UNIVERSITI TEKNOLOGI MARA

THE DISCOVERY OF A COMMON SINGLE NUCLEOTIDE POLYMORPHISM ASSOCIATED WITH MENTAL RETARDATION DISEASE AMONG CHILDREN IN MALAYSIAN POPULATION

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ABSTRACT

Single Nucleotide Polymorphisms (SNPs) are useful tools for genome wide mapping and study of disease genes. The previous polymorphism studies have focused on specific genes or SNPs pooled from a variety of different sources. We evaluated denaturing high performance liquid chromatography (DHPLC) as a scanning method for mutation detection in Fragile X syndrome, a well known mental retardation disorder (MRD). We screened 10 SNPs locations in fragile X mental retardation 1 (FMR1 gene) obtained from HGBASE database. All the suspected DNA fragment were amplified in a range between 150-300bp from normal patients as well as MRD patients which is suspected Fragile X syndrome and screened by DHPLC. Optimisation of DHPLC analysis of each PCR product was carried out by minimum variation in the melting temperature of the amplicon, and titration of temperature. All of these variants were detected by DHPLC as homoduplexes chromatogram and no significant polymorphisms were found in the samples. Because of this initial DHPLC analysis failed to detect several SNPs deposited in HGBASE (Human Genic Bi Allelic Sequences) database, we chose to use microarray which we shifted the strategy in exploring SNPs in Fragile X syndrome to an approach of genome scan in order to pattern the SNPs distribution in mental retardation. In the mean time, we performed southern blot as a screening procedure to detect Fragile X patient. We addressed the issue of SNPs in patients with mental retardation disorder using GeneChip® Human Mapping 10K Array Xba 131 (Mapping 10K Array). We present here the results of the genome scan. A common SNP pattern was identified in a total of 24 SNP arrays which are duplicates. Samples from patients with putative and suspected Fragile X syndrome were screened together with samples from normal individuals. The reference DNA from Affymetrix GeneChip® Reagent was used as a control. Result for each of the allelic loci was determined by the GeneChip® DNA Analysis Software. The 10K Mapping Assay revealed genotype calls (AA, BB or AB) from 84% to 97% (average 91.73± 3.82%) of the 11560 SNPs in the SNP array for DNA from all samples.We identified 201 differentials SNP loci among the samples. Our study indicated that the occurrence of SNPs was lower in untranslated regions, UTR (3.48%) but highest in introns (30.85%). Wherelse, the ratio of nonsynonymous to synonymous changes was equal as well as the lowest (0.50%) functional SNP class in this study. By sequencing validation, we identified and characterised four candidate loci on chromosome X, potentially as disease causing loci and probably become a disease marker for MRD. There was no LOH found in the samples. We concluded that 10K mapping assay microarray is superior for detection of DNA sequence variation in XLMR disorders particularly for single base substitution mutations. These variables sites are present with high density in the genome, making them powerful tools for mapping and diagnosing disease related alleles.

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Read in the name of your Lord who created, created man from clots of blood. Read! And your Lord is the Most Bounteous, who has taught the use of the pen, has taught man what he did not know.

Quran 96: 1-5

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CHAPTER ONE

INTRODUCTION

Mental retardation is the most common developmental disorder in childhood and is a complex disease that cause learning disorders, attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD) and mental impairment (Batshaw & Perret, 1992). Mental retardation can happen before a child is born or during childhood. It can be caused by injury, disease or a brain abnormality. In many cases, these disorders can persist into adulthood resulting in lifelong morbidity. Many of these disorders present therapeutic challenges due to clinical heterogeneity, frequent lack of diagnostic markers, and inadequate understanding of their underlying pathophysiology (Jin & Warren, 2000).

For many children, the cause of their mental retardation is various. Through genetics a number of specific disorders have been identified as being genetically caused. Some of the most common known genetic causes of mental retardation are Fetal Alcohol Disease, Down syndrome and Fragile X syndrome (FXS). Fragile X syndrome is a X-linked inheritance caused by the presence of FMR1 gene located at a locus Xq27.3 (Claudia, Ashley & Warren, 1995, Warren & Sherman, 2001). This single non working gene leads to mental retardation. The affected children normally have a long face, long ears, high forehead and macroorchidism (enlarged testicles) (Claudia, et al., 1995; Warren & Sherman, 2001; Phadke, 2005). However the associated dysmophic features are subtle and making the patient especially man (Lachiewicz, Dawson, & Spiridigliozzi, 2000; Phadke, 2005) are difficult to be clinically diagnosed (Lachiewicz, et al., 2000; Warren & Sherman, 2001).

There is no prevention to most conditions that cause mental retardation. Only a special therapy in special schools has been practiced to mentally challenged student (Warren & Sherman, 2001). In United Kingdom (UK), British Institute for Brain Injured Children (BIBIC) provides a service to help maximise the potential of