Detection of DNA Copy Number Imbalance Using Array CGH

A thesis submitted for the degree of Doctor of Philosophy to the University of Adelaide

By

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SUMMARY

The association of constitutional chromosome imbalance in patients with intellectual disability with or without related dysmorphism and malformations is well established. The resolution of conventional cytogenetic examination is limited to imbalances of 5-10Mb. Patients with characteristic phenotypes which allude to a specific microdeletion or duplication syndrome may be investigated using locus specific fluorescent in situ hybridisation (FISH). Subtelomere FISH, a recently new improvement for cytogenetics screening, detects subtelomeric rearrangements in around 6% of patients with idiopathic disability. However it is evident that for these patients, most do not have a recurrent pattern of dysmorphism or malformations suggesting imbalance in a particular chromosome region.

Array CGH has the potential to detect chromosome imbalances beyond that of current technology allowing the whole genome can be screened in a single hybridisation at a resolution limited only by the genomic distance between the arrayed target clones. The aim of this study was to develop a custom whole genome array and utilize this array to screen a number of diverse patient groups.

Rather than immediately begin with the development of a whole genome array a smaller pilot study was initiated, in so enabling the efficacy of the methodology to be tested. A small clinical/ subtelomere array was designed and constructed to screen for cytogenetic imbalances within the first 5Mb of each chromosome end (excluding acrocentric chromosomes) together with the number of known clinically significant regions.

This clinical/subtelomere array (chapter 3), was ulilised to map the extent of deletion and/or duplication in patients with previously determined subtelomere abnormalities. This was followed with the screening of a small group of patients with idiopathic intellectual disability (chapter 4). Novel Copy number changes were identified together with a number of changes determined to be non-pathogenic variants.

The methodology used in the utilization of this array could determine copy changes in patients, however it became clearly evident that the effective resolution was compromised

when a number of clones were shown to map to other sites of the genome or cross hybridise to multiple sites.

With this knowledge the next stage of the project, a whole genome array, primarily constructed from a FISH validated clone set, reduced the possibility of mapping discrepancies. These FISH mapped clones did not give a even genomic coverage. All clones from this set were mapped using Ensembl resources.Any identified gaps (greater than 1Mb) were covered using clones from a second clone set (32K), giving a resolution of ~0.2-1Mb.

Three diverse groups of patients were screened using the whole genome array. Using a novel scoring system which evaluated degree of intellectual disability/developmental delay, dysmorphism, presence of malformations and the presence of an apparently balanced cytogenetic rearrangement, twenty five patients with idiopathic disability were assessed. Four novel copy number changes were determined of which three were determined likely to contribute to the phenotype of the patient. The fourth patient is under investigation.

Chapter 6 saw the application of the whole genome array to a second patient group; medically terminated pregnancies, in utero fetal deaths or newborn infants with one or more facial dysmorphism or fetal malformation. One potentially pathogenic copy number change was ascertained from this study.

The third patient group, those with retractable epilepsy (chapter 7) showed two copy number changes both of which will require further follow up and investigation, including screening with a higher density array platform.

The screening of more than seventy varied patients has shown the efficacy of these two custom platforms, to detect previously undetermined copy number changes.

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CHAPTER 2

MATERIAL AND METHODS

PART A SUBTELOMERE CLINICAL ARRAY

CHAPTER 3

TESTING THE EFFICACY OF THE SUBTELOMERE CLINICAL ARRAY: BY MAPPING UNBALANCED CYTOGENETIC REARRANGEMENTS.