Nicotine Withdrawal Sensitivity, Linkage to chr6q26, and Association of OPRM1 SNPs in the SMOfamilly in SMOking in FAmilies (SMOFAM) Sample

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Abstract

Background: Nicotine withdrawal symptoms are related to smoking cessation. A Rasch model has been used to develop a unidimensional sensitivity score representing multiple correlated measures of nicotine withdrawal. A previous autosomal-wide screen identified a nonparametric linkage (NPL) log-likelihood ratio (LOD) score of 2.7 on chromosome 6q26 for the sum of nine withdrawal symptoms.

Methods: The objectives of these analyses were to (a) assess the influence of nicotine withdrawal sensitivity on relapse, (b) conduct autosomal-wide NPL analysis of nicotine withdrawal sensitivity among 158 pedigrees with 432 individuals with microsatellite genotypes and nicotine withdrawal scores, and (c) explore family-based association test with the nicotine withdrawal sensitivity score identified eight tagging SNPs with global P values <0.05 and false discovery rate Q values <0.06.

Results: An increased risk of relapse was associated with nicotine withdrawal sensitivity score (odds ratio, 1.25; 95% confidence interval, 1.10-1.42). A maximal NPL LOD score of 3.15, suggestive of significant linkage, was identified at chr6q26 for nicotine withdrawal sensitivity. Evaluation of 18 OPRM1 SNPs via the family-based association test with the nicotine withdrawal sensitivity score identified eight tagging SNPs with multiple correlated measures of nicotine withdrawal. A Rasch-modeled nicotine withdrawal sensitivity scores in a multiplex smoking pedigree sample. Future studies should attempt to replicate these findings and investigate the relationship between nicotine withdrawal symptoms and variation at OPRM1.

Introduction

Smoking was reported to underlie 4.8 million premature deaths in the world in 2000, which were evenly split between developing countries and industrialized countries (1, 2). Most smokers who attempt to quit smoking have difficulty remaining tobacco free due to the large number of nicotine withdrawal symptoms, and the severity of these symptoms has been reported to be related to smoking cessation success (3).

Nicotine withdrawal symptoms have been characterized using more than two dozen research-based methods (4, 5). Withdrawal symptoms characterize smokers’ levels of irritability, restlessness, insomnia, depression, concentration, appetite, and cravings and urges for tobacco over many differing time periods ranging from weeks to months (7, 8). Nicotine withdrawal symptoms are highly correlated, and methods have been used to summarize these symptoms, including the use of latent class analyses (9, 10) and Rasch models (11). A unidimensional Rasch model of nicotine withdrawal sensitivity was constructed in a sample of 1,644 smokers reporting quitting for >3 months at least once (11). The Rasch-modeled nicotine withdrawal sensitivity score enables analysis of multiple highly correlated nicotine withdrawal symptoms in a single analysis, and is significantly associated with an increased hazard ratio with respect to the duration of these attempts, as well as to smoking intensity and a shorter time to first cigarette in the morning (11). Rasch models have also been previously used to evaluate the unidimensionality and associations of established measures of nicotine dependence (12), and of additional measures of nicotine dependence and their relationship to items within established measures of nicotine dependence (13).

This study utilized a Rasch-modeled nicotine withdrawal sensitivity score constructed from eight quit attempt symptoms obtained from participants in a longitudinal family sample called the SMOking in FAmilies study (SMOFAM; ref. 14). SMOFAM is a community-based sample of pedigrees, ascertainment via an adolescent proband, and longitudinally followed via annual assessments over a period of nearly two decades to study the...
social and behavioral risk factors for tobacco use (15). Multiplex ever smoking (≥100 cigarettes smoked) pedigrees, drawn from those pedigrees where the proband had completed at least seven of the first ten assessments on tobacco use and elected to provide a blood sample for DNA analysis, were recruited for an integrated study of the genetics of smoking behavior and nicotine metabolism. These pedigrees were administered a family history of tobacco use questionnaire that included cigarette smoking quit history and withdrawal symptoms (16). Specifically, respondents were asked: “During the first few days after you quit smoking cigarettes (the first or the most recent time) for at least 3 months, did you feel or experience any of the following?” Items presented were irritable or angry, restless, increased appetite, depressed mood, difficulty sleeping, craving to use tobacco, anxious, and difficulty concentrating. Previously (14), a withdrawal severity scale that included these eight symptoms of withdrawal, as well as a ninth symptom, decreased heart rate, was used in a linkage analysis. The Rasch-modeled nicotine withdrawal sensitivity score did not include the ninth withdrawal symptom due to reduced fit in a number of correlation, factor, and item response analyses (data not shown). These analyses use the Rasch-modeled nicotine withdrawal sensitivity score for analyses with relapse, defined as re-engaging in smoking after quitting for ≥1 wk, and for autosomal-wide linkage analysis and association analysis with common variation at a candidate gene of importance for smoking behavior, the mu opioid receptor (MOR) gene (OPRM1).

