Association of α4β2 nicotinic receptor and heavy smoking in schizophrenia

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Introduction: Previously we suggested that the *CHRNA7* polymorphism in nicotinic receptor genes, in particular the D15S1360 in *CHRNA7*, is associated with smoking in schizophrenia. Schizophrenia patients are usually heavy smokers. In this study we hypothesized that high-affinity nicotinic receptors are associated with smoking in such patients. **Objective:** To investigate the role of α4 (Ch 20) and β2 (Ch 1) genes in conferring a risk for smoking and for smoking a large number of cigarettes daily in subjects with schizophrenia. **Methods:** Our study sample consisted of 241 white European schizophrenia patients (157 smokers and 84 nonsmokers) from the Toronto area. Current smoking status was assessed by the medical history. We investigated 4 markers located in the *CHRNA4* gene and 3 markers located in the *CHRNB2* gene. **Results:** There was no difference in age or ethnicity between the 2 groups and the population was not stratified ($\lambda = 0.4527$). We found a significant association between the *CHRNA4* rs3746372 allele 1 and a large number of cigarettes smoked daily ($p = 0.0203$). The intragenic interaction between rs3787116 and rs3746372 ($p = 0.0050$) in *CHRNA4* showed a significant interaction for the number of cigarettes smoked. **Conclusion:** Although our findings suggest an association between rs3746372 allele 1 and heavy smoking, further study is warranted to investigate the relation between smoking and high-affinity nicotinic receptor genes in schizophrenia.

Introduction : Nous avons suggéré antérieurement que le polymorphisme du *CHRNA7* des gènes des récepteurs nicotiniques, et en particulier le D15S1360 du gène *CHRNA7*, est associé au tabagisme dans les cas de schizophrénie. Les patients schizophrènes sont habituellement de gros fumeurs. Au cours de cette étude, nous avons posé en hypothèse qu'il y a un lien entre les récepteurs nicotinique à grande affinité et le tabagisme chez ces patients. **Objectif :** Étudier le rôle des gènes α4 (Ch 20) et β2 (Ch 1) dans le risque de tabagisme et de consommation d'un grand nombre de cigarettes par jour chez les sujets schizophrènes. **Méthodes :** Notre échantillon d'étude regroupait 241 patients schizophrènes européens blancs (157 fumeurs et 84 non-fumeurs) de la région de Toronto. On a évalué le statut de fumeur en analysant les antécédents médicaux. Nous avons étudié quatre marqueurs situés sur le gène *CHRNA4* et trois marqueurs situés sur le gène *CHRNB2*. **Résultats :** Il n'y avait pas de différence sur les plans de l'âge ou de l'origine ethnique entre les deux groupes et l'on n'a pas stratifié la population ($\lambda = 0,4527$). Nous avons constaté un lien important entre l'allèle 1 rs3746372 du gène *CHRNA4* et le nombre élevé de cigarettes fumées par jour ($p = 0,0203$). L'interaction intragénique entre rs3787116 et rs3746372 ($p = 0,0050$) dans le gène *CHRNA4* a montré qu'il y avait une interaction importante dans le cas du nombre de cigarettes fumées. **Conclusion :** Même si nos constatations indiquent l'existence d'un lien entre l'allèle 1 rs3746372 et le degré de tabagisme chez les gros fumeurs, une étude plus poussée s'impose pour analyser le lien entre le tabagisme et les gènes des récepteurs nicotiniques à grande affinité dans les cas de schizophrénie.

