

NONINVASIVE PRENATAL TESTING: DETECTION OF FETAL DNA IN MATERNAL PLASMA

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1. INTRODUCTION

All started in 1997 with the discovery of cell-free DNA (cfDNA) fragments of fetal Y-chromosome in the plasma of pregnant women with male fetuses. The door for the development of non-invasive prenatal testing (NIPT) using maternal blood was opened to be thoroughly passed through sparking a new phase of innovation.

NIPT, is a sophisticated blood test that examines fetal DNA in the maternal bloodstream to determine whether the baby is at risk of Down syndrome, extra sequences of chromosome 13, chromosome 18 or a sex chromosome abnormality, such as Turner syndrome. The testing can also be used to determine a baby's sex, rhesus (Rh) type and a whole genome recovery by massive parallel sequencing. Fetal DNA can be detected in as little as 10 µL of maternal plasma and serum from as early as four gestational weeks.

NIPT has higher detection rates than the invasive techniques and the risks are lower, that is why it is increasing the interest not only of doctors, researchers and parents but also of businessmen.

The American Congress of Obstetricians and Gynecologists and other organizations recommend cfDNA testing only for high-risk pregnancies, and specify that abnormal results should be confirmed by invasive testing before any action is taken.

2. MATERIALS AND METHODS

I did my bibliographic research using NCBI (national center for biotechnology information) searching for articles and reviews in PubMed data base. For the selection I only choose articles from 2012 and later with an exception for the ethical issues which is from 2010.

The search was limited to English language publications.

Key words: noninvasive prenatal testing, fetal DNA in maternal plasma

3. RESULTS

1. The Technique

There are currently two primary next generation sequencing approaches for gathering genetic data from cfDNA. The first, massively parallel shotgun sequencing (MPSS), sequences DNA fragments from the whole genome, whereas the second, targeted sequencing (TS), selectively sequences specific genomic regions.

For Aneuploidy

- **Allelic ratio:** Assume that both alleles are transcribed at an equal rate and quantify SNPs → RT-PCR and mass spectrometry

Restrictions: heterozygous fetuses and monosomies

- **Chromosome dose:** Next generation sequencing is the most promising and it works with the principal that in a normal fetus the ratio of two chromosomes should be 2:2. It is polymorphism-independent.

For X-linked diseases and Rh type

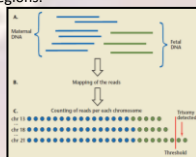
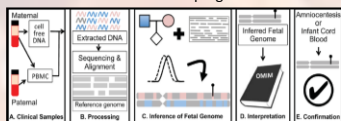
- **Sex determination for high risk of X-linked diseases:** TS of Y chromosome sequences, most commonly SRY or DYS14. It has reduced the need for invasive testing by 45%.

- **Determining Rh blood type:** Best results achieved by qRT-PCR for exons 4, 5 and 10 of RHD gene.

Whole fetal genome recovery

- The use of MPSS and the prediction about inheritance of **haplotype blocks** from parental genomes as they are not inherited as independent sites allows grater statistical power. Identification of fetal de novo mutations, microdeletions and genetic disease screening are possible.

Restrictions: epigenetic modifications and twin pregnancies



2. Data Analysis and Interpretation

The **z-score** reflects the number of standard deviations the proportion of reads from a particular chromosome (in relation to the proportion of reads from all other chromosomes) is above the mean.

$$Z\text{-score} > 2.5 \rightarrow \text{trisomy}$$

The score does not account for the variation in GC base content from chromosome caused by the different polymerase chain reaction conditions. To solve this, some approaches have been made:

- Z-score with GC correction
- Z-score with GC correction using an internal control

3. Commercial landscape

Company	Technical approach	Accuracy of detection		
		Anomaly	Sensitivity (%)	Specificity (%)
Sequenom	MPSS	T21	98.5	99.5
		T18	100	99.7
		T13	91.7	99.0
		45,X	--	--
		45,XXX	47	--
Verinata Health	MPSS	T21	100	100
		T18	97.2	99.8
		T13	78.6	99.4
		45,X	93.8	99.8
		45,XXX	--	--
Ariosa Diagnostics	TS+DANSR +FORTE	T21	100	100
		T18	98	100
		T13	--	--
		45,X	--	--
		45,XXX	--	--
Natera	TS+PS	T21	100	100
		T18	100	100
		T13	100	100
		45,X	100	100
		45,XXX	100	100



4. CONCLUSIONS

- NIPT is playing and will increasingly play an **important role** in the future practice of prenatal testing and it is in constant development.
- It has three **main applications**; X-linked diseases, Aneuploidies and Whole fetal Genome Recovery which is the most revolutionary and controversial
- **Data analysis and interpretation** are a key factor for the technique effectiveness and efficacy.
- **Competition between companies** can be a great incentive for the technique improvement but also a hurdle because of IP (intellectual propriety) issues.
- **MPSS** will be available and effective for all fetal aneuploidies, sub-chromosomal deletions and duplications, monogenic disorders and eventually the entire fetal genome.
- There is a need to address the **ethical, legal and social issues** surrounding such developments and the placental origin of the DNA.
- **Guidelines** from several professional societies now exist to aid women's healthcare providers in determining under which circumstances patients should be offered such testing.

5. REFERENCES

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