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Comparison of Polymorphisms in the α7 Nicotinic Receptor Gene and Its Partial Duplication in Schizophrenic and Control Subjects

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The hypothesis that the 15q13-15 region of chromosome 15 contains a gene that contributes to the etiology of schizophrenia is supported by multiple genetic linkage studies. The α7 neuronal nicotinic acetylcholine receptor (*CHRNA7*) gene was selected as the best candidate gene in this region for molecular investigation, based on these linkage findings and biological evidence in both human and rodent models. CHRNA7 receptors are decreased in expression in postmortem brain of schizophrenic subjects. A dinucleotide marker, D15S1360, in intron two of the *CHRNA7* gene is genetically linked to an auditory gating deficit found in schizophrenics and half of the first-degree relatives of patients. Single strand conformation polymorphism (SSCP) and sequence analyses of DNA from schizophrenic and control individuals identified 33 variants in the coding region and intron/exon borders of the *CHRNA7* gene and its partial duplication, *dupCHRNA7*; common polymorphisms were mapped. Twenty-one variants were found in the exons, but non-synonymous changes were rare. Although the expression of *CHRNA7* is decreased in schizophrenia, the general structure of the remaining receptors is likely to be normal. © 2003 Wiley-Liss, Inc.

KEY WORDS: schizophrenia; mutation; promoter; nicotinic receptor; sensory processing; auditory gating

INTRODUCTION

A significant genetic influence in the common mental illness, schizophrenia, is indicated from twin studies, which show a higher pair wise concordance rate for schizophrenia in identical twins (28%) than in fraternal twins (6%) [Torrey, 1992], and also from adoption studies [Kety et al., 1994]. The disease is thought to be oligogenic where the etiology may involve a subset of genes predisposing to the illness [Gershon, 2000; Freedman et al., 2001b]. Indeed, Online Mendelian Inheritance in Man (OMIM™) now lists 11 replicated genetic linkage sites, one of which is 15q14-q15. Our laboratory studies the expression, function, and molecular structure of a candidate gene in the region, the α7 nicotinic acetylcholine receptor subunit gene, *CHRNA7*.

Analysis of an auditory evoked potential deficit (P50) in nine large families with schizophrenic members resulted in the identification of genetic linkage of the auditory gating deficit to the 15q13-q14 region (lod score of 5.3, $\theta = 0.00$) [Freedman et al., 1997]. In the families described in the original linkage report, there were many siblings unaffected with schizophrenia that appeared to have the P50 auditory gating deficit, suggesting an autosomal dominant pattern of inheritance. *CHRNA7* maps to the 15q13-q14 region and was selected as the best candidate gene for the gating deficit found in individuals with schizophrenia. Pharmacological characterization of the P50 auditory gating deficit shows that nicotine transiently normalizes the deficit in human subjects [Adler et al., 1992, 1993]. It has been

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suggested that use of tobacco products may be an attempt by schizophrenics to self-medicate [Adler et al., 1998]. The incidence of smoking in the mentally ill is approximately 40%, but in schizophrenia it is much higher, approaching 80% [Leonard and Bertrand, 2001]. The candidate gene *CHRNA7* modulates auditory evoked responses in an auditory gating pathway in the rat, equivalent to the P50 deficit in man [Adler et al., 1992; Luntz-Leybman et al., 1992; Rollins et al., 1993]. A mouse model of the auditory gating deficit is also consistent with a role for the CHRNA7 receptor. DBA/2j mice have reduced expression of the CHRNA7 receptor and exhibit a gating deficit compared to the C3H mouse strain, which has normal levels of receptor expression [Stevens et al., 1996]. Further, the deficit in the DBA/2j strain is normalized by GTS-21, a specific agonist for the α7 nicotinic receptor [Stevens et al., 1998]. Binding studies with CHRNA7 receptor antagonists suggest that CHRNA7 is found at reduced levels in postmortem hippocampus of schizophrenic subjects compared to controls with a history of mental illness [Freedman et al., 1995], as well as in the reticular nucleus of the thalamus [Court et al., 1999], and in cortex [Guan et al., 1999]. Functional variants in the proximal promoter region of the *CHRNA7* gene have recently been found that are consistent with reduced expression of the receptor in schizophrenics and that are associated with both the P50 deficit and with the disease [Leonard et al., 2002].

These results suggest that the α7 nicotinic receptor gene *CHRNA7*, mapping in the replicated linkage region on 15q13-q14, represents an excellent candidate gene for investigation of other mutations in the coding region and introns that might also be associated with the biological findings. The human α7 neuronal nicotinic acetylcholine receptor functions as a ligand-gated ion channel that binds nicotine with low affinity and can function as an homomeric ion channel in vitro [Leonard and Bertrand, 2001]. Nicotinic receptors assemble as pentamers [Bertrand and Changeux, 1992]. The subunit peptides are thought to cross the plasma membrane four times, with the second trans-membrane domain forming the walls of the ionic pore. Characterization of the genomic structure of the human *CHRNA7* gene led to the discovery of its partial duplication [Gault et al., 1998]. Exons 5–10 and the intervening introns are duplicated and map approximately 1.5 Mb proximal to the full-length *CHRNA7* gene. Five non-α7 exons (D, D′, C, B, and A) are transcribed with the partially duplicated α7 sequences in human brain and immortalized lymphocytes. These novel exons are part of a gene of unknown function, the primordial copy of which is on chromosome 3. The gene on chromosome 3 was partially duplicated several times on chromosome 15 and then was interrupted by the partial duplication of the *CHRNA7* gene [Riley et al., 2002]. Function of the partially duplicated α7 gene (*dupCHRNA7*) is being investigated. In order to identify variants that might disrupt the function of the *CHRNA7* gene cluster, the coding regions and intron/exon splice junctions were screened in genomic DNA isolated from schizophrenic and control individuals.

MATERIALS AND METHODS

Subjects Used for Mutation Screening

Samples from 171 families with schizophrenic members and 185 samples from controls were available for screening. The sample population included 86 families from the NIMH Schizophrenia Genetics Initiative; 16 of these families had been used in a sib pair analysis showing greater than 50% inheritance-by-descent to a dinucleotide marker D15S1360 in the *CHRNA7* gene (0.58; $P < 0.0024$) [Leonard et al., 1998]. Nine probands from the P50 linkage analysis [Freedman et al., 1997] were also included and the remaining samples were collected in the Denver Schizophrenia Center. When postmortem brain samples were used, diagnosis was based upon review of medical records and family and physician interviews. Of the controls, 166 were interviewed and found to have no evidence for current or past psychosis, using a Structured Clinical Interview for DSM-IV Axis I Disorders-Non-Patient Edition (SCID-I/NP, Version 2.0) [First et al., 1996]. They also received a Family History-Research Diagnostic Criteria interview (FH-RDC, third edition) [Endicott et al., 1978]. Auditory evoked potentials were recorded on controls, using published methods [Freedman et al., 1991].

