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Invited review

Natural genetic variability of the neuronal nicotinic acetylcholine receptor subunit genes in mice: Consequences and confounds

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ABSTRACT

Recent human genetic studies have identified genetic variants in multiple nicotinic acetylcholine receptor (nAChR) subunit genes that are associated with risk for nicotine dependence and other smoking-related measures. Genetic variability also exists in the nAChR subunit genes in mice. Most studies on mouse nAChR subunit gene variability to date have focused on *Chrna4*, the gene that encodes the α4 nAChR subunit and *Chrna7*, the gene that encodes the α7 nAChR subunit. However, genetic variability exists for all nAChR genes in mice. In this review, we will describe what is known about nAChR subunit gene polymorphisms in mice and how it relates to variability in nAChR expression and function in brain. The relationship between nAChR genetic variability in mice and the effects of nicotine on several behavioral and physiological measures also will be discussed. In addition, an overview of the contribution of other genetic variation to nicotine sensitivity in mice will be provided. Finally, the potential for natural genetic variability to confound and/or modify the results of studies that utilize genetically engineered mice will be considered. As an example of the ability of a natural genetic variant to modify the effect of an engineered mutation, data will be presented that demonstrate that the effect of *Chrna5* deletion on oral nicotine intake is dependent upon naturally occurring variant alleles of *Chrna4*.

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1. Genetics of smoking in humans and nicotine sensitivity in mice

Data from humans indicate that there is a substantial genetic influence on smoking behavior (Heath et al., 1995; Kaprio, 2009). A large number of twin studies beginning with those of Fisher (1958) have demonstrated that the heritability of smoking ranges from 0.2 to 0.8 with a mean estimate of heritability of 0.53 (Li, 2006). In other words, approximately 50% of the variance of whether an individual is a smoker can be attributed to genetic factors. Genetic factors influence multiple aspects of tobacco use including initiation (Heath and Martin, 1993), persistence (Heath, 1990; Heath and Martin, 1993), number of cigarettes smoked (Carmelli et al., 1990) and the ability to stop smoking (Carmelli et al., 1992).

Similarly, the behavioral and physiological effects of nicotine, the major psychoactive component in tobacco, are influenced by genetic factors in mice (for an excellent review see (Marks, 2013)). For example, Marks et al. (1989) demonstrated that there is a 2–6-fold difference in ED_{50} values for a battery of tests for nicotine sensitivity across 19 inbred strains. These tests include nicotine effects on respiration, heart rate, startle response, locomotor activity and body temperature. Miner and Collins (1989) reported similar results for nicotine-induced seizure sensitivity for the same 19 inbred strains. Genetic influences on the development of tolerance to nicotine (Marks et al., 1986b; Marks et al., 1991) nicotine oral self-selection (Robinson et al., 1996; Li et al., 2007), the effects of nicotine on open field activity (Marks et al., 1986a) and conditioned place preference (Schechter et al., 1995; Harenza et al., 2014) have also been reported. Heritability estimates for some of these behaviors were obtained and found to range from 0.3 for nicotine-induced hypothermia (Marks et al., 1984) to 0.63 for nicotine-induced seizure sensitivity (Miner et al., 1984). It does not appear that the heritable differences in response to nicotine in mice are due to variations in nicotine metabolism. For example, Petersen et al. (1984) demonstrated that several mouse strains that vary

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considerably in their sensitivity to nicotine do not differ in nicotine metabolism. In addition, Miner et al. (Miner et al., 1984) determined that blood and brain levels of nicotine were not different between the mouse strains C3H/Ibg and DBA/2 following an i.p. injection of nicotine that elicits seizures in C3H/Ibg mice but not in DBA/2 mice.

Although it is clear that natural genetic variation in mice influences sensitivity to nicotine, there has not been an extensive effort to identify the specific genes that contribute to the natural variability in nicotine sensitivity in mice. In this review, those studies that have explored natural genetic variability in mice and its role in nicotine sensitivity will be described. In addition, examples will be provided of how natural genetic variability can confound the results of studies with nicotinic receptor null mutant mice. Such confounds include mice in which the knockout allele has linked genes that have different alleles between the knockout mouse and wild type control and modifier genes that alter the effect of the gene deletion. Being aware of the first confound is essential for interpretation of results from knockout mouse studies while identifying modifier genes may be of value in understanding the neurobiology of nicotine sensitivity.

2. Genetic variability in neuronal nicotinic acetylcholine receptor (nAChR) subunit genes

nAChRs are ligand-gated cation channels that are expressed in the brain and periphery (Gahring and Rogers, 2005; Gotti et al., 2009). The neuronal subfamily consists of the subunits α2-α10 (α8 exists only in avian species) and β2-β4. The nomenclature for the genes that encode the nAChR subunits is Chrnxy where Chrn stands for cholinergic receptor, nicotinic, and xy represents the subunit. For example, *Chrna2* is the gene for the α2 subunit. Most effort to identify genetic variants that affect nicotine sensitivity in mice has focused on the nAChR *Chrna7* and *Chrna4* subunit genes. Below is a summary of the findings with these genes as well as other nAChR genes in which natural genetic variability has been associated with sensitivity to nicotine and/or the expression of a given nAChR.

