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Heterogeneity in World Distribution of the Thermolabile C677T Mutation in 5,10-Methylenetetrahydrofolate Reductase

To the Editor:

Hyperhomocysteinemia now is recognized as an independent risk factor for vascular disease (Clarke et al. 1991) and defects of the neural tube (Mills et al. 1995).

Table 1

World Distribution of MTHFR T677

Population	No. of Chromosomes	Mutant Allele	
		No.	Frequency ± Standard Error
European:			
Italian (Calabria)	96	43	.448 ± .051
Spanish[a]	66	36	.545 ± .061
Sub-Saharan African:			
Dendi	24	0	0
Bariba	26	2	.077 ± .052
Berba	32	3	.094 ± .051
Fon	96	8	.083 ± .028
Total[b]	178	13	.073 ± .019
Asian:			
Tharu[a]	108	21	.194 ± .038
Chinese[a]	24	9	.375 ± .099
Indonesian	98	2	.020 ± .014
Amerindian:			
Cayapa[a]	114	49	.430 ± .046
Mixed:			
Ethiopian:			
Amhara	54	4	.074 ± .033
Oromo	54	3	.055 ± .031
Total[b]	108	7	.065 ± .024
African Ecuadoran:			
Viche[a]	82	13	.183 ± .043

[a] Testable population, except for Calabrians (Italy), in Hardy-Weinberg equilibrium.

[b] Populations were pooled when compatibility was verified by a χ^2 test of heterogeneity.

Genetic factors associated with hyperhomocysteinemia are mutations in genes coding for enzymes involved in the methionine-metabolism pathway, such as 5,10-methylenetetrahydrofolate reductase (MTHFR) and cystathionine β-synthase. Related environmental factors include low dietary intake or increased necessity of folate or vitamins B_6 and B_{12} (Fenton and Rosenberg 1995). Recently, a common thermolabile MTHFR variant, causing mild hyperhomocysteinemia in 30% of homozygotes, was detected. The C677T single-nucleotide substitution was found to cause an amino acid change, from alanine to valine (Frosst et al. 1995). The T677 allele is distributed widely among populations showing a high heterogeneity. The purpose of this study was to investigate the prevalence of the C677T MTHFR mutation among the major ethnic groups. The high prevalence of T677 homozygotes in preselected populations would result in the need for a higher dietary intake of folate, to prevent in utero neural-tube defects and long-term vascular damage (Molloy et al. 1997).

We studied a total of 437 unrelated, apparently healthy subjects from the major ethnic groups, categorized as follows: 81 Europeans (33 Spanish and 48 Italians); 89 sub-Saharan Africans (48 Fon, 13 Bariba, 16 Berba, and 12 Dendi); 115 Asians (49 Indonesians [Java], 12 Chinese [Peking area], and 54 Tharus); 57 Amerindians (Cayapa population [Ecuador]); and some mixed populations, namely, 54 Ethiopians (27 Amhara and 27 Oromo) and 41 African Ecuadorans (Viche area). All the subjects gave their informed consent for the study.

For information on the origins of the populations and their geographical distribution, see the article by Pepe et al. (1997) and the references cited therein. Genomic DNA was extracted from peripheral blood–cell lymphocytes, was amplified by PCR, and was digested with *Hin*fI restriction enzyme. The bands were visualized by ethidium-bromide staining, as described elsewhere (Abbate et al. 1998). The mutation creates a new *Hin*fI site, which is absent in the wild-type allele. Pooled frequencies were obtained as mean frequencies weighted for the sample size, after the compatibility of the allele frequencies was verified by the χ^2 test of heterogeneity. Results are reported in table 1.

Our data show a marked heterogeneity in the prevalence of the T677 MTHFR allele among the major ethnic groups surveyed and, in some cases, even *within* these groups. The frequency among Europeans ranged from .448 in Italian subjects to .545 in Spanish subjects, whereas the prevalence among Asians and Africans was within the ranges .041–.375 and 0–.094, respectively. For the Amhara and the Oromo, the frequency of the T677 allele was intermediate between that for the Europeans (~.5) and the sub-Saharan Africans (<.1); this also has been confirmed in previous studies on genetic admixture in these populations (Scacchi et al. 1994; O.R.

