

## Potential Genetic Risk Factors for Chronic TMD: Genetic Associations from the OPPERA Case Control Study

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**Abstract:** Genetic factors play a role in the etiology of persistent pain conditions, putatively by modulating underlying processes such as nociceptive sensitivity, psychological well-being, inflammation, and autonomic response. However, to date, only a few genes have been associated with temporomandibular disorders (TMD). This study evaluated 358 genes involved in pain processes, comparing allelic frequencies between 166 cases with chronic TMD and 1,442 controls enrolled in the OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment) study cooperative agreement. To enhance statistical power, 182 TMD cases and 170 controls from a similar study were included in the analysis. Genotyping was performed using the Pain Research Panel, an Affymetrix gene chip representing 3,295 single nucleotide polymorphisms, including ancestry-informative markers that were used to adjust for population stratification. Adjusted associations between genetic markers and TMD case status were evaluated using logistic regression. The OPPERA findings provided evidence supporting previously reported associations between TMD and 2 genes: HTR2A and COMT. Other genes were revealed as potential new genetic risk factors for TMD, including NR3C1, CAMK4, CHRM2, IFRD1, and GRK5. While these findings need to be replicated in independent cohorts, the genes potentially represent important markers of risk for TMD, and they identify potential targets for therapeutic intervention.

**Perspective:** Genetic risk factors for TMD pain were explored in the case-control component of the OPPERA cooperative agreement, a large population-based prospective cohort study. Over 350 candidate pain genes were assessed using a candidate gene panel, with several genes displaying preliminary evidence for association with TMD status.

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Supplementary data accompanying this article are available online at [jpain.org](http://jpain.org) and [sciencedirect.com](http://sciencedirect.com).

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**T**emporomandibular disorders (TMD) represent a group of musculoskeletal conditions characterized by orofacial pain and limitations in function. The etio-pathogenesis of TMD is complex and multifactorial. Proposed risk factors for TMD include joint and muscle trauma, anatomical factors, psychosocial profile, and sensitization of nociceptive pathways, but the relative importance of environmental versus genetic factors in explaining variability remains poorly understood. Although early twin and family studies failed to establish a genetic basis for the development of TMD,<sup>12,26</sup> they had insufficient statistical power to identify genetic factors for common disorders. Recent work has shown heritable factors play a role in both experimental pain as well as a wide variety of clinical pain conditions. A number of studies have estimated substantial heritability in pain disorders related to TMD, such as fibromyalgia at 51%,<sup>25</sup> headache at 34 to 58%,<sup>21,28</sup> and neck pain at 34 to 52%.<sup>11</sup>

The multifactorial nature of TMD suggests that a number of distinct genetic loci may play a role, each contributing small effects that interact with environmental exposures to determine the course and outcome of the disorder.<sup>6</sup> Commonly occurring genetic variants are best investigated using allelic association methods, in which frequencies of polymorphic genetic variants are compared between cases and controls.

Candidate gene studies have found several genes to be associated with TMD. The serotonergic system has received considerable attention, consistent with its known role in nociceptive and affective pathways. A variable number tandem repeat polymorphism (VNTR) in the serotonin transporter gene (SLC6A4) has been repeatedly associated with TMD.<sup>13,32</sup> The T102C single nucleotide polymorphism (SNP) of the serotonin receptor HTR2A has shown evidence of association,<sup>29</sup> and allele frequencies in the A218C SNP in the TPH1 gene, involved in the synthesis of serotonin from tryptophan, have also been shown to differ between TMD cases and controls.<sup>10</sup> Genes related to catecholamine neurotransmitter pathways have also been associated with TMD. Variants of the gene encoding the catabolic enzyme catechol-O-methyltransferase (COMT) are associated with risk of developing TMD,<sup>5,38</sup> and intriguingly, differential treatment outcomes.<sup>40</sup> Haplotypes of the beta-2 adrenergic receptor (ADRB2) have also been associated with TMD, and its psychological and physiological risk markers.<sup>6</sup> The greater occurrence of TMD in females has recently prompted investigators to examine the role of gonadal hormones in disease susceptibility. Such studies have replicated an association with estrogen receptor- $\alpha$  (ESR1) polymorphisms and TMD risk.<sup>15,34</sup>

This limited group of genes likely represents only the low-hanging fruit of genes contributing to TMD variability, as a growing number of genes have been identified as risk factors for other common pain conditions;<sup>8,17,20</sup> many of these genes have yet to be examined in TMD subjects.

A primary aim of the OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment) study is to examine the genetic basis of TMD by identifying genetic variants associated with the odds of chronic TMD and the risk of TMD development. Based on previous knowledge of chronic pain mechanisms, we hypothesized that the pathogenesis of TMD may result from disturbances in catecholamine, serotonin, opioid, and cytokine pathways.<sup>1,8</sup> Twenty-three genes belonging to these systems were chosen as candidates for a genetic association study. This candidate set was expanded with a discovery panel of over 350 known pain-related genes, to capture novel TMD genes previously unrecognized as risk factors for persistent pain conditions.

## Methods

### Study Setting and Participants

As described elsewhere,<sup>24</sup> the OPPERA baseline case-control study used advertisements, emails, flyers and word-of-mouth to recruit people who had chronic TMD (cases) and people who did not (controls). They were recruited between May, 2006 and November, 2008 from communities in and around academic health centers at 4 US study sites: Baltimore MD, Buffalo NY, Chapel Hill NC, and Gainesville FL. At each study site, the target was to recruit 800 controls and variable numbers of cases based on local operational requirements, for a total of 3,200 controls and 200 cases. The number enrolled was 3,263 controls and 186 cases.

The classification of TMD was based on the Research Diagnostic Criteria for Temporomandibular Disorder.<sup>9</sup> In summary, cases met all 3 of the following criteria: during the telephone interview: 1) pain reported with sufficient frequency in the cheeks, jaw muscles, temples or jaw joints during the preceding 6 months (at least 15 days in the preceding month and at least 5 days per month in each of the 5 months preceding that); 2) during the examination, pain reported in the examiner-defined orofacial region for at least 5 days out of the prior 30 days; and 3) pain reported in at least 3 masticatory muscles or at least 1 temporomandibular joint in response to palpation of the orofacial muscles or maneuver of the jaw. Examiners defined the orofacial region by touching the following anatomical areas bilaterally: temporalis, preauricular, masseter, posterior mandibular, and sub-mandibular. Controls met all 5 of the following criteria: during the telephone interview, 1) pain reported infrequently in the cheeks, jaw muscles, temples or jaw joints (no orofacial pain in the preceding month and no more than 4 days per month in any of the 5 months preceding that); 2) no more than 4 headaches per month within the preceding 3 months; 3) never diagnosed with TMD; 4) no use of night guard occlusal splint; and 5) during the examination, pain reported in the examiner-defined orofacial region for no more than 4 days in the prior 30 days.

