Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations1–3

Rosa-Maria Guéant-Rodriguez, Jean-Louis Guéant, Renée Debard, Sylvie Thirion, Lu Xiao Hong, Jean-Pierre Bronowicki, Farès Namour, Nicoëdème W Chabi, Ambaliou Sanni, Guido Anello, Paolo Bosco, Corrado Romano, Emile Amouzou, Heidy R Arrieta, Beatriz E Sánchez, Antonino Romano, Bernard Herbeth, Jean-Claude Guillard, and Osvaldo M Mutchinick

ABSTRACT
Background: Methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism is heterogeneous distributed worldwide, with the highest and lowest frequencies of the T allele in Mexico and Africa, respectively, and a south-to-north gradient in Europe. Distribution of MTHFR 1298A→C is less well known. It has been hypothesized that 677T frequency could result in part from gene-nutrient interactions.

Objective: The objective was to compare the association of 677T and 1298C alleles with plasma concentrations of homocysteine, folate, and vitamin B-12 in geographical areas with contrasting 677T allele frequencies.

Design: Healthy young adults (n = 1277) were recruited in Mexico City, the West African countries of Bénin and Togo, France, and Sicily (Italy). Homocysteine, folate, and vitamin B-12 were measured in plasma, and MTHFR polymorphisms were measured in genomic DNA.

Results: Mexico City and Sicily reported the highest and Bénin and Togo the lowest plasma concentrations of folate. Mexico City had the highest 677T allele prevalence and the lowest influence of 677TT genotype on homocysteine, whereas the opposite was observed in Africa. The prevalence of the 1298C allele was lowest in the Mexicans and Africans and highest in the French. The percentage of the 677T genotype was significantly associated with the folate concentrations in 677CC carriers in a univariate analysis (R = 0.976; 95% CI: 0.797, 0.996; P < 0.0002) and in a multiple regression model that included homocysteine, vitamin B-12, and age (P = 0.0002).

Conclusion: Our data agree with the hypothesis of a gene-nutrient interaction between MTHFR 677C→T polymorphism and folate status that may confer a selective advantage of TT-homozygous genotype when dietary intake of folate is adequate, at least in the areas studied.

KEY WORDS Methylenetetrahydrofolate reductase, polymorphism, folate, homocysteine, vitamin B-12

INTRODUCTION

Homocysteine is a non-protein-forming sulfur amino acid of one carbon metabolism that results from the demethylation of S-adenosylmethionine. Plasma concentrations of total homocysteine (tHcy) are influenced by nutritional predictors that include folate and vitamin B-12 and by polymorphisms of methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298A→C (1, 2). MTHFR catalyzes the synthesis of 5-methyltetrahydrofolate, the methyl donor for the vitamin B-12–dependent remethylation of homocysteine to methionine. The 677TT genotype, which leads to a substitution of an alanine residue for a valine residue in the amino acid consensus sequence, encodes for a thermolabile variant that reduces enzyme activity by 70% and increases tHcy, at least in the case of low dietary folate (1-3). Consequently, the 677C→T polymorphism is a key genetic predictor of 2 major functions of the one-carbon metabolism, DNA synthesis and DNA methylation, whose effects are modulated by folate status (4). Double 677CT/1298AC heterozygosity was found to be associated with moderate hyperhomocysteinemia, particularly in persons with low blood folate concentrations (5).

It may be that the MTHFR 677C→T polymorphism was produced by a single ancestral mutation, because it is strongly associated with a common haplotype of the gene (6). Its worldwide distribution is very heterogeneous: the highest and the

1 From INSERM U-724, Cellular and Molecular Pathology in Nutrition, Faculté de Médecine, University Henry Poincaré of Nancy, Vandoeuvre lès Nancy, France (RMG-R, J-LG, RD, ST, LXH, JPB, and FN); the Laboratory of Biochemistry and Nutrition, Lomé, Togo (NWC and AS); the IRCCS, Oasi Maria SS–Institute for Research on Mental Retardation, Triona, Italy (GA, PB, and CR); the Laboratory of Biochemistry and Molecular Biology, University of Cotonou, Cotonou, Benin (EA); the Department of Genetics, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico (HRB, BES, and OMM); the Department of Internal Medicine and Geriatrics, Università Cattolica del Sacro Cuore, Complesso Integrato Columbus, Rome, Italy (AR); the Preventive Health Care Center and INSERM U525, Nancy, France (BH); and the Department of Physiology and Nutrition, University of Burgundy, Dijon, France (JCG).

