Genetic Variation of the Mu Opioid Receptor (OPRM1) and Dopamine D2 Receptor (DRD2) is Related to Smoking Differences in Patients with Schizophrenia but not Bipolar Disorder

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Abstract

It is not known why mentally ill persons smoke excessively. Inasmuch as endogenous opioid and dopaminergic systems are involved in smoking reinforcement, it is important to study mu opioid receptor (OPRM1) A118G (rs1799971), dopamine D2 receptor (DRD2) Taq1A (rs1800497) genotypes, and sex differences among patients with schizophrenia or bipolar disorder. Smokers and nonsmokers with schizophrenia (n=177) and bipolar disorder (n=113) were recruited and genotyped. They were classified into three groups: current smoker, former smoker, and never smoker by tobacco smoking status self-report. The number of cigarettes smoked per day was used as the major tobacco smoking parameter. In patients with schizophrenia, tobacco smoking prevalence was greater in males than in females as expected, but women had greater daily cigarette consumption ($p<0.01$). Subjects with schizophrenia who had the OPRM1 *G genotype smoked more cigarettes per day than the AA allele carriers with schizophrenia ($p<0.05$). DRD2 Taq1A genotype differences had no effect on the number of cigarettes smoked per day. However, female smokers with schizophrenia who were GG homozygous of the DRD2 receptor smoked more than the *A male smokers with schizophrenia ($p<0.05$). In bipolar patients, there were no OPRM1 and DRD2 Taq1A genotype differences in smoking status. There also were no sex differences for smoking behavior among the bipolar patients. The results of this study indicate that single nucleotide polymorphism (SNP) of the less functional mu opioid receptor increases tobacco smoking in patients with schizophrenia. Alteration of DRD2 receptor function also increased smoking behavior in females with schizophrenia.

Key Words: Schizophrenia, Bipolar, Tobacco Smoking, Genetics, Mu Opioid Receptors

Introduction

It is well documented that persons with mental illness smoke more than normal controls (1-6). This includes patients with schizophrenia (7-15), depression (16-19) and bipolar disorder (20-22). Furthermore, tobacco smoking is the primary cause of preventable diseases and death in the United States (23). Although many individuals with a mental illness desire to stop using tobacco products (24), they are unable to easily do so, resulting in an increase of serious physical disease risk. For those with a mental illness, up to twenty-five years of life are lost when compared to the general population with diseases attributable to smoking (25).

Our research group has examined pharmacogenetic variability within the mu opioid receptor and dopamine D2 receptor genes as possible risk factors for cigarette smoking. Both receptors are involved in the reinforcing effects of smoking directly and indirectly (26). Previous work has shown that the OPRM1 A118G variant results in an amino acid change (Asn40Asp) on the N-glycosylation site of the receptor protein. This amino acid change produces decreased mRNA and receptor protein expression (27). It has

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Submitted: April 8, 2014; Revised: June 5, 2014; Accepted: June 6, 2014

Table 1 Demographics for All Subjects

Classifications	All	SCZ	BP
Age±SD	45.0±11.7	46.2±11.3	43.1±12.0
Sex			
Male (%)*	153 (52.8)	114 (64.4)	39 (34.5)
Female (%)*	137 (47.2)	63 (35.6)	74 (65.5)
Races			
Caucasian (%)*	194 (66.9)	102 (57.6)	92 (81.4)
African American (%)*	75 (25.9)	62 (35.0)	13 (11.5)
Other (%)	21 (7.2)	13 (7.3)	8 (7.0)
Smoking History			
Current smoker (%)*	135 (46.6)	95 (53.7)	40 (35.4)
Former smoker (%)	65 (22.4)	44 (24.9)	21 (18.6)
Never smoker (%)*	90 (31.0)	38 (21.5)	52 (46.0)
Overall pack-year history±SD†	19.9±22.2	21.0±22.7	17.3±21.1
Number of cigarettes/day±SD†	17.8±12.0	18.4±11.6	16.4±13.0

*The distributions of subjects were statistically different between the classifications in the diagnostic groups (p<.05). Detailed statistical analysis methods and p values are available upon request. †Never smokers were excluded to calculate the number of cigarettes smoked per day. SCZ=patients with schizophrenia, BP=patients with bipolar disorder.

