

SNP rs1137101 Leptin Receptor Gene LEPR as a Risk Factor for Type 2 Diabetes

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Abstract

Mutations of leptin gene resulting in leptin deficiency cause obesity, insulin resistance, and diabetes in animals and, in few cases, morbid obesity and hyperinsulinemia in humans. The distribution of single nucleotide polymorphism of the leptin receptor gene "*LEPR rs1137101* (Q223R)" 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)". In control group, the major allele in the studied population is Q, its frequency $pQ=0.57$. The difference is non-significant when comparing men (0.59) to women (0.56). There is high percent of homozygotes QQ in men than women. In diabetic group the major allele is R with a frequency = 0.59 with significant difference when comparing men 0.56 to women 0.63. There is a high percent of the genotype RR in women than men. The RR genotype increases the probability of contracting type 2 diabetes mellitus (Relative risk (RR) = 1.44) whereas QQ genotype decreases this probability (RR = 0.45). In conclusion, rs1137101 (Q223R) polymorphism of the LEPR gene is associated with susceptibility to type -2 diabetes. This polymorphism may represent genetic marker for the risk of type -2 diabetes.

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

1. Introduction

"Leptin is the hormone of adipose tissue and secreted by adipocytes, plays an essential function in directing body weight. In rodents with mutations in the gene for leptin receptor promote morbid obesity[1]. Leptin is required in appetite control, causing a decrease or an increase in food consumption while conserving body fat.

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The gene encoding leptin receptor is mapped on the short arm of chromosome first (1p31), composes of 20 exons, 19 introns, has a size of > 70 kilobases of DNA [2]. When a mutation in the gene for leptin or its receptor gene, all of the biological effects of the hormone are blocked.

In these patients, along with the development of obesity, delayed growth and puberty". Mutations in the leptin gene leading to leptin deficiency cause obesity, insulin resistance, and diabetes in animals [3] and, in few cases, morbid obesity and "hyperinsulinemia" in humans [4]. "Common genetic variants (e.g., SNPs) at the *LEPR* gene locus have been related with obesity, hyperinsulinemia, type 2 diabetes mellitus (T2DM), and alterations in leptin levels in different populations. For example, three non-synonymous SNPs (Arg109Lys, Arg223Gln, and Lys656Asn) have been evaluated for association studies " [5-9]. The study of population structure on the 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 in the genesis of which play the role of disturbances in metabolic pathways controlled by this gene. The goal of this study is to investigate for first time the single nucleotide polymorphism *rs1137101* (Q223R) in patients with type 2 diabetes and healthy individuals from Russia and Ukraine.

2. Materials and Methods

"DNA of 50 patients with type-2 diabetes mellitus (27 male and 23 female) as well as DNA of 100 persons (48 male and 52 female) – Russians and Ukrainians residents of Kharkov and Poltava cities have been investigated in this study. 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) [10-11]. Studied single nucleotide polymorphism of *C/G* is localized in

exon 6. Endonuclease (restriction enzyme *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*)".

3. Results and Discussion

Results of this study add further evidence for the reported "*LEPR* rs1137101 polymorphism" effect on the risk of type -2 diabetes. Major allele in the control group of studied population is Q, its frequency $p_Q=0.57$. The difference is non-significant when comparing men (0.59) to women (0.56) $P>0.05$ (Fig.1)(Table 1). There is high percent of homozygotes QQ in men than women. In diabetic group the major allele is R with a frequency of 0.59 with significant difference when comparing men 0.56 to women 0.63 $P<0.05$. There is a high percent of the genotype RR in women than men.

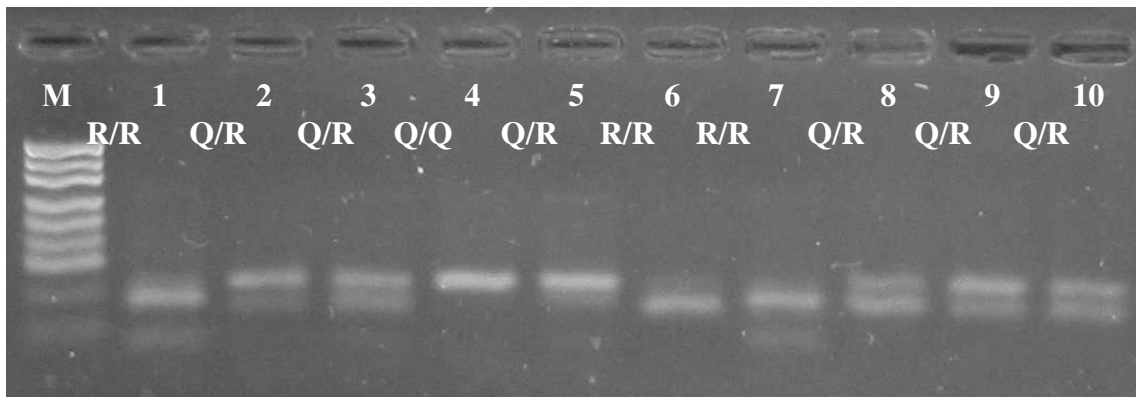


Figure 1: Electrophoregram products amplified by PCR with restriction fragment of the gene *LEPR* (M-DNA marker *pUC19*, hydrolyzed with endonuclease *MspI*, 1–10 – DNA donors; *RR*, *QQ*, *QR* – genotypes.

