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Received: 16 July 2023 | Revised: 22 January 2024 | Accepted: 5 February 2024

DOI: 10.1111/joor.13663

ORIGINAL ARTICLE

JOURNAL OF ORAL REHABILITATION WILEY

Association between oxidative stress and chronic orofacial pain and potential druggable targets: Evidence from a Mendelian randomization study

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Funding information
The Fundamental Research Funds for Central Universities of the Central South University, Grant/Award Number: 2022ZZTS0913

Abstract

Background: Oxidative stress indicators affect chronic orofacial pain (COFP), but how to reduce these effects is uncertain.

Objectives: 11 oxidative stress biomarkers were collected as exposures, while four forms of COFP were chosen as outcomes for Mendelian randomization (MR) study.

Methods: The effect estimates between oxidative stress and COFP were calculated using inverse variance-weighted MR (IVW-MR). Then, functional mapping and annotation (FUMA) was utilized in order to carry out SNP-based functional enrichment analyses. In addition, the IVW-MR method was applied to combine effect estimates when using genetic variants associated with oxidative stress biomarkers as an instrument for exploring potential druggable targets.

Results: The results indicated that oxidative stress biomarkers (causal OR of uric acid (UA), 0.998 for myofascial pain, 95% CI 0.996–1.000, $p < .05$; and OR of glutathione transferase (GST), 1.002 for dentoalveolar pain, 95% CI 1.000–1.003, $p < .05$) were significantly linked with the probability of COFP. Functional analysis also demonstrated that UA and myofascial pain genes were prominent in nitrogen and uracil metabolism, while GST and dentoalveolar pain genes were enriched in glutathione metabolism. Also, the study provided evidence that solute carrier family 2 member 9 (SLC2A9) and glutathione S-transferase alpha 2 (GSTA2) cause discomfort in the myofascial pain (OR = 1.003, 95% CI 1.000–1.006; $p < .05$) and dentoalveolar region (OR = 1.001, 95% CI 1.000–1.002; $p < .05$), respectively.

Conclusions: In conclusion, this MR study indicates that genetically predicted myofascial pain was significantly associated with decreased UA and dentoalveolar pain was significantly associated with increased GST level. SLC2A9 inhibitor and GSTA2 inhibitor were novel chronic orofacial pain therapies and biomarkers, but clinical trials are called to examine if these oxidative biomarkers have the protective effect against orofacial pain, and further research are needed to explore the underlying mechanisms.

KEYWORDS
chronic orofacial pain 2, mendelian randomization 5, molecular drug 4, oral disease 3, oxidative stress 1

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1 | INTRODUCTION

With a frequency of about 25% of the population, orofacial pain is one of the most serious pain disorders worldwide, including pain in the teeth and gums, the muscles, the temporomandibular joint, the cranial nerves, pain that is similar to primary headaches, and pain that has no known cause.[1] Orofacial pain has a mixed pain phenotype (nociceptive, neuropathic and neoplastic or dysfunctional pain), and the mechanism underlying their aetiology and pathogenesis is not well understood.[2] Latest research has revealed that because of the complexity and lack of clarity of the detailed mechanisms many pains are difficult to identify and treat conditions and become chronic.[3] It is estimated that 7%–11% of orofacial pain develops chronic because of poorly managed acute recurrent orofacial pain.[4] Patients suffering from orofacial neuropathic pain often do not find satisfactory alleviation despite the availability of many pain management options.[5] Since it can affect mood and severely worsen the patient's quality of life, the management of chronic orofacial pain is the most important factor in medicine and dentistry. Thus, the pathophysiology and effective treatment options for chronic orofacial discomfort are still being researched.

There is mounting evidence that one's oxidative stress level is crucial to health.[6] Hyperalgesia and allodynia are two painful conditions that patients experience because of oxidative stress. This stress can be caused by either an increase in the production of reactive oxygen species or a decrease in the body's antioxidant defences.[7] Recent research has shown that individuals with the temporomandibular joint disorder (TMD), one of the most common chronic orofacial pain disorders, have considerably increased levels of indicators of oxidative stress and a commensurate decrease in total antioxidant capacity.[8] Furthermore, hyperalgesia and allodynia in orofacial pain have been linked to elevated levels of prooxidative substances like nitric oxide, lipid peroxidation and mitophagy.[9] A previous study suggested chronic orofacial pain is significantly influenced by oxidative stress indicators.[10] These indicated that oxidative stress indicators may serve as essential mediators of chronic orofacial pain. In addition, antioxidant treatment has recently been shown to have new applications in the prevention and management of chronic orofacial pain patients. Improved treatment outcomes are discussed, including the use of dietary changes,[11] hyperbaric oxygen therapy[12] and antioxidative drugs.[13] According to these findings, reducing the harmful effects of oxidative stress on facial neurons may be an effective treatment of chronic orofacial pain.

The antioxidant defence system is important for regulating oxidative stress, including enzymatic and non-enzymatic antioxidants. The primary antioxidant systems in the cell are enzymatic antioxidants, which primarily include glutathione transferase (GST), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX).[14] Non-enzymatic antioxidants include the main extracellular fluid antioxidant system, glutathione (GSH), vitamins (such as vitamins A, E and C), uric acid (UA), albumin, total bilirubin and some metal ions, such as zinc. Above antioxidants have also been shown to be linked to pain oral facial. In a rat neuropathic pain model, CAT, GSH and GST levels significantly decreased.[15,16] Additionally, it was demonstrated that GPX decreased in female rats after stimulating nociceptive pain.[17] According to research by Hae et al., slice pretreatment with ROS scavengers like SOD or CAT reduced the nociceptive pain in the orofacial area in a rat model.[7] However, CAT, SOD and total antioxidant capacity between TMD patients and healthy controls did not differ significantly in clinical studies.[18] Following UA injection into the rabbit's temporomandibular joint, hyper-analgesic relief over the craniofacial region was found.[19] Rats' pain thresholds were upregulated along with a substantial increase in bilirubin in both their cerebrospinal fluid and serum.[20] In dental clinics, zinc is consistently and frequently used as an analgesic,[11] and anecdotal placebo-controlled studies using tocopherol revealed short-term pain alleviation,[21] the results listed above imply that enzymatic antioxidants and non-enzymatic antioxidants are involved in the mechanism of chronic pain and can be exploited as possible therapeutic targets; however, further clinical validation is required because the majority of research is done using animal models.

The Mendelian randomization (MR) study is a technique that analyses the probable causal relationships between environmental factors and outcomes by employing genetic variants in the role of instrumental variables (IVs) for those factors.[22] This strategy can reduce the influence of confounding factors and eliminate the reverse causation bias. Genetic variants are assumed to be inherited at random, and alleles are not influenced by diseases. As a result, in this study, we utilized a two-sample MR analysis to investigate whether or not oxidative stress biomarkers (enzymatic antioxidants and non-enzymatic antioxidants) and chronic orofacial pain are associated with one another. In addition, an investigation was carried out to determine the possible druggable targets of oxidative stress on chronic orofacial pain.

