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Genetic Variations in *Lep*tin and *Lep*tin Receptor and Susceptibility to Colorectal Cancer and Obesity

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Abstract

Background: Colorectal cancer (CRC) is the second most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality around the world.
Objectives: With regard to the role of obesity in colorectal cancer (CRC) and the role of *lep*tin in obesity, we investigated whether *leptin* (*LEP*) and *leptin* receptor (*LEPR*) gene variants are associated with CRC risk.
Patients and Methods: We evaluated *LEP* (rs7799039) and *LEPR* (rs1137101) gene variants by using PCR-RFLP method in 261 cases with CRC and 339 controls.
Results: No significant difference was found for rs7799039 and rs1137101gene variants between the cases with CRC and controls. However, the *LEPR* rs1137101 "GG" genotype compared with "AA" genotype and "AA + AG" genotype was associated with increased risks for obesity, and the differences remained significant after adjustment for confounding factors including age, sex, smoking status, and NSAID use (P = 0.015; OR = 2.42, 95%CI = 1.19 - 4.93 and P = 0.016; OR = 2.28, 95%CI = 1.17 - 4.48, respectively). In addition, the *LEPR* "G" allele compared with the "A" allele was associated with an increased risk for obesity (P = 0.024; OR = 1.44, 95%CI = 1.05 - 1.98).
Conclusions: Consistent with most previous studies, our findings found no association between *LEP* (rs7799039) and *LEPR* (rs1137101) gene variants and CRC risk. However, the *LEPR* rs1137101 "GG" genotype compared with the "AA" genotype and "AA+AG" genotype was associated with a 2.42-fold and a 2.28-fold increased risk for obesity, respectively.

Keywords: Colorectal Cancer, *LEP*, *LEPR*, Variant

1. Background

Colorectal cancer (CRC) is the second most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality around the world (1). Epidemiological reports have shown that CRC is associated with obesity (2-4), hyperinsulinemia and insulin resistance (3, 5). Previous studies have also demonstrated that leptin participate in body weight regulation. Furthermore, high leptin levels result in increased insulin resistance and obesity (6, 7).

Leptin, a 167-amino-acid peptide, mainly synthesized and released by adipocytes. This adipocytokine plays a very important role in body weight and energy homeostasis (8). *Leptin* also stimulates proliferation and inhibits apoptosis in human colorectal cancer cells (9) and exerts its physiological action through its receptor, which is expressed in human colon cancer cells lines (10). Epidemiologic evidence has also demonstrated higher serum levels of *leptin* (11, 12) in cases with CRC than controls. Furthermore, a positive association for *leptin*-adiponestin ratio and CRC risk has been reported (13). Finally, the potential associations between CRC risk and leptin (*LEP*) (14-18) and leptin receptor (*LEPR*) (5, 16, 19) gene variants have been investigated in several studies, but the results were contradictory.

2. Objectives

We therefore designed the present study to investigate the possible associations of *LEP* (rs7799039) and *LEPR* (rs1137101) gene variants with CRC risk. These SNPs were selected based on their commonly use in previous genetic epidemiology studies, degree of heterozygosity, position in the gene, and functional importance.

3. Patients and Methods

3.1. Participants

A total of 261 cases with CRC aged 22 to 84 years and 339 controls aged 19 to 85 years were enrolled into the study between March 2008 and June 2012. All the 600 subjects were Iranian and genetically unrelated. Both cases

and controls were recruited from individuals who were undergoing colonoscopy for various gastrointestinal complaints such as unexplained weight loss, rectal bleeding, change of bowel habits, long-term pain in the abdomen, chronic diarrhea or constipation. However, the case group consisted of all eligible colonoscopy patients with positive pathologic report for CRC, while only subjects whose colonoscopic results were negative for malignancy, adenomatous polyps, or other polyps were chosen as controls. Before subjects' colonoscopy, interviews were performed by using a self-administered questionnaire and information regarding the subjects' demographic and anthropometric characteristics was recorded. Prior entering the study, informed consent was obtained from each subject and the study was approved by the Ethical Committee of Gastroenterology and liver diseases research center, Shahid Beheshti University of Medical Sciences. Body mass index (BMI) of each subject was calculated as body weight divided by height squared (kg/m^2) and the subjects were divided in the subgroups based on the diagnosis of CRC and BMI values as follows: normal weight ($BMI < 25\ kg/m^2$) controls (n = 166); overweight/obese ($BMI \geq 25 kg/m^2$) controls (n = 173); normal weight cases with CRC (n = 120); and overweight/obese cases with CRC (n = 141).

