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NO ASSOCIATION OF THE *LEPTIN* (RS17151919) AND *LEPTIN RECEPTOR* (RS3790434) *GENE* POLYMORPHISMS WITH IRRITABLE BOWEL SYNDROME IN TURKISH POPULATION

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ABSTRACT

Background: Irritable bowel syndrome(IBS) is a gastrointestinal(GI) system disease that is characterized by altered bowel habits and abdominal pain in the absence of detectable structural abnormalities. Familial aggregation and twin studies suggest that there is a genetic contribution to the development of disease. Leptin and its receptors expressed in gastric mucosa and present in the GI system, especially in the proximal intestine, have complex effects on GI tract motility, nutrient absorption, and immune modulation of the GI system. **Material and methods:** *leptin* (rs17151919) and *leptin receptor* (rs3790434) polymorphisms were investigated in 159 patients with IBS and 104 healthy subjects in Turkish population using Real-time PCR. **Results:** Current findings display that there was no significant difference in leptin and leptin receptor genotype distribution or allele frequency between the patient and control groups. **Conclusion:** Our results indicate that leptin and leptin receptor gene polymorphisms may not be genetic risk factors for IBS development in a sample of Turkish population.

KEYWORDS: Leptin, rs17151919, Leptin Receptor, rs3790434, Polymorphisms, Irritable Bowel Syndrome.

1. INTRODUCTION

Irritable bowel syndrome (IBS), characterized by abdominal pain, swelling, and alterations in bowel movement, is one of the most common gastrointestinal (GI) diseases, which was defined by Powel in the 1940's but cases of patients with symptoms similar to IBS can be found in the literature prior to 1818. IBS affects up to 5–30% of the general population. Although the prevalence of IBS is greater in developed countries, the prevalence of this disease is increasing in the world. According to recent research, 60–75% of patients affected by IBS are women, indicating that women are more predisposed to IBS than men.[1–3]

Currently, there is no gold standard for defining IBS. The lack of standardized biomarkers and the fact that abnormalities cannot be detected by radiological or endoscopic tests renders IBS diagnosis difficult.[4,5] Therefore, current diagnosis is performed using a symptom-based strategy such as the Rome III criteria containing a combination of symptoms, of which abdominal pain and altered bowel movements are the most accepted criteria used in clinical practice. Considering the dominant symptoms, IBS can be categorized into four main groups according to the Rome III Criteria: IBS-D (diarrhea predominant), IBS-C (constipation predominant), IBS-M (mixed type), and IBS-U (unidentified type).[2,6]

The direct and indirect results of this syndrome have serious effects on the quality of life of individuals with IBS, who are also exposed to expensive diagnostic tests and treatment. Unfortunately, despite the high prevalence, the pathogenesis of this disease is not yet clearly understood. It is known that certain factors such as genetics, the environment, inflammatory and infective agents, and neurological and physiological issues cause abnormal gastrointestinal function and alterations in the brain–bowel axis; however, it is not clear which of these factors trigger IBS.[7] The possible roles of certain genes in the development of IBS suggest that many genetic factors may be involved in the etiology and clinical manifestation of IBS. To evaluate the effect of genetic factors on this disease, studies focusing on spontaneous

genetic variations and SNPs (single nucleotide polymorphisms) may provide novel insight into the mechanisms contributing to IBS.[3,8]

Leptin secreted by white adipocytes, first identified by Zhang in 1994, is the product of the *lep gene* (or *Ob-gene*; localized to chromosome 7) and is well described to regulate food intake and energy metabolism.[9,10] *Leptin*, which is primarily defined as a satiety factor, has been found in the hypothalamus, heart, placenta, lung, liver, muscle, kidney, thymus, spleen, prostate, testis, ovary, and intestine. This circulating protein also has a variety of endocrine functions and is involved in the modulation of immune and inflammatory responses, reproduction, haematopoiesis, bone formation, angiogenesis, and wound healing, suggesting that it not only regulates energy metabolism but also plays a regulatory role in many organs and systems.[11]

