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The Human *CHRNA7* and *CHRFAM7A* Genes: A Review of the Genetics, Regulation, and Function

Melissa L. Sinkus, M.D. [a], **Sharon Graw, Ph.D.** [a], **Robert Freedman, M.D.** [a,c], **Randal G. Ross, M.D.** [a], **Henry A. Lester, Ph.D.** [b], and **Sherry Leonard**[a,c,*]

Melissa L. Sinkus: Melissa.Sinkus@ucdenver.edu; Sharon Graw: Sharon.Graw@ucdenver.edu; Robert Freedman: Robert.Freedman@ucdenver.edu; Randal G. Ross: Randy.Ross@ucdenver.edu; Henry A. Lester: lester@caltech.edu

[a]Department of Psychiatry, University of Colorado Denver, Aurora, CO, 80045

[b]Division of Biology, California Institute of Technology, Pasadena, CA, 91125

[c]Veterans Affairs Medical Research Center, Denver, CO, 80262

Abstract

The human α7 neuronal nicotinic acetylcholine receptor gene (*CHRNA7*) is ubiquitously expressed in both the central nervous system and in the periphery. *CHRNA7* is genetically linked to multiple disorders with cognitive deficits, including schizophrenia, bipolar disorder, ADHD, epilepsy, Alzheimer's disease, and Rett syndrome. The regulation of *CHRNA7* is complex; more than a dozen mechanisms are known, one of which is a partial duplication of the parent gene. Exons 5-10 of *CHRNA7* on chromosome 15 were duplicated and inserted 1.6 Mb upstream of *CHRNA7*, interrupting an earlier partial duplication of two other genes. The chimeric *CHRFAM7A* gene product, dupα7, assembles with α7 subunits, resulting in a dominant negative regulation of function. The duplication is human specific, occurring neither in primates nor in rodents. The duplicated α7 sequence in exons 5-10 of *CHRFAM7A* is almost identical to *CHRNA7*, and thus is not completely queried in high throughput genetic studies (GWAS). Further, pre-clinical animal models of the α7nAChR utilized in drug development research do not have *CHRFAM7A* (dupα7) and cannot fully model human drug responses. The wide expression of *CHRNA7*, its multiple functions and modes of regulation present challenges for study of this gene in disease.

Keywords

Nicotinic receptor; Duplication; Schizophrenia; Alzheimer's; *CHRNA7*; *CHRFAM7A*; Gene mutation

[*]Correspondence: Sherry Leonard, Ph.D., Professor, Department of Psychiatry, University of Colorado Denver, Mailstop 8344, P.O. Box 6511, Aurora, Colorado 80045, Sherry.Leonard@ucdenver.edu, +1-720-216-0851.

1. The Human Alpha 7 Nicotinic Acetylcholine Receptor Gene Cluster on Chromosome 15

The α7 neuronal nicotinic receptor gene, *CHRNA7* on Chromosome 15, is widely expressed in both the brain and periphery with multiple important roles in cognition and the immune system. Decreased expression and function of *CHRNA7* have been associated with many diseases including schizophrenia, bipolar disorder, attention deficit hyperactivity disorder (ADHD), Alzheimer's disease, autism, epilepsy, and learning disorders. Regulation of *CHRNA7* expression and function is complex. More than a dozen different mechanisms are currently known, including a partial duplication of the parent gene.

1.1 The *CHRNA7* gene is partially duplicated, forming a new gene, *CHRFAM7A*

Gene duplication during evolution resulted in the formation of the nicotinic receptor gene family from the primal *CHRNA7* gene (Changeux, 2012; Le Novere et al., 2002; Ortells and Lunt, 1995). In addition, a new and relatively recent partial duplication of *CHRNA7* occurred, forming a new gene, *CHRFAM7A*. Construction of a yeast artificial chromosome map across the schizophrenia genetic linkage region on chromosome 15q13.3 resulted in the discovery of this partial duplication (Gault et al., 1998). The *CHRNA7* gene has 10 exons; exons 5-10 were duplicated along with additional DNA. The duplicon of ~250Kb was inserted centromeric to the *CHRNA7* gene by 1.6Mb, interrupting earlier partial duplications of two other genes (Gault et al., 1998; Riley et al., 2002). Prior to the partial duplication of *CHRNA7*, several exons of the unc-51 like kinase 4 gene (*ULK4*) at chromosome 3p22.1 were duplicated on chromosome 15 (Lang et al., 2014; Riley et al., 2002) (Figure 1A).

In initial studies, four exons were identified in *CHRFAM7A* (exons A, B, C, and D) (Gault et al., 1998). Subsequent use of mRNA from human cell lines THP1 and SHEP1 that do not express the *CHRNA7* gene, and Rapid Amplification of cDNA 5′-Ends (5′-RACE) identified an additional three exons, E, F, and G. The genomic order of the upstream exons is shown in Figure 2A and the exon sequences in 2B. Exons A, B, C, and E are copies of *ULK4* exons (yellow). Exons D, F, and G (green) share homology with the *GOLGA8B* gene, 2.5 Mb 3′ of *CHRNA7* (Stephens et al., 2012). Exons A-F constitute the genetic element FAM7A, which is duplicated at least four times on chromosome 15q13.3, FAM7A(1-4)(Figure 1A). Deletions in the *ULK4* gene were recently associated with schizophrenia (Lang et al., 2014). However, the duplicated *ULK4* exons in FAM7A(A, B, C, and E) are not included in these deletions, suggesting that generation of FAM7A was a separate event.

Subsequent to the formation of the FAM7A copies, *CHRNA7* exons 5-10 were duplicated and interrupted FAM7A(1), upstream, forming the new chimeric gene, *CHRFAM7A*. FAM7A(3) was also duplicated and contained in the 250 Kb duplicon with the *CHRNA7* exons. This partially duplicated *CHRNA7* cassette was inserted in a reverse orientation to the nearby parent gene (Figure 1B).

The formation of *CHRFAM7A* is human specific (Locke et al., 2005). *CHRFAM7A* is not found in either rodents or in primates, and likely occurred more than 3.5 million years ago when hominids evolved. The duplication is evolutionarily new; *CHRNA7* sequences in

*CHRFAM7*A are 99.9% identical to exons 5-10 of *CHRNA7* (Gault et al., 1998). The two genes, thus, cannot be adequately queried in genome wide association studies (GWAS). Large-scale genomic sequencing and SNP analysis methodology do not provide accurate mapping of polymorphisms in duplicated regions.

Another small deletion is even more recent, a 2bp deletion in exon 6 of *CHRFAM7A* (Gault et al., 1998; Sinkus et al., 2009). The 2bp deletion is not present in *CHRNA7*. Further, this version of the duplicated gene, *CHRFAM7AΔ2bp*, is accompanied by a gene inversion (Flomen et al., 2008), and has the same orientation as the parent gene, *CHRNA7* (Figure 1C).

The 2bp deletion is found more frequently in Caucasians (42%) than in African Americans (14%) ($P=1.98 \times 10^{-7}$) (Sinkus et al., 2009). The data, thus, suggest that the 2bp deletion and inversion occurred after the second migration from Africa and after the formation of *CHRFAM7A*.

The two genes, *CHRNA7* and its partial duplication *CHRFAM7A* are intimately related. This review reports on the expression, function, and regulation of each separately, followed by topics involving both genes.

2. The Alpha7 Nicotinic Acetylcholine Receptor Gene, *CHRNA7*

The α7 neuronal nicotinic acetylcholine receptor, α7nAChR, is a member of the nicotinic acetylcholine receptor family. These receptors are ligand-gated ion channels of five subunits, stimulated endogenously by acetylcholine, resulting in flux of the cations Na^+, K^+, and Ca^{++} (Changeux, 2012; Vijayaraghavan et al., 1992). Eleven nicotinic receptor subunit genes are expressed in the human brain, including α2-7, 9, 10, and β2-4 (Elgoyhen et al., 2009; Lindstrom, 1997), which form multiple pentameric heteromers and a homomeric receptor, usually with only α7 subunits (Gotti et al., 2007). Receptors containing both α and β subunits bind nicotine with high affinity (nM), but the low-affinity α7nAChRs require μM concentrations of nicotine for activation (Dani and Bertrand, 2007; Marks and Collins, 1982; Weiland et al., 2000). The α7 nicotinic receptor gene, *CHRNA7*, is ancient, homologs appearing in archaea (Tasneem et al., 2005), possibly as sensors for quaternary ammonium metabolites and osmolytes such as choline and glycine betaine (Lucht and Bremer, 1994). *CHRNA7* may be the evolutionary ancestor of multiple ligand gated ion channels including GABA, $5HT_3$, and the other nicotinic acetylcholine receptor subunit genes (Le Novere et al., 2002). Perhaps some of its ancient roles developed into peripheral functions, such as in inflammation, but as a ligand-gated channel it evolved as an important central nervous system receptor.

2.1. Localization and Function

High affinity nicotinic receptors are generally localized pre-synaptically in the CNS, but α7nAchRs have a much wider localization and function, being found both pre- and postsynaptically (Leonard and Bertrand, 2001; Lindstrom, 1997). They are also expressed in the periphery, including on neuroendocrine cells (Song and Spindel, 2008), macrophages (de Jonge and Ulloa, 2007), and sperm (Son and Meizel, 2003). The Ca^{++} permeability of

α7nAChRs exceeds that of other nicotinic receptor subtypes and also that of NMDA receptors (Albuquerque et al., 2009; Vijayaraghavan et al., 1992). Resulting depolarization of the cell activates voltage-dependent calcium channels (VDCCs), and induces Ca^{++} release from the endoplasmic reticulum (Shen and Yakel, 2009). The ion selectivity of the receptor appears to be regulated by glutamate residues in the intracellular face of transmembrane region (TM) TM2, which are not found in other alpha subunits (Corringer et al., 1999).

Normal function of α7nAChRs in mammalian cells requires the co-expression of chaperone proteins such as ric-3 (Castelan et al., 2008) and lynx1 (Ibanez-Tallon et al., 2002) that facilitate formation of surface receptors.

The principal endogenous ligand for most, if not all, α7nAChRs is acetylcholine. Choline, itself, is a specific agonist at α7nAChRs (Uteshev et al., 2003), and is particularly important during fetal development (Ross et al., 2010). Hippocampal choline acetyltransferase is not expressed until late prenatal or early postnatal development (Court et al., 1993). Thus, choline is likely to be the principal ligand for the α7nAChR during this important period of neuronal migration and synapse formation.

Following activation, all α7nAChRs then close to a large extent. This process, termed desensitization, occurs within just a few ms, faster than at other nAChRs or at other ligand-gated channels. Desensitization also occurs at hippocampal α7nAChRs (Dani et al., 2000). Desensitization probably consists of several processes, none well understood at a molecular level. Of some interest, desensitization is less complete at α7nAChRs than at other nAChRs, so that some channels remain open even during the so-called “desensitized” state. At nicotine concentrations in the brain during smoking (0.1 to 1 μM), this steady-state fractional activation may exceed 20% (Wang et al., 2014; Xiao et al., 2015). The response to agonists is also likely to be shaped by the recovery from desensitization, which is also faster than recovery at other nAChRs. Some experiments show that α7nAChRs recover from desensitization in just 15–30s (Frazier et al., 1998).

2.1.1. Central Nervous System Expression and Function—In the brain, presynaptic α7nAChR are localized on GABAergic and glutamatergic terminals in hippocampus and other regions, where influx of Ca^{++} leads to release of multiple types of neurotransmitters, including GABA, glutamate, acetylcholine, and dopamine (Albuquerque et al., 2009; Dani and Bertrand, 2007; Dickinson et al., 2008; Jones and Wonnacott, 2004). In human brain both *CHRNA7* mRNA and protein, measured by *in situ* hybridization and [^{125}I]-α-bungarotoxin binding, respectively, are expressed in most nuclei (Breese et al., 1997a). Highest expression is found in hippocampus, cingulate gyrus, lateral and medial geniculates, and the reticular nucleus of the thalamus (RTN). In hippocampus, α7nAChR-mediated release of glutamate is enhanced by the activity of protein kinase A (PKA) (Cheng and Yakel, 2014). Protein kinase A expression appears to be permanently up regulated in postmortem brain of smokers (Hope et al., 2007).

Postsynaptically, the α7nAChR is found in postsynaptic densities (PSD) (Levy and Aoki, 2002; Li et al., 2012), where Ca^{++} influx affects phosphorylation of CREB (Hu et al., 2002), leading to changes in gene expression. Treatment with α7nAChR agonists enhances

cognitive circuits through an NR2B-NMDAR mechanism (Yang et al., 2013). Expression of both NR2A and NR2B is up regulated in postmortem hippocampus of smokers (Mexal et al., 2005).

Astrocytes also express α7nAChR, but at lower levels than neurons (Shen and Yakel, 2012). The influx of Ca^{++} through α7nAChR promotes further release of calcium from intracellular stores, unlike in neurons where Ca^{++} influx activates voltage-gated calcium channels (Sharma and Vijayaraghavan, 2001). Stimulation of astrocytic α7nAChRs results in the recruitment of GluA1 and GluA2 AMPA receptors to post-synaptic sites (Wang et al., 2013).

Thus, α7nAChRs have an important role in the excitatory mechanisms that regulate cognitive processes (Lendvai et al., 2013; Wallace and Bertrand, 2013; Wallace and Porter, 2011). Both agonists and modulators of α7nAChR improve episodic and working memory and attention in multiple preclinical models.

2.1.2. Peripheral Expression and Function—The α7nAChR is found on multiple types of cells in the periphery including neuroendocrine cells in the lung, where Ca^{++} influx leads to release of bombesin and other peptides (Aguayo, 1994). An important role for α7nAChR in peripheral systems seems to be as a modulator of inflammatory responses, where activation of the receptor prevents release of cytokines such as TNFα, IL-6, IL-8 and high mobility group B protein-1 (HMGB1) (de Jonge and Ulloa, 2007; Wang et al., 2003). This response requires STAT3 protein expression in macrophages (Pena et al., 2010), and the Ly-6 protein family in keratinocytes (Chernyavsky et al., 2010). Additional sites of this ubiquitous receptor include in the skin (Ortiz and Grando, 2012), bone marrow (Pinheiro et al., 2011), fibroblast-like synoviocytes (van Maanen et al., 2009), and sperm (Son and Meizel, 2003). The wide expression of α7nAChR in neuroendocrine tissues suggests that Ca^{++} entry through this receptor may modulate a large variety of functions in the organism.

2.1.3. Expression in Development—The α7nAChR is expressed early in hippocampal development in the mouse at Embryonic Day 13 (E13) and peaks in the neonatal period at Postnatal Day 5 (P5) (Adams et al., 2002), suggesting that it influences early processes such as neuronal migration, dendritic formation and pruning (Aramakis et al., 2000; Catone and Ternaux, 2003; Morley and Mervis, 2013). Expression parallels the development of calcium binding proteins, probably because overexpression of *Chrna7* before these proteins are expressed is toxic (Berger et al., 1998).