The SMOFAM Study previously conducted a model-free linkage analysis with several nicotine dependence and tobacco use phenotypes (14). Swan et al. found a maximal log-likelihood ratio (LOD) score (MLS) of 2.7 on chromosome 6 for the Fagerström Test for Nicotine Dependence and a MLS score of 2.7 on chromosome 6 for withdrawal severity (the summation of nine withdrawal symptom scores, rated on a 4-point Likert scale at 0 for not at all to 3 for severe, with a range of 0 to 27), with the Fagerström Test for Nicotine Dependence MLS found in the more proximal region of 25 cM, from 148 to 173 cM. The support interval included the OPRM1 locus.

The MOR binds endogenous β-endorphin, the release of which increases upon nicotine administration; activation of the MOR results in dopamine release within the nucleus accumbens, possibly through inhibition of GABAergic inhibition of dopaminergic neurotransmission (18). The potential involvement of the OPRM1 gene in smoking-related phenotypes is supported by multiple analyses. A novel linkage analysis of existing data and bioinformatic analyses of extant literature reported positive correlations between linkage analyses of nicotine dependence, microarray/candidate gene studies, and biological pathway analyses (19). Analysis of seven OPRM1 SNPs identified several haplotypes significantly associated with smoking initiation and two haplotypes marginally associated with nicotine dependence (20). The OPRM1 nonsynonymous (Asn40Asp) SNP (rs1799971) has been significantly associated with long-term smoking cessation in interaction with gender (18).

Herein we present findings regarding an association between the Rasch-modeled nicotine withdrawal sensitivity score and smoking relapse. We also present the results of an autosomal-wide linkage analysis, and a family-based association analysis at the OPRM1 gene to the Rasch-modeled nicotine withdrawal sensitivity score using microsatellite genotypes and OPRM1 SNPs available in the SMOFAM dataset.

Materials and Methods

Cohort. Included in these analyses were subjects ages 11 to 15 y, and their parents and siblings from the longitudinal SMOFAM study of environmental and genetic determinants of tobacco use (15, 16). Characterization of phenotypes for tobacco use, including the acquisition and maintenance of smoking, as well as potential psychosocial and environmental predictors of substance use, were collected for this cohort. After identification and recruitment of probands and family members, a detailed intake assessment was done that included sociodemographics and a complete cigarette smoking history. Genomic DNA was extracted from venous whole blood using a standard salt-based precipitation method (21).

Phenotypes. The phenotypic outcome measure used in the logistic regression analyses was relapse, which was defined as re-engaging in smoking after quitting for ≥1 wk. Relapse was used as the dependent variable whereas the Rasch-modeled nicotine withdrawal sensitivity score was the independent variable in the logistic regression analysis. The phenotypic outcome measure used in the linkage and association analyses was the nicotine withdrawal sensitivity score formulated using the smoking withdrawal responses from the Smoking History Questionnaire, with details of the development of this measure described in (11). The unidimensional nicotine withdrawal sensitivity score summarizes the eight quit attempt symptoms. The nicotine withdrawal sensitivity score was examined for autosomal-wide linkage and for candidate gene association with SNPs of the OPRM1 gene.

