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Evidence of Association between Smoking and α7 Nicotinic Receptor Subunit Gene in Schizophrenia Patients

Vincenzo De Luca[1], Albert HC Wong[1], Daniel J Muller[1], Gregory WH Wong[1], Rachel F Tyndale[2] and James L Kennedy*,[1]

[1]Neurogenetics Section, Clarke Site, Centre for Addiction and Mental Health, Department of Psychiatry, Toronto, Ontario, Canada; [2]Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

A number of studies have suggested that the α-7-nicotinic receptor D15S1360 polymorphism is associated with schizophrenia and a deficiency in the normal inhibition of the P50 auditory-evoked response. Schizophrenia patients and some of their unaffected relatives show a failure of inhibition in their 50-ms response to the second of a pair of tones. Biochemical studies have suggested that the α7 nicotinic acetylcholine receptor is involved in this sensory gating deficit. Furthermore, high-dose nicotine transiently normalizes the abnormality in *P*50 inhibition in schizophrenic patients and in their relatives. Schizophrenic patients are unusually heavy smokers, and up to 85% of hospitalized schizophrenic patients smoke. This rate is three times higher than the general population, and may represent an attempt to self-medicate through this pathophysiologic mechanism. Despite schizophrenics' extremely heavy nicotine use, nicotinic receptor genes have not been previously investigated in relation to smoking in schizophrenia. In this study, we hypothesized that the D15S1360 marker is associated with smoking in patients with schizophrenia. We found an association between the homozygous 113 bp allele and smoking risk ($\chi^2 = 10.37$, 3 df, $p = 0.015$). Although this novel finding requires replication, it suggests that further study into the relationship between schizophrenia and nicotine system genes is warranted.
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INTRODUCTION

The incidence of cigarette smoking in psychiatric patients is approximately 40%, but in schizophrenia it is much higher, approaching 80% (Leonard and Bertrand, 2001), and the amount that schizophrenic patients smoke is also higher (de Leon *et al*, 1995). It has been suggested that smoking may be an attempt by schizophrenia patients to self-medicate (Adler *et al*, 1998), and various studies have explored the possible links between schizophrenia and smoking. Patients with schizophrenia have abnormal *P*50 responses (Freedman *et al*, 1994) and nicotine transiently normalizes the *P*50 auditory evoked-potential deficit in both humans and animals (Adler *et al*, 1992, 1993; Leonard *et al*, 1996). Genetic linkage analysis of the *P*50 auditory-evoked potential deficit in families of patients with schizophrenia found a peak LOD score at 15q13–q14. The LOD score was 5.3 (theta = 0.00) at the D15S1360 marker, which is located in intron 2 of the gene for the α7 nicotinic acetylcholine receptor subunit (CHRNA7), suggesting that the *P*50 deficit is linked to CHRNA7 (Freedman *et al*, 1997).

The CHRNA7 gene modulates evoked potentials in an auditory gating pathway in the rat (Adler *et al*, 1992; Luntz-Leybhan *et al*, 1992). A mouse model has been developed in which a similar inhibitory sensory gating is also correlated with the CHRNA7 expression. Mice with a knockdown for CHRNA7 (DBA/2j) show disinhibited sensory gating compared to the C3H mouse strain, which has normal levels of CHRNA7 mRNA. In addition, GTS-21, a specific agonist of α7 neuronal nicotinic receptors, normalizes this loss of inhibition in the DBA/2j strain (Stevens *et al*, 1996, 1998).

The nicotinic receptor containing the α7 subunit in human brain is assembled with other subunits as a pentameric holoreceptor (Lindstrom *et al*, 1996), but can function as a homomeric ion channel *in vitro* (Zhang *et al*, 1994). It belongs to the family of ligand-gated ion channels that bind nicotine with low affinity.

The CHRNA7 gene is located on chromosome 15q13–14, a region linked with schizophrenia in several earlier studies (Kauffman *et al*, 1998; Leonard *et al*, 1998). The α7 nicotinic receptor gene (CHRNA7) has a partial duplication of exons 5–10, including the intervening introns (CHRFAM7) that map approximately 0.5 Mb proximal to the full-length CHRNA7 gene (Gault *et al*, 1998).

