

Alpha-2-adrenergic receptor gene polymorphism in Turkish population with irritable bowel syndrome

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Background/aims: Irritable bowel syndrome is a multifactorial functional gastrointestinal disorder affecting more than 10% of world population. Genetic component in pathophysiology of irritable bowel syndrome is still unknown. The aim of this study was to examine the potential impact of C-1291G polymorphism in α 2-adrenergic receptor gene promoter region in the etiology of the disease. **Materials and Method:** This prospective case-control study included 100 irritable bowel syndrome patients and 100 healthy controls adjusted for sex and age. The subjects were genotyped by using polymerase chain reaction amplification of the promoter region of α 2-adrenergic receptor gene. Allele and genotype frequencies were compared in patient and control groups. The study was approved by the University ethics committee. **Results:** The frequency of C allele was 72% and 75%, G allele was 28% and 25% in patient and control groups, respectively ($p>0.05$). We found that the frequencies of C1291C, C1291G, and G1291G genotypes were 50, 44, and 6%, respectively, in the patient group and 51, 48, and 1%, respectively, in the control group ($p>0.05$). The subgroup analysis of patients revealed that 70 patients were constipation-predominant, 27 patients were alternating diarrhea and constipation, and 3 patients were diarrhea-predominant irritable bowel syndrome. **Conclusion:** No significant association was observed between α 2-adrenergic receptor gene C-1291G polymorphism and irritable bowel syndrome in Turkish population. The high number of constipation predominant irritable bowel syndrome and very low number of diarrhea predominant irritable bowel syndrome patients might be the reason for statistical non-significance since α 2-adrenergic receptor gene is found to be responsible for mediating intestinal antisecretory action and probably is involved in the pathogenesis of diarrhea predominant irritable bowel syndrome. Further investigations are needed.

Key words: Alpha-2-adrenergic receptor, irritable bowel syndrome, genetic polymorphism, Rome III criteria

İrritabl barsak sendrom tanılı Türk hastalarda alfa-2 adrenerjik reseptör gen polimorfizmi

Giriş ve Amaç: İrritabl barsak sendromu multifaktöryel fonksiyonel bir gastrointestinal sistem hastalığı olup dünya popülasyonunun %10'dan fazlasını etkilemektedir. İrritabl barsak sendromu patofizyolojisinde genetiğin rolü kesinleşmemiş bir konudur. Bu çalışmanın amacı, alfa-2 adrenerjik reseptör geni promoter bölgesinde yer alan C-1291G polimorfizminin hastalık etiolojisindeki yerini araştırmaktır. **Gereç ve Yöntem:** Bu prospektif vaka-kontrol çalışmasına Roma III kriterlerine göre seçilmiş 100 irritabl barsak sendromu hastası ve yaş ve cinsiyet uyumlu 100 sağlıklı kontrol dahil edilmiştir. Alfa-2 adrenerjik reseptör geni promoter bölgesi polimerize zincir reaksiyon amplifikasyon yöntemi ile genotiplendirilmiştir. Hasta ve kontrol grubunun hem allel, hem de genotip frekansları karşılaştırılmıştır. Çalışma öncesi Üniversite Etik Kurul onayı alınmıştır. **Bulgular:** Hasta ve kontrol gruplarında C allel frekansı sırası ile %72 ve %75, G allel frekansı %28 ve %25 olarak bulundu ($p>0.05$). Hasta grubunda C1291C, C1291G ve G1291G genotipleri sırası ile %51, 48 ve %1 olarak saptanırken, kontrol grubunda %50, 44 ve %6 idi ($p>0.05$). İrritabl barsak sendromu hastalarının alt grup analizi yapıldığında 70 hastanın kabızlık predominant irritabl barsak sendromu, 27 hastanın alterne form irritabl barsak sendromu ve 3 hastanın ishal predominant irritabl barsak sendromu olduğu görüldü. **Sonuç:** Sonuçlarımız, Türk popülasyonunda alfa-2 adrenerjik reseptör geni C-1291G polimorfizmi ile irritabl barsak sendromu arasında anlamlı ilişki olmadığının göstermektedir. Çalışmamızdaki kabızlık predominant irritabl barsak sendromlu hasta sayısının oldukça fazla ve ishal predominant irritabl barsak sendromlu hasta sayısının çok az olması, çalışmanın sonucunu etkilemiş olabilir, çünkü alfa-2 adrenerjik reseptör geni barsakta antisekretuar etki göstermekte ve muhtemelen ishal predominant irritabl barsak sendromu'nun patogenezinde etkili olmaktadır. Bu konuda yeni çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Alfa2 adrenerjik reseptör, irritabl barsak sendromu, gen polimorfizmi, Roma III kriterleri