been suggested the G allele is a "loss-of-function" variant. Ray et al. (28) reported that A118G variant carriers had a reduction in the binding potential of free mu opioid receptors in several brain areas involving the reward system. The DRD2 Taq1A (rs1800497) variant is a variant of the ankyrin repeat and kinase domain containing 1 (ANKK1) gene. The minor A1 (A) allele is associated with low expression of DRD2 protein in vitro (29), which regulates dopamine (DA) release as an autoreceptor (30, 31). This allele in particular has been examined for its role in both schizophrenia and bipolar disorder (32, 33) and been identified as a marker in antipsychotic drug response (34).

The present study was designed to confirm two hypotheses: 1) subjects with schizophrenia or bipolar disorder that carry the less active OPRM1 118G allele smoke more compared to subjects with the AA genotype; and, 2) subjects with the DRD2 Taq1A A1 (A) allele will exhibit greater tobacco smoking behaviors due to lower DRD2 protein expression resulting in more DA release. These hypotheses were examined with appropriate statistical analyses to compare their smoking differences. In addition, joint OPRM1 and DRD2 Taq1A genotype effects were determined on smoking behavior. These two genes are often discussed together, but the joint gene effects have not been well studied to date. The results imply that greater smoking prevalence in patients with schizophrenia is associated with genetic components. The present study contributes to further understanding of tobacco smoking among persons with mental illness.

Methods

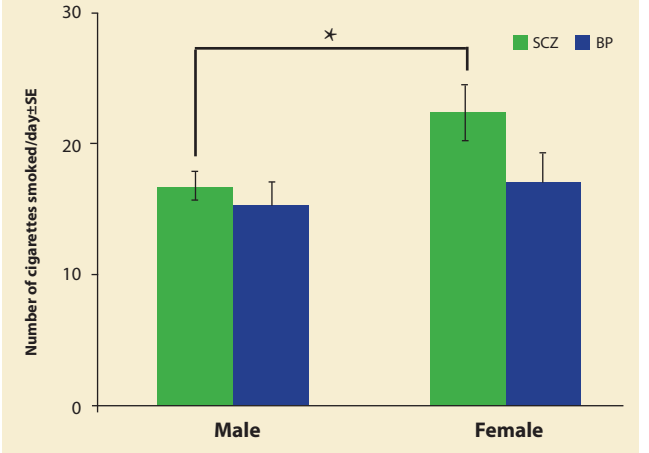
Subjects

The subjects in this study met the following inclusion criteria: 1) *DSM-IV* diagnosis of schizophrenia, schizophreniform disorder, schizoaffective disorder, or bipolar disorder I or II; 2) ≥18 years old; and, 3) treated with antipsychotic or mood-stabilizing medication as clinically indicated for at least six months. Subjects were excluded if they were unable to provide informed consent (assessed using a short questionnaire asking key questions about the study). Study subjects were recruited from ambulatory care mental health clinics and were included in a previous pharmacogenomic study related to the occurrence of atypical antipsychotic-associated metabolic complications (35). Subjects meeting inclusion and exclusion criteria underwent informed consent, including a brief assessment of the risks and benefits associated with study participation. Afterwards, a clinical interview—which included the Structured Clinical Interview for DSM Diagnoses (SCID) for schizophrenia patients (36) and the Diagnostic Interview for Genetic Studies (DIGS) for bipolar disorder subjects—was completed by a trained research associate and verified through chart review. Two different diagnostic assessments were utilized since subjects with schizophrenia versus bipolar disorder were initially recruited for separate, but similar, pharmacogenomic studies. The study protocols were approved by the University of Michigan Medical School Institutional Review Board (IRB).

Smoking Status Data Collection

Smoking status was assessed by self-report at the time of the study visit. Lifetime smoking status was classified as 1) current smoker; 2) former smoker; and, 3) never smoker based on patient self-report. Former smokers were also identified by self-report and subjects who had quit smoking more than twelve months previously were classified as former smokers. Upon classification, more information was obtained from subjects regarding former and current smoking habits as they were asked questions about smoking (number of cigarettes smoked per day, age at when smoking started, and quit date if applicable) to calculate a smoking pack-year history. Whenever possible these data were confirmed through documentation within their medical records.

