New Genetic Approaches to Prenatal Diagnosis: THE TRANSITION TO GENOMICS

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# Prenatal screening and diagnosis for chromosome disorders

## Screening

- **Primarily for Down syndrome**
- **1970’s**: Maternal age
- **1980’s**: Triple test
- **1990’s**: 1\textsuperscript{st} tri biochemistry
- **2000**: Combined screen
  - 1\textsuperscript{st} tri nuchal & biochemistry
- **2012**: NIPT

## Diagnosis

- **For chromosome disorders**
- **1970’s**: Amniocentesis & karyotype
- **2000’s**: CVS & aneuploidy qPCR
- **2010’s**: array CGH
Genetic technology - diagnostics

- Chromosome analysis
- Detects >5-10Mb

- Sanger sequencing & PCR
- Few hundred base pairs
- Single genes / exons
Roles of traditional genetic testing

• Diagnostic / confirmatory testing
• Prenatal testing (and PGD)

• Predictive testing
• Carrier testing
• Population screening – usually reserved for populations at high risk on basis of ethnicity
The clinical genetic approach

<table>
<thead>
<tr>
<th>Approach:</th>
<th>Unique aspects:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case history</td>
<td>Family history</td>
</tr>
<tr>
<td>Clinical examination</td>
<td>Dysmorphology</td>
</tr>
<tr>
<td>Investigations</td>
<td>Genetic tests --&gt; Genomic tests</td>
</tr>
<tr>
<td>Medical care</td>
<td>Where relevant...</td>
</tr>
</tbody>
</table>
| Genetic counselling           | **Information:**
|                                | • On clinical and lab findings
|                                | • On personal and familial implications              |
|                                | **Support:**
|                                | • To adjust to the condition
|                                | • To make decisions (e.g. regarding TOP)             |
|                                | **Family follow-up**                                 |
Genomics

A branch of biotechnology...
...applying new techniques of genetic testing...
...to sets of genes...
...or even the complete DNA sequence

... and analysis using ‘bioinformatic’ databases
and computational methods
Genomic diagnostic testing

- **Array CGH:**
  - ‘Comparative genomic hybridization’
  - ‘Molecular karyotyping’

- **2nd generation sequencing:**
  - Non-invasive prenatal testing
  - Whole genome sequencing
  - Whole exome sequencing
    (all protein coding regions)
Pro’s and cons of genomic approach

• Pro’s:
  – Lots more information:
    • New understanding of genome structure and function
    • New tests and treatments

• Cons:
  – Too much information:
    • What do detected genetic variants mean?
      – Particularly important in prenatal diagnosis
    • How much information should be known/disclosed?
Array CGH

• ‘High resolution karyotype’:
  – Detects very small chromosome deletions / duplications
    e.g. 10,000 - 500,000 base pairs in size

• Limitations:
  – As resolution increases so does detection of normal or unknown variants (higher false positive rate)
  – Less good than karyotype for a few indications:
    • Mosaicism
    • Triploidy
    • ‘Balanced’ chromosome abnormalities
Application of array CGH in paediatrics

• Now a first-line test in paediatrics for:
  – Intellectual disability
  – Autism
  – Multiple congenital abnormalities (MCA)

• Detection rate is 15-20% (vs ~3% for karyotype)
  
  (E.g. Lu et al, 2009)
Tygerberg experience with aCGH

In SA, availability of aCGH is still limited even in paediatrics

OUR EXPERIENCE:

• Carefully selected patients with:
  – MCA and/or intellectual disability
  – Normal karyotype

• 24% disease-causing variants
• 12% variants of unknown significance (VOUS)
Photos of dysmorphic baby

Karyotype: 46,XY
aCGH result

7Mb duplication

7Mb deletion
How did this test result help?

- Did not alter medical care
  (in some cases may affect management)
- Gave the parents an answer
- Reduced further investigations
- Informed us of possible recurrence risk
- Can offer parents testing for carrier status
- Option of prenatal diagnosis in future pregnancies
Prenatal use of aCGH

... a tale

• Fetal anomaly scan at 18 weeks:
  – Isolated CL&P

• Karyotype requested, but failed

• Lab arranged aCGH --> result:
  – Arr 9p22.3(14,210,804-15,317,498) x3
  – 1.1 Mb duplication of material from short arm of chromosome 9
• Paediatrician informed parents that aCGH showed duplication of 9p

• Referred to an excellent website - information:
  – Severe condition
  – Limited ability to communicate

Closer assessment...