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common multifactorial gastrointestinal disorder characterized by recurrent abdominal pain and altered bowel habits. It affects about 15% of world population. The clinical symptoms include recurrent abdominal pain, discomfort, bloating, and altered bowel habits such as diarrhea, constipation, and alteration between diarrhea and constipation (1). The etiology of IBS remains to be unclear, but some pathophysiological mechanisms have been proposed in IBS, including altered gut motility and visceral sensitivity as well as impaired barrier function, dysregulated interactions with intraluminal bacteria and alterations in local host immune responses (2).

The familial aggregation studies and twin studies suggested a heritable component of IBS, which played an important role in beginning of genetic investigations. The results of these investigations are candidate gene studies in which it is postulated that a specific gene, and typically a specific functional gene polymorphism that results in alterations in protein function or quantity, may play a role in disease pathophysiology (3). These gene polymorphic studies mostly investigate the possible associations with the clinical phenotype of IBS. The best studied polymorphisms are those related to serotonin, adrenergic system, cytokines, and recently, post-infectious IBS (4).

Today, it is clear that $\alpha 2$ -adrenergic receptors ($\alpha 2$ -AR) play a central role in the regulation of systemic sympathetic activity. $\alpha 2$ -AR are cell-surface receptors for catecholamines and are expressed in both central and peripheral nervous systems (5). Gastrointestinal system is extensively innervated by extrinsic noradrenergic neurons and that endogenous catecholamines modulate a variety of digestive functions through their interaction with $\alpha 2$ -AR (6). For example, $\alpha 2$ -AR activation in the stomach results in a reduction of acid secretion, motility, and emptying, whereas at intestinal level, $\alpha 2$ -AR mediate inhibitory effects on mucosal fluid secretion, colonic propulsion, and fecal excretion. Due to the variety of effects mediated by $\alpha 2$ -AR, these receptors may represent therapeutic targets for the development of drugs suitable for treatment of some digestive diseases.

Although adrenergic system plays an important role in regulation of gastrointestinal functions, surprisingly genetic contribution of this system has been evaluated only in a few studies. In recent

years, the genetic component in the pathophysiology of IBS has been investigated in different studies with conflicting results, probably due to racial and ethnic differences. The aim of this study was to examine the role of the gray area in the pathophysiology of IBS and the potential impact of C-1291G polymorphism in $\alpha 2$ -adrenergic receptor gene (ADRA2A) promoter region in the etiology of the disease.

MATERIALS and METHODS

Patients

The study included one hundred patients with IBS (16 men and 84 women, with a mean age of 45.80 years) who were referred to Gastroenterology clinic at Dokuz Eylul University Hospital from January to June 2011 and 100 healthy controls adjusted for sex and age. The diagnosis of IBS was made according to the Rome III criteria (7). The study was approved by the Ethics Committee of Dokuz Eylul University. Written informed consent was obtained from all subjects before sampling.

Genetic Analysis

Genomic DNA from peripheral blood cells was extracted using the Accuprep Genomic DNA Extraction Kit according to the manufacturer's instructions (Bioneer, South Korea). Polymerase chain reaction (PCR) was carried out in 50 μ L of solution containing 100 ng of genomic DNA, 0.1 μ mol/L of each primer, 100 μ mol/L of each dNTP, 20 mmol/L of TrisHCl (pH 8.5), 50 mmol/L of KCl, 3 mmol/L of MgCl₂, and 1.0 U of Taq polymerase (Nanohelix) using following pair of primers Forward: 5'-TCA CAC CGG AGG TTA CTT CCC TCG-3' and Reverse 5'-TCC GAC GAC AGC GCG AGT T-3'. The amplification was performed on thermocycler (Perkin Elmer 9700), with a predenaturing procedure for 3 minutes at 94°C for 35 cycles (denaturing at 94°C for 1 minute, annealing at 63°C for 1 minute, and extension at 72°C for 1 minute), followed by an additional 7-minute incubation at 72°C. PCR products were purified using PureHelix™ PCR Purification Kit according to the manufacturer's instructions (Nanohelix, South Korea) and subjected to automatic sequence analysis (Automated sequencer ABI 3130; Applied Biosystems, CA 94404, USA) by Big Dye terminator reaction according to the supplier's instructions (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits Version 3.1; Applied Biosystems, CA 94404, USA) using Forward: 5'-TCACACCGGAGGTTACTTCCCTCG-3' primer. The obtained sequences for C-1291G polymorphism

were analyzed using BioEdit software, version 7.0.5.3. (8-9).

