Influence of TNF-α-308 G/A gene polymorphism on temporomandibular disorder

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Introduction: Tumor necrosis factor alpha (TNF-α) levels are significantly upregulated in the synovial fluid of patients with temporomandibular joint disorder (TMD). The TNF-α influences pain generation and maintenance. Therefore, the aim of this study was to investigate the influence of single nucleotide polymorphism TNFA-308 (rs1800629) on TMD risk and on the pressure pain threshold. Methods: The genotypic and allelic frequencies of candidate single nucleotide polymorphisms were compared among 152 TMD patients and 91 sex- and age-matched healthy subjects in the control group using the real-time polymerase chain reaction technique. The pressure pain threshold in the temporomandibular joint, anterior fascicle of the temporal muscle, masseter muscle, and Achilles tendon were recorded with an algometer. After the pressure test, all participants received a complete physical examination, including masticatory muscle evaluation, temporomandibular joint palpation, and assessment of mandibular range of motion. Results: The TNF-308 polymorphism is positively associated with TMD. Subjects with TMD had a 2.87 (95% confidence interval, 1.256-6.569) times greater chance of having the GA genotype than did the control group. Rare A-allele homozygotes demonstrated decreased pain sensitivity for the temporomandibular joint and anterior fascicle of the temporal muscle in the pressure pain threshold test compared with ancestral allele homozygotes. Conclusions: This study presents an unprecedented association between the TNF-308 (rs1800629) polymorphism and TMD. Future studies are needed to enlighten the association between TNFA-308 G/A single nucleotide polymorphism and mechanical pain sensitivity. (Am J Orthod Dentofacial Orthop 2016;149:692-8)

Temporomandibular disorder (TMD) is a heterogeneous group of musculoskeletal disorders that affect the temporomandibular joint (TMJ), the masticatory muscles, and the neighboring structures of the TMJ. TMDs affect 5% to 15% of adults, are more prevalent in women between 20 and 40 years old,2-4 and cause billions of dollars in health care costs every year.5 According to project Orofacial Pain Prospective Evaluation and Risk Assessment, TMDs are associated with psychosocial factors, sleep apnea, history of trauma, parafunction, greater sensitivity to experimental pain, and other related painful disorders.6,7

However, TMD pathophysiology is not yet fully understood. TMJ inflammation, peripheral and central sensitization, uncontrolled autonomic nervous system stimulation, and inefficient descending pain modulation are considered major risk factors that trigger and maintain the disorder.6 The complex and multifactorial nature of the pathology of TMD suggests that environmental exposure and diverse genetic risk factors located in multiple loci could contribute to the disease pathogenesis.8,9

Interestingly, chronic TMD is considered a functional somatic pain syndrome, similar to irritable bowel syndrome, migraines, fibromyalgia, interstitial cystitis, and chronic fatigue syndrome.10 Functional somatic syndromes have chronic pain of undetermined organic origin as their distinctive feature and tend to respond
to antidepressant drug treatment and cognitive behavioral therapy. Furthermore, these seemingly unrelated conditions cumulatively affect certain persons, suggesting an underlying common etiologic factor. However, the exact pathophysiologic mechanisms explaining these conditions remain unknown but probably involve amplified pain perception, alterations in brain activity, or neuroendocrine and immunologic systems dysregulations, all of which could have a common genetic background.

The single nucleotide polymorphism (SNP) in the promoter region of the TNFA gene (responsible for codifying tumor necrosis factor alpha [TNF-α]) at position -308 (TNFA-308 G/A; rs1800629) could modulate the inflammatory response because rare A-allele carriers are associated with increased TNF-α production. Since TNF-α is strongly associated with various pain diseases and chronic pain defines functional somatic syndromes such as TMD, and the TNF-α levels are significantly up-regulated in the synovial fluid of TMD patients, the aim of this study was to investigate the influence of SNP TNFA-308 (rs1800629) on TMD risk and in the pressure pain threshold.

