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PATHCHAT

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Molecular chromosomal analysis
(array comparative genomic hybridisation; a-CGH)

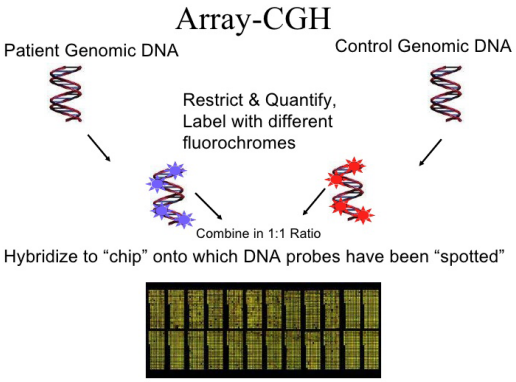
Background of cytogenetic and cytomolecular genetic advances

Chromosomal banding patterns, developed during the late 1960s and early 1970s, are used to elucidate the breakpoints and constituent chromosomes involved in chromosome translocations. Deletions and inversions within an individual chromosome can also be identified and described more precisely using standardised banding nomenclature. In the 1980s, a new technique became available. Using fluorescent labelled probes and hybridising them to chromosomal preparations came to be known as fluorescence *in situ* hybridisation (FISH). FISH is a cytogenetic technique where specified fluorescent probes are used that bind to only parts of the chromosome with a high degree of sequence complementarity. Fluorescence microscopy is used to visualise a target locus where a pre-specified fluorescent probe is bound on a chromosome. Traditional chromosomal analysis and FISH are limited by speed, throughput and resolution.

A new form of chromosomal analysis, array, comparative genomid hybridisation (CGH), enables higher-resolution genome analysis and allows the detection of submicro-scopic chromosomal copy number variants (CNVs) across the entire genome in one single experiment. Conventional chromosome analysis is limited by its capacity to detect only those copy number changes that are large enough to be microscopically visible (typically 5–6 Mb in size at the 500-band level). In contrast, array CGH analysis simultaneously evaluates regions across the entire genome and allows for the detection of unbalanced structural and numerical chromosome abnormalities of less than 100 kb. The main disadvantage of CGH is its inability to detect structural chromosomal aberrations without copy number changes, such as mosaicism, balanced chromosomal translocations, and inversions. *Combining* single nucleo-tide polymorphism (SNP)-CGH with conventional cyto-genetics in oncology patients where necessary provides comprehensive clinical information by detecting clonality, large balanced rearrangements, copy number aberrations, and copy number loss of heterozygosity.

Microarray-based comparative genomic hybridisation (array CGH) was made possible by the Human Genome Project, which generated a library of cloned DNA fragments with known locations throughout the human genome, with these fragments being used as probes on the DNA microarray.

How is array CGH done?



A chip reader and software programme is then used to extract the data. Automation of the generation of array data does not mean theat a straightforward result is produced, which can easily be incorporated into a clinical report. Expertise is required to compare CNVs that may be clinically relevant in view of the patient’s features. Several databases are incorporated into the software to assist the laboratory clinician with this. Interpretation of microarray data is complicated by the presence of both novel and recurrent copy number variants of unknown significance, but the greater diagnostic sensitivity arising from the application of array CGH outweighs interpretative issues for the reporting laboratory, arising from the detection of CNVs of uncertain significance. As knowledge is gained for each of the recurrent CNVs, this is rapidly translated to practical genetic counselling opportunities.

ANTENATAL DIAGNOSIS

What are the different applications for non-invasive foetal testing versus array CGH testing in antenatal medicine?

