See corresponding editorial on page 1495.

Transcobalamin 776C→G polymorphism is associated with peripheral neuropathy in elderly individuals with high folate intake[1–3]

*Hathairat Sawaengsri,[4] Peter R Bergethon,[5] Wei Qiao Qiu,[6] Tammy M Scott,[4] Paul F Jacques,[4] Jacob Selhub,[4] and Ligi Paul[4]**

[4]Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA; [5]Neuroscience and Pain Research Unit, Pfizer Inc., Cambridge, MA; and [6]Boston University School of Medicine, Boston, MA

ABSTRACT

Background: The 776C→G polymorphism of the vitamin B-12 transport protein transcobalamin gene (*TCN2*) (rs1801198; Pro259Arg) is associated with a lower holotranscobalamin concentration in plasma. This effect may reduce the availability of vitamin B-12 to tissues even when vitamin B-12 intake is adequate. Clinical outcomes associated with vitamin B-12 insufficiency could potentially be worsened by high folate intake.

Objective: We determined the association of the *TCN2* 776C→G polymorphism and folate intake with peripheral neuropathy in elders with normal plasma concentrations of vitamin B-12.

Design: Participants in this cross-sectional study (n = 171) were from a cohort of community-based, home-bound elderly individuals aged ≥60 y who underwent an evaluation by physicians including an assessment for peripheral neuropathy. Participants were administered food-frequency and general health status questionnaires, anthropometric measurements were taken, and a fasting blood sample from each subject was collected.

Results: Odds of neuropathy were 3-fold higher for GG genotypes than for CC genotypes (OR: 3.33; 95% CI: 1.15, 9.64). When folate intake was >2 times the Recommended Dietary Allowance (800 μg), GG genotypes had 6.9-fold higher odds of neuropathy than CC genotypes (OR: 6.9; 95% CI: 1.31, 36.36). There was no difference between the genotypes in the odds of peripheral neuropathy when folate intake was ≤800 μg (OR: 1.5; 95% CI: 0.18, 12.33).

Conclusion: The *TCN2* 776C→G polymorphism is associated with increased odds of peripheral neuropathy in the elderly, even with a normal vitamin B-12 status, especially if their folate intake is >2 times the Recommended Dietary Allowance. *Am J Clin Nutr* 2016;104:1665–70.

Keywords: folate, folic acid, peripheral neuropathy, *TCN2* 776C>G polymorphism, transcobalamin, vitamin B-12

INTRODUCTION

Transcobalamin is a vitamin B-12–binding protein that transports the vitamin from the ileum to the tissues (1). The 776C→G single nucleotide polymorphism in the gene for transcobalamin (*TCN2*) (rs1801198) results in a Pro259Arg substitution in the transcobalamin protein (2). This polymorphism affects the binding of vitamin B-12 by transcobalamin and is associated with a lower concentration of holotranscobalamin (vitamin B-12–bound transcobalamin) in plasma, which, in turn, may affect the availability of vitamin B-12 to the tissues (3–5). There are 2 vitamin B-12–dependent reactions in mammalian cells as follows: the synthesis of methionine from homocysteine and methyltetrahydrofolate catalyzed by the enzyme methionine synthase and the conversion of methylmalonyl CoA to succinyl CoA by methylmalonyl-CoA mutase. Methionine is the precursor of *S*-adenosyl methionine, which is the biological methyl donor for methylation reactions in the cell. *S*-adenosyl homocysteine, which is generated during every methylation reaction, is hydrolyzed to homocysteine. When vitamin B-12 availability is limited, homocysteine and methylmalonic acid concentrations increase in plasma. Thus, total homocysteine and methylmalonic acid in plasma serve as functional biomarkers for vitamin B-12 status in tissues. The GG genotype of the 776C→G polymorphism of *TCN2* is associated with a higher concentration of homocysteine in individuals with lower plasma vitamin B-12 (6). However, the G allele has also been reported to be associated with lower homocysteine under low vitamin B-12 status (7). In a population with normal plasma values for vitamin B-12, the concentration of methylmalonic acid was higher in GG genotypes (4). These studies indicate that the *TCN2* polymorphism is functional and affects the availability of vitamin B-12 in tissues for metabolic reactions.

A lack of sufficient vitamin B-12 is associated with peripheral neuropathy, anemia, depression, and cognitive impairment (8–10). Peripheral neuropathy with characteristic features is a frequent clinical outcome in vitamin B-12 deficiency, which has been observed even in the absence of anemia or macrocytosis (11). It has also been reported that older adults may present with

[1] Supported by the USDA (special cooperative agreement 58-1950-4-003) and the National Institute on Aging (grant AG21790-01).

