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Methylenetetrahydrofolate Reductase Thermolabile Variant and Human Longevity

To the Editor:

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Severe hyperhomocysteinemia due to MTHFR deficiency causes neurological abnormalities, mental retardation, arteriosclerosis, and thrombosis (Rosenblatt 1989). Mild hyperhomocysteinemia is considered to be an independent risk factor for occlusive arterial disease, including myocardial infarction (Kang et al. 1991; Stamfer et al. 1992). Hyperhomocysteinemia can be induced by disturbances in homocysteine remethylation and has been associated with a thermolabile variant of MTHFR (Kang et al. 1991; Engbersen et al. 1995). A C677T mutation in the MTHFR gene, which converts an alanine residue to a valine residue, results in an enzyme with decreased activity and increased thermolability (Frosst et al. 1995). Recent results show that this MTHFR C677T mutation is associated with hyperhomocysteinemia in cardiovascular patients (Frosst et al. 1995; Kluijtmans et al. 1996) and in subjects with risk factors for coronary artery disease (Jacques et al. 1996).

MTHFR thus has appeared to be a gene candidate for cardiovascular disease, one of the major causes of death. We therefore decided to investigate the relationship between longevity and MTHFR genetic variants in a centenarian population.

A population of 458 French centenarians (89% women and 11% men; mean age 100.71 ± 0.13 years), living at home or in institutions, was recruited through media advertising and organizations. They were compared to a control group of 374 French adults (49% women and 51% men; mean age 53.1 years; age range 20–70 years). All controls and centenarians were Caucasian and were living in France. Centenarians and controls from the CEPH pedigree (Schachter et al. 1994) were recruited throughout France. The regional distribution of centenarians and controls, respectively, was as follows: 34% and 18.7% from Paris and its suburbs; 17% and 14.3% from central France; 5% and 18.7% from northern France; 19% and 14.2% from eastern France; 20% and 21.9% from western France; and 5% and 12.2% from southern France. A population of 106 nonagenarians, recruited on the basis that they had at least one nonagenarian sibling still alive, also was studied. In each family, one of the siblings was selected at random so that all 106 nonagenarians were unrelated individuals.

The subjects were screened for the MTHFR C677T mutation by use of a PCR-based assay, as reported elsewhere (Frosst et al. 1995). The mutant allele (T677), denoted by a plus sign (+), gives *Hinf*1 restriction fragments of 175 bp and 23 bp, whereas the normal allele (C677), denoted by a minus sign (−), gives a single fragment of 198 bp.

Nonfasting blood samples were collected in EDTA-containing tubes and were centrifuged promptly. Plasma was stored at −70°C. Plasma folate levels were determined by use of radio-assay kits from Ciba-Corning. Total plasma homocysteine levels were determined by ion-exchange chromatography on a Beckman 6300 amino acid analyzer (Brattstrom et al. 1988).

The distribution of the MTHFR genotypes among the population is in Hardy-Weinberg equilibrium. The frequency of the +/+ genotype was lower among the centenarians (13.3%) than among the controls (18.5%), although the difference was not statistically significant ($\chi^2 = 4.31$; $P = .12$) (table 1). We tested a population of nonagenarians with a family history of longevity and also found that the frequency of the +/+ genotype was lower among the nonagenarians (11.4%) than among the controls ($\chi^2 = 3.33$; $P = .17$). When we pooled the

Table 1

Distribution of MTHFR Genotypes and Alleles Among Controls, Centenarians, and Nonagenarians

Subjects (*n*)	Age (Years)	Frequency of Genotype [*n*] (%)			Frequency of Allele (%)	
		+/+	+/−	−/−	+	−
Controls (374)	20–70	18.5 [69]	44.1 [165]	37.4 [140]	40.5	59.5
Centenarians (458)	>100	13.3 [61]	45.4 [208]	41.3 [189]	36.0	64.0
Nonagenarians (106)	>90	11.4 [12]	50.9 [54]	37.7 [40]	36.8	63.2

two populations with the longevity trait (nonagenarians and centenarians, $n = 564$) and compared them to the controls, we observed a trend toward significance ($\chi^2 = 5.34$; $P = .06$).

Individuals with low folate status, particularly the elderly, tend to present hyperhomocysteinemia (Selhub et al. 1993). We therefore investigated the relationships between these variables in 113 centenarians and in 85 controls selected at random (table 2). Mean plasma homocysteine levels were significantly higher in the centenarians (26.7 ± 9.6 μmol/liter) than in the younger, control population (10.65 ± 6.5 mol/liter; $P < .0005$), whereas mean plasma folate was significantly lower in the centenarians (5.15 ± 2.7 μmol/liter) than in the controls (14.5 ± 4.6 μmol/liter; $P < .0005$). There was an inverse relationship between homocysteine and folate levels ($r = .30$; $P < .05$).

