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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

Investigation of Serotonin 2A Receptor Gene 5-HTR2A rs6311 Polymorphism in Trigeminal Neuralgia Disease

Trigeminal Nevralji Hastalığında Serotonin 2A Reseptör Geni 5-HTR2A rs6311 Polimorfizminin Araştırılması

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ABSTRACT

Objectives: Trigeminal neuralgia (TN) is a disease that occurs in the distribution region of the trigeminal nerve and is frequently recurrent pain. Serotonin (5-HT) acts on neurotransmitters in the central nervous system. The study aims to examine the relationship between sensitivity and pain intensity of the serotonin receptor gene (5-HTR2A) and rs6311 polymorphism in patients by examining TN genetically.

Materials and Methods: The study consisted of 10 TN patients and 10 healthy individuals. Genotyping of 5-HTR2A rs6311 polymorphism using DNA isolated from blood, Real-Time PCR in StepOnePlus device, and TaqMan SNP Genotyping Tests were performed by manufacturers' protocols.

Results: In 5HTR2A rs6311 analysis, 3 of 10 TN patients were found to have CC, 5 had CT and 2 had TT genotype. In the control group, CC genotype was detected in 4 individuals, CT genotype in 4 individuals, and TT genotype in 2 individuals.

Conclusion: It was found that the TN genotype and allelic frequency differences between the patient and control groups were not significant. However, the CT genotype was found to be more common.

Keywords: Trigeminal neuralgia, polymorphism, serotonin, serotonin receptor

ÖZ

Amaç: Trigeminal nevralji (TN), trigeminal sinirin dağılım bölgesinde ortaya çıkan ve sıklıkla tekrarlayan ağrılarla karakterize bir hastalıktır. Serotonin (5-HT), merkezi sinir sistemindeki nörotransmitterlere etki eder. Çalışmada, TN'yi genetik olarak inceleyerek hastalarda serotonin reseptör geni (5-HTR2A) duyarlılığı ve ağrı şiddeti ile rs6311 polimorfizmi arasındaki ilişkiyi araştırmak amaçlanmıştır.

Gereç ve Yöntem: Çalışmada 10 TN hastası ve 10 sağlıklı birey yer almıştır. 5-HTR2A rs6311 polimorfizminin genotiplemesi kanlardan izole edilen DNA ile StepOnePlus cihazında Real-Time PCR ve TaqMan SNP Genotipleme Testleri kullanılarak üretici protokolüne ugun olarak yapılmıştır.

Bulgular: 5HTR2A rs6311 analizinde, 10 TN hastasından 3'ünde CC, 5'inde CT ve 2'sinde TT genotipinin varlığı tespit edildi. Kontrol grubunda ise 4 kişide CC genotipi, 4 kişide CT genotipi ve 2 kişide TT genotipi tespit edildi.

Sonuç: Hasta ve kontrol grupları arasındaki TN genotipi frekans farklılıklarının anlamlı olmadığı görüldü. Ancak CT genotipinin diğerlerine göre daha yaygın olduğu görüldü.

Anahtar Kelimeler: Trigeminal nevralji, polimorfizm, serotonin, serotonin reseptörü

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INTRODUCTION

Trigeminal neuralgia (TN) is a disease that has sudden, usually unilateral, short-term, stabbing, and recurrent pain that occurs in the distribution area of the branches of the trigeminal nerve (McMillan R, 2011). This pain originating from the trigeminal nerve, responsible for facial sensation, is in the form of sudden and severe attacks. The duration of the pain can be a few seconds or a few minutes. It mostly occurs on one side of the face, teeth, and jaw (Maarbjerg et al., 2017). To diagnose the disease, at least three of the four factors created according to the International Association for the Study of Pain (IASP) must be present. TN has been examined in many ways by several studies, however, there is no consensus on the causes and treatment yet (Küçükkurt et al., 2019).

Serotonin (5-hydroxytryptamine, 5-HT) is a hormone that regulates neurotransmitters in the central nervous system and also has psychological effects. It is one of the most important neuromodulators in the central nervous system. In previous studies, it has been found that serotonin plays a crucial role in many physiological processes such as cell division, neuronal migration, and differentiation (Paredes et al., 2019). In addition, 5-HT is an effective factor in pain disorders originating from the trigeminal system. The 5-HT receptor family is divided into seven subfamilies (5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6, 5-HT7) consisting of 14 receptor subtypes. Serotonin 2A receptor (5-HTR2A) can be found in almost all organs. They are effective in wound healing and blood coagulation, as well as taking part in many neural events in the central nervous system. Besides 5-HTR2A is effective in mental disorders and processes such as learning and memory, and also in the production of nerve cells (Ebdrup et al., 2011).

The study aims to examine the relationship between pain content and responses of the expanded *5-HTR2A* by examining the genetic aspects of trigeminal neuralgia.

