Preliminary evidence for conserved transcriptional response to adversity in adults with temporomandibular disorder

Christopher D. King, Ian A. Boggero, Grant S. Schulert, Hannah M. Pickerill, Steve Cole

Abstract

Introduction: Temporomandibular disorder (TMD) is one of the most common orofacial pain conditions. Alteration in immune functioning is one promising biological mechanism underlying pain in TMD. However, there is a gap in the understanding of molecular bases contributing to altered immune functioning in these patients.

Objectives: In the current study, we investigated whether individuals with TMD would exhibit differential activity of 3 specific transcription factors involved in inflammatory (nuclear factor-kappa B, NF-kB), antiviral (interferon-regulatory factors, IRF), and sympathetic (cAMP response element-binding protein, CREB) processes using a promoter-based bioinformatics analysis, which is characterized as the “Conserved Transcriptional Response to Adversity.”

Methods: Adults with TMD (n = 19) and without (n = 17) underwent a standardized clinical examination for TMD. A blood sample was collected for genome-wide transcriptional RNA profiling. Bioinformatic analyses tested for differential prevalence of proinflammatory and antiviral transcription factor activity in core promoter sequences from all genes showing >1.2-fold differential expression in TMD vs controls.

Results: Promoter-based bioinformatic analyses of genome-wide transcriptome profiles confirmed upregulation of genes bearing response elements for proinflammatory transcription factor (NF-kB, \( P = 0.002 \)) and downregulation of genes with response elements for IRF (\( P = 0.037 \)) in patients with TMD relative to controls. Results also indicated upregulated activity of CREB in patients with TMD (\( P = 0.08 \)), consistent with increased activity of the sympathetic nervous system.

Conclusion: These results provide initial support that the regulation of immune pathways is altered in individuals with TMD. A shift of transcriptional resources to a proinflammatory state may be driven by psychosocial stress and contributes to symptoms associated with TMD.

Keywords: Pain, Inflammation, TMD, Gene expression

1. Introduction

Chronic temporomandibular disorder (TMD), characterized by muscle and/or joint pain of the masticatory system lasting greater than 3 months, affects up to 5% of the population. Although a number of psychobehavioral (somatic awareness, stress, and sleep) stressors contribute to TMD, alterations in the immune system have been proposed as a key biological mechanism in chronic pain and TMD. However, there is a current gap in the understanding of the molecular basis contributing to alterations in immune system in individuals with chronic pain. The “Conserved Transcriptional Response to Adversity” (CTRA) profile, characterized by an upregulation of genes bearing response elements for the proinflammatory transcription factor

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expression of genes underlying the sympathetic nervous system
explored other aspects of the CTRA profile, including differential
for transcription factors, compared with controls. We also
would exhibit elevated CTRA expression, which will be charac-
current study tested the hypothesis that individuals with TMD
observed with peripheral measurements of cytokines. The
components of the immune system, which may not be readily
offer a unique window into the functioning of the cellular
proinflammatory gene regulation. Third, the CTRA profile may
relationship between stress and pain involves changes in
sensitive to psychosocial stress, and it is possible that the
syndrome.19 Second, pain conditions such as TMD are highly
psychosocial, behavioral, and/or environmental stress, and consequent increases in activity of the sympathetic
tissue (cAMP response element-binding protein, CREB). This proinflammatory/anti-interferon transcriptome bias,
in turn, can contribute to negative health outcomes related to stress.6,8,21,23,30
For the current study, we investigate whether the activity of 3
specific CTRA-related transcription factors, as indicated by
expression of genes bearing response elements for NF-κB, IRF,
and CREB, differed as a function of TMD status. This is important
for 3 reasons. First, little is known about the relationship of CTRA
to chronic pain states because only one study to date has tested
whether the CTRA profile is present in patients with irritable bowel
Second, pain conditions such as TMD are highly
sensitive to psychosocial stress, and it is possible that the
relationship between stress and pain involves changes in
proinflammatory gene regulation. Third, the CTRA profile may
offer a unique window into the functioning of the cellular
components of the immune system, which may not be readily
observed with peripheral measurements of cytokines. The
current study tested the hypothesis that individuals with TMD
would exhibit elevated CTRA expression, which will be charac-
terized by a differential expression of inflammation (upregulated)
and antiviral (downregulated) genes bearing response elements
for transcription factors, compared with controls. We also
explored other aspects of the CTRA profile including differential
expression of genes underlying the sympathetic nervous system
(SNS)-responsive CREB signaling pathway because altered
β-adrenergic signaling has been implicated in TMD.