2 | MATERIALS AND METHODS

2.1 | Study design

In order to identify the association and possible druggable targets for oxidative stress indicators in patients with chronic orofacial pain, a MR design was utilized (Figure 1). A collection of oxidative stress biomarkers included GST, CAT, SOD and GPX, as well as uric acid (UA), zinc, alpha-tocopherol, ascorbate, retinol, albumin and total bilirubin; chronic orofacial pain included: facial pains for 3+ months (facial pain), TMD muscular pain linked with fibromyalgia (myofascial pain), TMD- related pain (temporomandibular joint (TMJ) pain) and mouth/teeth dental problems: painful gums (dentoalveolar pain). To begin, an MR design was utilized in order to investigate the potential causes of three distinct types of chronic orofacial discomfort caused by 11 different oxidative stress indicators. The following is a list of the MR assumptions: (1) the single nucleotide polymorphisms (SNPs) from genome-wide

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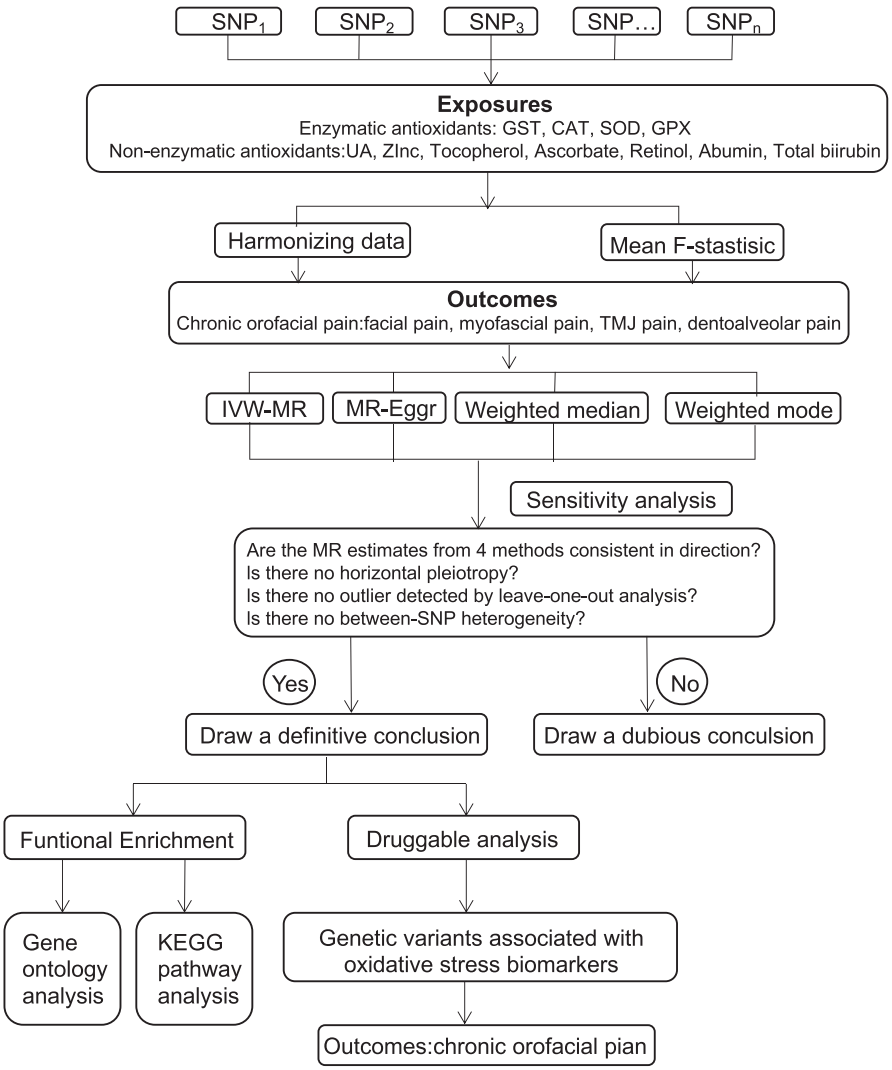


FIGURE 1 Flow chart of a Mendelian randomization study design. Notes: CAT, catalase; dentoalveolar pain, mouth/teeth dental problems: painful gums; facial pain, Facial pains for 3+ months; GPX, glutathione peroxidase; GST, glutathione S-transferase; IVW-MR, inverse variance weighted MR; MR, Mendelian randomization; myofascial pain, TMD muscular pain linked with fibromyalgia; SNP, single nucleotide polymorphism; SOD, superoxide dismutase; TMJ pain, TMD related pain; UA, uric acid.

association studies (GWAS) applied as instrumental variables (IVs) are related to exposures; (2) the IVs are not associated with the confounders; and (3) the IVs affect the risk of outcome only by the exposure. Following that, functional mapping and annotation (FUMA) was utilized in order to carry out SNP-based functional enrichment analyses. In addition, the inverse-variance-weighted (IVW-MR) method was applied in order to combine effect estimates when using genetic variants associated with oxidative stress biomarkers as an instrument for exploring potential druggable targets in chronic orofacial pain. This was done in order to determine whether or not these biomarkers could be treated with medication.

2.2 | Datasets

The available summarized data were obtained from the open database (The NHGRI-EBI Catalogue of human genome-wide association studies: https://www.ebi.ac.uk/gwas/, The Finngen GWAS summary statistics: https://www.finngen.fi/en/access_results and The IEU OPEN GWAS PROJECT: https://gwas.mrcieu.ac.uk/) (accessed on 1st April 2023). Only the data that were summarized for the European population were used in this study so that we could eliminate any potential for bias caused by the heterogeneity of the population. Table S1 contains a description of the information that is specific to the GWAS data sets.

2.2.1 | GWAS summary datasets with oxidative stress biomarkers

The research of Zhe et al. served as the basis for selecting the 11 oxidative stress biomarkers from the most recent GWAS.[23] Data of GST, CAT, SOD and GPX were obtained from the INTERVAL study[24]; UA data were referred from the UK biobank[25]; zinc data were derived from a data set that was established for MR-Base and was produced by a number of different consortia[26]; the information about tocopherol, ascorbate and albumin was taken from the Twins UK cohort and the KORA project[27]; the data collected on the retinal focused on older people of European descent[28]; The Vanderbilt University Medical Center provided the total bilirubin data that was used in this study.[29]

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2.2.2 | GWAS summary datasets with chronic orofacial pain

The criteria provided by the International Classification of Orofacial Pain (ICOP) served as the foundation for the compilation of summary data on chronic orofacial pain. Facial pain, myofascial pain, TMJ pain and dentoalveolar pain were selected as outcomes.[30] The following is a breakdown of the sample sizes for each category: facial pain (3107 cases and 3403 controls), myofascial pain (2530 cases and 216 262 controls) TMJ pain (8907 cases and 333 592 controls) and dentoalveolar pain (13 314 cases and 447 799 controls).

2.2.3 | Genetic correlation analysis

Through the use of linkage disequilibrium (LD) score regression, genetic correlations between oxidative stress biomarkers and the outcomes of patients with chronic orofacial pain were determined. It was possible to assess the LD structure of European populations because to the 1000 Genome Project Phase 3 data. SNPs that had a minor allele frequency of 0.05 or above were eliminated from the analysis using a subset of 1000 Genomes and HapMap3 which had 1.1 million variations. Independent SNPs ($p < 5 \times 10^{-6}$, $r^2 < .001$, and distance >10 000 kb) associated with exposures were acquired.[31] Statistics based on the letter F were utilized in order to look for weak instrumental variables. Moreover, each and every one of the included SNPs had an F statistic that was higher than 10. After removing the individuals who had conflicting alleles, the effects of SNPs, including their values and orientations, were compared and harmonized across each pair of data sets. The results, including all of their specifics, are available in the (Table S2).