Linkage. Genotypes were determined for 739 dinucleotide microsatellite markers (14) on 158 pedigrees containing 611 individuals with an average of 3.87 individuals genotyped per family (these figures apply to the entire SMOFAM dataset). A sex-averaged genetic map developed by Applied Biosystems, using 763 autosomal map positions generated from Centre d’Etude du Polymorphisme Humain (CEPH) genotype data, was used in the linkage analysis and all CM references herein are with respect to this map. All available genotypes were analyzed for each family using Pedigree RELationship Statistical Test (PREST) to validate the structure of pedigrees (22). Pedcheck was used to detect non-Mendelian inheritance patterns (23). The probability that each genotype was correct was assessed in the context of all other available genotypes using the error-checking algorithm implemented in Merlin (24). Less than 0.5% of all genotypes were excluded after these quality controls were applied.

Autosomal multipoint nonparametric linkage analysis (NPL) was done on the final genotype data in Merlin with the nicotine withdrawal sensitivity score as the phenotype (24, 25). Merlin (24) was used to identify the number of times a LOD score as significant as the MLS observed in this linkage analysis was observed in 1,000 simulated genome scans.

SNP Selection, Genotyping, and Linkage Disequilibrium Analyses. Existing OPRM1 SNP data were available from a candidate gene panel designed to interrogate nicotinic acetylcholine receptor, dopaminergic, and neuro-peptide candidate genes for studies of nicotine addiction and treatment-related phenotypes (26-29). As described in Conti et al., genotyping of DNA samples used the GoldenGate assay (Illmina), with quality control procedures that included automated sample handling protocols and the inclusion of replicate DNA samples to aid in identifying genotyping errors (28). The online databases dbSNP (30) and Genewindow (31) were used to assign chromosome position and genomic annotation for OPRM1 SNPs. The program HAPLOVIEW (32) was employed to examine the extent of linkage disequilibrium between pairs of OPRM1 SNPs as well as to determine the haplotype block structure within the SMOFAM sample. Blocks were defined using the criteria proposed by Gabriel et al. (33). The tagging SNP selection program Tagger (34) was used to select a subset of 21 SNPs representing the genetic information contained in the 46 OPRM1 SNPs genotyped in the SMOFAM sample.

Association Testing. Logistic regression as implemented in Stata Version 9 (35) was used to assess the association between relapse and the Rasch-modeled nicotine withdrawal sensitivity score. The family-based association test (36), as implemented within the GeneticsBase package from Bioconductor (37) of the R programming language (38), was used to test for association between OPRM1 SNPs in the pedigrees between the nicotine withdrawal sensitivity score and 18 candidate SNPs. We considered a P value < 0.05 to represent nominal statistical significance.

False Discovery Rate Analysis. To assign false discovery probabilities to individual OPRM1 SNPs included in the family-based association test, false discovery rate (FDR) Q values were calculated (39). Briefly, whereas P values express the probability of a single false-positive result among all tests, Q values estimate the proportion of results declared interesting that are actually false (40). As suggested by Storey et al. (41) for analyses with few numbers of P values, the bootstrap option was utilized in preference to the smoother option in generation of the Q values. The authors suggest that the bootstrap method provides more reliable estimates under these conditions (41). We opted to not employ a specific Q value threshold but rather to present the Q values in relationship to P values and biological plausibility.

Results

The sample for these genetic analyses consisted of 224 pedigrees (n = 520 individuals) with complete nicotine withdrawal data, where 158 pedigrees had linkage scan genotype data and 172 pedigrees had OPRM1 SNP data. The sample of SMOFAM subjects with complete OPRM1 genotype and nicotine withdrawal sensitivity scores (n = 419) used in these analyses was approximately equally divided by gender and by smoking status (current versus former smokers with a small proportion of experimenters; Table 1). In these analyses 45% of subjects stated that they had relapsed due to withdrawal symptoms (data not shown). In the sample of 520 subjects, the nicotine withdrawal sensitivity score distribution was approximately normal (Fig. 1), and had a mean value of -0.014 with SD of 1.84. An increased risk of relapse was associated with the nicotine withdrawal sensitivity score (odds ratio, 1.25; 95% confidence interval, 1.10-1.42; data not shown). Models testing for associations between gender and the nicotine withdrawal sensitivity score were null, and therefore adjustments for gender were considered unnecessary in these analyses.

