Helicobacter pylori serologic status has no influence on the association between fucosyltransferase 2 polymorphism (*FUT2 461 G→A*) and vitamin B-12 in Europe and West Africa[1–4]

Abderrahim Oussalah, Cyril Besseau, Céline Chery, Elise Jeannesson, Rosa-Maria Guéant-Rodriguez, Guido Anello, Paolo Bosco, Maurizzio Elia, Antonino Romano, Jean-Pierre Bronowicki, Philippe Gerard, Justine Paoli, Patrice Hodonou Avogbe, Nicodème Chabi, Ambaliou Sanni, Emile Amouzou, Laurent Peyrin-Biroulet, and Jean-Louis Guéant

ABSTRACT

Background: Genomewide association studies have shown a relation between plasma vitamin B-12 concentration and the *461G→A* polymorphism of fucosyltransferase 2 (*FUT2*), a gene associated with susceptibility to *Helicobacter pylori* infection.

Objective: We evaluated in 2 populations the association of *FUT2 461 G→A* polymorphism with vitamin B-12 and related metabolic markers and investigated whether the influence of *FUT2* on *H. pylori* serology is part of the mechanisms that underlie these associations.

Design: The study included 1282 ambulatory subjects from Europe and West Africa. Blood concentrations of vitamin B-12, folate, homocysteine, and methylmalonic acid were measured. Genotyping was performed by real-time polymerase chain reaction. *H. pylori* serology testing was performed by using ELISA.

Results: In univariate analysis, *FUT2 461 A/A* genotype was associated with higher plasma vitamin B-12 concentration in the total population ($P = 0.0007$) as well as in Europe ($P = 0.0009$) and in West Africa ($P = 0.0015$). Positivity for *H. pylori* serology was higher in West Africa ($P < 0.0001$) and was not associated with low plasma vitamin B-12. The prevalence of *H. pylori*–positive patients did not differ among *FUT2 461 G→A* genotypes ($P = 0.2068$). In multivariate analysis, *FUT2 461 G→A* genotype ($P = 0.0008$), but not positive *H. pylori* serology, was an independent predictor of plasma vitamin B-12 concentration.

Conclusion: This study confirms the influence of *FUT2 461 G→A* polymorphism on plasma vitamin B-12 concentration and showed no influence of *H. pylori* serologic status on this association in ambulatory subjects from Europe and West Africa. *Am J Clin Nutr* 2012;95:514–21.

INTRODUCTION

Vitamin B-12 (cobalamin) is a water-soluble vitamin that is required in one-carbon metabolism (1). Cobalamin is found in animal foods, meat, milk, egg, fish, and shellfish (2). Uptake of dietary vitamin B-12 and its delivery to cells is a complex process, requiring 3 cobalamin-binding proteins—intrinsic factor, haptocorrin (previously known as R-binder), and transcobalamin—and their cell membrane receptors (1). Vitamin B-12 is a cofactor required for the remethylation of homocysteine to methionine by methionine synthase and is part of one-carbon metabolism, which is essential for purine and thymidylate synthesis, which is necessary for maintenance of genomic integrity (3). Data from genomewide association studies suggested that plasma vitamin B-12 concentration could be influenced by genetic polymorphisms (4–6). Hazra et al (4) showed that white women who were homozygous for fucosyltransferase 2 (*FUT2*) rs492602 G (NG_007511.1, located on chromosome 19 at 19q13.3.), which is in strong linkage disequilibrium with the *FUT2* rs601338 [*461 G→A* (NM_000511) according to the Human Genome Variation Society, also known as *428 G→A*] nonsecretor variant allele (W143X variant), had higher plasma vitamin B-12 concentrations.

It has been suggested that the association between *FUT2* and low plasma vitamin B-12 concentration may be the consequence of susceptibility to *Helicobacter pylori* infection in carriers of the *FUT2* secretor status as compared with individuals with the nonsecretor status (5). However, no study to date has evaluated the relation between *FUT2* polymorphism and *H. pylori* serologic status. Furthermore, data regarding the influence of *FUT2* polymorphism on the overall profile of one-carbon metabolism markers

[1] From INSERM U954, Cellular and Molecular Pathology in Nutrition, Henri Poincaré University Nancy 1, and University Hospital of Nancy, Vandoeuvre-lès-Nancy, France (AO, CB, CC, EJ, R-MG-R, LP-B, J-PB, PHA, PG, JP, and J-LG); Istituto di Ricovero e Cura a Carattere Scientifico, Oasi Maria SS–Institute for Research on Mental Retardation, Troina, Italy (GA, PB, ME, AR, and J-LG); the Department of Internal Medicine and Geriatrics, Università Cattolica del Sacro Cuore, CI Columbus, Rome, Italy (AR); the Laboratory of Biochemistry and Molecular Biology, Université de Cotonou, Cotonou, Benin (NC and AS); and the Laboratory of Biochemistry and Nutrition, Lomé, Togo (EA).

[2] AO, CB, and CC contributed equally to this article.

[3] Supported by grants from the French National Institute for Health and Medical Research (INSERM) and the French National Agency for Research (ANR; Nutrivigène program). Mast Diagnostic (Amiens, France) kindly provided Mastazyme *Helicobacter pylori* IgG ELISA kits for *H. pylori* serology testing.