The stomach is the major source of leptin in the GI tract. Gastric endocrine and exocrine cells of the mucosa produce leptin; however, exocrine cells produce more.[12] Leptin and the soluble leptin receptors are secreted in the gastric mucosa and remain stable in the acidic stomach environment, crossing the duodenum as protein-bound and free forms.[13] Leptin has complex effects on the motility of the GI system; in the small intestine, leptin can cause excitatory and inhibitory effects on mechanoreceptors.[14,15]

Moreover, leptin has multifaceted effects on macronutrient absorption in physiological and pathological states. In the physiological state, luminal leptin produced by the gastric mucosa reduces the absorption of glucose by rapidly decreasing the expression and activity of sodium–glucose transporter-1, which is the major transporter of glucose in the preprandial state. However, in the postprandial state, leptin enhances the uptake of glucose, whereas systemic leptin has a slower indirect effect that is at least in part mediated by cholecystokinin-1 (CCK-1).[16,17] Leptin increases the absorption of small peptide products of protein digestion via an increase in mRNA production of di-/tripeptide transporter (PepT1) and protein expression in the brush border of intestinal cells found in the proximal small bowel lumen.[18] This effect of leptin is modulated by the ERK-1/2 cell signalling pathway.[19] Leptin may also participate in the uptake, intracellular metabolism, and transport of long-chain fatty acids.[20]

Another important function of leptin is to modulate inflammatory and anti-inflammatory responses in the GI system as an immune modulator. Leptin executes these functions by directly stimulating the expression and release of IL-1 and TNF-α by T cells, facilitating type 1 T-helper cells, and stimulating the expression and production of the IL-1 receptor antagonist in human monocytes.[21] This immune function is an indication that leptin is important during intestinal inflammation in patients with inflammatory bowel disease.[22]

Leptin performs its biological functions through leptin receptors that belong to the class I cytokine receptor family. Leptin receptors are abundant in the GI system, especially in the proximal part of the intestine. These receptors can be found in the luminal and basolateral borders of intestinal cells. Due to alternative mRNA splicing, there are least six leptin receptors that have different intracellular and extracellular domains. According to the length of their cytoplasmic domain, leptin receptors are classified into three categories consisting of the long form (OB-Rb), short form (OB-Ra, OB-Rc, OB-Rd, and OB-Rf), and soluble form (OB-Re). The binding of leptin to leptin receptors activates various signalling pathways, including JAK/STAT (Janus kinase/Signal transducer and activator of transcription) via OB-Rb, and MAPK (Mitogen-activated protein kinase), AMPK (5'-adenosine monophosphate-activated protein kinase), and PI3K (Phosphatidylinositol 3 kinase) via OB-Ra, c, d, and f, exerting biological functions such as cell proliferation, oxidation of fatty acids, and regulation of food intake and body mass.[23,24]

Considering the interaction of leptin with leptin receptors at the cellular level, it is thought that gene polymorphisms causing loss of function may contribute to the pathogenesis of many GI diseases such as cancers of the GI tract, inflammatory bowel diseases, and IBS. Therefore, the genes encoding leptin and leptin receptors may be candidates for involvement in IBS. In the present study, we aimed to evaluate whether there is any association of the *leptin (LEP)* (rs17151919) (G>A) missense variant SNP positioned on chr7:128254539 and the *leptin receptor (LEPR)* (rs3790434) (G>A) intronic variant SNP positioned on chr1:65420765 with IBS in the Turkish population.

2. MATERIALS AND METHODS

2.1. Participants

The present cross-sectional genetic predisposition study, which was performed in Mersin, situated in the eastern Mediterranean region of Turkey, included 159 IBS patients diagnosed and classified using the Rome III criteria as a case group, who were referred to the Department of Gastroenterology, Mersin University Faculty of Medicine Hospital between 2010 and 2012. The IBS group was not divided into subgroups (IBS-C, D, M), since there were not enough patients in each group for statistical analysis. The control group consisted of 104 healthy individuals (with no other GI or chronic diseases). Thus, a total of 263 individuals were recruited to the present study. All genetic analyses were performed by the Department of Medical Biology and Genetics, Mersin University Faculty of Medicine Hospital, Turkey. Approval of the study was received from the Ethics Committee of Mersin University Clinical Research. All patients or their caregivers were informed of the study and their consent to take part was obtained.

