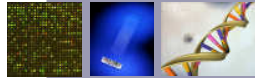


## UCSF Antepartum Intrapartum Management Course

June 2008

### Prenatal Diagnosis: Are There Microarrays in Your Future?



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## Financial Disclosure

I have no financial relationship with any aspect of private industry

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## 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

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## Case presentation

**8 year old girl with severe short stature, exotropia, developmental delay, and history of atrial septal defect repair and G-tube requirement**

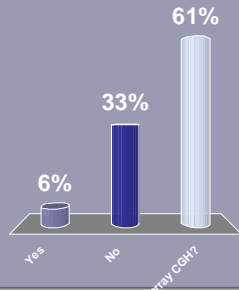
- Born to 33 year-old G2 P1→2 at term with normal amniocentesis performed for "maternal anxiety"
- Initially presented at 6 months of age with feeding difficulties and severe short stature
- After numerous evaluations over several years, including a normal repeat (postnatal) karyotype, ultimately found 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."

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### 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?



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### 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

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### Chromosomal abnormalities

	Incidence <sup>1</sup>
First trimester spontaneous abortions	1 / 2.5
Neonatal deaths and stillbirths	1 / 16
Live births	1 / 156

<sup>1</sup>South ST, Chen Z, Brothman AR. Genomic medicine in prenatal diagnosis. Clin Obstet and Gynecol. 2008;51(1):62-73.

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### Invasive prenatal diagnosis

- **Amniocentesis**
  - 15 weeks' gestation
  - Procedure-related loss rate < 1:300 – 1:500<sup>1</sup>
- **Chorionic villous sampling (CVS)**
  - 10 – 14 weeks' gestation
  - Procedure-related loss rate: similar to amniocentesis in experienced centers and individuals<sup>1</sup>
  - Risk of confined placental mosaicism: 1-2%

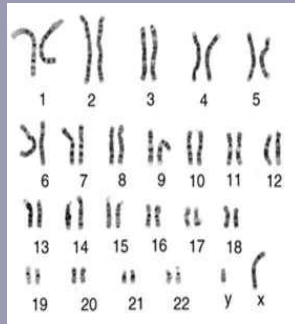
<sup>1</sup>American College of Obstetricians and Gynecologists. Invasive prenatal testing for aneuploidy. ACOG Practice Bulletin No. 88. Obstet Gynecol 2007; 110:1459.

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## Current state of prenatal diagnosis

### Karyotype analysis

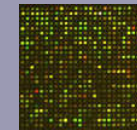
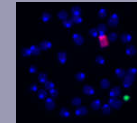
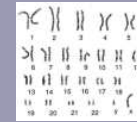
- Cultured amniocytes or chorionic villi
- G-banded karyotype
  - ~ 400 bands
  - ~ 10 – 15 Mb resolution (~5 Mb for postnatal)
- Turnaround time: up to 14 days



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## Prenatal diagnosis: Evolving techniques

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



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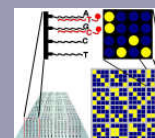
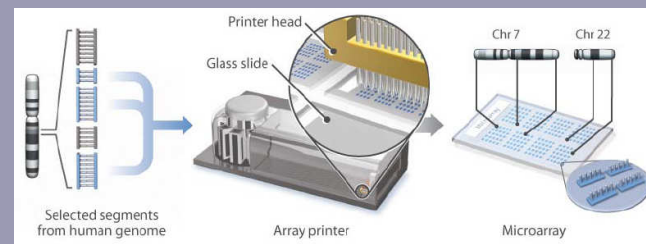
## Array comparative genomic hybridization

- Also known as microarray CGH
- Array [*uh-rey*]: to place in proper or desired order<sup>1</sup>
- Multiple targets (eg, DNA) fixed (“spotted” or “arrayed”) on a solid support (eg, 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

<sup>1</sup>www.dictionary.com

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## Array comparative genomic hybridization



BAC array\*

SNP array

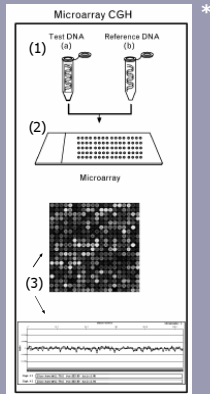


Array chips

\*Used with permission from Signature Genomic Labs educational materials

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## 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

\*Lapierre JM, Tachdjian G. Detection of chromosomal abnormalities by comparative genomic hybridization. 2005. Curr Opin Obstet Gynecol 17:171-177.

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## 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

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## 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 ~1:3,000 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

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## Disadvantages of array CGH

- Will not detect balanced translocations (including triploidy) or inversions
- Will not detect low level mosaicism (< 10 – 20%)

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## Non-prenatal use of array CGH

Detection rate for chromosomal imbalances in patients with mental retardation<sup>1</sup>:

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

<sup>1</sup>deRavel TJJ et al. What's new in karyotyping? The move towards array comparative genomic hybridisation (CGH). 2007. Eur J Pediatr 166:637-643.

