[Frontiers in Bioscience 13, 477-491, January 1, 2008]

Naturally occurring genetic variability in the nicotinic acetylcholine receptor alpha4 and alpha7 subunit genes and phenotypic diversity in humans and mice

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1. ABSTRACT

Through the use of pharmacological and molecular strategies, the identities of the nicotinic acetylcholine receptor (nAChR) subtypes that modulate different behaviors and physiological measures are being revealed. However, little is known with respect to how naturally occurring genetic variability in the genes that encode the members of the neuronal nAChR family contribute to phenotypic diversity in humans and research organisms. Because behavior, physiology and disease susceptibility in humans and other species are influenced to some extent by genetic factors and nAChRs contribute to a wide range of phenotypes, it is likely that polymorphisms in the genes that encode the nAChR subunit family contribute to phenotypic variability among individuals in a population. Over the past decade, data have accumulated that support the premise that naturally occurring nAChR subunit gene variability contributes to phenotypic diversity in both humans and mice. In this review, current evidence for the role of variability in the genes that encode the two major brain-expressed alpha subunits, alpha4 and alpha7, in modulating behavior, physiology and disease risk in both humans and mice will be discussed.

2. INTRODUCTION

Neuronal nicotinic receptors (nAChRs) are pentameric ligand-gated cationic channels that are distributed throughout the central and peripheral nervous

systems. Recent reports also indicate that nAChRs are expressed in non-neuronal cells (1). Due to their widespread distribution, nAChRs likely contribute to a wide range of normal and pathological behavioral and physiological conditions. Neuronal nAChRs are comprised of an unknown number of combinations of the subunits alpha2-10 (alpha8 is only found in avian species) and beta2-beta4 (2). The predominant nAChR subtypes in brain are thought to be alpha4beta2* and homomeric alpha7 whereas the major nAChR subtype in the periphery is likely alpha3beta4*. Through the use of pharmacological tools and genetically engineered mice, the influence of individual nAChR subtypes on behavior, physiology and pathology is slowly being revealed. For example, using the alpha4beta2* selective antagonist dihydro-beta-erythroidine, Corrigall *et al* and others provided evidence that the reinforcing properties of nicotine are mediated, in part, by alpha4beta2* nAChRs (3-6). Further support for a role of alpha4beta2* nAChRs in modulating the reinforcing properties of nicotine has come from genetically engineered mice. Picciotto *et al.* and Grabus *et al.* utilized nAChR beta2 subunit null mutant mice to demonstrate that the nAChR beta2 subunit is required for nicotine self–administration and nicotine conditioned place preference, respectively (7,8). Using a mouse engineered to express a nAChR alpha4 subunit that is hypersensitive to activation by agonists, Tapper *et al.* also reported that activation of nAChRs containing the alpha4 subunit is sufficient for nicotine reward (9). The sum of these data has provided convincing evidence that alpha4beta2* nAChRs are critical for nicotine reinforcement.

Despite the fact that we are beginning to gain an appreciation of which nAChR subtypes modulate different behaviors and physiological measures, we still know little with respect to how naturally occurring genetic variability in the genes that encode the members of the neuronal nAChR family influence individual differences in behavior, physiology and susceptibility to disease. Since virtually all behaviors, physiological traits and pathologies are influenced to some extent by genetic factors and nAChRs contribute to a wide range of phenotypes, it is likely that polymorphisms in the genes that encode the nAChR subunit family contribute to individual differences in behavior, physiology and disease susceptibility. Over the past decade, data have accumulated that support the premise that naturally occurring nAChR subunit gene variability contributes to phenotypic diversity in both humans and mice. In this review, current evidence for the role of variability in the genes that encode the two major brain-expressed alpha subunits, alpha4 and alpha7, in modulating behavior, physiology and disease risk in both humans and mice will be discussed.

3. CHRNA4/Chrna4

3.1. CHRNA4/Chrna4 basics

The gene that encodes the nAChR alpha4 subunit (CHRNA4 in humans, Chrna4 in mice) is located on chromosome 20q13.2-q13.3 in humans and chromosome 2 at 108 cM in mice. Two splice variants of alpha4, termed alpha4-1 and alpha4-2 have been reported by Connolly *et al* (10). The alternatively spliced products exhibit altered sensitivity to allosteric modulation by steroids (11). CHRNA4/Chrna4 contains 6 known coding exons. Exon 6 is alternatively spliced to generate the alpha4-1 and alpha4-2 products.

3.2. Human studies

3.2.1. CHRNA4 polymorphisms

The first reported polymorphism in any nAChR subunit gene was a CfoI restriction fragment length polymorphism (RFLP) located in exon 5 of CHRNA4 (12) (Table 1). Today, there are 33 verified single nucleotide polymorphisms (SNPs) listed in the SNP database (http://www.ncbi.nlm.nih.gov/SNP) for CHRNA4 which includes 6 synonymous (non-amino acid altering) exonic and 27 intronic SNPs. In addition, 3 non-synonymous SNPs (S248F, S252L and T265I) and one trinucleotide insertion (L259ins) have been reported for CHRNA4 (13-16).

3.2.2. CHRNA4 and epilepsy

The first evidence that genetic variability in a nAChR subunit gene contributed to phenotypic variability in the human population was provided by Steinlein et al. (13). This group discovered that a non-synonymous SNP in CHRNA4, which led to a serine to phenylalanine substitution at amino acid position 248 (S248F), was linked to autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) in a large Australian pedigree. CHRNA4 was the first gene identified that underlies an inherited idiopathic partial epilepsy. Since this initial discovery, three additional CHRNA4 mutants (two non-synonymous and one insertional mutation) have been linked to ADNFLE (17). Although it has been known for some time that over-stimulation of the nicotinic cholinergic system by high dose nicotine leads to seizure activity, linkage of CHRNA4 mutations to ADNFLE provided the first evidence of the involvement of the nicotinic cholinergic system in the etiology of human epilepsy (18). Interestingly, each of the CHRNA4 mutants leads to a change in the amino acid sequence within the ion channel-forming second transmembrane domain of the subunit. Not surprisingly, each of the CHRNA4 ADNFLE mutants affects nAChR function. However, inconsistencies exist in the literature with respect to how each of the mutations affects nAChR function. As a consequence, there is debate regarding the mechanism through which the CHRNA4 mutations lead to ADNFLE (19). However, based on data from mice engineered to express either the S248F or 259insL Chrna4 mutations, Klaassen *et al.* suggest that the mechanism underlying ADNFLE seizures may involve inhibitory synchronization of cortical networks (20). The potential role of genetic variability in CHRNA4 in contributing to other seizure phenotypes comes from studies by Steinlein *et al.* and Chou *et al* (21,22) . These groups reported that synonymous SNPs within CHRNA4 are associated with idiopathic generalized epilepsies and febrile convulsions, respectively.

3.2.3. CHRNA4 and attention

Parasuraman *et al* and Greenwood *et al* recently reported that a synonymous SNP, rs1044396 (referred to as

Table 1. Polymorphisms in CHRNA4 (human) and Chrna4 (mouse)

Gene	Polymorphism	Location of polymorphism/ type	Phenotypes	Reference	SNP functionally characterized?	Reference
CHRNA4	S248F	Exon 5, NS[1]	ADNFLE[3]	13, 106, 107, 108	YES	111-118
	S252L	Exon 5, NS	ADNFLE	15, 109, 110, 116	YES	115, 117, 118
	L259ins	Exon 5, insertion	ADNFLE	14	YES	111, 113-115, 117, 118
	T265I	Exon 5, NS	ADNFLE	16	YES	16
	rs1044394 (C594T)	Exon 5, S[2]	Generalized idiopathic epilepsy	14	NO	
	rs1044394 (C594T)	Exon 5, S	Febrile convulsions	22	NO	
	rs1044396 (C1545T)	Exon 5, S	Visuospatial attention	23-25	NO	
		Intron 2	Attentional deficit	28	NO	
	rs1044394 (C594T)	Exon 5, S	Lack of association with Attention deficit hyperactivity disorder	27	NO	
	G145-55A C105T C555T	Intron 2 Exon 2, S Exon 5, S	Alzheimer's disease	33	NO NO NO	
	rs1044394	Exon 5, S	Alzheimer's disease	34	NO	
	rs1044394	Exon 5, S	Lack of association with Alzheimer's disease	36	NO NO NO	
	rs1044394	Exon 5, S	Alcoholism	42	NO	
	rs1044396 rs1044397	Exon 5, S Exon 5, S	Nicotine dependence	37	NO NO	
	rs1044396 rs2236196 rs2273504 rs3787137	Exon 5, S 3' UTR Intron 2 Intron 5	Nicotine dependence	38	NO YES NO NO	39
	rs6122429 rs2236196	Promoter 3'UTR	Nicotine dependence	39	YES YES	39
Chrna4	rs27680364	Exon 5, NS	Nicotine-induced convulsions	51	YES	47, 49, 60
			Nicotine effects on locomotor activity, body temperature, respiration rate and startle reflex	52, 56		
			Oral nicotine consumption	54		
			Withdrawal from ethanol	58		
			Ethanol effects of startle reflex	59		
			Ethanol consumption	56		
			Ethanol consumption	54		
			Ethanol consumption	55		
			Enhancement of $\alpha 4\beta 2$ function by ethanol	61		

NS = non-synonymous polymorphism; S = synonymous polymorphism; ADNFLE = autosomal dominant nocturnal frontal lobe epilepsy

C1545T in these manuscripts), located in exon 5 of CHRNA4 is associated with individual differences in performance of a visuospatial attention task (23,24). In both reports, visuospatial task performance increased as the number of "C" alleles of the CHRNA4 SNP increased. Espeseth *et al* also described an interaction between CHRNA4 C1545T genotype and alleles of the apolipoprotein epsilon gene, APOE, in the modulation of visuospatial attention (25). Adults carrying the APOE-4 allele, which has been associated with increased risk for late-onset *al*zheimer's disease, exhibited a more profound reduction in visuospatial attention if they also were homozygous for the "T" allele of CHRNA4 (26). This gene-gene interaction was seen in middle aged adults but not older adults indicating an age by genotype interaction. Kent *et al*, however, did not detect a significant association between the C1545T CHRNA4 SNP and susceptibility to attention deficit hyperactivity disorder (ADHD) although Todd *et al* reported that an intronic SNP (intron 2) in CHRNA4 is associated with severe inattention in ADHD subjects (27,28). Thus, variability in CHRNA4 may not be a risk factor for ADHD but may contribute to the attentional deficits in individuals with ADHD. The observation that genetic variability in CHRNA4 may lead to individual differences in attention is consistent with reports that the administration of agonists that are presumed to be selective for alpha4beta2* type nAChRs improves performance on attentional tasks in rodents, primates and humans (29-32).