[4] Address correspondence to J-L Guéant, AGAF, INSERM U954, Cellular and Molecular Pathology in Nutrition, Henri Poincaré University Nancy 1, and University Hospital of Nancy, Rue du Morvan, 54500, Vandoeuvre-lès-Nancy, France. E-mail: jean-louis.gueant@medecine.uhp-nancy.fr.

Received March 22, 2011. Accepted for publication November 1, 2011.

First published online January 11, 2012; doi: 10.3945/ajcn.111.016410.

Supplemental Material can be found at:
http://www.ajcn.org/content/suppl/2012/01/24/ajcn.111.016410.DC1.html

are lacking, and no study has evaluated in multivariate analysis the influence of *FUT2* polymorphism on vitamin B-12 concentration.

The aims of the study were as follows: *1*) to appraise the distribution of *FUT2 461 G→A* (rs601338) polymorphism and to assess its influence on one-carbon metabolism markers (vitamin B-12, folate, homocysteine, and methylmalonic acid) and *2*) to investigate the potential association between *H. pylori* infection and *FUT2 461 G→A* polymorphism in the general population.

SUBJECTS AND METHODS

Study populations

Between January 1999 and December 2006, ambulatory subjects were included from 3 countries: Italy [OASI cohort, Sicily (7)], Benin (coastal urban area of Cotonou), and Togo (the coast, Lomé, and the Savanna). These populations have been previously reported on in 2 studies (8, 9). Italian subjects were considered representative of the European continent, and those from Benin and Togo were considered representative of the West African region. Institutional review board approval was obtained from the ethical committees of the Medical Centre of Troina (Instituto di Ricovero e Cura a Carattere Scientifico, the Oasi Maria Institute, Institute for Research on Mental Retardation, Troina, Sicily, Italy), the University Hospital of Nancy (Vandoeuvre-lès-Nancy, France), the University of Benin (Cotonou, Benin), and the University of Lomé (Lomé, Togo) (8–10). Written informed consent was obtained from participants. All participants had received a systematic clinical evaluation to rule out cancer, cardiovascular, renal, hepatic, or genetic disease and any vitamin supplementation before inclusion in the study.

Blood sampling

Phlebotomy was performed, and fasting venous blood was collected in EDTA-containing tubes. Samples were centrifuged immediately at 2500 *g* for 15 min at room temperature. Aliquots were stored at −70°C until analysis.

Vitamin B-12, folate, homocysteine, and methylmalonic acid assays

All specimens were tested in a single laboratory (Vandoeuvre-lès-Nancy, France). Homocysteine and methylmalonic acid were determined by ultra-performance liquid chromatography–tandem mass spectrometry procedure as previously published (11), with an Acquity ultra-performance liquid chromatography bridged ethylsiloxane/silica hybrid (UPLC BEH) C18 column (1.7 μm, 2.1 × 50 mm; Waters Corporation). Methylmalonic acid was assessed only in the European population. Plasma vitamin B-12 and folate concentrations were assayed with a vitamin B-12 and folate immunoassay kit by means of automated chemiluminescence (ASC:180 Automated Chemiluminescence Systems; Chiron Diagnostics). The intraassay CVs for vitamin B-12, folate, homocysteine, and methylmalonate were 2.8%, 5.1%, 4.5%, and 7.3%, respectively.

H. pylori testing

Serum specimens were tested for the presence of IgG antibodies against *H. pylori* by using ELISA (Mastazyme *Helicobacter*; Mast Diagnostic) according to the manufacturer's instructions. Classification of infection status followed the manufacturer's instructions.

Analysis of genomic DNA

Genomic DNA was isolated from peripheral leukocytes by using a Qiagen kit (Qiagen-France). Fluorescence resonance energy transfer real-time polymerase chain reaction (PCR) was performed for the genotyping of *FUT2 461 G→A* (rs601338) polymorphism by using a LightCycler 480 instrument (Roche Molecular Biochemicals). The 5′ to 3′ DNA sequences of forward primer, reverse primer, sensor probe, and anchor probe used for the *FUT2 461 G→A* polymorphism were AGATTCAAGCCATGTGGGAGTTA, CTTCCACACTTTTGGCATGAC, CCTGCTCCTACACCTTCTACC, and CCACCTCCGCCAGGAGATCCTC, respectively. PCR was performed in a 96-well plate in a total volume of 10 μL. The final reaction contained 10 ng genomic DNA, 0.5 μmol/L each of forward and reverse primers, 0.2 μmol/L each of the sensor and anchor probes (TIB MOLBIOL Syntheselabor GmbH), and 2 μL of LightCycler® 480 Genotyping Master kit (kit for 384 reactions). Real-time PCR for *FUT2 461 G→A* (rs601338) genotyping was performed by an initial denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 10 s, 59°C for 10 s, and 72°C for 20 s. To determine the melting points after the amplification phase, a melting curve analysis was performed at 95°C for 20 s and at 40°C for 20 s, followed by slow heating at the rate of 0.2°C/s until reaching 85°C.

