UNIVERSITI PUTRA MALAYSIA

ANALYSIS OF GENETIC POLYMORPHISMS OF *TBX5*, *NKX2-5* AND *GATA4* CARDIAC TRANSCRIPTION FACTOR GENES IN MALAYSIAN NON SYNDROMIC CONGENITAL HEART DISEASE SUBJECTS

NORA F. KADHIM

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By

NORA F. KADHIM

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

May 2012

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DEDICATIONS

This thesis is dedicated to my beloved parents who motivate me to have a higher education , my wonderful husband Hussein and sweet daughter Rewan for their patient and extreme encouragement for me to accomplish my study and finally to my best friend and sister Nada , who always inspires me .

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Abstract of the thesis presented to the School of Graduate Studies of University Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ANALYSIS OF GENETIC POLYMORPHISMS OF *TBX5*, *NKX2-5* AND *GATA4* CARDIAC TRANSCRIPTION FACTOR GENES IN MALAYSIAN NON SYNDROMIC CONGENITAL HEART DISEASE SUBJECTS

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May 2012

Chair: Prof. Patimah Ismail, PhD

Faculty: Faculty of Medicine and Health Sciences

Congenital heart disease (CHD) is the most common congenital anomaly of the new born infants. The underlying etiology of CHD is unrecognized in the majority of cases. Cardiac transcription factor genes have a crucial role in the cardiogenesis process during the embryonic period, hence a number of single nucleotide polymorphisms (SNPs) have been identified to cause CHD in many populations but there have been no studies that had been found among Malaysian CHD subjects. Hence, this study was initiated to determine the allelic and genotypic frequencies of three important polymorphisms of cardiac transcription factor genes, namely the intronic polymorphism rs11067075 of *TBX5* gene, R25C of *NKX2-5* gene and G296S of *GATA4* gene. We conducted a cross-sectional unmatched genetic association study

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between cases with CHD and healthy control subjects to determine the association of these polymorphisms and their genotype-phenotype correlation. A total of 150 non syndromic CHD subjects and 150 normal healthy individuals were recruited to this study with no matching for age and gender between cases and controls. We designed a protocol for genotyping of those three polymorphisms by real time-PCR-high resolution melt (HRM) analysis. Our study results shows that, the frequency of the polymorphism rs11067075 of *TBX5* gene was 4.7% in CHD subjects versus a frequency of 0.7% in the healthy controls showed a significant association with the development of CHD ($p<0.05$). *NKX2-5* gene heterozygote R25C (c.73.C>T) polymorphism was totally absent from both the cases and the control groups while genotyping of this polymorphism was incidentally accompanied by genotyping of a common variant of *NKX2-5* gene (c.63A>G). Nevertheless, the genotype and allele frequencies of the polymorphism c.63A>G of *NKX2-5* gene showed no difference between the cases and control groups ($p=0.893$). *GATA4* gene heterozygote G296S polymorphism was also not detected in this study cohort. The association of *TBX5* gene intronic polymorphism (rs11067075) with the development of CHD in this study emphasizes the role of TBX5 gene in the pathogenesis of non-syndromic CHD. The selected polymorphisms of *NKX2-5* gene (R25C) and *GATA4* gene (G296S) were not associated with the development of CHD in Malaysian subjects. However, investigating *GATA4* and *NXK2-5* genes in a bigger sample size for different variants might reveal an association of those gene polymorphisms with the development of CHD in Malaysian subjects. High resolution melting (HRM) analysis was used as a new technology for detecting those polymorphisms and had shown its power in an efficient genotyping and had the advantage of simultaneous genotyping and screening for sequence variants of R25C of *NKX2-5* gene.