The number of pedigrees with linkage scan data was 158, and the number of individuals in those pedigrees with nicotine withdrawal sensitivity score data was 432. The autosomal-wide MLS for the nicotine withdrawal sensitivity score linkage analysis was 3.15 at 164.5 cM on chr6q26 (Fig. 2), with a multipoint P of 7 × 10^-5 (Supplementary Table S1). A LOD score result of this magnitude was observed 37 times in 1,000 simulated genome scans. The 1 and 2 LOD support intervals ranged from 152 to 172, and from 147 to 178 cM, respectively. The average numbers of pedigrees and subjects with complete linkage scan genotype data and nicotine withdrawal sensitivity score data at the two markers flanking the MLS were 154 and 397, respectively. No other chromosomal regions with LOD scores >2.0 other than chr6q26 were observed. Chromosomal regions with LOD scores >1.0 were observed on chr1 at 112.1 cM (P = 0.009), chr4 at 144.1 cM (P = 0.0015), chr13 at 13.4 cM (P = 0.004), and chr15 at 74.8 cM (P = 0.011), in addition to chr6q26 (see Supplementary Table S1 for a complete list of LOD scores >1 associated with the nicotine withdrawal sensitivity score).

Forty-six genotyped SNPs covering the OPRM1 genetic region in the SMOFAM sample were available for analysis (Fig. 3). SNPs in intervening sequence 1 (IVS1) of OPRM1 and SNPs in IVS3 through IVS5 were in significant linkage disequilibrium in this sample, where SNPs in IVS2 seemed to be in linkage disequilibrium with SNPs in either the 5’ or 3’ linkage disequilibrium block (Fig. 3). Because there was substantial pairwise linkage disequilibrium between adjacent markers and to reduce redundant

Table 1. Characteristics of subjects with complete genotype and Rasch-modeled nicotine withdrawal score data

<table>
<thead>
<tr>
<th>Gender</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>204 (48.7)</td>
</tr>
<tr>
<td>Female</td>
<td>215 (51.3)</td>
</tr>
<tr>
<td>Smoker status</td>
<td></td>
</tr>
<tr>
<td>Experimenter*</td>
<td>9 (2.2)</td>
</tr>
<tr>
<td>Current‡</td>
<td>216 (51.6)</td>
</tr>
<tr>
<td>Former†</td>
<td>194 (46.3)</td>
</tr>
</tbody>
</table>

*Experimenter smoker: smoking ≥ 100 cigarettes in a lifetime and not ever smoking on a daily basis.
‡Current smoker: ever smoking a cigarette even a puff and smoking ≥100 cigarettes in a lifetime and currently smoking ≥1 cigarettes on most days or currently smoking ≥1 cigarettes on most days and not having quit smoking cigarettes completely.
†Former smoker: ever smoking a cigarette even a puff and ever smoking on a daily basis and quitting smoking cigarettes completely.
multiple testing, a subset of 21 SNPs that captured 85% of all SNPs in the region (minor allele frequency ≥ 0.05) at \( r^2 > 0.8 \) were selected (Fig. 3). Three SNPs with minor allele frequencies <0.05 (rs12210856, rs1799972, and rs9282819) and seven subjects with genotypes inconsistent with Mendelian inheritance were removed from the data-set prior to family-based association test analyses. In this family-based association test analysis, there were 172 pedigrees (\( n = 419 \) subjects) with complete OPRM1 SNP genotypes and nicotine withdrawal sensitivity scores. Eight of eighteen SNPs evaluated for family based association with the nicotine withdrawal sensitivity score had global \( P \) values of <0.05 (Table 2). All eight SNPs with \( P \) values <0.05 had \( Q \) values <0.06. Five of these eight SNPs are located in IVS1, with one each in IVS3, IVS4, and IVS5. The nonsynonymous SNP rs1799971, which has been investigated in numerous studies of substance dependence, had \( P = 0.07 \) and \( Q = 0.08 \) in this analysis.

Discussion

Our analyses of a multiplex smoking pedigree sample identified an increased risk of relapse associated with nicotine withdrawal sensitivity score. We found suggestive evidence for an autosome-wide linkage with nicotine withdrawal sensitivity score. We identified a significant association and a nominal FDR between common sequence variants at the MOR gene locus, OPRM1, and nicotine withdrawal sensitivity score.