Statistical analysis

All quantitative variables are described as medians and percentiles (IQR, 25th–75th percentile). All proportions and genotype frequencies are expressed as percentages with 95% CIs. Genotype distributions were tested for consistency with expected Hardy-Weinberg equilibrium proportions, including analysis for ascertainment bias, according to the method proposed by Rodriguez et al (12). Proportions were compared by using the chi-square test or Fisher's exact test, as appropriate. All correlations were studied by using Spearman's rank-order correlation coefficient. The comparison of plasma concentrations of vitamin B-12 (pmol/L), folate (nmol/L), homocysteine (μmol/L), and methylmalonic acid (μmol/L) between *FUT2 461 G→A* genotypes, was performed by using the Kruskal-Wallis test; post hoc analysis for pairwise comparison of subgroups was performed according to Conover, to avoid multiple testing problems (13). To evaluate the influence of baseline characteristics and *FUT2 461 G→A* polymorphism on plasma concentrations of vitamin B-12 (pmol/L), folate (nmol/L), homocysteine (μmol/L), and methylmalonic acid (μmol/L), univariate analyses were carried out by using Spearman's rank-order correlation coefficient, the Mann-Whitney *U* test, or the Kruskal-Wallis test, as appropriate. All significant items obtained in univariate analyses were integrated into a multiple regression model for multivariate analysis by using a stepwise method. All variables with a *P* value <0.1 were included in the model, and the variables with *P* values <0.05 were retained in the model. Correction for multiple comparisons was performed by using the Bonferroni test. All of the reported *P* values were 2-sided, and *P* values

<0.05 were considered significant. Statistical analyses were performed by using MedCalc software, version 11.4.4 (MedCalc Software).

RESULTS

Baseline characteristics

A total of 1282 ambulatory participants were included in the study (Italy, n = 657; West Africa, n = 625: Benin, n = 517; Togo, n = 108). The median age of the study population at inclusion was 60 y, and the median plasma concentrations of vitamin B-12 and folate were within the reference values (**Table 1**). As expected, plasma concentrations of vitamin B-12 and folate were significantly and inversely correlated with those of homocysteine [ρ = −0.259 (95% CI: −0.310, −0.206; $P <$ 0.0001) and ρ = −0.286 (95% CI: −0.336, −0.233; $P <$ 0.0001) for vitamin B-12 and folate, respectively]. Vitamin B-12 was inversely correlated with methylmalonic acid concentration (ρ = −0.187; 95% CI: −0.261, −0.111; $P <$ 0.0001). Homocysteine was positively correlated with methylmalonic acid (ρ = 0.180; 95% CI: 0.105, 0.254; $P <$ 0.0001). As expected, positive *H. pylori* serology was more prevalent in West African subjects than in European subjects (92.1% compared with 81.0% for West Africa and Europe, respectively; $P <$ 0.0001) (Table 1).

TABLE 1
Characteristics of the 1282 healthy individuals included in the study[1]

Characteristics	n	Values
Vitamin B-12 (pmol/L)[2]		
Total population	1226	345.0 (239.0–500.0)
Europe	650	297.0 (209.1–401.2)
West Africa	576	418.0 (293.1–589.5)
P (Europe compared with West Africa)		<0.0001[3]
Folate (nmol/L)[2]		
Total population	1225	10.0 (6.8–14.0)
Europe	648	12.0 (8.8–15.8)
West Africa	577	7.7 (5.4–11.5)
P (Europe compared with West Africa)		<0.0001[3]
Homocysteine (μmol/L)[2]		
Total population	1278	14.3 (11.4–18.7)
Europe	654	14.4 (11.8–18.1)
West Africa	624	14.1 (10.7–19.4)
P (Europe compared with West Africa)		0.11[3]
Methylmalonic acid (μmol/L)[2]		
Europe	648	0.205 (0.135–0.298)
West Africa	0	
Positive *Helicobacter pylori* serology[4]		
Total population	1036	85.1 (83.0, 87.3)
Europe	654	81.0 (78.0, 84.1)
West Africa	382	92.1 (89.4, 94.9)
P value (Europe compared with West Africa)		<0.0001[5]
Male sex[4]		
Total population	683	53.4 (50.6, 56.1)
Europe	262	39.9 (36.1, 43.6)
West Africa	421	67.6 (63.9, 71.3)
P (Europe compared with West Africa)		<0.0001[5]

[1] Total population (Europe and West Africa), n = 1282; Europe, n = 657; West Africa, n = 625.
[2] Values are medians; IQRs (25th–75th percentile) in parentheses.
[3] Mann-Whitney U test.
[4] Values are percentages; 95% CIs in parentheses.
[5] Chi-square test.

Influence of *H. pylori* serologic status on one-carbon metabolism markers in European and West African populations

Total population

In the total population, *H. pylori*–positive serology was not associated with a lower vitamin B-12 concentration. Conversely, plasma folate concentration was significantly higher in *H. pylori*–negative subjects (11.4 nmol/L; IQR: 8.5–15.9 nmol/L) than in *H. pylori*–positive subjects (9.8 nmol/L; IQR: 6.7–13.9 nmol/L) ($P <$ 0.0001) (**Table 2**).

Europe

In Europe, *H. pylori*–positive subjects had significantly lower plasma folate (P = 0.03) and methylmalonate concentrations (P = 0.0008) than did *H. pylori*–negative subjects. Plasma concentrations of vitamin B-12 and homocysteine did not differ according to *H. pylori* serologic status (Table 2).

West Africa

In the West African population, *H. pylori* serologic status did not influence the plasma concentrations of vitamin B-12, folate, homocysteine, and methylmalonic acid (Table 2).

