Brief report

Transcobalamin II 775G>C polymorphism and indices of vitamin B12 status in healthy older adults

Joshua W. Miller, Marisa I. Ramos, Marjorie G. Garrod, Margaret A. Flynn, and Ralph Green

A common polymorphism (775G>C) in the vitamin B12 transport protein, transcobalamin II (TCII), has been identified in which proline replaces arginine at codon 259. We determined the influence of TCII genotype on indices of B12 status, including total serum B12, the amount of B12 bound to TCII (holoTCII), methylmalonic acid, and homocysteine, in 128 healthy older adults (ages 40-88 years). Mean total B12 and homocysteine concentrations were not significantly different among the 3 genotypes. Mean holoTCII concentration was significantly higher in those subjects homozygous for the proline form of TCII (PP) compared with those homozygous for the arginine form (RR) and heterozygotes (PR) (P < .006). In addition, mean methylmalonic acid concentrations were significantly lower in the PP and PR groups compared with the RR group (P < .02). The PP genotype may be more efficient in delivering B12 to tissues, resulting in enhanced B12 functional status. TCII genotype may thus influence susceptibility to B12 deficiency.

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Introduction

The serum protein, transcobalamin II (TCII), transports vitamin B12 (B12) from the ileum to the tissues. The B12-TCII complex (holoTCII) is then taken up into cells by receptor-mediated endocytosis. In the 1970s and 1980s, 2 research groups independently identified distinct isopeptide forms of TCII by polyacrylamide gel electrophoresis and isoelectric focusing. More recently, DNA sequencing has revealed that the isopeptide forms of TCII are the result of single nucleotide polymorphisms. The most common polymorphism in white populations is a G-to-C substitution at base position 775 (775G>C), which results in the replacement of proline with arginine at codon 259. Recently, the potential influence of the 775G>C polymorphism on indices of vitamin B12 status has been investigated. Persons homozygous for the proline form of the protein (PP) tend to have higher holoTCII but similar total B12 concentrations compared with those homozygous for the arginine form (RR). One group has found that homocysteine, a functional indicator of B12 status, is higher in heterozygous persons (PR) than in PP and RR persons, but this finding was not confirmed. Notably, the relationship between TCII genotype and methylmalonic acid, potentially a more specific indicator of B12 status than homocysteine, has not been reported. Therefore, we assessed the relationship between 775G>C TCII genotype and methylmalonic acid and between total B12, holoTCII, and homocysteine in a cohort of healthy older adults.

Study design

Subjects

The study sample consisted of 108 men and 20 women (mean age, 67 years; range, 40-88 years). Subjects are current participants in the Longitudinal Aging Study, initiated in 1969 and continuing through the present at the University of Missouri-Columbia. The study population consisted primarily of University of Missouri faculty and staff who were physically active and in apparent good health, with no evidence of decreased intrinsic factor secretion or gastrointestinal B12 malabsorption. The project was approved by the University of Missouri-Columbia Institutional Review Board and all participants provided their informed consent.

TCII genotyping

Polymerase chain reaction (PCR) product was amplified using an Eppendorf Mastercycler (Brinkmann, Westbury, NY) in a total reaction volume of 25.0 μL containing 7.0 μL genomic DNA, 1.5 mM MgCl2, 0.2 mM dNTP mix, 2.0 μM forward primer (5’-GTC-AGG-TGC-AGC-ACC-TAG-3’), 2.0 μM reverse primer (5’-CGT-TCT-GAA-CCA-GAA-GAC-CTA-3’), 2.5 μL 10 × PCR buffer, and 1 U Taq polymerase (Gibco BRL/Life Technologies, Rockville, MD). The amplification consisted of initial denaturation (94°C, 2 minutes); 35 cycles consisting of denaturation (94°C, 1 minute), annealing (64°C, 1 minute), and extension (72°C, 2 minutes); and final extension (72°C, 2 minutes). PCR product was digested at 37°C overnight in a total reaction volume of 20 μL containing 15 μL PCR product, 2 μL 10 × digestion buffer, and 20 U MvaI restriction enzyme (Roche Molecular Biochemicals, Indianapolis, IN). Digested samples were electrophoresed on 1% agarose and 2% NuSieve GTG (FMC Bioproducts, Rockland, ME) gels and were visualized (Figure 1) using the NucleoVision Gel Documentation System (NucleoTech Corporation, San Mateo, CA).

Metabolite assays

Fasting serum B12 and RBC folate were determined by automated chemiluminescence assay (ACS180; Bayer Diagnostics, Tarrytown, NY); methylmalonic acid by gas chromatography–mass spectrometry; homocysteine by high-performance liquid chromatography with fluorescence detection; creatinine by standard spectrophotometric assay; and holoTCII by indirect assay using anti-TCII antibodies as follows: activated Sepharose beads were coupled with polyclonal antibody raised in goats inoculated

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with rabbit TCII purified by photo-dissociative affinity chromatography.\textsuperscript{16} The antibody shows immunologic cross-reactivity with human holoTCII.\textsuperscript{17} In the assay, goat anti-rabbit TCII antibody–coated Sepharose beads were washed and resuspended as a 5\% mixture in phosphate-buffered saline. One-milliliter aliquots of the washed beads were transferred to microfuge tubes and centrifuged at 3000g (5 minutes). After aspiration of the supernatant, serum samples (500 \( \mu \)L) were added to the bead pellet, mixed for 2 hours at room temperature, and centrifuged at 3000g (5 minutes). B12 concentrations were determined in the supernatants by radioassay (Simultaneously chromatography.\textsuperscript{16} Radiodissociative affore TCII.\textsuperscript{16} Purification of rabbit TCII puriﬁed by photo-dissociative af for TCII (University of Missouri, Columbia). We thank Lisa M. Rogers, Rebecca F. Cotterman, and Autumn Nguyen for conducting metabolic reactions. PP genotype is associated with higher homocysteine than either homozygous genotype,\textsuperscript{8,11} a finding conﬁrmed in 2 other reports.\textsuperscript{9,10} It has been suggested that the discrepancy between the studies with respect to homocysteine levels is related to differences in the age of the study subjects, with younger subjects exhibiting the homocysteine difference and older subjects not.\textsuperscript{11} A more likely explanation is that the higher homocysteine observed in PR subjects in one study\textsuperscript{11} was related to some other uncontrolled determinant of homocysteine, such as sex, B vitamin levels (folate, B12, B6), kidney function, thyroid function, and other genetic factors.\textsuperscript{10} In this regard, methylmalonic acid may be better than homocysteine as an indicator of the effect of TCII genotype on functional B12 status because methylmalonic acid is influenced by fewer confounding factors. Notably, in the present study, significant differences in methylmalonic acid among the genotypes were observed after controlling for age, sex, serum creatinine and total B12 level.

We conclude that the TCII 775G>C genotype signiﬁcantly inﬂuences tissue B12 delivery and functional B12 status. Because none of the subjects in the study sample exhibited evidence of hematologic abnormalities, the differences among the genotypes in methylmalonic acid and holoTCII may represent preclinical alterations in B12 status and function. It remains to be determined whether TCII genotype ultimately inﬂuences the susceptibility of persons to develop the overt clinical manifestations of B12 deﬁciency, including hematologic and neurologic sequelae.

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References


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