The early switch of chloride channels, regulating the change of positive to negative stimulation and accompanied by down regulation of *NKCC1* and up regulation of *KCC2*, is controlled by the expression of α7nAChR (Liu et al., 2006). The switch does not occur in murine models with decreased *Chrna7* expression.

Less is known about the localization of *CHRNA7* gene expression in human development. Nicotinic receptors, including α7nAChR, are present in human prenatal brain and spinal cord at 4–6 weeks gestation, comparable to that in rodents based on hippocampal development (Hellstrom-Lindahl and Court, 2000). *CHRNA7* mRNA declines with age. In

adult postmortem brain, *CHRNA7* mRNA and protein are expressed in most brain regions, with highest expression in nuclei involved in cognitive processing (Breese et al., 1997a). The developmental gene expression switch from *NKCC1* to *KCC2*, described above, that regulates the inhibitory effects of GABA is impaired in schizophrenic individuals (Arion and Lewis, 2010; Morita et al., 2014; Tao et al., 2012). In humans the role of α7nAChR is likely to be important for competent development of both synaptic and peripheral mechanisms.

2.2 Regulation of Human CHRNA7

2.2.1 Regulation by Nicotine—It has been known since 1983 that chronic exposure to nicotine increases the number of high-affinity nicotinic receptors. In human brain, this is measured by [^{3}H]-nicotine or [^{3}H]-epibatidine binding (Breese et al., 1997b; Perry et al., 1999), or by positron emission tomography scanning using the α4β2* nAChR ligand 2-[18F]fluoro-A-85380 (Brody et al., 2013). As in rodents, this “up regulation” occurs in humans in a dose-dependent manner in which heavier smokers have much higher levels of [^{3}H]-nicotine binding, essentially doubling the number of receptors (Breese et al., 1997b). [^{3}H]-Nicotine binding is also detected in polymorphonuclear cells (PMN) isolated from human blood, where a similar up regulation in the number of receptors is found in smokers and the binding is also dose dependent (Benhammou et al., 2000). In schizophrenic smokers, high-affinity nicotine binding does not increase in postmortem brain (Breese et al., 2000), or in leukocytes (Leonard, 2014).

The mechanism for up regulation by nicotine in rodent brain is thought to occur at the protein level rather than the transcriptional level (Henderson and Lester, 2014; Pauly et al., 1991). While nicotine binding may promote protein stabilization, there is evidence that nicotine binding in the endoplasmic reticulum may act as a pharmacological chaperone to bring receptors out of the endoplasmic reticulum, eventually increasing function and receptor binding at the plasma membrane (Kuryatov et al., 2005; Lester et al., 2009).

As in rodents, the human α7nAChR is less sensitive to up regulation by nicotine than is the high-affinity receptor, probably due to the increased IC_{50} (Marks et al., 1986). While high-affinity nicotine binding is almost doubled in hippocampus of smokers [^{125}I]-α-bungarotoxin binding, specific for α7nAChRs, is increased only in very heavy smokers (Breese et al., 2000).

Nicotine intake in humans results in changes in hippocampal gene expression of more than 200 genes (Mexal et al., 2005). Both NR2A and NR2B NMDA receptor subunits are up regulated in postmortem brain of smokers, as are other genes in the NMDA postsynaptic density. Some of the changes in smokers appear to be permanent (Hope et al., 2007). Regulation of gene expression by smoking is different in schizophrenic patients (Mexal et al., 2005). While many genes are up or down regulated in a similar manner to control subjects, more than 75 genes are differentially regulated in patients. One of these genes is *CHRNA7*. Expression of both mRNA and protein for *CHRNA7* is low in schizophrenic non-smokers, compared to controls, but is brought to control levels in smokers (Mexal et al., 2010). Schizophrenic smokers, thus, appear to express equivalent α7 protein to that in control smokers. However, schizophrenics have 50% less [^{125}I]-α-bungarotoxin binding in

hippocampus (Freedman et al., 1995), in cortex (Guillozet-Bongaarts et al., 2014; Marutle et al., 2001), and in the reticular nucleus of the thalamus (Court et al., 1999). These results suggest there may be aberrant assembly or trafficking of receptors to neuronal membranes in schizophrenic patients.

2.2.2. Regulation by Transcriptional Mechanisms—Promoter polymorphisms have been identified in *CHRNA7*. In a proximal promoter of 300bp, immediately upstream of the translation start site, 21 polymorphisms are known (Leonard et al., 2002), most of which decrease transcription in an *in vitro* assay. This is a large number of polymorphisms for such a small DNA fragment, considering that the average mutation rate is 1 in 10^8 nucleotides per generation (Nachman and Crowell, 2000), and promoter sequence is usually conserved. *CHRNA7* promoter mutations are associated with schizophrenia and with sensory processing deficits (Leonard et al., 2002).

The *CHRNA7* proximal promoter can also be methylated, which decreases transcription (Canastar et al., 2012). In SHEP cells, a human permanent neuroblastoma cell line commonly used in neuroscience research (Biedler et al., 1978), the *CHRNA7* promoter is heavily methylated, preventing expression of this gene. Further, in human tissues, *CHRNA7* mRNA levels are correlated with the extent of promoter methylation (Canastar et al., 2012).

The *AP2-α* gene has been identified as a potent repressor of *CHRNA7* transcription (Finlay-Schultz et al., 2011). AP-2α is a transcription factor that binds in the proximal promoter of *CHRNA7*.

The neuregulin gene, *NRG1*, regulates *CHRNA7* transcription; polymorphisms in the promoter region of *NRG1* are associated with levels of *CHRNA7* mRNA (Mathew et al., 2007).

2.2.3. Regulation of Function—Although α7nAChR generally assemble as homomers, a heteromeric α7 receptor containing β2 subunits has been reported in human basal forebrain (Liu et al., 2009). This heteromer has slower kinetics with nicotinic receptor agonists and is inhibited by oligomeric amyloid beta ($A\beta_{1\text{-}42}$), suggesting the heteromer might be involved in decreased cholinergic function in Alzheimer's disease. Binding of amyloid may increase the trafficking of α7nAChR to the plasma membrane, inducing a hyperexcitability and/or excitotoxity (Liu et al., 2013).

The tryptophan metabolite kynurenic acid, an NMDA receptor antagonist, decreases function of α7nAChR and increases expression of high-affinity nicotinic receptors (Hilmas et al., 2001). Schizophrenic patients have higher levels of kynurinine (Erhardt et al., 2001; Schwarcz et al., 2012), which could disrupt cognitive and sensory processes (Albuquerque and Schwarcz, 2013; Pocivavsek et al., 2012).

There is evidence for regulation by neurosteroids (Bullock et al., 1997). Steroids appear to be non-competitive antagonists, binding in the channel of the receptor (Grun et al., 1995). Chronic corticosterone treatment in adrenalectomized mice reduces [^{125}I]-α-bungarotoxin binding and auditory gating in C3H mice (Stevens et al., 2001). Other compounds, including antidepressant drugs such as fluoxetine and bupropion also bind in the channel of

α7nAChR, resulting in inhibition (Fryer and Lukas, 1999a, b). Functional promoter mutations that decrease *CHRNA7* transcription are associated with a decreased cortisol response to a blood draw stressor (Sinkus et al., 2010). These results suggest that regulation of α7nAChR by steroids is likely to be important for the role of this receptor in infection and stress responses (Rosas-Ballina and Tracey, 2009).

The peptide product of the *CHRFAM7A* gene also regulates function of α7nAChRs, as discussed in the Section 3.

3. The Duplicated Alpha 7 Gene, *CHRFAM7A*

As described in Section 1, The *CHRNA7* gene was partially duplicated relatively late in evolutionary history, forming a new gene, *CHRFAM7A*, mapping 1.6 Mb centromeric to the parent gene. As a recent discovery, much less is known about the duplication.

3.1 Localization and Expression of the CHRFAM7A Gene

The *CHRFAM7A* gene is expressed in both human brain and in the periphery. *CHRFAM7A* mRNA represents approximately 10–20% of the α7 sequence in the mRNA of human brain (de Lucas-Cerrillo et al., 2011; Gault et al., 1998). Most of the transcripts begin in exon D and contain only sequence from exons D, C, B, and A. Rare transcripts contain exon E or begin in exon F or G.

CHRFAM7A is transcribed efficiently, but is translated poorly. Compared to translation of *CHRNA7* mRNA, translation of *CHRFAM7A* mRNA occurs with a 5% efficiency (Araud et al., 2011; Wang et al., 2014). Putative translation start sites are shown in Figure 3, and amino acid sequences of the peptides in Figure 4. *In silico* analysis of the *CHRFAM7A* mRNA suggests that translation from the AUG codon in exon B results in *CHRNA7* sequence from amino acid 117 (Figure 3B.1). The translated peptide subunit, dupα7, would be missing one glycosylation site, but retain the cysteine bridge and vicinal cysteines of the agonist binding site (Figure 4B). If the 2bp deletion in exon 6 is present, a translational start in exon B would lead to a truncated peptide (Figure 3B.2). However, there are two AUG codons in exon 6 (Figure 2B.3). It is, thus, likely that translation starts from one of these codons, resulting in a peptide that is out of frame for either 5 or 12 amino acids (Araud et al., 2011). Then the 2bp deletion would change the reading frame back to that of *CHRNA7* (Figure 3B.3). This peptide, dupΔα7, would be missing all of the glycosylation sites and the cysteine bridge (Figure 4C). Assembly of only one dupα7 subunit with four α7 subunits would remove two of the five agonist binding sites (Figure 4D).

3.2 Function of the CHRFAM7A Gene

3.2.1 The CHRFAM7A and CHRFAM7AΔ2bp Gene Products, dupα7 and dupΔα7 Assemble with α7 Subunits—Assembly of the chimeric gene products dupα7 and dupΔα7 with α7 subunits to form the receptor (α7dupα7*) was studied utilizing Förster fluorescence energy transfer (FRET) (Wang et al., 2014). FRET was measured with an acceptor photobleaching method, previously adapted for nAChR subunits (Drenan et al., 2008; Nashmi et al., 2003), and with a fluorescent lifetime imaging technique newly applied to nAChRs (Wang et al., 2014). Positive FRET is detected only when subunits are <12 nM

apart. Several fluorescent moieties were cloned into the large cytoplasmic loop portion of the genes. After transfection of N2A cells with either dupα7 or dupΔα7, and α7, FRET measurements show that the duplicated and WT subunits are in close proximity and likely assemble together (Wang et al., 2014). Competition with unlabeled subunits confirmed the result. The stoichiometry is not yet known. Interestingly, FRET measurements show that the *CHRFAM7A* gene products, dupα7 and dupΔα7, also assemble with α4 and α3 nicotinic receptor subunits.

Electrophysiological experiments utilized the substituted cysteine accessibility mutagenesis technique (Akabas et al., 1994; Akabas et al., 1992). A dupα7 or dupΔα7 subunit, Cys-mutated in the M2 region, was coexpressed with α7. As a result, ACh responses were decreased by alkylation of the Cys residue with ethylammonium methanethiosulfonate (MTSEA). This shows that dupα7 co-assembles with native α7 subunits to form functional nAChRs; likewise for dupΔα7 subunits. Functional coassembly is likely possible if only a single dupα7 or dupΔα7 subunit is present in the pentamer (dupα7:α7 or dupΔα7:α7 subunit molar ratio < 1)(Wang et al, 2014). Inspection of Figure 4D indicates that incorporation of two adjacent dupα7 or dupΔα7 subunits would decrease the number of agonist binding sites in the pentamer to only two; and if the two dupα7 or dupΔα7 subunits are non-adjacent, only a single binding site survives. Earlier experiments show that α7nAChRs are poorly activated with only one or two of the binding sites occupied (Murray et al., 2012).

3.2.2. The Duplicated Gene Products are Dominant Negative Regulators of α7nAChR Function—The *CHRFAM7A* gene product, dupα7, was identified in lymphocytes but does not exhibit acetylcholine or nicotine binding when expressed alone (Villiger et al., 2002). Expression of α7 and dupα7 in a 1:1 molar ratio in oocytes resulted in a decrease of acetylcholine-stimulated current by more than 30% (Araud et al., 2011; de Lucas-Cerrillo et al., 2011), suggesting that the *CHRFAM7A* gene product (dupα7) is a dominant-negative regulator of ion channel function. The dupΔα7 subunit, containing a 2bp deletion in exon 6, is a more potent inhibitor than the wild-type dupα7 subunit (Araud et al., 2011). Thus, the number of copies and mutation status of *CHRFAM7A* can regulate *CHRNA7* function.

Binding of [^{125}I]-α-bungarotoxin to the α7dupα7* receptor was also decreased, compared to oocytes expressing only *CHRNA7* (Araud et al., 2011). It is not known whether the decreased toxin binding is due to sequestration of the receptor in the endoplasmic reticulum or because two [^{125}I]-α-bungarotoxin binding sites are lost for each dupα7 or dupΔα7 subunit incorporated in the α7dupα7* heteromer (Figure 4D). The ratio of α7 to dupα7 and the presence of the 2bp deletion (dupΔα7) is, therefore, important for evaluating the overall function of α7nAChR in human patients.

3.3 Regulation of the CHRFAM7A Gene

A promoter for the *CHRFAM7A* gene has not been characterized, but may contain exon D, which has Sp-1 and AP2α binding sites. Promoter investigation in *CHRFAM7A* is complicated by a gene inversion that is present in alleles containing the 2bp deletion in exon 6 (CHRFAM7AΔ2bp; dupΔα7)(Flomen et al., 2008). Regulatory regions may differ for this

allele, present in 42% of Caucasian subjects. Further, long distance regulation for both *CHRNA7* and *CHRFAM7A* has recently been reported. Chromosome conformation capture on chip (4C) analysis was utilized to show genomic interaction of DNA sequence at chromosome 15q11.2 with both *CHRFAM7A* and *CHRNA7* at 15q13.3 (Yasui et al., 2011).

Very little is known about the effects of nicotine on the *CHRFAM7A* gene. In macrophages 1μM nicotine down regulates *CHRFAM7A* mRNA by approximately 50% (de Lucas-Cerrillo et al., 2011). A quantitative determination of *CHRFAM7A* mRNA levels in primary lymphocytes from control and schizophrenic smokers and non-smokers is shown in Figure 5. In human lymphocytes from non-mentally ill control subjects, *CHRFAM7A* mRNA levels are not significantly decreased by nicotine, as is seen in macrophages. However, schizophrenic individuals have significantly more *CHRFAM7A* mRNA than do controls and smoking does not down regulate the higher mRNA levels in patients. *CHRFAM7A* mRNA is also up regulated in the prefrontal cortex (PFC) of schizophrenic patients and the up regulation is not changed in schizophrenic smokers (Dr. Barbara Lipska, NIMH, personal communication). Thus, schizophrenic patients may have increased levels of *CHRFAM7A* expression in multiple central and peripheral tissues that are not affected by smoking.