FUT2 461 G→A genotype frequency distribution

The distribution of *FUT2 461 G→A* genotype frequencies in European and West African populations is reported in **Table 3**. This distribution of *FUT2 461 G→A* genotype is consistent with the Hardy-Weinberg equilibrium model (*see* Supplemental Table 1 under "Supplemental data" in the online issue). The homozygous *FUT2* wild-type genotype (*G/G*) was more prevalent in West Africa than in Europe (36.0% compared with 21.2%, $P <$ 0.0001). Conversely, the heterozygous genotype (*FUT2 461 G/A*) was more frequent in Europe than in West Africa (52.0% compared with 42.7% for Europe and West Africa, respectively; P = 0.001) (Table 3). The proportion of the *FUT2* nonsecretor genotype (*A/A*) did not differ between European and West African participants (26.8% compared with 21.2% for Europe and West Africa, respectively; P = 0.0502) (Table 3). It should be noted that the prevalence of the FUT2 secretor phenotype—corresponding to *FUT2 461 G/G* or *G/A* genotypes—did not differ between European (73.2%) and West African (78.8%) populations (P = 0.0502) (Table 3).

Influence of *FUT2 461 G→A* polymorphism on *H. pylori* serologic status in European and West African populations

The prevalence of positive *H. pylori* serology did not differ across *FUT2 461 G→A* genotypes in the total population or in Europe and West Africa (P = 0.2068, P = 0.4712, and P = 0.8785, respectively) (**Table 4**). Consistently, the prevalence of positive *H. pylori* serology did not differ between FUT2 secretor and FUT2 nonsecretor groups in the total population (P = 0.18) or in Europe (P = 0.27) and West Africa (P = 0.80) (Table 4).

TABLE 2
Influence of *Helicobacter pylori* serologic status on one-carbon metabolism markers in European and West African populations

	Negative *Helicobacter pylori* serology			Positive *Helicobacter pylori* serology			
One-carbon metabolism marker	*n*	Median	IQR[1]	*n*	Median	IQR[1]	*P*[2]
Total population							
Vitamin B-12 (pmol/L)	143	306.0	198.9–436.3	807	338.0	239.0–468.1	0.0500
Folate (nmol/L)	143	11.4	8.5–15.9	808	9.8	6.7–13.9	0.0001
Homocysteine (μmol/L)	144	14.6	11.9–19.0	844	14.3	11.6–18.0	0.4340
Methylmalonic acid (μmol/L)	—	—	—	—	—	—	—
Europe							
Vitamin B-12 (pmol/L)	119	281.3	188.0–398.4	524	298.5	216.0–402.3	0.2613
Folate (nmol/L)	119	12.8	9.8–16.5	524	11.6	8.6–15.5	0.0293
Homocysteine (μmol/L)	118	14.6	12.0–19.2	519	14.1	11.600–17.0	0.0591
Methylmalonic acid (μmol/L)	117	0.237	0.153–0.394	519	0.195	0.132–0.283	0.0008
West Africa							
Vitamin B-12 (pmol/L)	24	412.5	313.0–631.5	283	428.0	297.0–569.3	0.7410
Folate (nmol/L)	24	7.1	5.3–8.9	284	6.5	4.8–9.1	0.4528
Homocysteine (μmol/L)	26	12.9	11.3–17.1	325	14.8	11.7–19.8	0.3047
Methylmalonic acid (μmol/L)	0	—	—	0	—	—	—

[1] 25th–75th percentile.
[2] For positive compared with negative *Helicobacter pylori* serology (Mann-Whitney *U* test).

Influence of *FUT2 461 G→A* polymorphism on one-carbon metabolism markers in European and West African populations

In the total population, vitamin B-12 concentration was influenced by *FUT2 461 G→A* polymorphism, with median concentrations of 310, 325, and 378 pmol/L in subjects with *FUT2 461 G/G*, *G/A*, and *A/A* genotypes, respectively (P = 0.0007) (**Table 5**). The effect of *FUT2 461 G→A* polymorphism on the concentration of vitamin B-12 was consistently observed in both European (P = 0.0009) and West African (P = 0.0015) populations (Table 5). In *H. pylori*–positive participants, plasma vitamin B-12 concentration was significantly influenced by *FUT2 461 G→A* polymorphism in the total population (P = 0.0016) (*see* Supplemental Table 2 under "Supplemental data" in the online issue). In the *H. pylori*–negative group, the limited sample size allowed the evaluation of this association only in the European subjects. In this *H. pylori*–negative European subgroup, there was an influence of *FUT2 461 G* and *A* alleles on plasma vitamin B-12 concentration, with vitamin B-12 concentrations of 247.8 pmol/L (IQR: 181.8–299.0 pmol/L) and 323.5 pmol/L (IQR: 196.9–423.6 pmol/L) for *G/G* and *A/A* homozygous genotypes, respectively (P = 0.0063). Plasma folate concentration was significantly associated with *FUT2 461 G→A* polymorphism in the total population but not in European (P = 0.14) and West African (P = 0.10) populations (Table 5). Plasma homocysteine concentration was significantly associated with *FUT2 461 G→A* polymorphism in West Africa (P = 0.02) but not in Europe (P = 0.21) (Table 5). Plasma methylmalonic acid concentration was not influenced by *FUT2 461 G→A* polymorphisms in the European population (Table 5).

FUT2 secretor phenotype influenced only vitamin B-12 concentrations, with significantly higher vitamin B-12 concentrations in the FUT2 nonsecretor group than in the FUT2 secretor group in the total population (P = 0.0004) as well as in Europe (P = 0.0005) and West Africa (P = 0.0092) (*see* Supplemental Table 3 under "Supplemental data" in the online issue).

