Array-CGH

What is array-CGH and why has it replaced karyotyping as the front line test for patients with neurodevelopmental disorders?

INTRODUCTION

In the past karyotyping has been the “gold standard” test for detecting chromosome abnormalities particularly in patients ascertained with intellectual deficit, developmental delay, autism spectrum disorder, moderate to severe learning difficulties, with or without dysmorphic features or congenital abnormalities. Current karyotyping can only detect relatively large chromosome abnormalities, but technological advances now allow us to detect pathogenic chromosome gains and losses which cannot be seen by conventional karyotyping. The technique used in place of karyotyping is called array comparative genomic hybridization (array-CGH) which accurately identifies both the location and gene content of pathogenic chromosome imbalances. International studies, including work carried out in this laboratory, have shown that array-CGH doubles the detection of underlying chromosome imbalances in the cohort of patients summarised above.

WHAT IS ARRAY-CGH?

Array CGH (also known as microarray, or chromosome microarray (CMA)) is an ultra-high resolution way of objectively and quantitatively detecting whether a patient’s DNA has losses (deletions) or gains (duplications, triplications etc) which are pathogenic and therefore explain their clinical problems.

- The WRGL is a founder member of the Clinical Genome Resource (ClinGen) consortium (previously known as ISCA). For further details please see the ClinGen website (**www.clinicalgenome.org/**). This laboratory uses an array based on that originally designed by ClinGen which utilises 60,000 oligonucleotide probes in multiples of eight arrays per slide (8x60 K format). The content of this design has been updated by Oxford Gene Technology to include single exonic copy number variant detection in the genes identified as most relevant to cause developmental delay by both the Deciphering Developmental Disorders (DDD) study and ClinGen. (https://www.ogt.com/products/1073_cytosure_constitutional_v3_and_constitutional_v3_loh_arrays)

- Array-CGH can detect genomic deletions or duplications with far greater sensitivity (>100 times) than can be achieved by chromosome analysis using light microscopy.

- Array-CGH *cannot* detect; (1) “balanced” chromosome rearrangements (e.g. translocations or inversions), (2) low frequency mosaicism (<30% abnormal cells for deletions and duplications, <10% abnormal cells for aneuploidy), (3) polyploidy, and (4) nucleotide sequence variants.

- Karyotyping will continue for some referrals e.g. patients with suspected Down (+21), Edwards (+18), Patau (+13) syndromes from QF-PCR, or Turner (45,X) or Klinefelter (47,XXY) syndromes.

- Array-CGH will also detect "normal" deletions and duplications in the genome known as benign "Copy Number Variation" or CNVs. Data are now available to help us distinguish between "normal" and "pathogenic" deletions and duplications.

WHAT CATEGORY OF PATIENT IS MOST SUITABLE FOR ARRAY-CGH?

- Patients presenting with intellectual deficit, developmental delay, with or without dysmorphism or multiple (3 or more) congenital abnormalities will be eligible for array-CGH. A blood sample taken into an EDTA tube is required together with a completed WRGL postnatal referral form. The array report should be received within the current best practice reporting time guidelines. In urgent cases (newborn infants with a suspected chromosome syndrome, prenatal cases or where clinical management is dependent on the array-CGH test results) the array can be fast tracked and reported within 14 days.

QUESTIONS FREQUENTLY ASKED ABOUT ARRAY-CGH

- **Q.** *If a patient has the appropriate referral criteria (see introduction), how frequently will array-CGH detect an underlying pathogenic abnormality?*

- **A.** We would expect ~20% of referrals to have imbalances which require further follow-up, we will then request blood samples from both parents to assist interpretation. Overall 10-15% of imbalances turn out to be pathogenic many of which are *de novo* (i.e. found for the first time in the patient) and therefore likely to explain the clinical phenotype.

- **Q.** *What chromosome abnormalities will array-CGH not detect?*

- **A.** Array-CGH cannot detect "balanced" chromosome rearrangements such as reciprocal translocations and inversions and polyploidy. However, ~40% of patients with apparently balanced *de novo* chromosome rearrangements and abnormal clinical phenotypes will have imbalances detected by arrays which often involve chromosomes not involved in the cytogenetically visible chromosome rearrangement. Array-CGH will also detect imbalances which are the unbalanced products of balanced parental chromosome rearrangements. Array-CGH will not necessarily detect either mixtures of normal and abnormal cells (mosaicism) and will not detect triploidy, however, mosaicism and triploidy are both extremely rare conditions and, if suspected, can be tested with other methods. It should be noted that we have been able to pick up mosaic aneuploidy present in >10% cells and mosaic deletions/duplications present in >30% cells.

- **Q.** *What tests will be used if we suspect one of the trisomy syndromes eg. Down, Edwards, Patau, Turner or Klinefelter syndromes?*

- **A.** QF-PCR test will be used initially for suspected Down, Edwards, and Patau syndromes followed by karyotyping if a positive result is obtained. In cases of suspected Turner and Klinefelter syndrome, karyotyping is still the most appropriate test.

- **Q.** *How long will it take to receive a report?*

- **A.** The array report should be received within the current best practice reporting time guidelines. If follow-up testing is required, testing may be delayed until all the samples that have been requested have been received at the laboratory or we have been informed that they are not available.

- **Q.** What tube should be used for collecting blood samples for array-CGH?

- **A.** 5ml of blood should be taken into an EDTA tube for the initial test. Some follow-up tests also require blood to be taken into a lithium heparin tube or a combination of both EDTA and lithium heparin tubes but the samples required will be clearly indicated in the array-CGH report.