

The allele frequencies of three polymorphisms in genes involved in homocysteine metabolism in a group of unrelated healthy Singaporeans

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Abstract. The cystathionine β -synthase (CBS) 844ins68 polymorphism, methionine synthase (MS) A2756G SNP, and 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T SNP are associated with homocysteine (Hcy) level in humans. Elevated Hcy level is considered a risk factor for atherosclerotic diseases among Asian populations. Therefore, the three polymorphisms may vary the risk for developing such diseases in Singaporeans. In this study, the three polymorphisms were determined in a group of unrelated healthy Singaporeans (273 Chinese, 127 Indians, and 156 Malays). Regarding allele frequencies, Indians had the highest frequencies of the CBS insertion allele (2.0%) and the MS 2756G allele (26.4%), while Chinese had the highest MTHFR 677T allele frequency (27.5%). In addition, the MTHFR 677T allele was found significantly lower in Chinese males than in their female counterparts. As the CBS insertion allele was suggested to be associated with lower Hcy level, whereas the MS 2756G allele and the MTHFR T/T genotype were related to higher Hcy level, the MS A/G or G/G genotype and the MTHFR T/T genotype were considered double genetic risk factors for elevated Hcy level. The frequency of such double genetic risk was 0.7% (4 subjects) in the total population consisting of 3 Chinese (1.1%) and 1 Malays (0.6%). No MTHFR T/T genotype was found in Indians. Such results suggested that Chinese could have higher Hcy levels than Malays while the situation for Indians was complicated. Since human Hcy levels are also affected by environmental factors, further studies are required to better evaluate the association between these three polymorphisms and Hcy levels and/or disease susceptibilities in Singaporeans.

Keywords: Cystathionine β -synthase, Methionine synthase, 5,10-Methylenetetrahydrofolate reductase, Polymorphism, Homocysteine, Singaporean

1. Introduction

Homocysteine is a sulfhydryl-containing amino acid derived from the metabolic demethylation of dietary methionine. In humans, Hcy can either be converted to cystathionine by the enzyme cystathionine β -synthase (CBS) (the transsulfuration pathway), or it can be methylated to form methionine by the enzyme methion-

ine synthase (MS) (the remethylation pathway), using 5-methyltetrahydrofolate as a methyl donor, which is converted from 5,10-methylenetetrahydrofolate by the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) [1].

Since CBS, MS, and MTHFR are all involved in the conversion of Hcy to other metabolites, polymorphisms located on these genes have been extensively studied and some of them are found associated with varied levels of plasma Hcy. At the CBS gene, a 68-bp insertion between base 844 and 845 (844ins68) at the junction of intron 7 and exon 8 has been associated with lower post-methionine load increase in total Hcy concentra-

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tions [2,3]. At the *MS* gene, an A to G transition at nucleotide 2756 (A2756G), which results in the substitution of glycine for aspartic acid at amino acid position 919 (D919G), has been described [4]. In Indian [5] and Korean [6] populations, the 2756G allele was found to be associated with increased Hcy levels. Finally at the *MTHFR* gene, a common C to T transition at nucleotide 677 (C677T), which leads to the substitution of valine for alanine, has been reported to impair the enzymatic activity of MTHFR [7], and the homozygous form of the 677T allele (T/T genotype) is therefore associated with higher Hcy concentrations than the other two genotypes among major Asian populations including Japanese [8,9], Koreans [6], and Chinese [10,11].

Among Asian populations, elevated tHcy level has been suggested to relate to some atherosclerotic diseases, such as cardiovascular disease (CVD) in Indians [12] and Chinese [13] and coronary artery disease (CAD) in Taiwanese Chinese [14,15], as well as to thrombotic disease, i.e., venous thromboembolism in Taiwanese Chinese [16]. In this sense, screening the aforementioned three polymorphisms among general populations may help to define the possible genetic risk factors that affect the susceptibility to these diseases. The objectives of the present study are to: (1) determine the allele frequency of the three polymorphisms, namely, the *CBS* 844ins68 polymorphism, the *MS* A2756G SNP, and the *MTHFR* C677T SNP, in unrelated healthy individuals of Singaporean Chinese, Indians, and Malays; and (2) evaluate the carriage rate of multiple genetic risk factors for elevated Hcy concentrations among Singaporeans.

