

## The C677T Mutation in the Methylenetetrahydrofolate Reductase Gene among the Indonesian Javanese Population

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**The presence of the C677T mutation in the methylenetetrahydrofolate reductase (*MTHFR*) gene has been regarded as a genetic risk factor for coronary artery diseases and neural tube defects. Although the prevalence of this mutation has been reported from various ethnic populations, few data concerning Indonesian populations are available. We have investigated the frequency of the mutation in 68 Indonesian Javanese (residents of Java Island) and compared it with the data from 244 Japanese (residents of Honshu Island). The frequencies of the three genotypes in Javanese were C/C 0.84, C/T 0.16 and T/T 0.00, whereas those in Japanese were C/C 0.39, C/T 0.48 and T/T 0.13. The rarity of the T/T genotype in the Indonesian Javanese population may be due to malnutrition in pregnant women, because insufficient intake of folate is considered to be a survival disadvantage for fetuses with the T/T genotype. In conclusion, homozygosity for the C677T mutation in the *MTHFR* gene does not constitute a genetic risk factor for coronary artery diseases and neural tube defects in the Indonesian Javanese population.**

Methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme in folate metabolism. It catalyzes reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and a carbon donor for the remethylation of homocysteine to methionine.

The common C677T missense mutation in the *MTHFR* gene, which converts an alanine to a valine residue, decreases the enzymatic activity and leads to high homocysteine and low folate levels in plasma (3,11,14,33). This mutation has been regarded as a genetic risk factor for various disorders, such as coronary artery diseases (1,3,9,10,11,14,21) and neural tube defects (20,31,32,34), although conflicting results have been reported (33,6,12,22,37). This discrepancy could be partly explained by recent studies that have revealed that the frequency of C677T differs widely among areas and ethnic populations (7,23).

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A number of studies have reported the frequencies of C677T in European and American Caucasian populations, but there are few studies concerning Southeast Asian populations. Here, we examined the distribution of the C677T mutation of the *MTHFR* gene among the Indonesian Javanese population (resident on Java Island), and compared the results with data reported from other ethnic populations.

## SUBJECTS AND METHODS

**Subjects:** Sixty-eight Indonesian Javanese (28 males and 40 females living in Java Island) were enrolled in this study after obtaining their informed consent. Two hundred forty-four Japanese (174 males and 70 females living in Honshu Island) volunteered to participate in this study.

**PCR and enzyme digestion:** Genomic DNA was extracted from fresh blood by use of a SepaGene Kit (Sanko Junyaku Co., Ltd., Tokyo, Japan). Polymerase chain reaction (PCR) was carried out with a PC-700 thermal cyclor (Astec, Tokyo, Japan) in thirty microliters of reaction mixture containing 200 ng of genomic DNA in 1 x PCR buffer (Applied Biosystems, Foster City, CA) with 1.5 mM MgCl<sub>2</sub>, 200 μM dNTPs, 0.15 μM primers, and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). The sequences of the primers were: 5'-TGA AGG AGG TGT CTG CGG GA-3' and 5'-AGG ACG GTG CGG TGA GAG TG-3'. The conditions for PCR included an initial denaturation at 94°C for 7 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 7 min. The amplified products were digested with 10 U of *Hinf*I (New England Biolabs Inc., USA) at 37°C for 4 hours. The digested products were electrophoresed in an agarose gel containing ethidium bromide. Individuals with the C/C (wild-type homozygosity) genotype show a single band of 198 bp, those with the C/T (heterozygosity) genotype show bands of 198 bp and 175 bp, and those with the T/T (mutated homozygosity) genotype show a single band of 175 bp.

**Statistics:** The T allele frequencies and frequencies of homozygosity with 95% confidence intervals (95% CI) were calculated using the normal approximation. Exact confidence intervals were used when observed frequencies were zero. The frequencies of T allele and genotypes among different populations were compared by a chi-squared test. Tests for Hardy-Weinberg equilibrium were carried out by a chi-squared test. A *P* value below 0.05 was taken as significant.

## RESULTS

Table I presents the genotype distributions, the mutated allele (T allele) frequencies, the frequency of the mutated homozygosity (T/T) genotype, and Hardy-Weinberg equilibrium analysis in different ethnic populations. The T allele frequencies were 0.08 (95% CI = 0.035 – 0.127) in Indonesian Javanese and 0.37 (95% CI = 0.326 – 0.413) in Japanese. Figure 1 shows the 95% CI of the T allele frequency in different ethnic populations; Indonesian Javanese were among the populations with the lowest T allele frequency.

