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Association of the 5′-upstream regulatory region of the α7 nicotinic acetylcholine receptor subunit gene (*CHRNA7*) with schizophrenia

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ABSTRACT

Background: The α7 neuronal nicotinic acetylcholine receptor subunit gene (*CHRNA7*) is localized in a chromosomal region (15q14) linked to schizophrenia in multiple independent studies. *CHRNA7* was selected as the best candidate gene in the region for a well-documented endophenotype of schizophrenia, the P50 sensory processing deficit, by genetic linkage and biochemical studies.

Methods: Subjects included Caucasian-Non Hispanic and African-American case-control subjects collected in Denver, and schizophrenic subjects from families in the NIMH Genetics Initiative on Schizophrenia. Thirty-five single nucleotide polymorphisms (SNPs) in the 5′-upstream regulatory region of *CHRNA7* were genotyped for association with schizophrenia, and for smoking in schizophrenia.

Results: The rs3087454 SNP, located at position −1831 bp in the upstream regulatory region of *CHRNA7*, was significantly associated with schizophrenia in the case-control samples after multiple-testing correction ($P = 0.0009$, African American; $P = 0.013$, Caucasian-Non Hispanic); the association was supported in family members. There was nominal association of this SNP with smoking in schizophrenia.

Conclusions: The data support association of regulatory region polymorphisms in the *CHRNA7* gene with schizophrenia.

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1. Introduction

Schizophrenia is a complex neuropsychiatric disorder partially characterized by sensory processing and cognitive deficits (Leonard et al., 2001; Light and Braff, 2003; George et al., 2006; Adams and Stevens, 2007; Martin and Freedman, 2007). Sensory processing deficits are normalized in schizophrenic patients and first-degree relatives by smoking (Adler et al., 1993, 1998; Olincy et al., 1998; Light and Braff, 2003; Leonard et al., 2007b), and cognitive deficits are improved by nicotine administration (Levin et al., 1998, 2006; Rezvani and Levin, 2001). These discoveries led to a hypothesis of self-medication wherein schizophrenics smoke to correct an underlying biological deficit (Leonard, 2003; Leonard et al., 2007a; Adler et al., 1998; Kumari and Postma, 2005). The hypothesis is supported by studies demonstrating that smoking alters gene expression in normal individuals and differentially regulates gene expression in schizophrenics (Mexal et al., 2005, 2008; Kuehn, 2006).

Nicotine exerts its effect through neuronal nicotinic acetylcholine receptors expressed in the brain and periphery

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(Leonard and Bertrand, 2001; Gotti et al., 2006). The α7 neuronal nicotinic receptor subunit gene (*CHRNA7*), localized at 15q14, was first genetically linked to the P50 auditory sensory processing deficit in schizophrenia (Freedman et al., 1997), and then to schizophrenia as a disease in multiple independent studies. These reports included cohorts of European American, African American, South African Bantu, Azorean, and Han Chinese ancestry, indicating that the linkage is valid across ethnicities (Coon et al., 1994; Kaufmann et al., 1998; Leonard et al., 1998; Riley et al., 2000; Freedman et al., 2001; Freedman and Leonard, 2001; Gejman et al., 2001; Liu et al., 2001; Tsuang et al., 2001; Xu et al., 2001; Fallin et al., 2003). Activation of the homopentameric α7* receptor results in the influx of Ca^{++} and neurotransmitter release (Vijayaraghavan et al., 1992; Aramakis and Metherate, 1998; Berg and Conroy, 2002; Dajas-Bailador and Wonnacott, 2004). Binding studies with an α7* receptor antagonist (α-bungarotoxin) show there are 50% fewer receptors in postmortem hippocampus of individuals with schizophrenia compared to control subjects (Freedman et al., 1995). Decreased expression has also been found in cortex (Guan et al., 1999; Marutle et al., 2001) and in the reticular thalamic nucleus (Court et al., 1999). Low levels of the α7* receptor may have downstream consequences for multiple neurotransmitter systems, altering the balance of neurotransmitter release and activation (Leonard, 2003). The *CHRNA7* gene is now considered one of the important candidate genes for schizophrenia (Harrison and Weinberger, 2005), and α7* receptor agonists are ranked as the most promising targets for development of a drug to treat cognitive impairments in the disorder (Psychiatric News, 2006). Recent Phase I and Phase II studies of an α7* receptor partial agonist, DMXB-A (Martin et al., 2004) resulted in improvements of both sensory processing and attention in non-smoking schizophrenics (Olincy et al., 2006; Freedman et al., 2008).

