Association of PPARG rs3856806 C>T Polymorphism With Body Mass Index, Glycaemia and Lipid Parameters in Serbian Adolescents

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Abstract

Background/Aim: Peroxisome proliferator-activated receptor gamma (PPARγ) belongs to a family of nuclear hormone receptors and ligand-activated transcription factors. PPARG gene is expressed in many tissues including adipose tissue where it plays a crucial role in differentiation of adipocyte, insulin resistance, blood glucose levels and lipid metabolism. The aim of the study was to examine the association of rs3856806 polymorphism with the body mass index (BMI), fasting glucose levels and lipid parameters in Serbian adolescents.

Methods: This research included 287 adolescents of both genders (143 boys and 144 girls), 14-15 years of age. Genotype detection was done by polymerase chain reaction-restriction fragment length polymorphism (RFLP) assay.

Results: Results showed statistically significant difference in terms of fasting glucose levels among girls (p = 0.013) depending on their genotype. Female carriers of CC genotype had significantly higher level of fasting glucose levels. Also, results showed that in the group of overweight and obese girls, carriers of CT or TT genotype had statistically significant lower values of HDL cholesterol compared to girls - carriers of CC genotype (p = 0.000). However, this result was not confirmed by multiple regression analysis. Statistically significant association of rs3856806 polymorphism was not observed with BMI nor with other lipid parameters.

Conclusion: This polymorphism is associated with fasting glucose level and HDL cholesterol among girls. To draw definite conclusions, further research should be conducted including non-genetic factors and other polymorphisms among this gene.

Keywords: PPARG; rs3856806; Lipid profile; BMI; Fasting glucose level.

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Introduction

Childhood and adolescent obesity is becoming a major problem worldwide, reaching epidemic proportions. It is estimated that one out of five youth is overweight and out of them 9.5 % are severely obese.[1, 2] Adolescent obesity is associated with various disorders of which the most common are metabolic disorders and diabetes mellitus type II.[3] Recently, many studies are focused on the molecular-genetic causes of obesity and their comorbidities. To the already known factors, brown adipose tissue (BAT) is gaining an increased interest since the discovery that this type of tissue is present not only in newborns but also in adolescents and adults. Due to this fact many studies were conducted in order to give answers on its activation, physiology, impact on

human health and possible treatment options.[4, 5] The main role of BAT is to dissipate chemical energy to produce heat.[6] Its activation depends on gender, age, temperature and obesity. However, activation and volume of BAT increases during puberty, probably due to effects of sex steroids and growth hormone.[7, 8] It has been shown that adults with detectable BAT had significantly lower levels of total cholesterol, low-density lipoprotein cholesterol and glycaemia in comparison to the individuals without BAT.[9–11]

Differentiation and activation of BAT is very complex mechanism which involves multiple transcriptional factors, among which peroxisome proliferator-activated receptor gamma (PPARγ) is a crucial regulatory factor. PPARγ is essential not only for differentiation of BAT, but also for differentiation and activation of white adipose tissue.[12] PPARγ belongs to family of nuclear hormone receptors and ligand-activated transcription factors and is encoded by PPARG gene. PPARG gene is expressed in many tissues including adipose tissue where it has a key role in lipid metabolism, adipocyte differentiation, regulation of insulin resistance and blood glucose levels.[13]

Preadipocyte differentiation into either white or brown adipose tissue depends on PPARγ and its coactivators. PPARγ in interaction with Positive regulatory domain containing 16 (PRDM16) transcriptional factor leads to the expression of genes specific for BAT and beige adipose tissue and to the expression of uncoupling protein 1 (UCP1).[14, 15] On the other side, binding to the Transducin-like enhancer protein 3, PPARγ is able to suppress expression of brown and beige adipose tissue and to stimulate expression of selective genes for white adipose tissue.[14, 16] In humans, obesity leads to the decreased expression of PPARG, thus causing an increased degree of inflammation, angiogenesis and fibrosis in white adipose tissue. In line with these facts, individuals with mutations within the PPARG gene are prone to insulin resistance and lipodystrophy. However, increased PPARG expression results in improved insulin sensitivity.[17]

Within the PPARG gene, several mutations have been described which are linked to the metabolic disorders and lipodystrophy.[18] One of the common polymorphic variants in this gene is C1431T silent mutation (rs3856806) found in the exon 6.

