Whole Genome Chromosomal Microarrays for Prenatal Diagnosis
Experience from a Reference Laboratory

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Chromosomal Microarrays in Clinical Diagnosis

• Genome-wide copy-number analysis
• Detects submicroscopic chromosomal imbalances
• Yields clinically significant findings in 10-20% of cases
  – Developmental delay
  – Congenital anomalies
  – Autism
  – Syndromic and nonsyndromic intellectual disability
• First-tier cytogenetic diagnostic test

Chromosomal Microarrays (CMA) in Clinical Diagnosis

CMA has transformed chromosome analysis into a DNA-based discipline.
Objectives

• Understand use of microarrays in prenatal setting
• Appreciate detection rates of fetal chromosome abnormalities
• Appreciate future of microarrays in prenatal testing

Chromosomal Microarray in Prenatal Diagnosis

• Use still being investigated
• Studies show significant improvement in detection of clinically significant cytogenetic information relative to karyotyping
  – Detects clinically significant copy number variations (CNVs) in >6% of cases
  – Detection highest in patients with abnormal ultrasound
  – Detects copy number variations with unknown clinical significance in ~1-4% of cases


Chromosomal Microarray in Prenatal Diagnosis

Clinical Use

• Follow up abnormal ultrasound findings
• Further characterize an abnormal karyotype
• Address advanced maternal age, any parental concern
• History of previous child with cytogenetic abnormality
• Cases of parental rearrangement
### Quest Diagnostics Experience

#### 2010
- Targeted BAC array
- 3200 clones

#### 2011
- Affymetrix 6.0
- Oligo-SNP: 1.8M probes

#### 2012
- Cytoscan HD
- Oligo-SNP: 2.7M probes

- Information provided
  - Copy number variations
  - Segments of homozygosity-UPD
  - SNP genotypes

- Thresholds for genome-wide screening
  - >50 kb for losses
  - >200 kb for gains
  - 5 Mb for homozygosity
  - Thresholds may be lower in cytogenetic relevant regions

- Not reported
  - Gains or losses <1 Mb and not encompassing genes
  - Gains <0.5 Mb encompassing genes, not dosage sensitive, and of unknown clinical significance

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- Targeted BAC array
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- Cytoscan HD
- 3200 clones
- Oligo-SNP: 1.8M probes
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- Data for 1,000 cases
  - BAC array: 350 cases
  - Affymetrix arrays: 650 cases

- Sample type
  - Amniotic fluid (direct and cultured): 932 (93%)
  - Chorionic villi (direct and cultured): 68 (7%)

### Possible Results

<table>
<thead>
<tr>
<th>Normal</th>
<th>No reportable copy number variants; recommend karyotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal (clinically significant)</td>
<td>Provides diagnosis; directs genotype-phenotype correlation; parental studies to determine recurrence risk</td>
</tr>
<tr>
<td>Unclear clinical significance</td>
<td>No clear interpretation of results due to size and/or gene content; parental studies very valuable</td>
</tr>
</tbody>
</table>
**Quest Diagnostics Experience**

**Clinical Indication**

- Abnormal Ultrasound: 14%
- Abnormal Chromosomes: 15%
- Abnormal US and Chromosomes: 3%
- Advanced Maternal Age: 5%
- Other or N/A: 63%

**Quest Diagnostics Experience: Results**

**Overall Results**

- Normal: 3%
- Abnormal: 82%
- Unclear Clinical Significance*: 9%

*4 cases with segments of homozygosity >5 Mb

**ABNORMAL CMA, N=92**

- Abnormal Ultrasound (US): 10%
- Abnormal Chromosomes: 3%
- Abnormal US and Chromosomes: 34%
- Advanced Maternal Age: 18%
- Other or N/A: 35%

**Abnormal Microarray and Chromosomes**

- Normal karyotype (19 recurrent microdeletion/microduplication syndrome): 26
- Alternative: 6
- Marker chromosome: 16
- Deletion/Duplication/other: 16
- Unbalanced translocation: 16
**CNV of Unclear Clinical Significance, n=94**

- Abnormal Ultrasound (US)
- Abnormal Chromosomes
- Abnormal US and Chromosomes
- Advanced Maternal Age
- Other or N/A

- Abnormal chromosomes: 4 inversions, 4 marker chromosomes (origin of 2 identified by microarray)

• Parental studies in 28 cases: 25 confirmed inherited; 3 confirmed de novo
• May reduce cases with copy number variants of unclear significance from 9% to 6.9%

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**Normal Microarray Results, n=814**

- Abnormal Ultrasound (US)
- Advanced Maternal Age
- Abnormal Chromosomes*
- Other

- Abnormal chromosomes:
  - 12 balanced translocations
  - 7 inversions

*Abnormal chromosomes:
  - 6 marker chromosomes
  - 6 other

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**Recurrent Microdeletion**

- Abnormal U/S; Abnormal chromosome analysis
- Chromosomes: 47,XX; +del(15)(q11.2), ish pus del(15)(q11.2)(D15Z1++, SNRPN+)
- Microarray: arr 16p13.11(14,866,263-16,391,045)x1 (1.5 Mb)
**Recurrent Microdeletion Syndrome**

- Abnormal ultrasound: Chromosomes 46,XX
- arr 22q11.21(18,916,842-21,465,659)x1
- 2.5 Mb; includes DGS/VCFS

**Rare, Novel Microdeletion**

- Abnormal ultrasound: hypoplastic right heart, thick nuchal area, shortened long bones, absent nasal bone, short-wide feet, abnormal contours of soft tissues/bulges, pectus excavatum, microopen.
- arr 2q36.3q37.3(228,558,473-238,544,102)x1 [hg19] (10 Mb)
- 46,XY,del(2)(q36.3q37.3)

**Mosaic Gain—Confirm Karyotype**

- Abnormal ultrasound: omphalocele
- arr 2p25.3p11.2(12,771-89,129,064)x2-3
- 47,XY,+i(2)(p10)[9]/46,XY[36]
Complex Mosaic Abnormality

- arr 4p16.3p15.33(970,878-12,433,910)x1
- arr 1p41.2p14q12(37,936,771 - 55,287,697)x2-3

Finding of Unclear Clinical Significance

- Abnormal ultrasound - cystic hygroma
- arr 12q24.32q24.33(129,068,615-130,178,142)x1 (1.1 Mb)
- Genes involved within variation: TMEM132C, SLC15A4, GLT1D1, TMEM132D

Multiple Segments of Homozygosity

Previous child with arr 17p11.2:[216,772,264-20,413,564]x1 and multiple segments of HMZ

Homzygosity >5 Mb detected

Total length of homozygous segments: ~154.4 Mb (5-6% of genome)
Conclusions

• Chromosomal microarray can identify a substantially higher percentage of clinically relevant abnormalities in prenatal samples.
• Deletions, duplications, and unbalanced rearrangements can be identified at a high resolution and with high sensitivity and specificity.
• Resolution has improved from 10 Mb by karyotyping to a few kb by CMA.

Conclusions

• Microarray cannot detect balanced rearrangements, some cases of uniparental disomy, and low-level mosaic abnormalities.
• Challenges for pretest and posttest counseling continue to evolve.

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