3.2.4. CHRNA4 and Alzheimer's disease

Several reports have implicated CHRNA4 polymorphisms in susceptibility to Alzheimer's disease (AD). Kawamata and Shimohama reported that three SNPs in CHRNA4 (two synonymous and one intronic SNP), were associated with AD while Vasto *et al* found that the

"T" allele of a synonymous SNP in exon 5 (referred to as 594 C/T (rs2229960)) was significantly increased in Alzheimer's patients relative to controls in two cohorts (33,34). Among Alzheimer's patients, CHRNA4 exon 5 SNPs were more prevalent among individuals who expressed higher levels of markers of oxidative DNA damage (35). However, not all studies have supported CHRNA4 variability as a risk factors for AD. Namely, Cook *et al* did not find a significant association between two exon 5 SNPs (C1545T and 145^{-55} G/A (rs2273502)) and AD (36). This divergent finding might be due to the fact that only late onset *al*zheimer's (onset ≥ age 65) patients were included in the Cook *et al* study while the other studies did not exclude individuals diagnosed with the disease who were under 65. Another possible explanation is that the influence of CHRNA4 allelic variants on Alzheimer's risk is dependent upon or influenced by population-based genetic differences. The sample pool for the Cook *et al.* study was Australian while the other studies included subjects of Japanese, Italian, or Polish descent (33-36).

3.2.5. CHRNA4 and nicotine dependence

Feng *et al.* reported that two CHRNA4 SNPs, rs1044396 and rs1044397 are significantly associated with three separate measures of nicotine dependence (Fagerström test for nicotine dependence (FTND), Revised Tolerance questionnaire (RTQ) and nicotine addiction (a dichotomous variable based upon FTND scores)) in Chinese male subjects (37). In addition, this group also found that a CHRNA4 haplotype based upon six SNPs was associated with nicotine dependence. In both the individual SNP and haplotype analyses, the less common genotype/haplotype was associated with reduced liability for nicotine dependence. A study by Li *et al* also provided evidence for the involvement of genetic variability in CHRNA4 in influencing individual differences in nicotine dependence in American subjects of African or European descent (38). However, their results were more complex. Whether a particular CHRNA4 SNP was associated with nicotine dependence was contingent upon the phenotypic measure, ethnicity and gender of the subjects. For example, one SNP (rs2236196) was associated with all three indices of nicotine dependence examined (smoking quantity (SQ), heaviness of smoking index (HSI) and FTND) in African American women, another SNP (rs2273504) was associated with one measure of nicotine dependence (FTND) in both African American and European American women, a third SNP (rs3787137) was associated with one measure of nicotine dependence (SQ) in European American men and a fourth SNP (rs1044396) was associated with one measure of nicotine dependence (SQ) in the pooled European American sample. Recently, Hutchison *et al.* reported that two CHRNA4 SNPs, rs6122429 and rs2236196 were significantly associated with subjective responses to smoking (39). One of the SNPs (rs2236196) also was associated with smoking cessation outcome. Moreover, *in vitro* analysis of these SNPs indicated that they are functional. The SNP rs6122429, which is located in the promoter of CHRNA4 differentially affected the binding of transcription factors and also altered promoter activity as measured by a luciferase reporter assay. Although the influence of CHRNA4 genotype on nicotine dependence appears to be dependent upon several factors including gender, ethnicity and measure of nicotine dependence, the potential involvement of CHRNA4 variants in influencing individual variability in liability to nicotine dependence is consistent with both pharmacological and genetic studies in rodents that indicate that alpha4beta2 nAChRs are involved in the reinforcing properties of nicotine (3-9).

3.2.6. CHRNA4 and alcoholism.

Studies have shown that a low level of response to alcohol early in an individual's drinking career is a strong predictor of the risk for developing alcoholism (40). The first evidence that CHRNA4 may contribute to individual differences in susceptibility to alcoholism was provided by Schuckit *et al* who reported that a genetic marker, D20S94 (now referred to as D20S443), near CHRNA4 on human chromosome 20q13, is linked to low responsiveness to alcohol (41). The observation by Kim *et al.* that the CfoI polymorphism in CHRNA4 is associated with alcoholism in Korean subjects supports this possibility (42). Interestingly, it is well established that smoking prevalence among alcoholics is significantly greater than in the population as a whole (43). In addition, both smoking and alcoholism are strongly influenced by genetic factors and there appears to be some overlap in the genetics of smoking and alcoholism (44-46). Since CHRNA4 is associated with both nicotine dependence and alcoholism, it may be a common genetic link between these two behaviors.

3.3. Mouse studies

3.3.1. Chrna4 polymorphisms in mice

According to the mouse SNP database, there are 154 verified SNPs in Chrna4 including three that are non-synonymous, seven that are synonymous, sixty-one located in the 3' UTR and sixteen that are immediately upstream. The majority of these SNPs occur between the common laboratory strain C57BL/6 (the strain used for the mouse genome project) and wild-derived mouse strains. However, one of the three non-synonymous SNPs is well represented among commonly used inbred mouse strains (47-49). This non-synonymous SNP leads to a threonine to alanine substitution at amino acid position 529 (T529A) in the mouse nAChR alpha4 subunit. This variation is located within the large intracellular loop between transmembrane domains three and four in a region of the loop that is relatively highly conserved across species. A mouse strain also has been described that has a 300 kb deletion on chromosome 2 that encompasses Chrna4 and other genes (50,51).

3.3.2. Chrna4 behavior in mice

The first evidence that genetic variability in Chrna4 may contribute to individual differences in behavior in mice came from a study by Stitzel *et al* (51). This group reported that a RFLP in Chrna4 is associated with sensitivity to nicotine-induced seizures in a panel of recombinant inbred mouse strains derived from Long Sleep (LS) and Short Sleep (SS) selected lines. A subsequent study demonstrated that this RFLP was in high linkage

disequilibrium with the non-synonymous T529A SNP (52). This same study also reported that the Chrna4 polymorphism as defined by either the RFLP or T529A SNP is significantly associated with several additional responses to nicotine including the effect of nicotine on respiration rate, acoustic startle response, locomotor activity and body temperature. A preliminary report also suggested that the T529A polymorphism might influence cognition (53). In this study, mice possessing the A529 allele of Chrna4 required a significantly greater number of days to learn to associate the auditory cue with a reward than those carrying the T529 allele.

Recently, Butt *et al* and Li *et al* reported that the T529A SNP is linked to individual differences in oral nicotine consumption (54,55). Both the Butt *et al* and Li *et al* studies reported that mouse strains that express the T529 variant of alpha4 consume more nicotine than do mice that express the A529 variant. A previous study by Tritto *et al.* also reported that mice homozygous for the T529 allele consumed more nicotine by choice than did A529 homozygotes although the difference was not statistically significant (56). Nonetheless, all studies that have examined the relationship between Chrna4 genotype and nicotine consumption have found that the T529 allele is consistently associated with higher nicotine consumption. Using mice carrying a null mutation for Chrnb2, the gene that encodes the nAChR beta2 subunit, Butt *et al.* also demonstrated that the relationship between Chrna4 genotype and nicotine consumption is dependent upon the presence of the nAChR beta2 subunit (54). The beta2 subunit combines with the alpha4 subunit to form the most abundant nAChR in brain. In the absence of the β2 subunit there is no measurable function from alpha4 nAChRs (57). The fact that the loss of alpha4beta2 function leads to a loss in the linkage between Chrna4 T529A genotype and nicotine consumption provides strong evidence that the linkage between Chrna4 genotype and nicotine consumption is due to genetic variability in Chrna4 and not a gene linked to Chrna4.

The studies by Butt *et al.*, Li *et al.*, and Tritto *et al*, also examined the relationship between Chrna4 genotype and ethanol consumption. However, these groups obtained conflicting results (54-56). Butt *et al.* reported that the T529 allele was linked to increased alcohol consumption while Tritto *et al.*, and Li *et al.* reported that the A529 allele was linked to increased alcohol consumption. Moreover, the linkage between Chrna4 genotype and alcohol consumption did not require the presence of the beta2 subunit. The observation that the relationship between Chrna4 genotype and alcohol consumption did not require the presence of the beta2 subunit suggests that either a nAChR subtype containing alpha4 but not beta2 (alpha4beta4?) or a gene linked to Chrna4 is responsible for the relationship between the T529A polymorphism and alcohol consumption. This result may explain why the relationship between Chrna4 genotype and ethanol consumption varied between the studies. Each group used mice of different genetic backgrounds. Therefore, the opposite relationships observed between Chrna4 genotype and ethanol consumption across studies could be due to strain specific differences in an allele or alleles of a gene or genes that are linked to Chrna4.

Using the same basic strategy of combining mouse strains that are polymorphic for the T529A variants of Chrna4 with beta2 subunit null mutant mice, Butt *et al* demonstrated that withdrawal from alcohol is influenced by the Chrna4 variants in mice (58). Mice homozygous for the A529 allele of Chrna4 were more sensitive to handling induced convulsions during alcohol withdrawal than were animals homozygous for the T529 allele of the gene. This effect of Chrna4 genotype was not observed in beta2 null mutant mice. Additional support for the potential role of Chrna4 variability in modulating individual differences in sensitivity to the effects of alcohol was provided by a study by Owens *et al* (59). Using the LS x SS recombinant inbred strains, Owens *et al* found that mice homozygous for the A529 allele of Chrna4 were significantly more sensitive to the depressant effects of ethanol on the acoustic startle response than were mice homozygous for the T529 allele of the gene.