Population Demographics

Caucasians accounted for approximately 65% of the samples from individuals with schizophrenia and 61% of the controls, and African Americans approximately 31% of the schizophrenic sample and 34% of the controls. Samples from Hispanics were 4% of samples from individuals with schizophrenia and 5% of controls.

Mutation Analysis

All schizophrenic subjects in each family were screened for polymorphisms to detect the possible presence of different variants in related individuals. Initially, a strategy was used to screen genomic DNA from 96 samples from individuals where postmortem brain tissue or lymphoblasts were available. This was done as mRNA would be needed for the mapping of variants to either the full-length *CHRNA7* or its duplication (*dupα7*). In the initial gene mutation screen, all the exons, intron/exon boundaries, and the 3′ untranslated region (UT) were examined by means of single-strand conformation polymorphism (SSCP) analysis using the primers shown for exons 1–10 in Table I. Exon 10 and the 3′UT were divided into an additional eight overlapping PCR fragments of approximately 200 bp, designed from *CHRNA7* sequence (Genbank Accession No. U40583). For SSCP analysis, the primer sets were kinased using [γ-^{33}P] ATP with Promega T4 kinase, then used to amplify regions of the *CHRNA7* gene using the polymerase chain reaction (PCR). PCR was done using *Taq* Gold™ and GeneAmp® PCR System 9600 (Perkin–Elmer, Foster City, CA) with the following program: 95°C-3′; 95°C-30″, 58°C-30″, 72°C-30″ ×35;

TABLE I. PCR Primers for Amplification of the *CHRNA7* Gene

Products	Variants detected	Primers	T_A (°C)	SSCP (°C)
Exon 1	45, +82	S GCGGCGAGGTGCCTCTGT	60	25
		AS GGATCCCACGGAGGAGTGGAG		
Exon 2		S CCTGCCCGGGTCTTCTCTCCT	58	25
		AS AACTAGAGTGCCCCAGCCGAGCT		
Exon 3		S AACAACGCTCTCGACAGTCAGATC	58	25
		AS AAGATCTTGCAGCCCATGGGAG		
Exon 4	334	S GGAATTCTCTTTGGTTTTGCAC	58	6
		AS ACATATCCAGCATCTCTGTGA		
Exon 5	370	S TCATGCAGTCCTTTTCCTGTTTC	60	6
		AS CTCGCTTCAGTTTTCTAACATGG		
Exon 6		S GGAACTGCTGTGTATTTTCAGC	58	Both
		AS TTAAAGCTTGCCCAGGAATAGG		
Exon 7		S GCTTGTGTGTGGTATACACATTG	58	Both
		AS TCCAGAGCTGATCTCAGCAGAAG		
Exon 8	861	S GAGGAACCGCTGTGTGTTTAT	58	25
		AS CTGGGCACACTCTAACCCTAACC		
Exon 9		S TGTGACGTGCAGTGCCACAGGA	60	25
		AS AAACCCTAGGAGGAGCCTCCTT		
Exon 10		S GATCAGCCCGTTTCCGCCTCAG	58	Both
		AS CCGATGTACAGCAGGTTCCCGTTGC		
Exon 6[a]	497–498	S CAGTACCTGCCTCCAGG	58	25
		AS TCCAAGGACCAGCCTCCGTAAGA		
Exon 7[a]	654/690	S CTATGAGTGCTGCAAAGA	58	25
		AS CAGGGGATCAGCAGGTT		
Exon 7[a]	698/+21	S GCCGCAGGACACTCTAC	58	25
		AS TCCAGAGCTGATCTCAGCAGAAG		
Intron 7[a]	−11, −20, −29	S GCCCCTCGTTAGACAGAATTGAG	58	25
		AS CTGGGCACACTCTAACCCTAACC		
Exon 10[a]	1,044, 1,116	S GATCAGCCCGTTTCCGCCTCAG	58	25
		AS CCGATGTACAGCAGGTTCCCGTTGC		
Exon 10[a]	1,335	S TCCCGACCCCCGACTCT	58	6
		AS TGATGGTGAAGACCGAGAAGG		
Exon 10[a]	1,269, 1,354, 1,456	S TCCCGACCCCCGACTCT	58	25
		AS TGATGGTGAAGACCGAGAAGG		
Exon 10[a]	1,466	S CCTTCTCGGTCTTCACCATC	58	25
		AS GCCTCCACGAAGTTGGGAGC		
Exon 10[a]	1,487	S GGTCCGCTACATTGCCAA	58	25
		AS CCTTGCCCATCTGTGAGTT		
3′UT[a]	1,737, 1,837	S GTGTTGCTTACGGTTTCTT	58	25
		AS TTTCAGGTAGACCTTCATGCAGACA		
cDNA[b] 1–10		S TGCCCATCTGTGAGTTTTCCACATG	72–68	
		AS CGCTGCAGCTCCGGGACTCAACATG		
cDNA[b] D-10		S CTCGGTGCCCCTTGCCATTT	72–68	
		AS CCTTGCCCATCTGTGAGTTTTCCAC		

T_A, annealing temperature.
[a]Primers used to PCR specific variants.
[b]Primers used in primary RT-PCR for mapping.

72°C-3′ (see Table I for specific annealing temperatures). The products, amplified and analyzed separately, were denatured with loading dye (7.26 M urea, 60% formamide, 22 mM EDTA, 32 mM NaOH, 0.25% bromophenol blue, 0.25% xylene cyanol), and separated on GeneAmp detection gels (Perkin-Elmer) run at 25 and 6°C using a BioRad Power Pac 3000 with a temperature probe. Samples with unique SSCP patterns were sequenced and polymorphisms were correlated with the SSCP patterns. Identified variants were subsequently screened in additional genomic samples from controls, individuals with schizophrenia, and family members, using the appropriate primers and gel conditions (see Table I; the additional primer sets used to detect specific variants are indicated).

Variant Mapping

CHRNA7 exons 5–10 are duplicated and nearly homologous (>99%), complicating the mutation screen [Gault et al., 1998]. However, the duplicated exons are transcribed with different 5′ sequence and could be isolated as unique mRNA species. The cDNA primer sets, used to specifically amplify full-length cDNA from either *CHRNA7* or its duplication (*dupCHRNA7*), are listed as the last two entries in Table I. These cDNA templates were then used to map the variants in exons 5–10, using RT-PCR and subsequent SSCP and sequence analysis of the RT-PCR products.

Eighty-four samples from this mutation screening study were used for cDNA mapping of the eight common

variants. Immortalized cell lines were not available from the NIMH schizophrenia initiative samples and, thus, postmortem brain and immortalized lymphoblasts collected locally in the Denver Schizophrenia Center were utilized.

