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POSSIBLE INFLUENCE OF *MTHFR* C677T POLYMORPHISM ON SERUM LIPID LEVELS IN SERBIAN SCHOOL CHILDREN

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Abstract - The effect of 5,10-methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism on serum lipid profiles is controversial. We randomly selected 530 healthy schoolchildren that were included in the Yugoslav Study of the Precursors of Atherosclerosis in School Children (YUSAD). The detected frequencies of MTHFR 677TT genotypes and MTHFR 677T allele were 13.4% and 35.5%, respectively. The mean levels of total cholesterol, low-density lipoprotein cholesterol and triglycerides were higher and high-density lipoprotein cholesterol was lower in the group of children with TT genotype compared with remaining genotypes. All differences were without statistical significance. Our findings suggest that there is no influence of MTHFR C677T gene variants on the lipid parameters in the population of Serbian children.

Key words: Atherosclerosis, lipid levels, schoolchildren, *MTHFR* gene, *MTHFR* 677T allele, polymorphism

INTRODUCTION

Atherosclerosis is one of the major causes of mortality in industrialized countries and has become a global problem. Although the clinical manifestations of atherosclerosis generally occur during the middle age it is documented that disease pathogenesis begins in childhood (Anand, 2009).

Levels of serum cholesterol, triglycerides and the various classes of lipoproteins change during childhood, adolescence and young adulthood. Cholesterol levels in childhood and adolescence predict early adult cholesterol levels (Brotons et al. 1998). Risk factors of atherosclerosis, such as high serum total cholesterol (TC) and triglycerides, low-density lipoprotein (LDL) cholesterol (März et al., 2004) or low high-density lipoprotein (HDL) cholesterol (Boden, 2000) and hypertension have been found in children and adolescents with familial predisposition (Berenson et al., 2003; Labarthea et al., 2003).

The 5,10 methylenetetrahydrofolate reductase (MTHFR) enzyme is involved in the reduction of 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate. Substitution of thymine for cytosine in the *MTHFR* gene at position 677 (C677T; rs1801133) results in a thermolabile enzyme form

that causes a reduced enzymatic activity and consequently an elevated plasma homocysteine level. A higher level of homocysteine may alter vascular function and thus promote atherosclerosis. According to several studies, the *MTHFR* 677T allele is associated with unfavorable serum lipid profile (Zhang et al., 2010) but there are opposite results (Pallaud et al., 2001).

The aim of the present study was to evaluate the association of *MTHFR* C677T polymorphism with serum lipid levels in Serbian schoolchildren (Yugoslav Study of the Precursors of Atherosclerosis in School Children- YUSAD).

MATERIALS AND METHODS

YUSAD is a prospective multicentric study that began in 1998. The study included 530 randomly selected healthy children of both genders from 11 Serbian centers (Čukarica, Palilula, Požarevac, Užice, Kraljevo, Knjaževac, Bor, Niš, Subotica, Arilje and Despotovac). At the time of physical examination and blood sample collection for genetic analysis, all children were 14-15 years of age.

For each child written informed consent was obtained from parents or guardians. The study protocol was approved by the Ethical Committee of the Faculty of Medicine, University of Belgrade. Data were collected using standardized questionnaires and clinical examinations (height, body mass, blood pressure). Body mass index (BMI) was calculated as the participant's weight in kilograms divided by the square of their height in meters. Detailed medical histories of children were taken.

Each individual fasted for 12 h before sample administration. Whole blood samples were collected in EDTA-containing tubes and serum samples in tubes without any anticoagulant. Serum glucose, TC, HDL cholesterol and triglyceride levels were measured as described previously (Majkic-Singh et al., 2006). LDL cholesterol concentrations in samples were calculated using Friedewald's equation. Levels of apolipoprotein AI, apolipoprotein AII, apolipoprotein B, apolipoprotein (a) and apolipoprotein E were measured by immunoturbidimetric methods (Majkic-Singh et al., 2006).

Genomic DNA was extracted from peripheral blood leukocytes by the salting out method (Miller et al., 1988). *MTHFR* C677T genotypes were analyzed by the polymerase chain reaction restriction-fragment length polymorphism (PCR-RFLP) method, as previously described (Frosst et al., 1995).

