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

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INTRODUTION

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

Non-invasive techniques	• Ultrasound and maternal serum screening • Assesment pregnant women at risk of for several abnormalities (chromosomal aneuploidies, single-gene disorders • Limited sensitivity and high false-positive rates.
Invasive procedures	• Amnioscentesis and chorionic villus sampling (CVS) • Provide fetal tissue for genetic diagnosis • Significant risk of fetal loss (1%)

Many researchers have focused on finding alternative source 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).**

Cell-free fetal DNA in maternal plasma

- **ORIGIN:** CffDNA originates from the placenta tissue, the syncytiotrophoblast cells of the chorionic villi, which undergo apoptosis and release it into maternal circulation.
- **QUANTIFICATION:** CffDNA is present in maternal in a proportion of 10-20%. It is detected as early as the 7th week of gestation and its concentration increased as pregnancy progressed.
- **KINETICS:** The mean half-life of cffDNA concentration IS1 hour, and it IS undetectable at 1 or 2 days after delivery.
- **SIZE:** cffDNA fragment are significantly shorter (<313bp) than the background of circulating maternal DNA.

NIPT: TECHNIQUES AND APPLICATIONS

Fetal Sex Determination

- Ultrasound can be helpful to confirm fetal sex in the second trimester, except when there is a suspicion of genital ambiguity.
- Clinically indicated to those pregnant women at risk of having a fetus with a X-linked genetic disorders.
- 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 sequences (SRY or DYS14) 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-linked disease and should perform an invasive procedure for definitive diagnosis.
- A negative result would be indicative of carrying a female fetus, who would be able to avoid invasive techniques, because of not being at risk of the sex-linked disease.

RhD Genotyping

- Indicated n pregnancies of RhD negative women whose partners are heterozygous for the RhD gene. If the fetus is RhD positive, there is a risk that the mother gets sensitized and attacks the fetus blood, causing an hemolytic disease.
- Nowadays, many countries are performing NIPT for fetal RhD genotyping to assess the real need of anti-D immunoglobin us a prophylactic treatment.
- Technique: multiplex real-time PCR targeting multiple RhD exons 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 paternally inherited autosomal dominant diseases and autosomal recessive diseases:

- PCR assay to detect mutant paternally inherited alleles.
- When both mother and father carried different mutant alleles: Detection or absence of the paternal mutation in maternal plasma.
- When both mother and father carried 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 (RMD) approach: Detecting the ratio between mutant alleles and wild-type alleles in maternal plasma.

Development of MPS technology

- At the present, the genome-wide profile of the fetus can be deduced from the maternal plasma.
- It is possible to sequence a whole gene and also several targeted gene can be analyzed in the same sample.

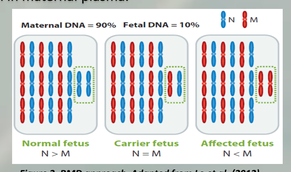

Figure 2. RMD approach. Adapted from Lo et al. (2012)

Chromosomal Aneuploidies

First strategies to quantify chromosomal dosage:

- Epigenetic allelic ratio (EAR) approach: quantification of maspin gene promoter, which is located in chromosome 18 and has differential methylation pattern between placenta and maternal blood cells.
- Polymorphism allelic ratio analysis on PLAC4 mRNA, a placental-specific mRNA transcribed from a chromosome 21 gene
- Digital relative chromosome dosage (RCD) analysis.: A polymorphisms-independent approach to compare chromosome 21 dosage from trisomy 21 fetuses with a reference sample.
- Several limitation, such as the lack of reproducibility, complex experimental and data analysis procedures and suboptimal diagnostic results.

MPS technology for NIPT of chormosomal Aneuploidies:

- Large-scale clinical trials reported a sensitivity >98,6% and specificity >97,9%.
- NIPT for chromosomal aneuploidies is clinically available from 2011.
- Professional societies recommend it for screening of fetal aneuploidies in high-risk pregnant women.
- Patients with positive NIPT results are recommended to undergo an invasive diagnostic procedures for an confirmatory diagnosis.

NEXT STEP: Microdeletion Syndromes

Clinically significant microdeletions and duplications occur in 1-1.7% of all structurally normal pregnancies and they are independent of maternal age.