VOUS (variant of unknown significance)
Genetic counselling

• Parents informed of uncertain significance

• For them:
  – Better than expected
  – Continued pregnancy
  – Ongoing anxiety
  – ‘An emotional rollercoaster thanks to conflicting opinions they received’

At 3 ½ months age

Photo of normal looking baby with cleft lip
Current aCGH in prenatal diagnosis

• aCGH is increasingly recommended as primary genetic diagnostic test for investigation of ultrasound abnormalities (ACOG, Dec 2013)

• Still debate about:
  – Array resolution
  – Reporting of results
  – Clinical criteria for testing:
    • E.g. for single or multiple sonar abnormalities?

• Still very limited access in South Africa
One approach: Guy’s Hospital

<table>
<thead>
<tr>
<th>Dx test</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLD APPROACH</td>
<td></td>
</tr>
<tr>
<td>Amnio &amp; karyotype</td>
<td>• High risk Down syndrome</td>
</tr>
<tr>
<td></td>
<td>• Fetal anomalies</td>
</tr>
<tr>
<td>NEW APPROACH</td>
<td></td>
</tr>
<tr>
<td>CVS and Aneuploidy qPCR</td>
<td>• High risk Down syndrome</td>
</tr>
<tr>
<td>(Since 2007)</td>
<td></td>
</tr>
<tr>
<td>Array CGH</td>
<td>• Fetal anomalies</td>
</tr>
<tr>
<td>(Since 2012)</td>
<td>• NT&gt;3,0mm</td>
</tr>
<tr>
<td></td>
<td>• IUGR...</td>
</tr>
</tbody>
</table>

Array CGH:
• Limit resolution to predefined level (currently 3Mb)
• Except for known microdeletions regions (e.g. DiGeorge)

Detection rate: ~7%

Courtesy of Dr Christine Patch
Guy’s and St Thomas’ NHS Foundation Trust
Non-invasive prenatal testing (NIPT) for fetal chromosome abnormalities
Cell-free DNA in Maternal Blood

- Short DNA fragments in plasma
- In pregnancy, cell-free fetal DNA (cffDNA):
  - Comprises ~10% of total cell-free DNA (after ~10 weeks gestation)
  - Probably mainly placental in origin
  - Rapidly cleared after birth
- The entire genome of a fetus has been sequenced from cffDNA
Clinical availability of cffDNA tests

Available for 5-10 years (internationally):
• Fetal sexing
• Fetal Rh status in Rh- women (Caucasian)

Available for 2 years:
• Aneuploidy

In the future?
• Other chromosome abnormalities
• Other genetic disorders
## NIPT methods for aneuploidy

<table>
<thead>
<tr>
<th>Test* (Company)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Harmony</strong> (Ariosa)</td>
<td>Comparison of counts of targeted chromosomes</td>
</tr>
<tr>
<td><strong>Verifi</strong> (Verinata/Illumina)</td>
<td>Comparison of counts of all chromosomes</td>
</tr>
<tr>
<td><strong>Panorama</strong> (Natera)</td>
<td>Analysis of SNPs (single nucleotide polymorphisms)</td>
</tr>
</tbody>
</table>

*These tests are mentioned because they are available through local ‘agents’

Note that different tests have different methods, and different pros and cons
In practice NIPT for aneuploidy is currently a:

• highly accurate...
• screening test...
• for Down syndrome
NIPT can detect...

- Trisomy 13
- Trisomy 18
- Down syndrome
- Turner syndrome
- 22q11 del/DiGeorge
## Accuracy of NIPT

<table>
<thead>
<tr>
<th></th>
<th>Detection rate</th>
<th>False pos rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trisomy 21</strong>*</td>
<td>99.3%</td>
<td>0.16%</td>
</tr>
<tr>
<td><strong>Trisomy 18</strong>*</td>
<td>97.3%</td>
<td>0.15%</td>
</tr>
<tr>
<td><strong>Trisomy 13</strong>*</td>
<td>79%</td>
<td>0.41%</td>
</tr>
<tr>
<td><strong>Sex chromosome</strong></td>
<td>~80%</td>
<td>~0.2%</td>
</tr>
<tr>
<td><strong>abnormalities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other chromosome</strong></td>
<td>Limited data</td>
<td>?</td>
</tr>
<tr>
<td><strong>abnormalities</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data from ~6000 pregnancies