For statistical analyses, the statistical software package SAS and Statistica 10 were used. Quantitative variables were expressed as mean ± standard deviation. Genotype frequencies were determined by direct counting, and the standard fit test was used to test the Hardy-Weinberg equilibrium. The association of genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). In order to evaluate the association of serum lipid levels with gender, BMI and *MTHFR* C677T genotypes, multivariate regression analysis was also performed.

RESULTS

All children included in the study were healthy. Table 1 presents the mean characteristics of the children cohort analyzed in the YUSAD study. Data are presented separately for girls and boys. Systolic and diastolic blood pressures were significantly higher in boys than in girls. The means of serum TC, LDL cholesterol, ApoAI and ApoB levels and BMI were significantly higher in girls than in boys.

Frequencies of genotypes and alleles of the *MTHFR* C677T polymorphism are shown in Table 2. The genotype distribution was in Hardy-Weinberg equilibrium, and no significant difference in genotypes and allele frequencies between boys and girls has been observed.

As shown in Table 3, the mean measured levels of TC, LDL cholesterol and triglycerides were higher in the group of children with TT genotype compared with the remaining genotype groups. Additionally,

Table1. Characteristics of the studied cohort of children (YUSAD study participants).

Parameter	boys	girls	Comparison of gender F, p
BMI (kg⁄m^2)	20.6±3.7	21.3±3.8	1.962, p< 0.05
SBP(mmHg)	115.0±12.5	110.0±11.3	4.212, p< 0.05
DBP (mmHg)	73.0±8.5	72.0±8.4	2.158, p< 0.05
TC (mmol⁄L)	4.22±0.9	4.54±0.9	4.082, p< 0.05
Triglyceride (mmol⁄L)	0.93±0.5	0.96±0.4	0.617, p> 0.05
HDL cholesterol (mmol⁄L)	1.43±0.5	1.47±0.4	0.891, p> 0.05
LDL cholesterol (mmol⁄L)	2.39±0.8	2.58±0.9	2.389, p< 0.05
apoAI (g⁄L)	1.35±0.3	1.42±0.3	2.527 ,p< 0.05
apoAII (g⁄L)	0.32±0.3	0.30±0.2	0.624, p> 0.05
apo(a) (g⁄L)	0.20±0.2	0.24±0.2	1.184, p> 0.05
apoB (g⁄L)	0.71±0.3	0.75±0.2	1.994, p< 0.05
apoE g⁄L)	0.32±0.3	0.30±0.2	0.624, p> 0.05

BMI – Body mass index, SBP – Systolic blood pressure, DBP – Diastolic blood pressure, TC – Total cholesterol. Results are presented as mean ±standard deviation

Table 2. Genotypes and alleles frequencies of the MTHFR C677T polymorphism in analyzed YUSAD study cohort of children **n (%)**.

Genotype	n (%)	Allele	%
	530		
CC	225 (42.4)	C	64.5
CT	234 (44.2)	T	35.5
TT	71 (13.4)		

Results are presented as numbers and percents (n, %).

Table 3. The mean values of serum lipid levels by *MTHFR C677T* genotype (mean ±standard deviation).

Parameter	genotype	all	boys	girls
TC	CC	4.39±0.91	4.26±0.88	4.54±0.,93
mmol/l	CT	4.31±0.81	4.11±0.78	4.54±0.79
	TT	4.56±1.02	4.51±0.99	4.63±1.06
HDL	CC	1.44±0.39	1.41±0,39	1.47±0,39
mmol/l	CT	1.48±0.48	1.46±0.55	1.49±0.40
	TT	1.40±0.33	1.40±0.,33	1.40±0.33
LDL	CC	2.52±0.91	2.43±0.87	2.61±0.94
mmol/l	CT	2.39±0.83	2.29±0.84	2.52±0.80
	TT	2.70±1.04	2.65±1.03	2.81±1.05
Triglycerides	CC	0.91±0.39	0.86±0.39	0.97±0.39
mmol/l	CT	0.94±0.47	0.94±0.53	0.94±0.40
	TT	0.99±0.48	0.99±0.58	0.99±0.65

the HDL cholesterol was lower in the group of children with TT genotype. All observed differences were without statistical significance.

No statistical significance in mean values of BMI between groups of children with different *MTHFR* genotypes was observed (boys, p=0.46; and girls, p=0.39).