However, controls could be positive or negative with respect to pain in response to palpation or jaw maneuver and with respect to the examination criteria. Additional studywide criteria for all study participants were: aged 18 to 44 years; fluent in English; negative responses to each of 10 questions about significant medical conditions; no history of facial injury or surgery; not receiving orthodontic treatment; not pregnant or nursing.

This analysis uses data from all 186 recruited TMD cases and one half of the 3,263 recruited controls (1,633 people). The controls for this analysis were selected at random so that data from people in the reserved sample could be used for validation studies that will be reported elsewhere. The accompanying paper<sup>39</sup> gives a more detailed account of study recruitment, case-classification methods, and inclusion and exclusion criteria.

### **Ethical Conduct of Research With Humans**

The OPPERA study was reviewed and approved by institutional review boards at each of the 4 study sites and at the data coordinating center, Battelle Memorial Institute. All participants verbally agreed to a screening interview done by telephone and they provided informed, signed consent for all other study procedures.

### **Genotyping**

At each OPPERA site, whole blood was collected by venipuncture from study participants who provided consent for genotyping. Blood was collected into 5 mL EDTA containing polyethylene vacutainers, which were stored at  $-80^{\circ}\text{C}$ . Each sample was labeled with a unique, barcoded identifier label. Genomic DNA was purified utilizing protocols based on Qiagen<sup>TM</sup> Extraction Kits at Cogenics, Inc. (now Beckman-Coulter Genomics, Morrisville, NC).

Samples were genotyped using the Algnomics (Chapel Hill, NC) Pain Research Panel, a dedicated chip-based platform utilizing the Affymetrix MegAllele technology. The Pain Panel assesses 3,295 single nucleotide polymorphisms (SNPs) representing 358 genes known to be involved in systems relevant to pain perception (complete list provided through <http://www.algnomics.com/pain-research-panel.html>). Pathways assessed by the Pain Panel represent 1 or more of 3 broad domains and include genes that: 1) mediate the transmission of pain signals by sensory nerve fibers and by central nervous system neural pathways that mediate the perception of pain; 2) mediate peripheral and central inflammatory responses to tissue injury or psychological stress; and 3) influence mood and affective states associated with chronic pain conditions. The Panel also includes genes that influence the pharmacokinetics and dynamics of analgesic compounds and includes ancestry-informative markers. Within each gene, SNPs were prioritized for inclusion based on known functionality (ie, they result in nonsynonymous amino acid changes, expression level differences, or disrupted alternative splicing). Other SNPs were selected as representative markers of regions with high linkage disequilibrium (LD), containing many corre-

lated SNPs that are inherited in blocks, in order to tag untyped SNPs.

Selected duplicate study samples and HapMap reference DNA were genotyped concurrently with each batch in order to examine consistency of genotype calls throughout the study, and genotyping was monitored for batch or site effects in call rates that might introduce bias into the association tests. All samples were clustered together at the conclusion of the genotyping process, using the manufacturer's supplied software in accordance with Affymetrix protocols. Genotyping results were returned for 3,221 unique samples, representing enrollees in the prospective cohort study and in the case-control study. The overall call rate was 99.1% and repeated sample concordance was 99.8%.

Raw genotypes were filtered for quality using utilities implemented in PLINK v.1.07 (Broad Institute, Cambridge MA).<sup>33</sup> An identity-by-state analysis was performed to cluster individuals according to racial heritage by multidimensional scaling (MDS) (Supplementary e-Fig 1). Samples were dropped from the study due to: 1) call rate  $<.95$  ( $n = 38$ ); 2) duplicate genotypes ( $n = 20$ ); 3) cryptic relatedness ( $n = 84$ ); 4) mismatch between genotypic and self-reported sex and race ( $n = 29$ ); and 5) case misclassification ( $n = 14$ ). SNPs were filtered for: 1) call rate  $<.95$  ( $n = 170$ ); 2) repeated sample concordance rate  $<.99$  ( $n = 58$ ); 3) minor allele frequency (MAF) in the full cohort  $<1\%$  ( $n = 101$ ); and 4) Hardy-Weinberg equilibrium  $P$ -value  $<1 \times 10^{-5}$  in either non-Hispanic whites or African-Americans separately ( $n = 42$ ).

### **Statistical Analysis**

The final cleaned dataset included 2,924 SNPs assayed in 3,050 subjects, with a completeness rate of 99.7%. Baseline data from half of the people enrolled into the prospective cohort were reserved for later replication studies, leaving 1,442 people from the cohort who did not have TMD when enrolled. Their data were compared to data from the 166 people with chronic TMD enrolled for the baseline case-control study (Table 1). Chi-square analysis detected no significant differences between the 2 split halves in genotyping quality characteristics.

Association tests assuming codominant effects were performed using logistic regression in PLINK, in which the number of copies of the rare allele was the genetic predictor variable and TMD case-status was the dependent variable. As reported in an accompanying paper in this volume<sup>39</sup> the percentage of enrollees who were TMD cases varied among study sites to meet operational requirements: 11% at Baltimore, 6% at Buffalo, 12% at Chapel Hill and 12% at Gainesville. To account for differences in study sites, dummy variables coding for the 4 recruitment sites were introduced as covariates in the regression model. Also included as covariate terms were sex and the first 2 dimensions of variance (eigenvectors) in the MDS analysis, to adjust for population stratification. After adjustment for study site, age and gender, non-Whites had significantly lower odds of TMD (OR = .2, 95% CI = .2, .3) than non-Hispanic Whites. Effect sizes were estimated using odds ratios (OR) with their

**Table 1. Genotyped OPPERA Subjects**

RACE	GENDER	NUMBER OF PEOPLE		
		CASES	CONTROLS	ALL
Caucasian	Male	24	308	332
	Female	103	423	526
	All	127	731	858
African American	Male	3	183	186
	Female	16	236	252
	All	19	419	439
Other/mixed	Male	1	144	145
	Female	19	148	167
	All	20	292	312
All	Male	28	635	663
	Female	138	807	945
	All	166	1,442	1,608

NOTE. Numbers of subjects used for genetic association, stratified by racial background, sex, and case status.

corresponding 95% confidence intervals (CI 95%). To evaluate stratum-specific genetic effects, additional tests were performed as above in males and females separately (without adjustment for sex), and in Caucasians and African Americans separately (without adjustment by racial eigenvectors).

The OPPERA project, as originally conceived and powered, included a candidate gene study evaluating 23 strong candidates (see [Supplementary e-Table 1](#) for a list of Tier 1 genes). Adopting the Pain Research Panel as the genotyping platform provided access to 336 additional known pain-related genes, greatly increasing the exploratory value of the study. To preserve the intent and power of the original study design, we retained the original, high priority gene set as a separate first tier analysis, and analyzed the full Panel as a second discovery tier with a more conservative significance threshold.

