

Association of PRDM16 rs12409277 and CtBP2 rs1561589 gene polymorphisms with lipid profile of adolescents

Nela Maksimovic¹, Vanja Vidovic², Tatjana Damnjanovic¹, Biljana Jekic¹, Nada Majkic Singh³, Slavko Simeunovic⁴, Dara Savic Bozovic⁵, Stojko Vidovic², Ivana Novakovic¹

¹Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Serbia

²Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

³Society of Medical Biochemists, Belgrade, Serbia

⁴University Children's Hospital, Belgrade, Serbia

⁵Uzice General Hospital, Uzice, Serbia

Submitted: 25 April 2019

Accepted: 18 October 2019

Arch Med Sci

DOI: <https://doi.org/10.5114/aoms/113174>

Copyright © 2020 Termedia & Banach

Corresponding author:

Nela Maksimovic

Institute of Human Genetics

Faculty of Medicine

University of Belgrade

26 Visegradska St

11000 Belgrade, Serbia

Phone: +381 11 3607 040

E-mail:

nela.maksimovic@med.bg.ac.rs

Abstract

Introduction: Positive regulatory domain containing 16 (PRDM16) protein represents the key regulator of brown adipose tissue (BAT) development. It induces brown fat phenotype and represses white adipose tissue specific genes through the association with C-terminal binding co-repressor proteins (CtBP1 and CtBP2). In healthy adults presence of BAT has been associated with lower glucose, total cholesterol and LDL (low-density lipoprotein) cholesterol levels. Our aim was to analyze the association of *PRDM16* gene (rs12409277) and *CtBP2* gene (rs1561589) polymorphisms with body mass index (BMI), fasting glucose level and lipid profile of adolescents.

Material and methods: Our study included 295 healthy school children, 145 boys (49.2%) and 150 girls (50.8%), 15 years of age. Genotypes for the selected polymorphisms were detected by the real-time PCR method. Age, gender, height, weight, lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) and fasting glucose levels were recorded.

Results: We did not find a statistically significant association of rs12409277 and rs1561589 polymorphisms with BMI, fasting glucose and lipid profile of adolescents. We further analyzed the combined effect of the two SNPs and the statistical analysis showed that carriers of CT genotype of rs12409277 polymorphism and GG genotype of rs1561589 polymorphism had significantly lower total cholesterol ($p = 0.001$) and LDL cholesterol ($p = 0.008$) levels compared to all other groups of genotypes.

Conclusions: Our study suggests that rs12409277 and rs1561589 polymorphism might have an influence on total and LDL cholesterol levels in adolescents. Larger studies should be performed in order to confirm our results.

Key words: polymorphisms, BMI, lipids, adolescents, brown adipose tissue.

Introduction

During childhood, brown adipose tissue (BAT) dissipates chemical energy to produce heat as a defense against hypothermia and obesity and has an important role in weight determination and musculoskeletal development [1, 2]. Activity of BAT may vary depending on age, gender, adiposity and temperature [3]. It has been shown that volume and activity of BAT especially increases during puberty [4], probably because of the effect of sex

steroids and growth hormone [5, 6]. Discovery of presence of BAT in adolescents and adults raised many questions about its physiology, function, possible influence on human health and possible treatment options. The activity of BAT in adults has been associated with body mass index (BMI), body fat mass and glucose metabolism [7]. It has been observed that healthy adults with BAT have lower glucose, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels compared to individuals without BAT [7, 8]. Brown adipose tissue volume was significantly associated with increased whole-body lipolysis, triglyceride fatty acid cycling, triglyceride-free fatty acid (FFA) oxidation and adipose tissue insulin sensitivity [9].

Positive regulatory domain containing 16 (PRDM16) protein, a 140 kDa zinc-finger protein, is a key regulator of BAT development. Its expression is highly elevated in BAT, where it induces brown fat phenotype as a co-activator of PGC1 α , PPAR α , PPAR γ , C/EBP and thyroid receptor [10]. PRDM16 also acts as a repressor of white fat cells expressed genes through association with C-terminal binding co-repressor proteins (CtBP1 and CtBP2) [11].

