The present study showed that amniocentesis is associated with a significant increase in the fetal DNA concentrations, representing a transfer of either fetal cells or fetal DNA to the maternal circulation. However, in 21% of our study participants, the concentration of the SRY sequence decreased after amniocentesis, which could be associated with uterine contraction in response to the procedure or may reflect assay imprecision. Frequent uterine contraction may inhibit fetal DNA transfer into the maternal circulation for a few minutes because fetal DNA is cleared from the maternal circulation, with a mean half-life of 16.3 min (19).

The limitation of the current method in assessing subclinical fetal-maternal hemorrhage is that only women carrying a male fetus can provide useful information because the fetal origin of the DNA is based on the presence of the SRY gene on the Y chromosome. A system that uses polymorphic markers outside the Y chromosome (20, 21) or epigenetic markers (22) must be developed before this technique can be applied to mothers carrying female fetuses.

In conclusion, amniocentesis significantly disturbs the maternal-placental interface; further studies are needed to determine whether fetal DNA is a sensitive marker for fetal-maternal hemorrhage or whether its increase after amniocentesis reflects transfer of DNA from amniotic fluid to the maternal circulation.

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 13770921).

References

The Transcobalamin (TC) Codon 259 Genetic Polymorphism Influences Holo-TC Concentration in Cerebrospinal Fluid from Patients with Alzheimer Disease, Henrik Zetterberg,1* Ebba Nexö,2 Björn Regland,3 Lennart Minthon,4 Roberta Boson,4 Mona Palme,5 Lars Rymo,6 and Kai Bliemel7,8 (1 Department of Clinical Chemistry and Transfusion Medicine, 2 Institute of Clinical Neuroscience, Psychiatry Section, and 3 Institute of Clinical Neuroscience, Department of Experimental Neuroscience, Sahlgrenska University Hospital, Göteborg University, S-413 45 Gothenburg, Sweden; 4 Department of Clinical Biochemistry, AKH, Aarhus University Hospital, DK 8000 Aarhus C, Denmark; 5 Neuropsychiatric Clinic, Malmö University Hospital, S-205 02 Malmö, Sweden; * author for correspondence: fax 46-31-828458, e-mail henrik.zetterberg@clinchem.gu.se)

Two proteins bind vitamin B12 in plasma: haptocorrin (transcobalamin I) and transcobalamin (transcobalamin II; TC). The latter is the critical transporter that delivers vitamin B12 to peripheral tissues. TC carries one-third of the circulating B12 (holo-TC), but most TC is unsaturated (apo-TC) (1, 2). Polyacrylamide gel electrophoresis has revealed two common TC isoforms, M and X, and two rare variants, S and F (3, 4), that may influence the cellular availability of vitamin B12 (5, 6). The phenotypic variability is a multifactorial phenomenon that probably includes cell-type-specific processing of translated TC (5), but the substitution of proline (P) for arginine (R) at codon 259 of the TC gene is the major determinant of the TC variability, at least in Caucasians (5, 7), and affects TC concentrations.
in plasma (5, 8). Most 259PP individuals have the TC M phenotype, whereas most 259RR individuals have the X phenotype.

Vitamin B₁₂ is essential for the function of the central nervous system (CNS) (9). Little is known about vitamin B₁₂ transport in the human brain, but data in vitro indicate that TC plays a central role (10). Cerebrospinal fluid (CSF) contains both haptocorrin and TC, with the latter predominating (11). The CSF:plasma ratio of TC is high compared with other plasma proteins (12), which suggests an active transport mechanism or synthesis by cells in the CNS. Cultured astrocytes have been shown to produce and secrete TC in vitro (13), indicating that at least some of the TC in CSF originates from within the CNS. However, because vitamin B₁₂ is not synthesized in human cells, it must enter the brain and CSF from the blood across the blood–brain barrier, conceivably via interaction between holo-TC and the TC receptor. In the present investigation, we hypothesized a correlation between the TC P259R polymorphism and the holo-TC concentration in CSF.

We studied 78 outpatients (27 men and 51 women) being evaluated for cognitive dysfunction at the Neuropsychiatric Clinic, Malmö University Hospital. All of the outpatients underwent a thorough clinical examination and fulfilled the DSM-IV criteria for primary degenerative dementia of Alzheimer type (14) and National Institute of Neurological and Communicative Disorders and Stroke criteria for probable Alzheimer disease (15). Blood and CSF samples were taken as part of the diagnostic procedure, which was the main reason that this group of patients was chosen as the study population. The patients’ mean age (SD) was 74 (8) years, and the mean (SD) age at disease onset was 73 (8) years. Patients receiving vitamin supplementation were excluded. For the plasma analyses, EDTA blood was collected, immediately placed on ice, and centrifuged within 30 min. Plasma, whole blood, and CSF samples were stored at −80 °C until further processing. The study was approved by the Ethics Committee at the Malmö University Hospital, and written informed consent was obtained from all of the patients or the closest relatives if a patient could not give valid consent.

