Published in final edited form as:

Schizophr Res. 2009 April ; 109(1-3): 102–112. doi:10.1016/j.schres.2008.12.017.

Association of the 5'-upstream regulatory region of the $\alpha 7$ nicotinic acetylcholine receptor subunit gene (*CHRNA7*) with schizophrenia

Sarah H. Stephens^a, Judith Logel^a, Amanda Barton^a, Alexis Franks^a, Jessica Schultz^a, Margaret Short^a, Jane Dickenson^a, Benjamin James^a, Tasha E. Fingerlin^b, Brandie Wagner^b, Colin Hodgkinson^d, Sharon Graw^a, Randal G. Ross^a, Robert Freedman^{a,c}, and Sherry Leonard^{a,c,*}

^a Department of Psychiatry, University of Colorado at Denver, United States

^b Department of Preventive Medicine and Biometrics, University of Colorado at Denver, United States

^c The Veterans Affairs Medical Research Center, Denver, Colorado 80045, United States

^d NIH/NIAAA, Rockville, MD, United States

Abstract

Background—The $\alpha 7$ 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.

*Corresponding author. Department of Psychiatry University of Colorado at Denver, Mailstop 8344, P.O. Box 6511, Aurora, CO 80045, United States. Tel.: +1 303 724 4426; fax: +1 303 724 4425. Sherry.Leonard@UCHSC.edu (S. Leonard).

Contributors

Dr. Leonard designed the study and edited the manuscript. Dr. Sarah Stephens wrote the first draft of the paper and Dr. Sharon Graw provided editorial assistance. Dr. Sarah Stephens, Alexis Franks, Judith Logel, Amanda Barton, Jane Dickenson, and Benjamin James performed the genotyping. Jessica Schultz and Margaret Short performed the functional studies. Drs. Tasha Fingerlin and Brandie Wagner provided statistical expertise. Dr. Colin Hodgkinson graciously shared his admixture markers. Drs. Randal Ross and Robert Freedman recruited the Denver patients participating in the study.

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2008.12.017.

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

Keywords

Nicotinic receptor; Schizophrenia; Polymorphism; Association; Sensory Processing; Alpha 7 nicotinic receptor; Regulatory region; Promoter

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 (Leonard and Bertrand, 2001; Gotti et al., 2006). The $\alpha 7$ 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 $\alpha 7^*$ receptor results in the influx of Ca^{++} and neurotransmitter release (Vijayaraghavan et al., 1992; Aramakis and Metharate, 1998; Berg and Conroy, 2002; Dajas-Bailador and Wonnacott, 2004). Binding studies with an $\alpha 7^*$ 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 $\alpha 7^*$ receptor may have downstream consequences for multiple neuro-transmitter 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 $\alpha 7^*$ 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 $\alpha 7^*$ 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 $\alpha 7$ 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.

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 individuals collected in our laboratory, and schizophrenic subjects from the NIMH families. Ethnicities of case-control subjects were recorded from self-report or family interview. The Caucasian-Non Hispanic case-control sample was comprised of 307 schizophrenic patients, 56 childhood onset schizophrenics, and 99 controls. The African-American case-control sample included 103 schizophrenics, 2 childhood onset schizophrenics, and 45 controls. P50 data were available for 89 Caucasian-Non Hispanic controls subjects and 43 African-American controls. Adult case subjects were chosen based on a diagnosis of schizophrenia, utilizing a Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1996b). Control subjects were interviewed and found to have no evidence for current or past psychosis, using a Structured Clinical Interview for DSM-IV Axis I Disorders for non-patients (First et al., 1996a). Childhood onset schizophrenia cases were diagnosed utilizing the K-SADS-PL (Kaufman et al., 1997). Smoking history was determined on local subjects utilizing a detailed questionnaire (Breese et al., 1997; Breese et al., 2000) and for the NIMH Genetics Initiative subjects, from the web site. P50 auditory evoked potentials were recorded on controls, using published methods (Freedman et al., 1991).

2.2. DNA isolation

Nucleated cells were obtained from anticoagulated blood (EDTA) via lysis with a high sucrose solution (0.3 M sucrose, 0.01 M Tris-HCl, pH 7.5, 0.005 M MgCl₂, and 1% Triton X-100) and subsequent centrifugation. DNA was then isolated from these cells as previously described (Miller et al., 1988). DNA samples for the NIMH Genetics Initiative subjects were obtained from the NIMH collection at Rutgers (<http://www.rucdr.org/ASHG/Schizophrenia.htm>).

2.3. Genotyping and fragment analysis

Eleven amplicons were designed to screen 2.0 kb of DNA in the 5'-upstream regulatory region of the *CHRNA7* gene. The Polymerase Chain Reaction (PCR) with AmpliTaq GoldTM and GeneAmp[®] PCR System 9600 (Perkin-Elmer, Foster City, CA) was used for fragment amplification; thermal cycler conditions unique to each amplicon are shown in Table 2. Fragments were characterized using temperature modulated heteroduplex analysis with Denaturing High-Performance Liquid Chromatography (DHPLC; Transgenomic WAVETM) (Transgenomic Inc., San Jose, California) as previously described (Leonard et al., 2002; Gault et al., 2003). Samples were “spiked” with control DNA to ensure that a homozygous variant that might migrate as a single peak would be detected. When a sample resulted an unusual WAVETM pattern, automated DNA sequencing on an Applied Biosystems 3100 Avant DNA Sequencer (Applied Biosystems, Foster City, CA) was employed for genotyping. For most SNPs, a specific and recognizable WAVETM pattern was generated.

2.4. Functional studies

2.4.1. Cell culture—The human neuroblastoma cell lines SH-SY5Y (a gift from Dr. June Biedler) (Biedler et al., 1978), and SK-N-BE (ATCC number CRL-2271; ATCC, Manassas, VA) were grown in 1:1 Ham F12 Dulbecco Eagle medium supplemented with 15% fetal bovine serum, 100 µg/ml streptomycin, 100 units/ml penicillin, and 2 mM L-glutamine (Invitrogen, Carlsbad, CA) at 37 °C in a 5% CO₂ incubator.

2.4.2. Plasmid constructs—Isolation and characterization of 2.6 kb of 5'-flanking sequence in the human *CHRNA7* subunit gene was described previously (Gault et al., 1998; Leonard et al., 2002). The promoter-luciferase construct Pr2B contains a *CHRNA7* 5'-upstream regulatory region fragment from -2004 bp to the translation start site at +1 bp inserted upstream of the luciferase gene in the pGL3-Basic vector. Pr2B was generated by first TA cloning a 2004 bp PCR fragment from the previously cloned 2.6 kb promoter into pDrive, according to manufacturer's instructions (Promega, Madison, WI). Restriction enzymes (*HindIII* and *KpnI*) were then used to isolate the 2004 bp fragment and insert it into the pGL3 reporter plasmid. The promoter-luciferase fusions were confirmed by DNA sequencing.