In the 5'-flanking region the *PRDM16* gene contains the polymorphism rs12409277. It has been suggested that T to C change in this polymorphism might influence the transcriptional activity of the gene [12].

CtBP2 controls cellular processes by acting as a transcriptional corepressor, transcriptional activator and regulator of the cytoskeleton [13]. An intronic polymorphism within the *CtBP2* gene, rs1561589, has been previously associated with BMI in females [14].

The aim of our research was to investigate the association of rs12409277 polymorphism within the *PRDM16* gene and rs1561589 polymorphism within the *CtBP2* gene with BMI, fasting glucose level and lipid profile of adolescents.

Material and methods

Study design and study population

Our study included 295 randomly selected healthy school children of both genders, 15 years of age, who participated in Yugoslav Study of the Precursors of Atherosclerosis in School Children (YUSAD). Anthropometric, demographic and medical data including age, gender, height, weight, lipid profile (TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, triglycerides (TG)) and fasting glucose were recorded. Children were classified as normal weight, overweight (above 85th percentile) and obese (above 97th percentile) according to BMI charts published for Serbian children 15 years of age [15]. Criteria for exclusion from the study were type 1 or type 2 diabetes, generalized inflammation, cardiovascular diseases, malignant diseases, genetic syndromes and chronic immobility or cerebral palsy. A signed informed consent form was obtained from each child's parent or guardian and the study protocol was approved by the Ethics Committee of Faculty of Medicine, University of Belgrade, Serbia.

Genotyping

Molecular-genetic analyses were performed at the Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Serbia. DNA was extracted from 5 ml of peripheral blood by the salting out method [16]. Genotypes of the selected polymorphisms were detected by the real-time PCR method using pre-designed TaqMan SNP Genotyping assays. PCR amplification included an initial step at 95°C for 10 minutes and 40 cycles of 15 seconds at 92°C and 1 minute at 60°C. PCR and post-PCR fluorescence analyses were performed on the Applied Biosystems 7500 Real Time PCR System (Applied Biosystems, Foster City, CA), and the results were analyzed using the Applied Biosystems 7500 software v2.0.6 (Applied Biosystems, Foster City, CA).

Table I. Main characteristics of adolescents included in the study

Parameter	All	Boys	Girls	P-value
BMI [kg/m ²]	22.08 ±4.31	21.86 ±4.33	22.26 ±0.63	0.244
SBP [mm Hg]	114.77 ±12.53	116.64 ±14.22	112.96 ±10.37	0.012*
DBP [mm Hg]	72.91 ±9.12	73.09 ±9.63	72.74 ±8.62	0.745
FPG [mmol/l]	4.72 ±0.58	4.79 ±0.53	4.64 ±0.63	0.005*
Tg [mmol/l]	0.99 ±0.55	0.99 ±0.63	1.00 ±0.47	0.213
TC [mmol/l]	4.36 ±0.87	4.21 ±0.79	4.50 ±0.92	0.003*
HDL-C [mmol/l]	1.42 ±0.41	1.38 ±0.43	1.45 ±0.39	0.164
LDL-C [mmol/l]	2.48 ±0.87	2.40 ±0.84	2.55 ±0.90	0.122

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, FPG – fasting plasma glucose, Tg – triglycerides, TC – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol. *P-value < 0.05.

Biochemical analysis

Each individual fasted for 12 h before sample collection. Serum glucose, total cholesterol, HDL-C and triglyceride levels were measured as described previously [17]. LDL-C concentrations in samples were calculated using Friedewald's equation ($LDL-C = TC - HDL-C - TG/5$) [18].

