Array CGH for developmental delay, learning difficulties and congenital anomalies.

Caroline Ogilvie
Chromosome imbalance (copy number variation; CNV)

normal copy number = 2
(except for sex chromosomes)

deletions  → x1
           or x0

duplications  → x3
triplications  → x4
........
Copy number imbalance may cause:

- learning difficulties
- mental retardation
- developmental delay
- language delay
- dysmorphism
- syndromic disease
Detection of imbalance can be:

*genome-wide*

- karyotyping
- array CGH

*targetted at specific sequence(s)*

- FISH (eg microdeletion loci)
- MLPA (eg subtelomeres)
Karyotype analysis

Pros:
- traditional
- familiar
- cheap consumables
- detects triploidy and balanced rearrangements

Cons:
- sensitivity ~3-5Mb
- subjective – depends on the skill of the individual analyst
- labour-intensive
Array = many DNA probes attached to a glass slide

DNA probes bind to complementary DNA sequences
CGH arrays (aCGH, microarrays, etc.)

Test DNA

Reference DNA
Procedure

- test DNA for quality and quantity
- label with fluorochrome
- mix with control DNA, differentially labelled
- pipette onto array slide
- hybridize
- wash and dry
- scan
Oligonucleotide arrays

60,000 probes
Array CGH vs karyotyping:

- ≥20kb vs 3~5Mb
- objective vs subjective
- costs can be comparable if innovative strategies used (array consumable costs will fall; karyotyping staff costs will not)
Chromosome size (100 Mb)  

Conventional G-banding karyotype resolution (10 Mb)  

Molecular karyotype resolution (100 kb)

Resolution  
G-banding  
array CGH

From: Stephenson et al J R Soc Interface. Sep 8, 2010
International agreement that arrays should replace karyotyping for genome-wide detection of imbalance

→ ~ 3Mb – 5Mb (1Mb = 1 million base pairs)

→ ~ 20kb – 100kb (1kb = 1 thousand base pairs)
Commissioning

How to fund?

i) find extra money to fund “add on” array testing following karyotyping for highly indicative cases

ii) commission array testing as a first line test in place of karyotyping

Commissioned as a first line test at Guy’s in April 2009.

First line testing at Guy’s

- n=8,794
- CNV detection rate=25%
- 87% too small to be detected by G-banded chromosome analysis
- **33% of imbalances are definitely pathogenic**
- 34 different established genomic disorders detected in 430 patients
- Imbalance for 6 different susceptibility loci detected in 205 patients
- Most common genomic disorder: 22q11.2 deletion syndrome (n=64)
- Most common susceptibility locus imbalance: 16p11.2 (n=60)
- Average reporting time: 21 days from receipt of sample
- Overall success rate: 99%
Human variation

- research studies using array CGH have shown that “normal” individuals carry multiple small CNVs.
- different combinations of these CNVs may contribute to phenotypic variation between individuals.
Imbalance detected:

- “benign” CNVs – published as present in normal individuals and/or common in our population
- known regions (e.g., microdeletion syndrome loci)
- unknown CNVs – not in DGV

Inheritance studies
NO ABNORMALITY DETECTED

arr(1-22)x2,(XY)x1

Array CGH analysis of DNA from XXX has been carried out using oligonucleotide arrays with ~60,000 probes across the genome. No imbalance was detected (excluding established population polymorphisms).

The results are consistent with a normal male chromosome complement.

Array CGH is a technique for detecting abnormalities of genomic copy number. It has a higher resolution than karyotype analysis, and will therefore detect regions of imbalance too small to be detected by analysis of G-banded chromosomes. It will not detect balanced chromosome rearrangements or ploidy abnormalities such as triploidy.

arr(1-22)x2,(XY)x1
arr(1-22,X)x2

arr
(1-22)x2
(XY)x1
i.e. male: 46,XY

OR

arr
(1-22,X)x2
i.e. female: 46,XX

array karyotype
2 copies each of chromosomes 1 to 22
1 copy each of chromosomes X and Y

i.e. male: 46,XY

array karyotype
2 copies each of chromosomes 1 to 22, and X

i.e. female: 46,XX
CHROMOSOME IMBALANCE DETECTED

arr 4p16.3(72,446-156,159)x1

Preliminary report:

Array CGH analysis of DNA from XXX has been carried out using oligonucleotide arrays with ~44,000 probes across the genome.

