Am. J. Hum. Genet. 70:758–762, 2002

Report

The Frequent 5,10-Methylenetetrahydrofolate Reductase C677T Polymorphism Is Associated with a Common Haplotype in Whites, Japanese, and Africans

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The common 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism causes decreased activity of this enzyme and can be associated with mild-to-moderate hyperhomocysteinemia in homozygotes, particularly when there is folic acid deficiency, as well as with vascular dementia, arterial thrombosis, venous thrombosis, neural-tube defects, and fetal loss. When folic acid intake is sufficient, homozygotes for MTHFR 677T appear to be protected against colon cancer and acute lymphatic leukemia, and fetuses bearing this genotype have an augmented survival. The distribution of MTHFR 677T is worldwide, but its frequency in different populations varies extensively. In the present study, we addressed the question of whether the MTHFR 677T alteration has an ancestral origin or has occurred repeatedly. We analyzed the frequency distribution of the previously described polymorphism A1298C in exon 7 and of three intronic dimorphisms, in white Israelis (Jews and Arabs), Japanese, and Ghanaian Africans. The 677T allele was, remarkably, associated with one haplotype, G-T-A-C, in white and Japanese homozygotes. Among the Africans, analysis of maximum likelihood also disclosed an association with the G-T-A-C haplotype, although none of the 174 subjects examined was homozygous for MTHFR 677T. These results suggest that the MTHFR 677T alteration occurred on a founder haplotype that may have had a selective advantage.

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR [MIM 236250]) catalyzes the conversion of 5,10-methylenetetrahydrofolate (methylene-THF) to 5-methyltetrahydrofolate, which is the carbon donor for methylation of homocysteine to methionine (Rosenblatt and Fenton 2001). The human gene for MTHFR has been mapped to chromosomal region 1p36.3 and consists of ~17 kb, which include 11 exons spanning 2.2 kb (cDNA GenBank accession number U09806) (Goyette et al. 1994, 1998). A common alteration in the MTHFR gene, C677T (dbSNP cluster ID rs1801133), converts an alanine to a valine at position 222 and is associated with reduced specific activity and increased thermolability of the enzyme, causing mild hyperhomocysteinemia in homozygotes (Frosst et al. 1995). Homozygosity for MTHFR 677T was shown to increase the risk of neural-tube defects and vascular dementia (van der Put et al. 1997; Yoo et al. 2000), and, according to some—but not all—studies, to confer a risk of arterial and venous thrombosis (Brattstrom et al. 1998; Gemmati et al. 1999). These deleterious effects contrast with the possible protective effects of MTHFR 677T homozygosity against colon cancer and acute lymphatic leukemia (Ma et al. 1997; Skibola et al. 1999; Wiemels et al. 2001). The protective effect is probably related to the contribution of methylene-THF to proper DNA synthesis, in that it provides a methyl group for the conversion of dUMP to dTMP, thereby preventing misincorporation of dUMP into DNA, which can cause double-strand DNA breaks (Blount et al. 1997).

The frequency of the MTHFR 677T allele varies substantially in different regions of the world and among ethnic groups. For example, the allele frequency is 0.07

Received September 27, 2001; accepted for publication November 29, 2001; electronically published January 4, 2002.

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0002-9297/2002/7003-0019$15.00

Table 1

Conditions for Identification of Three Novel Polymorphisms in the MTHFR Gene

Location	Primer		Fragment Length (bp)	Annealing Temperature (°C)	Restriction Enzyme
	Forward	Reverse			
Intron 2	TCCTCTTCCCACTGGTCACC	GGCCTGAAGAACATCATGGCG	893	61	*Pae*I
Intron 6	CTTGTCTCAATTCTCTGTCCC	TCCCGCTCCCAAGAACAAACAT[a]	222	59	*Afl*III
Intron 10	CGTAGTGGATCCCGTCAGC	CCACCGCTCAATCCACAGG	371	61	*Mnl*I

[a] The underlined letter denotes a modified base necessary for the creation of an *Afl*III recognition site.

in sub-Saharan Africans and 0.06 in Canadian Inuit, whereas in whites, Japanese, and Chinese, the allele frequencies are 0.24–0.54 (Hegele et al. 1997; Pepe et al. 1998). Interestingly, a north-to-south increase in allele frequency has also been observed in Europe (Botto and Yang 2000).