Statistical methods

For statistical analyses, the statistical software package SPSS 17 was used. Quantitative variables were expressed as mean \pm standard deviation. The association of genotypes and BMI, fasting glucose level and serum lipid levels was tested by analysis of variance (ANOVA) or the Kruskal-Wallis test. After the grouping of genotypes Student's *t*-test or the Mann-Whitney test was used, depending on the variable distribution. In order to evaluate the association of serum lipid levels and PRDM16 and CtBP2 genotypes with gender, fasting glucose level and BMI as covariates, multivariate linear regression analysis was also performed.

Results

Our study included 295 school children 15 years of age. There were 145 boys (49.2%) and 150 girls

(50.8%). Among boys, 26 (17.9%) were overweight and 20 (13.8%) were obese. In the group of girls, 29 (19.3%) were overweight and 17 were obese (11.3%). The main characteristics of adolescents are presented in Table I. There was a statistically significant difference in mean values of systolic blood pressure, fasting glucose level and TC level between the groups of boys and girls.

Frequencies of genotypes and alleles of rs12409277 and rs1561589 polymorphisms are presented in Table II. The distributions of genotypes

Table II. Frequencies of rs12409277 and rs1561589 genotypes and alleles, *n* (%)

Genotypes	Frequency, <i>n</i> (%)	Alleles	Frequency
rs12409277			
TT	242 (82.03)	T	0.91
CT	52 (17.63)	C	0.09
CC	1 (0.34)		
rs1561589			
GG	128 (43.39)	G	0.65
GA	129 (43.73)	A	0.35
AA	38 (12.88)		

Table III. Mean values of analyzed parameters by PRDM16 rs12409277 genotype (mean \pm SD)

Parameter/Genotype	All	<i>P</i> -value	Boys	<i>P</i> -value	Girls	<i>P</i> -value
BMI [kg/m ²]						
TT	21.99 \pm 4.30	0.327	21.74 \pm 4.22	0.474	22.21 \pm 4.39	0.434
CT+CC	22.54 \pm 4.38		22.47 \pm 4.92		22.45 \pm 3.96	
FPG [mmol/l]						
TT	4.72 \pm 0.60	0.790	4.79 \pm 0.52	0.919	4.65 \pm 0.67	0.793
CT+CC	4.70 \pm 0.48		4.79 \pm 0.52		4.59 \pm 0.40	
Tg [mmol/l]						
TT	1.00 \pm 0.58	0.442	0.99 \pm 0.66	0.555	1.02 \pm 0.49	0.229
CT+CC	0.92 \pm 0.38		0.99 \pm 0.46		0.90 \pm 0.38	
TC [mmol/l]						
TT	4.40 \pm 0.87	0.067	4.21 \pm 0.79	0.739	4.57 \pm 0.92	0.037*
CT+CC	4.20 \pm 0.84		4.17 \pm 0.81		4.21 \pm 0.86	
HDL-C [mmol/l]						
TT	1.42 \pm 0.42	0.930	1.40 \pm 0.44	0.328	1.43 \pm 0.39	0.340
CT+CC	1.41 \pm 0.36		1.30 \pm 0.36		1.51 \pm 0.34	
LDL-C [mmol/l]						
TT	2.51 \pm 0.88	0.170	2.39 \pm 0.85	0.828	2.61 \pm 0.90	0.054
CT+CC	2.36 \pm 0.82		2.41 \pm 0.80		2.29 \pm 0.85	

**P*-value < 0.05

Table IV. Mean values of analyzed parameters by CtBP2 rs1561589 genotype (mean \pm SD)

