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

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By

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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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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.
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

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Congenital heart disease (CHD) is the most common congenital anomaly of the newborn 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
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 \( NKX2-5 \) 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.
Abstrak tesis yang dikemukakan kepada Sekolah Pengajian Siswaah 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

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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 genotyping 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 NKX2-5 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 am grateful to my parents, my dear husband, my daughter and my family for their love, support and extreme encouragement throughout the duration of my study.
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.

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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.

___________________________
NORA F. KADHIM

Date: 10 May 2012
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