Immortalized lymphocytes were cultured 6 hr with 1 mg/ml cyclohexamide before RNA isolation. Total RNA was isolated from postmortem human hippocampus or cyclohexamide treated immortalized lymphocytes, using TRIzol reagent (Life Technologies, Gibco-BRL). RNA was reverse transcribed (500 ng) using Superscript II reverse transcriptase components (Gibco-BRL) with 8 μM random hexamers (Pharmacia & Upjohn Diagnostics, Kalamazoo, MI), and 0.5 U/μl placental RNase inhibitor (Boehringer-Mannheim, Indianapolis, IN). A primary PCR was performed using specific primers designed with Oligo software 4.1 (National Biosciences, Inc., Plymouth MN) (Table I). Full-length *CHRNA7* transcripts were amplified using 1 M GC-melt and 10× cDNA buffer (Clontech, K1905-1) from the Advantage cDNA PCR kit (CLONTECH, Laboratories, Inc., Palo Alto, CA) and a two-step program with annealing temperatures from 72 to 68°C. Partially duplicated *dupCHRNA7* transcripts were amplified using 1 M GC melt and 5× cDNA buffer from the Advantage-GC cDNA PCR kit (K1907-1). These primary reactions were then analyzed using SSCP and sequence analysis.

Statistical Analysis

Chi square statistics or Fisher's exact tests were used to determine whether a variant was found more frequently in the schizophrenic sample than the control sample. Allele frequencies were calculated for variants in exons 1–4, but could not be determined for polymorphisms in the duplicated exons. A case-control study was done. All schizophrenic subjects in each family were screened for polymorphisms to determine if variants cosegregate with affected family members and to ensure no mutations were missed. Total counts from schizophrenic individuals include one schizophrenic individual from each family unless other schizophrenic family members differed from the proband at that nucleotide position. When this occurred, the other family members were counted, as well. The sample size provided sufficient power to detect a 0.11 difference in allele frequency between the schizophrenic and control groups at a $P < 0.05$ for an allele with population frequency 0.050.

Two population-specific loci, FY-null and RB2300, were used to estimate the degree of admixture in African American samples of schizophrenic individuals and controls [Parra et al., 1998]. The FY-NULL*1 allele is the normal allele with a C at −46 in the promoter of the DARC gene (Duffy antigen receptor of chemokines). The FY NULL*1 allele has an allele frequency of 1.0 in European populations, 0 in African populations, and 0.06–0.2 in African American populations [Parra et al., 1998]. FY-NULL*1 allele frequencies did not vary significantly between our African American controls (0.2) and schizophrenic individuals (0.18). The RB2300*1 allele has an allele frequency of 0.900–0.944 in African populations, 0.776–0.888 in African American populations, and 0.287–0.588 in European populations [Parra et al., 1998]. The RB2300*1 allele does not have a BamHI polymorphism in intron 1 of the human retinoblastoma gene. The RB2300*1 allele was found at a frequency of 0.82 in our African American controls and 0.86 in the African American subjects with schizophrenia; this was not significantly different. These data suggest that there is a similar degree of admixture in our African American control and schizophrenia samples and that differences in variant frequencies between these samples should not reflect ethnic bias.

RESULTS

Mutation analysis of the α7 nicotinic receptor gene *CHRNA7* and its partial duplication *dupCHRNA7* was carried out using SSCP, and sequence analyses. Figure 1a depicts the 15q13-q14 region containing *CHRNA7* and *dupCHRNA7*. The unique dinucleotide marker D15S1360, used in several linkage studies [Freedman et al., 1997, 2001a,b; Leonard et al., 1998], lies in intron two of *CHRNA7* [Leonard et al., 2002]. D15S1031 and D15S144, also single copy, flank the full-length *CHRNA7* gene and duplicated cassette (duplicon). Unique loci D15S1043 and D15S165 flank the proximal duplicon. The duplicon contains exons 5–10 of the *CHRNA7* gene, the dinucleotide repeat L76630, exons D′–D–C–B–A, and the expressed sequence tag (EST) WI13983. The transcripts from both α7 containing genes are shown with their unique 5′ ends and the number of variants mapped to each exon (Fig. 1b). The orientation of the duplicon is shown as head to tail, determined from yeast artificial chromosome (YAC) mapping from two separate YAC libraries [Gault et al., 1998]. A head to head orientation has been reported based on BAC clone mapping from a single library [Riley et al., 2002], suggesting that the orientation of this duplicon may be polymorphic.

Thirty-three variants in the *CHRNA7* gene cluster were identified in genomic DNA from individuals with schizophrenia and controls of Caucasian, African American and Hispanic descent (Table II). Twenty-one different variants were found in the coding region of the α7 genes, including ten non-synonymous variants. Base pair numbering is from the first base pair in exon 1.

Allele frequencies for 14 of the rare variants were calculated and are shown in Table III. Allele frequencies for the more common variants could not be determined because they could be homozygous in either *dupCHRNA7* or full-length *CHRNA7* genes.

Six variants were found more frequently in the African Americans than the Caucasians (Table IV). Three variants at 497–498 (2 bp deletion), 654, and 1,466 bp were found more frequently in Caucasians than in African Americans. Two rare, but non-synonymous variants in exon 5 at 370 bp, and in exon 7 at 698 bp, were found only in Hispanics (Table II).

Mapping of Variants

Exons 5–10 of the α7 nicotinic receptor subunit gene are duplicated. Genomic variants in these exons,

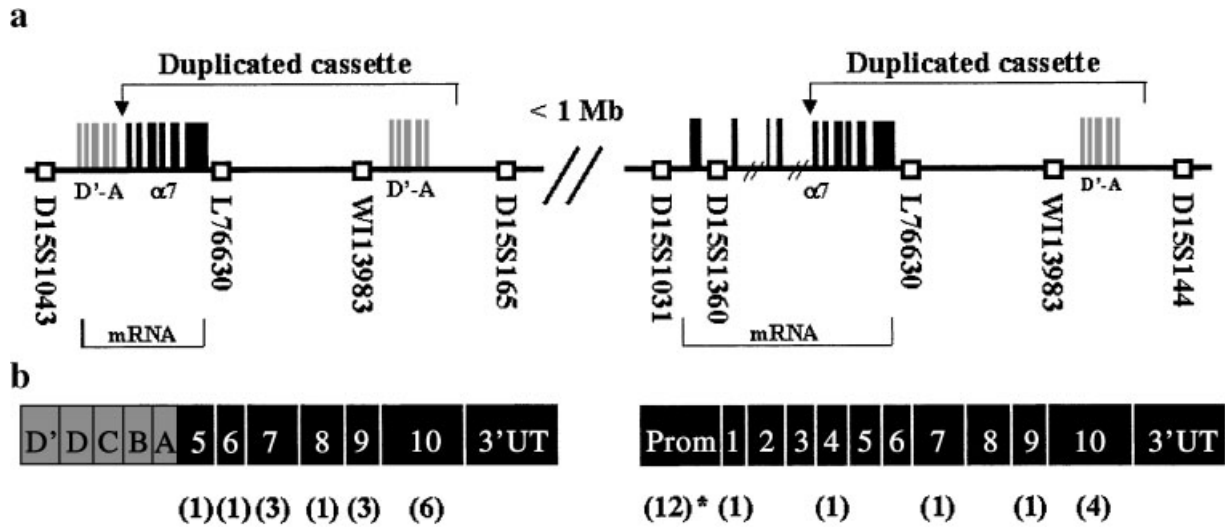

Fig. 1. Genomic 15q13-14 region that contains the *CHRNA7* gene and its partial duplication, *dupCHRNA7*. **a**: Structure of the duplicated region containing *CHRNA7* sequence for exons 5–10. Black vertical bars indicate α7 exons; gray vertical bars indicate novel exons D′–A. Note multiple copies of novel exons D′, D, C, B, and A and the presence of a duplicated EST WI13983. **b**: Structure of the transcripts with the number of exon variants mapped to each gene.

therefore, could be present in either the full-length *CHRNA7* gene or in *dupCHRNA7*. Polymorphisms were mapped, when possible, to one of the two duplicons, utilizing mRNA isolated from either immortalized lymphoblasts or postmortem brain and gene specific PCR, as described in Materials and Methods. In some cases, a given variant was present in both duplicons. In others, only tissue from a schizophrenic subject was available for mapping. In this case, we cannot be certain of the map site, since gene rearrangements or conversions could have occurred. Mapping for these variants is indicated as provisional in Table II.

