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High prevalence of hyperhomocysteinemia related to folate deficiency and the 677C→T mutation of the gene encoding methylenetetrahydrofolate reductase in coastal West Africa[1–3]

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ABSTRACT

Background: Moderate hyperhomocysteinemia is a risk for neural tube defect and neurodegenerative and vascular diseases and has nutritional, metabolic, and genetic determinants. Its prevalence in sub-Saharan Africa remains unknown.

Objective: Our goal was to evaluate the prevalence of hyperhomocysteinemia and the influence of nutritional, metabolic, and genetic determinants in savanna and coastal regions of Togo and Benin.

Design: Volunteers were recruited from coastal (C groups; $n = 208$) and savanna (S group; $n = 68$) regions. Vitamin B-12, folate, total homocysteine (tHcy), cystatin C (a marker of glomerular filtration), and inflammatory and nutritional protein markers were measured in plasma, and the methylenetetrahydrofolate reductase (*MTHFR*) 677C→T and 1298A→C polymorphisms and the methionine synthase 2756A→G polymorphism were examined in genomic DNA.

Results: Moderate hyperhomocysteinemia (tHcy > 15 μmol/L) was recorded in 62.3% and 29.4% of the subjects from the coast and savanna, respectively ($P < 0.0001$). A histogram distribution of tHcy in the coastal groups showed a distinct group, C2 (15% of the total group), with tHcy > 28 μmol/L. Folate < 6.75 nmol/L (lower quartile) and *MTHFR CT/TT* genotype were the 2 main risk factors for moderate hyperhomocysteinemia in the whole population [odds ratios: 5.3 (95% CI: 2.5, 11.2; $P < 0.0001$) and 4.9 (1.6, 14.8; $P = 0.0048$), respectively] and in the C2 group [odds ratios: 15.9 (4.5, 56.8; $P < 0.0001$) and 9.0 (2.3, −35.2; $P = 0.0017$), respectively]. Cystatin C was another potent risk factor in the C2 group.

Conclusion: A high prevalence of hyperhomocysteinemia in coastal West Africa, related to folate concentrations and the *MTHFR 677 T* allele, suggests the need to evaluate the influence of hyperhomocysteinemia on disease in this area. *Am J Clin Nutr* 2004;79:619–24.

KEY WORDS Homocysteine, folate, vitamin B-12, methylenetetrahydrofolate reductase, cystatin C

INTRODUCTION

Homocysteine is a circulating sulfur amino acid, concentrations of which depend on folate and vitamin B-12 nutritional status; metabolic disorders, such as chronic renal disease; and genetic factors. The prevalence of moderate hyperhomocysteinemia in North America and Europe has been widely evaluated as a risk factor for coronary artery disease, neurodegenerative diseases, and spina bifida (1). Differences in the prevalence of hyperhomocysteinemia have been reported in various ethnic groups. Asian Americans have significantly lower total homocysteine (tHcy) concentrations than do whites (2), whereas Hispanic Americans have intermediate values. Another study reported higher concentrations of tHcy in African Americans than in European Americans, which may be related to the prevalence of nutrient deficiencies (3). In South Africa, plasma homocysteine concentrations were reported to be significantly lower in traditionally living adult black men than in whites (4). Blacks metabolized homocysteine more efficiently than did whites, even after vitamin supplementation, which suggests the influence of genetic factors. The prevalence of moderate hyperhomocysteinemia in sub-Saharan Africa is unknown.

The known genetic determinants of tHcy are polymorphisms of the genes encoding enzymes involved in one-carbon metabolism. Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MTR) are 2 key enzymes in the folate- and vitamin B-12–dependent transmethylation of homocysteine into methionine (5, 6). Three single-nucleotide polymorphisms, 677C→T, 1298A→C, and 1317T→C (a silent mutation), have been identified in the *MTHFR* gene (7). The 677C→T mutation leads to moderate hyperhomocysteinemia when associated with low plasma folate (8–10). The 1298A→C mutation was shown to be related to hyperhomocysteinemia in association with the *MTHFR* 677C→T genotype (11). Another polymorphism, 2756A→G, has been identified in the *MTR* gene, but its association with tHcy was not established (12). The percentage of individuals homozygous for the 677C→T mutation ranges between 14% and 18% among whites but is considerably lower in African Americans (on the order of 0–2%) (13).

