Prenatal cytogenetic diagnosis: laboratory aspects

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Centre of Medical Genetics
Introduction

0.6 % of newborns show clinically relevant chromosomal aberrations

Prenatal diagnosis (PND) : important tool to identify chromosomal abnormalities
Different tissues used for PND

- **Chorionic villus sampling:**
  - First used for PND in 1984
  - From 11 - 12th week of gestation
    → placental biopsy

- **Amniocentesis:**
  - First used for PND in 1967
  - From 14 - 16th week of gestation
    → aspiration of amniotic fluid

- **Cordocentesis:**
  - After 20th week of gestation
    → fetal blood
Embryological origin

Bianchi et al. (1993) AJMG 46:542-550
Conventional karyotype - CVS

Cell types:
- Syncytiotrophoblast
- Cytotrophoblast
- Mesenchyme core
- Maternal decidua

Coral-like projections which surround the embryonic sac and form the placenta
Conventional karyotype - AC

Cell types: heterogeneous population of cells from
- amnion
- skin
- urogenital
- respiratory systems
- alimentory

- maternal blood cells
PND: technique for chromosomal analysis

- **Until 2012-2013:**

- **From 2013:**
Conventional karyotype and FISH
CVS: direct method: cytотrophoblast cells

Dissection of decidua

In culture dish

FdU

Overnight incubation

Thymidine

Fixation

Analyses

Hypotonic shock

Colcemid

Karyotype
CVS: culture method: mesenchymal core cells

Dissection of decidua
In culture dish
Collagenase/Trypsin
Analyses
Hypotonic shock
Fixation
Karyotype
Colcemid
<table>
<thead>
<tr>
<th>Direct</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Cytotrophoblasts</td>
<td>● Mesenchymal core</td>
</tr>
<tr>
<td>● Result: 48h</td>
<td>● Result: 12 – 20 days</td>
</tr>
<tr>
<td>● Short chromosomes</td>
<td>● Long chromosomes</td>
</tr>
<tr>
<td>● Banding resolution: &gt; 10 Mb</td>
<td>● Banding resolution: 7.5 - 10 Mb</td>
</tr>
<tr>
<td>● Minimal problems with maternal contamination</td>
<td>● Risk of maternal cell contamination (1%)</td>
</tr>
<tr>
<td></td>
<td>● Derived from inner cell mass: more likely to reflect the fetal karyotype</td>
</tr>
</tbody>
</table>
Karyotype: CVS

**Direct**

Resolution: > 10 Mb

**Culture**

Resolution: 7.5 – 10 Mb
Karyotype: mother

46,XX,t(3;21)(p24?q22)
FISH result

3pterGreen
CEP 3 spectrum orange
Paint 21 spectrum red
AC: FISH and culture

Amniotic fluid

13q14 green / 21q22 orange

Normal 21
Trisomy 21

Karyotype
Diagnostic problems in conventional karyotyping

- Mosaicism and pseudomosaicism (CVS – AC)
- Confined placental mosaicism (CVS)
- Maternal cell contamination
- Unexpected adverse findings
Mosaicism and pseudomosaicism

- Mosaicism: presence of 2 or more cell lines in an individual or tissue sample

- When mosaicism is found in cultured fetal cells: problems in differentiating culture artefacts from real fetal mosaicism.
I. **Single abnormal cell (SC)**
   - single cell pseudomosaicism
   - frequency approx. 3.5%

II. **Multiple cells with the same abnormality in a single clone or flask (MC)**
   - multiple cell pseudomosaicism
   - frequency approx. 1.0%

III. **Multiple cells with the same abnormality in multiple clones or flasks**
   - true mosaicism
   - frequency approx. 0.25%
Confined placental mosaicism (CPM)

- Discrepancy between the chromosomal make-up of the cells in the placenta and the cells in the fetus (Kalousek and Dill, 1983)
- In approximately 1-2% of ongoing pregnancies that are studied by CVS at 10 to 12 weeks of pregnancy (Ledbetter, 1992)
- Most commonly CPM represents a trisomic cell line in the placenta and a normal diploid chromosome complement in the fetus (Robinson et al., 1997)
Confined placental mosaicism (CPM)

- **Mitotic CPM** - Mitotic non-disjunction can occur in a trophoblast cell or a non-fetal cell from the inner cell mass creating a trisomic cell line in the tissue which is destined to become the placental mesoderm.

