Molecular Cytogenetics Using Microarrays

*In a nutshell:*

- Molecular evaluation by microarrays is an alternative that has several advantages over traditional karyotyping.
- Is quickly becoming the primary tool for chromosomal evaluation after birth.
- Has been demonstrated to add important information to the evaluation of fetal anomalies and stillbirth.
- Still some unanswered questions regarding some aspects of clinical implementation of this technique.
What is a Microarray?

- A **microarray** is a “lab-on-a-chip”
- Can be used to study
  - gene expression
  - single nucleotide polymorphisms (SNPs)
  - whole genome comparative genomic hybridization (CGH)
    - Copy number variation (extra or missing pieces) of the genome
- Microarrays are a significant advance both because they may **contain a very large number of genes or gene regions** and because of their **small size**
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Comparative Genomic Hybridization (CGH) using Microarrays

- **A.K.A.**: Chromosomal microarray analysis, CMA, microarray-based comparative genomic hybridization, array CGH, a-CGH, aCGH
- A technique to detect genomic copy number variations at a higher resolution than can be seen by routine karyotype
Microarray Technology

Array CGH Maps DNA Copy Number Alterations to Positions in the Genome

Test Genomic DNA (your patient)  Reference Genomic DNA (normal control)

Gain of DNA copies  Loss of DNA copies
Array CGH: Trisomy 21

Normal

Trisomy 21

log2 ratio

log2 ratio

CHROMOSOME 7

MICROARRAY COVERAGE
BAC clones (blue) / oligo probes (orange)

TYPES OF MICROARRAYS

BAC Array
An array of thousands of BAC clones representing regions of interest in the genome

Oligo Array
An array of tens of thousands of oligo nucleotide probes representing regions of interest in the genome, as well as tile-like coverage at a lower density for most other regions of the genome.
Types of CGH arrays

- Oligonucleotide ("oligo") arrays
  - Made of short (30-50 bp) segments
- BAC arrays
  - Made of larger (150-750 bp) segments
- Targeted arrays
  - Fewer probes, maximal coverage of regions known to have genes with potential to cause problems
- Whole genome arrays
  - More dense coverage of the whole genome

Array Platform Comparison
Backbone and Resolution

der(4)t(4q;12p)

<table>
<thead>
<tr>
<th>Backbone</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>SignatureChipWG™</td>
<td>Chromosome 4</td>
</tr>
<tr>
<td>SignatureChipOS™</td>
<td>Chromosome 4</td>
</tr>
<tr>
<td></td>
<td>Chromosome 12</td>
</tr>
<tr>
<td></td>
<td>Chromosome 12</td>
</tr>
</tbody>
</table>
Copy Number Variants (CNVs)

- Copy number variants (CNVs) are regions of DNA usually larger than a kilobase (1000 base pairs) in size that are present at an altered copy number in comparison with a normal reference genome
  - Extra or missing pieces of chromosomes

- CNVs can be
  - Benign, often inherited
  - Pathologic
  - Of unknown significance

- In general, large alternations in gene-rich areas that are not inherited have the greatest likelihood of causing disease

Ultrasound Guided Amniocentesis

Amniocentesis with the Vidoson
History of Cytogenetics

- The Dark Ages (<1952)
  - Humans have 48 chromosomes
- The Hypotonic Period (late ’50’s)
  - Improved techniques: 46 chromosomes
- The Trisomy Period: 1959
  - Down syndrome is trisomy 21, etc
- The Banding Era: 1970
  - Chromosomes could be distinguished from each other
- The Molecular Age: current

Chromosome Analysis

- Labor intensive, subjective
- Can be used to “screen” for chromosomal abnormalities
Standard karyotype

G-banded analysis of metaphase chromosomes

Resolution:
5-10 Mb per G-band

High resolution:
3-5 Mb per G band

Fluorescence in situ Hybridization (FISH)

- Less labor intensive, rapid, high resolution, objective
- Need to have prior knowledge of regions to interrogate
Array CGH

- Phenotypic abnormality occurs below the 5-10 Mb resolution of karyotype
- Submicroscopic deletions, duplications and cryptic rearrangements increasingly recognized as cause of genetic disease
- aCGH can identify chromosome abnormalities that are 100x smaller than what can be seen by karyotype
- Although small, these abnormalities can still be responsible for phenotypic abnormalities