This polymorphic variant is related with lower body mass index and favourable lipid profile.[19, 20] Even though this polymorphic variant is associated with better metabolic state, results obtained from different research are still contradictory.

Methods

Study design

Participants enrolled in this study (n = 287) were healthy adolescents, 15 years of age. Participants were selected randomly from a total of 6000 adolescents who were included in Yugoslav Study of the Precursors of Atherosclerosis in School Children (YUSAD). YUSAD study lasted for 21 years and it ended in 2008. Data of anthropometric measurements (height and weight), gender, fasting blood glucose (FBG) levels and values of lipid parameters such as total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were recorded during 2003 at 11 paediatric departments of primary health care centres (Čukarica, Palilula, Požarevac, Užice, Kraljevo, Knjaževac, Bor, Niš, Subotica, Arilje and Despotovac) in Serbia. According to the previously published BMI charts for Serbian adolescents, participants were classified in 3 groups: normal weight (< 85th percentile), overweight (≥ 85th percentile) and obese (≥ 95th percentile).[21] Criteria for exclusion from the study were presence of neurodegenerative malformations, malignant and cardiovascular disease, congenital genetics malformations, cerebral palsy, chronic immobility and diabetes mellitus type 1 or 2.

Ethical principles

This research was permitted by the Ethics Committee of Faculty of Medicine, University of Belgrade, Serbia. Signed authorisation form was obtained from each participant's parent or guardian.

Genotyping

Genotype analysis were conducted at the Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Serbia. DNA extraction was performed from 5 mL of peripheral blood by salting out method.[22] Genotypes of rs3856806 polymorphism were investigated by classical PCR followed

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by restriction fragment length polymorphism (RFLP) analysis. The cycling conditions were performed by the standard genotyping protocol which included 37 cycles (5 min at 95 °C, 30 s at 94 °C, 30 s 63 °C, 30 s at 72 °C and 7 min at 72 °C). Two set of primers were used (Forward: 5'-CAG GTT TGC TGA ATG TGA AGC-3', and Reverse: 5' -TGG CTC AGG ACT CTC TGC TAG -3'). After PCR, fragments of 191 bp were digested with NlaIII restriction enzyme at 37 °C for 2 hours. The 8.0 % polyacrylamide gel electrophoresis was used to detect fragments of 197 bp and 62 bp which corresponds to CC genotype, and fragments of 156, 62 and 41 bp were noticed in TT genotype.

Blood analysis

Before blood sampling, each participant fasted for 12 h. Levels of TG, TC, HDL-C were measured as described previously.[21] Friedewald's equation was used to calculate levels of LDL-C concentrations.[23]

Statistical methods

Numerical variables were presented as mean ± standard deviation. Student`s t-test or Mann-Whitney U-test were used to examine the association of selected parameters with rs3856806 genotypes. Multivariate linear regression analysis was also performed to assess the association of lipid levels and PPARG genotypes with gender, using BMI and FBG as covariates.

Results

This research included 287 randomly selected school children 15 years of age. There were 143 boys (49.8 %) and 144 girls (50.2 %). In the group of boys 24 (16.8 %) had BMI ≥ 85th percentile, and 18 (12.6 %) had BMI ≥ 95th percentile. Among girls

Table 1: Analysis of selected parameters according to gender

Parameters	Total	Boys	Girls	p
BMI (kg/m²)	21.99 ± 4.30	21.64 ± 4.26	22.34 ± 4.32	0.088
Fasting blood glucose (mmol/L)	4.69 ± 0.65	4.78 ± 0.61	4.61 ± 0.67	0.031
Triglycerides (mmol/L)	0.99 ± 0.56	1.00 ± 0.64	0.98 ± 0.47	0.213
Total cholesterol (mmol/L)	4.34 ± 0.86	4.17 ± 0.75	4.50 ± 0.94	0.001
HDL-Cholesterol (mmol/L)	1.41 ± 0.41	1.38 ± 0.42	1.45 ± 0.39	0.171
LDL-Cholesterol (mmol/L)	2.44 ± 0.84	2.33 ± 0.74	2.54 ± 0.91	0.033

