The concept of a single temporomandibular joint (TMJ) syndrome or disorder has been replaced with a series of diagnostic categories (such as myofascial pain and dysfunction, internal derangement, arthritic disorders, and muscle hyperactivity disorders). Although many pain syndromes can affect the face or the head and neck region, the more prevalent conditions include TMJ disorders and atypical facial pain.1,2 Previously, the myofascial pain dysfunction syndrome included all TMJ/masticator muscle pain, jaw dysfunction and joint-clicking conditions.3,4

The pathophysiologic mechanisms underlying TMJ dysfunction and pain have been linked to disturbance in serotonin (5-HT) metabolism and transmission.5-8 The activity of the 5-HT is regulated by the gene known as the serotonin transporter (5-HTT) gene that has 2 identified polymorphic regions (Fig 1). The 5-HTT gene is located on chromosome 17q 11.2, and a functional 44-base pair (bp) deletion/insertion polymorphism has been identified in the 5´-flanking promoter region of 5-HTT gene (5-HTTLPR), which can create a short (S) and a long (L) allele.9,10 The short variant, S, is associated with the reduced transcriptional efficiency of the 5-HTT gene promoter that results in lowered serotonin uptake activity, when compared with the long, L, variants.9-11 Recently, another polymorphic region that conforms a 17-bp variable number of tandem repeats (VNTR) in the second intron of the 5-HTT gene was also described.12,13

The purpose of this study was to evaluate the relationship between temporomandibular joint pain and dysfunction and serotonin transporter (5-HTT) gene polymorphism. Forty-eight patients with temporomandibular joint pain and 111 healthy control subjects were examined. The results for the patients and control subjects were not significantly different (P > .05). The analysis of genotype distribution (homozygous for STin 2.10 genotypes of the variable-number tandem-repeat polymorphism) showed significant differences between the patients and control subjects (P = .003). ST 2.10 allele was more frequent in the patients with temporomandibular joint pain and dysfunction. In the control group, however, STin 2.12/12 genotype was significantly higher (P = .017). In the patients who were homozygous or heterozygous for variable-number tandem-repeat variants of 5-HTT STin 2.12 copies, the average scores of somatization and anger were significantly higher than those who were homozygous for STin 2.10 variant (P < .05). The patients who were homozygous for STin 2.10 genotype were also homozygous for “L” genotype (P = .019). However, this was not the condition in the control subjects. This study does not provide evidence to support the involvement of 5-HTT gene-linked polymorphic region in temporomandibular joint pain and dysfunction. Our findings indicated that only the presence of the homozygous STin 2.10 genotype of variable-number tandem-repeat is likely to play a substantial role in the genetic predisposition to temporomandibular joint pain and dysfunction and that the STin 2.12/12 genotype may have a protective role against temporomandibular joint pain and dysfunction.
The 5-HTT gene proved to be associated with a number of conditions (such as autism, severe alcoholism, anxiety disorder, seasonal affective disorder, fibromyalgia, migraine, depression, schizophrenia, and bipolar affective disorder). Although the 5-HTT has been proposed as a possible candidate for its involvement in the pathogenesis of major psychosis and bipolar affective disorders, the gene was not proved to be involved in the pathogenesis of mood disorders.

Because of the presence of a possible pathophysiological role of the 5-HT in TMJ dysfunction and pain, we thought that the polymorphism in the 5-HTT gene, which determines the 5-HT activity, could also have a role in this disease state. For this reason, the aim in this study was to clarify the association of the 5-HTT gene with TMJ pain disorder (TMJP) by examining the polymorphisms in the promoter region (5-HTTLPR) and in the second intron (VNTR).

**MATERIAL AND METHODS**

Forty-eight patients with TMJP who were consecutively admitted to the Department of Orthodontics & Oral and Maxillofacial Surgery Clinic, Faculty of Dental School, Selcuk University, Turkey, were included in the study. The diagnoses of TMJP were assigned according to our standard examination protocol that was modified from the previously described diagnostic criteria (Fig 2). The control group consisted of 111 individuals who represented different social groups. The patients and healthy subjects who participated in the study were volunteers.

