Utility of Array Comparative Genomic Hybridization as a Primary Analysis for the Indication of Developmental Delay/Mental Retardation

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This speaker has nothing to disclose.
What is cytogenetics

- The original whole genome analysis
  - Analysis of chromosomes from a tissue of interest to identify large scale genomic alterations
    - G-banded chromosome analysis (karyotype)

- Molecular cytogenetics analyzes smaller regions for imbalances and rearrangements
  - FISH and Array CGH
Pediatric indications for a cytogenetic analysis

- Growth abnormality
  - Small/large for age
- Neurologic impairment
  - mental retardation / seizures / microcephaly / hypotonia / psycho-emotional dysfunction
- Dysmorphic features
- Cardiovascular malformations
- Other congenital anomalies

Chromosomal anomalies are responsible for birth defects in ~0.2% of live births

Most common tissue studied: peripheral blood
Standard Chromosome Analysis

- G-banding (Giemsa) chromosomes in metaphase

- Benefits:
  - Viewing entire genome
  - Can visualize individual cells and individual chromosomes

- Limits:
  - Limit of resolution around 5-10 Mb (depending on region of genome and length of chromosomes)
  - Need an actively growing source of cells
Common types of chromosome abnormalities detected with standard chromosome analysis:

- aneuploidies
- deletions, duplications
- inversions

- Balanced and unbalanced translocations

Trisomy 21
Terminal deletion of 11
Pericentric inversion of 18

Cell 2

Reciprocal translocation between 3 and 6

Unbalanced translocation between 13 and 14
Fluorescence *in situ* hybridization (FISH)

- First described by Pinkel, Straume, & Gray in 1986
- Label DNA with fluorescent molecule and hybridize to human chromosomes on a slide

**Benefits:**
- Can turn almost any DNA into a probe
- For clinical use, most probes 100-500 kb
- Much higher resolution as compared to G-banding for identifying deletions, insertions, and translocation breakpoints
- Can use cells in any state of the cell cycle as well as archived tissue
- Can analyze results on a cell-by-cell basis
- Shorter TAT since tissue does not need to be cultured for metaphase cells

**Limits:**
- Only going to see the region of the genome complementary to your probe
Example of FISH to detect a small deletion

- Microdeletion of 4p detected by FISH using a probe for the Wolf-Hirschhorn syndrome (WHS) critical region (red) and chromosome 4 centromere (green)

![Image of normal appearing 4s]

![Image of FISH result: del(4)]

deletion between 2-4 Mb in 25-30% of patients with WHS

Must have suspicion of WHS to run this probe
Comparative Genomic Hybridization (CGH) Microarray

Test Sample (Genomic DNA)

Exp. 1

Test
Cy3

Reference
Cy5

Exp. 2

Test
Cy5

Reference
Cy3

true gain

artifact

true loss

Cy3/Cy5 Ratios
Array CGH data from a BAC-based chip with dye-swap experiment

Gain of terminal end of chromosome 2

Array CGH data from an oligo-based chip without dye-swap

Loss of interstitial region in chromosome 2
Copy Number Array Platforms

- **Oligo Arrays**
  - Agilent
  - Nimblegen
  - Signature Genomics

- **BAC Arrays**
  - BlueGnome
  - Signature Genomics
  - Spectral Genomics

- **SNP Arrays**
  - Affymetrix
  - Illumina

**GENOMIC COORDINATES**
CGH Microarray

Benefits

- Can customize array to concentrate clones in areas of interest (targeted regions) and/or spread clones throughout genome (backbone)
- Resolution will depend on density of clones in region of interest, but can be as good as 5 kb
- Detection of smaller abnormalities
- Detection of cryptic abnormalities
- Better definition of cytogenetic abnormalities
- Interpretation usually less subjective than standard chromosome analysis
- Can use on archived or non-growing tissue
CGH microarray

- **Limits**
  - Will not detect balanced rearrangements
  - May uncover copy number changes of unclear clinical significance
  - Will not detect copy number changes in regions of the genome that are not on the array platform (chip)
Detection rate for each technology

- **Routine G-banded chromosome analysis**
  - 5-10% (depending on severity of MR and MCA)

- **Subtelomeric FISH (screening) after normal chromosomes**
  - 2-3%

- **Array CGH after normal chromosomes**
  - 10-15%
Why the increased detection?