**MATERIAL AND METHODS**

The study protocol was approved by the Bauru School of Dentistry of the University of São Paulo’s ethics committee (protocol 118/2010) and was in accordance with the ethical principles of the World Medical Association’s Declaration of Helsinki. The study group was recruited from subjects seeking treatment at the dental clinic of the Bauru School of Dentistry. The recruitment strategy was not randomized by consecutive patients and enrolled 152 (136 female) TMD patients diagnosed according to the Guidelines for Diagnosis and Treatment of Orofacial Pain of the American Academy of Orofacial Pain. Patients diagnosed with articular disc displacement (with or without reduction), inflammatory articular disease, or masticatory muscle disorders (local myalgia, myofascial pain, and centrally mediated myalgia) were selected for the study. Regardless of the type of pathology, painful symptoms were mandatory for the subject to be included. The exclusion criteria were congenital or developing disorders (aplasia, hyperplasia, dysplasia, and neoplasm), neuropathies, burning mouth syndrome, toothache, and otitis. The control group comprised 91 sex- and age-matched healthy subjects (82 female). A basic assessment, including questionnaires and a clinical examination, was performed for all participants at recruitment.

The pressure pain threshold values in the TMJ, anterior fascicle of the temporal muscle, masseter muscle, and Achilles tendon (extra trigeminal site) were recorded with an algometer (Kratos Medical Supply, King of Prussia, Pa) equipped with a 1 cm² round tip calibrated at 0.5 kg per square centimeter per second applied perpendicular to the skin surface. The pressure was applied until it became painful. At this point, the participant was instructed to push a hand-held button attached to the algometer so that the device automatically recorded the amount of pressure. After the pressure tests, all participants received a complete physical examination, including masticatory muscle evaluation, TMJ palpation, and assessment of mandibular range of motion.

Saliva was collected from all participants at the enrollment session using a DNA Oragene OG-500 kit (DNA Genotek, Ottawa, Ontario, Canada) following the manufacturer’s instructions. DNA was extracted from each participant’s saliva using a commercially available QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s guidelines. A spectrophotometer (Nanodrop 1000; Thermo Scientific, Waltham, Mass) was used to quantify and qualify the DNA samples. All isolated DNA samples were between 1.7 and 1.9 (260/280 nm ratio) and 1.9 and 2.1 (260/230 nm ratio). TNF-α-308 genotyping was performed using a TaqMan SNP genotyping assay (Applied Biosystems, Foster City, Calif) containing a 20-times mix of unlabeled polymerase chain reaction forward and reverse primers (5′–AGGCAATAGGTGTTTGGAGGCCAT-3′ and 5′–TCCTCCCTGCTCGATTCCG-3′), as well VIC/FAM labeled allele discrimination probes (GAGGCAA TAGGTTTGAGGGCATG/A[G]GACGGGTCAGCCT CCAGGGTCC). Quantitative polymerase chain reaction was carried out in a 5-μL reaction mixture with 20 ng of genomic DNA and 2.5 μL of the TaqMan genotyping polymerase chain reaction master mix (Applied Biosystems). Amplification and detection were performed using the Viia 7 platform (Applied Biosystems). Thermal cycling conditions were 10 minutes at 95°C followed by 45 cycles (2 steps), including 15 seconds of denaturation at 92°C and 60 seconds of annealing and extension at 60°C. All reactions were set manually in duplicate, and allele calling was done using SDS software (version 1.1; SDS Software, Bala Cynwyd, Pa).

**Statistical analysis**

The data distribution was tested by the Shapiro-Wilk normality test. The differences for the pressure pain thresholds between groups and for the different TNF-308 genotypes were assessed by unpaired t tests, Mann-Whitney U tests, analysis of variance, post hoc Bonferroni tests, or Kruskal-Wallis post hoc Dunn tests. The differences between polymorphism frequencies...
RESULTS

The mean ages for the TMD and control groups were 36 ± 11.00 years and 34 ± 11.47 years, respectively. There were no significant differences for age or sex distribution between the groups (P = 0.354). Complete demographic information and clinical parameters for the study populations are shown in Tables I and II.

The pressure pain threshold was significantly lower in the TMD group than in the controls for the TMJ, the anterior fascicle of the temporal muscle, the masseter muscle, and the Achilles tendon (Fig 1).

The genotype and allele frequencies of the investigated polymorphisms were in Hardy-Weinberg equilibrium. The TNFA-308 polymorphism is positively associated with TMD because 26.97% of TMD patients have the rare A-allele, whereas it was found in just 13.18% of healthy patients, with an odds ratio of 2.454 (95% CI, 1.212-4.968). Subjects suffering from TMD had a 2.87 (95% CI, 1.256-6.569) times greater chance of having the GA genotype for the TNF-308 polymorphism than the control group (Table III). From the genetic viewpoint, the study power calculation demonstrated (performed with 0.05-0.15 values for prevalence, 0.16 for disease allele frequency, and 2.45 for genotype relative risk) the trustworthiness of the study because of the power values of 99% to 100% for the multiplicative model, 96% to 99% for the additive model, and 90% to 96% for the dominant model.