The α7 nicotinic receptor subunit gene, *CHRNA7*, was found to be partially duplicated, with both loci mapping to the 15q14 region (Gault et al., 1998; Riley et al., 2002). The partially duplicated gene (*CHRFAM7A*) is located 1.6 Mb centromeric to the full-length gene; its function remains unknown. Mutation screening of the coding region and intron/exon borders of *CHRNA7* and of *CHRFAM7A* identified 33 polymorphisms (Gault et al., 2003). Three of the polymorphisms were non-synonymous and mapped to the full length *CHRNA7* coding region. These polymorphisms, however, were very rare and were not associated with either schizophrenia or the P50 gating deficit (Gault et al., 2003). Mutation screening of the core promoter in the *CHRNA7* gene identified a large number of polymorphisms (Leonard et al., 2002). Functional analysis of polymorphisms in the 231 base pairs upstream of the translation initiation site, the core promoter region, demonstrated that most decrease transcription. These polymorphisms were also found to be statistically more prevalent in schizophrenics than in control subjects ($P=0.007$) (Leonard et al., 2002). Further, the presence of a promoter polymorphism in non-schizophrenic controls was associated with a P50 gating deficit ($P<0.0001$) (Leonard et al., 2002). Reporter gene assays with fragments 1.0 kb and 2.6 kb proximal to the translation initiation site exhibit less activity than the core promoter, suggesting that repressor elements may lie upstream of the core promoter.

Utilizing overlapping genomic fragments, we have identified 35 SNPs in the 2 kb upstream regulatory region of *CHRNA7*. SNPs were genotyped utilizing a combination of heteroduplex analysis by denaturing high-performance liquid chromatography and sequencing, and were analyzed for association with schizophrenia in Caucasian-Non Hispanic and African-American subjects. Smoking history was considered as a secondary outcome. The results show significant association of a specific SNP, rs3087454 (−1831 bp), in the 5' upstream regulatory region of the *CHRNA7* gene with schizophrenia.

Table 1a
Number of individuals for case-control and family-based association studies with the *a priori* outcome of schizophrenia.

Case-control	Denver SZ	NIMH SZ	COS	Total SZ	Total controls
African-American	56	47	2	105	45
Caucasian	234	73	56	363	99
NIMH Families	Schizophrenics				Family Members
African-American	113				32
Caucasian	142				42

Denver SZ = schizophrenics collected locally in Denver, NIMH SZ = schizophrenic individuals from the NIMH Schizophrenia Genetics Initiative included in case-control association studies, COS = Childhood onset schizophrenics collected in Denver. Family Members = NIMH family members with a diagnosis of never mentally ill.

Table 1b
Number of individuals for case-control and family-based association studies with the outcome of smoking status.

Case-control	Denver Sm	NIMH Sm	Total Sm	Denver NS	NIMH NS	Total NS
African-American	54	5	59	49	15	64
Caucasian	160	10	170	112	13	125
NIMH Families	Smokers (Sm)			Non-Smokers (NS)		
African-American	17			45		
Caucasian	25			51		

Sm = smokers, NS = non-smokers.

2. Materials and methods

2.1. Subjects

Characteristics of study participants are shown in Tables 1a and 1b. DNA samples from both case-control subjects collected in Denver, and schizophrenic subjects from NIMH families were included in the association studies. For the family-based study, a total of 329 African-Americans (47 nuclear families) and Caucasian-Non Hispanic subjects (73 nuclear families) from the NIMH Schizophrenia Genetics Initiative were chosen based on a diagnosis of schizophrenia. This cohort has previously shown positive LOD scores and association to markers at the 15q14 locus in both African-American and Caucasian individuals (Kaufmann et al., 1998; Leonard et al., 1998; Freedman et al., 2001). Detailed information on the NIMH family structure is available from the web site (http://www.nimhgenetics.org).

Case-control association studies of 612 African-American and Caucasian-Non Hispanic schizophrenics and controls included