Microarrays: What Do They Add?

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Disclosures
• I have no relevant financial relationships

Objectives
• Prenatal chromosomal assessment: the basics
  • Traditional karyotype
  • Array CGH, CMA
• Traditional karyotype vs. Array CGH
  • Benefits & Limitations; Array choices
• Professional organization recommendations
  • ACOG, ACMG
• Utility in certain clinical circumstances
  • Screening
  • Fetal anomalies
  • NICHD prenatal array trial

Case 1
26 yo G2P1 presents for initial prenatal care visit
  US reveals missed AB
  Prior neonatal death
  Child with cognitive disability
  Fetus with congenital malformation(s) - heart defect

She asks why?
By traditional karyotype - What is the burden of chromosomal disease in each of these circumstances?
**Impact of chromosomal abnormality by traditional karyotype**

- Miscarriage ~30%
- Infant and childhood death 5-7%
- Structural congenital malformation 4-8%
  - Congenital heart defects 13%
  - Multiple birth defects and Cog disability 5.5%
  - Cognitive Disability 3-35%
  - Multiple miscarriage 2-5%
- Risk of chromosomal abnormality at term with normal phenotype <1%
- Why?
  - Refine prognosis, tailor treatment, provide recurrence

EB Hook 1992; Gardner and Sutherland 2004

**Development of Cytogenetics**

Chromosome “colored body”
- 19th century - “stuff of heredity”
- 1956 - Hypotonic wash
- 1959 - Down syndrome (Lejeune)

Cytogenetics
- Cultured cells
- Banding
- Structural abnormalities

**Attempts to Refine Analyses of Chromosomes (1980-1990s)**

Prometaphase – prophase banding
G-band: 400-450
Turn-around: 7-14d
Resolution: 3-10MB
Disease occurs at a lower resolution
- Single gene
- Deletion/duplication

Reverse banding

Centromere banding

Telomere banding

Fluorescence in situ hybridization (FISH)
Case 2

41 yo G2P1 at 9 3/7 weeks, spontaneous conception

- Prior Pregnancy
  - Amniocentesis for “AMA” – 46, XY (450 bands)
  - Term SVD
  - Murmur in postnatal period
    - Williams syndrome – 1.55MB deletion 7q11.2
    - Incidence ~1 in 7500
- Current pregnancy: Desires invasive testing

Invasive prenatal testing - Indications

- “Advanced maternal age”
- Abnormal fetal US findings
- Abnormal screening (serum, NT, sequential screen)
- Previous pregnancy with chromosomal abnormality
- Parental balanced rearrangement
- Family history of a genetic disorder putting fetus at risk
- Anyone?
  - “Maternal anxiety”

Deletion/Duplication Disorders

- Deletion Syndrome
  - Chromosomal copy number variant (CNV)
    - Deletion typically spanning several genes
  - Cannot detect most with conventional cytogenetic methods.
    - <3MB of missing/extra material
    - Accounts for up to 15% of genetic disease burden*
  - FISH or Microarray CGH can detect chromosomal deletions
    - 22q11 deletion syndrome – conotruncal, palate, craniofacial, etc
    - Smith-Magenis del(17)(p11.2)
    - Williams-Beuren del (7)(q11.23)  
* Vissers LE 2005
Deletion/Duplication Disorders

Duplication Syndrome
- Chromosomal copy number variant (CNV)
- Additional material → Consistent abnormal phenotype
- In general, less severe
  - Tandem duplications and *most are inherited* from one parent
- Individual disorders rare
- Aggregate - about 1/1000 livebirths
  - Microduplication syndromes - Reciprocal to microdeletion syndromes
    - DiGeorge/Velocardiofacial (del 22q11) → dup(22)(q11.2)
    - Smith-Magenis del(17)(p11.2) → dup (17)(p11.2)
    - Williams-Beuren del (7)(q11.23) → dup (7) (q11.23)

III. Microdeletion

Test type: Molecular cytogenetic
Test method: Fluorescent in situ hybridization (FISH)

IV. Deletion of a portion of a chromosome

Test type: Chromosome analysis
Comparative Genomic Hybridization (CGH)

1. Subject & reference DNA are differentially labeled
2. Samples compete to hybridize (bind) to referent segments (normal metaphase chromosomes)
   - An “array” consists of DNA fragments of known sequence printed on a platform (a glass slide or chip)
3. Genomic imbalances produce differential fluorescent signals
   - Analyzed by computer and laser scanner
4. “virtual or molecular karyotype” Resolution: 3-10MB

