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A CHRNA5 Allele Related to Nicotine Addiction and Schizophrenia

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Abstract

Schizophrenia and nicotine addiction are both highly heritable phenotypes. Because individuals with schizophrenia have a higher rate of smoking than those in the general population, one could hypothesize that genes associated with smoking might be over-represented in schizophrenia and thus help explain their increased smoking incidence. Although a number of genes have been proposed to explain the increased smoking risk in schizophrenia, none of them have been consistently linked to smoking and schizophrenia and thus difficult to explain the increased smoking in schizophrenia. A functional smoking-related nicotinic acetylcholine receptor $\alpha 5$ subunit gene (CHRNA5) nonsynonymous SNP rs16969968 (Asp398Asn) has recently been discovered and replicated. As such, we tested whether this variant contributes to smoking in schizophrenia in a sample of 313 schizophrenia patients and 525 controls. The Asp398Asn risk allele is significantly associated with smoking severity independently in schizophrenia patient smokers ($p=0.001$) and in control smokers ($p=0.029$). Furthermore, the same risk allele is significantly associated with schizophrenia in both Caucasian ($p=0.022$) and African American ($p=0.006$) nonsmoker schizophrenia patients compared to control nonsmokers. Intriguing, this SNP was not significantly associated with smoking status (smokers vs. nonsmokers) in either schizophrenia patients or controls. Therefore, our study identifies a genetic variant that is simultaneously linked to smoking and schizophrenia in the same cohort, but whether and how this SNP contributes to the increased smoking prevalence in schizophrenia patients requires additional studies.

Keywords

smoking; nicotine addiction; schizophrenia; nAChR; alpha5; comorbidity

Introduction

Smoking rate in schizophrenia is about three times higher than that in the general population (Hughes *et al.* 1986; Chapman *et al.* 2009). Most patients smoke prior to psychosis onset (Kelly and McCreadie, 1999; Diaz *et al.* 2008), suggesting an increased risk for

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smoking prior to psychosis onset. Schizophrenia and nicotine addiction are highly heritable: the heritability is 60-75% for nicotine dependence (Sullivan and Kendler, 1999) and 60-80% for schizophrenia (Cannon *et al.* 1998). Thus, these two discrete yet tightly coupled phenotypes are also highly heritable.

Indeed, a genetic attribute to this smoking-schizophrenia comorbidity has often been proposed. However, testing whether a genetic variant contributes to smoking increases in schizophrenia is more intricate than a simple case-control association test. A candidate variant could cause the increased smoking rate by either: 1) contributing to both smoking and schizophrenia in an additive pattern, leading to the increased smoking; or 2) contributing specifically to smoking in schizophrenia, distinct from other genetic variants for smoking. Accordingly, such a variant should show 1) association with smoking in controls, smoking in patients, and simultaneously also with schizophrenia diagnosis regardless of smoking, thus possibly accounting for the increased smoking risk; or 2) association specifically and more robustly with schizophrenia smokers.

It has been suggested that increased smoking in schizophrenia is mediated by the nicotinic acetylcholine receptor (nAChR) $\alpha 7$ subunit gene *CHRNA7* (Freedman *et al.* 1996; Leonard *et al.* 2002). However, evidence for its association with smoking itself is weak (Faraone *et al.* 2004; Zammit *et al.* 2007; Sanders *et al.* 2008) and none of the GWAS on smoking has implicated *CHRNA7* (Thorgeirsson *et al.* 2008). *CHRNA7* variants may be associated with schizophrenia and not with smoking in the disorder (Stephens *et al.* 2009). *CHRNA4* and *CHRNA2* have been associated with smoking (Li *et al.* 2005; Hutchison *et al.* 2007; Conti *et al.* 2008), although no association with schizophrenia or smoking status in schizophrenia has been identified with the exception of an association with smoking quantity (De, V *et al.* 2006; Voineskos *et al.* 2007). Dysbindin polymorphisms have been associated with both schizophrenia and smoking although association with smoking in schizophrenia patients was not reported (Voisey *et al.* 2010). A dopamine-related gene *NR4A3* was associated with smoking severity in schizophrenia smokers but not in controls (Novak *et al.* 2010). The lack of consistent association of these variants with smoking itself, let alone stronger association with smoking in schizophrenia, indicating that we have yet identified the key genetic variants, if exist, for the comorbidity.