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3 Address reprint requests and correspondence to J-L Guéant, INSERM U-724, Faculté de Médecine, Université Henri Poincaré, BP 184, 54000, Vandoeuvre lès Nancy, France. E-mail: Jean-Louis.Gueant@medecine.uhp-nancy.fr.

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lowest T allele prevalences are reported in Mexico and sub-Saharan Africa, respectively (7–12). A north-to-south gradient of allele 677TT prevalence has been observed in Western Europe, and the highest frequency occurs in Sicily (13). The mechanisms generating this gradient are not known and could involve, at least in part, gene-nutrient interactions. Folate intake is lower in the diet of Northern Europe than in that of Mediterranean countries (14–18). It was therefore hypothesized that dietary folate is one of the factors that has influenced the prevalence of T allele in Europe (1). Indeed, the T allele is associated with a greater risk of genetic birth defects, but that risk seems to be neutralized by a diet rich in folate (14). Two studies evaluated the worldwide distribution of the T allele, one in adults (11) and the other in newborns (12). However, the efforts to test the validity of the above hypothesis have not included evaluation of the respective relation of the allele with homocysteine and its 2 main nutritional predictors, folate and vitamin B-12, in geographical areas with contrasting 677TT allele frequencies. In the current study, we examined the distribution of allele frequencies of the 2 polymorphisms of MTHFR in a country with a high 677T allele frequency (Mexico), 2 countries with an intermediate frequency [Italy (Sicily) and France], and 2 countries with a low frequency (Benin and Togo), and we evaluated the association of these distributions with folate and 2 other markers of homocysteine metabolism, tHcy and vitamin B-12.

SUBJECTS AND METHODS

Recruitment of subjects

The study was carried out in a population sample of 1277 healthy persons. All subjects were examined medically before inclusion and recruited on the basis of absence of any known disease. Particular attention was given to the absence of reported cancer; of cardiovascular, renal, hepatic, or genetic disease; and of vitamin supplementation. The 300 volunteers from Mexico were blood donors at the National Institute of Medical Sciences and Nutrition in Mexico City. The 146 volunteers from Sicily were from the rural area around the village of Troina in the northeastern part of Sicily, and they attended the medical center in Troina for preventive care. The 366 apparently healthy volunteers from France were recruited either in the northeast (Lorraine) or east (Burgundy and Franche Comté). A total of 195 volunteers was recruited in Togo, including 127 volunteers from the coast (Lomé) and 68 from the interior savanna. The 270 volunteers from Benin were recruited in the coastal city of Cotonou. Part of the African population was described previously (8).

Written informed consent was obtained from subjects. Institutional review board approval was obtained from the ethics committees of the National Institute of Medical Sciences and Nutrition (Mexico City, Mexico), the medical center of Troina (IRCCS Associazione Oasi Maria SS, Troina, Sicily, Italy), the University Hospital Center of Nancy (Nancy, France), the University of Bénin (Lomé, Togo), and the University of Cotonou (Cotonou, Bénin).

