Cigarette smoking is a leading cause of preventable death throughout the world. Nicotine, the primary addictive compound in tobacco, plays a vital role in the initiation and maintenance of its use. Nicotine exerts its pharmacological roles through nicotinic acetylcholine receptors (nAChRs), which are ligand-gated ion channels consisting of five membrane-spanning subunits. Besides the CHRNA4, CHRN2 and CHRNAS/A3/B4 cluster on chromosome 15, which has been investigated intensively, recent evidence from both genome-wide association studies and candidate gene-based association studies has revealed the crucial roles of the CHRN3–CHRNAS gene cluster on chromosome 8 in nicotine dependence (ND). These studies demonstrate two distinct loci within this region. The first one is tagged by rs13277254, upstream of the CHRN3 gene, and the other is tagged by rs4952, a coding single nucleotide polymorphism in exon 5 of that gene. Functional studies by genetic manipulation in mice have shown that α6*-nAChRs, located in the ventral tegmental area (VTA), are of great importance in controlling nicotine self-administration. However, when the α6 subunit is selectively re-expressed in the VTA of the α6−/− mouse by a lentiviral vector, the reinforcing property of nicotine is restored. To further determine the role of α6*-nAChRs in the process of nicotine-induced reward and withdrawal, genetic knock-in strains have been examined, which showed that replacement of Leu with Ser in the 9′ residue in the M2 domain of α6 produces nicotine-hypersensitive mice (α6 L9′S) with enhanced dopamine release. Moreover, nicotine-induced upregulation may be another ingredient in the pathology of nicotine addiction although the effect of chronic nicotine exposure on the expression of α6-containing receptors is controversial. To gain a better understanding of the pathological processes underlying ND and ND-related behaviors and to promote the development of effective smoking cessation therapies, we here present the most recent studies concerning the genetic effects of the CHRN3–CHRNAS gene cluster in ND.

Translational Psychiatry (2016) 6, e843; doi:10.1038/tp.2016.103; published online 21 June 2016
nicotinic receptors in the brain and their high affinity for nicotine, a large body of research has focused primarily on these subunits. Recently, several genetic variants located in nAChR subunit encoding genes other than 

\[ \text{CHRNB3} \] 

or \n
\[ \text{CHRNA2} \]

were detected by genome-wide association studies (GWAS) and various candidate gene-based association and functional studies. For example, the most compelling SNP rs16969968 in 

\[ \text{CHRNA5} \]

leading to an amino acid change in the position 398 (D398N) of the \[ \alpha5 \] subunit protein, has been consistently demonstrated to be a major biological contributor to ND. For details on this part of the research, please refer to recent reviews. NICOTINE DEPENDENCE After analyzing 3713 SNPs in >300 candidate genes for their association with ND, Saccone et al. reported that rs6474413 \((P = 9.36 \times 10^{-5})\) and rs10958726 \((P = 1.33 \times 10^{-5})\) in \n
\[ \text{CHRNB3} \]

are significantly associated with ND. Both SNPs are located in the putative 5′ promoter region of the gene, with rs6474413 being 2 kb away from the start codon and 15 kb from rs10958726. Because of the high linkage disequilibrium between the two SNPs, they may contribute to a single association signal. Using a sample of 1050 ND cases and 879 non-ND controls of European descent, the same population as used in the study of Saccone et al., another study revealed a significant locus, tagged by rs13277254 at the 5′ end of \n
\[ \text{CHRNB3} – \text{CHRNA6} \]

that influences the transition from smoking to ND. This finding was replicated in the follow-up study, which considered peer smoking as a social environmental risk factor for smoking behavior.

On the basis of the previous association results of a high-density study covering the complete family of 16 \n
\[ \text{CHRN} \]

genes in a population of European ancestry, Saccone et al. extended their research to determine whether variants in the \n
\[ \text{CHRNB3} – \text{CHRNA6} \]

gene cluster also are associated with ND in African-Americans (AAs). They did not detect any associated SNP in their AA sample with a sample size of 710. It was suggested that there might exist at least two distinct loci in the \n
\[ \text{CHRNB3} – \text{CHRNA6} \]

gene cluster that are associated with ND in European Americans (EAs). The first one was tagged by rs13277254, upstream of the gene cluster, together with additional associated SNPs in this region constituted Signal 1. Signal 2 was tagged by rs4952, the only known coding SNP in the exon 5 of \n
\[ \text{CHRNB3} \]

which had a low correlation with rs13277254 (Figure 1).