- The estimated per locus mutation rate for genomic rearrangements is approximately three to four orders of magnitude greater than that of single nucleotide substitution.
Detection of small gains and losses: Microdeletion on 4p detected by CGH microarray

normal appearing chromosome 4s

reciprocal deviation at distal end of 4p showing a loss

Array CGH results and patient’s phenotype (growth retardation, distinctive facial features, seizures) consistent with WHS
Detection of large cryptic abnormalities

Chromosome analysis normal. Array CGH showed a 21 Mb duplication of 2qter and a 16 Mb deletion of 5pter – likely an unbalanced translocation with 2q “replacing” 5p.
Better definition of cytogenetic abnormalities

G-band designation vs. Array CGH and Database mapping

7q34 (+/- a band = +/- 5 Mb) vs. 7q35 – q36.1, size defined +/- 75 kb

Slide courtesy of CL Martin
Combination of better definition of visible abnormality and identification of cryptic abnormalities in same patient

- 5 yo male
- developmental delay
- cytogenetic analysis showed a t(2;18) that looked balanced
Microarray revealed three significant abnormalities

- Terminal deletion of 9p – 5.9 Mb
- Terminal duplication of 18p – 6.0 Mb
- Interstitial deletion of 18q – 1.2 Mb
- **18q11.2** LOSS
  - Suggests loss at the breakpoint of the t(2;18)
- **9p24** LOSS
- **18p11.3** GAIN
  - Suggests an unbalanced translocation with 18p gain on deleted 9p

Normal appearing 9s

Image showing normal 9s and derivative 9 (der(9)) with 18p on 9p.
Less subjective analysis of chromosome rearrangements

- Prenatal and postnatal growth retardation
- Unusual facial features
- Hip dislocations
- Required G-tube for feeding
- At 3 years of age, functioning in the moderate range of mental retardation
- Both parents apparently phenotypically normal
Interpretation:
Both proband and mother have a paracentric inversion in the long arm of 9:
inv(9)(q32q34.3)
But this does not explain differing phenotypes
Differing array CGH results despite identical banding patterns

Hypothesis – Mom’s abnormal but balanced 9 underwent a complex recombination event during meiosis to become unbalanced, but coincidentally the banding pattern was retained.
Detection of abnormalities of unknown clinical significance

- 3 y.o. female referred for microarray analysis
- Developmental delay
- Right polycystic kidney

LOSS
chr2:59,900,000-62,600,000
Benign vs. Pathogenic

1. Size
2. Location in the Genome
3. Genomic Content
4. Comparison with other Cases
5. Inherited or *de novo*
~12 genes

No seg dups
Comparison with other cases:
del(2)(p15p16.1) – Literature Search

Clinical and molecular cytogenetic characterisation of a newly recognised microdeletion syndrome involving 2p15-16.1
E Rajcan-Separovic, C Harvard, X Liu, B McGillivray, J G Hall, Y Qiao, J Hurlburt, J Hildebrand, E C R Mickelson, J J A Holden, M E S Lewis

A newly recognised microdeletion syndrome involving 2p15p16.1: narrowing down the critical region by adding another patient detected by genome wide tiling path array comparative genomic hybridisation analysis

The facial dysmorphism in the newly recognised microdeletion 2p15 p16.1 refined to a 570 kb region in 2p15
E Chabchoub, J R Vermeesch, T de Ravel, P de Cock and J-P Fryns
2\textsuperscript{nd} case with abnormality of unknown clinical significance

