UDC: 575.17(477)

Structure of Ukrainian population on SNP *rs1137101* of leptin receptor gene *LEPR*

Z.H.Ghali[1,2], I.H.Ahmed[1,2,] M.Yu.Gorshunska[3], A.K.Pochernyaev[4], L.A.Atramentova[2,4]

[1]Department of Biology, College of Science, University of Wasit (Wasit, Iraq);
[2]V.N.Karazin Kharkiv National University (Kharkiv, Ukraine);
[3]Kharkiv Postgraduate Medical Academy (Kharkiv, Ukraine);
[4]S.I. "V.Danilevsky Institute of Endocrine Pathology Problems of NAMS of Ukraine" (Kharkiv, Ukraine)
atramentova@yandex.ru

The distribution of single nucleotide polymorphism of the leptin receptor gene *LEPR* in the Slavonic population (Ukrainian and Russian) from Kharkov and Poltava was investigated. Identification of single nucleotide polymorphisms *C/G LEPR* leptin receptor gene was performed using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). Major allele in the studied population is *Q*, its frequency p_Q=0.57. The difference is no significance when comparing men (0.59) to women (0.56). There is a high percent of homozygotes. In men, *QQ* homozygous at 30% is higher than the theoretically expected value and 2.5 times more than *RR* homozygotes. In women, *QQ* homozygous at 40% is higher than the theoretically expected value and homozygotes *RR* almost at 80%. Heterozygotes *QR* constitute 36% and 41% of the theoretically expected value for males and females respectively. The marriage structure of the studied population is not random mating.

Key words: *leptin receptor gene LEPR, single nucleotide polymorphism, structure of Ukrainian population.*

Структура украинской популяции по SNP *rs1137101* гена рецептора лептина *LEPR*

З.Х.Гали, И.Х.Ахмед, М.Ю.Горшунская, А.К.Почерняев, Л.А.Атраментова

Методом ПЦР-ПДРФ в славянском населении (украинцы и русские) Харькова и Полтавы изучено распределение однонуклеотидного полиморфизма *C/G* гена рецептора лептина *LEPR*. Полиморфизм *C/G* приводит к аминокислотной замене *Q/R*. Мажорным аллелем в исследуемом населении является *Q*, его частота p_o=0,57 (у мужчин 0,59, у женщин 0,56). У мужчин доля гомозигот *QQ* на 30%, а гомозигот *RR* в 2,5 раза выше, чем при панмиксии. У женщин гомозигот *QQ* на 40%, а гомозигот *RR* почти на 80% больше, чем теоретически ожидаемое значение при случайном скрещивании. Гетерозиготы *QR* составляют 36% (у мужчин) и 41% (у женщин) от теоретически ожидаемого значения при равновесном состоянии.

Ключевые слова: *ген рецептора лептина LEPR, однонуклеотидный полиморфизм, структура украинской популяции.*

Структура української популяції за SNP *rs1137101* гена рецептора лептину *LEPR*

З.Х.Галі, І.Х.Ахмед, М.Ю.Горшунська, А.К.Почерняєв, Л.О.Атраментова

Методом ПЦР-ПДРФ в слов'янському населенні (українці і росіяни) Харкова і Полтави вивчено розподіл однонуклеотидного поліморфізму *C/G* гена рецептора лептину *LEPR*. Поліморфізм *C/G* призводить до амінокислотної заміни *Q/R*. Мажорним алелем в досліджуваному населенні є *Q*, його частота p_o=0,57 (у чоловіків 0,59, у жінок 0,56). У чоловіків частка гомозигот *QQ* на 30%, а гомозигот *RR* у 2,5 рази більше, ніж при панміксії. У жінок гомозигот *QQ* на 40%, а гомозигот *RR* майже на 80% більше, ніж теоретично очікуване значення при випадковому схрещуванні. Гетерозиготи *QR* становлять 36% (у чоловіків) і 41% (у жінок) від теоретично очікуваного при стані рівноваги.