TABLE 3
Distribution of *FUT2 461 G→A* polymorphism and FUT2 secretor status according to geographic area[1]

		Genotype counts										
		FUT2 461, G/G			*FUT2 461, G/A*			*FUT2 461, A/A*[2]			FUT2 secretor status[3]	
Geographic area	Total *n*	*n*	Percentage	95% CI	*n*	Percentage	95% CI	*n*	Percentage	95% CI	*n*	Percentage	95% CI
			%	%		%	%		%	%		%	%
Total population	1047	282	26.9	24.2, 29.6	507	48.4	45.4, 51.5	258	24.6	22.0, 27.3	789	75.4	72.7, 78.0
Europe	642	136	21.2	18.0, 24.4	334	52.0	48.2, 55.9	172	26.8	23.4, 30.2	470	73.2	69.8, 76.6
West Africa	405	146	36.0	31.4, 40.7	173	42.7	37.9, 47.6	86	21.2	17.2, 25.2	319	78.8	74.8, 82.8
P (Europe compared with West Africa)[4]			<0.0001			0.0014			0.0502			0.0502	

[1] *FUT2*, fucosyltransferase 2.
[2] *FUT2 461 A/A* genotype corresponds to FUT2 nonsecretor status.
[3] FUT2 secretor status corresponds to *FUT2 461 G/G* or *G/A* genotypes.
[4] Derived by using chi-square test.

The American Journal of Clinical Nutrition

TABLE 4
Prevalence of positive *Helicobacter pylori* serology according to *FUT2 461 G→A* polymorphism or FUT2 secretor phenotype[1]

Geographic areas	*FUT2 461, G/G*				*FUT2 461, G/A*				*FUT2 461, A/A*[2]				FUT2 secretor status[3]				
	n	Hp+	Percentage	95% CI	*n*	Hp+	Percentage	95% CI	*n*	Hp+	Percentage	95% CI	P^4	*n*	Hp+	Percentage	95% CI	P^5
		n	%	%		*n*	%	%		*n*	%	%			*n*	%	%	
Total population	259	229	88.4	84.5, 92.3	475	407	85.7	82.5, 88.8	239	198	82.8	78.0, 87.7	0.2068	734	636	86.6	84.2, 89.1	0.1761
Europe	134	112	83.6	77.2, 89.9	325	269	82.8	78.6, 86.9	170	134	78.8	72.6, 85.0	0.4712	459	381	83.0	79.6, 86.5	0.2744
West Africa	125	117	93.6	89.2, 98.0	150	138	92.0	87.6, 96.4	69	64	92.8	86.5, 99.0	0.8785	275	255	92.7	89.6, 95.8	0.8012

[1] *FUT2*, fucosyltransferase 2; Hp+, patients with positive *Helicobacter pylori* serology in each group.
[2] *FUT2 461 A/A* genotype corresponds to FUT2 nonsecretor status.
[3] FUT2 secretor status corresponds to *FUT2 461 G/G* or *G/A* genotypes.
[4] *FUT2 461 G/G* compared with *G/A* and *A/A* (chi-square test).
[5] FUT2 secretor status compared with nonsecretor status (chi-square test).

Independent predictors in multivariate analysis of vitamin B-12 concentration in European and West African populations

In multiple regression analysis, *FUT2 461 G/G* genotype was negatively and independently associated with plasma vitamin B-12 concentration in the total population (β coefficient = −57.64; SE: 17.19; $P = 0.0008$) (**Table 6**). Consistently, when the "*FUT2 461 G/G* or *G/A* genotype" item—which corresponds to FUT2 secretor phenotype—was considered in the regression model, it was independently and negatively associated with vitamin B-12 concentration in the total population (β coefficient = −57.89; SE: 17.13; $P = 0.0008$) (Supplemental Table 4 under "Supplemental data" in the online issue). Male sex was negatively and independently associated with plasma vitamin B-12 (Table 6). Conversely, the West African geographic area was positively and independently associated with plasma vitamin B-12 concentration (Table 6 and Supplemental Table 4 under "Supplemental data" in the online issue). Positive *H. pylori* serology was not an independent predictor of plasma vitamin B-12 concentration (Table 6 and Supplemental Table 4 under "Supplemental data" in the online issue). No interaction was found between *FUT2 461 G→A* polymorphism and *H. pylori* serologic status.

DISCUSSION

This study, which was performed in a general population setting encompassing West African and European ambulatory subjects, showed that *FUT2 461 G→A* polymorphism was an independent predictor of plasma vitamin B-12 concentration. These results are consistent with the data from 2 genomewide association studies conducted in white populations (5, 6). The family of α-1,2-fucosyltransferases (also known as the Se enzyme) catalyzes the addition of fucose—a hexose deoxy sugar—in α-1,2 linkage to the galactose of types 1 and 2 disaccharide to form H type 1 and H type 2 antigens, respectively (14). The FUT2 variant is a physiologic trait that regulates the expression and secretion of ABH blood group antigens in epithelial cells of glands (15). Approximately 20% of whites are nonsecretors who do not express ABO blood group antigens in saliva because they are homozygous for the *FUT2 461* null allele (*A/A*) (15). In Europeans, Africans, and Iranians, the *FUT2* nonsecretor genotype variant was associated with higher vitamin B-12 concentrations than in carriers of the secretor genotype (5, 15).

