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Transcobalamin Polymorphism 67A->G, but Not 776C->G, Affects Serum Holotranscobalamin in a Cohort of Healthy Middle-Aged Men and Women[1–3]

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Abstract

Two polymorphic variants in the gene coding for transcobalamin II (*TCN2*), *TCN2* 776C- > G and *TCN2* 67A- > G, may alter serum holotranscobalamin (holoTC), which in turn may affect cellular uptake of cobalamin (Cbl) and thereby Cbl status indicators. We studied the effects of *TCN2* 776C- > G and *TCN2* 67A- > G on blood concentrations of holoTC, Cbl, methylmalonic acid (MMA), and total homocysteine (tHcy) in 2411 individuals (50–64 y) that had been selected on the basis of these *TCN2* genotypes from 10601 Norwegian inhabitants. The serum holoTC concentration was lower in *TCN2* 67AG (55 ± 0.75 pmol/L) and 67GG (48 ± 2.14 pmol/L) than in 67AA (62 ± 0.67 pmol/L) ($P < 0.001$) but did not differ among *TCN2* 776C- > G genotypes. The polymorphisms interacted as serum holoTC determinants ($P = 0.001$) and the presence of *TCN2* 67AG and GG in strata of 776CC and CG, but not 776GG, increased the risk of having serum holoTC < 45.6 pmol/L [tertile 1 vs. tertiles 2 and 3: OR = 2.5 (95% CI 1.8–3.5) for 67AG; OR = 5.7 (95% CI 3.5–9.1) for 67GG in 776CC; OR = 2.1 (95% CI 1.6–2.9) for 67AG; and OR = 4.5 (95% CI 2.4–8.2) for 67GG in 776CG; all $P < 0.001$]. Plasma MMA, tHcy, and Cbl were not affected by either polymorphism. In summary, serum holoTC, but not plasma Cbl, MMA, or tHcy, varied according to *TCN2* 67A- > G genotypes. It remains to be determined whether this polymorphic effect on serum holoTC alters its diagnostic utility as Cbl status indicator. J. Nutr. 141: 1784–1790, 2011.

Introduction

Vitamin B-12 (Cbl)[12] is bound to 2 transport proteins in plasma. Most circulating Cbl is bound to transcobalamin I. Up to 37% is bound to TC that transports Cbl to the cells (1), where the Cbl-TC (holoTC) complex is taken up by receptor-mediated endocytosis (2). Intracellular Cbl serves as coenzyme for the 2 enzymes methionine synthase and methylmalonyl-CoA mutase that are involved in Hcy and MMA metabolism, respectively. This explains why insufficient cellular availability of Cbl leads to increased plasma concentrations of MMA and tHcy (3).

Analysis of Cbl in plasma or serum is the most commonly used biochemical test for diagnosing Cbl deficiency, although it has a lower diagnostic sensitivity and specificity than plasma MMA and tHcy to detect mild or subclinical Cbl deficiency (4). Recently, routine assays for analysis of holoTC have become available and serum holoTC has been demonstrated to be a better indicator of Cbl status than plasma Cbl (5–7) and is more strongly associated with conditions related to impaired Cbl function (8,9).

To date, at least 11 polymorphisms in the gene encoding TC (*TCN2*; MIM#275350) have been identified [(10) and refs. cited within], of which *TCN2* 776C- > G (rs1801198; p.Pro259Arg) has been extensively investigated in relation to transport of Cbl in plasma and Cbl delivery into the cell. However, published data on the influence of this polymorphism on serum holoTC and associations with plasma concentrations of Cbl, MMA, or tHcy are conflicting (11–20). Moreover, knowledge of the associations

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[3] This trial was registered at ClinicalTrials.gov as NCT00119912.
[12] Abbreviations used: Cbl, cobalamin; holoTC, holotranscobalamin; Hcy, homocysteine; MMA, methylmalonic acid; NORCCAP, Norwegian Colorectal Cancer Prevention; tHcy, total homocysteine; TC, transcobalamin II.
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between indicators of Cbl status and *TCN2* 67A- > G (p.Ile23Val) is scarce. No change in serum holoTC (12) or plasma Cbl (16) according to *TCN2* 67A- > C genotypes has been reported, and differences in plasma concentrations of MMA and tHcy across allelic variants were of borderline significance (16) or significant only when plasma Cbl was high (21).

