Short Communication

Association between C677T/MTHFR genotype and homocysteine concentration in a Kazakh population

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We recently suggested that due to insufficient intake of vegetables, low folate status and mild homocysteinemia might exist in the Kazakh population. To clarify the determinants of homocysteine concentrations among this population, we determined concentrations of serum folate, albumin, creatinine, vitamin B_{12}, and the *C677T/MTHFR* genotype in 110 Kazakh individuals and compared these with plasma total homocysteine. In Kazakh, after adjustment for age and sex, folate was correlated with plasma total homocysteine, whereas concentrations in those with the *TT* genotype was almost twice as high as in those with the *CC* and *CT* genotypes (19.7±1.8 mol/L vs. 10.7±0.5 mol/L, p<0.001). Our results suggest that the *C677T/MTHFR* genotype is associated with homocysteine concentrations in this population and this association might be affected by other factors, such as folate status.

Key Words: folate, homocysteine, Kazakh, 5,10-methylenetetrahydrofolate reductase

INTRODUCTION

Awareness of the health benefits of folate intake has considerably increased; research has shown that insufficient levels of this vitamin may contribute to neural tube defects such as spina bifida and Down syndrome.[1] Periconceptional daily supplementation with synthetic folic acid has been recommended in many developed countries.[2] Results of intervention studies have shown that periconceptional use of folic acid supplements alone, or multivitamins combined with folic acid, can lower the risk of neural tube defects by 40% to 80%.[3]

Also, it is well known that folate influences homocysteine (Hcy) metabolism as a cosubstrate,[4] and elevated plasma total Hcy is an independent risk factor for cardiovascular disease (CVD) and stroke.[5] Data from several, but not all, prospective studies show a reduced risk of CVD and stroke associated with high intakes or blood concentration of folate.[5]

In addition to folate intake, plasma Hcy levels are regulated mainly by 5,10-methylenetetrahydrofolate reductase (MTHFR), which is involved in the folate-dependent remethylation of Hcy.[6,7] In particular, the 677 C to T polymorphism of the MTHFR gene (*C677T/MTHFR*) has been investigated most extensively in relation to its effects on the total Hcy (tHcy) concentration. The prevalence of *C677T/MTHFR* is relatively high in the general population. It was reported that the prevalence of this allele is 0.34 (0.29-0.39) in whites, 0.42 (0.34-0.50) in the Japanese, and 0.08 (0.06-0.12) in Africans,[8] but there is no report on its frequency in the Kazakh.

Recently, we showed that folate deficiency might exist among the Kazakhs, probably due to their traditional diet.[9,10] We screened serum folate and plasma Hcy in the general population of Kazakh adults, and fifty of 61 (82.0%) people tested, showed low concentrations of folate. In order to reduce the risk of neural tube defects and future cardiovascular disease due to atherosclerosis, supplementation of folic acid is definitely needed in Kazakhstan. However, for the appropriate implementation of this health policy, identification of Hcy determinants in the Kazakh population is needed.

In this study, we screened biochemical and genetic markers linked to folate and Hcy metabolism in order to identify the determinants of Hcy concentration among the Kazakhs. The results obtained should be useful for future folic acid supplementation in a population with a low folate status.

MATERIALS AND METHODS

Prior to this study, ethical approval was obtained from the special committee of Semipalatinsk State Medical Academy.We collected blood samples in Semipalatinsk, Republic of Kazakhstan. Before the study, participants with

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an apparent past or present history of atherosclerotic diseases (including cerebral infarction or hemorrhage or ischemic heart disease), current pregnancy, psoriasis, seizures, or use of methotrexate or phenytoin were excluded. After these exclusions, 110 healthy Kazakh adults (61 men and 49 women, age 20-77 years) were included in this study. The average age was 37.9±16.1 years. For comparison, we also analyzed serum folate, plasma tHcy levels, and *C677T/MTHFR* genotype with the same protocol in 110 sex- and age-matched volunteers from the Japanese general population (61 men and 49 women, age 20-76 years), who were recruited during the "National Insurance Health-Up Project" performed at Nagasaki Prefecture, Japan. Before the study, written informed consent was obtained from all participants.

Fasting blood samples were obtained, and after the separation from whole blood, serum and plasma were kept at -20°C and -80°C until assay, respectively. Serum folate and vitamin B_{12} (VB_{12}) were measured using chemiluminescent immunoassay radioimmunoassay methods. Serum creatinine and albumin were measured by enzyme and BCG methods, respectively. Plasma tHcy was measured using high performance liquid chromatography. The normal ranges of serum folate, VB_{12}, creatinine, and albumin were 3.6-12.9 g/L, 233-914n g/L, 6.5-10.9 mg/L, and 3.7-5.5 g/dL, respectively. The normal range of plasma tHcy was 6.3-8.9mol/L in men and 5.1-11.7 mol/L in women.