Pr2B contains the (C) allele at -1831 bp. The -1831(A) polymorphism was introduced into Pr2B by PCR, utilizing the QuikChange II XL Site-Directed Mutagenesis Kit, according to the manufacturer's protocols (Stratagene). The mutagenesis primers were: a7Prom-1831A forward (5'-gccca-tacatactccagaaaaatAaataattcccttgccc-3') and a7Prom-1831A reverse (5'-ggccaagggaattattTatttttctggagtatg-tatggc-3'); the mutant construct was confirmed by DNA sequencing and named Pr2B-1831A.

2.4.3. Transient transfection and reporter gene assay—SH-SY5Y and SK-N-BE cells grown to subconfluency were transfected with the ProFection Mammalian Transfection Calcium Phosphate System (Promega). A pGL3-Control construct in which the luciferase gene is regulated by the SV40 promoter and enhancer was used as a positive control to measure the maximum reporter gene activity. A pRL-TK construct (Promega) with the Renilla luciferase gene, controlled by the HSV thymidine kinase promoter, served as an internal control for normalization of transfection efficiency. 2×10⁵ cells/35 mm plate were incubated with molar

equivalents of Pr2B, Pr2B-1831A, pGL3-Control, or pGL3-Basic luciferase reporter constructs and 1 μ g of pRL-TK vector as an internal control for the transfection efficiency. Cells were harvested after 48 h and luciferase activity was determined with the Dual-Luciferase Reporter Assay System according to the manufacturer's instructions (Promega). Each transfection was performed using eight separate plates in triplicate.

2.5. Statistical analysis

2.5.1. Case-control data—The case group of schizophrenic individuals included one randomly chosen schizophrenic individual per family. For each marker, allele frequencies were estimated and conformance of genotype frequencies with Hardy–Weinberg Equilibrium (HWE) expected proportions was tested via a 1 *df* chi-squared goodness-of-fit test using Haploview (Barrett et al., 2005). Linkage disequilibrium parameters D' and r^2 were calculated for each pair of markers and tests for differences in allele frequencies between cases and controls were computed using a chi-squared test (1 *df*) for each SNP in Haploview. Since several of the SNPs were rare, empirical *P*-values were utilized rather than relying on the large-sample approximation. Further, since several SNPs were tested for each outcome, empirical *P*-values were generated via permutation that were corrected for the number of SNPs tested. For each permutation, the minimum *P*-value over all SNPs was compared to the minimum *P*-value over all SNPs in the original data. Chi-squared tests of association (2 *df*) between each SNP and schizophrenia under a genotypic model were calculated using the UNPHASED software version 3.0.13 (Dudbridge, 2003); a similar permutation procedure was applied. Corrected *P*-values are indicated with an (*). Odds ratio estimates (OR) and 95% confidence intervals (CI) for comparing relevant genotype or allele classes were computed with SPSS 16 software (SPSS Inc., Chicago IL).

Analysis of the P50 gating deficit in controls was conducted using simple linear regression models that tested the mean differences in P50 ratios under both additive and genotypic models of association separately for each SNP. In the absence of evidence for ethnic-specific effects, ethnicities were combined for most SNPs in order to gain power to detect association, and ethnicity was included as a covariate. All analyses were performed using SAS version 9.1 software (SAS Institute Inc.: Cary, NC, 2004).

Power estimates (at a significance level of 5%) for case-control analyses were obtained with the Quanto software (Gauderman, 2002a,b). Results indicated that the Caucasian schizophrenic/control sample size had power between 0.74 and 0.99 to detect a genotype relative risk (GRR) ≥ 2.0 over a range of marker allele frequencies between 0.10 and 0.40. At GRRs < 2.0 , power was substantially lower (0.12–0.88) for most allele frequencies. The African-American schizophrenic/control sample size had less power to detect a GRR ≥ 2.0 (0.38–0.87) over a range of marker allele frequencies between 0.10 and 0.40. Similarly, at GRRs < 2.0 , power was considerably lower (0.07–0.49) for all allele frequencies.

2.5.2. Admixture analysis—For both ethnicities, case-control samples were evaluated for the presence of admixture by genotyping 176 ancestry-informative SNP markers (Hodgkinson et al., 2008). Within each ethnic group, relative frequencies of alleles between the cases and controls were compared using a chi-squared test (Pritchard and Rosenberg, 1999). There were no significant differences from what was expected, indicating a similar degree of admixture between cases and controls in both ethnic groups. These conclusions were corroborated by estimating individual admixture proportions within each ethnic group using *structure* (Pritchard et al., 2000). These analyses indicated no significant differences in average admixture proportions between cases and controls within each ethnic group.

2.5.3. Family-based data—Prior to data analysis, the PEDCHECK software was utilized to detect marker allele errors in Mendelian inheritance (O’Connell and Weeks, 1998). Pedigree Disequilibrium Test(s) (PDT) of association at SNP markers were implemented in the UNPHASED software version 3.0.13 (Dudbridge, 2003). Similar to the case-control analyses, UNPHASED was used to generate an empirical *P*-value corrected for the number of SNPs.

3. Results

3.1. Hardy Weinberg Equilibrium (HWE) conformance and linkage disequilibrium estimations

Thirty-five polymorphisms were identified in the 2 kb of *CHRNA7* 5’-upstream regulatory sequence, screened in a multi-ethnic cohort in this study. Many of these SNPs reside in transcription factor binding sites (Fig. 1). Twenty-six of these SNPs were present in Caucasian-Non Hispanic subjects and/or in African-American subjects. The minor allele frequencies (MAF) for the 26 SNPs are shown in Table 3. Genotype frequencies of all SNPs were consistent with HWE (all $P > 0.05$; data not shown). Only SNPs with MAFs ≥ 0.05 in at least one ethnic population were considered for statistical analyses.

Of note, the minor alleles for rs3087454 (–1831 bp) were different in the African-American and Caucasian-Non Hispanic samples. The frequency of the rs3087454 (–1831 bp) “C” allele was 0.360 in the Caucasian-Non Hispanic sample, and 0.350 for the “A” allele in the African-American sample.

Fig. 2 shows the LD plot for common SNPs in the core promoter of *CHRNA7* (Leonard et al., 2002) and in the upstream regulatory region of the gene. LD parameter estimates were generated using the case-control data. Many of the 26 polymorphisms are rare and were not included in the LD plots. In the Caucasian sample, markers rs3087454 (–1831 bp) and rs6494165 (–1512 bp) were designated as a haplotype block using the Gabriel definition (Gabriel et al., 2002). Two SNP marker pairs, rs3087454 (–1831 bp)/rs3826029 (–1313 bp) ($D' = 0.89$), and rs6494165 (–1512 bp)/rs3826029 (–1313 bp) ($D' = 1.0$) were in particularly strong LD as they are all contained within a 500 bp region (see Table 3). In the African-American sample, SNP marker rs3826029 (–1313 bp) was in strong LD with marker –1252 bp ($D' = 1.0$), as well as two core promoter polymorphisms [–190 bp ($D' = 1.0$) and –46 bp ($D' = 1.0$)]. Additionally, in both sample populations, SNP markers at –905 bp/–768 bp were in LD with one another ($D' = 1.0$) and with a core promoter polymorphism at –191 bp ($D' = 1.0$).

