# Genetic Polymorphisms of Catechol-O-Methyltransferase: Association with Temporomandibular Disorders and Postoperative Pain

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**Aims:** To evaluate the association between catechol-O-methyltransferase (COMT) gene polymorphisms and temporomandibular disorders (TMD), TMD pain, psychosocial impairment related to TMD, and postoperative pain. **Methods:** A total of 90 patients with a diagnosis of painful TMD and 92 matched controls were investigated for the presence of TMD, TMD pain, and psychosocial variables by the Research Diagnostic Criteria for TMD. In a prospective cohort study of 40 subjects who underwent extraction of at least one fully impacted mandibular third molar, subjects had 6 months post-surgery follow-up of postoperative pain. DNA extracted from peripheral blood was genotyped for three COMT polymorphisms (rs4680, rs6269, and rs165774) by real-time TaqMan method. The association between COMT polymorphisms and clinical variables was determined by calculating odds ratios (OR) and their 95% confidence intervals (CI). **Results:** Homozygous AA genotype and heterozygous variant A allele carriers (genotype AG/AA) for rs165774 polymorphism were associated with increased risk of TMD compared to wild type (wt) GG genotype (OR = 9.448, $P$ = .006; OR = 2.088, $P$ = .017, respectively). In addition, AA genotype was associated with increased risk of arthralgia (OR = 4.448, $P$ = .011), myofascial pain (OR = 3.543, $P$ = .035), and chronic TMD pain (OR = 6.173, $P$ = .006), compared to wt genotype. AA genotype for rs6269 polymorphism was related to less postoperative chronic TMD pain ($P$ = .025) and lower postoperative acute pain at the extraction site ($P$ = .030). No associations with depression and somatization were observed. **Conclusion:** AA genotype of rs165774 could be a significant risk factor for the development of TMD and TMD pain, while AA genotype of rs6269 presents less postoperative chronic TMD pain and acute pain at a dental extraction site. *J Oral Facial Pain Headache 2016;30:302–310. doi: 10.11607/ofph.1688*

**Keywords:** *COMT, genetic polymorphisms, pain, temporomandibular disorders, third molar surgery*

Temporomandibular disorders (TMD) include a number of clinical conditions that involve the masticatory muscles, temporomandibular joint (TMJ), and adjacent structures.[1] The primary characteristic of TMD is pain[2]; TMD represent the main cause of pain of nondental origin in the orofacial region[3] and are often characterized by a persistent facial pain and heightened sensitivity to painful stimuli in several areas.[2]

TMD are multifactorial in origin and several variables, including trauma and structural and psychosocial factors, may be involved in their etiology.[4] During the last decade, a number of studies have indicated that TMD are influenced by both genetic polymorphisms and diverse environmental factors.[4–7]

Single nucleotide polymorphisms (SNPs) in the gene that encodes for the catabolic enzyme catechol-O-methyltransferase (COMT), located in chromosome 22q11, have been largely investigated in relation to pain sensitivity.[8–11] COMT inactivates biologically active catechols, including the neurotransmitters dopamine, noradrenaline, and adrenaline. These neurotransmitters are involved in numerous physiologic processes, including pain modulation.[11] COMT gene polymorphisms may cause a decrease in COMT enzyme activity, resulting in an elevated

level of catecholamines and ultimately decreased tolerance to pain.[10] A common functional polymorphism, Val158Met (rs4680), leads to a three- to four-fold variation in the activity of the COMT enzyme.[12] The Met158 (A) allele has been linked to a thermolabile enzyme with lower COMT activity, which in turn has been associated with a pronounced response to acute pain[13] and an increased risk of chronic pain.[14] The combination of several SNP variants within a haplotype causing low COMT activity is more often associated with pain than any single SNP variant.[8] Diatchenko et al[15] have demonstrated that three major haplotypes formed by four COMT SNPs (rs6269, rs4633, rs4818, and rs4680) are associated with pain sensitivity and the risk of developing TMD. Not all studies, however, have confirmed the association between these haplotypes and various pain phenotypes.[9,10,16] In addition, a potential association between TMD and other SNPs in the COMT locus has been observed.[4,17] A recent study showed an association between COMT SNP rs165774 and TMD, suggesting that this polymorphism encodes for a truncated COMT isoform with higher efficiency in metabolizing dopamine.[18]

Since COMT affects dopamine and noradrenaline catabolism, variations in the COMT gene have been studied in relation to depression[19–21] and somatic symptoms.[22,23] However, the findings have been inconclusive.

