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Original Paper

Associations between *LEP* G2548A polymorphisms and lipids metabolism

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The aim of this study was to determine the population structure based on identification of G2548A polymorphism in leptin gene and analyse its impact on selected traits related with lipids metabolism as well as obesity. Totally, 236 human samples were included in SNP genotyping using PCR-RFLP method and endonuclease *Hha*I. The impact of locus was assessed for biochemical parameters: BMI, level of total, HDL and LDL cholesterol. In population the prevalence of A allele was detected. The highest proportion was found for heterozygous individuals (0.44). The observed heterozygosity indicated relative sufficient proportion of heterozygotes but the F_{IS} index showed positive value (0.1) that can signalize the increase of homozygotes proportion. The association analysis using GLM procedure proved significant impact of *LEP* genotypes on each of selected parameter. The results showed the significant role of leptin in human nutritional status and confirmed its value as candidate gene of lipids metabolism disorders.

Keywords: fat metabolism, human, leptin, obesity related gene, SNP

1 Introduction

The obesity as result from the interaction between genetic variability, environment and sedentary lifestyle is one of the most challenging health problems of the last century with tremendous increase in the prevalence (Chavarria-Avila et al., 2015; Becer et al., 2016). It is also known, that obesity is an important risk factor for developing type 2 *diabetes mellitus*, hypertension and cardiovascular diseases (Brunkwall et al., 2013; Cahill et al., 2014). The increasing prevalence of obesity in worldwide human population resulted in many studies on candidate genes that may contribute to its development. Most obesity predisposing genes encode the molecular components of physiological systems related to energy balance (Duarte et al., 2007). The leptin (*LEP*) is a protein hormone with structural similarities to long-chain helical cytokines that play a key role in regulation of food intake, increase energy expenditure, and modulate immune and inflammatory responses by binding and activating the leptin receptor (Poeggeler et al., 2010; Roszkowska-Gancarz et al., 2014).

The *LEP* gene, human homologue of the rat obese gene cloned and sequenced by Zhang et al. (1994), is located on autosome 7 (7q31.3) and the length of its mRNA in adipose tissue is 4.5 kb (Mohammadzadeh et al., 2015). There are several studies indicating the significant association between polymorphisms in *LEP* gene and obesity (Duarte et al., 2006), hypertension (Mendoza-Núñez et al., 2006), coronary artery diseases and metabolic syndrome (Kim et al., 2008), insulin resistance (Chiu et al., 2004), type 2 *diabetes mellitus* (Han et al., 2008) and cancer (Mohammadzadeh et al., 2015). The G2548A polymorphism (rs7799039: guanine > adenine) are studied widely because it has been associated with leptin production and secretion (Suriyaprom et al., 2014).

The aim of this study was to detect the population genotype structure based on determination of *LEP* G2548A polymorphisms in human population and assess its impact on selected traits related to lipids metabolism.

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2 Material and methods

2.1 Biological samples and SNP genotyping

In total 236 human blood samples were used to analyse the impact of *LEP* gene on selected biochemical parameters. The genomic DNA was extracted from each sample using the commercial kit NucleoSpin Blood (Macherey Nagel) according to manufacturer's instruction. The concentration and quality of extracted DNA were tested by NanoPhotometer (Implen) measuring of the optical density at wave length of 260 nm. The identification of *LEP* G2548A polymorphism was carried out based on use of PCR-RFLP method and restriction endonuclease *Hha*I according to Ren et al. (2004). The PCR products and restriction fragments were visualised by horizontal electrophoresis in 2 % agarose gels in 0.5 x TBE (140 V for 36 min) (Brody and Kern, 2004) stained by GelRed (Biotium) prior to visualization under UV light.

2.2 Genotype structure

The allele and genotype frequencies of *LEP* G2548A polymorphism were evaluated in order to determine the population structure by use of Genalex version 6.1 (Peakell and Smouse, 2012). The significance of differences between observed and expected genotype frequencies were tested based on Chi-square test. The population genetic indices, including observed (H_o) and expected (H_e) heterozygosity, effective allele numbers (N_e) and Wright's F_{IS} index were calculated to assess the state of genetic diversity in population.

2.3 Association analysis

The analysis of *LEP* G2548A genotypes impact on selected biochemical parameters (BMI, total cholesterol, HDL cholesterol and LDL cholesterol) was performed using GLM procedure adopted in SAS Enterprise Guide 4.2 software (SAS Institute Inc. 2009). The significance of genotypes effect on biochemical parameters was tested with the involvement of other fixed effects according to following general linear models:

$$Y_{ijklm} = G_i + G_j + A_k + CH_l + T_m + e_{ijklm}$$

where:

Y_{ijklm} – BMI, G_i – effect genotypes, G_j – gender, A_k – age, CH_l – level of total cholesterol, T_m – level of total triacylglycerols, e_{ijklm} – random error.

$$Y_{ijklm}^{1, 2, 3} = G_i + G_j + A_k + B_l + T_m + e_{ijklm}$$

where:

$Y_{ijklm}^{1, 2, 3}$ – level of total cholesterol, HDL and LDL, G_i – effect genotypes, G_j – gender, A_k – age, B_l – level of BMI, T_m – level of total triacylglycerols, e_{ijklm} – random error.