Large-scale studies are required to investigate the phenotypic effects of genotypes or genotype combinations of low prevalence. We therefore selected 2411 middle-aged or elderly men and women from a population-based cohort comprising 10,601 presumptively healthy Norwegians, based on the presence of the rare *TCN2* 67G allele. In this population, we investigated the isolated and combined effects of the *TCN2* 776C- > G and 67A- > G polymorphisms on serum holoTC and other indicators of Cbl status.

Materials and Methods

Study population. The NORCCAP cohort was established to study the utility of sigmoidoscopy and fecal occult blood testing as screening modalities for early detection of colorectal cancer in men and women aged 50–64 y. Study individuals were randomly selected from the population registries in the city of Oslo and the county of Telemark and were recruited to 3 study centers from 1999 to 2001 (22). Those with the following were excluded: former colonic resections, need of long-lasting attention and nursing services, ongoing treatment for malignant disease, severe heart or lung disease (New York Heart Association III-IV), lifelong anticoagulant therapy, a coronary episode requiring hospital admission during the last 3 mo, or a cerebrovascular accident during the last 3 mo (16,22). With these criteria, 535 individuals were excluded and for 10,601 eligible participants we obtained blood samples for analysis of metabolites and genotypes. The Regional Ethics Committee and the Data Inspectorate approved the study, which was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participating individuals.

Selection of participants. The participants were selected from the NORCCAP cohort (n = 10,601) on the basis of blood samples that had been genotyped with respect to the *TCN2* 776C- > G and 67A- > G polymorphisms (16). We included all individuals from the cohort that had the 67GG genotype (n = 176) and the 67AG/776GG genotype (n = 235). For each of the remaining 5 genotype combinations, we drew a random sample of 400 individuals. In total, 2411 individuals were selected from the NORCCAP cohort for further subgroup analyses. Due to low volume in 32 blood samples, serum holoTC could be analyzed in only 2379 individuals. A complete data set of all Cbl nutritional status indicators was then obtained in 2374 of 2379 individuals.

Sample collection and laboratory methods. Nonfasting venous blood samples were collected at inclusion into the study. EDTA samples were immediately placed on ice and serum samples were allowed to clot for 1 h at room temperature. Samples were centrifuged at 1100 × g for 10 min at 23°C and subsequently stored at −80°C until analysis. DNA samples were analyzed for *TCN2* 776C- > G and *TCN2* 67A- > G by multiplex genotyping using matrix-assisted laser desorption/ionization time-of-flight MS (23). The determination of holoTC was carried out by a newly developed monoclonal immunoassay on an Abbot AxSYM instrument (24). The interassay CV on pooled serum controls was ≤13%. Serum folate was analyzed by a *Lactobacillus casei* microbiologic assay (25,26) and plasma Cbl by a *Lactobacillus leichmannii* microbiological assay (27). The assays were performed on a microtitre plate platform adapted to a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz). Plasma concentrations of MMA, tHcy, and Met were simultaneously measured by an isotope dilution GC-MS method (28), whereas plasma creatinine was determined by HPLC-MS/MS (29).

Reference concentrations for blood analytes. Low holoTC was defined as serum concentrations ≤ 40 pmol/L (30). The lower reference value for plasma MMA was ≤ 0.28 μmol/L (31), and for plasma tHcy ≤ 15 μmol/L, as presented for nonfolate-supplemented adults aged 15–65 y (32). Low Cbl and folate were defined as plasma concentrations ≤ 150 pmol/L and ≤ 7.5 nmol/L, respectively, and high creatinine as plasma concentrations above age- and gender-specific reference ranges (>90 μmol/L for women and >105 μmol/L for men), all of which are in line with the reference values used by the Laboratory of Clinical Chemistry at the Haukeland University Hospital.