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The frequencies of the *MTHFR* 1298A→C and *MTR* 2756A→G mutations and their influence on plasma homocysteine concentrations have never been evaluated in a black population from Africa concurrently with nutritional determinants of hyperhomocysteinemia. In the present study, we evaluated the influence of nutritional (folate and vitamin B-12), metabolic, and genetic (*MTHFR* and *MTR* polymorphisms) determinants of homocysteine concentrations in Benin and Togo, 2 neighboring tropical countries of West Africa in which cardiovascular diseases are relatively less frequent than in Western countries. Special attention was paid when comparing the populations from coastal and savanna regions because of differences in diet between these 2 regions.

SUBJECTS AND METHODS

Subjects

A total of 276 volunteers were recruited after they provided informed consent. The volunteers from the coast were recruited in Togo (127 subjects) and in Benin (81 subjects). The volunteers from the savanna were recruited only in Togo (68 subjects). The local ethical committees approved the study protocol.

Materials and methods

Venous blood from fasting subjects was collected in EDTA-containing tubes. The samples were immediately centrifuged at $1500 \times g$ for 15 min at −4 °C and were stored at −30 °C until analyzed. Plasma concentrations of vitamin B-12 and folate were assayed with an immunoassay kit on an ACS 180 automated chemiluminescent system (Chiron Diagnostics Corporation, East Walpole, MA). Plasma homocysteine concentrations were measured by use of the Abbott fluorescence polarization immunoassay (Abbott Laboratories Diagnostics Division, Abbott Park, IL). Nutritional and inflammatory protein markers were determined by immunonephelometry with an Array Protein System analyzer (Beckman, Berkeley, CA). Serum cystatin C concentrations were measured by particle-enhanced immunonephelometry (Behring, Marburg, Germany). Serum creatinine (Jaffe method), uric acid, and urea concentrations were measured on a Hitachi model 917 multichannel analyzer (Hitachi, Tokyo).

Genomic DNA was isolated from the buffy coat layer of blood by using Qiagen kits according to the manufacturer's recommendations (Qiagen-France, Courtaboeuf, France). Polymerase chain reaction–based restriction fragment length polymorphism methods were used to determine genotypes. Previously described methods were used for the 677C→T and 1298A→C mutations of the *MTHFR* gene (7, 14, 15). The 677C→T mutation creates a *Hinf*I recognition site and the 1298A→C mutation creates a *Fnu*4HI recognition site. The 2756A→G mutation in *MTR* creates a *Hae*III recognition site. For all genotype determinations, each experimental batch of DNA was analyzed in parallel with control DNA to avoid misinterpretation from any lack of digestion of the experimental DNA (15).

Statistical analyses

Categorical variables are reported as *n*'s and percentages and continuous variables as medians with 10th and 90th percentiles. For categorical variables, a continuity-corrected chi-square test was used to assess differences between the groups. For continuous variables, Student's *t* test for unpaired data and Bonferroni's adjustment were used for the comparisons. In the case of skewed distributions of data, logarithmic transformations were carried out to normalize the distributions. Multiple regression analysis was used to estimate the relation among tHcy, folate, vitamin B-12, and the nutritional and metabolic markers. Significance and odds ratios (ORs) of independent categorical and continuous determinants of tHcy were determined by multivariate logistic regression analysis. P values < 0.05 were considered to indicate statistical significance. The data were analyzed by using STATVIEW 5 software for WINDOWS (SAS Institute Inc, Berkley, CA).

