Prenatal Diagnosis: Are There Microarrays in Your Future?

Susan Tran, MD
Division of Medical Genetics, and
Division of Maternal-Fetal Medicine
University of California, San Francisco

Financial Disclosure
I have no financial relationship with any aspect of private industry

Learning Objectives
- Define array comparative genomic hybridization
  - Methodology
  - Limitations
- Review literature on array CGH for prenatal use
- Discuss potential prenatal applications of array CGH
- Review key points for counseling patients

Case presentation
8 year old girl with severe short stature, exotropia, developmental delay, and history of atrial septal defect repair and G-tube requirement
- Born to 33 year-old G2 P1 at term with normal amniocentesis performed for “maternal anxiety”
- Initially presented at 6 months of age with feeding difficulties and severe short stature
- After numerous evaluations over several years, including a normal repeat (postnatal) karyotype, ultimately found to have de novo submicroscopic distal deletion of chromosome 15q on FISH subtelomere analysis
- Family relieved but devastated by diagnosis: “We got the amnio hoping to avoid this.”
Question
Had the technology been available 8 years ago, would you have offered this less than 35 year-old patient with “maternal anxiety” a microarray comparative genomic hybridization (array CGH) analysis?

1. Yes
2. No
3. What is array CGH?

Indications for prenatal diagnosis

- Advanced maternal age
- Abnormal fetal ultrasound findings
- Abnormal maternal serum screening
- Previous pregnancy or child with chromosome abnormality
- Chromosome rearrangement in either parent
- Increased risk for X-linked or single gene disorder

Chromosomal abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Incidence¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester spontaneous abortions</td>
<td>1 / 2.5</td>
</tr>
<tr>
<td>Neonatal deaths and stillbirths</td>
<td>1 / 16</td>
</tr>
<tr>
<td>Live births</td>
<td>1 / 156</td>
</tr>
</tbody>
</table>

Invasive prenatal diagnosis

- Amniocentesis
  - 15 weeks’ gestation
  - Procedure-related loss rate < 1:300 – 1:500¹

- Chorionic villous sampling (CVS)
  - 10 – 14 weeks’ gestation
  - Procedure-related loss rate: similar to amniocentesis in experienced centers and individuals¹
  - Risk of confined placental mosaicism: 1-2%


Current state of prenatal diagnosis

Karyotype analysis
- Cultured amniocytes or chorionic villi
- G-banded karyotype
  - ~ 400 bands
  - ~ 10 – 15 Mb resolution (~5 Mb for postnatal)
- Turnaround time: up to 14 days

Prenatal diagnosis: Evolving techniques

- Standard karyotype (G-banded karyotype)
- Fluorescence in situ hybridization (FISH)
- Microarray comparative genomic hybridization (array CGH)

Array comparative genomic hybridization
- Also known as microarray CGH
- Array [uh-rey]: to place in proper or desired order
- Multiple targets (e.g., DNA) fixed ("spotted" or "arrayed") on a solid support (e.g., glass slide)
  - Bacterial artificial chromosome (BAC): Short segments of DNA; ~ 80,000 – 200,000 bp long
  - Oligonucleotide: Short fragment of a single-stranded DNA; ~ 25 – 60 bp long
- Types of arrays
  - Targeted: Includes probes to assess only specific regions of interest within genome
  - Whole genome scanning: Probes evenly spaced throughout genome

Array comparative genomic hybridization
- Used with permission from Signature Genomic Labs educational materials

Array chips

BAC array

SNP array

www.dictionary.com
**Array CGH procedure**

1) Patient ("test") and control ("reference") DNA are fluorescently labeled
2) Samples compete to hybridize (bind) to corresponding DNA segments
3) Resulting fluorescent signals analyzed by computer program to detect DNA dosage alterations


**Advantages of array CGH**

- Potential for automation
- Rapid turnaround time
- Small sample requirement
- Higher resolution than standard karyotype
  - Can detect imbalances as small as 6 kb – 1 Mb (versus ~10 Mb with standard prenatal karyotype)
  - Detects microduplications / microdeletions

**Microdeletion / microduplication syndromes**

- Syndrome caused by chromosomal deletion involving several genes (contiguous gene deletion) that is too small to be detected by standard karyotype
  - Example: velocardiofacial syndrome (del 22q11.2)
    - Incidence ~13,000 live births
    - Variable phenotype: Cardiac defects, thymic aplasia, hypocalcemia, possible cognitive deficits and psychiatric/social disorders
  - Example: Smith-Magenis (del 17p11.2)
    - Incidence ~1:25,000 live births
    - Self-injurious behavior, sleep disturbance, stereotypic behaviors, ADD, facial features can resemble Down syndrome
- Individually rare but collectively 1:500 – 1:1,000 live births