Surgical procedures in the orofacial region may affect several structures, including the TMJ and masticatory muscles, and may cause chronic pain.[24] Huang and Rue estimated that 23% of all TMD cases in young adults could be attributed to third molar extraction.[25] However, various factors, including preoperative TMD symptoms, might be associated with TMD risk after third molar removal.[24,25] Variation in the COMT gene has been associated with postoperative pain sensitivity[10] and acute pain after third molar extraction.[26,27] Therefore, the aim of the current study was to evaluate the associations of COMT gene polymorphisms with TMD, TMD pain, psychosocial impairment related to TMD, and postoperative pain. The investigated rs6269 SNP is located in the promoter region of the COMT gene, rs165774 in the fifth intron,[28] and the nonsynonymous rs4680 is located in exon 4.[15] It was hypothesized that the COMT polymorphisms rs6269, rs165774, and rs4860 could be related to TMD, TMD pain, depression, somatization, and/or postoperative pain after third molar surgery.

## Materials and Methods

The study protocol was approved by the Ethical Committee of the Faculty of Medicine and conformed to the principles embodied in the Declaration of Helsinki. All participants received detailed information about the study and signed an informed consent form.

### Cross-sectional study

This cross-sectional, case-control study involved two groups of subjects: 90 TMD patients and 92 control subjects of the same population, all Caucasians of the same ethnicity. A level of significance of 5% and a power of 80% were adopted, and sample size was calculated according to the genetic models. With a case-control ratio of 1:1 and heterozygous odds ratios (ORs) calculated using a single SNP marker, and with the assumption of a disease prevalence of 5% (as previously indicated for TMD), 5% minor allele frequency, and complete linkage disequilibrium (LD), the dominant model required 90 cases.[29]

A total of 90 TMD patients of both genders (68 women, 22 men) and aged 19 to 45 years (mean age: 28.37 ± 5.85 years) were selected for the study group from patients of the Department of Prosthodontics (Faculty of Medicine, University of East Sarajevo, Foca, BiH) during the period from October 2012 to October 2014. The subjects had to fulfill the following criteria to be included in the study group: aged from 18 to 45 years and diagnosed as having painful TMD according to the Research Diagnostic Criteria for TMD (RDC/TMD).[30] The control group consisted of 92 subjects attending yearly routine checkups at the Department of Restorative Dentistry (Faculty of Medicine, University of East Sarajevo, Foca, BiH) who did not fulfill the criteria for RDC/TMD diagnosis. Subjects were selected to match the patients in the study group with an overall gender and age (mean age: 28.83 ± 6.34) distribution similar to the cases. Exclusion criteria for all study participants, including the controls, were: a current or past medical history of systemic disease, other chronic painful physical conditions in the orofacial region, pregnancy, lactation, irregularity in menstrual cycle, any medication intake in the past 6 months (in the cases where the subject was taking analgesics, the examination was done 12 hours after taking the last dose), presence of unstable occlusal contacts or prosthetic restorations, and previous or current orthodontic treatment.

### Clinical Examination and Assessment of TMD, TMD Pain, Depression, and Somatization

The assessment of TMD, TMD pain, depression, and somatization was performed in accordance with the RDC/TMD.[30] Information about remote pain within the prior 6 months (susceptibility to painful symptoms in other parts of the body; eg, head, chest, muscles, and back) was collected from the Symptom Report Questionnaire (SRQ).[31]

**Table 1 Demographic Data, Axis II Psychological Characteristics, and Pain in Other Body Regions in the TMD Group (n = 90) and the Control Group (n = 92)**

| | TMD group | | Control group | | *P* |
|---|---|---|---|---|---|
| Age (y, mean ± SD) | 28.37 ± 5.85 | | 28.83 ± 6.34 | | .612 |
| | n | (%) | n | (%) | |
| Gender | | | | | .056 |
| Female | 68 | 75.6 | 57 | 62.0 | |
| Male | 22 | 24.4 | 35 | 38.9 | |
| Highest level of education | | | | | .402 |
| Elementary school | 0 | 0.0 | 0 | 0.0 | |
| High school | 50 | 55.6 | 59 | 65.6 | |
| College | 1 | 1.1 | 3 | 3.3 | |
| Faculty | 28 | 31.1 | 21 | 22.8 | |
| Postgraduate studies | 10 | 11.1 | 8 | 8.7 | |
| Marital status | | | | | .905 |
| Single | 58 | 64.4 | 60 | 65.2 | |
| Married | 29 | 32.2 | 28 | 30.4 | |
| Divorced | 1 | 1.1 | 1 | 1.1 | |
| Widowed | 0 | 0.0 | 1 | 1.1 | |
| Other | 2 | 2.2 | 2 | 2.2 | |
| Smoking status | | | | | .831 |
| Never | 51 | 56.7 | 49 | 53.3 | |
| Former | 12 | 13.3 | 15 | 16.3 | |
| Current | 27 | 30.0 | 28 | 30.4 | |
| Depression[a] | | | | | **.002** |
| Normal | 40 | 44.4 | 62 | 67.4 | |
| Moderate | 32 | 35.6 | 21 | 22.8 | |
| Severe | 18 | 20.0 | 9 | 9.8 | |
| Somatization[b] | | | | | **.000** |
| Normal | 40 | 44.4 | 65 | 70.7 | |
| Moderate | 27 | 30.0 | 19 | 20.7 | |
| Severe | 23 | 25.6 | 8 | 8.7 | |
| Headache | 74 | 82.2 | 59 | 64.1 | **.006** |
| Chest pain | 22 | 24.4 | 18 | 19.6 | .427 |
| Back pain | 48 | 53.3 | 43 | 46.7 | .374 |
| Body muscle pain | 46 | 51.1 | 31 | 33.7 | **.017** |
| Total number of painful places | | | | | .073 |
| 0 | 6 | 6.7 | 12 | 13.0 | |
| 1 | 27 | 30.0 | 36 | 39.1 | |
| 2 | 19 | 21.1 | 23 | 25.0 | |
| 3 | 25 | 27.8 | 14 | 15.2 | |
| 4 | 13 | 14.4 | 7 | 7.6 | |