TABLE 1
Clinical and biological data for the 276 subjects studied[1]

					P[2]			
	Group S	Group C	Group C1	Group C2	S vs C	S vs C1	S vs C2	C1 vs C2
No. of subjects [*n* (%)]	68 (24.6)	208 (75.4)	166 (60.2)	42 (15.2)				
Sex ratio (F/M)	0.70	0.59	0.76	0.24	0.5412	0.7570	0.0120	0.0025
Age (y)	29.5 (20–54)[3]	26 (22–48)	25 (20–50)	27 (20–50)	0.0225	0.0218	0.124	0.4858
BMI (kg/m^2)	23.5 (17.5–34.9)	22.2 (19.3–26.3)	22.2 (17–31)	21.7 (17–31)	0.0087	0.0142	0.0612	0.8250
Homocysteine (μmol/L)	11.6 (7.1–21.8)	14.1 (8.4–40.6)	12.6 (8.1–20.5)	40.3 (30.9–70.2)	0.0036	0.5550	0.0001	0.0001
Folate (nmol/L)	8.4 (4.09–18.2)	9.2 (5.05–17.65)	10.0 (5.5–19.1)	6.1 (3.9–11.8)	0.3317	0.0646	0.0363	0.0001
Vitamin B-12 (pmol/L)	551.6 (282.1–907.1)	451.15 (213.7–916.3)	524.6 (237–1128.5)	354.4 (171.1–1064.6)	0.2049	0.8574	0.0030	0.0018
Albumin (mmol/L)	0.58 (0.44–0.69)	0.62 (0.58–0.77)	0.62 (0.44–0.77)	0.65 (0.42–0.75)	0.0001	0.0001	0.0001	0.1358
Prealbumin (μmol/L)	3.8 (1.2–5.4)	4.5 (1.2–41.8)	4.5 (1.2–41.8)	4.5 (1.2–41.8)	0.0001	0.0001	0.0003	0.4977
Retinol-binding protein (μmol/L)	1.32 (0.80–2.12)	1.41 (0.81–2.21)	1.37 (0.78–2.21)	1.59 (1.03–3.59)	0.1601	0.3797	0.0321	0.1246
C-reactive protein (nmol/L)	0.3 (0.3–0.9)	0.3 (0.3–2.7)	0.3 (0.3–2.7)	0.3 (0.3–2.7)	0.7534	0.6559	0.8591	0.6433
Creatinine (μmol/L)	0.07 (0.06–0.12)	0.08 (0.06–0.11)	0.08 (0.04–0.3)	0.08 (0.07–0.54)	0.0017	0.0126	0.0005	0.1432
Cystatine C (nmol/L)	0.06 (0.03–0.08)	0.06 (0.02–0.13)	0.06 (0.02–0.12)	0.07 (0.04–0.13)	0.3874	0.6757	0.1010	0.2096

[1] S, savanna; C, coast; C1, subjects from the coast with homocysteine concentrations < 28 μmol/L; C2, subjects from the coast with homocysteine concentrations > 28 μmol/L.
[2] Student's *t* test with Bonferroni adjustment of log-transformed data.
[3] Median; 10th–90th percentile in parentheses (all such values).

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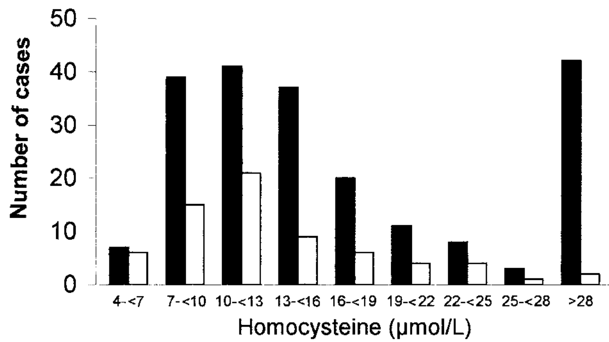

FIGURE 1. Comparative distribution of plasma homocysteine in volunteers from the coast (■; $n = 208$) and savanna (□; $n = 68$) of Western Africa. A distinct group with homocysteine concentrations > 28 μmol/L was identified among the volunteers from the coast.