2.2.4 | Selection of genetic instruments for potentially druggable genes

We additionally proposed an instrument by selecting SNPs within 100 kb windows from target genes for oxidative stress biomarkers that were significantly associated with chronic orofacial pain at a genome-wide significance level ($p < 5 \times 10^{-8}$) to proxy the exposure of oxidative stress drugs.[32] Only common SNPs with MAFs greater than 1% were considered for inclusion. This was done in order to further explore the potential causal effects of potentially druggable genes of oxidative stress biomarkers on chronic orofacial pain.

2.3 | Statistical analysis

The Two-Sample MR package found in the R program, version 4.1.0, was utilized in order to carry out the processes as follows:

2.3.1 | Primary MR analysis

The IVW model was utilized for most investigations because it presupposes an intercept value of zero and determines the level of causality through a fixed-effect meta-analysis. In order to evaluate the sensitivity of the causal linkages, multiple MR approaches that are complementary to one another were utilized, such as the MR-Egger method, the weighted median method and the weighted mode method. A comparison was made between the directions and magnitudes of their effect estimations and those of the IVW (Table S3).[33] *P*-values that were less than .05 was taken into consideration as possible evidence of a causal connection between the two variables.

2.3.2 | Sensitivity analysis

MR-Egger regression and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) analysis was used to assess the potential horizontal pleiotropy of the SNPs used as instrument variants. And we examined the possibility of heterogeneity by employing Cochran's *Q* test. A significance level of $p < .05$ suggests that there is evidence of horizontal pleiotropy.[34] The leave-one-out sensitivity method was utilized in order to ascertain whether or not a particular genetic locus had an effect on the random estimates. In addition, scatter plots and funnel plots were constructed for the purpose of additional analysis. The condition that the outcome can only be affected by the exposure-related SNPs themselves is an essential component of the prerequisite for the MR technique.

2.4 | Functional enrichment analysis

In the first step of our research, we constructed oxidative stress biomarker-driven pathways in order to regulate chronic orofacial pain. We began by performing functional annotation analysis with FUMA, which was accessed on 1 April 2023. As a criterion for classifying lead SNPs, we decided to use a threshold of r^2 less than 0.1.[35] For the purpose of this study, the LD estimates were computed using the reference panel from phase 3 of the 1000 genomes project, which consisted of 503 European people. Only gene–trait relationships that were significant in both the primary and sensitivity analyses were taken into consideration as probable causative genes.[36] Then, using the Kyoto Encyclopedia of Genes and Genomes, we found significant Gene Ontology (GO) terms associated with chronic orofacial pain. These terms included biological processes, molecular functions, cellular components and potential pathways (KEGG). For the purposes of the functional enrichment study, those GO keywords and KEGG pathways were considered significant if they achieved adjusted *p*-values of less than .05.

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3 | RESULTS

In order to identify the association and possible druggable targets for oxidative stress indicators in patients with chronic orofacial pain, a MR design was utilized (Figure 1).

3.1 | Causal effect of genetically predicted oxidative stress biomarkers on chronic orofacial pain

The primary MR results showed there was no significant correlation effect of oxidative stress biomarkers on facial pain (all $p > .05$) (Table S3). However, part of the oxidative stress biomarkers was associated with myofascial pain and dentoalveolar pain (Figure 2). Genetically predicted UA was associated with low odds myofascial pain (OR = 0.998, 95% CI 0.996–1.000; $p < .05$), while GST was associated with high odds dentoalveolar pain (OR = 1.002, 95% CI 1.000–1.003; $p < .05$). The multiple complementary MR methods aligned in their directions and magnitudes of effects (MR-Egger, weighted median and weighted mode), providing no evidence for pleiotropy.

Pleiotropy and heterogeneity analyses are shown in Table 1. None of the MR-Egger intercepts significantly deviated from 0, with *p*-values of .216 for UA on myofascial pain, and .529 for GST on dentoalveolar pain. By using the MR-PRESSO test, there is no outliers were detected, and the magnitude of all these associations persisted were consistent (OR = 0.998, 95% CI 0.996–1.000 for UA on myofascial pain and OR = 1.002, 95% CI 1.000–1.003 for GST on dentoalveolar pain). Cochran's Q statistic indicated low heterogeneity and more reliability of these SNPs (all $p > .05$). Furthermore, the findings of the sensitivity analysis between UA on myofascial pain, and GST on dentoalveolar pain were consistent (Figure 3). The leave-one-out sensitivity method is shown in Figures S1 and S2.

3.2 | Construction of oxidative stress-driven pathways regulating orofacial pain

There were 746 genes shared between UA and myofascial pain from extensive functional annotation for lead SNPs between UA and myofascial pain (Table S4). GO enrichment analysis showed the genes were significantly enriched in Urate metabolic process (Biological processes), Nucleoplasm part (Cellular component), Active transmembrane transporter activity (Molecular functions), and so on. Specifically, Solute carrier family 2 member 9 (SLC2A9) gene was closely related to the above pathways. KEGG pathways analysis showed that Maturity-onset diabetes of the young pathways and the nitrogen metabolism pathway were related to UA regulation on myofascial pain.

Eight potential associated genes were shared between GST and dentoalveolar pain (Table S5). GO enrichment analysis showed the genes were significantly enriched in glutathione metabolic process (Biological processes), DNA packaging complex details (Cellular component) and glutathione transferase activity details (Molecular functions) (Figure 4). KEGG pathways analysis showed four pathways relating to oxidative stress regulation, including glutathione metabolism, drug metabolism-cytochrome P450 and 1 pathway relating to Systemic lupus erythematosus (Figure 4). The associated pathway to oxidative stress regulation was driven by three genes (glutathione S-transferase alpha 1 (GSTA1), GSTA2 and glutathione S-transferase alpha 1 (GSTA5).

3.3 | Causal effect of potentially druggable genes of oxidative stress biomarkers on chronic orofacial pain

Table 2 is a description of the information that is available regarding genetic tools for possible druggable genes. We selected SLC2A9 as a target gene for UA level. 23 SNPs were chosen from the SLC2A9 gene as potential UA inhibitors (Table S6). We chose the risk genes that were shared between GST and dentoalveolar pain, and the GSTA1, GSTA2 and GSTA5 genes are the ones that would be investigated further. Five SNPs from the GSTA1 gene, seven from the GSTA2 gene and two from the GSTA5 gene were chosen as potential GST accelerators (Table S7).

IVW-MR results found suggestive evidence for the association between SLC2A9-against UA and the increasing risk of myofascial pain (OR = 1.003, 95% CI 1.000–1.006; $p < .05$). MR-Egger, weighted

Exposure	Outcome	N SNPs	Method	OR (95% CI)	P value
UA	Myofascial pain	348	Inverse variance weighted	0.998 (0.996 to 1.000)	0.048
		348	MR Egger	1.000 (0.996 to 1.003)	0.825
		348	Weighted median	1.000 (0.996 to 1.003)	0.806
		348	Weighted mode	0.999 (0.996 to 1.003)	0.711
GST	dentoalveolar pain	6	Inverse variance weighted	1.002 (1.000 to 1.003)	0.041
		6	MR Egger	1.001 (0.997 to 1.004)	0.798
		6	Weighted median	1.001 (1.000 to 1.003)	0.089
		6	Weighted mode	1.001 (1.000 to 1.003)	0.150

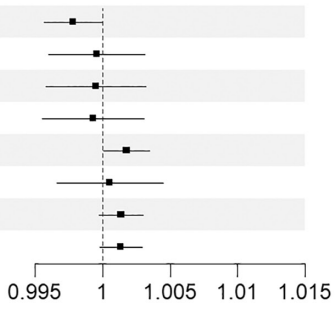


FIGURE 2 Forest plot showing association UA and myofascial pain, and GST and dentoalveolar pain from MR-Egger, weighted median, inverse variance weighted and weighted mode analyses. Notes: dentoalveolar pain, mouth/teeth dental problems: painful gums; GST, glutathione S-transferase; myofascial pain, TMD muscular pain linked with fibromyalgia; UA, uric acid.