The *CHRFAM7A* gene product, dupα7, is a dominant negative regulator of α7nAChR function and results in decreased binding of [^{125}I]-α-bungarotoxin to the heteromeric receptor α7dupα7* (Araud et al., 2011). Therefore, increased expression of the *CHRFAM7A* gene in schizophrenic individuals may account for the decreased [^{125}I]-α-bungarotoxin binding seen in these patients. Dupα7 and dupΔα7 also assemble with α4 and α3 subunits (Wang et al., 2014). It is possible that the increased expression of *CHRFAM7A* in schizophrenics might also account for the failure to up regulate nicotine binding to high affinity receptors (Breese et al., 2000).

In the immune system, infection regulates levels of *CHRFAM7A* mRNA. Lipopolysaccharide (LPS) treatment of the monocytic cell line, THP-1, decreases transcription of *CHRFAM7A* (Benfante et al., 2011). This would increase the function of α7nAChRs and decrease levels of cytokines (de Jonge and Ulloa, 2007; Pavlov et al., 2009).

Changes in expression of *CHRFAM7A* and *CHRFAM7AΔ2bp* may, thus, be critical for multiple functions in human subjects.

4.0 Mutation in the *CHRNA7/CHRFAM7A* gene cluster

Mutation screening in both of these genes was done in mRNA as there is more than 99% conservation in the duplicated *CHRNA7* exons 5-10 (Gault et al., 2003).

4.1 Mutation in CHRNA7

An mRNA screen in 300 schizophrenics and control subjects found only a rare mutation in the coding region of the *CHRNA7* gene and none of these were associated with schizophrenia (Gault et al., 2003). The proximal promoter contains a large number of polymorphisms, which are associated with schizophrenia and also with the P50 auditory gating deficit (Leonard et al., 2002). Promoter mutation generally down regulates

transcription of the *CHRNA7* gene. A polymorphism 1830bp upstream in the 5′-regulatory region (not duplicated) of *CHRNA7* (rs3087454) is strongly associated with schizophrenia (Stephens et al., 2009). This same SNP is associated with improvement in the fMRI default network with an α7nAChR partial agonist, DMXB-A (Tregellas et al., 2011), and with normal infant P50 gating following perinatal choline administration (Ross et al., 2013). These results suggest that rs3087454 can be used successfully in pharmacogenomics studies.

4.2 Mutation in CHRFAM7A

In the *CHRFAM7A* gene, a 2bp deletion in exon 6 was found (Gault et al., 1998). This allele, *CHRFAM7AΔ2bp*, is present much more frequently in Caucasian individuals (42%) than in African Americans (14%) and is significantly associated with schizophrenia (Sinkus et al., 2009). The 2bp deletion is associated with the P50 deficit (Flomen et al., 2013; Raux et al., 2002), with poor episodic memory (Dempster et al., 2006), and is inversely associated with idiopathic generalized epilepsy (Rozycka et al., 2013). The 2bp deletion in *CHRFAM7A* is found less frequently in individuals with a *CHRNA7* promoter mutation, and is in linkage disequilibrium with a 3bp intronic insertion in *CHRNA7* exon 7, -11insGTT, the latter of which might lead to alternative splicing (Gault et al., 2003; Rozycka et al., 2013). *CHRFAM7AΔ2bp* (dupΔα7) is a more potent dominant negative regulator of α7nAChR function than the normal copy, *CHRFAM7A* (dupα7) (Araud et al., 2011).

5.0 Copy Number Variation in *CHRNA7* and *CHRFAM7A*

CHRFAM7A varies in copy number; some individuals have only one copy of *CHRFAM7A* and a rare subject has no copies. Copy number of the *CHRNA7* gene is not as variable; deletion and duplication are rare. We developed a copy number assay for alleles of *CHRFAM7A*, utilizing real-time quantitative PCR and exon-specific primers (Flomen et al., 2006; Sinkus et al., 2009). Table 1 shows copy number data for the *CHRNA7* gene (7) and the *CHRFAM7A* gene (7A) in 772 individuals, 322 control subjects and 450 schizophrenics. Copy number variations are found in both cohorts. The most common genotype is two copies of each gene, 7(2)/7A(2) (78%). Approximately 20% of individuals have only one copy of *CHRFAM7A*, and only 1% have no copies. Other subjects (3%) have extra copies of *CHRNA7* and/or *CHRFAM7A*. There are no significant differences in copy number in the schizophrenic patients in this cohort.

Deletions involving the *CHRNA7* and *CHRFAM7A* genes are rare but quite strongly associated with schizophrenia (Stefansson et al., 2008; Stone et al., 2008)(Table 2). The *CHRNA7* gene is deleted in these cases but the *CHRFAM7A* gene is usually present, leaving the patient with only one copy of *CHRNA7* and two copies of the dominant-negative regulatory gene, *CHRFAM7A*.

Similar and rare deletions and duplications in this same region have been reported in mental retardation, autism, seizures, and bipolar disorder (Masurel-Paulet et al., 2010). Rare, large duplications of 15q13.3 are found in ADHD (Williams et al., 2012b). In all of these reports, the large deletions and duplications are rare, but strongly associated with disease. There are six repetitive regions on Chromosome 15 identified as breakpoint regions (BP) in Prader-Willi Syndrome (Mewborn et al., 2001; Miller et al., 2009). Approximately 20% of Prader-

Willi patients exhibit psychoses (Boer et al., 2002; Vogels et al., 2004). Deletions found in schizophrenia and other mental disorders involve BP4 and BP5 (Mewborn et al., 2002), which contain the *CHRNA7/CHRFAM7A* gene cluster.

Mapping of the chromosomal aberrations in most of these studies generally do not make it clear whether both the *CHRNA7* and *CHRFAM7A* genes are affected; they are only 1.6Mb apart. Since the *CHRFAM7A* gene product, dupα7, inhibits α7nAChRs, the number of *CHRFAM7A* copies remaining could have an important effect on function of α7nAChRs in these subjects.

6.0 Genetics of *CHRNA7* and *CHRFAM7A* in Mental Illnesses

The possible polymorphisms that can be used for genetic studies in these two genes are limited and complex. The *CHRNA7* proximal promoter (not duplicated) is polymorphic with 21 known mutations (Leonard, 2014; Leonard et al., 2002), most of which decrease transcription, suggesting that heterogeneity at this locus is an important consideration. In initial studies, we did find association of a single promoter SNP at −86C/T to schizophrenia, but the association was stronger for grouped functional variants both to schizophrenia and to the P50 deficit (Leonard et al., 2002).

The genetic association of most of the *CHRNA7* gene with mental disorders is complicated by the partial duplication of this gene (Gault et al., 1998). Exons 5-10 of the parent gene are duplicated, incorporated in a new, expressed gene, *CHRFAM7A*, mapping 1.6Mb from *CHRNA7*. The duplicated sequence in *CHRFAM7A* is 99.9% identical to that in *CHRNA7*. Therefore, markers in exons 5-10 cannot be correctly used for genetic studies unless they have been mapped. The *CHRNA7* gene does have two very large introns (introns 2 and 4). Intron 2 is not duplicated and much of intron 4 is not duplicated. SNPs in this part of the gene can be queried. However, the remaining half of the gene cannot be examined in the newer high-throughput genetic studies such as genome wide association studies (GWAS). This has likely resulted in a gross underestimation of association of the *CHRNA7* gene with mental illness and other diseases. Table 2 summarizes positive genetic results, utilizing mapped markers in non-duplicated regions, for linkage and association of *CHRNA7* and *CHRFAM7A* in mental illness.

6.1. Schizophrenia

The expression of α7nAChRs is decreased in multiple regions of postmortem brain in schizophrenic subjects, including the hippocampus (Freedman et al., 1995), cortex (Guan et al., 1999; Guillozet-Bongaarts et al., 2014; Marutle et al., 2001), and the reticular thalamic nucleus (Court et al., 1999).

Genetically, the *CHRNA7* gene was initially linked to schizophrenia, utilizing a dinucleotide marker, D15S1360, in intron 2. Linkage was more significant to a sensory deficit, the P50 (LOD = 5.3), which is inherited as an autosomal dominant trait (Freedman et al., 1997). Subsequent positive linkage to schizophrenia was found to other microsatellite markers near the *CHRNA7* gene at 15q13.3 that are not in duplicated regions (Faraone et al., 2004; Freedman et al., 2001; Gejman et al., 2001; Kaufmann et al., 1998; Liu et al., 2001; Riley et

al., 2000; Tsuang et al., 2001)(Table 2). Other groups did not find linkage to this region using similar methodology (Curtis et al., 1999; Iwata et al., 2007; Neves-Pereira et al., 1998). An association study of 14 candidate genes with schizophrenia in a large European cohort failed to find significant association to any of these genes, highlighting the importance of heterogeneity in the genetics of mental disorders (Sanders et al., 2008).

A SNP, rs3087454, in the 5′-UT of *CHRNA7* was associated with schizophrenia (Stephens et al., 2009), with a positive fMRI improvement in the default network with DMXB-A treatment in schizophrenia (Tregellas et al., 2011), and with a positive effect on the P50 sensory processing measure in newborns following perinatal choline administration (Ross et al., 2013)(Table 2).

Several studies support the association of the 2bp deletion in exon 6 of the *CHRFAM7A* gene, with schizophrenia (Table 2). Although the 2bp deletion is more common in Caucasians, association to schizophrenia is found in both African Americans and Caucasians (Sinkus et al., 2009). It is also a risk factor for having a P50 sensory processing deficit (Flomen et al., 2013; Raux et al., 2002) and poor episodic memory (Dempster et al., 2006). It seems to be protective for some forms of epilepsy (Rozycka et al., 2013). Lai et al. did not find association in schizophrenia, but copy number was not determined in this study (Lai et al., 2001).

6.2. Bipolar Disorder

The expression of α7nAChRs, as measured by [^{125}I]-α-bungarotoxin binding, appears to be increased in hippocampus of bipolar patients (Thomsen et al., 2011). The association of the *CHRNA7* gene cluster with bipolar disorder has been supported in multiple studies, including genetic linkage (Turecki et al., 2000), association (Ancin et al., 2011)(Table 2), and in neurobiological studies of the P50 deficit (Leonard et al., 2001; Martin et al., 2007). Patients with bipolar disorder have cognitive deficits involving attention that are similar to patients with schizophrenia (Ancin et al., 2010b). Bipolar patients with psychotic symptoms also have deficits in the sensory processing deficit phenotype, P50 gating (Sanchez-Morla et al., 2008). In bipolar type schizoaffective disorder, P50 deficits are associated with the presence of grouped functional mutations in the *CHRNA7* proximal promoter (Martin et al., 2007).

SNP analysis in the non-duplicated region of *CHRNA7* shows association for decreased risk of bipolar disorder with rs6494223 in intron 3 of *CHRNA7* (Ancin et al., 2010a), a haplotype containing this SNP is associated with impaired attention in the disorder (Ancin et al., 2011). A single promoter SNP in *CHRNA7*, −86C/T, was also genotyped, but no association was found. As previously mentioned, the proximal promoter is extremely heterogeneous, suggesting that depending on the population single SNPs are not common enough to produce positive results.

The presence of the 2bp deletion in the duplicated alpha 7 gene, *CHRFAM7A*, that functionally inhibits α7nAChR function is significantly associated with bipolar disorder as well as schizophrenia (Hong et al., 2004)(Table 2). This study also found more than two alleles of the 2bp deletion genotype in several bipolar subjects.

6.3 Autism

Expression of the α7nAChR is decreased in postmortem brain of both Rett syndrome and in autism (Ray et al., 2005; Yasui et al., 2011). As in schizophrenia and bipolar disorder, auditory evoked responses are abnormal (Dinstein et al., 2012; Orekhova et al., 2008). A large genetic linkage study of autism pedigrees in Utah shows significant linkage at 15q13.1-q14, the locus of the *CHRNA7/CHRFAM7A* gene cluster (Allen-Brady et al., 2010) (Table 2). Rare chromosomal deletions in autistic patients have also been reported at this locus (Pinto et al., 2010).

These results suggest that common deficits in attention and focus are found in schizophrenia, bipolar disorder and autism, and further that the *CHRNA7/CHRFAM7A* gene locus may have an important role.

6.4 Alzheimer's disease

Alzheimer's disease (AD) is denoted by cholinergic denervation of the cortex, which most severely affects the hippocampus and temporal lobes (Craig et al., 2005). Pathological findings include extracellular plaques containing β amyloid (Aβ) peptide and neurofibrillary tangles of hyperphosphorylated tau protein (Sivaprakasam, 2006). $A\beta_{1\text{-}42}$ and α7nAChR are co-localized in many regions, including the hippocampus, where they associate with pM affinity (Wang et al., 2000). In normal brain, soluble $A\beta_{1\text{-}42}$ is present in pM concentrations, binding near the agonist site and activating α7nAChR. However, as AD progresses $A\beta_{1\text{-}42}$ concentrations increase to the nM range, resulting in inactivation of α7nAChRs. For a review, see (Parri et al., 2011). A recent report shows that α7 subunits assemble with β2 subunits to form α7β2* receptors in select regions such as the basal forebrain, where they exhibit increased affinity for $A\beta_{1\text{-}42}$ and are up regulated by this peptide (Liu et al., 2009; Murray et al., 2012). Binding of high (nM) concentrations of $A\beta_{1\text{-}42}$ induces a hyperexitability, which may affect receptor up regulation and development of AD symptomatology (Liu et al., 2013). The gene product of *CHRFAM7A*, dupα7 only found in human subjects, also assembles with α7 subunits forming functional α7dupα7* receptors (Wang et al., 2014). It will be of interest to investigate possible association of Aβ peptides with this newly characterized receptor.

Multiple genetic studies suggest an important role for *CHRNA7* in Alzeimer's disease (Table 2). A haplotype block in the 5′-UT and intron 2 of *CHRNA7*, containing rs1514246, rs2337506, and rs8027814, is associated with decreased risk of AD (Carson et al., 2008b). The same investigative group found association of another *CHRNA7* SNP, rs6494223 in intron 3, to AD with delusions (Carson et al., 2008a). This latter SNP was also associated with impaired attention in bipolar disorder, discussed above (Ancin et al., 2011). Acetylcholinesterase inhibitors (AEI), such as galantamine are frequently utilized for treatment of AD (Taylor, 1998). Women with AD carrying the polymorphism rs8024987 in intron 2 of *CHRNA7* show improved cognitive symptoms with AEI (Weng et al., 2013).

