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Full Length Research Paper

Prevalence of methylenetetrahydrofolate reductase (*MTHFR*) and cytosolic serine hydroxymethyltransferase (*cSHMT*) genes polymorphisms in healthy Malaysian population

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Methylenetetrahydrofolate reductase (MTHFR) and Cytosolic serine hydroxymethyltransferase (cSHMT) are enzymes involve in folate regulation in human. The C to T transition of the *cSHMT* and *MTHFR* genes at the 1420 as well as 677 nucleotides both carries TT genotype respectively. These enzymes have direct and indirect relations with the production of homocysteine. TT genotypes of both genes give rise to high level of homocysteine which in turn is linked to cardiovascular diseases susceptibility. This study evaluates MTHFR 677C-T and cSHMT 1420C-T polymorphisms and the distribution of their genotypes in a Malaysian study population emphasizing on the gender and major ethnics. Three hundred and ten (310) healthy subjects were recruited and genotyping of the variants were performed using PCR-restriction fragement length polymorphism (RFLP) method. Our results showed that for *MTHFR* gene, the frequency of TT genotype on the whole study population was 0.17±0.374. Females (21.08%) were identified to carry higher frequency of TT genotype while the Chinese (28.83%) had the highest frequency of TT genotype compared to Malay and Indian ($p < 0.05$). The frequency of TT genotype of *cSHMT* gene on the other hand was 0.24±0.425. Females (16.32%) and the Chinese displayed highest frequency of cSHMT TT genotype ($p < 0.05$). Our preliminary results showed that female and the Chinese display higher prevalence of TT genotype of both *MTHFR* and *cSHMT* genes in Malaysia.

Key words: methylenetetrahydrofolate reductase (MTHFR), cytosolic serine hydroxymethyltransferase (*cSHMT*), polymorphisms, PCR-RFLP, Malaysian population.

INTRODUCTION

Methylenetetrahydrofolate reductase (MTHFR) is one of the key enzymes involves folic acid metabolism. The human *MTHFR* gene is located at chromosome 1p36.3 and consists of 11 exons (Goyette et al., 1998). This enzyme reduces 5,10-methylenetetrahydrofolate to 5 methyltetahydrofolate which is essential for homocysteine remethylation to methionine in the folate cycle. The most common polymorphism in *MTHFR* gene is the transition of C to T located at the 677 nucleotide (Frost et al., 1995). This substitution leads to the synthesis of a thermolabile form of MTHFR which has decreased enzymatic activity. Therefore, the individual with this genotype has low ability to methylate homocysteine to methionine resulting in increased level of total plasma homocysteine (De Bree et al., 2002). Previous researches suggested that this particular MTHFR polymorphism accelerates the progression of cardiovascular disease (CVD) in patients with familial hypercholesterolemia or previous history of myocardial infarction (Kawashiri et al., 2000). There are also reports on significant associations

of MTHFR polymorphism with essential hypertension and ischemic stroke (Malinowska and Chmurzynska, 2009).

Serine hydroxymethyltransferase (SHMT) catalyzes the interconversion reaction of serine to glycine with tetrahydrofolate serving as the one carbon acceptor (Capelluto et al., 1999). cSHMT is located in the cytosol and the gene is localized at chromosome 17p11.2 (Schirch and Peterson, 1980). A single nucleotide polymorphism (SNP) in the *cSHMT* gene at nucleotide 1420 as either C or T will result in the amino acid leucine or phenylalanine, respectively (Lim et al., 2005). The role of cSHMT is to provide one-carbon unit for the remethylation of homocysteine in the folate cycle. Therefore, any disturbance in the gene expression or enzyme activity due to this polymorphism could mimic a folate deficiency symptom (Skibola et al., 2007).

Homocysteine is a naturally occurring amino acid that could cause vessel damage through several mechanisms. Several clinical reports have shown that elevated level of homocysteine (hyperhomocysteinemia) is a risk factor for CVD in human (Alam et al., 2008). Homocysteine is also a good biomarker in predicting mortality risks independently from other traditional risk factors in patients with heart conditions (Aydin et al., 2009). Hyperhomocysteinemia results from polymerphism in the genes of the enzymes that participate in the synthesis and metabolism of folate. It also could be caused by deficiency of folic acid, vitamins B6 and B12 (Makedos et al., 2007). Therefore, the purpose of this study was to determine the prevalence of the MTHFR 677CT and cSHMT 1420CT polymorphisms emphasizing on different gender and major ethnics in Malaysian healthy population.

