Clinical applications of non-invasive prenatal diagnosis from maternal blood: What will bring the future

PD Dr. rer. nat. Markus Stumm
Center for Prenatal Diagnosis and Human Genetics Kudamm 199
Kurfürstendamm 199, 10719 Berlin, Germany
www.kudamm-199.de
Actual situation

- Placental, embryonic and fetal material can be obtained \textit{invasively through chorionic villi sampling, amniocentesis or chordocentesis}.
- The invasive diagnostic techniques are generally safe and accurate, but harbor a small but measureable chance of miscarriage.
- Cell-free DNA and RNA molecules that circulate in the maternal blood can be obtained \textit{noninvasively by maternal venipuncture} and therefore, has no associated risk of fetal loss.
- However maternal blood contains a mixture of both embryonic/fetal cell free DNA (predominantly trophoblastic/placental DNA) and maternal DNA, which increases the downstream analytical complexity.
Clinical applications of cffDNA

Complications of pregnancy

- RhD diagnostics by RT-PCR
- Pre-eclampsia
- Intrauterine Growth retardation

- RhD-testing with specific primer and probe combinations detects RhD+ fetuses in RhD- women with low false positive results (~0,2%).
- NIPD limits RhD prophylaxis only to women who carry a RhD+ fetus.
- Transition to clinical care e.g. in UK and Denmark

- subject of research
- potential biomarkers for early detection: e.g. microRNAs are exported by exosomes from syncytiotrophoblast to maternal blood and therefore, may have a functional role in feto-maternal communication or the development of immune tolerance
Clinical applications of cffDNA

Genetic diseases

Autosomal diseases by RT-PCR and RMD
• Few cases described for the qualitative detection of paternally inherited or de novo mutations
• Few cases described for the quantitative detection of maternally inherited mutations by RMD = digital relative mutation dosage testing

Identification of sex by RT-PCR
• Fetal sex determination for the management of X-linked diseases or of ambiguous genitalia detected by sonography.
• Knowledge of fetal sex to determine which women has to take steroids to prevent masculanization of a female fetus that is at risk for congenital adrenal hyperplasia (CAH)
• Transition to clinical care e.g. in UK

Chromosomal aberrations by NGS
• Autosomal Aneuploidies
• Trisomy 21 testing is offered in China (2011), USA (2011) and Germany (2012)
• Trisomy 13 and 18 testing is also offered in China and USA, but it is more challenging because the diagnostic accuracy is affected by the GC content of an individual chromosome
## MPS for non-invasive detection of fetal trisomy 21

<table>
<thead>
<tr>
<th>Study</th>
<th>cases</th>
<th>Trisomy 21</th>
<th>Sensitivity (False neg.)</th>
<th>Specificity (False pos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>proof-of-concept</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fan et al. 2008</td>
<td>18</td>
<td>9</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Chiu et al. 2008</td>
<td>28</td>
<td>14</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Chiu et al. 2010</td>
<td>15</td>
<td>5</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Sehnert et al. 2011</td>
<td>47</td>
<td>13</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Sparks et al. 2012</td>
<td>298</td>
<td>89</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Stumm et al. 2012</td>
<td>42</td>
<td>8</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>clinical setting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiu et al. <em>BMJ</em> 2011</td>
<td>232</td>
<td>86</td>
<td>100%</td>
<td>97.9% (3)</td>
</tr>
<tr>
<td>Ehrich et al. <em>AJOG</em> 2011</td>
<td>449</td>
<td>39</td>
<td>100%</td>
<td>99.7% (1)</td>
</tr>
<tr>
<td>Palomaki et al. 2011</td>
<td>1696</td>
<td>212</td>
<td>99.1% (2)</td>
<td>99.9% (1)</td>
</tr>
<tr>
<td>Bianchi et al. 2012</td>
<td>532</td>
<td>89</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
## MPS for non-invasive detection of trisomy 18

<table>
<thead>
<tr>
<th>Study</th>
<th>cases</th>
<th>Trisomy 18</th>
<th>Sensitivity (False neg.)</th>
<th>Specificity (False pos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fan et al. <em>PNAS</em> 2008</td>
<td>18</td>
<td>2</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Sehnert et al. 2011</td>
<td>47</td>
<td>8</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Chen et al. 2011</td>
<td>392</td>
<td>37</td>
<td>91,9% (3)</td>
<td>98,0% (5)</td>
</tr>
<tr>
<td>Sparks et al. 2012</td>
<td>298</td>
<td>7</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Palomaki et al. 2012</td>
<td>1971</td>
<td>59</td>
<td>100%</td>
<td>99,7% (5)</td>
</tr>
<tr>
<td>Bianchi et al. 2012</td>
<td>532</td>
<td>36</td>
<td>97,2% (1)</td>
<td>100%</td>
</tr>
</tbody>
</table>

