Journal of Craniomaxillofacial Research

Vol. 10, Issue. 4 Autumn 2023

Evaluation Of The Effect Of Comt Gene Polymorphism In Rs4680 On The Occurrence Of Concomitant Head, Neck And Back Pain In Patients With Temporomandibular Joint Disorders

Shamsoulmolouk Najafi [1], Hasan Roudgari [2,3], Nafise Sheykhbahaei [1], Amir Mohammad Oloumi [4*]

1. Department of Oral Medicine, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.

2. Genomic Research Center, Taleghani Hospital, Shahid Beheshti Medical University, Tehran, Iran.

3. Department of Applied Medicine, Polwarth Building, Medical School, Foresterhill Campus, University of Aberdeen, AB25 2ZD, Uk.

4. School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.

ARTICLE INFO

Article Type:
Original Article

Received: 1 July 2023
Revised: 5 September 2023
Accepted: 18 November 2023

**Corresponding author:*
Amir Mohammad Oloumi

School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.

Tel: +98-21-84902473
Fax: +98-21-84902473
Email: am-oloumi@student.tums.ac.ir

ABSTRACT

Introduction: Temporomandibular disorders (TMD) include a number of clinical conditions involving the masticatory muscles, temporomandibular joint (TMJ), and adjacent structures. The main feature of TMD is pain. According to past studies, TMD can have genetic causes. One of the genes that seems to be important in this regard is COMT. This study aims to compare the effect of COMT gene polymorphism rs4680 on the occurrence of simultaneous head, neck and back pain in patients with TMD.

Materials and Methods: This study was conducted as a case-control study on patients aged between 18 and 65 with TMD disorder. After meeting the conditions for entering the study, a written informed consent was obtained from the people participating in this project. Then a blood sample of 5cc was prepared from each person and poured into tubes containing EDTA anticoagulant and used for DNA extraction and identification. The COMT gene was sent to the genetics laboratory and was compared in terms of the polymorphism of the COMT gene using the PCRARMS technique.

Results: The sample size was 100 people aged 18 to 65 years. Variables with normal distribution were analyzed with Chi-Square test and variables with non-normal distribution with Mann-Whitney test. After genetic analysis in terms of rs4680 gene polymorphism, 18 people (18%) were identified with AA, 80 people (80%) with GA and 2 people (2%) with GG. "temporal headache in the last 30 days", "temporal headache change with chewing hard foods", "temporal headache change with opening the mouth or moving the jaw forward or to the sides", " Temporal headache changes with jaw habits such as teeth touching, pressing or grinding teeth together, chewing gum ", " Temporal headache changes with jaw activities such as talking, kissing or yawning ", " having Back pain during the last 4 weeks", having pain in the arm, leg, joints (neck) during the last 4 weeks", "headache during 4 last weeks" and "time of onset of headache in the temporal region" did not have significant relationships with COMT gene polymorphism rs4680 in patients with TMD. (repectively, p value=0.873، p value=0.658، p value=0.518 p value=0.685، p value=0.884،p value=0.654، p value=0.723، p value=0.831، p value=0.692).

Conclusion: According to previous studies, there is a significant relationship between rs4680 polymorphism in the COMT gene and the occurrence of temporomandibular disorders (TMD), although in our study, no significant relationship was found between headache and neck and back pain with this polymorphism.

Keywords: Temporomandibular joint disorder; Headache; COMT gene; Rs4680.

Please cite this Article as:
Najafi Sh, Roudgari H, Sheykhbahaei N, Oloumi AM. Evaluation Of The Effect Of Comt Gene Polymorphism In Rs4680 On The Occurrence Of Concomitant Head, Neck And Back Pain In Patients With Temporomandibular Joint Disorders. J Craniomaxillofac Res 2023; 10(4): 146-152. DOI: 10.18502/jcr.v10i4.15305

Introduction

Temporomandibular disorders (TMD) encompass a range of clinical conditions that can involve the jaw muscles, the temporomandibular joint (TMJ), and adjacent structures. TMD is the primary cause of non-dental, orofacial pain and is often characterized by persistent facial pain and increased sensitivity to painful stimuli. Various factors, including psychosocial disorders, parafunctional habits, and trauma, are recognized as the etiology of TMD [1].