Data analysis. Continuous data were given as mean ± SE or as OR (CI 95%) and categorical data were presented as absolute counts or proportions. Statistical analyses were adjusted for study centers at which the blood samples were taken to correct for variation due to preanalytical conditions, and by age, sex, and creatinine, where appropriate. The Cbl nutritional status indicators (Cbl, holoTC, MMA, tHcy), folate, and methionine were skewed (right-tailed), and they were log-transformed to approximate normal distribution before inclusion in linear analyses. As such, the obtained P values referred to the differences in log-transformed values.

For evaluation of differences in continuous data between the selected subgroup and the remaining NORCCAP group and between men and women in the subgroup, we employed ANOVA. Differences in categorical variables between the groups were assessed by the chi-squared test. Partial Pearson correlation coefficients for associations between blood concentrations of holoTC, Cbl, folate, MMA, tHcy, tMet, creatinine, and age were calculated separately for men and women.

We obtained trend estimates by univariate linear modeling of an outcome effect related to the G-allelic genotypes of either *TCN2* 67A- > G or *TCN2* 776C- > G. In secondary analyses, we stratified the data by *TCN2* 776C- > G variants and tested for linear trends in the estimated mean of the outcome variable across *TCN2* 67A- > G variants and for interaction between genotypes by including a product term in the model. We conducted 4 sets of analysis, the outcomes being serum holoTC and plasma concentrations of Cbl, MMA, and tHcy, respectively. Independent covariates included age, sex, and plasma creatinine in addition to study center. With univariate linear modeling, we obtained the estimated differences in means of the outcome variable between factors. To estimate differential effects of the polymorphisms at different concentrations of the outcome variable, we applied quantile regression analysis, which allows inference about the effect of the G-allelic variants of the *TCN2* 67A- > G polymorphism compared to homozygous 67AA (reference) at specific quantile (decile) cutpoints of the distribution of serum holoTC and plasma concentrations of Cbl, MMA, and tHcy.

We then estimated the effect of *TCN2* 67AG and 67GG in strata of *TCN2* 776C- > G genotypes on the OR of low (tertile 1: <45.6 vs. ≥45.6 pmol/L) and high (tertile 3: ≥68.8 vs. <68.8 pmol/L) serum holoTC, low (tertile 1: <267 vs. ≥267 pmol/L) and high (tertile 3: ≥354 vs. <354 pmol/L) plasma Cbl, high plasma MMA (tertile 3: ≥0.20 vs. <0.20 μmol/L), and high plasma tHcy (tertile 3: ≥11.4 vs. <11.4 μmol/L). Analyses were performed by binary logistic regression and *TCN2* 67AA was the reference category.

We used the open source statistical program environment R (33) with the package "quantreg" to obtain quantile regression estimates for the outcome variables and the package "sampling" version 2.3 to draw a random sampling without replacement, using the "srswor" command. For other analyses, we used SPSS, version 15.0. Tests were 2-tailed and P values ≤ 0.05 were considered significant.

Results

Characteristics of the study population. The selected population comprised of 2411 individuals (48.6% males) with a mean age of 56.2 y (**Table 1**). Concentrations (adjusted mean ± SE) of vitamins and metabolites were within normal reference ranges and did not differ from the remaining individuals in the NORCCAP cohort (n = 8190, mean age 56.2 y, 49.3% male). Serum concentrations of holoTC and folate were higher in women (59 ± 0.72 pmol/L and 19.2 ± 0.39 nmol/L, respec-

TABLE 1 Characteristics of individuals selected for measurement of serum holoTC[1]

	Selected	Not selected	
	n = 2411	*n* = 8190	*P*
Plasma concentrations			
Cbl, *pmol/L*	333 ± 3.90	332 ± 2.27	0.20
Folate, *nmol/L*	17.5 ± 0.24	17.1 ± 0.12	0.09
MMA, *μmol/L*	0.18 ± 0.002	0.18 ± 0.001	0.64
tHcy, *μmol/L*	10.8 ± 0.07	10.8 ± 0.04	0.58
Met, *μmol/L*	23.4 ± 0.11	23.5 ± 0.06	0.19
Creatinine, *μmol/L*	69.7 ± 0.34	70.0 ± 0.15	0.36
Characteristics			
Age, *y*	56.2 ± 0.08	56.2 ± 0.04	0.64
Smokers, *%*	30.9	30.7	0.61
Sex, *% males*	48.6	49.2	0.55