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Introduction

Schizophrenia is a relatively common severe psychiatric disorder, affecting about 1% of the general population, and remains one of the more serious problems facing psychiatrists. The disease is characterized by a wide spectrum of symptoms that include thought disorder, perceptual aberrations, cognitive difficulties, apathy and social withdrawal. Although genetic and environmental factors contribute to the etiology of schizophrenia,[2] the exact causes are unclear. Addiction to tobacco is, perhaps, the most common of all addictions. The prevalence of smoking is declining in the general population; however, the same trend is not seen in subjects with schizophrenia. Mentally ill subjects purchase 45% of the tobacco products sold in the United States.[3] A "typical" person with schizophrenia is young, male, single, unemployed and living in social housing. This double blow of smoking and unemployment hits the patients very hard financially.[4] About 40% of psychiatric patients are cigarette smokers, but the rate in schizophrenia patients is much higher, approaching 80%,[5] and the number of cigarettes that patients with schizophrenia smoke is also greater.[6] Patients with schizophrenia often smoke to relax or calm themselves.[7] It has been suggested that smoking may be an attempt by schizophrenia patients to self-medicate,[8] and various studies have explored possible links between schizophrenia and smoking. Normally, there is a substantial increase in high-affinity nicotinic receptors in smokers.[9] However, when compared with matched control smokers, the high-affinity nicotine binding in smokers with schizophrenia is decreased by about one-half in multiple regions of the postmortem brain, including the hippocampus, caudate and cortex,[10] suggesting the possibility of a gene–drug interaction. The decrease in high-affinity nicotine binding in schizophrenic smokers may be related to impaired plasticity of nicotinic receptors in the face of chronic nicotine use, and it is unknown whether specific receptor subunits are affected in this aberrant regulation. However, since decreased binding has been found in many different brain regions, we speculate that α4β2, which are the most abundant high-affinity receptors in the brain,[11] could be the target of this aberrant regulation.

In an animal study using mice, Tapper and colleagues[12] demonstrated that activation of mutant α4 receptors with low doses of agonists induces the same tolerance and sensitization elicited by chronic nicotine administration. However, it has been found that nicotine stimulates dopamine release in the ventral striatum of wild-type mice but not in the ventral striatum of β2-mutant mice.[13] Further, an increasing body of evidence, for the most part derived from neurophysiological, animal model and genetic studies, has provided compelling evidence for major involvement of low-affinity nicotinic receptors in the neurobiology of schizophrenia.[14]

Despite many genetic studies that investigated the low-affinity α7-nicotinic receptor located at 15q13–15 in schizophrenia,[15–19] to date, no clinical genetic studies investigating the possible role of variants of the high-affinity nicotinic receptor have been reported in schizophrenia, apart from that of De Luca and associates.[20]

High-affinity nicotinic receptors in the brain are controlled mainly by 2 genes, α4 and β2.[5] In humans, the *CHRNA4* gene is assigned to chromosome 20q13.2–13.3 and *CHRNB2* to chromosome 1q21. Further, *CHRNA4* spans more than 17 kb, whereas *CHRNB2* is smaller, spanning only 14 kb.

In this paper, we aim to explore the hypothesis that these 2 genes confer a risk for smoking in schizophrenia.

Methods

To select only the single nucleotide polymorphisms (SNPs) with minor allele frequency higher than 0.1, we selected only SNPs with high heterozygosity from dbSNP database. On this basis, we chose 4 SNPs in the *CHRNA4* region (rs3746372, rs3787116, rs4522666, rs755203) and 3 SNPs located in *CHRNB2* region (rs3926124, rs1127309, rs1127314). To test for a gene-specific effect, we analyzed the haplotypes created by the combination of these markers across the 20q13.2 region and 1q21 region. For genomic control and structure analysis, we analyzed 16 alleles from 4 micosatellites on chromosome 4 (D4S1616, D4S2987, D4S2989, D4S3004) as biallelic markers. We also examined the hypothesis that the interaction between polymorphisms located in the *CHRNA4* and *CHRNB2* genes confers susceptibility to smoking in schizophrenia.

For example, in a similar analysis model, specific gene–gene combined association for the *G72/DAAO* gene has been shown in a case–control study of schizophrenia, highlighting the role of genetic interactions within proteins that are functionally related.[21]

The procedures for this study were approved and monitored by the Centre for Addiction and Mental Health's (CMAH's) Research Ethics Board. We determined diagnoses by using best-estimate procedures incorporating the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I)[1,22] and medical records when they were available. Written informed consent to participate in the study was obtained from all participants.

