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The relation between temporomandibular disorders, mood status and serotonin transporter gene polymorphism. Study in Syrian population.

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Abstract

The purpose of this study was to evaluate the relationship between temporomandibular disorders, mood status and serotonin transporter (5-HTT) gene polymorphisms in Syrian population. Twenty patients with temporomandibular joint disorder (TMD), and 36 healthy control subjects were examined. The sample consists of 23 males, and 33 females aged between (19-30 years old), visited the department of fixed prosthodontic of the faculty of dentistry, Damascus University. This study shows that S/L allele of 5-HTTLPR was the most frequently observed allele in TMD patients; whereas statistical analysis did not show significant difference between patient and control groups by neither genotype distribution analysis nor allele frequency analysis. S\S allele analysis showed a relation between it and mood status. Although the study did not show significant relation between TMD and serotonin transporter polymorphism gene, but according to the importance of serotonin transporter polymorphism gene and its relation to psychological factors we suggest other studies which focus on the relation between the genetics, psychology and TMD.

Keywards: temporomandibular disorder, serotonin transporter gene.

Introduction

Temporomandibular disorder (TMD) is a heterogeneous group of disorders affect the temporomandibular joints, the masticator muscles, or both. TMD generally develops orofacial pain as a primary symptom. Approximately 10 % of all orofacial pain patients who complain of tenderness in the masticator muscles for more than one month are diagnosed as TMD [1,2]. TMD patients often complain of psychosomatic symptoms such as sleep disorder, headache, fatigue, and depression, which are characterized as functional somatic syndromes (FSS) [3,4]. The pathophysiologic mechanisms of TMD and pain have been linked to disturbance in serotonin (5-HT) metabolism and transmission [5,6]. Also the 5-HT neuronal system regulates diverse physiologic functions including sleep, respiration, appetite, pain, motor function, cognition, sexual activity, as well as emotions such as mood and anxiety. The activity of the 5-HT is regulated by a gene known as the 5-HT transporter (5HTT) gene which has two identified polymorphic regions (Fig 1[7]). The 5-HTT gene is located on chromosome 17q 11.2. A

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functional 44-base pair (bp) deletion/insertion polymorphism has been identified in the 5'-flanking promoter region of 5-HTT gene (5-HTT gene-linked polymorphic region [5-HTTLPR]), which can create a short (S) and a long (L) allele. The 5-HTTLPR polymorphism is situated in a guanine cytosine (GC)-rich region composed of 20- to 23-bp repeating units, (Fig1[7]).

The S and L alleles have 14 and 16 repeated elements respectively[7,8]. In comparison with the long (L) variants, the short variant (S) is associated with a reduced transcriptional efficiency of the 5-HTT gene promoter which cause decrease in serotonin uptake activity[9,10]. We think that the polymorphism in 5-HTT gene, which determines the 5-HTT activity, could also have a role in the TMD status or degree. For this reason, the aim from this study is to clarify the association between the 5-HTT gene and TMD by examining the polymorphisms in the promoter region (5-HTTLPR).

Material and methods

The twenty patients with TMD of our study were consecutively admitted to the Department of Fixed Prosthodontic. They were diagnosed with TMD by chronic and localized myalgia and tenderness to palpation lasted for more than one month, the diagnosis of TMD was assigned according to clinical examination and the dental history questionnaire.

The control group consisted of 36 individuals representing different social levels. Patients and control groups were volunteers, and well informed of the procedures by obtaining written consents according to ethical principles that approved by the committee of scientific research at international university for science & technology. The age, sex, 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, somatic or neurologic illnesses (eg, hypothyroidism mimicking a depressive state, positive toxicologic findings) that could impair the psychiatric evaluation were excluded from the study. Subjects whose first-degree relatives had endogenous psychoses or alcoholism were also excluded.

Molecular analysis

Genomic DNA was extracted from a whole blood samples taken from all participants using a Genomic DNA isolation Kit (NucleoSpin® Blood, Macherey - Nagel CO. Germany) following the standard technique. The extracted DNA was 1% Agarose electrophoresed and visualized under UV using Ethidium Bromide staining in order to confirm extraction and to exclude contamination . Concentration and purity were checked using BioPhotometer® (Eppendorf). Then the DNA was amplified by polymerase chain reaction (PCR) to detect serotonin-related polymorphisms. For serotonin transporter protein promoter polymorphism (5-HTTLPR) analysis, the extracted DNA samples were amplified by polymerase chain reaction (PCR) basically according to the method of Lesch et al[8]. Oligonucleotide primers flanking the 5-HTTLPR and corresponding to the nucleotide positions -1416 to -1397 (5'-GGCGTTGCCGCTCTGAATGC) and -910 to 88 (5'-GAGGGACT-GAGCTGGACAACCAC) of the 5-HTT gene 5'-flanking regulatory region were used to generate 484(s) and 528(l), bp fragments.