Parameter/Genotype	All	P-value	Boys	P-value	Girls	P-value
BMI [kg/m²]						
GG	21.90 \pm 3.89	0.897	21.58 \pm 3.78	0.928	22.19 \pm 4.00	0.516
GA	22.15 \pm 4.79		22.13 \pm 4.86		22.18 \pm 4.74	
AA	22.26 \pm 4.20		21.38 \pm 4.10		23.22 \pm 4.20	
FPG [mmol/l]						
GG	4.69 \pm 0.57	0.259	4.80 \pm 0.55	0.153	4.59 \pm 0.57	0.578
GA	4.76 \pm 0.61		4.80 \pm 0.52		4.71 \pm 0.70	
AA	4.58 \pm 0.77		4.52 \pm 0.93		4.64 \pm 0.56	
Tg [mmol/l]						
GG	0.93 \pm 0.39	0.397	0.88 \pm 0.37	0.564	0.97 \pm 0.40	0.703
GA	1.06 \pm 0.66		1.10 \pm 0.84		1.01 \pm 0.40	
AA	1.01 \pm 0.62		0.93 \pm 0.33		1.10 \pm 0.82	
TC [mmol/l]						
GG	4.32 \pm 0.91	0.164	4.17 \pm 0.77	0.625	4.47 \pm 1.00	0.191
GA	4.45 \pm 0.86		4.28 \pm 0.84		4.64 \pm 0.85	
AA	4.17 \pm 0.78		4.13 \pm 0.62		4.21 \pm 0.93	
HDL-C [mmol/l]						
GG	1.42 \pm 0.36	0.743	1.38 \pm 0.35	0.993	1.46 \pm 0.37	0.511
GA	1.41 \pm 0.46		1.37 \pm 0.51		1.45 \pm 0.40	
AA	1.37 \pm 0.35		1.38 \pm 0.36		1.35 \pm 0.34	
LDL-C [mmol/l]						
GG	2.46 \pm 0.82	0.293	2.39 \pm 0.71	0.634	2.52 \pm 0.91	0.445
GA	2.55 \pm 0.96		2.46 \pm 1.00		2.64 \pm 0.91	
AA	2.31 \pm 0.76		2.26 \pm 0.66		2.35 \pm 0.86	

of analyzed polymorphisms were in Hardy-Weinberg equilibrium. There was no statistically significant difference between male and female adolescents in frequencies of *PRDM16* ($p = 0.476$) and *CtBP2* ($p = 0.816$) genotypes. Mean values of analyzed parameters depending on the *PRDM16* and *CtBP2* genotypes are shown in Table III and Table IV respectively.

Statistical analysis has shown that there is a statistically significant difference in mean values of TC ($p = 0.037$) and borderline significance in mean values of LDL-C ($p = 0.054$) depending on *PRDM16* genotype in the group of girls. Girls carrying TT genotype had a significantly higher TC level and a tendency towards a higher LDL-C level. However, when we performed multiple linear regression analysis using fasting glucose level and BMI as covariates we could not confirm these results. Children were further grouped according to

their *PRDM16* and *CtBP2* genotypes in groups with TT+GG genotypes, CT+GG genotypes, TT+GA/AA genotypes and CT+GA/AA genotypes, respectively. Combination of CC+GA genotypes was observed in just one patient while other combinations of genotypes were not recorded in our study group. Statistical analysis showed that carriers of CT+GG genotype have significantly lower TC ($p = 0.005$) and LDL-C ($p = 0.032$) levels compared to all other groups (Table V). These results were confirmed by multiple linear regression analysis for both TC ($\beta = -0.198$, $p = 0.001$) and LDL-C ($\beta = -0.159$, $p = 0.008$) using gender, BMI and fasting glucose level as covariates.

Discussion

It has been previously reported that BAT has an important role in weight determination [2, 19].

Table V. Mean values of analyzed parameters by combined PRDM16 and CtBP2 genotypes (mean \pm SD)

Genotypes	n (%)	BMI	FPG	Tg	TC	HDL-C	LDL-C
TT+GG	101 (34.35)	21.86 \pm 3.94	4.70 \pm 0.59	0.94 \pm 0.40	4.47 \pm 0.92	1.43 \pm 0.37	2.58 \pm 0.83
CT+GG	27 (9.18)	22.01 \pm 3.82	4.67 \pm 0.48	0.86 \pm 0.34	3.81 \pm 0.57	1.40 \pm 0.36	2.04 \pm 0.58
TT+GA/AA	141 (47.96)	22.12 \pm 4.62	4.75 \pm 0.61	1.06 \pm 0.69	4.35 \pm 0.85	1.40 \pm 0.45	2.46 \pm 0.92
CT+GA/AA	25 (8.51)	23.01 \pm 5.00	4.71 \pm 0.50	0.96 \pm 0.41	4.53 \pm 0.88	1.45 \pm 0.37	2.62 \pm 0.88
P-value		0.805	0.897	0.458	0.005*	0.897	0.032*

