Prenatal chromosome microarray

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Prenatal chromosome microarray

- Technique
- Benefits/literature of clinical utility
- Limitations
- Pretest counselling considerations
- Current Guidelines
- Audit.
Chromosome microarray (CMA)

• High resolution, whole genome technique.
• Used to identify imbalanced chromosomal abnormalities including most detectable by conventional karyotype techniques and submicroscopic deletions or duplications (copy number variants)
Figure 1. Comparative genomic hybridization array


DNA from a fetal sample, such as CVS or amniocentesis, is hybridized to an array platform consisting of DNA probes on a solid surface, such as a microscope slide or a silicon chip.

CGH compares the fetal DNA sample with a normal reference DNA sample.

The test DNA and the reference DNA samples are labelled with 2 different-coloured fluorescent dyes, then combined and hybridized to an array platform.

The relative intensities of the different colours are compared with bioinformatics tools.

Cases with duplications will have a greater hybridization signal, whereas cases with deletions will have a lower hybridization signal compared to the reference sample.
DNA from a fetal sample, such as CVS or amniocentesis, is hybridized to an array platform consisting of DNA probes on a solid surface, such as a microscope slide or a silicon chip.

A SNP is a variation at a single position in a DNA sequence among individuals.

With SNP arrays, only the DNA test sample is hybridized to the array platform.

SNP arrays detect CNVs by measuring probe signal intensities as used in the CGH approach.

Although CGH arrays are only able to detect CNVs, SNP arrays also can detect triploidy and regions on the 2 homologous chromosomes that are identical to each other, as occurs with uniparental disomy (UPD) and consanguinity.

With UPD, both copies of a chromosome are inherited from the same parent instead of 1 from each parent.

SNP arrays also can detect some cases of maternal cell contamination and mosaicism.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Aneuploidy</th>
<th>Balanced translocations and inversions</th>
<th>Unbalanced translocations</th>
<th>Triploidy</th>
<th>AOH/consanguinity</th>
<th>CNVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional karyotype</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CGH array</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>SNP array</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Benefits of microarray

- Precise definition of a region of imbalance.
- Resolution: KARYOTYPE 5-10MB, CMA 50-100kb
- Identify submicroscopic imbalance
- Potentially identify CNV near breakpoints of apparently balanced Karyotype.
- Delineate origin of marker chromosome
- Can be performed on uncultured DNA samples (CVS/amniocentesis) leading to quicker turnaround.
- Potential greater likelihood of obtaining a result due to ability to analyze ‘non-viable’ tissue.
Prenatal microarray

- Postnatal microarray gold standard for investigation of undiagnosed developmental disorders.
- Incremental value recognized in CMA versus karyotype alone in analysis of stillbirth. (*Reddy et al NEJM 2012*)
- Evidence of utility evolved from small studies in women whose fetus had high likelihood of having chromosome abnormalities to large blinded studies of prenatal diagnostic samples to assess ability to detect common chromosomal abnormalities and gauge extent of additional information.
• 4406 women undergoing prenatal diagnosis compared Chromosome microarray to karyotype

• Indications for Prenatal diagnosis:
  - advanced maternal age (46.6%)
  - abnormal DSS (18.8%)
  - structural anomalies on ultrasound (25.2%)
  - other indications (9.4%)

• Microarray successful in 98.8% samples
• Detected all the chromosomal abnormalities detected on KT (except balanced translocations and triploidy)

• in samples with a normal karyotype:
  - microarray analysis revealed clinically relevant deletions or duplications in 6.0% with a structural anomaly
  - in 1.7% of those whose indications were advanced maternal age or positive screening results.
Prenatal CMA diagnostic yield.

- SNP array study of 1,033 fetuses with US anomalies reported pathogenic CNV in 5.5% of cases. (Srebniak et al EJHG 2016)
- Study 5,000 fetuses showed incidence of 6.6% in 2,462 cases with US anomalies. (Shaffer et al prenatal diagnosis 2012)
- 2 meta-analysis, 2013 demonstrated increased diagnostic yield of 7-10% over karyotype in pregnancies with structural fetal anomalies (Callman et al prenatal diagnosis 2013, Hillman et al Ultrasound obstet and gynaecol 2013)
- Overall CMA estimated to provide additional information over karyotype in about 6-7% pregnancies when the fetus has an anomaly identified on US.
Limitations of Chromosome microarray

Because CMA looks for genomic imbalance, this technique is not able to detect totally balanced chromosomal rearrangements, such as translocations or inversions.