All PCR amplification processes were carried out in a final volume of

20 µl consisting of 50 ng of genomic DNA, 2.5 mM deoxyribonucleotides, 20 pmol of forward and reverse primers, 10 mM tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, and 1 U of Taq DNA polymerase (Takara bio inc, Japan). Annealing was carried out at 61°C for 30 s, extension at 72°C for 1 min, and denaturation at 95°C for 30 s for 35 cycles.

PCR products were electrphoresed with suitable DNA ladder using 2 % agarose gel and visualized under Ultraviolet using ethidium bromide staining.

Psychiatric evaluation:

All participants were instructed to fill out a questionnaire (MOODS-SR) to evaluate their mood status. This questionnaire is a version of the SCI-MOODS that is psychopathology assessment instrument which found to discriminate between patients with mood disorders. The MOODS-SR questionnaire contains 161 items which help to put the diagnosis of the major depressive disorder DSM-IV and bipolar disorder (mania), and it helps to identify borderline and atypical manifestations including symptoms not listed within DSM criteria [5,8].

This questionnaire focuses on four kinds of disorders: (1) mood fluctuations and associated changes in the lifestyle and subject 's relations (mood), (2) energy levels (energy), (3) cognitive functions (cognition), and (4) changes in energy, physical well-being, mental and physical efficiency, related to the weather and the seasons, changes in eating, and sleep (rhythmicity and vegetative functions).

Each positive item in this questionnaire is given one point. Patients with a score of 63 points or more are considered to have a problem related to mood swing or psychiatric problem [12].

Statistical analysis

Statistical analysis were performed with SPSS for Windows (version 14; SPSS).Chi-square, Correlations (Kendall's tau_b) tests were used for the statistical analysis of data. Statistical significance was set at probability value of less than 0.05.

Result

Regarding to sex distribution, females were significantly more numerous in TMD group (3 males, 17 females). Table 1.

Table (2A) shows a summary of the genotype distribution analysis of 5HTTLPR. Genotypes including (S/L) alleles were increased in the TMD group compared to control group. Statistical analysis did not show significant difference between patient and control groups neither by genotype distribution analysis nor allele frequency analysis, S\S ($p = 0.727$ by Kendall's tau_b), S\L ($p = 0.752$ by Kendall's tau_b), and L\L ($p = 1.00$ by Kendall's tau_b) Table (2B).

Chi-spuare test showed significant differences among TMD group for the prevalence of mood psychopathology. As MOODS-SR above-threshold ratings were scored by 5 subjects of 36 (13.89%) in TMD-free, and 13 of 20 (65%) of TMD group. Table 3A.

According to the distribution of 5HTT gene alleles in mood psychopathology group, S\S allele has the highest prevalence (55.6%) in comparison with L\L, S\L alleles (27.7%, 16.7% respectively). While S\L allele showed the highest prevalence (52.6%) in healthy group, table 3B. Statistical analysis for the S\S allele revealed a significant difference between the two group by correlation analysis, while the others (L|L, S|L) alleles did not shows any significant difference, table 3C.

Discussion

This study showed that females are more prone to TMD which agree with many other researchers [13,14,15]; however many researchers did not support gender as a risk factor for TMD [13,16]. The S\L allele of 5-HTTLPR, which was the most frequently observed in TMD patients of this study, But this allele failed to prove a significant relation with TMD.

Many studies suggested that there are differences in psychometric characteristics among various groups of TMD patients. The recent investigations showed a relation between (S\S) allele and mood status. To date, many diseases have been proved to be associated with 5-HTTLPR. For example, the longer alleles (L and xL) were demonstrated to be significantly increased in victims of sudden infant death syndrome (SIDS)[17]. Anxiety trait [18], and mood disorder [19], have likewise been investigated, and an association with 5-HTTLPR was described. Some of these results were controversial and others need clarification. The results of present study showed a strong relation between 5HTTLPR and psychopathology mood. This has confirmed the findings of previous studies [20,21,22]. In addition this study demonstrated a strong relation between TMD and psychopathology mood which goes in line with reported previous findings [23,24].