MATERIALS AND METHODS

Study population

Three hundred and ten healthy subjects of different genders and ethnicity were recruited from the National Blood Bank and Universiti Putra Malaysia (UPM). This study was approved by the Ethics Committee of UPM and National Medical Research Registry (NMRR) of Malaysian Ministry of Health with the registration number of NMRR-08-1104-2259. Subjects were briefed about the study, given inform consents to sign and respondents background information sheets to fill up before 10 ml of blood were drawn by a medical officer. Blood in ethylene-diaminetetraacetic acid (EDTA) tubes were stored in an ice box for transportation and kept in 4°C refrigerator if not processed immediately. DNA extraction was carried out within 48 h to ensure satisfactory yield and purity. The plasma was separated beforehand by centrifugation at 9000 xg for 15 min. The separated plasma was transferred out and stored in 5ml normal tube in -80°C freezer for consequence biochemical analysis.

Clinical and biochemical analysis

In addition to personal cardiovascular event history and family history of cardiovascular disease, the following vascular risk factors were also recorded: body mass index, systolic blood pressure (SBP), diastolic blood pressure (SBP). Next, total plasma cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C) levels were determined by using Hitachi 902 analyzer.

MTHFR gene analysis

DNA extraction was carried out by using the procedure suggested by Miller et al. (1988) with some modifications. There are three MTHFR genotypes: variant homozygous (val/val, TT), variant heterozygous (val/ala, CT) and wild-type homozygous (ala/ala, CC) (Chen et al., 1996). Several polymerase chain reaction (PCR)-based methods have been used for the detection of genetic abnormalities. The original protocols used restriction fragment length polymorphisms (RFLP) in which PCR products were digested with restriction enzymes followed by gel electrophoresis (Agarwal et al., 2007). The primer sequences used in the PCR reactions were: 5'-CGAAGCAGGGAGCTTTGAGGCTG-3' and 5'-AGGACGGTGCGGTGAGAGTG-3' (Bioneer, Korea). PCR amplification cycles were modified as follows: 5 min of initial denaturation at 94°C followed by 30 cycles of 1 min of denaturation at 94°C, 30 s of annealing at 69.5°C, 30 s of extension at 72°C and a final extension of 5 min at 72°C in GeneAmp PCR System 9700. The amplified products were digested with *Hin*f1 (Fermentas, Lithuania) at 37°C overnight (the C to T transition at nucleotide position 677 creates a new *Hin*f1 site) (Chen et al., 1996). This generates a 198 bp fragment. The C-T substitution creates a *Hin*fI recognition site, and digestion of the PCR product results in 175 and 23 bp fragments. The digested fragments were separated by electrophoresis in 2% agarose gel (Promega, USA) and visualized under ultraviolet light (Frosst et al., 1995).

CSHMT gene analysis

Genotyping for the cSHMT variants was also carried out by using a standard restriction fragment length polymorphism method. The following primers were used to amplify a 292-bp fragment containing the loci of interest: 5'-GTG TGG GGT GAC TTC ATT TGT G-3' and 5'- GGA GCA GCT CAT CCA TCT CTC-3' (Bioneer, Korea). PCR cycling condition consisted of one 2-minute cycle at 50°C, one 10 min cycle at 95°C, followed by 38 cycles of 92°C for 15 s and 62°C for 1 min (Skibola et al., 2002). cSHMT 1420C-T polymorphism was performed by digesting the 292 bp PCR product into 179 bp and 113 bp fragments using *Ear1* (Fermentas, Lithuania) digestion enzyme. The digested fragments were separated by electrophoresis in 2% agarose gel (Preomega, USA) and visualized under ultraviolet light.

Statistical analysis

Clinical and biochemical characteristics of the study population

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Abbreviations: MTHFR, Methylenetetrahydrofolate reductase; **cSHMT,** cytosolic Serine hydroxymethyltransferase; **CVD,** cardiovascular disease; **PCR,** polymerase chain reaction; **RFLP,** restriction fragment length polymorphisms; **NCVD,** National cardiovascular disease database; **SNPs,** single nucleotide polymorphism.

Table 1. Clinical characteristics of the subjects.

Clinical characteristic	Male N = 163 Mean (SD)	Female N = 147 Mean (SD)	p-value	Total N = 310 Mean (SD)
Age (years)	33.57 (10.41)	30.41 (9.46)	0.006*	32.07 (10.08)
BMI (kg/m^2)	25.59 (5.14)	25.43 (4.89)	0.784	25.51 (5.02)
Systolic blood pressure (mmHg)	127.26 (11.37)	123.87 (11.99)	0.011*	125.65 (11.78)
Diastolic blood pressure (mmHg)	79.87 (9.05)	77.83 (7.54)	0.033*	78.80 (8.41)
Triglycerides (mmol/l)	1.49 (0.61)	1.61 (0.59)	0.001*	1.54 (0.60)
Cholesterol (mmol/l)	4.667 (0.83)	4.331 (0.88)	0.077	4.51 (0.86)
HDL-cholesterol (mmol/l)	1.228 (0.33)	1.281 (0.27)	0.089	1.25 (0.30)
LDL-cholesterol (mmol/l)	2.828 (0.59)	2.712 (0.49)	0.065	2.77 (0.55)

