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Absence of Association of the Serotonin Transporter Gene Polymorphism with the Mentally Healthy Subset of Fibromyalgia Patients

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Abstract: The serotonin transporter (5-HTT) gene is considered to be a promising candidate for genetic involvement in some mood disorders owing to its role in the regulation of serotoninergic neurotransmission. In this study, we aimed to assess the significance of the 5-HTT gene in fibromyalgia syndrome (FS) as well as to find out whether the 5-HTT gene polymorphism is associated with this disease. Fifty-three mentally healthy fibromyalgia patients and 60 unrelated healthy volunteer controls were included in the study. Symptom Checklist-90-Revised (SCL-90-R), Beck Depression Inventory (BDI), and State and Trait Anxiety Inventory tests (STAI-I and II) were applied to both patients and controls. A PCR analysis of 5-HTT gene polymorphism was performed, and the results of the patients with FS and healthy controls were compared. In both FS patients and healthy controls the S/S, S/L and L/L alleles of the 5-HTTLPR genotype were represented in 24.5 % and 33%, 56.6% and 38.3%, and 18.9% and 28.3%, respectively. Additionally, in FS patients and healthy controls the 10/10, 10/12 and 12/12 alleles of the VNTR variant were represented in 5.9% and 11.7, 51% and 36.7%, and 43.1% and 51.7%, respectively. The 5-HTTLPR and VNTR results of the patients and controls were not significantly different ($P > 0.05$). We concluded that neither 5-HTT nor its polymorphism is associated with FS. Our results also address the frequencies of 5-HTT gene alleles in our population. Further studies are required to better understand the genetic basis of FS.

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Introduction

Fibromyalgia syndrome (FS) is a musculoskeletal disease of unknown aetiology characterised by chronic widespread pain, increased tenderness on palpation, and additional symptoms such as disturbed sleep, stiffness, fatigue and psychological distress [1,2].

Although the biophysiological mechanisms underlying FS have not been elucidated precisely, neuroendocrine factors are thought to be of major importance. Furthermore, some genetically determined factors have also been suggested to be involved [3]. There is also a possible contribution from serotonin (5-HT) to the aetiology of FS because of the efficacy of 5-HT reuptake inhibitors in the management of chronic pain and the presence of low levels of serum 5-HT and 5-hydroxyindole acetic acid in the cerebrospinal fluid of idiopathic pain patients [4]. Recently, a negative correlation was shown between the serum concentrations of substance P and serum tryptophan in FS patients [5].

The serotonin transporter (5-HTT) gene has long been assumed to be involved in the pathogenesis of major psychiatric disorders, including anxiety, depression, schizophrenia, neuroticism, autism, bipolar affective disorder and seasonal affective disorder [6].

Human 5-HTT is encoded by a single gene (SLC6A4) mapped to chromosome 17q12. Offenbaecher et al. showed that there was a polymorphism in the serotonin transporter gene in FS [7]. A 44-bp insertion or deletion in the 5′-flanking promoter region of HTT gene (5-HTT

gene-linked polymorphic region of 5-HTTLPR) can create a short (S) and a long (L) allele. The 5-HTTLPR polymorphism is situated in a 6C-rich region composed of 20–23 bp repeating units. The S and L alleles have 14 and 16 repeat elements, respectively [6,8]. The short variant designated 'S' is associated with reduced transcriptional efficiency of the 5-HTT gene promoter, resulting in lowered 5-HT reuptake activity compared to the long form (L) variant [6,9].

Recently another polymorphic region, that is 17-bp VNTR in the second intron, was found to be associated with a major depressive disorder. The gene proved to be associated with a number of other conditions, such as autism, FS and migraine [7], although the subsequent studies failed to replicate these results [10,11].

In this study we aimed to assess the significance of the 5-HTT gene in FS as well as to find out whether the 5-HTT gene polymorphism is associated with this disease.

Patients and Methods

The diagnosis of FS was made according to the criteria of the American College of Rheumatology 1990, in the Department of Physical Medicine and Rehabilitation, University Hospital, Gaziantep. The patients were contacted by telephone and were asked whether they were willing to participate in the study, and 53 agreed. The mean age of the patients was 26.5 ± 6.7 years, and they had been suffering from FS for a mean of 9.1 ± 6.2 years. Sixty unrelated healthy volunteers were also included in the study, and constituted the control group. The mean age of the control subjects was 29 ± 10 years. Patients and controls were of the same ethnic origin and from the same geographical area. All were female.