Intriguingly, rare A-allele homozygotes demonstrated decreased pain sensitivity for TMJ and the anterior fascicle of the temporal muscle in the pressure pain threshold test compared with ancestral allele homozygotes. When A-allele carriers (AA + GA) where clustered together and compared with the ancestral G-allele carriers, there was no statistical difference between groups for pain response (Fig 2).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the contribution of TNFA-308 SNP in the occurrence and clinical symptomatology of TMD. Since we observed significantly higher frequencies of the rare A-allele in TMD patients compared with the control group, we hypothesize that the polymorphic rare allele comprises a risk determinant for TMD.

Our results showed that patients with the GA genotypes have a 2.87 times greater chance of developing TMDs, whereas the overall risk of patients with the genotypes with the A-allele (GA + AA) is 2.54 higher compared with the GG genotype. Interestingly, homozygosity for the A-allele was rare in our study population, diminishing the chances of finding an association with TMD; nevertheless, being a carrier of just 1 A-allele was enough to significantly increase the risk of TMD.

In opposition to our results, the authors of a recent prospective cohort study including 2737 subjects, of whom 185 developed TMD during the follow-up period, found no significant association between any of the 2942 SNPs tested and TMD. Even though the former is the larger prospective cohort investigating the relationship of genetic variants and TMD onset, the fraction of patients who effectively developed TMD symptoms is relatively small, and the necessary correction for multiple testing of a large set of SNPs reduces the chances of...
finding a true correlation. In contrast, although a direct comparison is unsuitable because of methodologic differences, our subject group was comparably quite large, including 152 TMD patients, and the fact that we did not have the imperative to perform a Bonferroni correction favored the discovery of genetic and phenotypical

Fig 1. Pressure pain threshold in control and TMD groups. TMJ, Temporomandibular joint; AFMT, anterior fascicle temporal muscle; MM, masseter muscle; AcT, Achilles tendon. *P < 0.05.

Table III. Genotype and allele frequencies for SNP TNFA-308 (rs1800629) of the groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control, n = 91 (%)</th>
<th>TMD, n = 152 (%)</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>79 (86.81)</td>
<td>111 (73.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>8 (8.79)</td>
<td>32 (21.05)</td>
<td>0.0116*</td>
<td>2.873 (1.256-6.569)</td>
</tr>
<tr>
<td>AA</td>
<td>4 (4.4)</td>
<td>9 (5.93)</td>
<td>0.5649</td>
<td>1.616 (0.4804-5.435)</td>
</tr>
<tr>
<td>GA + AA</td>
<td>12 (13.18)</td>
<td>41 (26.97)</td>
<td>0.0107*</td>
<td>2.454 (1.212-4.698)</td>
</tr>
</tbody>
</table>

Allele distribution

<table>
<thead>
<tr>
<th>Allele</th>
<th>Control, n = 91 (%)</th>
<th>TMD, n = 152 (%)</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>166 (91.2)</td>
<td>254 (83.55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>16 (8.8)</td>
<td>50 (16.45)</td>
<td>0.3087</td>
<td>1.427 (0.779-2.612)</td>
</tr>
</tbody>
</table>

OR, Odds ratio.
*P < 0.05.
correlations. Also, it was previously demonstrated that differences in the inclusion and exclusion criteria, as well the strictness of disease phenotype determinations, can be key factors in the determinations of power and odds of genetic association studies.\textsuperscript{27}

In accordance with our results, a recent systematic review and meta-analysis including 19 studies, 2584 subjects, and 3254 controls established that the A-allele and the AA/AG genotype for TNFA-308 were strongly correlated with the occurrence of rheumatoid arthritis in Latin Americans, representing a risk factor in this particular population.\textsuperscript{28} Even though TMD and rheumatoid arthritis are not closely related conditions, both have chronic pain and loss of gating of the inflammatory response as their defining characteristics. Since TNF-$\alpha$ plays ubiquitous and pleiotropic roles in acute and chronic inflammatory processes, we hypothesize that there must be a yet not fully characterized common pathologic mechanism linking increased levels of expression of TNF-$\alpha$ and the occurrence of both TMD and rheumatoid arthritis. In an analog fashion, the TNF-308 polymorphism has been associated with several other pathologic conditions, including diabetes mellitus,\textsuperscript{29} Alzheimer’s disease,\textsuperscript{30} autoimmune thyroid disease,\textsuperscript{31} migraine,\textsuperscript{11} irritable bowel syndrome,\textsuperscript{32} and multisomatoform disorders.\textsuperscript{12}