[2] Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA.

[3] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: ligi.paul_pottenplackel@tufts.edu.

Received May 27, 2016. Accepted for publication September 8, 2016.

First published online October 12, 2016; doi: 10.3945/ajcn.116.139030.

clinical conditions and elevated concentrations of plasma biomarkers that are associated with vitamin B-12 insufficiency when their plasma concentrations of vitamin B-12 are low but in the normal range (8, 9, 12, 13). The objective of this study was to investigate the association between the *TCN2* polymorphism and peripheral neuropathy in individuals aged ≥60 y with normal plasma concentrations of vitamin B-12. Early reports on folic acid supplementation of vitamin B-12–deficient patients have shown that folic acid ameliorates the megaloblastic anemia and hematologic abnormalities seen in severe vitamin B-12 deficiency but has no effect on the neurologic conditions that are associated with the deficiency (14). Current evidence has suggested that excess folate intake worsens the clinical and metabolic manifestations of vitamin B-12 insufficiency as indicated by increases in the prevalences of anemia and cognitive dysfunction and the concentrations of homocysteine and methylmalonic acid in plasma (15–19). Hence, we hypothesized that high folate intake can modify the association between the *TCN2* polymorphism and peripheral neuropathy.

METHODS

Study population

This cross-sectional study consisted of participants from the Nutrition, Aging, and Memory in Elders study, which is a cohort of community-based, home-bound elderly participants aged ≥60 y who were recruited during 2003–2007 from the Boston area as described in detail previously (20). Participants were recruited from Boston's 3 Aging Services Access Points, which are home care agencies that provide services to the low-income elderly to facilitate independent living. The exclusion criteria were a lack of English fluency, severe auditory or visual impairment, brain tumor, severe mental retardation, schizophrenia, bipolar disorder, HIV or AIDS, or a diagnosis of epilepsy as documented in the case records of Aging Services Access Points. Informed consent for all tests including genotyping was obtained. Individuals with severe cognitive impairment or dementia [a score ≤10 on Mini-Mental State Examination (score range: 0–30)] or who had an estimated intelligence quotient <75 (as measured with the use of the North American Adult Reading Test) were excluded from the study. The participants were administered food-frequency and general health status questionnaires, anthropometric measurements were taken, and a fasting blood sample was collected from each subject. From the total 1248 participants in the cohort, a planned subset of 366 participants who consented to additional tests and were able to visit the hospital by taxi or wheelchair van underwent an evaluation by physicians that included an assessment of peripheral neuropathy and gait and balance at Tufts Medical Center (20). Participants were excluded from the current study if data were missing for the *TCN2* genotype ($n = 53$) or any of the covariates ($n = 86$), if they had a stroke ($n = 43$), or if they had a plasma vitamin B-12 concentration <148 pmol/L ($n = 2$), which was indicative of deficiency, or >701 pmol/L ($n = 11$), which may have been indicative of malignancies or abnormalities (21–23). A total of 171 participants who underwent an evaluation by the physicians met the criteria for the current study (**Supplemental Figure 1**). The study was approved by the Institutional Review Board at Tufts University and Tufts Medical Center.

Blood analyses

Blood samples were collected after ≥7 h of fasting. Concentrations of vitamin B-12 and folate in plasma were measured with the use of Quantaphase II vitamin B-12/folate radioassay (BioRad Laboratories) (interassay CVs of 4.3% for vitamin B-12 and 7.1% for folate), plasma total homocysteine was determined with the use of an HPLC method (24) (CV: 6%), and serum creatinine was determined with the use of a modified Jaffe reaction (25) (CV: 4%). The plasma concentration of pyridoxal 5′-phosphate, which is the functional form of vitamin B-6, was determined with the use of a tyrosine-decarboxylase-apoenzyme method (CV: 8%) (26). Blood glucose was measured with the use of a hexokinase method with a Beckman Coulter AU400e instrument (Beckman Coulter Inc.) (CV: 3.4%).

DNA isolation and genotyping

DNA was isolated with the use of QIAmp DNA Blood Mini Kit (Qiagen). The *TCN2* 776C→G polymorphism was determined with the use of a Taqman allelic discrimination assay, with primers and probes that were specific for the polymorphism, and Taqman Universal Master Mix (Applied Biosystems). No-template controls and duplicate DNA samples were included in the assays for quality control.