[1] Values are mean ± SE, or percent. Means were adjusted for study center. Cbl, cobalamin; holoTC, holotranscobalamin; Met, methionine; MMA, methylmalonic acid; tHcy, total homocysteine.

tively) than in men (56 ± 0.67 pmol/L and 15.5 ± 0.27 nmol/L, respectively) ($P < 0.002$; $P < 0.001$, respectively), whereas plasma concentrations of tHcy, Met, and creatinine were lower in women (10.2 ± 0.11, 22.5 ± 0.16, and 63 ± 0.29 μmol/L, respectively) than in men (11.4 ± 0.11, 24.3 ± 0.17, and 77 ± 0.56 μmol/L, respectively) ($P < 0.001$).

In men and women, serum holoTC and plasma Cbl were strongly related to each other and inversely correlated to plasma concentrations of MMA and tHcy (**Table 2**). Both serum holoTC and plasma Cbl were positively associated with plasma concentrations of folate and Met and these relationships were stronger in women. Serum holoTC was positively correlated with plasma creatinine only in men and with age only in women. Plasma Cbl was not associated with plasma creatinine in either gender but was related to age in women ($P = 0.015$).

Genotype frequencies of the TCN2 776C- > G and TCN2 67A- > G polymorphisms. In the NORCCAP study (n = 10,601), the respective genotype frequencies for the *TCN2* 776C- > G polymorphism were 32.5, 47.7, and 19.8% for CC, CG, and GG, and for the *TCN2* 67A- > G polymorphism 78.2, 20.0, and 1.8% for AA, AG, and GG. *TCN2* 776C- > G and *TCN2* 67A- > G were in linkage disequilibrium with D' of −0.41 (−0.35, −0.46) (16).

Concentrations of Cbl nutritional status indicators according to genotypes. The serum holoTC concentration was lower in *TCN2* 67AG (55 ± 0.75 pmol/L) and 67GG (48 ± 2.1 pmol/L) compared to 67AA (62 ± 0.67 pmol/L) ($P < 0.001$) but did not differ according to *TCN2* 776C- > G genotypes (CC = 54 ± 0.67 vs. CG = 54 ± 1.2 vs. GG = 56 ± 1.8 pmol/L). The differences in serum holoTC across *TCN2* 67A- > G genotypes were larger in the stratum of *TCN2* 776CC (22 pmol) than in strata 776CG (14 pmol) and 776GG (7 pmol) ($P < 0.001$) (**Table 3**).

Neither polymorphism was associated with significant differences in plasma Cbl. There was a trend for a lower plasma Cbl concentration according to the number of 67G-alleles in individuals with the *TCN2* 776GG genotype ($P = 0.06$) (Table 3) and there was a slightly higher plasma Cbl concentration according to the number of 776G-alleles in individuals with the *TCN2* 67AA genotype ($P = 0.07$; data not shown). However, the *TCN2* 67A- > G and *TCN2* 776C- > G had no overall interactive effect on plasma Cbl ($P = 0.14$) (Table 3).

Differences in plasma MMA concentrations among genotypes were borderline significant ($P = 0.06$) for *TCN2* 67A- > G but not for *TCN2* 776C- > G ($P = 0.26$). Neither polymorphism was associated with significant differences in plasma tHcy or Cbl concentrations ($P > 0.10$) (Table 3). There were no interactive effects of *TCN2* 67A- > G and *TCN2* 776C- > G on plasma concentrations of MMA or tHcy ($P > 0.10$; data not shown).