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TABLE 1 Pleiotropy and heterogeneity analyses of UA and myofascial pain, and GST and dentoalveolar pain.

Exposures	Outcomes	Nsnp	MR-Egger regression		MR-PRESSO		Heterogeneity analyses		
			Intercept	p_intercept	Global test p	Correct p	Method	Q	Q_value
UA	Myofascial pain	348	−0.004	0.216	0.186	.080	MR-Egger	365.809	0.222
							Inverse variance weighted	367.435	0.216
GST	Dentoalveolar pain	6	0.000	0.529	0.364	.096	MR-Egger	6.813	0.146
							Inverse variance weighted	7.620	0.178

Note: dentoalveolar pain, Mouth/teeth dental problems: Painful gums; GST, glutathione S-transferase; myofascoal pain, TMD muscular pain linked with fibromyalgia; UA, uric acid.

median and weighted mode MR methods aligned in their directions and magnitudes of effects, which further supported a possible dangerous effect of the SLC2A9 gene against myofascial pain (Figure 5). Also, the primary MR results showed that there was no significant association effect of GSTA1 and GSTA5 on dentoalveolar pain (Table S8), while the association between GSTA2-related GST and the increasing risk of dentoalveolar pain (OR = 1.001, 95% CI 1.000–1.002; $p < .05$) was proved by IVW-MR methods (Figure 5).

Furthermore, MR-Egger intercepts indicated no horizontal pleiotropy, with *p*-values of .413 for SLC2A9 on TMD pian and .793 for GSTA2 on dentoalveolar pain (Table 3). By using the MR-PRESSO test, the significance and magnitude of all these associations persisted were consistent (OR = 1.003, 95% CI 1.002–1.005; $p < .05$ for SLC2A9 on TMD pain, and OR = 1.001, 95% CI 1.001–1.002; $p < .05$ for GSTA2 on dentoalveolar pain). Cochran's Q statistic indicated low heterogeneity and more reliability of these SNPs (all $p > .05$). The leave-one-out sensitivity method was shown in Figures S3 and S4.

4 | DISCUSSION

An MR analysis was conducted in the current study to assess the causative relationships and possible therapeutic targets between 11 oxidative stress biomarkers and 4 chronic orofacial pian. The results showed that genetically predicted myofascial pain was significantly associated with lower total UA and dentoalveolar pain was substantially associated with increased GST level. Functional studies also demonstrated that genes associated with UA and myofascial pain were considerably enriched in the pathways controlling nitrogen metabolism and uracil metabolism, while genes associated with GST and dentoalveolar pain were significantly enriched in the glutathione metabolism. The study also offered suggestive evidence for a possible protective effect of SLC2A9 inhibition against myofascial pain and a potential protective effect of GSTA2 antagonist against dentoalveolar pain. These findings pointed to a possible protective effect of SLC2A9 inhibition against myofascial pain and a potential protective effect of GSTA2 antagonist against dentoalveolar pain.

There have been relatively few MR investigations on the topic, despite the fact that several observational studies have highlighted the link between oxidative stress and orofacial pain. These MR studies usually concentrated on antioxidant nutrition, like dried fruit and 25-hydroxyvitamin D, on chronic pain and only performed unidirectional MR studies.[37,38] This is the first time to comprehensively evaluate the significant associations between 11 oxidative stress biomarkers and 3 chronic orofacial pian using an MR analysis. Suggestive correlation was found between UA and the risk of myofascial pain, as well as between GST and the risk of dentoalveolar pain. These correlations suggest that chronic orofacial pain may result from an imbalance between peroxides and antioxidant defence. A previous study discovered a rare case of gout involving the temporomandibular joint in a 51-year-old woman, which showed that UA may be a risk factor for TMD pain.[39] However, under physiological conditions, uric acid can directly remove oxygen free radicals,

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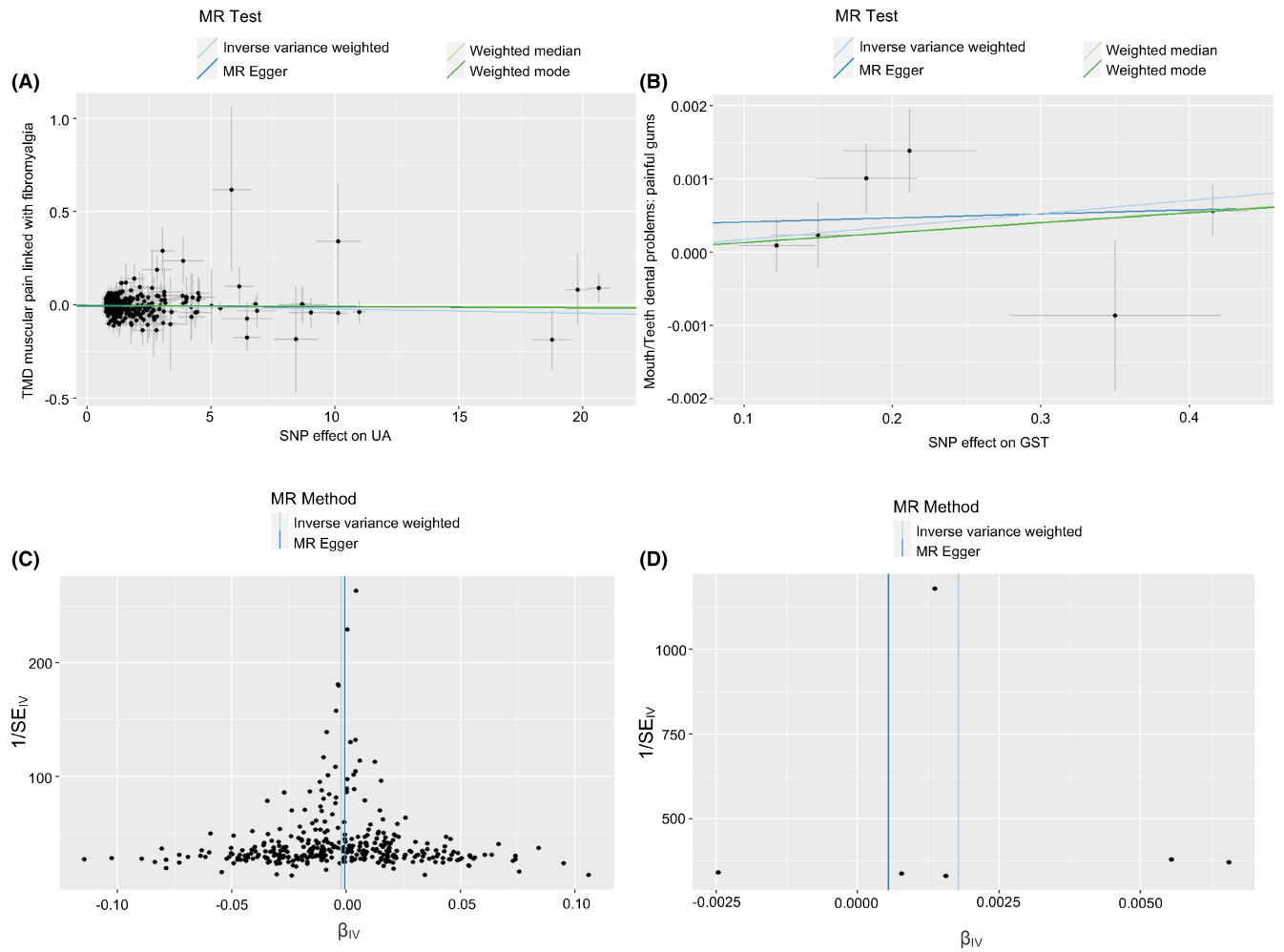