RESULTS

The main characteristics of the population (age, sex, and biological data) are presented in **Table 1**. The median tHcy concentration in the whole population was 13.5 μmol/L. No significant differences in plasma tHcy were found in relation to sex or age. Because the distributions of age and sex and the biological data for subjects from the Benin and Togo coastal regions did not differ significantly, we considered these subjects as a single population (group C). Plasma tHcy exceeded the threshold value for moderate hyperhomocysteinemia of 15 μmol/L (1) in 56.2% of the whole population and in a much higher proportion of subjects from the coast (group C) than from the savanna (group S): 62.3% and 29.4%, respectively ($P < 0.0001$). Median tHcy concentrations were also significantly higher in the C group than in the S group: 14.1 and 11.6 μmol/L, respectively ($P = 0.0036$). The same was observed for albumin and creatinine, whereas BMI was higher in the S group than in the C group. No significant differences in vitamin B-12 or folate were observed between the C and S groups.

The histogram distribution of tHcy in the S group ($n = 68$) was continuous and nearly symmetrical for 66 subjects, with a upper tHcy concentration of 28 μmol/L. The data points for 2 subjects were outliers, with respective values of 32 and 69 μmol/L. By comparison, the tHcy distribution of the coastal population showed 2 distinct groups: C1 ($n = 166$; 85% of the subjects in the C group), which had tHcy concentrations less than the 28-μmol/L upper limit observed in the S group and a continuous distribution similar to that of the S group, and C2 ($n = 42$; 15% of the subjects in the C group), which had a skewed distribution of tHcy concentrations exceeding the threshold value (**Figure 1**). All of the subjects from group C2 were recruited in 2 cities, Cotonou and Lomé.

No significant difference in either BMI or protein nutritional markers was found between the C1 and C2 groups (Table 1). In contrast, the sex ratio, folate, and vitamin B-12 were lower in group C2 than in group C1(Table 1). This suggests that the hyperhomocysteinemia in group C2 was due, at least in part, to a deficient status of these vitamins. Blood folate concentrations were below the lower quartile (6.75 nmol/L) in as many as 51% of the subjects from group C2 and in 20.5% and 33.3% of the subjects from groups C1 and S, respectively. In fact, 52% of the subjects from group C2 had blood folate concentrations less than the 7.0-nmol/L threshold value defined for folate deficiency, whereas only 5% had a vitamin B-12 concentration less than the 150-pmol/L threshold value (1).

Blood concentrations of cystatin C, folate, and albumin were the 3 significant independent determinants of tHcy in multiple regression analysis of the whole population (**Table 2**). RBP, cystatin C, and uric acid were the 3 determinants of tHcy in group C1 (Table 2). Folate was a weaker determinant of tHcy in group C2 than in group S (Table 2), but in logistic regression analysis, a concentration below the lower quartile (6.75 nmol/L) generated a potent risk of tHcy > 28 μmol/L (**Table 3**).

We investigated the genetic polymorphism of *MTHFR* and *MTR* as determinants of homocysteine concentrations in the coastal and savanna regions. The distributions of all of the polymorphisms were in Hardy Weinberg equilibrium. The genotypes and allele frequencies of the *MTHFR* 677C→T and 1298A→C and the *MTR* 2756A→G mutations are presented in **Tables 4** and **5**, respectively. No significant difference in frequency was observed for the 2 *MTHFR* polymorphisms and the *MTR* polymorphism between the coast (C1 + C2 groups) and savanna regions. However, we found a clear difference in the *MTHFR* 677→T polymorphism distribution when we compared group C2 with groups C1 and S (Tables 4 and 5). In fact, the *MTHFR 677 TT* mutant homozygous genotype was observed only in group C2, and the heterozygous *CT* genotype was also significantly more frequent in group C2, suggesting a role of this polymorphism as a determinant of hyperhomocysteinemia. In logistic regression analysis, the *MTHFR* 677 *T* mutated allele was a potent risk factor for mild hyperhomocysteinemia in the whole population and in group C2 (Table 3). The other determinants that increased the risk of having a tHcy concentration > 28 μmol/L were, by decreasing order of odds ratios, folate less than the lower quartile, cystatin C, and albumin (Table 3). The concentration of tHcy of

TABLE 2
Stepwise multiple regression analysis of independent determinants (continuous variables) of total homocysteine (tHcy)[1]

Group	Residual determinants	Coefficient	P
Whole population	Folate	−0.353	0.0005
	Albumin	0.689	0.0008
	Cystatin C	10.424	< 0.0001
Coast, group C1 (tHcy < 28 μmol/L)	Retinol-binding protein	−0.084	0.0054
	Cystatin C	4.930	0.0022
	Uric acid	0.098	0.0011
Coast, group C2 (tHcy > 28 μmol/L)	Folate	−1.463	0.0432
Savanna	Folate	−0.549	0.0028

[1] Nonsignificant determinants excluded were age, BMI, vitamin B-12, C-reactive protein, albumin, prealbumin, retinol-binding protein, cystatin C, creatinine, and uric acid.