Genomic DNA was automatically extracted from blood cells separated from plasma using a MagExtractor MFX® (TOYOBO, Osaka, Japan). For the determination of *C677T/MTHFR* genotypes, we used the TaqMan polymerase chain reaction (PCR) method (Applied Biosystems Japan, Tokyo, Japan). In the current investigation, we prepared 2 probes: the C allele-specific probe, 5' – Tet-TCT GCC GGA GcC GAT TTC ATC ATC – Tamra-3', and the T allele-specific probe, 5' – Fam-TCT GCG GGA GtC GAT TTC ATC ATC - Tamra-3'. The primer design for PCR of the flanking region of *C677T/MTHFR* was as follows: forward, 5' – CTG GGA AGA ACT CAG CGA AC – 3'; reverse, 5' – GGA AGG TGC AAG ATC AGA GC – 3'. PCR was carried out with a thermal cycler (Bio-Rad Laboratories, Hercules, USA). PCR was performed according to the following conditions: initial denaturation at 95ºC for 10 minutes, followed by 35 cycles of 95ºC for 15 seconds and 60ºC for 60 seconds. The fluorescence level of PCR products was measured with an ABI PRISM 7900 Sequence Detector (Applied Biosystems Japan, Tokyo, Japan), resulting in clear identification of the three *C677T/MTHFR* genotypes (*CC, CT, and TT*).

Since tHcy levels were skewedly distributed, logarithmic transformation was performed for the following statistical analysis Multiple linear regression analysis was performed for the identification of determinants of tHcy levels, adjusted for age and sex. Average values ± S.D. for men (n=49) and women (n=61), and multiple linear regression analysis for the association with plasma tHcy levels after adjustment for age and sex were determined. Furthermore, the tHcy level of each *C677T/MTHFR* genotype adjusted by age and sex was evaluated with the use of the ANCOVA test.

A probability value of less than 0.05 was considered to indicate significance. All statistical analyses were performed with SPSS 14.0® (SPSS Japan Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

Serum folate concentrations of the Kazakh participants ranged from 0.7 to 13.5 g/L and 72 of 110 (65.4 %), which indicated low folate concentrations (<3.6 g/L). Plasma tHcy levels of the Kazakh participants ranged from 5.5 to 41.1 mol/L. Multiple regression analysis adjusted by age and sex showed that serum creatinine and albumin did not correlate with plasma tHcy concentration. Serum VB_{12} was relatively correlated with tHcy (β=0, *p*=0.076, Table 2) and serum folate was significantly cor-

Table 1. Clinical characteristics of the Kazakh and Japanese study participants.

	Kazakh	Japanese	*p*-value†
Age (yrs)	37.9±16.1	38.1±14.9	0.92
tHcy‡ (μmol/L)	11.4±6.0	11.7±3.4	0.09
Creatinine (mg/L)	7.3±1.2	7.0±1.7	0.01
Vitamine B12 (ng/L)	337.8±110.9	310.9±90.2	0.24
Folate (μg/L)	3.6±2.1	10.4±5.3	<0.001
Albumin (g/dL)	4.8±0.3	4.6±0.3	0.67

† Data are expressed as mean ± S.D. Differences between the Kazakh and Japanese were evaluated by Mann-Whitney's U test.
‡tHcy: total homocysteine

Table 2. Multiple linear regression analysis for the association with plasma log (tHcy) levels after adjustment for age and sex.

	Kazakh			Japanese		
	β†	95%CI‡	*p*-value	β	95%CI	*p*-value
Creatinine (mg/L)	0.41	-0.14,0.96	0.14	0.26	0.18,0.35	0.01
Vitamin B12 (ng/L)	0	-0.001,0	0.076	0	-0.002,0.001	0.089
Folate (μg/L)	-0.26	-0.034,-0.006	0.007	0.01	0,0.003	0.060
Albumin (g/dL)	0.091	-0.077,0.26	0.28	0.099	-0.080,0.28	0.30

†The regression coefficient (β) is the average amount that the dependent variable increases when the independent variable increases by one unit and other variables are held constant.
‡95% confidence interval (CI) represents the plus/minus range around the observed sample regression coefficients. If the coefficient interval includes 0, there is no significant

Table 3. Plasma tHCY concentrations by *C677T/MTHFR* genotype in the Kazakh and Japanese

	MTHFR Genotype		
	TT	*CC&CT*	*p*-value
Not adjusted			
Kazakh			
log not transformed	19.5±1.8†	9.7±0.5	<0.001
log transformed	1.2±0.05	1.0±0.02	<0.001
Japanese			
log not transformed	12.6±0.6	11.6±0.2	0.13
log transformed	1.1±0.02	1.1±0.01	0.11
Age and sex adjusted			
Kazakh			
log not transformed	19.7±1.8	9.7±0.5	<0.001
log transformed	1.2±0.05	1.0±0.015	<0.001
Japanese			
log not transformed	12.7±0.6	11.6±0.2	0.09
log transformed	1.1±0.02	1.1±0.01	0.08

†Values are expressed as mean ± standard deviation

related with tHcy (β=-0.26, p<0.01).