3.2. Association of rs3087454 (–1831 bp) with schizophrenia

3.2.1. Case-control results—Eight regulatory region SNPs, –46 bp, –86 bp, –178 bp, –190 bp, –1252 bp, rs3826029 (–1313 bp), rs6494165 (–1512 bp), and rs3087454 (–1831 bp), were analyzed for association with schizophrenia and smoking in schizophrenia. Other SNPs were too rare for analysis. *P*-values were adjusted by generating empirical *P*-values via permutation that were corrected for the number of SNPs tested and are indicated by an (*) (Table 4). Genotype and allele counts for the association studies are shown in Supplementary Tables 1–6.

A significant difference was found in the genotype distribution of the rs3087454 (–1831 bp C/A) polymorphism between control and schizophrenic subjects (Table 4). In the Caucasian-Non Hispanic sample, the “C/C” genotype was associated with schizophrenia ($P = 0.0009^*$) (OR=1.57; 95% CI=1.31–2.19) and in the African-American sample, the “A/A” genotype was associated with schizophrenia ($P = 0.013^*$) (OR=2.0; 95% CI=1.19–3.35). Under an allelic model, the “C” allele was only nominally associated with schizophrenia ($P = 0.005$) in the Caucasian-Non Hispanic sample, and the “A” allele was nominally associated with schizophrenia in the African-American sample ($P = 0.005$) (Table 5). rs3087454 (–1831 bp C/

A) was not significantly associated with the outcome of smoking status in either ethnic population.

Fisher's method (Fisher, 1948) was employed to combine P -values for the ethnic groups, to determine the overall significance of the finding (Table 6). As expected, this meta-analysis of the rs3087454 (−1831 bp) SNP indicated a significant association with schizophrenia ($P = 0.0001$) under a genotypic model, but not under an allelic model ($P = 0.124$).

The 58 childhood-onset subjects included in this study were analyzed separately for association with rs3087454 (−1831 bp), but there was no evidence for association ($P = 0.560^*$). COS subjects were excluded from the Caucasian-Non Hispanic case-control sample (a majority of COS subjects were Caucasian), the P -value for an allelic model of association decreased from $P = 0.135^*$ to $P = 0.033^*$. Similarly, the P -value for a genotypic model of association decreased from $P = 0.0009^*$ to $P = 0.0002^*$.

In an attempt to further refine the schizophrenia phenotype, beyond the results shown in Tables 4 and 5, additional analyses were included under the assumption that schizophrenic smokers may be biologically distinct from schizophrenic non-smokers. These analyses included the following dependent variables; schizophrenic smokers versus control smokers, schizophrenic non-smokers versus control non-smokers, and schizophrenic smokers versus all controls. In the African-American sample, nominally significant P -values at rs3087454 (−1831 bp) were found with the outcomes of schizophrenic smokers versus control smokers ($P = 0.018$, genotype; $P = 0.508$, allele), schizophrenic non-smokers versus control non-smokers ($P = 0.0025$, genotype; $P = 0.008$, allele), and schizophrenic smokers versus all controls ($P = 0.001$ genotype; $P = 0.606$ allele). In the Caucasian-Non Hispanic sample, a nominally significant P -value at rs3087454 (−1831 bp) was found with the outcome of schizophrenic smokers versus all controls ($P = 0.041$ genotype; $P = 0.179$ allele).

3.3. Relationship of the schizophrenia “risk” allele rs3087454 to rare *CHRNA7* 5' regulatory region polymorphisms

The frequency of a polymorphism is an important factor in determining the power to detect the risk allele(s) in a given association study. Many of the *CHRNA7* 5' regulatory region polymorphisms are very rare with minor allele frequencies (MAF) < 0.001 . With this information and the recent hypothesis that the ‘common disease–rare alleles’ model might explain many cases of schizophrenia (McClellan et al., 2007), we examined the relationship between the rs3087454 (−1831 bp) “risk” genotype and rare proximal promoter polymorphisms. The number of individuals positive for both a proximal promoter polymorphism and the schizophrenia “risk” genotype at rs3087454 (−1831 bp) were compared against those with only proximal promoter polymorphisms (Table 7). Of the rare promoter SNPs in the Caucasian-Non Hispanic sample population, the SNP at −194 bp occurs more often in individuals with the “C” risk allele and the “CC” risk genotype. The D' value for −194 bp and rs3087454 is 0.73, however, the confidence bounds are large (0.40–0.89). Of the rare SNPs in the African-American sample population, the SNP at −190 bp occurs more often in individuals with the “A” risk allele and the “AA” risk genotype. The D' value is 0.20 for −190 bp and rs3087454, with confidence bounds of 0.02–0.75. Because these SNPs are rare and their D' values are not well estimated, the confidence bounds are large. With many rare SNPs and relatively low D' values, haplotype analyses would have limited power to detect a risk haplotype due to the presence of many rare haplotypes and the error associated with haplotype frequency estimation. However, observations provided by Table 7 suggest rare *CHRNA7* proximal promoter polymorphisms may be relevant to schizophrenia as a haplotype with rs3087454 (−1831 bp) or as single markers.

3.4. Family-based results

Results of the family-based PDT analysis in a second cohort supported the majority of results obtained in case-control studies (not shown). At rs3087454 (−1831 bp) in the Caucasian-Non Hispanic sample, the “C” allele was found to be linked/associated with schizophrenia (nominal $P=0.048$). The rs3087454 SNP was not associated with schizophrenia in the African-American sample. The rs6494165 (−1512 bp) SNP was nominally associated with smoking ($P=0.045$) when Caucasian-Non Hispanic schizophrenic non-smokers and smokers were compared, as was the proximal promoter SNP −86 bp ($P=0.026$). These results were not replicated in the African-American sample.

3.5. Association studies with P50 ratios in control subjects

Linear regression models were estimated separately for the two ethnic groups for the SNP at rs3087454 (−1831 bp) because different alleles were associated with schizophrenia in African-Americans (“A” allele) and Caucasian-Non Hispanics (“C” allele) at this biallelic marker. For all other SNPs, ethnic groups were combined in order to gain statistical power and ethnicity was included as a covariate. Two proximal promoter SNPs were nominally associated with higher P50 ratios in control subjects (SNP −86 bp, $P=0.03$; SNP −191 bp, $P<0.01$). This study did not identify significant association with the P50 deficit at rs3087454 (−1831 bp).

3.6. Functional analysis of rs3087454 (−1831 bp)

SNP rs3087454 (−1831 bp) was examined for effects on $\alpha 7$ nicotinic receptor gene expression in a luciferase reporter gene assay. Results of functional studies in two cell lines (SH-SY5Y and SK-N-BE) found no significant difference in the functional expression of either the “A” or “C” alleles for this SNP (data not shown).