The age, gender, and duration of illness were documented. Personnel interviews were conducted, and blood samples were obtained after physical examination. Those candidates who had mental retardation, drug dependence, or somatic or neurologic illnesses (eg, hypothyroidism mimicking a depressive state, positive toxicologic findings) that could impair the psychiatric evaluation were excluded. Persons whose first-degree relatives had endogenous psychoses or alcoholism were also excluded.

**Psychiatric evaluation**

The patients were evaluated with the Symptom Checklist 90 Revised (SCL-90-R), Beck Depression Scale (BDS), and State-Trait Anxiety Inventory I and II (STAI-I and -II). Minnesota Multiphasic Personality Inventory, a personality questionnaire used to identify personal traits of patients, was performed on the patients and healthy controls.

The SCL-90-R measures somatization, obsession, compulsion, depression, anxiety, sensitivity and hostility between people, phobic and paranoid thinking, psychoticism, and it includes a global severity index. We used a Turkish version of the SCL-90-R.

The BDS evaluates depressive symptoms and atti-
tudes. A single summed score can range from 0 to 63; a higher score indicates greater depression. We used a Turkish version of the BDS.

The STAI I-II is a 2 × 20-item inventory and gives scores that index the current anxiety level of the subject ("state") and degree to which the subject is prone to experience anxiety ("trait"). We used a Turkish version of the STAI I-II.

Molecular analysis

DNA was extracted from patients and control subjects from whole blood by standard techniques. A functional 44 bp insertion/deletion polymorphism in the promoter region of the SERT gene was typed by polymerase chain reaction (PCR) amplification of DNA with flanking primers 5′-TGGATTTCCTTCTCAGTGATTGG-3′ (forward) and 5′-TCATGTTCCTAGTCTTACGCCAGTG-3′ (reversed). PCR was performed with GC Rich PCR system (Roche Molecular Biochemical) in a 50 µL reaction mixture that contained 100 to 200 ng DNA, 100 µL deoxyribonucleoside triphosphate, 20 pmol of each primer, and 1.5 mmol/L MgCl₂. DNA was denatured at 95°C for 3 minutes and 35 cycles at 95°C for 1 minute for denaturation, 1 minute at 60°C for annealing and 1 minute at 72°C for extension, followed by 7 minutes at 72°C for final extension. Amplification products were resolved by electrophoresis on 2% agarose gels next to a DNA molecular weight standard and visualized with ultraviolet ethidium bromide staining. Alleles were designated S (484 bp) and L (528 bp), as was previously described.9

The 17-bp VNRT polymorphism in the second intron of the SERT gene was typed by PCR with primers 5′-TGGATTTCCTTCTCAGTGATTGG-3′ (forward) and 5′-TCATGTTCCTAGTCTTACGCCAGTG-3′ (reversed). PCR was performed in a 50 µL volume with 20–100 ng DNA, 100 µL deoxyribonucleoside triphosphate, 20 pmol of each primer, 1.5 mmol/L MgCl₂, 20 µmol/L Tris-HCl pH 8.6, 50 µmol/L KCl, 0.2% (weight/vol) bovine serum albumin, and 1 U Taq polymerase (MBI Fermentas). PCR conditions were 2 minutes for initial denaturation at 94°C, 40 cycles at 94°C for 1 minute for denaturation, 1 minute at 57°C for annealing, and 2 minutes at 72°C for extension, followed by 10 minutes at 72°C for final extension. Amplification products were resolved by electrophoresis on 2% agarose gels next to a DNA molecular weight standard and visualized with ultraviolet ethidium bromide staining. Alleles were designated 390 bp (12 copy, STin 2.12) 360 bp (10 copy, STin 2.10), or 345 bp (9 copy, STin 2.9) as was previously described.30

Statistical analysis

Statistical analyses were performed with SPSS for Windows (version 7.5; SPSS, Chicago, Ill). T test, chi-squared test, and 1-way analysis of variance (ANOVA) tests were used for the statistical analyses of data. A probability value of less than .05 was considered statistically significant.