BMI - body mass index; HDL - high-density lipoprotein; LDL - low–density lipoprotein; red colour - statistically significant

29 (20.1 %) had BMI ≥ 85th percentile and 18 were with BMI ≥ 95th percentile (12.6 %). Average values of selected parameters between group of boys and girls are summarised in Table 1.

Statistically significant differences were observed in mean values of glycaemia, TC and LDL-C between males and females. Observed differences in values of total and LDL-C (p = 0.01; p = 0.033, respectively) means that girls had higher values of these parameters in comparison to boys. On the other side, statistically significant difference was noticed in the mean values of FBG in the group of boys. Boys had statistically higher mean values of FBG in comparison to girls (p = 0.031). Allele and genotype frequencies of rs3856806 are summarised in Table 2.

Table 2: Genotypes and alleles frequencies of PPARG polymorphism

PPARG rs3856806 Genotypes	Frequency n (%)	Alleles	Frequencies %
CC	233 (81.2)	C	90.1
CT	52 (18.1)	–	–
TT	2 (0.7)	T	9.9

The distributions of genotypes were in Hardy-Weinberg equilibrium. No statistically significant difference was observed between male and female adolescents in frequencies of PPARG genotypes (p = 0.527).

Average values of BMI, FBG, TG, TC, HLD-C, and LDL-C according to PPARG genotype on whole sample as well as in the group of overweight and obese adolescents are summarised in Tables 3 and 4. Since only two adolescents were carriers of TT genotype, grouped genotypes as CC/CT+TT were grouped.

Lower mean values of FBG are noticed in carriers of CT or TT genotype compared to carriers of CC genotype (p = 0.054). Multiple linear regression analysis was performed to evaluate this result. The obtained p value on the total sample was p = 0.07. Since it has been previously observed that FBG levels are influenced by gender the association between the PPARG genotype and FBG separately in the groups of boys and girls was analysed. However, statistical significance was noticed only in the group of girls. No statistical association was found in mean values of other analysed parameters.

Furthermore, the association of selected parameters with PPARG polymorphism was analysed in

Table 3: Mean values of BMI and biochemical parameters according to PPRAG rs3856806 genotype

Parameters	Genotype	Total	p	Boys	p	Girls	p
BMI (kg/m²)	CC	21.98 ± 4.20	0.716	21.50 ± 4.00	0.810	22.46 ± 4.36	0.426
	CT+TT	22.01 ± 4.73		22.19 ± 5.20		21.80 ± 4.18	
Fasting blood glucose (mmol/L)	CC	4.73 ± 0.65	0.054	4.79 ± 0.64	0.689	4.67 ± 0.66	0.013
	CT+TT	4.54 ± 0.62		4.74 ± 0.50		4.30 ± 0.67	
Triglycerides (mmol/L)	CC	1.00 ± 0.59	0.968	1.01 ± 0.68	0.583	0.98 ± 0.49	0.617
	CT+TT	0.94 ± 0.41		0.99 ± 0.44		0.97 ± 0.37	
Total cholesterol (mmol/L)	CC	4.33 ± 0.86	0.796	4.15 ± 0.77	0.535	4.50 ± 0.92	0.987
	CT+TT	4.36 ± 0.87		4.25 ± 0.68		4.50 ± 1.06	
HDL-Cholesterol (mmol/L)	CC	1.43 ± 0.41	0.111	1.40 ± 0.44	0.214	1.46 ± 0.39	0.364
	CT+TT	1.33 ± 0.38		1.29 ± 0.37		1.38 ± 0.39	
LDL-Cholesterol (mmol/L)	CC	2.41 ± 0.85	0.198	2.29 ± 0.76	0.179	2.52 ± 0.92	0.498
	CT+TT	2.57 ± 0.76		2.50 ± 0.66		2.66 ± 0.87	