Epidemiological studies have shown that TMD is a chronic pain condition that can be associated with other common chronic pains, especially headaches, neck pain, shoulder pain, and lower back pain. It has been suggested that headaches are the most common symptom in 22% of TMD patients, while 55% of individuals with chronic headaches complain of TMD symptoms [2]. In most individuals suffering from lower back pain, there is no specific cause, and it is assumed that this pain may have a mechanical or non-specific origin [3]. Chronic pains and TMD have been considered as separate phenomena that may interact with each other as perpetuating and exacerbating factors. Many researchers have proposed the triad relationship hypothesis between parafunctional habits, TMD, and chronic somatic pains. The phenomenon of central sensitization or central sensitization can be influential in the development of effective pain. Heterotopic pains, especially referred pain found in headaches and neck pain, should be successfully treated by identifying and addressing the primary source of pain. Additionally, the central body muscles, including the head, neck, and spine muscles, contract and interact with the surrounding environment to maintain balance, and the contraction of each of these muscles is usually accompanied by the contraction of other muscles.

Various factors can influence pain processing in humans. One of these factors is genetics [16]. Over the past decade, the impact of genetic polymorphisms, alongside environmental factors, has received more attention in the context of TMD. In some genetic epidemiology studies, familial aggregation in certain types of headaches has been examined. The catechol-O-methyltransferase (COMT) gene has been recognized as a potential determinant of pain sensitivity in humans. The protein encoded by COMT is an important enzyme in the catabolic pathway of several pain-related neurotransmitters, including norepinephrine, adrenaline, and dopamine [4]. The activity of the COMT gene is influenced by various polymorphisms, and rs4680 is one of the most common polymorphisms in the COMT gene that has been investigated in previous studies in relation to chronic pain [5]. The findings indicate that haplotypes encompassing this SNP are associated with an increase in pain experience, as well as an increase in postoperative pain intensity and related hyperalgesia [6]. However, it appears that the association of this polymorphism with concurrent head, neck, and back pain has not been studied. This study was conducted with the aim of comparing the effect of the COMT gene polymorphism at rs4680 on the occurrence of concurrent head, neck, and back pain in patients with temporomandibular joint disorders (TMD).

Materials and Methods

The current study received approval from the Medical Ethics Committee of Tehran University of Medical Sciences, with the study protocol being granted approval under reference number IR.TUMS.DENTISTRY.REC.1400.192. Furthermore, all participants provided written informed consent.

Case-control study

We performed a cross-sectional study involving 100 TMD patients. These participants were randomly selected over a period of six months, spanning from October 2019 to April 2020. Based on the research by Furquim et al. (2019) and using a significance level of 5% and a power of 80%, the sample size was determined by assuming 91% dominance of the allele in the case group. Consequently, the minimum required sample size for the study group was 93 participants. A single trained investigator conducted a clinical assessment of all participants using the Axis 1 tool from the research diagnostic criteria for temporomandibular disorders (RDC/TMD), a validated method for diagnosing TMD. Additionally, for TMD patients, psychosocial well-being and factors related to pain, such as headaches, low back pain, and neck pain experienced in the past four weeks, were assessed using Axis II of RDC/TMD.

TMD patients were chosen from those referred to the Department of Oral and Maxillofacial Diseases and Pain at the Faculty of Dentistry, Tehran University of Medical Sciences. Table 1 displays the clinical examination findings for these TMD patients. Inclusion criteria encompassed confirmed TMD diagnoses according to RDC/TMD and obtaining informed consent. Exclusion criteria involved individuals with systemic joint disorders like rheumatoid arthritis, systemic lupus erythematosus, and fibromyalgia, as well as those with a history of orofacial surgery or trauma. Pregnant or

nursing women and individuals with significant medical conditions such as diabetes, cardiovascular diseases, or cancer were also excluded. Subsequently, venous blood samples of 5cc were collected from the antecubital vein using a syringe. The separation of hematocrit from plasma was achieved by centrifuging at 2000 rpm for 10 minutes. These blood samples, stored in special tubes containing 3% citric acid, were maintained at -20°C and sent to a laboratory within 2 hours.

Genotyping

The COMT gene polymorphism rs4680 was amplified and identified using the Tetra ARMS-polymerase chain reaction (T-ARMS-PCR) method, which employed specific internal and external primers (as shown in Table 2). The PCR reaction was conducted in a total volume of 25µL, which included 12.5µL of PCR master mix, 0.5µL for each of the primers, 2.2M betaine, 2µL of the DNA sample, and 7µL of distilled water. This procedure was carried out using the ABI VERRITI thermocycler. The procedure involved a series of denaturation cycles, initially at 95°C for 5 minutes, followed by 10 cycles of 95°C for 30 seconds, 63°C for 35 seconds, and 72°C for 45 seconds. This was followed by an additional 20 cycles of denaturation at 95°C for 30 seconds. Subsequently, 10µl of the PCR product underwent electrophoresis on a 2% agarose gel to determine the genotype. Afterward, a UV transilluminator was employed for gel analysis.