Figure 1



Genotyping

DNA was extracted from a whole blood sample using a Puregene kit (Qiagen, Valencia, California). After the samples were processed, they underwent spectrophotometry to establish purity and yield and were then frozen at -80°C. Polymerase chain reaction (PCR) and sequencing primers for the OPRM1 A118G (rs1799971) and DAD2 Taq1A (rs1800497) were designed using Pyrosequencing SNP Primer Design Version 1.01 software (http://www.pyrosequencing.com). The PCRs were performed using One Taq 2X Master Mix with Standard Buffer (Biolabs Inc.) with the forward primer (5'-TGA TGC CTT GGC GTA CTC A -3') and biotinylated reverse primer (5'-5Biosg/GCC GTG ATC ATG GAG GGA CT -3') for OPRM1 A118G variant. The PCRs were performed using Platinum PCR Super-Mix with the biotinylated forward primer (5'-/5Bio/CAA GGG CAA CAC AGC CAT C -3') and reverse primer (5'-CAA GGG CAA CAC AGC CAT C-3') for DRD2 Taq1A variant. PCR products were visualized by electrophoresis on 1.8% agarose gels stained with ethidium bromide before Pyrosequencing. Genotyping was done with Pyrosequencing™ Technology.

Statistical Analyses

The OPRM1 genotypes were grouped based on previous studies (26, 37-40) as OPRM1 AA and AG/GG (*G allele carriers). The DRD2 Taq1A genotypes were also classified according to previous studies with respect to the presence of A1 (A) allele as AA, AG and GG; also, the genotypes were grouped AA+AG (*A) and GG in most of the statistical comparisons, as previously described (41, 42).

The major tobacco smoking parameter used for this study was number of cigarettes smoked per day, as well as current smoking status at the time of assessment. Current and former smokers were included to examine the number of cigarettes smoked per day for all statistical analyses unless otherwise specified because no significant differences were detected in smoking habits in separated groups. The numbers of cigarettes per day were analyzed with a two-tailed Student t-test and one-way ANOVA for the genotypes and sex differences. A linear regression model was used to estimate the impact of the each factor (e.g., OPRM1 and DRD2 Taq1A genotype, diagnosis, sex, age and race) on tobacco smoking behavior. The genotypes, race and sex frequency differences were examined by χ^2 and Hardy-Weinberg analyses. All groups in the present study included all sex, medication groups, and ethnicity unless otherwise specified. The nonsmoker data were excluded for all statistical analyses to compare the number of cigarettes unless otherwise mentioned. These analyses were performed with the IBM SPSS (Statistical Package for Social Sciences) statistics version 20 for Windows. A $p<.05$ was considered significant.

Results

Study Population Characteristics

A total of 290 smoking and nonsmoking patients with schizophrenia (n=177) or bipolar disorder (n=113) were recruited. Table 1 summarizes the demographic differences between groups. It is important to note that the number of males and females were significantly different in opposite directions in the diagnostic groups ($p<.05$ for both groups). Overall, their mean age was 45.0±11.7 (range=19–71) years. Caucasians were the racial majority (n=194, 66.9%) followed by African Americans (n=75, 25.9%). Males comprised the majority, with a total of 52.8% (n=153). Within the whole group, 135 (46.6%) were current smokers, 65 (22.4%) were previous smokers and 90 (31.0%) were never smokers. Current smokers smoked an average of 17.3±10.8 cigarettes per day, which resulted in a mean pack-year history of 23.0±21.9. Former smokers smoked an average of 19.0±14.1 cigarettes per day, which resulted in a mean pack-year history of 14.1±21.8. Although there were no sex differences for the overall demographic variables, surprisingly female