Hazra et al (4) showed that white women who were homozygous for the *G* allele of rs492602, which is in strong linkage disequilibrium with rs601338, had higher vitamin B-12 concentrations. Here, we performed the direct genotyping of rs601338 and showed its association with plasma vitamin B-12 concentration among 1282 adult subjects from Europe and West Africa. Although the difference in *H. pylori* prevalence between European and West African populations was statistically significant, it was not dissimilar enough to be clinically relevant. It has been suggested that the reduced vitamin B-12 concentration in carriers of the FUT2 secretor phenotype is a consequence of increased susceptibility to *H. pylori* infection (5). Indeed, *FUT2*-null mice displayed an altered glycosylation profile and impaired BabA-mediated *H. pylori* adhesion to gastric mucosa (16). In a prospective study in 304 *H. pylori*–infected individuals, Azevedo et al (17) showed that FUT2 secretor

TABLE 5
Comparison of plasma concentrations of vitamin B-12, folate, homocysteine, and methylmalonate according to *FUT2 461 G→A* polymorphism[1]

One-carbon metabolism markers	Geographic area	*FUT2 461, G/G*			*FUT2 461, G/A*			*FUT2 461, A/A*			
		n	Median	IQR[2]	*n*	Median	IQR[2]	*n*	Median	IQR[2]	*P*[3]
Vitamin B-12 (pmol/L)	Total population	257	310.0	223.0–420.3	488	324.5	222.2–469.9	249	378.3	250.0–516.0	0.0007
	Europe	134	270.0	191.0–374.0	332	283.4	199.9–394.4	170	345.5	231.1–443.2	0.0009
	West Africa	123	382.0	263.8–505.5	156	443.5	309.5–580.0	79	486.0	328.3–645.0	0.0015
Folate (nmol/L)	Total population	256	8.7	5.8–12.4	488	10.7	7.1–14.2	249	10.0	6.8–14.4	0.0001
	Europe	132	11.0	8.6–14.4	332	12.2	9.4–15.9	170	11.7	8.3–16.3	0.1351
	West Africa	124	6.0	4.1–8.7	156	6.7	5.1–10.4	79	7.4	5.0–10.3	0.0986
Homocysteine (μmol/L)	Total population	281	15.0	12.1–19.3	505	14.2	11.3–17.9	258	14.8	12.2–19.7	0.0054
	Europe	136	14.8	12.3–17.5	332	14.2	11.6–17.9	172	14.6	12.1–19.7	0.2068
	West Africa	145	15.8	11.9–21.8	173	14.1	10.9–18.2	86	15.1	12.3–20.7	0.0148
Methylmalonic acid (μmol/L)	Europe	134	0.202	0.112–0.296	329	0.204	0.133–0.297	170	0.205	0.141–0.305	0.6129
	West Africa	0	—	—	0	—	—	0	—	—	—

[1] *FUT2*, fucosyltransferase 2.
[2] 25th–75th percentile.
[3] Kruskal-Wallis test.

phenotype was associated with *H. pylori* infection and with severity of *H. pylori*–induced gastric lesions.

During the gastric phase of vitamin B-12 absorption, the low pH of the stomach and the peptic acid digestion of food protein–bound cobalamin are necessary for the release of cobalamin (18–20). *H. pylori*–induced chronic atrophic gastritis may cause a disturbance of acid/peptic secretion, which can in turn cause food-cobalamin malabsorption (20, 21). This was suggested by a Finnish population-based study that reported a high prevalence of *H. pylori* infection among subjects with chronic atrophic gastritis and low vitamin B-12 status (22). By contrast, in a case-control study, Stettin et al (23) evaluated one-carbon metabolism markers in 90 healthy subjects, of whom 69 had positive *H. pylori* serology, and did not find any significant impact of *H. pylori*–positive serology on vitamin B status. Consistent with Stettin et al (23), vitamin B-12 concentration was not influenced by *H. pylori* serology in our study of ambulatory subjects with no reported digestive disorder. This can be explained by the fact that *H. pylori*–positive serologic status does not necessarily imply the presence of chronic atrophic gastritis. In a large, prospective, population-based cohort from Germany, among *H. pylori*–positive subjects only 2.7% developed chronic atrophic gastritis after 5 y of follow-up (24). Moreover, in a prospective study in 202 subjects, most *H. pylori*–positive subjects had normal egg yolk–cobalamin absorption test results (20).

TABLE 6
Predictors of plasma vitamin B-12 concentration in multivariate analysis in the total study population[1]

	P value			
	Univariate analysis	Multivariate analysis[2]	Coefficient (*β*)	SE
FUT2 461 G→A	0.0007[3]	0.0008[4]	−57.64[4]	17.19[4]
Male sex	0.0035[5]	<0.0001	−63.19	15.48
Geographic area, Africa	<0.0001[5]	<0.0001	147.71	16.68
Age (y)	<0.0001[6]	Not retained	—	—
Positive *Hp* serology	0.0500[5]	Not retained	—	—

[1] *FUT2*, fucosyltransferase 2; *Hp*, *Helicobacter pylori*; Not retained, not retained in the multiple regression model.
[2] Multiple regression analysis (stepwise method).
[3] Kruskal-Wallis test.
[4] For *FUT2 461, G/G* genotype.
[5] Mann-Whitney *U* test.
[6] Spearman's rank-order correlation coefficient.

In our study, *H. pylori*–positive subjects had lower plasma folate concentrations. This result is in agreement with a previous report (25), suggesting a role of *H. pylori*–related gastritis in modulating folate status. In our study, male sex was negatively and independently associated with plasma vitamin B-12 concentration. These results are in line with a previous study that reported a higher frequency of mild cobalamin deficiency in elderly white men in comparison with black and Asian American women (26).