Statistics

The results were expressed as mean $\pm$ SD and as number. The differences in alleles and genotype frequencies between patients and controls were analyzed using the χ^2 test (or the Fisher's exact test). C/G and G/G genotype frequencies were collapsed into one group to form 2x2 tables of C/C and non-C individuals. Statistical analysis was performed using SPSS for Windows version 15.0. In all procedures, $p < 0.05$ was considered the level of significance.

RESULTS

Characterization of Patients

A total of 100 patients with a diagnosis of IBS based on the Rome III criteria and 100 healthy patients were enrolled in this study. The mean age of the patient and control groups were 45.80 and 48.98 years, respectively ($p = 0.091$). IBS patients were classified into constipation-predominant IBS

(C-IBS) ($n = 70$), diarrhea-predominant IBS (D-IBS) ($n = 3$), and alternating diarrhea and constipation IBS (A-IBS) ($n = 27$). Control and patient groups were similar with respect to age and sex (Table 1).

Alleles Frequencies

The frequencies of the C alleles in patient and control groups were 75 and 72%, whereas the G allele frequencies were 25 and 28%, respectively. The results were not statistically different between the patient and control groups ($\chi^2: 0.462$; $p = 0.497$) (Table 2).

Genotype Frequencies

The frequencies of C1291C, C1291G, and G1291G genotypes were 50, 44 and 6%, respectively, in the patient group and 51, 48 and 1%, respectively, in the control group. We did not find a significant difference in $\alpha 2A$ -1291 C > G genotype between total IBS and control groups ($\chi^2: 0.20$, $p = 0.888$). The analysis between IBS subtypes groups and controls also did not reveal a significant association ($p > 0.05$). We could not also find a significant difference in the wild and non-wild type gene status between the IBS groups and controls (Table 2).

DISCUSSION

The presence of $\alpha 2$ -AR in the digestive system has been observed in 1969 when it was demonstrated that prejunctional adrenergic receptors are localized on cholinergic axon terminals of guinea-pig ileum myenteric plexus which were able to mediate the inhibitory control of noradrenaline on acetylcholine release (10). These observations lead to a large number of investigations about the regulatory actions of $\alpha 2$ -AR throughout the digestive tract. In this case-control study, we evaluated the

Table 1. Basal characteristics of the patients and controls.

	IBS (n:100)	Control (n:100)
Age	45,8 $\pm$ 11,6	48,9 $\pm$ 14,6
Male	16 (16%)	16 (16%)
Female	84 (84%)	84 (84%)
Duration of disease	12,28 $\pm$ 11,7	(-)
C-IBS	70 (70%)	(-)
A-IBS	27 (27%)	(-)
D-IBS	3 (3%)	(-)

C-IBS: Constipation-predominant IBS.
A-IBS: Alternating diarrhea and constipation.
D-IBS: Diarrhea-predominant IBS.

Table 2. Distribution of genotype and allele frequency of $\alpha 2A$ -1291 C > G polymorphism in IBS patients and controls.

Genotype	IBS n=100 (%)	C-IBS n=70 (%)	A-IBS n=27 (%)	D-IBS n=3 (%)	Controls n=100 (%)
Wild type C/C	50 (50%)	32 (45.7%)	18 (66.7%)	0	51 (51%)
Heterozygous C/G	44 (44%)	33 (47.1%)	8 (29.6%)	3 (100%)	48 (48%)
Homozygous polymorphism G/G	6 (6%)	5 (7.1%)	1 (3.7%)	0	1 (1%)
Allele frequency					
C Allele	144 (72%)	97 (63.3%)	44 (81.5)	3 (50%)	150 (75%)
G Allele	56 (28%)	43 (30.7%)	10 (18.5%)	3 (50%)	50 (25%)

No significant difference was observed in $\alpha 2A$ -1291 C > G in any of the groups.

C-IBS: Constipation-predominant IBS.
A-IBS: Alternating diarrhea and constipation.
D-IBS: Diarrhea-predominant IBS.

possible role of C-1291G polymorphism in $\alpha 2$ -adrenergic receptor gene (ADRA2A) promoter region in the pathophysiology of IBS and found that this polymorphism was not different in patient and control groups.