3.3.3. Chrna4 and nAChR function in mice

An initial report suggested that variability in Chrna4 influences the function of alpha4beta2* nAChRs in thalamic synaptosomes prepared from two mouse lines (LS and SS) that differ in T529A genotype (60). In a follow-up study, Dobelis *et al.* compared nicotine-stimulated $^{86}Rb^{+}$ efflux from synaptosomes prepared from eight brain regions (cerebral cortex, thalamus, hippocampus, striatum, hindbrain, midbrain, septum, and hypothalamus) isolated from fourteen mouse strains, eight that possess the T529 allele of Chrna4 and six strains that carry the A529 allele of the gene (47). In every brain region evaluated, mean nicotine-stimulated $^{86}Rb^{+}$ efflux was greater in the A529 allele strains relative to the T529 allele strains. This study also reported that Chrna4 T529A genotype did not influence the expression of alpha4beta2* nAChRs. Consistent with this observation, Gahring *et al* used immunohistochemical methods to show that Chrna4 genotype is not associated with the neuroanatomical distribution or expression of the alpha4 subunit in brain across seven strains of mice (48). These combined data suggest that the influence of Chrna4 genotype on nicotine-stimulated $^{86}Rb^{+}$ efflux is the result of an effect on receptor function, not receptor expression. In support of this possibility, Kim *et al* reported that the T529A polymorphism influences function but not expression of mouse alpha4beta2 nAChRs heterologously expressed in HEK293T cells (49). According to Kim *et al*, the T529A polymorphism affects the ratio nAChRs that exhibit low and high sensitivity to activation by nicotine. The majority of T529 alpha4beta2 nAChRs appear to exhibit high sensitivity to activation while the majority of A529 alpha4beta2 nAChRs occur as a population with low sensitivity to activation by nicotine. If the functional *in vitro* data on the T529A variants of alpha4 nAChRs are indicative of *in vivo* function, then genetic variability that alters the ratio of high to low sensitivity alpha4beta2 nAChRs may lead to individual differences in sensitivity to nicotine.

Table 2. Polymorphisms in CHRNA7 (human) and Chrna7 (mouse)

Gene	Polymorphism	Location of polymorphism/type	Phenotypes	Reference	SNP functionally characterized?	Reference
CHRNA7	D15S1360	Intron 2	P50 auditory evoked potential	68	NO	
	-86C→T -92G→A -143G→A -178-G -194G→C -241A→G	Promoter	Schizophrenia, P50 auditory evoked potential	66	YES	66
	-194G→C	Promoter	P50 auditory evoked potentail	72	YES	66
	D15S1360	Intron 2	Smoking among schizophrenics	75	NO	
	Linkage		Smoking in schizophrenic families	76		
	D15S1360 L76630	Intron 2 FAM7A	Not associated with smoking in schizophrenics	77	NO	
	Linkage		Juvenile myoclonic epilepsy	78		
	Linkage		Rolandic epilepsy	79		
Chrna7	PvuII RFLP	Intron 9	Nicotine-induced seizures	86	NO	
	Entire Chrna7 allelic variant	Many	P30 auditory evoked potential	87	NO	
	Multiple Chrna7 RFLPs	Majority located in intron 9	Level of αBTX binding in mouse brain	81	NO	
	Entire Chrna7 allelic variant	Many	Neuroanatomy of αBTX binding in adult hippocampus	88	NO	
	Entire Chrna7 allelic variant	Many	Neurodevelopment of αBTX binding sites in hippocampus	89	NO	
	-491C→T -583C→T	Promoter	Chrna7 Promoter activity	82	YES	82

The T529A Chrna4 polymorphism not only appears to affect the function of alpha4beta2* nAChRs, it also may influence the ability of this nAChR subtype to be potentiated by ethanol. Butt *et al* observed that ethanol potentiation of nicotine-stimulated $^{86}Rb^{+}$ efflux from synaptosomal alpha4beta2* nAChRs occurs in synaptosomes prepared from mouse strains expressing the A529 variant of the alpha4 subunit but not in synaptosomes prepared from alpha4 T529 expressing strains (61). This genotype-dependent potentiation provides a potential explanation for the linkage of T529A genotype and alcohol withdrawal; mouse strains that express the allelic variant of Chrna4 that is associated with ethanol potentiation of alpha4beta2* nAChR function exhibit more severe ethanol withdrawal symptoms than do strains that possess the alpha4 variant that is not associated with ethanol potentiation. Nonetheless, the mechanism through which the polymorphism affects receptor function and potentiation by ethanol remains unknown.

4. CHRNA7/Chrna7.

4.1. CHRNA7/Chrna7 basics

The gene that encodes the nAChR alpha7 subunit (CHRNA7 in humans, Chrna7 in mice) is located on chromosome 15q14 in humans and chromosome 7 at 30 cM in mice. In humans, there also is a partial duplication of CHRNA7 which includes exons 5-10 of CHRNA7 and exons A-E of a novel gene from the family with sequence similarity 7 (FAM7A). This duplication is termed CHRFAM7A and is located on chromosome 15q13.1. Currently, there is no evidence that this hybrid between CHRNA7 and FAM7A forms a functional protein. A similar duplication has not been found among other species. CHRNA7/Chrna7 contains 10 known coding exons. Several putative splice variants of alpha7 have been described in humans although their function has not been evaluated (62). Three splice variants have been identified for alpha7 in non-human mammals. Two of the splice variants (isolated from bovine adrenal chromaffin cells and mouse brain) do not form functional receptors when expressed alone but do act as dominant-negatives when co-expressed with normal alpha7 *in vitro* (63,64). The third splice variant (isolated from rat brain) does form a functional receptor with properties that are distinct from the normal alpha7 nAChR (65).

4.2. Human studies.

4.2.1. CHRNA7 polymorphisms

For the purposes of this review, only polymorphisms in CHRNA7 will be discussed as the functional relevance of the partially duplicated CHRFAM7A is not known and an equivalent partial duplication does not exist in mice or other species (Table 2). Two hundred and thirty-three verified SNPs currently are listed in the SNP database for CHRNA7. All of the SNPs listed in the database are intronic. However, Leonard *et al.* have reported twelve polymorphisms in the minimal promoter region of CHRNA7 in an ethnically mixed sample of control and schizophrenic subjects (66). Nine of these polymorphisms are SNPs, two are single nucleotide deletions/insertions and one is a six base pair insertion/deletion. Gault *et al* also identified three non-synonymous and seven synonymous SNPs in Chrna7 in a similar sample (62). Recently, Tsuneki *et al* reported a non-synonymous SNP in a Japanese sample that leads to a glycine/serine variation at amino acid position 423 (G423S) (67). Based upon *in vitro* analysis, the G423S variants of alpha7 differ in protein kinase C-dependent, agonist-induced receptor desensitization.

4.2.2. CHRNA7, auditory gating and schizophrenia

The first evidence that genetic variability in CHRNA7 may contribute to schizophrenia came from a

study by Freedman *et al* (68). This group demonstrated that a polymorphic marker within CHRNA7 (D15S1360) was linked to an auditory gating deficit that is common among schizophrenic patients and their first degree relatives. Subsequently, a large number of studies have been reported that have assessed the association between the region of chromosome 15 that contains CHRNA7 (15q13-14), auditory gating deficits and schizophrenia. Although the results are mixed, a majority of the studies show a positive relationship between this region of the genome with gating deficits and schizophrenia (69). Moreover, Leonard *et al* described several SNPs in the proximal promoter of CHRNA7 and found that schizophrenic patients had a significantly greater frequency of promoter SNPs than did control subjects (70). Functional analysis of the CHRNA7 promoter SNPs indicated that of the eight CHRNA7 promoter SNPs tested, six led to reduced promoter activity while the remaining two had no effect. The observation that schizophrenic patients had a significantly greater number of promoter activity-reducing SNPs than did the controls is consistent with the finding that schizophrenic patients have reduced levels of expression of alpha7 nAChRs (71). Houy *et al* also reported an association between one of the CHRNA7 promoter activity-reducing SNPs and auditory gating (72). However, this group observed that the CHRNA7 promoter SNP was associated with protection from the auditory gating deficit.

4.2.3. CHRNA7 and smoking

Smoking among schizophrenics is substantially greater than in the general population (73). Although the reason for this high rate of smoking among schizophrenics is not completely understood, it may serve as a form of self-medication to normalize some of the neurophysiological deficits common among schizophrenics or to reduce side effects of common anti-psychotic medications (74). The potential role of CHRNA7 in smoking among schizophrenics was assessed by De Luca *et al* and Faraone *et al* (75,76). Both of these groups found evidence that smoking status among schizophrenic patients is associated with genetic variability in CHRNA7. However, Stassen *et al* did not find an association between CHRNA7 genotype and smoking among healthy controls (77). Therefore, the influence of genetic variability in CHRNA7 on smoking may be influenced by whether an individual has a neuropsychiatric disorder.

4.2.4. CHRNA7 and epilepsy

At least two studies have found linkage between CHRNA7 and idiopathic epilepsy. Elmslie *et al* reported that among the families they studied, the majority had a major susceptibility locus for juvenile myoclonic epilepsy located at chromosome 15q14, which contains CHRNA7 (78). In addition, Neubauer *et al* found linkage between the same region of chromosome 15 and rolandic epilepsy (79). Neither of these studies used polymorphic markers specific to CHRNA7. In addition, these types of epilepsy, like all idiopathic epilepsies, are heterogeneous and likely due to a wide range of causes. Nonetheless, these studies indicate that CHRNA7 should be considered as a candidate gene for these forms of idiopathic epilepsy.

4.3. Mouse studies.

4.3.1. Chrna7 polymorphisms

The first evidence of genetic variability in Chrna7 in mice was reported by Nagavarapu and Boyd (80). This group identified a Sac I RFLP in Chrna7 across several inbred mouse strains. Additional Chrna7 RFLPs in mice were described by Stitzel *et al* (81). According to the most recent SNP database, there are 292 verified SNPS in Chrna7. This includes four 3' UTR SNPs and 2 synonymous SNPs. The remaining SNPs are in intronic regions. Mexal *et al* also has reported that there are fourteen SNPs in an approximately 1000 bp region immediately upstream of the Chrna7 translation start site (82).