Biological and genetic analyses

All specimens were tested in a single laboratory (Nancy, France). Fasting venous blood was collected in EDTA-containing tubes and, after immediate centrifugation, aliquots were stored at −70 °C until analysis. Plasma tHcy concentrations (protein-bound and free homocysteine) were determined by using the fluorescence polarization immunoassay (FPIA IMx homocysteine method; Abbott Laboratories Diagnostics Division, Abbott Park, IL). Plasma vitamin B-12 and folate concentrations were assayed by using a VB12 and Folates immunoassay kit on an ACS 180 automated chemiluminescent system (both: Bayer Health Care Diagnostics, Cergy Pontoise, France) with threshold values established at 100 pmol/L and 7 nmol/L, respectively, according to World Health Organization criteria (1). Buffy coat was prepared from the previously collected blood, and genomic DNA was isolated with the use of a kit according to the manufacturer’s recommendations (Qiagen-France, Courtaboeuf, France). A polymerase chain reaction–based RFLP method was used to determine the genotypes of MTHFR, as described previously (3, 5, 13). The 677C→T polymorphism creates a Hinf I recognition site in a 198-bp amplified DNA fragment that was used to differentiate the various genotypes: 677CC, 677CT, and 677TT. The MTHFR 1298A→C polymorphism was analyzed by using a previously described protocol (13). The enzyme Fnu4HI cuts the amplified DNA of C allele carriers. Electrophoresis with 15% polyacrylamide gel showed a single 138-bp fragment for the 1298AA homozygotes, two 119- and 19-bp fragments for the 1298CC homozygotes, and three 138-, 119-, and 19-bp fragments for the 1298AC heterozygotes.

Statistical analysis

Allele and genotype frequencies were reported as percentages and 95% CIs. A continuity-corrected chi-square test and Bonferroni adjustment were used to assess differences between groups. Continuous variables were reported as medians and 25th and 75th percentiles. A Student’s t test for unpaired data and Bonferroni adjustment were used to compare continuous variables. In the case of skewed data distributions, logarithmic transformations were carried out to normalize the distributions. Univariate regression analyses were performed by transformed correlation (Fisher z transformation) and stepwise multiple regressions with models that included age, tHcy, vitamin B-12, and folate. A P value ≤ 0.05 was considered significant. Data were analyzed by using STATVIEW for WINDOWS software (version 5.0.0.0; SAS Institute Inc, Berkeley, CA) and EPIINFO for MS-DOS software (version 6.0; World Health Organization, Geneva, Switzerland).

RESULTS

As expected, the highest and lowest frequencies of the 677T allele and the 677TT genotype were observed in Mexico and West Africa, respectively (Table 1). The T allele was also significantly more frequent in Sicily than in France (Table 1). We found no significant difference in T allele frequency [36.1% (95% CI: 28.7%, 44.2%) and 36.0% (31.2%, 40.8%); P = 0.6725] or 677TT genotype [13.9% (7.8%, 23.8%) and 14.3% (9.9%, 19.8%); P = 0.9262] between the subsets of the French population from the northeastern and eastern parts of France, respectively. West African countries and Mexico were the 2 areas with the lowest frequencies of the 1298C allele and the 1298CC genotype, and the highest frequency was reported in France (Table 1). The distributions of the 3 genotypes of MTHFR 677C→T and 1298A→C polymorphisms observed in the 7 areas
of recruitment did not differ significantly from those expected under Hardy-Weinberg equilibrium (data not showed).

The plasma concentrations of tHcy, folate, and vitamin B-12 are shown in Table 2. Mexico had the highest folate concentration, and the concentration did not differ significantly between France and Sicily. The highest concentrations of tHcy and the lowest concentrations of folate were registered in West African countries. The highest rate of folate deficiency was recorded in Sicily. The highest concentrations of tHcy and the concentration did not differ significantly between Togo and coastal Benin, but significant elevation of tHcy in France and Italy and no effect in Mexico (Figure 1). In contrast, the 677CT/1298AC double heterozygosity did not have a significantly greater effect on total homocysteine than did the 677CT/1298AA genotype [10.0 (7.9, 12.2) μmol/L and 10.0 (8.1, 14.0) μmol/L, respectively; P = 0.0952]. This finding was confirmed when the analysis was stratified by area.