2.2. Isolation of Genomic DNA and Genotyping

Approximately 7–8 mL peripheral blood from case and control individuals was collected into tubes containing 2% EDTA (Sigma E-5134, Germany). DNA was extracted from whole blood using Miller's salting out procedure.[25] Each DNA sample was genotyped for *leptin (LEP)* (rs17151919) (G>A) and *leptin receptor (LEPR)* (rs3790434) (G>A) genes using a TaqMan SNP genotyping assay in the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The total real-time PCR was performed in 25 µL with 30 ng DNA, 12.5 µL 2X TaqMan Universal PCR Master Mix, 2.5 µL 900 nmol each primer *(LEP-F, LEP-R, LEPR-F, LEPR-R)*, 0.4 µL 200 nmol Yakima Yellow™ probe *(LEP-PrA, LEPR-PrA)*, 0.4 µL 200 nmol FAM-labelled probe *(LEP-PrG, LEPR-PrG)*, and 4.2 µL distilled water (Applied Biosystems, Foster City, CA, USA). Primers and probes were designed using the Primer Express 3.0 software (Applied Biosystems, Foster City, CA). For PCR amplification, the primers and fluorogenic probes for *LEP* (rs17151919) were: Forward 5'-ACTGGCAGTCTACCAACAGATCCT-3'; Reverse 5'-AGGTTCTCCAGGTCG-TTGGA-3'; 5'-YY™-TT(pdC)(pdC)AGAAA(pdC)ATGAT(pdC)AAA-BHQ-1-3', and 3'-FAM-T(pdC)(pdC)AGAAA(pdC)GTGAT(pdC)(pdC)AA-BHQ-1-3', respectively. The primers and fluorogenic probes for *LEPR* (rs3790434) were: Forward 5'-TTGACCGGAACGGAGGTG-3'; Reverse 5'-AGACATGGCGGGCGTTAA-3'; 5'-YY™-CA(pdC)A(pdC)GACAAG(pdC)GAG(pdC)C-BHQ-1-3'; and 3'-FAM-A(pdC)A(pdC)GACGAG(pdC)GAG(pdC)C-BHQ-1-3', respectively (underlined nucleotides in the probe sequences indicate SNPs and the substitution of C-5 with dC, which is an effective strategy for enhancing base pairing (pdC: propynyl-dC; YY: Yakima Yellow™). All probes are labelled with fluorogenic Yakima Yellow™ stain, which was assessed as VIC in the testing process, since they emit at the same wavelength. The reaction conditions consisted of a 2 min preincubation at 50°C, a 10 min activation at 95°C, and 40 cycles of 15 s at 95°C for denaturation and 1 min at 60°C for annealing and extension. The reactions were analyzed using the Sequence Detection Software (SDS) Version 2.0.3 for allelic discrimination (Applied Biosystems, Foster City, CA, USA). All reactions were performed in triplicate.

2.3. Statistical analysis

The Hardy–Weinberg equilibrium of groups regarding genotypes was evaluated. An independent sample *t*-test was used for the comparison of patient and control groups with respect to age. The relationships between disease and genotypes or alleles were separately evaluated by a chi-square test. The odds ratio was calculated for significant genotype and allele frequencies (95% confidence interval). Statistical analysis was performed using the Statistical Package for Social Sciences, version 11.5 (SPSS, Inc., Chicago, IL, USA). $p < 0.05$ is considered statistically significant.

3. RESULTS

The IBS patient group consisted of 117 women and 42 men (19 to 89 years old), with a mean age of 44.30 and the control group consisted of 46 women and 58 men whose ages ranged from 33 to 81 (mean age of 50.19). There was no statistical difference in terms of the mean age between patient and control groups (p = 0.001). The *LEP* (G>A, rs17151919) GG genotype frequencies was determined as 100% in patient and control groups. There was no G>A polymorphism in the *LEP* gene in both groups. The frequencies of GG, GA, and AA genotypes for the *LEPR* (rs3790434) gene were similar in patient and control groups and the frequencies of *LEPR* A allel in both group was higher than G allel. The genotypes of *LEP* and *LEPR* in the patient and control groups were in Hardy–Weinberg equilibrium. Real-time PCR multicomponent plots of genotype distributions in the *LEP* and *LEPR* gene are shown in figure 1 and 2, respectively. The genotype distribution between *LEP* (G>A, rs17151919) and *LEPR* (G>A, rs3790434) was similar in the patients with IBS and control groups. As a result, there was no association of *LEP* (G>A, rs17151919) and *LEPR* (G>A, rs3790434) polymorphisms with IBS ($p > 0.05$), as shown in table 1.

