It is an enigma that individuals can vary considerably in their sensory and emotional responses to clinical conditions that have apparently similar potential to cause pain. This phenomenon is observed frequently in temporomandibular disorders (TMD), a group of chronic orofacial pain conditions that affect approximately 10% of adults in developed nations (Dworkin and LeResche, 1992; Okeson, 1996). Human experimental studies confirm extensive inter-individual variability in reported levels of pain elicited by standardized noxious stimuli (Gracely, 1999), suggesting that genetic and environmental factors influence pain perception. TMD, in turn, appears to be related to a general state of pain amplification (Maixner et al., 1998). A systematic review of epidemiological studies found that pre-existing pain conditions, depression, and female sex were consistent risk factors for TMD (Drangsholt and LeResche, 1999).

Associations between psychological characteristics and pain potentially could be explained by variations in people's genetic characteristics. Variation in a single nucleotide polymorphism (SNP) of the gene encoding catechol-O-methyltransferase (COMT) is both predictive of anxiety (Enoch et al., 2003) and associated with pain regulation in the central nervous system (Zubieta et al., 2003). The same SNP has been implicated as a risk factor for schizophrenia (Egan et al., 2001), although a systematic review found the evidence to be inconsistent (Glatt et al., 2003). However, when Shifman et al. (2002) analyzed a panel of SNPs in the gene encoding COMT, they identified a haplotype that was associated with a 1.5-fold increase in odds of schizophrenia (95% CI = 1.4-1.6). The same haplotype has been shown to code for relatively lower expression of COMT mRNA in human brain tissue (Bray et al., 2003).

Recently, we have identified 3 prevalent COMT haplotypes that accounted for 11% of variability in experimental pain perception in women, and which were predictive of TMD risk (Diatchenko et al., 2005). However, the potential for variations in this gene to mediate relationships among psychological characteristics, pain sensitivity, and TMD risk has not been examined.

The overall goal of this study was to determine if psychological characteristics associated with pain sensitivity are predictive of TMD risk independently of any effects of COMT haplotype. We aimed to answer 4 related research questions:

1. Is sensitivity to standardized noxious stimuli predictive of TMD risk?
2. Which psychological characteristics are associated with pain sensitivity?
3. Are those same psychological characteristics predictive of TMD risk?
4. Is the magnitude of TMD risk associated with those psychological characteristics attenuated after adjustment for variants of the gene encoding COMT?
MATERIALS & METHODS

We undertook a three-year, prospective cohort study of first-onset TMD among healthy, female volunteers aged 18-34 years at the time of recruitment. We aimed to follow 238 participants for up to 3 yrs, this being the number calculated to provide statistical power of 0.80 to detect risk ratios of at least 2.7, assuming a three-year cumulative incidence of 9%, which we estimated based on results reported by Von Korff et al. (1993).

Prior to enrollment in the study, participants were screened and underwent a baseline physical examination of the head and neck, conducted according to the research diagnostic criteria (RDC) for TMD (Dworkin and LeResche, 1992). Individuals were excluded if they had a history of TMD, were diagnosed with TMD at the baseline examination, or if they reported a significant medical history including traumatic facial injuries or use of centrally acting medications (see APPENDIX Table 1). At baseline, peripheral blood samples were collected from enrolled participants, and they completed psychological questionnaires and psychophysical pain assessments. For up to 42 mos after their baseline assessment, participants were contacted every 3 mos by research staff, who administered a medical history update questionnaire. Any participants responding positively to key questions about TMD symptoms were re-examined according to the RDC protocol. Additionally, each year, all participants were invited to attend for RDC examination. New cases of TMD were defined according to RDC criteria (Dworkin and LeResche, 1992) for Group I Myofascial Pain and/or Group III Arthralgia, both of which include reported pain and pain on palpation. New cases were confirmed additionally by two examiners (W.M., A.S.).

COMT Genotyping

We used peripheral blood samples for genotyping 4 COMT SNPs: rs6269, rs4633, rs4818, and val158met (see APPENDIX Table 2). Haplotypes were constructed, and participants were selected for this analysis if they had combinations of the 3 most prevalent haplotypes:

- Participants carrying solely haplotypes ACCG or ATCA for 4 SNPs (rs6269, rs4633, rs4818, and val158met, respectively) were classified as having pain-sensitive haplotypes (PSH);
- Remaining participants, who had at least 1 haplotype (GCGG), were classified as having pain-resistant haplotypes (PRH).

The labels "sensitive" and "resistant" were based on our observed associations of these haplotypes with sensitivity to experimental pain, supported additionally by the results of in vitro and in vivo experiments (Diatchenko et al., 2005).

Psychological Measures

Four psychological questionnaires, which assessed a broad range of psychological characteristics, were administered at the time of participant recruitment. The Brief Symptom Inventory (BSI) consists of 53 items designed to assess 9 subscales of psychological function (Derogatis and Melisaratos, 1983). The Perceived Stress Scale (PSS) asks about 14 sources of stress, to yield a single, overall rating (Cohen et al., 1983). The Profile of Mood States-Bi-Polar (POMS-Bi) consists of 72 mood-related items yielding 6 subscales measuring affective dimensions of mood (Lorr and McNair, 1988). The State-Trait Anxiety Inventory (STAI) contains 20 statements evaluating levels of state and trait anxiety separately (Spielberger et al., 1983).