Microarray CGH, CMA

- Multiple targets (eg, DNA) fixed (“spotted” or “arrayed”) on a solid support (eg, glass slide, chip)
- Substantial improvement in resolution
  - Improved detection of disease causing chromosomal changes compared with traditional karyotype
- Higher referent segment density = higher accuracy
  - Multiple probe deletions or duplications – better reproducibility
  - Increased chance of finding CNV

Array platforms

- Several varieties of CMA
- Targeted arrays
  - Bacterial artificial chromosome (BAC)
    - Short segments of DNA
    - ~ 80-150 kb
- Whole genome
  - Oligonucleotide
    - Short fragments of a single-stranded DNA
    - ~ 50-60 bp long
  - Single nucleotide polymorphisms (SNP)
Array Platforms: Clinical choices

• BAC vs. Oligonucleotide array
  • Oligo’s: smaller segments of DNA
    • Increased resolution, breakpoint specificity & reproducibility
    • Pathogenic CNV and CNV of uncertain significance
  • Targeted: More reliable clinical phenotype
    • Penetrance, expressivity
    • Few pathogenic CNV and CNV of uncertain significance

• SNP Arrays
  • Assess contiguous regions of homozygosity
    • Uniparental disomy, consanguinity

Microarray CGH

• Interrogation many DNA probes
  • Detect all aneuploidies
  • Detect additions & deletions 100-200 kb vs. traditional karyotype ~5 Mb

Advantages – Microarray CGH

• Higher resolution
  • Detect disorders typically missed by conventional cytogenetics
    • Smith-Magenis, 22q11, Angleman, Prader Willi, etc

• Versatility
  • Designed as targeted arrays - Prenatal/Pediatric chips
    • ~200 recognized disorders
    • Sensitivity for detection is variable
      • Based on contribution of deletion/duplication to underlying disorder

• Faster: TAT (5 to 9 days)
  • Smaller amounts of DNA required – Cell culture is not required
    • Direct CVS, amniocytes, IUFD, SAB, cell free DNA in AF

Disadvantages - Microarray CGH

• Cannot detect balanced structural rearrangements
  • Balanced translocations, inversions, triploidy
  • Multiple SABs

• Cannot differentiate trisomy 21 from Roberstonian TL
• Cannot detect low-level mosaicism (10-20%)
• Cost: $$$, insurance, access
• No single gene disorders (e.g. point mutation, CF)
• Copy number variations (CNVs) of unclear significance
  • Regions of DNA loss or gain which are not known to alter phenotype pathologically
CNVs of undetermined significance

• Significance of CNV
  • Estimated – 800 per person (Albertson 2003)
  • Outside of gene rich & highly conserved regions of genome
    • Most located in “gene deserts”
  • Few code for proteins of development
  • “Environmental sensor genes” – cell adhesion, sensory perception, chemical stimuli and neurophysiologic processes
  • Contribute to human phenotypic diversity

• When identified
  • Often benign if median size < 0.43 Mb
  • More often relevant if median size >2.76 Mb
  • Must compare to parental studies, phenotype
  • Compare with published databases of known CNVs
    • DECIPHER, ECARUCA, UC Santa Cruz, Toronto database of genomic variants
  • No guarantees – may or may not - adverse effect
    • May lead to considerable parental anxiety

Application of aCGH in Pediatrics

• Cognitive disability +/- dysmorphic features and congenital anomalies with normal traditional karyotype
  • 4-20% with pathologic imbalances (1MB platform)
• Array CGH is 1st Tier (not traditional karyotype) for evaluation of a child with:
  • Multiple anomalies not specific to a genetic syndrome
  • Nonsyndromic developmental delay/intellectual disability
  • Autism spectrum disorders

• When the first line for multiple congenital anomalies alone?

( Menten, 2006; Shaffer 2006; de Vries 2005; Shaw-Smith 2004; Manning, 2010)

Case 3

• 36 yo G1P0 at 9 weeks – presents to initiate care
  • Cousin with cognitive disability
  • “I want invasive testing and an array”
  • Array CGH?
    • Targeted vs. Genome wide?
    • If you offer array – what are the risks and benefits?
  • Pathologic CNV vs. CNV of uncertain significance
    • What are chances of both?
    • What do ACOG and ACMG suggest?
Array or not? What does ACOG say:

**Recommendations**
- 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. Follow-up genetic counseling is required for interpretation of array CGH results. 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.