Table 2 Demographics with OPRM1 A118G Genotypes

Classifications	All		SCZ		BP	
OPRM1	AA	G*	AA	G*	AA	G*
N (%)	220 (77.5)	64 (22.5)	138 (79.3)	36 (20.7)	82 (74.5)	28 (25.5)
Sex						
Male (%)	120 (42.3)	30 (10.6)	92 (52.9)	20 (11.5)	28 (25.5)	10 (9.1)
Female (%)	100 (35.2)	34 (12.0)	46 (26.4)	16 (9.2)	54 (49.1)	18 (16.4)
Race						
Caucasian (%)	137* (48.2)	53* (18.7)	72* (41.4)	29* (16.7)	65 (59.1)	24 (21.8)
African American (%)	69* (24.3)	4* (1.4)	57* (32.8)	3* (1.7)	12 (10.9)	1 (0.9)
Other (%)	14* (4.9)	7* (2.5)	9* (5.2)	4* (2.3)	5 (4.5)	3 (2.7)
Smoking History						
Current smoker (%)	111 (39.1)	22 (7.7)	81* (46.6)	13* (7.5)	30 (27.3)	9 (8.2)
Former smoker (%)	46 (16.2)	17 (6.0)	34* (19.5)	9* (5.2)	12 (10.9)	8 (7.3)
Never smoker (%)	63 (22.2)	25 (8.8)	23* (13.2)	14* (8.0)	40 (36.4)	11 (10.0)
Number of cigarettes smoked/day±SD†	17.28±12.1	19.91±11.7	17.54±11.4‡	22.89±12.1‡	16.58±14.1	16.06±10.1

*The distributions of subjects were statistically different between the genotypes within the diagnosis group (p<.05). Detailed statistical analyses methods and p value are available upon request. †Never smokers were excluded to calculate the number of cigarettes smoked per day. ‡The number of cigarettes smoked per day by the patients with schizophrenia was statistically different between the OPRM1 genotype (see the Results section, Figure 1). SCZ=patients with schizophrenia, BP=patients with bipolar disorder.

subjects with schizophrenia smoked more cigarettes per day (cpd) compared to the male subjects (22.4±13.3 cpd vs. 16.8±10.5 cpd; t(135)=2.621, p=.010; see Figure 1). No sex differences were found among those with bipolar disorder related to number of cigarettes smoked per day.

OPRM1 A118G Genotype

A total of 290 subjects were genotyped for the OPRM1 A118G variant. Three subjects with schizophrenia and three subjects with bipolar disorder were not genotyped due to assay failure. There were 220 subjects (77.5%) with the AA genotype, 58 subjects (20.4%) with the GA genotype and 6 subjects (2.1%) with the GG genotype. The genotypes were within the Hardy-Weinberg distribution in the whole and both diagnostic groups. In the entire group, the G allele was more common in the Caucasian subjects (χ^2=16.7, p=0.0002; see Table 2). Similar racial differences were found in the whole group and the subjects with schizophrenia (χ^2=13.8, p=0.001; see Table 2) but not in the bipolar disorder group. The genotype distributions were not associated with the subject's sex.

DRD2 Taq1A Genotype

A total of 290 subjects were genotyped for the DRD2 Taq1A variant. There were 26 subjects (9.0%) with the AA genotype, 87 subjects (30.0%) with the AG genotype and 177 subjects (61.0%) with the GG genotype. In the whole group, the G allele was more common in Caucasian subjects (χ^2=10.8, p=0.03; see Table 3). Also there was a trend for racial differences among the subjects with schizophrenia (χ^2=14.1, p=0.07; see Table 3), but not bipolar disorder. As Parsons et al. previously described (43), a lower prevalence of AA genotype carriers was confirmed in the subjects with schizophrenia (χ^2=6.6, p=0.01; see Table 3). The Taq1A genotypes were within the Hardy-Weinberg distribution for the subjects with bipolar disorder.

Correlations between Genotypes and Tobacco Smoking

When examining the group as a whole, no differences in number of cigarettes smoked per day were found with either genotype. However, when separated by diagnosis, the schizophrenia subjects—including current and former smokers—who had the OPRM1 *G allele smoked more cigarettes per day compared to the AA genotype group (22.9±12.1 cpd compared to AA who smoked 17.5±11.4 cpd [t[135]=2.000, p=.047; see Figure 2). The subjects diagnosed with the bipolar disorder carrying the *G allele versus the AA genotype smoked a similar number of cigarettes per day (16.1±10.1 and 16.6±14.1, respectively).