In a study that characterized nicotine dependence and nicotine withdrawal symptoms among adolescent smokers, seven withdrawal symptoms were more common among persons who had not successfully quit smoking than among successful quitters (42). In a Dutch sample of adolescent smokers, higher levels of nicotine craving at the beginning of the prequit week and on the target quit day decreased the odds of being abstinent during the last week of the study (3). In another Rasch model analysis of nicotine withdrawal sensitivity, increased nicotine withdrawal scores were associated with a shorter duration of quitting and with measures of nicotine dependence (11). In our analysis of nicotine withdrawal sensi-

tivity score in a sample of multiplex smoking pedigrees (16), an increased nicotine withdrawal sensitivity score was associated with a greater likelihood of relapse.

Our linkage analyses identified a MLS of 3.15 at 164.5 cM on chr6q26 for the nicotine withdrawal sensitivity score. Swan et al. previously found a MLS of 2.7 at 159 cM on chr6q26 for withdrawal severity (the summation of nine withdrawal symptom scores, rated on a 4-point scale at 0 for not at all to 3 for severe, with a range of 0 to 27) in the SMOFAM sample (14). The use of the Rasch-modeled nicotine withdrawal sensitivity score constructed from eight nicotine withdrawal symptoms as the phenotype in the present analysis resulted in an increase in the multipoint MLS of 0.45 units over the use of the sum of nine withdrawal symptoms as the phenotype in the previous analysis (14).

This suggests that the use of the Rasch model using eight withdrawal symptoms may be a more precise measure of a nicotine withdrawal trait linked to chr6q26. The multipoint \( P \) and the empirical \( P \) suggest that a linkage analysis result of this magnitude is expected by chance approximately 5% of the time in this sample (43). Existing linkage studies using nicotine withdrawal symptoms include only the studies of Swan et al., and a linkage study in two European ancestry populations that identified a genome-wide significant linkage on chr11p15 in the region of several monoamine and nicotinic acetylcholine candidate genes, as well as two other regions of interest on chromosomes 6p and 11q (44). Other linkage analyses have identified suggestive linkage evidence (43) for smoking-related behaviors on chr6q. Evidence of suggestive linkage was found for a combined dependence phenotype for any drug class and/or regular tobacco usage (every day for at least 1 month), analyzed as a threshold or continuous trait.
(LOD of 2.54 or 3.3) in a linkage sample of 49 pedigrees at 157 cM on chr6q25.3 (45), using the same linkage scan panel as used in this analysis. Analysis of a combined trait of body mass index, drug dependence, and/or regular tobacco use in the same sample reached a MLS of 4.1 at 151 cM on chr6q25.2. A search of the Sullivan Lab Evidence Project psychiatric genetics database (46) for genome-wide linkage and association findings in the 2 LOD support interval flanking our MLS annotates findings with bipolar, schizophrenia, major depressive disorder, Attention Deficit Hyperactivity Disorder, and autism disorders (Supplementary Table S2). Psychiatric disorders are well known to be comorbid with cigarette smoking; it has been estimated that the majority of cigarettes smoked in the United States are smoked by individuals fulfilling standardized criteria for nicotine dependence and a diagnosis of a psychiatric disorder (47).

Our candidate gene analysis suggests possible association with the OPRM1 A118G nonsynonymous (N40D) SNP rs1799971 and the nicotine withdrawal sensitivity score ($P = 0.07$, $Q = 0.08$). This SNP has been associated with functional differences in physiologic (48) or therapeutic (49, 50) responses to opioids in vivo and in vitro (51), although there are negative studies (52, 53). In addition to increased affinity for endorphin, an endogenous MOR ligand, and increased postsynaptic potassium current (51), differential allelic expression of the gene has been another mechanism hypothesized to account for potential functional differences between alleles (54). A meta-analysis of rs1799971 allelic association utilizing 28

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**Table 2. OPRM1 SNPs and association with Rasch-modeled nicotine withdrawal sensitivity**