4. Discussion

The current study expands our original data implicating core promoter polymorphisms in the $\alpha 7$ nicotinic acetylcholine receptor gene, *CHRNA7*, in susceptibility for schizophrenia. The rs3087454 (−1831 bp) polymorphism, located in a putative repressor region upstream of *CHRNA7* was significantly associated with schizophrenia in both the Caucasian-Non Hispanic and African-American case-control samples. Nominal P -values for family-based analyses supported results in the case-control sample. Notably, different “risk” alleles were associated with schizophrenia in African-Americans (“A” allele) and Caucasian-Non Hispanics (“C” allele) at this biallelic marker. Associations of different alleles at the same locus with the same disease have been reported across different ethnic groups (Singleton et al., 2003; Tan et al., 2003) and within the same ethnic group (Park et al., 2002; Glatt et al., 2003; Wonodi et al., 2003; Chen et al., 2004; Sanders et al., 2005). This could be explained by population differences where heterogeneous effects of the same polymorphism are caused by differences in genetic background. Alternatively, in complex diseases such as schizophrenia, in which multiple loci act jointly to confer susceptibility for disease, associations of different alleles at the same locus could be attributed to a correlation with a causal polymorphism at another locus (Lin et al., 2007). The correlation can be due to either an interaction between the loci or to linkage disequilibrium. The finding that transcription levels were not changed in an *in vitro* reporter gene analysis of the rs3087454 (−1831 bp) polymorphism suggests that the results of our genetic association studies are likely due to linkage disequilibrium.

Attempts to refine the schizophrenia phenotype utilizing smoking history did not improve evidence for association, although nominal significance was noted in several comparisons. This indicates that the principal finding in this study is association with schizophrenia and not with smoking in the disorder.

Exploratory examination of *CHRNA7* proximal promoter polymorphisms and their relationship with rs3087454 (−1831 bp) suggests that rare *CHRNA7* promoter polymorphisms may occur more frequently in individuals with the rs3087454 (−1831 bp) risk genotype and could be relevant to schizophrenia as a haplotype with rs3087454 (−1831 bp) or as single markers in some individuals.

Exclusion of the COS subjects in the Caucasian case-control sample resulted in a more significant *P*-value for both allelic and genotypic models of association with schizophrenia. It is, therefore, likely that the rs3087454 “risk” allele is more relevant to the etiology of adult onset schizophrenia.

Results of this study continue to support the $\alpha 7$ nicotinic receptor gene (*CHRNA7*) as a candidate gene for schizophrenia. Future studies to investigate the *CHRNA7* gene will attempt to capture all genetic variance in the linkage peak region by fine mapping with a combination of SNPs and microsatellite markers. Such studies could uncover a causal SNP, correlated with rs3087454, and reveal whether variance in the candidate gene *CHRNA7*, its upstream regulatory regions, or a relevant SNP in the partial duplication (*CHRFAM7A*) is associated with schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Role of funding source

This research was supported by National Institutes of Health grants DA09457 and MH081177 (SL), MH068582 (RF), and the Veterans Affairs Medical Research Service (SL, RF).

We acknowledge the valuable contributions of clinical information and blood by all the participants in the genetic study. We would like to thank Sarah Bulman, Philippa Kirby, Bernadette Sullivan, and Shannon Straub for technical assistance.

References

- Adams CE, Stevens KE. Evidence for a role of nicotinic acetylcholine receptors in schizophrenia. *Front Biosci* 2007;12:4755–4772. [PubMed: 17485411]
- Adler LE, Hoffer LD, Wiser A, Freedman R. Normalization of auditory physiology by cigarette smoking in schizophrenic patients. *Am J Psych* 1993;150:1856–1861.
- Adler LE, Olincy A, Waldo MC, Harris JG, Griffith J, Stevens K, Flach K, Nagamoto H, Bickford P, Leonard S, Freedman R. Schizophrenia, sensory gating, and nicotinic receptors. *Schizophr Bull* 1998;24:189–202.
- Aramakis VB, Metherate R. Nicotine selectively enhances NMDA receptor-mediated synaptic transmission during postnatal development in sensory neocortex. *J Neurosci* 1998;18:8485–8495. [PubMed: 9763491]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265. [PubMed: 15297300]
- Berg DK, Conroy WG. Nicotinic alpha 7 receptors: synaptic options and downstream signaling in neurons. *J Neurobiol* 2002;53:512–523. [PubMed: 12436416]
- Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Res* 1978;38:3751–3757. [PubMed: 29704]
- Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, Leonard S. Effect of smoking history on [3H]nicotine binding in human postmortem brain. *J Pharmacol Exp Ther* 1997;282:7–13. [PubMed: 9223534]

- Breese CR, Lee MJ, Adams CE, Sullivan B, Logel J, Gillen KM, Marks MJ, Collins AC, Leonard S. Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. *Neuropsychopharmacology* 2000;23:351–364. [PubMed: 10989262]
- Chen WY, Shi YY, Zheng YL, Zhao XZ, Zhang GJ, Chen SQ, Yang PD, He L. Case-control study and transmission disequilibrium test provide consistent evidence for association between schizophrenia and genetic variation in the 22q11 gene ZDHC8. *Hum Mol Genet* 2004;13:2991–2995. [PubMed: 15489219]
- Coon H, Jensen S, Holik J, Hoff M, MylesWorsley M, Reimherr F, Wender P, Waldo M, Freedman R, Leppert M, Byerley W. Genomic scan for genes predisposing to schizophrenia. *Am J Med Genet* 1994;54:59–71. [PubMed: 7909992]
- Court J, Spurdin D, Lloyd S, McKeith I, Ballard C, Cairns N, Kerwin R, Perry R, Perry E. Neuronal nicotinic receptors in dementia with Lewy bodies and schizophrenia: alpha-bungarotoxin and nicotine binding in the thalamus. *J Neurochem* 1999;73:1590–1597. [PubMed: 10501205]
- Dajas-Bailador F, Wonnacott S. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends pharmacol Sci* 2004;25:317–324. [PubMed: 15165747]
- Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003;25:115–121. [PubMed: 12916020]
- Fallin MD, Lasseter VK, Wolyniec PS, McGrath JA, Nestadt G, Valle D, Liang KY, Pulver AE. Genomewide linkage scan for schizophrenia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 10q22. *Am J Hum Genet* 2003;73:601–611. [PubMed: 12929083]
- First, MB.; Gibbon, M.; Spitzer, RL.; Williams, JBW. Structured Clinical Interview for Axis I DSM-IV Disorders—Non-patient Edition—(SCID-I/NP, Version 2.0). Biometrics Research Department, New York State Psychiatric Institute; New York: 1996a.
- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for Axis I DSM-IV Disorders—Patient Edition (With Psychotic Screen)—(SCID-I/P, W/PSYCHOTIC SCREEN) (Version 2.0). Biometrics Research Department, New York State Psychiatric Institute; New York: 1996b.
- Fisher RA. Combining independent tests of significance. *Am Stat* 1948;2:30.
- Freedman R, Leonard S. Genetic linkage to schizophrenia at chromosome 15q14. *Am J Med Genet* 2001;105:655–657. [PubMed: 11803510]
- Freedman R, Waldo M, Bickford-Wimer P, Nagamoto H. Elementary neuronal dysfunctions in schizophrenia. *Schizophr Res* 1991;4:233–243. [PubMed: 1645590]
- Freedman R, Hall M, Adler LE, Leonard S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biol Psychiatry* 1995;38:22–33. [PubMed: 7548469]
- Freedman R, Coon H, MylesWorsley M, OrrUrtreger A, Olincy A, Davis A, Polymeropoulos M, Holik J, Hopkins J, Hoff M, Rosenthal J, Waldo MC, Reimherr F, Wender P, Yaw J, Young DA, Breese CR, Adams C, Patterson D, Adler LE, Kruglyak L, Leonard S, Byerley W. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A* 1997;94:587–592. [PubMed: 9012828]
- Freedman R, Leonard S, Gault JM, Hopkins J, Cloninger CR, Kaufmann CA, Tsuang MT, Farone SV, Malaspina D, Svrakic DM, Sanders A, Gejman P. Linkage disequilibrium for schizophrenia at the chromosome 15q13–14 locus of the alpha 7-nicotinic acetylcholine receptor subunit gene (CHRNA7). *Am J Med Genet* 2001;105:20–22. [PubMed: 11424985]
- Freedman R, Olincy A, Buchanan RW, Harris JG, Gold JM, Johnson L, Allensworth D, Guzman-Bonilla A, Clement B, Ball MP, Kutnick J, Pender V, Martin LF, Stevens KE, Wagner BD, Zerbe GO, Soti F, Kem WR. Initial phase 2 trial of a nicotinic agonist in schizophrenia. *Am J Psychiatry* appi. 2008
- Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science* 2002;296:2225–2229. [PubMed: 12029063]
- Gauderman WJ. Sample size requirements for association studies of gene–gene interaction. *Am J Epidemiol* 2002a;155:478–484. [PubMed: 11867360]