Peripheral neurologic examination

A diagnosis of peripheral neuropathy was made by a board-certified neurologist from the neurologic history and a complete elementary neurologic examination was performed that included both lower limbs. The sensory examination included a timed vibration with a 128-Hz tuning fork that was measured bilaterally at the distal phalanges of great toe. The distal vibration test was scored on the basis of the report of a loss of vibration by the participant <10 s before the examiner perceived the loss of vibration (normal to mild), ≥10 s (mild to moderate), or the inability of the patient to feel the maximum vibration of the tuning fork (severe to total). The predictive value and reproducibility of the tuning fork test for peripheral neuropathy has been shown to be better than that of a monofilament test (27). In addition, subjects were evaluated for slow and fast proprioceptive sensations at the distal phalange of the great toe. Proprioceptive loss in either of these modalities was considered to be evidence of large-fiber neuropathy. Sensitivity to a pinprick, temperature, and a light touch were also determined and mapped to define a level of involvement that was typically in a distal to proximal pattern. Deep-tendon reflexes at the Achilles, patella, biceps, and triceps were tested. Peripheral neuropathy was diagnosed as the presence of a consistent pattern of large-fiber polyneuropathy that was characterized by a loss of either vibratory sense or proprioception. Typically, these subjects had decreased or absent Achilles deep-tendon reflexes. Neuropathy was also diagnosed if signs of small-fiber loss that were characterized by a loss of pain or temperature sensation, numbness, or dysesthesia were shown on the examination, which was typically in a distal-to-proximal pattern of loss. This pattern of sensory loss along with a loss of deep-tendon reflexes in the Achilles as well as the patella was considered evidence of small-fiber polyneuropathy. All subjects were examined by the same neurologist.

Other covariates

Folate and vitamin B-12 intakes were determined with the use of a validated semiquantitative food-frequency questionnaire (28). Information on the other covariates that were used in this study were collected as follows: age, sex, race or ethnicity, education, and smoking status (on the basis of a participant self-report); diabetes (on the basis of a blood glucose concentration >7 mmol/L and reported use of diabetes medication); hypertension (on the basis of systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg and reported use of medications for hypertension); and BMI (in kg/m^2), which was calculated as measured weight divided by the square of measured height).

Statistical analysis

ANOVA was used to describe participant characteristics by genotype categories for continuous outcomes, and Pearson's chi-square test was used for categorical variables. For diabetes and hypertension, adjustments were made for age, sex, and race or ethnicity. The association of the *TCN2* 776C→G polymorphism with plasma concentrations of vitamin B-12 and total homocysteine was determined with the use of ANCOVA. Logistic regression was used to determine the OR with 95% CIs of having peripheral neuropathy in the GG and CG genotypes compared with in the reference genotype CC. In a secondary analysis to determine whether folate intake modified the association of peripheral neuropathy with the *TCN2* polymorphism, we stratified the cohort on the basis of folate intake >2 times the Recommended Dietary Allowance (>800 compared with ≤800 μg dietary folate equivalent/d) or on the basis of the median plasma folate concentrations (>27.9 compared with ≤27.9 nmol/L) before calculating the association or OR as previously described. The interaction between the *TCN2* genotype and folate intake in relation to risk of peripheral neuropathy was determined with the use of the interaction term genotype × folate intake in a logistic regression analysis. For all analyses, adjustments were made for the following covariates: age, sex, BMI, race or ethnicity, education (as an indicator of socioeconomic status), serum creatinine concentration (as an indicator of kidney function), smoking status, alcohol consumption, diabetes, hypertension, and vitamin B-12 intake. We adjusted for vitamin B-12 intake in the analyses to distinguish the effect of the *TCN2* polymorphism from that of vitamin B-12 intake on the outcomes. In addition, plasma concentrations of folate and pyridoxal 5′-phosphate were used as covariates for the plasma total homocysteine concentration to identify the effect of the *TCN2* polymorphism separate from that of these vitamins. Plasma concentrations of homocysteine were log transformed before analyses because of a skewed distribution. Adjustments were made for multiple comparisons (Tukey's test). There were 10 participants whose vitamin B-12 intakes were less than the recommended 2.4 μg/d. All the analyses were repeated after the exclusion of these participants. All analyses were performed with SAS 9.3 software (SAS Institute Inc.), and a 2-sided $P < 0.05$ was considered significant.