To maintain a desired experiment-wide  $\alpha = .05$ , it is necessary to correct for the large number of multiple comparisons in both Tier 1 and Tier 2 phases; this is customarily performed by applying a Bonferroni correction according to the number of SNPs tested. Due to LD structure between neighboring SNPs, many of the tested SNPs are correlated and thus violate the assumption of independent statistical tests, rendering the Bonferroni method overly conservative. We first calculated the number of effectively independent SNPs in each tier, taking LD structure into account, by the spectral decomposition method,<sup>23,31</sup> before applying the Bonferroni correction. The number of independent SNPs in the Tier 1 analysis was estimated to be 151 (from 211 that passed quality control filters), for a corrected threshold for significance of  $P < 3.4 \times 10^{-4}$ . For the larger Tier 2 SNP set, the number of effectively independent SNPs was 1,911 (of 2,657 total), giving a corrected threshold of  $P < 2.6 \times 10^{-5}$ .

In order to increase power, subjects from an additional TMD case-control study were added to the OPPERA cohort. This study was conducted at 1 of the OPPERA sites (UNC) by members of the OPPERA team, with genotyping and phenotyping procedures largely consistent (and contemporaneous) with OPPERA protocols. Recruit-

ment for the UNC cohort differed from OPPERA in that all subjects in the UNC cohort were non-Hispanic white females, aged 18 to 45; cases were 182 TMD cases recruited through the UNC Orofacial Pain Clinic (not population-based, as in OPPERA); and controls were 170 healthy women recruited by community-wide advertisements. After combining studies, the analytical dataset included 1,961 subjects (348 cases, 1,612 controls), with 2,657 SNPs in common between cohorts. The linear regression models using the UNC cohort included a covariate term for this additional study. Secondary analyses assuming dominance and recessive models of inheritance were performed to characterize genetic effects.

For genes with suggestive single marker associations, multimarker tests were performed using haplotype analysis. Haplotypes were constructed and phased in PLINK, and statistical comparison of each identified haplotype with frequency >5% against all others was performed by logistic regression, adjusted for sex, racial eigenvectors, and site as in the single SNP analyses.

## Results

### Quality of Genotype Data

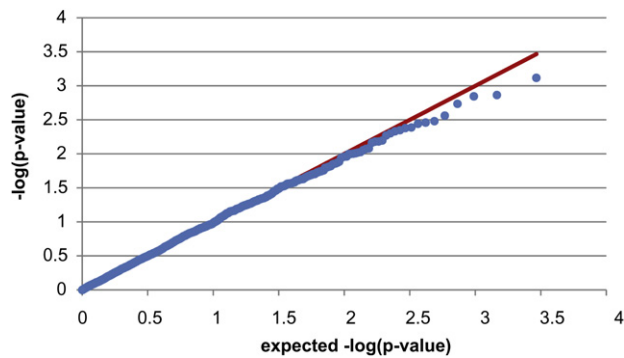
The genotyping completeness rate in the cleaned dataset approached 99.7% with very strong correspondence between replicate samples, indicating a high level of confidence in accurate and unbiased genotyping. OPPERA was designed to be racially inclusive, raising the possibility that population stratification might lead to systematic allele frequency differences that could be misinterpreted as association signals. The genomic inflation factor  $\lambda_{GC}$  was calculated as a measure of systematic deviation from the null hypothesis of no association<sup>4</sup> for all association tests. Only a minimal elevation of test statistics was detected when adjusting for site and race in the OPPERA cohort ( $\lambda_{GC} = 1.03$ ) and OPPERA and UNC combined analyses ( $\lambda_{GC} = 1.00$ ).

### TMD Associations

In the analysis of 358 candidate genes (Tier 2 SNP set) using OPPERA cases and controls, no SNPs exceeded the threshold of statistical significance. The quantile-quantile (Q-Q) plot of the distribution of *P*-values did not deviate from that expected under the null hypothesis ([Fig 1](#)). Odds ratios and *P*-values for the top 20 results are provided in [Table 2](#), for the full cohort and by sex and racial strata. The majority of SNPs exhibited stronger associations in females compared to males, but due to the smaller sample size of males it is inconclusive whether this trend is indicative of sex-specific genetic effects. Similarly, stronger associations generally were observed in non-Hispanic whites compared with African Americans, consistent with the higher numbers of cases and controls in the former group.

The addition of 182 TMD cases and 170 healthy controls from the UNC cohort improved the resolution of genetic effects, with a number of SNPs elevated above the expected *P*-value on the Q-Q plot ([Fig 2](#)), although no SNPs exceeded the strict Bonferroni correction threshold





**Figure 1.** Genetic Association Test for 358 candidate genes from 166 temporomandibular disorder (TMD) cases and 1,442 controls in the OPPERA study. Q-Q plot of case-control association test within OPPERA cohort only. Each blue dot represents a single SNP. The observed  $-\log_{10}(P\text{-values})$  on the y-axis are ranked and plotted against the expected  $-\log_{10}(P\text{-values})$  under the null hypothesis on the x-axis; the null distribution is represented by the red line. Since no SNP elevated significantly above the expected line, we were not able to reject the null hypothesis for SNPs with  $-\log_{10}(P\text{-values})$ . SNP, single nucleotide polymorphism.

(Fig 3). There were 9 SNPs with  $P$ -values lower than would be expected by chance, representing 6 separate genes (Table 3).

The first 3 ranked SNPs were located within a 130-kb long block of high LD within the glucocorticoid receptor gene *NR3C1* on Chr 5. The SNP most strongly associated with TMD status (rs2963155, minor allele (MA) = G,  $P = 6.15 \times 10^{-5}$ , OR = .62, 95% CI .50–.79) was the most common of the 3 polymorphisms, with a minor allele frequency (MAF) of 20 to 24% in both Caucasians and African Americans (see Table 3 for MAF of SNPs not given in the text). The next 2 SNPs were in almost perfect LD with each other in Caucasians, with MAF of about 13% (rs9324918, MA = C,  $P = 8.41 \times 10^{-5}$ , OR = .56, 95% CI .42–.75; and rs33389, MA = T,  $P = 2.17 \times 10^{-4}$ , OR = .57, 95% CI .43–.77). The minor alleles of these SNPs all exerted a protective effect against TMD. Conditioning on rs2963155 eliminated the associations with the other SNPs in the region. These SNPs all were located within the long intron of the *NR3C1* gene and therefore are likely markers of the true effect polymorphism.

