Prenatal cytogenetic diagnosis: laboratory aspects

Ann Van Den Bogaert, PhD
Centre of Medical Genetics
Goal of prenatal diagnosis

To inform couples about the risk of a birth defect or genetic disorder in their pregnancy

To provide them with informed choices on how to manage that risk (genetic counseling)
Principal indications

Known family history -> elevated risk for a specific genetic disorder

Ultrasound abnormalities

Advanced maternal age

Data from Benn PA, Hsu LY, 2003
Invasive testing

- Chorionic villus sampling
- Amniocentesis

- Cordocentesis: after 20th week of gestation
  → fetal blood

- Preimplantation genetic diagnosis
  → another presentation
Evolution of prenatal diagnosis

- **<2010**
  - All invasive samples

- **2010**
  - Ultrasound anomalies
  - Other indications

- **2013**
  - All invasive samples
Consensus 8 genetic centres in Belgium

- From 2013 in Belgium: for all prenatal samples = aCGH

  → Consensus:
  - Use 60K arrays (or comparable resolution)
  - Always test for maternal cell contamination
  - Always obtain a parental blood sample
  - Always have at least 1 backup flask in culture
  - Testing for triploidy is done (FISH, STR, SNP array)
  - A rapid aneuploidy test is not necessary if the TAT is less than one week
    - Batching samples -> benefits for cost (lab work)
Invasive testing

- Chorionic villus sampling (CVS): From 11 - 12th week of pregnancy
- Amniocentesis: From 14 - 16th week of pregnancy

in our laboratory
Chorionic villus sampling (CVS)
Prenatal culture room-CVS

Microscopic dissection chorionic villi
1 villi (uncultured): array CGH + MCC
1 villi: if necessary for DNA/stock
1 villi -> short-term culture (overnight) for FISH + back-up culture (long-term, > 1 week)
Prenatal culture room-AC

1 tube (10 ml): array CGH + MCC
1 tube: if necessary for DNA/stock
1 tube: FISH (3 ml) + back-up culture (7 ml)
Fluorescence in situ hybridisation
Fluorescent in situ hybridisation

Aneuploidy screening (interphase nuclei: direct test)

- Echographical abnormalities: X, Y, 13, 18, 21
- No echographical abnormalities: X, Y, 21

Normal result (XX, 21)  
Trisomy 21 (XX)
Array CGH-Principal

1. Reference DNA
2. Test DNA
3. Labeling
   - Cy 5
   - Cy 3
4. Mix
5. Hybridisation
6. Scan
   - Log 2 test/reference
   - Gain
   - Loss
   - Chromosomal position
   - Analysis

Prenatale diagnostiek cytogenetica
Array CGH prenatal result

- In Belgium 2013: aCGH for all prenatal samples
  - consensus: to use 60K arrays (60,000 probes) or an equivalent for an average resolution of 400 kb
  - Additional diagnostic yield (compared to conventional kayotyping; Shaffer et al. 2012; Wapner et al. 2012):
    - ±10% in fetuses with multiple ultrasound abnormalities
    - ±1% in lower risk women, such as those of advanced maternal age
  - Drawback: introduce CNVs of uncertainty into the diagnostic interpretation
National consensus guideline between the 8 Centres for Medical Genetics in Belgium

- Practical recommendation of pre- and post-counselling
  - can we expect parents to make ‘on spot’ decisions on what they do and do not want to know?
  - should we confront parents with questions that are unlikely to be relevant for them?

- How to interpret and report prenatal array results
Review

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{b}, 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{e}, Annelies Dheedene\textsuperscript{a}, Stéphane Gaillez\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 Ravoot\textsuperscript{h}, Nicole Revenç\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{f,}\textsuperscript{x}

\textsuperscript{a}Center for Medical Genetics, Universiteit Gent, Belgium
\textsuperscript{b}Center for Medical Genetics, Universiteit Antwerpen, Belgium
\textsuperscript{c}Center for Medical Genetics, Vrije Universiteit Brussel, Belgium
\textsuperscript{d}Center for Medical Genetics, Université Libre de Bruxelles, Belgium
\textsuperscript{e}Center for Medical Genetics, IPA, Charleroi, Belgium
\textsuperscript{f}Center for Medical Genetics, Katholieke Universiteit Leuven, Belgium
\textsuperscript{g}Center for Medical Genetics, Université de Liège, Belgium
\textsuperscript{h}Center for Medical Genetics, Université Catholique de Louvain, Belgium
Prenatal array guidelines

