Redefining the genetic basis of learning disability

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About one in 200 babies worldwide is born with a chromosomal abnormality and many of these children have mental retardation and learning disabilities. Chromosomal imbalances, such as deletions, microdeletions, translocations or inversions, underlie some of these disorders and the genetic diagnosis of these patients has conventionally relied on cytogenetic chromosomal analysis techniques, such as karyotyping, fluorescence in situ hybridization (FISH) or conventional comparative genomic hybridization (CGH). However, the limited resolution of these approaches means that subtle chromosomal rearrangements involving fewer than 5-10 mega base pairs (Mb) cannot be detected, and many affected children go undiagnosed.

Over the past few years, scientists have started to develop microarray-based technologies, such as array-based CGH (aCGH), to investigate genetic changes in samples from patients with learning disabilities, since these techniques have the potential to detect abnormalities with much greater resolution than standard analysis methods. Conventional CGH experiments compare genomic DNA from patient samples with reference DNA. The two sets of DNA are labeled with different fluorochromes and competitively hybridized to normal human metaphase chromosomes so that copy number changes can be easily identified. By applying CGH to micro-arrays, the test and reference materials are hybridized to thousands of defined DNA probes concurrently, so that a high resolution screen of all chromosomes can be completed in a single, cost effective experiment.

At the West Midlands Regional Genetics Laboratory, we are using a Tecan HS 4800™ Pro hybridization station to automate aCGH for screening of patients with learning disability and dysmorphology. The laboratory is the largest NHS diagnostics genetic unit in the UK, processing over 27,000 samples each year from the local population as well as from medical institutions in the UK and Europe, covering a full spectrum of diagnoses for genetic diseases.

There are some classic chromosomal abnormalities which we know how to detect, and we largely use FISH for those. But we are particularly interested in the very large proportion of mental retardation patients who do not have a genetic diagnosis for their disease, and started using aCGH, which is becoming a frontline approach now.

The patients have been referred to the laboratory following assessment by clinical geneticists and are typically moderately affected children with documented developmental delays. The extent to which a person is affected by a genetic copy number imbalance depends substantially but not wholly on the exact genetic material lost or gained. Being able to pinpoint the exact genetic changes in a patient, and knowing the genes' functions, might allow doctors to give parents a better prediction of a child's future development. Ultimately this may also impact on treatment.

The application of microarrays in clinical diagnostics is fundamentally reliant on accurate results delivered automatically within a comprehensive quality control framework. Through their collaboration,

Dom McMullan loads slides into the Tecan HS 4800 Pro

Tecan and BlueGnome have optimized the Tecan HS Pro series of automated hybridization stations and BlueGnome protocols for automated processing of the CytoChip™ diagnostic bacterial artificial chromosome (BAC) microarrays, which we are using for our screening.

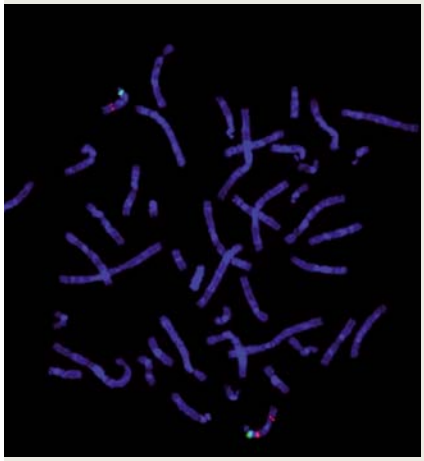
We had some initial aCGH pilot studies using manual methods, which sometimes produced uneven hybridization, and we straightaway saw the need for automation, largely because of the increased reliability and reproducibility it offers. Also, because we are relying on commercially available BAC arrays, we cannot afford to have any failings with them - we want to maximize our success rate as far as we can, which is why we turned to the HS 4800 Pro, chosen for several reasons. One thing that really

helped us to make the decision is the automated slide drying, which no other instrument on the market has. After injecting our probe, the operator walks away, leaving the machine to hybridize, wash and dry the slides, ready for scanning, so the whole process becomes less dependent on core lab hours and operator-related inconsistency. The recent development of segmented chambers allows us to perform two separate hybridizations on one slide without any crossover. This is very important for aCGH, because we can test patient- and reference-labeled DNA in opposing fluorochromes on two different arrays, so removing any potential dye effects or bias. The HS Pro is easy to use and its inherent agitation makes the hybridization very even across the slide, increasing the signal-to-noise ratio. The agitation setting allows more viscous hybridization buffers to be used than previously, which is key, because aCGH buffers contain dextran sulfate and so are more viscous.

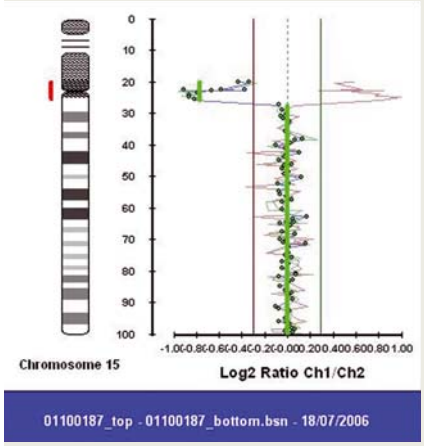
The CytoChip™ BAC microarrays are spotted with 3,200 BAC clones to give a spatial resolution of approximately 1 Mb; any abnormalities detected by the arrays we then directly confirm with FISH. Its resolution means that, effectively, there is a BAC for every 1 Mb of the genome. On current evidence, the majority of pathogenic imbalances should be detectable, given high quality raw data. The CytoChip™ is unusual in that it is tiled with increased BAC density across areas of certain well-characterized cytogenetic syndromes relating to recurrent chromosomal abnormalities. Our laboratory was involved in this design feature, which enables pick-up of potentially missed changes as well as detection of novel changes. In other words some patients might not have a classic phenotype, but may have a classic abnormality. For the patients that we are looking at, we should have up to a 20% pick-up rate using this technique, according to previously published studies; i.e. up to 20% of those patients should have something that we could not detect with traditional methods. We have found that we are now picking up genomic changes that previously would not be detectable; we are almost starting again, in terms of redefining new syndromes, by increasing the resolution.

CytoChip is a trademark of BlueGnome.
At present, the HS 4800 Pro is intended to be used for general purpose in Europe and for research use only in the USA.

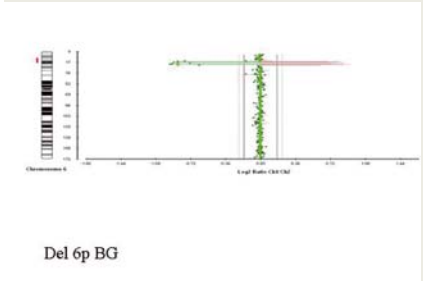
The HS 4800 Pro



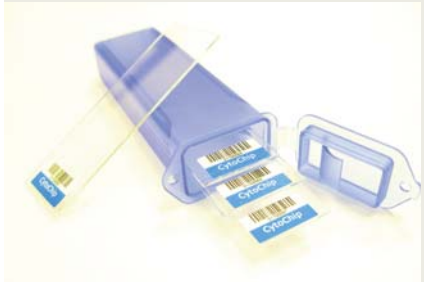
Metaphase FISH image of chromosome 15q deletion



Plot of aCGH showing chromosome 15q deletion



BlueGnome CytoChip™: typical result of a dye swap experiment showing a deletion of 7 clones on the p arm of chromosome 6



The BlueGnome CytoChip™