Table1: Distribution of genotypes and allele frequency of SNP *rs1137101* gene in investigated population

Group	Gender	Number	Genotypes, <i>n</i>			Allele frequencies	
			<i>RR</i>	<i>QR</i>	<i>QQ</i>	<i>R</i>	<i>Q</i>
Control	Male	48	13	13	22	0,41	0,59
	Female	52	12	22	18	0,44	0,56
	Total	100	25	35	40	0,43	0,57
T2DM	Male	27	8	14	5	0,56	0,44
	Female.	23	10	9	4	0,63	0,37
	Total	50	18	23	9	0,59	0,41

In control group of the investigated population, there is a high percent of homozygotes 40% indicating that there is a population subdivision (Vahlunda effect), a kinship or positive assortative mating [23]. In T2DM group the heterozygotes have a high percent with 46%. Furthermore, the *RR* genotype frequency of *rs1137101* in the T2DM group was lower than that in the control group with nonsignificant difference ($P = 0.05$). Table2 shows the frequencies of alternative allele *R* with a maximum frequency of this allele (0.89) is present in the indigenous population of Australia, the nations of Asia - the Japanese and Koreans (0.85). The most rare allele that Pima Indians (0.32), and the inhabitants of Greece (0.32).

"There were conflicting works of association of *rs1137101* polymorphism with obesity and type II diabetes. Researchers have indicated reasons for hiding the effect of *rs1137101* polymorphism, and they relate that to the differences in the background variation in other genes among studied populations" (24,25,26).

"There is positive assortative by genotype: the number of married couples in which husband and wife have the same genotype, higher than expected for a random combination of genes. The reasons for this are unclear. It is possible that there is a hidden kinship spouse, and possibly true assortative mating on the grounds on which this affects genotype"[27].

Table2: 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 and his colleagues 1997
England	White	0.44	Gotoda and his colleagues 1997
France	White	0.44	Mammes and his colleagues 2001
Belgium	White	0.48	Wauters and his colleagues 2001
Netherlands	White	0.44	van Rossum and his colleagues 2002
Sweden	White	0.50	Rosmond and his colleagues 2000
Denmark	White	0.41	Echwald and his colleagues 1907
Greece	White	0.32	Yiannakouris and his colleagues 2001
Australia	White	0.58	De Silva and his colleagues 2001
Japan	Japanese	0.85	Matsuoka and his colleagues 1997
Korea	Korean	0.85	Koh and his colleagues 2002
USA	Pima Indians	0.32	Stefan and his colleagues 2002
USA	Brazilians	0.40	Mattevi and his colleagues 2002
Australia	Native people	0.89	DeSilva and his colleagues 1999

Moreover, the LEPR Q223R SNP showed a significant association with type-2 diabetes mellitus (RR, QR and QQ (95% CI): 23.3-49.8, 32.4-59.9 and 8.6-29.9 Table 3.

Confidence intervals of relative risk (RR) as revealed in Table 3, the RR genotype increases the probability of contracting type 2 diabetes mellitus (Relative risk (RR) = 1.44) whereas QQ genotype decreases this probability (RR = 0.45).

Table3: The relative risk RR in studied groups

Group	Genotypes, (%)			Allele frequencies	
	RR	QR	QQ	R	Q
Control	25,0	35,0	40,0	0,43	0,57
95 % CI	16,9 – 34,1	25,8-44,5	30,4-49,9	0,36-0,50	0,08-0,17
	60.0		40.0		
T2DM, %	36,0	46,0	18,0	0,59	0,41
95% CI	23,3-49,8	32,4-59,9	8,6-29,9	0,49-0,69	0,31- 0,51
	82.0		18.0		
RR	1.44	1.31	0.45	1.37	0.72

4. Conclusions

We conclude that rs1137101 (Q223R) polymorphism of the LEPR gene is associated with susceptibility to type -2 diabetes. This polymorphism may represent genetic marker for the risk of type -2 diabetes.

5. Recommendations

Study of population structure on the polymorphisms of this gene in different populations 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 in the genesis of which play the role of disturbances in metabolic pathways controlled by this gene.

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