Following the *NR3C1* SNPs, the next most strongly associated SNP was an intronic polymorphism of the serotonin 2A receptor *HTR2A* (rs9316233, MA = G,  $P = 3.44 \times 10^{-4}$ , OR = .64, 95% CI .50–.82). The minor G allele was protective against TMD in our cohort. A dominant model of inheritance appeared to describe the data better than the additive model ( $P = 4.96 \times 10^{-5}$ , OR = .55, 95% CI .42–.74, Supplementary e-Table 2). In the analysis of OPPERA subjects, the effect of this SNP was not observed in males. There was also evidence of a race-specific effect, as the effect was not observed in African Americans (OR = .90,  $P = .77$ ), in whom the minor allele is almost twice as common as in non-Hispanic whites (OR = .56,  $P = .0053$ ). In white females alone, the protective effect of the minor G allele was even more evident (OR = .39,  $P = 2.3 \times 10^{-4}$ ).

The next findings were from an intronic SNP in the muscarinic cholinergic receptor 2 (*CHRM2*) gene with allele counts almost evenly divided between the 2 alleles (rs7800170, MA = A,  $P = 6.20 \times 10^{-4}$ , OR = .72, 95% CI

.59–.87). The effect of this SNP was better modeled by dominance (OR = .53, Supplementary e-Table 2).

The next 2 SNPs were located in the calcium/calmodulin-dependent protein kinase 4 gene (*CAMK4*). The first was found between the second and third exons (rs3756612, MA = G,  $P = 6.37 \times 10^{-4}$ , OR 1.51, 95% CI 1.19–1.92) while the second was located almost 70 kb downstream between the fifth and sixth exons (rs10491334, MA = T,  $P = 8.84 \times 10^{-4}$ , OR = .63, 95% CI .48–.83). Although they had similar allele frequencies (about 19% in whites, 5% in African Americans), they were not in LD and had opposite effects on TMD risk in our cohort, and therefore represent distinct risk factors (test of allelic interaction  $P = .73$ ). Again, there was evidence for a dominant model of inheritance for these 2 SNPs (OR = 1.62 and .59, respectively, Supplementary e-Table 2). Because these 2 minor alleles did not coexist in these subjects, haplotype analysis did not provide any additional information.

The risk allele G of the next SNP, located in the first intron of the interferon-related developmental regulator 1 (*IFRD1*) gene, was the more common allele in African Americans but the less common allele in whites (rs728273, MA = G,  $P = .0012$ , OR = 1.38, 95% CI 1.13–1.67). In the OPPERA cohort alone, the G allele was weakly associated with increase in risk for TMD in Caucasians ( $P = .01$ , OR = 1.42), but no effect was observed in African Americans ( $P = .79$ , OR = 1.11).

The last SNP with suggestive association with TMD was located in the second intron of the G protein-coupled receptor kinase 5 (*GRK5*) gene (rs12415832, MA = A,  $P = .0013$ , OR = 2.40, 95% CI 1.40–4.08). Although uncommon in both races, it was more rare in whites, with a MAF = 2%. In the OPPERA only analysis stratified by race, the OR was pronounced in whites (OR = 3.92,  $P = 2.52 \times 10^{-4}$ ), whereas the association was weak in African Americans (OR = 1.51,  $P = .53$ ). To assess whether this result was a statistical limitation of the regression model, which is sensitive to low MAF, Fisher's exact test was used because it is more robust for rare alleles, albeit with no capacity to adjust for covariates. However, the  $P$ -value was very similar ( $P = 3.05 \times 10^{-4}$ ).

### Tier 1 SNPs

The 23 genes hypothesized a priori as high priority candidates were intended to mitigate the stringent Bonferroni correction requirement of correcting for the entire set of SNPs tested. While no Tier 1 SNPs surpassed the Bonferroni corrected threshold for significance, there was clear divergence from the  $P$ -value distribution expected under the null (Fig 4). Eight Tier 1 SNPs showed suggestive evidence for association with TMD.

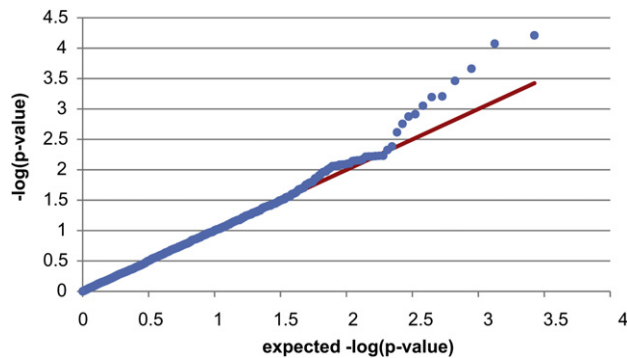
Two SNPs flanking the interleukin 10 (*IL10*) gene (rs3024496, MA = G,  $P = .0059$ , OR = .76, 95% CI .63–.93; rs1800896, MA = C,  $P = .0086$ , OR = .77, 95% CI .64–.94) were in strong LD with each other, suggesting they are both markers of a single effect.

Three SNPs tag adrenergic receptor genes: 12 kb upstream from the alpha-2C (*ADRA2C*) gene (rs7696139,

