UNIVERSITI PUTRA MALAYSIA

INTERACTION OF GENE POLYMORPHISMS IN THE RISK OF CORONARY ARTERY DISEASE AND RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS OF CORONARY ARTERY DISEASE

CHU WERN CUI

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By

CHU WERN CUI

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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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July 2015

Chair: Cheah Yoke Kqueen, PhD
Faculty: Medicine and Health Sciences

Genetic variants of *methylenetetrahydrofolate reductase* (*MTHFR*), *endothelial nitric oxide synthase* (*eNOS*), and *cholesteryl ester transfer protein* (*CETP*), influence homocysteine, nitric oxide synthesis, and high-density lipoprotein cholesterol (HDL-C) metabolism, respectively and might increase the risk of coronary artery disease (CAD). It is becoming increasingly proven that polymorphisms in multiple genes are involved in the pathogenesis of CAD. Therefore, this study was conducted to investigate the association and interaction of *MTHFR C677T, eNOS G894T, eNOS 4a4b* and *CETP TaqIB* polymorphisms with the risk of CAD in multi-ethnics Malaysian population and the usefulness of random amplified polymorphic DNA (RAPD) analysis in discriminating CAD patients. A total of 344 subjects including angiographically confirmed 243 CAD patients and 101 control subjects were genotyped. The presence of *MTHFR 677T* allele was significantly associated with the increased risk of CAD and it was associated with higher total cholesterol and low-density lipoprotein cholesterol levels in the Chinese group. The presence of *eNOS 4a* allele was significantly associated with the increased risk of CAD in Malay and Indian groups. The *CETP B2B2* genotype was significantly associated with higher HDL-C and associated with decreased risk of CAD in the Malay group. Moreover, the concomitant presence of *MTHFR 677T* and *CETP B1* alleles was significantly increased the risk of CAD in Malay group and Chinese group but not Indian. At the same time, the concomitant presence of both *CETP B1* and *eNOS 4a* alleles was significantly increased the risk of CAD in Malay group and Indian group but not Chinese. The RAPD analysis show that, under certain conditions, genetic polymorphisms in genomic DNA of CAD patients could be detected by using RAPD analysis and enable the discrimination of the CAD patients from the controls. In conclusion, this study shows that gene polymorphisms differ in both distributions and association with CAD among different ethnic groups in Malaysia. Moreover, this study has identified a novel ethnic-specific gene-gene interactions, suggested that the role of gene-gene interaction in the pathogenesis of CAD might be ethnic specific. The detected polymorphisms by the arbitrary primers OPO 02 and OPO 10 enable the discrimination of the CAD patients from the controls. These findings can be further analysed for biomarker development.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk Ijazah Master Sains

INTERAKSI POLIMORFISME GENE PADA RISIKO PENYAKIT CORONARI ARTERI DAN ANALISIS AMPLIFIKASI ACAK POLIMORFISME DNA PADA PENYAKIT CORONARI ARTERI