There also exist many other common variants in the \n
\[ \text{CHRNB3} – \text{CHRNA6} \]

gene cluster that show a significant association with ND in multiple ethnic populations, including Han Chinese, AAs, EAs, and Israelis. We performed a meta-analysis of variants in \n
\[ \text{CHRNB3} \]

in relation to ND by combining data from the studies of subjects of different ethnicities. Although allele frequencies in AAs were different from those in EAs and subjects of Asian ancestry, where the last two ethnic samples appeared to be similar, we found that the genetic effect of seven SNPs in \n
\[ \text{CHRNB3} \]

are in the same direction among the three populations. More importantly, all these SNPs showed a significant association with ND. However, because of the different genetic structures of various ancestries, inconsistent results were found at the SNP level. We detected only four of seven SNPs in the samples of African origin, whereas the associations of all SNPs in the samples of European and Asian ancestry were significant. In contrast, none of these SNPs was reported to be associated with ND among three other studies in Finnish, Swiss and Czech populations. Hubacek attributed this discrepancy partly to socioeconomic status in that the prevalence of smoking was higher in post-Communist countries than in western European countries, and this fact could mask the real effect of each SNP. Thus, further replication studies in additional independent samples of different origins are warranted.
Table 1. Replicated SNPs in the CHRNB3 gene cluster associated with ND-related behaviors

