Associations between Genetic Variants and Clinical, Psychosocial and Pain Sensitivity Risk Factors of Chronic Temporomandibular Disorders

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Introduction

The purpose of this article is to examine the potential associations between the 2657 single nucleotide polymorphisms (SNPs) and 16 possible risk factors for temporomandibular disorders (TMD) selected from two genetic association studies: Orofacial Pain Prospective Evaluation and Risk Assessment (OPPERA) study and COMT study.

OPPERA Study:

The Orofacial Pain Prospective Evaluation and Risk Assessment (OPPERA) study is a large prospective case control study. One of the purposes of OPPERA was to examine the influences of genetic factors on the onset and persistence of temporomandibular disorders (TMD)\(^1\).

The OPPERA baseline case control study used advertisement methods to recruit people who had chronic TMD and people who did not in 2006-2008 from four academic health centers in Baltimore MD, Buffalo NY, Chapel Hill NC, and Gainesville FL. After selection based on several criteria, the final number of participants OPPERA included are 166 cases and 1442 controls. Also, 3295 single nucleotide polymorphisms assessed, representing 358 genes\(^2\).

COMT Study:

In order to increase the power of the OPPERA study, additional subjects from the COMT cohort, which was conducted by UNC, were added to the OPPERA datasets. The 182 cases in the COMT cohort were recruited through the UNC Orofacial Pain Clinic, and 170 controls were recruited by community wide advertisement. Both cases and controls in the COMT cohort were non-Hispanic white females\(^2\).
These two genetic association studies of TMD collected a large set of intermediate phenotypes, including clinical, psychosocial, autonomic and sensory variables. The four criteria for intermediate phenotypes are: associated with TMD in the population; heritable; manifests in an individual independent of active disease; cosegregates with TMD in families. The 16 risk factors that are investigated in this research satisfied the criteria of intermediate phenotypes, and are listed below.

The clinical intermediate phenotypes include count of non-specific orofacial symptoms and count of comorbid conditions.

The psychosocial intermediate phenotypes include the Pennebaker Inventory of Limbic Languidness, the depression, anxiety and somatization subscales of the SCL 90R, the perceived stress scale, state-trait anxiety inventory, physical health composite score of Rumination, and the rumination, magnification and helplessness subscales of the pain catastrophizing scale.

The pain sensitivity intermediate phenotypes include pressure main threshold (measured at trapezius), thermal tolerance, heat pain sensitivity measured at 50 degrees Celsius, and the sum of 10 heat pain measures at 50 degrees Celsius.

After combining the datasets of the OPPERA and COMT studies, the analysis dataset consists of 2657 SNPs and the 16 risk factors (intermediate phenotypes). Since the data was collected during two case control studies, the data is not a random sample from the population, so our estimates of the effect of SNPs on the intermediate phenotypes could be biased. The sample can be seen as a stratified sample with two strata: cases and controls of TMD. Inverse probability of sampling weighted regression model (IPW) was used to address the potential
bias in this stratified sample. The output table for regression analysis consists of the p values, t values, confidence intervals and estimates for each SNP and each secondary trait variable.

QQ plots was used to investigate the significance p values that may be of interest. The results show that three observations lie between false discovery rate (FDR) = 0.2 and FDR = 0.1 line for heat pain sensitivity, which can be interpreted as significant. Also, one observation lies between FDR = 0.05 and FDR = 0.1 line in the QQ plot of heat pain windup. However, the plot shows an obvious inflation so more investigation should be performed before reaching conclusions.

Our next steps include investigations of the three SNPs whose p values are extremely low for heat pain sensitivity to find out if they have significant associations, given the inflation and correlation between SNP’s. Also, we will check the causes the inflation in the QQ plot of heat pain windup.

Analysis method

Data Management

16 intermediate phenotypes were selected from both the OPPERA and the COMT datasets, with TMD status (0=control, 1=case). The specific construct and meaning of these intermediate phenotypes are listed below:

Trapezius algometer⁶: the variable was defined by the pain pressure thresholds of the center of trapezius assessed using a commercially available pressure algometer. The examiner would
increase the pressure at a steady rate of 30 kPa/s, until the participant indicated first pain sensation by pressing a button.