[a]Depression: normal < 0.535; moderate depression 0.535 to 1.10; severe depression > 1.105.
[b]Somatization (pain items included): normal < 0.500; moderate somatization 0.500 to 1.000; severe somatization > 1.000.
Significant values are presented in bold.

## Genotyping

Genomic DNA was extracted from a total of 182 blood samples (from all 90 cases and all 92 controls) by using the Blood Prep Isolation Kit (Qiagen) according to the manufacturer's instructions. Three COMT SNPs, rs6269 (A > G, located in the 5'UTR), rs4680 (A/G, missense158Val/Met), and rs165774 (G > A, located in an intronic region) were genotyped using the TaqMan SNP genotyping assays (Applied Biosystems) according to the manufacturer's protocols. Allelic discrimination was performed by a real-time polymerase chain reaction (PCR) 7500 (Applied Biosystems).

## Prospective Study

In a prospective cohort study, out of the 182 subjects initially genotyped, a subset of 40 subjects underwent extraction of at least one fully impacted mandibular third molar under local anesthesia. The group consisted of 31 females and 9 males ranging in age from 18 to 41 years (mean age: 27.65 ± 5.91 years). Of these subjects, 22 were diagnosed as having TMD. The subjects underwent standardized surgical technique by an experienced oral surgeon (I.S.) removing at least one fully impacted mandibular third molar. Local anesthesia was obtained with 4% articaine with 1:200,000 adrenaline (Ubistesin Forte, 3M ESPE AG). For treatment of postoperative pain, a loading dose of paracetamol up to 2 g (Panadol, GlaxoSmithKline) was administered, followed by ibuprofen 400 mg up to six times daily if necessary. Each subject underwent two postoperative RDC/TMD assessments, the first at 1 week and the second at 6 months after surgery. Questions 8 through 12 and question 20 in the RDC/TMD questionnaire were adjusted for the examination performed 1 week postoperatively.[24] In addition, the subjects rated pain intensity in the third molar extraction site prior to surgery, 1 week after surgery, and 6 months after surgery on a 0–10 numeric verbal rating scale. They were also asked about the duration of pain in the third molar region and the duration of analgesic medication 6 months after surgery.

TMD, TMD pain, depression, and somatization were assessed by an examiner (I.M.) while genotyping was performed by other investigators (G.S. and B.M.) who were blinded to patient status and outcomes.

## Statistical Analyses

Statistical calculations were performed by the SPSS 20.0 package. The Kolmogorov-Smirnov test showed that data related to pain intensity and duration were not normally distributed ($P > .05$). The Kruskal-Wallis and Mann-Whitney tests were used to compare these and ranked variables. Categorical variables were analyzed with the chi-square or Fisher exact probability tests while numeric variables were compared with Student *t* test. The association of gene variants with investigated clinical variables was determined by calculating ORs and their 95% confidence intervals (CI).

The Haploview 4.2 software was used to estimate departure from the Hardy–Weinberg

equilibrium, haplotype frequencies and pairwise LD, based on the expectation maximization algorithm.[32]

All reported *P* values were two-sided and associations were considered significant when *P* values were less than .05.

## Results

### Cross-sectional study

No significant differences in gender, age, smoking, marital status, or education level were observed between the TMD cohort and control groups (Table 1). Approximately half of the subjects in the TMD group and about one third of the control group suffered from depression (55.6%, 32.6%, respectively) and reported somatic symptoms (55.6%, 29.4%, respectively). Both somatization and depression levels were significantly higher in the TMD group (*P* = .002, *P* = .000, respectively). A large majority of TMD patients (82.2%) had regular headaches and about half of them reported body muscle pain (51.1%), a prevalence that was significantly higher than in controls (*P* = .006, *P* = .017, respectively). There was no difference in frequency of pain symptoms in other body regions between the groups, but a tendency for a higher total number of pain sites was observed in the TMD group in comparison with the control group (*P* = .073).

