Jpn J Human Genet **41**, 247-251, 1996

Short Communication

A COMMON MUTATION IN METHYLENETETRAHYDROFOLATE REDUCTASE GENE AMONG THE JAPANESE POPULATION

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Summary Hyperhomocysteinemia has been reported as an independent risk factor for atherosclerotic cerebrovascular and coronary heart diseases. 5,10-Methylenetetrahydrofolate reductase (MTHFR) is one of the enzymes responsible for hyperhomocysteinemia. The C to T transition of the MTHFR gene at nucleotide position 677 results in decreasing the enzymatic activity and increasing the plasma homocysteine level. We studied the distribution of the MTHFR gene mutation among the Japanese population. The subjects were 129 Japanese males (aged 40-59 years). The allele frequency of the mutation was 0.38. The frequencies of the three genotypes were as follows: +/+, 11%; +/−, 54%; −/−, 35% (+ and − indicate the presence and absence of the mutation, respectively). We also studied the frequency of the MTHFR gene mutation in the middle-aged Japanese males with hypertension to investigate the possibility that this mutation is related to essential hypertension. The normotensive and hypertensive subjects were identical in the distribution of the mutated allele and the frequencies of the three genotypes. Furthermore, the prevalence of hypertension in each genotype group was same, although the mean diastolic pressure of the group with homozygous mutation was significantly higher than that of other groups ($p<0.05$).

Received December 28, 1995; Revised version accepted March 7, 1996.
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Therefore, we concluded that there was no significant relationship between the MTHFR gene mutation and hypertensive subjects studied in this study.

Key Words hyperhomocysteinemia, 5,10-methylenetetrahydrofolate reductase, hypertension, transition mutation

Introduction

Hyperhomocysteinemia has recently been established as an independent risk factor for atherosclerotic disorders such as myocardial infarction, cerebral arterial occlusive disease and peripheral arterial occlusive disease (Boers *et al.*, 1985; Clarke *et al.*, 1991; Selhub *et al.*, 1995; Kluijtmans *et al.*, 1996). 5,10-Methylenetetrahydrofolate reductase (MTHFR) is one of the enzymes responsible for hyperhomocysteinemia. MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is a carbon donor in the methylation of homocysteine to methionine. MTHFR gene mutations result in decreasing enzymatic activity of MTHFR and hyperhomocysteinemia (Kang *et al.*, 1991a, b; Goyette *et al.*, 1995; Engbersen *et al.*, 1995). Frosst *et al.* reported a very frequent mutation among the French Canadian population, a C to T transition of the MTHFR gene at nucleotide position 677 (Frosst *et al.*, 1995). The C to T transition converts an alanine to a valine residue of the MTHFR protein. The amino acid substitution decreases the enzymatic activity of MTHFR and increases the plasma homocysteine level in the individual (Frosst *et al.*, 1995; Kluijtmans *et al.*, 1996). Kluijtmans *et al.* (1996) reported that the homozygous mutation in the MTHFR gene is associated with a threefold increase in risk for premature cardiovascular disease.

In this study, we explored the distribution of the C to T transition of the MTHFR gene at nucleotide position 677 among the Japanese population. We also studied the relationship between the MTHFR gene mutation and essential hypertension. Haffner *et al.* suggested that the cluster of atherogenic findings (atherogenic lipid and lipoprotein profile, glucose intolerance, and hyperinsulinemia) actually precede the development of the hypertensive state (Haffner *et al.*, 1992). MTHFR gene mutation as an atherogenic risk factor may contribute to development of hypertensive state. To examine this hypothesis, we studied the frequencies of genotypes in normotensive and hypertensive subjects, and the prevalence of hypertension in each genotype.

Materials and Methods

The subjects were 129 Japanese males (aged 40–59 years) randomly selected from the register of the Jichi Medical School (JMS) cohort study. The mutated allele was detected by the method of Frosst *et al.* (1995). Polymerase chain reaction (PCR) was carried out on genomic DNA extracted from the peripheral blood cells. The sequences of primers used in this study were: 5′-TGAAGGAGA-

AGGTGTCTGCGGGA-3′, and 5′-AGGACGGTGCGGTGAGAGTG-3′. The amplified products were digested with *Hin* f I (the C to T transition at nucleotide position 677 creates a new *Hin* f I site). The digested fragments were separated by electrophoresis in 4% agarose gel and visualized with ethidium bromide.

To examine the relationship between the MTHFR gene mutation and essential hypertension, we reviewed the individual data including histories, physical findings and the results of the blood chemistry tests. Body mass index was calculated as weight (kg)/height $(m)^2$. Blood pressure was measured with the subject seated after more than 5 min rest. A subject, who showed more than 140 mmHg in systolic blood pressure and/or more than 90 mmHg in diastolic blood pressure, and/or who was on antihypertensive therapy, was registered as a hypertensive subject. The numbers of normotensive and hypertensive subjects were 82 and 47, respectively. No subjects had any factors in their histories or blood chemistry findings suggesting secondary hypertension. Blood samples were collected after an overnight fast. Data are shown as the mean $\pm$ standard deviation. One-way analysis of variance and the unpaired Student's *t*-test were used for comparison of the mean values in independent groups. A p value less than 0.05 was taken as significant.