Ключові слова: *ген рецептора лептину LEPR, однонуклеотидний поліморфізм, структура української популяції.*

Introduction

Leptin is an adipocyte-secreted hormone that regulates energy homeostasis through central and peripheral mechanisms (Mantzoros, 1999; Wauters et al., 2000). Leptin and the leptin receptor (LEPR) are involved in satiety and energy expenditure via central and peripheral mechanisms. The primary site of leptin

action is the hypothalamus where the leptin receptor interacts with the adipocyte-derived leptin signal to regulate appetite, energy balance, and metabolism. LEPRs also regulate energy homeostasis in peripheral tissues including skeletal muscle, liver, pancreas, and adipose tissue. Leptin prevents obesity via LEPRs by stimulating glucose uptake and fatty acid oxidation in skeletal muscle and liver (Aiston, Agius, 1999; Wauters et al., 2000; Minokoshi et al., 2002), and inhibits insulin secretion of pancreatic β-cells (Seufert, 2004). Mutations in the leptin gene resulting in leptin deficiency cause obesity, insulin resistance, and diabetes in animals (Zhang et al., 1994) and, in rare cases, morbid obesity and hyperinsulinemia in humans (Montague et al.,1997). Common genetic variants (e.g., SNPs) at the *LEPR* gene locus have been associated with obesity, hyperinsulinemia, type 2 diabetes mellitus (T2DM), and variations in leptin levels in different populations. For example, three non-synonymous SNPs (Arg109Lys, Arg223Gln, and Lys656Asn) have been evaluated for association studies (Rosmond et al., 2000; Takahashi-Yasuno et al., 2003; van Rossum et al., 2003; Loos et al., 2006; Park et al., 2006). The study of population structure polymorphisms of this gene is of practical importance, since it may serve as the basis of studies similar to the distribution of polymorphisms in patients with cardiovascular, endocrine and other diseases. This may lead to disturbances in metabolic pathways that are controlled by this gene. The objective was to investigate the distribution of single nucleotide polymorphism of the leptin receptor gene *LEPR* in the Slavonic population (Ukrainian and Russian) from Kharkov and Poltava.

Materials and methods

DNA of 100 persons (48 men and 52 women) – Russians and Ukrainians residents of Kharkov and Poltava cities have been investigated. Samples of blood and epithelium of inner side of cheek were obtained with the written agreement of people. DNA was separated from leukocytes by ion-exchange gum Chelex-100 method. Identification of single nucleotide polymorphisms *C/G of* leptin receptor gene *LEPR* was performed using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) (Walsh et al., 1999; Gotoda et al., 1997). Studied single nucleotide polymorphism of *C/G* is localized in exon 6. Endonuclease (restrictase *MspI*) recognizes the DNA sequence 5 '... CCGG ... 3' and cuts it into two fragments in both DNA strands between nucleotides *CC*, resulting in formation of fragments of length 80 and 40 bp. In the absence of restriction site PCR product is a fragment of 120 bp that was visualized as the presence of one band. Changes in DNA associate with the arginine-glycine substitution in the 233 position of leptin receptor (*Q233R*).

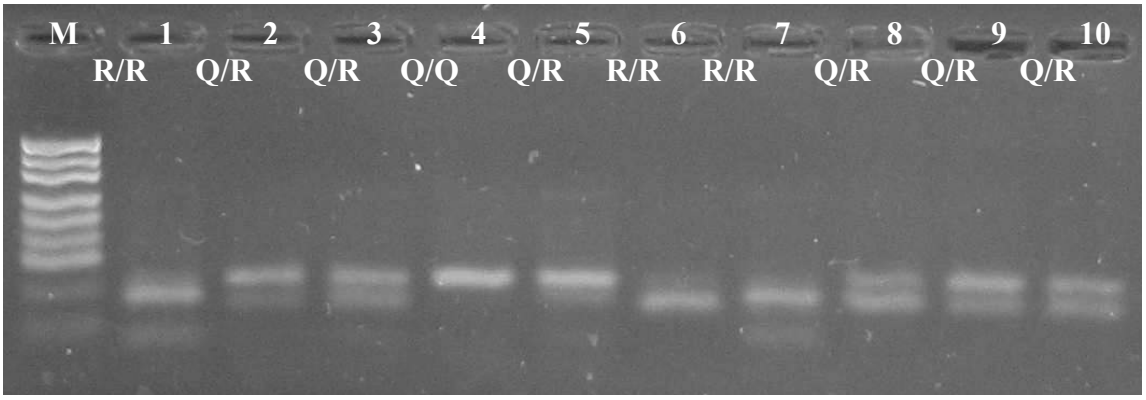

Fig. 1. Electrophoregram products amplified by PCR with restriction fragment of the gene *LEPR* (M-DNA marker *pUC19*, hydrolyzed with endonuclease *MspI*, 1–10 – DNA donors; *RR, QQ, QR* – genotypes)

Results and discussion

Major allele in the studied population is *Q*, its frequency p_Q=0.57. The difference is no significance when comparing men (0.59) to women (0.56) (Table 1). Table 2 shows the frequencies of minor alleles *R* in different populations. The maximum frequency of this allele is present in the indigenous population of Australia (0.89) and in the populations of Asia – the Japanese and Koreans (0.85). The rarest allele is observed in Pima Indians (0.32) and the inhabitants of Greece (0.32).

