Non-Invasive Prenatal Diagnosis

Damien Bruno, PhD
VCGS Pathology
Landmarks in NIPD Research

- **1893**: Schmorl shows presence of trophoblasts in maternal lung, where they form emboli
- **1979**: Herzenberg and Bianchi show evidence of fetal cells in maternal blood
- **1990**: Mueller et al. isolate fetal cells from maternal blood
- **1993**: Fetal RHD typing using maternal blood
- **1996**: Massively parallel sequencing of maternal plasma
- **2007**: PLAC4 – plasma RNA allelotyping approach
- **2008**: Liao et al. Targeted sequence capture Proof of Concept
- **2011**: MPS: Prenatal detection of trisomy 21 Validation Studies
- **2011**: Whole-Genome Fetal Genotyping using MPS

Lo et al. uses PCR to detect fetal DNA in maternal blood
Mueller et al. isolate fetal cells from maternal blood
PLAC4 – plasma RNA allelotyping approach
Liao et al. Targeted sequence capture Proof of Concept
MPS: Prenatal detection of trisomy 21 Validation Studies
Papageorgiou et al. methylated DNA immunoprecipitation
NIPD

• Background
• Clinical Applications (Current)

• Down syndrome
• the ‘fetal karyotype’
• the ‘fetal genome’
Current prenatal diagnosis requires invasive procedures
Combined first trimester screening / Second trimester screening

In 2003-2004, 52% of women in Victoria had a ‘prenatal test’:
  - 93% had a screening test
  - 7% had an invasive procedure

False negative rate: **0.02%** (Combined 1st TS), 0.05% (2nd TS)

False positive rate: **4.5%** (Combined 1st TS), 7.8% (2nd TS)

Sensitivity: 91.8% (Combined 1st TS), 72.7% (2nd TS)

Only 6.5% of increased risk results (Combined 1st TS) were confirmed with Down syndrome

Pregnancy losses / Down syndrome births
Fetal-Maternal Interface
NIPD: Current Clinical Applications

• Management of X-linked conditions by fetal sexing

• Screening for fetal RHD in RHD- women

• Some single gene disorders
cffDNA

Lo et al. 1998 Am J Hum Genet
Isolating cfDNA

- Isolate plasma (centrifugation, filtration)

- DNA isolation (20ng from 5ml blood)

- Take place as soon as possible after phlebotomy, to prevent breakdown of leucocytes and an increase in the proportion of maternal DNA
NIPD for DS

- Analysis of SNP allele ratios from cffRNA chromosome 21 transcripts using MALDI-TOF mass spectrometry (SEQUENOM) or digital PCR
- Relative chromosome dosage by digital PCR
- Analysis of fetal specific epigenetic markers
- Massively parallel whole genome sequencing of total maternal cell free plasma DNA
Microchimerism

cffRNA Allelotyping

Normal

SNP → A G

Fetal Genome

Trisomy

A G G

Transcription

cff-RNA in maternal blood

PLACENTAL RNA PLAC4

RT-PCR

Quantitation of Allelic Ratio

1:1

AG

1:2

G

A
Limitations / Concerns

- Stability of RNA
  - Intermediate fixation step
  - Logistics of transport service

- Sequenom Experience

- Test performance (as reported by Lo et al) approached that of current screening methods

- SNP informativeness / availability of markers
Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21

Elisavet A Papageorgiou¹, Alex Karagrigoriou², Evdokia Tsaliki¹,³,⁴, Voula Velissariou³, Nigel P Carter⁵ & Philippos C Patsalis¹

Figure 3 Box plot representation of the results obtained from four DMRs. EP1, EP4, EP7 and EP10 in 20 normal and 20 trisomy 21 cases. The box plots depict the five-number summaries, namely the minimum and maximum values, the upper (Q3) and lower (Q1) quartiles and the median. The median is identified by a line inside the box. The length of the box represents the interquartile range. Values more than three IQRs from either end of the box are labeled as extremes and are denoted by an asterisk (*). Values more than 1.5 IQRs but less than three IQRs from either end of the box are labeled as outliers (o).
MASSIVELY PARALLEL SEQUENCING
Optimal Detection of Fetal Chromosomal Abnormalities by Massively Parallel DNA Sequencing of Cell-Free Fetal DNA from Maternal Blood

Amy J. Sehnert, Brian Rhees, David Comstock, Eileen de Feo, Gabrielle Heilek, John Burke, and Richard P. Rava

Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study

Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting

Mathias Erich, MD; Cosmin Deciu, MSc; Tricia Zwiefelhofer; John A. Tynan, DPhil; Lesley Cagasan, MSc; Roger Tim, DPhil; Vivian Lu; Ron McCullough, DPhil; Erin McCarthy; Anders O. H. Nygren, DPhil; Jarrod Dean; Lin Tang, DPhil; Don Hutchison, MSc; Tim Lu, DPhil; Huiquan Wang, DPhil; Vach Angkachatchai, DPhil; Paul Oeth, MSc; Charles R. Cantor, DPhil; Allan Bombard, MD; Dirk van den Boom, DPhil
Fig. 2. NCVs for the 47 samples in the test set. The last 13 samples in the chromosome 21 data (NCVs 5–14) and the last 8 samples in the chromosome 18 data (NCVs 8.5–22) were correctly classified as T21 and T18, respectively. The last sample in the chromosome 13 data set (NCV of approximately 3) was classified as a “no call.”

Fig 2 | Z scores of percentage chromosome 21 (proportion of sequenced plasma DNA molecules originating from chromosome 21) determined by the 8-plex and 2-plex sequencing protocols. Broken lines indicate the z score cut-off value of 3.

Chiu et al. 2011, BMJ
Non-normalized fractional representation of chromosome 21 stratified by multiplexing level and sample type. The data for reference sets are slightly different. In particular, the estimated variance of the reference set for monoplex samples is lower than for tetraplex samples (both n = 24). Therefore, after standardization to the appropriate reference set, the trisomy 21 (T21) sample with the lowest chromosome 21 representation in monoplex can still be correctly classified.

Table 3 | Probabilities for a trisomy 21 fetus in women by age alone and according to result of maternal plasma DNA sequencing test

<table>
<thead>
<tr>
<th>Maternal age (years)</th>
<th>Pretest probability*</th>
<th>8-plex sequencing</th>
<th>Post-test probability</th>
<th>2-plex sequencing</th>
<th>8-plex sequencing</th>
<th>Post-test probability</th>
<th>2-plex sequencing</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Positive test result†</td>
<td>Negative test result</td>
<td>Positive test result†</td>
<td>Negative test result</td>
<td>Positive test result†</td>
<td>Negative test result</td>
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<td>20</td>
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<td>1 in 177</td>
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</tr>
</tbody>
</table>

*Pretest probabilities are based on prevalence of fetal trisomy 21 at 12th week of gestation.\(^{28}\)
†A positive test result is a sample with a z score for percentage chromosome 21 >3.

Chiu et al. 2011, BMJ
Normalisation / Statistical Approach to Enumeration

Sehnert et al. 2011 Clin Chem

Using method of Chiu et al.

Table 1. Chromosome ratio calculation rules.

<table>
<thead>
<tr>
<th>Chromosome of interest</th>
<th>Numerator (chromosome mapped sites)</th>
<th>Denominator (chromosome mapped sites)</th>
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<tr>
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<td>21</td>
<td>9</td>
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<tr>
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<td>X</td>
<td>6</td>
</tr>
<tr>
<td>Y</td>
<td>Y</td>
<td>Sum (2–6)</td>
</tr>
</tbody>
</table>
SureSelect X-chromosome array (Agilent)

Genome Analyzer II (Illumina)

36bp (paired end)

14.5 million PE-reads per sample (86% on target)

213-fold enrichment of reads (mapping to chromosome X)

Targeted Region Read Depth: 60-100 reads per base

Fetal specific allele detection: 96% of paternally inherited alleles detected within 3.05Mb targeted region
Maternal Plasma DNA Sequencing Reveals the Genome-Wide Genetic and Mutational Profile of the Fetus


Sci Transl Med
Ethical Issues (NIPD by MPS)

Centre around:

• Informed consent

• Enlarging the scope of prenatal testing
  - much broader range of genetic abnormalities

• An increase in uptake (non-invasive) and more selective abortions*

‘Microarrays in Prenatal Diagnosis’
Fig. S1. Predicted coverage of fetal alleles against number of sequenced reads produced. The prediction is based on the Poisson distribution with the assumptions of fractional concentration of fetal DNA being 11.4%.

Fetal/Placental Cells

• Number of fetal cells in maternal blood is very low
  – 1 part per 10 million
  – 20 fetal cells in 20ml of maternal blood

• Trophoblasts more prevalent in transcervical samples

• Lack of robust *in situ* assay or immunohistochemistry to definitively identify fetal/placental cells

• Laborious, operator variability, biological variation
Only 6.5% of increased risk results (Combined 1\textsuperscript{st} TS) were confirmed with Down syndrome

This indicates that approximately 94% of these invasive procedures were performed to exclude rather than confirm a Down syndrome diagnosis

Based on the 1\% risk of miscarriage* with invasive procedures, there would be an estimated 13 associated pregnancy losses