The G allele of transcobalamin 2 c.776C→G is associated with an unfavorable lipoprotein profile

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Abstract: BACKGROUND/AIM: Recent studies have suggested a relation of homocysteine with lipid metabolism. The aim of this study was to analyze a possible genetic basis for such a relation in 504 individuals including 135 consecutive Caucasian patients diagnosed with cerebrovascular disease as well as the patients' healthy spouses (n = 100) and offspring (n = 269). METHODS: We analyzed the association of plasma levels of lipoprotein(a), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides with plasma homocysteine levels and with the following 7 variants of homocysteine metabolism: dihydrofolate reductase c.594 + 59del19bp, cystathionine -synthase c.844$_8$55ins68, $methioninesynthasec.2756AG$, $methylenetetrahydrofolatereductasec.677CTandc.1298AC$, $reducedfolate$ $Linearregressionanalysisshowedanassociationof Tc2c.776CGwithLDL(p = 0.010), HDL(p = 0.009), andTG(p =$ $0.007), withtheGalleleof Tc2c.776CGassociatedwithanunfavorablebloodlipidprofile.Moreover, theGalleleof Tc2c.776C$ $0.013, 1-wayANOVA).CONCLUSION : Thesedatasupportthehypothesisthatalterationsinhomocysteinemetabolisma$

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The G Allele of Transcobalamin 2 c.776C→G Is Associated with an Unfavorable Lipoprotein Profile

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Key Words
Homocysteine · Transcobalamin 2 c.776C→G · Lipoprotein profile

Abstract
Background/Aim: Recent studies have suggested a relation of homocysteine with lipid metabolism. The aim of this study was to analyze a possible genetic basis for such a relation in 504 individuals including 135 consecutive Caucasian patients diagnosed with cerebrovascular disease as well as the patients' healthy spouses (n = 100) and offspring (n = 269). ***Methods:*** We analyzed the association of plasma levels of lipoprotein(a), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides with plasma homocysteine levels and with the following 7 variants of homocysteine metabolism: dihydrofolate reductase c.594 + 59del19bp, cystathionine β-synthase c.844_855ins68, methionine synthase c.2756A→G, methylenetetrahydrofolate reductase c.677C→T and c.1298A→C, reduced folate carrier 1 c.80G→A, and transcobalamin 2 (Tc2) c.776C→G. ***Results:*** Linear regression analysis showed an association of Tc2 c.776C→G with LDL (p = 0.010), HDL (p = 0.009), and TG (p = 0.007), with the G allele of Tc2 c.776C→G associated with an unfavorable blood lipid profile. Moreover, the G allele of Tc2 c.776C→G was associated with higher homocysteine plasma levels in the subgroup of patients (p = 0.013, 1-way ANOVA). ***Conclusion:*** These data support the hypothesis that alterations in homocysteine metabolism and an unfavorable blood lipoprotein profile may have a common genetic basis. Such conditions may be relevant for studies investigating independent risk factors for vascular disease.

Introduction

Hyperhomocysteinemia and altered plasma lipid levels are considered to be independent risk factors for the development of vascular disease. Recent studies, however, have suggested a relation between homocysteine metabolism and lipid metabolism [1]. Patients with severe hyperhomocysteinemia due to a cystathionine β-synthase (CBS) deficiency (OMIM 236200) develop a wide range of symptoms including arteriosclerosis and hepatic steatosis (fatty degeneration of liver tissue) [2]. CBS-deficient mice also show hepatic steatosis associated with an abnormal lipid metabolism [3]. A correlation of plasma homocysteine levels with the blood lipid profile was found in patients with moderate hyperhomocysteinemia [4, 5] as well as in animal models [6, 7] and cell cultures

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[8]. In wild-type and APOE-deficient mice, a depletion of dietary folate (a cofactor of homocysteine metabolism) leads to increased levels of serum and liver cholesterol and an altered expression profile of the cholesterol biosynthesis pathway [9]. A meta-analysis by Sharma et al. [10] demonstrated 112 genes that are modulated by elevated levels of homocysteine. Mapping these genes to their pathways suggested that hyperhomocysteinemia might induce arteriosclerosis by directly affecting lipid metabolism. To test for a possible interaction between homocysteine and plasma lipid metabolism on the genetic level, we examined 7 functionally relevant genetic variants of homocysteine metabolism for a possible influence on the plasma lipid profile.