*P-value < 0.05

In the study of Matsushita *et al.* [7] healthy, adult subjects with and without detectable BAT were compared. Subjects with detectable BAT were younger and showed lower adiposity-related parameters such as BMI, body fat mass, and abdominal fat area. It has also been observed that blood glucose, total cholesterol and LDL cholesterol were significantly lower in the BAT-positive group. Our aim was to investigate whether polymorphisms of genes important for expression of BAT and repression of white adipose tissue (WAT) formation may influence BMI, glucose level and lipid profile of adolescents. PRDM16 is a co-regulatory protein crucial for BAT development. Its main role is to induce BAT formation through co-activation of PGC1 α , PPAR α , PPAR γ , C/EBP and thyroid receptor genes [10] and repress the expression of white cells specific genes by interacting with CtBP1 and CtBP2 co-repressors [20]. The results of Urano *et al.* showed that the rs12409277 single nucleotide polymorphism (SNP) influences transcriptional activity of the PRDM16 gene. The presence of the C allele corresponds with higher expression activity [12] and therefore higher volume and activity of BAT/beige WAT. In our study we did not find a statistically significant association between this polymorphism and BMI, fasting glucose and lipid profile of all adolescents. In the group of girls an association between PRDM16 rs12409277 polymorphism and TC level was observed. During puberty there is an increase in production of growth hormone, growth factors, gonadotropins and sex steroid hormones that might influence BAT activity [4]. Therefore, the possible explanation for the gender-specific result we obtained might be the difference in growth and sexual maturity between boys and girls in our study group. However, our result was not confirmed by multiple linear regression analysis.

The same polymorphism was analyzed [12] in the group of postmenopausal women. Results of this study have shown that presence of CC or CT genotypes is significantly associated with higher lean body mass. In the study of AlAmrani *et al.* wild type genotype of PRDM16 rs2651899 polymorphism was associated with higher blood cho-

lesterol, HDL-C and LDL-C and with lower triglyceride level [21]. Also, it has been suggested that presence of mutated alleles (GA and AA genotype) increases the risk of obesity. Yue *et al.* investigated the rs2236518 polymorphism located in the 3'UTR region of the PRDM16 gene and detected an association with BMI in both young males (20–40 years) and older males (50–80 years) [22]. Park *et al.* tested the association of PRDM16 rs17390167 and susceptibility to metabolic syndrome (MetS) and found a significant trend but the results did not reach the statistical threshold in cohorts from the Framingham Heart Study [23]. Zhang *et al.* in their study observed a negative association between rs2236518 and susceptibility to MetS in Uygur Chinese subjects [24].

Our analysis of CtBP2 rs1561589 polymorphism also did not reveal a statistically significant association with any of the analyzed parameters. The same polymorphism was investigated in the genome-wide association study (GWAS) of Hinney *et al.* [14]. This study revealed the association of rs1561589 as well as two other intronic polymorphisms (rs126681170 and rs126674064) with susceptibility to anorexia nervosa and increased BMI, predominantly in females. They also analyzed these SNPs in the Early Growth Genetics Consortium data set that includes only children and adolescents and no significant findings were observed, which is in accordance with our results [25].

