Leptin Receptor and Ghrelin Genes Polymorphisms in Relation to the Metabolism of Lipids

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Abstract

The aim of this work was to analyse genetic polymorphisms in genes encoding leptin receptor (*LEPR*) and ghrelin (*GHR*) as genetic markers of metabolic disorders in human nutrition. Genomic DNA was obtained from in total 84 human blood samples. Effect of analysed genetic markers was evaluated for three biochemical parameters: total cholesterol, HDL and LDL cholesterol. The PCR-RFLP method was used for identification of SNPs in *LEPR* (Gln223Arg) and *GHR* (171T/C) genes. In analysed population prevalence of heterozygous $LEPR^{AG}$ (47.62%) and GHR^{CT} (40.48%) genotypes was observed. Frequency of $LEPR^{A}$ and $LEPR^{B}$ alleles were 0.55 and 0.45, respectively. Similar the GHR^{C} allele had only slight predominance than GHR^{T} allele (0.54/0.46). In population was found higher level of observed heterozygosity across loci (0.44). For both SNPs was found high effective allele number (1.98) which was also transferred to the median level of polymorphic information content (0.37). Association analysis of *LEPR* and *GHR* genotypes effect on selected biochemical parameters was performed using GLM procedure. Significant association was found only for levels of LDL cholesterol ($P<0.01$). Our study shows that both genes are involved in nutritional status and therefore can be considered as candidate genes of lipids metabolism disorders and obesity.

Keywords: genotyping, GHR gene, LEPR gene, lipids metabolism, polymorphism

1. Introduction

Lipids alongside proteins and carbohydrates are fundamental components of the human nutrition. Lipids as one of the essential nutrients are important mainly based on their high energy value and the content of essential fatty acids. The increase of lipids intake in human nutrition can lead to the occurrence of different health disorders such as overweight and obesity. The number of obese people in worldwide population grows rapidly. The overweight is basically resulted from the disruption of the balance between food intake and energy expenditure. The obesity as important health problem encourages the development of significant diseases of cardiovascular and respiratory system, such as heart disease, diabetes or spinal disorders. Obesity is heterogeneous disorder that is affected by complex of many genes, environmental factors, and also by their interactions.

The gene encoding leptin (*LEP*) is one of the genes that are involved in control of energy balance in organism. Leptin is released from adipocytes as a signal of body fat stores and acts as a satiety factor with its receptor located mainly in the hypothalamus, a brain area known to be involved in the regulation of food intake. In addition to regulating satiety, leptin increases thermogenesis via sympathetic nervous system activity [1]. The hypothalamus which is the centre of energy balance regulation plays in this system key role. In hypothalamus leptin binds to his receptors (*LEPR*) what lead to the decrease of appetite and increase of energy expenditure [2]. The concentration of leptin circulating in plasma is signal of sufficient energy reserve and depends

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on the amount of adipose tissue. The leptin receptor (*LEPR*) is described as factor affecting mainly the energy homeostasis. The mutations in gene encoding leptin receptor can lead to the loss of transmembrane and intracellular domains what resulting in the phenotype changes and development of obesity and infertility [3]. Moreover, the high concentration of *LEPR* was also associated with sleep apnea [4]. The gene encoding leptin receptor located on chromosome 1 consists of 17 exons [5]. In many studies was suggested that the mutations in the *LEPR* gene caused severely changes in phenotype due to the modification of his functions [6]. Zhang et al. [7] and Strobel et al. [8] indicated a direct relationship between *LEP* gene and obesity. Rosmond et al. [9] and Guízar-Mendoza et al. [1] described associations between the most prevalent single nucleotide polymorphisms in *LEPR* gene (Lys109Lys and Gln223Arg) and blood pressure and level of BMI and obesity.

Ghrelin as the endogenous ligand for the growth hormone–secretagogue receptor (ghrelin receptor) is mainly secreted by the stomach in response to fasting, but it is also synthesized locally in the hypothalamus and various peripheral tissues [10]. Ghrelin is involving in the stimulation of growth hormone trough the effect on growth hormone–releasing hormone cells and on pituitary somatotrophs [11]. The orexigenic effect of ghrelin was found also in regulation of energy homeostasis leading to increase of body weight and adiposity [12]. The GHR gene located on human chromosome 3 (p26-p25) consist from the 6 exons and 5 introns. The GHR gene encodes the preprotein ghrelin-obestatin which gives rise to the production of two protein – ghrelin and obestatin [13]. The previous studies of ghrelin genetic variants provided contradictory findings of its role in occurrence of obesity [14]. In associations with body mass index and obesity risk three polymorphisms was described [15]: a single base substitution G152A, with Gln replacing Arg at codon 28 of mature ghrelin, C214A with Met replacing Leu at codon 72, or A269T with Leu replacing Gln at codon 90 in the prepro-ghrelin. The genetic variants of GHR gene have been associated with earlier onset of obesity and also as signal of the fat accumulation [14].

The aim of this study was to analyse the impact of SNPs in *LEPR* (Gln223Arg) and *GHR* (171C/T) genes on selected biochemical parameters in relation to the metabolisms of lipids.