The genotype frequencies of each of the COMT SNPs tested in the groups are shown in Table 2. COMT SNP rs165774 polymorphism showed a significant difference in distribution in the variant A allele (*P* = .006) and A allele carrier genotypes (AG/AA; *P* = .015) between the groups; the variant A allele and genotypes containing the A allele showed up more in the TMD group than in the control group. Homozygous AA genotype was associated with an increased risk of TMD compared to wild type (wt) GG genotype (OR = 9.448, CI = 1.913 to 46.652, *P* = .006), and variant A allele carriers (AG/AA) were also associated with an increased risk of TMD compared to wt GG genotype (OR = 2.088, CI = 1.138 to 3.829, *P* = .017). The distribution of rs4680 and rs6269 SNPs did not show a significant difference between the TMD group and the control group.

The associations between COMT genotypes and TMD subtypes/TMD pain in all subjects genotyped are presented in Table 3.

**Table 2 COMT Genotype Distribution in the TMD Group (n = 90) and the Control Group (n = 92)**

| COMT SNPs | TMD | Controls | *P* | OR[a] (95% CI) | *P* |
|---|---|---|---|---|---|
| rs4680 | | | | | |
| wt (GG) | 17 | 23 | NS | Ref | |
| het (AG) | 55 | 52 | | 1.407 (0.670 to 2.952) | NS |
| mut (AA) | 18 | 17 | | 1.427 (0.567 to 3.597) | NS |
| rs6269 | | | | | |
| wt (GG) | 1 | 5 | NS | Ref | |
| het (AG) | 57 | 60 | | 4.316 (0.483 to 38.535) | NS |
| mut (AA) | 32 | 27 | | 5.320 (0.578 to 48.928) | NS |
| rs165774 | | | | | |
| wt (GG) | 30 | 47 | **0.006** | Ref | |
| het (AG) | 49 | 43 | **0.015**[b] | 1.769 (0.949 to 3.298) | .072 |
| mut (AA) | 11 | 2 | | **9.448 (1.913 to 46.652)** | **.006** |
| | | | | **2.088 (1.138 to 3.829)**[b] | **.017**[b] |

SNPs = single nucleotide polymorphisms; wt = wild type; het = heterozygous; mut = mutant; OR = odds ratio; 95% CI = 95% confidence interval.
Ref = referent genotype (wt). NS = nonsignificant at *P* > .10;
significant values (*P* < .05) are presented in bold.
[a]Adjusted for age and sex.
[b]A allele carriers vs GG genotype (het/mut vs wt).
Significant values are presented in bold.

The reported relationship between rs165774 polymorphism and arthralgia (*P* = .037) was observed, and the rs165774 AA genotype showed an association with an increased risk of arthralgia (OR = 4.448, 95% CI = 1.311 to 15.089, *P* = .011) and myofascial pain (OR = 3.543, 95% CI = 1.038 to 12.092, *P* = .035) compared to the wt genotype. SNP rs165774 was also related to the level of chronic pain (*P* = .010) with significantly higher levels of chronic TMD pain in subjects with the AA genotype compared to the wt genotype (*P* = .004), and the AA genotype also had an increased risk of chronic TMD pain compared to the wt genotype (OR = 6.173, CI = 1.565 to 24.353, *P* = .006). For pain scale measures used in this study, a relationship between SNP rs165774 and both worst and average pain intensity was observed (*P* = .021 and *P* = .007, respectively). Other COMT SNPs showed no evidence of a relationship with TMD pain.

Depression and somatization levels and susceptibility to remote pain in relation to the examined polymorphisms are presented in Table 4. SNP rs6269 showed a significant relationship with the total number of remote pain sites (*P* = .041) and a trend toward a relationship with body muscle pain (*P* = .062). In general, for both depression and somatization levels for all investigated COMT polymorphisms, homozygous wt genotype subjects showed the highest values, followed by heterozygous genotype subjects; the lowest scores were presented by homozygous mutant genotype subjects. However, none of the studied SNPs showed any evidence of an association with the psychological components of the RDC/TMD Axis II, either in the whole sample or in the TMD group.

The haplotype frequency estimates of LD were obtained by Haploview v. 4.2, while Thesias software v. 3.121 was used to determine the potential association of specific haplotypes with TMD risk, determined over the haplotype odds