Oleh

CHU WERN CUI

Julai 2015

Pengerusi : Cheah Yoke Kqueen, PhD
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Variasi genetik *metilenatetrahidrofolat reduktase* (*MTHFR*), *endotelial nitrik oksida synthase* (*eNOS*), dan *protein cholesteryl ester transfer* (*CETP*), mempengaruhi sintesis homocysteine, nitirk oksida, dan metabolisme kolesterol lipoprotein berkepadatan tinggi (HDL-C), masing-masing, dan mungkin meningkatkan risiko penyakit arteri koronari (CAD). Semakin banyak bukti menunjukkan bahawa polimorfisme dalam pelbagai gen terlibat dalam patogenesis CAD. Oleh itu, kajian ini telah dijalankan untuk menyiasat hubungan dan interaksi polimorfisme *MTHFR C677T, eNOS G894T, eNOS 4a4b and CETP TaqIB* dengan risiko CAD dalam pelbagai etnik penduduk Malaysia dan kegunaan analisis amplifikasi acak polimorfisme DNA (RAPD) dalam membezakan pesakit CAD. Seramai 344 subjek termasuk 243 pesakit CAD dan 101 subjek kawalan yang disahkan secara angiografi telah digenotipkan oleh kaedah PCR-RFLP. Kehadiran alel *MTHFR 677T* menyebabkan peningkatan risiko CAD dan ia dikaitkan dengan tahap jumlah kolesterol dan kolesterol lipoprotein berketumpatan rendah yang lebih tinggi dalam kumpulan Cina. Kehadiran alel *eNOS 4a* menyebabkan peningkatan risiko CAD dalam kumpulan Melayu dan India. Genotip *CETP B2B2* mempunyai kaitan dengan tahap HDL-C yang lebih tinggi dan dikaitkan dengan penurunan risiko CAD dalam kumpulan Melayu. Selain itu, kehadiran alel *MTHFR 677T* dan alel *CETP B1* sama-sama telah meningkat risiko CAD dalam kumpulan Melayu dan kumpulan tetapi bukan India. Pada masa yang sama, kehadiran kedua-dua alel *CETP B1* dan alel *eNOS 4a* sama-sama telah meningkat risiko CAD dalam kumpulan Melayu dan kumpulan India. Analisis RAPD menunjukkan bahawa, dengan syarat-syarat tertentu, polimorfisme genetik dalam DNA genomik pesakit CAD boleh dikesan dengan menggunakan analisis RAPD dan membolehkan pesakit CAD dibezakan daripada kawalan. Kesimpulannya, kajian ini menunjukkan bahawa polimorfisme gen berbeza dalam kedua-dua taburan dan hubungan dengan CAD dalam kalangan kumpulan etnik yang berbeza di Malaysia. Selain itu, kajian ini telah mengenal pasti interaksi gen-gen etnik khusus yang novel, mencadangkan bahawa peranan interaksi gen-gen dalam patogenesis CAD berkemungkinan khusus etnik. Polimorfisme dikesan oleh primer-primer arbitari OPO 02 dan 10 OPO membolehkan pembezaan pesakit CAD daripada kawalan. Penemuan ini boleh dilakukan kajian lanjut untuk pembangunan penanda bio.

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I certify that a Thesis Examination Committee has met on 2nd July 2015 to conduct the final examination of Chu Wern Cui on her thesis entitled "Interaction of gene polymorphisms in the risk of coronary artery disease and random amplified polymorphic DNA analysis of coronary artery disease" 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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The 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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This is to confirm that:

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LIST OF SYMBOLS, UNITS, ABBREVIATIONS AND TERMS

WHO	World Health Organization
CAD	coronary artery disease
MTHFR	*methylenetetrahydrofolate reductase*
eNOS	*endothelial nitric oxide synthase*
CETP	*cholesteryl ester transfer protein*
HDL-C	high-density lipoprotein cholesterol
RAPD	random amplified polymorphic DNA
PCR	polymerase chain reaction
CVD	cardiovascular diseases
%	percentage
NHMS	National Health and Morbidity Survey
TC	total cholesterol
TG	truglycerides
LDL-C	low-density lipoprotein cholesterol
BMI	body-mass index
≥	more than or equal to
PCSK9	*proprotein convertase subtilisin/kexin type 9*
DNA	deoxyribonucleic acid
RFLP	restriction fragment length polymorphism
FAD	flavin adenine dinucleotide
VNTR	variable number of tandem repeat
BH4	etrahydrobiopterin
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
ApoE	*apolipoprotein E*
ApoB	*apolipoprotein B-100*
CBS	*cystathionine β-synthase*
NMRR	National Medical Research Registry
EDTA	ethylenediaminetetraacetic acid
°C	degree Celcius
rpm	revolution per minute
w/v	weight/volume
dNTP	dinucleotide triphosphate
UPGMA	unweighted paired group method with arithmetic mean
HWE	Hardy-Weinberg equilibrium
ORs	odds ratios
CI	confidence intervals
ND	not determined

CHAPTER 1

INTRODUCTION

1.1 Background of the study

According to the World Health Organization (WHO) data published in April 2011, coronary artery disease (CAD) death in Malaysia reached 22,701 or 22.18% of total deaths. CAD also is the number one cause of premature deaths in Malaysia (Yusoff et al., 2013). This high rate could have been due to late presentation and diagnosis, leading to delayed treatment.