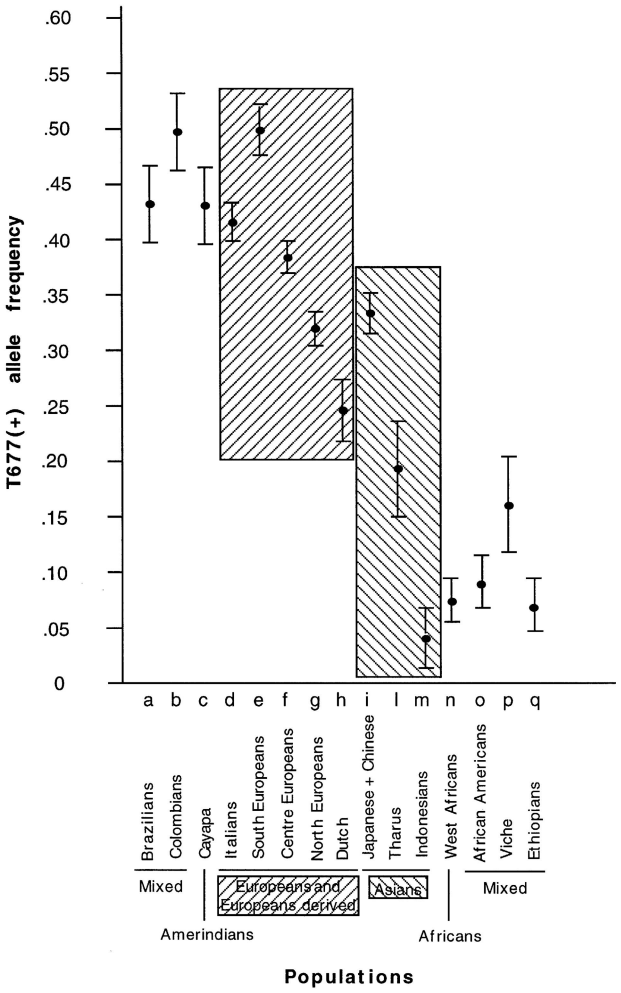

Figure 1 Allele frequencies of the T677 allele, with 1 standard error. The populations, of which all are in Hardy-Weinberg equilibrium, were pooled when possible, after a heterogeneity test. Mixed populations: a = Brazilians (Arruda et al. 1997); b = Colombians (Camacho Vanegas et al. 1998); c = Amerindians, from Cayapa population (Ecuador) (this report); o = African Americans (McAndrew et al. 1996; Austin et al. 1997); p = African Ecuadorans, from Viche area (this report); and q = Ethiopians, from Amhara and Oromo (this report). European and European-derived populations: d = Italians, from Puglia (Grandone et al. 1997) and Campania (De Franchis et al. 1996); e = southern Europeans, from Toscany (this report), Lombardia (Cattaneo et al. 1997), and Spain (this report); f = central Europeans, from France (Brulhart et al. 1997; Faure-Delanef et al. 1997), and French Canadians (Frosst et al. 1995; Deloughery et al. 1996); g = northern Europeans, from Great Britain (Narang et al. 1996; Papapetrou et al. 1996) and Ireland (Harmon et al. 1996; Kirke et al. 1996; Molloy et al. 1997), Australians (Wilcken et al. 1996; van Bockxmeer et al. 1997), and Americans (Deloughery et al. 1996; Ma et al. 1996; McAndrew et al. 1996; Austin et al. 1997); and h = Dutch (Kluijtmans et al. 1996). Asian populations: i = Japanese (Izumi et al. 1996; Morita et al. 1997) and Chinese (this report); l = Tharu (this report); and m = Indonesians (this report). Western African populations: n = Bariba, Berba, Dendi, and Fon (this report). Data for the European and European-derived and the Asian populations are within hatched boxes in order to show a possible north-south cline.

and G.P., unpublished data). Furthermore, whereas all the sub-Saharan African populations had a low frequency of T677, the Asian population displayed a remarkable heterogeneity, with a very low value for Indonesians (.041), a high value for the Chinese (.375), and a value in between for the Tharus (.194). The Spanish population displayed a T677-allele frequency that is even higher than that for the wild-type allele.

By pooling our frequency data on the T677-allele distribution among controls with those in the literature, we confirmed the marked heterogeneity of the allele (fig. 1). Therefore, this allele also is a good anthropological marker for studies of genetic admixture. Among the hybrid populations, Colombians displayed a frequency rate (.487) between that for the European and Amerindian populations (fig. 1). It is noteworthy that in Europe, as well as in Asia, the frequency of T677 seems to have an increasing north-to-south cline and also displays microheterogeneity in a single population (e.g., frequency in the Italian population is .448–.556). Because the amount of data available at present is small, this nonrandom distribution cannot be assayed statistically.