Risk estimates for low and high Cbl nutritional status indicators. In strata *TCN2* 776CC and CG, but not in GG, the *TCN2* 67G allele significantly increased the OR for low serum holoTC and decreased the OR for high serum holoTC ($P < 0.001$) (**Table 4**). In *TCN2* 776CG and GG, but not in CC, *TCN2* 67AG was borderline significantly associated with a risk of having high plasma MMA ($P = 0.06$) and low plasma Cbl

TABLE 2 Associations of circulating holoTC and Cbl with plasma nutritional markers and age in healthy middle-aged men and women[1]

	Men, *n* = 1159		Women, *n* = 1215	
Variable	Serum holoTC	Plasma Cbl	Serum holoTC	Plasma Cbl
	r			
Cbl	0.60**		0.65**	
Folate	0.16**	0.14**	0.22**	0.20**
MMA[3]	−0.27**	−0.23**	−0.29**	−0.22**
tHcy	−0.26**	−0.28**	−0.30**	−0.29**
Met	0.07*	0.07*	0.11**	0.07*
Creatinine	0.11**	0.01	0.02	−0.03
Age, *y*	0.03	0.01	0.07*	0.07*

[1] Partial correlation coefficients, adjusted for study center. Coefficients are significant, *$P \leq 0.05$, **$P \leq 0.001$. Cbl, cobalamin; holoTC, holotranscobalamin; Met, methionine; MMA, methylmalonic acid; tHcy, total homocysteine.

TABLE 3 Serum holoTC and plasma Cbl by *TCN2* 776C- > G or *TCN2* 67A- > G genotype in healthy, middle-aged men and women[1]

TC genotypes			Serum holoTC,	Plasma Cbl,
776C- > G	67A- > G	*n*	*pmol/L*	
	AA	390	64 ± g1.2	316 ± 9
CC	AG	394	55 ± 1.2	329 ± 9
	GG	103	43 ± 2.3	327 ± 17
P-trend			<0.001	0.49
	AA	394	62 ± 1.2	328 ± 9
CG	AG	396	53 ± 1.2	326 ± 9
	GG	50	49 ± 3.3	315 ± 25
P-trend			<0.001	0.81
	AA	394	59 ± 1.2	353 ± 9
GG	AG	232	57 ± 1.5	312 ± 11
	GG	21	53 ± 5.0	312 ± 39
P-trend			0.18	0.06
P-interaction			0.001	0.14

[1] Values are means ± SE. The statistical model was adjusted for study center, age (56.2 y), sex, and creatinine. Cbl, cobalamin; holoTC, holotranscobalamin.

TABLE 4 OR (95% CI) for low or high serum holoTC for *TCN2* 67 A- > G genotypes in strata of *TCN2* 776C- > G genotypes[1]

TCN2 genotypes				Serum holoTC[2], OR (95% CI)[3]	
776C- > G	67A- > G	NORCCAP, *n* (%)	Study group, *n* (%)	<45.6 pmol/L	>68.8 pmol/L
	AA	2322 (71.9)	390 (43.9)	1.0 (reference)	1.0 (reference)
CC	AG	805 (24.9)	394 (44.5)	2.5 (1.8–3.5)*	0.5 (0.3–0.6)*
	GG	103 (3.2)	103 (11.6)	5.7 (3.5–9.0)*	0.2 (0.1–0.3)*
	AA	3745 (78.9)	394 (46.9)	1.0 (reference)	1.0 (reference)
CG	AG	952 (20.0)	396 (47.1)	2.1 (1.6–2.9)*	0.5 (0.4–0.7)*
	GG	52 (1.1)	50 (6.0)	4.5 (2.4–8.2)*	0.4 (0.2–0.7)*
	AA	1711 (87.0)	394 (61.0)	1.0 (reference)	1.0 (reference)
GG	AG	235 (11.9)	232 (35.8)	1.2 (0.8–1.7)	0.9 (0.7–1.3)
	GG	21 (1.1)	21 (3.2)	1.7 (0.7–4.2)	0.3 (0.1–1.1)

[1] holoTC, holotranscobalamin; NORCCAP, Norwegian Colorectal Cancer Prevention.
[2] Tertiles 1 and 3.
[3] Adjusted for study, center, age, sex, and creatinine. *Different from reference, $P < 0.001$.