As this is the first Syrian study investigating the relation between genetics and TMD, We suggest that a further study with a larger sample size could be carried out to clarify the transitively relation between the gene 5-HTT, TMD, and psychopathology factors.

Conclusion

Although this study did not show significant relation between TMD and serotonin transporter polymorphism gene, we have to be very careful in our evaluation of these findings because of the small sample size, even though the P value is small. Further independent studies will be necessary to investigate the relation between the genetic, psychology, and temporomandibular disorder factors in Syrian population.

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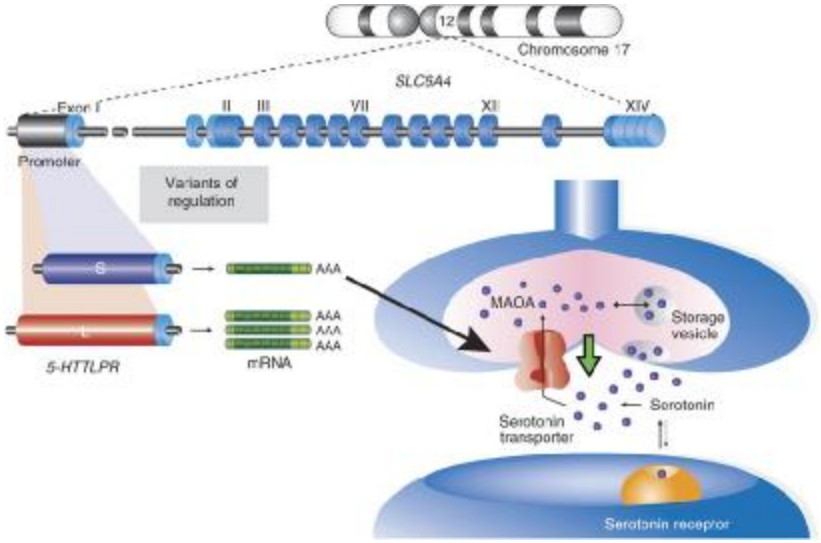

Fig1. Illustrate the region 11.2 of the chromosome 17 and 5HTT polymorphisms (Turhan & Klaus-Peter, 2007).

Table 1: Distribution for Gender in patients and healthy control subjects

Gender	**TMD-free**	**TMD**
Male	n = 20	n = 3
Female	n = 16	n = 17
P value		
Chi-square		0.002

Table2A. Genotype distribution allele frequency for 5HTTLPR from TMD patients and control

Genotype	**control**	**TMD**
5HTTLPR	n = 36	n = 20
S/ S	11 (30.6%)	5 (25%)
S /L	14 (38.8%)	9 (45%)
L/L	11 (30.6%)	6 (30%)

Table2B. Correlation between allele frequency for 5HTTLPR and TMD patients

Genotype	**TMD**
5HTTLPR	n = 20
S/ S	**5 (25%)**
P value	
Correlations (Kendall's tau_b)	0.727
S/L	**14 (38.8%)**
P value	
Correlations (Kendall's tau_b)	0.752
L/L	**11 (30.6%)**
P value	
Correlations (Kendall's tau_b)	1.000

Table 3A: Distribution for mood status in patients and healthy control subjects

Mood status	**TMD-free**	**TMD**
No mood psychopathology	n =31 (86.11%)	n =7 (35%)
Mood psychopathology	n = 5 (13.89%)	n = 13 (65%)
P value		
Chi-square		0.001

Table 3B. Genotype distribution allele frequency for 5HTTLPR from moody and control individuals.

Genotype	**No mood psychopathology**	**mood psychopathology**
5HTTLPR	n = 38	n = 18
S/S	6 (15.8%)	10 (55.6%)
S/L	20 (52.6%)	3 (16.7%)
L/L	12 (31.6%)	5 (27.7%)

Table 3C. Correlation between allele frequency for 5HTTLPR and mood psychopathology patients

Genotype	**mood psychopathology**
5HTTLPR	n = 18
S/S	10 (55.6%)
P value	
Correlations (Kendall's tau_b)	0.001
S/L	3 (16.7%)
P value	
Correlations (Kendall's tau_b)	0.987
L/L	5 (27.7%)
P value	
Correlations (Kendall's tau_b)	0.787