BMI - body mass index; HDL - high-density lipoprotein; LDL - low–density lipoprotein; red colour - statistically significant

Table 4: Mean values of biochemical parameters according to PPRAG rs3856806 genotype in adolescents with BMI ≥ 85th percentile

Parameters	Genotype	BMI ≥ 85th pc	p	Boys	p	Girls	p
Fasting blood glucose (mmol/L)	CC	4.80 ± 0.51	0.104	4.90 ± 0.44	0.446	4.73 ± 0.55	0.030
	CT+TT	4.55 ± 0.60		4.77 ± 0.54		4.18 ± 0.49	
Triglycerides (mmol/L)	CC	1.11 ± 0.67	0.164	1.12 ± 0.74	0.538	1.12 ± 0.65	0.561
	CT+TT	1.15 ± 0.45		1.15 ± 0.51		1.04 ± 0.40	
Total cholesterol (mmol/L)	CC	4.41 ± 0.86	0.796	4.11 ± 0.66	0.492	4.61 ± 0.95	0.419
	CT+TT	4.24 ± 1.01		4.27 ± 0.63		4.24 ± 1.50	
HDL-Cholesterol (mmol/L)	CC	1.33 ± 0.35	0.052	1.22 ± 0.28	0.620	1.41 ± 0.38	0.000
	CT+TT	1.14 ± 0.25		1.29 ± 0.46		1.08 ± 0.11	
LDL-Cholesterol (mmol/L)	CC	2.54 ± 0.84	0.847	2.31 ± 0.70	0.512	2.65 ± 0.94	0.964
	CT+TT	2.58 ± 0.93		2.47 ± 0.65		2.67 ± 1.34	

BMI - body mass index; HDL - high-density lipoprotein; LDL - low–density lipoprotein; red colour - statistically significant

the group of overweight and obese adolescents. Statistically significant lower mean values of HDL-C were noticed in adolescents with CC+CT genotype in comparison to adolescents with CC genotype (p = 0.052). The result is on the border of statistical significance and multiple linear regression analysis did not confirm these results (β = -0.125, p = 0.315).

However, overweight and obese girls who were carriers of CT+TT genotype revealed statistically significant lower mean values of fasting blood glucose and HDL-C in comparison to the girls who were carriers of CT+TT genotype (p = 0.030; p = 0.000, respectively). Multiple linear regression analysis confirmed only the results for FBG (β = -0.302, p = 0.050). Statistically significant difference in mean values of other analysed parameters was not observed.

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Discussion

BAT is very important organ which role is reflected on the lipid and glucose metabolism, energy homeostasis and weight regulation. Its differentiation and activation is a complex mechanism in which PPARγ has very important role.[5] Researches have shown that lean adolescents have more functional and non-functional BAT compared to children and adolescents with BMI ≥ 85th percentile.[24] Overweight and obese adolescents with no metabolically active BAT had three times more subcutaneous fat, and six times more visceral fat compared to children and adolescents with metabolically active BAT.[5, 25, 26] Bearing in mind that volume and activation of BAT increases during puberty, the aim of this research was to investigate whether single nucleotide polymorphism rs3856806 within PPARG gene could affect the mean values of TC, HLD-C, LDL-C, TG, FBG levels and BMI in adolescents. Even though results from previous researches showed an association of rs3856806 polymorphism with favourable metabolic traits and lower BMI, studies are still rare especially in children and adolescent population.[9, 20]

Results from this study revealed statistically significant difference in mean values of FBG, TC and LDL-C between the group of boys and girls. Higher mean values of fasting blood glucose were noticed in boys, while girls had higher mean values of TC and LDL-C. These differences could be explained by the increase in production of growth hormone, growth factors, gonadotropins and sex steroid hormones. Thus, the differences in obtained results between the group of boys and girls might come from difference in growth and sexual maturity between these two groups.[4]