4.3.2. Chrna7 polymorphisms and behavior

Several studies suggested that genetically-determined differences in the expression of alpha7 nAChRs contributed to individual differences in sensitivity to nicotine. For example, Miner *et al* reported that alpha7 expression, as measured by [^{125}I] α-bungarotoxin (αBTX), correlated with sensitivity to nicotine-induced seizures in a classic genetic cross between the inbred mouse strains C3H and DBA/2 and across 19 different inbred mouse strains (83,84). In addition, Stevens *et al* found that mouse strain variations in hippocampal αBTX binding were correlated with strain differences in inhibitory auditory gating (85). In 1996, Stitzel *et al* reported that polymorphisms in Chrna7 between the inbred mouse strains C3H and DBA/2 are linked to individual differences in the levels of αBTX binding in mouse brain (81). Subsequently, this group reported that the variant alleles of Chrna7 are linked to individual differences in sensitivity to the seizure-inducing effects of nicotine in a C3H x DBA/2 F2 intercross (86). This was the first study to demonstrate that genetic variability in a nAChR subunit gene might contribute to individual differences in sensitivity to nicotine. More recently, a preliminary report by Stevens *et al* found that the allelic variants of Chrna7 contribute to individual differences in auditory gating (87). In this study, Stevens used congenic strains in which the C3H allele of Chrna7 was introgressed onto a DBA/2 genetic background and the DBA/2 Chrna7 allele was introgressed onto a C3H genetic background. In short, Stevens *et al* found that auditory gating phenotype was dependent upon Chrna7 genotype and not genetic background. Moreover, the effect of genotype on auditory gating was dominant. For example, mice that were either heterozygous or homozygous for the DBA/2 allele of Chrna7 exhibited significantly reduced auditory gating relative to animals homozygous for the C3H allele regardless of whether the genetic background was C3H or DBA/2. These findings convincingly demonstrate that genetic variability in Chrna7 or at the least, a gene linked to Chrna7, contributes to individual differences in auditory gating.

4.3.3. Chrna7 polymorphisms and alpha7 expression

Polymorphisms that empirically might be predicted to affect the function of alpha7 nAChRs have not been found. However, the allelic variants of Chrna7 do appear to have a considerable impact on the expression, neuroanatomical distribution and developmental profile of

alpha7 nAChRs. Studies have shown that C3H and DBA/2 mice differ in the expression and neuroanatomical distribution of αBTX binding sites (83,88). As mentioned previously, Stitzel *et al* reported that Chrna7 polymorphisms are linked to alpha7 expression levels in mouse brain in an F2 cross derived from C3H and DBA/2 parents. Using the C3H Chrna7 congenic mouse strain described above, Adams *et al* also determined that the alleles of Chrna7 are linked to mouse strain differences in the neuroanatomical distribution of alpha7 nAChRs in the hippocampus of adult animals (88). Despite the fact that the congenic animals were greater than 95% C3H genetically, the neuroanatomical distribution of αBTX binding sites depended upon Chrna7 genotype; animals homozygous for the DBA/2 allele of Chrna7 possessed the DBA/2 distribution pattern of alpha7 nAChRs while animals homozygous for the C3H allele exhibited the C3H distribution of alpha7 nAChRs. Recently, Adams *et al* used the C3H and DBA/2 congenic lines to demonstrate that the embryonic development of alpha7 nAChR expression is dependent upon Chrna7 genotype (89). Again, the developmental profile of alpha7 expression was dependent upon Chrna7 genotype but not genetic background; embryos that were homozygous for the DBA/2 allele displayed the DBA/2 developmental pattern regardless of genetic background and mice homozygous for the C3H allele of Chrna7 presented with the C3H developmental pattern regardless of genetic background.

Recently, Mexal *et al.* characterized 947 bp Chrna7 proximal promoter fragments that contain 14 SNPs between from C3H and DBA/2 mice (82). Initial analysis indicated that the fragment derived from C3H had greater promoter activity than did the fragment from DBA/2. Through a series of experiments, Mexal *et al.* were able to demonstrate that two SNPs located at 491 and 583 bp upstream of the Chrna7 initiation codon were responsible for the difference in Chrna7 promoter activity between the two strain-derived fragments. Both SNPs may introduce binding sites for the transcription factor c-myb in the DBA/2 allele of Chrna7. The data from the mouse studies is consistent with human studies in that the strain (DBA/2 mice) with reduced αBTX binding and poor auditory gating is the strain with polymorphisms that reduce Chrna7 promoter activity.

5. DISCUSSION

This review has described the potential role of genetic variability in the human and mouse CHRNA4/Chrna4 and CHRNA7/Chrna7 nAChR subunit genes in influencing individual differences in behavior and susceptibility to disease. However, polymorphisms in virtually all nAChR subunit genes in both humans and mice have been linked to behavioral traits or disease risk. Nonetheless, CHRNA4/Chrna4 and CHRNA7/Chrna7 are the focus of this review for two reasons. First, these genes encode the most abundant alpha subunits expressed in the brain, the alpha4 and alpha7 subunits. Second, there is considerably more published data on polymorphisms in these genes in both humans and mice than for any of the other nAChR subunit genes. Therefore, these two genes allow for a cross-species comparison of the role of genetic variability in nAChR subunit genes in influencing behavior, physiology and pathology. Perhaps the most striking aspect of the studies on genetic variability in CHRNA4/Chrna4 and CHRNA7/Chrna7 is the observation that similar phenotypes appear to be influenced by polymorphisms in these genes in both humans and mice. For example, in humans, genetic variability in CHRNA4 is associated with epilepsy, attentional aspects of cognition, nicotine dependence and alcoholism. Similarly in mice, polymorphisms in Chrna4 are associated with nicotine-induced seizures, learning, nicotine consumption, alcohol preference, and withdrawal from alcohol. Likewise, CHRNA7 is associated with an auditory gating deficit, levels of alpha7 nAChR expression and two forms of epilepsy in humans while Chrna7 is linked to an auditory gating deficit, levels of alpha7 nAChR expression and nicotine-induced seizures in mice. Moreover, SNPs in the human CHRNA7 promoter that lead to reduced promoter activity are associated with reduced auditory gating and SNPs in the mouse Chrna7 promoter that result in reduced promoter activity are from a mouse strain with a deficit in auditory gating.

Also noteworthy is the fact that the cross-species similarities in genotype-phenotype relationships are observed despite the fact that there are no common polymorphisms in CHRNA4/Chrna4 and CHRNA7/Chrna7 between humans and mice. This suggests that polymorphisms within a gene need not be the same across species to elicit a common phenotypic effect. For example, none of the SNPs in CHRNA4 that are associated with nicotine dependence, attention or alcoholism in humans affect the amino acid sequence of CHRNA4. In contrast, a functional non-synonymous SNP in mouse Chrna4 is associated with nicotine consumption, cognition and alcohol-related phenotypes. One potential explanation as to why the human and mouse SNPs affect the same phenotypes is that the net effect of the polymorphisms is the same. Perhaps the CHRNA4 SNPs alter the expression level of alpha4 nAChRs to an extent that there is a net change in response of the affected neurons to agonist. This change in response could be similar to the difference in function observed in the mouse variants of alpha4. However, what effect, if any, the non-coding CHRNA4 SNPs have on alpha4 nAChR expression in human brain has not been determined.

Genetic studies in humans are restricted to polymorphisms present in the population of interest. As described in this review, naturally occurring genetic variability in the mouse also can be used as a genetic tool to assess the role of genetic variability on phenotypic diversity. However, various other genetic approaches can be exploited in the mouse to evaluate genotype-phenotype relationships. For example, molecular manipulation of genes, such as knock-out, knock-in or transgenic over-expression can be used to assess the role of a gene in regulating a phenotype in mice. In addition, chemical induction of mutations can be used to produce novel gain or loss of function alleles of a gene. Each of these genetic approaches has strengths and weaknesses. Recent reviews

have discussed the pros and cons of genetically engineered mice and chemical mutagenesis (90,91). Some of the strengths and weaknesses of using naturally occurring genetic variation as a genetic tool to understand the genetics of behavior, physiology and disease will be discussed below.

5.1. Advantages of using naturally occurring polymorphisms in mice to understand the genetics of behavior, physiology and disease

The most obvious advantage of studying naturally occurring genetic variation in mice is that it will lead to a better understanding of how genetic variability contributes to trait variance in a natural population. Most behavioral and physiological traits in natural populations likely are influenced by many genes of modest or small effect. However, mutations in genetically engineered mice are typically designed with the intention of having a robust effect on phenotypic measures: knockout strategies completely eliminate a gene product and knock-in strategies often lead to the expression of a protein product with substantially altered function. Of course, these types of mutations are made for practical reasons. If the time and funds are going to be invested in making such a mouse, then it is only reasonable to manipulate a gene in such a way as to provide the best chance of seeing an effect. Moreover, studies with genetically engineered mice generally are geared towards asking the question: is the gene of interest necessary for or contribute to a phenotype or phenotypes of interest? In contrast, investigations into the role of naturally occurring genetic variability in modulating behavior and physiology typically address the question: does existing variation in a gene or genes of interest influence individual phenotypic differences in the population. Although studies with genetically engineered mice no doubt are essential for the identification of genes that contribute to a phenotype, the magnitude of the effect of the introduced mutations on gene expression or function is rarely seen in natural populations. For example, the knockin mouse used by Tapper *et al* to demonstrate that alpha4 nAChRs play a role in nicotine reinforcement, carry a mutant allele of Chrna4 that is 20 times more sensitive to activation by nicotine than wild-type nAChRs (9). In contrast, the naturally occurring T529A variants of mouse alpha4 nAChRs only differ about two fold in sensitivity to activation by nicotine (49). Consequently, despite the fact that genetically engineered mice provide invaluable information as to which genes might contribute to phenotypic differences in natural population, only studies that examine naturally occurring genetic variation will lead to the identification of the types of genetic variants that contribute to phenotypic diversity in a population. Because genetic influences on behavior, physiology and disease susceptibility in humans is a result of naturally occurring genetic variants, understanding how such variants in an animal model contribute to phenotypic variation in a population should provide insight into the human condition.

Another benefit of studying naturally occurring genetic variations is that this approach likely will lead to the discovery of new functional domains in gene products and novel molecular mechanisms through which gene expression in regulated. For example, the T529A variant of mouse Chrna4 occurs in a region of the alpha4 subunit not previously known to influence nAChR function. Yet *in vitro* data demonstrated that the polymorphism does affect the function of alpha4beta2 nAChRs by altering the ratio of alpha4beta2 nAChRs that exhibit high sensitivity to activation by nicotine. Several studies had previously demonstrated that heterologously expressed alpha4beta2 nAChRs exist as two populations with low or high sensitivity to activation by agonists (49,92-95). However, the study by Kim *et al* was the first to identify a region in an nAChR subunit that regulates the intrinsic ratio of high and low sensitivity alpha4beta2 nAChRs (49). These results along with the behavioral data linking Chrna4 genotype to several measures of nicotine sensitivity provided the first evidence that genetic variation that alters the ratio of high to low sensitivity alpha4beta2* nAChRs may lead to individual differences in sensitivity to the effects of nicotine. Therefore, understanding the molecular mechanism responsible for the effect of the T529A polymorphism on receptor function may lead to new insights into the biological underpinnings of nicotine sensitivity.