We investigated whether the 677T allele frequency was associated with folate status and other predictors of homocysteine metabolism in the 7 areas (Table 4). In univariate analysis, we found a correlation between the T allele frequency and, in order of significance, the median folate, tHcy, and vitamin B-12 concentrations reported in the 7 areas. The association of the T allele with folate status and other predictors of homocysteine metabolism in the 7 areas was also associated with vitamin B-12 in Sicilians and Mexicans (Table 3). The TT genotype was associated with a dramatically higher concentration of tHcy in West Africans. It produced a mild but significant elevation of tHcy in France and Italy and no effect in Mexico (Figure 1). In contrast, the 677CT/1298AC double heterozygosity did not have a significantly greater effect on total homocysteine than did the 677CT/1298AA genotype [10.0 (7.9, 12.2) μmol/L and 10.0 (8.1, 14.0) μmol/L, respectively; P = 0.0952]. This finding was confirmed when the analysis was stratified by area.

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frequency with folate remained significant when the folate concentration of 677CC carriers was considered (Table 4). To avoid bias due to the effect of TT genotype on folate concentration, we considered the folate concentration only in 677CC homozygotes. A close inverse correlation was also found between 677T allele frequency and the percentage of subjects with low plasma folate (<7 nmol/L) (Figure 2). Folate in 677CC carriers and, to a lesser extent, fHcy were the 2 residual significant predictors of T allele frequency in a stepwise multivariate regression analysis that also included age and vitamin B-12 (Table 4). The same results were obtained when the association of folate concentration in 677CC carriers with the frequency of TT genotype was considered (Table 4). In contrast, there was no significant association between the frequency of the combined 677CT/1298AC genotype and plasma folate in 677CC carriers (P = 0.2450).

**DISCUSSION**

The current study was performed on a population sample of reproductive age, which avoided any influence of a possible association of 677C→T and 1298A→C genetic polymorphisms with a risk of decreased longevity (19). As expected, the T allele frequency was highest in Mexico City, intermediate in eastern France and Sicily, and lowest in West Africa. The frequencies of the 677TT genotype and the 677 allele observed in Mexican and Sicilian newborns in a study by Wilcken et al (11) did not differ significantly from those we observed in young adults in Mexico (P = 0.6429) and Sicily (P = 0.5619). In the current study, such frequencies in newborns also did not differ significantly from those Wilcken et al (11) found or from the frequencies in young adults in northeastern France (P = 0.9318). Assuming that a very long-term change in the nutritional environment may influence 677T and 1298C allele frequencies in migrants, we found it interesting to compare the allele frequencies in young adults with

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**TABLE 3**

<table>
<thead>
<tr>
<th>Area and independent predictor</th>
<th>Initial</th>
<th>Residual</th>
<th>β-coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Africa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.5626</td>
<td></td>
<td>−0.316</td>
</tr>
<tr>
<td>Folate</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>−0.316</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0.4698</td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.9177</td>
<td></td>
<td>−0.181</td>
</tr>
<tr>
<td>Folate</td>
<td>0.0690</td>
<td>0.0064</td>
<td>−0.181</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0.3466</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.1007</td>
<td></td>
<td>−0.444</td>
</tr>
<tr>
<td>Folate</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>−0.444</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0.0262</td>
<td>0.0118</td>
<td>−0.244</td>
</tr>
<tr>
<td>Mexico City</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.1770</td>
<td></td>
<td>−0.299</td>
</tr>
<tr>
<td>Folate</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>−0.299</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0.0161</td>
<td>0.0111</td>
<td>−0.142</td>
</tr>
</tbody>
</table>

1 Stepwise multiple regression was performed with models that included age, vitamin B-12, and folate.

**TABLE 4**

Predictors of the frequency of MTHFR 677T allele and 677TT genotype among 7 areas of West Africa, France, Italy, and Mexico in univariate and backward stepwise multiple regression analyses

<table>
<thead>
<tr>
<th>Dependent predictor</th>
<th>Univariate analysis</th>
<th>Multiple regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>β-coefficient</td>
</tr>
<tr>
<td>Frequency of 677T allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.1961</td>
<td>0.555</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>0.0161</td>
<td>−0.847</td>
</tr>
<tr>
<td>Folate</td>
<td>0.0015</td>
<td>0.943</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0.0293</td>
<td>−0.804</td>
</tr>
<tr>
<td>Frequency of 677TT genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.2453</td>
<td>0.507</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>0.0523</td>
<td>−0.750</td>
</tr>
<tr>
<td>Folate</td>
<td>0.0002</td>
<td>0.976</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0.0789</td>
<td>−0.702</td>
</tr>
</tbody>
</table>

