

## Comparison of Beta-2 Adrenergic Receptor Gene Polymorphisms Between Patients with Fibromyalgia Syndrome and Healthy Controls

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### ABSTRACT

**Objectives:** This study aims to compare the beta-2 adrenergic receptor (ADRB2) gene polymorphisms of patients with fibromyalgia syndrome (FMS) with those of healthy control subjects, and to investigate the possible relationship between symptoms of FMS and polymorphisms of the ADRB2 gene.

**Patients and methods:** The study included 170 females (mean age 47.8±10.3 years; range, 21 to 75 years) diagnosed with FMS according to the 2010 American College of Rheumatology criteria and 170 healthy females (mean age 47.2±8.8 years; range, 20 to 72 years) as the control group. Several clinical symptoms of the participants related to FMS were questioned and recorded. The visual analog scale (VAS) and Fibromyalgia Impact Questionnaire (FIQ) scores of the fibromyalgia group were recorded. In both groups, the ADRB2 (rs1042717) single-nucleotide polymorphism was detected by way of a real-time polymerase chain reaction. The wild-type (Guanine/Guanine), the mutant type (Adenine/Adenine) and heterozygous type (Adenine/Guanine) were detected. The sample power was calculated considering the minor allele frequency.

**Results:** The comparison of the ADRB2 gene polymorphism between patients with FMS and the control subjects showed that the groups were similar in terms of ADRB2 gene polymorphism and genotype ( $p>0.05$ ). There was no significant difference in terms of genotype when the ADRB2 gene polymorphisms in patients with FMS were compared in terms of clinical symptoms, VAS and FIQ scores ( $p>0.05$ ).

**Conclusion:** Beta-2 adrenergic receptor (rs1042717) gene polymorphisms and genotype distribution are no different between patients with FMS and healthy individuals. ADRB2 gene polymorphisms in patients with FMS have no effect on clinical symptoms and VAS and FIQ scores. The results of the present study will light the way for future research into ADRB2 gene polymorphisms in the pathogenesis of FMS.

**Keywords:** Beta-2 adrenergic receptor, fibromyalgia syndrome, polymorphism.

Fibromyalgia syndrome (FMS) is a clinical condition characterized by widespread pain, fatigue, sleep disturbance and cognitive dysfunction.<sup>1</sup> Genetic, environmental and immunological factors, as well as peripheral and central mechanisms, are considered to play a role in its etiopathogenesis.<sup>2,3</sup>

Previous clinical studies into the pathogenesis of FMS have identified disturbances in the sympathetic and parasympathetic systems and associated dysfunctions in the autonomic nervous system. These studies have also shown that a number of clinical findings in FMS could be

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associated with autonomic dysfunction.<sup>4-7</sup> That said, various other studies have provided evidence that gene polymorphisms in serotonergic and catecholaminergic systems, along with strong familial predisposition, could play a role in the etiopathogenesis of FMS.<sup>8-10</sup>

Catecholamines are sympathetic neurotransmitters that exert their effects by binding to adrenergic receptors (ARs). Catechol-O-methyltransferase (COMT) is the main enzyme involved in the breakdown of catecholamines, and it has been reported previously that COMT plays a role in pain modulation.<sup>5</sup> Another study has shown that the polymorphism of the gene that encodes the COMT enzyme may also be involved in the pathogenesis of FMS.<sup>11-13</sup> The fact that catecholamines exert their effects by binding to ARs, and the demonstration of increased pain sensitivity through the activation of ARs due to low COMT activity in these studies have prompted further research into AR gene polymorphisms in various diseases.

Polymorphisms are defined as nucleotide changes that do not cause, but may predispose to a disease condition.<sup>14</sup> Polymorphic changes can occur in various ways, with single-nucleotide polymorphisms (SNPs) being the most common.<sup>15</sup> AR gene polymorphisms substantially affect the sensitivity and functions of ARs. AR polymorphisms have been shown to be associated with various disorders, such as cardiovascular diseases, asthma, obesity and chronic pain.<sup>16-18</sup>

Dysfunctional ARs have been implicated in the pathogenesis of chronic painful conditions.<sup>19</sup> Adrenergic receptors are divided into two main groups, as alpha and beta. Beta-adrenergic receptors (ADRBs) are important components of the sympathetic nervous system.<sup>20</sup> The haplotypes made up of the gene variants of beta-2 adrenergic receptors (ADRB2) have been shown to be associated with chronic pain by affecting receptor expression levels and ligand binding.<sup>21</sup>