$\alpha 2$ -AR is coded by ADRA2A, which is a small, intronless gene mapped to 10q24-26, with a single nucleotide polymorphism (1291 C>G) on its promoter region (11). In the literature, there are many studies about the relation of ADRA2A C1291G polymorphism with some pathological conditions such as obesity, tobacco smoking, and some psychiatric conditions such as schizophrenia.

The relation between ADRA2A C1291G polymorphism and IBS has been studied in several studies. Sikander investigated the C1291G polymorphism in clinical subtypes of IBS, ulcerative and microscopic colitis patients and showed a significant association between the ADRA2A C1291G polymorphism and D-IBS (12), whereas Kim found an association between this polymorphism and C-IBS (13). In our study, we evaluated the possible effects of ADRA2A C1291G polymorphism in different phenotypes of IBS patients in Turkish population and, to the best of our knowledge, this is the first study in this area. Our data showed that C-1291G polymorphism in ADRA2A was not different in the patient and control groups, so there was no significant association between ADRA2A C-1291G polymorphism and IBS in Turkish population. Our results were similar to those of Camilleri, that he could not detect any relation between $\alpha 2A$ genotype and subtypes of IBS (14). The high number of C-IBS and very low number of D-IBS patients might be the reason for statistical non-significance since ADRA2A is found to be responsible for mediating intestinal antisecretory action and probably is involved in pathogenesis of D-IBS (15-19). Liu and Coupar showed that administration of selective $\alpha 2$ -AR agonist increased the rate of basal fluid absorption in the small intestine, whereas $\alpha 2$ -AR antagonist decreased the basal absorption rate (17). From that point, it can be concluded that any change occurring in ADRA2A can lead to a decrease in fluid and electrolyte absorption and can predispose to diarrhea. It was also shown by Camilleri that clonidine, which is an ADRA2A agonist, causes the relief of symptoms in diarrhea-dominant IBS patients without any change in gastrointestinal transit time (18).

In the literature, there are also some studies about the differences in autonomic functions among

IBS subtypes that can be important in understanding the effects of ADRA2A in D-IBS. Aggarwal found the vagal dysfunction of IBS patients to be particularly associated with constipation-predominant symptoms, whereas they found adrenergic sympathetic dysfunction to be associated especially with diarrhea-predominant symptoms (20). Elsenbruch reported that D-IBS patients differed from C-IBS patients that D-IBS patients were shown to have autonomic and cortisol hyper-responsiveness unlike that of C-IBS patients and controls (21). Robert observed elevated sympathetic dominance and vagal withdrawal during non-REM and REM sleep in D-IBS but not in A-IBS (22). Nevertheless, although these studies support the effect of sympathetic nervous system in D-IBS, IBS is a multifactorial disease and detailed mechanism of the disease still remains to be further evaluated.

The small sample sizes of D-IBS and A-IBS is the drawback of our study. In the literature, genetic polymorphism studies about the D-IBS are also limited and do not support each other, since they study different types of polymorphism. In a study of Pata (23), D-IBS was associated with SERT-P1/s genotype, whereas Park (24) found that the s/s genotype is an important factor in patients with D-IBS. High producer tumour necrosis factor alpha (TNF- α) and low producer IL-10 genotype were significantly associated with D-IBS compared with other IBS subgroups (25) but this result was not supported with other studies (26). B3-adrenoceptor, cholinergic receptor muscarinic 3 polymorphisms, and tryptophan hydroxylase gene polymorphisms were also studied in IBS patients (27,28). Very recent studies about D-IBS patients address the genes regulating hepatic bile acid synthesis but more studies are needed to state an association (29,30).

Today, genetic polymorphism studies have become the major area in defining the etiology of IBS. Serotonergic system, inflammation, microflora, and intestinal barrier systems are the other areas of genetic investigations. However, it should be remembered that the allele frequencies and genetic distributions of these polymorphisms differ in different geographic areas. Several studies have shown that the frequency of 1291G allele is high in Japanese (31), Taiwan (32), Swedish (33), and Chinese (34) population (72, 70, 77, and 70%, respectively), whereas it is only 1% in Germans (35). This broad spectrum of allele frequencies shows the importance of ethnic control over the genetic differences.

In conclusion, our data showed that there was no significant association between ADRA2A C-1291G polymorphism and IBS in Turkish population. Since most of our patients were constipation-predominant type, we think that ADRA2A C-1291G polymorphism is not an important factor in the

pathogenesis of C-IBS, but also the very low number of D-IBS patients might be the reason for statistical non-significance. Further investigations with a higher number of D-IBS patients might be useful in identifying the genetic contribution of adrenergic system to the pathogenesis of IBS.

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