CytoScan Optima

Izak Storm
BIOCOM diagnostics
Introduction
### Prenatal Research

<table>
<thead>
<tr>
<th><strong>Who?</strong></th>
<th>Obstetric and Gynecologic clinical researchers, clinical research labs, cytogeneticists, assisted reproductive technology (ART) laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Need</strong></td>
<td>Specific, sensitive test to detect chromosomal abnormalities typically associated with advanced maternal age (AMA), abnormal ultrasound, NIPT confirmation. May be concerned about data complexity and VOUS as they don’t know how to report them</td>
</tr>
</tbody>
</table>
In most pregnancies with proper care, the mother will go through a number of routine screening tests. Positive results indicate a need to move towards diagnostic procedures.
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Positive results indicate a need to move towards diagnostic procedures.
1. NICHD ~4,000 cases 5 yr pros. trial
   - Ultrasound abnormalities >6.0% incremental diagnostic yield (at low resolution screen)
   - ~90 genomic regions

2. Liao et.al 2014 & 2015
   - >10-13% clinical yield
   - Genome Wide (CytoScan)

3. Rauch et al 2014
   - +17% vs traditional
   - Genome Wide (CytoScan)

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Dec 2013

The Use of Chromosomal Microarray Analysis in Prenatal Diagnosis

ABSTRACT: Chromosomal microarray analysis is a technique that identifies chromosomal abnormalities, including submicroscopic abnormalities that are too small to be detected by conventional karyotyping. Like conventional fetal karyotyping, prenatal chromosomal microarray analysis requires direct testing of fetal tissue and thus can be offered only with chorionic villus sampling or amniocentesis. Based on the results of a Eunice Kennedy Shriver National Institute of Child Health and Human Development multicenter trial and of prior studies, prenatal chromosomal microarray analysis is most beneficial when ultrasonographic examination identifies fetal structural anomalies. The potential for complex results and detection of clinically uncertain findings identified by prenatal chromosomal microarray testing can result in substantial patient anxiety. This underscores the critical need for comprehensive patient pretest and posttest genetic counseling from qualified personnel about the benefits, limitations, and results of testing so that patients can make informed decisions. The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine offer background information as well as recommendations regarding the application of chromosomal microarray technology in the prenatal setting.
### Table 2. Evolution of SNP arrays used in our report.

<table>
<thead>
<tr>
<th>Comparisons with array types</th>
<th>SNP6.0</th>
<th>Cytogenetic2.7M</th>
<th>CytoScan HD</th>
<th>CytoScan 750K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2008</td>
<td>2009</td>
<td>2010-2011</td>
<td>2012-2013</td>
</tr>
<tr>
<td>Constitutional gene coverage</td>
<td>83%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>OMIM morbid gene coverage</td>
<td>78%</td>
<td>100%</td>
<td>98%</td>
<td>83%</td>
</tr>
<tr>
<td>RefSeq gene coverage</td>
<td>82%</td>
<td>100%</td>
<td>94%</td>
<td>80%</td>
</tr>
<tr>
<td>X-chromosome gene coverage</td>
<td>76%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Scanner</td>
<td>Scan3000</td>
<td>Scan3000</td>
<td>Scan3000 DX2</td>
<td>Scan3000 DX2</td>
</tr>
<tr>
<td>Software package</td>
<td>genotyping console</td>
<td>CHAS1.0</td>
<td>CHAS1.2</td>
<td>CHAS1.2</td>
</tr>
<tr>
<td>Number of fetuses with ultrasound malformations and normal karyotypes</td>
<td>38</td>
<td>76</td>
<td>189</td>
<td>143</td>
</tr>
<tr>
<td>Percentage of cases with a pathogenic CNV (n)</td>
<td>7.9% (3)</td>
<td>10.5% (8)</td>
<td>13.2% (25)</td>
<td>10.5% (15)</td>
</tr>
<tr>
<td>Percentage of cases with a VOUS (n)</td>
<td>2.6% (1)</td>
<td>5.3% (4)</td>
<td>1.6% (3)</td>
<td>0.7% (1)</td>
</tr>
</tbody>
</table>

VOUS: variations of unknown

Implementation of high-resolution SNP arrays in the investigation of fetuses with ultrasound malformations: five years of clinical experience

Can Liao et al., Guangzhou, China
Prenatal screening vs confirmation

- In most pregnancies with proper care, the mother will go through a number of routine screening tests.

Positive results indicate a need to move towards diagnostic procedures.
The False Positive term is frequently used, but as the Positive rate is very low, it is very low... a different term should be used:

- PPV (positive predictive value) is “false positives” but taking into account only positive cases (not all cases)

- When results are presented using PPV...

**False Positives (PPV):**

- T21: 5.6%
- T18: 40.5%!!!!!!!
- T13: 55.6%!!!!!!!
- SCA: 62.1%!!!!!!!!!!!!

**Confirmation!**
- **Triploidy:**
  - 1-2% in all conceptions but...
  - 10 (-25%) in all spontaneous abortions
- ❌ Some NIPS might miss Triploidies!
- ✔ SNP-A can pick them up easily
ACOG Recommendations

- If a fetal structural anomaly is identified on ultrasound examination, invasive prenatal diagnosis should be offered.

- A negative cell free fetal DNA test result does not ensure an unaffected pregnancy.

- A patient with a positive test result should be referred for genetic counseling and offered invasive prenatal diagnosis for confirmation of test results.