- Classification of variants with regard to pathogenicity:
  - Pathogenic
  - Benign variants without functional consequences
  - Unclassified variants (UV)
Pathogenic CNV

- known to be associated with a phenotype (e.g. del22q11.2)
- resulting in a known effect on gene function and known phenotypic effect

Are communicated
Benign CNV without functional consequences

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

Are NOT communicated
Unclassified variants (UV)

- In principle, UVs are NOT communicated and parental analysis is not performed.
  - unless one expects that this will add to the interpretation of the UV and to the decision to communicate this CNV.

Examples include CNVs with a higher degree of suspicion that they may cause a phenotype, the presence of ultrasound anomalies, family history etc.
Analysis prenatal arrays

1. Unclassified variant
   - Intragenic Del/dup
     - Known (haploinsufficient) gene
       - yes
       - no
     - Search literature/databases for similar CNV
       - yes
       - no
   - Del/dup > 18 genes
     - yes
     - no
   - X-linked gene in a XY fetus
     - yes
     - no

2. Evaluate likeliness of pathogenicity
   - Strong arguments for pathogenicity
     - Inherited
     - Test parents
     - Depending on parental phenotype
     - De novo
     - Test parents for genetic counseling if not previously done
   - No strong arguments for pathogenicity
     - NORMAL

REPORT
SUSCEPTIBILITY CNVs

• CNVs that are risk factors for developmental disorders

NOT communicated

• unless the risk is large enough and/or the CNV is associated with structural malformations for which ultrasound follow-up is indicated