($P = 0.07$), respectively. The risk estimates of having low or high plasma Cbl or high plasma MMA or tHcy concentrations were not increased ($P > 0.10$; data not shown).

The effect of TCN2 67A- > G across distribution of Cbl nutritional status indicators. The effect profiles of the different variants of the *TCN2* 67A- > G polymorphism across the plasma concentration range of holoTC (**Fig. 1***A*), Cbl (Fig. 1*B*), MMA (Fig. 1C), and tHcy (Fig. 1*D*) are given as percentage difference from the reference (67AA) for each decile of the outcome variable. Serum holoTC (Fig. 1*A*) was ~15 and 30% lower in 67AG and 67GG individuals, respectively ($P < 0.001$). Within each variant, the percentage difference of serum holoTC from the reference (67AA) tended to be largest ($P = 0.08$) at the lower range of serum holoTC. The percentage difference of plasma MMA (Panel C) was slightly above the reference for 67AG ($P = 0.07$), but not for 67GG individuals ($P = 0.13$). This effect related to heterozygous 67AG was attenuated after additional adjustment for serum holoTC (data not shown). None of the plasma Cbl and tHcy concentrations differed from the reference concentrations.

Discussion

Principal findings. We demonstrated that serum holoTC decreased according to the number of rare alleles of the *TCN2* 67A- > G polymorphism. The genotype-related decrease in serum holoTC was not associated with an increase in plasma MMA or plasma tHcy, and plasma Cbl remained essentially unchanged. There was a considerable interaction between the polymorphisms with the strongest effect of *TCN2* 67A- > G on serum holoTC in the *TCN2* 776CC stratum. Serum holoTC and plasma concentrations of Cbl, MMA, and tHcy were not affected by *TCN2* 776C- > G.

Study design. This is a large study that allows the detection of phenotypic effects of rare alleles on markers of Cbl nutritional status. We increased the statistical power of our analyses by including all individuals in the NORCCAP cohort ($n = 10,601$) with the 4 genotype-combinations with lowest prevalence ($n = 406$) (16). There was no difference between the selected study group and the NORCCAP population (Table 1) with respect to demographic data, such as sex, age, and smoking, and the plasma concentrations of vitamins, metabolites and creatinine, which reduced the possibility of selection bias. Circulating total TC and transcobalamin I were not measured, nor was their saturation with Cbl. This is a weakness of the present study, because no strong inference can be made about the concentration of the Cbl binding proteins and the plasma distribution of their ligands in relation to *TCN2* 67A- > G and 776C- > G genotypes.

Relation between vitamins and metabolites. Serum holoTC was slightly higher in women than in men, which is in accordance with some (24,34) but not all data (35). As expected, plasma tHcy was lower in women than in men [(36) and refs. cited within], whereas plasma concentrations of Cbl and MMA did not show gender-specific differences (37). HoloTC and Cbl concentrations were strongly correlated in both men and women, as previously shown (34). Serum holoTC was positively associated with age (in women only), creatinine (in men only), and plasma concentrations of folate but inversely related to plasma concentrations of MMA and plasma tHcy, which is in line with published data (1). The moderate association between holoTC and Met has not to our knowledge been described earlier but may reflect Cbl-dependent remethylation of Hcy to Met.

Effect of TCN2 67A- > G and TCN2 776C- > G on serum holoTC and plasma Cbl. Serum holoTC was ~30% lower in 67GG and 15% lower in 67AG variants than in the homozygous 67AA variant of the *TCN2* 67A- > G polymorphism. This observation is in contrast with an earlier study of 115 women (12). In this study, serum holoTC did not significantly differ between *TCN2* 67A- > G genotypes but was slightly higher in *TCN2* 67AG. The investigators suggested that the heterozygous variant of this polymorphism improved Cbl binding to *TCN2*. The discrepancy between our results and the findings in the above-mentioned study may be due to chance finding given the small number of individuals carrying the variant genotypes ($n = 24$ for 67AG, $n = 3$ for 67GG) (12).