3.2. Genotype Analysis

5 ml of peripheral blood samples from all 600 subjects were collected in tubes containing EDTA as an anticoagulant and store at 4°C. Genomic DNA was isolated from peripheral blood leucocytes using standard methods, and genotyping was done by PCR-RFLP method. Characteristics of the studied gene variants, PCR primers, and PCR and RFLP conditions are summarized in Table 1. The PCR products were digested overnight with the appropriate restriction enzymes (Fermentas, Leon-Rot, Germany) and the digested products were run on 3% agarose gels. Bands in gels stained with ethidium bromide for visualization under ultraviolet light. The concordance of genotyping was confirmed by duplicate analysis of approximately 10% of the randomly selected samples.

3.3. Statistical Methods

Differences in demographic or anthropometric factors were calculated using t-test or chi-square test when appropriate. Testing Hardy-Weinberg equilibrium (HWE) for both of the two gene variants among cases and controls, separately, and comparisons of the distribution of the allele frequencies between the groups were performed using the chi-square test. Logistic regression was used to examine genotype frequencies between the different groups. We also used logistic regression analysis to adjust for confounding factors. All statistical analyses were conducted by using SPSS software (version 15.0; SPSS Inc. Chicago, IL, USA) and P values less than 0.05 were considered statistically significant.

4. Results

4.1. Clinicopathological Analysis

Selected characteristics of the study population and their statistical significance are summarized in Table 2. In general, cases with CRC were older and less likely to use NSAIDs when compared with their control counterparts. However, there were no significant differences between the cases with CRC and the controls in terms of sex, BMI, smoking status, and family history of CRC.

4.2. LEP and LEPR SNPS Analysis

The distribution of genotypes and alleles of the *LEP* (rs7799039) and *LEPR* (rs1137101) gene variants in cases with CRC and controls are provided in Table 3. None of the genotype frequency distributions for the rs7799039 and rs1137101 variants deviated significantly from the Hardy-Weinberg equilibrium in both cases and controls ($P > 0.05$), suggesting that the alleles are in equilibrium. Furthermore, no significant difference was observed in genotype and allele frequencies between the cases and controls for *LEP* and *LEPR* genes either before or after adjustment for confounding factors.

In this study the risk of obesity in relation to the two gene variants was also examined. These data indicated that the *LEPR* rs1137101 "GG" genotype compared with "AA" genotype was associated with an increased risk for obesity in controls after adjustment for age, sex, smoking status, and NSAID use (P = 0.015; OR = 2.42, 95%CI = 1.19 - 4.93). In addition, the carriers of the *LEPR* "GG" genotype compared with "AA + AG" genotype occurred more frequently in overweight/obese controls than normal weight controls, and the difference remained significant after adjustment for confounding factors (P = 0.016; OR = 2.28, 95%CI = 1.17 - 4.48). Finally, the *LEPR* "G" allele compared with the "A" allele was significantly overrepresented in overweight/obese controls than normal weight controls (P = 0.024; OR = 1.44, 95%CI = 1.05 - 1.98).

5. Discussion

We conducted a case-control study to explore the possible association between the *LEP* (rs7799039) and *LEPR* (rs1137101) gene variants and CRC risk among Iranians. No significant difference was found for *LEP* and *LEPR* gene variants in either genotype or allele frequencies between the cases with CRC and the controls. However, the *LEPR*

Table 1. Information for the Studied Markers in Leptin (*LEP*) and Leptin Receptor (*LEPR*) Genes