The frequencies of *C677T/MTHFR* genotypes were 41.8% for *CC*, 44.5% for *CT,* and 13.7% for *TT* in the Kazakh. With regard to the Japanese, the frequencies were 36.4% for *CC*, 48.1% for *CT,* and 15.5% for *TT*. Plasma tHcy levels in participants with the *TT* genotype were significantly higher than those in participants with *CC* and *CT* genotypes (19.5±1.8 mol/L vs. 9.7±0.5 mol/L; p<0.001; Table 3). When adjusted by age and sex, tHcy levels in the *TT* genotype were almost twice as high as in subjects with *CC* and *CT* genotypes in the Kazakh (19.7±1.8 mol/L vs. 9.7±0.5 mol/L; p<0.001). On the other hand, tHcy levels in the *TT* genotype were relatively, but not significantly elevated in comparison with *CC* and *CT* genotypes in Japanese (12.7±0.6 mol/L vs. 11.6±0.2 mol/L; p=0.09).

In this study, we showed that serum folate concentration was independently correlated with tHcy (p=0.007) and that the *TT* genotype of *C677T/MTHFR* had tHcy levels almost twice as high as the *CC* and *CT* genotypes in the Kazakh. In the Republic of Kazakhstan, which is located in central Asia, because the cultural roots of the Kazakh people (60% of the population) are nomadic, the traditional diet mainly consists of meat, such as mutton and beef, and vegetable intake tends to be deficient, particularly in rural areas, due to insufficient distribution. In our current study, 65.4% of the Kazakh participants showed low folate concentrations. This suggests that effective supplementation of folic acid is needed in this area.

Several studies showed that higher dietary folate intake is associated with lower tHcy levels in adults, independent of other dietary factors.[11,12] However, these studies have mainly been conducted in developed areas such as Western Europe and the USA, and there have been few reports on populations with low folate intake. In this study, we showed that serum folate was significantly correlated with tHcy in the Kazakh, which suggest that appropriate folate supplementation will be effective to control the Hcy status of the population in this area.

Furthermore, our current study shows that tHcy levels in participants with the *TT* genotype of *C677T/MTHFR* were almost twice as high as those of participants with the *CC* and *CT* genotypes in the Kazakh. On the other hand, tHcy levels in the *TT* genotype were not significantly elevated in subjects with *CC* and *CT* genotypes in the Japanese. Recently, Casas *et al.* reviewed the association between *C677T/MTHFR* genotype and Hcy concentrations, and reported that the weighted mean difference in homocysteine concentration between *TT* and *CC* homozygous was 1.93 mol/L in normal subjects.[13] However, differences ranged widely from -1.30 mol/L to 11.30 mol/L and only two of forty-one studies have shown an over 10.0 mol/L differences between *TT* and *CC* homozygous individuals. In the Kazakh, tHcy concentrations adjusted for age, sex in *TT* and *CC* homozygous individuals were 19.6±1.8 mol/L and 10.7±0.5 mol/L, respectively, which is a difference of 10.0 mol/L. On the other hand, in the Japanese, tHcy concentrations adjusted for age, sex in *TT* and *CC* homozygous individuals were 12.7±0.6 mol/L and 11.5±0.2 mol/L, respectively, which is a difference of only 1.2 mol/L. Other than different assay conditions, contributing factors such as races, daily lifestyle, including the diet should be considered in order to evaluate the association between Hcy and the *C677T/MTHFR* genotype.

There are several limitations to our study. Since the number of *TT* genotype of Kazakh and Japanese were only 15 and 17, respectively, further sample collection will be needed. Also we need to evaluate other polymorphisms in *MTHFR*, such as A1298C.

In conclusion, our results suggest that appropriate supplementation of folic acid would be effective to improve Hcy, as well as folate conditions in Kazakh. Furthermore, *C677T/MTHFR* is strongly related to tHcy concentration with low folate status. Further studies are needed to outline the procedures for effective supplementation of folic acid in Kazakh individuals.

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AUTHOR DISCLOSURES

Ainur Akilzhanova, Noboru Takamura, Yosuke Kusano, Ludmila Karazhanova, Shunichi Yamashita, Hiroshi Saito and Kiyoshi Aoyagi, no conflicts of interest.

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一個哈薩克族群之 C677T/MTHFR 基因型與同半胱胺酸濃度的關係

近來，我們認為由於蔬菜攝取不足，在哈薩克人中可能有低葉酸以及輕微的同半胱胺酸血症的問題存在。為釐清哈薩克族群中同半胱胺酸濃度的決定因素，我們測定 110 位哈薩克人的血清葉酸、白蛋白、肌酸酐、維生素 B12 的濃度及 *C677T/MTHFR* 基因型，並且與血漿中總同半胱胺酸濃度作比較。在哈薩克，校正了年齡及性別之後，葉酸與血漿總同半胱胺酸有關，*TT* 基因型者其濃度幾乎是 *CC* 以及 *CT* 基因型的兩倍高（19.7±1.8 mol/L vs. 10.7±0.5 mol/L, p<0.001）。我們的結果顯示，在這個族群中 *C677T/MTHFR* 基因型與同半胱胺酸濃度之間是有關聯性的，而這個關聯性可能藉由其他因子，例如葉酸狀態，而被影響。

關鍵字：葉酸、同半胱胺酸、哈薩克、5,10 –次甲基四氫葉酸還原酶