Genetics and functional studies, thus, indicate an important role for the *CHRNA7/CHRFAM7A* gene group in Alzheimer's disease.

7.0 Cognition and the *CHRNA7/CHRFAM7A* Gene Cluster

Expression and function of α7nAChR affects multiple disorders. All of the mental illnesses discussed above are characterized by cognitive disorders, such as attention and working memory, in which α7nAChRs are known to play a role. Additional mental disorders in which cognition and the α7nAChR have been implicated include Down's syndrome (Deutsch et al., 2014), and Parkinson's disease (Quik et al., 2013). There are common and measurable phenotypes for cognitive deficits that will be useful for drug development (Levin, 2013), such as episodic memory (acquisition, consolidation, retrieval), working memory (temporary information storage), attention (ability to focus), and sensory processing (P50 gating), reviewed in (Wallace and Bertrand, 2013; Wallace and Porter, 2011). Medications developed to treat cognitive deficits will likely be helpful across disorders.

8.0 Drug Development

The efficacy of therapeutic agents targeted toward the α7nAChR was first suggested in studies of atypical neuroleptics. The atypical antipsychotic, clozapine, although having adverse side effects on neutrophil count and also on weight gain, is the most effective treatment for schizophrenia to date (McEvoy et al., 2006; Miyamoto et al., 2012). Clozapine, has a complex pharmacology with a higher affinity for $5HT_{2A}$ serotonin receptors than for dopamine D_2, which partially defines atypicals (Meltzer et al., 1989; Miyamoto et al., 2012). Of clozapine's multiple targets, it is an antagonist of the $5HT_3$ serotonin receptor, blockade of which releases high levels of acetylcholine (Shirazi-Southall et al., 2002). The effects of clozapine on the positive symptoms of psychosis, such as hallucinations and delusions, are most likely mediated through these three receptors. The IC_{50} for $5HT_3$ is 0.9μM in oocytes. Clozapine inhibits α7nAChR at higher concentrations, IC_{50}=3.2μM, also in oocytes (Singhal et al., 2007). At therapeutic doses in humans (0.5–2μM) clozapine has positive effects on sensory processing deficits (Nagamoto et al., 1996) and decreases smoking in schizophrenic patients (McEvoy et al., 1995). These results suggest that increased release of acetylcholine from the $5HT_3$ blockade may be targeting nicotinic receptors. In support of this hypothesis, other $5HT_3$ blockers such as olanzapine (Simosky et al., 2003) and tropisetron (Koike et al., 2005; Zhang et al., 2012) also normalize P50 sensory processing. Currently, little is known about the pharmacology of α7nAChRs containing the *CHRFAM7A* subunit, dupα7. Coassembly of α7 and dupα7 results in decreased [^{125}I]-α-bungarotoxin binding (Araud et al., 2011) and altered sensitivity to both varenicline and choline (Wang et al., 2014). It is reasonable to suggest that clozapine might have an altered affinity for an α7dupα7* receptor in humans. Relative effects of clozapine at α7dupα7*, compared to its known effects on other receptors, such as $5HT_3$, might explain the positive outcome for the P50 deficit. For example, clozapine may preferentially block α7dupα7* receptors, diverting acetylcholine to receptors containing only α7 subunts.

Varenicline, a full agonist at α7nAChR (Mihalak et al., 2006), improves cognition and has anti-smoking effects in schizophrenic patients (Smith et al., 2009; Williams et al., 2012a). Receptors containing the *CHRFAM7A* gene products, dupα7 and dupΔα7 have increased sensitivity to varenicline, which may account for reports of exacerbation of psychosis in a

few cases (Freedman, 2007; Wang et al., 2014). The increased sensitivity also suggests that varenicline or related compounds may be helpful for smoking cessation in this disorder.

The first clinical trial of an α7nAChR targeted drug, the partial agonist 3-[(2,4-dimethoxy) benzylidene] anabaseine (DMXB-A, GTS-21) (Mahnir et al., 1998) resulted in positive results for both sensory processing deficits and attention measures in schizophrenia (Freedman et al., 2008; Olincy et al., 2006). The default network in human brain, aberrant in schizophrenic patients, shows improvement following treatment with DMXB-A (Tregellas et al., 2011). The improvement is associated with a SNP (rs3087454) in the 5′-upstream regulatory region of the *CHRNA7* gene, previously associated with schizophrenia (Table 2) (Stephens et al., 2009). DMXB-A may also be useful in Parkinson's disease in a neuroprotective role (Suzuki et al., 2013).

A new therapeutic approach of treating sensory deficits prenatally with choline, a specific agonist of the α7nAChR, is showing some promise. The α7nAChR is expressed very early in development, during the time of neuronal migration and synapse formation (Adams et al., 2002; Broide et al., 1995). Choline is essential for brain development (Zeisel, 2006) and levels are often low in pregnancy (Ross et al., 2010). A recent study, utilizing infant sensory processing where P50 suppression is measured when the baby is sleeping (Hunter et al., 2008), shows that prenatal choline during fetal development results in normal P50 measures in the infant (Ross et al., 2013). The positive effects are associated with the risk allele for SNP rs3087454 in the *CHRNA7* gene.

α7nAChR containing the *CHRFAM7A* gene products dupα7 and dupΔα7 have altered sensitivities to choline (Wang et al., 2014). Heteromers of α7dupα7* have an increased sensitivity to choline, but heteromers containing dupΔα7 with the 2bp deletion in exon 6 do not demonstrate this sensitivity. Thus, *CHRFAM7AΔ2bp* carriers may have altered responses to choline early in development and benefit from dietary choline supplements.

Heteromeric α7dupα7* receptors are also potentiated efficiently by Type II positive allosteric modulators (PAMs), such as 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea (PNU-120596), which acts in the transmembrane portion of the receptor, affecting the duration of channel opening (Araud et al., 2011; Bertrand et al., 2008). Type II PAMs have the advantage that they do not up regulate receptors, as do agonists of the α7nAChR (Christensen et al., 2010).

One of the major issues with the development of drugs targeted to the α7nAChR is the partial duplication of *CHRNA7* to form a chimeric gene, *CHRFAM7A*, which functions as a dominant negative regulator (Araud et al., 2011). The gene duplication is not found in either rodents or in primates (Locke et al., 2005) and, thus, no animal models exist to act as surrogates for drug testing of an α7dupα7* receptor. While drugs targeted to the α7nAChR have and are being developed, it will be difficult to determine efficacious doses in preclinical testing until animal models expressing *CHRFAM7A* can be generated. Testing in animals expressing only the *Chrna7* gene has likely resulted in fewer positive results in some clinical trials, where more optimum dosing might have been effective.

8.0 Summary and Future Directions

The *CHRNA7* gene cluster is ubiquitously expressed in the human body and has roles in CNS and peripheral development, cognitive performance, and inflammation. The early appearance of α7nAChRs in evolution as an important source of calcium entry into the cell, may explain its residual peripheral functions and development of synaptic roles. The *CHRNA7* gene is the parent of other nicotinic receptors, and of a recent additional duplication to form the new gene, *CHRFAM7A*, only found in humans. Regulation of expression and function of the α7nAChR is complex with more than a dozen mechanisms known to date, including regulation by its own duplication. Each of these requires further investigation, particularly in human cohorts, and studies on the regulation and expression of the *CHRFAM7A* gene have only just begun.

The α7nAChR plays a strong role in cognitive phenotypes, which are aberrant in both mental and degenerative diseases. Preclinical drug evaluation will need to address altered efficacy of compounds at α7dupα7* receptors, which are not found in current animal models. Focus on the development of α7dupα7* receptor agonists and modulators that can enhance cognition in human subjects may benefit multiple disorders.

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Abbreviations

dupα7	gene product of *CHRFAM7A*
dupΔα7	gene product of *CHRFAM7AΔ2bp*
α7dupα7*, α4β2*, α7β2*	heteromeric receptors with unknown stoichiometry
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors

References

Adams CE, Broide RS, Chen YL, Winzer-Serhan UH, Henderson TA, Leslie FM, Freedman R. Development of the alpha 7 nicotinic cholinergic receptor in rat hippocampal formation. Dev Brain Res. 2002; 139:175–187. [PubMed: 12480132]

Aguayo SM. Determinants of susceptibility to cigarette smoke. Potential roles for neuroendocrine cells and neuropeptides in airway inflammation, airway wall remodeling, and chronic airflow obstruction. Am J Resp Crit Care Med. 1994; 149:1692–1698. [PubMed: 7911710]

Akabas MH, Kaufmann C, Archdeacon P, Karlin A. Identification of acetylcholine-receptor channel-lining residues in the entire M2 segment of the alpha-subunit. Neuron. 1994; 13:919–927. [PubMed: 7524560]

Akabas MH, Stauffer DA, Xu M, Karlin A. Acetylcholine-receptor channel structure probed in cysteine-substitution mutants. Science. 1992; 258:307–310. [PubMed: 1384130]

Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: From structure to function. Physiolog Rev. 2009; 89:73–120.

Albuquerque EX, Schwarcz R. Kynurenic acid as an antagonist of alpha 7 nicotinic acetylcholine receptors in the brain: Facts and challenges. Biochem Pharmacol. 2013; 85:1027–1032. [PubMed: 23270993]

Allen-Brady K, Robison R, Cannon D, Varvil T, Villalobos M, Pingree C, Leppert MF, Miller J, McMahon WM, Coon H. Genome-wide linkage in Utah autism pedigrees. Mol Psych. 2010; 15:1006–1015.

Ancin I, Barabash A, Vazquez-Alvarez B, Santos JL, Sanchez-Morla E, Martinez JL, Aparicio A, Pelaez JC, Diaz JAC. Evidence for association of the non-duplicated region of *CHRNA7* gene with bipolar disorder but not with Schizophrenia. Psych Gen. 2010a; 20:289–297.

Ancin I, Cabranes JA, Santos JL, Sanchez-Morla E, Vazquez-Alvarez B, Rodriguez-Moya L, Pousada-Casal A, Fernandez C, Aparicio A, Barabash A. CHRNA7 haplotypes are associated with impaired attention in euthymic bipolar disorder. J Affect Dis. 2011; 133:340–345. [PubMed: 21550667]

Ancin I, Santos JL, Teijeira C, Sanchez-Morla EM, Bescos MJ, Argudo I, Torrijos S, Vazquez-Alvarez B, De La Vega I, Lopez-Ibor JJ, Barabash A, Cabranes-Diaz JA. Sustained attention as a potential endophenotype for bipolar disorder. Acta Psych Scand. 2010b; 122:235–245.

Aramakis VB, Hsieh CY, Leslie FM, Metherate R. A critical period for nicotine-induced disruption of synaptic development in rat auditory cortex. J Neurosci. 2000; 20:6106–6116. [PubMed: 10934260]

Araud T, Graw S, Berger R, Neveu E, Bertrand D, Leonard S. The duplicated alpha 7 nicotinic receptor gene *CHRFAM7A* is a dominant negative regulator of *CHRNA7* expression. Biochem Pharmacol. 2011; 82:904–914. [PubMed: 21718690]

Arion D, Lewis DA. Altered expression of regulators of the cortical chloride transporters NRCC1 and KCC2 in schizophrenia. Arch Gen Psych. 2010; 68:21–31.

Benfante R, Antonini RA, De Pizzol M, Gotti C, Clementi F, Locati M, Fornasari D. Expresssion of the α7 nAChR subunit duplicate form (*CHRFAM7A*) is down-regulated in the monocytic cell line THP-1 on treatment with LPS. J Immunol. 2011; 230:74–84.

Benhammou K, Lee MJ, Strook M, Sullivan B, Logel J, Raschen K, Gotti C, Leonard S. [^{3}H]Nicotine binding in peripheral blood cells of smokers is correlated with the number of cigarettes smoked per day. Neuropharmacol. 2000; 39:2818–2829.

Berger F, Gage FH, Vijayaraghavan S. Nicotinic receptor-induced apoptotic cell death of hippocampal progenitor cells. J Neurosci. 1998; 18:6871–6881. [PubMed: 9712657]

Bertrand D, Bertrand S, Cassar S, Gubbins E, Li JH, Gopalakrishnan M. Positive allosteric modulation of the alpha 7 nicotinic acetylcholine receptor: Ligand interactions with distinct binding sites and evidence for a prominent role of the M2-M3 segment. Mol Pharmacol. 2008; 74:1407–1416. [PubMed: 18678621]

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]

Boer H, Holland A, Whittington J, Butler J, Webb T, Clarke D. Psychotic illness in people with Prader Willi syndrome due to chromosome 15 maternal uniparental disomy. Lancet. 2002; 359:135–136. [PubMed: 11809260]

Breese CR, Adams C, Logel J, Drebing C, Rollins Y, Barnhart M, Sullivan B, DeMasters BK, Freedman R, Leonard S. Comparison of the regional expression of nicotinic acetylcholine receptor α7 mRNA and [^{125}I]-α-bungarotoxin binding in human postmortem brain. J Comp Neuro. 1997a; 387:385–398.

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. Neuropsychopharmacol. 2000; 23:351–364.

Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, Leonard S. Effect of smoking history on [^{3}H]nicotine binding in human postmortem brain. J Pharm Exper Ther. 1997b; 282:7–13.

Brody A, Mukhin A, La Charite J, Ta K, Farahi J, Sugar C, Mamoun M, Vellios E, Archie M, Kozman M, Phuong J, Arlorio F, Mandelkern M. Up-regulation of nicotinic acetylcholine receptors in menthol cigarette smokers. Int J Neuropsychopharmacol. 2013; 16:957–966. [PubMed: 23171716]

Broide RS, O'Connor LT, Smith MA, Smith JA, Leslie FM. Developmental expression of alpha 7 neuronal nicotinic receptor messenger RNA in rat sensory cortex and thalamus. Neuroscience. 1995; 67:83–94. [PubMed: 7477913]

Bullock AE, Clark AL, Grady SR, Robinson SF, Slobe BS, Marks MJ, Collins AC. Neurosteroids modulate nicotinic receptor function in mouse striatal and thalamic synaptosomes. J Neurochem. 1997; 68:2412–2423. [PubMed: 9166735]

Canastar A, Logel J, Graw S, Finlay-Schulz JF, Osborne C, Palionyte M, Drebing C, Plehaty M, Wilson L, Eyeson R, Leonard S. DNA methylation and tissue-specific transcription of the α7 nicotinic receptor gene (*CHRNA7*). J Mol Neurosci. 2012; 47:389–400. [PubMed: 22052086]

Carson R, Craig D, Hart D, Todd S, McCuinness B, Johnston JA, O'Neill FA, Ritchie CW, Passmore AP. Genetic variation in the alpha 7 nicotinic acetylcholine receptor is associated with delusional symptoms in alzheimer's disease. Neuromol Med. 2008a; 10:377–384.