Gene (SNP)	Location (Base Change)	Forward Primer Reverse Primer	PCR Program (35 Cycles)	PCR Fragment Size (Bp)	Restriction Enzyme, Incubation Temperature	Alleles: RFLP Fragments Size (Bp)
***LEP* (rs7799039)**	Promoter (G > A)	5' - TTTCCTGTAATT TTCCCGTGAG-3' 5' - AAAGCAAAGACA GGCATAAAAA-3'	93 °C 45s, 61 °C 30s, 72 °C 35s	242	HhaI, 37 °C	Allele A: 242 Allele G: 181 + 61
***LEPR* (rs1137101)**	Exon 6 (A > G)	5'-AAACTCAACGA CACTCTCCTT-3' 5'-TGAACTGACATT AGAGGTGAC-3'	93 °C 45s,57 °C 30s, 72 °C 30s	80	MspI, 37 °C	Allele A: 80 Allele G: 59 + 21

Table 2. Selected Characteristics of the Cases with Colorectal Cancer and Controls[a]

Variables	Controls (N = 339)	Cases (N = 261)	P Value
Age (years)	44.3 (16.3)	56.1 (12.6)	< 0.001
BMI (kg/m^2)	25.2 (4.2)	25.6 (4.9)	0.261
Gender			
Men	164 (48.4)	146 (55.9)	
Women	175 (51.6)	115 (44.1)	0.066
Smoking history			
No	290 (85.5)	214 (82.0)	
Former	39 (11.5)	32 (12.3)	
Current	10 (3.0)	15 (5.7)	0.261
Regular NSAID use			
No	270 (79.6)	248 (95.0)	
Yes	69 (20.4)	13 (5.0)	< 0.001
Family history of colorectal cancer			
No	303 (89.4)	229 (87.7)	
Yes	36 (10.6)	32 (12.3)	0.530
Tumor site			
Colon	-	167 (63.9)	
Rectal	-	94 (36.1)	-
Metastasis			
No	-	176 (89.3)	
Yes	-	21 (10.7)	-

[a] Variables presented as mean (SD) or number (%).

rs1137101 "GG" genotype and the *LEPR* "G" allele were risk factors for obesity in the controls.

5.1. LEP Gene Rs7799039 Variant

Complex diseases such as CRC are influenced by interacting networks of genetic and environmental factors. One method of identifying novel susceptibility genes for the diseases is to study polymorphisms in candidate genes and therefore the association between DNA sequence variations and CRC has become a subject of interest in recent years. On the other hahnd, adipokine genes including *LEP* and *LEPR* are strong candidates for the link between obesity and risk of CRC and it has been suggested that leptin and its receptor may be involved in cancer development. The association between *LEP* gene variants and CRC risk

Table 3. Association Between Genotypes and Alleles of Leptin (*LEP*) and Leptin Receptor (*LEP*) Gene Variants and Risk of Colorectal Cancer[a]

Gene (Variant)	Controls (N = 339)	Cases (N = 261)	Or (95% Ci) P Value[b]
***LEP* (rs7799039) Genotype-wise comparison**			
AA	113 (33.3)	76 (29.1)	1.0 (reference)
AG	154 (45.4)	135 (51.7)	1.44 (0.96 - 2.15) 0.076
GG	72 (21.3)	50 (19.2)	1.07 (0.65 - 1.77) 0.791
AG and GG	226 (66.7)	185 (70.9)	1.32 (0.90 - 1.92) 0.152
GG versus others	72 (21.2)	50 (19.2)	0.86 (0.56 - 1.33) 0.497
Allele-wise comparison			
A	380 (56.0)	287 (55.0)	1.0 (reference)
G	298 (44.0)	235 (45.0)	0.96 (0.76 - 1.21) 0.712
***LEPR* (rs1137101) Genotype-wise comparison**			
AA	146 (43.1)	127 (48.7)	1.0 (reference)
AG	147 (43.3)	101 (38.7)	0.76 (0.52 - 1.10) 0.141
GG	46 (13.6)	33 (12.6)	0.75 (0.44 - 1.27) 0.305
AG and GG	193 (56.9)	134 (51.3)	0.75 (0.53 - 1.07) 0.114
GG versus others	46 (13.6)	33 (12.6)	0.86 (0.51 - 1.44) 0.564
Allele-wise comparison			
A	439 (64.7)	355 (68.0)	1.0 (reference)
G	239 (35.3)	167 (32.0)	0.87 (0.68 - 1.10) 0.237

[a] Variables presented as number (%).
[b] Adjusted for age, BMI, sex, smoking status, NSAID use, and family history in genotype-wise comparisons.

