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T102C polymorphism of the 5-HT2A receptor gene may be associated with temporomandibular dysfunction

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OBJECTIVE: To assess whether a relationship existed between the T102C polymorphism of 5-HT2A receptor gene and temporomandibular dysfunction.
METHODS: Sixty-three patients with temporomandibular dysfunction, and 54 healthy volunteer controls were included in the study. Molecular analysis of the T102C polymorphism of the 5-HT2A receptor gene was performed using PCR technique.
RESULTS: The C/C genotype was over represented in the patients whereas T/T genotype was over represented in the controls ($P < 0.05$). The genotype distribution of the patients who had temporomandibular dysfunction was not different than those who did not have temporomandibular dysfunction ($P > 0.05$).
CONCLUSION: The T102C polymorphism may be involved in the etiology of temporomandibular dysfunction. The overrepresentation of the C/C variant of 5-HT2A receptor gene in temporomandibular dysfunction suggests a possible role of the serotonergic system in this disease, particularly at the receptor level.
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Keywords: temporomandibular dysfunction; serotonin; 5-HT2A receptor; polymorphism

Introduction

The myofacial pain syndrome (MFPS) includes a spectrum of disorders that cause facial pain. The majority of these disorders originate from temporomandibular joint (TMJ) disorders or masticator muscle spasm, and manifest by facial pain, jaw dysfunction or joint clicking (Laskin, 1969; Lerman, 1973; Eversole *et al*, 1985).

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The temporomandibular dysfunction (TMD), which can cause MFPS, can result from internal derangements in the joint, arthritic disorders, muscle hyperactivity disorders or neurobiological predispositions, or bruxism. MFPS can be due to muscle spasm rather than TMJ disorder. The triggering mechanism of this condition is unclear. This may be due to neurobiological predispositions or some other pathologic events causing muscle spasm. Neurotransmitters in the central or peripheral nervous system can be involved not only in muscular contractibility, but also in some neurobiological disorders. Thus, changes in the neurotransmitters can lead to changes in muscular tone.

Serotonin (5-HT) is a neurotransmitter that occupies a uniquely important place in neurobiology because of its role in many physiological processes such as sleep, appetite, thermoregulation, pain perception, hormone secretion, and sexual behavior. Abnormality of the serotonergic system has been implicated in a number of human diseases such as mental depression, migraine, epilepsy, obsessive-compulsive behavior, and affective disorder. Like other neurotransmitters, 5-HT is released into the synaptic junction, and exerts its effect on specific receptors on the postsynaptic membranes. Based on differential radioligand binding affinities, at least six types of 5-HT receptors have been identified: 5-HT-1A, 5-HT-1B, 5-HT-1C, 5-HT-1D, 5-HT-2 and 5-HT-3 (Peroutka, 1988).

The activity of serotonergic system is under the control of the genes like 5HT1D (1p36.3-34.3), 5HT1B (6q13), 5HT2A (13q14-21), 5HT transporter (17q11.2-12), CACNLB1 (17q11.2-22) and FHM (19p13) (Monari *et al*, 1997).

Polymorphisms of many of the genes involved in 5-HT biosynthesis, catabolism, and response have been reported, suggesting that genetic variability may underlie the development of diseases such as schizophrenia, obsessive-compulsive disorder, and suicide (Marshall *et al*, 1999).

The 5-HT2A receptor gene is mapped to chromosome 13q14-21 (Blairy *et al*, 2000). Recently, a silent polymorphism in the 5-HT2A receptor gene was identified

which is defined by a T to C transition at position 102. This mutation does not alter the amino acid composition and has therefore has no influence on receptor protein (Bondy *et al*, 1999).

Anxiety, depression and imbalance in the autonomic nervous system are the components that should be considered in chronic pain. Therefore, antidepressants and when necessary nerve blocks should also be considered as an adjunct in the treatment of chronic pain. Serotonergic system may be important at this point as it is involved in a variety of neural functions. Since polymorphism in serotonin related genes could affect the functional status of the system, it is likely that 5-HT may be related to TMD. In this study, our purpose was to assess whether a relationship existed between the T102C polymorphism of 5-HT2A receptor gene and the TMD.

Materials and methods

Eighty-eight patients with TMD who were admitted consecutively to the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, between October 2001 and October 2002, were included in this study. Among these patients, 25 of them were excluded from the study based on the exclusion criteria. There were 22 (34.9%) female and 41 (65.1%) male patients, with a mean age of 23.4 (s.d. 12.0) years, in the research group. Ethical permission was obtained from local ethical committee. The diagnosis of TMD was made on the basis of anamnestic questionnaire (Table 1) and clinical investigation (manual functional analysis, Table 2) as well as magnetic resonance imaging of the left and right joints. Patients' anamnestic properties are given in the Table 1 for each category as *n* (%).

The age, gender, and duration of the illness [5.3 (s.d. 2.1) years] were documented in all patients, and the patients with mental retardation or drug dependence, and somatic or neurological illnesses (e.g. hypothyroidism mimicking, positive toxicological findings) were excluded.