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Abstrak tesis yang dikemukakan kepada Sekolah Pengajian Siswazah Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS GEN POLIMORFISME TERHADAP GEN FAKTOR TRANSKRIPSI JANTUNG *TBX5*, *NKX2-5* DAN *GATA4* DI KALANGAN PESAKIT JANTUNG KONGENITAL BUKAN SINDROMIK DI MALAYSIA

Oleh

NORA F. KADHIM

Mei 2012

Pengerusi: Prof. Patimah Ismail, PhD

Fakulti: Fakulti Perubatan Dan Sains Kesihatan

Penyakit jantung kongenital (CHD) adalah penyakit kongenital anomali yang paling kerap berlaku di kalangan bayi yang baru lahir. Etiologi yang berdasarkan CHD adalah tidak dikenalpasti dalam kebanyakan kes. Gen faktor transkripsi jantung mempunyai peranan yang penting dalam proses kardiogenesis semasa tempoh embrio, maka beberapa nukleotida polimorfisme (SNPs) telah dikenal pasti menjadi punca CHD dalam beberapa populasi tetapi tidak terdapat sebarang kajian yang telah ditemui di kalangan subjek CHD di Malaysia. Oleh itu, kajian ini telah dimulakan untuk menentukan frekuensi alel dan genotip tiga polimorfisme yang penting dalam gen faktor transkripsi jantung iaitu polimorfisme rs11067075 dalam gen *TBX5*, R25C dalam gen *NKX2-5* dan G296S dalam gen *GATA4*. Kami telah menjalankan kajian perkaitan genetik yang tidak seimbang diantara subjek kes CHD dengan subjek kawalan yang sihat untuk menentukan hubungkait polimorfisme ini dan korelasi

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genotip-fenotip mereka. Sebanyak 150 yang mempunyai CHD bukan sindromik dan 150 orang individu normal yang sihat telah direkrut untuk kajian ini dengan tidak memadankan umur dan jantina antara subjek kes dan kawalan. Kami telah mereka protokol untuk genotyping tiga polimorfisma ini menggunakan analisis masa nyata-PCR-resolusi leburan tinggi (HRM). Hasil kajian kami menunjukkan bahawa, frekuensi polimorfisma rs11067075 terhadap gen *TBX5* adalah 4.7% dalam subjek CHD dibandingkan dengan frekuensi 0.7% dalam subjek kawalan. Ini telah menunjukkan hubungan yang signifikan dengan perkembangan CHD ($p<0.05$). Gen heterozygot *NKX2-5* R25C (c.73.C>T) polimorfisma tidak didapati dalam kedua-dua kes dan kawalan manakala genotpyping terhadap polimorfisme ini kebetulan disertai oleh genotyping gen varian biasa *NKX2-5* (c.63A>G). Walau bagaimanapun, frekuensi genotip dan alel bagi polimorfisme c.63A>G dalam gen *NKX2-5* tidak menunjukkan perbezaan di antara kumpulan kes dan kawalan ($p= 0.893$). Gen heterozygot *GATA4* bagi polimorfisme G296S juga tidak dapat dikesan dalam kajian kohort ini. Hubungan antara gen intronik polimorfisma (rs11067075) dengan perkembangan CHD dalam kajian ini menekankan peranan gen *TBX5* dalam patogenisis CHD bukan sindromik. Semua polimorfisma yang dipilih iaitu polimorfisma gen *NKX2-5* (R25C) dan *GATA4* (G296S) tidak ada korelasi bagi perkembangan CHD di kalangan subjek di Malaysia. Walau bagaimanapun, kajian terhadap gen *GATA4* dan *NXK2-5* dalam saiz sampel yang lebih besar untuk varian yang berbeza mungkin akan mendedahkan hubungan gen polimorfisme ini dengan perkembangan CHD di kalangan subjek di Malaysia. Analisis HRM telah digunakan sebagai satu teknologi baru bagi mengesan polimorfisme dan ia mempunyai kelebihan melakukan genotyping dan saringan untuk varian urutan R25C dalam gen *NKX2-5* secara serentak.

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I Certify that a Thesis Examination Committee has met on to conduct the final examination of Nora Fawzi Kadhim Al-Shawee on her thesis entitled **"Analysis of Genetic Polymorphisms of *TBX5*, *NKX2-5* And *GATA4* Cardiac Transcription Factor Genes In Malaysian Congenital Heart Disease Subjects"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory 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 to any other degree at Universiti Putra Malaysia or at other institution.

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NORA F. KADHIM

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