SEE list
# List of susceptibility loci

<table>
<thead>
<tr>
<th>Chr</th>
<th>Start in Mb (hg19)</th>
<th>Stop in Mb (hg19)</th>
<th>Size in kb</th>
<th>CNV</th>
<th>Gene</th>
<th>Phenotype</th>
<th>Morph. Anomaly</th>
<th>Return?</th>
<th>OMIM</th>
<th>Update May 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>146.57</td>
<td>147.39</td>
<td>820</td>
<td>distal 1q21.1 dup</td>
<td>GJAS (CX40)</td>
<td>ID, DD, ASD, schizophrenia</td>
<td>macrocephaly, CHD</td>
<td>YES</td>
<td>612475</td>
<td>YES</td>
</tr>
<tr>
<td>1</td>
<td>146.57</td>
<td>147.39</td>
<td>820</td>
<td>distal 1q21.1 del</td>
<td>GJAS (CX40)</td>
<td>ID, DD, ASD, SZ, facial dysmorphism</td>
<td>microcephaly, CHD, renal and urinary tract anomalies</td>
<td>YES</td>
<td>612474</td>
<td>YES</td>
</tr>
<tr>
<td>1</td>
<td>171.81</td>
<td>172.38 (?)</td>
<td>57</td>
<td>1q24.3 del</td>
<td>DNM3</td>
<td>ID</td>
<td>IUGR, microcephaly, brachydactyly</td>
<td>YES</td>
<td>612001</td>
<td>YES</td>
</tr>
<tr>
<td>15</td>
<td>31.13</td>
<td>32.48</td>
<td>1350</td>
<td>15q13.3 del</td>
<td>CHRNA7</td>
<td>DD, ID, ASD, epilepsy, SZ</td>
<td>microcephaly, CHD</td>
<td>YES</td>
<td>613444</td>
<td>YES</td>
</tr>
<tr>
<td>15</td>
<td>99.36</td>
<td>102.52</td>
<td>3160</td>
<td>15q26 del</td>
<td>IGFR1</td>
<td>MR</td>
<td>IUGR</td>
<td>YES</td>
<td>614671</td>
<td>moved to YES since actionable; penetrance del and dup comparable</td>
</tr>
<tr>
<td>16</td>
<td>28.74</td>
<td>28.96</td>
<td>220</td>
<td>16p11.2 distal del</td>
<td>SH2B1</td>
<td>obesity, DD, ID, SZ</td>
<td>none</td>
<td>YES</td>
<td>614527</td>
<td>YES</td>
</tr>
<tr>
<td>16</td>
<td>29.59</td>
<td>30.19</td>
<td>600</td>
<td>16p11.2 proximal dup</td>
<td>TBX6</td>
<td>ASD, ID, DD, SZ, anorexia</td>
<td>microcephaly</td>
<td>NO</td>
<td>611913</td>
<td>YES</td>
</tr>
<tr>
<td>16</td>
<td>29.59</td>
<td>30.19</td>
<td>600</td>
<td>16p11.2 proximal del</td>
<td>TBX6</td>
<td>ID, DD, ASD, obesity, SZ, speech delay</td>
<td>macrocephaly, vertebra</td>
<td>YES</td>
<td>614332</td>
<td>NO</td>
</tr>
<tr>
<td>17</td>
<td>34.82</td>
<td>36.21</td>
<td>1390</td>
<td>17q12 deletion syndrome, RCAD (renal cysts &amp; diabetes)</td>
<td>TCF2</td>
<td>facial dysmophory, genital abnormalities, ID, DD, ASD, MODY</td>
<td>renal anomalies</td>
<td>YES</td>
<td>608363</td>
<td>YES</td>
</tr>
<tr>
<td>22</td>
<td>19.02</td>
<td>20.29</td>
<td>1270</td>
<td>22q11.2 dup</td>
<td>TBX1</td>
<td>ASD, ID, DD, dysmorphic features</td>
<td>microcephaly, CHD</td>
<td>YES</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>1</td>
<td>144.97</td>
<td>146.61</td>
<td>1640</td>
<td>1q21.1 dup</td>
<td>HFE2</td>
<td>DD, ASD</td>
<td>CHD</td>
<td>NO</td>
<td>6136570</td>
<td>NO</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>51.11</td>
<td>1110</td>
<td>2p16.3 del</td>
<td>NRXN1</td>
<td>ID, ASD, SZ, DD, dysmorphic features</td>
<td>none</td>
<td>NO</td>
<td>614332</td>
<td>NO</td>
</tr>
<tr>
<td>2</td>
<td>110.87</td>
<td>110.98</td>
<td>110</td>
<td>2q13 dup</td>
<td>NPHP1</td>
<td>ASD, ID</td>
<td>none</td>
<td>NO</td>
<td>614332</td>
<td>NO</td>
</tr>
<tr>
<td>3</td>
<td>197.2</td>
<td>198.84</td>
<td>1600</td>
<td>3q29 dup</td>
<td>NIPA1</td>
<td>MR, DD</td>
<td>none</td>
<td>NO</td>
<td>614332</td>
<td>NO</td>
</tr>
<tr>
<td>13</td>
<td>20.81</td>
<td>21.01</td>
<td>1200</td>
<td>13q12 dup</td>
<td>CRYL1</td>
<td>DD, motor delay, speech delay, ASD</td>
<td>none</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>15</td>
<td>22.8</td>
<td>23.09</td>
<td>290</td>
<td>15q11.2 dup</td>
<td>NIPA1</td>
<td>ID, DD, epilepsy</td>
<td>CHD</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>15</td>
<td>22.8</td>
<td>23.09</td>
<td>290</td>
<td>15q11.2 del</td>
<td>NIPA1</td>
<td>ID, DD, epilepsy</td>
<td>CHD</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>15</td>
<td>31.13</td>
<td>32.48</td>
<td>1350</td>
<td>15q13.3 del</td>
<td>CHRNA7</td>
<td>ADHD, ID, DD, ASD</td>
<td>none</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>16</td>
<td>14.98</td>
<td>16.48</td>
<td>1500</td>
<td>16p13.11 del</td>
<td>MYH11</td>
<td>ID, ASD, SZ, ADHD</td>
<td>aorta dilatation</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>16</td>
<td>14.98</td>
<td>16.48</td>
<td>1500</td>
<td>16p13.11 del</td>
<td>MYH11</td>
<td>ID, DD, ASD, epilepsy</td>
<td>microcephaly</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>16</td>
<td>21.94</td>
<td>22.46</td>
<td>520</td>
<td>16p12.2 dup</td>
<td>EEF2K, CDR2</td>
<td>DD, speech delay</td>
<td>craniofacial and skeletal abnormalities, CHD</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
<tr>
<td>16</td>
<td>21.94</td>
<td>22.46</td>
<td>520</td>
<td>16p12.2 del</td>
<td>EEF2K, CDR2</td>
<td>DD, speech delay</td>
<td>craniofacial and skeletal abnormalities, CHD</td>
<td>NO</td>
<td>615656</td>
<td>NO (likely benign)</td>
</tr>
</tbody>
</table>
Incidental findings