CMA does not provide information about the chromosomal mechanism of a genetic imbalance e.g. cannot distinguish between trisomy 13 and an unbalanced Robertsonian translocation.

CMA will not detect all CNVs, such as those that are in regions not represented on the array platform and very small CNVs that are below the level of detection.

In some cases, a postnatal CMA may identify a CNV that was not identified prenatally because of the greater resolution of postnatal arrays.

In addition, CMA will not detect point mutations within single genes.
CMA Pretest counselling issues

• Scope of genomic imbalance detectable (compared e.g. NIPT)
• Potential of Variant of unknown significance (estimated incidence 1.0%)
• Concept of phenotypic heterogeneity-variable penetrance/expressivity. Potential unpredictable clinical spectrum in fetus – or uncover affected parent with milder phenotype.
• Potential late onset condition detected, unrelated to test indication. Implications for future health/family health.
• Potential for detection of neurosusceptibility locus. Postnatal phenotype of abnormality that may predispose to developmental disability or neuropsychiatric illness.
• Identification of consanguinity/non paternity.
Recommendations for the use of chromosome microarray in pregnancy

Prenatal microarray indications.

• In fetuses where conventional Karyotype by amniocentesis or chorionic villus sampling has been indicated and qfPCR is normal, CMA is indicated if:

1. One or more structural anomalies identified on an ultrasound scan
2. An isolated nuchal translucency NT> 3.5mm when crown-rump length measures from 45mm to 84mm (at approximately 11 weeks 0 days to 13 weeks 6 days)
3. Fetuses with a sex chromosome aneuploidy that is unlikely to explain the ultrasound anomaly (e.g. XXX, XXY and XYY)
Variants to be always reported:

Includes pathogenic variants related to indication for CMA but may also include:

- High penetrance neuro-susceptibility loci that are associated with a risk of a severe phenotype.
- Neuro-susceptibility loci associated with an increased incidence of anomalies detectable on scna, as reporting these may help direct further scanning.

Any variant that will potentially inform the management of the pregnancy, or of the family in the clinical context in which CMA was done/in the future, should be reported regardless of size of imbalance.
Variant s to be always reported:

Unsolicited pathogenic findings fulfilling the above criteria-

Deletion of known cancer predisposition gene e.g. BRCA1. may enable parents to benefit from screening or prophylactic treatments if available.

-rare occurrence: 27 CNVs affecting cancer genes among 9005 subjects in one study: incidence 0.30%.

Deletion of the dystrophin gene in a female fetus-allowing mother to be tested for carrier status and inform future reproductive choices.
Incidental findings not to be reported

- Any finding not linked to potential phenotypes for the pregnancy or has no clinically actionable consequence for that child or family in the future, e.g. VUS that cannot be linked to a potential phenotype on the basis of genes involved, low penetrance neuro-susceptibility loci and unsolicited pathogenic variants for which there is no available intervention.
Incidental findings not to be reported

**Specific variants routinely falling into this category:**

- 15a13.1q13.3 duplications

- 15q11 BP1-BP2 duplications or deletions

- Xp22.31 (STS) duplications

- 16p13 duplications

Heterozygous deletion of recessive genes that cannot be linked to the presenting phenotype.
Survey on Current Reporting Practices in Genetic Services for Prenatal Microarray

UK Clinical Genetics services. 2018
Survey on Current Reporting Practices in Genetic Services for Prenatal Microarray

AIM: Evaluate current reporting practices in UK clinical genetics departments utilising prenatal chromosome microarray.

METHOD: Questionnaire distributed to all UK Clinical Genetics services.
1. Which Clinical Genetics Service are you reporting from?

ARE YOU OFFERING PRENATAL MICROARRAY TESTING-IF SO ON WHICH PATIENTS?

All CVS and amnios including those referred for a single gene test or raised SS only?

Only those with an anomaly on scan or a raised NT?