<table>
<thead>
<tr>
<th>Name</th>
<th>Position*</th>
<th>Genomic†</th>
<th>MAF‡</th>
<th>Chisq</th>
<th>Global P</th>
<th>FDR Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799971</td>
<td>154452910</td>
<td>Ex1-173</td>
<td>0.13</td>
<td>3.28</td>
<td>0.0702</td>
<td>0.0821</td>
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<tr>
<td>rs477292</td>
<td>154455500</td>
<td>IVS1+218</td>
<td>0.226</td>
<td>5.73</td>
<td>0.0167</td>
<td>0.0586</td>
</tr>
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<td>rs4870266</td>
<td>154461111</td>
<td>IVS1+8029</td>
<td>0.066</td>
<td>0.018</td>
<td>0.8937</td>
<td>0.5534</td>
</tr>
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<td>rs610231</td>
<td>154470390</td>
<td>IVS1+17508</td>
<td>0.113</td>
<td>2.19</td>
<td>0.1390</td>
<td>0.1463</td>
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<tr>
<td>rs3823010</td>
<td>154471265</td>
<td>IVS1+1813</td>
<td>0.178</td>
<td>4.14</td>
<td>0.0418</td>
<td>0.0599</td>
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<td>rs589046</td>
<td>154485251</td>
<td>IVS1+17823</td>
<td>0.261</td>
<td>5.90</td>
<td>0.0152</td>
<td>0.0586</td>
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<td>rs563649</td>
<td>154500080</td>
<td>IVS1-2994</td>
<td>0.085</td>
<td>0.29</td>
<td>0.5316</td>
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<td>rs9322446</td>
<td>154500815</td>
<td>IVS1-2259</td>
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<td>4.79</td>
<td>0.0296</td>
<td>0.0599</td>
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<tr>
<td>rs510587</td>
<td>154505541</td>
<td>IVS3+711</td>
<td>0.054</td>
<td>4.00</td>
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<td>0.0599</td>
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<td>rs9282821</td>
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<td>0.413</td>
<td>0.13</td>
<td>0.7173</td>
<td>0.5188</td>
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<tr>
<td>rs3796683</td>
<td>154503527</td>
<td>IVS4+3799</td>
<td>0.144</td>
<td>4.29</td>
<td>0.0384</td>
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<tr>
<td>rs599548</td>
<td>154510674</td>
<td>IVS4-3936</td>
<td>0.133</td>
<td>&lt;0.01</td>
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<td>0.5592</td>
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<td>rs623956</td>
<td>154522138</td>
<td>IVS4-9792</td>
<td>0.238</td>
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<td>0.6276</td>
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<tr>
<td>rs606148</td>
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<td>0.347</td>
<td>0.24</td>
<td>0.6234</td>
<td>0.5082</td>
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</table>

*Chromosome positions are based on NCBI Human Genome Assembly, chr6.
†Genomic annotation obtained at genewindow.nci.nih.gov.
‡Minor allele frequency of the SNP in the SMOFAM sample.
samples of individuals fulfilling DSM-III, DSM-IV, or ICD-10 criteria for alcohol, opioid, methamphetamine, or mixed substance dependence reported no association (55). Because no exclusions for smoking behaviors were made in case or control samples, this particular meta-analysis may not be informative for substance dependence that involves nicotine dependence alone or in combination with other substance dependence. The SNP rs1799971 was not significantly associated with nicotine dependence in some population-based (20, 56) or treatment-seeking smoker (27) samples. With respect to smoking-related behaviors, the OPRM1 SNP rs1799971 minor allele (A) homozygote has been associated with an increased reinforcing value of nicotine in women and a tendency to prefer nicotine over denicotinized cigarettes in both sexes (2), and with endorsing cigarette liking after negative mood induction (57). In a clinical trial studying the effects of transdermal nicotine and nicotine nasal spray, smokers with one or more copies of the major allele (G) were more likely to be abstinent and experienced less mood disturbance and short-term weight gain compared with smokers with two copies of the minor allele (58). The study also found that the gene main effect on treatment outcome was most pronounced in smokers receiving transdermal nicotine (58). Note, however, that the minor allele homozygote has been associated with increased long-term smoking cessation with the use of transdermal nicotine patch versus placebo (18).