Statistical Analysis

Descriptive statistics and frequency counts were computed to characterize the participants. Chi-square and Fisher's exact tests were employed to assess the relationship between categorical variables. Variables with a normal distribution were analyzed using the Chi-Square test, while variables with a non-normal distribution were analyzed using the Mann-Whitney test. All analyses were performed using STATA version 14. A P-value<0.05 was considered as the threshold of rejection area in all analyses and all reported P-values were two-sided.

Results

In this Cross-sectional study, 100 patients including 23 men and 77 women with a mean age of 36 years (18-65 years) entered the study. After genetic analysis for the rs4680 gene polymorphism, it was determined that 18 individuals (18%) had the AA genotype, 80 individuals (80%) had the GA genotype, and 2 individuals (2%) had the GG genotype. Additionally, allele A (n=115) was more prevalent than allele G (n=85). Among the TMD patients, 18 individuals did not experience headaches, lower back pain, or neck pain, while 25 individuals simultaneously had headaches, lower back pain, and neck pain. No significant relationship was observed between "lower back pain in the past 4 weeks" in TMD patients and the rs4680 gene polymorphism in COMT (p value=0.654). 50% of individuals with the AA genotype had experienced either low or high levels of lower back pain in the past month. 43% of individuals with the GA genotype had experienced either low or high levels of lower back pain in the past month.

No significant relationship was found between "neck pain in the past 4 weeks" in TMD patients and the rs4680 gene polymorphism in COMT (p value=0.723). 44.4% of individuals with the AA genotype had experienced either low or high levels of neck pain in the past month. 49.4% of individuals with the GA genotype had experienced either low or high levels of neck pain in the past month. Similarly, no significant relationship was observed between "headache in the past 4 weeks" in TMD patients and the rs4680 gene polymorphism in COMT (p value=0.831). 55.6% of individuals with the AA genotype had experienced either low or high levels of headache in the past month. 59.5% of individuals with the GA genotype had experienced either low or high levels of headache in the past month (Table 3). A significant relationship between the components of the Graded Chronic Pain Scale Version 2.0 questionnaire and the rs4680 gene polymorphism in COMT in patients was not observed (Table 4).

Table 1. Clinical characteristics of TMD patients (n=100).

Features	*Cases*
*Pain in jaw, temple, ear or front of ear **	*76/5%*
*Recurrent pain**	*59/8%*
*Permanent pain **	*12/7%*
*Mean severity of pain in face according to VAS **	*>5*
Chew tough food #	*33/3%*

DOI: 10.18502/jcr.v10i4.15305

Features	Cases
Opening, lateral and protrusion movement #	17/6%
Press, touch, or hold teeth together other than while eating or chewing gum #	28/4%
Yawn, talk, kiss#	12/7%
Maximum mouth opening without pain ¥	43/78mm
Maximum mouth opening with pain ¥	48mm
Midline deviation to left ¥	2/6 mm (5 cases)
Midline deviation to right ¥	0/82 mm (17 cases)
Pain of temporal muscle during jaw movement ¥	12.32%
Pain of masseter muscle during jaw movement ¥	36.96%
Pain of TMJ during jaw movement ¥	46.36%
Click with or without pain ¥	16.92%
Pain of Posterior mandibular region	43.65%
Pain of Submandibular region ¥	19.1%
Pain of Lateral pterygoid area ¥	42.65%
Pain of Temporalis tendon ¥	38.25%
Straight Opening pattern ¥	70.6%
Corrected deviation ¥	24.8%
Uncorrected deviation ¥	1%

TMD: temporomandibular disorder, VAS: visual analogue scale, *: In the last month, #: the pain gets worse during …, ¥: The average of.

Table 2. PCR primers of COMT gene.

	COMT rs4680
Forward inner primer	GGATGGTGGATTTCGCTGTCA
Reverse inner primer	AGGCATGCACACCTTGTCCTTAAC
Forward outer primer	GAGGGGCTCCTGCTCTTTGG
Reverse outer primer	CTGGGGGGGTCTTTCCTCAG

Table 3. The association between headache, back pain, and neck pain in TMD patients with COMT gene polymorphism (rs4680).

