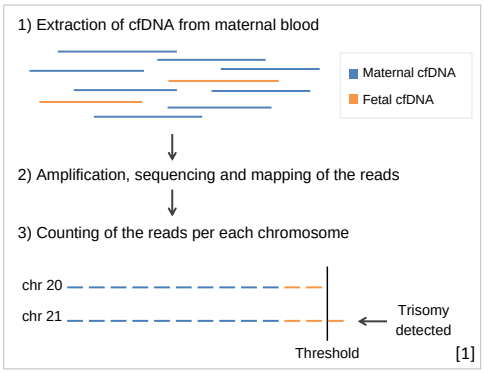
The risk of miscarriage of invasive prenatal tests, like amniocentesis or chorionic villus sampling, has led to the development of non-invasive prenatal tests. These tests are only screening tests but they assess the risk of carrying a fetus with an anomaly, reducing the number of invasive tests that have to be performed. In 1997, the presence of fetal cell-free DNA (cfDNA) in maternal plasma during pregnancy was demonstrated (aprox. 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.

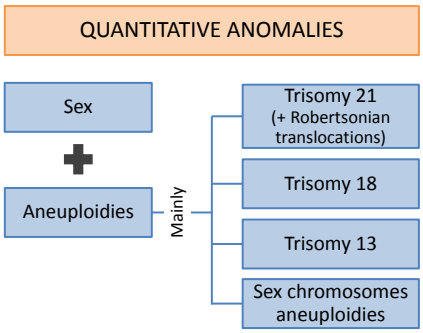
What is Massively Parallel Sequencing?

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 stablish the quantity of reads at which one chromosome is considered over- or under-represented.



What anomalies can be detected?



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

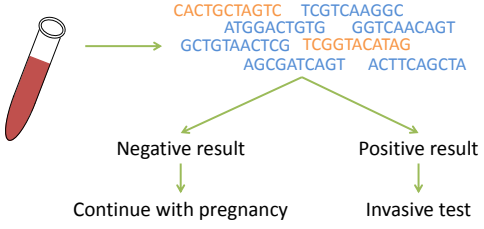
Subchromosomal abnormalities
Detection of abnormalities that only affect a part of the chromosome.
✓ Detection at a higher resolution than traditional karyotyping
✗ Requires high fractions of fetal cfDNA

Molecular karyotype
Determination of the ploidy of every chromosomic 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

[1] Adaptation of Norwitz ER, Levy B. Noninvasive prenatal testing: the future is now. *Reviews in Obstetrics & Gynecology* (2013) 6(2): 48-62