- Gauderman WJ. Sample size requirements for matched case-control studies of gene–environment interaction. *Stat Med* 2002b;21:35–50. [PubMed: 11782049]
- Gault J, Robinson M, Berger R, Drebing C, Logel J, Hopkins J, Moore T, Jacobs S, Meriwether J, Choi MJ, Kim EJ, Walton K, Buiting K, Davis A, Breese CR, Freedman R, Leonard S. Genomic organization and partial duplication of the human $\alpha 7$ neuronal nicotinic acetylcholine receptor gene. *Genomics* 1998;52:173–185. [PubMed: 9782083]
- Gault J, Hopkins J, Berger R, Drebing C, Logel J, Walton K, Short M, Vianzon R, Olincy A, Ross RG, Adler LE, Freedman R, Leonard S. Comparison of polymorphisms in the $\alpha 7$ nicotinic receptor gene and its partial duplication in schizophrenic and control subjects. *Am J Med Genet* 2003;123B:39–49. [PubMed: 14582144]
- Gejman PV, Sanders AR, Badner JA, Cao QH, Zhang J. Linkage analysis of schizophrenia to chromosome 15. *Am J Med Genet* 2001;105:789–793. [PubMed: 11803532]
- George TP, Termine A, Sacco KA, Allen TM, Reutenauer E, Vessicchio JC, Duncan EJ. A preliminary study of the effects of cigarette smoking on prepulse inhibition in schizophrenia: involvement of nicotinic receptor mechanisms. *Schizophr Res* 2006;87:307–315. [PubMed: 16854565]
- Glatt SJ, Faraone SV, Tsuang MT. Association between a functional catechol *O*-methyltransferase gene polymorphism and schizophrenia: meta-analysis of case-control and family-based studies. *Am J Psychiatry* 2003;160:469–476. [PubMed: 12611827]
- Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends pharmacol Sci* 2006;27:482–491. [PubMed: 16876883]
- Guan ZZ, Zhang X, Blennow K, Nordberg A. Decreased protein level of nicotinic receptor $\alpha 7$ subunit in the frontal cortex from schizophrenic brain. *Neuroreport* 1999;10:1779–1782. [PubMed: 10501574]
- Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry* 2005;10:40–68. [PubMed: 15263907]
- Hodgkinson CA, Yuan QP, Xu K, Shen PH, Heinz E, Lobos EA, Binder EB, Cubells J, Ehlers CL, Gelernter J, Mann J, Riley B, Roy A, Tabakoff B, Todd RD, Zhou ZF, Goldman D. Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol Alcohol* 2008;43:505–515. [PubMed: 18477577]
- Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, Williamson D, Ryan N. Schedule for affective disorders and schizophrenia for school-age children present and lifetime version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psych* 1997;36:980–988.
- Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD, Meyer J, Zambuto CT, Schmitt K, Matise TC, Harkavy-Friedman JM, Hampe C, Lee H, Shore D, Wynne D, Faraone SV, Tsuang MT, Cloninger CR. NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. *Am J Med Genet* 1998;81:282–289. [PubMed: 9674972]
- Kuehn BM. Link between smoking and mental illness may lead to treatments. *JAMA, J Am Med Assoc* 2006;295:483–484.
- Kumari V, Postma P. Nicotine use in schizophrenia: the self medication hypotheses. *Neurosci Biobehav Rev* 2005;29:1021–+. [PubMed: 15964073]
- Leonard S. Consequences of low levels of nicotinic acetylcholine receptors in schizophrenia for drug development. *Drug Dev Res* 2003;60:127–136.
- Leonard S, Bertrand D. Neuronal nicotinic receptors: from structure to function. *Nicotine Tob Res* 2001;3:203–223. [PubMed: 11506765]
- Leonard S, Gault J, Moore T, Hopkins J, Robinson M, Olincy A, Adler LE, Cloninger CR, Kaufmann CA, Tsuang MT, Faraone SV, Malaspina D, Svrakic DM, Freedman R. Further investigation of a chromosome 15 locus in schizophrenia: analysis of affected sibpairs from the NIMH Genetics Initiative. *Am J Med Genet* 1998;81:308–312. [PubMed: 9674976]
- Leonard S, Adler LE, Benhammou K, Berger R, Breese CR, Drebing C, Gault J, Lee MJ, Logel J, Olincy A, Ross RG, Stevens K, Sullivan B, Vianzon R, Vernich DE, Waldo M, Walton K, Freedman R. Smoking and mental illness. *Pharmacol Biochem Behav* 2001;70:561–570. [PubMed: 11796154]
- Leonard S, Gault J, Hopkins J, Logel J, Vianzon R, Short M, Drebing C, Berger R, Venn D, Sirota P, Zerbe G, Olincy A, Ross RG, Adler LE, Freedman R. Association of promoter variants in the $\alpha 7$