Carson R, Craig D, McGuinness B, Johnston JA, O'Neill FA, Passmore AP, Ritchie CW. The alpha 7 nicotinic acetylcholine receptor gene and reduced risk of Alzheimer's disease. J Med Gen. 2008b; 45:244–248.

Castelan F, Castillo M, Mulet J, Sala S, Sala F, del Toro ED, Criado M. Molecular characterization and localization of the RIC-3 protein, an effector of nicotinic acetylcholine receptor expression. J Neurochem. 2008; 105:617–627. [PubMed: 18179477]

Catone C, Ternaux JP. Involvement of the alpha 7 subunit of the nicotinic receptor in morphogenic and trophic effects of acetylcholine on embryonic rat spinal motoneurons in culture. J Neurosci Res. 2003; 72:46–53. [PubMed: 12645078]

Changeux JP. The nicotinic acetylcholine receptor: The founding father of the pentameric ligand-gated ion channel superfamily. J Biol Chem. 2012; 287:40207–40215. [PubMed: 23038257]

Cheng Q, Yakel JL. Presynaptic alpha 7 nicotinic acetylcholine receptors enhance hippocampal mossy fiber glutamatergic transmission via PKA activation. J Neurosci. 2014; 34:124–133. [PubMed: 24381273]

Chernyavsky AI, Arredondo J, Galitovskiy V, Qian J, Grando SA. Upregulation of nuclear factor-kappa B expression by SLURP-1 is mediated by alpha(7)-nicotinic acetylcholine receptor and involves both ionic events and activation of protein kinases. Am J Physiol-Cell Physiol. 2010; 299:C903–C911. [PubMed: 20660165]

Christensen DZ, Mikkelsen JD, Hansen HH, Thomsen MS. Repeated administration of alpha 7 nicotinic acetylcholine receptor (nAChR) agonists, but not positive allosteric modulators, increases alpha 7 nAChR levels in the brain. J Neuroch. 2010; 114:1205–1216.

Corringer PJ, Bertrand S, Galzi JL, Devillers-Thiery A, Changeux JP, Bertrand D. Mutational analysis of the charge selectivity filter of the alpha 7 nicotinic acetylcholine receptor. Neuron. 1999; 22:831–843. [PubMed: 10230802]

Court J, Spurden 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]

Court JA, Perry EK, Johnson M, Piggott MA, Kerwin JA, Perry RH, Ince PG. Regional patterns of cholinergic and glutamate activity in the developing and aging human brain. Develop Brain Res. 1993; 74:73–82.

Craig D, Mirakhur A, Hart DJ, McIlroy SP, Passmore AP. A cross-sectional study of neuropsychiatric symptoms in 435 patients with Alzheimer's disease. Am J Ger Psych. 2005; 13:460–468.

Curtis L, Blouin JL, Radhakrishna U, Gehrig C, Lasseter VK, Wolyniec P, Nestadt G, Dombroski B, Kazazian HH, Pulver AE, Housman D, Bertrand D, Antonarakis SE. No evidence for linkage between schizophrenia and markers at chromosome 15q13-14. Am J Med Gen. 1999; 88:109–112.

Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Ann Rev Pharmacol Toxicol. 2007; 47:699–729. [PubMed: 17009926]

Dani JA, Radcliffe KA, Pidoplichko VI. Variations in desensitization of nicotinic acetylcholine receptors from hippocampus and midbrain dopamine areas. Eur J Pharmacol. 2000; 393:31–38. [PubMed: 10770995]

de Jonge WJ, Ulloa L. The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. Brit J Pharmacol. 2007; 151:915–929. [PubMed: 17502850]

de Lucas-Cerrillo AM, Maldifassi MC, Arnalich F, Renart J, Atienza G, Serantes R, Cruces J, Sanchez-Pacheco A, Andres-Mateos E, Montiel C. Function of partially duplicated human alpha 7 nicotinic receptor subunit *CHRFAM7A* Gene: Potential implications for the cholinergic anti-inflammatory response. J Biol Chem. 2011; 286:594–606. [PubMed: 21047781]

Dempster EL, Toulopoulou T, McDonald C, Bramon E, Walshe M, Wickham H, Sham PC, Murray RM, Collier DA. Episodic memory performance predicted by the 2bp deletion in exon 6 of the "alpha 7-like" nicotinic receptor subunit gene. Am J Psych. 2006; 163:1832–1818.

Deutsch SI, Burket JA, Benson AD. Targeting the alpha(7) nicotinic acetylcholine receptor to prevent progressive dementia and improve cognition in adults with Down's syndrome. Prog Neuro-Psychopharmacol Biol Psych. 2014; 54:131–139.

Dickinson JA, Kew JNC, Wonnacott S. Presynaptic alpha 7- and beta 2-containing nicotinic acetylcholine receptors modulate excitatory amino acid release from rat prefrontal cortex nerve terminals via distinct cellular mechanisms. Mol Pharmacol. 2008; 74:348–359. [PubMed: 18445710]

Dinstein I, Heeger DJ, Lorenzi L, Minshew NJ, Malach R, Behrmann M. Unreliable evoked responses in autism. Neuron. 2012; 75:981–991. [PubMed: 22998867]

Drenan RM, Nashmi R, Imoukhuede P, Just H, McKinney S, Lester HA. Subcellular trafficking, pentameric assembly, and subunit stoichiometry of neuronal nicotinic acetylcholine receptors containing fluorescently labeled alpha 6 and beta 3 subunits. Mol Pharmacol. 2008; 73:27–41. [PubMed: 17932221]

Elgoyhen AB, Katz E, Fuchs PA. The nicotinic receptor of cochlear hair cells: A possible pharmacotherapeutic target? Biochem Pharmacol. 2009; 78:712–719. [PubMed: 19481062]

Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. Neurosci Lett. 2001; 313:96–98. [PubMed: 11684348]

Faraone SV, Su J, Taylor L, Wilcox M. A novel permutation testing method implicates sixteen nicotinic acetylcholine receptor genes as risk factors for smoking in schizophrenia families. Hum Hered. 2004; 57:59–68. [PubMed: 15192278]

Finlay-Schultz J, Canastar A, Short M, El Gazzar M, Coughlan C, Leonard S. Transcriptional repression of the alpha 7 nicotinic acetylcholine receptor subunit gene (*CHRNA7*) by Activating Protein-2 alpha (AP-2 alpha). J Biol Chem. 2011; 286:42123–42132. [PubMed: 21979958]

Flomen RH, Collier DA, Osborne S, Munro J, Breen G, St Clair D, Makoff AJ. Association study of CHRFAM7A copy number and 2bp deletion polymorphisms with schizophrenia and bipolar affective disorder. Am J Med Gen Part B-Neuropsych Gen. 2006; 141B:571–575.

Flomen RH, Davies AF, Di Forti M, La Cascia C, Mackie-Ogilvie C, Murray R, Makoff AJ. The copy number variant involving part of the alpha 7 nicotinic receptor gene contains a polymorphic inversion. Eur J Hum Gen. 2008; 16:1364–1371.

Flomen RH, Shaikh M, Walshe M, Schulze K, Hall MH, Picchioni M, Rijsdijk F, Toulopoulou T, Kravariti E, Murray RM, Asherson P, Makoff AJ, Bramon E. Association between the 2-bp deletion polymorphism in the duplicated version of the alpha7 nicotinic receptor gene and P50 gating. Eur J Hum Gen. 2013; 21:76–81.

Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV. Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. J Neurosci. 1998; 18:1187–1195. [PubMed: 9454829]

Freedman R. Exacerbation of schizophrenia by varenicline. Am J Psych. 2007; 164:1269–1269.

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 Nat Acad Sci. 1997; 94:587–592. [PubMed: 9012828]

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

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

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 Psych. 2008; 165:1040–1047.

Fryer JD, Lukas RJ. Antidepressants noncompetitively inhibit nicotinic acetylcholine receptor function. J Neurochem. 1999a; 72:1117–1124. [PubMed: 10037483]

Fryer JD, Lukas RJ. Noncompetitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. J Pharmacol Exper Therapeut. 1999b; 288:88–92.

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 α7 nicotinic receptor gene and its partial duplication in schizophrenic and control subjects. Am J Med Gen Part B. 2003; 123:39–49.

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 α7 neuronal nicotinic acetylcholine receptor gene. Genomics. 1998; 52:173–185. [PubMed: 9782083]

Gejman PV, Sanders AR, Badner JA, Cao QH, Zhang J. Linkage analysis of schizophrenia to chromosome 15. Am J Med Gen. 2001; 105:789–793.

Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M. Heterogeneity and complexity of native brain nicotinic receptors. Biochem Pharmacol. 2007; 74:1102–1111. [PubMed: 17597586]

Grun EU, Pauly JR, Bullock AE, Collins AC. Corticosterone reversibly alters brain alpha-bungarotoxin binding and nicotine sensitivity. Pharmacol Biochem Behav. 1995; 52:629–635. [PubMed: 8545485]

Guan ZZ, Zhang X, Blennow K, Nordberg A. Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. Neuroreport. 1999; 10:1779–1782. [PubMed: 10501574]

Guillozet-Bongaarts AL, Hyde TM, Dalley RA, Hawrylycz MJ, Henry A, Hof PR, Hohmann J, Jones AR, Kuan CL, Royall J, Shen E, Swanson B, Zeng H, Kleinman JE. Altered gene expression in the dorsolateral prefrontal cortex of individuals with schizophrenia. Mol Psych. 2014; 19:478–485.

Hellstrom-Lindahl E, Court JA. Nicotinic acetylcholine receptors during prenatal development and brain pathology in human aging. Behav Brain Res. 2000; 113:159–168. [PubMed: 10942042]

Henderson BJ, Lester HA. Inside-out neuropharmacology of nicotinic drugs. Neuropharmacol. 2014 This issue.

Hilmas C, Pereira EFR, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha 7 nicotinic receptor activity and increases non-alpha 7 nicotinic receptor expression: Physiopathological implications. J Neurosci. 2001; 21:7463–7473. [PubMed: 11567036]

Hong CJ, Lai IC, Liou LL, Tsai SJ. Association study of the human partially duplicated alpha 7 nicotinic acetylcholine receptor genetic variant with bipolar disorder. Neurosci Lett. 2004; 355:69–72. [PubMed: 14729237]

Hope BT, Nagarkar D, Leonard S, Wise RA. Long-term upregulation of protein kinase A and adenylate cyclase levels in human smokers. J Neurosci. 2007; 27:1964–1972. [PubMed: 17314292]

Hu M, Liu QS, Chang KT, Berg DK. Nicotinic regulation of CREB activation in hippocampal neurons by glutamatergic and nonglutamatergic pathways. Mol Cell Neurosci. 2002; 21:616–625. [PubMed: 12504594]

Hunter SK, Corral N, Ponicsan H, Ross RG. Reliability of P50 auditory sensory gating measures in infants during active sleep. Neuroreport. 2008; 19:79–82. [PubMed: 18281897]

Ibanez-Tallon I, Miwa JM, Wang HL, Adams NC, Crabtree GW, Sine SM, Heintz N. Novel modulation of neuronal nicotinic acetylcholine receptors by association with the endogenous prototoxin lynx1. Neuron. 2002; 33:893–903. [PubMed: 11906696]

Iwata Y, Nakajima M, Yamada K, Nakamura K, Sekine Y, Tsuchiya KJ, Sugihara G, Matsuzaki H, Suda S, Suzuki K, Takei N, Mori N, Iwayama Y, Takao H, Yoshikawa T, Riley B, Makoff A, Sham P, Chen R, Collier D. Linkage disequilibrium analysis of the *CHRNA7* gene and its partially duplicated region in schizophrenia. Neurosci Res. 2007; 57:194–202. [PubMed: 17113175]

Jones IW, Wonnacott S. Precise localization of alpha 7 nicotinic acetylcholine receptors on glutamatergic axon terminals in the rat ventral tegmental area. J Neurosci. 2004; 24:11244–11252. [PubMed: 15601930]

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]

Koike K, Hashimoto K, Takai N, Shimizu E, Komatsu N, Watanabe H, Nakazato M, Okamura N, Stevens KE, Freedman R, Iyo M. Tropisetron improves deficits in auditory P50 suppression in schizophrenia. Schiz Res. 2005; 76:67–72.

Kuryatov A, Luo J, Cooper J, Lindstrom J. Nicotine acts as a pharmacological chaperone to up-regulate human alpha 4 beta 2 acetylcholine receptors. Mol Pharmacol. 2005; 68:1839–1851. [PubMed: 16183856]

Lai IC, Hong CJ, Tsai SJ. Association study of a nicotinic receptor variant with schizophrenic disorders. Neuropsychobiol. 2001; 43:15–18.

Lang B, Pu J, Hunter I, Liu M, Martin-Granados C, Reilly TJ, Gao GD, Guan ZL, Li WD, Shi YY, He G, He L, Stefansson H, St Clair D, Blackwood DH, McCaig CD, Shen SB. Recurrent deletions of *ULK4* in schizophrenia: a gene crucial for neuritogenesis and neuronal motility. J Cell Sci. 2014; 127:630–640. [PubMed: 24284070]

Le Novere N, Corringer PJ, Changeux JP. The diversity of subunit composition in nAChRs: Evolutionary origins, physiologic and pharmacologic consequences. J Neurobiol. 2002; 53:447–456. [PubMed: 12436412]

Lendvai B, Kassai F, Szajli A, Nemethy Z. Alpha 7 Nicotinic acetylcholine receptors and their role in cognition. Brain Res Bull. 2013; 93:86–96. [PubMed: 23178154]

Leonard, S. Nicotinic Receptors and Mental Illness. In: Lester, R., editor. Nicotinic Receptors. Springer; New York: 2014. p. 417-434.

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, Bertrand D. Neuronal nicotinic receptors: from structure to function. Nic Tob Res. 2001; 3:203–223.

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 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. Arch Gen Psych. 2002; 59:1085–1096.

Lester HA, Xiao C, Srinivasan R, Son CD, Miwa J, Pantoja R, Banghart MR, Dougherty DA, Goate AM, Wang JC. Nicotine is a selective pharmacological chaperone of acetylcholine receptor number and stoichiometry: Implications for drug discovery. AAPS J. 2009; 11:167–177. [PubMed: 19280351]

Levin ED. Complex relationships of nicotinic receptor actions and cognitive functions. Biochem Pharmacol. 2013; 86:1145–1152. [PubMed: 23928190]

Levy RB, Aoki C. alpha 7 nicotinic acetylcholine receptors occur at postsynaptic densities of AMPA receptor-positive and -negative excitatory synapses in rat sensory cortex. J Neurosci. 2002; 22:5001–5015. [PubMed: 12077196]

Li S, Li ZX, Pei L, Le AD, Liu F. The α7nACh-NMDA rceptor complex is involved in cue-induced reinstatement of nicotine seeking. J Exp Med. 2012; 209:2141–2147. [PubMed: 23091164]

Lindstrom J. Nicotinic acetylcholine receptors in health and disease. Mol Neurobiol. 1997; 15:193–222. [PubMed: 9396010]

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 Gen. 2001; 105:658–661.