FIGURE 3 Sensitivity analyses on oxidative stress injury biomarkers and the risk of chronic orofacial pain. (A,B) Scatterplots showing association of single variants and slopes from inverse variance weighted, MR-Egger, weighted median analyses and Weighted mode, for UA exposure and myofascial pain outcome, and GST exposure and dentoalveolar pain outcome. (C,D) A funnel plot was applied to detect whether the observed association was along with obvious heterogeneity on UA versus myofascial pain and GST versus dentoalveolar pain. dentoalveolar pain, mouth/teeth dental problems: painful gums; GST, glutathione S-transferase; myofascial pain, TMD muscular pain linked with fibromyalgia; UA, uric acid.

peroxides and singlet oxygen from the body, increase the activity of superoxide dismutase and increase the antioxidant capacity of the body.[40] It is found that peripheral nerve injury usually leads to abnormal chronic pain, and oxidative stress is an important factor leading to neuropathic injury.[41,42] Oxidative stress can activate the calcium/calmodulin-dependent protein kinase II (CAMK2/CaMKII) of glutamatergic neurons in the spinal cord, causing the increase of neuropathic hyperalgesia and the expression of proinflammatory factors in mice.[41] In addition, it is found that the increase in oxidative stress can aggravate the pain by increasing the excitability of neurons.[42] Donghoon et. al found that autophagy damage of astrocytes increased the level of oxidative stress in neurons, which may be caused by the decrease of glutathione level released by astrocytes, and this process plays an important role in maintaining neuropathic pain.[43] Oxidative stress is considered as the inducement of many nervous system diseases, especially migraine.[44] The decrease of antioxidant level will destroy the oxidative balance of migraine patients, which may trigger migraine attacks by causing neurogenic inflammation.[45] Yasin et al. found that UA level decreased in migraine patients, and UA was an effective extracellular free radical scavenger.[46] This evidence suggests UA is more likely to play a protective role as an antioxidant agent in the pathogenesis of oxidative stress and orofacial pain. Recently studies mainly focus on evaluating the effective index of medicine in the treatment of chronic pain through GST levels. For example, the natural compounds in Paclitaxel increased the protective GST levels in the sciatic nerve and spinal cord,[47] as well as the therapeutic effects of Atorvastatin[15] and Streptozotocin.[48] The fact that our findings also suggested that GST was associated with an increased incidence of dentoalveolar pain, while additional epidemiological research and more focused laboratory investigations are required to understand the biochemical and chemical biological underpinnings for these correlations.

Functional analyses showed that 746 genes shared between UA and myofascial pain were significantly enriched in Urate metabolic

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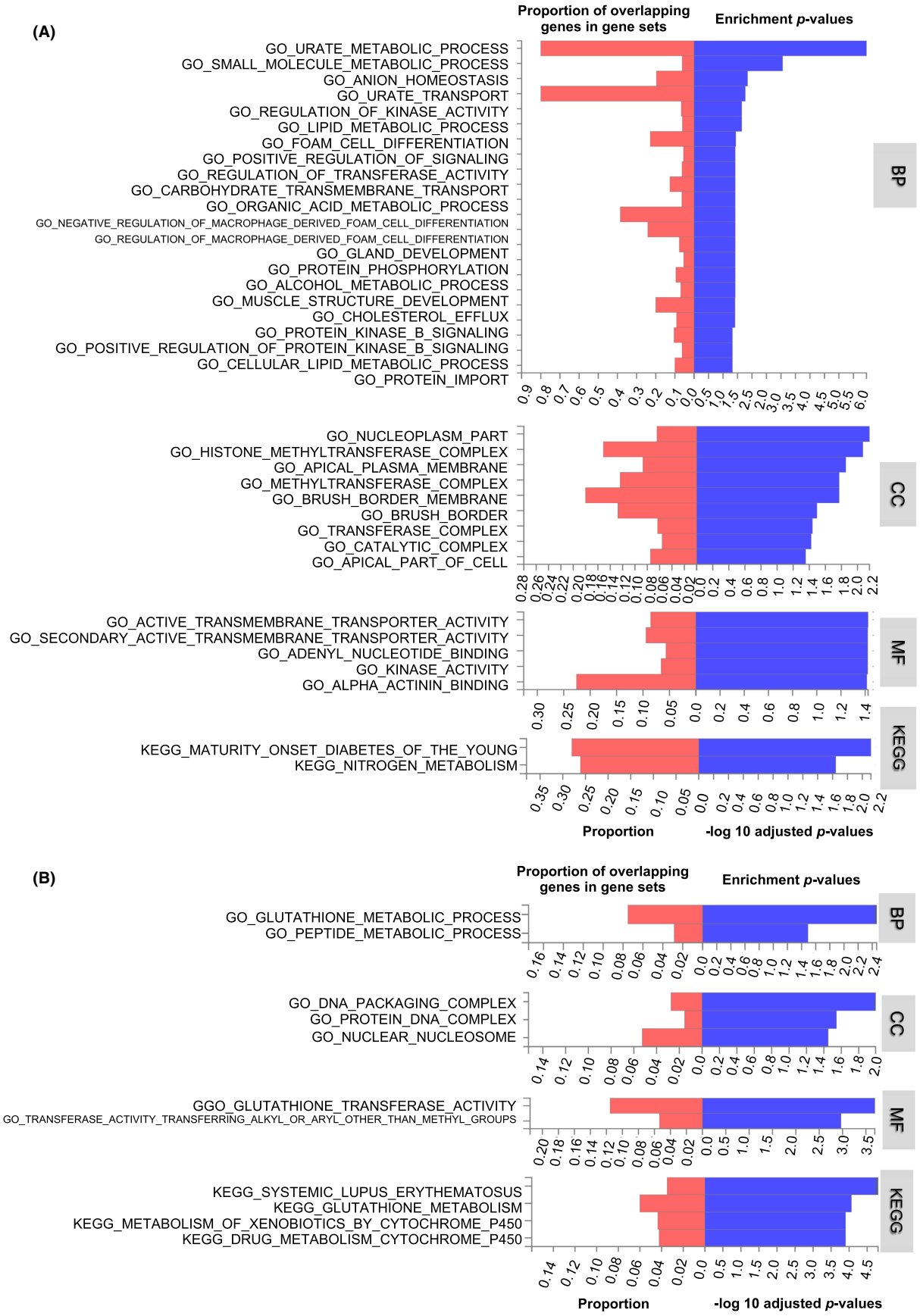


FIGURE 4 GO/KEGG-based pathway analyses from FUMA. (A) GO/KEGG-based pathway analyses of the genes associated with UA and myofascial. (B) GO/KEGG-based pathway analyses of the genes associated with both GST and dentoalveolar pain. Notes: dentoalveolar pain, mouth/teeth dental problems: painful gums; GST, glutathione S-transferase; myofascial pain, TMD muscular pain linked with fibromyalgia; UA, uric acid.