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TABLE 3
Evaluation by logistic regression analysis of the risk (odds ratio) of mild hyperhomocysteinemia related to genetic and nutritional determinants[1]

Dependent determinant	Residual determinants	Odds ratio (95% CI)	P
Homocysteine > 13.5 μmol/L (median, whole population)	Folate < 6.75 nmol/L (lower quartile)	5.3 (2.5, 11.2)	< 0.0001
	MTHFR 677 *T* allele	4.9 (1.6, 14.8)	0.0048
	Creatinine	1.3 (1.0, 1.6)	0.0330
Homocysteine > 28 μmol/L (group C2)	Folate < 6.75 nmol/L (lower quartile)	15.9 (4.5, 56.8)	< 0.0001
	MTHFR 677 *T* allele	9.0 (2.3, 35.2)	0.0017
	Cystatin C	7.7 (2.0, 29.9)	0.0030
	Albumin	1.2 (1.00, 1.3)	0.0451

[1] Nonsignificant determinants excluded were age, BMI, vitamin B-12, C-reactive protein, prealbumin, retinol-binding protein, and uric acid. MTHFR, methylenetetrahydrofolate reductase. Group C2, subjects from the coast with homocysteine concentrations > 28 μmol/L.

the whole population was significantly lower in the subjects with the *MTHFR* 677 *CC* genotype than in those with either the *CT* or *TT* genotypes, with respective values (median and 10th–90th percentiles) of 12.6 (8.0–30.5), 19.1 (9.9–46.2), and 86.4 (59.1–95.5) μmol/L (P = 0.0100 and P = 0.0011, respectively), whereas no significant difference was observed for folate. In contrast, tHcy concentrations of subjects with the 677 *CT* and wild-type *MTHFR* genotypes were not significantly different in group C2 [39.1 (31.9–66.4) and 39.0 (30.8–53.9) μmol/L, respectively]. The other polymorphisms from *MTHFR* and *MTR* had no significant influence on tHcy.

DISCUSSION

In the present study, we observed a high prevalence of age-independent moderate hyperhomocysteinemia in the population from the coast of Togo and Benin. The 13.5-μmol/L median concentration of tHcy is 3.5 and 1.5 μmol/L higher than average tHcy values recorded in northern and southern Europe, respectively (16). There is a north-to-south increase in the frequency of the *MTHFR 677 T* allele in Europe, which may explain in part the tHcy gradient (16, 17). However, the *MTHFR* polymorphism corresponds to only 12.3% of the total sample variance in tHcy (16). Another determinant of tHcy, folate, may limit this gradient, in regard to the apparently higher folate content of the food of Mediterannean populations (18). In the present study, folate was the main determinant of mild hyperhomocysteinemia (Table 3).

Blood folate concentrations were lower in group C2 than in groups C1 and S, and this low folate concentration (lower quartile) generated a 15.9-fold increased risk of having homocysteine concentrations > 28 μmol/L in this group. The subjects of group C2 were recruited in Lomé and Cotonou, in contrast with the rural origin of the group from the savanna. Our data show, therefore, that dietary folate intake was deficient mostly in a defined population from these 2 towns, as illustrated by the distinct plasma tHcy distributions in groups C1 and C2 (Figure 1). This difference was not explained by differences in traditional diet between the coast and the savanna, even though the traditional diets of the 2 regions differ in sources of micronutrients (19). Cereals (corn, millet, and sorghum) are predominant in the savanna, whereas starchy foods (cassava, yams, and sweet potatoes) are predominant on the coast. Daily folate intake is nearly identical in the coastal and savanna regions, with respective estimated values of 280.5 and 312.6 μg/d (19).