• Only highly penetrant monogenic disorders are considered, with validated evidence on the phenotype associated with the deletion or duplication.
Incidental findings

Four categories are distinguished:

- **Late-onset genetic disorders with clinical utility**
  - will be communicated (typically cancer caused by the deletion of a tumor suppressor gene)

- **Late onset disease without therapeutic possibilities**
  - the decision after consulting the ad hoc committee

- **Carrier for X-linked recessive disorders**
  - will be communicated

- **Carrier for autosomal recessive disorders**
  - will not be communicated, unless the disorder is frequent (carrier frequency >1/50: CFTR, SMA and Connexin)
Analysis prenatal arrays

Prenatal microarray

- Normal
- Benign
- Non-actionable incidental finding
- Unclassified variant

Known pathogenic variant or Known risk factor with high penetrance or US anomalies or Actionable incidental findings

Intragenic Del/dup?
Known (haploinsufficient) gene? yes

Search literature/databases for similar CNV
none yes

Del/dup > 18 genes
no

X-linked gene in a XY fetus
no

No strong arguments for pathogenicity

Evaluation likelihood of pathogenicity

Strong arguments for pathogenicity

Inherited Test parents

Test parents for genetic counseling if not previously done

De novo
Implementation of an *Ad Hoc* committee

- 2 clinical geneticists and 2 cytogeneticist from each center = 32 individuals
- Cases are presented to the committee through e-mail
- AIM: to reach a consensus decision within 24-48h

- Less subjective
- More consistent counselling in case of second opinion in another centre
- Rapid learning curve on evaluation of ‘difficult’ CNVs

Advisory role

Clinician holds responsibility on final decision
To Do / Ongoing national guidelines

- Regular re-evaluation to further optimize the consensus approach
- Mining of the national BEMAPRE database
- Address several outstanding questions
  - proportion of cases with unclassified variant?
  - % detection of causal CNVs in different indications?
  - % of incidental findings?
  - how often is parental analysis indicated?
  - incidence of susceptibility loci?
  - detection of causal CNVs postnatally?
  - postnatal follow-up
Conclusion national guidelines

- The National consensus approach solves:
  - technical issues (resolution, what to test for, etc..)
  - variation in interpretation amongst laboratories
  - variation of reporting
  - issues related to liability

Practical aid for those routinely using prenatal arrays
Conclusion national guidelines

Belgian Society for Human Genetics

Good Practice Guidelines

- Guidelines for CFTR gene analysis
- Guidelines for FMR1 analysis
- Guidelines for gene analysis for HBOC
- Guidelines for postnatal karyotyping
- Summary of prenatal array guidelines
- Summary of NIPT guidelines
- NIPT guidelines for incidental findings

National guidelines
http://www.beshg.be
Mosaicism in prenatal diagnosis

- Mosaicism
  - Is difficult for making a conclusion
  - The presence of two or more cell lines in a tissue sample
  - Three categories
    - Confined placental mosaicism
    - True Constitutional fetal mosaicism
    - Pseudomosaicism refers to an abnormality that arose during tissue culture in vitro (cultural artifact)
Confined placental Mosaicism

- Confined placental mosaicism
  - An abnormal cell line may only exist in the extra-embryonic tissues of the placenta
  - Is encountered at CVS rather than AC
  - It is uncommon that mosaicism at CVS reflects a true constitutional mosaicism of the fetus
    - More than 50000 procedures (grati et al. 2014)
      - In 2.2% of CVS mosaicism was seen -> 0.3% proved to have true fetal mosaicism
True fetal Mosaicism?

- Mosaic CVS -> follow-up AC
- Chorion Villi Sampling
  - Samples more distantly related from the fetus
- Amniocentesis
  - Cells closely reflect the true constitution of the fetus
Confined placental mosaicism (CPM)

- **2 types of CPM:**
  - *Mitotic CPM*: Mitotic errors create a trisomic cell line in the placenta (normal conceptus)
  - *Meiotic CPM*: can occur through the mechanism of trisomy rescue (trisomic conceptus)
    - If a trisomic conception undergoes trisomic rescue in certain cells, including those that are destined to become the baby, then the remaining trisomy cells may be confined to the placenta
    - trisomy rescue -> can cause UniParental Disomy (chr 6,7,11,14,15,16,and 20: imprinted genes)