Deep sequencing approaches:

- Detection of a 4.2Mb deletion on chromosome 12 in maternal plasma samples obtained at the 35th week of gestation (Peters et al. 2011)
- Detection of significant decrease in the representation of 3 Mb, corresponding 22q11.2 region (Di George syndrome). (Jensen et al. 2012)
- Detection of seven cases of microdeletions, duplications, translocations and trisomy 20.at a 100 kb resolution. (Srinivasan et al. 2013)

} high coverage of sequence reads, clinically suitable because of the high costs.

Low coverage MPS assay with FCAPS bioinformatics method

- Detecting fetal large deletions/duplications in maternal plasma of >10 Mb in fetal genome with a 100% of sensitivity and 99.92% of specificity. (Chen et al 2013)

Targeted SNP-based approach: (Wapner et al. 2015)

- Detection of the 5 microdeletion syndromes that have more severe phenotypes: 22q11.2 (Di George syndrome), 1p36, Cri-du-Chat, Prader-Willi and Angelman deletions.
- Technique: targeted multiplex PCR, sequencing and NATUS algorithm .
- Results:

Estimated positive predictive value and negative predictive value

Disorder	Incidence (1:n)	Frequency of deletion evaluated	Positive predictive value,[a] %	Negative predictive value,[b] %
22q11.2 del	2000	0.87	5.3	>99.99
Prader-Willi	10,000	0.28	4.6	>99.99
Angelman	12,000	0.28	3.8	>99.99
1p36 del	5000	0.60	17.0	>99.99
Cri-du-chat	20,000	0.65	5.3	>99.99

[a] Calculated by multiplying population incidence, the frequency of the deletion evaluated, and the positive likelihood ratio (detection rate/false-positive rate); [b] Calculated by multiplying population incidence, the frequency of the deletion evaluated, and the negative likelihood ratio ((1-detection rate)/(1-false-positive rate)).

Combined detection rate and false-positive rate for pregnancy plasmas and PlasmArt samples

	Effective detection rate[a]			False-positive rate		
Variable	n/N	95% CI	Net detection rate,[b] %	n/N	%	95% CI
22q11.2 del	45/47	95.7: 85.5—99.5	83.3	3/422	0.71	0.1—2.1
Larger deletions combined	61/63	96.8: 89.0—99.6	45.5	1/1813	0.06	0.0—0.3

Samples for which the algorithm did not receive a result are treated conservatively as negatives.

CI, confidence interval.

[a] Detection rates for the specific detected deletions; [b] Net detection rates for each syndrome that take into account the prevalence of each detected deletion.

Figure 3: Results of the SNP-targeted approach. Adapted from Wapner et al. (2015)

- Conclusion: Targeted method detects specifically microdeletions with high sensitivity with no need to increase the depth of sequence reads. Also, allows to focus the specific genomic regions with known clinical significance and avoids incidental findings. One important limitation for this approach is the lack of an important number of maternal plasma samples from affected pregnant women.

CONCLUSIONS

- Since the discovery of the cffDNA in maternal plasma in 1997, the field of noninvasive prenatal testing has suffered a very huge 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 next year, the detection of fetal subchromosomal abnormities by NIPT would also be translated into clinical practice after large-scale clinical trial validation.
- invasive procedure, such CVS and amniocentesis, are still necessary to make the definitive diagnostic when the NIPT show a positive result.
- Ethical and social issues about the consequences of NIPT have not been considered enough. Antenatal and specialist genetic services should be well trained in order to provide counseling to those couples at risk of carrying a fetus with a genetic condition.

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- 1997 — Discovery of cffDNA
- 1998 — First quantification of cffDNA
- 1999 — Description of rapid clearance of cffDNA after delivery
- 2001 — Introduction of fetal RhD genotyping NIPT in UK clinical service.
- 2002 — Discovery of fetal mathylation markerf
- 2003 — Detection of placental mRNA in maternal plasma
- 2004 — Size difference between fetal and maternal DNA
- 2005 — First unversal fetal marker (maspin gene)
- 2006 — Development of epigenetic allelic ratio method
- 2007 — Development of digital PCR for fetal aneuploidies
- 2008 — Development of MPS for aneuploidies and single-gene disorders
- 2010 — First fetal genome sequencing from maternal plasma
- 2011 — Large-scale validation of MPS for aneuploidies. Introduction in clinical service.
- 2014 — Detection of subchrmosomal abnormalities.

Figure I: Time-line of the key developments in the field of NIPT. Adapted from Lo et al. (2012)