The hyperhomocysteinemia observed in as much as 15% of the population of the coast could therefore correspond to an inadequate intake of folate related to a high frequency of poverty in the 2 towns. Animal sources of micronutrients, in contrast with vegetal sources, are predominant on the coast, explaining why the dietary intake of vitamin B-12 is higher on the coast than in the savanna, with respective estimated values of 5.7 and 3.5 μg/d (19). This difference in the average dietary intake of vitamin B-12 could account for the higher concentrations of this vitamin in the subjects from the coast (Table 1). Blood concentrations of vitamin B-12 were not a determinant of hyperhomocysteinemia and were below the threshold value indicating vitamin B-12 deficiency in only 12 subjects in the whole population. In addition, 10 of these 12 subjects belonged to group C1 and only 2 to belonged to group C2, which confirms that vitamin B-12 deficiency was not a frequent cause of hyperhomocysteinemia in the coastal population.

TABLE 4
Frequency of methylenetetrahydrofolate reductase (*MTHFR*) 677C→T and 1298A→C and methionine synthase (*MTR*) 2756A→G polymorphisms in the populations from the coast and the savanna[1]

Geographic origin	*MTHFR* 677 *CC*	*MTHFR* 677 *CT*	*MTHFR* 677 *TT*	*MTHFR* 1298 *AA*	*MTHFR* 1298 *AC*	*MTHFR* 1298 *CC*	*MTR* 2756 *AA*	*MTR* 2756 *AG*	*MTR* 2756 *GG*
		n (%)			*n (%)*			*n (%)*	
Savanna, group S (n = 68)	60 (88.2)	8 (11.7)	0	50 (73.5)	15 (22.1)	3 (4.4)	41 (60.2)	21 (30.8)	6 (8.8)
Coast, group C1 (n = 166)	144 (86.7)	22 (13.2)	0	115 (69.4)	48 (28.8)	3 (1.7)	78 (46.9)	79 (47.6)[2]	9 (2.4)
Coast, group C2 (n = 42)	23 (54.7)	16 (38.2)[3,4]	3 (7.1)	29 (69.1)	12 (28.5)	1 (2.4)	23 (54.7)	18 (42.8)	1 (2.3)

[1] Group C1, subjects with homocysteine concentrations < 28 μmol/L; group C2, subjects with homocysteine concentrations > 28 μmol/L.
[2] Significantly different from group S, chi-square = 12.2, P = 0.0005 (Bonferroni corrected).
[3] Significantly different from group C1, chi-square = 16.1, P < 0.0001 (Bonferroni corrected).
[4] Significantly different from group S, chi-square = 4.8, P = 0.0277 (Bonferroni corrected).

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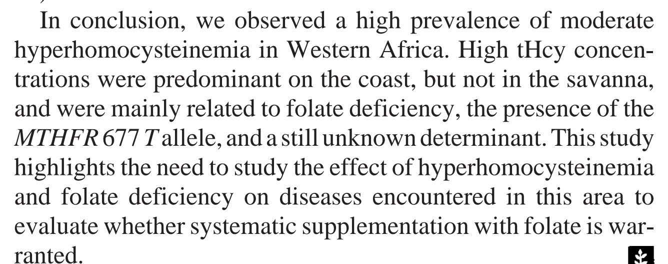
TABLE 5
Mutated allele frequency of methylenetetrahydrofolate reductase (*MTHFR*) 677C→T and methionine synthase (*MTR*) 2758A→G polymorphisms in the populations from the coast and the savanna[1]

Geographic origin	*MTHFR* 677 *T* allele	*MTHFR* 1298 *C* allele	*MTR* 2756 *G* allele
	%	%	%
Savanna, group S (n = 68)	5.9 (0.3, 11.4)	15.4 (6.5, 23.9)	24.3 (13.8, 34.1)
Coast, group C1 (n = 166)	6.6 (3.1, 10.8)	16.3 (10.5, 21.6)	29.2 (19.5, 32.8)
Coast, group C2 (n = 42)	26.2[2,3] (13.0, 39.6)	16.7 (5.4, 27.9)	23.8 (13.7, 40.5)

[1] All values are frequencies; 95% CI in parentheses. Group C1, subjects with homocysteine concentrations < 28 μmol/L; group C2, subjects with homocysteine concentrations > 28 μmol/L.
[2] Significantly different from group S, chi-square = 18.2, $P < 0.0001$ (Bonferroni corrected).
[3] Significantly different from group C1, chi-square = 16.1, $P < 0.0001$ (Bonferroni corrected).