A third advantage of the study of naturally occurring polymorphisms is that such studies can provide data that implicate a gene of interest in modulating a phenotype that previously was not thought to be influenced by the gene. The best example of this is ADNFLE. Prior to the linkage of human chromosome 20q13 to ADNFLE, the nicotinic cholinergic system was not typically considered to be a major contributor to epilepsy phenotypes. However, after the initial discovery that a mutation in CHRNA4 was the likely cause of ADNFLE in an Australian pedigree, several subsequent studies have provided evidence ADNFLE in other family pedigrees are due to mutations in CHRNA4 as well as in CHRNB2, the gene that encodes the nAChR beta2 subunit. Moreover, recent data support the possibility that genetic variability in CHRNA2, which encodes the nAChR alpha2 subunit, is responsible for a sleep-related familial idiopathic epilepsy (96). Furthermore, CHRNA7 is a candidate for juvenile myoclonic epilepsy and rolandic epilepsy (78,79).

5.2. Disadvantages of using naturally occurring polymorphisms to understand the genetics of behavior, physiology and disease.

The major disadvantage of using the natural variant approach to studying genetics is that it is exceedingly difficult to prove cause-effect relationships between a specific polymorphism or polymorphisms and a phenotype. This is due to the fact that several polymorphisms, including ones that have not been identified, are in linkage disequilibrium (co-segregate) with the "marker" polymorphism. Since the polymorphism may have some value as a predictive marker of disease risk this may not be viewed entirely as a disadvantage. However, from a mechanistic point of view, it remains a drawback. This disadvantage also exists with both knockout and knockin mice (97). Any engineered gene that arose from homologous recombination in embryonic stem cells will be flanked by genetic material from the ES cell line.

Therefore, if the mouse strain from which the ES cell line was derived possesses different alleles in this flanking DNA relative to the background strain onto which the genetic mutant was bred, then it is possible that any effect of the engineered mutation is actually due to a gene linked to the mutation. For example, Bolivar et el reported that differences in open field activity between an IL10 knockout mouse on a C57BL/6 background and C57BL/6 inbred mice is due to a gene linked to the null IL10 allele, not the null allele (98). Due to the presence of ES cell line-linked DNA, it has been proposed that knockout/knockin mutants might be a valuable resource to assess the role of naturally occurring variability in the DNA flanking the engineered mutation in modulating behavior (99).

As previously mentioned, naturally occurring genetic variants typically have a small effect on behavioral and physiological measures. Although a major reason for studying naturally occurring variants is to gain insight into the genetic architecture of how variants of small individual effect lead to phenotypic diversity, analysis of variants that only have a small effect on behavioral and physiological measures is not trivial. In order to confidently determine whether a genetic variant of small effect is linked to a phenotype, relatively large sample sizes may be required. This may be particularly true when the variant of interest is on a mixed genetic background that contains many polymorphic genes that affect the phenotype under investigation.

A final disadvantage of using naturally occurring genetic variability to examine the genetics of behavior and physiology is that studies are limited to known polymorphisms. Although the density of SNP maps is increasing daily for both humans and mice and SNP "chips" that can type 10^6 SNPs from an individual are now available, large-scale SNP identification is from a limited number of individuals (or mouse strains). In addition, other forms of genetic variability such as micro deletions, micro insertions and copy number variants are distributed throughout the genome and most certainly contribute to phenotypic diversity (100-102).

6. FUTURE DIRECTIONS.

One of the key challenges in genetics today is to identify the "functional" polymorphism (or polymorphisms) in a gene that is responsible for linkage to a phenotype of interest. Although *in vitro* studies can be used to assess the influence of an obvious candidate polymorphism, such as a non-synonymous SNP or promoter variants on protein function or expression, these studies, by themselves, do not prove a cause-effect relationship. Moreover, such studies exclude the vast majority of polymorphisms from analysis. Nonetheless, an ever increasing arsenal of methods is being developed to zero in on functional polymorphisms in the mouse (103). These methods can be used to determine whether polymorphisms in a gene of interest, such as the nAChR subunit genes, or a polymorphism linked to these genes is responsible for the association between genotype and the phenotypes discussed in this review. However, the ultimate test of whether polymorphisms in a gene influence a phenotype is to test the polymorphisms in genetic isolation. In mice this can readily be accomplished using currently available knock-in strategies; mice can be engineered that differ genetically only for the polymorphism of interest. If the two lines of mice that are genetically identical except for a single polymorphism differ in behavior or physiology, a definitive effect of the SNP will have been established. For example, if the T529A Chrna4 SNP described in this review does, in fact, influence oral nicotine consumption, mice engineered to differ at this single SNP should show differences in nicotine consumption. The use of knock-in mice also can be used to test human polymorphisms so long as the region of the gene containing the human polymorphism is conserved between humans and mice and the phenotype of interest can be reasonably modeled in the mouse. Such mice have been made for more than one of the CHRNA4 polymorphisms that have been linked to ADNFLE (20). These mice exhibit abnormal electroencephalograms, spontaneous seizures and increased inhibitory post synaptic potentials in cortex. Based upon their findings, Klaassen *et al* hypothesized that the CHRNA4 ADNFLE mutations in humans cause epilepsy due to synchrony in the frontal cortex produced by interneuronal hyperactivity. These data demonstrate that approaches such as knock-in mice of naturally occurring genetic variants not only can identify functional polymorphisms but also can lead to mechanisms underlying genotype-phenotype relationships.

In many cases, the identity of the "functional" SNP or SNPs is not known. In these instances, knockin strategies are not a reasonable option. Among the SNPs discussed in this review, the CHRNA4 SNPs that are linked to nicotine dependence, alcoholism and cognition, and all of the CHRNA7 SNPs are included in this category. In mice, several strategies can be used to zero in on the functional SNP (103). However, there are few strategies for the identification of functional SNPs in a human gene. Nonetheless, BAC (bacterial artifical chromosome) transgenic mice are an emerging approach that can be used to assess whether allelic variants of a gene lead to phenotypic differences among individuals (104). In this method, BAC clones containing different alleles for the gene of interest are introduced into a mouse that is a null mutant for the gene of interest. These mice then would express the human versions of the gene of interest. If BAC transgenic mice carrying the different allelic variants of the human gene of interest exhibit phenotypic differences, evidence that polymorphisms in the gene do influence the phenotype of interest is provided. Subsequent studies to identify the specific "functional" polymorphism or polymorphisms within the BAC would follow.

Unfortunately, the BAC transgenic approach is not without problems. Investigators cannot control copy number, site of integration and position effects in independently generated transgenic mice. Therefore, it may be impossible to determine whether any phenotypic differences in transgenic mice that harbor different alleles of a human gene are due to the human genetic variants or unavoidable variability in copy number, integration site and position effects of the human genes. However, a more

elegant approach to evaluate human genes in mice has recently been described. The strategy, termed recombinase-mediated genomic replacement, allows for homologous replacement of a mouse gene with the human ortholog of interest (105). Moreover, different alleles of the human gene can be introduced into separate mice through this method. Each human gene allele will, therefore, be present in a single copy in the same genomic location. Although this approach requires significantly greater molecular genetic expertise than does the standard BAC transgenic approach, it eliminates the problems associated with the BAC transgenic approach and should allow for the analysis of the potential effects of human polymorphisms on a phenotype of interest.

7. PERSPECTIVE

In summary, naturally occurring genetic variability in the nAChR subunit genes CHRNA4/Chrna4 and CHRNA7/Chrna7 in both humans and mice have been discussed. Studies to date indicate that genetic variability in these genes influences various traits including cognition, nicotine addiction and alcoholism as well as susceptibility to epilepsy, Alzheimer's, and schizophrenia. Moreover, genetic variability in these genes tends to influence similar phenotypes across the two species despite the fact that there are no common polymorphisms in the nAChR subunit genes between humans and mice. Nonetheless, further studies clearly are needed to establish whether polymorphisms in these genes or other genes linked to the nAChR subunit genes are responsible for the genotype-phenotype associations.

8. ACKNOWLEDGEMENTS

This work was supported, in part, by NIH (DA014369)

9. REFERENCES

1. Sharma, G. and S. Vijayaraghavan: Nicotinic receptor signaling in nonexcitable cells. *J Neurobiol* 53, 524-534 (2002)
2. Gotti, C., M. Zoli, & F. Clementi: Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci* 27, 482-491 (2006)
3. Corrigall, W.A., K.M. Coen, & K.L. Adamson: Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res* 653, 278-284 (1994)
4. Yeomans, J. and M. Baptista: Both nicotinic and muscarinic receptors in ventral tegmental area contribute to brain-stimulation reward. *Pharmacol Biochem Behav* 57, 915-921 (1997)
5. Watkins, S.S., M.P. Epping-Jordan, G.F. Koob, & A. Markou: Blockade of nicotine self-administration with nicotinic antagonists in rats. *Pharmacol Biochem Behav* 62, 743-751 (1999)
6. Kenny, P.J. and A. Markou: Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. *Neuropsychopharmacology* 31, 1203-1211 (2006)
7. Picciotto, M.R., M. Zoli, V. Zachariou, & J.P. Changeux: Contribution of nicotinic acetylcholine receptors containing the beta 2-subunit to the behavioural effects of nicotine. *Biochem Soc Trans* 25, 824-829 (1997)
8. Grabus, S.D., B.R. Martin, S.E. Brown, & M.I. Damaj: Nicotine place preference in the mouse: influences of prior handling, dose and strain and attenuation by nicotinic receptor antagonists. *Psychopharmacology (Berl)* 184, 456-463 (2006)
9. Tapper, A.R., S.L. McKinney, R. Nashmi, J. Schwarz, P. Deshpande, C. Labarca, P. Whiteaker, M.J. Marks, A.C. Collins, & H.A. Lester: Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science* 306, 1029-1032 (2004)
10. Connolly, J., J. Boulter, & S.F. Heinemann: Alpha 4-2 beta 2 and other nicotinic acetylcholine receptor subtypes as targets of psychoactive and addictive drugs. *Br J Pharmacol* 105, 657-666 (1992)
11. Paradiso, K., J. Zhang, & J.H. Steinbach: The C terminus of the human nicotinic alpha4beta2 receptor forms a binding site required for potentiation by an estrogenic steroid. *J Neurosci* 21, 6561-6568 (2001)
12. Steinlein, O.: Detection of a CfoI polymorphism within exon 5 of the human neuronal nicotinic acetylcholine receptor alpha 4 subunit gene (CHRNA4). *Hum Genet* 96, 130 (1995)
13. Steinlein, O.K., J.C. Mulley, P. Propping, R.H. Wallace, H.A. Phillips, G.R. Sutherland, I.E. Scheffer, & S.F. Berkovic: A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet* 11, 201-203 (1995)
14. Steinlein, O.K., A. Magnusson, J. Stoodt, S. Bertrand, S. Weiland, S.F. Berkovic, K.O. Nakken, P. Propping, & D. Bertrand: An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. *Hum Mol Genet* 6, 943-947 (1997)
15. Hirose, S., H. Iwata, H. Akiyoshi, K. Kobayashi, M. Ito, K. Wada, S. Kaneko, & A. Mitsudome: A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy. *Neurology* 53, 1749-1753 (1999)
16. Leniger, T., C. Kananura, A. Hufnagel, S. Bertrand, D. Bertrand, & O.K. Steinlein: A new Chrna4 mutation with low penetrance in nocturnal frontal lobe epilepsy. *Epilepsia* 44, 981-985 (2003)
17. Steinlein, O.K.: Nicotinic receptor mutations in human epilepsy. *Prog Brain Res* 145, 275-285 (2004)
18. GOKHALE, S.D. and O.D. GULATI: Nicotine convulsions and their modification by reserpine and chlorpromazine. *Indian J Physiol Pharmacol* 6, 154-160 (1962)
19. Combi, R., L. Dalpra, M.L. Tenchini, & L. Ferini-Strambi: Autosomal dominant nocturnal frontal lobe epilepsy--a critical overview. *J Neurol* 251, 923-934 (2004)
20. Klaassen, A., J. Glykys, J. Maguire, C. Labarca, I. Mody, & J. Boulter: Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy. *Proc Natl Acad Sci U S A* (2006)
21. Steinlein, O., T. Sander, J. Stoodt, R. Kretz, D. Janz, & P. Propping: Possible association of a silent polymorphism