In all subjects medical and psychiatric examinations were carried out together with routine laboratory screening (complete blood count, blood chemistry analyses, sedimentation rate, antinuclear antibody) to exclude severe physical or psychiatric disorders. For this reason, the patients and controls were also evaluated using psychiatric tests, including the Symptom Checklist-90-Revised (SCL-90-R), the Beck Depression Inventory (BDI), and the State and Trait Anxiety Inventory tests (STAI-I and II). Those who had high score with these tests or had a psychiatric disorder as determined by DSM IV criteria [12] were not included in the study.

Molecular Analysis

High molecular weight genomic DNA was prepared from venous blood using the standard phenol chloroform extraction. Laboratory staff were blind to the psychiatric observations. Analysis of the polymorphism of the 5-HT transporter gene took place in a manner modified according to Lesch et al.'s 44-bp insertion/deletion and Ogilvie et al. [13] 17-bp VNTR. All amplification reactions were performed in a total volume of 25 µl containing 100 ng DNA, 140 µM dNTP, 1 µM of each primer, 50 mM KCL, 10 mM Tris-HCI (pH 8.0) 1 mM Mg^{2+} and 1 unit of Ampli Taq polymerase (Perkin Elmer Roche, USA). For the PCR reaction of the 44-bp insertion/deletion variant of the 5-HT transporter, dNTP was replaced by 200 µM dATP, dCTP, dTTP, 100 µM dGTP, and 100 µM 7-deaza-GTP (Boehringer Mannheim, Germany). Applied primer sequences were GGC GTT GCC GCT CTG AAT GC (forward) and GAG GGA CTG AGC TGG ACA ACC AC (reverse). The 44-bp insertion/deletion polymorphism, as well as G GTC AGT ATC ACA GGC TGC GAG TAG (forward) and TGT TCC TAG TCT TAC GCC AGT GAA G (reverse) for the 17-bp VNTR. The PCR program of both reactions consisted of 35 cycles, an initial denaturation at 94°C for 2 min, and a final extension period of 72°C using a GeneAmp 9600 Perkin Elmer Cetus PCR machine. The various conditions of the cyclic PCR reactions were as follows: 44-bp insertion/deletion: 95°C, 30 s, 62°C 30 s, 72°C I min, 17-bp VNTR 94°C 30 s, 62°C 20 s, 72°C I min. All PCR products were separated by 3.5 % agarose gel electrophoresis and stained with ethidium bromide for UV visualisation.

Sequence Analysis

The VNTR of the second intron was sequenced with a Dye deoxy-terminator cycle sequencing kit (Applied Biosystem). Amplicons of individuals being heterozygous for the STin2.12,2.10,2.9,2.7 allele were excised from the gel, purified by 0.45 µM Ultrafree and 30000 NMWL filter units (Millipore) and sequenced with 5′ TGATTGGCTATGCTGTGG 3′ as upper and 5′ TGTTCCTAGTCTTACGCCAGTG 3′ as lower primer. The PCR program of the sequencing reaction consisted of 25 cycles, an initial denaturation at 96°C for 30 s, annealing at 50°C for 5 s, and extension at 60°C for 4 min using a GeneAmp 9600 Perkin-Elmer Cetus PCR machine. Analysis was performed using a Dye Primer cycle sequencing kit on an ABI 377 automated DNA sequencer (Perkin Elmer) and compared with the published sequence [3].

Statistical Analysis

Statistical analyses were performed using SPSS for Windows (version 7.5). The results were reported as mean ± SD. Student's *t,* and χ^2 and one-way ANOVA tests were used for the statistical analysis of data. A P value < 0.05 was considered statistically significant.

Results

The ages and sexes of the patients and controls were similar (test, $P > 0.05$). None of the patients or controls had a psychiatric disorder, and their psychiatric test results were also similar (test, $P > 0.05$). The test results are summarised in Table 1.