TABLE 1
Characteristics of study participants by *TCN2* 776C→G genotype[1]

Characteristic	Total (n = 171)	CC (n = 79)	CG (n = 61)	GG (n = 31)	P
Age, y	73.5 ± 8.0[2]	73.2 ± 7.9	73.8 ± 8.3	73.8 ± 8.0	0.90
Women, n (%)	121 (70.8)	55 (69.6)	41 (67.2)	25 (80.7)	0.39
BMI, kg/m^2	31.9 ± 8.6	32.6 ± 9.9	31.7 ± 7.1	30.5 ± 7.7	0.50
Education, n (% within genotype)					0.66
Kindergarten through grade eight	21 (12.3)	10 (12.7)	7 (11.5)	4 (12.9)	
Grades 9–11	19 (11.1)	11 (13.9)	6 (9.8)	2 (6.5)	
Grade 12 or high school	47 (27.5)	25 (31.7)	17 (27.9)	5 (16.1)	
Any undergraduate	70 (40.9)	28 (35.4)	26 (42.6)	16 (51.6)	
Any graduate	14 (8.2)	5 (6.3)	5 (8.2)	4 (12.9)	
Race or ethnicity, n (% within genotype)					<0.001
American Indian or Alaskan Native	3 (1.8)	3 (3.8)	0 (0)	0 (0)	
Asian	3 (1.8)	1 (1.3)	0 (0)	2 (6.5)	
Non-Hispanic black	58 (33.9)	39 (49.4)	16 (26.2)	3 (9.7)	
Hispanic	2 (1.2)	0 (0)	1 (1.6)	1 (3.2)	
Non-Hispanic white	105 (61.4)	36 (45.6)	44 (72.1)	25 (80.7)	
Current smoker, n (%)	39 (22.8)	18 (22.8)	14 (23.0)	7 (22.6)	1.00
Alcohol, g/d	2.0 ± 5.4	1.3 ± 4.3	3.2 ± 7.2	1.4 ± 3.2	0.11
Vitamin B-12 intake, μg/d	16.6 ± 16.6	14.8 ± 16.8	18.2 ± 16.0	17.8 ± 17.5	0.44
Folate intake, μg DFE/d	928.3 ± 576.0	863.5 ± 616.0	985.4 ± 543.0	980.8 ± 531.5	0.40
Plasma metabolites					
Folate, nmol/L	32.0 ± 19.7	31.0 ± 18.8	31.5 ± 21.1	34.4 ± 20.2	0.71
Pyridoxal 5′-phosphate, nmol/L	79.7 ± 83.34	76.5 ± 89.0	82.9 ± 76.9	82.1 ± 83.8	0.89
Creatinine, μmol/L	78.7 ± 26.5	79.6 ± 26.5	77.8 ± 35.4	75.1 ± 26.5	0.68
Diabetes, n (%)[3]	49 (28.7)	20 (25.3)	20 (32.8)	9 (29.0)	0.54
Hypertension, n (%)[3]	142 (83.0)	68 (86.1)	53 (86.9)	21 (67.7)	0.21

[1] Between-genotype differences were analyzed with the use of Pearson's chi-square test for categorical variables and a 1-factor ANOVA for continuous variables. DFE, dietary folate equivalent; *TCN2*, transcobalamin gene.
[2] Mean ± SD (all such values).
[3] ANCOVA adjusted for age, sex, and race or ethnicity.

TABLE 2
Concentrations of plasma metabolites by *TCN2* 776C→G genotype[1]

Metabolite	Total (n = 171)	CC (n = 79)	CG (n = 61)	GG (n = 31)	P
Vitamin B-12,[2] pmol/L	366.0 ± 9.8	398.0 ± 30.5	336.5 ± 31.7*	317.0 ± 33.9**	<0.01
Homocysteine,[3] μmol/L	10.7 ± 1.0	9.9 ± 1.1	10.1 ± 1.1	10.7 ± 1.1	0.61

[1] *TCN2*, transcobalamin gene.
[2] All values are means ± SEs. ANCOVA was used to determine the association of the *TCN2* polymorphism with the plasma concentration of vitamin B-12. Adjustments were made for sex, age, BMI, education, alcohol intake, race or ethnicity, smoking status, vitamin B-12 intake, and serum creatinine concentration as well as for multiple comparisons. *,**Compared with the CC genotype, *$P < 0.02$; **$P = 0.01$.
[3] All values are geometric means ± SEs. ANCOVA was used to determine the association of the *TCN2* polymorphism with the plasma concentration of homocysteine. Adjustments were made for sex, age, BMI, education, alcohol intake, race or ethnicity, smoking status, vitamin B-12 intake, serum concentrations of creatinine, and plasma concentrations of folate and pyridoxal 5′-phosphate as well as for multiple comparisons.

RESULTS

The prevalence of homozygosity for the variant allele G of the *TCN2* 776C→G polymorphism was 17.2% in this cohort (**Table 1**). Individuals with the GG and CG genotypes had significantly lower plasma vitamin B-12 than that of individuals with the CC genotype even after adjusting for vitamin B-12 intake ($P < 0.02$) (**Table 2**). The concentration of plasma total homocysteine was similar for all genotypes after adjusting for the covariates (Table 2).