PRDM16 and CtBP2 form complexes that bind directly to the promoters of white fat tissue specific genes such as resistin and angiotensinogen and repress their expression [11]. Based on the knowledge of interaction between PRDM16 and CtBP2 proteins we investigated the combined effect of two analyzed polymorphisms. Our results have shown that carriers of CT genotype for the PRDM16 gene rs12409277 and GG genotype of CtBP2 gene rs1561589 polymorphism have significantly lower levels of TC and LDL-C compared to all the other combinations of genotypes. Presence of the minor C allele increases expression of the PRDM16 gene. The effect of intronic polymorphism in the CtBP2 gene has not yet been confirmed. However, based on the current knowledge

it is possible that the presence of the minor allele reduces the expression or activity of CtBP2 either directly or by being in linkage disequilibrium with another, still unknown functional polymorphism. Therefore, it is possible that carriers of CT+GG genotypes have higher expression of PRDM16 and CtBP2 and consequently less WAT and more BAT. To our knowledge this is the first study that has investigated the combined effect of *PRDM16* and *CtBP2* gene polymorphisms.

One limitation of our research is the lack of data about non-genetic factors that could influence the BMI, glucose level and lipid profile of adolescents. It is known that lifestyle, nutritional behavior and medications can modify lipid status [25]. The studies of Bergier *et al.* and Junger *et al.* have shown that boys are more active than girls, while girls, who in a very small percentage fulfill the physical activity recommendations, have better nutritional behavior [27, 28]. Also, an association between the *PRDM16* and *CtBP2* genotypes and TC was observed only in the population of healthy adolescents, and the long-term health impact of this association is not clear. Some studies investigated the tracking of blood lipid changes from adolescence to adulthood and their results supported the importance of measuring lipids during adolescence for identifying individuals with high risk for cardiovascular disease [29, 30]. Still, there is not enough evidence about the importance of early measurement of lipids [31].

In order to draw definite conclusions it would be of great interest to further analyze the association of combined *PRDM16* and *CtBP2* genotypes with lipid profile of adolescents and adults on a larger population, taking into consideration non-genetic factors as well.

Acknowledgements

This work was supported by the Serbian Ministry of Education, Science and Technological development (Grant 175091).

Conflict of interest

The authors declare no conflict of interest.

References

- Gilsanz V, Hu HH, Kajimura S. Relevance of brown adipose tissue in infancy and adolescence. *Pediatr Res* 2013; 73: 3-9.
- Sharp LZ, Shinoda K, Ohno H, et al. Human BAT possesses molecular signatures that resemble beige/brite cells. *PLoS One* 2012; 7: e49452.
- Kajimura S, Seale P, Spiegelman BM. Transcriptional control of brown fat development. *Cell Metab* 2010; 11: 257-62.
- Gilsanz V, Smith ML, Goodarzi F, Kim M, Wren TA, Hu HH. Changes in brown adipose tissue in boys and girls during childhood and puberty. *J Pediatr* 2012; 160: 604-9.
- Rodriguez-Cuenca S, Monjo M, Frontera M, Gianotti M, Proenza AM, Roca P. Sex steroid receptor expression profile in brown adipose tissue. Effects of hormonal status. *Cell Physiol Biochem* 2007; 20: 877-86.
- Hioki C, Yoshida T, Kogure A, et al. Effects of growth hormone (GH) on mRNA levels of uncoupling proteins 1, 2, and 3 in brown and white adipose tissues and skeletal muscle in obese mice. *Horm Metab Res* 2004; 36: 607-13.
- Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M. Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. *Int J Obes* 2014; 38: 812-7.
- Ozguven S, Ones T, Yilmaz Y, Turoglu HT, Imeryuz N. The role of active brown adipose tissue in human metabolism. *Eur J Nucl Med Mol Imaging* 2016; 43: 355-61.
- Chondronikola M, Volpi E, Borsheim E, et al. Brown adipose tissue activation is linked to distinct systemic effects on lipid metabolism in humans. *Cell Metab* 2016; 23: 1200-6.
- Seale P. Transcriptional regulatory circuits controlling brown fat development and activation. *Diabetes* 2015; 64: 2369-75.
- Kajimura S, Seale P, Tomaru T, et al. Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes Dev* 2008; 22: 1397-409.
- Urano T, Shiraki M, Sasaki N, Ouchi Y, Inoue S. Large-scale analysis reveals a functional single-nucleotide polymorphism in the 5'-flanking region of PRDM16 gene associated with lean body mass. *Aging Cell* 2014; 13: 739-43.
- Chinnadurai G. CtBP family proteins: more than transcriptional corepressors. *Bioessays* 2003; 25: 9-12.
- Hinney A, Kesselmeier M, Jall S, et al. Evidence for three genetic loci involved in both anorexia nervosa risk and variation of body mass index. *Mol Psychiatry* 2017; 22: 321-2.
- Nedeljkovic S, Vukotic M, Simeunovic S, et al. Antropometrijski parametri kod školske dece stare 10 i 15 godina u JUSAD. In: Nedeljkovic S, Simeunovic S, Vukotic M, editors. *Jugoslovenska studija prekursora ateroskleroze kod školske dece*. Belgrade: Faculty of Medicine, Belgrade University; 2006, pp. 429-38.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.
- Majkic-Singh N, Ilic M, Jankovic O, Ignjatovic S, Obradovic I, Trendovi u nalazima lipidnih frakcija u JUSAD studiji. In: Nedeljkovic S, Simeunovic S, Vukotic M, editors. *Jugoslovenska studija prekursora ateroskleroze kod skolske dece*. Belgrade: Faculty of Medicine, Belgrade University; 2006, pp. 323-34.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.
- Garcia CA, Van Nostrand D, Atkins F, et al. Reduction of brown fat 2-deoxy-2-[F-18]fluoro-D-glucose uptake by controlling environmental temperature prior to positron emission tomography scan. *Mol Imaging Biol* 2006; 8: 24-9.
- Chi J, Cohen P. The multifaceted roles of PRDM16: adipose biology and beyond. *Trends Endocrinol Metab* 2016; 27: 11-23.
- AlAmrani A, AbdelKarim M, AlZoghaibi M. PRDM16 gene polymorphism is associated with obesity and blood lipids profiles in Saudi population. *J Clin Med* 2018; 7: 141.