2. Methods
Detailed information about participants and methods can be
found in Supplementary Information (available at http://links.lww.
com/PR9/A89).

2.1. Participants
After informed consent and health history review, all participants
underwent a standardized clinical examination (DC-TMD) by a
calibrated investigator for TMD.5,34 In participants with TMD (n =
19) and pain-free healthy controls (HCS, n = 17), a blood sample
was collected for gene expression profiling (Fig. 1A). Information
about sex, age, and history of smoking and alcohol consumption
were collected and used as covariates (Table 1).

2.2. Gene expression profiling and analysis
After RNA extraction (Fig. 1B), genome-wide transcriptional
profiling of RNA samples was performed (Fig. 1C). For the gene
expression analysis, we first excluded genes with minimal level (or
variation) in expression and then identified a set of genes with a
>1.2-fold differential expression in TMD vs controls (Supple-
mental Tables, available at http://links.lww.com/PR9/A89). Due
to the limited size of the sample and the hypothesis-testing nature
of this study, we did not perform any exploratory/discovery
analysis or statistical testing of individual gene transcripts, but
rather this gene set served as input to the Transcription Element
Listening System (TELiS) promoter-based bioinformatic analy-
ses15 to explore associated transcription factor activity (Fig. 1D)
and the CTRA profile (Fig. 1E). As in previous research, point
estimates of differential expression served as input because previous research has found such lists to generate more reliable
downstream bioinformatics results than gene lists derived from statistical p-/q-values.17,36,47

3. Results
3.1. Covariates
Sex ($\chi^2 = 0.56, P = 0.46$), history of alcohol use ($\chi^2 = 2.5, P =
0.11$), BMI ($t = 0.3, P = 0.79$), and age ($t = 1.9, P = 0.07$) were
similar between the 2 groups. Differences were observed with
race ($\chi^2 = 4.5, P = 0.03$) with a higher percentage of Whites in the
TMD group. None of the participants reported a history of smoking (current or past), so this variable was not included as a
covariate in the transcriptome analysis.

3.2. Transcription factor activity
Genome-wide transcriptional profiling by RNA sequencing
identified 1173 gene transcripts showing >1.2-fold differential
expression in TMD vs HCs (80 upregulated and 1093 down-
regulated). TELiS bioinformatics analysis of transcription control
pathways (Fig. 2) indicated significant upregulation of genes with
response elements for the proinflammatory transcription factor
NF-κB (2.138 log2 TFBM ratio $\pm$ 0.694 standard error, $P = 0.002$
and significant downregulation of genes with IRF response
elements ($-1.135 \pm 0.541, P = 0.037$) in adults with TMD
compared with HCs. Results also indicated a nonsignificant trend
upregulation of the SNS-responsive CREB signaling
pathway in patients with TMD ($1.092 \pm 0.624, P = 0.08$).

4. Conclusions
In this small pilot study, we found preliminary evidence that patients
with TMD show a CTRA-characteristic shift in gene regulation
within the basal leukocyte transcriptome including greater proin-
flammatory activity, reduced antiviral activity, and greater adren-
ergic signaling. These 3 transcription control pathways were
targeted a priori based on previous hypotheses linking pain to
stress biology and inflammation and were quantified by asymmet-
rical distribution of binding sites for the targeted transcription
factors in upregulated vs downregulated genes.4,8,21,23,30 Consis-
tent with the classic CTRA profile, patients with TMD showed
upregulation of genes bearing response elements for the proin-
flammatory transcription factor NF-κB, suggesting elevated levels
of NF-κB activation and a propensity for proinflammatory
responses. In addition, patients with TMD showed downregulation
of genes bearing response elements for interferon regulatory
factors (IRF), and the primary inverse component of the CTRA
profile.9,10 These results were observed after controlling for
demographic and behavioral factors that might otherwise con-
found relationships between TMD and gene expression.
Evidence for proinflammatory shift in our current sample is supportive of a growing literature about the presence of low-grade inflammation (eg, basal inflammation and exaggerated inflammatory activity)\(^1\)–\(^3\),\(^4\) in chronic pain. Although some of this literature is conflicting,\(^1\) low-grade inflammation can be driven by toll-like receptor-4 (TLR4) signaling,\(^6\),\(^18\) which induces the transcription factor NF-κB. Our preliminary data support the presence of low-grade inflammation, as reflected by NF-κB, in TMD. Also, limited evidence suggests that interferon signaling pathways can distinguish individuals with pain,\(^27\) and infections have been shown to be predictive of widespread pain.\(^22\) Finally, these CTRA-characteristic shifts may be related to altered β-adrenergic signaling due to chronic activation of the SNS by psychobehavioral stressors\(^11\),\(^14\),\(^15\),\(^20\),\(^39\),\(^48\). Recent studies have highlighted favorable impacts of β-adrenergic antagonist medication in TMD.\(^42\),\(^43\) Our preliminary data found directional evidence for CREB activation in TMD, which would be consistent with increased SNS activity in TMD.