ACOG Committee Opinion #446 2009

And ACMG?

2. Microarray CGH should not be used as a first-tier test in prenatal diagnosis. Limited use of the targeted array may be helpful in the evaluation of fetuses with structural anomalies and normal chromosome analysis or of marker chromosomes.33

Manning 2007

Microarray CGH—in addition to karyotype

- Meta-analysis (8 studies, 798 pregnancies)
- Invasive testing
  - Indications: AMA (60%), ultrasound abnormalities, anxiety, family hx
- Normal karyotype, 8 studies, 798 pregnancies
- All indications
  - 3.6% pathogenic (1-33%)
  - 1.1% uncertain significance
- Similar pick-up compared to traditional karyotype

Hillman 2011

Microarray CGH—in addition to karyotype

- Cross-sectional (3171 pregnancies)
- Invasive testing
  - Indications: AMA, ultrasound, abnl karyotype, anxiety
  - Different platforms (1MB BAC, 60k oligo)
  - In addition to abnormalities detected on traditional karyotype
    - 1.1% pathologic CNV (total cohort)
    - 11.8% pathologic CNV (balanced rearrangements/marker)
    - 10.5% pathologic CNV (major anomaly on US)
    - 0.2% uncertain significance

C-N Lee BJOG 2012
NIH Prenatal array trial

- Sequential patients (n=4401) with CVS/Amnio
- Indications
  - AMA (46%), Abnl screening (18%) US findings (26%)
- Results in 99%, uncultured in 88%
- Results
  - Normal
  - CNV – benign
  - CNV – pathogenic
  - CNV – unknown clinical significance

- Traditional karyotype
  - 7.3% aneuploidy
  - 1.3% sex chromosome aneuploidy
- Array CGH
  - Detected all of the above
    - US abnormalities – 5.8% “potential or known clinical significance”
    - AMA, + screening – 1.7% “potential or known clinical significance”

- Balance of increased resolution with variants of uncertain significance
- Maternal/family anxiety/stress – short and long term
Case 3 - revisited

- 36 yo G1P0 at 9 weeks – presents to initiate care
  - Cousin with Cognitive disability
  - Choosing invasive testing
  - Array?
    - ACOG & ACMG do not recommend
    - Targeted vs. Genome wide?
    - “pick-up” of pathologic genomic imbalance – 1-6%
    - CNV of unknown but potential significance – 0.2-3%
  - How does this compare?

Microarray CGH – Obstetric applications

- Tissue/sample at high risk of culture failure
  - Miscarriage, IUFD, ?late amniocentesis
- SABs – karyotype vs. targeted array
  - Additional 9.8% del/dups; detected all chromosomal abnls
- IUFD/Stillbirth
  - Retrospective, two anomalies present
  - 13% additional pathologic abnormalities
    - Prior sample classified as normal, or no results
- Preimplantation Genetic Diagnosis
  - Aneuploidy

(Raca, 2009)

Microarray CGH – Ethical considerations

- Information of unknown clinical significance could lead to 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?

Array – Genetic Counseling

- Pretest counseling
  - Objective of testing – etiology, refine prognosis, therapy
  - Methodology
    - Limitations – not all genetic abnormality is eliminated
  - Obtaining samples (amnio, CVS, blood, tissue)
  - Potential for results of unclear significance
    - Potential need perform parental sample testing (~10%)
    - Incomplete penetrance/Variable expressivity not predictable
  - Parental Consent (both)
  - Post test: Appointment to Review / Interpret results
aCGH in prenatal diagnosis: Summary

- Increased resolution compared with traditional karyotype
  - Traditional karyotype is still first tier in prenatal diagnosis
  - ~6% pathologic CNV with US abnormality, normal karyotype
    - Refine prognosis, therapy and offer recurrence
- Rapid TAT when compared with karyotype
- Useful with non-dividing cells
  - Small amount of DNA
  - IUFD, SAB, late amniocentesis
- May be useful in certain clinical circumstances
  - US anomalies associated with deletion/duplication
  - de novo balanced rearrangements, marker chromosomes

Microarray CGH – Summary

- CNV of uncertain significance
  - May be as high as 1.5-3%
    - Lower rates at centers/labs with highest volume?
    - Will decrease with time/experience
- Appropriate counseling of risks and benefits
  - Formal genetic counseling
  - Geneticist
  - Perinatologist/MFM
- When will the microarray CGH replace traditional karyotype in prenatal diagnosis?

Thank you!

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