- 7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Arch Gen Psychiatry* 2002;59:1085–1096. [PubMed: 12470124]
- Leonard S, Mexal S, Berger R, Olincy A, Freedman R. Smoking and schizophrenia: evidence for self medication. *Schizophr Bull* 2007a;33:262–263.
- Leonard S, Mexal S, Freedman R. Genetics of smoking and schizophrenia. *J Dual Diag* 2007b;3:43–59.
- Levin ED, Conners CK, Silva D, Hinton SC, Meck WH, March J, Rose JE. Transdermal nicotine effects on attention. *Psychopharmacology* 1998;140:135–141. [PubMed: 9860103]
- Levin ED, McClernon FJ, Rezvani AH. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology* 2006;184:523–539. [PubMed: 16220335]
- Light GA, Braff DL. Sensory gating deficits in schizophrenia: can we parse the effects of medication, nicotine use, and changes in clinical status? *Clin Neurosci Res* 2003;3:47–54.
- Lin PI, Vance JM, Pericak-Vance MA, Martin ER. No gene is an island: the flip-flop phenomenon. *Am J Hum Genet* 2007;80:531–538. [PubMed: 17273975]
- Liu CM, Hwu HG, Lin MW, Ou-Yang WC, Lee SFC, Fann CSJ, Wong SH, Hsieh SH. Suggestive evidence for linkage of schizophrenia to markers at chromosome 15q13–14 in Taiwanese families. *Am J Med Genet* 2001;105:658–661. [PubMed: 11803511]
- Martin LF, Freedman R. Schizophrenia and the alpha7 nicotinic acetylcholine receptor. *Int Rev Neurobiol* 2007;78:225–246. [PubMed: 17349863]
- Martin LF, Kem WR, Freedman R. Alpha-7 nicotinic receptor agonists: potential new candidates for the treatment of schizophrenia. *Psychopharmacology* 2004;174:54–64. [PubMed: 15205879]
- Marutle A, Zhang X, Court J, Piggott M, Johnson M, Perry R, Perry E, Nordberg A. Laminar distribution of nicotinic receptor subtypes in cortical regions in schizophrenia. *J Chem Neuroanat* 2001;22:115–126. [PubMed: 11470559]
- McClellan JM, Susser E, King MC. Schizophrenia: a common disease caused by multiple rare alleles. *Br J Psychiatry* 2007;190:194–199. [PubMed: 17329737]
- Mexal S, Frank M, Berger R, Adams CE, Ross RG, Freedman R, Leonard S. Differential modulation of gene expression in the NMDA postsynaptic density of schizophrenic and control smokers. *Mol Brain Res* 2005;139:317–332. [PubMed: 16122832]
- Mexal S, Berger R, Pearce L, Barton A, Logel J, Adams CE, Ross RG, Freedman R, Leonard S. Regulation of a novel alpha N-catenin splice variant in schizophrenic smokers. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:759–768. [PubMed: 18163523]
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215. [PubMed: 3344216]
- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998;63:259–266. [PubMed: 9634505]
- Olincy A, Ross RG, Young DA, Roath M, Freedman R. Improvement in smooth pursuit eye movements after cigarette smoking in schizophrenic patients. *Neuropsychopharmacology* 1998;18:175–185. [PubMed: 9471115]
- Olincy A, Harris JG, Johnson LL, Pender V, Kongs S, Allensworth D, Ellis J, Zerbe GO, Leonard S, Stevens KE, Stevens JO, Martin L, Adler LE, Soti F, Kem WR, Freedman R. Proof-of-concept trial of an alpha 7 nicotinic agonist in schizophrenia. *Arch Gen Psychiatry* 2006;63:630–638. [PubMed: 16754836]
- Park TW, Yoon KS, Kim JH, Park WY, Hirvonen A, Kang D. Functional catechol-O-methyltransferase gene polymorphism and susceptibility to schizophrenia. *Eur Neuropsychopharmacol* 2002;12:299–303. [PubMed: 12126868]
- Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet* 1999;65:220–228. [PubMed: 10364535]
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;155:945–959. [PubMed: 10835412]
- Psychiatric, News. Molecular Targets Ranked. *Psych News* 2006;41:16–1a.
- Rezvani AH, Levin ED. Cognitive effects of nicotine. *Biol Psychiatry* 2001;49:258–267. [PubMed: 11230877]

- Riley BP, Makoff AM, Magudi-Carter M, Jenkins TJ, Williamson R, Collier DA, Murray RM. Haplotype transmission disequilibrium and evidence for linkage of the CHRNA7 gene region to schizophrenia in Southern African Bantu families. *Am J Med Genet* 2000;96:196–201. [PubMed: 10893497]
- Riley B, Williamson M, Collier D, Wilkie H, Makoff A. A 3-Mb map of a large segmental duplication overlapping the alpha 7-nicotinic acetylcholine receptor gene (CHRNA7) at human 15q13–q14. *Genomics* 2002;79:197–209. [PubMed: 11829490]
- Sanders AR, Rusu I, Duan J, Vander Molen JE, Hou C, Schwab SG, Wildenauer DB, Martinez M, Gejman PV. Haplotypic association spanning the 22q11.21 genes COMT and ARVCF with schizophrenia. *Mol Psychiatry* 2005;10:353–365. [PubMed: 15340358]
- Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 2003;302:841. [PubMed: 14593171]
- Tan EK, Tan C, Shen H, Chai A, Lum SY, Teoh ML, Yih Y, Wong MC, Zhao Y. Alpha synuclein promoter and risk of Parkinson's disease: microsatellite and allelic size variability. *Neurosci Lett* 2003;336:70–72. [PubMed: 12493604]
- Tsuang DW, Skol AD, Faraone SV, Bingham S, Young KA, Prabhudesai S, Haverstock SL, Mena F, Menon AS, Bisset D, Pepple J, Sauter F, Baldwin C, Weiss D, Collins J, Boehnke M, Schellenberg GD, Tsuang MT. Examination of genetic linkage of chromosome 15 to schizophrenia in a large veterans affairs cooperative study sample. *Am J Med Genet* 2001;105:662–668. [PubMed: 11803512]
- Vijayaraghavan S, Pugh PC, Zhang ZW, Rathouz MM, Berg DK. Nicotinic receptors that bind a-bungarotoxin on neurons raise intracellular free Ca⁺⁺ *Neuron* 1992;8:353–362. [PubMed: 1310863]
- Wonodi I, Stine OC, Mitchell BD, Buchanan RW, Thaker GK. Association between Val108/158 Met polymorphism of the COMT gene and schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2003;120:47–50. [PubMed: 12815739]
- Xu JZ, Pato MT, Dalla Torre C, Medeiros H, Carvalho C, Basile VS, Bauer A, Dourado A, Valente J, Soares MJ, Macedo AA, Coelho I, Ferreira CP, Azevedo MH, Macciardi F, Kennedy JL, Pato CN. Evidence for linkage disequilibrium between the alpha 7-nicotinic receptor gene (CHRNA7) locus and schizophrenia in Azorean families. *Am J Med Genet* 2001;105:669–674. [PubMed: 11803513]