MATERIALS AND METHODS

Study Group

The study consisted of 10 individuals diagnosed with trigeminal neuralgia (TN group) and 10 healthy individuals without TN (C group). The study protocol was prepared by the Helsinki Declaration 2 (2015) guidelines and was approved by Marmara University Ethics Committee (protocol code: 09.2021.323).

Study inclusion criteria: TN diagnosis by a neurologist, maxilla/mandible attacks, unilateral neuralgia in 2nd/3rd trigeminal branches, no genetic disease in family/self, age 18-65. Exclusion criteria: Presence of organic factors such as tumors or other brain lesions (eg multiple sclerosis), presence of atypical facial pain with symptoms similar to TN, presence of a genetic disease in the family, not being 18-65 years old range.

DNA Isolation and Genotyping

For genomic DNA isolation, 200 µL peripheral blood samples were collected in the EDTA-containing tubes and a PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, CA, USA) was used for isolation, as previously stated (Kazancı et al., 2021). All the samples were isolated at the same day, and the isolated DNAs were kept at – 20^0 C until the genotyping process was carried out.

Real Time – PCR Analyses

Genotyping of the *5-HTR2A* rs6311 polymorphism was performed using Real-Time PCR on the StepOnePlus (Thermo Fisher Scientific, Inc.) instrument. Commercially available TaqMan SNP Genotyping Assays genotyping kits were used according to manufacturers' protocols (cat. no. 4371355, Thermo Fisher Scientific, Inc.).

Statistical Analysis

Statistical analyses of the results were performed using the SPSS 25.0 program and by the $\chi 2$ (chi-square) test. The P-value of less than 0.05 was regarded as statistically significant.

RESULTS

Genotype and allele distributions of individuals with and without TN disease are given in Table 1 and 2, respectively. In the *5-HTR2A* rs6311 analysis, 3 (30%) TN patients had CC, 5 (50%) CT, and 2 (20%) TT genotypes (Table 1). When the distributions of alleles were examined, it was seen that it was 55% for the C allele and 45% for the T allele (Table 2). In the C group, 4 individuals were CC, 4 were CT and 2 were TT genotype. When the distributions of alleles were examined, it was seen that it was 60% for the C allele and 40% for the T allele.

The quantitative Real-Time PCR amplification of the *5-HTR2A* rs6311 polymorphism in the CC, CT, and TT genotypes is shown in Fig. 1. FAM (blue curve) indicates the T allele, while VIC (green curve) indicates the C allele. (A) The

single green curve shows the homozygous genotype of CC (Fig. 1A), the green and blue curves show the heterozygous genotype of CT (Fig. 1B), and the single blue curve shows the homozygous genotype of TT (Fig. 1C).

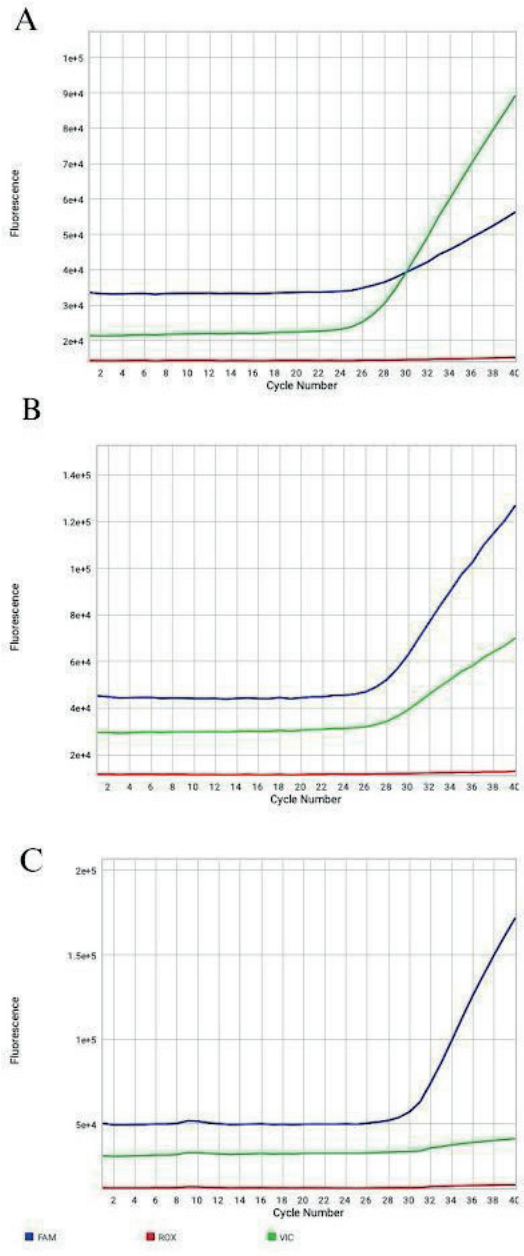

Figure 1. Real Time – PCR image of *5-HTR2A* rs6311 polymorphism. Genotype A is CC homozygous. For this genotype, a high fluorescence signal is observed in the FAM channel and a low fluorescence signal in the VIC channel. Genotype B is CT heterozygous. For this genotype, a moderate fluorescence signal is observed in FAM and VIC channels. Genotype C is TT homozygous. For this genotype, a low fluorescence signal is observed in the FAM channel and a high fluorescence signal in the VIC channel.

