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ORIGINAL ARTICLE

Association of leptin receptor gene Gln223Arg polymorphism with susceptibility to colorectal cancer

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ABSTRACT

Aim: Leptin is a 16 kDa polypeptide hormone which secreted by adipose tissue and has an important role in energy balance, insulin pathway and inflammation, because of that it may play an important role in colorectal cancer (CRC). Leptin exerts its effect through the leptin receptor (LEPR) a member of the class I cytokine receptor family.

Background: We have investigated whether glutamine to arginine substitution (Gln223Arg) in exon 6 of the leptin receptor gene, has implications for susceptibility to CRC.

Patients and methods: Polymerase chain reaction (PCR) and restriction enzyme digestion (RFLP) was performed to evaluate the association between the Gln223Arg polymorphism of the LEPR and CRC risk in a case-control study in 346 subjects involving 173 cases with CRC and 173 controls.

Results: There was no statistically evidence of significant difference in genotype and allele frequencies between the cases with CRC and controls for the Gln223Arg polymorphism of LEPR, before or after adjusting for confounders (age, BMI, sex, and smoking status). Furthermore, no significant difference was observed between the CRC cases and controls by BMI, sex and smoking status.

Conclusion: Our findings suggest that the LEPR Gln223Arg polymorphism is not associated with the risk of CRC in Iranian population.

Keywords: Colorectal cancer, LEPR gene, PCR-RFLP, Single nucleotide polymorphism.

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Introduction

Colorectal cancer (CRC) is the third most common type of cancer in both men and women in the world and it causes around 650,000 deaths worldwide per year (1). Leptin (LEP) is a 16 KDa glycoprotein, expressed by adipose tissues and plays its role via leptin receptor (LEPR), a member of Il-6 cytokine receptor family. LEPR expressed in colon cancer cell lines as well as in human normal colonic tissue and adenomatous polyps (2,3). When LEP bind to its receptor a signaling pathway from adipose tissue to the brain is started, involves in regulation of feeding and energy homeostasis (4). LEP activates cytokine signaling transduction by inducing Janus kinase (JAK2) pathways through LEPR (Figure 1). Activation of this tyrosin kinase (JAK2) initiates down stream signaling of suppressor of cytokine signaling 3 (SOCS3) and signal transducer and activator of transcription-3 (STAT3). SOCS3 has been linked to leptin and insulin resistance because of its ability to inhibit leptin and insulin signaling pathways (5, 6). As demonstrated in previous studies

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leptin signaling pathway stimulates several proinflammatory cytokines by activating Janus kinase and STAT3 that appear to influence on Il-6 and CRP mediated transcriptional activation (7,8). Energy balance, adiposity, insulin resistance, and inflammation have been associated with CRC and consequently related to LEP and LEPR (9-12). However, only a few studies have addressed the association between LEPR and CRC, their results are in agreement (13-15). The studies that have done by Slattery et al. (13), Uddin et al. (14), and Pechlivanis et al. (15) reported significant associations between LEPR SNPs and the risk of CRC. Polymorphism in the LEPR gene that has been studied in conjunction with obesity and insulin resistance might also provide insight into associations with cancer (16-20). To obtain a better understanding of this association between LEPR and CRC, we evaluated one of the most common and functionally importance variant (Gln223Arg), located in exon6 of LEPR gene (16,18).

Patients and Methods

Total of 346 recruited subjects, including 173 CRC patients (age range24-83) as cases and 173 controls (age range13-84), were evaluated in a case-control study. The patients who were undergoing colonoscopy for various gastrointestinal complain were recruited by the Research Center for Gastroenterology and Liver Diseases (RCGLD). Cases were defined as the patients with positive pathological reports for CRC. 33 of 173 cases (19.1%) presented positive family history (first-degree relatives, including parents, siblings, and children) for CRC. Control participants showed negative colonoscopy report for malignancy or polyps (including adenomatous and other polyps) and 36 of the 173 controls (20.8 %) had positive family history for CRC. At colonoscopy, anthropometric measurements, smoking habits, and family history for CRC were recorded. The recruitment of the participants was between July 2009 and February 2011 (all of them were Iranian and genetically unrelated). Informed consent was provided from all the subjects at recruitment and the Ethical Review Boards of the Institution approved the study protocol. The body mass index (BMI) of each subject was calculated by weight (kg)/height (m^2) formula. Subjects were divided into subgroups, on the basis of their BMI values denoted as following: normal-weight (BMI <25 kg/m2) cases (n=76), overweight/obese (BMI ≥25 kg/m2) cases (n=95), normal-weight (BMI <25 kg/m2) control (n=89) and overweight/obese (BMI ≥25 kg/m2) control (n=84)