Liu Q, Huang Y, Xue FQ, Simard A, DeChon J, Li G, Zhang JL, Lucero L, Wang M, Sierks M, Hu G, Chang YC, Lukas RJ, Wu J. A novel nicotinic acetylcholine receptor subtype in basal forebrain cholinergic neurons with high sensitivity to amyloid peptides. J Neurosci. 2009; 29:918–929. [PubMed: 19176801]

Liu Q, Xie XT, Lukas RJ, St John PA, Wu J. A novel nicotinic mechanism underlies beta-amyloid-induced neuronal hyperexcitation. J Neurosci. 2013; 33:7253–7263. [PubMed: 23616534]

Liu ZP, Neff RA, Berg DK. Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. Science. 2006; 314:1610–1613. [PubMed: 17158331]

Locke DP, Jiang Z, Pertz LM, Misceo D, Archidiacono N, Eichler EE. Molecular evolution of the human chromosome 15 pericentromeric region. Cytogen Genome Res. 2005; 108:73–82.

Lucht JM, Bremer E. Adaptation of Escherichia coli to high osmolarity environments: osmoregulation of the high-affinity glycine betaine transport system proU. FEMS Microbiol Rev. 1994; 14:3–20. [PubMed: 8011357]

Mahnir V, Lin B, Prokai-Tatrai K, Kem WR. Pharmacokinetics and urinary excretion of DMXBA (GTS-21), a compound enhancing cognition. Biopharmaceut Drug Dispos. 1998; 19:147–151.

Marks MJ, Collins AC. Characterization of nicotine binding in mouse brain and comparison with the binding of α-bungarotoxin and quinuclidinyl benzilate. Mol Pharmacol. 1982; 22:554–564. [PubMed: 7155123]

Marks MJ, Stitzel JA, Romm E, Wehner JM, Collins AC. Nicotinic binding sites in rat and mouse brain: Comparison of acetylcholine, nicotine and α-bungarotoxin binding. Mol Pharmacol. 1986; 30:427–436. [PubMed: 3534542]

Martin LF, Leonard S, Hall MH, Tregellas JR, Freedman R, Olincy A. Sensory gating and alpha-7 nicotinic receptor gene allelic variants in schizoaffective disorder, bipolar type. Am J Med Gen. 2007; 144B:611–614.

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]

Masurel-Paulet A, Andrieux J, Callier P, Cuisset JM, Le Caignec C, Holder M, Thauvin-Robinet C, Doray B, Flori E, Alex-Cordier MP, Beri M, Boute O, Delobel B, Dieux A, Vallee L, Jailliard S, Odent S, Isidor B, Beneteau C, Vigneron J, Bilan F, Gilbert-Dussardier B, Dubourg C, Labalme A, Bidon C, Gautier A, Pernes P, Pinoit JM, Hute F, Mugneret F, Aral B, Jonveaux P, Saniaville D, Faivre L. Delineation of 15q13.3 microdeletions. Clin Genetics. 2010; 78:149–161.

Mathew SV, Law AJ, Lipska BK, Davila-Garcia MI, Zamora ED, Mitkus SN, Vakkalanka R, Straub RE, Weinberger DR, Kleinman JE, Hyde TM. Alpha 7 nicotinic acetylcholine receptor mRNA expression and binding in postmortem human brain are associated with genetic variation in neuregulin 1. Hum Mol Gen. 2007; 16:2921–2932. [PubMed: 17884806]

McEvoy J, Freudenreich O, McGee M, Vanderzwaag C, Levin, Rose J. Clozapine decreases smoking in patients with chronic schizophrenia. Biol Psych. 1995; 37:550–552.

McEvoy JP, Lieberman JA, Stroup TS, Davis SM, Meltzer HY, Rosenheck RA, Swartz MS, Perkins DO, Keefe RSE, Davis CE, Severe J, Hsiao JK. Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. Am J Psych. 2006; 163:600–610.

Meltzer HY, Matsubara S, Lee JC. Classification of typical and atypical antipsychotic-drugs on the basis of dopamine D-1, D-2 and serotonin 2 PKI values. J Pharmacol Exper Ther. 1989; 251:238–246. [PubMed: 2571717]

Mewborn SK, Fantes JA, Miley NL, Brown R, Christian SL, Ledbetter DH. Chromosome 15 duplicons mediate both Class I and Class II common deletions of Prader-Willi and Angelman syndrome patients. Am J Hum Gen. 2001; 69:810.

Mewborn SK, Miley NL, Fantes JA, Brown RL, Butler MG, Christian SL, Ledbetter DH. Breakpoint junction fragments in Prader-Willi and Angelman syndrome (PWS/AS) deletion patients reveal variable breakpoints within large duplicons. Am J Hum Gen. 2002; 71:736.

Mexal S, Berger R, Logel J, Ross RG, Freedman R, Leonard S. Differential regulation of alpha 7 nicotinic receptor gene (CHRNA7) expression in schizophrenic smokers. J Mol Neurosci. 2010; 40:185–195. [PubMed: 19680823]

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]

Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at alpha 4 beta 2 and a full agonist at alpha 7 neuronal nicotinic receptors. Mol Pharmacol. 2006; 70:801–805. [PubMed: 16766716]

Miller DT, Shen Y, Weiss LA, Korn J, Anselm I, Bridgemohan C, Cox GF, Dickinson H, Gentile J, Harris DJ, Hegde V, Hundley R, Khwaja O, Kothare S, Luedke C, Nasir R, Poduri A, Prasad K, Raffalli P, Reinhard A, Smith SE, Sobeih MM, Soul JS, Stoler J, Takeoka M, Tan WH, Thakuria J, Wolff R, Yusupov R, Gusella JF, Daly MJ, Wu BL. Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders. J Med Gen. 2009; 46:242–248.

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psych. 2012; 17:1206–1227.

Morita Y, Callicott JH, Testa LR, Mighdoll MI, Dickinson D, Chen Q, Tao R, Lipska BK, Kolachana B, Law AJ, Ye T, Straub RE, Weinberger DR, Kleinman JE, Hyde TM. Characteristics of the cation cotransporter NKCC1 in human brain: Alternate transcripts, expression in development, and potential relationships to brain function and schizophrenia. J Neurosci. 2014; 34:4929–4940. [PubMed: 24695712]

Morley BJ, Mervis RF. Dendritic spine alterations in the hippocampus and parietal cortex of alpha7 nicotinic acetylcholine receptor knockout mice. Neurosci. 2013; 233:54–63.

Murray TA, Bertrand D, Papke RL, George AA, Pantoja R, Srinivasan R, Liu Q, Wu J, Whiteaker P, Lester HA, Lukas RJ. α7β2 nicotinic acetylcholine receptors assemble, function, and are activated primarily via their α7-α7 interfaces. Mol Pharmacol. 2012; 81:175–188. [PubMed: 22039094]

Nachman MW, Crowell SL. Estimate of the mutation rate per nucleotide in humans. Genetics. 2000; 156:297–304. [PubMed: 10978293]

Nagamoto HT, Adler LE, Hea RA, Griffith JM, McRae KA, Freedman R. Gating of auditory P50 in schizophrenics: Unique effects of clozapine. Biol Psych. 1996; 40:181–188.

Nashmi R, Dickinson ME, McKinney S, Jareb M, Labarca C, Fraser SE, Lester HA. Assembly of alpha 4 beta 2 nicotinic acetylcholine receptors assessed with functional fluorescently labeled subunits: Effects of localization, trafficking, and nicotine-induced upregulation in clonal mammalian cells and in cultured midbrain neurons. J Neurosci. 2003; 23:11554–11567. [PubMed: 14684858]

Neves-Pereira M, Bassett AS, Honer WG, Lang D, King NA, Kennedy JL. No evidence for linkage of the CHRNA7 gene region in Canadian schizophrenia families. Am J Med Gen. 1998; 81:361–363.

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 Psych. 2006; 63:630–638.

Orekhova EV, Stroganova TA, Prokofyev AO, Nygren G, Gillberg C, Elam M. Sensory gating in young children with autism: Relation to age, IQ, and EEG gamma oscillations. Neurosci Lett. 2008; 434:218–223. [PubMed: 18313850]

Ortells MO, Lunt GG. Evolutionary history of the ligand-gated ion-channel superfamily of receptors. Trends Neurosci. 1995; 18:121–127. [PubMed: 7754520]

Ortiz A, Grando SA. Smoking and the skin. Int J Dermatol. 2012; 51:250–262. [PubMed: 22348557]

Parri HR, Hernandez CM, Dineley KT. Research update: Alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease. Biochem Pharmacol. 2011; 82:931–942. [PubMed: 21763291]

Pauly JR, Marks MJ, Gross SD, Collins AC. An autoradiographic analysis of cholinergic receptors in mouse brain after chronic nicotine treatment. J Pharmacol Exper Ther. 1991; 258:1127–1136. [PubMed: 1890617]

Pavlov VA, Parrish WR, Rosas-Ballina M, Ochani M, Puerta M, Qchani K, Chavan S, Al-Abed Y, Tracey KJ. Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. Brain Behav Immun. 2009; 23:41–45. [PubMed: 18639629]

Pena G, Cai BL, Liu J, van der Zanden EP, Deitch EA, de Jonge WJ, Ulloa L. Unphosphorylated STAT3 modulates alpha7 nicotinic receptor signaling and cytokine production in sepsis. Eur J Immunol. 2010; 40:2580–2589. [PubMed: 20706987]

Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ. Increased nicotinic receptors in brains from smokers: Membrane binding and autoradiography studies. J Pharm Exper Ther. 1999; 289:1545–1552.

Pinheiro ML, Nijhuis LEJ, Duarte JM, Ribeiro A, de Jonge WJ, Palermo-Neto J. Alpha 7 nicotinic receptor stimulation modifies cytokine production and alpha 7 nAChR expression in bone marrow-derived dendritic cells. Neuroimmunomod. 2011; 18:398–398.

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bolte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BHY, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, Piven J, Ponting CP, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Sequeira AF, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stein O, Sykes N, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang ZZ, Wassink TH, Webber C, Weksberg R, Wing K, Wittemeyer K, Wood S, Wu J, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Devlin B, Ennis S, Gallagher L, Geschwind DH, Gill M, Haines JL, Hallmayer J, Miller J, Monaco AP, Nurnberger JI, Paterson AD, Pericak-Vance MA, Schellenberg GD, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Scherer SW, Sutcliffe JS, Betancur C. Functional impact of global rare copy number variation in autism spectrum disorders. Nature. 2010; 466:368–372. [PubMed: 20531469]

Pocivavsek A, Wu HQ, Elmer GI, Bruno JP, Schwarcz R. Pre- and postnatal exposure to kynurenine causes cognitive deficits in adulthood. Eur J Neurosci. 2012; 35:1605–1612. [PubMed: 22515201]

Quik M, Campos C, Grady SR. Multiple CNS nicotinic receptors mediate L-dopa-induced dyskinesias: Studies with parkinsonian nicotinic receptor knockout mice. Biochem Pharmacol. 2013; 86:1153–1162. [PubMed: 23831952]

Raux G, Bonnet-Brilhault F, Louchart S, Houy E, Gantier R, Levillain D, Allio G, Haouzir S, Petit M, Martinez M, Frebourg T, Thibaut F, Campion D. The-2 bp deletion in exon 6 of the 'alpha 7-like' nicotinic receptor subunit gene is a risk factor for the P50 sensory gating deficit. Mol Psych. 2002; 7:1006–1011.

Ray MA, Graham AJ, Lee M, Perry RH, Court JA, Perry EK. Neuronal nicotinic acetylcholine receptor subunits in autism: An immunohistochemical investigation in the thalamus. Neurobiol Dis. 2005; 19:366–377. [PubMed: 16023579]

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]

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 Gen. 2000; 96:196–201.

Rosas-Ballina M, Tracey KJ. Cholinergic control of inflammation. J Intern Med. 2009; 265:663–679. [PubMed: 19493060]

Ross RG, Hunter SK, McCarthy L, Beuler J, Hutchison AK, Wagner BD, Leonard S, Stevens KE, Freedman R. Perinatal choline effects on neonatal pathophysiology related to later schizohrenia risk. Am J Psych. 2013; 170:290–298.

Ross RG, Stevens KE, Proctor WR, Leonard S, Kisley MA, Hunter SK, Freedman R, Adams CE. Research Review: Cholinergic mechanisms, early brain development, and risk for schizophrenia. J Child Psychol Psych. 2010; 51:535–549.

Rozycka A, Dorszewska J, Steinborn B, Lianeri M, Winczewska-Wiktor A, Sniezawska A, Wisniewska K, Jagodzinski PP. Association study of the 2-bp deletion polymorphism in exon 6 of the *CHRFAM7A* gene with Idiopathic Generalized Epilepsy. DNA Cell Biol. 2013; 32:640–647. [PubMed: 24024466]

Sanchez-Morla EM, Garcia-Jimenez MA, Barabash A, Martinez-Vizcaino V, Mena J, Cabranes-Diaz JA, Baca-Baldomero E, Santos JL. P50 sensory gating deficit is a common marker of vulnerability to bipolar disorder and schizophrenia. Acta Psych Scand. 2008; 117:313–318.

Sanders AR, Duan J, Levinson DF, Shi J, He D, Hou C, Burrell GJ, Rice JP, Nertney DA, Olincy A, Rozic P, Vinogradov S, Buccola NG, Mowry BJ, Freedman R, Amin F, Black DW, Silverman JM, Byerley WF, Crowe RR, Cloninger CR, Martinez M, Gejman PV. No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: Implications for psychiatric genetics. Am J Psych. 2008; 165:497–506.

Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci. 2012; 13:465–477. [PubMed: 22678511]

Sharma G, Vijayaraghavan S. Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. Proc Nat Acad Sci. 2001; 98:4148–4153. [PubMed: 11259680]

Shen JX, Yakel JL. Nicotinic acetylcholine receptor-mediated calcium signaling in the nervous system. Acta Pharmacolog Sin. 2009; 30:673–680.

Shen JX, Yakel JL. Functional alpha 7 nicotinic ACh receptors on astrocytes in rat hippocampal CA1 slices. J Mol Neurosci. 2012; 48:14–21. [PubMed: 22351110]

Shirazi-Southall S, Rodriguez DE, Nomikos GG. Effects of typical and atypical antipsychotics and receptor selective compounds on acetylcholine efflux in the hippocampus of the rat. Neuropsychopharmacol. 2002; 26:583–594.