Regarding the DRD2 Taq1A genotype, the mean ± SD number of cigarettes smoked per day by the patients with schizophrenia with AA, AG and GG genotypes was 15.19±7.6, 16.65±10.6 and 19.55±12.3, respectively, which was not statistically different. Following the approach by Munafo et al. (41), the genotypes were grouped as AA+AG (*A

Table 3 Demographics with DRD2 Taq1A Genotypes

Classifications	All			SCZ			BP		
DRD2 Taq1A	AA	AG	GG	AA	AG	GG	AA	AG	GG
N (%)	26* (9.0)	87* (30.0)	177* (61.0)	15* (8.5)	50* (28.2)	112* (63.3)	11 (9.7)	37 (32.7)	65 (57.5)
Sex									
Male (%)	9 (3.1)	51 (17.6)	93 (32.1)	7 (4.0)	36 (20.3)	71 (40.1)	2 (1.8)	15 (13.3)	22 (19.5)
Female (%)	17 (5.9)	36 (12.4)	84 (29.0)	8 (4.5)	14 (7.9)	41 (23.2)	9 (8.0)	22 (19.5)	43 (38.1)
Race									
Caucasian (%)	11* (3.8)	55* (19.0)	128* (44.1)	4* (2.3)	24* (13.6)	74* (41.8)	7 (6.2)	31 (27.4)	54 (47.8)
African American (%)	11* (3.8)	24* (8.3)	40* (13.8)	8* (4.5)	20* (11.3)	34* (19.2)	3 (2.7)	4 (3.5)	6 (5.3)
Other (%)	4* (1.4)	8* (2.8)	9* (3.1)	3* (1.7)	6* (3.4)	4* (2.3)	1 (0.9)	2 (1.8)	5 (4.4)
Smoking History									
Current smoker (%)	15 (5.2)	42 (14.5)	78 (26.9)	9 (5.1)	29 (16.4)	57 (32.2)	6 (5.3)	13 (11.5)	21 (18.6)
Former smoker (%)	5 (1.7)	16 (5.5)	44 (15.2)	4 (2.3)	8 (4.5)	32 (18.1)	1 (0.9)	8 (7.1)	12 (10.6)
Never smoker (%)	6 (2.1)	29 (10.0)	55 (19.0)	2 (1.1)	13 (7.3)	23 (13.0)	4 (3.5)	16 (14.2)	32 (28.3)
Number of cigarettes smoked/day±SD†	15.25±7.5	16.87±10.2	18.68±13.2	15.20±7.6	16.65±10.6	19.55±12.3	15.36±7.96	17.25±9.65	16.34±15.4

*The distributions of subjects were statistically different between the genotypes within the diagnosis group (p<.05). Detailed statistical analyses methods and p value are available upon request.
†Never smokers were excluded to calculate the number of cigarettes smoked per day. SCZ=patients with schizophrenia, BP=patients with bipolar disorder.

allele carriers) and GG alleles. The data indicate that females with schizophrenia and the GG genotype smoked more cigarettes than male smokers with schizophrenia and the *A genotype (F[3, 135]=3.114, p=.028); see Figure 3). The GG female schizophrenia patients, including current and former smokers, smoked 24.1±14.4 cpd compared to the male A allele carriers who smoked 15.8±9.5 cpd. With the bipolar patients, there was no difference in the number of cigarettes smoked per day based upon the DRD2 Taq1A genotype.

The relationship between current smoking status (current, previous, never) and genotype was examined. Subjects with schizophrenia and the OPRM1 G allele seemed more likely to be never or former smokers, compared to AA subjects with schizophrenia: 13% of those with the G allele were current smokers compared to 21% of former smokers and 38% of never smokers (χ^2=9.32, p=0.009; see Table 2). No DRD2 Taq1A genotype effect on smoking status was found among those with schizophrenia and bipolar disorder. OPRM1 and DRD2 Taq1A joint genotype effect on tobacco smoking was found. The joint genotypes were classified into 4 groups: 1) OPRM1 AA + DRD2 Taq1A *A; 2) OPRM1 AA + DRD2 Taq1A GG; 3) OPRM1 *G + DRD2 Taq1A *A; and, 4) OPRM1 *G + DRD2 Taq1A GG. The numbers of cigarettes smoked per day were compared using one-way ANOVA. The results showed that the OPRM1 *G + DRD2 Taq1A GG group had the highest consumption (26.27±12.74 cpd) and the OPRM1 AA + DRD2 Taq1A *A group had the lowest consumption (15.98±10.02 cpd) in smokers with schizophrenia but not those with bipolar disorder (F[3, 135]=2.671, p=.050).