Genotyping of the TC codon 259 polymorphism and a novel 2-bp deletion of nucleotides 45 and 46 (A and G) relative to the first nucleotide in intron 5 of the TC gene was performed by solid-phase minisequencing (16).

Genomic DNA was amplified by PCR with the sense primer 5′-GTCCGAGAGGAGATCTTGAA-3′ and the antisense primer 5′-GTAGGTCTTGTGTTCCAGAA-3′. After amplification, biotinylated PCR products were bound to streptavidin-coated microtiter plates (Wallac) and denatured with NaOH. After washing, Thermosequenase DNA polymerase (Amersham Biosciences), fluorescent dyeoxynucleotide triphosphates (NEN), and the antisense detection primers were added. The detection primers were 5′-CTGTCCCCAATTTCTGCCCAC-3′ for the codon 259 polymorphism and 5′-TTTTTTTTTACCTGACCCACTTCCCCAC-3′ for the intronic deletion. The poly(T) sequence of the latter was added to modify the electrophoretic mobility of the primer. After the minisequencing reaction, the plates were washed, and the extended sequence primers were released from the PCR products by incubation with formamide. The primers were separated and analyzed in the same reaction by capillary electrophoresis and laser-induced fluorescence in an ABI 310 genetic analyzer (PE Applied Biosystems).

Plasma total homocysteine (tHcy) was measured by HPLC with fluorescence detection (17). Plasma total vitamin B₁₂ and whole-blood folate were determined by commercial immunoassays (Bayer Corporation) on a Beckman-Coulter instrument. Plasma and CSF holo- and total TC were measured by ELISA as described previously in detail (18, 19). Day-to-day imprecision (CV) for all biochemical analyses was <10%.

Because distributions of most of the biochemical variables were skewed, the nonparametric Kruskal–Wallis test was used throughout to compare results for the different genotypes. Statistical significance was defined as P < 0.05. Rank correlation coefficients were calculated by the Spearman method. All of the analyses were performed with SYSTAT (SPSS Inc.).

The distribution of TC P259R genotypes among the patients was 30.8% PP, 43.6% PR, and 25.6% RR, similar to distributions in other Caucasian populations (6, 7). There were no associations between the TC P259R polymorphism and age, gender, minimental score, age of onset of Alzheimer disease, or duration of disease (data not shown). The vitamin B₁₂ concentration range was 142-1013 pmol/L with three values >600 pmol/L and two <150 pmol/L. tHcy, vitamin B₁₂, and folate were not significantly associated with the P259R genotype (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>259PP (n = 24)</th>
<th>259PR (n = 34)</th>
<th>259RR (n = 20)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy, μmol/L</td>
<td>14.0 (7.00–29.0)</td>
<td>12.0 (5.00–36.0)</td>
<td>12.0 (6.00–28.0)</td>
<td>0.288</td>
</tr>
<tr>
<td>B₁₂, pmol/L</td>
<td>259 (148–1013)</td>
<td>264 (161–412)</td>
<td>248 (142–702)</td>
<td>0.941</td>
</tr>
<tr>
<td>Folate, nmol/L</td>
<td>181 (132–344)</td>
<td>170 (100–668)</td>
<td>211 (88.0–957)</td>
<td>0.261</td>
</tr>
<tr>
<td>Plasma total TC, pmol/L</td>
<td>1011 (693–1490)</td>
<td>949 (640–1340)</td>
<td>757 (628–1090)</td>
<td>0.002</td>
</tr>
<tr>
<td>Plasma holo-TC, pmol/L</td>
<td>83.0 (49.0–186)</td>
<td>80.0 (20.0–154)</td>
<td>64.5 (21.0–216)</td>
<td>0.375</td>
</tr>
<tr>
<td>CSF total TC, pmol/L</td>
<td>600 (370–830)</td>
<td>485 (220–810)</td>
<td>380 (210–550)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF holo-TC, pmol/L</td>
<td>30.0 (12.0–54.0)</td>
<td>24.0 (12.0–45.0)</td>
<td>18.0 (12.0–51.0)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

*a Values are presented as medians (range).

* By Kruskal–Wallis test.
Because TC P259R genotype might influence the holo-TC concentration in CSF, we analyzed plasma and CSF samples from patients with the different TC P259R genotypes. The 259R allele was associated with significantly lower plasma and CSF total TC (P = 0.002 and P < 0.001, respectively) and CSF holo-TC (P = 0.010) in a distinct gene dose-dependent manner compared with the 259P allele (Table 1), but did not affect plasma holo-TC concentration significantly. Plasma and CSF holo-TC were strongly correlated irrespective of P259R genotype, but the correlation between plasma and CSF concentrations of total TC was disrupted by the 259R allele (Fig. 1).