To our knowledge, our study is the first that has examined the influence of *FUT2* polymorphism on *H. pylori* serologic status. Note that *FUT2 461 G→A* polymorphism as well as FUT2 secretor phenotype were not predictive of *H. pylori* positive serology. In addition, *FUT2 461 G/G* genotype was an independent predictor of vitamin B-12 concentration. It may be assumed that subjects with negative *H. pylori* serology had no reactive contact with *H. pylori*. Accordingly, a part of the influence of *FUT2* polymorphism on vitamin B-12 concentration was independent from *H. pylori*–related mechanisms. Hence, an alternative hypothesis could be that the reduced vitamin B-12 concentration in carriers of the FUT2 secretor phenotype results in part from an influence of *FUT2* on other mechanisms related with either absorption or transport of vitamin B-12. This needs to be investigated further in experimental models.

It should be noted that, similar to our study, the study by Hazra et al (4) showed that vitamin B-12 concentration remained within the normal range in each of the 3 genotypes of the *FUT2* rs601338 polymorphism. Hence, even though the *FUT2* polymorphism is involved in the variation of vitamin B-12 concentration, it is not by itself a predictor of vitamin B-12 deficiency. In our study, *FUT2* polymorphism consistently showed no effect on methylmalonate concentration in the 2 populations; it had

a very weak effect on homocysteine concentration in West African participants, and no effect in Europeans. Our results confirm those from 2 previous studies that found no influence of *FUT2* polymorphism on plasma homocysteine concentration (5, 6).

The higher concentration of vitamin B-12 observed in West African subjects, compared with Europeans, was presumably not related to environmental exposure, because this difference was previously observed between whites and Africans living in the United Kingdom (27). In line with our findings, a study in 30 Nigerians and 10 Europeans living in northern Nigeria found higher vitamin B-12 plasma concentrations in Nigerians than in Europeans (28). Interestingly, the plasma concentration of transcobalamin II was higher in Nigerian subjects than in Europeans subjects living in Nigeria (28). The reasons for this difference in the concentrations of vitamin B-12 have not been fully elucidated and might be related to genetic factors (29). Among these, *FUT2* would have had a limited influence to explain this higher concentration of vitamin B-12, because the frequency of FUT2 nonsecretor phenotype (*FUT2 461*, *A/A* genotype) was not significantly different in the 2 populations. Furthermore, *H. pylori* serologic status was not a contributing factor to this difference between Africans and Europeans, because it was not a predictor of vitamin B-12 concentration in either population.

In conclusion, this is the first study performed in a general population setting that evaluated the influence of *H. pylori* serologic status on the association between *FUT2 461 G→A* polymorphism and vitamin B-12 concentration. We found that *H. pylori* serologic status had no influence on this association. This suggests the involvement of another mechanism of *FUT2* in its influence on vitamin B-12 concentration, in addition to its possible effect on *H. pylori* gastritis, at least in ambulatory subjects from Europe and West Africa.

The authors' responsibilities were as follows—AO: conducted research, performed biochemical assays, analyzed data, performed statistical analyses, and contributed to writing the manuscript; CB: conducted research, analyzed data, performed genotyping analyses, and contributed to writing the manuscript; CC: analyzed data, performed genotyping analyses, and contributed to writing the manuscript; EJ: conducted research and performed biochemical assays; R-MG-R: conducted research, provided essential materials, participated in genotyping analyses, and analyzed data; GA: conducted research, recruited subjects, provided essential materials, and performed biochemical assays; PB: conducted research, recruited subjects, provided essential materials, and participated in genotyping analyses; ME, NC, AS, and EA: conducted research, recruited subjects, and provided essential materials; AR and PA: conducted research and provided essential materials; J-PB: conducted research; PG and JP: conducted research and performed biochemical assays; LP-B: conducted research, analyzed data, and contributed to writing the manuscript; and J-LG: designed and coordinated research, provided essential materials, analyzed data, contributed to writing the manuscript, and had primary responsibility for final content. None of the authors declared a conflict of interest.