Statistically significant association of rs3856806 with BMI was not observed in this study, which corresponds to the data obtained in research of Leon-Mimila et al conducted in population of 1218 healthy children 6-18 years of age.[27] Besides, no association of this polymorphism with BMI was found in the study of Luo et al,[28] Parra et al[29] and in the research of Kim et al.[19] Analysing the obtained results regarding lipid parameters, the authors came to the conclusion that in the group of overweight and obese adolescents, carriers of CT or TT genotype had lower mean values of HDL-C compared to the carriers of CC genotype, although significance was borderline. The reasonable ex-

planation for the statically significance found only among girls could be derived from the fact that secretion of oestrogen and other sex hormones is increasing during puberty. Due to rise of oestrogen, girls tend to have higher BMI compared to boys as well as increase of subcutaneous adiposity. On the other hand, testosterone has impact on lipase activity of liver, thus causing lower levels of lipid parameters in boys. Also, there were no available data on eating habits or level of physical activity of adolescents, which are also very important parameters when assessing lipid parameters.[30, 31]

To the authors' knowledge, there is no available studies which examined the effects of this polymorphism on metabolic parameters in the population of children and adolescents. However, the study of Wei-min Wei and colleagues[32] investigated the association of rs3856086 with ischaemic stroke and lipid status in patients with this disease as well as with normal controls. Results revealed that in both groups carriers of CC genotype had significantly higher values of total and LDL-C compared to carriers of CT or TT genotype, while the association between this polymorphism and mean values of TG and HDL-C was not observed.[32] Research of Butt et al showed that higher mean values of LDL-C were recorded in carriers of CC genotype in comparison with carriers of CT or TT genotype.[33] In the same research the association of rs3856806 with levels of TG and HDL-C was not found. On the other side, some studies showed opposite results. Also, results from study of Fan et al showed that carriers of CT or TT genotype had higher values of LDL-C compared to carriers of CC genotype.[34] However, study of Dujic et al conducted in the population of Bosnia and Herzegovina in patients with metabolic syndrome and healthy controls did not find association of rs3856806 with lipid profile parameters in neither group.[35]

Regarding association of this polymorphism with FBG, these results showed that girls who were carriers of T allele had lower values of FBG in comparison to the girls who revealed CC genotype.

Relieving the literature results obtained in previous studies, data are contradictory. However, Zhou et al revealed association of this polymorfism with lower values of blood glucose in patients with coronary artery diesease, but not in healthy controls.[36] The study of Butt et al and Grygiel-Górniak et al results have shown that carriers of CC genotype had significanlty lower values of FBG

compared to carriers of T allele.[33, 37] On the other hand, Parra et al investigated the association of 11 different polymorphisms including rs3856806 with diabetes mellitus type 2 and BMI in the Latinoamerican population. Their results have shown that presence of T allele is significantly associated with diabetes mellitus type 2, but haplotype analysis with another polymorphisms within this gene did not show any significant association.[29]

However, inconsistencies in results obtained from different studies in terms of metabolic traits might be explained by the fact that this polymorphism is silent and is not causing change in amino acid sequence, thus is unlikely to have any direct link to certain phenotype, or it could be in linkage disequilibrium with another functional polymorphism such as Pro12Ala polymorphism, or with still unidentified functional variation. Also, other reasons for contradictory results could be different study designs, the selection criteria of patients as well as differences in ethics origins.

Conclusion

Taking into account all available data of this polymorphism and its correlation with the levels of lipid parameters and FBG it could be concluded that rs3856806 plays significant role in lipid and glucose metabolism. However, the present study is limited with data about non-genetic factors that could influence the BMI, glucose level and lipid profile of adolescents. It is known that lifestyle, primarily eating habits, physical activity and usage of certain drugs can modify lipid status. Thus, to draw definite conclusions it would be of great interest to further investigate the impact of this polymorphism on lipid parameters of adolescents and adults on a larger population with taking in consideration the non-genetic factors as well.

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Conflict of interest

None.

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