Eight of the more common variants were mapped in 32 samples from individuals with schizophrenia and 52 samples from control individuals (total of 84). Four common variants, the 497–498 2 bp deletion, the neutral variant at 654 bp, the neutral variant at 1,044 bp, and the amino acid changing variant at 1,466 bp all mapped only to *dupCHRNA7* (Table V). The 2 bp deletion in exon 6 was found in 15 out of the 32 Caucasians with schizophrenia, 29 out of the 49 Caucasian control samples, 1 out of the 4 African Americans with schizophrenia, and 2 out of the 3 African American controls.

Three common neutral variants, at 690, 1,269, and 1,335 bp mapped to both duplicons. The very common variant at 690 bp mapped primarily to the duplicated gene (69 out of 72 individuals). The 1,269 bp variant mapped to both *CHRNA7* genes in 14 individuals out of 54; the neutral variant at 1,335 bp maps primarily to the full-length *CHRNA7*gene, and variant 933 bp only to the full-length gene. Variant 933 bp G→A is in linkage disequilibrium with an intronic variant, discussed below, and may involve splicing.

Ten of the thirty-three variants in Table II were not mapped. Seven of the unmapped variants lie in introns and could not be mapped using the cDNA specific RT-PCR methodology. One unmapped variant in exon 10 was discovered late in the screen and was synonymous.

A large number of variants (12)* were found in a short proximal promoter region 5′ of the translation start shown in Figure 1a. This complex grouping of polymorphisms is reported in a previous publication [Leonard et al., 2002]. Many of the variants were found to functionally reduce transcription in a reporter gene assay and to be associated with both the P50 auditory gating deficit and with schizophrenia. The relationship of these promoter polymorphisms to some of the variants in the coding and non-coding sequence are discussed below.

Non-Synonymous Variants

The full-length *CHRNA7* gene coding region consists of ten exons. Eleven variants mapping to the full-length gene are reported in Table II, three of which are non-synonymous. The A→G variant at 334 bp in exon 4 results in a conservative amino acid change of an isoleucine to a valine at amino acid (aa)112. However, this aa lies in the putative agonist binding site where a conformational alteration could result in a change in agonist affinity [Galzi et al., 1991]. The 334 bp rare variant was found in one African American schizophrenic but not in an affected sibling and in one Hispanic control subject. The control subject exhibited abnormal auditory evoked potential responses, having a P50 test to conditioning ratio of 1.91. Both subjects with this rare 334 bp variant also have a rare insertion in the α7 core promoter (−190 +G) [Leonard et al., 2002], suggesting that this might represent a minor haplotype. The schizophrenic, however, also carries a core promoter mutation on the other chromosome (−178 −G).

The G→A variant at 1,354 bp in exon 10 changes a glutamic acid to a lysine in the large intracellular loop of

TABLE II. Variants Identified in the *CHRNA7* Gene and its Partial Duplication

		Schizophrenics						Controls						Map	
		Caucasian		Af. Am.		Hispanic		Caucasian		Af. Am.		Hispanic			
Exon/intron	Variant	V	T	V	T	V	T	V	T	V	T	V	T	α7	dα7
Non-synonymous variants															
E-4	334 A→G I112V	0	113	1	43	0	6	0	103	0	55	1	8	X	
E-5	370 G→A A124T	0	112	0	42	1	7	0	100	0	53	2	8		X[c]
E-6[a]	497-8 -TG L166	68	96	15	50	5	7	48	71	12	54	4	4		X
E-7	698 A→G Y233C	0	85	0	38	1	7	0	58	0	4	0	4		X[c]
E-9	970 G→A G324R	0	110	10	52	0	6	0	79	4	52	0	7		X
E-10	1116 C→G S372R	0	106	0	36	0	6	0	71	1	49	0	4		X
E-10	1354 G→A E452K	1	102	0	41	0	6	1	63	0	3	0	3	X	
E-10	1456 A→G I486V	0	91	0	40	0	6	1	58	0	4	0	3		X
E-10	1466 C→T S489L	23	110	7	49	1	7	27	82	3	52	3	7		X
E-10	1487 C→A A496D	0	62	1	10	0	6	0	12	0	50	0	3	X	
Synonymous variants															
E-1	45 G→A	1	99	0	41	0	6	0	64	0	3	0	3	X	
E-7	654 C→T	77	90	32	47	5	6	57	70	3	4	3	3		X
E-7	690 G→A	82	83	36	36	6	6	59	59	4	4	3	3	X	X
E-8	861 C→T	4	98	1	40	1	7	1	59	0	4	0	3		X
E-9	921 G→A	2	112	1	45	0	6	4	77	0	50	0	7		X
E-9	933 G→A	56	127	28	53	6	8	39	79	18	50	6	7	X	
	933 A/A	2	127												
E-9	966 C→T	1	110	6	46	0	6	0	79	2	52	0	7		X[c]
E-10	1044 C→T	12	123	3	43	0	6	9	72	1	55	1	5		X
E-10	1116 C→T	2	107	8	44	0	6	0	71	6	54	1	5	—	—
E-10	1269 C→T	75	95	29	40	5	6	47	57	2	3	3	3	X	X
	1269 T/T	2	95	1	40			1	57						
E-10	1335 C→T	32	74	3	11	2	7	30	65	2	4	1	3	X	X
Non-coding variants															
I-2	+75 G→A	0	87	1	38	0	6	1	50	0	3	0	1	X	
I-2	+82 A→C	0	87	2	38	0	6	0	50	0	3	0	1	X	
I-3	−9 A→G	0	113	3	45	0	6	0	103	1	55	0	8	X	
I-7	+21 C→T	21	31	1	6	1	3	3	3	0	0	0	0	—	—
I-7	−11+GTT	10	38	2	4	0	4	5	10	0	1	0	1	—	—
I-7	−20 G→A	15	37	1	4	2	4	5	10	1	1	1	1	—	—
I-7	−29 T→G	1	37	0	4	0	4	0	10	0	1	0	1	—	—
I-9	+19 C→T	0	43	0	5	0	7	1	78	4	54	0	7	—	—
I-9	+27 −TCGGAG	0	110	1	44	0	6	0	78	2	54	0	7	—	—
I-9[b]	+37 G→C	56	126	36	58	6	8	38	79	17	53	6	7	—	—
3′UT	1737 C→A	1	34	0	5	0	2	0	33	0	1	0	1	—	—
3′UT	1837 T→G	0	34	1	6		2	0	33	0	1	0	0	—	—