-2040 gtcctacatt gtaagctgag tgagtggact gcgcttgacg gtcctccagg tgcttagcga
 -1980 gaacagagga caaataaata ttacgaatt gcttgctca cctgaaatg gtttatttct
 -1920 aggtttctga tattatggg tgcaatggcg gtaagaagc agttctggt tcaggaaagt
 -1860 gatcctgata gccatactcc agaaaaatc aataaattcc cttggcccca tgggctcatg
 -1800 ctcttctaga aggaagaca gggctcttag gtactttcag cgctcgtaga agagtgtgt
 -1740 tacagtccca tgaccagtgc aggggatgtg cactgagaa tcttttact gatgctcat
 -1680 gggctttctc tattctgcta ctgggtttta ttcccttct tctaattctc ctttaacca
 -1620 caactaatcc cctgtagata attaatcat caatgcctg ctctgtgatg tccggactgc
 -1560 tagaagtgt aggggactc aagagccaga tgaagctaa ggcacgctg tctgctctcc
 -1500 agggaccctc ggcgtccctt tctcctggca gaatgactgc tatccttga ggtgaatcca
 -1440 gttcagctgt cactcttctt attaaccact ctccaaaac agctaactct tcttctagc
 -1380 tcttaccgca gttatgaaag cctatgctga cctttgttt aaacatgtgt acattaacag
 -1320 taatacatctt aagacacttc atggcaaggg caataactg cgttattctt ccaaatcaaa
 -1260 tagttgggt cagtcctcca ttctgctac tgggttacag tcaagctcag tcacctttt
 -1200 gtgagcctt cctagtctt tggagtctta aaagaatccc gtggtttctg gcagttcaga
 -1140 aaccagcca ttgcgctgc gtgtccacg ggagttgctc tggtgagct cggatgccg
 -1080 ggggtgcag gaaagaaggt ggcagcggc cctacggga cgcaggggc tgcgtgctc
 -1020 agcagaagg agcaaatgg atggagctc agccacctg gaagcggcc cttggcgct
 -960 tcctccctc cttctcttt ccaaaatcaa cccctctt caacatcaag aactcccg
 -900 actccctgga cctctcagag cctctctca ttactctt ccaatgcgt ggtcaaaag
 -840 agcctagata agaaccacaa gttctgctg tcctccagc aaagagttag gagttaact
 -780 ttcaatctt tttaatctc tttaaaaag aatgagccat acattagggt aaccactggg
 -720 aatcccatca cacacattg cggcatctc cctcccgac aggggtgctc cagcacttca
 -660 gateccagc gagatctg ctgctggcg ccagcaaac gtgcggaaag caaacgggg
 -600 ctgcggaaa gcgggaggag gggggtctc tcgggtctgt ttgtctggt tggcaagact
 -540 tccgaagcct ggttccctat actgccacc cgtcgtctg cgtggaggag ggagtcggg
 -480 aagactggac ccagaattg tcccgctt cctccgagtg ccagcgcag cttctggctg
 -420 agagcgggag cgggtgagt ggggacaaag aacgcaagg agaggtagag cctggcctg
 -360 ggcagccctc ggctgcca gaggcgcga gccgagagc cgtcagtag agactgggg
 -300 tggaggtgcc cggagcgtac ccagcgcgg gactacctc cgtcacacc tcgggctcga
 -240 gttccctggg tggcgccga gacgtggc cgggtggaq gqatgaggg qcggggacg
 -180 gggcggggc gggctcgtc acgtggagag cgcgcggag cggggcggg cggggcgcg
 -120 cgcccgctc cttaaaggc cgcgagcga cggcgaggt gctctgtgg ccgagggcg
 -60 agggccggc gacagccga acgtggagc gcgggctc ctgcagctc gggactcaac
 +1 ATG

Fig. 1.

Polymorphisms identified in the *CHRNA7* proximal promoter and 5'-upstream regulatory region. Polymorphisms identified are in red. The polymorphism associated with schizophrenia in the current study is highlighted in yellow. Transcription factor binding sites are indicated by blue bars.

Fig. 2. Haploview graph of the pairwise linkage disequilibrium (LD) between SNPs in the proximal promoter of *CHRNA7*, and SNPs in the upstream regulatory region of the gene. A) African-American sample population and B) Caucasian sample population. Numbers inside the squares are $D' \times 100$.

Table 1

Table 1a. Number of individuals for case-control and family-based association studies with the <i>a priori</i> 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	

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		

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

Sm=smokers, NS=non-smokers.

Table 2

Amplicons utilized in the current study.

Products	Polymorphisms detected	Size (bp)	Primers	Melting temp	Thermal profile
Amplicon 1	-46,-86,-92,-178 -190,-191,-194,-241	271	5' AGTACCTCCCGCTCACACCTCG 3' 3' ATGTTGAGTCCCGAGCTGCAG 5'	70.2 °C	95 °C 3 min, (95 °C 30 s, 56 °C 30 s, 72 °C 1 min) ×38 cycles, hold 4 °C
Amplicon 3	-316,-322	195	5' CGGGAGCGGGCTGGAGTGGGGACAAA 3' 5' CGGCGGCCACCCAGGGAACTGC 3'	65.7 °C	95 °C 3 min, (94 °C 30 s, 70 °C 1 min) × 5 cycles (94 °C 30 s, 65 °C 1 min) × 5 cycles (94 °C 20 s, 66 °C 1 min) × 25 cycles, 68 °C 3 min, hold 4 °C
Amplicon 4	-322,-518	373	5' CAGACCTTCAGATCCCGAGCGGAGAG 3' 5' CCACCCCAAGTCTCCACCGA 3'	64.4 °C	95 °C 3 min, (94 °C 30 s, 68 °C 1 min) × 5 cycles (94 °C 30 s, 66 °C 1 min) × 5 cycles (94 °C 20 s, 64 °C 1 min) × 25 cycles, 68 °C 3 min, hold 4 °C
Amplicon 5	-653,-704	215	5' GAATGAGCATACATTAGGTAACCC 3' 5' CGGAAGTCTTGCCACCGAGACAA 3'	61.7 °C	95 °C 10 min, [94 °C 20 s, 58 °C (-0.5 °C to 55 °C) 1 min, 72 °C 1 min] ×14 cycles (94 °C 20 s, 51 °C 1 min, 72 °C 1 min) ×20 cycles, 72 °C 10 min, hold 4 °C
Amplicon 6	-768,-813	178	5' TCCTTTCCAAATGGGCTGGCTCAAAAAG 3' 5' AGGAGAGATGCCGCCCAATGTGTG 3'	58.1 °C	95 °C 2 min, [95 °C 30 s, 67 °C (-0.5 °C to 60 °C) 30 s, 72 °C 30 s] ×14 cycles (95 °C 30 s, 60 °C 30 s, 72 °C 30 s) ×19 cycles, 72 °C 5 min, hold 4 °C
Amplicon 7	-905,-929	209	5' GGAGCAATGGGATGGAGCTTCA 3' 5' GGAAGGACAGCCAGAAC TTGGTGT 3'	61.5 °C	95 °C 2 min, [95 °C 30 s, 67 °C (-0.5 °C to 60 °C) 30 s, 72 °C 30 s] × 14 cycles (95 °C 30 s, 60 °C 30 s, 72 °C 30 s) ×19 cycles, 72 °C 5 min, hold 4 °C
Amplicon 9	-1247,-1248,-1252 rs3826029(-1313) -1372,-1382,-1452	340	5' CTGTCTGCTCTCCAGGGAGCC 3' 3' GACTCCAAAGAACTAGGGAAGGC 5'	57.6 °C	95 °C 10 min, [94 °C 20 s, 62 °C (-0.5 °C to 55 °C) 1 min, 72 °C 1 min] ×15 cycles (94 °C 30 s, 55 °C 1 min, 72 °C 1 min) ×20 cycles, 72 °C 10 min, hold 4 °C
Amplicon 10	rs6494165(-1512) -1587	269	5' CTCCTTTTACCACACACTAATCCC 3' 3' CATAACTGCGGTAAAGAGCCTAGA 5'	61.6 °C	95 °C 2 min, [95 °C 30 s, 64.4 °C (-0.5 °C to 55 °C) 30 s, 72 °C 30 s] ×15 cycles (95 °C 30 s, 57.4 °C 30 s, 72 °C 30 s) ×20 cycles, 72 °C 5 min, hold 4 °C
Amplicon 11	rs3087454(-1831)	162	5' AAGCAGTTCTGTTTCAGGAATCTG 3' 3' ACTGGTATGGGACTGTAAACACAC 5'	56.6 °C	95 °C 10 min, [94 °C 20 s, 62 °C (-0.5 °C to 55 °C) 1 min, 72 °C 1 min] ×14 cycles (94 °C 20 s, 55 °C 1 min, 72 °C 1 min) ×20 cycles, 72 °C 10 min, hold 4 °C