Whereas chromosome 21 has a midrange percentage of GC content, chromosome 13 and 18 have a lower percentage, which increases the coefficient of variation in the sequencing reactions of these chromosomes.
MPS for non-invasive detection of fetal trisomy 13

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases</th>
<th>Trisomy 13</th>
<th>Sensitivity (False neg.)</th>
<th>Specificity (False pos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fan et al. <em>PNAS</em> 2008</td>
<td>18</td>
<td>1</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Sehnert et al. 2011</td>
<td>47</td>
<td>1</td>
<td>0% (1)</td>
<td>100%</td>
</tr>
<tr>
<td>Chen et al. 2010</td>
<td>392</td>
<td>25</td>
<td>100%</td>
<td>98.9% (3)</td>
</tr>
<tr>
<td>Palomaki et al. 2012</td>
<td>1971</td>
<td>12</td>
<td>91.7% (1)</td>
<td>99.1% (16)</td>
</tr>
<tr>
<td>Bianchi et al. 2012</td>
<td>532</td>
<td>14</td>
<td>78.6% (3)</td>
<td>100%</td>
</tr>
</tbody>
</table>

Specific quantitative correction of the GC content bias in the sequencing data using modified z-score equations has resulted in improved sensitivity and specificity of trisomy 13 and 18.
Clinical applications of cffDNA

Genetic diseases

Autosomal diseases by RT-PCR and RMD
- Few cases described for the qualitative detection of paternally inherited or de novo mutations
- Few cases described for the quantitative detection of maternally inherited mutations by RMD = digital relative mutation dosage testing

Identification of sex by RT-PCR
- Fetal sex determination for the management of X-linked diseases or of ambiguous genitalia detected by sonography.
- Knowledge of fetal sex to determine which women has to take steroids to prevent masculanization of a female fetus that is at risk for congenital adrenal hyperplasia (CAH).
- Transition to clinical care e.g. in UK

Chromosomal aberrations by random MPS
- Autosomal Aneuploidies
- Trisomy 21
- Trisomy 13 and 18
- Other chromosomal aberrations, e.g. mosaic trisomy 9, deletions, duplications and gonosomal aneuploidies where also detected in single studies
Cell-free DNA in placenta

Cell-free DNA in maternal plasma

Maternal DNA
Red blood cell
Fetal DNA

Monogenic disorders

Aneuploidies

Massively parallel sequencing of total DNA present in maternal plasma.

Alignment of sequencing reads to human genome sequence and determination of relative chromosome representation

Detection of aneuploidy e.g. trisomy 21

Conventional or real-time PCR using primers to genes unique to the fetus and not present in the mother

Detection of PCR products corresponding to fetal-specific genes such as RHD
Future developments

2010 First successful construction of a genetic map of a fetus by sequencing (65-fold coverage) of cell free fetal DNA from maternal plasma (Lo et al. *Sci Transl Med*)
  • status of a familiar ß-thalassemia mutation detectable

2011 Detection of a familiar inherited microdeletion (Peters et al. *JAMA*)
  • 4.2 Mbp deletion in chromosome 12p detectable

2012 Complete whole-genome sequence reconstruction of two fetal genomes by genome sequencing of two parents, genome wide maternal haplotyping (32-fold coverage) and deep sequencing of cell free DNA from maternal plasma (72-fold coverage) (Kitzman et al. *Sci Transl Med*)
  • genome wide detection of fetal de novo mutations becomes possible

2012 Analysis of the complete fetal genome by massively parallel sequencing of cell free maternal plasma DNA (Fan et al. *Nature*)
  • 2,85 Mbp deletion in chromosome 22q11.2 detectable
  • non-invasive exome screening of all clinical relevant and deleterious alleles becomes possible
Future developments

• It is theoretically possible to noninvasively screen maternal blood for both fetal DNA copy number variation and single gene disorders by random MPS strategies.

• But in the near future, targeted MPS approaches may be applied for the NIPD of groups of common single-gene disorders, microdeletion/-duplication syndromes and aneuploidies.
From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges

Diana W Bianchi

Thus far, the focus of personalized medicine has been the prevention and treatment of conditions that affect adults. Although advances in genetic technology have been applied more frequently to prenatal diagnosis than to fetal treatment, genetic and genomic information is beginning to influence pregnancy management. Recent developments in sequencing the fetal genome combined with progress in understanding fetal physiology using gene expression arrays indicate that we could have the technical capabilities to apply an individualized medicine approach to the fetus. Here I review recent advances in prenatal genetic diagnostics, the challenges associated with these new technologies and how the information derived from them can be used to advance fetal care. Historically, the goal of prenatal diagnosis has been to provide an informed choice to prospective parents. We are now at a point where that goal can and should be expanded to incorporate genetic, genomic and transcriptomic data to develop new approaches to fetal treatment.
Thank you for your attention!