Of the 171 participants who were assessed for peripheral neuropathy, 75 individuals were diagnosed with peripheral neuropathy (**Table 3**). The odds of having peripheral neuropathy were 3.3-fold higher in individuals with the GG genotype than in individuals with the CC genotype (OR: 3.33; 95% CI: 1.15, 9.64) (Table 3). There was no difference between the CG genotype and the CC genotype in odds of peripheral neuropathy (OR: 1.26; 95% CI: 0.56, 2.81) (Table 3).

Because high folate intake can potentially worsen conditions that are associated with vitamin B-12 insufficiency (15), we determined if the association between the *TCN2* polymorphism and peripheral neuropathy was modified by folate intake in a secondary analysis. There was no significant interaction between the *TCN2* genotype and folate intake on risk of peripheral neuropathy ($P = 0.8$). However, because we had an a priori hypothesis that high folate intake may modify the effect of the *TCN2* polymorphism, we stratified the samples on the basis of total folate intake. When folate intake was >2 times the Recommended Dietary Allowance (800 μg/d), GG genotypes had 7-fold higher odds of neuropathy than CC genotypes (OR: 6.9; 95% CI: 1.31, 36.36) (Table 3).There was no significant difference between genotypes in odds of peripheral neuropathy when folate intake was ≤800 μg/d (OR: 1.49; 95% CI: 0.18, 12.33) (Table 3). The mean natural folate and folic acid intake of subjects who consumed >800 μg total folate/d was higher than that of those who consumed ≤800 μg total folate/d [in >800- and ≤800-μg folate intake/d categories, means ± SDs of natural folate intakes of 312.6 ± 124 compared with 245 ± 87 μg ($P < 0.001$), respectively, and of folic acid intakes of 572 ± 204 compared with 149.5 ± 53 μg ($P < 0.0001$), respectively]. In individuals in the high-folate group, 93% of subjects were multivitamin users. The median plasma folate concentration in individuals with total folate intake ≤800 μg/d was 19.5 nmol/L, and for subjects whose intake was >800 μg/d, the median plasma folate concentration was 40 nmol/L. However, the plasma folate concentration did not modify the odds of peripheral neuropathy in GG genotypes when the analyses were conducted after stratifying by median plasma folate. When the analyses were limited only to subjects whose dietary intakes of vitamin B-12 met the Recommended Dietary Allowance, the results for all of the outcomes studied were not changed.

DISCUSSION

In this study, we report the association of the 776C→G polymorphism in the transcobalamin gene *TCN2* with peripheral neuropathy in older adults with normal plasma concentrations of vitamin B-12. Peripheral neuropathy associated with vitamin

TABLE 3
Association between peripheral neuropathy and *TCN2* 776C→G genotype[1]

	CC genotype (reference group)		CG genotype		GG genotype (variant)	
	Cases/total, n	OR	Cases/total, n	OR (95% CI)	Cases/total, n	OR (95% CI)
Overall	33/79	1	25/61	1.26 (0.56, 2.81)	17/31	3.33 (1.15, 9.64)*
Folate intake,[2] μg DFE/d						
≤800	24/47	1	11/26	1.11 (0.31, 3.93)	7/12	1.49 (0.18, 12.33)
>800	9/32	1	14/35	1.30 (0.38, 4.51)	10/19	6.90 (1.31, 36.36)**

[1] Logistic regression analyses were adjusted for sex, age, BMI, education, alcohol intake, serum creatinine concentration, race or ethnicity, smoking status, vitamin B-12 intake, diagnosis of diabetes, and diagnosis of hypertension. *$P = 0.03$, **$P = 0.02$. DFE, dietary folate equivalent; *TCN2*, transcobalamin gene.
[2] *TCN2* genotype × folate intake, P-interaction = 0.8.

B-12 insufficiency is usually reversible with vitamin B-12 treatment (11). In our cohort, despite having a normal plasma concentration of vitamin B-12, the odds of development of peripheral neuropathy was 3.3-fold higher in GG genotypes than in CC genotypes of the *TCN2* polymorphism. The association of the *TCN2* polymorphism with peripheral neuropathy was independent of vitamin B-12 intake.