Racial Differences for Tobacco Smoking and Genotype Distributions

Caucasian was the majority ethnic group (n=194, 66.9%) followed by African Americans (n=75, 25.9%), and others including Asian and Hispanic (n=21, 7.2%). Within each ethnic group, no genetic and sexual effects on tobacco smoking were found. However the amount of daily tobacco cigarette consumption by current and former smokers was significantly different between ethnic groups. Caucasians smoked more than the current and former African-American smokers in the whole group (F[2, 193]=6.646, p=.002) as well as the subjects with schizophrenia (F[2, 134]=6.000, p=.003). The number of cigarettes smoked per day was 20.1±13.4 and 13.6±7.3 for the Caucasians and African- Americans, respectively, in the whole group. A difference in daily tobacco consumption was not found among the bipolar patients. The genotype distributions of the two genes in each ethnicity groups were consistent with the previous studies (44, 45).

Linear Regression Analyses

Variables such as diagnosis, sex, OPRM1 genotype, DRD2 Taq1A genotype and race were used as independent factors to determine the number of cigarettes smoked per

Figure 2

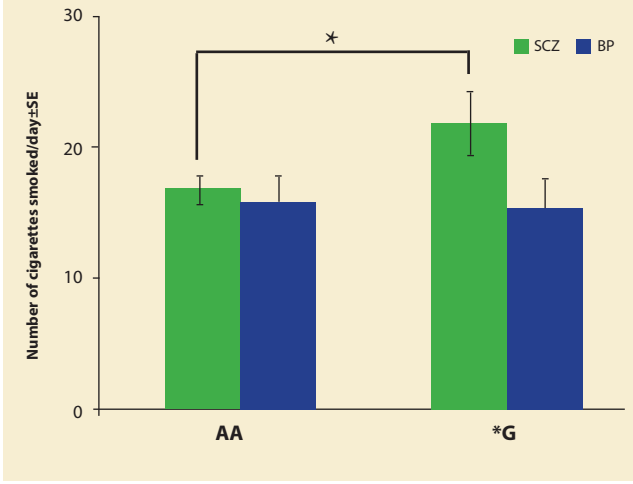
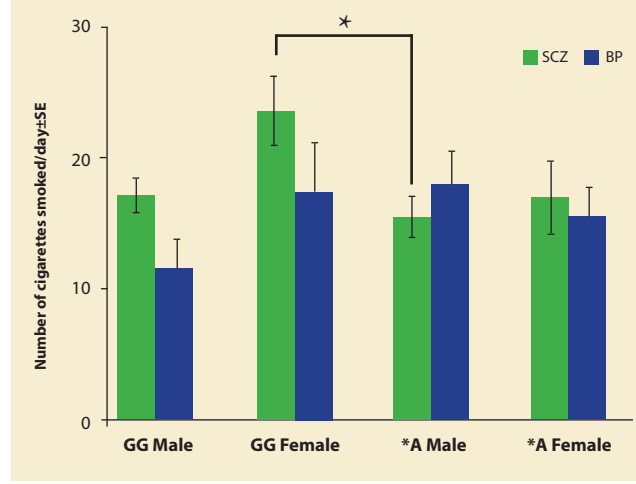


Figure 3



day. The threshold for variable entry was p<.05 and that of retention was p<.10 in the model. The most fitted model was described by predictors such as sex, age, race, OPRM1 and DRD2 Taq1A genotypes for the subjects with schizophrenia (adjusted R^2=.170, p=.000). For the entire group, the daily tobacco consumption was well predicted by diagnosis, sex, age, race, OPRM1 and DRD2 Taq1A genotypes. When the whole group included all smoking status, adjusted R^2=0.86, p=.000. When only current and former smokers were included, adjusted R^2=.155, p=.000. Surprisingly, the demographic and genetic factors did not have significant effects for the number of cigarettes per day in the bipolar group. Medication effects were not considered as a factor of tobacco smoking because there was no significant difference between the daily tobacco consumption and the smoking status among each medication group. However, the groups studied may not be large enough to detect any effects.