2. Materials and methods

2.1. Experimental subjects

A total of 556 unrelated Singaporeans (273 Chinese, 127 Indians, and 156 Malays) were enrolled. They were apparently healthy and not on medication by self-indication. The majority of participants were polytechnic and college students and trainee teachers. The average age (Mean \pm S.D.) of the three ethnic groups were 27.6 ± 9.7 yr (17 to 71 yr) for Chinese, 31.0 ± 10.7 (15 to 60 yr) for Indians, and 29.4 ± 13.5 (15 to 68 yr) for Malays. Written informed consents for DNA analysis were obtained from all participants and the study was approved by the local ethics committee.

2.2. Genomic DNA extraction

Genomic DNA was isolated from buccal cells. Briefly, each participant vigorously swished 10 ml of sterile saline solution for 30 s after rinsing his/her mouth with water. One milliliter of the solution was centrifuged at room temperature (13,000 rpm, 2 min). The buccal cell pellet was re-suspended in 50 μ l of saline solution and then transferred into 100 μ l of 10% (w/v) Chelex (Bio-Rad, Hercules, CA, USA) solution (1 g of Chelex 100 in 10 ml of 50 mM Tris; pH 11). The Chelex solution with buccal cells was heated to 99°C for 10 min before it was centrifuged at room temperature (13,000 rpm, 2 min). After centrifuge, 100 μ l of the supernatant, containing the genomic DNA from buccal cells, was collected and stored at -20°C for later use.

2.3. Genotyping

The *CBS* 844ins68 polymorphism was genotyped by PCR, while the *MS* A2756G SNP and the *MTHFR* C677T SNP were genotyped by PCR-RFLP analysis. For all the three polymorphisms, PCR was carried out in a volume of 25 μ l containing 3 μ l of genomic DNA solution, 200 μ M of each dNTP, 1 \times ThermoPol Reaction Buffer (10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 20 mM Tris-HCl, 2 mM MgSO_4 , 0.1% Triton X-100, pH 8.8), 0.5 U of *Taq* DNA polymerase (New England BioLabs, Ipswich, MA, USA) and 9 pmol of each primer (Research Biolabs, Singapore). Primer sequences, PCR cycle conditions, and size of products were summarized in Table 1. To detect the *MS* A2756G SNP, 5 μ l of PCR products were digested with 2 U of HaeIII restriction enzyme (New England BioLabs) in a 10 μ l reaction system at 37°C for 3 h. For the detection of the *MTHFR* C677T SNP, 8 μ l of PCR products were digested with 1 U of HinfI restriction enzyme (New England BioLabs) in a 16 μ l reaction system at 37°C for 3 h. The products were electrophoresed in 2% (w/v) agarose (Bio-Rad) gels and the bands were visualized by ethidium bromide staining.

2.4. Data analysis

Genotype and allele frequencies were determined by direct counting. Genotype frequencies of each ethnic group were tested for Hardy-Weinberg Equilibrium (HWE) using goodness-of-fit Chi-square test. HWE was assumed when $p > 0.05$. Comparisons of genotypic and allelic distributions were done by Chi-square test using SPSS 11.0 for windows (SPSS Inc., Chicago, IL, USA). The differences was considered significant if $p \leq 0.05$.

Table 1
Primer sequences, PCR cycle conditions and sizes of products for the genotyping of polymorphisms

Gene	Primer sequence	PCR cycle condition			Restriction enzyme	Size of products	
<i>CBS</i>	5'-GTTGTTAACGGCGGTATTGG-3'	94°C 30s			—	N allele ^a 240 bp	
	5'-GTTGTCTGCTCCGTCTGGTT-3'	58°C 40s 72°C 60s 35 cycles				I allele ^b 172 bp	
<i>MS</i>	5'-GGTGTGTTCCAGCTGTTAGATG-3'	94°C 10 min	94°C 30s	72°C	HaeIII	A allele 265 bp	
	5'-GACACTGAAGACCTCTGATTGAAC-3'		56°C 40s 72°C 30s 38 cycles	10 min		G allele 180 bp, 85 bp	
<i>MTHFR</i>	5'-TGAAGGAGAAGGTGTCTGCGGGA-3'	94°C 30s			HinfI	C allele 198 bp	
	5'-AGGACGGTGCGGTGAGAGTG-3'	60°C 40s 72°C 40s 35 cycles				T allele 175 bp, 23 bp	

^aNon-insertion allele; ^b Insertion allele.