The α7 nicotinic receptor is expressed at low levels in schizophrenia postmortem brain. Regions of lower

*Correspondence: Dr JL Kennedy, Neurogenetics Sections, Clarke Site, Centre for Addiction and Mental Health, 250 College Street, R-30, Toronto, Ontario, Canada M5T 1R8. Tel: +416 979 4987, Fax: +416 979 4666, E-mail: James_Kennedy@camh.net
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expression compared to psychiatric controls include those involved in processing of sensory information, such as the hippocampus and the reticular nucleus of the thalamus (Freedman *et al*, 1995; Court *et al*, 1999).

The D15S1360 microsatellite repeats, in intron two of the CHRNA7 gene, cosegregates with an auditory gating deficit in family linkage studies of schizophrenic patients (Freedman *et al*, 1997). This marker lies 33 531 bp downstream relative to the end of exon 2.

Mutation screening of CHRNA7 from schizophrenia and control individuals identified promoter polymorphisms associated with schizophrenia that decreased the subunit transcription and *P*50 inhibition (Leonard *et al*, 2002). Recently, Gault *et al* (2003) described new SNPs in the alpha7 nicotinic receptor subunit gene; however, in contrast to D15S1360, they are not related to *P*50 deficit. Since these variants are rare and thus uninformative in currently available samples, we chose to genotype the D15S1360 marker, which has common variants, and is not duplicated, making the result easier to interpret in this unstable genomic region.

PATIENTS AND METHODS

We recruited 177 schizophrenic patients (130 men, 47 women) from Toronto and Central Canada. All patients were diagnosed on the basis of the Structured Clinical Interview for DSM-IV Axis I/Patients (SCID-I/P) (APA, 1994), and each subject gave a 30-ml blood sample for DNA extraction. All subjects gave written informed consent. In addition, a medication history questionnaire was administered to each patient, in which current tobacco consumption was also addressed. If the subjects identified themselves as a smoker, then further questions determined the number of cigarettes per day. If the subject was currently a nonsmoker, no other question were asked regarding previous smoking behavior including whether or not they had quit smoking. Thus, quitters would be classified as nonsmokers. Based on the questionnaire responses, 108 (85 men, 23 women) were identified as 'current smokers', while 69 (43 men, 26 women) were classified as 'current nonsmokers'. In all, 93% of the smokers and 91% of the nonsmokers were of Caucasian ethnicity (Table 1). The nonsmokers fraction in our sample is 38% a number higher than the reported 15% of hospitalized schizophrenics; however, our sample consists of mostly outpatients who are less prone to smoke (Uzun *et al*, 2003).

All subjects were unrelated, with the exception of two sib pairs that were included in the study. The smokers *vs* nonsmokers were group matched for ethnicity and age to reduce the potential effect of population stratification.

Table 1 Demographic Difference between Two Groups

	Smokers	Nonsmokers
Sample size	108	69
Male	87 (81%)	43
Female (%)	21 (19%)	26 (38%)
Age	41.0 ± 10.7	38.2 ± 10.9
Caucasian %	93%	91%

Age reliability $t = -1.678$; 174 df, $p = 0.09$; gender ratio reliability $\chi^2 = 5.96$, 1 df, $p = 0.01$.

We genotyped the D15S1360 dinucleotide polymorphism in the α7 nicotinic receptor gene (CHRNA7), as described in Freedman *et al* (1997), using the ABI 3100 (Applied Biosystem, Foster City, CA). Gault *et al* (1998) described the structural organization of the CHRNA7 gene as being partially duplicated; however, the locus D15S1360 appears to be unique in the genome, and therefore provides genotypes easier to interpret than markers in the duplicated region. The genotype data were analyzed using χ^2 test and logistic regression.