**Table 2. Top 20 Association Results for OPPERA Cohort**

SNP	GENE	CHR	BP	TIER	ALL	WHITE	AF-AMER	ALL OPPERA			MALE ONLY			FEMALE ONLY			WHITE ONLY			AF-AMER ONLY		
					MAF	MAF	MAF	A1	OR	P	A1	OR	P	A1	OR	P	A1	OR	P	A1	OR	P
rs11466066	NGF	1	115682889	2	.02	.00	.06	A	4.19	.00076	A	3.57	.29	A	4.47	.0011	A	NA	.99	A	5.23	.0023
rs12415832	GRK5	10	121102317	2	.03	.02	.05	A	2.51	.0014	A	1.47	.61	A	2.83	.0012	A	3.92	.00025	A	1.51	.53
rs1563826	EREG	4	75454388	2	.31	.21	.49	T	.61	.0014	T	.97	.93	T	.56	.00070	T	.49	.00037	A	1.14	.72
rs1076292	CRHR2	7	30679226	2	.49	.36	.19	G	1.50	.0018	G	2.40	.0073	G	1.37	.029	C	.71	.020	G	1.59	.25
rs2072100	TAC1	7	97199720	2	.44	.46	.34	C	.69	.0027	C	.74	.29	C	.68	.0048	C	.73	.028	C	.30	.013
rs728273	IFRD1	7	111855337	2	.50	.41	.31	C	.69	.0033	C	.66	.14	C	.69	.011	G	1.42	.015	C	.90	.79
rs3782221	NOS1	12	116280264	2	.24	.22	.26	A	.63	.0035	A	.66	.27	A	.62	.0065	A	.66	.019	A	.52	.15
rs2367707	EREG	4	75467298	2	.21	.21	.24	A	.62	.0036	A	1.21	.58	A	.52	.00067	A	.55	.0021	A	.76	.56
rs1448239	GRIN2A	16	10094936	1	.19	.14	.34	C	.59	.0041	C	.83	.64	C	.54	.0029	C	.59	.022	C	.33	.017
rs255097	CRHR2	7	30693484	2	.46	.39	.17	A	1.44	.0042	A	2.00	.025	A	1.36	.031	G	.75	.045	A	1.72	.18
rs6967334	CACNA2D1	7	81899704	2	.31	.40	.11	C	1.45	.0045	C	1.17	.58	C	1.51	.0051	C	1.53	.0036	C	.40	.23
rs3804452	MAPK14	6	36184912	2	.08	.11	.02	A	1.73	.0047	A	3.02	.0028	A	1.48	.085	A	1.63	.018	A	.00	.99
rs3782202	NOS1	12	116204763	2	.18	.22	.11	G	.61	.0050	G	.74	.41	G	.58	.0061	G	.68	.038	G	.47	.32
rs4883544	P2RX2	12	131712020	2	.33	.41	.22	T	.69	.0054	T	.49	.033	T	.75	.043	T	.69	.011	T	.95	.91
rs1550798	KCNJ3	2	155387083	2	.44	.47	.27	T	.71	.0064	T	.97	.92	T	.64	.0021	A	1.40	.020	T	.89	.78
rs7800170	CHRM2	7	136274860	2	.50	.48	.37	C	1.41	.0066	A	.64	.12	C	1.35	.033	C	1.34	.038	C	1.26	.51
rs7687621	EREG	4	75468690	2	.18	.20	.14	T	.63	.0066	T	1.25	.52	T	.53	.0014	T	.56	.0034	T	.52	.30
rs2363561	KCNK2	1	213321930	2	.43	.38	.46	T	1.40	.0069	T	1.37	.27	T	1.39	.018	T	1.41	.016	T	1.49	.25
rs3787535	NTSR1	20	60823966	2	.25	.30	.15	A	1.40	.0083	A	1.02	.94	A	1.51	.0044	A	1.45	.010	A	1.30	.58
rs1557545	GRIA3	23	122148800	2	.28	.26	.31	A	1.45	.0084	A	.68	.41	A	1.57	.0026	A	1.24	.20	A	1.80	.12

Abbreviations: SNP, single nucleotide polymorphism; CHR, chromosome ; BP, location in base pairs; MAF, minor allele frequency; A1, minor allele; OR, odds ratio ; P, P-value of logistic regression association test.



**Figure 2.** Genetic Association Test for 358 candidate genes from 348 temporomandibular disorder (TMD) cases and 1,612 controls in the combined OPPERA and UNC cohorts. Q-Q plot of case-control association test within combined OPPERA and UNC cohorts. Each blue dot represents a single SNP. The observed  $-\log_{10}(P\text{-values})$  on the y-axis are ranked and plotted against the expected  $-\log_{10}(P\text{-values})$  under the null hypothesis on the x-axis; the null distribution is represented by the red line. Eleven SNPs showed elevation above the expected line, thus rejecting the null hypothesis for SNPs with  $-\log_{10}(P\text{-values})$ . SNP, single nucleotide polymorphism.

MA = G,  $P = .0072$ , OR = .74, 95% CI .60–.92), and 2 closely spaced within the long intron of the alpha-1D (ADRA1D) gene (rs1556832, MA = T,  $P = .0082$ , OR = 1.29, 95% CI 1.07–1.56; rs946188, MA = G,  $P = .018$ , OR = .76, 95% CI .61–.95). Additionally, an intronic SNP in COMT, an enzyme that catabolizes the catecholamine ligands of these receptors, was also represented among this list (rs174697, MA = A,  $P = .0099$ , OR = 1.62, 95% CI 1.12–2.34).

One SNP was located in the long first intron of the delta opioid receptor (OPRD1) gene (rs2236857, MA = C,  $P = .0087$ , OR = 1.32, 95% CI 1.07–1.63). The remaining SNP was located within an intron of the GRIN2A ionotropic N-methyl-D-aspartate (NMDA) receptor 2A gene (rs1448239, MA = C,  $P = .012$ , OR = .71, 95% CI .54–.93).

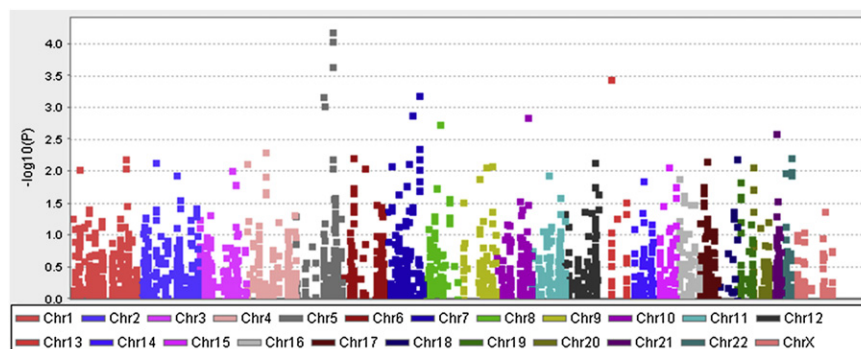
## Discussion

The OPPERA study's investigation of 358 genes offers opportunities for deeper insight into the genetic influences on TMD than previous studies that have targeted 1 or a few genetic markers. This is, to our knowledge, the first large-scale candidate gene study to assess genetic

mediators of TMD in both genders and all races. However, a gene panel of this size also creates limitations, primarily because of the Bonferroni adjustment of  $P$ -value thresholds, which is the conventional method used to adjust for multiple tests. The initial results reported here describe the effects of individual SNPs on odds of TMD, after adjustment for potential confounding effects of study site, sex, and race. We also examined the effect of these SNPs across strata, as a major goal of OPPERA is to discover how these variables interact. In general, though, this stratification decreased statistical power compared to analysis of the complete sample, with the result that no SNPs achieved a strict experiment-wide significance threshold. However, we believe that the evidence of association of the top associated SNPs is strong enough to warrant further study and replication of these genes in other cohorts. The OPPERA investigative group is also currently expanding the number of TMD cases in order to perform a genomewide association study. This approach will further improve statistical power and provide for unbiased assessment of the genetic contribution to TMD.

We observed association with TMD in a number of genes previously shown to influence TMD risk. The strongest such association was for rs9316233 of the HTR2A serotonin receptor gene, where the minor G allele showed a protective effect against TMD risk. This gene was previously associated with TMD based on another of its SNPs, rs6313, a synonymous polymorphism in the first exon of the gene.<sup>29</sup> It lies 40 kb upstream from rs9316233, and is not in strong LD with it ( $D' = .29$ ,  $r^2 = .02$ ). A tag SNP in strong LD with rs6313 was also assessed in this study (rs4941573,  $r^2 = 1.0$ ); the G allele corresponding to the protective T allele in rs6313 showed a trend toward greater risk (recessive test OR = 1.34,  $P = .11$ ). More recently, 2 other SNPs in the HTR2A gene were associated with chronic widespread musculoskeletal pain in a prospective, population-based cohort study.<sup>30</sup> These SNPs were not genotyped or tagged at  $r^2 > .80$  in the present study, and are not in LD with rs9316233 ( $r^2 < .02$  for both SNPs). The risk variant discovered in this study is therefore not a replication of the earlier HTR2A findings, but rather represents a novel genetic risk factor.