Simosky J, Stevens K, Adler L, Freedman R. Clozapine and olanzapine improve insufficient inhibitory processing of the P20-N40 auditory evoked potential evidence for a nicotinic mechanism. Schiz Res. 2003; 60:260–260.

Singhal SK, Zhang L, Morales M, Oz M. Antipsychotic clozapine inhibits the function of alpha(7)-nicotinic acetylcholine receptors. Neuropharmacol. 2007; 52:387–394.

Sinkus ML, Lee MJ, Gault J, Logel J, Short M, Freedman R, Christian SL, Lyon J, Leonard S. A 2-base pair deletion polymorphism in the partial duplication of the alpha 7 nicotinic acetylcholine gene (*CHRFAM7A*) on chromosome 15q14 is associated with schizophrenia. Brain Res. 2009; 1291:1–11. [PubMed: 19631623]

Sinkus ML, Wamboldt MZ, Barton A, Fingerlin TE, Laudenslager ML, Leonard S. The alpha 7 nicotinic acetylcholine receptor and the acute stress response: Maternal genotype determines offspring phenotype. Physiol Behav. 2010; 104:321–326. [PubMed: 21073885]

Sivaprakasam K. Towards a unifying hypothesis of Alzheimer's disease: Cholinergic system linked to plaques, tangles and neuroinflammation. Curr Med Chem. 2006; 13:2179–2188. [PubMed: 16918347]

Smith RC, Lindenmayer JP, Davis JM, Cornwell J, Noth K, Gupta S, Sershen H, Lajtha A. Cognitive and antismoking effects of varenicline in patients with schizophrenia or schizoaffective disorder. Schiz Res. 2009; 110:149–155.

Son JH, Meizel S. Evidence suggesting that the mouse sperm acrosome reaction initiated by the zona pellucida involves an alpha 7 nicotinic acetylcholine receptor. Biol Reprod. 2003; 68:1348–1353. [PubMed: 12606407]

Song P, Spindel ER. Basic and clinical aspects of non-neuronal acetylcholine: Expression of non-neuronal acetylcholine in lung cancer provides a new target for cancer therapy. J Pharmacolog Sci. 2008; 106:180–185.

Stefansson H, Rujescu D, Cichon S, Pietilainen OPH, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE, Hansen T, Jakobsen KD, Muglia P, Francks C, Matthews PM, Gylfason A, Halldorsson BV, Gudbjartsson D, Thorgeirsson TE, Sigurdsson A, Jonasdottir A, Bjornsson A, Mattiasdottir S, Blondal T, Haraldsson M, Magnusdottir BB, Giegling I, Moller HJ, Hartmann A, Shianna KV, Ge DL, Need AC, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Paunio T, Toulopoulou T, Bramon E, Di Forti M, Murray R, Ruggeri M, Vassos E, Tosato S, Walshe M, Li T, Vasilescu C, Muhleisen TW, Wang AG, Ullum H, Djurovic S, Melle I, Olesen J, Kiemeney LA, Franke B, Sabatti C, Freimer NB, Gulcher JR, Thorsteinsdottir U, Kong A, Andreassen OA, Ophoff RA, Georgi A, Rietschel M, Werge T, Petursson H, Goldstein DB, Nothen MM, Peltonen L, Collier DA, St Clair D, Stefansson K. Large recurrent microdeletions associated with schizophrenia. Nature. 2008; 455:232–237. [PubMed: 18668039]

Stephens SH, Franks A, Berger R, Palionyte M, Fingerlin TE, Wagner B, Logel J, Olincy A, Ross RG, Freedman R, Leonard S. Multiple genes in the 15q13-q14 chromosomal region are associated with schizophrenia. Psych Gen. 2012; 22:1–14.

Stephens SH, Logel J, Barton A, Franks A, Schultz J, Short M, Dickenson J, James B, Fingerlin T, Wagner B, Hodgkinson CA, Graw S, Ross RG, Freedman R, Leonard S. Association of the 5′-upstream regulatory region of the α7 nicotinic acetylcholine receptor subunit gene (*CHRNA7*) with schizophrenia. Schiz Res. 2009; 109:102–112.

Stevens KE, Bullock AE, Collins AC. Chronic corticosterone treatment alters sensory gating in C3H mice. Pharmacol Biochem Behav. 2001; 69:359–366. [PubMed: 11509192]

Stone JL, O'Donovan MC, Gurling H, Kirov GK, Blackwood DHR, Corvin A, Craddock NJ, Gill M, Hultman CM, Lichtenstein P, McQuillin A, Pato CN, Ruderfer DM, Owen MJ, St Clair D, Sullivan PF, Sklar P, Purcell SM. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature. 2008; 455:237–241. [PubMed: 18668038]

Suzuki S, Kawamata J, Matsushita T, Matsumura A, Hisahara S, Takata K, Kitamura Y, Kem W, Shimohama S. 3-(2,4-dimethoxy)benzylidene - anabaseine dihydrochloride protects against 6-hydroxydopamine-induced parkinsonian neurodegeneration through alpha 7 nicotinic acetylcholine receptor stimulation in rats. J Neurosci Res. 2013; 91:462–471. [PubMed: 23239187]

Tao R, Li C, Newburn EN, Ye T, Lipska BK, Herman MM, Weinberger DR, Kleinman JE, Hyde TM. Transcript-specific associations of SLC12A5 (KCC2) in human prefrontal cortex with development, schizophrenia, and affective disorders. J Neurosci. 2012; 32:5216–5222. [PubMed: 22496567]

Tasneem A, Iyer LM, Jakobsson E, Aravind L. Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal Cys-loop ion channels. Genome Biol. 2005; 6:R4. [PubMed: 15642096]

Taylor P. Development of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease. Neurology. 1998; 51:S30–S35. [PubMed: 9674760]

Thomsen MS, Weyn A, Mikkelsen JD. Hippocampal alpha 7 nicotinic acetylcholine receptor levels in patients with schizophrenia, bipolar disorder, or major depressive disorder. Bipolar Dis. 2011; 13:701–707.

Tregellas JR, Tanabe J, Rojas DC, Shatti S, Olincy A, Johnson L, Martin LF, Soti F, Kem WR, Leonard S, Freedman R. Effects of an alpha 7-nicotinic agonist on default network activity in schizophrenia. Biol Psych. 2011; 69:7–11.

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 Gen. 2001; 105:662–668.

Turecki G, Grof P, Grof E, D'Souza V, Cavazzoni P, Duffy A, Brewer C, Hudson T, Rouleau GA, Alda M. A genome scan using a pharmacogenetic approach indicates a susceptibility locus for BD on 15q14. Biol Psych. 2000; 47:69S.

Uteshev VV, Meyer EM, Papke RL. Regulation of neuronal function by choline and 4OH-GTS-21 through alpha 7 nicotinic receptors. J Neurophysiol. 2003; 89:1797–1806. [PubMed: 12611953]

van Maanen MA, Stoof SP, van der Zanden EP, de Jonge WJ, Janssen RA, Fischer DF, Vandeghinste N, Brys R, Vervoordeldonk MJ, Tak PP. The alpha 7 nicotinic acetylcholine receptor on fibroblast-like synoviocytes and in synovial tissue from rheumatoid arthritis patients: A possible role for a key neurotransmitter in synovial inflammation. Arth Rheum. 2009; 60:1272–1281. [PubMed: 19404936]

Vijayaraghavan S, Pugh PC, Zhang ZW, Rathouz MM, Berg DK. Nicotinic receptors that bind alpha-bungarotoxin on neurons raise intracellular free Ca^{++} Neuron. 1992; 8:353–362. [PubMed: 1310863]

Villiger Y, Szanto I, Jaconi S, Blanchet C, Buisson B, Krause KH, Bertrand D, Romand JA. Expression of an alpha 7 duplicate nicotinic acetylcholine receptor-related protein in human leukocytes. J Neuroimmunol. 2002; 126:86–98. [PubMed: 12020960]

Vogels A, De Hert M, Descheemaeker MJ, Govers V, Devriendt K, Legius E, Prinzie P, Fryns JP. Psychotic disorders in Prader-Willi syndrome. Am J Med Gen Part A. 2004; 127A:238–243.

Wallace TL, Bertrand D. Importance of the nicotinic acetylcholine receptor system in the prefrontal cortex. Biochem Pharmacol. 2013; 85:1713–1720. [PubMed: 23628449]

Wallace TL, Porter RHP. Targeting the nicotinic alpha7 acetylcholine receptor to enhance cognition in disease. Biochem Pharmacol. 2011; 82:891–903. [PubMed: 21741954]

Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang HC, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ. Nicotinic acetylcholine receptor alpha 7 subunit is an essential regulator of inflammation. Nature. 2003; 421:384–388. [PubMed: 12508119]

Wang HY, Lee DHS, D'Andrea MR, Peterson PA, Shank RP, Reitz AB. Beta-amyloid(1-42) binds to alpha 7 nicotinic acetylcholine receptor with high affinity - Implications for Alzheimer's disease pathology. J Biol Chem. 2000; 275:5626–5632. [PubMed: 10681545]

Wang X, Lippi G, Carlson DM, Berg DK. Activation of alpha 7-containing nicotinic receptors on astrocytes triggers AMPA receptor recruitment to glutamatergic synapses. J Neurochem. 2013; 127:632–643. [PubMed: 24032433]

Wang Y, Xiao C, Indersmitten T, Freedman R, Leonard S, Lester HA. The duplicated α7 subunits assemble and form functional nicotinic receptors with the full-length α7. J Biol Chem. 2014; 289:26451–26463. [PubMed: 25056953]

Weiland S, Bertrand D, Leonard S. Neuronal nicotinic acetylcholine receptors: from the gene to the disease. Behav Brain Res. 2000; 113:43–56. [PubMed: 10942031]

Weng PH, Chen JH, Chen TF, Sun Y, Wen LL, Yip PK, Chu YM, Chen YC. *CHRNA7* polymorphisms and response to cholinesterase inhibitors in Alzheimer's disease. Plos One. 2013; 8:12.

Williams JM, Anthenelli RM, Morris CD, Treadow J, Thompson JR, Yunis C, George TR. A randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of varenicline for smoking cessation in patients with schizophrenia or schizoaffective disorder. J Clin Psych. 2012a; 73:654–660.

Williams NM, Franke B, Mick E, Anney RJL, Freitag CM, Gill M, Thapar A, O'Donovan MC, Owen MJ, Holmans P, Kent L, Middleton F, Zhang-James Y, Liu L, Meyer J, Nguyen TT, Romanos J, Romanos M, Seitz C, Renner TJ, Walitza S, Warnke A, Palmason H, Buitelaar J, Rommelse N, Vasquez AA, Hawi Z, Langley K, Sergeant J, Steinhausen HC, Roeyers H, Biederman J, Zaharieva I, Hakonarson H, Elia J, Lionel AC, Crosbie J, Marshall CR, Schachar R, Scherer SW, Todorov A, Smalley SL, Loo S, Nelson S, Shtir C, Asherson P, Reif A, Lesch KP, Faraone SV. Genome-wide analysis of copy number variants in attention deficit hyperactivity disorder: The role of rare variants and duplications at 15q13.3. Am J Psych. 2012b; 169:195–204.

Xiao C, Miwa J, Henderson BJ, Wang Y, Deshpande P, McKinney S, Lester HA. Nicotinic receptor subtype-selective circuit patterns in the subthalamic nucleus. J Neurosci. 2015 (in press).

Yang Y, Paspalas CD, Jin LE, Picciotto MR, Arnsten AFT, Wang M. Nicotinic α7 receptors enhance NMDA cognitive circuits in dorsolateral prefrontal cortex. Proc Natl Acad Sci. 2013; 110:12078–12083. [PubMed: 23818597]

Yasui DH, Scoles HA, Horike S, Meguro-Horike M, Dunaway KW, Schroeder DI, LaSalle JM. 15q11.2-13.3 chromatin analysis reveals epigenetic regulation of CHRNA7 with deficiencies in Rett and autism brain. Hum Mol Gen. 2011; 20:4311–4323. [PubMed: 21840925]

Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. Ann Rev Nutr. 2006; 26:229–250. [PubMed: 16848706]

Zhang XY, Liu L, Liu S, Hong X, Chen DC, Xiu MH, Yang FD, Zhang Z, Zhang X, Kosten TA, Kosten TR. Short-term tropisetron treatment and cognitive and P50 auditory gating deficits in schizophrenia. Am J Psych. 2012; 169:974–981.

Highlights

- The α7 nicotinic receptor gene, *CHRNA7*, is associated with many mental illnesses
- *CHRNA7* on chromosome 15 was partially duplicated forming a new gene, *CHRFAM7A*
- The gene product of *CHRFAM7A*, dupα7, assembles with α7 subunits (α7dupα7*)
- Dupα7 is a dominant negative regulator of α7nAChR function
- *CHRFAM7A* is not present in either rodents or primates, only in humans

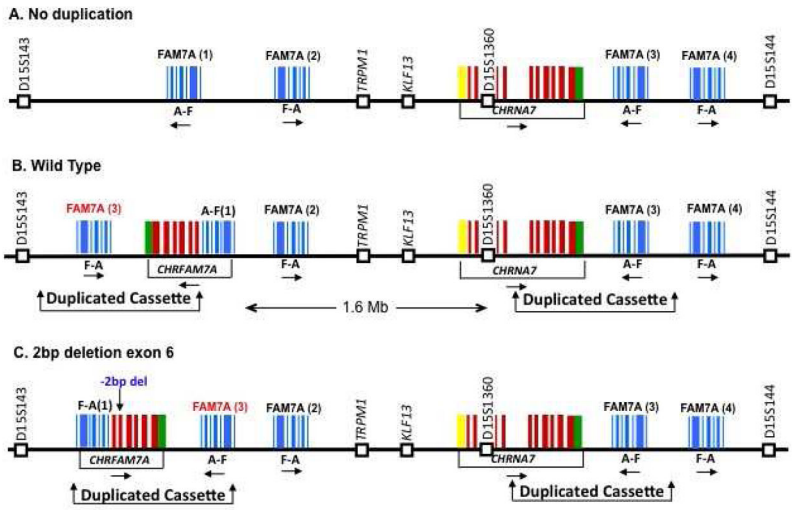

Figure 1.