Another arrangement? Please describe.
<table>
<thead>
<tr>
<th>Response from 9 services:</th>
</tr>
</thead>
<tbody>
<tr>
<td>West of Scotland Regional Genetics Service</td>
</tr>
<tr>
<td>Nottingham</td>
</tr>
<tr>
<td>North West Thames Regional Genetics service</td>
</tr>
<tr>
<td>Northern Genetics service</td>
</tr>
<tr>
<td>Oxford Centre for Genomic medicine</td>
</tr>
<tr>
<td>Wessex Clinical Genetics Service</td>
</tr>
<tr>
<td>Exeter and Bristol Clinical Genetics Service</td>
</tr>
<tr>
<td>Manchester</td>
</tr>
<tr>
<td>West Midlands Regional Genetics Service.</td>
</tr>
</tbody>
</table>
Survey 1. Results

All 9 centres responding offered prenatal CMA.

All 9 gave indication as: **only those with an anomaly on scan or a raised NT.**

5 centres gave additional detail of indication including:

- Additionally sex aneuploidy unlikely to explain phenotype.
- Fetal growth restriction not due to placental insufficiency
- Known parental copy number variation/balanced rearrangement
- Exception of fetal anomaly such as isolated talipes.
- Targeted testing in pregnancies for patients with child with developmental disorder and array finding
Survey 2.

2. If you offer testing, when did you start doing this testing?

How many tests have you done in total?

How many samples do you analyse on average over one year- 01/04 to 31/03?

How many pathogenic CNVs (pCNV) have you reported in total?

How many pCNVs have you reported on average in one year 01/04-31/03?
When did testing start?

- West of Scotland Regional Genetics Service: Dec-17
- Nottingham: May-16
- North West Thames Regional Genetics Service: Sep-14
- Northern Genetics service: Nov-14
- Oxford Centre for Genomic medicine: Apr-13
- Wessex Clinical Genetics Service: Jun-14
- Exeter and Bristol Clinical Genetics Service: Aug-12
- Manchester: Nov-14
- West Midlands Regional Genetics Service: Sep-14
Total number of test results and total number of pathogenic copy number variants (pCNV) by service.
Percentage of pCNV of total results by service.

- WEST MIDLANDS REGIONAL GENETICS SERVICE: 7%
- MANCHESTER: 5%
- EXETER AND BRISTOL: 10%
- WESSEX: 7%
- OXFORD: 10%
- NORTHERN: 8%
- NORTH WEST THAMES: 6%
- NOTTINGHAM: 7%
- WOS: 14%
Number of pCNV year: 17/18 by service.
Percentage pCNV year 17/18 by service.

Percentage pCNV of annual results 17/18

- West Midlands Regional Genetics Service: 9
- Manchester: 6
- Exeter and Bristol: 14
- Wessex: 7
- Oxford: 11
- Northern: 10
- North West Thames: 7
- Nottingham: 7
- WOS: 14
pCNV total and year 17/18. All services.
3. How do you decide on reporting CNVs that may not linked to the fetal phenotype e.g. neuro-susceptibility loci?

How many of these have you reported since you started analysis

On average how many of these do you report annually- 01/04-31/03?
All services reported that they decided on reporting CNVs ‘unlinked’ to the fetal phenotype by adherence to the 2015 National guidelines, in addition many commented that they use an MDT discussion forum/consultant review.
Proportion 'unlinked' CNV.
All services.

Proportion 'unlinked' CNV year 17/18.
All services.
4. How many actionable incidental findings have you reported since you started analysis? e.g. BRCA1 deletions.

How many have you reported on average annually 01/04-31/03
## Incidental actionable findings.

<table>
<thead>
<tr>
<th>Service</th>
<th>Total actionable incidental findings. All years.</th>
<th>Actionable incidental findings year 17/18.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nottingham</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>North West Thames</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Northern</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Oxford</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wessex</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exeter and Bristol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Manchester</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>West Midlands Regional Genetics Service</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>All services TOTAL</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>
5. What do you do about reporting results where the significance is uncertain?
What do you do about reporting results where the significance is uncertain?

