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C677T MUTATION OF THE METHYLENETETRAHYDROFOLATE REDUCTASE GENE AMONG THE KOREAN INFANTS IN SEOUL CITY

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INDEXING WORDS

methylenetetrahydrofolate reductase; C677T mutation; Korean; Japanese; Hardy-Weinberg equilibrium

SYNOPSIS

The missense mutation, C677T (Ala--->Val), in the methylenetetrahydrofolate reductase (MTHFR) gene, is related to hyperhomocysteinemia and is regarded as a risk factor for coronary artery disease and neural tube defects. The prevalence of this mutation was reported to differ among various ethnic groups, but there are few reports concerning Asian populations. We have investigated the frequencies of C677T mutation in 124 Korean infants (residents in Seoul city, Korea) and 115 Japanese adults (residents in Kobe city, Japan), and compared them with the reported data from other ethnic groups. The frequencies of the three genotypes in Koreans were as follows: C/C (wild homozygosity) 0.27, C/T (heterozygosity) 0.66, T/T (mutated homozygosity) 0.07, while those in Japanese were as follows: C/C 0.44, C/T 0.40, T/T 0.16. There was a marked difference in the genotype frequencies between the two populations (chi-square=16.67, P=0.0002), even though they are closely related in genetic background. The high C/T

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genotype frequency led to significant deviation from Hardy-Weinberg equilibrium (chi-square=17.35, P=0.00003). Deviation from Hardy-Weinberg equilibrium has not been found in any other ethnic groups. The high frequency of C/T genotype may offer Koreans a selective advantage.

INTRODUCTION

Mutations of the methylenetetrahydrofolate reductase (MTHFR) gene have been found to be a genetic risk factor for various disorders such as coronary artery disease[1,2,6,10,14] and neural tube defects.[19,23] MTHFR catalyzes 5,10-methylenetetrahydrofolate, reducing it to 5-methyltetrahydrofolate, the predominant circulatory form of folate and a carbon donor for the remethylation of homocysteine to methionine. Frosst et al[6] reported a very frequent mutation among the French-Canadian population, a C to T transition of the MTHFR gene at nucleotide 677 (C677T). As the C to T transition converts an alanine to a valine residue of the MTHFR, it decreases the enzymatic activity of MTHFR, leading to high homocysteine and low folate levels in plasma.[3,6,9,11] Recent reports revealed that frequencies of C677T were very diverse among areas and ethnic groups.[5,15]

A number of studies have been reported on frequencies of C677T in Europe and America, but there are few studies in Asia. This is the first report regarding the mutation of the MTHFR gene in the Korean population. We examined the distribution of C677T mutation of the MTHFR gene among the Korean and Japanese populations, and compared them with reported data from other ethnic groups. Moreover, we examined whether the observed genotype frequencies were similar to the expected ones based on Hardy-Weinberg equilibrium.[7]

MATERIALS AND METHODS

One hundred and twenty four non-related Korean infants (66 males and 58 females) were enrolled in this study after obtaining informed consent from their parents. One hundred and fifteen non-related Japanese adults (45 males and 70 females) were volunteered to participate in this study. Korean subjects were residents in Seoul city, the capital of Korea, and Japanese ones were residents in Kobe city in Hyogo prefecture, Japan.

Genomic DNA was extracted from fresh blood or dried blood spotted on filter paper using SepaGene Kit (Sanko Junyaku Co., Ltd., Tokyo, Japan) or RapidPrep™ Micro Genomic DNA Isolation Kits for Blood (Amersham

Pharmacia Biotech UK Ltd., Bucks, U.K.). Polymerase chain reaction (PCR) was done with a PCR thermal cycler (TP2000, Takara Biomedicals, Kyoto, Japan) in 30 μl reactions, using 200 ng of genomic DNA in 1 x Expand HF buffer (Boehringer Mannheim, Mannheim, Germany) with 1.5 mM $MgCl_2$, 250 μM dNTPs, 0.5 μM primers and 0.7 units of Expand™ High Fidelity PCR System Enzyme Mix (Boehringer Mannheim, Germany). The sequences of the primers were: 5'-TGAAGGAGGTGTCTGCGGGA-3' and 5'-AGGAC-GGTGCGGTGAGAGTG-3'.[6] The conditions for PCR included an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, extension at 72°C for 3 min, and a final extension at 72°C for 7 min. The amplified products were digested with 10 units of *Hinf* I (New England Biolabs Inc., USA) at 37°C for 4 hours. The digested products were electrophoresed in a 3% agarose gel.

The frequencies of T allele and genotypes among different populations were compared by a chi-square test. Tests for Hardy-Weinberg equilibrium were carried out by a chi-square test. A P value below 0.01 was taken as significant.

RESULTS

Figure 1 shows the results of the PCR-restriction enzyme analysis. A newly generated 175 bp fragment after *Hinf* I digestion indicates the presence of the C677T mutation. Individuals with C/C (wild homozygosity) genotype show a single band of 198 bp, those with C/T (heterozygosity) genotype show bands of 198bp and 175 bp, and those with T/T (mutated homozygosity) genotype show a single band of 175 bp.

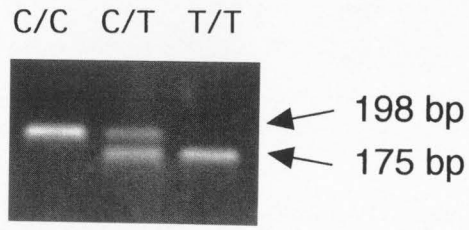

Figure 1. Agarose gel electrophoresis of *Hinf* I digested PCR products. The presence of a C to T transition at position 677 in the MTHFR gene creates a restrictive site for *Hinf* I, which digests the 198 bp fragment into 175 and 23 bp fragments; the latter fragment has been run off the gel. All three genotypes are shown.