Values are shown in mean (SD). Comparison between genders was made. Significant level of $p<0.05$ are shown in * using parametric independent t-test.

were analyzed and compared using *Chi-square* test and independent sample t-test. Multivariate analyses were performed by including the following covariants in the model: age, gender, ethnicity, smoking habit, exercise routine, blood pressure, body metabolic index (BMI), abdominal obesity and lipid profiles. The allelic frequency and genotypic distribution of the SNPs among the gender and ethnic groups were compared. A p-value < 0.05 was considered to be significant.

RESULTS

Clinical and biochemical characteristics

The clinical and biochemical characteristics of the subjects enrolled in this study are shown in Table 1. Out of 310 subjects, 163 (52.58%) were males and 147 (47.41%) were females. The mean age (SD) for the subjects was 32.07(10.08) years. Among the subjects were three major ethnics in Malaysian population which comprised Malay (n = 203, 33.23%), Chinese (n = 111, 35.80%) and Indian (n = 96, 30.97%). The BMI mean (SD) for the subjects was 25.51(5.02) kg/m^2. Abdominal obesity was detected in 39 subjects (12.6%) out of the total healthy studied population. The mean (SD) systolic and diastolic blood pressure were 125.65 (11.78) mmHg and 78.80 (8.4) mmHg respectively. Mean total cholesterol (SD) was 5.08 (1.04) mmol/L, triglycerides was 1.55 (0.69) mmol/L, HDL-C was 0.90 (0.31) mmol/L and LDL-C was 3.10 (0.67) mmol/L. Ninety seven (31.3%) of the subjects have family history of cardiovascular disease (CVD) while the other 213 (68.7%) do not have any family history of CVD. There are significant differences in age, systolic blood pressure, diastolic blood pressure, and triglycerides level between the males and females. However, all biochemical data were found to be in the normal ranges.

Genotyping results

The distribution of genotypes in these subjects showed deviation in Heidy-Weinberg equilibrium ($p < 0.05$). The genotypic and allelic distributions among the gender for *MTHFR* gene are shown in Table 2. The frequency of CC, CT and TT genotypes of *MTHFR* gene in males were 61.9, 42.6 and 38.8%, respectively. In females, 38.1, 57.4 and 61.3% were observed for CC, CT and TT genotypes, respectively.

The genotypic and allelic distributions among the gender for *cSHMT* gene are shown in Table 2. The prevalence for CC, CT and TT genotypes of *cSHMT* gene in males were 79.14, 9.20 and 11.66%, respectively. For females, the prevalence was 67.35, 16.32 and 16.32% for CC, CT and TT genotypes, respectively.

The genotypic and allelic distributions among major ethnics in healthy Malaysian population for *MTHFR* gene are shown in Table 3. The CC, CT and TT genotypes of *MTHFR* gene in Malays were 30.3, 56.8 and 28.8%, respectively. In Chinese, 33.9, 29.7 and 23.1% were observed for CC, CT and TT genotypes respectively. In Indian, the prevalence of CC, CT and TT genotypes were 35.7, 13.5 and 23.1%.

The genotypic and allelic distributions of *cSHMT* gene in healthy Malaysian population are shown in Table 3. The CC, CT and TT genotypes of *cSHMT* gene in Malay were 36.8, 25.9 and 26.3%, respectively; 37.5, 22.2 and 41.3% were observed in Chinese, respectively and 23.9, 51.9 and 32.5% were observed in Indian, respectively.

DISCUSSION

Up to recently, there was no previous study reported on *MTHFR* and *cSHMT* genes polymorphisms prevalence in Malaysian population. This study was carried out to determine the distribution of these genes polymorphisms in different gender and major ethnicity in Malaysia. Current molecular findings indicate that specific group characterized by predisposing genetic traits; ethnicity, age, gender as well as health and nutritional impairment

Table 2. Genotypic and allelic distributions of MTHFR and cSHMT genes in different gender.

Genotypes and alleles	Gender		Total N = 310 (%)
	Male N = 163 (%)	Female N = 147 (%)	
MTHFR C677T genotypes			
CC	144 (65.2)	77 (34.8)	221 (71.3)
CT	10 (27.0)	27 (73.0)	37 (11.9)
TT	9 (17.3)	43 (47.4)	52 (16.8)
MTHFR C677T alleles			
C	298 (91.4)	181 (61.6)	-
T	28 (8.6)	113 (38.4)	-
$\chi^2 = 14.471$, $p = 0.000$*			
cSHMT C1420T genotypes			
CC	109 (61.9)	67 (38.1)	176 (56.8)
CT	23 (42.6)	31 (57.4)	54 (17.4)
TT	31 (38.8)	49 (61.3)	80 (25.8)
cSHMT C1420T alleles			
C	241 (73.9)	165 (56.1)	-
T	85 (26.1)	129 (43.9)	-
$\chi^2 = 49.660$, $p = 0.000$*			

Data were analyzed using contingency χ2 test. Statistically significant value was observed at $p < 0.05$.