The *TCN2* 67A- > G polymorphism had a progressively greater effect on serum holoTC at its lower deciles, especially in *TCN2* 67GG individuals, as demonstrated by quantile regression analysis. A similar asymmetric effect of a *TCN2* polymorphism has not been described before, to our knowledge, and could be due to lower affinity of Cbl for TC in 67AG and 67GG individuals.

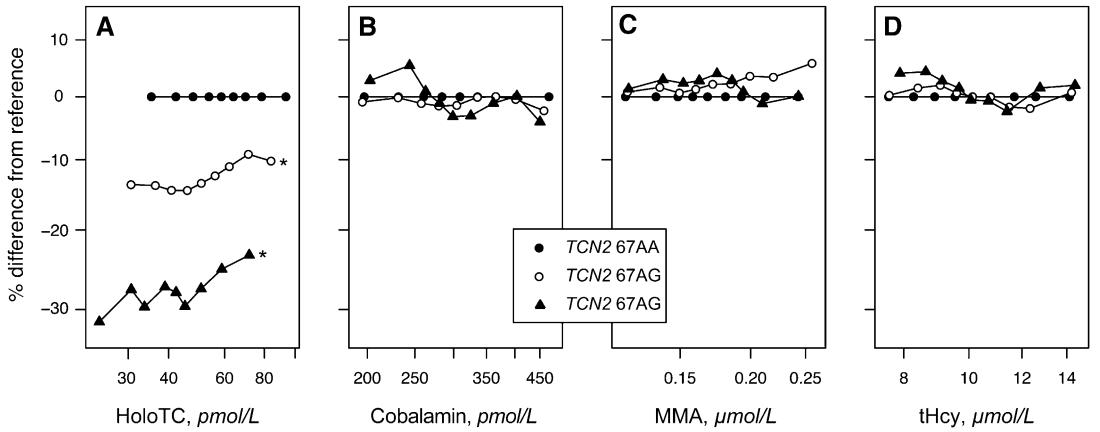

FIGURE 1 Associations between *TCN2* 67A- > G variants and serum holoTC (*A*), plasma Cbl (*B*), plasma MMA (*C*), and plasma tHcy (*D*) in healthy, middle-aged men and women by quantile regression analyses. Distributions of Cbl nutritional status indices in serum or plasma according to *TCN2* 67AA (reference; $n = 1178$), *TCN2* 67AG ($n = 1022$), and *TCN2* 67GG ($n = 174$) are shown on the *x*-axis. Dots connected with straight lines represent cutpoints for the 10th to the 90th percentiles estimated by the regression analysis. The percent difference from the reference (*TCN2* 67AA) for corresponding decile cutpoints is shown on the *y*-axis. The regression model included adjustment for study center, age, sex, and plasma creatinine. All decile cutpoints in *A* differed from the reference, $*P < 0.05$. holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.

We observed that serum holoTC did not differ across variants of the *TCN2* 776C- > G polymorphism, which is in contrast to the results of most (11–15,19) but not all studies (18,38). The discrepancy between the studies may be related to sample size or to the underlying effect of *TCN2* 67A- > G on serum holoTC in different *TCN2* 776C- > G variants.

The effect of allelic variants of *TCN2* 67A- > G on serum holoTC and the associated odds of having low serum holoTC differed across the *TCN2* 776C- > G genotypes in our study population. These observations demonstrate that the *TCN2* 67A- > G and *TCN2* 776C- > G polymorphisms interacted as predictors of serum holoTC, with *TCN2* 67A- > G as the strongest determinant.

Plasma Cbl did not change according to variants of the *TCN2* 67A- > G polymorphism, which is in agreement with previously published data (16). Nor was plasma Cbl associated with allelic variants of *TCN2* 776C- > G, which is consistent with results from most (11,13,15–19) but not all (20) studies. However, we observed an interaction that involved slightly lower plasma Cbl across variants of *TCN2* 67A- > G in stratum 776GG and slightly higher Cbl across variants of *TCN2* 776C- > G in stratum 67AA. These data and the data of others (18,20) suggest that *TCN2* 67A- > G and *TCN2* 776C- > G may affect the distribution and binding of circulating Cbl.