in the neuronal nicotinic acetylcholine receptor subunit alpha4 with common idiopathic generalized epilepsies. *Am J Med Genet* 74, 445-449 (1997)
22. Chou, I.C., C.C. Lee, C.C. Huang, J.Y. Wu, J.J. Tsai, C.H. Tsai, & F.J. Tsai: Association of the neuronal nicotinic acetylcholine receptor subunit alpha4 polymorphisms with febrile convulsions. *Epilepsia* 44, 1089-1093 (2003)
23. Parasuraman, R., P.M. Greenwood, R. Kumar, & J. Fossella: Beyond heritability: neurotransmitter genes differentially modulate visuospatial attention and working memory. *Psychol Sci* 16, 200-207 (2005)
24. Greenwood, P.M., J.A. Fossella, & R. Parasuraman: Specificity of the effect of a nicotinic receptor polymorphism on individual differences in visuospatial attention. *J Cogn Neurosci* 17, 1611-1620 (2005)
25. Espeseth, T., P.M. Greenwood, I. Reinvang, A.M. Fjell, K.B. Walhovd, L.T. Westlye, E. Wehling, A. Lundervold, H. Rootwelt, & R. Parasuraman: Interactive effects of APOE and CHRNA4 on attention and white matter volume in healthy middle-aged and older adults. *Cogn Affect Behav Neurosci* 6, 31-43 (2006)
26. Mahley, R.W., K.H. Weisgraber, & Y. Huang: Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A* 103, 5644-5651 (2006)
27. Kent, L., F. Middle, Z. Hawi, M. Fitzgerald, M. Gill, C. Feehan, & N. Craddock: Nicotinic acetylcholine receptor alpha4 subunit gene polymorphism and attention deficit hyperactivity disorder. *Psychiatr Genet* 11, 37-40 (2001)
28. Todd, R.D., E.A. Lobos, L.W. Sun, & R.J. Neuman: Mutational analysis of the nicotinic acetylcholine receptor alpha 4 subunit gene in attention deficit/hyperactivity disorder: evidence for association of an intronic polymorphism with attention problems. *Mol Psychiatry* 8, 103-108 (2003)
29. McGaughy, J., M.W. Decker, & M. Sarter: Enhancement of sustained attention performance by the nicotinic acetylcholine receptor agonist ABT-418 in intact but not basal forebrain-lesioned rats. *Psychopharmacology (Berl)* 144, 175-182 (1999)
30. Grottick, A.J. and G.A. Higgins: Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task. *Behav Brain Res* 117, 197-208 (2000)
31. Prendergast, M.A., W.J. Jackson, A.V. Terry, Jr., N.J. Kille, S.P. Arneric, M.W. Decker, & J.J. Buccafusco: Age-related differences in distractibility and response to methylphenidate in monkeys. *Cereb Cortex* 8, 164-172 (1998)
32. Wilens, T.E., J. Biederman, J. Wong, T.J. Spencer, & J.B. Prince: Adjunctive donepezil in attention deficit hyperactivity disorder youth: case series. *J Child Adolesc Psychopharmacol* 10, 217-222 (2000)
33. Kawamata, J. and S. Shimohama: Association of novel and established polymorphisms in neuronal nicotinic acetylcholine receptors with sporadic Alzheimer's disease. *J Alzheimers Dis* 4, 71-76 (2002)
34. Vasto, S., G. Candore, A. Aquino, M. Bulati, C.R. Balistreri, M.P. Grimaldi, V. Ditta, G. Colonna-Romano, D. Lio, S. Vitello, R. Barbieri, & C. Caruso: The nACHR4 594C/T polymorphism in Alzheimer disease. *Rejuvenation Res* 9, 107-110 (2006)
35. Dorszewska, J., J. Florczak, A. Rozycka, J. Jaroszewska-Kolecka, W.H. Trzeciak, & W. Kozubski: Polymorphisms of the CHRNA4 gene encoding the alpha4 subunit of nicotinic acetylcholine receptor as related to the oxidative DNA damage and the level of apoptotic proteins in lymphocytes of the patients with Alzheimer's disease. *DNA Cell Biol* 24, 786-794 (2005)
36. Cook, L.J., L.W. Ho, A.E. Taylor, C. Brayne, J.G. Evans, J. Xuereb, N.J. Cairns, A. Pritchard, H. Lemmon, D. Mann, D. St Clair, D. Turic, P. Hollingworth, P.J. Moore, L. Jehu, N. Archer, S. Walter, C. Foy, A. Edmondson, J. Powell, S. Lovestone, M.J. Owen, J. Williams, C. Lendon, & D.C. Rubinsztein: Candidate gene association studies of the alpha 4 (CHRNA4) and beta 2 (CHRNB2) neuronal nicotinic acetylcholine receptor subunit genes in Alzheimer's disease. *Neurosci Lett* 358, 142-146 (2004)
37. Feng, Y., T. Niu, H. Xing, X. Xu, C. Chen, S. Peng, L. Wang, N. Laird, & X. Xu: A common haplotype of the nicotine acetylcholine receptor alpha 4 subunit gene is associated with vulnerability to nicotine addiction in men. *Am J Hum Genet* 75, 112-121 (2004)
38. Li, M.D., J. Beuten, J.Z. Ma, T.J. Payne, X.Y. Lou, V. Garcia, A.S. Duenes, K.M. Crews, & R.C. Elston: Ethnic- and gender-specific association of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) with nicotine dependence. *Hum Mol Genet* 14, 1211-1219 (2005)
39. Hutchison, K.E., D. Allen, C. Jepson, C. Lerman, N. Benowitz, J. Stitzel, A. Bryan, J. McGeary, & H.M. Haughey: CHRNA4 and Tobacco Dependence: From Gene Regulation to Treatment Outcome. *Arch Gen Psychiatry* (2007)
40. Schuckit, M.A. and T.L. Smith: The relationships of a family history of alcohol dependence, a low level of response to alcohol and six domains of life functioning to the development of alcohol use disorders. *J Stud Alcohol* 61, 827-835 (2000)
41. Schuckit, M.A., H.J. Edenberg, J. Kalmijn, L. Flury, T.L. Smith, T. Reich, L. Bierut, A. Goate, & T. Foroud: A genome-wide search for genes that relate to a low level of response to alcohol. *Alcohol Clin Exp Res* 25, 323-329 (2001)
42. Kim, S.A., J.W. Kim, J.Y. Song, S. Park, H.J. Lee, & J.H. Chung: Association of polymorphisms in nicotinic acetylcholine receptor alpha 4 subunit gene (CHRNA4), mu-opioid receptor gene (OPRM1), and ethanol-metabolizing enzyme genes with alcoholism in Korean patients. *Alcohol* 34, 115-120 (2004)
43. Bobo, J.K.: Tobacco use, problem drinking, and alcoholism. *Clin Obstet Gynecol* 45, 1169-1180 (2002)
44. Madden, P.A. and A.C. Heath: Shared genetic vulnerability in alcohol and cigarette use and dependence. *Alcohol Clin Exp Res* 26, 1919-1921 (2002)
45. True, W.R., H. Xian, J.F. Scherrer, P.A. Madden, K.K. Bucholz, A.C. Heath, S.A. Eisen, M.J. Lyons, J. Goldberg, & M. Tsuang: Common genetic vulnerability for nicotine and alcohol dependence in men. *Arch Gen Psychiatry* 56, 655-661 (1999)
46. Bierut, L.J., M.A. Schuckit, V. Hesselbrock, & T. Reich: Co-occurring risk factors for alcohol dependence and habitual smoking. *Alcohol Res Health* 24, 233-241 (2000)