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TABLE 2 Information of genetic instruments for potential druggable genes.

Exposures	Genetic instruments	Outcomes
SLC2A9	Twenty-three common SNPs (MAF >1%) in low linkage disequilibrium ($r^2 < .3$), associated with UA ($p < 5.0 \times 10^{-8}$), located within ±100 kb windows from SLC2A9 region	Myofascial pain
GST	Twenty-three common SNPs (MAF >1%) in low linkage disequilibrium ($r^2 < .3$), associated with glutathione S-transferase ($p < 5.0 \times 10^{-8}$), located within ±100 kb windows from GSTA1, GSTA2 and GSTA5 region	Dentoalveolar pain

Note: dentoalveolar pain, Mouth/teeth dental problemds: Painful gums; GST, glutathione S-transferase; myofascial pain, TMD muscular pain linked with fibromylgia; UA, uric acid.

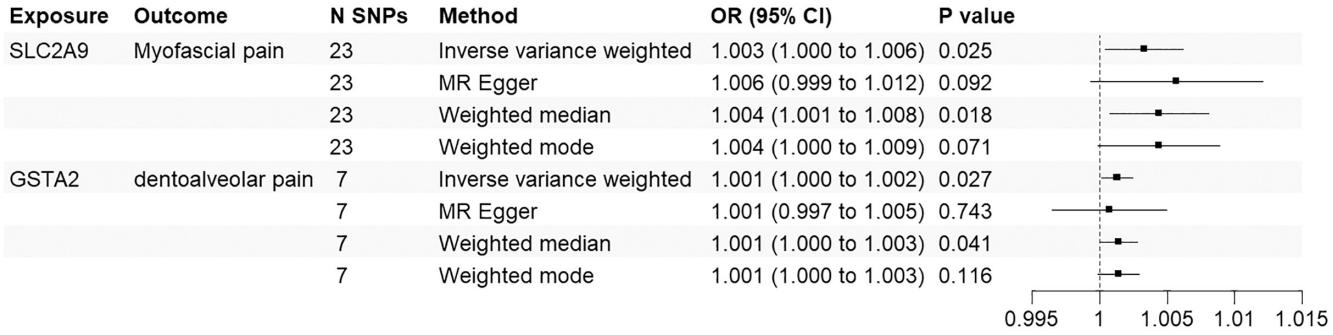


FIGURE 5 Forest plot showing associations between expression of SLC2A9 and GSTA2 for potential druggable genes and the risk of chronic orofacial pain from IVW-MR analysis. Notes: dentoalveolar pain, mouth/teeth dental problems: painful gums; GST, glutathione S-transferase; myofascial pain, TMD muscular pain linked with fibromyalgia; UA, uric acid.

process and regulating the nitrogen metabolism pathway. In human beings, the end product of purines being broken down by the liver, muscles and intestine is called UA, which maintains the equilibrium between generation and excretion and necessitates the presence of specialized transporters in renal proximal tubule cells, intestinal epithelial cells and vascular smooth muscle cells in order for excretion to occur.[49] A, a by-product of purine metabolism, has been linked to a variety of human diseases. There is a negative correlation between illness severity and an elevated blood uric acid level, especially in the context of cardiovascular disease, which is consistent with the tendency of UA to regulate myofascial pain.[50] The production of nitrogen species is characterized by a series of metabolic events that occur collectively in the absence of oxygen and lead to the contentious stimulation of oxidative metabolism.[51] A previous study indicated that UA is produced as a nontoxic soluble vehicle for the elimination of nitrogen originating from the catabolism of amino acids.[52] These indicated that Urate metabolic process and regulating the nitrogen metabolism pathway may be a potential mechanism for UA regulating myofascial pain. Furthermore, GO/KEGG enrichment analysis showed the genes associated with GST and dentoalveolar pain were significantly enriched in glutathione metabolic process, glutathione transferase activity, which was related to oxidative stress regulation. Maintaining antioxidant defence mechanisms in cells, such as redox management, signal transduction and gene regulation, which are disrupted in many disorders, is crucial for maintaining the ratio of glutathione in the reduced (GSH) and oxidized state (GSSG).[53] As an illustration, decreased GSH metabolism and a low GSH/GSSG ratio after oxidative stress are linked to mitochondrial dysfunctions and play a crucial role in the neuroinflammatory and neurodegenerative processes that go along with neurological disease, which is a putative explanation of dentoalveolar pain.[54]

According to the findings of this study, oxidative stress injury biomarkers can cause chronic orofacial pain by disrupting several redox-sensitive signalling pathways, resulting in an imbalance between pro-oxidant species and antioxidant systems. Thus, we address the most recent findings in order to identify novel molecular actors and pharmacological agents that could prevent chronic orofacial pain, which could be translated into novel chronic orofacial pain therapies and biomarkers for patient benefit. The study found suggestive evidence that SLC2A9 was positively related to myofascial pain, showing SLC2A9 inhibition could reduce the risk of myofascial pain. SLC2A9 was shown to transport essentially UA.[55] SLC2A9 constitutive deletion in whole mice causes hyperuricemia and hyperuricosuria.[56] SLC2A9 is a critical player in renal UA handling in vivo and a potential target for uricosuric drugs.[57] Previous study selected 195 participants to discover the possible influencing factors of TMJ pain.[58] Moreover, MR investigation has the potential to overcome the constraints of more conventional forms of epidemiological research, which are observational studies. In this MR study, the exposure to therapy was estimated

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TABLE 3 Pleiotropy and heterogeneity analyses of expression of SLC2A9 and GSTA2 genes and the risk of chronic orofacial pain.

Exposures	Outcomes	Nsnp	MR-Egger regression		MR-PRESSO		Heterogeneity analyses		
			Intercept	*p*_intercept	Global test p	Correct *p*	Method	Q	Q_value
SLC2A9	Myofascial pain	23	−0.029	0.413	0.996	.001	MR-Egger	7.025	0.998
							Inverse variance weighted	7.722	0.998
GSTA2	Dentoalveolar pain	7	0.000	0.793	0.974	.003	MR-Egger	1.122	0.952
							Inverse variance weighted	1.199	0.977

Note: dentoalveolar pain, Mouth/teeth dental problems: Painful gums; GST, glutathione S-transferase; myofascial pain, TMD muscular pain linked with fibromyalgia; UA, uric acid.

through the use of genetic variations associated to SLC2A9-mediated UA as proxies.[58] These results provided some evidence supporting the finding from the largest cohort study for patients with myofascial pain.[59] In the liver, GSTA2, which is a member of the alpha class of genes, is the glutathione S-transferase that is expressed at the highest level. The alpha class of these enzymes not only metabolizes bilirubin and certain anti-cancer medications in the liver,[60] but they also exhibit glutathione peroxidase activity, which protects the cells from reactive oxygen species and the products of peroxidation. GSTA2 is a member of glutathione s-transferase family, which plays an important role in cell antioxidant stress. Oxidative stress is related to ischemic injury. Choi et al. found that GSTA2 has a cell-permeable protein transduction domain, which can protect cell death by inhibiting the production of reactive oxygen species (ROS) and DNA damage induced by oxidative stress. In addition, by constructing an animal model of ischemia in vivo, it is found that GSTA2 can prevent the loss of hippocampal neurons and reduce the activation of microglia and astrocytes. These studies show that GSTA2 can inhibit hippocampal cell death. Therefore, GSTA2 protein may play an important role in ischemic injury induced by oxidative stress.[61] This study explored that GSTA2 genetic variations associated with dentoalveolar discomfort have been investigated as a potential instrument to imitate the exposure of GST. In spite of the fact that the findings suggested that inhibiting GSTA2 would be a potential target for reducing the risk of dentoalveolar discomfort, additional in vivo and in vitro investigations need to be done before the hypothesis can be verified.