The control group included 54 healthy volunteers [24 (44.5%) female and 30 (55.5%) male, with a mean age of 22.6 (s.d. 9.2) years] who were selected from the dental faculty students. Eighty students were approached but 26 students were excluded following the exclusion criteria. In healthy control subjects, personal interviews were conducted. The control subjects were selected according to the following criteria; (1) no history of muscle or joint pain or tenderness; (2) no history of joint noise; (3) demonstration of smooth and symmetric mandibular movement; (4) a maximum opening of the jaw 40 mm or greater; (5) maximum lateral movements of 10 mm or greater. The subjects whose first-degree relatives had endogenous psychoses or alcoholism were not included in TMD patients and the control group. Presence of a psychiatric problem was also a cause of exclusion.

The age and sex of the patients and controls were similar ($P > 0.05$). To assess the results of genetic association, it is important for research and control groups to come from same ethnic origin. Polymorphic configurations can be affected by ethnic differences. For that reason it is important to come from same ethnic origin as well as geographic origin for research and control groups. In this study the patients and controls were from Konya and all of them were Turkish. An informed consent to participate in the study was taken from all patients and healthy volunteers after explaining the study procedure.

Molecular analysis

A 5 ml blood sample was obtained and the DNA was extracted from the leucocytes using the QIAamp Blood Isolation kit (QIAGEN). Primers flanking the 5-HT2A polymorphic site at position 102 (5-HT2A-F 5′-CTGTCT GCT ACA AGT TCT GGC TTT-3′; 5-HT2A-R 5′-CTG CAG CTT TTT CTC TAG GG-3′) were used to generate a 342 bp fragment. PCR was performed in a final volume of 25 μl consisting of 50 ng DNA, 0.6 μmol l^{-1} of each primer, 200 μmol l^{-1} dNTPs, 10 mmol l^{-1} Tris-HCL (pH 8.3), 50 mmol l^{-1} KCL, 1.5 mmol l^{-1} $MgCl_2$, and 1.25 U AmpliTag Gold (Perkin Elmer). Annealing was carried out at 60°C for 30 s, extention at 72°C for 30 s, and denaturation at 95°C 30 s for 35 cycles. PCR products were digested with 10 U Mspl (Roche Molecular Biochemicals) for 14 h and seperated on a 2% agarose gel (FMC NuSieve 3:1, from Biozym). Allele 1 (T102C allele) was

Table 1 Questionnaire form for TMD

1. Do you have difficulty or pain, or both, when opening your mouth, as for instance, when yawning? $n = 52$ (82%)
2. Does your jaw get 'stuck', 'locked', or 'go out'? $n = 21$ (34%)
3. Do you have difficulty or pain, or both, when chewing, talking, or using your jaws? $n = 57$ (90%)
4. Are you aware of noises in the jaw joints? $n = 52$ (82%)
5. Do you have pain in or about the ears, temples, or cheeks? $n = 63$ (100%)
6. Does your bite feel uncomfortable or unusual? $n = 49$ (78%)
7. Do you have frequent headaches? $n = 62$ (98%)
8. Have you had a recent injury to your head, neck, or jaw? $n = 35$ (56%)
9. Have you previously been treated for a jaw joint problem? If so, when? $n = 41$ (65%)

McNeill *et al* (1990).

Table 2 Clinical examination procedures for TMD

1. Measure range of motion of the mandible on opening and right and left lateral movement
2. Palpate for preauricular TMJ tenderness
3. Palpate for TMJ crepitus
4. Palpate for TMJ clicking
5. Palpate for tenderness in the masseter and temporalis muscles
6. Note excessive occlusal wear, excessive tooth mobility, fremitus, or migration in the absence of periodontal disease, and soft tissue alterations, for example, buccal mucosal ridging, lateral tongue scalloping
7. Inspect symmetry and alignment of the face, jaws, and dental arches

McNeill *et al* (1990).

represented by the uncut 342 bp PCR product and allele 2 (C102 allele) consisted of two fragments at 216 and 126 bp.

Statistics

Statistical Package for Social Sciences (SPSS) 8.0 for Windows was used for the statistical analyses. Chi-square test was used to compare the genotypes of the patients and controls.

Results

The C/C genotype was over represented in the patients whereas T/T genotype was over represented in the controls ($\chi^2 = 7.67$, d.f. $= 2$, $P = 0.022$) (Table 3). In the patients, the frequencies of the T and C alleles were 38.1 and 61.9%, respectively. The corresponding frequencies were 55.5 and 44.5% in the controls, respectively. There was no significant difference between the allele frequencies of the patients and controls ($P > 0.05$).