Participants were from a community-based sample of 421 unrelated schizophrenia patients who were recruited through the CAMH in Toronto. Participants were categorized by ethnicity according to the self-reported ethnicity of the participants' grandparents. This sample is an enlargement of one we have studied before.[23] The analysis was limited to white Europeans to limit population stratification. Therefore, 241 participants with 4 white European grandparents were included in the α4β2 genotype and smoking analysis. Within this group, we were able to select 21 participants with all 4 grandparents of Italian heritage and 9 participants with all 4 grandparents of Portuguese heritage; all other participants were considered mixed.

The clinical information was collected from the SCID-I and we administered a medication history questionnaire in which current tobacco consumption and current antipsychotic regimen were also addressed. Within the sample of 241 patients there were 175 men and 66 women. The mean (standard deviation [SD]) age at the time of the inclusion in the study was 39.4 (10.8) years. At the time of the assessment, 156 of the patients were current smokers. The mean (SD) number of cigarettes smoked daily was 19.7 (11.2).

We used a high salt extraction method to extract genomic DNA from leukocytes.[24] The 7 markers were genotyped by the TaqMan fluorogenic detection method with the ABI7000 (Applied Biosystem, Foster City, Calif.). Population structure analysis was done with Structure 2.1.[25] We used GCF for genomic control analysis.[26] We used Helix Tree analysis (HelixTree Genetics Analysis Software, Golden Helix Inc., Bozeman, Mont.) for allelic association, genotype association, haplotype association and gene–gene interactions. For every test we calculated raw p values and adjusted p values. Multiplicity adjustment applied for smoking status and number of cigarettes smoked daily where multiple possible cut points or categories were searched through for an optimal split. This adjustment is less conservative than Bonferroni correction. Power analysis was performed with G*Power 2 (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/).

Results

The population structure analysis using all 23 biallelic markers did not find a genetic population substructure ($\lambda = 0.4527$) among mixed white Europeans ($n = 211$), Italians ($n = 21$) and Portuguese ($n = 9$). With Structure 2.1, the 3 inferred genetic clusters did not identify the ancestry of these 3 populations. The SNP *CHRNB2(*rs3926124) was not polymorphic in this population.

The GCF analysis[26] revealed a robust correction factor of $\lambda = 0.7245$ across the 23 biallelic markers in smokers versus nonsmokers; thus, we did not use a GCF correction for our analyses.

The analysis of smokers versus nonsmokers demonstrates that single genotypes of the 7 markers are not associated with smoking status.

Allelic association was also negative. Sex was strongly associated ($p = 0.008$), with 71% of men being smokers versus only 47% of women. Age at the time of assessment did not influence smoking status ($p = 0.61$).

We screened intergenic and intragenic interactions totalling 13 interactions. The interaction between *CHRNA4* (rs755203) and *CHRNB2*(rs1127309) was significantly associated with smoking status ($p = 0.003$), however, the adjusted p value was not significant ($p = 0.72$) (Table 1).

The 4 markers in α4 were not in linkage disequilibrium (LD), except for *CHRNA4*(rs755203) and *CHRNA4*(rs4522666), which showed a strong pairwise LD ($p < 0.001$, d' = 0.598, $r = 0.469$).

In *CHRNB2*, rs1127314 and rs1127309 showed strong LD ($p < 0.001$, d' = 0.999, $r = 0.983$).

The global p value for haplotype analysis combining *CHRNA4*(rs755203) and *CHRNA4*(rs4522666) was 0.97. The specific haplotype p values were negative as well. The global p value for haplotype analysis of *CHRNB2*(rs1127314) and *CHRNB2*(rs1127309) was 0.86.