The discovery of nicotinic acetylcholine receptor $\alpha 5$ subunit gene (*CHRNA5*) SNP rs16969968 (Asp398Asn) is compelling because the association between this or nearby SNPs with smoking related phenotypes is based on replicated genome-wide searches (Saccone *et al.* 2007; Thorgeirsson *et al.* 2008; Lips *et al.* 2010). The aspartic acid to asparagine substitution is functional such that the risk allele Asn reduces $\alpha 4\beta 2\alpha 5$ receptor functions (Bierut *et al.* 2008). It provides an opportunity to test whether a gene robustly contributing to smoking may explain the increased smoking risk in schizophrenia. To test this hypothesis, we examined whether the Asp398Asn association with smoking severity is replicable in our control sample, whether it can be extended to a schizophrenia sample and finally, whether it is over-represented in schizophrenia.

Materials and Methods

Schizophrenia patients were recruited from the outpatient clinics of the Maryland Psychiatric Research Center (MPRC) and from community clinics from which we receive referrals. Controls were recruited from the Baltimore area using local media advertisement. All participants gave written consent using local Institutional Review Board approved consent forms. The Structured Clinical Interview for DSM IV was administered to determine DSM-IV Axis I diagnosis. Patient participants were individuals with DSM-IV schizophrenia. Control subjects were screened and excluded for Axis I psychosis diagnoses.

The study included 313 unrelated schizophrenia patients and 525 healthy controls with genotyping information. A subgroup of these subjects (42 schizophrenia and 180 controls) and their genotyping information were used in a previous imaging study, although no schizophrenia-related analyses were conducted there (Hong *et al.* 2010). Genotyping of rs16969968 was performed using Taqman Assays-on-Demand (Applied Biosystems, Foster City, California) methods as previously described (Hong *et al.* 2008; Hong *et al.* 2010). Genotyping accuracy was determined by duplicate genotyping of 10% of the samples selected randomly. The error rate was <0.005.

Smokers (n=323, including schizophrenia patients and controls) were classified as individuals who smoked for over one year and were currently smoking. Fagerström Test for Nicotine Addiction (FTND) was used to measure nicotine addiction severity (0-10, 10 being most severe). Cigarettes per day (CPD) was included as an additional measure of smoking severity, given its use in previous GWAS studies. CPD data were not available for 2 smokers, while FTND data were not available for 16 smokers. Chronic cigarette exposure was estimated in pack-years. Age of regular smoking onset was also collected. Nonsmokers (n=379, including schizophrenia patients and controls) were defined by lifetime smoking of less than 100 cigarettes and currently not smoking. Past-smokers (n=67) were smokers who had quit smoking for any duration and were currently not smoking. Subjects' smoking status was designated as "undetermined" (n=69) when we could not reliably classify current, past, or nonsmoking status when the reported smoking history was deemed unreliable. Subjects with past or undetermined smoking status were included in genotype comparisons of schizophrenia vs. control, but were excluded from smoking-related analyses. Smoking behavioral characteristics from both case and controls are given in Table 1.

We compared observed genotype frequencies with those expected under Hardy-Weinberg equilibrium using Haploview (Barrett *et al.* 2005). Effects of genotype on quantitative smoking related measures were examined using linear regressions based on an additive model (coded as 0, 1, and 2 for the number of "risk allele"). Genotype and allelic tests for association between schizophrenia cases and controls or between smokers and nonsmokers were performed using χ^2 tests, separately in each ethnic subgroup to reduce bias from population stratification during case-control comparisons. Data were also re-examined using logistic regression where age and gender were entered as covariates and Wald's χ^2 , odds ratio [OR as calculated by $\text{Exp}(B)$] and its 95% confidence interval (CI) were calculated. Power analysis for single-locus main effects was performed using the Quanto program (version 1.2.4; <http://hydra.usc.edu/gxe/>).