Table 2
Genotype frequencies, allele frequencies, and test for HWE

Ethnic group	Genotype frequency (n/%)			Allele frequency (n/%)		Test for HWE	
<i>CBS</i>	N/N	I/N	I/I	I	N		
	Chinese	271/99.3	2/0.7 ^a	0	2/0.4 ^b	544/99.6	$p = 0.995$
	Indians	122/96.1	5/3.9	0	5/2.0	249/98.0	$p = 0.980$
Malays	151/96.8	5/3.2	0	5/1.6	307/98.4	$p = 0.985$	
<i>MS</i>	A/A	A/G	G/G	A	G		
	Chinese	214/78.4	58/21.2	1/0.4 ^c	486/89.0	60/11.0 ^d	$p = 0.365$
	Indians	73/57.5	41/32.3	13/10.2 ^e	187/73.6	67/26.4 ^f	$p = 0.167$
Malays	106/67.9	44/28.2	6/3.8	256/82.1	56/17.9	$p = 0.867$	
<i>MTHFR</i>	C/C	C/T	T/T	C	T		
	Chinese	140/51.3	116/42.5	17/6.2 ^g	396/72.5	150/27.5 ^h	$p = 0.550$
	Indians	96/75.6	31/24.4	0	223/87.8	31/12.2	$p = 0.377$
Malays	125/80.1	29/18.6	2/1.3	279/89.4	33/10.6	$p = 0.970$	

^aThe frequency of the *CBS* I/N genotype is significantly lower in Chinese than in Indians ($p = 0.023$).

^bThe frequency of the *CBS* I allele is significantly lower in Chinese than in Indians ($p = 0.024$).

^cThe frequency of the *MS* G/G genotype is significantly lower in Chinese than in Malays ($p = 0.006$).

^dThe frequency of the *MS* G allele is significantly lower in Chinese than in Malays ($p = 0.004$).

^eThe frequency of the *MS* G/G genotype is significantly higher in Indians than in Chinese ($p = 0.000$) and Malays ($p = 0.033$).

^fThe frequency of the *MS* G allele is significantly higher in Indians than in Chinese ($p = 0.000$) and Malays ($p = 0.016$).

^gThe frequency of the *MTHFR* T/T genotype is significantly higher in Chinese than in Indians ($p = 0.004$) and Malays ($p = 0.017$).

^hThe frequency of the *MTHFR* T allele is significantly higher in Chinese than in Indians and Malays (both $p = 0.000$).

3. Results

Genotype patterns of the three polymorphisms displayed by agarose gel electrophoresis were shown in Fig. 1. All the genotype frequencies were conforming to HWE (Table 2). As expected, there was no homozygous state of the insertion allele (I/I genotype) for the *CBS* 844ins68 polymorphism in the 556 individuals tested (Table 2). However, 0.7% of Chinese, 3.9% of Indians, and 3.2% of Malays were carriers of the I al-

lele, resulting in I allele frequencies of 0.4% in Chinese, 2.0% in Indians, and 1.6% in Malays. Accordingly, the *CBS* 844ins68 is not a polymorphism to our Chinese samples since the frequency of the minor allele (I allele) does not reach 1% in this group. In addition, both of the frequencies of insertion/non-insertion (I/N) genotype and I allele were significantly lower in Chinese than in Indians ($p = 0.023$ and 0.024 , respectively).

The *MS* 2756G and *MTHFR* 677T alleles were common in all the three ethnic groups (Table 2). The fre-

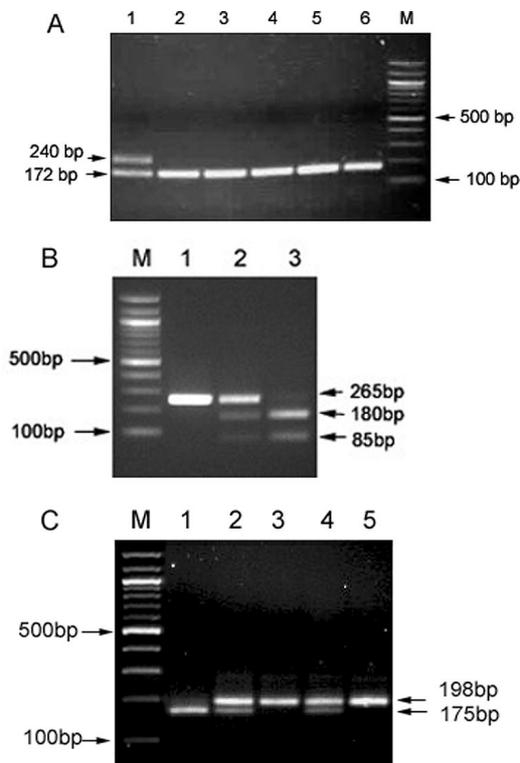


Fig. 1. Genotype patterns of the three polymorphisms displayed by agarose gel electrophoresis. M, 100 bp DNA ladder. (A) Genotype patterns of the *CBS* 844ins68 polymorphism. Lane 1, I/N genotype; lanes 2 to 6, N/N genotype. (B) Genotype patterns of the *MS* A2756G SNP. Lane 1, A/A genotype; lane 2, A/G genotype; and lane 3, G/G genotype. (C) Genotype patterns of the *MTHFR* C677T SNP. Lane 1, T/T genotype; lanes 2 & 4, C/T genotypes; and lanes 3 & 5, C/C genotypes.