We observed a suggestive association at a SNP in the COMT locus, rs174697. Previous reports have not tested its association with any pain phenotype. Diatchenko



**Figure 3.** Manhattan Plot of Genetic Association Test for 358 candidate genes from 348 temporomandibular disorder (TMD) cases and 1,612 controls in the combined OPPERA and UNC studies. Each dot represents a single SNP, mapped by genomic location on the x-axis and the observed  $-\log_{10}(P\text{-values})$  on the y-axis. Each chromosome is colored differently. SNP, single nucleotide polymorphism.

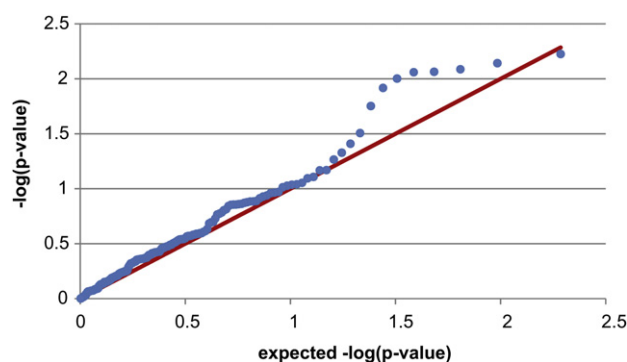
**Table 3. Top Association Results for Combined OPPERA and UNC Cohorts**

SNP	GENE	CHR	BP	TIER	OR_ADD	P_ADD	OR_DOM	P_DOM	OR_REC	P_REC	MIN	MAJ	MAF
rs2963155	NR3C1	5	142736197	2	.63	6.15E-05	.58	.00012	.47	.016	G	A	.22
rs9324918	NR3C1	5	142747353	2	.56	8.41E-05	.54	.00019	.29	.030	C	T	.14
rs33389	NR3C1	5	142680692	2	.57	.00022	.56	.00049	.30	.033	T	C	.13
rs9316233	HTR2A	13	46331356	2	.64	.00034	.55	4.96E-05	.87	.68	G	C	.25
rs7800170	CHRM2	7	136274860	2	.72	.00062	.53	3.65E-05	.79	.14	A	C	.50
rs3756612	CAMK4	5	110731386	2	1.51	.00064	1.62	.00058	1.61	.19	G	A	.16
rs10491334	CAMK4	5	110800303	2	.63	.00088	.59	.00045	.71	.45	T	C	.15
rs728273	IFRD1	7	111855337	2	1.38	.0012	1.43	.017	1.62	.0037	G	C	.48
rs12415832	GRK5	10	121102317	2	2.40	.0013	2.55	.00098	0	.99	A	C	.03

Abbreviations: SNP, single nucleotide polymorphism; CHR, chromosome; BP, genomic location in base pairs; OR\_ADD, odds ratio of TMD risk for additive model; P\_ADD, *P*-value of TMD risk for additive model; OR\_DOM, odds ratio of TMD risk for dominance model; P\_DOM, *P*-value of TMD risk for dominance model; OR\_REC, odds ratio of TMD risk for recessive model; P\_REC, *P*-value of TMD risk for recessive model; Min, minor allele; Maj, major allele; MAF, minor allele frequency.

et al<sup>5</sup> described 3 common haplotypes of COMT that predict low (LPS), average (APS), or high (HPS) pain sensitivity in white females. Consistent with the previous finding, in this study the HPS haplotype was significantly associated with a higher risk of TMD (OR = 1.28, 1-sided test *P* = .05) relative to the other haplotypes, although there was no significant difference between the APS and LPS haplotypes. The effect of rs174697 is independent of these haplotypes, which suggests that the haplotypic diversity of this gene is more complex than previously reported, although an effect of this SNP is consistent with an additional functional site at the 3' end of the COMT gene locus.<sup>35,36</sup>

Several genes selected as Tier 1 candidates showed associations that were stronger than expected by chance, and will be useful in OPPERA's additional studies of TMD onset. Evidence continues to accrue supporting a role for monoamine pathways in TMD susceptibility. Previously we observed suggestive associations between TMD and adrenergic receptors ADRA2A and ADRA1D as well as COMT. These results are consistent with the crucial role of catecholamines in persistent pain and chronic musculoskeletal pain conditions.<sup>3,5,22,41</sup>



**Figure 4.** Genetic Association Test for Tier 1 SNPs in 23 candidate genes from 348 temporomandibular disorder (TMD) cases and 1,612 controls in the combined OPPERA and UNC studies. Q-Q plot of case-control association test within combined OPPERA and UNC cohorts for Tier 1 genes. Each blue dot represents a single SNP. The observed  $-\log_{10}(P\text{-values})$  on the y-axis are ranked and plotted against the expected  $-\log_{10}(P\text{-values})$  under the null hypothesis on the x-axis; the null distribution is represented by the red line. Eight SNPs showed elevation above the expected line, thus rejecting the null hypothesis for SNPs with  $-\log_{10}(P\text{-values})$ . SNP, single nucleotide polymorphism.

Furthermore, our data suggest that genetic variations in OPRD1<sup>16,27</sup> and GRIN2A genes play a role in TMD development. Functional polymorphisms in these genes may result in disturbance of pain regulatory pathways, underlying a state of pain amplification leading to the development of persistent pain and thus contributing to TMD.<sup>7</sup> Similarly, alterations in the anti-inflammatory activity of IL10 may cause people to respond differently to trauma or stress.<sup>37</sup>

This study revealed a number of genes which, while known to be involved in nociceptive pathways, have not been shown previously to be involved in susceptibility to a chronic pain condition. These genes warrant further investigation as mediators of TMD risk and as potential targets for therapeutic intervention. The glucocorticoid receptor encoded by the NR3C1 gene is the binding site for cortisol and a major element of the hypothalamic-pituitary-adrenal (HPA) system. The HPA system is the primary endocrine stress axis in humans and has been implicated in the pathophysiologic development of TMD.<sup>19</sup> NR3C1 has been investigated, along with several other HPA axis genes, for association with somatic symptom count in a large population-based study, but no associations were discovered in this gene.<sup>14</sup>