2. Materials and methods

In total 84 genomic DNA samples was used to evaluate the impact of *LEPR* and *GHR* genotypes on selected biochemical parameters. The genomic DNA was extracted from blood samples using the commercial kit NucleoSpin Blood (Macherey Nagel). Subsequently, the concentration and the purity of DNA were estimated by NanoPhotometer (Implen) measuring of the optical density at wave length of 260 nm. The identification of SNPs in *LEPR* (Gln223Arg) and *GHR* genes (171T/C) was carried out according to [1] and [16], respectively. The PCR-RFLP methods and restriction enzymes *Hpa*II (*LEPR*/ Gln223Arg) and *Lwe*I (*GHR*/171T/C) were used to genotyping of analysed individuals. The results of PCR amplifications and digestion of PCR products were visualised by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (140 V for 40 min) [17] stained with GelRed (Biotium) prior to visualization under UV light.

The allele and genotype frequencies of SNPs in *LEPR* and *GHR* genes were calculated by direct counting to specify the genotype structure of analysed population. The differences between observed and expected genotype frequencies were tested using χ^2 analysis. Moreover, the population genetic indices including observed heterozygosity (H_o), effective allele numbers and polymorphic information content were calculated according [18].

The analysis of *LEPR* and *GHR* genotypes effects on selected biochemical parameters (BMI, total cholesterol, HDL cholesterol and LDL cholesterol) was performed using GLM procedure incorporated in SAS Enterprise Guide 4.2 software [19]. The significance of genotypes effect on biochemical parameters was tested with the involvement of other fixed effects by 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 single nucleotide polymorphisms located in genes encoding leptin receptor and ghrelin were successfully detected in analysed human population using PCR-RFLP methods. For both analysed SNPs all three genotypes were detected. Table 1 shows the observed allele and genotype frequencies. In the analysed population the heterozygous AG and CT genotypes were the most frequent. The lowest distribution was observed for individuals with $LEPR^{GG}$ and GHR^{TT} genotypes. The higher frequency was detected for $LEPR^{A}$ and GHR^{C} alleles. Based on differences between observed and expected allele frequency the Hardy-Weinberg equilibrium was detected in population ($P > 0.05$). The number of heterozygous individuals was also transferred to the relative higher observed heterozygosities. The polymorphic information content reached the medium level and based on the effective allele numbers was in population observed balanced activity of alleles.

Our results are in accordance with previously published studies. Guízar-Mendoza et al. [1] found in population of Mexican adolescent only slight prevalence of $LEPR^{A}$ allele in both obese individuals and individuals with normal body weight. The significantly higher frequency of $LEPR^{A}$ allele in non-obese individuals compared to the obese group was described in study Linjawi and Hussain [20]. Oliveira et al. [21] observed in group of non-obese and obese patient comparable distribution of $LEPR^{G}$ allele at the level of 30% with prevalence of homozygous AA individuals in both groups. For SNP *GHR*/171T/C was found the prevalence of heterozygous CT genotype in study of Miraglia del Giudice et al. [22] and Nass et al. [23]. In comparison the observed genotype structure in study of Wang et al. [16] indicated the prevalence of T allele in obese samples (0.75) than in the underweight individuals.

Table 2 describe the average value of analysed biochemical parameter divided based on analysed individuals' gender. The average value in both genders achieved the level of BMI on the border of slight overweight with average age 40 years. The maximum of observed BMI indicated that in some individuals was found the obesity type II. The average levels of HDL and LDL cholesterol are optimal in population of selected individuals. The slight increase was found only for the level of total cholesterol, but the average values showed only low risk for the analysed individuals. The statistical analyses of *LEPR* and *GHR* genotypes impact on selected biochemical parameters in analysed population indicated the significant associations only for the level of LDL cholesterol, with $LEPR^{G}$ and GHR^{C} as desirable alleles. The observed differences in average values of other analysed parameters in relation to the *LEPR* and *GHR* genotypes were low and statistically non-significant ($P > 0.05$). Based on the fixed effects involved in linear models we were able to estimate the variability of selected parameters in average on 50 %. Table 3 shows the average values of analysed biochemical parameters in relation to the specific *LEPR* and *GHR* genotypes and observed statistical significance for selected fixed effects. Alongside the *LEPR* and *GHR* genotypes the impact of gender, age and total level of triacylglycerols was also evaluated. For each of these parameters the significant effect was found. The level of BMI influenced significantly the value of observed total, HDL and LDL cholesterol.

A relationship between *LEPR*/Gln223Arg genetic variants and level of BMI was previously reported in Tunisian population, when the individuals with A allele in genotype had lower BMI than the homozygous GG individuals [24]. Yiannakouris et al. [25] found only the weak associations between BMI and Gln223Arg polymorphism in Greek population. Daghestani et al. [26] demonstrated the risk of increase in BMI and level of leptin concentration for individuals with GG and AG genotypes. Moreover, Duarte et al. [27] reported that the genotype combination of polymorphisms in *LEPR* and *LEP* genes can led to the increase in obesity risk. The SNP Gln223Arg was also reported in relation to the increase of leptin and insulin concentration in India population [28]. However, in several studies the significant effect of *LEPR*/Gln223Arg genotypes on the biochemical parameters associated with metabolisms of lipids was not found [21]. One of the reasons that may explain the differences in results reported for different human groups across

the world population can be the fact that the metabolism of lipids is controlled by the interaction with many genetic, epigenetic and environmental factors.