Discussion

Patients with facial pain can have extremely complex etiologies involving anatomical structures and central nervous system or psychological factors (Israel and Scrivani, 2000). MFPS is not uncommon, and can present with a variety of symptoms like atypical facial pain. In the majority of the patients these symptoms are attributed to muscular spasm due to dental or occlusive problems although there is no evidence of a detectable anatomic abnormality in some patients. The surgery is indicated in selected cases, and the majority of the patients are treated with anti-inflammatory or muscle relaxant drugs. Despite these facts, all people who have dental or occlusal problem are not sufferers of MFPS. This discordance between the subjective symptoms of the patients and objective findings of the physician is interesting, and attributable to the individual differences though the source of this difference is unclear.

One of the main determinants of the individual differences may be the genetic. With recent identification of polymorphism of the genes, we have understood better the genetic basis of the diseases, and individual differences in terms of symptomatology and treatment response.

The molecular basis of TMD is also largely unknown and worthy to search. One of the neurotransmitters, 5-HT, which is involved in a variety of disease states, may be one of the candidate mediators playing a role in TMD. There is evidence to support this contention that an association of the MFPS with the 5-HT transporter gene has been shown recently (Herken *et al*, 2001).

According to the results of this study in which the recently identified T102C polymorphism of the 5-HT2A receptor gene was studied, the C/C genotype was over represented in the patients whereas T/T genotype was over represented in the controls. Thus, it is apparent that a difference exists at molecular level in TMD patients and healthy subjects. We do not know whether this genotype difference creates a genetic predisposition to or is involved in the etiology of TMD. Although the frequencies of the T and C allels of the patients and controls were similar, homozygosity for T allele may have protective role in TMD while homozygosity for C allele may increase the risk of TMD. Since it is also unclear whether different variants of this gene possess different level of activities, we are unable to explain how C/C or T/T variants affect or modify the serotonergic activity, thereby exerting a function in TMD. Despite the differences between the patients and controls, the genotypes of the patients who had TMD were not different than those without TMD. The pain is a subjective symptom while TMD can be diagnosed by objective means. At this point, we propose that the polymorphism of the gene may be important in determining the subjective symptoms of MFPS while the coexisting objective findings like TMD are due to other factors. Owing to discordance in the symptoms and findings in some patients with TMD, this disease may be considered similar to fibromyalgia syndrome in a sense in which there is also discordance between the subjective symptoms and objective findings (Bayazıt *et al*, 2002). The T102C polymorphism of the 5-HT2A receptor gene was studied in fibromyalgia patients, and C/C and C/T genotypes were found to be over-represented in the patients while T/T genotype was high in the controls (Bondy *et al*, 1999). In this study, C/C genotype overrepresented in TMD while T/T variant was high in the controls. The social withdrawal and anxiety scores of the patients who had T/T or T/C genotypes were higher than those who had C/C genotype in TMD. These may suggest a similarity between fibromyalgia syndrome and TMD as well as the polymorphism of the gene is concerned.

According to our results, C/C genotype rather than T/T or T/C genotype is associated with TMD. The following comments may be made; (1) psychiatric factors are not pivotal in TMD; (2) psychiatric abnormalities seen in some patients with TMD may be due to presence of T/T genotype; (3) patients with T/T

Table 3 Distribution of the 5-HT2A receptor gene variants in TMD and healthy controls

	Genotype				
	T/T [n (%)]	*T/C [n (%)]*	*C/C [n (%)]*	*Total [n (%)]*	*Control of Hardy–Weinberg*
TMD ($n = 63$)	8 (12.7)	32 (50.8)	23 (36.5)	63 (100)	0.3725 ($P > 0.05$)
Control ($n = 54$)	15 (27.8)	30 (55.6)	9 (16.7)	54 (100)	0.8388 ($P > 0.05$)

genotype who express more psychiatric symptoms may lead to misunderstanding that TMD has a psychiatric background. Results of the fibromyalgia patients also seems in agreement with these contentions (Gursoy *et al*, 2001). It was suggested that the 5-HT has antinociceptive effects (Bardin *et al*, 2000). The T102 allele may also be involved in nociception in TMD. Because, it was shown that chronic paracetamol administration resulted in a significant decrease in the number of 5-HT2A binding sites with resultant decrease in the analgesic efficacy of paracetamol, and that plasticity of this neurotransmitter system may lead to loss of analgesic efficacy and, in its more extreme form, may produce analgesic-related painful conditions, for example, analgesic abuse headache (Srikiatkhachorn *et al*, 2000). It should also be remembered that the serotonergic system is a complex system and the factors which are related to 5-HT production, transportation or degredation may interact in an antagonist or agonist fashion.

In conclusion, the T102C polymorphism may be involved in the etiology of TMD. The over-representation of the C/C variant of 5-HT2A receptor gene in TMD suggest a possible role of the serotonergic system in this disease, particularly at the receptor level. Although this study was performed in a small sample of patients and healthy controls, this is the first study regarding the association of T102C polymorphism of 5-HT2A receptor gene with TMD. This study is a preliminary study and further studies performed in a larger series are needed to make this issue more clear.

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