There were no individuals homozygous for the mutation (T/T genotype) in the Indonesian Javanese population. The frequencies of the three genotypes in Indonesian Javanese were as follows: C/C 0.84 (57/68), C/T 0.16 (11/68) and T/T 0.00 (0/68), whereas those in Japanese were as follows: C/C 0.39 (96/244), C/T 0.48 (116/244) and T/T 0.13 (32/244). The frequencies of T/T genotype were 0 (95% CI = 0.000 – 0.053) in Indonesian Javanese and 0.13 (95% CI = 0.089 – 0.174) in Japanese. There was a significant difference in the genotype frequencies between the two populations (chi-squared = 43.22, df = 2, *P* <

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0.00001). Figure 2 shows the 95% CI of the frequency of T/T genotype in different ethnic populations; Indonesian Javanese were among the populations with the lowest frequency of the T/T genotype.

Hardy-Weinberg equilibrium analysis showed that genotype distributions for the *MTHFR* gene in Indonesian Javanese and Japanese were in accordance with the Hardy-Weinberg equilibrium.

### DISCUSSION

The frequency of the C677T mutation differs among ethnic populations. The T allele frequency ranges from 0.06 to 0.59, and the frequency of the T/T genotype (homozygosity for the C677T mutation) ranges from 0.00 to 0.35 (Table I). Indonesian Javanese population, as well as sub-Saharan African and Canadian Inuit populations, show the lowest T allele frequency, 0.06, and there was no Indonesian Javanese individual with the T/T genotype in this study. The Japanese population showed a relatively high T allele frequency of 0.37 (95% CI = 0.326 – 0.413) and a relatively high T/T genotype frequency of 0.13 (95% CI = 0.089 – 0.174). According to a previous report (16), the Mexican population has the highest T allele frequency of 0.59 (95% CI = 0.543 – 0.629), and the highest T/T genotype frequency of 0.35 (95% CI = 0.289 – 0.407).

Table I. Prevalence of the MTHFR gene C677T mutation in different ethnic populations.

Country	Ethnic populations (References)	Number of Subjects	Genotype frequency			Allele frequency (%)		Homozygosity frequency (%)		HWE*
			C/C	C/T	T/T	Frequency	95% CI	Frequency	95% CI	
Indonesia	Javanese (this study)	68	57	11	0	8.1	3.5, 12.7	0	0.0, 5.3	0.47
Japan	Mongolian (this study)	244	96	116	32	36.9	32.6, 41.3	13.1	8.9, 17.4	0.74
Australia	Caucasian (35)	225	88	113	24	35.8	31.2, 40.1	10.7	6.6, 14.7	0.16
Brazil	Amerindian (7)	129	77	42	10	24	18.6, 29.1	7.8	3.1, 12.4	0.22
Canada	Caucasian (3,5**)	414	172	183	59	36.3	33.0, 39.6	14.3	10.9, 17.6	0.53
Canada	Inuit (8)	174	155	17	2	6	3.5, 8.5	1.1	0.0, 2.7	0.07
China	Mongolian (13)	121	51	53	17	35.9	29.9, 42	14	7.9, 20.2	0.6
England	Caucasian (1)	222	96	97	29	34.9	30.4, 39.2	13.1	8.6, 17.5	0.57
Holland	Caucasian (2)	503	224	234	45	32.2	29.3, 35.0	5.4	1.2, 9.6	0.77
Korea	Mongolian (38)	124	33	82	9	40.3	34.2, 46.4	7.3	2.7, 11.8	0.00003***
Ireland	Caucasian (2)	1,309	600	568	141	32.5	30.7, 34.2	10.8	9.1, 12.5	0.41
Italy (North)	Caucasian (29)	130	42	71	17	40.4	34.2, 46.2	13.1	7.3, 18.9	0.13
Italy (South)	Caucasian (4)	431	130	223	78	44	40.6, 47.2	18.1	14.4, 21.7	0.3
Mexico	Mexican (16)	250	44	119	87	58.6	54.3, 62.9	34.8	28.9, 40.7	0.76
South Africa	African (30)	107	85	22	0	10.3	6.2, 14.3	0	0.0, 3.4	0.24
Sub-Sahara	African (2)	301	263	38	0	6.3	4.3, 8.2	0	0.0, 1.2	0.24
USA	African-American (2)	496	363	127	6	14	11.8, 16.1	1.2	0.2, 2.2	0.16
USA	Hispanics (26)	169	63	71	35	41.7	36.3, 46.8	20.7	14.6, 26.6	0.08

\* HWE = *P* value in chi-square test for the accordance with Hardy-Weinberg Equilibrium.

\*\* Data of Canadian Caucasian is combined data from two papers.

\*\*\* Significantly different and already described elsewhere.