47. Dobelis, P., M.J. Marks, P. Whiteaker, S.A. Balogh, A.C. Collins, & J.A. Stitzel: A polymorphism in the mouse neuronal alpha4 nicotinic receptor subunit results in an alteration in receptor function. *Mol Pharmacol* 62, 334-342 (2002)
48. Gahring, L.C., K. Persiyanov, D. Dunn, R. Weiss, E.L. Meyer, & S.W. Rogers: Mouse strain-specific nicotinic acetylcholine receptor expression by inhibitory interneurons and astrocytes in the dorsal hippocampus. *J Comp Neurol* 468, 334-346 (2004)
49. Kim, H., B.A. Flanagin, C. Qin, R.L. Macdonald, & J.A. Stitzel: The mouse Chrna4 A529T polymorphism alters the ratio of high to low affinity alpha 4 beta 2 nAChRs. *Neuropharmacology* 45, 345-354 (2003)
50. Yang, Y., B.J. Beyer, J.F. Otto, T.P. O'Brien, V.A. Letts, H.S. White, & W.N. Frankel: Spontaneous deletion of epilepsy gene orthologs in a mutant mouse with a low electroconvulsive threshold. *Hum Mol Genet* 12, 975-984 (2003)
51. Stitzel, J.A., M. Jimenez, M.J. Marks, T. Tritto, & A.C. Collins: Potential role of the alpha4 and alpha6 nicotinic receptor subunits in regulating nicotine-induced seizures. *J Pharmacol Exp Ther* 293, 67-74 (2000)
52. Tritto, T., J.A. Stitzel, M.J. Marks, E. Romm, & A.C. Collins: Variability in response to nicotine in the LSxSS RI strains: potential role of polymorphisms in alpha4 and alpha6 nicotinic receptor genes. *Pharmacogenetics* 12, 197-208 (2002)
53. Balogh, S.A., B.J. Bowers, S.F. Logue, J. Ernisse, & J.M. Wehner: Alpha4-Containing Neuronal Nicotinic Receptors Modulate Appetitive Learning. *Society for Nicotine and Tobacco Research* New Orleans, LA, (2003)
54. Butt, C.M., N.M. King, S.R. Hutton, A.C. Collins, & J.A. Stitzel: Modulation of nicotine but not ethanol preference by the mouse Chrna4 A529T polymorphism. *Behav Neurosci* 119, 26-37 (2005)
55. Li, X.C., M.S. Karadsheh, P.M. Jenkins, & J.A. Stitzel: Genetic correlation between the free-choice oral consumption of nicotine and alcohol in C57BL/6JxC3H/HeJ F2 intercross mice. *Behav Brain Res* 157, 79-90 (2005)
56. Tritto, T., R.J. Marley, D. Bastidas, J.A. Stitzel, & A.C. Collins: Potential regulation of nicotine and ethanol actions by alpha4-containing nicotinic receptors. *Alcohol* 24, 69-78 (2001)
57. Marks, M.J., P. Whiteaker, J. Calcaterra, J.A. Stitzel, A.E. Bullock, S.R. Grady, M.R. Picciotto, J.P. Changeux, & A.C. Collins: Two pharmacologically distinct components of nicotinic receptor-mediated rubidium efflux in mouse brain require the beta2 subunit. *J Pharmacol Exp Ther* 289, 1090-1103 (1999)
58. Butt, C.M., N.M. King, J.A. Stitzel, & A.C. Collins: Interaction of the Nicotinic Cholinergic System with Ethanol Withdrawal. *J Pharmacol Exp Ther* (2003)
59. Owens, J.C., S.A. Balogh, T.D. McClure-Begley, C.M. Butt, C. Labarca, H.A. Lester, M.R. Picciotto, J.M. Wehner, & A.C. Collins: Alpha4beta2* nicotinic acetylcholine receptors modulate the effects of ethanol and nicotine on the acoustic startle response. *Alcohol Clin Exp Res* 27, 1867-1875 (2003)
60. Stitzel, J.A., P. Dobelis, M. Jimenez, & A.C. Collins: Long sleep and short sleep mice differ in nicotine-stimulated 86Rb+ efflux and alpha4 nicotinic receptor subunit cDNA sequence. *Pharmacogenetics* 11, 331-339 (2001)
61. Butt, C.M., S.R. Hutton, J.A. Stitzel, S.A. Balogh, J.C. Owens, & A.C. Collins: A polymorphism in the alpha4 nicotinic receptor gene (Chrna4) modulates enhancement of nicotinic receptor function by ethanol. *Alcohol Clin Exp Res* 27, 733-742 (2003)
62. Gault, J., J. Hopkins, R. Berger, C. Drebing, J. Logel, C. Walton, M. Short, R. Vianzon, A. Olincy, R.G. Ross, L.E. Adler, R. Freedman, & S. Leonard: Comparison of polymorphisms in the alpha7 nicotinic receptor gene and its partial duplication in schizophrenic and control subjects. *Am J Med Genet* 123B, 39-49 (2003)
63. Garcia-Guzman, M., F. Sala, S. Sala, A. Campos-Caro, W. Stuhmer, L.M. Gutierrez, & M. Criado: alpha-Bungarotoxin-sensitive nicotinic receptors on bovine chromaffin cells: molecular cloning, functional expression and alternative splicing of the alpha 7 subunit. *Eur J Neurosci* 7, 647-655 (1995)
64. Saragoza, P.A., J.G. Modir, N. Goel, K.L. French, L. Li, M.W. Nowak, & J.A. Stitzel: Identification of an alternatively processed nicotinic receptor alpha7 subunit RNA in mouse brain. *Brain Res Mol Brain Res* 117, 15-26 (2003)
65. Severance, E.G., H. Zhang, Y. Cruz, S. Pakhlevaniants, S.H. Hadley, J. Amin, L. Wecker, C. Reed, & J. Cuevas: The alpha7 nicotinic acetylcholine receptor subunit exists in two isoforms that contribute to functional ligand-gated ion channels. *Mol Pharmacol* 66, 420-429 (2004)
66. Leonard, S., J. Gault, J. Hopkins, J. Logel, R. Vianzon, M. Short, C. Drebing, R. Berger, D. Venn, P. Sirota, G. Zerbe, A. Olincy, R.G. Ross, L.E. Adler, & R. Freedman: Association of promoter variants in the alpha7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Arch Gen Psychiatry* 59, 1085-1096 (2002)
67. Tsuneki, H., S. Kobayashi, K. Takagi, S. Kagawa, M. Tsunoda, M. Murata, T. Matsuoka, T. Wada, M. Kurachi, I. Kimura, & T. Sasaoka: Novel G423S mutation of human {alpha}7 nicotinic receptor promotes agonist-induced desensitization by a protein kinase C-dependent mechanism. *Mol Pharmacol* (2006)
68. Freedman, R., H. Coon, M. Myles-Worsley, A. Orr-Urtreger, A. Olincy, A. Davis, M. Polymeropoulos, J. Holik, J. Hopkins, M. Hoff, J. Rosenthal, M.C. Waldo, F. Reimherr, P. Wender, J. Yaw, D.A. Young, C.R. Breese, C. Adams, D. Patterson, L.E. Adler, L. Kruglyak, S. Leonard, & W. Byerley: Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A* 94, 587-592 (1997)
69. Leonard, S. and R. Freedman: Genetics of chromosome 15q13-q14 in schizophrenia. *Biol Psychiatry* 60, 115-122 (2006)
70. Leonard, S., J. Gault, J. Hopkins, J. Logel, R. Vianzon, M. Short, C. Drebing, R. Berger, D. Venn, P. Sirota, G. Zerbe, A. Olincy, R.G. Ross, L.E. Adler, & R. Freedman: Association of promoter variants in the alpha7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Arch Gen Psychiatry* 59, 1085-1096 (2002)

71. Freedman, R., M. Hall, L.E. Adler, & S. Leonard: Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biol Psychiatry* 38, 22-33 (1995)
72. Houy, E., G. Raux, F. Thibaut, A. Belmont, C. Demily, G. Allio, S. Haouzir, G. Fouldrin, M. Petit, T. Frebourg, & D. Campion: The promoter -194 C polymorphism of the nicotinic alpha 7 receptor gene has a protective effect against the P50 sensory gating deficit. *Mol Psychiatry* 9, 320-322 (2004)
73. Hughes, J.R., D.K. Hatsukami, J.E. Mitchell, & L.A. Dahlgren: Prevalence of smoking among psychiatric outpatients. *Am J Psychiatry* 143, 993-997 (1986)
74. Kumari, V. and P. Postma: Nicotine use in schizophrenia: the self medication hypotheses. *Neurosci Biobehav Rev* 29, 1021-1034 (2005)
75. De Luca, V., A.H. Wong, D.J. Muller, G.W. Wong, R.F. Tyndale, & J.L. Kennedy: Evidence of association between smoking and alpha7 nicotinic receptor subunit gene in schizophrenia patients. *Neuropsychopharmacology* 29, 1522-1526 (2004)
76. Faraone, S.V., J. Su, L. Taylor, M. Wilcox, P. Van Eerdewegh, & M.T. Tsuang: A novel permutation testing method implicates sixteen nicotinic acetylcholine receptor genes as risk factors for smoking in schizophrenia families. *Hum Hered* 57, 59-68 (2004)
77. Stassen, H.H., R. Bridler, S. Hagele, M. Hergersberg, B. Mehmann, A. Schinzel, M. Weisbrod, & C. Scharfetter: Schizophrenia and smoking: evidence for a common neurobiological basis? *Am J Med Genet* 96, 173-177 (2000)
78. Elmslie, F.V., M. Rees, M.P. Williamson, M. Kerr, M.J. Kjeldsen, K.A. Pang, A. Sundqvist, M.L. Friis, D. Chadwick, A. Richens, A. Covanis, M. Santos, A. Arzimanoglou, C.P. Panayiotopoulos, D. Curtis, W.P. Whitehouse, & R.M. Gardiner: Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q. *Hum Mol Genet* 6, 1329-1334 (1997)
79. Neubauer, B.A., B. Fiedler, B. Himmelein, F. Kampfer, U. Lassker, G. Schwabe, I. Spanier, D. Tams, C. Bretscher, K. Moldenhauer, G. Kurlemann, S. Weise, K. Tedroff, O. Eeg-Olofsson, C. Wadelius, & U. Stephani: Centrotemporal spikes in families with rolandic epilepsy: linkage to chromosome 15q14. *Neurology* 51, 1608-1612 (1998)
80. Nagavarapu, U. and R.T. Boyd: Distribution of neuronal nicotinic acetylcholine receptor gene restriction fragment length polymorphisms in inbred and partially inbred mice. *Pharmacogenetics* 5, 326-331 (1995)
81. Stitzel, J.A., D.A. Farnham, & A.C. Collins: Linkage of strain-specific nicotinic receptor alpha 7 subunit restriction fragment length polymorphisms with levels of alpha-bungarotoxin binding in brain. *Brain Res Mol Brain Res* 43, 30-40 (1996)
82. Mexal, S., P.M. Jenkins, M.A. Lautner, E. Iacob, E. Crouch, & J.A. Stitzel: alpha 7 nicotinic receptor gene promoter polymorphisms in inbred mice affect expression in a cell type-specific fashion. *J Biol Chem* (2007)
83. Miner, L.L., M.J. Marks, & A.C. Collins: Genetic analysis of nicotine-induced seizures and hippocampal nicotinic receptors in the mouse. *J Pharmacol Exp Ther* 239, 853-860 (1986)
84. Miner, L.L. and A.C. Collins: Strain comparison of nicotine-induced seizure sensitivity and nicotinic receptors. *Pharmacol Biochem Behav* 33, 469-475 (1989)
85. Stevens, K.E., R. Freedman, A.C. Collins, M. Hall, S. Leonard, M.J. Marks, & G.M. Rose: Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. *Neuropsychopharmacology* 15, 152-162 (1996)
86. Stitzel, J.A., J.M. Blanchette, & A.C. Collins: Sensitivity to the seizure-inducing effects of nicotine is associated with strain-specific variants of the alpha 5 and alpha 7 nicotinic receptor subunit genes. *J Pharmacol Exp Ther* 284, 1104-1111 (1998)
87. Stevens, K.E., J.A. Stitzel, M. Jimenez, & A.C. Collins: Transferring the alpha7 nicotinic receptor between different mouse backgrounds alters auditory gating. *Society for Neuroscience Annual Meeting* Abstract viewer/Itinerary planner, Program Number: 145.4 (2001)
88. Adams, C.E., J.A. Stitzel, A.C. Collins, & R. Freedman: Alpha7-nicotinic receptor expression and the anatomical organization of hippocampal interneurons. *Brain Res* 922, 180-190 (2001)
89. Adams, C.E., J.C. Yonchek, & J.A. Stitzel: Development of hippocampal alpha7 nicotinic receptors in C3H and DBA/2 congenic mice. *Brain Res* 1122, 27-35 (2006)
90. Hickman-Davis, J.M. and I.C. Davis: Transgenic mice. *Paediatr Respir Rev* 7, 49-53 (2006)
91. Williams, R.W., L. Flaherty, & D.W. Threadgill: The math of making mutant mice. *Genes Brain Behav* 2, 191-200 (2003)
92. Covernton, P.J. and J.G. Connolly: Multiple components in the agonist concentration-response relationships of neuronal nicotinic acetylcholine receptors. *J Neurosci Methods* 96, 63-70 (2000)
93. Buisson, B. and D. Bertrand: Chronic exposure to nicotine upregulates the human (alpha)4((beta)2 nicotinic acetylcholine receptor function. *J Neurosci* 21, 1819-1829 (2001)
94. Vallejo, Y.F., B. Buisson, D. Bertrand, & W.N. Green: Chronic nicotine exposure upregulates nicotinic receptors by a novel mechanism. *J Neurosci* 25, 5563-5572 (2005)
95. Nelson, M.E., A. Kuryatov, C.H. Choi, Y. Zhou, & J. Lindstrom: Alternate stoichiometries of alpha4beta2 nicotinic acetylcholine receptors. *Mol Pharmacol* 63, 332-341 (2003)
96. Aridon, P., C. Marini, C. Di Resta, E. Brilli, M. De Fusco, F. Politi, E. Parrini, I. Manfredi, T. Pisano, D. Pruna, G. Curia, C. Cianchetti, M. Pasqualetti, A. Becchetti, R. Guerrini, & G. Casari: Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. *Am J Hum Genet* 79, 342-350 (2006)
97. Gerlai, R.: Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends Neurosci* 19, 177-181 (1996)
98. Bolivar, V.J., M.N. Cook, & L. Flaherty: Mapping of quantitative trait loci with knockout/congenic strains. *Genome Res* 11, 1549-1552 (2001)