Heat pain sensitivity and Heat pain windup\textsuperscript{6}: the variables defined by the heat pain thresholds of the forearms assessed using a commercially available thermal stimulator. The examiner placed the ATS thermode in contact with the skin at 32 °C, and then would increase the temperature at a rate of 0.5 °C/second, until the participant indicated the first pain sensation by pressing a button. Heat pain sensitivity was evaluated using the first pain rating in the series of 10, and heat pain temporal summation was evaluated by taking the sum of the 10 pain ratings.

Thermal tolerance\textsuperscript{6}: the variable was defined by the heat pain tolerance of the forearms assessed using the same thermal stimulator following the similar protocol as the measure of heat pain sensitivity. The participant would press the button to indicate s/he could no longer tolerate the pain. For both heat pain thresholds and heat pain tolerance, the ceiling temperature was set at 52 °C and was recorded when participant fail to press the button in each trial.

Pennebaker Inventory of Limbic Languidness (PILL)\textsuperscript{5}: The PILL assesses the frequency with which individuals are bothered by each of 54 common physical symptoms and sensations on a five-category scale.

Measures of psychological distress (depression, anxiety, somatization)\textsuperscript{5}: these three variables are among the 90 psychological symptoms evaluated by the Symptom Checklist 90-Rivised (SCL-90R). Participants report the extent to which they have been bothered by each symptom.
The Perceived Stress Scale (PSS): The variable assesses the perception of stress. For each of the 10 items, participants described how often in the past five months they felt stressful. The perceived stress core was evaluated by summing the numerical weight of each item.

State-trait anxiety inventory (Y1 and Y2): The state-trait anxiety inventory consists of two 20-items questionnaires: state anxiety inventory and trait anxiety inventory. State anxiety inventory asks participants to describe their feelings (for the 20 items) at their current state, while trait anxiety inventory asks participants to describe their general feelings.

The Pain Catastrophizing Scale (Rumination, Magnification, and Helplessness): Rumination, magnification and helplessness are three subscales of the pain catastrophizing scale. Participants indicate the degree to which they have specified thoughts and feelings when experiencing pain, and the scores were calculated for each subscale.

Count of comorbid conditions: the count of comorbid conditions was one of the measure of health status, which was assessed by a checklist inquiring into past or current conditions. The comorbidities include overlapping pain condition or other functional disorders.

Count of non-specific orofacial symptoms: the count of non-specific orofacial symptoms was one of the measures of clinical status. Six jaw symptoms (such as ache or fatigue) were evaluated, the total count of symptoms was used a summary score.

After selecting these 16 intermediate phenotypes, the two datasets were then merged by ID, generating the intermediate phenotypes dataset. The intermediate phenotypes dataset was then merged with the genetics analytical dataset (which was generated by combining the SNPs from the two cohorts) by ID. After removing participants that only exist in one of the datasets,
the final analytical dataset for this study consists of 1946 participants, with their TMD status, covariates (C1, C2, site1, site2, site2, site4, sex), 16 interested variables and 2657 SNPs. A spreadsheet of allele frequencies was generated, and SNP’s with a minor allele frequency (MAF) of less than 0.02 were included to increase the power of the study.

Inverse-probability-of-sampling-weighted (IPW) regression

The explicit goal of OPPERA project and COMT study is to analyze the association between genotypes and TMD case status, which adopted a case control design that implicitly conditions on a primary disease (TMD) outcome. The data from thesis two studies investigates the associations between the intermediate phenotypes and SNPs based on stratified sample (two strata, one for cases and one for controls). The prevalence of cases in the sample is much greater than that in the general population. Common analysis approaches which ignore the ascertainment or adjust the ascertainment by TMD status will lead to inflated type I error rate for tests of association between genotypes and intermediate phenotypes, and also provide biased estimates of the effects of marker genotypes on the intermediate phenotypes.

The purpose of this study is to analyze the associations between marker genotypes and intermediate phenotypes (the 16 intermediate phenotypes as we described above). These associations can be viewed as an analysis of case control data with biased sampling. One possible approach to deal with this situation is the inverse probability of sampling weighted regression model. The IPW regression model is a linear model with two different weights to the two strata (cases and controls of TMD in this case). The weights come from the reciprocal of the
selection probability of TMD status, which is given by the number of observations in each condition times the prevalence of the condition:

Case: \( w_1 = p_1 \times \frac{1}{n_1} = \frac{0.05}{n_1} \)

Control: \( w_0 = p_0 \times \frac{1}{n_0} = \frac{0.95}{n_0} \)

Where \( p_1 \) represents the prevalence of cases in general population, \( p_0 \) represents the prevalence of controls in general population, \( n_1 \) represents the number of cases in the sample and \( n_0 \) represents the number of controls in the sample.