	AA (n=18)	GA (n=80)	GG (n=2)	P.value
Headache	55.6% (n=10)	59.5% (n=48)	100% (n=2)	0.831
Back pain	50% (n=9)	43% (n=27)	100% (n=2)	0.654
Neck pain	44.4% (n=8)	49.4% (n=39)	0% (n=0)	0.723

Table 4. Association between components of the graded chronic pain scale version 2.0 and the rs4680 comt gene polymorphism in TMD patients.

Graded Chronic Pain Scale Version 2.0	Mann-Whitney U Asymp. Sig. (2-tailed)
How many days in the last 6 months have you had facial pain	0.981
How would you rate your facial pain right now?	0.525
In the last 30 days, how would you rate your worst facial pain?	0.770
In the last 30 days, on average, how would you rate your facial pain?	0.764

Graded Chronic Pain Scale Version 2.0	Mann-Whitney U Asymp. Sig. (2-tailed)
In the last 30 days, how many days did your facial pain keep you from doing your usual activities?	0.828
In the last 30 days, how much has facial pain interfered with your daily activities?	0.473
In the last 30 days, how much has facial pain interfered with your recreational, social and family activities?	0.360
In the last 30 days, how much has facial pain interfered with your ability to work, including housework?	0.664

Discussion

Based on the findings of the current study, a significant relationship between the COMT gene polymorphism (rs4680) and headaches, lower back pain, and neck pain in TMD patients was not observed. The GA, AA, and GG genotypes had the highest prevalence among TMD patients, respectively. Additionally, allele A had a higher frequency compared to allele G. It appears that the substitution of allele G with A (substituting methionine for valine) may increase the risk of TMD. TMD is a primary cause of non-dental orofacial pain and is often characterized by persistent facial pain and heightened sensitivity to painful stimuli in different areas. Patients with persistent facial pain are alerted to oral dysfunction and an increased sensitivity to painful stimuli in various regions. There is evidence to suggest that genetic factors may explain the variability in pain sensitivity in TMD patients. The catechol-O-methyltransferase gene (COMT), which encodes an enzyme involved in metabolizing catecholamines such as epinephrine, norepinephrine, and dopamine, is one of the genes implicated in regulating pain perception. TMD patients demonstrate the lowest COMT activity compared to pain-free controls [7]. Different mutations, functional SNPs, and common haplotypes in the COMT gene have been associated with neuropsychiatric disorders like schizophrenia or mood disorders [8]. Depression and psychiatric disorders are considered one of the main areas of TMD disease, exacerbated by parafunctional oral-dental habits.

The polymorphism of the COMT gene rs4680 leads to the substitution of an amino acid in the enzyme's peptide chain, resulting in lower thermal stability and reduced enzyme activity [9]. In previous studies, the association between TMD and SNPs in the COMT gene has been investigated. In 2010, Nackley demonstrated that some COMT gene haplotypes containing rs4680 had a pronounced association with pain and sensitivity in TMD patients. In 2016, Irena Mladenovic and her colleagues found no significant difference in the distribution of SNPs rs4680 and rs6269 between the TMD group and the control group [10]. In the current study, all participants had TMD, and we did not find a significant relationship between the COMT gene polymorphism (rs4680) and the occurrence of head, neck, and back pain in these TMD patients. In a study conducted by K. Hagen and his colleagues, which investigated the rs4680 polymorphism of the COMT gene in relation to migraines, no significant association was found between migraine headaches and this polymorphism [11]. Hagen's study also did not find a significant relationship between chronic musculoskeletal pain and the COMT gene polymorphism (rs4680). Although another study reported a significant association between migraine with or without aura and the Val158Met polymorphism in the COMT gene [12]. However, in a study by Omair and his colleagues, a significant association was reported between the COMT rs4680 polymorphism and long-term pain reduction after back surgery in patients with chronic back pain [13].

Studies have shown that three genotypes of rs4680 have different effects on human pain perception. Homozygous (A/A) Met/Met individuals are more sensitive to pain compared to (G/G) Val/Val individuals, while heterozygotes have moderate pain sensitivity [14]. The increased activity of the enzyme has an inverse relationship with pain sensitivity, and it has been reported that Val/Val homozygotes produce an effective enzyme, whereas heterozygotes express moderate COMT activity [9]. In this study, we investigated the relationship between different genotypes of the rs4680 COMT gene polymorphism and the severity of facial pain in TMD patients using the visual analog scale (VAS) questionnaire. Our study did not show a significant association, and in a study conducted by Erdel and colleagues [15], no association was found between myofacial pain syndrome and COMT gene polymorphisms. However, Marbach and Levitt [17] reported that patients with TMD-related facial pain exhibit elevated urinary levels of catecholamine metabolites and

 DOI: 10.18502/jcr.v10i4.15305

reduced COMT activity in red blood cells, indicating the role of COMT in the persistent pain associated with TMD. Genetic factors play a role in the etiology of chronic pain by regulating processes such as nociceptive sensitivity, psychological well-being, inflammation, and autonomic responses. Currently, only a limited number of genes have been associated with TMD. Implementing preventive strategies such as education and self-management in individuals carrying predisposing genes for TMD can be effective in reducing the incidence and severity of the disease in the future.