Table 1: The frequency of distribution of *LEP* and *LEPR* genotypes and alleles in IBS and control groups.

Gene			IBS n(%)	Control n(%)
LEP G>A (rs17151919)	Genotype	GG	159 (100)	104 (100)
		GA	-	-
		AA	-	-
	Allele	G	318(100)	208 (100)
		A	-	-
LEPR G>A (rs3790434)	Genotype	GG	37 (23.3)	22 (21.1)
		GA	73(45.9)	49 (47.1)
		AA	49 (30.8)	33 (31.8)
	Allele	G	147 (46.2)	93 (44.7)
		A	171 (53.8)	115 (55.3)

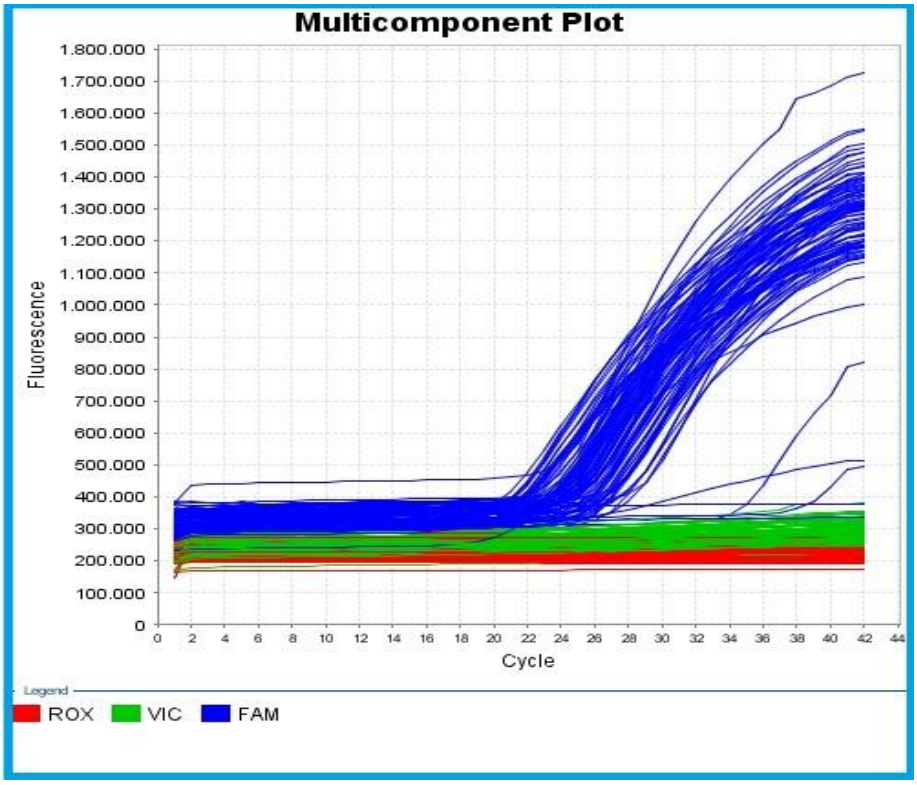

Figure 1: Multicomponent graphic of *LEP* gene Real-time PCR (Applied Biosystems, Foster City, CA, USA) analysis. (Green: Adenine labeled with Yakima Yellow™ (YY), Blue: Guanine labeled with FAM, Red: Reference stain ROX. (IBS and control groups)(Wave length of YY and VIC is similar).