**Table 3 COMT Genotype Distribution in Relation to TMD Diagnosis and TMD Pain Symptoms (n = 182)**

| | | COMT SNPs | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | rs4680 | | | | rs6269 | | | | rs165774 | | | |
| | | wt | het | mut | | wt | het | mut | | wt | het | mut | |
| Clinical variables | | 40 | 107 | 35 | *P* | 6 | 117 | 59 | *P* | 77 | 92 | 13 | *P* |
| Myofascial pain | + | 9 | 26 | 8 | NS | 1 | 30 | 12 | NS | 15 | 22 | 6 | .111 |
| | – | 31 | 81 | 27 | | 5 | 87 | 47 | | 62 | 70 | 7 | |
| Arthralgia | + | 7 | 30 | 8 | NS | 1 | 32 | 12 | NS | 16 | 22 | 7 | **.037** |
| | – | 33 | 77 | 27 | | 5 | 85 | 47 | | 61 | 70 | 6 | |
| Chronic TMD pain | + | 15 | 46 | 13 | NS | 1 | 49 | 24 | NS | 27 | 37 | 10 | **.017** |
| presence | – | 25 | 61 | 22 | | 5 | 68 | 35 | | 50 | 55 | 3 | |
| Chronic TMD pain grade[a] | | 87.9 | 94.3 | 87.0 | NS | 68.2 | 93.6 | 89.8 | NS | 85.2 | 92.3 | 123.0 | **.010** |
| Present pain[b] | | 0.6 | 0.9 | 0.6 | NS | 0.0 | 0.9 | 0.7 | NS | 0.6 | 0.8 | 1.6 | NS |
| Worst pain[b] | | 1.7 | 2.2 | 1.8 | NS | 0.5 | 2.2 | 1.9 | NS | 1.7 | 2.1 | 3.8 | **.021** |
| Average pain[b] | | 1.3 | 1.6 | 1.6 | NS | 0.3 | 1.6 | 1.5 | NS | 1.2 | 1.6 | 3.2 | **.007** |
| Pain duration (mo)[b] | | 13.1 | 19.6 | 10.2 | NS | 0.5 | 18.4 | 13.9 | NS | 14.2 | 17.3 | 22.7 | .079 |

SNPs = single nucleotide polymorphisms; wt = wild type; het = heterozygous; mut = mutant.
NS = not significant at $P > .10$; significant values ($P < .05$) are presented in bold.
[a]Mean rank of scores (RDC/TMD[30]).
[b]Mean value (RDC/TMD[30]).

**Table 4 COMT Genotype Distribution in Relation to Psychosocial Variables and Remote Pain (n = 182)**

| | | COMT SNPs | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | rs4680 | | | | rs6269 | | | | rs165774 | | | |
| | | wt | het | mut | | wt | het | mut | | wt | het | mut | |
| Clinical variables | n | 40 | 107 | 35 | *P* | 6 | 117 | 59 | *P* | 77 | 92 | 13 | *P* |
| Depression grade[a] | | 101.2 | 89.3 | 87.1 | NS | 96.9 | 91.8 | 90.4 | NS | 94.5 | 90.0 | 84.4 | NS |
| Somatization grade[a] | | 93.8 | 90.9 | 90.8 | NS | 103.6 | 88.6 | 96.0 | NS | 90.9 | 92.9 | 85.0 | NS |
| Headache | + | 30 | 80 | 23 | NS | 5 | 83 | 45 | NS | 56 | 70 | 7 | NS |
| | – | 10 | 27 | 12 | | 1 | 34 | 14 | | 21 | 22 | 6 | |
| Chest pain | + | 13 | 23 | 4 | .088 | 3 | 22 | 15 | NS | 19 | 19 | 2 | NS |
| | – | 27 | 84 | 31 | | 3 | 95 | 44 | | 58 | 73 | 11 | |
| Back pain | + | 17 | 53 | 21 | NS | 2 | 55 | 34 | NS | 35 | 51 | 5 | NS |
| | – | 23 | 54 | 14 | | 4 | 62 | 25 | | 42 | 41 | 8 | |
| Body muscle pain | + | 15 | 44 | 18 | NS | 3 | 42 | 32 | .062 | 29 | 42 | 6 | NS |
| | – | 25 | 63 | 17 | | 3 | 75 | 27 | | 48 | 50 | 6.7 | |
| Total number of remote pain sites[b] | | 91.4 | 90.6 | 94.3 | NS | 101.4 | 84.4 | 104.6 | **.041** | 88.6 | 95.8 | 78.3 | NS |

SNPs = single nucleotide polymorphisms; wt = wild type; het = heterozygous; mut = mutant; OR = odds ratio.
NS = not significant at $P > .10$ level; significant values ($P < .05$) are presented in bold.
[a]Mean rank of scores (RDC/TMD[30]); [b]Mean rank of scores (Symptom Report Questionnaire[31]).