Results and Discussion

Figure 1 shows the results of PCR-restriction enzyme analysis. A newly generated fragment of 175 bp after *Hin* f I digestion reflects the presence of the mutated allele. Tables 1 and 2 summarize the frequencies of the transition mutation and the clinical findings of the subjects examined in this study.

Our data demonstrated that the mutated allele frequency was 0.38, and that the homozygous and heterozygous forms have incidences of 11% and 54% among the Japanese population (see the right column "Total" in Table 1). The distribution of the mutated allele and the three genotypes among the Japanese population

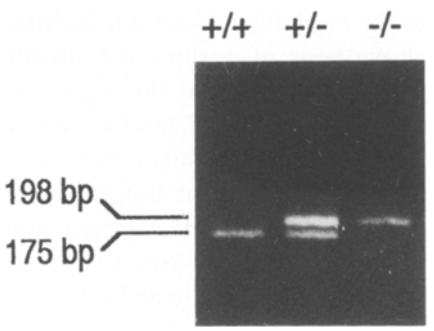

Fig. 1. *Hin* f I digestion for detection of the C to A transition at nucleotide position 677. The transition creates a *Hin* f I recognition sequence which digests the 198 bp fragment into 175 and 23 bp fragments; the latter fragment has been run off the gel. All three genotypes are shown.

Table 1. C to T transition of the MTHFR gene among the Japanese population.

Subjects	Normotensive (n=82)	Hypertensive (n=47)	Total (n=129)
Allele frequency	0.38	0.38	0.38
Frequencies of genotypes			
+/+	0.11	0.11	0.11
+/−	0.54	0.55	0.54
−/−	0.35	0.34	0.35

+ and − indicate the presence and absence of the C to T transition at nucleotide position 677, respectively.

Table 2. Relationship between the genotypes and clinical findings.

Genotypes	+/+	+/−	−/−
Number of subjects	14	70	45
Age (years)	49.2±5.2	50.2±6.4	51.0±5.9
Body mass index (kg/m^2)	23.2±2.7	23.9±3.2	23.0±2.5
Systolic pressure (mmHg)	146.6±26.2	134.1±23.3	132.7±22.2
Diastolic pressure (mmHg)	90.9±19.3*	81.0±12.6	79.0±11.1
Prevalence of hypertension	0.36	0.37	0.36
Total cholesterol (mg/dl)	187.2±36.6	196.1±36.2	189.6±29.0
HDL-cholesterol (mg/dl)	46.6±10.7	48.7±11.9	48.0±14.0
Triglycerides (mg/dl)	119.0±62.3	121.5±70.7	107.0±60.5

+ and − indicate the presence and absence of the C to T transition at nucleotide position 677, respectively. Each value represents mean±standard deviation. The unpaired Student's *t*-test were used for comparison of the mean values in independent groups. * $p<0.05$ for +/+ group versus +/− group or −/− group.

was the same as those among the French Canadian population (Frosst *et al.*, 1995). Such a high frequency of the transition suggests that the mutated MTHFR gives concomitant advantages to the population. Engbersen *et al.* conceive that, in times of starvation, a reduced MTHFR activity decreases homocysteine remethylation and that it preserves the available one-carbon moieties of the tetrahydrofolate derivatives for the vital synthesis of purines and thymidine (Engbersen *et al.*, 1995). Even so, a question remains as to why this transition is so widespread in the two different races, Japanese and French Canadian. The C to T transition of the MTHFR gene at nucleotide position 677 might be a shared compensatory mechanism to survive the starvation period of the human history.

We studied the frequency of the MTHFR gene mutation in the middle-aged Japanese males with hypertension. The normotensive and hypertensive subjects were identical in the distribution of the mutated allele and the frequencies of the three genotypes (Table 1). We also studied the prevalence of hypertension in each genotype. The prevalence of hypertension in each group was same, although the mean diastolic pressure of the group with homozygous mutation was significantly higher than that of other groups ($p<0.05$) (Table 2). Furthermore, from the results

of blood chemistry tests it appeared that there was no difference in the levels of total cholesterol, HDL-cholesterol, triglycerides among the three genotype groups. In conclusion, we found neither significant relationship between the MTHFR gene mutation and essential hypertension, nor between the MTHFR gene mutation and hyperlipidemia. However, the number of the subjects surveyed in this study was small, and the age of the subjects was limited to 40–59 years old. Large-scale case-control studies in aged people may be necessary to evaluate the contribution of the MTHFR gene mutation to essential hypertension.

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