Table I presents the genotype distribution and T allele frequencies in different ethnic groups. The T allele frequencies of C677T mutation were 0.40 in Koreans and 0.36 in Japanese. The frequencies of the three genotypes in Koreans were as follows: C/C 0.27, C/T 0.66, T/T 0.07, while those in Japanese were as follows: C/C 0.44, C/T 0.40, T/T 0.16. There was a significant difference in the genotype frequencies between the two populations (chi-square=16.67, P=0.0002).

Table I. Prevalence of the MTHFR gene C 677T mutation in different ethnic groups.

Country	Race	Genotype Frequency			T Allele Frequency	References
		C/C	C/T	T/T		
Korea	Asian	0.27	0.66	0.07	0.40	in this paper
Japan	Asian	0.44	0.40	0.16	0.36	in this paper
Italy	Caucasian	0.35	0.50	0.15	0.40	(16)
Canada	Caucasian	0.37	0.51	0.12	0.38	(6)
Australia	Caucasian	0.39	0.50	0.11	0.36	(24)
England	Caucasian	0.43	0.44	0.13	0.35	(1)
USA	Caucasian	0.44	0.43	0.13	0.35	(18)
Ireland	Caucasian	0.51	0.43	0.06	0.28	(23)
Holland	Caucasian	0.57	0.38	0.05	0.24	(10)
USA	African-American	0.79	0.21	0.00	0.11	(18)
USA	African-American	0.80	0.20	0.00	0.10	(12)
Canada	Inuit	0.89	0.10	0.01	0.061	(8)

Table II lists Hardy-Weinberg equilibrium analysis in various ethnic groups. Statistically significant deviation from the Hardy-Weinberg equilibrium towards excess heterozygotes was identified only in the Korean population (chi-square=17.35, P=0.00003). In the other populations, the genotype distribution for the MTHFR gene was in accordance with Hardy-Weinberg equilibrium.

Table II. Hardy-Weinberg equilibrium analysis of various ethnic groups.

Country	Race	Number of Subjects	Genotype (observed/expected)			P	References
			C/C	C/T	T/T		
Korea	Asian	124	33/44	82/60	9/20	0.00003	in this paper
Japan	Asian	115	51/48	46/53	18/15	0.17	in this paper
Italy	Caucasian	258	90/93	129/124	39/42	0.51	(16)
Canada	Caucasian	57	21/22	29/27	7/8	0.53	(6)
Australia	Caucasian	225	88/93	113/103	24/29	0.16	(24)
England	Caucasian	222	96/94	97/101	29/27	0.57	(1)
USA	Caucasian	151	66/64	65/68	20/18	0.53	(18)
Ireland	Caucasian	99	50/52	43/40	6/8	0.41	(23)
Holland	Caucasian	111	63/64	42/41	6/7	0.77	(10)
USA	African-American	146	115/117	31/28	0/2	0.15	(18)
USA	African-American	102	82/83	20/18	0/1	0.27	(12)
Canada	Inuit	174	155/154	17/20	2/1	0.07	(8)

DISCUSSION

The frequencies of C677T mutation are quite different among ethnic groups. As shown in Table I, the T allele frequencies ranged from 0.061 to 0.40. C/T genotype frequencies ranged from 0.10 to 0.66 and T/T genotype frequencies from 0.00 to 15.7. Among these ethnic groups, Canadian Inuit and African-American revealed quite low T allele frequencies (0.06 and 0.11, respectively). The range of T allele frequencies of Caucasians was from 0.24 to 0.40. The Japanese population showed a relatively high T allele frequency (0.36), which was consistent with other studies.[13,14]

The Korean population revealed a very unique genotype distribution. The first characteristic feature was that C/T genotype frequency of Koreans was significantly higher than those of any other ethnic group including Japanese. It is an interesting finding that Koreans and Japanese were significantly different in C677T genotype distribution, although they are closely related in genetic background.[17] Due to this high frequency of C/T genotype, genotype distribution in the Korean population deviated from

Hardy-Weinberg equilibrium (Table Ⅱ). The deviation from Hardy-Weinberg equilibrium indicated that the Korean population had genetic characteristics different from other ethnic groups, although the number of Korean subjects involved in this study was limited.

Preservation of the excess heterozygosity might have some merit. Mutated heterozygosity proved to decrease the activity of MTHFR to 66% .[3] Engbersen et al suggested that, in times of starvation, reduced MTHFR activity decreases homocysteine remethylation and preserves available one-carbon moieties of the tetrahydrofolate derivatives for the vital synthesis of purines and thymidine.[4] Therefore, the potential positive effect of mutated heterozygosity might have been related to the survival of the Korean population during the starvation period.

The second characteristic feature of the Korean genotype distribution was a low frequency of the homozygous mutation. The Japanese population showed a relatively high T/T frequency (0.16), which was consistent with other studies,[13,14] while the T/T frequency of the Korean population was 0.07.Mutated homozygosity is being reported to be associated with arteriosclerotic disorders,[1,2,10] although heterozygous mutation is not a significant genetic risk factor for these disorders. The death rate of acute myocardial infarction in Korea was 10.2 per 100,000 population, which was about 32% of that in Japan (32.1 per 100,000 population) and less than 15% of that of Caucasian (67.0 to 189.7 per 100,000 population) .[25] The low death rate of this disease in Korea may reflect the low frequency of homozygous mutation among the Korean population.

In conclusion, the genotype distribution in the Korean population was very unique and deviated from Hardy-Weinberg equilibrium. The high C/T genotype might have given Koreans a selective advantage.

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