**Disadvantages of array CGH**

- Will not detect balanced translocations (including triploidy) or inversions
- Will not detect low level mosaicism (< 10 – 20%)
Non-prenatal use of array CGH

Detection rate for chromosomal imbalances in patients with mental retardation\(^1\):

- Standard karyotype and FISH: ~10%
- Array CGH: additional 10% detected


Array CGH in prenatal specimens

- Products of conception from spontaneous abortions (SABs)
- Cell-free fetal DNA
- Fetuses with multiple malformations
- Cultured amniocytes and chorionic villi
- Direct analysis of prenatal specimens

Array CGH and spontaneous abortions


- Cultured tissue from spontaneous abortions (n = 41)
- GenoSensor Array 300 (targeted array)
- Detected all abnormalities previously identified by G-banding karyotype analysis
- 4 additional abnormalities not previously identified
  - Trisomy 21 found to be mosaic for trisomy 20
  - Duplication of 10q telomere region
  - Interstitial deletion of chromosome 9p
  - Interstitial duplication PWS/Angelman syndr (chr 15q)

Array CGH and cell-free fetal DNA


- Cell-free fetal DNA (cffDNA) from frozen amniotic fluid supernatant
- GenoSensor Array 300 (targeted array)
- 17 / 28 (~61%) cffDNA microarrays informative for fetal sex and aneuploidy
- Noninvasive, high-resolution prenatal diagnosis
### Array CGH and SABs

**Benkhalifa M et al (Prenatal Diagnosis, 2005)**
- Spontaneous abortion specimens that failed to grow in culture ($n = 26$)
- Human BAC Array - 1MB resolution
- 15 / 26 cases (58%) had chromosomal imbalances by array
  - Trisomy 8, 13, 18, 21
  - Monosomy 1, 16, 21
  - Deletion 22q
  - Duplication 1p
  - Double aneuploidy

### Array CGH and multiple malformations

**Le Caignec et al (J Med Genet, 2005)**
- Fetuses with 3 or more anomalies and normal karyotype ($n = 49$)
- DNA extracted from frozen lung tissue
- GenoSensor Array 300 (targeted array)
- Detected 5 chromosomal abnormalities (~10%)
  - Deletion 15q telomere
  - Interstitial deletion 16q
  - Deletion 22q11.2
  - Mosaicism for a rearranged chromosome 18
  - Deletion 6q subtelomere

### Array CGH & cultured prenatal specimens

- Cultured prenatal and postnatal samples with unbalanced rearrangements ($n = 30$)
- Custom targeted vs. 1 Mb resolution genome-wide array
- Custom array detected 29 / 30 abnormalities; 1 Mb array detected 22 / 30 abnormalities
- Small, targeted arrays preferable for prenatal screening
  - Less prone to technical error
  - Produce fewer false positives
  - Less expensive
  - Can be designed to avoid genomic regions with known polymorphic copy number variation (CNV)

### Array CGH & direct prenatal specimens

**Sahoo T et al (Genet Med, 2006)**
- Prospective study of uncultured prenatal samples ($n = 98$)
  - 57% amnio
  - 43% CVS
- Baylor targeted array (BACs, 366 clones, 55 disorders)
- Results of array CGH vs. standard cytogenetic techniques were 100% concordant (5 abnormalities)
- Results of array CGH on cultured vs. uncultured (direct) specimens were 100% concordant
- 12 / 98 cases (12%) found to have copy number variants
Copy number variation (CNV)

The human genome contains hundreds of segmental duplications and deletions (CNVs)

- AKA, copy number polymorphisms (CNPs)
- Several to hundreds of kilobases of genomic DNA among phenotypically normal individuals
- Can comprise ~12% of genome
- Unknown significance

Issues to consider regarding CNVs:

- Test parental specimens
- Check growing database of CNVs
- Consider variable / incomplete penetrance

UCSF pilot study of prenatal aCGH

- Evaluate the utility and power of array CGH in a clinical diagnostic setting
- ~1.5 Mb resolution BAC array
- 26 direct prenatal specimens compared with conventional cytogenetic analysis
  - 13 amniocenteses
  - 13 chorionic villus sampling
- Samples:
  - Cytogenetically normal karyotypes
  - Balanced translocation
  - Whole chromosome aneuploidies
  - Microscopic deletion
  - Submicroscopic deletion