CAD is considered to be a complex disease that is caused by multiple genetic factors, environmental factors, and interactions among these factors. These factors may vary depending on ethnic group (Tanus-Santos, Desai, & Flockhart, 2001). Traditional risk factors such as hypertension, obesity, dyslipidaemia, smoking status, and physical activity have been studied in Malaysia (Zain, Ooyub, & Rahmat, 2007; Chin & Pengal, 2009; Rampal et al., 2010; Thon, Yein, & Lian, 2012). However, a quarter of the risk of CAD is unexplained by traditional risk factors (Dent, 2010). Some studies have showed that genetic variants can improve CAD assessment beyond the use of traditional risk factors (Damani & Topol, 2007). Therefore, understanding genetic variants that associated with CAD in Malaysian may contribute to better prevention, diagnosis and treatment of CAD in Malaysian population.

Methylenetetrahydrofolate reductase (MTHFR) C677T (rs1801133) that results in thermolabile *MTHFR* has been suggested to increase total plasma homocysteine and the risk of CAD (Sakowicz, Fendler, Lelonek, Sakowicz, & Pietrucha, 2013). Moreover, variants of the *endothelial nitric oxide synthase (eNOS)* gene have been associated with reduced concentration of nitric oxide (NO) and increased risk of CAD (Casas, Bautista, Humphries, & Hingorani, 2004). *Cholesteryl ester transfer protein (CETP) TaqIB* (rs 708272) polymorphism has been associated with the high-density lipoprotein (HDL) levels and the risk of CAD (Boekholdt et al., 2005). The effects of these gene polymorphisms on the risk of CAD have been reported in many countries but could not be generalised across ethnic groups. There is no data available regarding the association of these gene polymorphisms with the risk of CAD in Malaysian population. Hence, the association of these gene polymorphisms with the risk of CAD in Malaysian population should be studied.

CAD is contributed by interplay of complex biochemical processes which includes lipid and apolipoprotein metabolism, inflammatory response, endothelial dysfunction, homocysteine metabolism, thrombosis, insulin sensitivity, and blood pressure regulation (Scheuner, 2004). However, most studies of CAD focus on single-marker based analysis ignoring the interactions between markers causing the identified markers often only explain a small fraction of the phenotypic variation (Chikkagoudar, Wang, & Li, 2011). It is becoming increasingly evident that gene-gene interaction plays an important role in the etiology of complex disease such as CAD (Poduri, Khullar, Bahl, Sharma, & Talwar, 2009). Therefore, identification of the gene-gene interaction may help us to understand more on the pathological mechanisms of the CAD.

Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction – based fingerprinting technique that amplifies random DNA fragments with single short primers of arbitrary nucleotide sequence under low annealing stringency. RAPD can detect genetic alterations in the entire genome (including point mutations, microsatellite instability, chromosome alterations). RAPD method has been applied as a mean for identifying the genomic instability in brain tumours (Dil-Afroze et al., 1998), liver cancer (Zhang, Cong, Xian, Dong, & Wu, 2004), skin cancer (Ribeiro et al., 2004), and breast cancer (El-Assal, El-Tarras, & Abd-alla, 2011). There is increasing evidence that multiple mutations are responsible for the development of CAD. Therefore, RAPD analysis might be useful to detect the genetic polymorphisms that occur in CAD patients and potentially use for the detection of CAD.

1.2 Objectives

The general objectives of this study are to determine the association and interaction of specific gene polymorphisms with the risk of CAD in Malaysian population and the usefulness of RAPD analysis in discriminating CAD patients.

Hence, the specific objectives of this study are:
1. To profile specific gene polymorphisms for *CETP*, *MTHFR*, and *eNOS*.
2. To correlate these gene polymorphisms with the risk of CAD.
3. To determine the interaction of these gene polymorphisms in the development of CAD.
4. To compare the amplification profiles of genomic DNA in CAD patients with controls using RAPD analysis.

1.3 Hypothesis

Polymorphisms of *MTHFR*, *eNOS* and *CETP* genes may have an association with the risk of CAD in Malaysian population. There are possible interactions between these gene polymorphisms on the risk of CAD. Moreover, RAPD analysis might able to discriminate the CAD patients from the control.

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