For the *CHRNA4* SNPs that were not in strong LD, we applied the 2-loci interaction analysis as if they were on 2 different chromosomes. The α4 intragenic interaction was significant between *CHRNA4*(rs755203) and *CHRNA4*(rs3787116) ($p = 0.005$), but the adjusted p value was 0.81 (Table 1).

When we analyzed the number of cigarettes smoked, we found that the age of the subject was significant ($p < 0.001$); in particular, age is a risk factor with patients older than 43 years, smoking an average of 25.0 cigarettes daily. Further, the sex of the subject was significant ($p = 0.027$). Women smoked an average of 16.0 cigarettes daily, whereas men smoked 21.0 cigarettes daily. The SNP *CHRNA4*(rs3746372) was found to be significant ($p = 0.01$), and those with genotype (1,1) smoked 22.0 cigarettes daily, whereas the other patients smoked an average of 17.0 cigarettes daily.

In regard to the allele effect, we found that allele 1 (frequency 74.3%) had a specific effect ($p = 0.02$), with a mean number of 20.42 cigarettes smoked daily, compared with allele 2, which was associated with a mean number of 17.07 cigarettes smoked daily.

CHRNA4(rs3787116) showed a slight trend ($p = 0.059$), with the heterozygous genotype (1,2) protecting against heavy smoking (average 18.0 cigarettes daily), whereas subjects with homozygous genotypes smoked 21.0 cigarettes daily.

When we employed the Helix Tree 2-loci interaction analysis, we did not include the uninformative SNP *CHRNB2 (*rs3926124). The interaction of SNP *CHRNA4*(rs755203) and SNP *CHRNB2*(rs1127309) was significant ($p = 0.07$) (Table 2). However, after the multiple test correction, the adjusted p value became insignificant ($p = 0.06$). Surprisingly, both SNPs were not significant on their own.

The global p value for the haplotype analysis of *CHRNA4* (rs755203) and *CHRNA4*(rs4522666) was 0.98. The global p value for the haplotype analysis of *CHRNB2*(rs1127314) and *CHRNB2*(rs1127309) was 0.77.

Table 1: Smoker versus nonsmoker analysis: Helix Tree 2-loci interaction analysis using single nucleotide polymorphisms (SNPs) in *CHRNA4* and *CHRNB2* regions*

Gene name (SNP no.)	Gene name (SNP no.); interaction p values†					
	A4(rs3746372)	*A4*(rs3787116)	*A4*(rs4522666)	*A4*(rs755203)	*B2*(rs1127309)	*B2*(rs1127314)
B2(rs1127314)	0.0062	0.0048	0.0167	0.0052	NA	NA
B2(rs1127309)	0.0059	0.0080	0.0338	0.0028	NA	NA
A4(rs755203)	0.0085	0.0050	NA	NA	0.7205	0.7618
A4(rs4522666)	0.0122	0.0089	NA	NA	0.9808	0.8804
A4(rs3787116)	0.0085	NA	0.8421	0.8119	0.8722	0.8523
A4(rs3746372)	NA	0.8364	0.8825	0.8366	0.8762	0.8814

NA = not applicable.
*SNP *CHRNB2*(rs3926124) was not informative and was excluded from the table.
†Interaction p value for all possible combinations except for the linked markers; p values above the diagonal are uncorrected, p values below are adjusted for multiple test.

Table 2: Cigarettes smoked daily: Helix Tree 2-loci interaction analysis using single nucleotide polymorphisms (SNPs) in *CHRNA4* and *CHRNB2* regions*

Gene name (SNP no.)	Gene name (SNP no.); interaction *p* values†					
	A4(rs3746372)	*A4*(rs3787116)	*A4*(rs4522666)	*A4*(rs755203)	*B2*(rs1127309)	*B2*(rs1127314)
B2(rs1127314)	0.0148	0.0074	0.0066	0.0128	NA	NA
B2(rs1127309)	0.0087	0.0072	0.0069	0.0068	NA	NA
A4(rs755203)	0.0074	0.0072	NA	NA	0.0577	0.1209
A4(rs4522666)	0.0037	0.0013	NA	NA	0.0633	0.0603
A4(rs3787116)	0.0004	NA	0.0112	0.0656	0.0662	0.0674
A4(rs3746372)	NA	0.0026	0.0325	0.0680	0.0618	0.1084