Table 1. Genotype distributions of individuals with and without TN disease.

	Genotype			P value
	CC	**CT**	**TT**	
TN (n=10)	3	5	2	0.8807
Percentage	30%	50%	20%	
C (n=10)	4	4	2	
Percentage	40%	40%	20%	

TN: Trigeminal neuralgia group. C: Control group

Table 2. Allele distributions of individuals with and without TN disease.

	Allelic Frequency		P value
	C	**T**	
TN (n=10)	11	9	
Percentage	55%	45%	0.7491
C (n=10)	12	8	
Percentage	60%	40%	

TN: Trigeminal neuralgia group. C: Control group

DISCUSSION

Serotonin is a neurotransmitter that regulates neural activity and plays important roles both in the brain and many parts of the body through its receptors (Berger et al., 2017). Serotonin and its receptors have also been associated with the development of several diseases such as Alzheimer's disease (Thome et al., 2001), Parkinson's disease (Lee et al., 2012), migraine (Naito et al., 2010, Yücel et al., 2016), schizophrenia (Golimbet et al., 2007) and temporomandibular pain disorders (Cui et al., 2014) in humans and animal models, but the mechanisms by which they induce pain remain unclear.

In the study examining the effects of drugs targeting specific serotonin receptors on different diseases, it was stated that more than one serotonin receptor is expressed in all regions of the brain and that neurons can express multiple serotonin receptors (Berger et al., 2017). Di Stefano et al. (2020) conducted a genetic analysis of neuronal TN, suggesting that trigeminal neurons respond rapidly to stimuli in their study, in which they systematically described the clinical features of TN patients with the same characteristics. As a result of the study, it was pointed out that these patients displayed genetic variations in the genes responsible for voltage-gated ion channels and transient receptor channels. Besides, the study comparing the expressions of Nav1.3, Nav1.7, and Nav1.8 in TN patients with healthy individuals, the relationship between sodium channels and

acute and chronic neuropathic pain was evaluated and it was reported that Nav1.7 causes insensitivity to pain syndromes (Siqueria et al., 2009). This channel is referred to as Nav1.8. Nav1.3 is an embryonic channel expressed in neurons in neuropathic conditions and after injury. This study points to the expression of these molecules in individuals suffering from chronic pain. In the study examining the relationship between the serotonin transporter gene 5-HT and rs25531 polymorphism and pain severity in TN patients, it was determined that the genotype distribution of 5-HTTLPR between patients and healthy individuals was different and that the short-short genotype was higher in patients than in controls. However, it was suggested that the rs25531 polymorphism was not associated with TN susceptibility and/or pain severity (Cui et al., 2014).

Genetic variations play an important role in the diagnosis and treatment of chronic pain. In the case of chronic pain conditions like fibromyalgia, low back pain, migraine, and TN, it has been found that the serotonin transporter gene affects the release of neurotransmitters into the synaptic cleft and regulates neurotransmission (Knezevic et al., 2018). HTR2A gene c.102T> C (rs6313) and 1438A>G (rs6311) variants affect impulse control and the formation of repetitive behaviors. The T allele, which is associated with increased receptor expression, is linked to impulsivity and repetitive behaviors in diseases such as Parkinson's disease. The genetic influence leading to these behaviors significantly increases in the low levodopa equivalent dose groups, which raises the risk of developing the disease in CT and TT carriers by 2.8 and 6.9 times, respectively (Lee et al., 2012). Regina et al. (2007) determined the frequency and linkage disequilibrium for – 1438A/G, – 1420C/T, and – 783A/G in their study on promoter single nucleotide polymorphisms, and significant linkage disequilibrium was pointed between SNPs and – 1438A/G (rs6311) and – 783A/G (rs6312). Also, no significant difference in promoter activity between the A – and G-alleles of the – 1438 locus was seen when expressed with the – 1420C/T and – 783A/G major alleles. It was observed that the mRNA expression of the 5-HT2A receptor in the human fibroblast cell line, and the – 783A/G polymorphism significantly changed the effects of the – 1438A/G single nucleotide polymorphism.

CONCLUSION

In our study, no difference was found between genotype (p=0.8807) and allelic (p=0.7491) frequencies between TN patients and the control group. Accordingly, we found that the CT genotype was more common in the patients. In light of this information, we think that the rs6311 polymorphism in the *5-HTR2A* gene may be effective in TN patients, but more effective results can be obtained by increasing the study group.

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Limitation of the study: Since the sample collection phase of the study coincided with the COVID-19 pandemic, patients' arrival at the clinic was disrupted. For this reason, the number of individuals remained below the planned.

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