Comparatively little data exist linking CHRM2, CAMK4, IFRD1, and GRK5 to nociception. CHRM2 codes for muscarinic cholinergic receptor 2 that binds to acetylcholine and controls cellular responses such as adenylate cyclase inhibition, phosphoinositide degradation, and potassium channel mediation in the central and peripheral nervous system. The other candidate genes code for regulatory proteins. The CAMK4 gene codes for a multifunctional serine/threonine protein kinase with limited tissue distribution that has been implicated in transcriptional regulation in neurons. Its activity affects learning and memory,<sup>44</sup> and development of opioid analgesic tolerance.<sup>18</sup> IFRD1 is a histone-deacetylase-dependent transcriptional coregulator and is involved in control of inflammation, the growth and differentiation of specific cell types during embryonic development, and tissue regeneration. Mutations in this gene are associated with sensory/motor neuropathy with ataxia.<sup>2</sup> GRK5 is involved in the phosphorylation and regulation of activation of G protein-coupled receptors, including several that have been implicated in chronic pain, including ADRB2.<sup>42,43</sup>



Although several compelling genetic risk factors were revealed in this study, it will be necessary to confirm putative associations in independent cohorts. The power of this study was limited for several reasons. The number of cases in this study, even with the addition of the UNC cohort, was small relative to the number of controls, and to the number that would be needed to achieve the desired power. As implemented, this study had only 25% power to detect even the larger odds ratios observed ( $>1.4$ ) for a SNP with a median MAF of .25. Therefore, it is possible that we failed to detect additional genes with true effects on TMD risk, and it is also likely that the observed effect sizes of the top associated SNPs are inflated over their true values, due to the winner's curse phenomenon.<sup>45</sup> Heterogeneity in the subject population, while a valuable property for the epidemiological goals of the OPPERA project, likely impeded our ability to parse subtle genetic effects. This heterogeneity arose both from the racially inclusive recruitment, but also from the different sources which provided cases (population-based for OPPERA, pain clinic-based for UNC).

The OPPERA study offers considerable potential to investigate combined effects of genotypes and a rich set of baseline intermediate phenotypes, including clinical, psychosocial, autonomic, and sensory domains. In addition,

OPPERA Case-Control Gene Association Study of TMD data on first onset TMD from the OPPERA prospective cohort study will be analyzed to search for genes that predict onset of TMD after the 5 years of follow-up.

In summary, the OPPERA case-control study is the first genetic association study of TMD to have investigated a substantial number of pain-related candidate genes. While the results require replication, they provide tentative evidence that chronic TMD is influenced by genetic contributions within a number of gene loci, including NR3C1, CAMK4, CHRM2, IFRD1, GRK5, HTR2A and COMT.

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## Supplementary Data

The supplementary data accompanying this article are available online at [jpain.org](http://jpain.org) and [sciencedirect.com](http://sciencedirect.com).

## References

- Belfer I, Wu T, Kingman A, Krishnaraju RK, Goldman D, Max MB: Candidate gene studies of human pain mechanisms: Methods for optimizing choice of polymorphisms and sample size. *Anesthesiology* 100:1562-1572, 2004
- Brkanac Z, Spencer D, Shendure J, Robertson PD, Matsushita M, Vu T, Bird TD, Olson MV, Raskind WH: IFRD1 Is a Candidate Gene for SMNA on Chromosome 7q22-q23. *Am J Hum Genet* 84:692-697, 2009
- Coderre TJ, Basbaum AI, Dallman MF, Helms C, Levine JD: Epinephrine exacerbates arthritis by an action at presynaptic  $\beta_2$ -adrenoceptors. *Neuroscience* 34:521-523, 1990
- Devlin B, Roeder K: Genomic control for association studies. *Biometrics* 55:997-1004, 1999
- Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W: Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet* 14:135-143, 2005
- Diatchenko L, Anderson A, Slade G, Fillingim R, Shabalina S, Higgins T, Sama S, Belfer I, Goldman D, Max M, Weir B, Maixner W: Three major haplotypes of the  $\beta_2$  adrenergic receptor define psychological profile, blood pressure, and the risk for development of a common musculoskeletal pain disorder. *Am J Med Genet Part B* 141B:449-462, 2006
- Diatchenko L, Nackley A, Slade G, Fillingim R, Maixner W: Idiopathic pain disorders—pathways of vulnerability. *Pain* 123:226-230, 2006
- Diatchenko L, Nackley AG, Tchivileva IE, Shabalina SA, Maixner W: Genetic architecture of human pain perception. *Trends Genet* 23:605-613, 2007
- Dworkin S, LeResche L: Research diagnostic criteria for temporomandibular disorders: Review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6: 301-355, 1992
- Etoz OA, Ataoglu H, Erdal ME: Association between tryptophan hydroxylase gene polymorphism and painful non-osseous temporomandibular disorders. *Saudi Med J* 29:1352-1354, 2008
- Fejer R, Hartvigsen J, Kyvik KO: Heritability of neck pain: A population-based study of 33794 Danish twins. *Rheumatology* 45:589-594, 2006
- Heiberg A, Heloe B, Heiberg AN, Heloe LA, Magnus P, Berg K, Nance WE: Myofascial pain dysfunction (MPD) syndrome in twins. *Community Dent Oral Epidemiol* 8: 434-436, 1980
- Herken H, Erdal E, Mutlu N, Barlas O, Cataloluk O, Oz F, Güray E: Possible association of temporomandibular joint pain and dysfunction with a polymorphism in the serotonin transporter gene. *Am J Orthod Dentofacial Orthop* 120: 308-313, 2001
- Holliday KL, Macfarlane GJ, Nicholl BI, Creed F, Thomson W, McBeth J: Genetic variation in neuroendocrine genes associates with somatic symptoms in the general population: Results from the EPiFUND study. *J Psychosom Res* 68: 469-474, 2010
- Kang S-C, Lee D-G, Choi J-H, Kim ST, Kim Y-K, Ahn H-J: Association between estrogen receptor polymorphism and pain susceptibility in female temporomandibular joint osteoarthritis patients. *Int J Oral Maxillofac Surg* 36:391-394, 2007
- Kim H, Neubert JK, San Miguel A, Xu K, Krishnaraju RK, Iadarola MJ, Goldman D, Dionne RA: Genetic influence on variability in human acute experimental pain sensitivity

associated with gender, ethnicity and psychological temperament. *Pain* 109:488-496, 2004

17. Kim H, Clark D, Dionne RA: Genetic contributions to clinical pain and analgesia: Avoiding pitfalls in genetic research. *J Pain* 10:663-693, 2009

18. Ko SW, Jia Y, Xu H, Yim S-J, Jang D-H, Lee Y-S, Zhao M-G, Toyoda H, Wu L-J, Chatila T, Kaang B-K, Zhuo M: Evidence for a role of CaMKIV in the development of opioid analgesic tolerance. *Eur J Neurosci* 23:2158-2168, 2006

19. Korszun A, Young EA, Singer K, Carlson NE, Brown MB, Crofford L: Basal circadian cortisol secretion in women with temporomandibular disorders. *J Dent Res* 81:279-283, 2002