has been examined in several epidemiologic studies, and the results were conflicting (14-18). Our results are in line with studies by Slattery et al. (14), Vasku et al. (15), Pechlivanis et al. (16) and by He and Xu (18), where no association was found between *LEP* gene rs7799039 variant and CRC risk; nevertheless, Partida-Perez et al. (17) reported a significant association between other variants of this gene and CRC risk. The discrepancy in the reported associations between the *LEP* gene variants and CRC risk may be due to false positive results, small sample size, statistical methods, genotyped markers, population differences in allele frequencies, or differences in the dietary and lifestyle factors. Alternatively, the rs7799039 variant may be in linkage disequilibrium with another unknown functional variant of *LEP* gene that explains the discrepancy observed. However, to conclude that the gene is not involved in the pathogenesis of CRC, other *LEP* gene variants should also be investigated in larger studies.

5.2. LEPR Gene Rs1137101 Variant

In this study, we also analyzed the association between the rs1137101 (Gln223Arg) variant in the exon 6 of *LEPR* gene and CRC risk. Studies of the effect of *LEPR* gene variants on CRC risk have also been inconclusive (15, 16, 19). Consistent with our findings, Vasku et al. (15), and Pechlivanis et al. (16) found no association between *LEPR* gene rs1137101 variant and CRC risk. In contrast, in a study by Liu et al. (19) a significant association was found between other variants of *LEPR* gene (rs12037879) and CRC risk.

Interestingly, in the present study we also demonstrated that the *LEPR* rs1137101 "GG" genotype compared with the "AA" genotype and "AA + AG" genotype was associated with a 2.42-fold and a 2.28-fold increased risk for obesity, respectively. Furthermore, the *LEPR* rs1137101 "G" allele compared with the "A" allele was a risk factor for obesity. The *LEPR* rs1137101 variant leads to an amino acid change (Gln223Arg) in the extracellular domain of LepR that represents a typical leptin-binding site and therefore the variant results in charge (neutral to positive) and can affect the functionality of LepR. In accordance with our findings, Yiannakouris et al. (20) demonstrated that homozygosity for the "G" or "Arg" allele of *LEPR* rs1137101 variant was associated with obesity risk. Previous studies have also shown that individuals homozygous for the "G" or "Arg" allele had higher serum leptin-binding affinity (21) and higher serum levels of leptin (20) than carriers of the "A" or "Gln" al-

lele. Accordingly, our finding that the rs1137101 “GG” genotype and the "G" allele appeared to be markers of increased obesity susceptibility is consistent with the notions above. A plausible hypothesis is that the increased obesity risk in the individuals with *LEPR* rs1137101 “GG” genotype compared with those with the “AA” genotype or “AA + AG” genotype is result of the higher serum leptin-binding affinity and the higher serum levels of leptin in subjects carrying the “GG” genotype. Such mechanism is speculative at the present but biologically plausible.

5.3. Study Limitations

Although well-designed, our study has several limitations. One limitation is the modest sample size that precludes drawing strong conclusions and doing detailed analyses. Another limitation is the limited number of polymorphisms examined. The other potential limitation is our lack of information on serum levels of leptin, which could modify the effects observed here. However, this study provides interesting information.

In conclusion, our findings do not support a role for effect of the *LEP* or *LEPR* gene variants investigated on CRC susceptibility in Iranian population. However, this study demonstrates that the rs1137101 variant of *LEPR* gene may be a genetic contributor to obesity risk. The *LEPR* rs1137101 “GG” genotype and “G”allele appear to be markers of decreased obesity susceptibility. This observation is relevant from a scientific standpoint; however, further large-scale studies will be needed to firmly establish the relationships between *LEPR* gene and obesity risk.

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Footnotes

Authors’ Contribution: Study concept and design, acquisition of data, analysis and interpretation of data: Touraj Mahmoudi; acquisition of data, statistical analysis, administrative, technical, and material support: Hamid Farahani; acquisition of data; drafting of the manuscript: Hossein Nobakht; acquisition of data; drafting of the manuscript: Reza Dabiri; critical revision of the manuscript for important intellectual content, study supervision: Mohammad Reza Zali

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