This test identified an apparently terminal deletion of approximately 84 kb from band p16.3 in the short arm of chromosome 4, between base pair coordinates 72,446 and 156,159.

This finding may represent a benign copy number variant.

No other imbalance was detected (excluding established population polymorphisms). The results are consistent with a male chromosome complement.

Please send blood samples in EDTA from XXX’s parents for inheritance studies.

Once inheritance studies are complete, an appointment for genetic counselling can be made by writing to this family’s local Genetics Centre.
arr 4p16.3(72,446-156,159)x1

arr
array karyotype

4p16.3
chromosome band

(72,446-156,159)
base pairs from the end of the short arm

x1
number of copies
Inherited imbalance

Inheritance from a parent who does share the proband’s phenotype

? Significance
→ further family studies
Inherited imbalance

Inheritance from a parent who **does not** share the proband’s phenotype

? prob benign, but can’t rule out a contribution to the child’s phenotype, especially for susceptibility loci
Microdeletion/duplication syndromes

• submicroscopic chromosomal regions 100 kb – 3000 kb

• recognizable as syndromes because they recur at low frequency in all populations

• arise due to genomic structure at the disease locus – predisposes to gene deletion-duplication by unequal recombination

• mediated by ‘low copy repeats’

• new microdeletion loci uncovered since array CGH testing started
Unequal crossing over within low copy repeat

duplication
deletion
Recurrent 16p11.2 microdeletions in autism

Kumar et al  Hum Mol Genet 2007 17:628-638
Copy number imbalance may cause:

- learning difficulties
- mental retardation
- developmental delay
- language delay
- dysmorphism
- syndromic disease

and may also be associated with:

- epilepsy
- autism
- schizophrenia
- obesity
<table>
<thead>
<tr>
<th>Deletions</th>
<th>Duplications</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRXN1</td>
<td>VIPR2</td>
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Purpose and value of genetic testing

1. reach a diagnosis – good for families → prognostic info and ends the diagnostic odyssey
2. establish reproductive risks for proband and family
3. increase knowledge of genetic pathways and disease etiology, leading ultimately to treatment options
Challenges

- Interpretation
  - Inheritance
  - Incomplete penetrance
- Incidental findings
  - Cancer genes
  - Late-onset conditions
- Array CGH is amenable to large batch testing, especially with robotics, giving low staff costs per sample.
- An array CGH service requires staff with a detailed understanding of the theoretical basis of the software algorithms and their limitations.
- Experience of the challenges associated with interpreting and reporting of results is essential to maintain a robust and accurate service.
- Commissioning array CGH in every cytogenetics lab may not be the most cost-effective use of public money.
BB-GRE (Brain and Behaviour Genetic Resource)

Aims:

- to increase medical and scientific knowledge about the relationship between clinical phenotype and detectable genetic variants
- to improve medical care and genetic advice for individuals/families with detected genetic variants

Database of genetic variants with associated phenotypes. Those investigating eg the aetiology of epilepsy can find families who may be interested to participate in research projects.

http://bbgre.org
- Cognitive development
- Specific developmental disorder
- Neurodevelopmental / behavioural problems
- Neurological disorders
- Growth abnormalities
- Congenital malformations / dysmorphism
- Endocrine and metabolic conditions
- Cutaneous stigmata / skin lesions
- Hair, nail, teeth abnormalities
- Other skeletal abnormalities (eg scoliosis)

http://bbgre.org
G-band resolution

array CGH resolution

whole genome sequencing resolution

From: Stephenson et al J R Soc Interface. Sep 8. 2010