- **Meiotic CPM** - CPM can occur through the mechanism of *trisomy rescue*. If a trisomic conception undergoes trisomic rescue in certain cells, including those that are destined to become the fetus, then the remaining trisomy cells may be confined to the placenta.

  trisomy rescue → UPD
CVS: discrepancies between results direct and culture method

- CVS direct (cytotrophoblast) and culture (mesenchymal core) with 3 types:

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>abnormal</td>
<td>normal</td>
<td>abnormal*</td>
</tr>
<tr>
<td>normal</td>
<td>abnormal</td>
<td>abnormal*</td>
</tr>
</tbody>
</table>

* with one mosaic

Highest level of accuracy using CVS: direct and long-term culture
Maternal cell contamination

- Possible explanation of some cases with both 46,XX and 46,XY cells
- Is more common in the CVS culture than in the direct CVS and amniotic fluid cell cultures
- Other explanations for presence of XX and XY
  - Cells from an undiagnosed (vanishing) twin pregnancy
  - Cross contamination in the laboratory
  - True fetal chimerism
IVF-MCBA twin / after transfer of 3 embryos

- **Prenatal:**
  - **BB1**
    - FISH: XX [53] / XY [47]
  - **BB2**
    - FISH: XX [50]
    - G-banding: XX [20]

- **Postnatal:**
  - **BB1**
    - Genitals: normal male
    - Karyotype
      - blood XX [20] / XY [80]
      - mucosa XX [57] / XY [143]
  - **BB2**
    - Genitals: normal female
    - Karyotype
      - blood XX [33] / XY [67]
      - mucosa XX [200]
      - urine XX [200]
Unexpected adverse findings

- A variant chromosome
- A balanced structural rearrangement
- A marker chromosome

De novo or inherited? → karyotyping both parents
Molecular karyotype
Molecular karyotyping (aCGH)

- Detects copy number variations (CNV) at a higher resolution than can be seen by routine karyotype.

- In Belgian: from 2013: aCGH for all the PND

- Genome – wide micro-array: different platforms (oligo/SNP) consensus: to use 60 k arrays (60 000 probes) or an equivalent for an average resolution of 400 kb.
Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges

Olivier Vanakker\textsuperscript{a}, Catheline Vilain\textsuperscript{d}, Katrien Janssens\textsuperscript{b}, Nathalie Van der Aa\textsuperscript{b}, Guillaume Smits\textsuperscript{d}, Claude Bandelier\textsuperscript{h}, Bettina Blaumeiser\textsuperscript{b}, Saskia Bulk\textsuperscript{g}, Jean-Hubert Caberg\textsuperscript{g}, Anne De Leener\textsuperscript{d}, Marjan De Rademaeker\textsuperscript{c}, Thomy de Ravel\textsuperscript{f}, Julie Desir\textsuperscript{e}, Anne Destree\textsuperscript{c}, Annelies Dheedene\textsuperscript{a}, Stéphane Gailliez\textsuperscript{g}, Bernard Grisart\textsuperscript{e}, Ann-Cécile Hellin\textsuperscript{g}, Sandra Janssens\textsuperscript{a}, Kathelijn Keymolen\textsuperscript{c}, Björn Menten\textsuperscript{a}, Bruno Pichon\textsuperscript{d}, Marie Ravoet\textsuperscript{h}, Nicole Renvcou\textsuperscript{h}, Sonia Rombout\textsuperscript{e}, Catherine Staessens\textsuperscript{c}, Ann Van Den Bogaert\textsuperscript{c}, Kris Van Den Bogaert\textsuperscript{f}, Joris R. Vermeesch\textsuperscript{f}, Frank Kooy\textsuperscript{b}, Yves Sznajer\textsuperscript{h}, Koen Devriendt\textsuperscript{a,f,*}

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\textsuperscript{i}Center for Medical Genetics, Université Catholique de Louvain, Belgium
AC - CVS

1 tube (10 ml): DNA extraction (aCGH + MCC)

1 tube (10 ml): FISH (3 ml) and back-up culture (7 ml)

Microscopic dissection chorionic villi

1 villi: DNA extraction (aCGH + MCC)

1 villi: FISH + back-up culture
Array CGH-Principle

Reference DNA

Test DNA

Labeling

Cy 5

Cy 3

Mix

Hybridisation

Scan

Log 2 test/referentie

gain

loss

Chromosomal position

Analysis
Data analysis

<table>
<thead>
<tr>
<th>Casus</th>
<th>Label</th>
<th>Regio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1924 - M14</td>
<td>[Deletion] 16: 15.048.751 - 15.111.247</td>
<td></td>
</tr>
<tr>
<td>1924 - M14</td>
<td>[Deletion] 17: 33.687.356 - 33.738.408</td>
<td></td>
</tr>
<tr>
<td>1924 - M14</td>
<td>[Multiplication] X: 2.700.316 - 151.903.977</td>
<td></td>
</tr>
<tr>
<td>1924 - M14</td>
<td>[Duplication] X: 151.959.017 - 154.841.396</td>
<td></td>
</tr>
</tbody>
</table>
Classification of variants with regard to pathogenicity

- Pathogenic
- Benign variants without functional consequences
- Unclassified variants (UV)
Pathogenic:

The observed CNV

- Is known to be associated with recurrent genomic disorders e.g.
  - del 22q11.2, del 15q11-13

- Results in a known effect on gene function and known phenotypic effect e.g.
  - deletion of a gene where haploinsufficiency causes a phenotype, or
  - duplication of entire gene causes a known phenotype
Benign variants without functional consequences

The observed CNV

- Is repeatedly found in the normal population and not enriched in individuals with certain abnormal phenotypes
Unclassified variants

All other CNVs that cannot be classified as pathogenic or benign must be considered as unclassified.