<table>
<thead>
<tr>
<th>SERVICE</th>
<th>RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOS</td>
<td>MDT DISCUSSION</td>
</tr>
<tr>
<td>Nottingham</td>
<td>CONSULTANT DISCUSSION</td>
</tr>
<tr>
<td>North West Thames</td>
<td>MDT DISCUSSION</td>
</tr>
<tr>
<td>Northern</td>
<td>MDT DISCUSSION/ VUSVREFERRED TO CLINICAL TEAM TO DISCUSS WITH FAMILY</td>
</tr>
<tr>
<td>Oxford</td>
<td>MDT DISCUSSION/NATIONAL DISCUSSION IF REQUIRED</td>
</tr>
<tr>
<td>Wessex</td>
<td>LOCAL PANEL/MDT DISCUSSION</td>
</tr>
<tr>
<td>Exeter and Bristol</td>
<td>MDT DISCUSSION</td>
</tr>
<tr>
<td>Manchester</td>
<td>CONSULTANT DISCUSSION</td>
</tr>
<tr>
<td>West Midlands Regional Genetics Service</td>
<td>LOCAL PANEL DISCUSSION</td>
</tr>
</tbody>
</table>
6. Do you have a local group that reviews these results?

If so, what health professionals are involved with this group?

Do you record discussions around these results?

If you record them, where do you record them
<table>
<thead>
<tr>
<th>SERVICE</th>
<th>LOCAL GROUP?</th>
<th>HEALTH PROFESSIONALS INVOLVED?</th>
<th>RECORD?</th>
<th>WHERE?</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERVICE</td>
<td>YES</td>
<td>CLINICIANS/SCIENTISTS</td>
<td>YES</td>
<td>PATIENT NOTES/LABORATORY MDT SPREADSHEET</td>
</tr>
<tr>
<td>WOS</td>
<td>YES</td>
<td>CONSULTANT ON CALL</td>
<td>YES</td>
<td>SHARED GENETICS DRIVE SPREADSHEET</td>
</tr>
<tr>
<td>Nottingham</td>
<td>NOT SPECIFIC</td>
<td>SENIOR CLINICAL SCIENTISTS/CLINICAL STAFF</td>
<td>YES</td>
<td>LABORATORY PATIENT RECORDS</td>
</tr>
<tr>
<td>North West Thames</td>
<td>YES</td>
<td>AT LEAST 2 CLINICAL SCIENTISTS AND 2 CLINICAL STAFF</td>
<td>YES</td>
<td>DATABASE DOCUMENT OF MDT DISCUSSION. UPLOADED TO PATIENT FILE.</td>
</tr>
<tr>
<td>Northern</td>
<td>YES</td>
<td>CLINICAL SCIENTIST/CLINICAL GENETICISTS/GENETIC COUSELLORS</td>
<td>YES</td>
<td>LAB WORKSHEETS</td>
</tr>
<tr>
<td>Oxford</td>
<td>YES</td>
<td>AT LEAST 2 PRINCIPAL CLINICAL SCIENTISTS AND 2 CLINICAL STAFF</td>
<td>YES</td>
<td>DEPARTMENTAL VARIANT INTERPRETATION PROFORMA. ALL ATTACHED TO PATIENT DATABASE FILE.</td>
</tr>
<tr>
<td>Wessex</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>EMAILS RELATED TO CNV REPORTING DECISIONS RECORDED IN PATIENT ELECTRONIC NOTES</td>
</tr>
<tr>
<td>Manchester</td>
<td>NO</td>
<td>N/A</td>
<td>N/A</td>
<td>ALL DISCUSSION RECORDED IN PATIENT LAB RECORD</td>
</tr>
<tr>
<td>West Midlands Regional Genetics Service</td>
<td>YES</td>
<td>CONSULTANT CLINICAL SCIENTISTS/CLINICAL GENETICIST/FETAL MEDICINE CONSULTANT</td>
<td>YES</td>
<td>LAB RECORD.</td>
</tr>
</tbody>
</table>

Manner in which different services deal with reporting results where the significance is uncertain?
Conclusion

• Growing number of years experience in use of prenatal microarray in prenatal diagnosis in UK genetics services responding to survey (range 9 months-6 years)
• Pathogenic copy number variants (pCNV) account for 7% of total results obtained by all services.
• Consistency amongst services in utilising national guidelines for reporting of CNVs not linked to the fetal phenotype.
• ‘Unlinked’ and actionable incidental findings account for a small proportion of overall results.
• Majority of services deal with reporting results in an MDT setting
• Variation in health professionals involved and recording practices.
• Future aim to standardise result discussion/recording practice to facilitate exome use.