REFERENCES

1. Li F, Watkins D, Rosenblatt DS. Vitamin B(12) and birth defects. Mol Genet Metab 2009;98(1-2):166–72.
2. Watanabe F. Vitamin B12 sources and bioavailability. Exp Biol Med (Maywood) 2007;232(10):1266–74.
3. Van den Veyver IB. Genetic effects of methylation diets. Annu Rev Nutr 2002;22:255–82.
4. Hazra A, Kraft P, Selhub J, Giovannucci EL, Thomas G, Hoover RN, Chanock SJ, Hunter DJ. Common variants of FUT2 are associated with plasma vitamin B12 levels. Nat Genet 2008;40(10):1160–2.
5. Hazra A, Kraft P, Lazarus R, Chen C, Chanock SJ, Jacques P, Selhub J, Hunter DJ. Genome-wide significant predictors of metabolites in the one-carbon metabolism pathway. Hum Mol Genet 2009;18(23):4677–87.
6. Tanaka T, Scheet P, Giusti B, Bandinelli S, Piras MG, Usala G, Lai S, Mulas A, Corsi AM, Vestrini A, et al. Genome-wide association study of vitamin B6, vitamin B12, folate, and homocysteine blood concentrations. Am J Hum Genet 2009;84(4):477–82.
7. Guéant JL, Elia M, Romano A. Associations between folate, vitamin B12, homocysteine and pathologies related to aging: the need to consider complex nutrient-nutrient and gene-nutrient interactions and the functional and socio-economic determinants in population-based studies. Clin Chem Lab Med 2007;45:127–9.
8. Gueant-Rodriguez RM, Gueant JL, Debard R, Thirion S, Hong LX, Bronowicki JP, Namour F, Chabi NW, Sanni A, Anello G, et al. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations. Am J Clin Nutr 2006;83(3):701–7.
9. Gueant JL, Chabi NW, Gueant-Rodriguez RM, Mutchinick OM, Debard R, Payet C, Lu X, Villaume C, Bronowicki JP, Quadros EV, et al. Environmental influence on the worldwide prevalence of a 776C->G variant in the transcobalamin gene (TCN2). J Med Genet 2007;44(6):363–7.
10. Amouzou EK, Chabi NW, Adjalla CE, Rodriguez-Gueant RM, Feillet F, Villaume C, Sanni A, Gueant JL. High prevalence of hyperhomocysteinemia related to folate deficiency and the 677C→T mutation of the gene encoding methylenetetrahydrofolate reductase in coastal West Africa. Am J Clin Nutr 2004;79:619–24.
11. Battaglia-Hsu SF, Akchiche N, Noel N, Alberto JM, Jeannesson E, Orozco-Barrios CE, Martinez-Fong D, Daval JL, Gueant JL. Vitamin B12 deficiency reduces proliferation and promotes differentiation of neuroblastoma cells and up-regulates PP2A, proNGF, and TACE. Proc Natl Acad Sci USA 2009;106(51):21930–5.
12. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 2009;169(4):505–14.
13. Conover WJ. Practical nonparametric statistics. 3rd ed. New York, NY: John Wiley, 1999.
14. Mollicone R, Bara J, Le Pendu J, Oriol R. Immunohistologic pattern of type 1 (Lea, Leb) and type 2 (X, Y, H) blood group-related antigens in the human pyloric and duodenal mucosae. Lab Invest 1985;53:219–27.
15. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB. Sequence and expression of a candidate for the human secretor blood group alpha (1,2)fucosyltransferase gene (FUT2): homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J Biol Chem 1995;270:4640–9.
16. Magalhaes A, Gomes J, Ismail MN, Haslam SM, Mendes N, Osorio H, David L, Le Pendu J, Haas R, Dell A, et al. Fut2-null mice display an altered glycosylation profile and impaired BabA-mediated Helicobacter pylori adhesion to gastric mucosa. Glycobiology 2009;19(12):1525–36.
17. Azevedo M, Eriksson S, Mendes N, Serpa J, Figueiredo C, Resende LP, Ruvoen-Clouet N, Haas R, Boren T, Le Pendu J, et al. Infection by Helicobacter pylori expressing the BabA adhesin is influenced by the secretor phenotype. J Pathol 2008;215:308–16.
18. Seetharam B, Alpers DH. Absorption and transport of cobalamin (vitamin B12). Annu Rev Nutr 1982;2:343–69.
19. Aimone-Gastin I, Pierson H, Jeandel C, Bronowicki JP, Plenat F, Lambert D, Nabet-Belleville F, Gueant JL. Prospective evaluation of protein bound vitamin B12 (cobalamin) malabsorption in the elderly using trout flesh labelled in vivo with 57Co-cobalamin. Gut 1997;41:475–9.
20. Carmel R, Aurangzeb I, Qian D. Associations of food-cobalamin malabsorption with ethnic origin, age, Helicobacter pylori infection, and serum markers of gastritis. Am J Gastroenterol 2001;96(1):63–70.
21. Annibale B, Capurso G, Delle Fave G. Consequences of Helicobacter pylori infection on the absorption of micronutrients. Dig Liver Dis 2002;34(suppl 2):S72–7.
22. Sipponen P, Laxen F, Huotari K, Harkonen M. Prevalence of low vitamin B12 and high homocysteine in serum in an elderly male

population: association with atrophic gastritis and Helicobacter pylori infection. Scand J Gastroenterol 2003;38:1209–16.
23. Stettin D, Waldmann A, Strohle A, Hahn A. Association between Helicobacter pylori-infection, C-reactive protein and status of B vitamins. Adv Med Sci 2008;53:205–13.
24. Adamu MA, Weck MN, Rothenbacher D, Brenner H. Incidence and risk factors for the development of chronic atrophic gastritis: five year follow-up of a population-based cohort study. Int J Cancer 2011;128:1652–8.
25. Tamura A, Fujioka T, Nasu M. Relation of Helicobacter pylori infection to plasma vitamin B12, folic acid, and homocysteine levels in patients who underwent diagnostic coronary arteriography. Am J Gastroenterol 2002;97:861–6.
26. Carmel R, Green R, Jacobsen DW, Rasmussen K, Florea M, Azen C. Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. Am J Clin Nutr 1999;70:904–10.
27. Moore SE, Mansoor MA, Bates CJ, Prentice AM. Plasma homocysteine, folate and vitamin B(12) compared between rural Gambian and UK adults. Br J Nutr 2006;96:508–15.
28. Fleming AF, Ogunfunmilade YA, Carmel R. Serum vitamin B12 levels and vitamin B12-binding proteins of serum and saliva of healthy Nigerians and Europeans. Am J Clin Nutr 1978;31:1732–8.
29. Carmel R. Ethnic and racial factors in cobalamin metabolism and its disorders. Semin Hematol 1999;36:88–100.

The American Journal of Clinical Nutrition