Referred for developmental delay and multiple congenital anomalies

Loss chr4:189,477,805-191,411,218
Low # genes

Some segmental duplications, Telomere associated repeats
Database of Genomic Variants on Human Genome Assembly Build 35 (hg17): Locus Summary

**Locus:** Locus1062

**Genome context (see the graphic below):**

<table>
<thead>
<tr>
<th>Chr</th>
<th>191170k</th>
<th>191180k</th>
<th>191190k</th>
<th>191200k</th>
<th>191210k</th>
<th>191220k</th>
<th>191230k</th>
<th>191240k</th>
<th>191250k</th>
<th>191260k</th>
</tr>
</thead>
</table>

**Cytogenetic Bands**
- 4q35.2

**RefSeq Genes**
- FRG1 | NM_004477

**Segmental Duplications (TCAG)**
- DC1315
- DC1316

**Genomic variants**
- Variation_4426|CTD-2032D23|Wong et al. (2007)
- InDels < 1kb

**Variation:** Variation_4426

**Landmark:** CTD-2032D23 (Genome Browsers: [TCAG Segmental Duplication](http://tcagensembl.sourceforge.net), [UCSC](http://genome.ucsc.edu), [Ensembl](http://www.ensembl.org))

**Variation Type:** CopyNumber

**Overlap with TCAG Segmental Duplication:** Yes

**Gap within 100k:** No

**Known Genes:** FRG1

**Method:** Array CGH

**Reference:** Wong et al. (2007)

**Pub Med ID:** 17160887

**Frequency Information:**
- **Subject Cohort:** Control
- **Sample Size:** 95 individuals
- **Normal Gain:** 1
- **Normal Loss:** 2
- **Total Gain/Loss:** 3
### Comparison with other Cases

**Original Article**

Subtelomere FISH analysis of 11,688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities

J B Ravnæn, J H Tepperberg, P Papenhausen, A N Lamb, J Hedrick, D Eash, D H Ledbetter, C L Martin


<table>
<thead>
<tr>
<th>Case</th>
<th>Karyotype</th>
<th>Chromosome</th>
<th>Father</th>
<th>Sex</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>del(4)(qter)</td>
<td>4q</td>
<td>Father same</td>
<td>Yes</td>
<td>DD, MR, obese, upper palpebral fissures, 5th finger clinodactyly, chorea movements</td>
</tr>
<tr>
<td>61</td>
<td>del(4)(qter)</td>
<td>4q</td>
<td>Father same</td>
<td>Yes</td>
<td>DD, MR</td>
</tr>
</tbody>
</table>

These cases were detected by FISH; Size not determined
The same size deletion was subsequently identified in the proband’s phenotypically normal father.

Is the 4q deletion pathogenic or a benign familial variant?

- Imprinting
- Penetrance
- Genetic background
As with many new technologies, array CGH has provided data that challenges old paradigms.

Expansion in Size of a Terminal Deletion: a Paradigm Shift for Parental Follow-up Studies
RB came to the clinic as a 3½-year-old female with hypomyelination, ataxia, anal stenosis and a history of growth retardation (first noticed at 6 months), and mild developmental delay.

No other birth defects were recognized.

For family history, the mother reported having anal stenosis which required rectal dilatation as a child, two previous miscarriages, and a nephew with cleft lip and palate, but mother was phenotypically normal.
Proband was found to have a terminal deletion of chromosome 18q on a 550 band karyogram, confirmed by the 18q subtelomere probe.