Table 1. Psychiatric test results of fibromyalgia patients and controls

	Patients		Controls	
Tests	Min.	Max.	Min.	Max.
BDI	(4)	(43)	(3)	(21)
STAI-I	(38)	(71)	(28)	(41)
STAI-II	(35)	(74)	(26)	(47)
Somatisation	(0.8)	(3.4)	(0.3)	(1.2)
Anxiety	(0.4)	(3.6)	(0.2)	(1.7)
Psychoticism	(0)	(2.8)	(0)	(0.8)
Obsessive-compulsive	(0.4)	(3.4)	(0.3)	(1.6)
Hostility	(0.2)	(2.7)	(0.1)	(1.1)
Interpersonality sensitivity	(0.8)	(3.8)	(0.4)	(1.2)
Phobic anxiety	(0)	(2.9)	(0)	(1.3)
Depression	(0.8)	(4)	(0.3)	(1.9)
Paranoid ideation	(0)	(3)	(0.1)	(0.7)
Global severity index	(0.8)	(2.9)	(0.4)	(1.1)

Table 2. The results of molecular analyses of 5-HTT gene polymorphism

	Serotonin transporter gene region					
	5-HTTLPR			VNTR		
Genotype	S/S	S/L	L/L	10/10	10/12	12/12
Patients *n* (%)	13 (24.5)	30 (56.6)	10 (18.9)	3 (5.9)	26 (51)	22 (43.1)
Controls *n* (%)	20 (33.3)	23 (38.3)	17 (28.3)	7 (11.7)	22 (36.7)	31 (51.7)

The results of molecular analyses of the patients and controls are summarised in Table 2, and were not different (test $P > 0.05$).

Discussion

There is a growing interest in the genetics of FS, which is a common rheumatologic condition characterised by chronic widespread musculoskeletal pain and tenderness at multiple sites on palpation [14]. Virtually nothing is known about the pathogenesis and there is no objective biochemical or imaging technique to substantiate the diagnosis [15]. It was claimed that a genetic susceptibility to FS may be linked to HLA on the basis of the reported association of HLA with FS and linkage between HLA and depression. The biophysiological mechanism in FS was proposed to be similar to that in depression, and suggested that this was likely to result from a neuroendocrine/neurotransmitter dysregulation [3]. It was shown that the decreased pain perception threshold during depression is likely to result from dysfunction in several neurotransmitter systems, especially the serotonergic one, which is also involved in the pathophysiology of depression [5]. A number of naturally occurring polymorphisms involved in 5-HT biosynthesis, catabolism or response have been reported which showed an association between HLA-DR4 and FS [3,14]. The studies also showed that there is a silent polymorphism (T102C) in the 5-HT2A receptor gene, but failed to show that this polymorphism is directly involved in the aetiology of FS [13].

On the other hand, according to our results, the 5-HTT gene polymorphism was not associated in FS in the patients who had normal psychiatric status. Therefore, these biophysiological mechanisms, which were proposed to be similar in FS and depression, are not attributable to the factors being related to the 5-HTT polymorphism. Some other factors may be involved in this similarity, which needs further investigation.

This study was performed in a mentally healthy subset of fibromyalgia patients. However, if it had been performed in another subset of fibromyalgia patients who scored differently on psychiatric tests, the results might also have been different. Such a condition could have prognostic significance or alert the physician to the possible development of depression or anxiety. This issue also needs further investigation.

To our knowledge, there is only one report of 5-HTT gene polymorphism in FS, in which the frequencies of S/S, L/L and L/S genotypes of the 5-HTTPR in patients and controls were 31% and 16%, 27% and 34%, and 42% and 50%, respectively [7]. In this report, the authors found a significant difference between patients and controls regarding the S/S genotype of 5-HTTPR, but they did not search for the VNTR variant of the 5-HTT gene. In our study, we assessed both the HTTPR and VNTR variants of the 5-HTT gene. However, we found no significant differences between patients and controls. Although these results seem controversial, this is not the case as there is ethnic diversity, and it is possible that the genetic basis of diseases can differ between various regions and populations. The influence of ethnic diversity may be important and should be borne in mind when performing genetic studies in FS.

It was reported that the patients with seasonal affective disorder were less likely to have the L/L genotype and more likely to have the 'S' allele [17]. It is also possible that psychological factors can affect the results of genetic studies in FS [14]. In order to avoid this drawback, we performed a psychiatric evaluation in both patients and controls. None of the subjects included in this study were psychologically impaired, and the results of the patients and controls were similar as well. Thus, we eliminated the impact of psychological factors in this study.

In conclusion, neither 5-HTT nor its polymorphism is associated with FS in a mentally healthy subset of patients. Our results also address the frequencies of 5-HTT gene alleles in our population. Further studies are required to better understand the genetic basis of FS.

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