- Cell free fetal DNA does not replace the accuracy and diagnostic precision of prenatal diagnosis with CVS or amniocentesis, which remain an option for women.

Pre-natal Array testing is likely to be used as confirmation of NIPT as once an invasive procedure is offered, the best option should be chosen
ACMG - 2015

Resolution of Conflicting Results from Non-Invasive Prenatal Screening by Cytogenetic Approaches

Abstract Number:
711
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Description:
Although a relatively recent development, the implementation of non-invasive prenatal screening (NIPS) is rapidly changing the approach to prenatal screening, testing and decision making. However, when these studies provide complex or conflicting information, more traditional approaches to prenatal diagnosis are required for interpretation. We have recently evaluated two such situations, using chromosomal microarray analysis, karyotype analysis and fluorescence in situ hybridization (FISH) to clarify NIPS findings.

The first patient was a 27 yr old, whose 1st trimester NIPS result indicated a 99% risk for trisomy 13 as well as a 1/19 risk for Cri du Chat syndrome (deletion 5p). As the incidence of trisomy 13 in first trimester screening of a patient this age is about 1/4,844 and the incidence of Cri du Chat syndrome is at most 1/20,000, the likelihood of both conditions occurring concurrently was very low. FISH, with probes that map to 13q14 and 21q22.13-q22.1, on uncultured chorionic villi showed the normal two signals for both chromosomes 13 and 21, with no evidence of trisomy 13. Chromosomal microarray analysis on DNA from this same sample revealed a 15.4 Mb loss of material at 5p15.33 to 5p15.1, which overlap the critical region for Cri du Chat syndrome, as well as a 44 Mb gain of material at 13q21.33 to 13q34 (a region distal to the FISH probe used). Karyotype analysis demonstrated an unbalanced 5;13 translocation with partial monosomy 5 and partial trisomy 13. Subsequently, the mother was shown to have the balanced form of this translocation, t(5;13) (p15.1;q22).

- Complex rearrangements can be cryptic to FISH, karyotype or qPCR.
- Micro deletions might be missed by karyotype
- The higher the resolution of NIPS the higher the FPR
Prenatal arrays

- Genome-wide unbiased
- Easy analysis
- Should yield low VOUS
- High success rate
- Shorter TaT
- Reliable results, Low false positives
Triploidy detection is a MUST

"...a post hoc review determined that had the SNP data been analyzed, the triploid cases would have been detected. We therefore suggest that arrays used for prenatal testing should contain SNP probes that can reliably identify triploidy."

1-2% triploidies in all conceptions but 10-25% in all spontaneous abortions
The power of SNPs

High density SNPs

- Identification of genomic contamination (MCC)
- Identification of AOH/LOH
- Aid in breakpoint determination
- Aid in the confirmation of copy-number Events
- Mosaicism events corroboration
- Mendelian consistency checking
- Triploidy

High-density SNPs allow for all of these performance attributes to be measured at gene-level resolution.
Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory

Boris T Wang, Thomas P Chong, Fatih Z Boyar, Kimberly A Kopita, Leslie P Ross, Mohamed M El-Naggar, Trilochan Sahoo, Jia-Chi Wang, Morteza Hemmat, Mary H Haddadin, Renius Owen and Arturo L Anguiano

- Approximately 60-70% of first trimester miscarriages are being caused by chromosomal abnormalities
- Traditional cytogenetic analysis of these samples is challenging due to high rates of culture failure and maternal contamination.
- Chromosomal microarray analysis overcomes these limitations and has proven to be an excellent tool for detection of chromosomal aberrations in these samples
SNP-A on POC

- POC have high rates of culture failure (successful 38.4% of times) and Maternal contamination
- SNP-A facilitates discovery of the “true” result
- CMA in POC has higher Diagnostic return

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Prenatal arrays

- Genome-wide unbiased
- Easy analysis
- Should yield low VOUS
- High success rate
- Shorter TaT
- Reliable results, Low false positives
- No Cell Culture required
- Need SNPS
  - Triploidy
  - MCC (maternal cell contamination)
  - Confirmation
CytoScan® Optima Suite
CytoScan Optima Suite: designed and optimized from empirical experience

1. NICHD ~4,000 cases 5 yr pros. trial
   - Ultrasound abnormalities >6.0% incremental diagnostic yield (at low resolution screen)
   - ~90 genomic regions

2. KOLs ~15,000 CytoScan (HD) cases
   - 396 genes & regions identified by Prenatal KOLs

Array content:
315,608 total probes:
148,450 SNP markers x 2
18,018 CN markers
Control features
CytoScan Optima Suite: designed and optimized from empirical experience

- Including amplification Reagents
- Including a new optimized reference file for prenatal & miscarriage applications
- Essentially, same lab protocol as CytoScan HD/750K
- Same analysis Software (ChAS) and tools
CytoScan Optima

Attributes
- Developed for Prenatal and POC samples
- Cost convenient
- All reagents included
- Easy Data analysis

Resolution and Coverage
- 2Mb Gains and 1 Mb for losses
- Increased probe density for 396 regions relevant for prenatal research (25 markers in 100kb).
- 5Mb for LOH across the genome

Design
- Optimized designed from the CytoScan HD array
- Allows the detection of CNVs, allelic imbalance and LOH
- Easy to use software includes features such as Trio analysis tool for Mendelian consistency checking, diploid correction, and easy data export tools for reporting.