Materials and Methods

Patients and Study Participants

Overall, 504 individuals were included in this study. We recruited 135 consecutive Caucasian patients (mean age 64.2 ± 8.8 years, 27.4% female) from the ultrasound division of the Department of Neurology of the University of Bonn, Germany. Patients had been referred due to cerebrovascular, cardiovascular, or peripheral vascular disease and had been diagnosed with at least unilateral 30% carotid stenosis. Additionally, we recruited the patients' spouses (n = 100; mean age 61.8 ± 8.7 years, 79% female) and their offspring (n = 269, mean age 35.9 ± 8.4 years, 53% female). Personal data, ultrasonic findings, medical history, tobacco smoking, and laboratory findings including fasting blood lipid profile parameters were determined as described previously [11]. Of the enrolled subjects, 17.9% received medication with an HMG-CoA reductase inhibitor (statin). Patients with abnormal renal parameters or an intake of (multi)vitamin preparations were excluded from the study. Data about physical activity or lifestyle were not available. The study was approved by the local ethics committee, and all participants gave their written informed consent.

Genotyping

Genomic DNA prepared from peripheral leukocytes was used for genotyping by PCR amplification and restriction analysis of 7 genetic variants of homocysteine metabolism including the intronic deletion dihydrofolate reductase c.594 + 59del19bp (affecting the transcript level; GenBank NM_000791.3); the splice alteration CBS c.844_855ins68 (affecting the transcript level; GenBank S78267.1); the missense mutations (i.e. leading to amino acid exchanges) methionine synthase c.2756A→G (p.D919G; rs1805087), methylenetetrahydrofolate reductase (MTHFR) c.677C→T (p.A222V; rs1801133), and c.1298A→C (p.E429A; rs1801131); reduced folate carrier 1 c.80G→A (p.R27H; rs1051266), and transcobalamin 2 (Tc2) c.776C→G (p.P259R; rs1801198) [12].

Statistics

Deviations from the Hardy-Weinberg equilibrium were separately analyzed using a χ^2 goodness-of-fit test comparing observed and expected numbers for each genetic variant ($\alpha = 0.05$). As some of the analyzed variables were not normally distributed, a log transformation was carried out for statistical testing. Linear regression with lipoprotein(a), total cholesterol (TC), LDL, HDL, and triglyceride (TG) fasting plasma levels as dependent variables and age, gender, statin therapy, smoking, family relationship, and the 7 examined polymorphisms as independent variables was utilized to test for independent associations of the polymorphisms with the plasma lipid profile. Due to multiple testing, the threshold was defined as $\alpha = 0.01$ in accordance with the Bonferroni correction. Additionally, linear regression was used to analyze the association of homocysteine levels with the 7 polymorphisms. One-way ANOVA was used for univariate descriptive analysis of the association of genotypes with plasma lipoprotein parameters.

Results

The distribution of genotypes did not deviate from the Hardy-Weinberg equilibrium. Linear regression showed a correlation of the Tc2 c.776C→G polymorphism with the plasma levels of LDL ($p = 0.010$), HDL ($p = 0.009$), and TG ($p = 0.007$) when all study participants were analyzed (table 1). Separate analyses for patients, spouses and offspring, and participants without arteriosclerosis yielded nonsignificant results (data not shown). TC plasma levels showed an association with the Tc2 c.776C→G polymorphism for trend ($p = 0.019$). Lipoprotein(a) plasma levels were not associated with Tc2 c.776C→G. The MTHFR c.677C→T polymorphism correlated with HDL plasma levels ($p = 0.008$). None of the other polymorphisms analyzed was associated with the blood lipid profile.

The plasma homocysteine level was associated with age ($\beta = 0.312$; $p < 0.001$), gender ($p < 0.001$), and tobacco smoking ($p = 0.012$), but not with plasma lipoproteins. MTHFR c.677C→T was associated with homocysteine plasma levels (MTHFR c.677CC: homocysteine 12.90 μmol/l ± 4.00; MTHFR c.677CT: 13.65 μmol/l ± 4.02; MTHFR c.677TT: 15.20 μmol/l ± 5.37; $p < 0.001$). The G allele of Tc2 c.776C→G was associated with higher homocysteine levels in the subgroup of patients ($p = 0.013$, 1-way ANOVA). None of the other polymorphisms analyzed was associated with the homocysteine level (data not shown).