1 The 7 areas are West Africa, coastal and savannah areas of Togo, coastal Benin, France, Sicily, and Mexico City. Regression analyses were performed by univariate and multiple regression with models that included age, total homocysteine, vitamin B-12, and folate in MTHFR 677CC carriers.
those published in newborns in corresponding migrant populations (11). We reported a 677T allele frequency in Benin and Togo that was significantly lower than that in black newborns in Atlanta, GA [9.0% (95% CI: 7.1%, 11.2%)] and 13.9% (11.2%, 17.1%), respectively; \( P = 0.0269 \) (Table 1; 8, 11). The 677T allele in Hispanic newborns in Atlanta (41.1%; 95% CI: 32.8%, 49.8%) was also significantly less frequent than that reported in the young adults in our population (\( P = 0.0006 \); Table 1) and newborns [57.0% (95% CI: 53.9%, 60.0%); \( P = 0.0008 \)] in another study in Mexico (11). These differences in allele frequency between migrant and settled populations may derive from differences in the study design or the age of the subjects studied, or from partial ethnic mixing. However, the influence of a change in gene pressure on the nutritional environment should be also considered.

Population genetic studies are one way to highlight geographical and ethnic differences that suggest evolutionary pressures generated by environmental factors. Such possible pressures on the nutritional environment remain speculative in the case of MTHFR polymorphisms. We evaluated the influence of the nutritional environment on ethnic differences in MTHFR variants by comparing populations that have different T allele frequencies and distinct traditional diets. Our results showed that the highest T allele frequency was in the area where influence of the 677TT genotype on tHcy was the lowest (Figure 1 and Table 1), whereas the lowest frequency was registered in Africa, where the genotype influence on tHcy was the most evident. For example, a high tHcy concentration was observed in the 4 patients in Africa with a 677TT genotype, and all of these patients had a low plasma concentration of folate and no other known genetic, metabolic, or nutritional characteristic that influenced tHcy.

In all 7 of the areas studied, we observed a close correlation of the T allele frequency with both the plasma concentration of folate and the percentage of persons with low plasma folate (Figure 2). Because we excluded the subjects who recurrently used vitamin supplements, the plasma folate concentration of 677CC carriers can be considered a biological marker of short-term dietary folate intake. However, a limitation of the current study was that we could not consider the long-term effect of folate status on T allele frequency. Despite limited ethnic commonalities in these areas, the highest concentration of folate and the lowest rate of folate deficiency were recorded in the 2 areas, Mexico City and Sicily, that had the highest T allele frequency. In fact, the main similarity between those 2 populations is their typical diet, which is poorer in animal proteins than the so-called Western diet but much richer in wheat, corn, beans, lentils, and fresh fruit and vegetables (17, 18, 20). Therefore, it would be particularly interesting to compare the 2 diets to estimate the dietary intake and bioavailability of folate and other nutrients with respect to homocysteine metabolism. Several estimates in reference samples of European populations showed south-to-north decreasing gradients of dietary folate intake and of T allele frequency. For example, dietary folate was estimated at 190 \( \mu \)g/d in females in Netherlands, 268.2 ± 91.5 \( \mu \)g/d in females in northeastern or eastern France, and 323 ± 87 \( \mu \)g/d in nonelderly females in central Italy (14, 15, 17). The high prevalence of folate deficiency in West Africa may be involved in the evident phenotypic effect of T allele on tHcy, which showed interaction between the environment and genetic pressure (8). Indeed, up to 26.4% of the African population in the current study had a plasma folate concentration below the 7 nmol/L threshold, whereas only 2.0% of the Mexico City population did so (Table 2). However, 9.7% of the Mexican group had a vitamin B-12 concentration of <100 pmol/L, which confirmed the high rate of vitamin B-12 deficiency reported in a recent review of vitamin B status in the Americas (Table 2; 21). In addition, vitamin B-12 was a predictor of tHcy in the 2 populations with the highest folate status, ie, Sicilians and Mexicans (Table 2), as previously reported in subjects supplemented with folate (22).