Aqua – 18 centromere
Red/Green Fusion – 18q
Parental chromosomes were normal, but the mother was surprisingly found to have an 18q subtelomere deletion.
Comparison of proband and mother with array CGH shows expansion of terminal deletion

### A.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Cyto band</th>
<th>Location of most distal normal probe (bp)</th>
<th>Location of most proximal deleted probe (bp)</th>
<th>Location of most distal deleted probe (bp)</th>
<th># of deleted probes</th>
<th>Approximate size of deletion (megabases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>proband</td>
<td>chr18</td>
<td>q23</td>
<td>72,268,375</td>
<td>72,366,480</td>
<td>76,083,258</td>
<td>42</td>
</tr>
<tr>
<td>mother</td>
<td>chr18</td>
<td>q23</td>
<td>75,544,270</td>
<td>75,641,908</td>
<td>76,083,258</td>
<td>9</td>
</tr>
</tbody>
</table>

### B.

- q22.1
- q22.3
Other family members had normal array CGH results

- normal 18q
- normal 18q
- 0.44 Mb deletion
- 3.72 Mb deletion

Other family members had normal array CGH results.
STR markers confirm deletion in proband expanded from smaller deletion in mother

<table>
<thead>
<tr>
<th>Marker</th>
<th>Location</th>
<th>Proband’s alleles</th>
<th>Mother’s alleles</th>
<th>Normal sibling’s alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>D18S1161</td>
<td>Proximal to proband deletion</td>
<td>231, 231</td>
<td>231, 231</td>
<td>231, 231</td>
</tr>
<tr>
<td>D18S462</td>
<td>Proximal to mother’s deletion, within proband’s deletion</td>
<td>306</td>
<td>304, 306</td>
<td>304, 304</td>
</tr>
<tr>
<td>D18S70</td>
<td>Within both mother’s and proband’s deletion</td>
<td>113</td>
<td>114</td>
<td>112, 114</td>
</tr>
</tbody>
</table>
Old Paradigm

- If parents are normal, then pure terminal deletions very likely de novo and parental studies not necessary
- Deletions are stable in size through generations; therefore, family studies can use a marker within the abnormality

New Possibility

- Parental studies should always be done
- Deletion size can expand between generations
### Old Paradigm

- Chromosome studies are sufficient for parental follow-up of a visible terminal deletion.
- Differences in phenotype between a parent and offspring with a known, but unsized, deletion is likely due to differences in environment, genetic background, penetrance, epigenetic differences, or deletion unrelated to proband phenotype.

### New Possibility

- Chromosome studies may not be sufficient for parental studies since they may not recognize smaller deletions.
  - Array CGH of parents may be needed.
- Differences in phenotype between a parent and a child with a deletion may be due to alterations in the genetic content (size) of the deletion.
Array CGH will not detect balanced rearrangements that may be clinically important

Chromosome analysis detected a balanced translocation.

High resolution array CGH analysis was normal (no loss or gain at breakpoints).
Characterization of breakpoints by FISH revealed likely genetic etiology.

Translocation disrupted HOXD gene cluster.
But, not all de novo balanced translocations are responsible for the observed phenotype:


- 31 phenotypically normal carriers of reciprocal translocation
  - No genomic imbalances at the breakpoints or elsewhere in the genome detected by array
  - 16/31 (52%) cases the breakpoint did disrupt a gene

- 14 abnormal carriers of reciprocal translocations
  - 4/14 (27%) cases showed disease causing imbalances by array
  - 5/14 (36%) cases the breakpoint did disrupt a gene
Abnormalities of regions of the genome not represented on the array platform will be missed.

Targeted array detected a deletion of the region around the Sotos syndrome gene, but it was missed on the “1 Mb” chip.

Targeted array missed a deletion within chromosome 10 (backbone too sparse) but it was detected on the “1 Mb” chip.
Conclusions

- Microarray technology is a powerful tool for the detection of the etiology of developmental delay and multiple congenital anomalies.

- The detection rate for these indications using microarray alone is 15-20%.
  - ~1% of clinically significant alterations can be detected by a chromosome analysis and not a microarray analysis (example: balanced translocations and perhaps some cases of mosaicism).

- Microarray provides a more detailed, automatable and less subjective analysis of abnormal DNA copy number compared to standard chromosome analysis.

- Proper counseling and follow-up is extremely important as a copy number change of unknown clinical significance can be identified (~ 5-10%).
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