E, exon; I, intron; Af. Am., African American. Numbering for exons and 3′UT is from the ATG start. Numbering for introns is from the 5′ donor splice site (+) or 3′ acceptor splice site (−). V, number of individuals with the variant; T, total individuals. α7, full-length gene. dα7, duplicated gene.
[a]Caucasian subjects $\chi^2 = 48.66,1$, $P < 0.0001$.
[b]Intron 9, $\chi^2 = 9.986$, 1, $P = 0.0016$.
[c]Mapping provisional.

the protein. A glutamic acid at this position is conserved across species. In rat, a large deletion of sequence including this codon resulted in a twofold increase of both α-bungarotoxin binding and current in transfected oocytes [Valor et al., 2002]. However, the single nonconservative change from an acidic to a basic residue, found in our current study, might be expected to effect a functional change in the receptor and is under investigation. The rare 1,354 bp variant was found in one Caucasian schizophrenic and in one Caucasian control subject. Both of these subjects have normal core promoter sequence. Although not having the 1,354 bp variant, an affected brother of the schizophrenic has a mutation in the core α7 promoter (−86 bp), suggesting the possibility of two α7 alleles for schizophrenia in this family.

The C→A variant at base pair 1,487 in exon 10 changes an alanine to an aspartic acid in the extracellular carboxyl terminus. The 1,487 bp variant was found in one African American schizophrenic but not in an affected child. A family member with an abnormal P50 test to conditioning ratio of 61.7 carried an α7 core promoter mutation (−191 G→A), again suggesting two alleles for schizophrenia.

Sixteen variants found in α7 exons 5–10 mapped to the duplicated gene *dupCHRNA7*, which is also in the region of chromosome 15q14 genetically linked to schizophrenia (Table II, Fig. 1a). The mRNA for *dupCHRNA7* is expressed in multiple tissues, including brain [Drebing et al., 1998]. *DupCHRNA7* is present in only one copy in approximately 30% of the general population, but is homozygotically deleted in 5% of

TABLE III. Allele Frequencies of the Rare Variants

Exon/ intron	Variant	Ethnicity	Allele frequency schizophrenics	Allele frequency controls	Map	
					α7	dα7
E-1	45 G → A	Caucasian	0.005	0	X	
I-2	+75 G → A	Caucasian	0	0.01	X	
I-2	+75 G → A	Af. Am.	0.013	0	X	
I-2	+82 A → C	Af. Am.	0.026	0	X	
I-3	−9 A → G	Af. Am.	0.033	0.009	X	
E-4	334 A → G I112V	Af. Am.	0.012	0	X	
E-4	334 A → G I112V	Hispanic	0	0.063	X	
E-8	861 C → T	Caucasian	0.020	0.008		X
E-8	861 C → T	Af. Am	0.013	0		X
E-8	861 C → T	Hispanic	0.071	0		X
E-9	921 G → A	Caucasian	0.009	0.026		X
E-9	921 G → A	Af. Am.	0.011	0		X
I-9	+19 C → T	Caucasian	0	0.006	—	—
I-9	+19 C → T	Af. Am	0	0.037	—	—
I-9	+27 −TCGGAG	Af. Am.	0.011	0.019	—	—
E-10	1116 C → G S372R	Af. Am.	0	0.010	—	X
E-10	1354 G → A E452K	Caucasian	0.005	0.008	X	—
E-10	1456 A → G I486V	Caucasian	0	0.009		X
3′UT	1737 C → A	Caucasian	0.015	0	—	—

E, exon; I, intron; Af. Am., African American. Numbering for exons and 3′UT is from the ATG start. Numbering is from the 5′ donor splice site (+) or 3′ acceptor splice site (−).

schizophrenic subjects [Gault et al., 1998; Leonard et al., 2001b]. Recent evidence suggests that *dupCHRNA7* transcripts are translated, but the function of this protein is not yet known [Lee et al., 2001]. Six single nucleotide polymorphisms (SNP) change amino acids in a putative ORF found in *dupCHRNA7* (370 bp in exon 5, 698 bp in exon 7, 970 bp in exon 9, and 1,116, 1,456, and 1,466 bp in exon 10).

A 2 bp deletion at bases 497–498 in exon 6 was found in one copy of the duplicated gene in 57.5% of schizophrenic subjects and 49.6% of controls, not a significant difference. It was, however, found more frequently in Caucasian control subjects than in African American controls ($\chi^2 = 25.31$, $P < 0.0001$). This deletion, found only in *dupCHRNA7*, shifts the reading frame, resulting in three stop codons within the next 53 codons. These stop codons, however, are the most frequently skipped during translation [MacBeath and Kast, 1998]. Further, the site surrounding the deletion in exon 6 is a consensus exon splice enhancer site (ESE) for enhancer factor SC35 [Cartegni et al., 2002]. Deletion of the two base pairs (TG) would disrupt this site, suggesting that exon 6 might be spliced out in these subjects, leaving an exon 5/exon 7 junction. Although there is not yet evidence that this occurs, the splice variant would leave the coding sequence in frame. Deletion of exon 6 removes the cysteine bridge and part of a putative ligand binding site; the remainder of the α7 coding sequence would be intact. In the analysis of the *CHRNA7* proximal promoter [Leonard et al., 2002], subjects with a promoter variant were much less likely to have a 2 bp deletion in exon 6 of the *dupCHRNA7* gene ($\chi^2 = 16.46$, 1; $P < 0.0001$). There was also an interesting relationship with a three base pair insertion in intron 7. Every subject (50 out of 50) with a 2 bp deletion in exon 6 of the *dupCHRNA7* gene, also had this insertion (+GTT) at the

TABLE IV. Variants With Significantly Different Frequencies in Ethnic Groups

Exon/ intron	Variant	Schizophrenics and controls combined					Map	
		Caucasian		Af. Am.				
		V	T	V	T	*P* values	α7	dα7
I-3	−9 A → G	0	216	4	100	0.0104	X	
E-6	497-8 -TG L166	116	167	27	104	<0.0001		X
E-7	654 C → T	134	160	35	51	<0.0001		X
E-9	966 C → T	1	189	8	98	0.0010		X
E-9	970 G → A G324R	0	189	14	104	<0.0001		X
I-9	+19 C → T	0	121	4	59	0.0475	—	—
I-9	+27 -TCGGAG	0	188	3	98	0.0394	—	—
E-10	1116 C → T	2	178	14	98	<0.0001	—	—
E-10[c]	1466 C → T S489L	50	192	10	101	0.0011		X

E, exon; I, intron; Af. Am., African American. Numbering for exons and 3′UT is from the ATG start. Numbering for introns is from the 5′ donor splice site (+) or 3′ acceptor splice site (−). V, number of individuals with the variant; T, total individuals; α7, full-length gene; dα7, duplicated gene.