RESULTS

The D15S1360 marker exhibited seven different dinucleotide repeat lengths (99, 109, 111, 113, 115, 117, and 125 bp), with a heterozygosity of 43% (calculated as number of heterozygous subjects in the total sample). Two major alleles, 113 and 115 bp, accounted for nearly 90% of the alleles. This finding is quite similar to that reported by Freedman *et al* (1997), although they did not find the very rare alleles. We tested whether the empirically derived genotype frequencies were in Hardy–Weinberg equilibrium at D15S1360 using the public domain program HWE by Ott (1999). Theoretically, there should be 28 individual genotypes composed of all the possible combinations of the seven observed alleles; however, only 12 different genotypes were represented in our sample population. The D15S1360 marker exhibited no deviation from Hardy–Weinberg equilibrium ($\chi^2 = 11.14$, 21 df, $p = 0.95$). These individuals were divided into four groups according to the genotype they possessed, that is, 113/113, 113/115, 115/115, and others (mixed groups). In spite of previous reports in literature (Stassen *et al*, 2000), we found highly significant differences ($\chi^2 = 10.37$, 3 df, $p = 0.015$) between the allele distributions of smokers and nonsmokers among schizophrenia patients. The genotype counts for this polymorphism were significantly different between the two groups ($\chi^2 = 10.39$, 3 df, $p = 0.01$) (Table 2). The estimated OR was 1.60 (95% CI 1.04–2.46), $p = 0.04$. Ethnicity and age did not contribute to the differences between the two groups ($\chi^2 = 0.04$, 1 df, $p = 0.84$; $t = -1.678$, 175 df, $p = 0.09$) (Table 1). The largest contribution to the χ^2 was from the 113 and 115 bp alleles. Among smokers, the allelic frequencies were 53.7% for the 113 bp allele and 40.2% for the 115 bp allele compared to nonsmokers, where the frequencies were 42 and 46.3%, respectively. Power calculation revealed that, if the frequency of exposure is approximately 60%, our sample has a power $>80\%$, with $\alpha = 0.05$, to detect a genotype relative risk <2 (Risch, 2000).

The two groups were matched for age and ethnicity. The analysis of genotype and allelic distributions across the two ethnic groups demonstrates that this marker did not show ethnic stratification: $\chi^2 = 5.59$, 3 df, $p = 0.13$ and $\chi^2 = 4.02$, 2 df, $p = 0.13$, respectively. Our Caucasian population is mainly from Western Europe, and there are no major differences in the distribution of ethnicities between smokers and nonsmokers. Furthermore, to our knowledge, there are no reports that have found any differences in allelic distribution of D15S1360 across Caucasian samples.

Table 2 D15S1360 Differences in Smokers/Nonsmokers

Allele[a]	Smokers	Nonsmokers
Sample size	216	138
113 bp (%)	116 (53.7%)	58 (42.0%)
115 bp (%)	87 (40.2%)	64 (46.3%)
111 bp (%)	5	12
Others (%)	8	4
Genotype		
113/113	38 (36%)	11 (16%)
113/115	32 (29%)	28 (41%)
115/115	25 (23%)	15 (22%)
Others	13 (12%)	15 (22%)

[a]Allele counts include rarer genotypes not specified in the genotype table.

Table 3 D15S1360 Female Differences

Genotype	Female smokers ($n=21$)	Female nonsmokers ($n=26$)
113/113	6 (28%)	4 (15%)
113/115	7 (33%)	7 (27%)
115/115	8 (39%)	5 (19%)
Others	0 (0%)	10 (39%)
Allele[a]	**Smokers ($n=42$)**	**Nonsmokers ($n=52$)**
113 bp	19 (45%)	20 (38%)
115 bp	23 (55%)	21 (40%)
Others	0 (0%)	11 (22%)

Genotype: $\chi^2=10.68$, 3 df, $p=0.01$; allele: $\chi^2=10.17$, 2 df, $p=0.006$.
[a]Allele counts include rarer genotypes not specified in the genotype table.

Table 4 D15S1360 Male Differences

Genotype	Smokers ($n=87$)	Nonsmokers ($n=43$)
113/113	32 (37%)	7 (16%)
113/115	25 (29%)	21 (49%)
115/115	17 (19%)	10 (23%)
Others	13 (15%)	5 (12%)
Allele[a]	**Smokers ($n=174$)**	**Nonsmokers ($n=86$)**
113 bp	96 (55%)	38 (42%)
115 bp	64 (37%)	43 (50%)
Others	14 (8%)	5 (8%)

Genotype: $\chi^2=7.74$, 3 df, $p=0.05$; allele: $\chi^2=4.18$, 2 df, $p=0.12$.
[a]Allele counts include rarer genotypes not specified in the genotype table.

However, there were significant sex differences ($\chi^2=5.96$, 1 df, $p=0.01$). There were more males in among the smokers than nonsmokers (81 *vs* 62%) (Table 1). A logistic regression analysis incorporating genotype and sex showed a significant interaction between 113 bp (homozygous + heterozygous) allele and males with an estimated OR of 1.85 (95% CI 1.01–3.41), $p=0.04$. The association between D15S1360 and smoking was analyzed separately in males and females. There were significant differences observed between female smokers and nonsmokers (genotype: $\chi^2=10.68$, 3 df, $p=0.01$; allele: $\chi^2=19.17$, 2 df, $p=0.006$) (Table 3). For males, smoking was significantly associated with genotype: $\chi^2=7.74$, 3 df, $p=0.05$ (Table 4), with a trend towards an association between smoking and the 113-bp allele ($\chi^2=4.18$, 2 df, $p=0.12$) (Table 4).