Table 1.

Distribution of genotypes and allele frequency of SNP *rs1137101* gene in investigated population

Group	Number	Genotypes, n			Allele frequencies	
		RR	*QR*	*QQ*	*R*	*Q*
Men	48	13	13	22	0,41	0,59
Women	52	12	22	18	0,44	0,56
Total	100	25	35	40	0,43	0,57

Table 2.

SNP allele frequencies in different populations and ethnic groups

Country	Ethnic group	*223R* frequency	Author
Ukraine	Slavonic	0.37	Own data
USA	White	0.45	Silver et al., 1997
England	White	0.44	Gotoda et al., 1997
France	White	0.44	Mammes et al., 2001
Belgium	White	0.48	Wauters et al., 2001
Netherlands	White	0.44	van Rossum et al., 2002
Sweden	White	0.50	Rosmond et al., 2000
Denmark	White	0.41	Echwald et al., 1997
Greece	White	0.32	Yiannakouris et al., 2001
Австралия	White	0.58	De Silva et al., 2001
Japan	Japanese	0.85	Matsuoka et al., 1997
Korea	Korean	0.85	Koh et al., 2002
USA	Pima Indians	0.32	Stefan et al., 2002
USA	Brazilians	0.40	Mattevi et al., 2002
Australia	Native people	0.89	De Silva et al., 1999

Table 3.

Assortative mating by genotype

Mates	Frequencies	N			
		E		O	A
		P	N	N	
QQ* × *QQ	p^4	0.1575	**3.93**	**9**	**1.29**
QQ× *QR*/*QR* × *QQ*	$4p^3q$	0.3701	**9.25**	**3**	**-0.68**
QQ* × *RR*/*RR* × *QQ	$2p^2q^2$	0.1086	**2.72**	**6**	**1.21**
QR ×*QR*	$4p^2q^2$	0.2173	**5.43**	**3**	**-0.45**
QR × *RR*/*RR* × *QR*	$4pq^3$	0.1278	**3.20**	**0**	**-1**
RR* × *RR	q^4	0.0187	**0.47**	**4**	**7.51**
Total	1	1	25	25	
Statistics	df=8; $\chi^2_{0.001}$=26.1; χ^2=44.6; p<0.001				

Remarks: E – theoretical result, O – observed result, A – assortative index of mating A=(O-E)/E, N – number of mates, P – fraction, p – significant level.

In the investigated population, there is a high percent of homozygotes indicating that there is a population subdivision (Wahlund effect), a kinship or positive assortative mating (Templeton, 2006). In men, *QQ* homozygous at 30% is higher than the theoretically expected value and 2.5 times more than *RR* homozygotes. In women, *QQ* homozygous at 40% is higher than the theoretically expected value and

homozygotes *RR* almost at 80%. Heterozygotes *QR* constitute 36% and 41% of the theoretically expected value for male and female respectively.

The calculations show that the marriage structure of the studied population is not random mating. There is positive assortative by genotype: the number of married couples in which husband and wife have the same genotype, is higher than expected for a random combination of genes. The reasons for this are unclear yet and further investigations are necessary.

References

Aiston S., Agius L. Leptin enhances glycogen storage in hepatocytes by inhibition of phosphorylase and exerts an additive effect with insulin // Diabetes. – 1999. – Vol.48. – P. 15–20.

De Silva A.M., Walder K.R., Boyko E.J. et al. Genetic variation and obesity in Australian women: a prospective study // Obes. Res. – 2001. – Vol.9. – P. 733–740.

Echwald S.M., Sorensen T.D., Sorensen T.I. et al. Amino acid variants in the human leptin receptor: lack of association to juvenile onset obesity // Biochem. Biophys. Res. Commun. – 1997. – Vol.233. – P. 248–252.

Gotoda T., Manning B.S., Goldstone A.P. et al. Leptin receptor gene variation and obesity: lack of association in a white British male population // Hum. Mol. Genet. – 1997. – Vol.6. – P. 869–876.

Koh J.M., Kim D.J., Hong J.S. et al. Estrogen receptor alpha gene polymorphisms Pvu II and Xba I influence association between leptin receptor gene polymorphism (Gln223Arg) and bone mineral density in young men // Eur. J. Endocrinol. – 2002. – Vol.147. – P. 777–783.