Results

Demographics

The sample includes 426 Caucasians (155 schizophrenia patients, 271 controls), 360 African-Americans (149 patients and 211 controls) and 52 from other ethnic backgrounds. The distribution of genotypes was consistent with those predicted under Hardy Weinberg Equilibrium in all ethnic and diagnostic subgroups. Schizophrenia cases and controls differed in age and gender (both $p < 0.001$). There were no significant gender differences of the Asp398Asn genotype ($\chi^2 = 2.71$, $p = 0.26$). Gender was not significantly associated with nicotine addiction severity (FTND: $F = 1.84$, $p = 0.18$), daily cigarettes (CPD: $F = 0.08$, $p = 0.78$) or pack years ($F = 0.06$, $p = 0.80$). Males became regular smokers at a younger age (19.17 ± 0.48) than females (21.53 ± 1.05 , $F = 5.44$, $p = 0.021$). See Table 1 for these and other demographic and smoking related data.

Associations with schizophrenia status

The frequency of the Asp398Asp risk allele Asn was 37.1% in Caucasians and 5.6% in African Americans (combined schizophrenia patients and controls), similar to those reported in the NCBI SNP database (35.4% in Caucasians and 4.3% in African Americans). To reduce stratification bias, case-control analyses were carried out in separate ethnic groups. The frequency of the Asn allele was significantly higher in schizophrenia cases than controls in Caucasians ($p=0.025$), but not in African Americans ($p=0.419$, see Table 2 for allele frequency and number of subject information), and remained significant after Bonferroni correction for two comparisons (Caucasians and African Americans). As this effect could be biased by more smokers in schizophrenia patient, we re-analyzed case vs. control association by stratifying smoking. The overrepresentation of this allele in schizophrenia was significant in both Caucasians nonsmokers ($p=0.022$) and African American nonsmokers ($p=0.006$) (see Table 2 for allele frequency and number of subject information); both remained significant after Bonferroni correction for two comparisons. However, no significant relationship was found in smokers (Table 2). The data were also analyzed using logistic regression to account for age and gender. Here, the frequency of the Asn allele was significantly higher in cases than controls in both Caucasian nonsmokers (Wald's $\chi^2=3.87$, OR=1.91 for one copy and 3.47 for two copies of Asn, 95%CI=1.01-11.99) and African American nonsmokers [$\chi^2=8.77$, OR=4.80, 95%CI=1.57-14.72 for one copy of Asn (no homozygote Asn in this group)]. Therefore, the overrepresentation of this smoking-related risk allele in schizophrenia is not due to more smokers in the schizophrenia cohort and is present in nonsmoking schizophrenia patients in two cohorts with different ethnic backgrounds.

Association with smoking severity

The Asp398Asn genotype was significantly associated with nicotine addiction severity as measured by FTND, and explained 3.3% (R^2 change; $F=10.44$, $p=0.001$) of the addiction severity variance in the entire smoker sample and 2.5% ($F=4.12$, $p=0.044$) and 4.4% ($F=6.67$, $p=0.011$) of the variances in control smokers and patient smokers analyzed separately (Figure 1). Although more robust in patients, the R^2 changes were not significantly different between the two groups ($z=-0.43$, $p=0.67$). The findings were similar for CPD, with genotype explaining 4.6% ($F=15.60$, $p<0.001$), 2.8% ($F=4.86$, $p=0.029$), and 7.3% ($F=11.53$, $p=0.001$) of the CPD variances in the combined, control, and patient samples, respectively. Again, genotype contribution to CPD was not significantly different between patients and controls ($z=-0.85$, $p=0.40$), although the relationship is numerically more robust in patients. After Bonferroni corrections for three calculations (combined, patient and control samples), genotype association with FTND and CPD were significant in the combined and patient sample, but not in the control sample alone.