Discussion

This study has two limitations: 1) lack of medication control group and 2) self-reporting smoking data collection instead of biological smoking markers. Nevertheless OPRM1 and DRD2 Taq1A genotypes, sex and race were significant predictors for tobacco smoking in the present study. Female smokers with schizophrenia consumed more cigarettes than the males per day despite a smaller number of current and former smokers. Smokers with schizophrenia and the OPRM1 G allele smoke more cigarettes than the AA genotype patients. Furthermore, females with schizophrenia and DRD2 Taq1A GG genotype consumed more cigarettes per day than males with schizophrenia and the *A genotype. Surprisingly, in the bipolar patients, there were no differences between genotype and sex. The tobacco reward system in patients with bipolar disorder appears to differ. Although many social and environmental factors have been associated with why people start smoking, the results indicate that genetic differences related to the opioid and dopamine neurotransmitter systems are implicated in tobacco smoking among subjects with schizophrenia.

Sex Differences and Tobacco Smoking Status

Sex differences in smoking behavior among the general population have been reported by many researchers (46-48). Males have been reported to smoke more than females (49). Female smokers have more difficulties quitting smoking (50) and seem to be less sensitive to nicotine cues than males and are more influenced with non-nicotine factors (51). Surprisingly, in the present study the females with schizophrenia smoked more than the male patients. Interestingly, Benowitz and Hatsukami (50) reported that, within the general population, females smoked more than males when they were under stress. It is possible that the diagnosis of schizophrenia is related to higher stress levels in females. Unfortunately, we did not obtain standardized rating scales, such as the Brief Psychiatric Rating Scale (BPRS) to estimate current symptom severity, which may contribute to potential stress. Also in the present study, the number of males and females were significantly different in the two diagnostic groups. Especially in the group of subjects with bipolar disorder, females were the majority. Many studies have reported on the influence of menstrual cycle phases on females' smoking behavior (52-56). Unfortunately, there were no menstrual phase controls in this study. Thus, methodological bias may contribute to the negative results in the bipolar disorder group.

Ethnic Differences for the Genotypes and Smoking Behavior

It is well known that there are significant ethnic genetic distributions. For the OPRM1 genotype, there are almost 50% of *G genotype in the Japanese while in the African-American population the genotype is less than 5% (44). The DRD2 Taq1A genotype is also differently distributed among various ethnicities (45). In the present study, the OPRM1 and DRD2 Taq1A genotype distributions in the ethnic population were consistent with previous studies. The number of daily cigarettes, pack year and smoking status were compared with genotypes within the ethnic groups, but significant differences were not found. However, the number of cigarettes smoked per day of the African-American group was significantly less than that of the Caucasian group. This difference was found only in the current smoker group, and the group combined current and former smokers in the whole group and the group with schizophrenia. After regrouping by race, the sample data are too small to examine the smoking behavior in the each ethnic group.

OPRM1 A118G Genotype Differences in Tobacco Smoking

Although the function of the OPRM1 A118G variant is still very controversial, it is well accepted that the mu (μ) opioid receptor on the GABAergic interneuron is associated with the dopaminergic reward system (57, 58). Alcohol and nicotine release endogenous opioids (58-61), which activate mu opioid receptors on GABAergic interneurons (62). Opioid receptor activation inhibits GABAergic interneurons (inhibition of inhibition) to increase DA release in the ventral tegmental area (VTA) (63). In vitro, this variant demonstrates increased binding affinity with β-endorphin (64, 59) and greater inhibition of cAMP accumulation in HEK293 and AV-12 cells (65). On the other hand, Ramchandani et al. (66) reported that humans and humanized mice with the GG genotype had greater DA response to alcohol than the AA genotype. Furthermore, mentally normal smokers with OPRM1 *G have more striatal DA release in response to average nicotine tobacco cigarette smoking compared with those homozygous for the A allele (67). Based on the latter, albeit very limited data, the *G variant is associated with greater DA release. Hence, the presence of this allele may result in greater daily cigarette consumption. Certainly other possible explanations for the greater cigarette consumption among *G patients may also exist. According to the study of Ray et al., female smokers with the OPRM1 *G were less likely to distinguish the difference between denicotinized and average nicotine cigarettes (39). These authors concluded that the G allele is associated with a fewer number of mu opioid receptors, resulting in being less sensitive to nicotine, which may contribute to the greater cigarette consumption because the *G allele subjects need more nicotine to satisfy their brain needs.