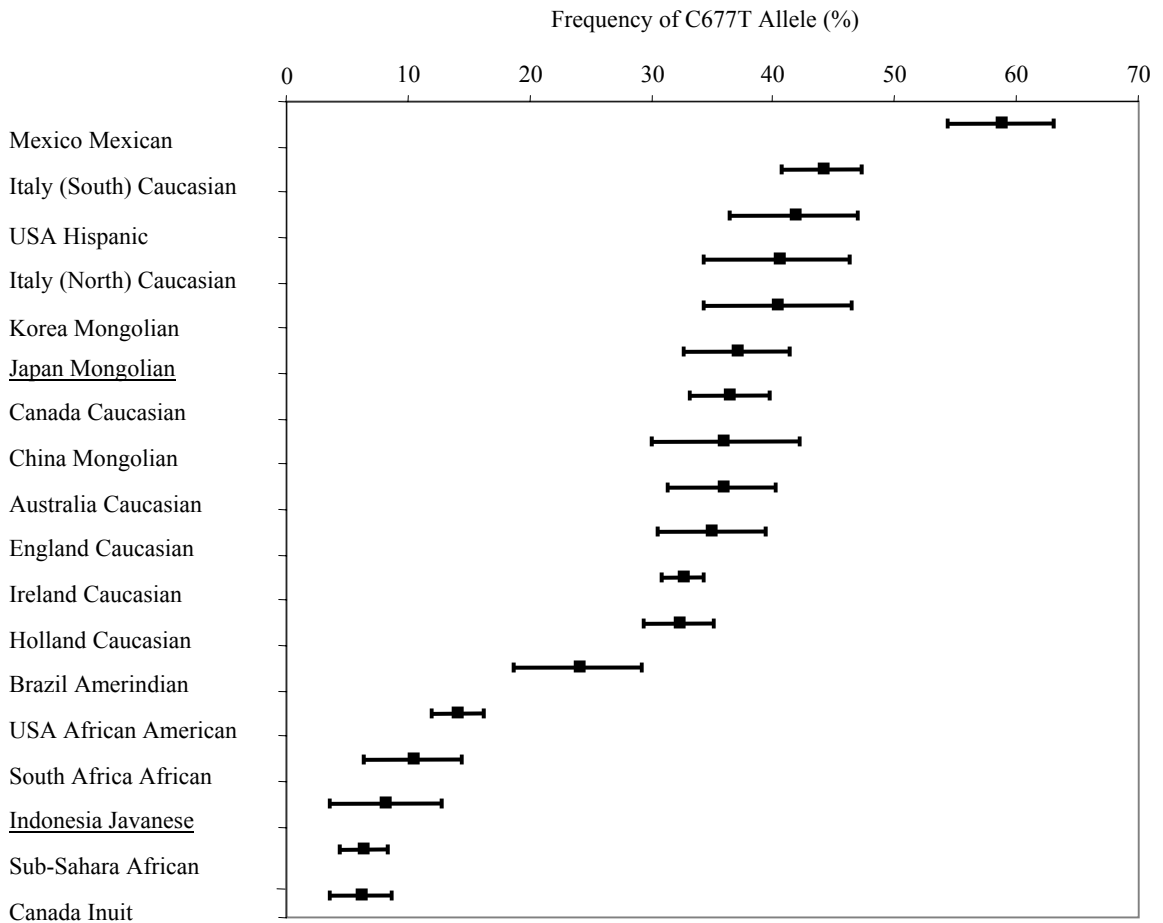


FIG. 1. Population frequency of the C677T allele of the *MTHFR* gene, by geographic area and ethnicity, 1996-2002. Data were obtained from Table I. Underlined populations are present study.

It has been reported that coronary artery diseases are increasing in the Indonesian Javanese population (27). In addition, the Indonesian Javanese population has a high incidence of a form of neural tube defect, frontoethmoidal encephalocele (28). However, since no Indonesian Javanese individuals with T/T genotype were detected in this study, we can state that homozygosity for the C677T mutation in the *MTHFR* gene is very rare, and does not constitute a genetic risk factor for coronary artery diseases and neural tube defects, in this population.

Next, the question arises as to how the Indonesian Javanese population acquired the extremely low T/T genotype frequency. Homozygosity for the C677T mutation in the *MTHFR* gene is associated with a two- to three-fold increased risk of recurrent early pregnancy loss (REPL), probably because of hyperhomocysteinemia in the absence of folate supplementation (19). Hyperhomocysteinemia is known to be a risk factor in women with unexplained REPL (36,17,24). Increased levels of maternal plasma homocysteine may cause

