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.
with rabbit TCII purified by photo-dissociative affinity chromatography. The antibody shows immunologic cross-reactivity with human holoTCII. 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 μL) 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 (Simultrac Radioassay, ICN Pharmaceuticals, Orangeburg, NY). The difference in B12 concentration between untreated serum and bead-treated serum represented the holoTCII level. In this assay, antibody-coated beads consistently remove more than 98% of 57Co-cyanocobalamin–labeled holoTCII (data not shown). Mean (± SD) for holoTCII in 22 nondeficient, healthy volunteers was 104.1 (± 66.7) pg/mL (range, 38-305 pg/mL). Within and between assay coefficients of variation in nondeficient samples were 15% and 17%, respectively.

Statistical analyses
Mean (± SD) for each metabolite was compared by analysis of variance, controlling for age, sex, and other covariates as indicated, followed by Scheffé F-test.

Results and discussion
Characteristics of the study sample divided by TCII genotype are presented in Table 1. The distribution of genotypes among the subjects was 30% PP, 50% PR, and 20% RR, similar to previous reports. No differences among the genotypes were observed for hematocrit and MCV, and no subjects had evidence of macrocytic anemia. The mean holoTCII concentration was significantly higher in the PP subjects than in the PR and RR subjects, but no differences in mean total B12 were observed. These results are consistent with previous reports. The mean methylmalonic acid concentration was significantly higher in the PR and RR subjects than in the PP and PR subjects. Taken together, these findings suggest that TCII genotype influences the cellular delivery of B12 and directly impacts 1 of the 2 biochemical reactions in which B12 participates as a cofactor—the conversion of methylmalonyl CoA to succinyl CoA catalyzed by the enzyme methylmalonyl CoA mutase. PP genotype was also associated with a higher percentage of total B12 bound to TC compared with the other genotypes. This suggests that the PP genotype has higher affinity for B12 than the RR genotype, but remains to be definitively determined.

No differences in mean homocysteine concentrations were observed among the genotypes. This is in contrast to a previous finding that the PR genotype is associated with higher homocysteine than either homozygous genotype, a finding unconfirmed in 2 other reports. 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. A more likely explanation is that the higher homocysteine observed in PR subjects in one study 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. 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.

Acknowledgments
We thank Genevieve Hill for coordinating subject participation and Wally Thomas for supervision of phlebotomy and blood processing (University of Missouri, Columbia). We thank Lisa M. Rogers, Rebecca F. Cotterman, and Autumn Nguyen for conducting methylmalonic acid and holoTCII assays and Angela Devlin for assistance in developing the TCII genotyping assay (University of California, Davis).

Table 1. Characteristics of study sample by transcobalamin II genotype

<table>
<thead>
<tr>
<th>TCII genotype</th>
<th>N</th>
<th>PP</th>
<th>PR</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men/women)</td>
<td>39</td>
<td>54/9</td>
<td>21/5</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>66 (± 11)</td>
<td>67 (± 11)</td>
<td>67 (± 11)</td>
<td></td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td>433 (± 177)</td>
<td>415 (± 168)</td>
<td>383 (± 191)</td>
<td></td>
</tr>
<tr>
<td>HoloTCII (pg/mL)</td>
<td>150 (± 81)</td>
<td>113 (± 56)</td>
<td>104 (± 83)</td>
<td></td>
</tr>
<tr>
<td>% Total B12 on TCII†</td>
<td>34.3 (± 9.5)</td>
<td>27.8 (± 9.9)</td>
<td>24.6 (± 10.1)</td>
<td></td>
</tr>
<tr>
<td>Methylmalonic acid (nm)</td>
<td>208 (± 96)</td>
<td>206 (± 80)</td>
<td>264 (± 138)‡</td>
<td></td>
</tr>
<tr>
<td>Homocysteine (μM)</td>
<td>10.3 (± 2.6)</td>
<td>10.7 (± 2.4)</td>
<td>11.2 (± 2.8)</td>
<td></td>
</tr>
<tr>
<td>RBC folate (ng/mL)</td>
<td>363 (± 97)</td>
<td>378 (± 106)</td>
<td>396 (± 106)</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.1 (± 0.9)</td>
<td>1.1 (± 1.0)</td>
<td>1.1 (± 0.6)</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.4 (± 3.2)</td>
<td>43.8 (± 3.5)</td>
<td>43.6 (± 3.3)</td>
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</tr>
<tr>
<td>MCV (μm³)</td>
<td>93.9 (± 3.2)</td>
<td>92.9 (± 3.2)</td>
<td>92.2 (± 5.1)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent means (± SD).
†Calculated using the equation: 100 × (holoTCII/total B12).
‡Significantly greater than PP and PR genotypes after controlling for potential confounding by age, sex, and total B12 (P = .006).
§Significantly different from total B12 (P = .02).

Figure 1. Representative gel illustrating PCR and MvaI digestion products indicative of the 3 775G>C TCII genotypes. PCR products measured 218 bp. Complete digestion of the PCR product with MvaI led to 2 fragments measuring 128 and 90 bp. Complete digestion of the PCR product (2 bands) was indicative of the PP genotype (sample 1), partial digestion (3 bands) was indicative of the PR genotype (sample 2), and no digestion (1 band) was indicative of the RR genotype (sample 3).
References


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

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