Asp398Asn was not associated with chronic cigarette exposure as measured by pack year in non-psychiatric controls (R^2 change=0.2%, $F=0.33$, $p=0.57$), although the association was significant in patients (6.9%, $F=10.39$, $p=0.002$). Finally, on age to becoming a regular smoker, the risk allele was significantly associated with younger age of regular smoking onset in the combined sample (3.6%, $F=6.25$, $p=0.012$). Although the effect was in the same directions in both groups, it was not significant in patients (1.9%, $F=1.80$, $p=0.18$) but was nominally significant in controls (5.8%, $F=4.47$, $p=0.038$). This finding may be related to previous data showing that this SNP is associated with heavy smoking only in those with younger smoking onset (Weiss *et al.* 2008).

Association with smoking status

When comparing smokers vs. nonsmokers (allele frequencies in Table 2), Asp398Asn was not significantly associated with smoking status in the combined patient and control sample

in Caucasians ($\chi^2=0.24$, $p=0.621$) or African Americans ($\chi^2=0.64$, $p=0.425$), consistent with previous data that have shown that this SNP or SNPs in LD is associated with smoking or addiction severity but is not associated with smoking status (Thorgeirsson *et al*, 2008; Lips *et al*, 2010). Logistic regression with age and gender as covariates showed similar findings in Caucasians ($p=0.665$) and African Americans ($p=0.474$). In separate diagnostic group, Asp398Asn was nominally overrepresented in smokers ($\chi^2=3.92$, $p=0.048$) compared with nonsmokers in Caucasian controls; but not overrepresented in nonsmokers compared with smokers in Caucasian patients ($\chi^2=3.70$, $p=0.054$). None of the above remained significant after Bonferroni correction. There were no significant findings in African American controls ($\chi^2=0.40$, $p=0.529$) or African American patients ($\chi^2=0.95$, $p=0.329$).

Posterior power calculations with Quanto for main effects of schizophrenia, assuming average rs16969968 genotype relative risk for schizophrenia to be similar to that for heavy smokers in several 15q25.1 SNPs, i.e., around 1.4 (Schlaepfer *et al*, 2008; Saccone *et al*, 2009), with MAFs at 37.1% in Caucasians and 5.6% in African Americans, and under an additive model, showed that the sample has 73% power for alpha of 0.05 for Caucasians but only 30% power for African Americans. Power is only 43% for Caucasian smokers and 29% for Caucasian nonsmokers under the assumption of the same genotype relative risk, so the lack of significant findings in smokers could be due to insufficient power. Power for smoking status was expected to be insufficient given the lack of significant findings in much larger samples (e.g., 13,945 smokers vs. 4,203 nonsmokers, $p=0.60$, in Thorgeirsson *et al*, 2008), unless this locus confers a much higher risk in schizophrenia smokers vs. schizophrenia nonsmokers as compared to control smokers vs. control nonsmokers. However, this does not appear to be the case ($\chi^2=3.92$, $p=0.048$ in non-schizophrenia samples and $\chi^2=3.70$, $p=0.054$ in schizophrenia samples, see above). Power for the quantitative trait FTND was estimated to be 40% for R^2 change of 0.7% (Thorgeirsson *et al*, 2008) but 90% for the observed R^2 change of 3.3% in the combined smoker groups.