Table 3Chromosomal location and minor allele frequencies for SNPs in the regulatory region of *CHRNA7*.

SNP marker	rs #	Chromosomal location (bp)	Minor allele frequency (Caucasian-Non-Hispanic)	Minor allele frequency (African-American)
-46 G/T		30110044	0.003	0.097
-80 G/A		30110010	0.003	0.0
-86 C/T		30110004	0.064	0.004
-92 G/A		30109998	0.006	0.004
-93 C/G		30109997	0.003	0.0
-178 -G		30109912	0.004	0.076
-190 +G		30109900	0.001	0.05
-191 G/A		30109899	0.001	0.0
-194 G/C		30109896	0.042	0.02
-241 A/G		30109849	0.001	0.0
-316 C/A		30109778	0.004	0.0
-518 C/T		30109306	0.0	0.017
-653 G/A		30109441	0.001	0.0
-704 C/A		30109390	0.007	0.0
-768 T/A		30109326	0.003	0.003
-813 C/A		30109281	0.0	0.001
-905 T/C		30109189	0.001	0.003
-929 C/G		30109165	0.0	0.002
-1247 T/A		30108843	0.001	0.0
-1248 G/C		30108842	0.001	0.0
-1252 C/A		30108838	0.0	0.053
-1313 C/T	rs3826029	30108777	0.205	0.436
-1382 G/A		30108708	0.001	0.0
-1452 G/C		30108638	0.001	0.0
-1512 T/G	rs6494165	30108578	0.059	0.081
-1831 C/A ^a	rs3087454	30108259	0.360	0.350

^aThe minor alleles are different in African-Americans and Caucasians at -1831 bp C/A (rs3087454). In African-Americans, C is the most common allele and A is the minor allele. In Caucasians, A is the most common allele and C is the minor allele.

Table 4
Case-control analyses under a genotypic model of association.

SNP	Schizophrenia vs No diagnosis		Smokers vs Non-smokers		SZ smokers vs SZ non-smokers	
	AA	Cauc	AA	Cauc	AA	Cauc
-46 bp	0.309	0.325	0.501	0.098	0.096	0.134
-86 bp	0.397	0.521	1	0.167	1	0.302
-178 bp	0.319	0.633	0.50	0.575	0.618	0.372
-190 bp	0.699	0.325	0.482	0.099	0.619	0.134
-1252 bp	0.639	1	0.564	1	0.601	1
rs3826029 (-1313 bp)	0.864	0.733	0.288	0.209	0.653	0.265
rs6494165 (-1512 bp)	0.134	0.638	0.228	0.505	0.937	0.229
rs3087454 (-1831 bp)	0.000017, 0.0009*	0.0005, 0.013*	0.349	0.204	0.06	0.734

AA=African-American, Cauc=Caucasian-Non Hispanic. Adjusted *P*-values are indicated with an (*).

Table 5
Case-control analyses under an allelic model of association.

SNP	Schizophrenia vs No diagnosis		Smokers vs Non-smokers		SZ smokers vs SZ non-smokers	
	AA	Cauc	AA	Cauc	AA	Cauc
-46 bp	0.331	0.326	0.519	0.099	0.104	0.135
-86 bp	0.398	0.411	1	0.798	1	0.389
-178 bp	0.139	0.634	0.521	0.576	0.595	0.373
-190 bp	0.695	0.326	0.342	0.69	0.610	0.135
-1252 bp	0.648	1	0.573	1	0.610	1
rs3826029 (-1313 bp)	0.592	0.652	0.120	0.992	0.410	0.724
rs6494165 (-1512 bp)	0.149	0.445	0.247	0.817	0.940	0.824
rs3087454 (-1831 bp)	0.005, 0.198*	0.005, 0.135*	0.952	0.649	0.083	0.958

AA=African American, Cauc=Caucasian non-Hispanic. Adjusted *P*-values are indicated with an (*).

Table 6

Combined *P*-values at SNP rs3087454 for case-control analyses using Fisher's method with the outcome of schizophrenia versus controls.

Polymorphism	Model of association	Sample	<i>P</i> ^a
rs3087454 C/A (-1831 bp)	Genotypic	Controls	–
		SZ	0.0001
	Allelic	Controls	–
		SZ	0.124

^a*P*-values were combined with the formula:

$$X_{2k}^2 = -2 \sum_{i=1}^k \log_e(pi).$$

Where *k*=degrees of freedom, and *p_i* = *P*-value.

Table 7

Comparison of the number of individuals positive for both 5' polymorphisms AND the schizophrenia "risk" genotype at rs3087454 against those with only 5' polymorphisms and without the "risk" genotype in the case-control sample.

Ethnicity	Caucasian			African-American		
	C/C (N=43)	A/C (N=193)	A/A (N=153)	A/A (N=7)	C/A (N=55)	C/C (N=41)
Genotype at rs3087454 (-1831)						
CHRNA7 5' Polymorphisms						
-46 G/T	1	1	0	0	8	11
-86 C/T	2	20	24	0	0	0
-92 G/A	0	2	2	0	0	0
-178 -G	1	1	1	2	6	6
-190 +G	0	1	0	1	8	0
-191 G/A	0	1	0	0	1	5
-194 G/C	10	22	0	0	2	1
-241 A/G	0	2	0	0	0	0
-316 G/A	0	1	3	0	0	0
-518 C/T	0	0	0	0	2	0
-653 G/A	0	1	0	0	0	0
-704 C/A	0	3	0	0	0	0
-768 T/A	0	1	0	0	1	2
-813 C/T	0	0	0	0	1	0
-905 T/C	0	1	0	0	1	3
-929 C/G	0	0	0	0	1	3
-1247 T/A	0	1	0	0	0	0
-1252 C/A	0	0	0	0	3	5
rs3826029 C/T (-1313)	29	118	0	2	24	36
-1382 G/A	0	1	0	0	0	0
-1452 G/C	0	0	1	0	0	0
rs6494165 T/G (-1512)	11	30	0	0	3	9

The CC genotype is associated with schizophrenia in the Caucasian sample population and the AA genotype is associated with schizophrenia in the African-American sample population.