99. Peirce, J.: Looking at old tools in new ways: using knockouts as congenics to study QTLs. *Genome Res* 11, 1469-1471 (2001)
100. Redon, R., S. Ishikawa, K.R. Fitch, L. Feuk, G.H. Perry, T.D. Andrews, H. Fiegler, M.H. Shapero, A.R. Carson, W. Chen, E.K. Cho, S. Dallaire, J.L. Freeman, J.R. Gonzalez, M. Gratacos, J. Huang, D. Kalaitzopoulos, D. Komura, J.R. MacDonald, C.R. Marshall, R. Mei, L. Montgomery, K. Nishimura, K. Okamura, F. Shen, M.J. Somerville, J. Tchinda, A. Valsesia, C. Woodwark, F. Yang, J. Zhang, T. Zerjal, J. Zhang, L. Armengol, D.F. Conrad, X. Estivill, C. Tyler-Smith, N.P. Carter, H. Aburatani, C. Lee, K.W. Jones, S.W. Scherer, & M.E. Hurles: Global variation in copy number in the human genome. *Nature* 444, 444-454 (2006)
101. Sharp, A.J., S. Hansen, R.R. Selzer, Z. Cheng, R. Regan, J.A. Hurst, H. Stewart, S.M. Price, E. Blair, R.C. Hennekam, C.A. Fitzpatrick, R. Segraves, T.A. Richmond, C. Guiver, D.G. Albertson, D. Pinkel, P.S. Eis, S. Schwartz, S.J. Knight, & E.E. Eichler: Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. *Nat Genet* 38, 1038-1042 (2006)
102. Sharp, A.J., Z. Cheng, & E.E. Eichler: Structural variation of the human genome. *Annu Rev Genomics Hum Genet* 7, 407-442 (2006)
103. Flint, J., W. Valdar, S. Shifman, & R. Mott: Strategies for mapping and cloning quantitative trait genes in rodents. *Nat Rev Genet* 6, 271-286 (2005)
104. Jiang, Z., T.P. Dalton, L. Jin, B. Wang, Y. Tsuneoka, H.G. Shertzer, R. Deka, & D.W. Nebert: Toward the evaluation of function in genetic variability: characterizing human SNP frequencies and establishing BAC-transgenic mice carrying the human CYP1A1_CYP1A2 locus. *Hum Mutat* 25, 196-206 (2005)
105. Wallace, H.A., F. Marques-Kranc, M. Richardson, F. Luna-Crespo, J.A. Sharpe, J. Hughes, W.G. Wood, D.R. Higgs, & A.J. Smith: Manipulating the mouse genome to engineer precise functional syntenic replacements with human sequence. *Cell* 128, 197-209 (2007)
106. Saenz, A., J. Galan, C. Caloustian, F. Lorenzo, C. Marquez, N. Rodriguez, M.D. Jimenez, J.J. Poza, A.M. Cobo, D. Grid, J.F. Prud'homme, & d.M. Lopez: Autosomal dominant nocturnal frontal lobe epilepsy in a Spanish family with a Ser252Phe mutation in the CHRNA4 gene. *Arch Neurol* 56, 1004-1009 (1999)
107. Steinlein, O.K., J. Stoodt, J. Mulley, S. Berkovic, I.E. Scheffer, & E. Brodtkorb: Independent occurrence of the CHRNA4 Ser248Phe mutation in a Norwegian family with nocturnal frontal lobe epilepsy. *Epilepsia* 41, 529-535 (2000)
108. McLellan, A., H.A. Phillips, C. Rittey, M. Kirkpatrick, J.C. Mulley, D. Goudie, J.B. Stephenson, J. Tolmie, I.E. Scheffer, S.F. Berkovic, & S.M. Zuberi: Phenotypic comparison of two Scottish families with mutations in different genes causing autosomal dominant nocturnal frontal lobe epilepsy. *Epilepsia* 44, 613-617 (2003)
109. Phillips, H.A., C. Marini, I.E. Scheffer, G.R. Sutherland, J.C. Mulley, & S.F. Berkovic: A de novo mutation in sporadic nocturnal frontal lobe epilepsy. *Ann Neurol* 48, 264-267 (2000)
110. Rozycka, A., E. Skorupska, A. Kostyrko, & W.H. Trzeciak: Evidence for S284L mutation of the CHRNA4 in a white family with autosomal dominant nocturnal frontal lobe epilepsy. *Epilepsia* 44, 1113-1117 (2003)
111. Weiland, S., V. Witzemann, A. Villarroel, P. Propping, & O. Steinlein: An amino acid exchange in the second transmembrane segment of a neuronal nicotinic receptor causes partial epilepsy by altering its desensitization kinetics. *FEBS Lett* 398, 91-96 (1996)
112. Kuryatov, A., V. Gerzanich, M. Nelson, F. Olale, & J. Lindstrom: Mutation causing autosomal dominant nocturnal frontal lobe epilepsy alters Ca2+ permeability, conductance, and gating of human alpha4beta2 nicotinic acetylcholine receptors. *J Neurosci* 17, 9035-9047 (1997)
113. Bertrand, D., F. Picard, S. Le Hellard, S. Weiland, I. Favre, H. Phillips, S. Bertrand, S.F. Berkovic, A. Malafosse, & J. Mulley: How mutations in the nAChRs can cause ADNFLE epilepsy. *Epilepsia* 43 Suppl 5, 112-122 (2002)
114. Figl, A., N. Viseshakul, N. Shafaee, J. Forsayeth, & B.N. Cohen: Two mutations linked to nocturnal frontal lobe epilepsy cause use-dependent potentiation of the nicotinic ACh response. *J Physiol* 513 (Pt 3), 655-670 (1998)
115. Bertrand, S., S. Weiland, S.F. Berkovic, O.K. Steinlein, & D. Bertrand: Properties of neuronal nicotinic acetylcholine receptor mutants from humans suffering from autosomal dominant nocturnal frontal lobe epilepsy. *Br J Pharmacol* 125, 751-760 (1998)
116. Matsushima, N., S. Hirose, H. Iwata, G. Fukuma, M. Yonetani, C. Nagayama, W. Hamanaka, Y. Matsunaka, M. Ito, S. Kaneko, A. Mitsudome, & H. Sugiyama: Mutation (Ser284Leu) of neuronal nicotinic acetylcholine receptor alpha 4 subunit associated with frontal lobe epilepsy causes faster desensitization of the rat receptor expressed in oocytes. *Epilepsy Res* 48, 181-186 (2002)
117. Rodrigues-Pinguet, N., L. Jia, M. Li, A. Figl, A. Klaassen, A. Truong, H.A. Lester, & B.N. Cohen: Five ADNFLE mutations reduce the Ca2+ dependence of the mammalian alpha4beta2 acetylcholine response. *J Physiol* 550, 11-26 (2003)
118. Rodrigues-Pinguet, N.O., T.J. Pinguet, A. Figl, H.A. Lester, & B.N. Cohen: Mutations linked to autosomal dominant nocturnal frontal lobe epilepsy affect allosteric Ca2+ activation of the alpha 4 beta 2 nicotinic acetylcholine receptor. *Mol Pharmacol* 68, 487-501 (2005)

Key Words: Review, Polymorphism, Neuronal Nicotinic Receptor, Allele, Genetics, Review

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