The authors therefore hypothesized that the ADRB2 gene polymorphisms that were shown to be associated with chronic pain may also be involved in the pathogenesis of FMS. There is a paucity of studies in the literature evaluating the relationship between FMS and ADRB2 gene polymorphisms,<sup>22,23</sup> and the relevant studies in the literature have yet to make a clear identification of

the relationship between the two. To the best of our knowledge, ADRB2 gene polymorphisms (rs1042717 Adenine/Guanine [AG]) are yet to be studied in FMS. Therefore, in this study, we aimed to compare the ADRB2 gene polymorphisms (rs1042717G/A) of patients with FMS with those of healthy control subjects, and to investigate the possible relationship between symptoms of FMS and polymorphisms of the ADRB2 gene.

## PATIENTS AND METHODS

The study was conducted at the Physical Medicine and Rehabilitation Clinic of Sivas Cumhuriyet University Medical Faculty between March 2017 and March 2018 and included 170 female patients (mean age 47.8±10.3 years; range, 21 to 75 years) who were newly diagnosed with FMS according to the 2010 American College of Rheumatology<sup>24</sup> criteria, and 170 female healthy control subjects (mean age 47.2±8.8 years; range, 20 to 72 years). The entire study group was composed of female patients and control subjects so as to eliminate possible differences in genetic and clinical parameters caused by sex, and to ensure homogeneous study groups. Patients with malignancy, inflammatory rheumatic disease (rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, etc.) and those with a known history of systemic disease (hypertension, diabetes mellitus, neurological or psychiatric disease, etc.) were excluded. The control group consisted of female respondents with no systemic, genetic, hormonal or psychiatric diseases, including FMS and regional pain syndrome. The study protocol was approved by the Cumhuriyet University Clinical Research Ethics Committee (Approval #: 2017-02/25, dated: 28.02.2017). A written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Clinical symptoms (sleep disturbances, morning fatigue, paresthesia, and feeling of swelling in soft tissues) and family history of FMS were inquired and recorded. The visual analog scale (VAS) and Fibromyalgia Impact Questionnaire (FIQ) were administered to the patients with FMS, and their scores were recorded.

Venous blood samples of all participants were collected into sterile citrate tubes, and genomic deoxyribonucleic acid (DNA) was isolated from the collected blood samples. Following DNA isolation, the blood samples in the citrate tubes were stored at  $-20^{\circ}\text{C}$ .

A 20-item FIQ was used to assess the patients' physical function, occupational status, depression, anxiety, sleep, pain, rigidity, fatigue and healthiness as a means of evaluating the functional status of the patients and the progression and outcomes of the disease.<sup>25</sup> The validity and reliability of the study were demonstrated in our country by Sarmer et al.<sup>26</sup>

The VAS was used to examine the pain levels of the patients. On a 10-cm scale on which 0 indicates no pain and 10 indicates the most severe pain, the patients were asked to mark the point that best corresponds to the level of pain they experience.

The blood samples of patients with FMS and of the control subjects were collected into 4-mL sodium citrate tubes. Genomic DNA was isolated via the DNA isolation method using a high salt concentration.<sup>27</sup>

A Nanodrop Spectrophotometer (ND-1000 NanoDrop Technologies Inc. Wilmington, DE, USA) was used to quantify and determine the quality of the DNA. The concentration of DNA samples was expressed as  $\text{ng}/\mu\text{L}$ . After the quantification of the DNA samples via the Nanodrop spectrophotometer, the best results were observed at a concentration of  $5 \text{ ng}/\mu\text{L}$  during optimization studies in DNA samples for ADRB2 (rs1042717), and the concentration was set to this value.

The detection of the Rs1042717 SNP was performed by real-time polymerase chain reaction using FAM-VIC-labeled primers. The wild-type FAM probe (Guanine/Guanine [GG]) generates green fluorescence and the mutant VIC probe (Adenine/Adenine [AA]) generates yellow fluorescence, while heterozygous (AG) samples generate both yellow and green fluorescence. We also tested for contamination of the disposed materials or experimental environment for control number 4 (Figure 1).