Discussion

In this sample of 525 non-psychiatric control subjects, rs16969968 was associated with smoking severity as previously shown in multiple replications (Saccone *et al*, 2007; Bierut *et al*, 2008; Berrettini *et al*, 2008; Stevens *et al*, 2008; Thorgeirsson *et al*, 2008; Lips *et al*, 2010). We also expanded these findings in a sample of 313 schizophrenia patients. This SNP was also significantly associated with schizophrenia in Caucasians, and interestingly also in nonsmokers in two ethnic groups. However, the sample sizes were small and findings in schizophrenia smokers were not significant, therefore replication studies are needed. Nevertheless, a genetic contribution to the schizophrenia-smoking comorbidity has been repeatedly hypothesized, although we are not aware of evidence of any functional genetic variant that has been associated with nicotine addiction in controls, nicotine addiction in schizophrenia patients, and association with schizophrenia itself in the same cohort beside the data on rs16969968 described here.

However, these findings occur in a pattern that the overrepresentation in schizophrenia was not due to more risk allele in schizophrenia smokers, which could have been a more straightforward explanation to the increased smoking rate in schizophrenia. Rather, the increased risk for schizophrenia occurs in the Caucasian sample and in nonsmokers in both Caucasians and African Americans. The lack of significant association with smoking status in schizophrenia is perhaps not surprising: rs16969968 and other SNPs in LD have been repeatedly associated with smoking related phenotypes that are related to smoking quantity and severity (Berrettini *et al*, 2008; Thorgeirsson *et al*, 2008; Bierut *et al*, 2008; Grucza *et al*, 2008; Chen *et al*, 2009; Le Marchand *et al*, 2008; Sherva *et al*, 2008; Saccone *et al*, 2007; Amos *et al*, 2008). However, it is typically not associated with smoking status (i.e.,

not significant if compared smokers vs. nonsmokers) (Thorgeirsson *et al*, 2008; Lips *et al*, 2010).

While most linkage evidence on chromosome 15q in schizophrenia have been reported on the 15q13-14 region given the strong interest in CHRNA7, some studies have shown linkage signals with psychosis at 15q25, an area where the CHRNA5 is located (Gejman *et al*, 2001; Park *et al*. 2004; Vazza *et al*. 2007), suggesting that a schizophrenia link to this genomic region is also possible. The over-representation of a smoking-related risk allele in nonsmoking schizophrenia patients remains intriguing, although an explanation is not yet clear. Interestingly, another study on association of CHRNB3 polymorphisms in bipolar disorder and nicotine addiction found that the CHRNB3 association with nicotine dependence was not different between bipolar disorder patients and the general population; although two synonymous SNPs in CHRNB3, rs4952 and rs4953, were significantly associated with bipolar disorder even in nonsmokers (Hartz *et al* 2010). Petrovsky *et al* showed an association between CHRNA3 polymorphisms and prepulse inhibition in schizophrenia patients although schizophrenia case vs. control association was not significant (Petrovsky *et al* 2010). Winterer *et al* showed that rs16969968 was associated with working memory and other cognitive phenotypes that have been related to schizophrenia (Winterer *et al* 2010). We also note that rs16969968 was protective for cocaine dependence (Gruza *et al*, 2008), despite the fact that cocaine dependence is also associated with an increased smoking rate (Lai *et al*. 2000). Therefore, while evidence for the association of rs16969968 and other smoking-related variants with nicotine addiction in psychiatric illnesses is accumulating, the field has yet demonstrated clear evidence that any of the variants is contributing to the *increased* smoking in schizophrenia or other psychiatric conditions.

We started with a candidate gene shown to be consistently related to smoking and then examined whether it is also overrepresented in schizophrenia itself. Using such strategy, our data suggest that the CHRNA5 variant Asp398Asn is an important genetic variant in our consideration for the genetic basis of smoking in schizophrenia, and should be considered along with other candidate genes in future studies.

In summary, our study shows that Asp398Asn is significantly associated with the severity of smoking in schizophrenia patients similar to (and numerically more robust than) findings in multiple non-psychiatric samples. While a genetic attribute to the high risk of smoking has been repeatedly suggested, this is the first time that a functional genetic variant has been simultaneously linked to smoking and schizophrenia in the same cohort. However, the finding did not provide immediate evidence that Asp398Asn is contributing to the cause of the *increased* smoking prevalence in schizophrenia patients because this SNP was not significantly more frequent in schizophrenia smokers. Our study suggests that this SNP is significantly contributing to nicotine addiction severity in schizophrenia smokers. Whether and how this finding is related to the increased smoking rate in schizophrenia cannot be determined using the present evidence alone.