This study has several limitations. Firstly, although level of biomarkers in the blood is a way to analyse the oxidative stress, there are no available eQTLs for GSTA2 gene from eQTLGen Consortium, we were unable to investigate the association between GSTA2 expression in blood and dentoalveolar pain outcomes. In addition, although there are available eQTLs in blood for SLC2A9, the top SNP is rs10805346, which appears to have a less significant relationship to the expression of the genes in the PhenoScanner and may provide evidence that is less convincing of the observed association. Secondly, the efficacy of SLC2A9 inhibition and GSTA2 antagonist most likely differs between different subgroups. However, since the use of summary-level data prevented us from performing subgroup analyses, additional MR research utilizing individual-level data is required in order to provide additional information of a more specific nature. Thirdly, although we have undertaken a variety of sensitivity analyses in order to evaluate the assumptions of the MR study, confounding bias and/or horizontal pleiotropy cannot be totally ruled out as causes of the results. Fourthly, the GWAS data that were employed in this investigation were largely collected from European population ancestry; these findings should be regarded with caution when generalizing to other populations. Hence, additional epidemiological research and more focused laboratory investigations are needed to convince evidence linking changes in oxidative stress status to experiencing chronic orofacial pain.

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5 | CONCLUSIONS

In conclusion, this MR study indicates that genetically predicted myofascial pain was significantly associated with decreased UA and dentoalveolar pain was significantly associated with increased GST level. SLC2A9 inhibitor and GSTA2 inhibitor were novel chronic orofacial pain therapies and biomarkers, but clinical trials are called to examine whether these oxidative biomarkers have the protective effect against orofacial pain, and further research are needed to explore the underlying mechanisms.

AUTHOR CONTRIBUTIONS

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used: Conceptualization, validation and formal analysis, S-H.Z. and Y.F.; methodology, M-M.Z.; software, investigation and resources, J-H.X.; data curation, X.X.; writing—original draft preparation and writing—review and editing, S-H.Z.; visualization, M-M.Z.; supervision and project administration, W.X.; funding acquisition, Y.F. All authors have read and agreed to the published version of the manuscript.

FUNDING INFORMATION

The Fundamental Research Funds for the Central Universities of Central South University, grant number 2022ZZTS0913.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interests.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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REFERENCES

1. Ananthan S, Benoliel R. Chronic orofacial pain. *J Neural Transm (Vienna)*. 2020;127(4):575-588.
2. Renton T. Chronic orofacial pain. *Oral Dis*. 2017;23(5):566-571.
3. Matsuka Y. Orofacial pain: molecular mechanisms, diagnosis, and treatment 2021. *Int J Mol Sci*. 2022;23(9):4826.
4. Benoliel R, Sharav Y. Chronic orofacial pain. *Curr Pain Headache Rep*. 2010;14(1):33-40.
5. Fitzcharles MA, Cohen SP, Clauw DJ, Littlejohn G, Usui C, Häuser W. Nociplastic pain: towards an understanding of prevalent pain conditions. *Lancet*. 2021;397(10289):2098-2110.
6. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44-84.
7. Yowtak J, Lee KY, Kim HY, et al. Reactive oxygen species contribute to neuropathic pain by reducing spinal GABA release. *Pain*. 2011;152(4):844-852.
8. de Almeida C, Amenábar JM. Changes in the salivary oxidative status in individuals with temporomandibular disorders and pain. *J Oral Biol Craniofac Res*. 2016;6(Suppl 1):S1-S4.
9. Assavarittirong C, Samborski W, Grygiel-Górniak B. Oxidative stress in fibromyalgia: from pathology to treatment. *Oxid Med Cell Longev*. 2022;2022:1582432.
10. Salvemini D, Little JW, Doyle T, Neumann WL. Roles of reactive oxygen and nitrogen species in pain. *Free Radic Biol Med*. 2011;51(5):951-966.
11. Raza A, Karimyan N, Watters A, Emperumal CP, Al-Eryani K, Enciso R. Efficacy of oral and topical antioxidants in the prevention and management of oral mucositis in head and neck cancer patients: a systematic review and meta-analyses. *Support Care Cancer*. 2022;30(11):8689-8703.
12. Hanna R, Dalvi S, Bensadoun RJ, Benedicenti S. Role of Photobiomodulation therapy in modulating oxidative stress in temporomandibular disorders. a systematic review and meta-analysis of human randomised controlled trials. *Antioxidants (Basel)*. 2021;10(7):1028.
13. Derwich M, Górski B, Amm E, Pawłowska E. Oral glucosamine in the treatment of temporomandibular joint osteoarthritis: a systematic review. *Int J Mol Sci*. 2023;24(5):4925.
14. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. *Eur J Med Chem*. 2015;97:55-74.
15. Pathak NN, Balaganur V, Lingaraju MC, et al. Atorvastatin attenuates neuropathic pain in rat neuropathy model by down-regulating oxidative damage at peripheral, spinal and supraspinal levels. *Neurochem Int*. 2014;68:1-9.
16. Viggiano E, Monda M, Viggiano A, Viggiano A, Aurilio C, De Luca B. Persistent facial pain increases superoxide anion production in the spinal trigeminal nucleus. *Mol Cell Biochem*. 2010;339(1–2):149-154.
17. Rostami Z, Ghasemi S, Farzadmanesh H, Safari M, Ghanbari A. Sex difference in trigeminal neuropathic pain response to exercise: role of oxidative stress. *Pain Res Manag*. 2020;2020:3939757.
18. Madariaga VI, Jasim H, Ghafouri B, Ernberg M. Myogenous temporomandibular disorders and salivary markers of oxidative stress-a cross-sectional study. *J Oral Rehabil*. 2021;48(1):1-9.
19. Okuda-Akabane K. Hyperalgesic change over the craniofacial area following urate crystal injection into the rabbit's temporomandibular joint. *J Oral Rehabil*. 1994;21(3):311-322.
20. Kong E, Wang H, Wang X, et al. Bilirubin induces pain desensitization in cholestasis by activating 5-Hydroxytryptamine 3A receptor in spinal cord. *Front Cell Dev Biol*. 2021;9:605855.
21. Cabras M, Gambino A, Broccoletti R, De Paola S, Sciascia S, Arduino PG. Effectiveness of nonpharmacologic treatments of burning mouth syndrome: a systematic review. *J Oral Facial Pain Headache*. 2021;35(3):175-198.
22. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *JAMA*. 2017;318(19):1925-1926.
23. Lu Z, Pu C, Zhang Y, et al. Oxidative stress and psychiatric disorders: evidence from the bidirectional Mendelian randomization study. *Antioxidants (Basel)*. 2022;11(7):1386.