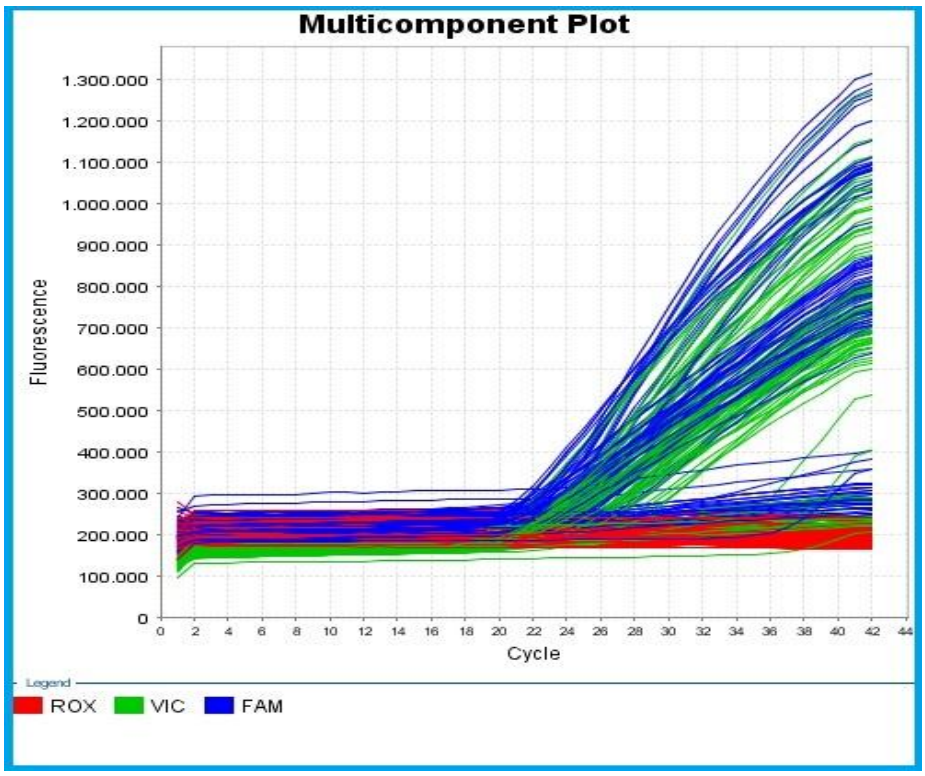

Figure 2: Multicomponent graphic of *LEPR* gene Real-time PCR (Applied Biosystems, Foster City, CA, USA) analysis. (Green: Adenine labeled with Yakima Yellow™ (YY), Blue: Guanine labeled with FAM, Red: Reference stain ROX. (IBS and control groups)(Wave length of YY and VIC is similar).

4. DISCUSSION

IBS is a member of the common gastrointestinal disorders and is characterized by abdominal pain or discomfort in combination with altered bowel habits. [26] A diagnosis of IBS can be established by the use of symptom-based criteria, the exclusion of concerning features, and the judicious use of diagnostic testing; [27] however, the elucidation of a novel diagnostic molecular biomarker is of great interest.

In developed countries, IBS symptoms are 1.5- to 2-times more prevalent among women than men, which is accordance with the present cross-sectional study, in which IBS prevalence was significantly greater (2.7:1) among women than men. This finding is also consistent with research published by Celebi et al.[28] indicating that the prevalence of IBS in Elazığ, situated in the eastern region of Turkey, was far more common among women. To the best of our knowledge, this is the first study to indicate the prevalence of this disease in Mersin, situated in the Mediterranean region of Turkey.

The discovery of both the *obese (Ob)* gene product, leptin, and leptin receptors in the digestive system suggests that leptin may play a role in the GI system, influencing intestinal function. Since leptin is released at the endocrine level after fasting or eating after fasting and large amounts of leptin receptors expressed in the proximal intestine have a regulatory function in nutrition and weight gain, this suggests that changes in leptin and leptin receptor activity may cause many GI diseases, such as IBS. Moreover, leptin is known to be involved in the etiology of inflammatory bowel diseases such as Crohn's disease and ulcerative colitis due to its immune functions.[29]

