UNIVERSITI PUTRA MALAYSIA

METHYLENETETRAHYDROFOLATE REDUCTASE AND *METHIONINE SYNTHASE REDUCTASE* GENE POLYMORPHISMS IN IRANIAN *VENTRICULAR SEPTAL DEFECT* SUBJECTS

SEYYED REZA PISHVA

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By

SEYYED REZA PISHVA

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

March 2013

DEDICATIONS

God grant me the serenity to accept the things I can't change; courage to change the things I can; and wisdom to know the difference.

I would like to dedicate this thesis to my lovely parents, for their kind consistent support throughout my whole life and their unconditional love and the sacrifice they made for me.

I would like to dedicate this thesis to my wonderful brothers, for all their on-going support and motivation which induced me to achieve a higher education.

There are many people I miss today, people who have made a difference to my life and influenced me to an extent. Foremost amongst them are my grandparents. I dedicate this thesis to their memories.

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

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March 2013

Chairman: Professor Patimah Ismail, PhD

Faculty: Medicine and Health Sciences

Congenital heart defects (CHD) are among the most pervasive congenital anomalies globally, occurring in an average of one in hundred living newborn babies. This occurrence of CHD is related to the relative frequency of ventricular septal defects (VSDs), which is the most common type of CHD. Heart defects at birth occur as an insular malformation, but are also linked with other anomalies or occur as part of a syndrome. There are vivid multifactorial causes for CHD in which both genetic and environmental risk factors are consequential in the development of CHD. Single nucleotide polymorphisms (SNPs) are the predisposing genetic risk factors for the contribution to major diseases. Genes involved in homocysteine/folate metabolism may play an important role in CHDs. Methionine synthase reductase (*MTRR*) and

Methylenetetrahydrofolate Reductase (*MTHFR*) are one of the key regulatory enzymes involved in the metabolic pathway of homocysteine. In this study, we determined the association of *A66G* and *C524T* polymorphisms of the *MTRR* gene and *C677T* polymorphism of the *MTHFR* gene with Iranian VSD subjects. A total of 123 children with VSDs and 125 healthy children were included in this study. Buccal cells were collected from subjects using cytology brush according to the inclusion and exclusion criteria. The PCR was carried out to amplify the *MTRR* and *MTHFR* genes. The amplified PCR products of *MTHFR C677T*, *MTRR C524T* and *MTRR A66G* gene polymorphisms were digested with *Hinf1*, *XHO1*and *Nde1*enzymes respectively using Restriction Fragment Length Polymorphism method. The distribution of genotypic and allelic frequency of polymorphisms was obtained using Chi square test. The genotype frequencies of CC,CT and TT of *MTRR* gene among cases were 43.1%, 40.7% and 16.3% respectively compared to 52.8%, 43.2% and 4.0% respectively among controls. For the *MTRR A66G* gene polymorphism, the genotypes frequencies of AA, AG and GG among cases were 33.3%, 43.9% and 22.8% respectively, while the frequencies were 49.6%, 42.4% and 8.0% among control subjects. The frequencies for CC and CT genotypes of the MTHFR gene were 51.2% and 48.8%, respectively, in VSD patients, and in control subjects were 56.8% and 43.2% respectively. Significant differences in the genotypic and allelic frequency distributions of both *MTRR C524T* and *MTRR A66G* polymorphisms between cases and controls were observed ($p<0.05$) whereas for *MTHFR C677T* polymorphism between cases and controls there was no significant association of neither genotypic nor allelic frequencies ($p>0.05$). In conclusion, the association of *C524T* and *A66G* gene polymorphisms of *MTRR* gene is considered as risk factors for the development of VSD in Iranian subjects.