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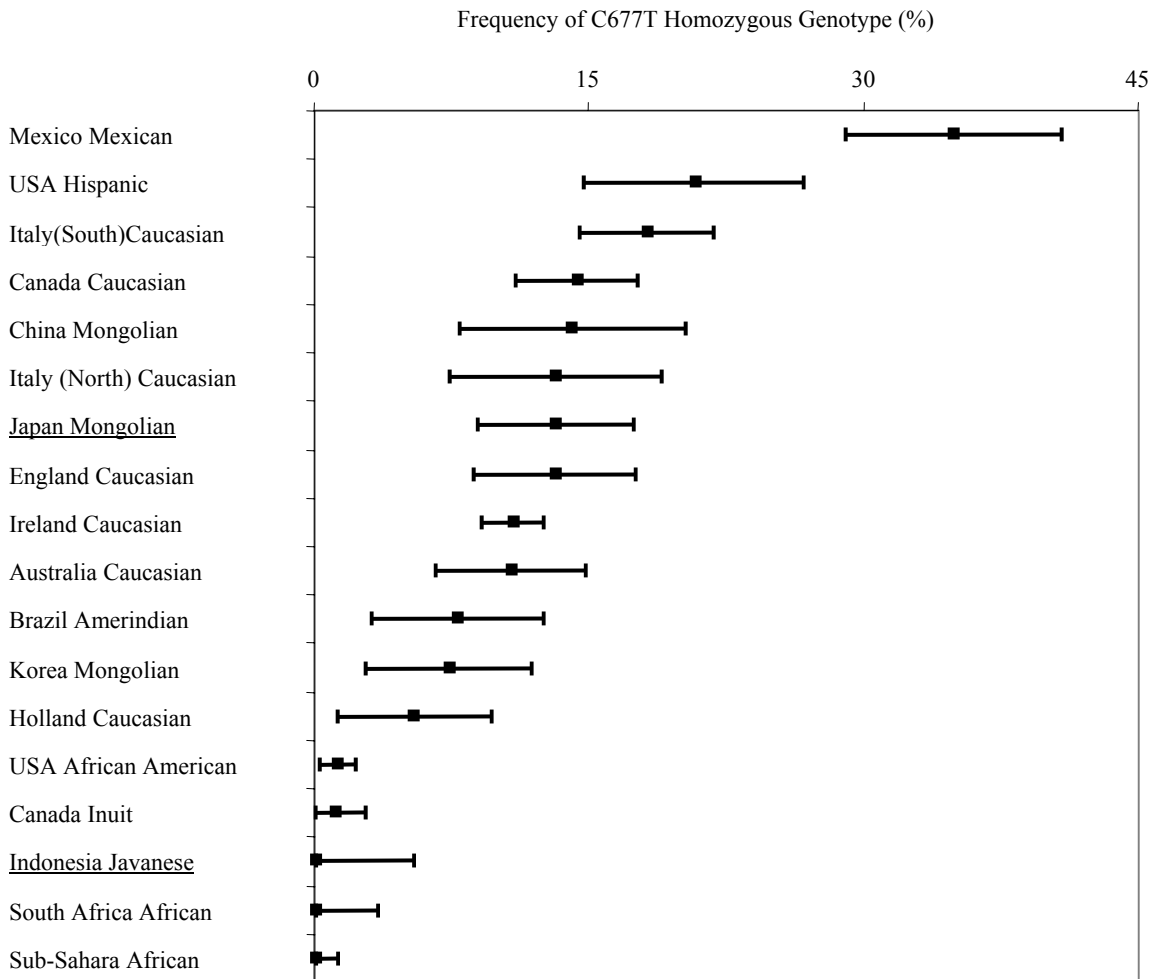


FIG. 2. Population frequency of homozygosity for the C677T allele of the *MTHFR* gene, by geographic area and ethnicity, 1996-2002. Data were obtained from Table I. Underlined populations are present study.

early damage of decidual or chorionic vessels, leading to spontaneous abortion (36,18).

Thus, insufficient intake of folate in the pregnant women is considered to be a survival disadvantage for fetuses homozygous for the T allele (25). The frequency of the T allele in the sub-Saharan African population (0.06) is lower than that in the African-American population (0.14), which may reflect REPL due to insufficient intake of folate in the sub-Saharan African population (25). The north-to-south increase in the prevalence of the T allele in Europe may be influenced by the apparent higher folic acid content in the food of the Mediterranean population compared with the northern European population (2,25).

Malnutrition among pregnant women is a common social problem in developing countries, including Indonesia. A folate supplementation program during pregnancy in such countries could protect fetuses homozygous for the T allele against REPL (24), resulting in an increase of the frequency of the T/T genotype (15).

In conclusion, we report a very low T allele frequency in the Indonesian Javanese population. Homozygosity for the C677T mutation in the *MTHFR* gene does not constitute a genetic risk factor for coronary artery diseases and neural tube defects in the Indonesian Javanese population.

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