Experimental Pain Procedures Used to Index Pain Phenotype

Each participant completed 13 pain perception assessments at baseline (see APPENDIX Table 3):

- Six measures were obtained by a modified "Marstock" procedure (Fruhstorfer et al., 1976; Fagius and Wahren, 1981) that measured threshold and tolerance to thermal pain, measured at each of 3 body sites with a thermode that increased in temperature at a rate of 0.5°C/sec. This excluded 3 measures of sensitivity to thermal pain increasing at 3°C/sec that we analyzed previously (Diatchenko et al., 2005), but which we now excluded based on comments by reviewers of another report from this study (Diatchenko et al., 2006).
- We assessed sensitivity to ischemic pain by rendering the arm hypoxic with a blood pressure cuff and recording elapsed time until the participant reported threshold and tolerance to ischemic pain (Maixner et al., 1990).
- Pressure pain thresholds in response to a hand-held pressure algometer (Jaeger and Reeves, 1986) were assessed at 4 sites: temporalis muscles, masseter muscles, temporomandibular joints, and ventral surfaces of the wrists.
- We computed a global measure of temporal perceptual responses to heat pain by summing visual analog scale ratings of 15 sequential 53°C heat pulses that were applied to the right hand (Price et al., 1977).

Data Analysis

We evaluated the 4 research questions in sequence, recognizing that there would be insufficient statistical power to evaluate all hypothesized relationships using multivariate modeling alone. We first quantified TMD risk by computing average incidence density rates of TMD. We measured pain sensitivity phenotype by summarizing responses to 13 standardized noxious stimuli, yielding a single index of pain sensitivity. The incidence density ratio (IDR) was computed for comparison of TMD risk between participants who had relatively high sensitivity and those who had relatively low sensitivity (research question 1). We then screened for bivariate associations by computing correlation coefficients between the index of pain sensitivity and each of 18 psychological subscales (research question 2). In this step, we evaluated statistical significance using the threshold of P < 0.0028, equivalent to Bonferroni’s correction of the conventional P < 0.05 for the 18 psychological subscales evaluated. We also made an initial assessment of potential for confounding due to haplotype by comparing mean psychological subscale scores between people with PSH and PRH haplotypes. We then dichotomized the psychological variables identified in those steps to assess associations with TMD risk (research question 3). We evaluated potential confounding by comparing prevalence of the PSH haplotype between dichotomized groups. Finally, we undertook multivariate modeling to determine the extent to which the association between TMD risk and depression was attenuated after adjustment for COMT genotype (research question 4). For a more detailed description of the study methods, see the online APPENDIX.

Ethical Conduct of Research

All participants provided signed, informed consent to participate in the study. The study was reviewed and approved by the UNC School of Dentistry’s Committee on Investigations Involving Human Subjects.

RESULTS

Two hundred and fifty-four females volunteered to take part in the study and completed baseline sensory assessments. Of
these, 212 (83%) provided a blood sample and written consent for genotyping. Of those genotyped, 194 carried the 3 most abundant COMT haplotypes. Follow-up data about clinical TMD status were obtained from 171 (88%) of them. The percentage of people with follow-up data was higher for single women compared with those who were not single (P < 0.01, Table 1). However, rates of follow-up did not vary significantly among subgroups classified by any other characteristics listed in Table 1.

Fifteen new cases of TMD were diagnosed according to RDC criteria among the 171 participants who were followed for an average of 30 mos (range = 8-42 mos). The 15 new cases of TMD represented a cumulative incidence of 8.8% (95%CI = 5.0-14.2%), and the average annual incidence rate of TMD was 3.5 cases per 100 person-years (95%CI = 2.4-5.1 cases per 100 person-years). Among the 55 participants whose index of pain sensitivity had a value of 3.0 or more, the incidence rate was 5.9 cases per 100 person-years, compared with 2.4 cases per 100 person-years among the 116 people with lesser values, yielding a statistically significant IDR of 2.5 (95%CI = 1.2-5.2) (Fig. 1). This represented an elevation of risk that was similar in magnitude to our previously reported finding that the pain-sensitive COMT haplotype was predictive of a 2.3-fold increase in risk of TMD (Diatchenko et al., 2005; data included in Fig. 1 for comparison).

Bivariate correlations ranging from 0.15 to 0.25 in absolute value were observed between 10 psychological scales and the index of pain phenotype (Table 2). Although they represented only weak levels of correlation, 3 of them were statistically significant at the threshold of P < 0.0028: BSI depression, POMS confident-unsure, and Perceived Stress. However, the mean values of psychological variables generally did not differ significantly between the 2 COMT haplotype groups, suggesting little potential for confounding (Table 2). The one exception was BSI depression, which had a marginally higher mean value in the PSH group compared with the PRH group (P = 0.05) (Table 2).