Duplication of exons 5-10 of the *CHRNA7* gene and formation of the chimeric gene *CHRFAM7A* on chromosome 15q13.3. (A) Prior to the duplication of *CHRNA7*, exons A, B, C, and E were duplicated from *ULK4* on chromosome 3 and exons D, F, and G were duplicated from other regions of chromosome 15. Copies of these exons, A-F (FAM7A) are present at least four times on chromosome 15. (B) Subsequently, exons 5-10 of *CHRNA7* and 3′ DNA (~250Kb) were duplicated, interrupting a copy of A-F, FAM7A(1), centromeric to *CHRNA7* by 1.6Mb forming the chimeric gene, *CHRFAM7A*. The original orientation of *CHRFAM7A* relative to *CHRNA7* was head to head. (C) A 2bp deletion in exon 6 of *CHRFAM7A* is associated with an inversion of the gene. *CHRFAM7AΔ2bp* is in the same orientation as *CHRNA7* (tail to head). *CHRNA7* exons in red; FAM7A exons in blue; *CHRNA7* exons in red; *CHRNA7* promoter in yellow.

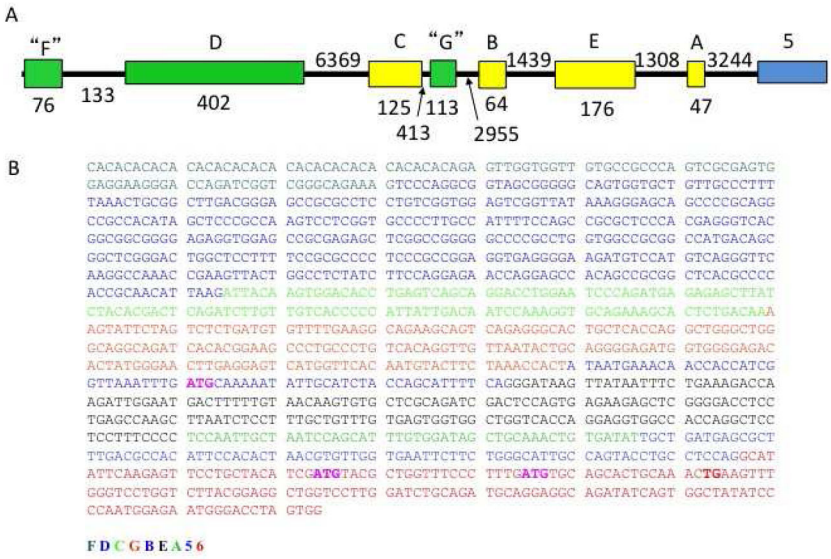

Figure 2.

Order and sequence of upstream exons of *CHRFAM7A*. (A) Genomic order of the upstream exons of *CHRFAM7A*. Diagram is not to scale. The sizes of the exons and the distance between are indicated in base pairs. Exons duplicated from the *ULK4* gene are shown in yellow; those with homology to *GOLGA8B* are in green; *CHRNA7* exon 5 in blue. (B) DNA sequence of the 5′ upstream exons of *CHRFAM7A* with exons indicated by color, below. Putative translation start codons are highlighted in fuchsia in exon B for *CHRFAM7A* and exon 6 for *CHRFAM7AΔ2bp*. The 2bp deletion (TG) in exon 6 is in bold.

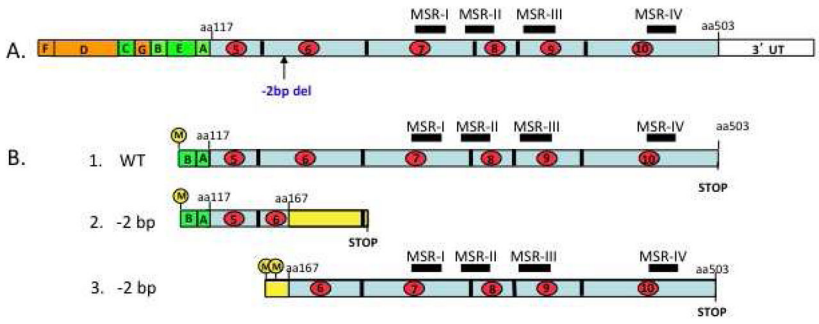

Figure 3.

Translation products from the *CHRFAM7A* gene. (A) *CHRFAM7A* mRNA. The most common transcript includes only upstream exons D, C, B, and A. (B) Putative translation products. (B.1) Normal translated sequence with methionine start codon in exon B. Peptide contains 26 aa coded by exons A and B before entering in frame sequence of *CHRNA7* at aa 117. (B.2) A truncated peptide is formed when the 2bp deletion is present if translation begins in exon B. The 2bp deletion is in the codon for aa167 and changes the reading frame, leading to a stop codon. (B.3) Initiation of translation in exon 6 in the *CHRFAM7AΔ2bp* mRNA. Two methionine codons are present in exon 6. Translation starts lead to either 6 or 13 aa out of frame before the 2bp deletion in the codon for aa167. After the 2bp deletion, aa sequence reverts to that of *CHRNA7*. MSR, membrane spanning region; aa, amino acid.

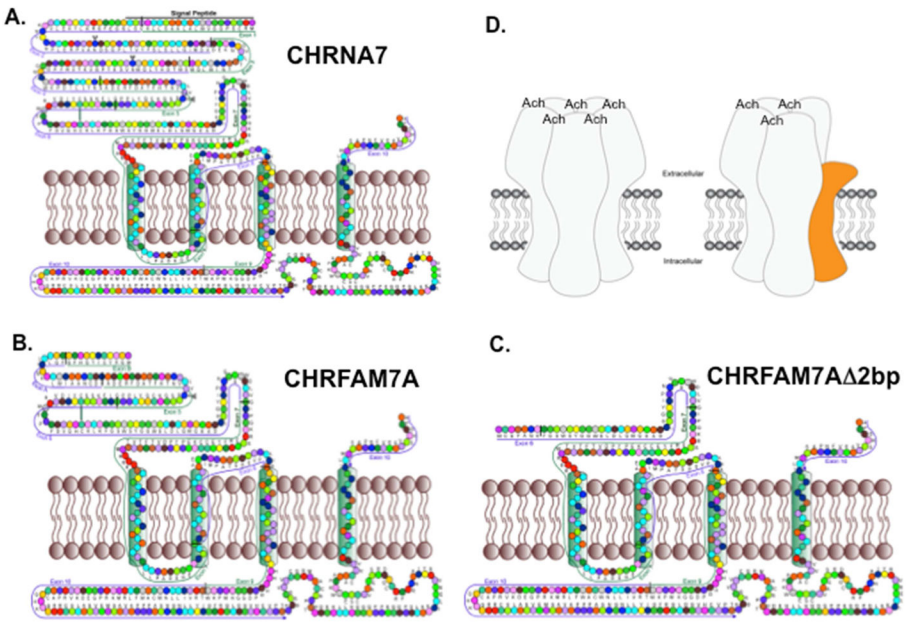

Figure 4.

Putative topology of *CHRNA7*, *CHRFAM7A*, and *CHRFAM7AΔ2bp* gene products. (A) The normal α7 subunit with three glycosylation sites and signal peptide. (B) *CHRFAM7A* gene product (dupα7), missing the signal peptide and two glycosylation sites. (C) *CHRFAM7AΔ2bp* gene product (dupΔα7), missing the signal peptide, all glycosylation sites, and the cysteine bridge. (D) Pentameric structure of the normal α7nAChR on the left and a pentamer containing the peptide of the duplicated subunit on the right. Note that incorporation of a single duplicated gene subunit eliminates two agonist binding sites, which are localized at the extracellular subunit interfaces.

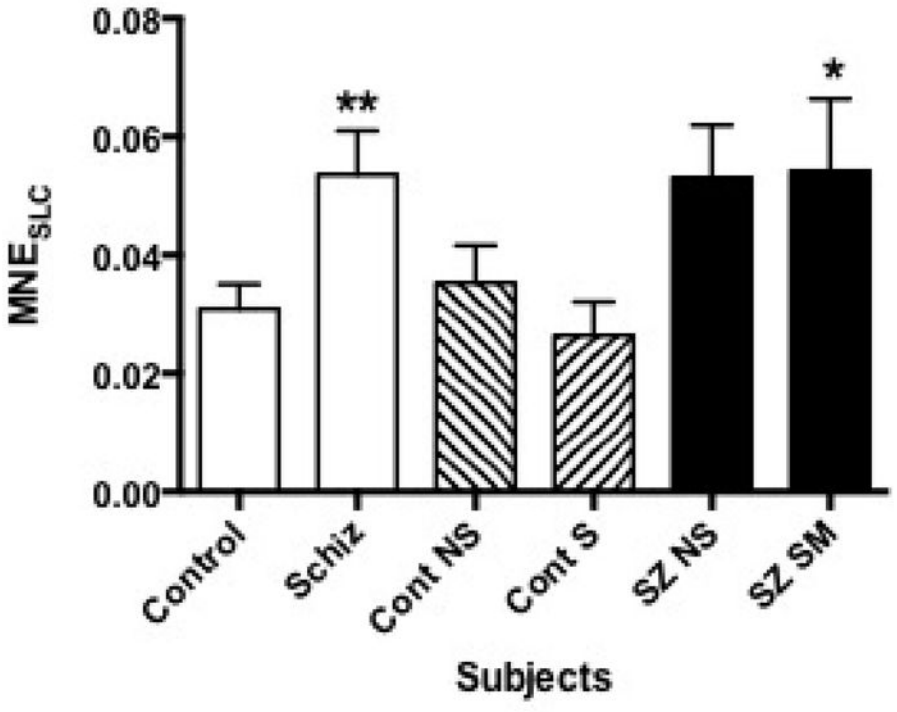

Figure 5.

Relative expression of *CHRFAM7A* in primary lymphocytes of control and schizophrenic subjects. Real-time PCR was utilized to quantify expression of *CHRFAM7A* in primary lymphocytes from control and schizophrenic, non-smokers and smokers. Fifteen subjects in each of the four groups were analyzed. Results were normalized to the housekeeping gene *SLC9A1*, a Na^+/H^+ antiporter using mean normalized expression (MNE_{SLC}) and actual efficiencies of each run. Expression of the *SLC9A1* gene was not changed in any of the groups. Expression of the *CHRFAM7A* gene was significantly increased in lymphocytes from schizophrenic patients. Smoking did not significantly decrease transcription of *CHRFAM7A* in controls. In schizophrenic smokers, the levels of *CHRFAM7A* mRNA were not changed, remaining higher than control smokers. (*, $p<0.05$; **, $p<0.01$)

Table 1

Copy Number: Copies of *CHRNA7*(7)/Copies of *CHRFAM7A*(7A)

Genotype	7(1)/7A(2)	7(2)/7A(0)	7(2)/7A(1)	7(2)/7A(2)	7(2)/7A(3)	7(2)/7A(4)	7(3)/7A(1)	7(3)/7A(2)	Total
Control	0	2	57	252	6	1	4	0	322
Schizophrenia	2	4	89	343	8	1	2	1	450
Total	2	6	146	595	14	2	6	1	772

Copy number variants in genomic DNA were detected with the ABI TaqMan™ Copy Number Assay run simultaneously with a TaqMan™ Copy Number Reference Assay in a duplex, real time PCR. The protocol was performed in 384-well plates on an ABI 7900HT Fast Real-Time PCR System (Foster City, CA). Each genomic DNA sample (10ng) was assayed with four replicates in a 10 µl volume. The assay contained TaqMan™ Genotyping Master Mix with two gene specific primers and a FAM dye-labeled MGB probe to detect DNA target sequences. The assay was run simultaneously with two gene specific primers and a VIC and TAMRA dye-labeled probe to detect the human Ribonuclease P RNA component H1 (H1RNA) gene (RPPH1) on chromosome 14 as a two copy reference sequence. After amplification, the C_T values for the copy number and RNase P reference assay were analyzed by the ABI Copy Caller software to determine copy number variation. Six ABI TaqMan™ assays were used to analyze copy number: Hs00059157_cn (exon 2), Hs03897718_cn (intron 3, 151 bp from 3′ end of exon 3), Hs00064302_cn (exon 4) and Hs3894862_cn (intron 4, 869 bp from 5′ end of exon 5). One custom ABI TaqMan™ assay was designed to detect exon 9 in both *CHRNA7* and *CHRFAM7A. CHRFAM7A* copy number was determined with a TaqMan™ assay spanning the breakpoint between *CHRFAM7A* exons F-A and the duplicated *CHRNA7* exons 5-10 (Sinkus et al, 2009; Flomen et al, 2006).

Table 2

Positive Genetic Linkage and Association with the *CHRNA7* and *CHRFAM7A* Genes

GENE	DISEASE	LINKAGE	ASSOC	LOCUS	PHENO	REFS
CHRNA7	Schiz	Microsatellites		15q13.3	Schiz	Kaufmann et al., 1998; Liu et al., 2001; Tsuang et al., 2001; Gejman et al., 2001; Riley et al., 2000; Freedman et al., 2001
				I-2, D15S1360	P50	Freedman et al., 1997
			rs3087454	5′-UT	Schiz	Stephens et al., 2009
			rs3087454	5′-UT	Default Network	Trigellas, et al., 2011
			rs3087454	5′-UT	P50 Newborns	Ross et al., 2013
	Bipolar	Microsatellites		15q13.3	Lithium Responsive Bipolar	Turecki et al., 2000
			−86C/T	5′UT	Improved	Ancin et al., 2011
			rs883473	I-2	Attention	
			rs6494223	I-3		
			rs904952	I-4		
			rs6494223	I-3	↓risk	Ancin et al., 2010a
	Autism	Microsatellites		15q13-q14	↑risk	Allen-Brady et al., 2010
	Alz		rs1514246	5′UT	↓risk	Carson et al., 2008b
			rs2337506	I-2		
			rs8027814	I-2		
			rs6494223	I-3	Delusions	Carson et al., 2008a
			rs8024987	I-2	CHEI response	Weng et al., 2013
CHRFAM7A	Schiz		2bp del	E-6	↑risk	Sinkus et al., 2009
			2bp del	E-6	P50	Raux et al., 2002; Flomen et al., 2013
			2bp del	E-6	Episodic memory	Dempster et al., 2006
	Bipolar		2bp del	E-6	↑risk	Hong et al., 2004
	Epilepsy		2bp del	E-6	↓risk	Rozycka et al., 2013
CHRNA7* and *CHRFAM7A	Schiz		Deletion	*CHRNA7*	↑risk	Stefansson et al., 2008; Stone et al., 2008
	Autism		Deletion	*CHRNA7 and CHRFAM7A*	↑risk	Pinto et al., 2010
	ADHD		Duplication	*CHRNA7 and CHRFAM7A*	↑risk	Williams et al., 2012b

Assoc, Association; Pheno, phenotype; Schiz, schizophrenia; Alz, Alzheimer's disease; ADHD, attention deficit hyperactivity disorder; CHEI, choline esterase inhibition; 5′UT, 5′untranslated region; I, intron; E, exon; del, deletion