TABLE V. Mapping of Common Variants Identified in the *CHRNA7* Gene and its Partial Duplication

		Schizophrenics								Controls							
		Caucasian				Af. Am.				Caucasian				Af. Am.					
		Genomic		cDNA		Genomic		cDNA		Genomic		cDNA		Genomic		cDNA		Map	
Exon/intron	Variant	V	T	α7	dα7	V	T	α7	dα7	V	T	α7	dα7	V	T	α7	dα7	α7	dα7
E-6	497-8 -TG L166	68	96	0	15	15	50	0	1	48	71	0	29	12	54	0	2		X
E-7	654 C→T	77	90	0	20	32	47	0	1	57	70	0	33	3	4	0	1		X
E-7	690 G→A	82	83	4	22	36	36	1	3	59	59	4	41	4	4	0	3	X	X
E-9	933 G→A	56	127	12	0	28	53	3	0	39	79	15	0	18	50	1	0	X	
	933 A/A	2	127																
E-10	1044 C→T	12	123	0	3	3	43	0	1	9	72	0	3	1	55	0	0		X
E-10	1269 C→T	75	95	9	13	29	40	2	3	47	57	18	22	2	3	0	1	X	X
	1269 T/T	2	95			1	40			1	57								
E-10	1335 C→T	32	74	6	2	3	11	0	0	30	65	17	3	2	4	2	0	X	X
E-10	1466 C→T S489L	23	110	0	4	7	49	0	0	27	82	0	12	3	52	0	0		X

E, exon; I, intron; Af. Am., African American. Numbering for exons and 3′UT is from the ATG start. α7, full-length gene. dα7, duplicated gene.

−11 bp position in intron 7. It is likely, therefore, that this intronic variant is in the gene duplication rather than in the full-length gene.

Synonymous Variants

Eleven SNPs were found in the coding regions that do not change an amino acid. Four conservative exon variants at bp 690, 1,269, and 1,335 appear to map to both the duplicated gene and the full-length *CHRNA7* genes. The variant at 690 bp in exon 7 is the most common variant found in the α7 nicotinic receptor genes; it is heterozygous in genomic DNA from 190 of 191 samples examined. The G primarily maps to *CHRNA7* and the A primarily maps to *dupCHRNA7*. The 1,269 and 1,335 bp variants were found in 80 and 43% of all subjects, respectively.

Another common synonymous variant in exon 9, at bp 933, is of interest. It was found only in the full-length gene and is also inversely associated with having a polymorphism in the proximal promoter, in all subjects examined, $\chi^2 = 6.916$, 1; $P = 0.0085$ [Leonard et al., 2002]. The association was significant in the controls ($\chi^2 = 5.183$, 1; $P = 0.0228$), but only suggestive in the schizophrenic subjects. The 933 G→C variant is found within the loop of a putative stem and loop structure formed by a tri-nucleotide repeat of $(GGT)_3$ and its complement repeat $(ACC)_3$ in exon 9 ($\Delta G = -16.2$ kcal/mol). The 933 bp variant is also in linkage disequilibrium with a common intronic variant in intron 9 discussed below.

Intronic Variants

Ten intron changes were identified, none of which change the consensus sequences at RNA splice junctions. However, a number of these variants may affect splicing by introducing a favorable splice site or affecting the binding sites of splice enhancer proteins. In intron 3, a variant at −9 g→a changes the sequence near the 3′ acceptor site to a sequence identical to 9 bp in exon 4, forming a cryptic splice site. Although found in only 3 of 45 African American schizophrenic families (3/90 alleles), this polymorphism was found in only 1 of 55 African American controls (1/110 alleles). This control subject had a P50 (test to conditioning ratio) of 0.32, in the unstable range, and had current major depression.

The intron 7−11 (+gtt) variant was mentioned above in relation to the 2 bp deletion in exon 6. Insertion of these three base pairs introduces additional pyrimidines into the splice acceptor site for exon 7, possibly increasing site use. Another intron 7 variant at −20 g→a, is inversely associated with the presence of proximal promoter variants. Only 1 of 29 subjects with the polymorphism had a promoter mutation; 20 of 58 subjects with the wild-type sequence had a promoter polymorphism ($\chi^2 = 10.17$, 1; $P = 0.0014$) [Leonard et al., 2002].

A variant in intron 9 (+37, counted from the splice donor site) was found more frequently in our African American schizophrenic sample than our control sample

($\chi^2 = 9.986$, 1; $P = 0.0016$). This same variant was not found at significantly different frequencies in the Caucasian schizophrenia sample. One unaffected family member was identified with a homozygous C at base pair +37. Interestingly, the variant E-9 933 bp G → A is in linkage disequilibrium with I-9 +37 bp g → c. Since the E-9 933 bp polymorphism appears to be in the full-length gene, it is likely that the I-9 variant is, as well. If an I-9 +37 bp variant is not associated with E-9 933 bp, there is a polymorphism present nearby, E-9 966 bp C → T, suggesting that E-9 966 is also in the full-length gene. However, this variant is rare and was only mapped in one individual who was a schizophrenic. The map location, therefore, is provisional.

DISCUSSION

Evidence for genetic linkage to schizophrenia in the 15q13-q14 region has grown as marker density on the human genomic map has improved. At least eight new studies over the last 3 years support a hypothesis of linkage at this site [Leonard et al., 1998; Riley et al., 2000; Stöber et al., 2000; Freedman et al., 2001a,b; Liu et al., 2001; Tsuang et al., 2001; Xu et al., 2001]. The region has also been linked to bipolar disorder [Turecki et al., 2001]. A candidate gene in this region, the α7 nicotinic acetylcholine receptor subunit gene *CHRNA7*, has been identified pharmacologically, as playing a role in an aberrant inhibitory pathway found in schizophrenia, the P50 auditory evoked potential deficit [Luntz-Leybman et al., 1992; Stevens et al., 1998; Leonard et al., 2000, 2001a]. The P50 deficit, an endophenotype of schizophrenia, is genetically linked to D15S1360, a dinucleotide marker in intron 2 of *CHRNA7* [Freedman et al., 1997]. Functional gene variants have been isolated in the proximal promoter region of *CHRNA7* that appear to be associated with both schizophrenia and with the P50 deficit [Leonard et al., 2002]. In this current report, we present the results of a mutation screening effort in the coding region and intron/exon borders of the *CHRNA7* gene cluster in schizophrenic and control subjects.

The mutation screening was complex, due to the partial duplication of the α7 gene [Gault et al., 1998]. Exons 5–10, and intervening introns, were duplicated and inserted with a large cassette of DNA into a position proximal to the full-length *CHRNA7*. The duplicated exons are expressed as mRNA with five non-α7 exons in several tissue types, including postmortem brain (*dupCHRNA7*; see GenBank Accession No. AF029838). Thus, mapping was required, for polymorphisms found in exons 5–10 in genomic DNA, to either the full-length *CHRNA7* or *dupCHRNA7*. Transcripts were isolated, specific for each gene, from either postmortem brain tissue or lymphoblasts.