Microdeletion Detection

- 22q microdeletion
- DiGeorge syndrome
Microdeletion Detection

- 22q microdeletion
- DiGeorge syndrome

del(22)(q11.2q11.2) (D22S75-)
Submicroscopic Copy Number Variants (CNVs)

- Present in 5-18% of children with developmental delay
- Also cause other abnormalities: structural birth defects, spontaneous abortion, stillbirth
- Most are very small, and not detectable by routine karyotype

Miller et al, AJHG, 2010

Birth defects association with cryptic subtelomeric chromosomal rearrangements

<table>
<thead>
<tr>
<th>Facial Abnormalities</th>
<th>Skeletal Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleft palate 19,23,24,30</td>
<td>Vertebral and rib anomalies 24,29</td>
</tr>
<tr>
<td>Cleft lip and palate 25,29</td>
<td>Rib anomalies 19</td>
</tr>
<tr>
<td>Cleft lip 30</td>
<td>Thoracic hemivertebrae 19</td>
</tr>
<tr>
<td>Micrognathia 19,23</td>
<td>Craniosynostosis 19</td>
</tr>
<tr>
<td>Microphthalmus 19,27</td>
<td>Talipes equinovarus 24,27,32</td>
</tr>
<tr>
<td>Anophthalmus 19</td>
<td></td>
</tr>
<tr>
<td>Cardiac Defects</td>
<td>Renal</td>
</tr>
<tr>
<td>Pulmonary stenosis 20,23</td>
<td>Polycystic dysplastic kidney 25</td>
</tr>
<tr>
<td>Tetralogy of Fallot 23,28</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Atrial septal defect 19,24,25,29</td>
<td>Duodenal atresia 25</td>
</tr>
<tr>
<td>Ventriculo-septal defect 19,20,23,29</td>
<td>Gastrochisis 19</td>
</tr>
<tr>
<td>Mitral valve insufficiency 24</td>
<td>Diaphragmatic hernia 27</td>
</tr>
<tr>
<td>Tricuspid insufficiency 29</td>
<td>Genital Abnormalities</td>
</tr>
<tr>
<td>Intra cranial Abnormalities</td>
<td>Hypospadias / micropenis 19</td>
</tr>
<tr>
<td>Complete or partial agenesis of the corpus colosum</td>
<td>Micropenis 39</td>
</tr>
<tr>
<td>Ventricleomegaly 23,24,33</td>
<td>Other</td>
</tr>
<tr>
<td>Arnold Chiari type 1 24</td>
<td>Two-vessel umbilical cord 30</td>
</tr>
<tr>
<td>Posterior fossa cyst 30</td>
<td>Increased nuchal thickness 25,33</td>
</tr>
<tr>
<td>Cerebellar arachnoid cyst 31</td>
<td>Intrauterine growth retardation 20,23,24,25,27,29,32</td>
</tr>
</tbody>
</table>
Submicroscopic Abnormalities

- FISH can be used to detect specific microdeletion syndromes
- aCGH screens genome for all submicroscopic abnormalities
- Can detect large aneuploidies and monosomies, as well as submicroscopic deletions, duplications, cryptic rearrangements

Microdeletion Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Location</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prader Willi</td>
<td>15q</td>
<td>1/25K</td>
</tr>
<tr>
<td>Angelman</td>
<td>15q</td>
<td>1/30K</td>
</tr>
<tr>
<td>Velocardiofacial</td>
<td>22q</td>
<td>1/3K</td>
</tr>
<tr>
<td>Smith-Magenis</td>
<td>17p</td>
<td>1/25K</td>
</tr>
<tr>
<td>Williams</td>
<td>7q</td>
<td>1/10K</td>
</tr>
<tr>
<td>Allagille</td>
<td>20p</td>
<td>1/70K</td>
</tr>
<tr>
<td>Rubenstein-Taybi</td>
<td>16p</td>
<td>1/100K</td>
</tr>
<tr>
<td>WAGR</td>
<td>11p</td>
<td>1/40K</td>
</tr>
<tr>
<td>Miller Dieker</td>
<td>17p</td>
<td>1/85K</td>
</tr>
<tr>
<td>Wolf-Hirschhorn</td>
<td>4p</td>
<td>1/50K</td>
</tr>
<tr>
<td>Cri-du-chat</td>
<td>5p</td>
<td>1/35K</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>13q</td>
<td>1/23K</td>
</tr>
</tbody>
</table>
Array CGH and Prenatal Testing