UCSF study results

<table>
<thead>
<tr>
<th>Conventional cytogenetics</th>
<th>Array CGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESULT</td>
<td>N</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
</tr>
<tr>
<td>47,XX,+22</td>
<td>1</td>
</tr>
<tr>
<td>47,XY,+21</td>
<td>2</td>
</tr>
<tr>
<td>16q-</td>
<td>1</td>
</tr>
<tr>
<td>22q-</td>
<td>1</td>
</tr>
<tr>
<td>Balanced translocation</td>
<td>1</td>
</tr>
</tbody>
</table>

Detection of whole chromosome aneuploidy

- DNA isolation from 2 mL of amniotic fluid

[Graph showing log2 mean raw ratio for chromosome 21]
Detection of a submicroscopic deletion

22q TUPLE1 deletion

Chromosome 22

Clarification of an interstitial deletion

del 16qter

Direct CVS

Interstitial 16q deletion

Direct CVS

Summary: aCGH clinical applications

Advantages
- Scan the entire genome for copy number changes
- Detection of constitutional chromosomal aberrations
  - Whole chromosome aneuploidies
  - Deletions & duplications
  - Submicroscopic deletions
  - Telomeric / cryptic rearrangements
- High resolution
- Further characterization of cytogenetic ambiguities
- Rapid turn-around
- Potential for automation
- Small amount of sample required
Summary: aCGH clinical applications (cont’d)

Disadvantages
- Cannot detect “balanced” translocations or low-level mosaicism (<10 – 20%)

Prenatal array CGH: Ethical issues

- Information of unknown clinical significance could lead to unnecessary anxiety
- Will microarray technology increase the number of TABs, particularly in phenotypically “normal” fetuses (eg, no ultrasound abnormality) found to have an abnormality on array CGH?
- Will this lead to eugenics?

Genetic counseling

1) Pretest counseling
   a) Objective of test
   b) Methodology
      i. Limitations
   c) Logistics of obtaining samples (amnio, CVS)
   d) Potential for results of unclear significance
      i. Potential need to run parental samples (~10%)
      ii. Variable expressivity not predictable

2) Informed consent

3) Review / interpret results

Counseling your prenatal patients

1) Array CGH is a relatively new technology that allows for higher resolution chromosome analysis
   a) Evaluates for microscopic and submicroscopic chromosomal imbalances that could lead to birth defects and/or mental retardation

2) The test has limitations and can result in anxiety regarding results of unclear significance that may not be resolvable
   a) Karyotype should still be performed
   b) Variable expressivity of a potential finding cannot be predicted

3) Genetic counseling should be obtained!
Labs offering prenatal diagnosis by aCGH*

<table>
<thead>
<tr>
<th></th>
<th>Signature Genomic Labs</th>
<th>Baylor College of Medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Array type</strong></td>
<td>BAC</td>
<td>oligonucleotide</td>
</tr>
<tr>
<td><strong># Clones or oligos</strong></td>
<td>1083 clones</td>
<td>44,000 oligos</td>
</tr>
<tr>
<td><strong># Loci</strong></td>
<td>367</td>
<td>1,476</td>
</tr>
<tr>
<td><strong>Turnaround time</strong></td>
<td>5 – 7 days</td>
<td>5 – 9 days (direct)</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>$1850 - $1950</td>
<td>$1695</td>
</tr>
<tr>
<td><strong>Conditions</strong></td>
<td>&gt;70</td>
<td>&gt;140</td>
</tr>
<tr>
<td></td>
<td>Subtelomeric and pericentromeric regions</td>
<td></td>
</tr>
<tr>
<td><strong>Website</strong></td>
<td><a href="http://www.signaturegenomics.com">http://www.signaturegenomics.com</a></td>
<td><a href="http://www.bcm.edu/cma">http://www.bcm.edu/cma</a></td>
</tr>
</tbody>
</table>

* According to respective websites as of April 2008

Conclusions

- Array CGH is a cytogenetic technique that can detect chromosomal imbalances not detectable by routine karyotype
- These imbalances can lead to potentially devastating conditions
- Array CGH may be applied to prenatal genetic samples (SABs and living fetuses), and prospective trials are underway
- Despite its advantages, array CGH’s current limitations necessitate pursuit of thorough genetic counseling
- Array CGH technology is evolving and could eventually become standard of care… Your patients WILL be asking about it!

Future directions

- Use of larger, genome-wide arrays as the issue of copy number variants is further clarified
- Should array CGH be offered to all women undergoing invasive testing?
- Noninvasive testing
  - Cell-free fetal DNA
  - Fetal DNA from trophoblast cells in cervical mucus

Thank you