22. Yue H, He JW, Ke YH, et al. Association of single nucleotide polymorphism Rs2236518 in PRDM16 gene with BMI in Chinese males. *Acta Pharmacol Sin* 2013; 34: 710-6.
23. Park YM, Province MA, Gao X, et al. Longitudinal trends in the association of metabolic syndrome with 550 k single-nucleotide polymorphisms in the Framingham Heart Study. *BMC Proc* 2009; 3 Suppl 7: S116.
24. Zhang JH, Li NF, Yan ZT, et al. Association of genetic variations of PRDM16 with metabolic syndrome in a general Xinjiang Uygur population. *Endocrine* 2012; 41: 539-41.
25. Bradfield JP, Taal HR, Timpson NJ, et al. A genome-wide association meta-analysis identifies new childhood obesity loci. *Nat Genet* 2012; 44: 526-31.
26. Soran H, Adam S, Mohammad J, et al. Hypercholesterolaemia – practical information for non-specialists. *Arch Med Sci* 2018; 14: 1-21.
27. Bergier J, Niżnikowska E, Bergier B, Acs P, Salonna F, Junger J. Differences in physical activity, nutritional behaviours, and body silhouette concern among boys and girls from selected European countries. *Hum Mov* 2018; 18: 19-28.
28. Junger J, Kačúr P, Tlučáková L, Čech P, Bebčáková V. Physical activity of female students in secondary schools in the context of physical activity recommendations fulfilment. *Hum Mov* 2018; 18: 67-73.
29. Lee JH, Kim HC, Kang DR, Suh I. The 23-year tracking of blood lipids from adolescence to adulthood in Korea: the Kangwha study. *Lipids Health Dis* 2017; 16: 221.
30. Adams C, Burke V, Beilin LJ. Cholesterol tracking from childhood to adult mid-life in children from the Buselton study. *Acta Paediatr* 2005; 94: 275-80.
31. US Preventive Services Task Force; Bibbins-Domingo K, Grossman DC, Curry SJ, et al. Screening for lipid disorders in children and adolescents: US preventive services task force recommendation statement. *JAMA* 2016; 316: 625-33.