It is well known that T677 may result in the need for a higher folate intake, to obtain normal homocysteine concentrations, and that homozygosity for T677, when associated with a decreased intake of or an increased requirement for folate, becomes pathogenic for vascular disorders, especially as the individual ages (Rozen 1997), and for particular conditions such as pregnancy, in which preconceptional folate intake prevents 70% of neural-tube defects. For these reasons and because of the high heterogeneity of T677, more information needs to be collected on the prevalence of homo- and heterozygosity of the T677 mutation, by means of population-based studies (Motulsky 1996). This information would encourage specific preventive measures that could be geared toward specific ethnic groups—for example, especially in large cosmopolitan cities, public-health education stressing the importance of optimal folic and B-vitamin intake. This might have a positive effect on prevention, clinical follow-up, and health care cost-benefit ratios.

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NTBC and Alkaptonuria

To the Editor:

La Du (1998) sounds an appropriate note of caution in posing the editorial question, "Are we ready to try to cure alkaptonuria?" (i.e., with homogentisate 1,2-dioxygenase [HGO] gene-replacement therapy). He suggests that localization of recombinant HGO to certain tissues might lead to accumulation of reactive intermediates of the tyrosine catabolic pathway. We would like to point out an alternative therapy for alkaptonuria (La Du 1995; MIM 203500) that obviates the problem of gene localization.

The potential treatment consists of oral administration of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, or NTBC, in combination with some dietary restriction of phenylalanine and tyrosine. NTBC is a member of the triketone class of herbicides, which cause plants to bleach. The triketone herbicides are inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Schulz et al. 1993), the enzyme that produces homogentisic acid (HA). Hence, NTBC should prevent the production of HA, which is believed to cause the signs and symptoms of alkaptonuria. NTBC binds to 4-HPPD in a rapid and avid (inhibitory concentration $[IC]_{50}$ = 40 nM), but reversible, fashion and inhibits both rat and human liver HPPD (Lindstedt et al. 1992; Schulz et al. 1993).

NTBC is the first effective drug therapy for the fatal hereditary disease tyrosinemia type I (Mitchell et al. 1997), which results from fumarylacetoacetate hydrolase deficiency. By reducing the supply of HA, NTBC partially blocks the formation of fumarylacetoacetate and thus lowers the concentration of oxidizing metabolites, which cause severe liver disease, hepatocellular carcinoma, and renal tubular dysfunction in infants and children with tyrosinemia type I (Mitchell et al. 1997). Early studies have shown both biochemical and clinical efficacy of NTBC (Lindstedt et al. 1992), and case reports indicate improvement in peripheral neuropathy (Gibbs et al. 1993) and renal disease (Pronicka et al. 1996). A murine model of tyrosinemia type I was treated with NTBC and responded with increased longevity, improved liver function, and partially normalized expression of hepatic mRNAs (Grompe et al. 1995). The only side effect was liver steatosis.

In humans, NTBC appears to be well tolerated for short and midterm administration and has been used at least since 1992 (Lindstedt et al. 1992). Complications—namely, photophobia and the presence of corneal crystals—have been observed in only one NTBC-treated child. These findings disappeared within 48 h, when a phenylalanine and tyrosine-restricted diet was introduced, and did not recur subsequently (Mitchell et al. 1997). Theoretically, NTBC therapy could result in neurological problems associated with tyrosinemia type II (tyrosine aminotransferase deficiency) or with tyrosinemia type III (4-HPPD deficiency) (Mitchell et al. 1997). Tyrosinemia type III has been diagnosed in only two patients, both of whom were ascertained because of neurological problems. One patient had mild ataxia, and the other had seizures and cerebral atrophy. Since these patients were evaluated because of their neurological symptoms, a causal relationship between their biochemical abnormalities and neurological symptoms cannot be determined. Patients treated with NTBC have not been reported to experience neurological problems, but dietary restriction of tyrosine and phenylalanine may be important for the prevention of any neurological, ophthalmological, and dermatological side effects of high tyrosine levels.

Because NTBC side effects have been reported for only a few patients, it may be prudent to await a more-detailed analysis of the entire group of tyrosinemia type I patients who have been treated with NTBC. However,