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24. Sun BB, Maranville JC, Peters JE, et al. Genomic atlas of the human plasma proteome. *Nature*. 2018;558(7708):73-79.
25. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet*. 2021;53(10):1415-1424.
26. Hemani G, Zheng J, Elsworth B, et al. The MR-base platform supports systematic causal inference across the human phenome. *Elife*. 2018;7:e34408.
27. Bulik-Sullivan B, Finucane HK, Anttila V, et al. An atlas of genetic correlations across human diseases and traits. *Nat Genet*. 2015;47(11):1236-1241.
28. Chen Y, Lu T, Pettersson-Kymmer U, et al. Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. *Nat Genet*. 2023;55(1):44-53.
29. Dennis JK, Sealock JM, Straub P, et al. Clinical laboratory test-wide association scan of polygenic scores identifies biomarkers of complex disease. *Genome Med*. 2021;13(1):6.
30. International Classification of Orofacial Pain, 1st edition (ICOP). *Cephalalgia* 2020; 40(2): 129–221.
31. Shin SY, Fauman EB, Petersen AK, et al. An atlas of genetic influences on human blood metabolites. *Nat Genet*. 2014;46(6):543-550.
32. Huang W, Xiao J, Ji J, Chen L. Association of lipid-lowering drugs with COVID-19 outcomes from a Mendelian randomization study. *Elife*. 2021;10:e73873.
33. Lawlor DA, Tilling K, Davey SG. Triangulation in aetiological epidemiology. *Int J Epidemiol*. 2016;45(6):1866-1886.
34. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50(5):693-698.
35. Liu Y, Shen H, Greenbaum J, et al. Gene expression and RNA splicing imputation identifies novel candidate genes associated with osteoporosis. *J Clin Endocrinol Metab*. 2020;105(12):e4742-e4757.
36. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74.
37. Jiang X, Zhou R, He Y, Zhu T, Zhang W. Causal effect of serum 25-hydroxyvitamin D levels on low back pain: a two-sample mendelian randomization study. *Front Genet*. 2022;13:1001265.
38. Huang J, Xie ZF. Dried fruit intake causally protects against low back pain: a Mendelian randomization study. *Front Nutr*. 2023;10:1027481.
39. Bhattacharyya I, Chehal H, Gremillion H, Nair M. Gout of the temporomandibular joint: a review of the literature. *J Am Dent Assoc*. 2010;141(8):979-985.
40. Virdis A, Masi S, Casiglia E, et al. Identification of the uric acid thresholds predicting an increased Total and cardiovascular mortality over 20 years. *Hypertension*. 2020;75(2):302-308.
41. Kim D, You B, Jo EK, Han SK, Simon MI, Lee SJ. NADPH oxidase 2-derived reactive oxygen species in spinal cord microglia contribute to peripheral nerve injury-induced neuropathic pain. *Proc Natl Acad Sci U S A*. 2010;107(33):14851-14856.
42. Grace PM, Gaudet AD, Staikopoulos V, et al. Nitroxidative signaling mechanisms in pathological pain. *Trends Neurosci*. 2016;39(12):862-879.
43. Li J, Tian M, Hua T, et al. Combination of autophagy and NFE2L2/NRF2 activation as a treatment approach for neuropathic pain. *Autophagy*. 2021;17(12):4062-4082.
44. Erdener ŞE, Kaya Z, Dalkara T. Parenchymal neuroinflammatory signaling and dural neurogenic inflammation in migraine. *J Headache Pain*. 2021;22(1):138.
45. Yazar HO, Yazar T, Aygün A, Kaygisiz Ş, Kirbaş D. Evaluation of simple inflammatory blood parameters in patients with migraine. *Ir J Med Sci*. 2020;189(2):677-683.
46. Yasin S, Altunisik E. Decreased serum uric acid level as an indicator of altered oxidative balance in patients with migraine. *Cureus*. 2022;14(12):e32136.
47. Faheem M, Khan AU, Saleem MW, et al. Neuroprotective effect of natural compounds in paclitaxel-induced chronic inflammatory pain. *Molecules*. 2022;27(15):4926.
48. Faheem M, Khan AU, Shah FA, Li S. Investigation of natural compounds for therapeutic potential in Streptozotocin-induced diabetic Neuroinflammation and neuropathic pain. *Front Pharmacol*. 2022;13:1019033.
49. Mandal AK, Mount DB. The molecular physiology of uric acid homeostasis. *Annu Rev Physiol*. 2015;77:323-345.
50. Jin M, Yang F, Yang I, et al. Uric acid, hyperuricemia and vascular diseases. *Front Biosci (Landmark Ed)*. 2012;17(2):656-669.
51. Blokhina O, Fagerstedt KV. Oxidative metabolism, ROS and NO under oxygen deprivation. *Plant Physiol Biochem*. 2010;48(5):359-373.
52. Guder WG, Häussinger D, Gerok W. Renal and hepatic nitrogen metabolism in systemic acid base regulation. *J Clin Chem Clin Biochem*. 1987;25(8):457-466.
53. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr*. 2004;134(3):489-492.
54. Bjørklund G, Peana M, Maes M, Dadar M, Severin B. The glutathione system in Parkinson's disease and its progression. *Neurosci Biobehav Rev*. 2021;120:470-478.
55. Clémençon B, Lüscher BP, Fine M, et al. Expression, purification, and structural insights for the human uric acid transporter, GLUT9, using the Xenopus laevis oocytes system. *PloS One*. 2014;9(10):e108852.
56. Preitner F, Bonny O, Laverrière A, et al. Glut9 is a major regulator of urate homeostasis and its genetic inactivation induces hyperuricosuria and urate nephropathy. *Proc Natl Acad Sci U S A*. 2009;106(36):15501-15506.
57. Auberson M, Stadelmann S, Stoudmann C, et al. SLC2A9 (GLUT9) mediates urate reabsorption in the mouse kidney. *Pflugers Arch*. 2018;470(12):1739-1751.
58. Wang L, Zhang T, Liu Y, Tang F, Xue F. Association of Serum Uric Acid with metabolic syndrome and its components: a Mendelian randomization analysis. *Biomed Res Int*. 2020;2020:6238693.
59. Myllymäki E, Heikinheimo K, Suominen A, et al. Longitudinal trends in temporomandibular joint disorder symptoms, the impact of malocclusion and orthodontic treatment: a 20-year prospective study. *J Oral Rehabil*. 2023;50:739-745.
60. Ng KT, Yeung OW, Lam YF, et al. Glutathione S-transferase A2 promotes hepatocellular carcinoma recurrence after liver transplantation through modulating reactive oxygen species metabolism. *Cell Death Dis*. 2021;7(1):188.
61. Choi YJ, Shin MJ, Youn GS, et al. Protective effects of PEP-1-GSTA2 protein in hippocampal neuronal cell damage induced by oxidative stress. *Int J Mol Sci*. 2023;24(3):2767.

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How to cite this article: Zhang S-H, Feng Y, Zhong M-M, Xie J-H, Xu W. Association between oxidative stress and chronic orofacial pain and potential druggable targets: Evidence from a Mendelian randomization study. *J Oral Rehabil*. 2024;00:1-12. doi:10.1111/joor.13663

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