quency of the *MS* 2756G allele was significantly different between each pair of the three ethnic groups, being lowest in Chinese (11.0%), moderate in Malays (17.9%), and highest in Indians (26.4%). The frequency of the *MS* G/G genotype was also significantly different between each pair of the three ethnic groups, which was lowest in Chinese (0.4%), moderate in Malays (3.8%), and highest in Indians (10.2%).

Regarding the *MTHFR* C677T SNP, the frequency of the 677T allele was significantly higher in Chinese than in Indians and Malays (both $p = 0.000$), as well as the frequency of the T/T genotype, which was also significantly higher in Chinese than in Indians ($p = 0.004$) and Malays ($p = 0.017$). Unexpectedly, there was no T/T genotype in Indians. In addition, gender bias in genotype and allele frequencies were also found in Chinese and Malays (Table 3). Specifically, the X/T genotype (C/T+T/T genotypes) was significantly less frequent in males of Chinese and Malays than in their

female counterparts ($p = 0.019$ and 0.049 , respectively). The frequency of the T allele was also significantly lower in males than in females of Chinese ($p = 0.015$), but the difference was not significant in the Malay group.

Since the possibly protective I allele of the *CBS* 844ins68 polymorphism was much less frequent than the N allele in Singaporeans, the *MS* A/G or G/G genotype and the *MTHFR* T/T genotype were considered as double genetic risk factors for elevated Hcy level in this study. Four individuals (0.7%) were carriers of such double risk factors (*MS* A/G + *MTHFR* T/T). Among them, 3 were Chinese (1.1%) and 1 was Malay individual (0.6%). No Indian individuals were carriers of the double risk factors since there was no *MTHFR* T/T genotype in the Indian group of this study.

4. Discussion

The *CBS* 844ins68 polymorphism was firstly reported as a novel mutation in an Italian patient with classic homocystinuria due to *CBS* deficiency [17]. The patient is heterozygous (I/N) for the mutation. Because the insertion (I allele) introduces a premature termination codon in the *CBS* mRNA, it is assumed that the truncated *CBS* protein is nonfunctional. However, a subsequent report [18] showed that individuals carrying the I allele of the mutation have normal-size mRNA. Later, large quantities of studies showed that the 844ins68 was commonly distributed among humans as a polymorphism. In this study, the I allele for the *CBS* 844ins68 polymorphism was only present as the I/N genotype in the 556 individuals. This is expected because the I/I genotype was only found in some Caucasians with varied low frequencies ranging between 0.6 and 2.8% [19–22]. The frequency of the I allele determined here for Singaporean Chinese, 0.4%, was the same as the frequency in Koreans [23], but was much lower than the 2.5% frequency in Southern Chinese [24], although our sample size (273 individuals) was more than twice larger than the Southern Chinese (100 individuals). Such finding indicated an intra-ethnic variation in terms of allelic distribution of the *CBS* 844ins68 polymorphism, which was also found in Indians and Italians. The I allele frequency determined in Singaporean Indians of this study ($n = 127$) was 2.0%, which was only half of the frequency determined in a group of Indians ($n = 138$) from different parts of India [25]. Also, the I allele frequency is 11.0% in Northern Italians and is only 4.3% in South-

Table 3
Distributions of genotypes and alleles of the *MTHFR* C677T SNP between genders

Ethnic group	Gender	Genotype frequency (n/%)			Allele frequency (n/%)	
		C/C	C/T	T/T	C	T
Chinese ^a	Male	61/61.6	35/35.4	3/3.0 ^b	157/79.3	41/20.7 ^c
	Female	76/46.6	75/46.0	12/7.4	227/69.6	99/30.4
Indian	Male	47/70.1	20/29.9	0	114/85.1	20/14.9
	Female	49/81.7	11/18.3	0	109/90.8	11/9.2
Malay	Male	69/86.25	10/12.5	1/1.25 ^d	148/92.5	12/7.5
	Female	56/73.7	19/25	1/1.3	131/86.2	21/13.8
Total	Male	177/72.0	65/26.4	4/1.6 ^e	419/85.2	73/14.8 ^f
	Female	181/60.5	105/35.1	13/4.3	467/78.1	131/21.9

^aOnly 262 Chinese participants (out of 273) provided their gender information.