Evidence suggests that high folate intake may worsen the clinical conditions associated with vitamin B-12 insufficiency in the elderly as indicated by the increased prevalence of anemia and cognitive impairment (15, 16, 19). The mechanism behind this association is still under investigation. The Recommended Dietary Allowance for folate is 400 μg dietary folate equivalent/d. The odds of neuropathy were significantly higher for GG genotypes than for CC genotypes when folate intakes were >2 times the Recommended Dietary Allowance (>800 μg/d) but not when the folate intakes were ≤800 μg/d. These results have been supported by a recent study on vitamin B-12 supplementation of elderly individuals who were deficient in the vitamin, in which the improvement of vitamin B-12 status after supplementation was impaired by high serum folate status (29). Both natural folate and synthetic folic acid from fortified foods and supplements contributed to total folate intake in our population. In this cohort, folic acid contributed to a large proportion of total folate intake in subjects who consumed >800 μg/d. Because 93% of the participants in the group with high folate intake also used folic acid–containing supplements, it appears that high total folate intake was mainly due to high folic acid intake. A previous study on folate intake and immune function suggested that folic acid supplementation may have a positive effect when food-folate intake is low, but when food-folate intake is adequate, supplementation may have a negative effect (30). The mean total folate intake of our cohort was 928.3 ± 576 μg dietary folate equivalent/d, which was similar to mean folate intake of the general US population aged ≥51 y as reported for the participants of the NHANES 2003–2006 (31), which indicated that excess folate intake is widely prevalent in older adults. The frequency of homozygosity for the G allele of the *TCN2* polymorphism in our cohort was similar to what has been previously reported in the United States (4). Thus, the results of our study are relevant to a large population of older adults who are at increased risk of neurological impairment because of the *TCN2* polymorphism.

When analyses were done after stratifying on the basis of the median plasma concentration of folate, higher plasma folate was not associated with increased odds of peripheral neuropathy in GG genotypes. This outcome might have been due to the fact that plasma folate does not always proportionately reflect total folate intake (32). In our cohort, 25.6% of subjects who consumed >800 μg folate/d had less than or equal to the median folate concentration, and 24.7% of subjects who consumed ≤800 μg/d had greater than the median folate concentration.

Despite the prevalence of peripheral neuropathy and its association with the *TCN2* polymorphism in the study population, macrocytic anemia, which is another condition that is frequently observed in vitamin B-12 insufficiency, was observed in only one participant. This finding was not unusual because previous studies have reported neurologic disorders that were associated with low vitamin B-12 in the absence of macrocytosis or anemia in the elderly (11).

Individuals with GG and CG genotypes had lower plasma concentrations of vitamin B-12 than did those with the CC genotype, although the values were in the normal range in our cohort. These results could be explained by the lower transcobalamin protein concentration, as well as its impaired binding of vitamin B-12 by the variant enzyme (3, 5), which may have resulted in the renal excretion of vitamin B-12. These results were different from those of previous studies that reported no differences between genotypes or that GG genotypes had higher vitamin B-12 concentrations in plasma (4, 6). We did not observe a relation between the *TCN2* polymorphism and plasma concentration of total homocysteine. There have been contradictory reports of higher homocysteine in individuals with GG genotypes (6, 33) as well as a lack of a genotype effect (4, 34).

A limitation of our study is the cross-sectional design of the cohort, which did not allow us to determine a cause-effect relation. Another limitation is the small sample size, as shown by the wide CI of the OR. In a larger sample size with a smaller CI, the odds of peripheral neuropathy due to the *TCN2* polymorphism might have been lower. The lack of an interaction between the *TCN2* genotype and folate intake in relation to risk of peripheral neuropathy could also have been due to the small sample size. The prevalence of neuropathy was 43.9% in the study participants who were assessed for neuropathy. This value was higher than the 23.8% that was reported for individuals aged ≥60 y in the general US population in the NHANES (1999–2000) (35). One possible reason for this discrepancy could have been the fact that the NHANES population was healthier than our cohort, which was comprised of individuals who needed assistance for independent living. There were more women than men in the subset who were assessed for neuropathy, but women were at lower risk of peripheral neuropathy in our population as well as in other populations (35); consequently, a study with more men would only have strengthened the association between the polymorphism and neuropathy. Our study population was comprised of individuals from different racial backgrounds, but they were predominantly non-Hispanic whites (61.4%) and non-Hispanic African Americans (33.9%). Although lower-extremity diseases such as peripheral neuropathy are more prevalent in non-Hispanic African Americans (35), the *TCN2* polymorphism is less prevalent in this group than in non-Hispanic whites (Table 1). Hence, the association between the *TCN2* polymorphism and peripheral neuropathy that we observed in this study was not driven by race. Diabetes increases risk of peripheral neuropathy, but we did not make a distinction between diabetic and nondiabetic patients in our analysis because of the small sample size. The prevalence of diabetes was similar in all genotypes (Table 1), and thus the association of the *TCN2* polymorphism and peripheral neuropathy was not significantly affected by the presence of diabetes.