- High resolution
- Simultaneously identifies both microscopic and submicroscopic alterations
- Can screen for a large number of abnormalities
- Comprehensive and phenotypic information is not necessary to perform array CGH

Microarray-based Cytogenetics

<table>
<thead>
<tr>
<th>Chromosome Analysis</th>
<th>Microarray Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic</td>
<td>Microscopic and submicroscopic</td>
</tr>
<tr>
<td>7-21 days</td>
<td>1-5 days</td>
</tr>
<tr>
<td>~4% detection rate</td>
<td>~20% detection rate</td>
</tr>
<tr>
<td>Low resolution (10 Mb)</td>
<td>High resolution (50Kb – 200Kb)</td>
</tr>
</tbody>
</table>
What Abnormalities Can be Detected by Microarray CGH?

WHOLE CHROMOSOME ANEUPLOIDY

Normal

Trisomy 21
Array CGH: Trisomy 21

What Abnormalities Can be Detected by Microarray CGH?

DELETIONS AND DUPLICATIONS
Chromosome 19 duplication

dup(19)(q13.2q13.4)

Array CGH: dup(19)(q13.2q13.4)

approx. 20 Mb duplication
Terminal and Interstitial Deletions of 1p36

1.6 Mb Terminal

2.6 Mb Interstitial

9.5 Mb Interstitial

Elastin

Chromosome 7

log₂ ratio

-1.5

-1

-0.5

0

0.5

1

1.5
What Abnormalities Can be Detected by Microarray CGH?

UNBALANCED STRUCTURAL REARRANGEMENTS
Ring 21 (r(21)(p11.2q22.3))

Array CGH: Ring 21 (r(21)(p11.2q22.3))

Chromosome 21

approx. 5 Mb deletion
### dup(4)(p15.2p16.3)

<table>
<thead>
<tr>
<th>Chromosome 4</th>
<th>Log_{2} ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approx. 29 Mb duplication AND 1.7 – 2.8 Mb deletion</td>
<td></td>
</tr>
</tbody>
</table>

#### Balanced Translocations

- 6% are associated with phenotypic abnormality
- Sometimes this is due to cryptic unbalance
- CGH can be useful in evaluation of breakpoints
Balanced Translocation

Karyotype: 46,XY,t(8;19)(q13;q13.1)
Other Benefits of array CGH

- Culture not required: faster time to results
- Automated: more objective assessment
- Better resolution: detection of submicroscopic rearrangements

Limitations of array CGH

- Balanced translocations will be missed
- Low level mosaicism will be missed
- Triploidy will be missed (or misclassified)
- Abnormalities of uncertain clinical significance may be detected
  - These may require parental samples to sort out
Limitations of aCGH: Translocations and Triploidy

- aCGH analysis will not detect balanced translocations
  - not clinically relevant for most prenatal cases
  - may provide important information for future generations and other family members
  - some de-novo translocations result in phenotypic consequence due to gene disruption
- Standard microarray analysis will also not identify triploidy because the relative gene content is balanced

Limitations of aCGH: Mosaicism

- Will not detect low-level mosaicism
  - Oligonucleotide array can detect mosaicism of 10% or greater
  - In some cases can detect mosaicism previously undetected by karyotype
- Standard karyotype detects 30–40% mosaicism with 95% certainty
- Importance of such low-level mosaicism when found prenatally is uncertain
Mosaicism

Results of Unknown Significance

- Results of unknown clinical significance are sometimes discovered
- With experience, these should become less frequent
- Some findings will always be difficult to interpret because of their variable phenotype
- A worldwide consortium (International Standards for Cytogenomic Arrays; ISCA) is collecting array findings and associated phenotypes and organizing them into a database within the National Institutes of Health (NIH).
### Indications for Prenatal Microarray

- **Evaluation of ultrasound abnormalities**
- **Identification of marker chromosomes**
  - Extra pieces of chromosomal material that can be hard to identify
  - Clinical significance usually depends on origin of marker
- **Evaluation of translocations**
  - Chance that a translocation will cause problems often depends on whether small pieces of material are missing
- **Evaluation of stillborn infants**
  - Tissue often doesn’t grow