Genomic DNA was obtained from 5ml EDTA-anticoagulated whole blood by using the standard "phenol chloroform" protocol. The LEPR Gln223Arg polymorphism was examined by means of PCR and amplified 80 bp fragment that used forward (5'-AAACTCAACGACACTCTCCTT-3') and reverse (5' –TGAACTGACATTAGAGGTGAC-3') primers for LEPR gene (21). The PCR reaction was started with an initial denaturation at 93°C for 10 min followed by 35 cycles of 93°C for 45 s, 57°C for 30 s, 72°C for 45 s, and final extension at 72°C for 10 min. To determine genotype differentiation within LEPR genes, restriction enzyme MspI was used to digest PCR products at 37°C for 3 hours. Digestion showed genotypes denoted: Gln/Gln (80bp), Gln/Arg (80,59 and 21bp), and Arg/Arg (59 and 21bp). Digested products were electrophoreses on 3% agarose gel. The concordance of genotyping was confirmed by duplicate analysis 10% of samples and DNA sequencing 5% of samples (figure 2,3) that all of them were selected randomly (all results were accurate).

The chi-square test was used to accurate genotype frequencies with Hardy-Weinberg equilibrium, also χ^2 test was applied for demonstrating differences between study groups for genotype and alleles frequencies. To adjust confounding factors including age, BMI, sex and smoking statues, logistic regression analysis was used. Odds ratios (OR) were given with the respective 95% confidence intervals (95% CI) to qualified the roll of distinctive genotypes in prevalence of CRC. The t-test was used to evaluate

Table 1. Study population characteristics

	Controls (n = 173)	**Cases** (n = 173)	P-value
Age (years)	44.8 ±17.2*	55.8±12.7	0.001
BMI (kg/m2)	25.1±5.3	26.2 ±7.19	0.11
Gender			0.28
Men	82(47.4)†	92(53.2)	
Women	91(52.6)	81(46.8)	
Smoking history			0.31
No	144(83.2)	146(85.4)	
Former	27(15.6)	20(11.7)	
Current	2(1.2)	5(2.9)	
Tumor site			-
Colon	-	98(60.1)	
Rectal	-	65(39.9)	
Metastasis			-
No	-	135(87.1)	
Yes	-	20(12.9)	

* Mean±standard deviation; † Number (%)

Table 2. Distribution of Leptin receptor (*LEPR*) genotypes and alleles in relation to CRC risk

SNP	Controls (n= 327)	Cases (n= 327)	OR (95%CI)	P-value
LEPR Gln 223Arg Polymorphism Genotype-wise comparison				
LEPR (Gln/Gln)	67(38.7)*	77(44.5)	1.0 (reference)	
LEPR (Gln/Arg)	80 (46.2)	75(43.4)	0.82 (0.52-1.29)	0.38
LEPR (Arg/Arg)	26 (15)	21(12.1)	0.703 (0.37-1.37)	0.3
LEPR (Gln/Arg) + LEPR (Arg/Arg)	106 (61.2)	96 (55.5)	0.890(0.59-1.36)	0.59
LEPR (Arg/Arg) versus others	26 (15)	21(12.1)	1.280(0.7-2.38)	0.43
Allele- wise comparision				
LEPR (Gln)	214 (61.8)	229 (66.2)	1.0 (reference)	
LEPR (Arg)	132 (38.1)	117(33.8)	1.097(0.943-1.277)	0.24

* Number (%)

the variations in demographic factors. Statistical tests were performed by SPSS software (version 15.0; SPSS, Chicago, IL, USA). Significance was assumed for $P < 0.05$.

Results

Characteristics of the subjects are presented in Table 1. There was no significant difference in BMI between cases (26.2) and the controls (mean 26.2 and 25.1 respectively, P=0.11). Cases were significantly older than controls (P<0.001) but were similar with respect to smoking history and gender. Furthermore in this population 60.1% of tumors were in colon and just 12.9 % of them had metastasis to other parts of the body.