The variable distribution of the MTHFR C677T transition and the pathophysiological importance of the MTHFR gene alteration prompted us to address the question of whether MTHFR C677T is a recurrent mutation or has a common ancestral origin. We hypothesized that if C677T alteration occurred many times, it would not be associated with specific intronic polymorphisms throughout the gene. If, however, a conserved haplotype is present for the MTHFR 677T allele in separate populations, it could suggest either an ancestral origin, a selective advantage, or both. To address these questions, we analyzed novel and previously described MTHFR polymorphisms in white, African, and Japanese subjects.

Blood samples were obtained from 188 white Israelis (Jews and Arabs), 82 Japanese, and 174 Ghanaian Africans, all unselected. Genomic DNA was isolated from whole blood or was extracted from dried blood drops mounted on cards (GENERATION; Gentra). All subjects gave their informed consent, and the study was approved by the institutional review boards in Israel, Japan, and Ghana.

A search for intronic polymorphisms by means of PCR amplification and direct sequencing of introns 2, 5–7, and 10 identified three new polymorphisms: nucleotide (nt) 533G→A in intron 2, nt31C→T in intron 6 (recently introduced to the dbSNP database as rs1994798), and nt262C→G in intron 10. Conditions for detection of these polymorphisms by PCR amplification and restriction-enzyme digestion are shown in table 1. The two known MTHFR polymorphisms, 677C→T in exon 4 and 1298A→C in exon 7 (dbSNP cluster ID rs1801131) were detected as described elsewhere (Frosst et al. 1995; Weisberg et al. 1998).

The frequencies of all MTHFR polymorphisms in the whites, Japanese, and Africans are presented in table 2. Differences in the frequencies of the polymorphisms in the three populations examined were determined by a two-tailed Fisher's exact test with 95% confidence intervals (CIs) (GraphPad Software). As in previous studies (Pepe et al. 1998; Botto and Yang 2000), we found a significant difference in the frequency of the MTHFR C677T polymorphism between Africans and non-Africans. Whereas the frequency of the 677T allele in whites and Japanese was similar (0.34 and 0.42, respectively; $P = .08$), the frequency in Africans (0.08) was signifi-

Table 2

Allele Frequencies of Five Polymorphisms in the MTHFR Gene in Whites, Japanese, and Africans

Polymorphism Location	Nucleotide Alteration[a]	Distance from nt677[b] (bp)	Allele Frequency (95% CI) in[c]		
			Whites ($N = 376$)	Japanese ($N = 164$)	Africans ($N = 348$)
Intron 2	533G→A	4,305	.65* (.6–.7)	.88 (.81–.92)	.62* (.57–.66)
Exon 4	677C→T	0	.34* (.29–.39)	.42* (.34–.50)	.08 (.06–.12)
Intron 6	31C→T	1,618	.49 (.44–.54)	.75 (.68–.81)	.39 (.34–.44)
Exon 7	1298A→C	1,902	.64 (.59–.69)	.79 (.72–.85)	.91 (.88–.94)
Intron 10	262C→G	5,378	.69 (.64–.73)	.81 (.74–.87)	.91 (.87–.93)

[a] Underlined bases denote the alleles for which the frequency is given. The nucleotide numbers in exons 4 and 7 were derived from the human cDNA sequence reported by Goyette et al. (1994). The nucleotide numbers in introns indicate their distance (in bp) from the 5′ boundaries of the respective introns.

[b] Distance calculated in accordance with Entrez-Nucleotide Database (locus gi 17444393).

[c] *N* represents the number of alleles examined. Asterisks (*) indicate nonsignificant differences between the depicted alleles, whereas allele frequencies without an asterisk represent a significant ($P \leq .01$) difference from the frequency in the other populations.