The association between SNP TNFA-308 with TMDs and other chronic pain comorbidities raises the question of whether TNFA is involved with central sensitization, which could explain why TNFA-308 G/A polymorphism may be not be restricted to articular TMDs with inflammatory pathophysiology.

**Fig 2.** Pressure pain threshold in the TMD group by genotype: A, TMJ; B, anterior fascicle temporal muscle; C, masseter muscle; D, Achilles tendon. Different letters indicate statistical difference ($P < 0.05$).
In addition to microscopic changes, such as increased spontaneous synaptic excitation, TNF-α may cause macroscopic changes identified by functional magnetic resonance. A-alleles of SNP TNFA-308 G/A were associated with lower hippocampal volumes in healthy people. As a component of the limbic system, the hippocampus plays a crucial role in the maintenance of cognitive functions as well as in controlling sleep and pain. TNF-α acts through 2 different cell surface receptors: TNFR1 (neurodegenerative) and TNFR2 (neuroproliferative). In the hippocampus, receptor affinity seems to be transferred to TNFR1, thus producing a more neurodegenerative effect. Thus, the lower hippocampal volume in subjects with A-alleles of TNFA-308 G/A polymorphism is explained by the high levels of TNF-α, which produces a neurodegenerative effect in this area of the brain. In rats, TNF-α sensitized other areas of the brain that are reactive to stress, such as the hypothalamus and the amygdala. For this reason, not only does it prove to be able to influence the response to traumatic or immunologic aggressions, but it also is relevant to behavioral pathologies. According to Wang et al. insecurely psychologically attached fibromyalgia patients had significantly higher levels of TNF-α than securely attached patients. Such evidence explains why the influence of the TNFA-308 G/A polymorphism is probably not restricted to articular inflammatory TMDs and could also influence muscle disorders with more complex pathophysiology related to cognition, stress, and sleep.

The fact that rare A-allele homozygotes exhibited a higher pressure pain threshold than the ancestral allele homozygotes in our study population is at first sight an intriguing result, since it apparently contradicts the current paradigm of the physiopathology of TMD, where the upregulation of proinflammatory cytokines plays a central role in joint degradation and hyperalgesia that characterize the disease. As previously noted, the rare occurrence of A-allele homozygotes (<6%) in our study population is a potential source of bias, and a larger well-characterized sample is required to confirm or contradict our results. Nevertheless, we could hypothesize that the upregulation in TNFA expression related to TNFA-308 SNP could cause a long-term reactive desensitization to chronic pain that leads to an increased pressure pain threshold in TMD subjects. In a recent report, a group of 754 subjects suffering from multisite musculoskeletal pain failed to exhibit the expected correlation between circulating inflammatory markers and clinical symptomatology after adjustment for lifestyle variables. The authors attributed the lack of correlation to the complex nature of chronic pain, where the interaction of the immune system with the hypothalamic-pituitary-adrenal axis and the autonomous nervous system, together with behavioral and lifestyle variables, cumulatively contribute to the disease phenotype and symptomatology. Similarly, in our study population, multiple unadjusted variables could contribute to the pressure pain threshold response in TMD subjects, and further studies taking these variables into account are needed to completely elucidate the exact contribution of genetic background to disease physiopathology.

TMD is a disease with complex traits, in which several other immunoregulatory cytokines and mechanisms are involved. Therefore, it is reasonable to expect that SNPs, even those functional as TNFA-308, exert a significant but not a major role in disease progression, as ascribed to other pathologies.

CONCLUSIONS
This study showed an increased risk of suffering TMD for polymorphic A-allele TNFA-308 carriers and decreased pain sensitivity in the pressure pain threshold test for TMD A-allele homozygotes compared with their ancient allele homozygote counterparts. Future longitudinal studies with large and adequately characterized groups of TMD patients and suitable controls are needed to unveil the precise contribution of the genetic variables to the onset and development of TMDs.

REFERENCES