Conclusion

Based on the results of the present study, allele A and the GA genotype had a higher frequency in TMD patients in relation to the COMT rs4680 polymorphism. However, no significant association was found between the rs4680 COMT polymorphism and the occurrence of headache, neck pain, and back pain in patients with temporomandibular disorders (TMD).

Conflict of Interest

There is no conflict of interest to declare.

References

[1] Ferrante T, Manzoni GC, Russo M, Camarda C, Taga A, Veronesi L, et al. Prevalence of tension-type headache in adult general population: the PACE study and review of the literature. Neurol Sci. 2013; 34 Suppl 1:S137-8.

[2] 55. Michelotti, A., et al., Catechol-O-methyltransferase (COMT) gene polymorphisms as risk factor in temporomandibular disorders patients from Southern Italy. The Clinical journal of pain, 2014. 30(2): p. 129-133.

[3] MacGregor AJ, Andrew T, Sambrook PN, Spector TD. Structural, psychological, and genetic influences on low back and neck pain: a study of adult female twins. Arthritis Rheum. 2004; 51(2):160-7.

[4] Tammimäki A, Männistö PT. Catechol-O-methyltransferase gene polymorphism and chronic human pain: a systematic review and meta-analysis. Pharmacogenet Genomics. 2012; 22(9):673-91.

[5] Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melén K, Julkunen I, et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry. 1995; 34(13):4202-10.

[6] Mladenovic I, Supic G, Kozomara R, Dodic S, Ivkovic N, Milicevic B, et al. Genetic Polymorphisms of Catechol-O-Methyltransferase: Association with Temporomandibular Disorders and Postoperative Pain. J Oral Facial Pain Headache. 2016; 30(4):302-10.

[7] Marbach, J.J. and M. Levitt, Erythrocyte catechol-O-methyltransferase activity in facial pain patients. Journal of dental research, 1976. 55(4): p. 711-711.

[8] Antonini, A., et al., COMT inhibition with tolcapone in the treatment algorithm of patients with Parkinson's disease (PD): relevance for motor and non-motor features. Neuropsychiatric disease and treatment, 2008. 4(1): p. 1.

[9] Männistö PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev. 1999; 51(4):593-628.

[10] Mladenovic, I., et al., Genetic Polymorphisms of Catechol-O-Methyltransferase: Association with Temporomandibular Disorders and Postoperative Pain. Journal of Oral & Facial Pain & Headache, 2016. 30(4).

[11] Hagen K, Pettersen E, Stovner LJ, Skorpen F, Zwart JA. The association between headache and Val-158Met polymorphism in the catechol-O-methyltransferase gene: the HUNT Study. J Headache Pain. 2006; 7(2):70-4.

[12] Park, J.W., et al., Genetic contribution of catechol-O-methyltransferase polymorphism in patients with migraine without aura. Journal of Clinical Neurology, 2007. 3(1): p. 24-30.

[13] Omair A, Lie BA, Reikeras O, Holden M, Brox JI. Genetic contribution of catechol-O-methyltransferase variants in treatment outcome of low back pain: a prospective genetic association study. BMC Musculoskeletal Disorders. 2012; 13(1):76.

[14] Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, et al. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science. 2003; 299(5610):1240-3.

[15] Erdal ME, Herken H, Mutlu MN, Bayazit YA. Significance of catechol-O-methyltransferase gene

polymorphism in myofacial pain syndrome. The Pain Clinic. 2003; 15(3):309-13.

[16] Najafi S, Gholizadeh N, Roudgari H, Sheykhbahaei N, Oloumi AM. Temporomandibular disorders is associated with genetic factors: A review. Journal of Craniomaxillofacial Research. 2022 Nov 19:69-80.

[17] Marbach JJ, Levitt M. Erythrocyte catechol-O-methyltransferase activity in facial pain patients. J Dent Res. 1976; 55(4):711.

 DOI: 10.18502/jcr.v10i4.15305