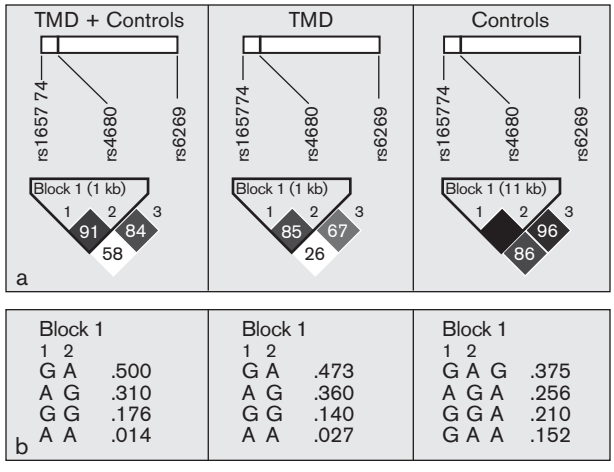


**Fig 1** Schematic diagram of linkage disequilibrium (LD) blocks within the COMT gene from the pooled study of 90 TMD cases and 92 controls. Triangles represent LD blocks. Block 1 includes rs165774 and rs4680 in the pooled study of TMD cases and controls and in the TMD cases. **(a)** The LD pattern represented by pairwise D' values between analyzed rs165774, rs4680, rs6269 polymorphisms in the COMT gene. Shades of grey: Logarithm of odds (LOD) > 2, D' < 1; black: LOD > 2, D' = 1; white: LOD < 2, D' < 1. **(b)** COMT haplotype distribution in the pooled study.

ratios (HOR). The two polymorphisms rs165774 and rs4680 were in strong LD and four haplotypes had a frequency > 1.0% (Fig 1). However, differences in haplotype distribution between TMD cases and controls were not significant (GA-: 47.3% vs 37.5%; AG-: 36% vs 25.6%; GG-: 14% vs 21%) and could

**Table 5 COMT Genotype Distribution in Relation to TMD and Pain Symptoms in Subjects with Third Lower Molar Removal Surgery (n = 40)**

| | | COMT SNPs | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | rs4680 | | | | rs6269 | | | | rs165774 | | | |
| | | wt | het | mut | | wt | het | mut | | wt | het | mut | |
| Clinical variables | | 10 | 22 | 8 | *P* | 1 | 28 | 11 | *P* | 17 | 19 | 4 | *P* |
| TMD preop | + | 5 | 13 | 4 | NS | 0 | 16 | 6 | NS | 7 | 12 | 3 | NS |
| | – | 5 | 9 | 4 | | 1 | 12 | 5 | | 10 | 7 | 1 | |
| TMD 1 w postop | + | 6 | 10 | 3 | NS | 0 | 16 | 3 | NS | 8 | 10 | 1 | NS |
| | – | 4 | 12 | 5 | | 1 | 12 | 8 | | 9 | 9 | 3 | |
| TMD 6 mo postop | + | 4 | 10 | 3 | NS | 0 | 14 | 3 | NS | 6 | 9 | 2 | NS |
| | – | 6 | 12 | 5 | | 1 | 14 | 8 | | 11 | 10 | 2 | |
| Chronic TMD pain 6 mo postop | + | 4 | 7 | 1 | NS | 0 | 12 | 0 | **.025** | 4 | 7 | 1 | NS |
| | – | 6 | 15 | 7 | | 1 | 16 | 11 | | 13 | 12 | 3 | |
| Chronic TMD pain grade 6 mo postop[a] | | 22.0 | 21.0 | 17.2 | NS | 15.0 | 23.4 | 13.7 | **.017** | 18.7 | 22.4 | 19.4 | NS |
| Present TMD pain 1 w postop[b] | | 1.9 | 2.4 | 1.3 | NS | 0.0 | 2.6 | 0.9 | .090 | 1.7 | 2.7 | 0.5 | NS |
| Worst TMD pain 1 w postop[b] | | 3.7 | 3.2 | 2.1 | NS | 0.0 | 3.9 | 1.6 | NS | 2.9 | 3.8 | 1.0 | NS |
| Average TMD pain 1 w postop[b] | | 2.6 | 2.4 | 2.1 | NS | 0.0 | 2.8 | 1.6 | NS | 1.9 | 3.1 | 1.0 | NS |
| Present TMD pain 6 mo postop[b] | | 0.3 | 0.7 | 0.3 | NS | 0.0 | 0.7 | 0.0 | NS | 0.4 | 0.6 | 0.5 | NS |
| Worst TMD pain 6 mo postop[b] | | 2.2 | 2.0 | 1.0 | NS | 0.0 | 2.5 | 0.4 | .099 | 1.6 | 2.1 | 2.0 | NS |
| Average TMD pain 6 mo postop[b] | | 1.4 | 1.3 | 0.9 | NS | 0.0 | 1.6 | 0.4 | NS | 0.7 | 1.6 | 1.8 | NS |
| Third molar pain intensity preop[b] | | 0.5 | 0.4 | 0.9 | NS | 0.0 | 0.4 | 0.7 | NS | 0.4 | 0.3 | 1.8 | NS |
| Third molar pain intensity 1 w postop[b] | | 5.0 | 4.6 | 3.9 | NS | 1.0 | 5.3 | 3.1 | **.030** | 4.4 | 4.8 | 4.0 | NS |
| Duration of postoperative pain (d)[b] | | 7.1 | 6.7 | 3.3 | NS | 1.0 | 7.2 | 3.9 | **.028** | 6.8 | 5.9 | 4.5 | NS |
| Duration of postoperative analgesics intake (d)[b] | | 4.3 | 4.4 | 1.5 | NS | 0.0 | 4.5 | 2.3 | **.017** | 3.8 | 4.2 | 2.0 | NS |

SNPs = single nucleotide polymorphisms; wt = wild type; het = heterozygous; mut = mutant; NS = not significant at $P > .10$; significant values ($P < .05$) are presented in bold.

