UNIVERSITI PUTRA MALAYSIA

PROFILING OF DNA COPY NUMBER MICROABERRATIONS ASSOCIATED WITH CONGENITAL DISORDERS AMONG MALAYSIAN CHILDREN USING ARRAY COMPARATIVE GENOMIC HYBRIDISATION

FATIM BINTI KHARUDDIN

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By

FATIM BINTI KHARUDDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair: Abhimanyu Veerakumarasivam, PhD

Faculty: Faculty of Medicine and Health Sciences

Copy number gains or losses of various chromosomal regions, whole chromosomes or subtelomeric rearrangements have been known to cause human mental retardation syndromes and congenital malformations. This study characterised chromosomal abnormalities associated with congenital disorders in Malaysian patients and identified novel microdeletions or microduplications that correlate with the phenotype. A high resolution method, array comparative genomic hybridization (aCGH) was used. DNA was extracted from blood and then quantified and qualified before proceeding with the technique. Samples with satisfactory DNA quality and quantity were then chosen to be analysed with aCGH. The DNA was labelled, combined, pre-hybridised, hybridised and scanned. The identification of genes located in the region of duplication and deletion was conducted using ENSEMBL!. The correlation between the genotype and phenotype was done using OMIM. One

hundred patients were analysed in this study. Chromosome specific deletions and duplications occurred most frequently in chromosomes 1 and 3. The reasons probably are because these chromosomes are large and harbour many loci and genes that are susceptible to changes. In this study, 496 clones harboured copy number changes. Of this, 88 copy number changes were found in more than one individual. A comparison of the most frequent changes associated with phenotypes was also executed. For example, chromosome 17q21.31 was identified as major cause for global delays observed in the patients within this study based on the comparitive analysis, previous literature and CRHR1 and MAPT genes functions. Furthermore, 10 patients were selected for an in depth analysis to identify the possible causal gene(s). Seven novel genes speculated to have significant effect on the phenotypes were identified. Amongst them were FBN2 gene for abnormal pinna, FUZ for holoprosencephaly, TMEM1 and ARFGEF2 for microcephaly, TM2D2 for developmental delay, SH3GL2 for autism and INPP5A for severe psychomotor delay and failure to thrive as observed in these patients. This study proves the importance of aCGH as a tool that refines birth defects diagnosis. However, due to the high-resolution output of the technique, the copy-number variable nature of the genome as well as the massive gaps in the knowledge of many regions of the genome and gene function; conclusive delineation of the genotype-phenotype correlation remains an arduous task. Nonetheless, aCGH helps to provide better insight into the patient diagnosis and helps greater understanding of the disease molecular pathogenesis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMPROFILAN MIKROPERUBAHAN SALINAN NOMBOR DNA DALAM PENYAKIT KONGENITAL DI KALANGAN PESAKIT KANAK-KANAK MALAYSIA DENGAN MENGGUNAKAN *'ARRAY COMPARATIVE GENOMIC HYBRIDISATION'*

Oleh

FATIM BINTI KHARUDDIN

Oktober 2010

Pengerusi: Abhimanyu Veerakumarasivam, PhD

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Penambahan atau kehilangan pelbagai kawasan spesifik pada kromosom dan penyusunan semula kromosom telah diketahui sebagai penyebab sindrom terencat akal dan malformasi. Kajian ini mencirikan kecacatan kromosom dalam penyakit kongenital di kalangan pesakit Malaysia dan mengenalpasti mikrodelesi dan mikroduplikasi yang berhubungkait dengan fenotip. Satu kaedah yang mempunyai resolusi tinggi '*array comparative genomic hybridisation' (aCGH)* telah digunakan. DNA telah diekstrak daripada sampel darah dan kemudian kualiti dan kuantitinya diperiksa sebelum ujikaji dijalankan. Sampel-sampel yang memenuhi kriteria yang telah ditetapkan dipilih untuk dianalisa dengan aCGH. Proses label, kombinasi, hibridisasi awal dan hibridisasi serta imbasan dilakukan. Pengenalan gen-gen yang terletak pada kawasan kromosom yang hilang atau bertambah dilakukan menggunakan Ensembl!. Korelasi antara genotip dan fenotip dilakukan

menggunakan OMIM. Kajian telah dijalankan ke atas 100 pesakit untuk dianalisis. Kromosom yang paling kerap mengalami perubahan sama ada hilang atau bertambah adalah 1 dan 3. Antara sebab yang mungkin ialah kerana kromosom-kromosom ini adalah antara yang terbesar dan mempunyai banyak *'loci'* dan gen-gen yang terdedah kepada perubahan. Dalam kajian ini, 496 klon menunjukkan perubahan bilangan. Terdapat 88 klon telah ditemui pada lebih daripada seorang individu. Perbandingan di antara perubahan klon yang kerap berlaku yang berkait dengan fenotip juga dijalankan. Sebagai contoh, kromosom 17q21.31 telah dikenal pasti sebagai penyebab kepada terencat akal dalam kajian ini berdasarkan kepada perbandingan analisis, kajian yang lepas dan juga fungsi gen-gen yang terlibat iaitu CRHR1 dan MAPT. Selain itu, 10 pesakit dipilih untuk kajian analisis secara mendalam untuk mengenalpasti gen-gen penyebab yang mungkin. Terdapat 7 gen-gen baru yang dispekulasi mempunyai kesan signifikan ke atas fenotip iaitu gen FBN2 untuk bentuk telinga yang tidak normal, FUZ untuk bentuk otak yang tidak normal, TMEM1 dan ARFGEF2 untuk saiz kepala yang kecil, TM2D2 untuk masalah kelewatan perkembangan kanak-kanak, SH3GL2 untuk autisme dan INPP5A untuk masalah otot pergerakan yang diperhatikan pada pesakit-pesakit. Kajian ini membuktikan kepentingan aCGH sebagai kaedah untuk pengdiagnosan kecacatan kelahiran. Walaubagaimanapun, disebabkan oleh hasil resolusi tinggi dari teknik ini, variasi semulajadi yang terdapat dalam genom dan juga pengetahuan yang kurang tentang lokasi-lokasi genom dan fungsi gen, hubungkait antara genotip fenotip menjadi satu tugas yang sukar. Namun, aCGH tetap membantu dalam memberi pengetahuan yang mendalam untuk diagnosis pesakit dan dalam memahami tentang penyebab suatu penyakit itu dari sudut molekular.

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I certify that an Examination Committee has met on **28 October 2010** to conduct the final examination of Fatim binti Kharuddin on her Master of Science thesis entitled 'Profiling of DNA Copy Number Microaberrations Associated with Congenital Disorders Among Malaysians Using Array Comparative Genomic Hybridisation' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science

Members of the Examination Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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FATIM BINTI KHARUDDIN

Date: 28 October 2010

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