DISCUSSION

Our results demonstrate a significant genetic difference between schizophrenia patients who smoke and those who do not. Our data need to be replicated in an independent sample population, and the genotyping of additional genetic markers would also further inform our results. The association between the 113-bp allele of the D15S1360 marker in the CHRNA7 gene and smoking may be related to the clinical observation that patients with schizophrenia are more likely to smoke and to smoke heavily. Schizophrenia is characterized by well-documented cognitive and neurophysiological deficits, including abnormal p50 auditory evoked potentials, which are mediated in part by the CHRNA7 receptor. Previous reports have shown a link between the D15S1360 marker in the CHRNA7 receptor gene and *P*50 (Freedman *et al*, 1997). The most salient question that arises from these results is whether the neurobiology of schizophrenia makes patients more vulnerable to nicotine addiction, and/or whether nicotine improves the cognitive or sensory-gating deficits. The finding of a greater genetic effect in women is interesting, as social convention protects them from heavy smoking to some extent. We did not study healthy control subjects, but one previous report comparing the CHRNA7 genotypes of healthy smokers and nonsmokers did not show significant differences (Stassen *et al*, 2000).

The increased proportion of the D15S1360 risk allele 113 bp in schizophrenic patients who smoke (especially in male smokers) suggests that smoking among schizophrenia patients may be mediated more by the addiction to nicotine. This hypothesis is further supported by a series of studies: smokers with schizophrenia are more likely to have had an earlier age of onset of schizophrenia, to have had a greater number of hospitalizations, to display significant reduction in Parkinsonism, to have more akathisia, and to have a higher total score on the BPRS (Goff *et al*, 1992).

The mechanism by which the D15S1360 marker may have an effect on smoking in schizophrenia is unclear and requires further experimental work; however, it is possible that the alleles of the D15S1360 marker confer different characteristics of mRNA stability and/or processing. It is also possible that 113 bp allele at this marker cosegregates with a nearby functional site that may affect the function of this receptor in schizophrenia. However, among the new variants found by Gault *et al* (2003), the closest one is located at least 30 kb from D15S1360 and with a minor allele frequency lower than 3%. Thus, it is unlikely to be informative, and unlikely to be in strong linkage disequilibrium with the 113 bp allele.

The definition of smoking status is not entirely consistent for subjects reported in the literature on CHRNA7 and *P*50, and this makes direct comparisons between studies difficult. Nevertheless, our data indicate that there are greater differences between smokers and nonsmokers among schizophrenics than among healthy control subjects (Stassen *et al*, 2000). The Stassen *et al* study was exclusively based on data derived from a relatively small number of smokers within a control group recruited to match with patients with psychiatric disorders, and was not in Hardy–Weinberg equilibrium. Our sample was in Hardy–Weinberg equilibrium, suggesting that population stratification was not a factor in our results.

One possible limitation to this study is the fact that some of our current nonsmokers may have been smokers and quit at some time in the past; thus, for future studies, examinations such as the Fagerstron Questionnaire (Heatherton *et al*, 1991) are warranted. We believe that this number of subjects will be fairly small, since these patients have multiple behavioral and cognitive impairments, quitting smoking may be very difficult for them (Ziedonis *et al*, 1994); furthermore, the lack of smoking cessation interventions for schizophrenics can explain the fact that these patients have less access to smoking cessation programs than the normal population (Addington *et al*, 1997). Addington *et al* (1998) have noted that schizophrenia patients have difficulty in complying with smoking cessation programs, and when they do complete the program, only 12% are successful at remaining abstinent at 6 months follow-up.

Despite the limitations, including a small sample size of the samples studied, our study suggests a role of the D15S1360 polymorphism in pathogenesis of nicotine addiction in patients with schizophrenia. Further investigations are needed in larger independent samples to confirm this factor in the complex mechanisms of heavy smoking in schizophrenics.

In summary, our findings suggest an association between a polymorphism in the CHRNA7 receptor gene and smoking status in schizophrenia. This may be relevant to the neurobiology of nicotine addiction, to the cognitive deficits seen in schizophrenia, or to the treatment of schizophrenia. The data may also represent a distinction between schizophrenic smokers and nonsmokers that could represent an endophenotype, suitable for further genetic studies.

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