[a]Mean rank of scores (RDC/TMD[30]).

[b]Mean value (numeric verbal rating scale).

not be associated with an increased risk of TMD determined with the HOR (AG- = 1.007, CI = 0.230 to 4.401, $P = .993$; and GG- = 0.541, CI = 0.204 to 1.436, $P = .2170$) in comparison to the most frequent haplotype, GA-. No significant associations between the COMT haplotypes and the clinicopathologic features were observed.

### Prospective Study

There was a response rate of 100% (40/40) in both postoperative evaluation terms. COMT genotype distribution in relation to TMD and pain symptoms in subjects who underwent third molar extraction is presented in Table 5. There was no significant difference in COMT genotype distribution between the patients who demonstrated preoperative TMD and those who did not. Significant differences between COMT rs6269 genotypes were observed, with the AA genotype showing significantly less postoperative chronic TMD pain ($P = .025$), chronic TMD pain grade ($P = .017$), postoperative third molar pain intensity ($P = .030$) and duration ($P = .028$), and duration of postoperative analgesic intake ($P = .017$). None of the patients reported pain at the extraction site 6 months postoperatively. The effect of rs6269 variants on postoperative pain risk could not be determined due to the small number of subjects carrying the wt genotype. Investigated clinical parameters were not significantly different in the rs4680 and rs165774 groups.

## Discussion

TMD are a set of conditions affecting the masticatory muscles or TMJs[1] and pain is the most common presenting symptom.[2] Genetics and sensory processing, as well as psychological and behavioral factors, are now considered to contribute to TMD and TMD pain.[4,33] TMD patients are reported to have higher levels of psychological and affective distress, greater perceived stress, and increased somatic awareness compared to control patients.[33] COMT metabolizes catecholamines and is a key regulator of pain perception, cognitive function, and affective mood[34]; polymorphisms of the human COMT gene have been associated with TMD,[4,15,17] postoperative pain,[10] and have been investigated for their role in depression[21] and somatic complaints.[22] To explore the effects of variations in the candidate gene for pain symptoms in TMD and related psychosocial impairment, the present study investigated three polymorphisms in the COMT gene: rs4680, rs6269, and rs165774. The

analysis showed COMT SNP rs165774 could be associated with TMD and TMD pain while rs6269 might be related to remote and postoperative pain.

The current study also revealed that AA genotype and variant A allele carriers of the synonymous SNP rs165774 were associated with an increased risk of TMD. In addition, an association was observed between this polymorphism and arthralgia, myofascial pain, and chronic pain. Subjects with the AA genotype also showed higher worst and average TMD pain intensity and higher pain duration than the wt GG genotype. The A allele carrier/AA genotype of the SNP rs165774, in an individual SNP analysis or within various haplotypes, has been previously associated with mental retardation,[35] experimental pain in women undergoing breast cancer surgery,[28] and persistent posttraumatic pain.[36] In accordance with the findings of the present study is the report that haplotypes containing the G allele of this SNP showed a pain-protective effect, evidenced by the low intensity of posttraumatic pain.[36] Recently, the rs165774 polymorphism was associated with the risk of TMD and individual variability in sensitivity to painful stimuli.[18] Data obtained in the current study support this hypothesis, suggesting that SNP rs165774 may predispose to painful TMD. However, in contrast to the present findings, A allele carriers of the SNP rs165774 have been previously associated with lower pain sensitivity in experimental and acute postoperative pain after breast cancer surgery.[28] In a recent study, rs165774 was linked to painful TMD and it was suggested that this polymorphism might have an important role in alternative splicing and production of the truncated COMT isoform previously associated with the higher efficiency in metabolizing dopamine.[18] Although mRNA stability is higher for COMT transcripts carrying the A allele of SNP rs165774, protein expression levels and enzymatic activity have been shown to be lower.[18] This provides an excellent example of how allelic variants can have opposite effects on mRNA stability and protein expression.