CCTGTGCTGATCTGGTCATGGGCCT[A/G]  
GCAGTGGTGCCCTTTGGGGCCGCC

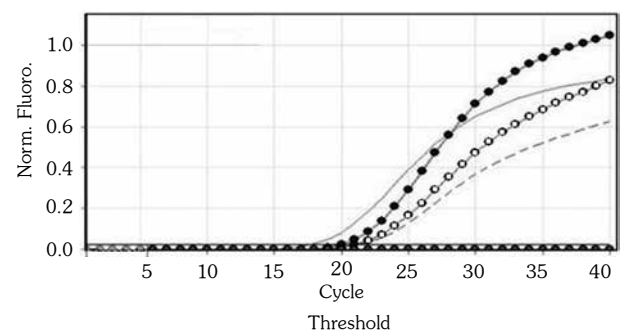
Chromosome: 5q32, Gene: ADRB2, Region: Intron, Base length: 1242 bp

### Statistical analysis

Analyses were performed using the IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA) software package. The normal distribution of the data was analyzed using Kolmogorov-Smirnov tests. Student's t test was applied when the parametric test assumptions were met. A Mann-Whitney U test was used when the parametric test assumptions could not be met, and a chi-square test was used to evaluate the categorical data. The data were expressed in tables in number, percentage and mean  $\pm$  standard deviation forms, and a  $p$  value of  $<0.05$  was considered statistically significant. The Chi square test was used to calculate Hardy-Weinberg equilibrium. The power of the study according to minor allele frequency<sup>28</sup> was found to be  $p=0.8109$  when the  $p1=0.98$ ,  $p^2=0.91$ ,  $\alpha=0.05$ , and  $\beta=0.20$ .

## RESULTS

Clinical symptoms of FMS patients are shown in Table 1. A comparison of the ADRB2 gene polymorphism (rs1042717) between patients with FMS and the control subjects showed that the



No	Colour	Name	Genotype	Cycling A. Green	Cycling A. Yellow
1	○	FMS 1	Heterozygous	Reaction	Reaction
2	●	FMS 2	Mutant	No reaction	Reaction
3	—	FMS 3	Wild type	Reaction	No reaction
4	▲	Control		No reaction	No reaction

**Figure 1.** Identification of beta-2 adrenergic receptor polymorphism (rs1042717) genotypes  
FMS: Fibromyalgia syndrome.

**Table 1.** Clinical symptoms of patients with fibromyalgia syndrome (n=170)

	n	%
Sleep disturbances	138	81.2
Fatigue	163	95.9
Paresthesia	127	74.7
Swelling of soft tissues	130	76.5
Family history of fibromyalgia	80	47.1

groups were similar in terms of rs1042717 gen polymorphism and genotype ( $p>0.05$ ) (Table 2). There was no significant difference in terms of genotype when the genotypes (GG, AG, AA) of the ADRB2 gene polymorphism (rs1042717) in patients with FMS were compared in terms of clinical symptoms and VAS and FIQ scores ( $p>0.05$ ) (Table 3).

## DISCUSSION

The present study has demonstrated that ADRB2 gene polymorphisms (rs1042717) and

the different genotypes of the ADRB2 gene polymorphism in patients with FMS are no different from those of the healthy control subjects. Furthermore, the clinical symptoms of FMS and VAS and FIQ scores were found to be similar in patients with FMS with different genotypes of the ADRB2 gene polymorphism (rs1042717). To the best of our knowledge, this is the first study to report these findings in Turkish patients with FMS.

Fibromyalgia syndrome is a significant health problem that restricts the daily life activities of its sufferers. There are various clinical symptoms in patients with FMS, although chronic pain is one of the most common. There is evidence suggesting that genetic polymorphisms, together with psychological and physiological components, contribute to the development of chronic pain.<sup>29,30</sup> Pain is affected by the ARs of the autonomic nervous system. Increasing levels of catecholamines result in the occurrence of persistent pain through stimulation of the ADRBs in the peripheral and central nervous system. For this reason,  $\beta$ -adrenergic antagonist propranolol has been suggested for the treatment

**Table 2.** Comparison of fibromyalgia syndrome and control groups in terms of beta-2 adrenergic receptor polymorphisms (rs1042717)

Rs1042717	Fibromyalgia group (n=170)		Control group (n=170)		p
	n	%	n	%	
Guanine/Guanine	25	14.7	15	8.8	0.174 $\chi^2=3.49$
Adenine/Guanine	123	72.4	126	74.1	
Adenine/Adenine	22	12.9	29	17.1	

**Table 3.** Comparison of clinical symptoms, Fibromyalgia Impact Questionnaire and visual analog scale scores among different genotypes of beta-2 adrenergic receptor gene polymorphism (rs1042717) in patients with fibromyalgia syndrome