When dichotomized at their approximate upper tertile, all 3 psychological variables identified in Table 2 were significant risk factors for TMD (Fig. 2). IDRs ranged from 2.6 for the Perceived Stress scale to 3.7 for the POMS confident-unsure subscale. However, the percentage of participants with PSH haplotypes did not differ significantly among psychological subgroups, as evidenced by prevalence ratios with 95%CIs that included unity, suggesting that they were not likely confounders of the haplotype-TMD association. As expected, the mean index of pain sensitivity differed significantly between each of the psychological subgroups (see APPENDIX, Table 4).

The BSI depression subscale was selected as the one psychological variable in which there was some potential for confounding due to COMT haplotype (Table 2). Multivariate analysis revealed independent effects on TMD risk of BSI depression (likelihood ratio statistic, 1 df, = 8.75, P = 0.004) and COMT haplotype (likelihood ratio statistic, 1 df, = 4.03, P = 0.046). In this multivariate model, which adjusted for COMT haplotype, the IDR for high BSI depression (subscale scores > 60) was 3.1 (95%CI = 1.5 6.4), compared with the reference group, which had low BSI depression scores (< 60). This IDR was not markedly different from the bivariate IDR of 3.2 for high BSI depression (Fig. 2).

**DISCUSSION**

In this prospective cohort study of otherwise-healthy females,
The observed IDRs were comparable with risk ratios reported for other multifactorial conditions, such as schizophrenia (Shifman et al., 2002), and for TMD (Von Korff et al., 1993). Nonetheless, these represent only moderately strong predictors, highlighting our belief that no single gene or psychological characteristic is sufficient to explain variability associated with a complex condition such as TMD.

The significance of these findings is strengthened by the prospective cohort study design, which overcomes a major limitation of previous case-control studies of TMD, in which it has been unclear whether putative risk factors such as pain phenotype and psychological characteristics associated with pain phenotype were both risk factors for first-onset TMD. The observed IDRs were comparable with risk ratios reported for other multifactorial conditions, such as schizophrenia (Shifman et al., 2002), and for TMD (Von Korff et al., 1993). Nonetheless, these represent only moderately strong predictors, highlighting our belief that no single gene or psychological characteristic is sufficient to explain variability associated with a complex condition such as TMD.

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sensitivity and depression existed in participants prior to the onset of TMD or arose as a consequence of it. Moreover, participants in this study were diagnosed independently by examiners using RDC guidelines. This provides confidence that the elevated risk of TMD is not simply an artifact of reporting bias among participants found to be at elevated risk.

Our results suggest that effects of psychological characteristics on TMD risk cannot be attributed to variants of the gene encoding COMT. Mean psychological scores generally did not vary among COMT haplotype groups, and prevalence of the PSH haplotype did not differ significantly among groups dichotomized according to psychological subscale scores, suggesting little, if any, potential for confounding. Furthermore, results from the multivariate analysis of the one psychological subscale that showed some potential for confounding revealed little attenuation of the IDR for BSI depression after adjustment for COMT haplotype. The lack of attenuation demonstrated that the relationship between BSI depression and TMD risk could not be attributed to the COMT haplotype. The implication is that there are separate etiological mechanisms by which these psychological characteristics and genetic variants of the COMT gene influence the risk of a person developing TMD.

When designing this study, we recognized that there would be a relatively small number of TMD cases diagnosed over 3 yrs. The observed cumulative incident of 8.8% is consistent with the rate of 7.7% observed during 3 yrs by Von Korff et al. (1993). Because there were few incident cases, we adopted a systematic analytic strategy to narrow the pool of candidate psychological characteristics by first screening for associations with pain phenotype, selecting only those variables that had statistically significant correlations.

One consequence of this analytic strategy is that psychological characteristics not associated with experimental pain have been omitted as potential risk factors in this analysis. This is one reason that we found no association between TMD risk and somatization or anxiety—2 risk factors reported in some previous studies (Drangsholt and LeResche, 1999). The limited number of TMD cases also precluded any evaluation of interactions between genetic and these psychological characteristics.

There are 2 principal clinical implications from these results. Most importantly, we interpret these findings as evidence of separate etiological pathways by which these psychological factors and COMT haplotypes increase risk of clinical pain. Second, the findings highlight a need for a multifaceted approach to treatment and prevention of TMD that is based on an understanding of its etiology. For example, based on these findings, control of psychological characteristics and COMT activity may be effective strategies to prevent or treat TMD. Existing treatments for TMD, such as analgesics, dental-orthopedic devices, and surgery, often are decided on empirically, and sometimes despite evidence that some such treatments are no better than a placebo (Dao et al., 1994). Indeed, Svensson and Graven-Nielsen (2001) have asserted that "the pathophysiology and etiology of craniofacial muscle pain are not known in sufficient detail to allow causal treatment." The findings from this study, if confirmed in other populations, would provide a rationale for the development and evaluation of the efficacy of interventions for TMD that target psychological characteristics or that compensate for decreased COMT activity.

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