Results from patients with TMD have shown that the majority of patients also suffer from remote pain.[31] According to a recent review on the heritability of TMD pain,[37] genes are not uniquely associated with TMD pain but rather with the individual vulnerability to pain in general. In the present study, the number of TMD patients who reported body muscle pain and regular headaches was significantly higher in comparison to controls. Although rs6269 was not related to TMD, rs6269 AA genotype was associated with a higher prevalence of body muscle pain and a higher total number of pain sites. Accordingly, Diatchenko et al[15] reported that the G allele and the GG genotype of rs6269 are associated with less experimental pain sensitivity. Results obtained in the present study support previous findings that the effect of COMT polymorphisms in chronic clinical pain depends on the different pain conditions.[8] Since the studied rs6269 polymorphism is located in the distal P2 promoter region that encodes both transcripts, soluble form (S-COMT) and a membrane-bound form (MB-COMT),[38,39] this polymorphism might have an important role in dopamine metabolism and in the variability of pain conditions.

No association between a preferred candidate gene variation for various pain phenotypes, COMT SNP rs4680 (Val108/158Met), and TMD or any of the pain characteristics was found in the present study. Despite evidence that rs4680 polymorphism influences pain sensitivity,[13,14] the current results are in accordance with findings reporting no difference in pain responses with respect to the rs4680 polymorphism.[16,26] These findings might be explained by the fact that the effect of rs4680 may depend on the genomic environment, including specific haplotype or co-occurring rare variants[15] and epistatic interactions between the rs4680 and other functionally-related genes.[40]

COMT gene variants have been studied regarding their role in depression[21] and somatization.[22] Higher COMT activity in the human brain results in lower synaptic dopamine and noradrenaline levels, which are linked to depression.[19] COMT also modifies the personality dimension conscientiousness, and thus may influence clinical complaints in patients with functional somatic disorders.[23] The present study revealed that wt subjects showed a trend toward the highest values of both depression and somatization grades for all investigated COMT polymorphisms. The lack of a significant association could be explained by the possible influence of modulators[21] and by the fact that the current investigation did not include inpatients but rather subjects in which depression and somatization were assessed by a tool for screening those symptoms in TMD patients. Accordingly, it has been suggested that psychosocial factors, rather than directly affecting psychosocial features in TMD patients, act either independently from COMT in influencing the risk of TMD[20] or as moderators of the COMT-TMD relationship.[41] The relationship between COMT activity, psychosocial parameters, and TMD seems to be quite complex and needs to be clarified in future research.

While a cross-sectional study cannot address changes in TMD signs and symptoms over time, the current prospective subset study included both patients with and without preoperative TMD. They had a relatively narrow age span and underwent standardized surgical procedure of third molar removal with a 100% response rate in postoperative evaluation. Regarding postoperative pain intensity, COMT

rs6269 AA-genotype subjects were characterized by markedly lower chronic TMD pain measures and also reported lower postoperative pain intensity and duration at the extraction site as well as a shorter period of postoperative analgesic intake. The contribution of other COMT genotypes to postoperative pain perception was not confirmed. In accordance with the present results are findings that homozygous COMT rs6269 AA patients demonstrated a trend toward lower pain intensity preoperatively as well as lower pain intensity at 1 year after lumbar discectomy in comparison with G allele carriers.[42] Conversely, Lee at al reported a lower pain sensitivity and higher prevalence of patients with adequate postoperative analgesia amongst patients with the GG genotype for the COMT SNP rs6269 in the first postoperative week after third molar removal.[27] An earlier study failed to show an association between the SNPS rs6269 and rs4680 and pain after third molar extraction.[26] The differences in the reported results may be explained by the present results as well as by the results of Rut et al[42] being related to both the short and long postoperative period, while the latter mentioned studies reported an association for a short postoperative course of 1 week. In addition, among individuals involved in the current prospective study, a single subject had COMT rs6269 wt genotype and the observed relationship between rs6269 and postoperative pain might be related to high values for pain intensity and duration obtained in heterozygotes. Higher pain levels after third molar surgery in rs6269 heterozygotes compared to homozygotes have previously been observed.[27] The present observations did not support the previous findings of associations between COMT rs4680 and rs165774 polymorphisms and persistent postoperative pain.[36,43] The contrasting effect of rs6269 for remote and postoperative pain in the current study supports the previous observation of the complex effect of lower COMT activity, as increased levels of catecholamines may have different or even opposite roles in different pain conditions.[8]

The major limitation of the present study was the relatively low number of patients. In addition, the presence of population substructure (various TMD subtypes) in any genotype-phenotype association study may reduce the power of the study. The findings reported here should be validated in other populations with different genetic backgrounds. Further studies on the functional relevance of the other COMT polymorphisms and their haplotypes may provide important information on the risk, etiology, and potential treatment of TMD and postoperative pain.

In summary, the results of the current study suggest that the variant AA genotype of COMT rs165774 polymorphism could be a significant risk factor for the development of TMD. Chronic TMD pain and acute pain at the extraction site after third molar removal showed a relationship with COMT rs6269 polymorphism. The appearance of depressive and somatic symptoms in TMD patients in relation to COMT activity also needs to be clarified.

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