A mutated *MTHFR* genotype was the single genetic determinant of hyperhomocysteinemia. The influence of this polymorphism on tHcy was also observed in the heterozygous subjects, contrary to what was observed in studies performed in Western countries. This may be related to a synergistic influence of the high prevalence of folate deficiency in our population (20, 21). The impairment of Hcy remethylation as a result of folate deficiency was potentiated by the *MTHFR* 677 *TT* genotype in one-quarter of the subjects from group C2, as observed previously in white populations (20, 21). Several studies have reported that the 677C→T mutation has a significantly heterogeneous distribution among different ethnic groups (22, 23). In 2 recent studies of Mexican populations, who are known to have a relatively high frequency of neural tube defects, the percentage of 677 *TT* homozygotes was $>$ 30% (24, 25). In contrast, Loktionov et al (26) found no homozygous 677 *TT* individuals among black Africans in a study comparing the frequency of the *MTHFR* 677C→T polymorphism between white and black South Africans. Plasma homocysteine concentrations were not evaluated in any of these comparative studies. The studies carried out in whites from Europe showed a North-to-South gradient, with a low allele frequency of 0.23 in the Baltic countries compared with an average frequency of 0.32 in the other regions (16) and of 0.42 in Sicily (15). In addition, it was recently suggested that the 677C→T mutation had occurred once on a *G-T-A-C* haplotype common to populations from Israel, Japan, and Ghana and that it may confer a survival advantage in populations with adequate folate intake (18). Considering our results on folate status and the dramatic synergic effect of folate deficiency and the *MTHFR T* allele on tHcy, this advantage could be limited in Africans because of a higher prevalence of folate deficiency and of infectious diseases, both of which impair folate assimilation (18, 27).

Cystatin C was another strong determinant of tHcy in the present study in the whole population but not in group C2. In a recent study of subjects from the savanna and coast of Togo, we showed that blood concentrations of cystatin C were not influenced by nutritional status (28). This protein is a marker of glomerular filtration and its association with tHcy has been described in healthy subjects and in patients with renal failure (29). Albumin and retinol-binding protein were 2 weak determinants of tHcy in the whole population and in group C1, respectively. These associations may reflect an influence of deficient nutritional status on tHcy, because these 2 proteins are sensitive markers of malnutrition (28). Uric acid was also a determinant of tHcy in group C1. This is not surprising because uric acid is an end product of tHcy catabolism (1). Of the subjects from the coast with tHcy concentrations $>$ 28 μmol/L, 33% had the wild-type *MTHFR* 677 *CC* genotype, folate and vitamin B-12 concentrations higher than the lower quartile, and a normal blood concentration of cystatin C, suggesting the involvement of another determinant. This determinant was not related to the *MTHFR* 1298A→C and *MTR* 2756A→G mutations because these polymorphisms were not related to the high tHcy concentration of the subjects from the coast. In addition, the percentage of *MTHFR* 1298 *CC* homozygotes was largely below that reported in white populations, and the frequency of the *MTR* 2756 *G* allele was similar to that commonly reported in the other ethnic groups (30, 31).


In conclusion, we observed a high prevalence of moderate hyperhomocysteinemia in Western Africa. High tHcy concentrations were predominant on the coast, but not in the savanna, and were mainly related to folate deficiency, the presence of the *MTHFR* 677 *T* allele, and a still unknown determinant. This study highlights the need to study the effect of hyperhomocysteinemia and folate deficiency on diseases encountered in this area to evaluate whether systematic supplementation with folate is warranted.

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