3 Results and discussion

The G2548A polymorphism in leptin gene has been successfully determined for each of analysed individual. Using PCR-RFLP methods all tree genotype were identified in population. The obtained allele and genotype frequencies are presented in table 1. In population the prevalence of A allele was found. The highest proportion reached heterozygous individuals. The lowest distribution was observed for GG homozygous genotype. The non-significant differences ($P > 0.05$) between observed and expected genotype frequencies indicated Hardy-Weinberg equilibrium in population. The proportion of heterozygous individuals was transferred to the relative higher observed heterozygosity. However, the positive value of Wright's F_{IS} index showed the increase of homozygote individuals in population. The effective allele number indicated balanced activity of both identified alleles. Our results are in accordance with previous reported studies for Turkish (Becer et al., 2016), Polish (Roszkowska-Gancarz et al., 2014), Thailand (Suriyaprom et al., 2014) or Mexican Mestizo (Chavarria-Avila et al., 2015) populations.

Table 1 Genotype structure of population based on LEP G2548A polymorphism

Genotypes frequency			Allele frequency		χ^2 test	H_o	H_e	N_e	F_{IS}
AA	AG	GG	A	G	ns	0.45	0.49	1.98	0.10
0.32	0.44	0.24	0.54	0.46					

H_o – observed homozygosity, H_e – observed heterozygosity, N_e – effective allele number, PIC – polymorphic information content, F_{IS} – fixation index; ns – not significant

Table 2 shows the summary statistic of evaluated biochemical parameters according to the individual's gender. The average BMI value obtained for both gender indicated the tendency of slight overweight across respondents in analysed sample. Moreover, the maximum BMI values signalized the risk of type II. obesity in some evaluated individuals. The average obtained levels of total and HDL cholesterol were across respondents optimal. The slight increase over the recommended limit was found only for the LDL cholesterol, but the average value signalize only low risk for individuals in analysed population.

Table 2 Basic statistical variation of analysed biochemical parameters in population

Parameter	Men (n=116)				Women (n=120)			
	$\bar{x}$	s.d.	x_{max}	x_{max}	$\bar{x}$	s.d.	x_{min}	x_{max}
Age	44.89	13.72	19.00	63.00	49.13	9.98	20.00	63.00
BMI [kg m^{-2}]	26.40	3.96	20.02	37.00	26.83	4.23	18.60	37.00
Total chol. [mmol l^{-1}]	5.04	1.09	2.93	7.18	5.27	0.99	2.93	7.18
HDL [mmol l^{-1}]	1.69	0.42	0.89	2.47	1.84	0.33	0.92	2.47
LDL [mmol l^{-1}]	2.69	0.96	0.93	4.53	2.82	0.90	0.93	4.53

The association analysis of *LEP* G2548A genotypes impact on selected biochemical parameters showed significant effect for each of trait (Table 3). Alongside the *LEP* genotype the effects of gender, age and total level of triacylglycerols were also analysed. Based on the involved fixed effects in linear models we were able to characterize the variability of parameters in average on 53.86%. Except total cholesterol, each of other analysed parameters in linear models showed statistically significant effect on BMI and level of total, LDL and HDL cholesterol.

Table 3 The impact of *LEP* G2548A polymorphisms on analysed biochemical parameters

Parameter	AA			AG			GG			Factors
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	
BMI	75	24.62***	2.24	105	26.86***	4.23	56	28.85***	4.52	Gender**
Total chol.	75	5.38**	1.05	105	5.14**	1.02	56	4.90**	1.03	Age***
HDL	75	2.96***	0.78	105	2.70***	1.05	56	2.56***	0.85	BMI**
LDL	75	1.88*	0.38	105	1.77*	0.34	56	1.63*	0.42	Total cholesterol Total triacylglycerols***
*P < 0.05, **P < 0.01, ***P < 0.0001										

These study support previously published results about the role of *LEP* G2548A polymorphism in control of lipids metabolism (Mammès et al., 2000; Constantin et al., 2010). The LEP gene has been investigated in the search for gene variants that are mainly related to pathophysiology of obesity and related disorders. Several single nucleotide polymorphisms have been described and one of these is G2548A (Becer et al., 2016). Alongside obesity, Hoffstedt et al. (2002) reported that the *LEP* G2548A variant can also affect the expression of leptin and its secretion in adipose tissue. Moreover, some of previous studies suggested its role also in the genetic prediction of other obesity related disorders including *diabetes mellitus* and cardiovascular diseases (Mendoza-Núñez et al., 2006; Han et al., 2008; Kim et al., 2008).

4 Conclusions

Our study confirmed the key role of leptin gene in control of nutritional organism status and indicate its significance in genetic evaluation in relation to the lipids metabolism disorders prediction. The association analysis support the previous published results that suggested the *LEP* G2548A polymorphism as genetic markers for the level of BMI and obesity related disorders. Because the obesity is considered as multifactorial disease and its genetic background is polygenic the involvement of other genes in the future research is needed.

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