Ampath Genetics will be offering nifty non-invasive foetal screening testing during the last quarter of 2015. This test and several similar offerings are often touted as non-invasive fetal diagnostic tests, but in reality remain screening tests only and, when "positive", still have to be followed up by an invasive diagnostic procedure such as amniocentesis for confirmatory proof of a genetic diagnosis. Their utility lies in a decrease in the number of invasive procedures required for negative screening results due to high test specificity. Array-CGH (a-CGH) enters the picture when whole-genome a-CGH offers a higher-resolution diagnostic capacity than conventional karyotyping for prenatal diagnosis either as a first-tier test or as a "further-test" for pregnancies with foetal ultrasound anomalies. A-CGH can be expected to eventually replace conventional cytogenetics for all pregnancies undergoing invasive diagnostic procedures after excluding common aneuploidies and triploidies by quantitative fluorescent polymerase chain reaction (PCR).

Miscarriages: Poor chromosome preparations, culture failure or maternal cell contamination may hamper conventional karyotyping. A technique such as a-CGH enables the detection of submicroscopic abnormalities with a higher yield than other current and readily available investigations.

A-CGH testing in paediatrics

In infants and children with congenital anomalies/dysmorphic features in whom the syndromic gestalt is not clinically recognisable, as well as in undifferentiated intellectual disability and autism spectrum disorders, array-CGH/SNP array has become a first-tier investigation. Detection rates for chromosome abnormalities with array CGH range from 5 to 17% in individuals with normal results from prior routine cytogenetic testing. Our platform has a resolution of ~40 kb in the backbone, and ~20 kb in the targeted regions. Molecular cytogenetic evaluation is possible of over 245 recognised genetic syndromes and over 980 gene regions of functional significance in human development, and the assay covers subtelomeric and pericentromeric regions. CNVs are also more common in children with a *combination* of intellectual disability and genetic generalised epilepsy rather than in those with either phenotype alone. The most common of the recurrent microdeletions associated with generalised epilepsy are typically seen at 15q13.3, 16p13.11 and 15q11.2 sites that also confer susceptibility for intellectual disability, autism and schizophrenia. In-house genetic counselling, which can also be done telephonically, is available to assist physicians and patients to translate results into practical clinical indicators.

ONCOLOGY

While FISH requires separate, targeted hybridisations for the detection of alterations at genomic loci of interest, in a single hybridisation reaction array, CGH allows identification of genomic alterations with prognostic significance. The combination of CGH and SNP probes on the same array allows the detection of copy number, as well as copy-neutral genomic aberrations, loss of heterozygosity (LOH) or uni-parental disomy (UPD) together. It also provides allelic information on deletions, duplications and amplifications. Our analysis covers over 2 410 cancer-relevant regions with coverage of genes present in the Cancer Cytogenomics Micorarray Consortium (CCMC) and Cancer Gene Census (http://www.sanger.ac.uk/genetics/CGP/census/) designs, genes cited more recently in the literature, and genes that are family members of known tumour-related factors.

Some examples where a-CGH is proving useful:

Diffuse large B-cell lymphoma (DLBCL), comprising of greater than 30% of adult non-Hodgkin lymphomas. Despite many classification efforts, most lymphomas remain undistinguishable and fall into DLBCL not otherwise specified (DLBCL-NOS). Recently, it has been shown that microarray data provides subtype specific classification models for DLBCL.

Myelodysplastic syndromes (MDS) are the most common clonal stem cell hematologic malignancies, and can result in rapid progression to acute myeloid leukemia. Approximately 40 to 50% of MDS patients do not have karyotypic abnormalities that are detectable using classical metaphase cytogenetic techniques. UPD that occurs without concurrent changes in the gene copy number is a common chromosomal defect in MDS and can be detected by array-CGH.

B cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia in the Western world. The detection rate for SNP-CGH was found to be 62.5% for MDS cases and 72.7% for CLL cases, which are significantly higher than the detection rates of chromosome G-banding and/or FISH (43.8% for MDS and 54.5% for CLL).

Specimen requirements:	3–5 ml EDTA blood, sent at room temperature.
TAT:	10 days from receipt of specimen at NRL.
Pricing:	For antenatal, miscarriage and paediatric specimens: similar to bone marrow chromosomal analysis For oncology specimens: similar to bone marrow chromosomal analysis x 2, due to denser probe spacing/high resolution chip, including analysis of SNPS.

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