RESULTS

There were 17 female and 31 male patients, with a mean age of 21.5 ± 3.87 years, in the study group. In the control group, there were 61 healthy female and 50 healthy male volunteers, with a mean age of 22.12 ± 4.08 years. The genders, ages, and psychiatric family history of the patients and control subjects were not significantly different (χ², P > .05).

The results of genetic analyses of the 5-HTT gene polymorphism are summarized in Table I. The 5-HTTLPR results of the patients and control subjects...
were not significantly different ($\chi^2, P > .05$). The analysis of genotype distribution, homozygous for STin 2.10 genotypes of the VNTR polymorphism, showed significant differences between the patients and control subjects ($\chi^2, 11.95$; degrees of freedom [df], 2; $P = .003$). STin 2.10 allele was more frequent in the patients with TMJD. In the control group, however, STin 2.12/12 genotype was significantly higher than in the patients ($\chi^2, 5.70$; df, 1; $P = .017$). One patient who was found to have STin 2.9 genotype was dropped from the study.

According to the SCL 90-R test, the average of somatization subscale scores of the patients who were homozygous for STin 2.12 variant was higher than for those patients who were homozygous and heterozygous for STin 2.10 variant (1-way ANOVA, $f = 3.76$; df, 2; $P = .034$).

In the patients who were homozygous or heterozygous for VNTR variants of 5-HTT STin 2.12 copies, the average scores of somatization and anger were significantly higher than for those patients who were homozygous for STin 2.10 variant (t, 2.83; $P = .008$ and t, 2.12; $P = .042$, respectively). However, their STAI-I point averages were not significantly different (t, 2.03; $P = .05$).

When the combined VNTR and 5-HTTLPR results were analyzed for the patient and control groups, it was found that the patients who were homozygous for STin 2.10 genotype were also homozygous for L genotype (Fisher exact test, $P = .019$), (Table II). However, this was not the condition in the control subjects (Table III).

### DISCUSSION

Several association studies with the 5-HTTLPR polymorphism have shown a higher frequency of the S allele in the individuals with affective disorders.$^{10,11,17}$ The 5-HTTLPR was also proposed as a possible candidate gene in major psychoses, because it modulates the 5-HTT gene at the transcriptional level, with the S allele corresponding to low 5-HTT uptake activity.$^{10,11}$ The individuals with either 1 or 2 copies of the S form had higher neuroticism scores than did the individuals who were homozygous for the L variant. Moreover, patients with seasonal affective disorder were less likely to have the L/L genotype and more likely to have the S allele, as compared with control subjects.$^{31}$ In this study, there was no relationship between the study and the control groups with regard to S and L allele variants of 5-HTTLPR.

A polymorphic region that contained a 17-bp VNTR in the second intron was found to be associated with bipolar disorders.$^{13}$ Liu et al,$^{17}$ and Gutierrez et al$^{32}$ found the frequency of allele 10 in bipolar affective disorder and major depression with melancholia to be significantly higher than in the control group. On the other hand, Rees et al$^{33}$ found a significant excess of 12 repeat

### Table I. Distribution for 5-HTTLPR and VNTR variants in patients and healthy control subjects

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>5-HTTLPR</th>
<th>VNTR genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/S (%)</td>
<td>S/L (%)</td>
</tr>
<tr>
<td>No. of patient$^*$ (%)</td>
<td>11 (22.9)</td>
<td>22 (45.8)</td>
</tr>
<tr>
<td>No. of control subjects$^+$ (%)</td>
<td>36 (32.4)</td>
<td>42 (37.8)</td>
</tr>
<tr>
<td>TOTALS (n)</td>
<td>47</td>
<td>64</td>
</tr>
</tbody>
</table>

S/S, Short/short; S/L, short/long; L/L, long/long.
$^*$N = 48 patients.
$^+$N = 111 healthy control subjects.