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### Prenatal array CGH

- Many studies have evaluated prenatal use of aCGH
- In cases of typical prenatal indications (maternal age, enlarged NT, ultrasound abnormalities), after a normal karyotype:
  - 5-6% will have a CNV (abnormal aCGH result)
  - 1-1.5% will have a CNV of uncertain significance

*Savage et al, Curr Opinion Ob Gyn, 2011*
Prenatal Utility of aCGH

<table>
<thead>
<tr>
<th>Indication</th>
<th># Cases</th>
<th>Total Abn</th>
<th>aCGH</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abn US</td>
<td>173</td>
<td>23</td>
<td>22(96%)</td>
<td>16 (69%)</td>
</tr>
<tr>
<td>Abn screening</td>
<td>235</td>
<td>12</td>
<td>12 (100%)</td>
<td>11 (92%)</td>
</tr>
<tr>
<td>Fam Hx</td>
<td>8</td>
<td>8</td>
<td>8 (100%)</td>
<td>5 (62%)</td>
</tr>
<tr>
<td>AMA</td>
<td>273</td>
<td>11</td>
<td>11 (100%)</td>
<td>8 (73%)</td>
</tr>
<tr>
<td>Other</td>
<td>20</td>
<td>2</td>
<td>2 (100%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>60</td>
<td>1</td>
<td>1 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>906</td>
<td>57</td>
<td>56 (98%)</td>
<td>42 (78%)</td>
</tr>
</tbody>
</table>

6.3% 6.2% 4.6%

Armengol L, Hum Genet, 2011

aCGH: Prenatal Experience

Large meta-analysis of 10 studies of aCGH for prenatal diagnosis

- Following a normal karyotype:
  - 3.6% had abnormal aCGH
  - 5.2% of structurally abnormal fetuses had abnormal aCGH
  - 1.1% had result of uncertain significance

- Karyotype did not find any chromosomal imbalance that aCGH did not

Hillman et al, US Ob/Gyn, 2011
The potential advantages of array CGH over conventional karyotyping in prenatal diagnosis include higher resolution, avoidance of culturing amniocytes or chorionic villi, automation, and faster turnaround times.

The disadvantages include the inability to detect balanced inversions or translocations as well as certain forms of triploidy, and costs.

The technique detects a large number of either benign copy number variants or copy number variants of uncertain clinical significance and is unlikely to detect mosaicism below 20%.

ACOG Recommendations: 2009

Conventional karyotyping remains the principal cytogenetic tool in prenatal diagnosis.

Targeted array CGH, in concert with genetic counseling, can be offered as an adjunct tool in prenatal cases with abnormal anatomic findings and a normal conventional karyotype, as well as in cases of fetal demise with congenital anomalies and the inability to obtain a conventional karyotype.

Couples choosing targeted array CGH should receive both pretest and posttest genetic counseling.

Couples should understand that array CGH will not detect all genetic pathologies and that array CGH results may be difficult to interpret.

Targeted array CGH may be useful as a screening tool; however, further studies are necessary to fully determine its utility and its limitations.
Recently published guidelines that specifically endorse using arrays in cases where individuals show ‘multiple anomalies not specific to a well delineated genetic syndrome [such as] nonsyndromic developmental delay and intellectual disability and autism spectrum disorders.’

Also recommend the use of arrays in evaluation of children with growth retardation, speech delay, and other less-well studied indications.

They do not give guidance for prenatal testing.

**American College of Medical Genetics**

**International Standard Cytogenetic Array (ISCA) Consortium**

Consensus panel supports use of chromosomal microarray as a first line standard approach for the genetic evaluation of children with a range of genetic disorders including developmental delay or intellectual disability, autism spectrum disorder, or multiple congenital anomalies (birth defects).

*Miller et al, AJHG, 2010*
Summary of Utility of aCGH

- Molecular evaluation by microarrays has several advantages over traditional karyotyping
- Is quickly becoming the primary tool for chromosomal evaluation after birth
- Will almost certainly become primary tool for prenatal diagnosis in *near* future
- Has been demonstrated to add important information with fetal anomalies and stillbirth
- Some unanswered questions remain regarding aspects of clinical implementation of this technique

Thank You!!