2.1. Genetic variation in Chrna7

2.1.1. Chrna7 alleles and α7 expression

The first evidence of genetic variability in mouse nAChR subunit genes was described by Nagavarapu and Boyd (1995). This group identified restriction fragment length polymorphisms (RFLPs) in *Chrna7*, the gene that codes for the α7 nAChR subunit. RFLPs are sequence differences between alleles of a gene that are detected because they either generate or disrupt a recognition sequence for a restriction endonuclease. RFLPs in *Chrna7* subsequently were shown to be linked to individual differences in the level of α-bungarotoxin binding (αBTX), a ligand that specifically labels α7 nAChRs in brain, in various brain regions of an F2 intercross between two mouse strains, C3H/Ibg and DBA/2 (Stitzel et al., 1996). Interestingly, the linkage of the alleles of *Chrna7* with αBTX levels was found to be brain region specific, with the C3H allele linked to higher levels of αBTX in hippocampus while the DBA/2 allele was linked to higher levels of αBTX expression in striatum. For some brain regions such as cortex, there was no relationship between αBTX levels and *Chrna7* genotype. This brain region-specific effect of *Chrna7* genotype on αBTX levels was confirmed in a later study (Brooks et al., 2009).

Adams et al. (2001) also examined the role of *Chrna7* genotype on the distribution of αBTX binding in the hippocampus. In this study, Adams et al. utilized a congenic mouse strain in which the DBA/2 allele was introgressed onto a C3H genetic background. Interestingly, results of this study demonstrated that *Chrna7* genotype not only has a quantitative effect on αBTX biding levels but also impacts the neuroanatomical distribution of αBTX-positive hippocampal interneurons. In short, mice that possessed the DBA/2 allele of *Chrna7* exhibited a DBA/2-like distribution of αBTX binding sites irrespective of the genetic background (C3H or DBA/2). Adams further explored the role of *Chrna7* genotype on the developmental profile of αBTX binding during embryonic and perinatal development. In an initial study (Adams, 2003), it was found that C3H and DBA/2 mice possess distinct developmental time courses of α7 expression, with DBA/2 mice showing a later appearance of αBTX binding (embryonic day 16) as compared to C3H mice (embryonic day 13). Using congenic strains in which both alleles of *Chrna7* were exchanged between genetic backgrounds (C3H *Chrna7* allele on a DBA/2 background and DBA/2 allele of *Chrna7* on a C3H genetic background), Adams later reported that the developmental profile of α7 expression was also dependent upon *Chrna7* genotype (Adams et al., 2006). Mice with the C3H allele of *Chrna7* had an earlier developmental onset of expression irrespective of whether the genetic background was C3H or DBA. Conversely, mice with the DBA/2 allele of *Chrna7* had a delayed onset of α7 expression irrespective of genetic background. The sum of these studies indicates that natural genetic variability in *Chrna7* has a substantial impact on α7 expression in mice at multiple levels, from developmental to anatomical distribution to overall levels. Importantly, these effects appear to be brain region specific.

2.1.2. Chrna7 alleles and response to nicotine

A few studies have attempted to assess the relationship between natural variability in *Chrna7* in mice and behavioral response to nicotine. In one study, it was reported that the allelic variants of *Chrna7* were linked to individual differences in sensitivity to nicotine-induced convulsions (Stitzel et al., 1998). These results were consistent with previous work demonstrating that the level of αBTX binding in hippocampus is correlated with nicotine-induced seizure sensitivity (Miner et al., 1984; Miner et al., 1985; Miner and Collins, 1989). Nonetheless, further studies are needed to validate the role of *Chrna7* variability in this measure of nicotine sensitivity particularly since deletion of *Chrna7* does not seem to affect seizure sensitivity (Franceschini et al., 2002) while gain of function increases sensitivity (Broide et al., 2002).

A recent study by Harenza (Harenza et al., 2014) utilized recombinant inbred (RI) strains derived from the parent strains C57BL/6 and DBA/2 to assess the relationship between strain differences in α7 mRNA expression and nicotine place conditioning. This group reported that there was an inverse relationship between α7 mRNA expression in the striatum and prefrontal cortex of the RI strains and their corresponding place conditioning score. That is, strains that had lower α7 mRNA expression tended to have greater risk for the rewarding properties of nicotine. Using α7 knockout mice, it was confirmed that reduced (eliminated) α7 expression increases nicotine reward as measured by place preference. Also as part of this study, it was reported that there is a provisional cis-expression quantitative trait locus (eQTL) for α7 expression in the nucleus accumbens. Although the eQTL spans a large region of chromosome 7, it is centered around the *Chrna7* locus suggesting genetic variability in or around *Chrna7* contributes to variability in α7 expression in the nucleus accumbens. Results also indicated, similar to the results with the previously described C3H and DBA/2 derived mice, that the DBA/2 allele of *Chrna7* is associated with higher levels of α7 expression in the nucleus accumbens (Brooks et al., 2009).These findings provide further support for the role of genetic variability in/around *Chrna7* in regulating variability in α7 expression in mice.

Mouse strain differences in hippocampal αBTX levels also have been implicated in strain variability in auditory gating. Mouse