In conclusion, our study shows that the *TCN2* 776C→G polymorphism is associated with increased risk of peripheral neuropathy in older adults despite having adequate vitamin B-12 status. The data also suggest that the odds of peripheral neuropathy are higher for GG genotypes if they consume excess folate. The prevalence of peripheral neuropathy in the US population in individuals aged ≥60 y is 23.8% and is higher at 34.7% in individuals aged ≥80 y (35). Intake of excess folate is also more prevalent in older adults in the United States (36). Our data suggest that increased risk of neuropathy in older adults because of the *TCN2* polymorphism may be avoided by limiting folate intake to be close to the Recommended Dietary Allowance and exercising caution with regards to the consumption of folic acid supplements.

The authors' responsibilities were as follows—HS, PRB, WQQ, TMS, and LP: conducted the research; HS, PRB, and LP: wrote the manuscript; HS and PFJ: performed statistical analysis; JS and LP: designed the research; LP: had primary responsibility for final content of the manuscript; and all authors: contributed to the critical revision of the manuscript and read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES

1. Seetharam B, Bose S, Li N. Cellular import of cobalamin (vitamin B-12). J Nutr 1999;129:1761–4.
2. Li N, Sood GK, Seetharam S, Seetharam B. Polymorphism of human transcobalamin II: substitution of proline and/or glutamine residues by arginine. Biochim Biophys Acta 1994;1219:515–20.
3. Namour F, Olivier J, Abdelmouttaleb I, Adjalla C, Debard R, Salvat C, Gueant J. Transcobalamin codon 259 polymorphism in HT-29 and Caco-2 cells and in Caucasians: relation to transcobalamin and homocysteine concentration in blood. Blood 2001;97:1092–8.
4. Miller JW, Ramos MI, Garrod MG, Flynn MA, Green R. Transcobalamin II 775G>C polymorphism and indices of vitamin B-12 status in healthy older adults. Blood 2002;100:718–20.
5. Afman LA, Lievers KJ, van der Put NM, Trijbels FJ, Blom HJ. Single nucleotide polymorphisms in the transcobalamin gene: relationship with transcobalamin concentrations and risk for neural tube defects. Eur J Hum Genet 2002;10:433–8.
6. Stanisławska-Sachadyn A, Woodside JV, Sayers CM, Yarnell JW, Young IS, Evans AE, Mitchell LE, Whitehead AS. The transcobalamin (TCN2) 776C>G polymorphism affects homocysteine concentrations among subjects with low vitamin B-12 status. Eur J Clin Nutr 2010;64:1338–43.
7. Garrod MG, Allen LH, Haan MN, Green R, Miller JW. Transcobalamin C776G genotype modifies the association between vitamin B-12 and homocysteine in older Hispanics. Eur J Clin Nutr 2010;64:503–9.
8. Lindenbaum J, Rosenberg IH, Wilson PW, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr 1994;60:2–11.
9. Bell IR, Edman JS, Marby DW, Satlin A, Dreier T, Liptzin B, Cole JO. Vitamin B-12 and folate status in acute geropsychiatric inpatients: Affective and cognitive characteristics of a vitamin nondeficient population. Biol Psychiatry 1990;27:125–37.
10. Stabler SP. Vitamin B12 deficiency. N Engl J Med 2013;368:2041–2.
11. Lindenbaum J, Healton EB, Savage DG, Brust JCM, Garrett TJ, Podell ER, Margell PD, Stabler SP, Allen RH. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988;318:1720–8.
12. Goodwin JS, Goodwin JM, Garry PJ. Association between nutritional status and cognitive functioning in a healthy elderly population. JAMA 1983;249:2917–21.
13. Smith AD, Refsum H. Vitamin B-12 and cognition in the elderly. Am J Clin Nutr 2009;89:707S–11S.
14. Will JJ, Mueller JF, Brodine C, Kiely CE, Friedman B, Hawkins VR, Dutra J, Vilter RW. Folic acid and vitamin B-12 in pernicious anemia; studies on patients treated with these substances over a ten year period. J Lab Clin Med 1959;53:22–38.
15. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. Am J Clin Nutr 2007;85:193–200.
16. Morris MS, Selhub J, Jacques PF. Vitamin B-12 and folate status in relation to decline in scores on the mini-mental state examination in the Framingham Heart Study. J Am Geriatr Soc 2012;60:1457–64.
17. Selhub J, Morris MS, Jacques PF. In vitamin B-12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. Proc Natl Acad Sci USA 2007;104:19995–20000.