* Benn et al, UOG 2013
Advantages of NIPT

**COMPARSED TO INVASIVE TESTS:**
- No risk of procedure-related miscarriage

**COMPARSED TO OTHER SCREENING TESTS:**
- Highly accurate for Down syndrome:
  - Low false negative rate (but some cases are missed)
  - Low false positive rate = far fewer invasive tests
- Anytime from 10 weeks onward
Comparison with combined screen

- Both figures have the same number of patients
  - 10 trisomy cases
  - 1,939 non-trisomy cases

Nicolaides et al., Am J Obstet Gynecol 2012
NIPT is not a diagnostic test

Example:

**Test accuracy:**
- 99% detection
- 0.1% false positive

**T21 prevalence:**
- 1 in 500

1,000 women

<table>
<thead>
<tr>
<th>2 T21</th>
<th>998 non-T21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test +</td>
<td>Test +</td>
</tr>
<tr>
<td>Test -</td>
<td>Test -</td>
</tr>
</tbody>
</table>

Positive test result is correct only 2/3 of the time
Clinical guidelines for NIPT

• ACOG guideline (Dec 2012):
  – Indications for NIPT (high risk of Down syndrome)

• ACMG guideline (Genet Med, 2013):
  – Pro’s and cons of NIPT
  – Pretest and post-test counselling

• Concerns about very rapid implementation esp. in low risk population:
  – But evidence is accumulating that NIPT accurately detects Down syndrome in this situation
Pretest counselling for NIPT

• **Assess if NIPT is appropriate:**
  – Check family history
  – Check why couple want NIPT
  – Check what they would do with NIPT result

• **Discuss each condition (implications & test accuracy):**
  – T21
  – T13/18
  – Turner and Klinefelter
  – 47,XXX and 47,XYY
  – Gender

• **Discuss / compare alternative approaches:**
  – Other screening tests
  – Invasive diagnostic testing

• **Discuss need for confirmatory invasive testing**
  – if ‘high risk’ NIPT result

• **Discuss failure rate and logistics**

• Couple decides whether to test, and what to test
Practical implementation of NIPT

Several options (not mutually exclusive)

• **10 weeks:**
  – Primary screening procedure
  – Allows for:
    • Modified first trimester scan
    • Early invasive testing

• **12 weeks:**
  – Contingent on NT/combined screen

• **Later gestation:**
  – Instead of triple screen
# Fetal Assessment Center

## NIPT implementation (Harmony)

<table>
<thead>
<tr>
<th>Numbers</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Offered NIPT</th>
<th>171</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declined NIPT after counselling</td>
<td>17 (10%)</td>
</tr>
<tr>
<td>Accepted NIPT:</td>
<td></td>
</tr>
<tr>
<td>10-11 weeks</td>
<td>41 (27% of tests)</td>
</tr>
<tr>
<td>12-13 weeks</td>
<td>60 (39%)</td>
</tr>
<tr>
<td>14-19 weeks</td>
<td>42</td>
</tr>
<tr>
<td>20+ weeks</td>
<td>12 (35%)</td>
</tr>
<tr>
<td>Results:</td>
<td></td>
</tr>
<tr>
<td>‘High risk’</td>
<td>1 =&gt; confirmed positive</td>
</tr>
<tr>
<td>‘Low risk’</td>
<td>150</td>
</tr>
<tr>
<td>Failed</td>
<td>3</td>
</tr>
</tbody>
</table>
## Emerging workflow for prenatal diagnosis

<table>
<thead>
<tr>
<th>SCREENING TEST(S)</th>
<th>RISK or SONAR FINDINGS</th>
<th>INVASIVE TESTING</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIPT</td>
<td>Risk Down syndrome</td>
<td>qPCR for aneuploidy</td>
</tr>
<tr>
<td>Combined screen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sonar</td>
<td>Fetal anomalies</td>
<td>Array CGH (Karyotype)</td>
</tr>
<tr>
<td></td>
<td>Increased nuchal</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from: Lichtenbelt et al, 2011
New directions for genomics

- Extended NIPT – microdeletions
- Extended carrier screening
Summary

• Genomics is superceding old genetic approaches:
  – Array CGH for prenatal diagnosis
  – NIPT for prenatal screening of Down syndrome

• Will continue to evolve rapidly

• Complementary to ultrasound
• Need for genetic counselling
Acknowledgements

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  – Dr H Ramdhani
  – Parents