- Criteria that can be used to try and classify a variant include size, number of genes, de novo versus inherited, containing regulatory sequences, presenting phenotype, may remain of uncertain clinical significance.
Implementation of an Ad Hoc committee

- Online Ad Hoc committee with 32 members (4 from each 8 Belgian genetic centers)
- Difficult cases: within 24-48 h response
Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

Frequency and clinical interpretation of micro-array in 3822 samples with a normal karyotype, according to indication for prenatal testing

<table>
<thead>
<tr>
<th>Indication for Prenatal Diagnosis</th>
<th>Normal Karyotype</th>
<th>Common Benign</th>
<th>Pathogenic</th>
<th>Uncertain Clinical Significance (N = 130)</th>
<th>Total Known Pathogenic and Potential for Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>no. (%)</td>
<td>no. (%)</td>
<td>no. (%)</td>
<td>no. (%) [95% CI]</td>
</tr>
<tr>
<td>Any</td>
<td>3822</td>
<td>1234 (32.3)</td>
<td>35 (0.9)</td>
<td>69 (1.8)</td>
<td>61 (1.6) [95% CI]</td>
</tr>
<tr>
<td>Advanced maternal age</td>
<td>1966</td>
<td>628 (31.9)</td>
<td>9 (0.5)</td>
<td>37 (1.9)</td>
<td>25 (1.3) [95% CI]</td>
</tr>
<tr>
<td>Positive on Down’s syndrome screening</td>
<td>729</td>
<td>247 (33.9)</td>
<td>3 (0.4)</td>
<td>13 (1.8)</td>
<td>9 (1.2) [95% CI]</td>
</tr>
<tr>
<td>Anomaly on ultrasonography</td>
<td>755</td>
<td>247 (32.7)</td>
<td>21 (2.8)</td>
<td>16 (2.1)</td>
<td>24 (3.2) [95% CI]</td>
</tr>
<tr>
<td>Other</td>
<td>372</td>
<td>112 (30.1)</td>
<td>2 (0.5)</td>
<td>3 (0.8)</td>
<td>3 (0.8) [95% CI]</td>
</tr>
</tbody>
</table>

\[\text{Wapner et al., 2012}\]
Diagnostic problems in molecular karyotyping

- Mosaicism (CVS – AC)
- Confined placental mosaicism (CVS)
- Triploidy
- Unexpected adverse findings
Mosaicism

- Preferentially from uncultured samples to avoid “pseudomosaicism”: artefact cell culture
- True mosaicism: 20 – 30% (depending quality aCGH)
Confined placental mosaicism (CVS)

- Uncultured chorionic villi: the genome content of both cell lineages (cytiotrophoblast – mesemchyme core) are present.

- First report of CPM explaining discordant result CVS and child born: false positive result for a submicroscopic cnv.

(Karampetsou et al., Prenatal diagnosis, 34, 98-101, 2014)
Not detected with aCGH

- Triploidy: exception SNP arrays or combination with FISH or QF-PCR technology

- Balanced translocation, or other structural abnormalities:
  - if inherited: no consequences
  - if de novo: further investigation is needed to determine the residual risk
Incidental findings

- Those which do not have a direct consequence for the foetus itself, but may have implications later for the individual or his/her relatives

Vanakker et al., EJMG, 57 (2014) 151-156
Molecular karyotyping

- Always test for maternal contamination
- A rapid aneuploidy test (FISH or QF-PCR) is necessary if the turnaround time is more than one week
- Testing for triploidy is performed (FISH – SNP array – STR multiplex system)
Conventional karyotype karyotype

Molecular

Genome – wide analysis

- Resolution 5-10 Mb
- Culture: 10 – 21 days
- Degree mosaicism detected: about 4%
- Detect:
  - balanced translocations
  - triploidy
- Resolution 400 Kb
- 2.5% extra del/dupl detected
- No culture: 3 – 5 days
- Degree mosaicism detected: 20 – 30%
- Do not detect:
  - balanced translocations
  - triploidy
- Abnormalities of uncertain clinical significance may be detected
- Incidental findings