Similarly the gene encoding ghrelin was evaluated as candidate for BMI and occurrence of obesity in several studies [29, 30]. Correll at al. [31] reported that the *GHR* gene may be candidate also for weight gain. In study of Yang et al. [32] was confirmed the significant effect of GHR/171T/C genotypes on weight gain and increase of BMI. In addition the 171T/C polymorphism has been linked with variation in blood pressure [31].

Moreover, the receptor of ghrelin seems to be involved in the determination of eating behaviour in terms of the overeating phenotype, and to a lesser degree, the snacking phenotype, as well as to obesity. A possible hypothesis for the manifestation of this eating behavior phenotype during the early years of life could be a predominant genetic effect relative to the common at-risk allele over environmental pressure [10].

Table 1. Genotype structure of population based on SNPs LEPR/Gln223Arg and GHR/171T/C

Genotype frequency			Allele frequency		χ^2 test	H_e	N_e	PIC
LEPR^{AA}	*LEPR*^{AG}	*LEPR*^{GG}	*LEPR*^{A}	*LEPR*^{G}	0.127	0.48	1.98	0.37
0.31	0.47	0.22	0.55 ± 0.03	0.45 ± 0.03				
GHR^{CC}	*GHR*^{CT}	*GHR*^{TT}	*GHR*^{C}	*GHR*^{T}	2.92	0.41	1.99	0.36
0.33	0.41	0.26	0.53 ± 0.03	0.47 ± 0.03				

P > 0.05

Table 2. Basic statistical variation measurements of biochemical parameters in analysed population

Parametre	Men (n=51)				Women (n=33)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Age	41.92	15.36	19.00	63.00	43.84	13.54	20.00	63.00
BMI [kg.m^{-2}]	26.26	4.71	20.02	37.00	26.58	5.81	18.60	37.00
Total chol. [mmol.l^{-1}]	4.74	1.00	2.93	6.82	4.91	0.86	2.93	6.82
HDL [mmol.l^{-1}]	1.59	0.43	0.89	2.47	1.78	0.41	0.92	2.47
LDL [mmol.l^{-1}]	2.48	0.80	0.93	3.65	2.47	0.74	0.93	3.65

Table 3. The effects of LEPR and GHR genes polymorphisms on selected parameters

Parameter	*LEPR*^{AA}			*LEPR*^{AG}			*LEPR*^{GG}			Factors
	n	mean	SD	n	mean	SD	n	mean	SD	
BMI [kg.m^{-2}]	26	26.70	2.06	40	24.66	1.90	18	29.77	3.84	Gender*
Total chol. [mmol.l^{-1}]	26	4.85	0.48	40	4.79	0.53	18	4.80	1.14	Age***
HDL [mmol.l^{-1}]	26	1.65	0.26	40	1.69	0.19	18	1.64	0.48	BMI*
LDL [mmol.l^{-1}]	26	2.62*	0.29	40	2.52*	0.56	18	2.18*	1.04	Total cholesterol*
										Total triacylglycerols**
	GHR^{CC}			*GHR*^{CT}			*GHR*^{TT}			
	n	mean	SD	n	mean	SD	n	mean	SD	
BMI [kg.m^{-2}]	28	30.32	3.84	34	24.27	1.82	22	24.66	2.06	Gender*
Total chol. [mmol.l^{-1}]	28	5.00	1.14	34	4.59	0.44	22	4.91	0.48	Age**
HDL [mmol.l^{-1}]	28	1.65	0.48	34	1.72	0.12	22	1.61	0.26	BMI*
LDL [mmol.l^{-1}]	28	2.67*	1.04	34	2.14*	0.29	22	2.75*	0.29	Total cholesterol
										Total triacylglycerols***

*P < 0.05, **P < 0.01, ***P < 0.0001

The results showed that the most frequent genotypes in analysed population were heterozygous *LEPR*^{AG} and *GHR*^{CT}. The higher distribution was found for *LEPR*^{A} and *GHR*^{C} alleles. Both of the analysed polymorphisms achieved the median level of polymorphic information content. The statistical analysis showed significant association only between the

LEPR and *GHR* genotypes and level of LDL cholesterol. The BMI and levels of total cholesterol was influenced by analysed genetic variants only non-significant. The recent studies of different human populations suggested both of the analysed genes as candidate for evaluation of variation in BMI index and occurrence of obesity. One of the problems that probably influenced our study was mainly the small sample size and therefore in the future the statistical analysis will be prepare on the greater sample of individuals in order to confirm the role of *LEPR* and *GHR* genes in increase of BMI index.

4. Conclusions

In our study was analysed the impact of two SNPs located in genes encoding leptin receptor (Gln223Arg) and ghrelin (171T/C) on selected biochemical parameters associated with metabolisms of lipids in human population.

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