Although findings provide initial support for the hypothesis of altered inflammatory and antiviral pathway activity in individuals with TMD, the study is preliminary and has several limitations. First, our current study was not large enough to support any hypothesis-free genome-wide discovery analysis. Consequently, any attempts to interpret the role of specific, differentially expressed genes will require a larger sample size. Second, our gene expression was measured in peripheral blood samples. Although the observed transcriptional shift occurred in total blood leukocytes, future studies should collect information about the leukocyte subset composition of peripheral blood (eg, by flow cytometry) and clarify whether other tissues associated with TMD also show similar transcriptional shifts.

Overall, a shift of transcriptional resources of immune cells in individuals with TMD to a proinflammatory state may contribute to somatic symptoms (pain and fatigue), although it is also possible that chronic pain causes SNS activation (through stressful experience). In addition to limiting healing of damaged tissue of the temporomandibular joint and muscle,\(^26\) this shift to a proinflammatory state of peripheral immune cells in individuals with TMD may result in a direct\(^45\),\(^48\) and/or indirect (eg, synovial fluid of the temporomandibular joint\(^25\)) enhancement of neuronal sensory processing. Preclinical evidence suggests that elevated levels of peripheral inflammation lead to activation of glial cells in peripheral (eg, trigeminal ganglia) and central nervous systems,\(^46\) which can increase release of inflammatory mediators and amplify neuronal excitability, which in turn leads to an increased hypersensitivity to painful stimuli within these neuronal structures.\(^28\),\(^44\) Future research blocking the pathways identified here and/or manipulating pain or distress may help clarify the causal

### Table 1

| Characteristic | TMD* (n = 19) | HCs (n = 17) | Cohen's d |
|---------------|--------------|-------------|-----------|
| Mean (SD) or % |              |             |           |
| Study covariates† |              |             |           |
| Age (Years) | 31.2 (6.4) | 27.4 (6.1) | 0.62      |
| BMI (kg/m\(^2\)) | 25.8 (7.7) | 25.2 (5.1) | 0.09      |
| Sex (% female) | 78.9% | 88.2% | —         |
| Race (% NHW)‡ | 89.5% | 58.8% | —         |
| Alcohol consumption (%yes) | 42.1% | 17.6% | —         |

*All participants with TMD meet criteria for mixed facial pain diagnosis (myofascial and arthralgia).
†Although the study did not restrict enrollment into the study for smoking, none of the enrolled participants report a history of smoking.
‡Significant group differences: \(P<0.05\).
TMD, temporomandibular disorder.
mechanisms involved. Despite these limitations, our results identify new candidate molecular pathways that can be targeted in future research examining pathogenesis of TMD pain.

Disclosures
G. Schulert has received consulting fees from Novartis and SOBI. The remaining authors have no conflicts of interest to declare.

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Appendix A. Supplemental digital content
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Transcription Factor Activity
(log2 TFBM ratio: high isolation / low isolation)

| Factor     | Activity (log2 TFBM ratio) |
|------------|----------------------------|
| NFkB       | -0.2                       |
| IRF        | -0.15                      |
| CREB       | -1.0                       |
| IRF        | -0.05                      |
| CREB       | 0.0                        |
| CREB       | 1.0                        |
| CREB       | 1.5                        |
| CREB       | 2.0                        |
| CREB       | 2.5                        |
| CREB       | 3.0                        |
| CREB       | 0.81                       |

Figure 2. Gene expression profiling of components of the CTRA Profile in adults with and without TMD. Data are represented as log2-transformed ratios of transcription factor binding motifs (TFBM) for proinflammatory (nuclear factor-kappa B, NF-kB), antiviral (interferon-regulatory factor, IRF), and sympathetic (cAMP response element-binding protein, CREB) transcription factors of differentially expressed genes, which showed a >1.2-fold difference in average transcript abundance between adults with TMD and controls. Because the study was interested in differences in molecular mechanisms (eg, transcription factors), we did not explore if individual genes were different between TMD and HCIs. IRF, interferon response factor; TMD, temporomandibular disorder.
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