### Table II. Combined analysis of the frequency of genotypes of 5-HTT gene in patients

<table>
<thead>
<tr>
<th></th>
<th>S/S (%)</th>
<th>S/L (%)</th>
<th>L/L (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STin 2.10/10</td>
<td>6 (40.0)</td>
<td>9 (60.0)</td>
<td>15 (100)</td>
<td></td>
</tr>
<tr>
<td>STin 2.10/12 and 12/12</td>
<td>19 (79.2)</td>
<td>5 (20.8)</td>
<td>24 (100)</td>
<td></td>
</tr>
<tr>
<td>TOTALS</td>
<td>25 (64.1)</td>
<td>14 (35.9)</td>
<td>39 (100)</td>
<td></td>
</tr>
</tbody>
</table>

S/S, Short/short; S/L, short/long; L/L, long/long. $^*Fisher exact test, P = .019.$

### Table III. Combined analysis of the frequency of genotypes of 5-HTT gene in control group

<table>
<thead>
<tr>
<th></th>
<th>S/S or S/L (%)</th>
<th>L/L (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STin 2.10/10</td>
<td>10 (62.5)</td>
<td>6 (37.5)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>STin 2.10/12 and 12/12</td>
<td>65 (70.7)</td>
<td>27 (29.3)</td>
<td>92 (100)</td>
</tr>
<tr>
<td>TOTALS</td>
<td>75 (69.4)</td>
<td>33 (30.6)</td>
<td>108 (100)</td>
</tr>
</tbody>
</table>

S/S, Short/short; S/L, short/long; L/L, long/long. Fisher exact test, $P = .56.$

$^*Fisher exact test, P = .019.$
alleles in bipolar patients. The functional polymorphisms located in the VNTR variants in intron-2 of the 5-HTT gene were found to be associated with TMJP in patients of Turkish origin. The homozygous STin 2.10 genotype of the VNTR was significantly higher in the patients with TMJP than in the control subjects.

Although some functional effects of 44-bp insertion/deletion with reduced 5-HTT uptake that are caused by lower expression have been well confirmed, there have been no in vitro data on the functional effects of VNTR in intron 2 as yet. Both polymorphisms may be working in an integrated fashion and may likewise be associated with transcriptional activity in a synergistic fashion. For this reason, we analyzed the combined results for patients and control subjects separately (Table III). Even though the number of citations that evaluated VNTR and 5-HTTLPR polymorphisms together has not been enough to draw a conclusion, when the 2 polymorphisms were evaluated together, the patients who were homozygous for the STin 2.10 genotype were also found to have homozygous L genotype.

It was shown that the frequency of the L genotype was high in depressive suicide victims. In the study, however, we found that patients with TMJP had been homozygous for both STin 2.10 and L genotypes. This finding should be taken into account in the further studies of this kind. On 1 side, there was no relationship between the psychiatric symptoms and 5-HTTLPR polymorphism. On the other side, the patients who were homozygous for STin 2.12 genotype were found to have significantly higher somatization scores than those patients with homozygous STin 1.10 genotype. Furthermore the somatization and anger scores of patients with homozygous or heterozygous STin 2.12 variant of VNTR were significantly higher than for those patients with the homozygous STin 2.10 variant. In the light of these findings, we propose that the VNTR polymorphism has an impact on the psychiatric symptoms of the patients.

It was proposed that individuals who have the tendency of somatization also have the tendency of myofascial pain. However, we found that the STin 2.12/12 genotype, which is thought to have a higher protective capacity, shows higher somatization tendency when compared with the STin 2.10/10 genotype. This suggests that there might be a weak relationship between somatization and TMJP and that genetic factors may be involved in the susceptibility to TMJP.

CONCLUSION

This study does not provide evidence to support the involvement of 5-HTTLPR in TMJP. Our findings indicated that only the presence of the homozygous STin 2.10 genotype of VNTR is likely to play a substantial role in the genetic predisposition to TMJP and that the STin 2.12/12 genotype may have a protective role against TMJP. Further studies are required for the confirmation of our results and to find the genetic basis of TMJP in other populations.

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