The genotype and allele frequencies for LEPR Gln223Arg gene polymorphism are presented in Table 2. Among both case and control population all the genotype frequencies for LEPR Gln223Arg SNP were distributed in compliance with Hardy-Weinberg equilibrium (p>0.05). The distributions of the genotypes of Gln/Gln, Gln/Arg, and Arg/Arg in CRC cases were 44.5%, 43.4%, and 12.1% compared with that of the controls, 38.7%, 46.2%, and 15%. No significant differences emerged for the LEPR gene Gln223Arg polymorphism in neither genotype nor allele frequencies between the patients and the controls. Additionally adjustment for covariates including age, BMI, sex, and smoking

status, did not significantly alter the association between this SNP and the risk of CRC.

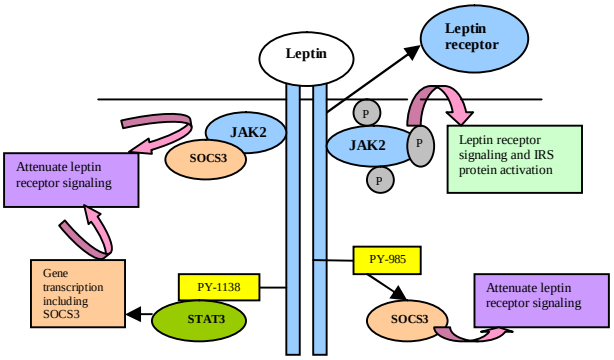


Figure1. Leptin receptor signaling. Leptin binding to the long isoform of the leptin receptor results in autophosphorylation and activation of JAK2, which then leads to phosphorylation of tyrosine residues located in the intracellular domain of the leptin receptor (pY-985 and pY-1138). pY-1138 recruits the transcription factor STAT3, which is appears to be crucial in the action of leptin on energy expenditure. STAT3 activation also results in the transcription of the signaling inhibitor SOCS3. Leptin receptor signaling also results in the activation of the IRS-phosphorylation–PI3-kinase pathway, probably through phosphorylation sites on JAK2. SOCS3 attenuates leptin receptor signaling by binding to pY-985 and to JAK2.

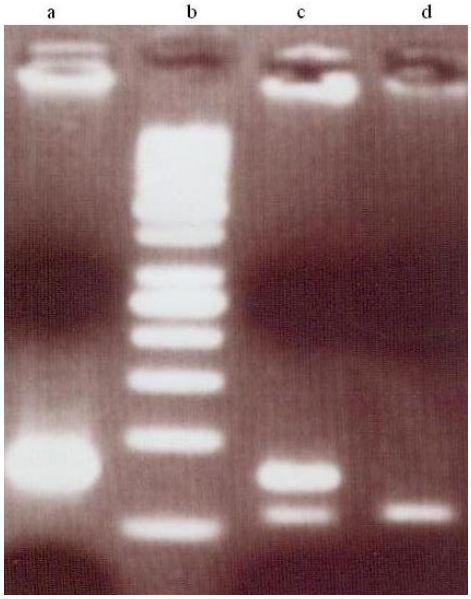
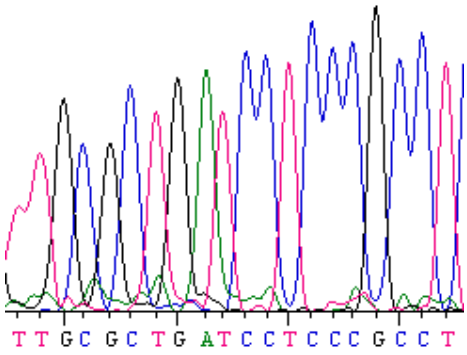
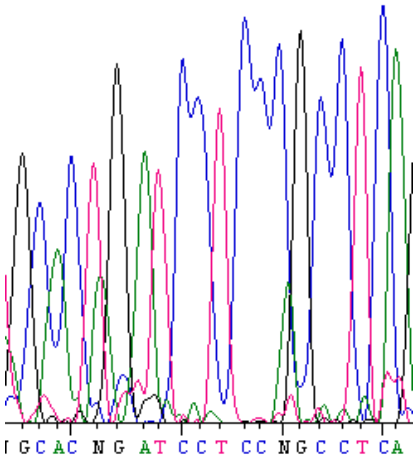