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## 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

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## Array CGH and spontaneous abortions

Schaeffer AJ et al (Am J Hum Genet, 2004)

- 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)

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## Array CGH and cell-free fetal DNA

Larrabee PB et al (Am J Hum Genet, 2004)

- 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

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## 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

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## 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

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## Array CGH & cultured prenatal specimens

Rickman L et al (J Med Genet, 2006)

- 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)

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## 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

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## 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

Iafrate AJ et al. Nat Genet, 2004  
Sebat J et al. Science, 2004

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## 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

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## UCSF study results

Conventional cytogenetics

RESULT	N
Normal	20
47,XX,+22	1
47,XY,+21	2
16q-	1
22q-	1
Balanced translocation	1

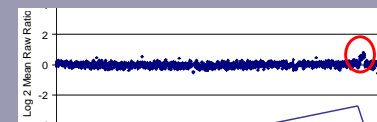
Array CGH

RESULT	N
Normal	21
47,XX,+22	1
47,XY,+21	2
16q-	1
22q-	1
Balanced translocation	0

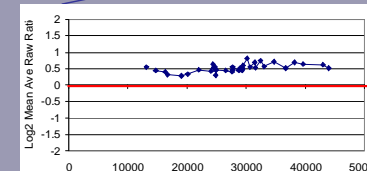
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## Detection of whole chromosome aneuploidy

- DNA isolation from 2 mL of amniotic fluid



Whole genome hybridization

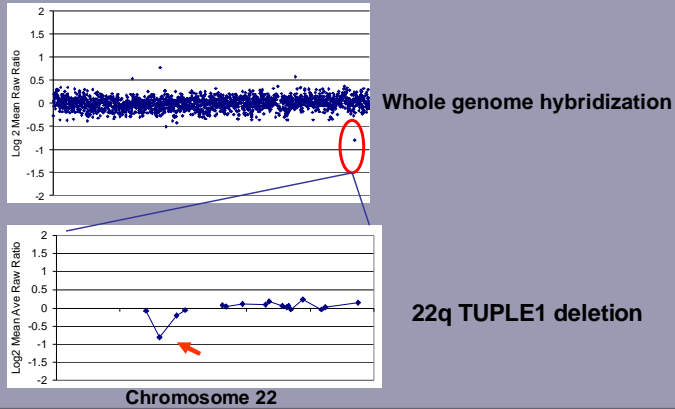


Trisomy 21

Chromosome 21

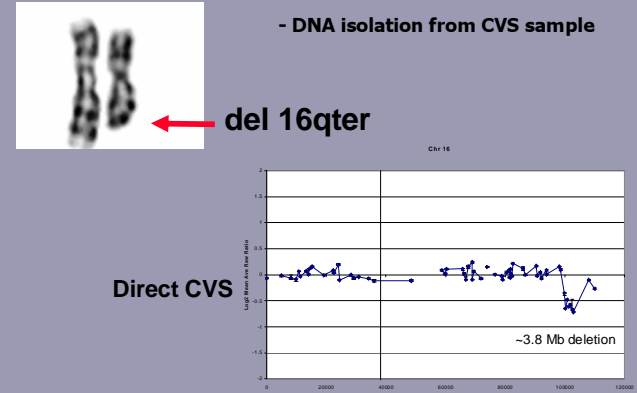
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## Detection of a submicroscopic deletion



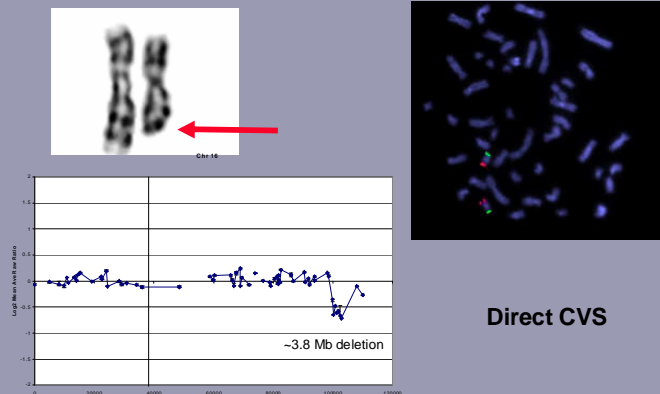
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## Clarification of an interstitial deletion



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## Interstitial 16q deletion



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## 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

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## Summary: aCGH clinical applications (cont'd)

### Disadvantages

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

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## 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?

Shuster E. Microarray genetic screening: A prenatal roadblock to life? 2007 Lancet 369(9560):526-9.

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## 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

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## 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!

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### Labs offering prenatal diagnosis by aCGH\*

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

\* According to respective websites as of April 2008

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### 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!

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### 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

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Thank you

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