We found a correlation of gender with HDL ($p < 0.001$) and TG ($p < 0.001$), with women yielding higher HDL levels and lower TG levels. Age correlated with TC ($\beta = 0.246$; $p < 0.001$) and LDL ($\beta = 0.206$; $p < 0.001$), with TC and LDL levels increasing with age. Study group (patients, spouses or offspring) was also associated with TC ($p < 0.001$) and LDL ($p < 0.001$) but not with the other lipoprotein parameters. A separate group analysis (patients, spouses and offspring) showed that the G allele of

Table 1. Linear regression with TC, LDL, HDL, TG, and Lpa fasting plasma levels (mg/dl) as dependent variables and Tc2 c.776C→G (the other polymorphisms are not shown); due to multiple testing (5 different plasma lipid levels), the threshold was defined as $\alpha = 0.01$

Tc2 c.776C→G	CC (n = 139)	CG (n = 229)	GG (n = 136)	Linear regression, p
Total blood cholesterol	236 ± 48	240 ± 45	248 ± 48	0.019
LDL cholesterol	154 ± 38	159 ± 40	168 ± 42	0.010
HDL cholesterol	61 ± 18	60 ± 18	56 ± 15	0.009
TG	100 ± 65	105 ± 60	120 ± 83	0.007
Lpa	34 ± 39	30 ± 32	32 ± 34	0.652

Values are shown as mean plasma levels ± standard deviation for each Tc2 genotype. All individuals were included in this analysis.

Tc2 c.776C→G was only associated with TC and LDL in the offspring, but not in patients or spouses or patients and spouses combined. Statin treatment correlated with TC ($p = 0.092$) and LDL ($p = 0.033$) only for trend.

Discussion

In our study sample, the G allele of Tc2 c.776C→G was associated with an unfavorable blood lipoprotein profile, i.e. with higher LDL plasma levels, lower HDL plasma levels, higher triglyceride plasma levels, and higher TC levels, for trend. Additionally, MTHFR c.677C→T correlated with HDL plasma levels. As this polymorphism is a major polymorphic determinant of homocysteine plasma levels and is associated with vascular disease, retesting this trend in additional studies may be worthwhile.

The G allele of Tc2 c.776C→G leads to the amino acid substitution p.P259R affecting the affinity of Tc2 to vitamin B_{12} and the ability to transport vitamin B_{12} into tissues [13–15]. The reported effects on homocysteine levels have been inconsistent [16, 17]. In our sample, the G allele of Tc2 c.766C→G was associated with higher homocysteine plasma levels in the patient subgroup but not in the whole study population. However, Tc2 c.766C→G has been reported to be associated with increased methylmalonic acid [17, 18], which is regarded as a very early indicator of functional vitamin B_{12} deficiency. This indicates a reduced intracellular homocysteine remethylation in association with the G allele. Olzewski et al. [19] described a correlation between plasma homocysteine levels and TC as well as TG plasma levels in middle-aged male probands. Treatment with vitamin B_{12}, vitamin B_6, and folic acid over 21 days significantly lowered homocysteine, but also TC, TG, and LDL plasma levels, in these individuals. Recently, it was shown that experimentally induced hyperhomocysteinemia in rats leads to significantly increased plasma cholesterol levels accompanied by an increased expression and activity of hepatic HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis [20–22]. In addition, it was demonstrated that homocysteine supplementation of yeast leads to an accumulation of S-adenosyl-homocysteine, which attenuates S-adenosyl-methionine-dependent methyltransferase activities and results in an inhibition of phospholipid methylation and finally triacylglycerol accumulation [23].

Therefore, the association of the G allele of Tc2 c.677C→G with an unfavorable lipoprotein profile may be explained by a functional vitamin B_{12} deficiency and a disturbed intracellular homocysteine and SAM metabolism [1].

The missing data about family relationship, physical activity, diet, and alcohol consumption are limitations of the present study. However, the results, though preliminary, suggest an intriguing genetic link between homocysteine metabolism and lipoprotein metabolism. Within the limitations of any association study and within the limitations given by the restricted representativity of our study sample concerning the general population, these data support the hypothesis that homocysteine metabolism interacts with lipoprotein metabolism. The missense polymorphism Tc2 c.776C→G may be a common genetic basis of elevated homocysteine plasma levels and an unfavorable blood lipoprotein profile. The observed biochemical and genetic linkage of homocysteine and lipoprotein plasma levels may be of importance for studies investigating risk factors for vascular disease and should be confirmed in independent studies.

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