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

REDUKTAS METILENETETRAHIDROFOLAT DAN *REDUKTAS SINTASE METHIONIN* GEN POLIMORFISME DALAM KECACATAN SUBJEK *SEPTAL VENTRIKULAR* DI IRAN

Oleh

SEYYED REZA PISHVA

March 2013

Pengerusi: Professor Patimah Ismail, PhD

Fakulti: Perubatan Dan Sains Kesihatan

Kecacatan jantung kongenital (CHD) adalah antara keganjilan kongenital paling semerbak di dunia yang dihidapi secara purata oleh satu dalam seratus bayi baru lahir. CHD berkaitan dengan frekuensi relatif kecacatan septal ventrikular (VSD), yang merupakan salah satu CHD yang lazim. Kecacatan jantung yang berlaku semasa kelahiran adalah kecacatan insular, tetapi juga adalah berkaitan dengan anomali-anomali lain ataupun sebagai sebahagian daripada suatu sindrom. Terdapat pelbagai penyebab untuk CHD, di mana faktor-faktor genetik dan persekitaran terlibat dalam perkembangan CHD. Polimorfisme nukleotida tunggal (SNP) adalah faktor-faktor genetik berkecenderungan untuk penyakit utama. Gen-gen yang terlibat dalam metabolisme homosistin/folat memainkan perana penting dalam CHD. Reduktas sintase methionin

(MTRR) dan reduktas metilenetetrahidrofolat (*MTHFR*) adalah salah satu enzim pengawal penting yang terlibat dalam laluan metabolic homosistin. Dalam kajian ini, kami telah menentukan kaitan antara polimorfisme *A66G* dan *C524T* dalam gen *MTRR* dan polimorfisme *C677T* gen *MTHFR* dalam subjek VSD warga Iran. Sejumlah 123 kanak-kanak dengan VSD dan 125 kanak-kanak sihat terlibat dalam kajian ini. Sel-sel bukal telah dikumpul daripada subjek-subjek menggunakan berus sitologi mengikut kriteria kemasukan dan pengecualian. PCR telah dilakukan untuk mengamplifikasi gen-gen *MTRR* dan *MTHFR*. Produk-produk PCR untuk polimorfisme *C677T MTHFR*, *C524T MTRR* dan gen *A66G MTRR* telah dihadamkan oleh enzim-enzim *Hinf1, XHO1* dan *Nde1* menggunakan kaedah RFLP. Frekuensi alel dan pengagihan genotip polimorfisme telah diperolehi menggunakan kaedah Chi-square.

Frekuensi genotip CC, CT dan TT bagi gen *MTRR* di kalangan kumpulan kes adalah 43.1%, 40.7% dan 16.3% berbanding 52.8%, 43.2% dan 4% setiap satunya bagi kumpulan kawalan.Bagi polimorfisme gen *MTRR A66G*, frekuensi genotip bagi AA, AG dan GG di kalangan kumpulan kes ialah 33.3%, 43.9% dan 22.8% setiap satunya manakala frekuensi di kalangan kumpulan kawalan ialah 49.6%, 42.4% dan 8.0%. Frekuensi bagi genotip CC dan CT gen *MTHFR* ialah 51.2% dan 48.8% di kalangan pesakit VSD serta 56.8% dan 43.2% dalam kumpulan kawalan. Perbezaan signifikan dalam pengagihan frekuensi genotip dan alel kedua-kedua polimorfisme *C524T MTRR* dan *A66G MTRR* antara kes dan kawalan telah diperhatikan ($p<0.05$), manakala tiada kaitan signifikan frekuensi genotip ataupun alel untuk polimorfisme *C677T MTHFR* antara kes dan kawalan ($p<0.05$). Kesimpulannya, kaitan polimorfisme-polimorfisme gen *MTRR C524T* dan *A66G MTRR* dianggap sebagai faktor-faktor risiko untuk perkembangan VSD dalam subjek-subjek warga Iran.

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I Certify that a Thesis Examination Committee has met on to conduct the final examination of Seyyed Reza Pishva on his thesis entitled **"ANALYSIS OF MTHFR AND MTRR GENE POLYMORPHISMS IN IRANIAN VENTRICULAR SEPTAL DEFECT 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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SEYYED REZA PISHVA

Date: 7 March 2013

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