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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 γ -synthase c.844g55ins68, methionine synthase c.2756AG, methylenetetrahydrofolate reductase c.677CT and c.1298AC, reduced folate carrier 1 c.1207G, and methylenetetrahydrofolate reductase c.1195G. RESULTS: Linear regression analysis showed an association of Tc2c.776CG with LDL (p = 0.010), HDL (p = 0.009), and TG (p = 0.007), with the G allele of Tc2c.776CG associated with an unfavorable blood lipid profile. Moreover, the G allele of Tc2c.776CG was associated with higher plasma homocysteine levels (p = 0.013, 1-way ANOVA). CONCLUSION: These data support the hypothesis that alterations in homocysteine metabolism are associated with unfavorable lipid profiles.

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

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Running title: Tc2 c.776C>G is associated with plasma lipoproteins

Keywords: homocysteine; transcobalamin 2 c.776C>G; lipoprotein profile

Abstract

Recent studies suggested a relation of homocysteine with lipid metabolism. Aim of this study was analyzing a possible genetic basis of 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). We analyzed the association of plasma levels of lipoprotein a (Lpa), total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (TG) with plasma homocysteine levels and with seven variants of homocysteine metabolism: dihydrofolate reductase (DHFR) c.594+59del19bp, cystathionine beta-synthase (CBS) c.844_855ins68, methionine synthase (MTR) c.2756A>G, methylenetetrahydrofolate reductase (MTHFR) c.677C>T and c.1298A>C, reduced folate carrier 1 (RFC1) c.80G>A, and transcobalamin 2 (Tc2) c.776C>G. 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, one-way ANOVA). These data support the hypothesis that alterations of 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, suggested a relation of homocysteine and lipid metabolism [1]. Patients with severe hyperhomocysteinemia due to 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 [8]. In wildtype and APOE-deficient mice, depletion of dietary folate (a cofactor of homocysteine metabolism) leads to increased levels of serum and liver cholesterol and altered expression profile of the cholesterol biosynthesis pathway [9]. A meta-analysis by Sharma et al 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 the lipid metabolism [10]. To test for a possible interaction between homocysteine and plasma lipid metabolism on the genetic level, we examined seven 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 \pm SD age, 64.2 ± 8.8 years, 27.4% female) from the ultrasound division of the Department of Neurology, University of Bonn, Germany. Patients had been referred due to cerebro-, cardiovascular or peripheral vascular disease and had been diagnosed with at least unilateral 30% carotid stenosis. Additionally, we recruited the patients' spouses (n = 100; 61.8

± 8.7 years, 79% female) and their offspring ($n = 269$, 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 a HMG-COA reductase inhibitor (statin). Patients with abnormal renal parameters or intake of (multi)vitamin preparations were excluded from the study. Data about physical activity or life-style were not available. The study was approved by the local ethics committee, and all participants gave written informed consent.

Genotyping

Genomic DNA prepared from peripheral leukocytes was used for genotyping by PCR amplification and restriction analysis of seven genetic variants of homocysteine metabolism including the intronic deletion dihydrofolate reductase (DHFR) c.594+59del19bp (affecting the transcript level; GenBank NM_000791.3), the splice alteration cystathionine beta-synthase (CBS) c.844_855ins68 (affecting the transcript level; GenBank S78267.1), and the missense mutations (i.e., leading to amino acid exchanges) methionine synthase (MTR) 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 (RFC1) c.80G>A (p.R27H; rs1051266), and transcobalamin 2 (Tc2) c.776C>G (p.P259R; rs1801198) [12].

Statistics

Deviations from 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) (Lpa), total cholesterol (TC), LDL, HDL, and triglyceride (TG) fasting plasma levels as dependent variables and age,

gender, statin therapy, smoking, family relationship, and the seven examined polymorphisms as independent variables was utilized to test for independent associations of the polymorphisms with the plasma lipid profile. Due to multiple testing, threshold was defined as $\alpha = 0.01$ in accordance to the Bonferroni-correction. Additionally, linear regression was used to analyze the association of homocysteine levels with the seven 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 analysed. Separate analysis for patients, spouses and offspring, and participants without arteriosclerosis yielded non-significant results (data not shown). TC plasma levels showed an association with the Tc2 c.776C>G polymorphism for trend ($p=0.019$). Lpa 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 with tobacco smoking ($p = 0.012$), but not with plasma lipoproteins. MTHFR c.677C>T was associated with the homocysteine plasma levels (MTHFR c.677CC: homocysteine = $12.90 \mu\text{mol/l} \pm 4.00$; MTHFR c.677CT: $13.65 \mu\text{mol/l} \pm 4.02$; MTHFR c.677TT: $15.20 \mu\text{mol/l} \pm 5.37$; $p < 0.001$). The G-allele of Tc2 c.776C>G was associated with higher homocysteine levels in the subgroup of patients ($p=0.013$, one-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. Group of study (patients, spouses or offspring) was also associated with TC ($p < 0.001$) and LDL ($p < 0.001$), but not with the other lipoprotein parameters. Separate group analysis (patients, spouses and offspring), showed that the G-allele of 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, this trend may be worthwhile to be retested in additional studies.

The G-allele of Tc2 c.776C>G leads to the amino acid substitution p.P259R affecting the affinity of the transcobalamin2 to vitamin B12 and the ability to transport vitamin B12 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 B12 deficiency. This indicates a reduced intracellular homocysteine remethylation in association with the G-allele. Olzewski et al described a correlation between plasma homocysteine levels and TC, as well as TG plasma

levels in middle-aged, male probands. Treatment with vitamin B12, vitamin B6 and folic acid over 21 days significantly lowered homocysteine, but also TC, TG and LDL plasma levels in these individuals [19]. Recently, it was shown that experimentally induced hyperhomocysteinemia in rats leads to significantly increased plasma cholesterol levels accompanied by 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 (SAH), which attenuates S-adenosyl-methionine (SAM)-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 functional vitamin B12 deficiency, and 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, although preliminary, suggest an intriguing genetic link between homocysteine and lipoprotein metabolism. Within the limitation of any association study and within the limitations given by 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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Tc2 c.776C>G	CC (n=139)	CG (n=229)	GG (n=136)	Linear Regression
total blood cholesterol	236 ± 48	240 ± 45	248 ± 48	p=0.019
LDL-Cholesterol	154 ± 38	159 ± 40	168 ± 42	p=0.010
HDL-cholesterol	61 ± 18	60 ± 18	56 ± 15	p=0.009
Triglycerides	100 ± 65	105 ± 60	120 ± 83	p=0.007
Lipoprotein a	34 ± 39	30 ± 32	32 ± 34	p=0.652

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 (five different plasma lipid levels), threshold was defined as $\alpha=0.01$. Values are shown as mean plasma level values in mg/dl \pm standard deviation for each TC2 genotype. All individuals were included in this analysis.