Table 3

Allele Frequencies of Four MTHFR Polymorphisms Associated with the 677C and 677T Alleles in White and Japanese Individuals Homozygous for these Alleles

		Allele Frequency (95% CI) in[b]			
		Whites		Japanese	
Location	Nucleotide Polymorphism[a]	677C (N = 262)	677T (N = 404)	677C (N = 50)	677T (N = 82)
Intron 2	533 G→A	.54 (.47–.60)	.998 (.987–.999)	.79 (.65–.90)	1.00 (.95–1.00)
Intron 6	31 C→T	.32 (.26–.38)	.979 (.96–.99)	.60 (.45–.74)	.95 (.88–.99)
Exon 7	1298 A→C	.52 (.46–.58)	.998 (.987–.999)	.64 (.49–.77)	.988 (.93–.999)
Intron 10	262 C→G	.57 (.50–.63)	.997 (.987–.999)	.76 (.62–.87)	.95 (.88–.99)

[a] Underlined bases denote the alleles for which the frequency is given.

[b] N denotes the number of alleles examined. Differences in allele frequencies within each population are significant at $P < .0001$, with the exception of the intron-10 262C→G polymorphism in the Japanese population, for which $P = .0018$.

cantly lower ($P < .0001$). There was also great variability in the frequencies of the four additional MTHFR polymorphisms.

For analysis of a possible association between the 677T or 677C allele and the other polymorphisms, we also used DNA samples previously obtained from 202 white and 41 Japanese individuals homozygous for MTHFR 677T and from 131 white and 25 Japanese individuals homozygous for 677C (Seligsohn and Zivelin 1997; Sonoda et al. 2000). Table 3 demonstrates that, in whites and Japanese, there was a remarkable association between the 677T allele and 533G in intron 2, 31T in intron 6, 1298A in exon 7, and 262C in intron 10. This finding defined a G-T-A-C haplotype that is seemingly associated with the 677T allele. None of the 174 African subjects was homozygous for the 677T allele, and, therefore, Africans could not be included in this analysis. However, analysis of the data in Africans by means of the maximum-likelihood method employing an expectation-maximization algorithm (Arlequin software; Excoffier and Slatkin 1995) showed that the G-T-A-C haplotype was significantly more common in association with the 677T allele than with the 677C allele ($P < .0001$). Table 4 summarizes the haplotype distribution in the three populations, as estimated by the maximum-likelihood method, which, again, reveals a strong association between MTHFR 677T and the G-T-A-C haplotype. Another haplotype (A-C-C-G) was common in whites and Japanese but was completely absent in Africans, whereas two other haplotypes (A-C-A-C and

Table 4

Frequency Distribution of Haplotypes in MTHFR 677C and 677T Alleles

	Haplotype Frequency ± SD in[b]					
	Whites		Japanese		Africans	
Haplotype[a]	677C (N = 250)	677T (N = 126)	677C (N = 95)	677T (N = 69)	677C (N = 319)	677T (N = 29)
G-T-A-C	.255 ± .04	.984 ± .106	.66 ± .09	.97 ± .13	.30 ± .03	1.00 ± .31
A-C-C-G	.43 ± .04	0	.17 ± .06	0	0	0
A-C-A-C	.02 ± .01	0	0	0	.30 ± .03	0
G-C-A-C	.075 ± .025	.016 ± .016	.04 ± .03	.03 ± .03	.27 ± .03	0
A-C-A-G	.06 ± .02	0	0	0	0	0
G-C-C-G	.06 ± .02	0	0	0	.02 ± .01	0
G-T-C-C	.02 ± .01	0	0	0	0	0
A-C-C-C	.01 ± .01	0	.09 ± .04	0	0	0
G-C-C-C	.035 ± .016	0	.04 ± .03	0	.05 ± .016	0
A-T-A-C	0	0	0	0	.04 ± .015	0
G-C-A-G	0	0	0	0	.01 ± .007	0
G-T-A-G	.035 ± .016	0	0	0	0	0

[a] Each haplotype represents states at the four polymorphic sites: intron 2, intron 6, exon 7, and intron 10.