^bThe frequency of the T-allele carriers (C/T+T/T) is significantly lower in males than in females ($p = 0.019$).

^cThe frequency of the T allele is significantly lower in males than in females ($p = 0.015$).

^dThe frequency of the T-allele carriers (C/T+T/T) is significantly lower in males than in females ($p = 0.049$).

^eThe frequency of the T-allele carrier (C/T+T/T) is significantly lower in males than in females ($p = 0.005$).

^fThe frequency of the T allele is significantly lower in males than in females ($p = 0.003$).

ern Italians. Moreover, even among Northern Italians, this frequency ranges between 3.5 and 14%, which is a four-fold difference. The frequencies among Southern Italians also vary a lot, which range between 2.7 and 7.8% [19]. Generally, among Caucasians, the frequencies of I allele range between 2.7% and 14% [19,20,26–28], and the highest frequency determined to date is 33.3% reported in Sub-Saharan Africans [29]. Therefore, although the I allele is associated with reduced level of plasma Hcy [2,3] and subsequently lowered risk of developing atherosclerotic and/or thrombotic diseases, similar study needs to be performed to evaluate the influence of the *CBS* 844ins68 polymorphism in particular sub-populations.

MS plays an important role in the Hcy metabolism as a key enzyme in the remethylation pathway. The *MS* gene has been cloned, sequenced and located, and several polymorphisms have also been identified [4,30–32]. The most prevalent polymorphism is the A2756G SNP that lead to a substitution of aspartic acid by glycine [4]. The *MS* 2756G allele in our Malay samples (17.9%) was similar to the frequencies in Japanese (17.3%) [33], Hispanics (18.8%), and Caucasians (19.9%) [34]. The *MS* 2756G allele frequency in our Chinese samples, 11.0%, was similar to the frequencies reported for Chinese (8.5%) [35], Thais (11.8%) [36] and Koreans (13.1%) [37]. Higher frequencies of the *MS* 2756G allele were detected in African Americans (23.8%) [34] and Northern Indians (24%) [38], which were similar to that in our Indian samples (26.4%). The *MS* 2756G allele thus appears to be commonly but also heterogeneously distributed among the world's populations.

MTHFR also plays a vital role in the remethylation pathway of the Hcy metabolism. The C677T transition in the *MTHFR* coding sequence, converting an alanine (the C allele) into a valine residue (the T allele) was first reported in 1995 [7]. The T/T genotype of the SNP shows increased thermolability and about 30% of the normal *MTHFR* enzyme activity [7]. The reduced enzyme activity led to a hypothesis that the T/T genotype might result in elevated Hcy level in humans, which was found to be true in subsequent studies, at least in some Asian populations [6,8–11]. In this study, the *MTHFR* 677T allele was less common than the 677C allele in Singaporeans, which is also the case in Koreans [37], Asian Indians living in South Africa [39] or UK [40], Japanese [41], and Caucasians [40,42]. However, in some groups of Northern Chinese [42,43], the 677T allele was more common than the 677C allele. In this study, the frequency of the 677T allele was most frequent in Chinese (27.5%), which was lower than the frequencies in other Chinese populations (30.7 to 44.6%) reported before [44–48]. In Singaporean Indians, the frequency of the 677T allele was 12.2%, being similar to the frequencies in Asian Indians living in South Africa (11%) [39] and UK (15.0%) [40], and to the frequency in Malays (10.6%) of this study.

As for the *MTHFR* C677T SNP, we even found that females were more likely to be carriers of 677T allele than their male counterparts in Chinese and Malays, because the combined frequencies of C/T and T/T genotypes were significantly higher in females of Chinese ($p = 0.019$) and Malays ($p = 0.049$). As such, the frequencies of T allele were significantly higher in females of Chinese ($p = 0.015$). While in the Malay

group, the difference was not significant. However, further analysis showed that there were no gender bias on the frequency of T/T genotype in Chinese and Malays. This result was consistent with the recent finding that genotype frequencies were similar between males and females [49], but was in contrast to a study reporting that two-thirds of newborn infants with the T/T genotype were males [50]. There is no suggested explanation for such conflicting results yet. Nonetheless, different influence of the *MTHFR* C677T SNP on males and females were found [51–53] regarding to diseases, suggesting a requirement of further studies on the SNP and, for example, sex-differentiated hormones.