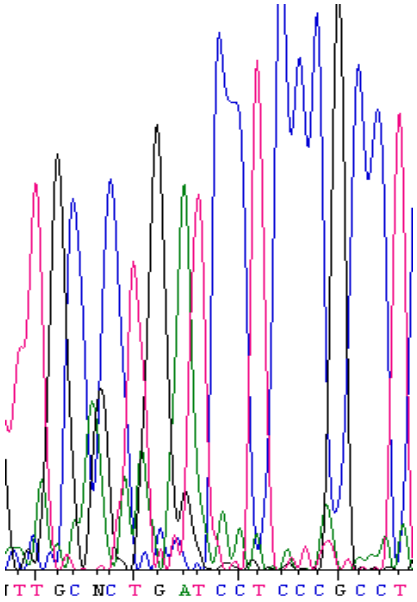
Figure2. Lane b: ladder 50bp, RFLP products: lane a: (Gln/Gln) 80bp, lane c :(Gln/Arg) 80, 59*, lane d: (Arg/Arg) 59*. *Determination of genotypes was based on recognizing 80 and 59 bp band on 3% agarose gel.



(A)



(B)



(C)

Figure3. Sequencing results for each genotype variation. **(A)** Arg, **(B)** Gln, **(C)** Gln/Arg.

Discussion

In this case–control study, we evaluated the associations between the LEPR Gln223Arg polymorphism and the risk of CRC in Iranian population. The findings of this investigation indicate that Gln223Arg polymorphism of the LEPR is not associated with susceptibility to CRC. Additionally our data suggested the LEPR polymorphism assessed do not interact with age, BMI, sex, and smoking status.

CRC is a disease with a high mortality and morbidity that have been repeatedly shown to be associated with obesity and diabetes (22-25). Leptin is one of the hormones that is produced and secreted by adipose tissues and has important roles in adiposity and insulin signaling pathway regulation. The leptin receptor is a single transmembrane domain receptor with two cytokine domains and has five alternatively spliced isoforms, which are distributed in many tissues and binds to leptin (26, 27).

Several single nucleotide polymorphisms (SNPs) in the LEPR gene have been described (28). Among them Gln223Arg polymorphism, results in an amino acid change within the region encoding the extracellular domain of the leptin receptor, lead to changes in its charge (neutral to positive), and most likely to has functional consequences. In the studies that have been conducted by Quinton et al. (29) and Mendoza et al. (30), higher levels of ligand binding affinity have been determined in individuals homozygous for the G (LEPR Arg223Arg) allele than for carriers of the A (LEPR223Gln) allele (29, 30). Yiannakouris et al. have found association between homozigosity for the G (LEPR Arg223Arg) allele and obesity (31), but in contrast Heo et al. and Paracchini et al. have failed to show an association between this polymorphism and obesity. An association between insulin sensitivity and the G (LEPR223Arg) allele has been reported by Chiu et al. (19). In contrast with the results obtained by Slattery et al. that reported the LEPR rs6588147 SNP reduced risk of colon cancer among men (13), we did not detect any significant difference in the distribution of LEPR Gln223Arg genotypes or alleles between cases with CRC and the controls. This study did not detect any association between gender and risk of CRC. In the study by Uddin et al. (14) it was shown LEPR significantly overexpressed in primary CRC compare with adenomas and normal colonic mucosa. Pechlivanis et al. reported (15) statistically, significant interactions were observed between the INS SNP rs3842754 (C1127INSPstI) genotypes and both the LEPR SNP rs1137101 (Q223R) and the ADIPOQ SNP rs266729 (C-11374G) genotypes. Different results such as these are common in genetic association studies (32). This discrepancy in the reported associations between LEPR gene polymorphisms and CRC implies the possibility that the effects of Q223R appear in relation with other genetic factors (such as genes involved in constitutive activation of Wnt/β-catenin signaling pathways), that were not examined in this study, that may influence the effect of Q223R or environmental factors which may also work as confounding variables. However, the small sample size or statistical methods are other reasons for our conflicting findings compare with others. Furthermore, previous investigations suggest that variants in the other adipokine genes in combination with variants in diabetes-related genes may influence on association between LEPR polymorphism and CRC risk (13, 14). In summary, the present observations suggest that in this case–control study, we were not able to demonstrate a significant association between LEPR Q223R polymorphism and the CRC risk in Iranian population. Our lack of association between the LEPR Q223R polymorphism and CRC risk may be due to the lack of functionality of the polymorphism. These results should be confirmed in additional investigations to further evaluate the

potential interaction between this polymorphism, obesity, insulin pathway and CRC.

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