20. LaCroix-Fralish ML, Mogil JS: Progress in genetic studies of pain and analgesia. *Annu Rev Pharmacol Toxicol* 49:97-121, 2009

21. Larsson B, Bille B, Pedersen NL: Genetic influence in headaches: A Swedish twin study. *Headache* 35:513-519, 1995

22. Levine J, Dardick S, Roizen M, Helms C, Basbaum A: Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis. *J Neurosci* 6:3423-3429, 1986

23. Li J, Ji L: Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* 95:221-227, 2005

24. Maixner W, Diatchenko L, Dubner R, Fillingim R, Greenspan J, Knott C, Ohrbach R, Weir B, Slade G: Orofacial Pain Prospective Evaluation and Risk Assessment Study - The OPPERA Study. *J Pain* 12(Suppl 3):T4-T11, 2011

25. Markkula R, Järvinen P, Leino-Arjas P, Koskenvuo M, Kalso E, Kaprio J: Clustering of symptoms associated with fibromyalgia in a Finnish Twin Cohort. *Eur J Pain* 13:744-750, 2009

26. Michalowicz BS, Pihlstrom BL, Hodges JS, Bouchard TJ: No heritability of temporomandibular joint signs and symptoms. *J Dent Res* 79:1573-1578, 2000

27. Mogil JS, Richards SP, O'Toole LA, Helms ML, Mitchell SR, Belknap JK: Genetic sensitivity to hot-plate nociception in DBA/2J and C57BL/6J inbred mouse strains: Possible sex-specific mediation by delta2-opioid receptors. *Pain* 70:267-277, 1997

28. Mulder EJ, Van Baal C, Gaist D, Kallela M, Kaprio J, Svensson DA, Nyholt D, Martin NG, MacGregor AJ, Cherkas LF, Boomsma DI, Palotie A: Genetic and environmental influences on migraine: A twin study across six countries. *Twin Res* 6:422-431, 2003

29. Mutlu N, Erdal M, Herken H, Oz G, Bayazit Y: T102C polymorphism of the 5-HT2A receptor gene may be associated with temporomandibular dysfunction. *Oral Dis* 10:349-352, 2004

30. Nicholl BI, Holliday KL, Macfarlane GJ, Thomson W, Davies KA, O'Neill TW, Bartfai G, Boonen S, Casanueva FF, Finn JD, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Silman AJ, Vanderschueren D, Wu FCW, Beth JM, Group tEMAS: HTR2A polymorphisms are associated with chronic widespread pain and the extent of musculoskeletal pain: Results from two population based cohorts. *Arthritis Rheum* 63:810-818, 2011

31. Nyholt D: A simple correction for multiple testing for SNPs in linkage disequilibrium with each other. *Am J Hum Genet* 74:765-769, 2004

32. Ojima K, Watanabe N, Narita N, Narita M: Temporomandibular disorder is associated with a serotonin transporter gene polymorphism in the Japanese population. *BioPsychoSoc Med* 1:3, 2007

33. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira M, Bender D, Maller J, Sklar P, de Bakker P, Daly M, Sham P: PLINK: A toolset for whole-genome association and population-based linkage analysis. *Am J Hum Genet* 81:559-575, 2007

34. Ribeiro-Dasilva MC, Peres Line SR, Leme Godoy dos Santos MC, Arthuri MT, Hou W, Fillingim RB, Rizzatti Barbosa CM: Estrogen receptor- $\alpha$  polymorphisms and predisposition to TMJ disorder. *J Pain* 10:527-533, 2009

35. Shibata K, Diatchenko L, Zaykin DV: Haplotype associations with quantitative traits in the presence of complex multilocus and heterogeneous effects. *Genet Epidemiol* 33:63-78, 2009

36. Shifman S, Bronstein M, Sternfeld M, Pisanté-Shalom A, Lev-Lehman E, Weizman A, Reznik I, Spivak B, Grisaru N, Karp L, Schiffer R, Kotler M, Strous RD, Swartz-Vanetik M, Knobler HY, Shinar E, Beckmann JS, Yakir B, Risch N, Zak NB, Darvasi A: 2002: A highly significant association between a COMT haplotype and schizophrenia. *Am J Hum Genet* 71:1296-1302, 2002

37. Shimizu K, Guo W, Wang H, Zou S, LaGraize S, Iwata K, Wei F, Dubner R, Ren K: Differential involvement of trigeminal transition zone and laminated subnucleus caudalis in orofacial deep and cutaneous hyperalgesia: The effects of interleukin-10 and glial inhibitors. *Mol Pain* 5:75, 2009

38. Slade G, Diatchenko L, Ohrbach R, Maixner W: Orthodontic treatment, genetic factors, and risk of temporomandibular disorder. *Semin Orthod* 14:146-156, 2008

39. Slade G, Bair E, By K, Mulkey F, Baraian C, Rothwell R, Reynolds M, Gonzalez Y, Gordon S, Ribeiro-Dasilva M, Lim P, Greenspan J, Dubner R, Fillingim R, Diatchenko L, Maixner W, Dampier D, Knott C, Ohrbach R: Study methods, recruitment, socio-demographic findings and demographic generalizability in the OPPERA study. *J Pain* 12(Suppl 3):T12-T26, 2011

40. Tchivileva IE, Lim PF, Smith SB, Slade GD, Diatchenko L, McLean SA, Maixner W: Effect of catechol-O-methyltransferase polymorphism on response to propranolol therapy in chronic musculoskeletal pain: A randomized, double-blind, placebo-controlled, crossover pilot study. *Pharmacogenet Genomics* 20:239-248, 2010

41. Torpy DJ, Papanicolaou DA, Lotsikas AJ, Wilder RL, Chrousos GP, Pillemer SR: Responses of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis to interleukin-6: A pilot study in fibromyalgia. *Arthritis Rheum* 43:872-880, 2000

42. Tran TM, Jorgensen R, Clark RB: Phosphorylation of the beta2-adrenergic receptor in plasma membranes by intrinsic GRK5 *Biochemistry* 46:14438-14449, 2007

43. Wang WCH, Muhlbachler KA, Bleeker ER, Weiss ST, Liggett SB: A polymorphism of G-protein coupled receptor kinase5 alters agonist-promoted desensitization of [beta]2-adrenergic receptors. *Pharmacogenet Genomics* 18:729-732, 2008

44. Wei F, Qiu C-S, Liauw J, Robinson DA, Ho N, Chatila T, Zhuo M: Calcium-calmodulin-dependent protein kinase IV is required for fear memory. *Nat Neurosci* 5:573-579, 2002

45. Xiao R, Boehnke M: Quantifying and correcting for the winner's curse in quantitative-trait association studies. *Genet Epidemiol* 35:133-138, 2011