Multivariate regression analysis showed significant positive influence of gender on TC and HDL cholesterol variation (β= 0.165, p=0.000; β=0.110, p=0.017), positive influence of BMI on triglycerides variation (β= 0.234, p=0.000), negative influence on HDL cholesterol variation (β= -0.162, p=0.000) and no influence of *MTHFR* C677T polymorphism genotypes on mean values of lipid levels.

DISCUSSION

Atherosclerosis is a heterogeneous disorder with various genetic and environmental factors that contribute to its development. Well-known risk factors for atherosclerosis are age, gender, hypertension, dyslipidemia, hyperhomocysteinemia, smoking habits and genetic predisposition (http://wwwcdcgov/ nccdphp/ burdenbook2004, Damnjanovic et al., 2006).

Elevated serum levels of TC, triglycerides, LDL cholesterol and apolipoprotein B (ApoB), or low HDL cholesterol and apolipoprotein AI (ApoAI) are important risk factors in the pathogenesis of atherosclerosis (Martin et al., 1986, Boden 2000, März et al., 2004, Hokanson et al., 1996, Kwiterovich et al., 1992). The lipid profile mentioned above depends on multiple genetic and environmental factors and their interactions (Heller et al., 1993, Ruixing et al., 2007). The presence of cardiovascular risk factors in children may be important for the development of atherosclerosis in adulthood.

Epidemiological studies of adolescents confirmed the differences in the lipid parameters between genders (Brotons et al., 1998). In the light of this finding, we compared the mean values of the lipid parameters measured in the boys' and girls' groups. Significantly higher mean values of TC and LDL cholesterol were observed in girls. Similar results were obtained in the entire cohort of children at the age of 15 included in YUSAD study (Simeunovic et al., 2011). These results are consistent with literature data for Caucasians, which suggests that girls between 12 to 16 years have higher TC levels than boys (Hickman et al., 1998).

As is well known, genetic polymorphisms account for 40-60% of the interindividual variation in plasma lipid levels (Heller et al., 1993, Pereira et al., 2004, Kathiresan et al., 2008). The MTHFR enzyme plays an important role in homocysteine metabolism. Literature data suggest an association of the *MTHFR* 677T allele with higher homocysteine levels in children and adults (Alessio et al., 2008, Ono et al., 2002, Pintó et al., 2001). Hyperhomocysteinemia plays a role in LDL oxidation and may influence plasma lipid profile. In concordance with this are findings that children with hyperhomocysteinemia had higher serum TC levels and low serum HDL cholesterol levels (Szamosi et al., 2004).

According to our study, the levels of TC, LDL cholesterol and triglycerides are higher in the group of children with TT genotype compared with the remaining genotypes. In contrast, the HDL cholesterol is lower in the TT group of children. The same results were obtained for both genders. Considering that the trend of lipid parameters depends on genotype, we assumed that the *MTHFR* 677TT genotype could influence the lipid parameter profile; however, all of the observed differences were without statistical significance. Multivariate regression analysis showed a positive influence of gender on TC and HDL cholesterol variation, positive influence of BMI on triglycerides and negative influence on HDL cholesterol variation. The influence of *MTHFR* C677T polymorphism genotypes on mean values of lipid levels was observed. In addition, T allele frequency between boys and girls was not significantly different.

Literature data indicate that the *MTHFR* 677T allele is associated with unfavorable serum lipid profiles in different populations (Huemer et al., 2006, Huang et al., 2008, Pitsavos et al., 2006, Zhang et al., 2010). Some results point on the *MTHFR* TT genotype as a predictor of cardiovascular risk in hypertensive adolescents (Koo et al., 2008). Furthermore, the T allele of the C677T *MTHFR* gene polymorphism was associated with hypertension in pregnancy (Kosmas et al., 2004), blood pressure variation (Yin et al., 2012) and increased risk of hypertension (Markan et al., 2007) in some populations.

Several studies have established an association between the *MTHFR* 677TT genotype and complications of atherosclerosis, venous thrombosis and cerebrovascular stroke (Gouveia and Canhão, 2010). In the Serbian population, children carriers of the 677CT genotype have a 3.62 times higher risk to develop stroke (Djordjevic et al., 2012).

According to our study, *MTHFR* C677T polymorphism genotypes are not relevant to the prediction of serum lipid levels in Serbian schoolchildren.

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