Variants in both coding region and introns were identified. We found 21 polymorphisms in the exons, 9 of which changed an amino acid. Three of these amino acid changes, although rare, mapped to the full-length gene. These three amino acids are conserved between humans, mouse (Genbank accession #A57175) and rat (Genbank accession #T01378). One, in exon 4 (Ile to Val, a.a. 112) lies in part of the putative agonist binding site [Galzi et al., 1991]. In the three families, in which these amino acid changes occurred, cosegregation with neither the P50 deficit nor with schizophrenia was observed. In such a complex disorder, bilineal inheritance or reduced penetrance could explain this result. However, we did find functional promoter variants in all three of these families [Leonard et al., 2002].

Ten intronic variants and two variants in the 3′-untranslated region were identified. Two polymorphisms in introns 2 and 3 were in the full-length gene, but the seven variants in introns 7 and 9 and those in the 3′-UT could not be easily mapped because of the gene duplication. One variant in intron 9 at +37 was associated with schizophrenia in African Americans ($\chi^2 = 9.986$, 1; $P = 0.0016$) and was in linkage disequilibrium with a synonymous variant mapped to *CHRNA7*. A number of the intronic polymorphisms either introduce a cryptic splice site or alter a splice site. The 497–498 bp deletion, present in the duplicated gene in more than 50% of subjects examined, disrupts an exonic splice enhancer site (EXE). If exon 6 were aberrantly spliced out in this gene variant, the translation of a putative protein would remain in frame, leading to speculation that this splice variant might be regulatory. Although each of these variants needs to be examined more carefully, we did identify multiple alternatively spliced transcripts in our initial studies of the α7 gene cluster [Gault et al., 1998]. Splice variants have been found to be a common causal element in disease [Ars et al., 2000; Grabowski and Black, 2001; Cartegni et al., 2002]. Since the CHRNA7 receptor assembles as a pentamer, the presence of splice variants represents a possible mechanism for dominant-negative decreased expression [Garcia-Guzman et al., 1995].

The partial duplication of exons 5–10 and flanking regions not only introduced complexity into the mutation screen, but suggests yet another mechanism of mutation. The duplicon containing α7 exons 5–10 was inserted 3′ of five exons, duplicated from another gene, and the chimera is transcribed in both lymphocytes and brain. This fusion gene or gene product might interfere with expression, assembly or function of the *CHRNA7* gene productin a manner similar to a splice variant. Variants in transcribed regions, common to both the *CHRNA7* and *dupCHRNA7* genes were mapped in mRNA from only a limited number of individuals. This also leaves open the possibility that gene conversion could be a mechanism of mutation resulting in disruption of full-length *CHRNA7* in some individuals, though we have no evidence for this occurrence to date.

Further, presence of the partial duplication could lead to deletion or additional duplication events. The duplicated sequence could prime misalignment, then recombination and subsequent deletion of the intervening sequences including part of the full-length gene. Deletions primed by duplications have been extensively characterized in Prader Willi and Angelman syndromes which map nearby at 15q11-q13 [Robinson et al., 1998]. In this regard, we have detected five schizophrenic subjects with homozygotic deletions of the duplicated gene, although none of these subjects appear to be missing any

part of the full-length gene. Deletion of both copies of *dupCHRNA7* has not yet been observed in controls.

Although a large number of polymorphisms were found in both the full-length *CHRNA7* gene and its partial duplication, no nucleotide changes that either cosegregate with the P50 gating deficit or schizophrenia, or that obviously disrupt the function of the full-length *CHRNA7* gene were isolated. We did not find any of the coding region variants to be in linkage disequilibrium with a functional promoter mutation. Previous work from our laboratory and other investigators show decreased expression of CHRNA7 receptors in several regions of postmortem brain in individuals with schizophrenia compared to control subjects. Since we find no prominent coding region mutations, the promoter polymorphisms recently reported suggest that further study of the regulatory regions, including the intronic variants in the gene, reported herein, is warranted. These results also indicate that α7 nicotinic receptors in schizophrenic subjects, though reduced in number, are likely to be functionally normal and may respond to therapies that modulate activity or response.

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REFERENCES

Adler LE, Hoffer LJ, Griffith J, Waldo MC, Freedman R. 1992. Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. Biol Psychiatry 32:607–616.

Adler LE, Hoffer LD, Wiser A, Freedman R. 1993. Normalization of auditory physiology by cigarette smoking in schizophrenic patients. Am J Psychiatry 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. 1998. Schizophrenia, sensory gating, and nicotinic receptors. Schiz Bull 24:189–202.

Ars E, Serra E, Garcia J, Kruyer H, Gaona A, Lazaro C, Estivill X. 2000. Mutations affecting mRNA splicing are the most common molecular defects in patients with neurofibromatosis type 1. Hum Mol Genet 9: 237–247.

Bertrand D, Changeux JP. 1992. Nicotinic receptor: An allosteric protein specialized for intercellular communication. Sem Neurosci 7:75–90.

Cartegni L, Chew SL, Krainer AR. 2002. Listening to silence and understanding nonsense: Exonic mutations that affect splicing. Nat Rev Genet 3:285–298.

Court J, Spurden D, Lloyd S, McKeith I, Ballard C, Cairns N, Kerwin R, Perry R, Perry E. 1999. Neuronal nicotinic receptors in dementia with Lewy bodies and schizophrenia: Alpha-bungarotoxin and nicotine binding in the thalamus. J Neurochem 73:1590–1597.

Drebing C, Logel J, Benhammou K, Robinson M, Gault J, Walton K, Meriwether J, Short M, Hopkins J, Berger R, Maslak M, Breese C, Freedman R, Leonard S. 1998. Expression of the human α7 neuronal nicotinic acetylcholine receptor and a partial gene duplication. Soc Neurosci Abstr 24:832.

Endicott J, Andreasen NC, Spitzer RL. 1978. Family history-research diagnostic criteria. New York: Research Assessment and Training Unit, New York State Psychiatric Institute.

First MB, Gibbon M, Spitzer RL, Williams JBW. 1996. Structured clinical interview for axis I DSM-IV disorders-non-patient edition—(SCID-I/NP, Version 2.0). New York: Biometrics Research Department, New York State Psychiatric Institute.

Freedman R, Waldo M, Bickford-Wimer P, Nagamoto H. 1991. Elementary neuronal dysfunctions in schizophrenia. Schiz Res 4:233–243.

Freedman R, Hall M, Adler LE, Leonard S. 1995. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. Biol Psychiatry 38:22–33.

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. 1997. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. Proc Natl Acad Sci 94:587–592.

Freedman R, Leonard S, Gault JM, Hopkins J, Cloninger CR, Kaufmann CA, Tsuang MT, Farone SV, Malaspina D, Svrakic DM, Sanders A, Gejman P. 2001a. Linkage disequilibrium for schizophrenia at the chromosome 15q13-14 locus of the alpha 7-nicotinic acetylcholine receptor subunit gene (CHRNA7). Am J Med Genet 105:20–22.