However, mean hyperhomocysteinemia was similar among the genotype subgroups (+/+, +/−, and −/−) of the centenarians and thus does not correlate with the C677T mutation. In contrast, in the population of controls, homocysteine levels were significantly higher in the +/+ genotype than in the +/− and −/− genotypes ($P < .025$). The −/− genotype was associated with higher folate levels in centenarians than were the +/− and +/+ genotypes ($P < .05$).

Thus our results show a trend toward a lower frequency of the MTHFR C677T +/+ genotype in both centenarians and nonagenarians than in the population of controls <70 years of age. These results could not be due to a difference between the distribution of women and men in our populations of centenarians and nonagenarians; indeed, at ages 70 years and 90 years, coronary artery mortality in women is close to that in men, at least in France (Michel et al. 1996).

The gene effect of the MTHFR +/+ genotype on the determination of the homocysteine levels was related strongly to folate levels, supporting the results of Jacques et al. (1996). Moreover, the association between the MTHFR genotype and folate levels is consistent with a direct interaction between the MTHFR phenotype and folate status.

This study shows that the centenarians have hyperhomocysteinemia associated with aging, which appears to be compatible with longevity and is associated with extremely low-folate nutritional status. It would be valuable to investigate the homocysteine metabolic pathway and its cofactors for other candidate genes and nutritional factors.

Table 2

Mean ± SD Plasma Homocysteine and Folate Levels in Centenarians and in Controls

Subjects (*n*) and Genotype	Mean ± SD Homocysteine Level[a] (μmol/liter)	Mean ± SD Folate Level[b] (nmol/liter)
Centenarians (113):		
+/+	25.1 ± 9.4	5.5 ± 2.4
+/−	27.4 ± 8.3	4.9 ± 3.2
−/−	27.1 ± 11.9	6.8 ± 2.8*
Controls (85):		
+/+	15.7 ± 8.3**	11.7 ± 6.6
+/−	10.3 ± 6.7	14.2 ± 8.1
−/−	10.2 ± 4.5	13.9 ± 5.8

[a] Normal levels are 5–14 μmol/liter.

[b] Normal levels are 5–28 nmol/liter.

* $P < .05$; significantly different from the folate levels in subjects with the +/+ or +/− genotype, by ANOVA.

** $P < .025$; significantly different from the homocysteine levels of subjects with the +/− or −/− genotype, by ANOVA.

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Reply to Stoneking: Ancient DNA—How Do You Really Know when You Have It?

To the Editor:

Stoneking's (1995) commentary on ancient DNA (in *The American Journal of Human Genetics,* December 1995) is welcome because it is important that the extensive difficulties involved in amplifying ancient human DNA are emphasized to archaeologists and other nonspecialists who may wish to utilize these techniques. However, several comments are potentially misleading.

Stoneking states that the ability to reproducibly amplify a different DNA sequence from each of several bones is an indication that the sequences are not due to contamination by a single contemporary DNA source. Strictly speaking this is true, but it conceals the potential problem of each bone being contaminated with different modern DNA sequences. This situation is quite plausible when a group of bones originates from differing archaeological sites or museums and are consequently handled (and contaminated) by different individuals. It is not commonly appreciated that contaminating DNA can deeply infiltrate ancient bone and tissue samples because of handling, washing, or preservative treatments. If this has occurred, removing the surface of an ancient sample may not totally remove modern contaminants (Richards et al. 1995; Handt et al. 1996).

The accompanying article in the *Journal* by Béraud-Colomb et al. (1995) describing the amplification of up to 380 bases of nuclear single-copy DNA from 12,000-year-old human bones is an interesting case in point. This is a potentially exciting finding because single-copy nuclear DNA sequences are thought to survive much less commonly than multicopy mitochondrial DNA. Unfortunately, there appears to be little reason to believe the results are authentic, rather than a consequence of each bone being uniquely contaminated during discovery and curation. The nuclear sequences do not permit a phylogenetic assessment of their authenticity, and when phylogenetically informative mitochondrial sequences were obtained they included one relatively uncommon haplotype (Francalacci et al. 1996) and that of the lead investigator. While the authors point out the latter is a common haplotype, the result is precisely what contamination could produce. The authors also report a single successful sexing experiment, but since this has an ~50% chance of success, and because the result again matched the lead investigator, this does not exclude contamination. Other recent studies of ancient human DNA have demonstrated the threat posed by multiple contaminants (Richards et al. 1995; Handt et al. 1996). Consequently it is important that research in this field is reviewed critically.