When sequencing the TC gene to confirm the specificity of the minisequencing method for the TC P259R polymorphism, we found a novel intronic deletion that was clearly polymorphic in the population studied. The polymorphism consisted of an intronic deletion of nucleotides 45 and 46 (A and G) relative to the first nucleotide in intron 5 of the TC gene compared with the published sequence (20), but it did not significantly influence any of the biochemical variables determined in this study (data not shown). We conclude that the intronic deletion influences neither expression nor function of the TC gene.

Vitamin B₁₂ is essential for many functions in CNS but cannot reach the brain directly without passing the blood–brain barrier. The molecular mechanism behind this passage is unknown, but TC appears to play a crucial role (10). Because vitamin B₁₂ is delivered to the cells in peripheral tissues bound to TC (holo-TC), which is bound to the TC receptor, a similar mechanism may be important for the transport of holo-TC across the blood–brain barrier.

In agreement with previously published studies, we found no associations between the TC P259R polymorphism and concentrations of tHcy, vitamin B₁₂, or folate (5, 21), but we did find a significant relationship of the polymorphism with total TC in plasma (5, 7, 8). The lower concentrations of total TC in both plasma and CSF in 259PR and 259RR individuals suggest that the 259R allele impairs TC expression, stability, and/or metabolism. We

![Fig. 1. Correlations between plasma and CSF total TC (A) and holo-TC (B).](image-url)

Rₛ, Spearman rank correlation coefficient. Diagonal lines, significant correlations (P < 0.05). NS, nonsignificant. The different TC P259R genotypes are designated PP, PR, and RR.
were, however, unable to confirm the previously reported association between the 259R allele and lower concentrations of holo-TC in plasma (8, 21). Nevertheless, holo-TC in CSF was reduced in patients with the 259PR and 259RR genotypes, showing that the polymorphism indeed affected holo-TC concentrations in CNS. Holo-TC concentrations in plasma and CSF were highly correlated, which is compatible with the notion that all holo-TC in CSF originates from plasma and needs to pass the blood–brain barrier to enter the CNS. Comparison of the slopes of the linear correlation equations for the homozygous 259RR and 259PP genotypes suggests that holo-TC encoded by the 259R allele crosses the blood–brain barrier less efficiently or is less stable in CSF than 259P-encoded holo-TC. Begley et al. (13) showed that human astrocytes synthesized and secreted TC. Thus, a certain proportion of total TC in CSF most likely originates from within the CNS. One is tempted to speculate that the disrupted correlation between plasma and CSF concentrations of total TC in individuals with the 259PR and 259RR genotypes may reflect up-regulation of TC synthesis within CNS in response to decreased import of B12 across the blood–brain barrier. A similar relationship between vitamin B12 and total TC was seen previously in plasma in which B12 concentrations were inversely correlated with total TC, possibly reflecting B12-mediated regulation of expression or clearance of the protein (22).

In conclusion, the TC 259R allele is associated with lower TC concentrations, especially in CSF in patients with Alzheimer disease. We plan to repeat the study in other populations and explore the possible association with Alzheimer disease. We plan to repeat the study in other populations and explore the possible association with Alzheimer disease.

This work was supported by grants from the Swedish Medical Research Council (project 12103), the Sahlgrenska University Hospital, EUREKA, and Biomedical Grant QLK3-2002-01775. We warmly acknowledge the technical assistance of Anna-Lisa Christensen and Jette Fisksr.

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Procedure for the Monitoring of Gabapentin with 2,4,6-Trinitrobenzene Sulfonic Acid Derivatization Followed by HPLC with Ultraviolet Detection, JoElta M. Jueneke,1,3 Paul I. Brown,1,3 Gwendolyn A. McCmillin,1,2 and Francis M. Urry1,2 1 ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories Inc, 500 Chipeta Way, Salt Lake City, UT 84108; 2 Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84108; * author for correspondence: fax 801-584-5207, e-mail: juenkejm@aruplab.com

Gabapentin is a novel anticonvulsant drug that was introduced in the early 1990s and later approved (1995) for use in the US as an adjunctive treatment of partial seizures with or without secondary generalization in persons >3 years of age. Although structurally similar to γ-aminobutyric acid (GABA), gabapentin does not interact with GABA receptors, nor is it converted to GABA or a GABA agonist (1). Gabapentin is widely studied therapeutically. Its initial and approved use as adjunctive epileptic therapy has been broadened, with many additional indications. These include treatment for neuropathic pain after spinal cord injury (2–4), posttraumatic stress disorder (5), poststroke pain syndrome (6–8), alcohol withdrawal (9), migraine therapy (10), hot flashes associated with prostate cancer treatment (11), and postoperative pain after cancer surgery (12, 13).