Freedman R, Leonard S, Olincy A, Kaufmann CA, Malaspina D, Cloninger CR, Svrakic D, Faraone SV, Tsuang MT. 2001b. Evidence for the multigenic inheritance of schizophrenia. Am J Med Genet 105:794–800.

Galzi J-L, Revah F, Bessis A, Changeux J-P. 1991. Functional architecture of the nicotinic acetylcholine receptor: From electric organ to brain. Annu Rev Pharmacol 31:37–72.

Garcia-Guzman M, Sala F, Sala S, Campos-Caro A, Stuhmer W, Gutierrez LM, Criado M. 1995. Alpha-bungarotoxin-sensitive nicotinic receptors on bovine chromaffin cells: Molecular cloning, functional expression, and alternative splicing of the alpha 7 subunit. Eur J Neurosci 7:647–655.

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. 1998. Genomic organization and partial duplication of the human α7 neuronal nicotinic acetylcholine receptor gene. Genomics 52:173–185.

Gershon ES. 2000. Bipolar illness and schizophrenia as oligogenic diseases: Implications for the future. Biol Psychiatry 47:240–244.

Grabowski PJ, Black DL. 2001. Alternative RNA splicing in the nervous system. Prog Neurobiol 65:289–308.

Guan ZZ, Zhang X, Blennow K, Nordberg A. 1999. Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. Neuroreport 10:1779–1782.

Kety SS, Wender PH, Jacobsen B, Ingraham LJ, Jansson L, Faber B, Kinney DK. 1994. Mental-illness in the biological and adoptive relatives of schizophrenic adoptees—replication of the Copenhagen study in the rest of Denmark. Arch Gen Psychiatry 51:442–455.

Lee MJ, Logel J, Gault J, Leonard S. 2001. Cloning and expression of variants of a partial duplication of the human α7 nicotinic receptor subunit. Soc Neurosci Abstr 27: Program Number 144.10.

Leonard S, Bertrand D. 2001. Neuronal nicotinic receptors: From structure to function. Nic Tob Res 3:203–223.

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. 1998. Further investigation of a chromosome 15 locus in schizophrenia: Analysis of affected sibpairs from the NIMH Genetics Initiative. Am J Med Genet 81:308–312.

Leonard S, Breese C, Adams C, Benhammou K, Gault J, Stevens K, Lee M, Adler L, Olincy A, Ross R, Freedman R. 2000. Smoking and schizophrenia: Abnormal nicotinic receptor expression. Eur J Pharm 393:237–242.

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. 2001a. Smoking and mental illness. Pharm Biochem Behav 70:561–570.

Leonard S, Gault J, Hopkins J, Logel J, Vianzon R, Drebing C, Short M, Walton K, Berger R, Ross R, Olincy A, Adler L, Freedman R. 2001b. DNA variants in the alpha 7 nicotinic receptor gene promoter are associated with schizophrenia. Biol Psychiatry 49:571.

Leonard S, Gault J, Hopkins J, Logel J, Drebing C, Vianzon R, Short M, Berger R, Robinson M, Freedman R. 2002. Promoter variants in the α7 nicotinic acetylcholine receptor subunit gene are associated with an inhibitory deficit found in schizophrenia. Arch Gen Psychiatry 59:1085–1096.

Liu CM, Hwu HG, Lin MW, Ou-Yang WC, Lee SFC, Fann CSJ, Wong SH, Hsieh SH. 2001. Suggestive evidence for linkage of schizophrenia to markers at chromosome 15q13-14 in Taiwanese families. Am J Med Genet 105:658–661.

Luntz-Leybman V, Bickford PC, Freedman R. 1992. Cholinergic gating of response to auditory stimuli in rat hippocampus. Brain Res 587:130–136.

MacBeath G, Kast P. 1998. UGA read-through artifacts—when popular gene expression systems need a pATCH. BioTechniques 24:789–794.

Parra EJ, Marcini A, Akey J, Martinson J, Batzer MA, Cooper R, Forrester T, Allison DB, Deka R, Ferrell RE, Shriver MD. 1998. Estimating African American admixture proportions by use of population-specific alleles. Am J Hum Genet 63:1839–1851.

Riley BP, Makoff AM, Magudi-Carter M, Jenkins TJ, Williamson R, Collier DA, Murray RM. 2000. Haplotype transmission disequilibrium and evidence for linkage of the CHRNA7 gene region to schizophrenia in Southern African Bantu families. Am J Med Genet 96:196–201.

Riley B, Williamson M, Collier D, Wilkie H, Makoff A. 2002. A 3-Mb map of a large segmental duplication overlapping the alpha 7-nicotinic acetylcholine receptor gene (CHRNA7) at human 15q13-q14. Genomics 79:197–209.

Robinson WP, Dutly F, Nicholls RD, Bernasconi F, Penaherrerra M, Michaelis RC, Abeliovich D, Schinzel AA. 1998. The mechanisms involved in formation of deletions and duplications of 15q11-q13. J Med Genet 35:130–136.

Rollins YD, Stevens KE, Harris KR, Hall ME, Rose GM, Leonard S. 1993. Reduction in auditory gating following intracerebroventricular application of α-bungarotoxin binding site ligands and α7 antisense oligonucleotides. Soc Neurosci Abstr 19:837.

Stevens KE, Freedman R, Collins AC, Hall M, Leonard S, Marks JM, Rose GM. 1996. Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. Neuropsychopharmacology 15:152–162.

Stevens KE, Kem WR, Mahnir VM, Freedman R. 1998. Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. Psychopharmacolgy 136:320–327.

Stöber G, Saar K, Rüschendorf F, Meyer J, Nürnberg G, Jatzke S, Franzek E, Reis A, Lesch KP, Wienker TF, Beckmann H. 2000. Splitting schizophrenia: Periodic catatonia-susceptibility locus on chromosome 15q15. Am J Hum Genet 67:1201–1207.

Torrey EF. 1992. Are we overestimating the genetic contribution to schizophrenia. Schiz Bull 18:159–170.

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. 2001. Examination of genetic linkage of chromosome 15 to schizophrenia in a large veterans affairs cooperative study sample. Am J Med Genet 105:662–668.

Turecki G, Grof P, Grof E, D'Souza V, Lebuis L, Marineau C, Cavazzoni P, Duffy A, Betard C, Zvolsky P, Robertson C, Brewer C, Hudson TJ, Rouleau GA, Alda M. 2001. Mapping susceptibility genes for bipolar disorder: A pharmacogenetic approach based on excellent response to lithium. Mol Psychiatry 6:570–578.

Valor LM, Mulet J, Sala F, Sala S, Ballesta JJ, Criado M. 2002. Role of the large cytoplasmic loop of the alpha 7 neuronal nicotinic acetylcholine receptor subunit in receptor expression and function. Biochemistry 41: 7931–7938.

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. 2001. Evidence for linkage disequilibrium between the alpha 7-nicotinic receptor gene (CHRNA7) locus and schizophrenia in Azorean families. Am J Med Genet 105:669–674.