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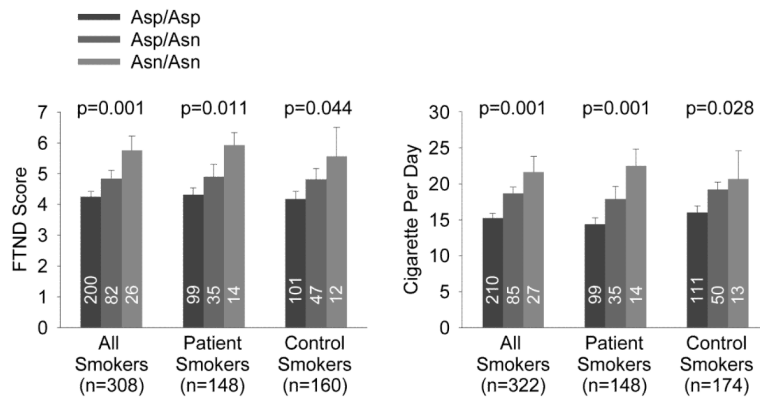


Figure 1. Asp398Asn genotype effect on nicotine addiction severity as measured by Fagerström Test for Nicotine Dependence (FTND) and cigarettes per day (CPD) in schizophrenia smokers and control smokers (mean±s.e.). Numbers in vertical bars are number of subjects in each genotype. The risk allele Asn is significantly associated with addiction severity and CPD, independently in patients and controls and in the combined sample. The significant associations with FTND and CPD remained after Bonferroni corrections for 3 calculations in the combined and the patient samples, but not in the control sample.

Table 1

Demographic and smoking related characteristics of the sample

	All schizophrenia	All controls	χ^2 or F	P value
N	313	525		
Gender (male: female)	226:87	280:245	29.19	0.000
Age	38.9±0.55	36.1±0.56	10.97	0.001
Race (Caucasian: African American: others) *	155:149:9	271:211:43	2.06	0.163
Current smoker : nonsmoker : past smoker #	149:65:30	174:314:37	73.37	0.000
FTND (current smokers)	4.60±0.19	4.41±0.20	0.47	0.49
Cigarettes per day (CPD) (current smoker)	16.00±0.78	17.13±0.71	1.17	0.28
Pack year (current smokers)	13.95±1.34	15.42±1.10	0.73	0.39
Age of regular smoking onset (current smoker)	20.37±0.81	19.38±0.50	1.17	0.28

* Statistics based on Caucasians vs. African Americans

Not including subjects with undetermined smoking status

Table 2

Frequencies of the Asn allele in schizophrenia patients and control subjects by ethnic subgroups and by smoking vs. non-smoking status

Ethnic groups	All subjects* (n=838)			Non-smokers (n=379)			Smokers (n=323)					
	Patient	Control	χ^2	p	Patient	Control	χ^2	p	Patient	Control	χ^2	p
Caucasian	0.406 (155)	0.331 (271)	4.98	0.025	0.481 (26)	0.318 (157)	5.22	0.022	0.403 (72)	0.346 (94)	1.14	0.286
African-American	0.064 (149)	0.050 (211)	0.65	0.419	0.105 (38)	0.029 (121)	7.50	0.006	0.040 (75)	0.071 (77)	1.42	0.233
Other	0.278 (9)	0.116 (43)	n/c	n/c	(1)	(36)	n/c	n/c	(2)	(3)	n/c	n/c

* All subjects regardless of smoking status, including smokers, nonsmokers, past-smokers, and those with smoking status undetermined. Number of subjects in each cell is in parenthesis. n/c: not calculated due to small numbers.