18. Miller JW, Garrod MG, Allen LH, Haan MN, Green R. Metabolic evidence of vitamin B-12 deficiency, including high homocysteine and methylmalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. Am J Clin Nutr 2009;90:1586–92.
19. Moore EM, Ames D, Mander AG, Carne RP, Brodaty H, Woodward MC, Boundy K, Ellis KA, Bush AI, Faux NG, et al. Among vitamin B-12 deficient older people, high folate levels are associated with worse cognitive function: combined data from three cohorts. J Alzheimers Dis 2014;39:661–8.
20. Scott TM, Peter I, Tucker KL, Arsenault L, Bergethon P, Bhadelia R, Buell J, Collins L, Dashe JF, Griffith J, et al. The Nutrition, Aging, and Memory in Elders (NAME) study: design and methods for a study of micronutrients and cognitive function in a homebound elderly population. Int J Geriatr Psychiatry 2006;21:519–28.
21. Carmel R, Vasireddy H, Aurangzeb I, George K. High serum cobalamin levels in the clinical setting-clinical associations and holotranscobalamin changes. Clin Lab Haematol 2001;23:365–71.
22. Ermens AA, Vlasveld LT, Lindemans J. Significance of elevated cobalamin (vitamin B12) levels in blood. Clin Biochem 2003;36:585–90.
23. Andrès E, Serraj K, Zhu J, Vermorken AJ. The pathophysiology of elevated vitamin B-12 in clinical practice. QJM 2013;106:505–15.
24. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987;422:43–52.
25. Husdan H, Rapoport A. Estimation of creatinine by the Jaffe reaction. A comparison of three methods. Clin Chem 1968;14:222–38.
26. Shin YS, Rasshofer R, Friedrich B, Endres W. Pyridoxal-5′-phosphate determination by a sensitive micromethod in human blood, urine and tissues; its relation to cystathioninuria in neuroblastoma and biliary atresia. Clin Chim Acta 1983;127:77–85.
27. Meijer JW, Smit AJ, Lefrandt JD, van der Hoeven JH, Hoogenberg K, Links TP. Back to basics in diagnosing diabetic polyneuropathy with the tuning fork! Diabetes Care 2005;28:2201–5.
28. Willett WC, Reynolds RD, Cottrell-Hoehner S, Sampson L, Browne ML. Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. J Am Diet Assoc 1987;87:43–7.
29. Brito A, Verdugo R, Hertrampf E, Miller JW, Green R, Fedosov SN, Shahab-Ferdows S, Sanchez H, Albala C, Castillo JL, et al. Vitamin B-12 treatment of asymptomatic, deficient, elderly Chileans improves conductivity in myelinated peripheral nerves, but high serum folate impairs vitamin B12 status response assessed by the combined indicator of vitamin B12 status. Am J Clin Nutr 2016;103:250–7.
30. Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B, Selhub J, McTiernan A, Yasui Y, Oral E, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 2006;136:189–94.
31. Bailey RL, Dodd KW, Gahche JJ, Dwyer JT, McDowell MA, Yetley EA, Sempos CA, Burt VL, Radimer KL, Picciano MF. Total folate and folic acid intake from foods and dietary supplements in the United States: 2003-2006. Am J Clin Nutr 2010;91:231–7.
32. Hao L, Yang QH, Li Z, Bailey LB, Zhu JH, Hu DJ, Zhang BL, Erickson JD, Zhang L, Gindler J, et al. Folate status and homocysteine response to folic acid doses and withdrawal among young Chinese women in a large-scale randomized double-blind trial. Am J Clin Nutr 2008;88:448–57.
33. Lievers KJ, Afman LA, Kluijtmans LA, Boers GH, Verhoef P, den Heijer M, Trijbels FJ, Blom HJ. Polymorphisms in the transcobalamin gene: association with plasma homocysteine in healthy individuals and vascular disease patients. Clin Chem 2002;48:1383–9.
34. von Castel-Dunwoody KM, Kauwell GP, Shelnutt KP, Vaughn JD, Griffin ER, Maneval DR, Theriaque DW, Bailey LB. Transcobalamin 776C->G polymorphism negatively affects vitamin B-12 metabolism. Am J Clin Nutr 2005;81:1436–41.
35. Gregg EW, Sorlie P, Paulose-Ram R, Gu Q, Eberhardt MS, Wolz M, Burt V, Curtin L, Engelgau M, Geiss L. Prevalence of lower-extremity disease in the U.S. adult population ≥40 Years of age with and without diabetes: 1999–2000 National Health and Nutrition Examination Survey. Diabetes Care 2004;27:1591–7.
36. Bailey RL, Mills JL, Yetley EA, Gahche JJ, Pfeiffer CM, Dwyer JT, Dodd KW, Sempos CT, Betz JM, Picciano MF. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged ≥60 y in the United States. Am J Clin Nutr 2010;92:383–9.