[b] N denotes the number of alleles examined. Haplotype frequencies were estimated using the maximum-likelihood method as implemented by the Arlequin software package. Differences in frequencies within each population are significant at $P < .0001$.

G-C-A-C) were frequent in Africans but rare in whites and Japanese. Taken together, the data suggest that the strong association between the 677T allele and the G-T-A-C haplotype stemmed from a founder effect, a strong selective advantage, or both.

Several advantages and disadvantages of 677T homozygosity have been reported. Clearly, protection against cancer could not have led to a significant evolutionary advantage, nor could vascular thrombosis or dementia constitute an evolutionary disadvantage. Two recent studies suggest that there is a survival advantage in fetuses who are homozygotes for MTHFR 677T. A fourfold-higher frequency of homozygous MTHFR 677T was observed in neonates in comparison with aborted fetuses (Isotalo et al. 2000). Another study from Spain showed that the prevalence of MTHFR 677T homozygosity was significantly higher in 0–20-year-old subjects (0.26) than in 21–40-year-old subjects (0.13) (Munoz-Moran et al. 1998). This profound difference was attributed to the use, during pregnancy, of folic acid, which became mandatory in Spain 20 years prior to the time of the study. In contrast, other studies demonstrated that 677T homozygosity in pregnant women was associated with recurrent early and late fetal loss, probably because of hyperhomocysteinemia in the absence of folic acid supplementation (Wouters et al. 1993; Nelen et al. 1997; Gris et al. 1999; Rosenblatt and Whitehead 1999). Taken together, these data suggest that there may be an overall survival advantage for fetuses homozygous for the 677T allele, when their mothers take sufficient folic acid during pregnancy.

The above considerations make it likely that adequate folic acid intake in a given population may give rise to an increase in the frequency of the MTHFR 677T allele, whereas an inadequate intake may result in decreased frequency. In this regard, the north-to-south increase in the prevalence of the MTHFR 677T allele in Europe is of interest (Botto and Yang 2000) and may be influenced by the apparent higher folic acid content in the food of Mediterranean populations compared with northern European populations. A similar gradient in the frequency of the MTHFR 677T allele has been described in the Americas. In the Inuit, the prevalence of the MTHFR 677T allele was 0.06, whereas in southern Amerindians prevalences of 0.21–0.68 have been observed (Hegele et al. 1997; Pepe et al. 1998); exceptions include the Tupi Parakana tribe of Brazil and the Cayapa population of Ecuador, in whom the prevalences were found to be 0.11 and 0.04, respectively (Botto and Yang 2000). Conceivably, the low frequency of MTHFR 677T in Africans is also related to folate deficiency. Malnutrition and infectious diseases impairing intestinal absorption of folic acid are still common in Africa (Rosenblatt and Whitehead 1999). Of note in this regard are the differences in 677T allele frequency between Africans and African Americans; a frequency of 0.06 has been observed in sub-Saharan Africans, and frequencies of 0.12–0.35 have been recorded in individuals of African descent from North and South America (Langman et al. 1998; Botto and Yang 2000). These differences may also stem from a variable intake of folic acid but could be related to population admixture.

Taken together, these data suggest that the MTHFR 677T alteration has occurred once on the G-T-A-C haplotype. We hypothesize that MTHFR homozygosity conferred a survival advantage in populations with adequate folic acid consumption, which may explain the currently observed variability in its prevalence in different populations.

Acknowledgments

We are indebted to Aviya Ifrah and Nurit Kornbrot for their skilled technical assistance.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

dbSNP Home Page, http://www.ncbi.nlm.nih.gov/SNP/ (for MTHFR polymorphisms [cluster IDs rs1801131, rs1801133, and rs1994798])

Entrez-Nucleotide Database, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide (for the MTHFR gene [locus gi 17444393])

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for MTHFR cDNA [accession number U09806])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MTHFR [MIM 236250])

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