NA = not applicable.
*SNP *CHRNB2*(rs3926124) was not informative and was excluded from the table.
†Interaction *p* value for all possible combinations except for the linked markers; *p* values above the diagonal are uncorrected, *p* values below are adjusted for multiple test.

CHRNA4(rs3787116) and *CHRNA4*(rs3746372) showed a significant interaction ($p = 0.004$). Further, the adjusted p value was also significant ($p = 0.03$). The interaction between *CHRNA4*(rs3787116) and *CHRNA4*(rs4522666) was significant ($p = 0.001$), as was the adjusted p value (0.001). Likewise, the interaction between *CHRNA4*(rs3746372) and *CHRNA4*(rs4522666) was significant ($p = 0.004$), as was the adjusted p value (0.033).

Discussion

Our results do not demonstrate a significant genetic difference in 7 markers from the α4 and β2 genes between schizophrenia patients who smoke and those who do not. On the other hand, we found that male sex is a risk factor for smoking and for heavy smoking (> 20 cigarettes daily) in schizophrenia patients.

The unrelated schizophrenia patients were all white Europeans, and when we analyzed genomic control markers, the structure analysis suggested no subpopulation stratification due to ethnicity or smoking status . The observed power considering the genotype (1,1) at *CHRNA4*(rs3746372) for heavy smoking and the observed effect size (d) of 0.4545 was 75.2% (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/).

One possible limitation to this study is the fact that some of our current nonsmokers could have been smokers at some time in the past; thus, for future studies, use of examinations such as the Fagerstrom Questionnaire[27] is warranted. We believe that this number of subjects will be fairly small, since quitting smoking may be very difficult for these patients, who have multiple behavioural and cognitive impairments.[28] Further, the lack of smoking cessation interventions for patients with schizophrenia can explain the fact that these patients have less access to smoking cessation programs than does the normal population.[29] Addington and colleagues[30] have noted that schizophrenia patients have difficulty in complying with smoking cessation programs, and when they do complete a program, only 12% are successful at remaining abstinent at 6-month follow-up.

We found a positive interaction between *CHRNA4* (rs755203) and *CHRNB2*(rs1127309), and more work needs to be done with these markers. Although some significant results were found, the adjusted p values for these tests turned out negative.

When we looked only at smokers and analyzed the number of cigarettes smoked, the age at time of assessment became a risk factor, with patients older than 43 years smoking an average of 25 cigarettes daily. The sex of subjects was also found to be significant; female sex was protective, with women smoking an average of only 16 cigarettes daily.

Because some SNPs in *CHRNA4* were found to be unlinked, intragenic interactions were investigated.

Even after the p value was adjusted for multiple testing, we found significant variance in the number of cigarettes smoked for 3 intragenic interactions: *CHRNA4*(rs3787116]) and *CHRNA4*(rs3746372), *CHRNA4*(rs3787116) and *CHRNA4*(rs4522666), and *CHRNA4*(rs3746372) and *CHRNA4*(rs4522666)). Our data need to be replicated in an independent sample population, and the genotyping of additional genetic markers would also further inform our results.

In conclusion, this study is the first to investigate allelic variants of nicotinic α4 and nicotinic β2 in schizophrenia with respect to smoking status. The most interesting finding was the intragenic α4 interaction. In fact, the subjects bearing the genotype (1,1) in rs3746372 and genotype (1,1) in rs3787116 were smoking an average of 24 cigarettes daily. However, this significant association needs to be interpreted very carefully given the multiple tests performed and the many negative findings of nearby markers.

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