The complicated distributions of the three polymorphisms stated above, although difficult to explain, could be possibly due to the differences in genetic drift or selection against a particular allele or genotype among populations. For example, mutations in these genes in some ethnic groups are so deleterious that they are lethal to the fetus and are thus not propagated or resulted in rare genotypes. Take the *MTHFR* C677T as an example. The frequency of the T/T genotype was 13.2% in Europe [54], whereas in our Indian samples, there was no T/T genotype at all. Even among other Indian populations, the frequency of the T/T genotype was lower than 3% [39,40]. It has been found that the *MTHFR* T/T genotype in women was associated with recurrent early pregnancy loss [55] and that the prevalence of the T/T genotype was increased following supplementation of pregnant women with multivitamins [56]. Compared to Europeans, Indians consumed significantly less B vitamin supplements [57]. Moreover, the majority of Indians are vegetarians due to cultural and religious reasons, and a strict vegetarian diet has been associated with an increased risk of vitamin B deficiency, especially B12 [58–60]. Therefore, it is reasonable that the frequency of *MTHFR* T/T genotype was significantly different between ethnic groups. Besides the possible genetic-environmental selection, differences in sample size or bias resulting from sample selection of the populations studied may play a role. For instance, it was found that the prevalence of the *MS* 2756G allele was significantly higher in elders than in younger individuals [61]. Therefore, average age of the participants in a study should be considered. In a word, in association studies between these three polymorphisms and diseases, many factors, i.e., genetic background, sample size, sample characteristics, and gender ratio, should be counted in with care.

Based on the three polymorphisms determined here, the risk for elevated Hcy level in Singaporeans was

about 0.7% (4 individuals carrying both *MS* A/G genotype and *MTHFR* T/T genotype out of 556 participants). When the ethnicity of the participants was considered, the result suggested that Singaporean Chinese were at higher risk for developing diseases related to higher Hcy level than Malays, because the combined frequency of the double risk factors was 1.1% in Chinese and 0.6% in Malays. Simply based on these three polymorphisms, it is difficult to compare the risk for elevated Hcy level between Indians and the other two ethnic groups. On one hand, Indians had much higher frequency of *MS* A/G + G/G genotypes that lead to higher Hcy level. On the other hand, Indians did not have *MTHFR* T/T genotype but had higher *CBS* 844ins68 I allele that related to lower Hcy concentrations [2,3]. Therefore, in order to better understand the relations of the three polymorphisms and plasma Hcy levels, further studies with blood samples from participants are required, although a previous general study found no significant differences of plasma total Hcy level among Singaporean Chinese, Indians, and Malays [62]. In this sense, it was a major limit that we can only get buccal cell samples instead of blood samples from the participants.

Among Asian populations, besides these three gene polymorphisms, human Hcy level was found to be significantly affected by many other non-genetic factors, such as gender, age, and plasma/serum levels of folate and vitamin B12 [57,63–65]. As such, to fully understand the gene polymorphisms involved in Hcy metabolism and Hcy level, all the aforementioned factors need to be considered and measured in later studies.

In summary, this report is the first to provide allele frequencies in randomly selected Singaporeans for the polymorphisms in three genes involved in the metabolism of Hcy. Although roughly, to screen the individuals that are at increased risk for elevated Hcy level (such as the genotypes of the three polymorphisms studied here) might provide an opportunity for public prevention and therapeutic intervention of diseases related to high Hcy level. This is possible because the human plasma total Hcy is inversely related with plasma and/or serum levels of folate and vitamin B12 [64–67] and sufficient intake of folate and vitamin B supplements can compensate the harmful high Hcy level [57, 63,65]. Among Singaporeans, the screening of risk markers for higher Hcy level seems more important in Indians, the majority of whom adhere to a vegetarian diet for cultural and religious reasons, because a strict vegetarian diet has been associated with an increased

risk of vitamin B12 deficiency [58–60]. Nonetheless, it should be noted that the genetic risk for high-Hcy related diseases could either be greater or lower than that measured by these three genes. Therefore, in order to be more convincing, it is undoubted that large-scale prospective population studies are in need to further elucidate the relationships between these polymorphisms, other polymorphisms, and the susceptibility to high-Hcy related diseases, such as CAD, CVD, venous thromboembolism, etc., in Singaporeans.

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