When any of the important host factors contributing to IBS symptoms are increased, it leads to intestinal permeability, gut mucosa immune activation, visceral hypersensitivity, dysbiosis, and unexplained weight loss.[27] Leptin, when bound to its soluble leptin receptor, decreases the phosphorylation of signal transducer and activator of transcription-3 (STAT-3) and blocks the regulatory effects of leptin on weight, food intake, hepatic glucose production, and gonadotropin secretion . STAT-5 is another downstream signalling pathway activated by leptin and leptin receptors. The increase in the absorption of peptides, the control of lipogenesis, the induction of arterial intima formation, and the pro inflammatory effects of leptin in the colon and liver have been suggested to be mediated by this pathway. On the other hand, leptin exerts complex effects on the motility of the GI system, since the afferent and efferent vagus nerve endings contain leptin receptors; in the small intestine, leptin can cause excitatory and inhibitory effects on mechanoreceptors. Moreover, it is known that leptin deficiency increases transit activity in the jejunum and shortens the total transit time in the small intestine.[11,30] The effect of leptin on nutrient absorption under physiological and pathological conditions is another important function. In the GI system, as a trophic factor, leptin has been shown to stimulate the development of intestinal mucosal morphometry, proliferation of intestinal mucosal epithelial cells, and enzymatic activity in the brush border of enterocytes in nutrient absorption. At the same time, leptin alters the innate and adaptive systemic immune responses and may participate systemically, or even locally, in diseases of the intestine and colon. Considering all these functions, leptin plays a key role in the etiology of diseases such as diabetes, hypertension, abnormal thyroid function, atherosclerosis, and GI diseases including IBS.[23]

Accordingly, we aimed to investigate the possible role of *LEP* (rs17151919) and *LEPR* (rs3790434) gene polymorphisms in the pathophysiology of IBS. The rs17151919 *leptin* polymorphism has been studied in relation to certain diseases such as obesity, cancer, hypertension, and cardiovascular disease. To the best of our knowledge, no previous studies have explored the role of *leptin* gene polymorphisms in IBS; thus, this is the first study regarding a possible relationship between *leptin* gene variants and IBS. Our data shows that there was no association of *LEP* (rs17151919) and *LEPR* (rs3790434) with IBS in the Turkish population. This polymorphisms has been shown to be associated with another diseases. In a Cleveland et al's study,[31] it was reported that this polymorphism increases the development of breast cancer, and Hansel et al.[32] also showed that this polymorphism is associated with chronic obstructive pulmonary disease. Li et al.[33] reported that there is no relationship between systemic lupus erythematosus (SLE) and the rs3790434 *leptin receptor* gene polymorphism in a Chinese population. Nevertheless, the association of *leptin* and *leptin receptor* genotypes with IBS has not previously been explored; therefore, we could not compare our results with others. More than 60 candidate genes have been studied in IBS. Current SNP studies may provide a novel insight into the ethiopathogenesis of IBS. The best-studied polymorphisms related to IBS are 5-hydroxytryptamine receptors (5-HT). In addition to 5-HT polymorphisms, certain studies have found an association of polymorphisms in the α2 adrenergic receptor, cytokine, and G-protein genes with IBS. These genetic polymorphisms in cytokines in the GI affect visceral hypersensitivity and motility.[11] The limitations of the present study were a lack of patient clinical features (weight, complaints, and genetic history) and no investigation of serum leptin levels; however, since the polymorphisms we investigated are not acquired, our results can contribute to the understanding of IBS independently of other clinical features.

5. CONCLUSION

IBS has been an elusive and controversial enigma for many years, with numerous theories regarding the etiology of related altered bowel habits. Moreover, since these genetic polymorphisms have been studied by other scientists, multivariate analysis would have contributed

to the elucidation of the relationship between various genetic factors and the independent effect of each gene. Furthermore, since IBS is a multifactorial polygenic disorder, in addition to genetic factors, the possible effect of environmental factors should be considered. It should not be forgotten that genetic distribution and allele frequencies of these polymorphisms vary in different geographical areas. Due to the small sample size, an independent study involving a large population should be performed to confirm the results of the present study. Finally, research regarding other polymorphisms of the leptin and leptin receptor genes and their expression levels will be useful for understanding the molecular ethiopathogenesis of IBS.

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Conflict of interest

The authors confirm that there are no conflict of interest.

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