The mechanism for the observed effects of the *TCN2* 67A- > G and 776C- > G polymorphisms on serum holoTC are not immediately apparent. Both the 67A- > G and the 776C- > G transition within the *TCN2*-gene have been shown to be associated with a lower concentration of TC (1,12,38). The substitution of proline by arginine in *TCN2* 776C- > G may change the secondary structure (12) and also alter the tertiary structure of the protein, thereby affecting its Cbl-binding properties (20). Other investigators do not support this notion (39,40). The amino acid exchange related to *TCN2* 67A- > G is considered to be of no significance for the secondary structure of the TC molecule (12) but might change Cbl affinity for TC, as indicated by molecular dynamic simulation studies (40). Further research may unveil whether conformational changes in the protein structure, reduced TC expression, or other conditions related to the kinetic properties of holoTC may explain the underlying mechanisms for the present observations.

Effect of TCN2 67A- > G and TCN2 776C- > G on plasma MMA and plasma tHcy. Lower serum holoTC according to the number of 67G-alleles was not associated with higher plasma tHcy or, although of borderline significance, with higher plasma MMA ($P = 0.06$). This observation is in agreement with published data (12,16). In one other study, plasma tHcy was found to be lower in individuals with the 67GG genotype. However, the difference was not significant and the sample size for the GG genotype was small ($n = 6$) (21).

The *TCN2* 776C- > G polymorphism was not associated with significant changes in the concentrations of plasma MMA and tHcy in this study. This is consistent with results in previous reports (13,15,18,19,38) but at variance with the results of others (11,16,17). The ambiguous results may be due to study design, including age, ethnicity, and nutritional factors.

The *TCN2* 776C- > G and 67A- > G polymorphisms did not interact as plasma MMA or plasma tHcy determinants and they did not increase the odds of having high plasma concentrations of either MMA or tHcy. *TCN2* 776CC individuals from a Hispanic population had a higher risk of having high tHcy (>13 μmol/L), not high MMA (>0.35 μmol/L), compared with carriers of the G-allele when circulating Cbl and holoTC were low (18). The prevalence and the allele distribution of the *TCN2* 67A- > G polymorphism in this ethnic population was not investigated. Also, other factors related to differences in demographic data may account for the observed discrepancy with our results.

Implication. Serum holoTC is affected by factors unrelated to the dietary availability or functional status of Cbl. The positive relationship of serum holoTC and creatinine may hamper the usefulness of the test in patients with severe renal dysfunction (7,41). Variations in plasma holoTC according to gender (1,34) and use of oral contraceptives (42) may also affect the diagnostic accuracy of holoTC. The main observation of the present study is that plasma holoTC could differ up to 33% between individuals harboring different variants of the *TCN2* gene. Lack of concordant variations in the metabolic markers of Cbl status, MMA and tHcy, suggests that such genetic variability may reduce the test specificity of holoTC. Alternatively, the *TCN2* 67A- > G polymorphisms may affect Cbl status to an

extent that is not detected by the possibly less sensitive metabolic markers. Finally, lower serum holoTC in non-European populations (6,43) at least in part may be related to population-related differences in the prevalence of *TCN2* 67A- > G.

In summary, in this study of middle-aged Norwegian men and women, serum holoTC was negatively associated with the number of G-alleles of *TCN2* 67A- > G but showed no relation to *TCN2* 776C- > G. The 2 polymorphisms interacted as determinants of serum holoTC but were not associated with plasma concentrations of Cbl, MMA, or tHcy in this population. Our data suggest that the *TCN2* 67A- > G and 776C- > G polymorphisms affect the distribution of circulating Cbl without having an effect on cellular Cbl uptake and availability. It remains to be determined whether the influence of these polymorphisms on serum holoTC alters the diagnostic utility of serum holoTC as a marker of Cbl status in a routine clinical setting.

Acknowledgments

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