In addition, the OPRM1 118G carriers with schizophrenia did not seem to start smoking and they were likely to have stopped smoking when compared to the AA genotype subjects with schizophrenia. Although, at first, these results may seem contradictory, these data are consistent with previous animal work showing that mu opioid receptor knockout mice are less likely to exhibit nicotine preference (68). Recently, Verhage et al. published a meta analysis about smoking initiation, nicotine dependence and smoking cessation. They concluded that subjects with the OPRM1 AA genotype had higher risk for nicotine dependence (69). Furthermore, Lerman et al. (37) and Ray et al. (39) have previously reported that the G allele carriers were more likely to quit smoking successfully using nicotine replacement therapy. Thus, the present data indicate additional positive evidence showing that the OPRM1 genotype affects smoking behavior and nicotine dependence.

Surprisingly, contrary to our hypothesis, there were no OPRM1 genotype smoking differences in the bipolar patients due to possible changes in dopamine neurotransmission, which may be dependent on the subject's current mood state (70). All of the bipolar patients included in this study were currently euthymic. The relationship between bipolar disorder moods, cigarette smoking and the dopaminergic reward system deserves further study.

DRD2 Taq1A Genotype Difference in Tobacco Smoking

DRD2 Taq1A is one of the genes associated with alcohol, drug abuse and nicotine dependence (71-73). Many studies have examined the relationship between the TaqA1 allele and tobacco smoking (41, 42, 74-77), but its relationship is still controversial. Our hypothesis was that the Taq1A AA genotype would be associated with greater smoking reinforcement due to less inhibition of DA release. However, the present data indicated a trend that the GG genotype is associated with greater daily tobacco consumption in patients with schizophrenia. In addition, female smokers with schizophrenia with GG genotype smoked more cigarettes than male smokers with the A allele (see Figure 3). The mean ± SD number of cigarettes smoked by male *A allele carriers was 15.8±9.5 cpd and the GG female patients smoked 24.1±14.4 cpd. These results agree with previous studies that female *A carriers were less likely to quit smoking with nicotine replacement therapy (41) and that GG females

have higher risk for smoking (78). Although Pearlson et al. (79) demonstrated that the DRD2 Bmax value is elevated in those with bipolar disorder and schizophrenia compared to controls, the mechanism of increased smoking reinforcement with GG allele is obscure. In contrast, Comings (75), Comings and MacMurray (74) and Lee et al. (42) found that males with the AG allele had a higher smoking prevalence among Korean patients with schizophrenia. Also Pohjalainen et al. (29) reported significantly decreased availability of DRD2 in the AG genotype. However, in the present study the AG genotype patients smoked a similar number of cigarettes per day among those with schizophrenia and bipolar disorder. Also, no significant genotype and sex differences were found among those with bipolar disorder.

OPRM1 and DRD2 genes are often discussed together for nicotine dependence and tobacco cessation (69, 80-83). However, most studies have not evaluated combined gene effects. In the present study, the OPRM1 and DRD2 Taq1A genotypes additively affected smoking habits. Integrated gene effects have been found in methadone maintenance therapy (84), and alcohol use and parental rule setting (85) to date. However, Hardman et al. reported a lack of association between these genes in obesity (86). The present study is preliminary; however, it contributes positive evidence of combined gene effects for tobacco smoking.

Acknowledgments

The present study was supported in part by the Department of Pharmacology Psychopharmacology Research Fund C361024, Education and Research Development Fund 276157 for EFD and NIMH (R01 MH082784), NIH-NCCR, GCRC Program (UL1RR024986), the Chemistry Core of the Michigan Diabetes Research and Training Center (NIH5P60 DK 20572) (all Bethesda, Maryland), and the Washtenaw Community Health Organization (WCHO, Ann Arbor, Michigan), The Brain and Behavior Research Foundation (formerly NARSAD, Great Neck, New York), and the Prechter Longitudinal Study of Bipolar Disorder (Ann Arbor, Michigan) for VLE.

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