The current study showed a high frequency of MTHFR 677TT in the presence of a high concentration of folate and a low frequency in the presence of low folate concentrations, but it could not ascertain whether MTHFR is an ancestral mutation. Rosenberg et al (6) showed that the MTHFR 677TT variant has occurred on a common haplotype in Israelis, Japanese, and Ghanaians. They (6) and others (23) suggested that MTHFR TT homozygosity may confer a survival advantage in populations with adequate dietary folate consumption. Our data are in accord with this hypothesis. All of the subjects in the current study were healthy young adults, and thus the differences in T allele frequency may be related to a long-term influence of diet on birth defects rather than on pathologic conditions that occur late in life. However, the association of MTHFR mutations with the viability of embryo or fetus may be difficult to estimate, because most spontaneous abortions occur before pregnancy is diagnosed. A study found a survival advantage related to MTHFR 677TT, with a frequency in newborns 4-fold that in aborted fetuses (24). In contrast, an increased frequency of both 677T and 1298C mutated alleles has been reported in a series of spontaneously aborted embryos (25). Increased tHcy and related genetic predictors have also been implicated as risk factors for neural tube defects, Down syndrome, trisomy 18, and recurrent embryo losses (26–31), and these associations seem to be influenced by the geographical location and nutritional status of the subjects (30–32). For both neural tube defects and Down syndrome, MTHFR polymorphisms are risk factors in northern Europe, with its low-folate diet and low 677T allele frequency, but are neutral in western and
southwestern Europe, where both dietary folate intake and T allele frequency are much higher (25, 26, 30–47). The risk of birth defects may be associated with other predictors of homocysteine metabolism interacting with MTHFR and folate. For example, the presence of transcobalamin (TCN) 776G allele and gene–gene interaction between MTHFR 677T and TCN 776G allele have been found to increase the risk of spontaneous abortion in Greece (48, 49). TCN 776G→G is a gene polymorphism influencing the expression of transcobalamin, the protein responsible for cell delivery of vitamin B-12 (50). The benefit deriving from a high dietary intake of folate by 677T allele carriers may be due to various mechanisms, such as the increased synthesis of purines and pyrimidines needed for DNA replication (2, 51). In addition, folate may neutralize the adverse cellular effects of the reduced activity of the thermolabile MTHFR variant, such as the defective remethylation of homocysteine, the subsequent DNA hypomethylation, and the uracil misincorporation (52). It may also influence epigenetic mechanisms regulating gene expression (53).

In 2 previous studies (54, 55), the 1298A→C polymorphism in combination with 677C→T appeared to affect enzyme activity and tHcy. However, the current study, as well as others (56–60)—including a biochemical study of the recombinant variants of MTHFR (60)—failed to find any association of the 677CT/ 1298AC combined genotype with tHcy. Finally, we also failed to find any influence of folate on the distribution of this genotype in the 7 areas studied.

In conclusion, comparing populations that have greatly differing allele 677T frequencies and folate status, we showed an association of 677T allele frequency with both the folate plasma concentration and the percentage of folate deficiency in 7 areas on 3 continents. This observation supports the hypothesis of a nutrient interaction between MTHFR and folate status.

R-MG-R and J-LG contributed equally to the conception and design of the study, statistical analysis and data interpretation, and manuscript revision. RD, ST, LHX, NWC, EA, GA, PB, HRA, and BES were responsible for the sampling, genotyping, and biological analyses. J-PB, FN, AS, EA, CR, AR, BH, and J-CG were responsible for local coordination of the study and recruitment of subjects in all areas except Mexico. OMM was responsible for local coordination of the study and recruitment of subjects in Mexico, for data interpretation, and for manuscript revision. J-LG was responsible for the general coordination of the study. None of the authors had any personal or financial conflict of interest.

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