In our analysis, there is slightly more evidence for association of OPRM1 SNPs to the nicotine withdrawal sensitivity score in OPRM1 introns, where four SNPs are nominally significantly associated with the nicotine withdrawal sensitivity score in each of the two linkage OPRM1 linkage blocks commonly observed in European ancestry populations (59). Evidence from other studies also suggests that there are potentially two regions of linkage disequilibrium at OPRM1 that may be associated with smoking-related phenotypes. In a study investigating associations between nine OPRM1 SNPs and nicotine dependence in interaction with gender, rs310769 (IVS1 +1050) was significantly associated with dichotomized nicotine dependence ($P = 0.000416$) in a sample of 1,929 unrelated ever-smokers with Fagerström Test for Nicotine Dependence scores of 0, or 4 or more (56). In a study investigating associations between 11 OPRM1 SNPs and nicotine dependence, rs10485057 (IVS3+538) was significantly associated ($P = 0.0297$) with dichotomized nicotine dependence in a sample of 430 unrelated ever-smokers with Fagerström Tolerance Questionnaire (60) scores of 0 to 2 or 7 to 11 (20). In addition to studies of OPRM1 variation association with nicotine withdrawal symptoms, dopamine receptor locus sequence variation has been evaluated for association with measures of nicotine withdrawal (61, 62); one of two SNPs at the D2 dopamine receptor locus (DRD2) was observed to be significantly associated with acute nicotine withdrawal in interaction with time in a clinical sample of 116 smokers quitting smoking (61); moreover, a trend towards significant association of the DRD4 exon 3 variable number of tandem repeat polymorphism with the question “Did you feel a strong desire or craving for a cigarette?” was observed in a national probability sample of 204 current and former smokers (62).

Limitations in our analyses include those related to phenotype, linkage, and association analyses. The original smoking withdrawal data were collected using a paper-based questionnaire to catalogue respondents’ withdrawal symptoms; respondents may have had trouble recalling their symptoms, and therefore these data may suffer from recall bias. Responses to nicotine withdrawal symptoms were characterized using a 0 to 3 Likert scale, and therefore measurement error may exist. It is possible that false-positive linkage or association results arose in these analyses, although we assessed the empirical significance of linkage and the likelihood of false positive associations to facilitate the interpretation of significance.

The selection of the OPRM1 gene for study is a limitation as there are other candidate genes located at chr6q25.2-q27 that may also contribute to the nicotine withdrawal sensitivity score phenotype. Although the map location of OPRM1 is within the proximal region of the 2 LOD support interval, OPRM1 is only one of several candidate genes potentially influencing the nicotine withdrawal sensitivity score trait located in the interval. Other candidate genes for future investigation in the 2 LOD support interval include the SLC22A1, SLC22A2, and SLC22A3 genes coding for the organic cation transporters that are responsible for cellular uptake of endogenous neurotransmitters such as acetylcholine and monamines, as well as xenobiotics.

Our family-based association test analyses focused on a subset of common tagging SNPs at the OPRM1 gene, and therefore it is possible that we did not include OPRM1 SNPs that may contribute to the nicotine withdrawal sensitivity score phenotype. In our study we used tagging SNPs and eliminated noninformative SNPs from analysis to reduce the likelihood of false associations due to multiple testing. Further examination of OPRM1 variation through the use of haplotype analyses and through analyses of less common and rare variants, in relation to the nicotine withdrawal sensitivity score should be pursued in SMOFAM and additional samples.

Although our study presents preliminary data with suggestive ($P$ values <0.05 and $Q$ values <0.06) evidence for 8 of the 18 SNPs tested for association with nicotine withdrawal sensitivity score, replication of our results in an independent sample is essential (63). These findings may stimulate future research on the role of variation, linkage disequilibrium, and population differences in genetic architecture at OPRM1 on nicotine withdrawal sensitivity score.

Disclosure of Potential Conflicts of Interest

Dr. Hops discloses consulting with the University of Washington Social Development Group and Dr. Swan discloses consulting (1 day) with Pfizer and support for participant recruitment for another, unrelated study. No other potential conflicts of interest were disclosed.

Acknowledgments

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Nicotine Withdrawal Sensitivity, Linkage to chr6q26, and Association of \textit{OPRM1} SNPs in the SMOking in FAMILies (SMOFAM) Sample

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