Table 3. Genotypic and allelic distributions of MTHFR and cSHMT among major ethnics.

Genotypes and alleles	Ethnic			Total N = 310 (%)
	Malay N = 103 (%)	Chinese N = 111 (%)	Indian N = 96 (%)	
MTHFR C677T genotypes				
CC	67(30.3)	75 (33.9)	79 (35.7)	221 (71.3)
CT	21(56.8)	11(29.7)	5(13.5)	37 (11.9)
TT	15 (28.8)	25 (48.1)	12 (23.1)	52 (16.8)
MTHFR C677T alleles				
C	155 (75.2)	161 (72.5)	163 (84.9)	-
T	51 (24.8)	61 (27.5)	29 (15.1)	-
$\chi^2 = 16.128$, $p = 0.003$*				
cSHMT C1420T genotypes				
CC	68 (38.6)	66 (37.5)	42 (23.9)	176 (56.8)
CT	14 (25.9)	12 (22.2)	28 (51.9)	54 (17.4)
TT	21 (26.3)	33 (41.3)	26 (32.5)	80 (25.8)
cSHMT C1420T alleles				
C	150 (72.8)	144 (64.9)	112 (58.3)	-
T	56 (27.2)	78 (35.1)	80 (41.7)	-
$\chi^2 = 49.660$, $p = 0.000$*				

Data were analyzed using contingency χ2 test. Statistically significant value was observed at $p < 0.05$.

were associated with higher risks as well as susceptibility towards certain disorders (Makpol et al., 2003).

In this study, we found that there were significant gender-mediated and ethnicity preferential distribution of *MTHFR* and *cSHMT* genes polymorphism in healthy Malaysian subjects ($p < 0.05$). The data were analyzed according to the three main ethnic races in Malaysian population. Our results showed that there were significant associations between both genes polymorphism with gender and ethnicity.

It has been reported that the MTHFR C677T polymorphism results in the reduction of MTHFR activity by about 30% in heterozygous and by 60% in homozygous individuals (Kalita et al., 2006). A study in India has reported that frequency of MTHFR C677T for homozygous alleles (TT) were at 3.5% whereby the heterozygous alleles (CT) were 40.8% of the subjects (Reddy and Jamil, 2006). In another study, distribution of *MTHFR C677T* gene polymorphism was reported at 3% for recessive homozygous and 18% for the heterozygous (Kaur and Sangha, 2006). A study conducted by Raganathan and co-workers on an African-American population in 2008 showed that there are differential effects of MTHFR polymorphism in different racial groups suggesting that race may significantly influence the C677T variant distributions in a population.

In this study, we found that the prevalence of TT genotype of *MTHFR* and *cSHMT* genes were higher in Chinese compared to Malay and Indian. A study conducted in China by Ho et al. (2005) found out that the TT genotype of MTHFR were mostly found in subjects with CVD and high level of homocysteine.

High regional and geographical variability of MTHFR polymorphism prevalence may be attributable to the racial and ethnic differences (Mattia and Toffoli, 2009). A pilot retrospective cross-validation study conducted by Ranganathan et al. (2008) has examined the pharmacogenetic association of C677T polymorphism with methotrexate toxicity in Caucasians and African–Americans with rheumatoid arthritis. The outcome of this investigation has shown that there are differential effects in these racial groups suggesting that race may significantly influence the C677T variant distributions. Thus, population genotyping is detrimental in detecting allele distribution in different ethnics and racial groups so that specific preventive or intervention measures that suit local needs can be strategically planned and carried out. Therefore, it is insightful if future research analyzing the prevalence of these genetic polymorphisms can be conducted in a case control study design, so that to the influence of these genetic variants to alter risk of developing CVD in Malaysian population can be determined.In terms of gender, female showed higher TT genotypes prevalence in both of the genes. This finding also does not correlate to the reports by NCVD which stated that the prevalence of CVD is almost the same in both gender with male at the higher end. Overall, men have a higher risk of developing CVD than women. But the difference narrows down after the women reach menopause stage. Because of their sex hormones, women are usually protected from heart disease but with the onset of menopause and depreciating sex hormone levels, their risks of developing CVD increase with age.

Conclusion

This study concludes that significant differences of MTHFR and cSHMT genotypes prevalence in different gender and ethnicity showed that they are significant important factors influencing genes polymorphisms distributions in a population.

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