Equivalently, we set the weight of TMD-free controls to 1. Then the weight of TMD cases is:

\( \frac{0.05}{n_1} \times \frac{n_1}{0.95} \cdot \frac{n_0}{n_0} \)

QQ plot for p values

Since we use IPW regression for each SNP and each secondary traits, the output table of results, which contains the estimates, confidence intervals, t values, p values for 2657 SNPs, is hard to interpret with the naked eye. Quantile-Quantile (QQ) plots of the p values were used to investigate if any special p value (extremely low) exists. The Quantile distribution of the observed -log p values are plotted on the y axis versus the quantile distribution of the expected -log p values, which are plotted on the x axis. The expected -log p values were determined by creating a vector of N values (N is the number of p values) evenly spaced from 1 to 1 / N, taking the -log of each of these values and ranking them from smallest to largest. If there are no associations between SNPs and intermediate phenotype, the p values will form a straight line.
on the QQ plots because the observed distribution of p values will follow a uniform distribution.

If there are such associations, which will generate extremely low p values, those p values would not follow the uniform distribution, and thus diverge from the straight line.

The false discovery rate (FDR) of a series of tests is the proportion of “significant” tests that are false positives. Three lines corresponding: FDR=0.2, FDR=0.1, FDR=0.05 are shown for in the QQ plot. If the observations on QQ plots lie above FDR=0.05 line, it means that if all tests corresponding to points above the line are treated as “significant”, then no more than 5% of these tests should be false positives. We can perform further investigations those p values that fall outside of the expected pattern. Observations that lie above 0.1 or 0.2 are more likely to be false positives, but we can still investigate these SNP’s in future studies.

Adjusting for covariates

The allele frequencies differences between cases and controls in genetic association study caused by population stratification can cause spurious associations in regression analysis. The following figure shows the QQ plots of the p values produced by performing an unadjusted regression analysis to evaluate the association between the SNP’s and the phenotype. We can see inflation in all of the plots, resulting in spurious associations between the SNPs and the intermediate phenotypes.
The race eigenvectors produced by EIGENSTRAT\textsuperscript{8}, adjust for population stratification since SNPs whose allele frequencies are different in different races be associated with C1 and C2. Also, as noted earlier, the regression analysis included covariates for sex and OPPERA study site.
Result

The 16 QQ plots were generated after adjusting for the covariates:
All plots except the plot of heat pain sensitivity and heat pain windup follow a straight line below FDR=0.2, which means that no significant associations were observed between the SNPs.
and the intermediate phenotypes. However, there were two QQ-plots that showed evidence of an association, namely the QQ plots for heat pain sensitivity and heat pain windup.

From the plots of heat pain sensitivity, 3 SNPs lie between the FDR=0.2 and FDR=0.1 lines, which suggests that 20% of these three SNP’s are false positives. Also, 1 observation lies between the FDR=0.1 and FDR=0.05 lines in the plot of heat pain windup, which suggests that there is a 10% change that this association is a false positive. Although these observations above three FDR lines may suggest relatively low p values, further investigation must be performed to confirm our conjecture that these associations are significant.

**Discussion**

Heat pain sensitivity contains three observations that lie above the FDR=0.2 line, which suggest that 20% of these SNP’s are false positives. However, concluding that these three observations are significant associations just based on the QQ plot is premature. Our next step will be to investigate which three SNPs these three observations represent. We will run the regression analysis for these three SNPs again to obtain their p values. Also, observations that lie near each other in a QQ plot for p values may be the result of some correlations between SNPs (like they are in the same chromosome, etc.). We can further investigate the SNP map to investigate the correlation between these SNPs. Based on the p values and our further investigation of SNP correlation, we seek to obtain a more information about the associations between these SNPs and heat pain sensitivity.
For the QQ plot for heat pain windup, although one observation passes the FDR=0.1 line, we can see clear evidence of inflation in the plot. Our next step is to investigate the causes of the inflation and add additional covariates (such as higher-order eigenvectors) to the regression models to attempt to reduce this inflation.
References