Loos R.J., Rankinen T., Chagnon Y. et al. Polymorphisms in the leptin and leptin receptor genes in relation to resting metabolic rate and respiratory quotient in the Québec Family Study // Int. J. Obes. – 2006. – Vol.30. – P. 183–190.

Mammes O., Aubert R., Betoulle D. et al. LEPR gene polymorphisms: associations with overweight, fat mass and response to diet in women // Eur. J. Clin. Invest. – 2001. – Vol.31. – P. 398–404.

Mantzoros C.S. The role of leptin in human obesity and disease: a review of current evidence // Ann. Intern. Med. – 1999. – Vol.130. – P. 671–680.

Matsuoka N., Ogawa Y., Hosoda K. et al. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity // Diabetologia. – 1997. – Vol.40. – P. 1204–1210.

Mattevi V.S., Zembrzuski V.M., Hutz M.H. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil // Int. J. Obes. Relat. Metab. Disord. – 2002. – Vol.26. – P. 1179–1185.

Minokoshi Y., Kim Y.B., Peroni O.D. et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase // Nature. – 2002. – Vol.415. – P. 339–343.

Montague C.T., Farooqi I.S., Whitehead J.P. et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans // Nature. – 1997. – Vol.387. – P. 903–908.

Park K.S., Shin H.D., Park B.L. et al. Polymorphisms in the leptin receptor (LEPR)-putative association with obesity and T2DM // J. Hum. Genet. – 2006. – Vol.51. – P. 85–91.

Rosmond R., Chagnon Y.C., Holm G. et al. Hypertension in obesity and the leptin receptor gene locus // J. Clin .Endocrinol. Metab. – 2000. – Vol.85. – P. 3126–3131.

Seufert J. Leptin effects on pancreatic beta-cell gene expression and function // Diabetes. – 2004. – Vol.53, (Suppl. 1). – S. 152–158.

Silver K., Walston J., Chung W.K. et al. The Gln223Arg and Lys656Asn polymorphisms in the human leptin receptor do not associate with traits related to obesity // Diabetes. – 1997. – Vol.46. – P. 1898–1900.

Stefan N.B., Vozarova A.D., Ossowski P.V. et al. The Gln223Arg polymorphism of the leptin receptor in Pima Indians influence on energy expenditure, physical activity and lipid metabolism // Int. J. Obes. Relat. Metab. Disord. – 2002. – Vol.26. – P. 1629–1632.

Takahashi-Yasuno A., Masuzaki H., Miyawaki T. et al. Leptin receptor polymorphism is associated with serum lipid levels and impairment of cholesterol lowering effect by simvastatin in Japanese men // Diabetes Res. Clin. Pract. – 2003. – Vol.62. – P. 169–175.

Templeton A.R. Population genetics and microevolutionary theory. – Hoboken, New Jersey: John Wiley & Sons, Inc., 2006. – 705p.

van Rossum C.T., Hoebee B., van Baak M.A. et al. Genetic variation in the leptin receptor gene, leptin, and weight gain in young Dutch adults // Obes. Res. – 2003. – Vol.11. – P. 377–386.

van Rossum C.T.M., Hoebee B., Seidell J.C. et al. Genetic factors and weight gain in Dutch men and women // Int. J. Obes. Relat. Metab. Disord. – 2002. – Vol.26. – P. 517–528.

Walsh P.S., Metzger D.A., Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material // BioTechniques. – 1999. – Vol.10. – P. 506–513.
Wauters M., Considine R.V., van Gaal L.F. Human leptin: from an adipocyte hormone to an endocrine mediator // Eur. J. Endocrinol. – 2000. – Vol.143. – P. 293–311.
Wauters M., Mertens I., Chagnon M. et al. Polymorphisms in the leptin receptor gene, body composition and fat distribution in overweight and obese women // Int. J. Obes. Relat. Metab. Disord. – 2001. – Vol.25. – P. 714–720.
Yiannakouris N., Yannakoulia M., Melistas L. et al. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability // J. Clin. Endocrinol. Metab. – 2001. – Vol.86. – P. 4434–4439.
Zhang Y., Proenca R., Maffei M. et al. Positional cloning of the mouse obese gene and its human homologue // Nature. – 1994. – Vol.372. – P. 425–432.

Представлено: О.В.Филипцова / Presented by: O.V.Filiptsova
Рецензент: Л.І.Воробйова / Reviewer: L.I.Vorobyova
Подано до редакції / Received: 29.03.2012