	AA (n=25)			AG (n=123)			GG (n=22)			p
	n	%	Mean $\pm$ SD	n	%	Mean $\pm$ SD	n	%	Mean $\pm$ SD	
Sleep disturbances	19	76		101	82		18	82		0.976*
Fatigue	22	88		123	100		22	100		0.522*
Paresthesia	18	72		93	76		16	73		0.902*
Swelling of soft tissues	17	68		94	76.4		19	86		0.498*
FIQ			63.7 $\pm$ 12.2			62.3 $\pm$ 11.9			59.3 $\pm$ 15.9	0.473**
Visual analog scale			6.9 $\pm$ 1.2			6.6 $\pm$ 1.4			6.6 $\pm$ 1.7	0.776***

AA: Adenine/Adenine; AG: Adenine/Guanine; GG: Guanine/Guanine; SD: Standard deviation; FIQ: Fibromyalgia Impact Questionnaire; \* Chi-square test was used; \*\* Mann-Whitney U test was used; \*\*\* T-test was used.

of migraine.<sup>31</sup> In another study, Wood et al.<sup>32</sup> have shown that pindolol may have beneficial effects on cardinal symptoms, including sleep and pain, in the treatment of FMS. ADRBs are membrane-integrated proteins that mediate the effects of catecholamines on the cell surface. ADRB2 is found in the central nervous system and peripheral tissues, and is responsible for pain signals.<sup>33</sup> In a previous study by Khasar et al.,<sup>34</sup> the activation of peripheral ADRB2 has been shown to cause increased pain sensitivity and hyperalgesia. In another study, the ADRB2 gene polymorphism was suggested to play a role in chronic musculoskeletal pain.<sup>19</sup> Vargas-Alarcón et al.<sup>23</sup> have evaluated different SNPs of alpha-1 (rs574584, rs1383914, rs1048101 and rs573542), beta-2 (rs1042713 and rs1042714) and beta-3 (rs4994) ARs in patients with FMS of Spanish and Mexican origin, and shown that the ADRB2 AC haplotype is more common in patients with FMS that are of Spanish and Mexican origin than in healthy control subjects. Furthermore, Vargas-Alarcón et al.<sup>23</sup> have found that alpha-1 AR SNP (rs1383914) is found more frequently in Spanish patients with FMS than in healthy control subjects. In another study evaluating the relationship between FMS and the ADRB2 polymorphism (rs1042713 and rs1042714), Xiao et al.<sup>22</sup> have reported no significant difference between patients with FMS and healthy control subjects. The findings of the present study are similar to those reported by Xiao et al.,<sup>22</sup> but different to those reported by Vargas-Alarcón et al.<sup>23</sup> The present study has also found no significant difference in the ADRB2 gene polymorphism (rs1042717) and haplotypes between patients with FMS and healthy control subjects. The difference between the present findings and those reported by Vargas-Alarcón et al.<sup>23</sup> may be attributed to be the differences in the ethnic origin of the studied patients with FMS and the number of studied SNPs being different in the two studies.

Aside from the risk of developing FMS, AR gene polymorphisms have also been associated with different clinical characteristics of FMS.<sup>22,23</sup> In their study, Vargas-Alarcón et al.<sup>23</sup> have shown that morning stiffness and fatigue are more frequent and FIQ scores are higher in Mexican patients with FMS carrying the GG genotype of the 1-AR polymorphism (rs574584) than in

other patients with FMS. In their study, Xiao et al.<sup>22</sup> have identified an association between the ADRB2 gene polymorphism in patients with FMS and sleep disturbances. The present study has detected no significant difference in the clinical symptoms or FMS, FIQ or VAS scores of patients with FMS carrying the ADRB2 gene polymorphism (rs1042717) and/or different genotypes of the ADRB2 gene polymorphism. The results of the present study differ from those reported in previous studies, which may be attributed to differences in the number of studied SNPs and the ethnic origin of the studied patients with FMS.

The limitations of the present study include the absence of male patients with FMS and healthy males as a separate group in addition to the relatively small sample size.

In conclusion, the ADRB2 (rs1042717) gene polymorphisms and genotypes do not differ between patients with FMS and healthy individuals. Furthermore, the ADRB2 (rs1042717) gene polymorphism in FMS has no effect on the symptoms of FMS. In addition, this study did not find any association between ADRB2 polymorphisms and FIQ or VAS score. However, the evaluation of only one SNP is not sufficient to determine the role of the ADRB2 polymorphism in the etiopathogenesis of FMS, and so further comprehensive clinical studies with larger sample size are required evaluating different SNPs.

#### **Declaration of conflicting interests**

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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