| dbSNP ID | Sample origin                  | Sample size | Phenotype                                 | Odds ratio or β-value | Reported P-value | Reference |
|----------|-------------------------------|-------------|-------------------------------------------|-----------------------|------------------|-----------|
| rs4950   | EA and Australian             | 1929        | ND (FTND)                                 | 1.38                  | 0.0001           | 30        |
|          | Ethnically diverse            | 1056        | Subjective responses to tobacco           | 4.88                  | 0.02             | 31        |
|          |                               |             | (adverse, negative physical, positive)    | 8.13                  | 0.004            |           |
|          | Ethnically diverse            | 1524 families| Subjective responses to tobacco           | NA                    | 0.043            | 31        |
|          | Caucasian, AA and Hispanic    | 1051        | Quit attempts                             | NA                    | 0.021            | 32        |
|          | Caucasian, AA and Hispanic    | 295         | ND                                        | 4.62                  | 0.007            | 32        |
|          | EA                            | 2062        | ND                                        | 0.78                  | 0.00143          | 33        |
|          | EA, AA and Asian (meta-analysis) | 22 654   | ND                                        | 0.1343                | 1.08E −05        | 34        |
|          | Ashkenazi                     | 591         | Smoking status                            | 1.94                  | 9.8E −05         | 35        |
| rs10958726| EA and Australian            | 1929        | ND (FTND)                                 | NA                    | 1.33E −04        | 19        |
|          | EA and Australian             | 1929        | ND (FTND)                                 | 1.38                  | 9.636E −05       | 30        |
|          | EA                            | 1600        | Early subjective response to tobacco (dizziness) | −0.126                | 0.005            | 36        |
|          | EA                            | 2062        | ND                                        | 0.77                  | 0.00113          | 33        |
|          | EA, AA and Asian (meta-analysis) | 22 654   | ND                                        | 0.1546                | 1.24E −07        | 34        |
| rs13280604| Ethnically diverse           | 1056        | Subjective responses to tobacco (adverse, negative physical, positive) | 5.00                  | 0.03             | 31        |
|          | Ethnically diverse            | 1524 families| Subjective responses to tobacco           | NA                    | 0.011            | 31        |
|          | Caucasian, AA and Hispanic    | 1051        | Quit attempts                             | NA                    | 0.024            | 32        |
|          | Caucasian, AA and Hispanic    | 295         | ND                                        | 4.67                  | 0.006            | 32        |
|          | EA, AA and Asian (meta-analysis) | 22 654   | ND                                        | 0.1362                | 7.77E −06        | 34        |
|          | Korean                        | 576         | NDSS (drive)                              | NA                    | 0.03             | 37        |
| rs6474413| EA and Australian             | 1929        | ND (FTND)                                 | NA                    | 9.36E −05        | 19        |
|          | EA and Australian             | 1929        | ND (FTND)                                 | 1.39                  | 6.26E −05        | 30        |
|          | EA                            | 1600        | Early subjective response to tobacco (dizziness) | −0.114                | 0.011            | 36        |
|          | EA                            | 2062        | ND                                        | 0.77                  | 9.26E −04        | 33        |
| rs13277254| EA and Australian            | 1929        | ND (FTND)                                 | 1.4                   | 4.02E −05        | 30        |
|          | EA                            | 1600        | Early subjective response to tobacco (dizziness) | −0.122                | 0.007            | 36        |
|          | EA                            | 2038        | ND (FTND)                                 | 0.79                  | 0.004            | 38        |
|          | EA                            | 2062        | ND                                        | 0.76                  | 6.2E −04         | 33        |
| rs6474412| EA and Australian             | 1929        | ND (FTND)                                 | 1.38                  | 1.126E −04       | 30        |
|          | EA                            | 1600        | Early subjective response to tobacco (dizziness) | −0.111                | 0.014            | 36        |
|          | EA                            | 2062        | ND                                        | 0.78                  | 0.00137          | 33        |
|          | EA, AA and Asian (meta-analysis) | 22 654   | ND                                        | 0.1548                | 5.34E −07        | 34        |
| rs4952   | EA and Australian             | 1929        | ND (FTND)                                 | NA                    | 0.0163           | 19        |
|          | EA and AA                     | 2772        | ND                                        | NA                    | 0.00881          | 33        |
|          | EA and AA (meta-analysis)     | 5092        | ND (FTND)                                 | 0.72                  | 0.02             | 39        |
| rs1955186| EA and Australian             | 1929        | ND (FTND)                                 | 1.38                  | 8.252E −05       | 30        |
|          | EA                            | 1600        | Early subjective response to tobacco (dizziness) | −0.119                | 0.009            | 36        |
|          | EA                            | 2062        | ND                                        | 0.77                  | 7.38E −04        | 33        |
| rs1955185| EA and Australian             | 1929        | ND (FTND)                                 | 1.38                  | 1.01E −04        | 30        |
|          | EA                            | 1600        | Early subjective response to tobacco (dizziness) | −0.118                | 0.009            | 36        |
|          | EA                            | 2062        | ND                                        | 0.78                  | 0.00117          | 33        |
| rs13277524| EA and Australian            | 1929        | ND (FTND)                                 | 1.39                  | 6.043E −05       | 30        |
|          | EA                            | 1600        | Early subjective response to tobacco (dizziness) | −0.121                | 0.007            | 36        |
|          | EA                            | 2062        | ND                                        | 0.77                  | 7.78E −04        | 33        |
| rs4953   | EA and Australian             | 1929        | ND (FTND)                                 | NA                    | 0.0162           | 19        |
|          | Ethnically diverse            | 1056        | Subjective responses to tobacco (adverse)  | 4.16                  | 0.04             | 31        |
| rs4954   | Han Chinese                   | 48          | ND (FTND)                                 | 2.18                  | 4.25E −07        | 40        |
|          | Korean                        | 576         | NDSS (drive)                              | NA                    | 0.02             | 37        |

Abbreviations: AA, African-American; EA, European-American; FTND, Fagerström Test for Nicotine Dependence; NA, not available; ND, nicotine dependence; NDSS, nicotine-dependence syndrome scale; SNP, single nucleotide polymorphism.
ND-RELATED PHENOTYPES

The early-subjective response to tobacco smoking is a subtype of SI, which can predict later persistence of smoking and addiction. DiFranza et al.\(^50\) reported that greater sensitivity to nicotine during early-smoking attempts, as manifested by relaxation, dizziness or nausea, was a determinant of later ND. Pomerleau et al.\(^51\) found that smokers who felt a pleasurable buzz during early smoking smoked much later than those who did not. Thus, it was reasonable to assume that genes, especially CHRN, associated with ND might also play a role in early-subjective responses to tobacco.

The first report concerning the association between the variants in CHRN3–CHRNA6 and subjective responses to tobacco was published by Zeiger et al.\(^31\) using as subjects 1056 ethnically diverse adolescents and a separate community sample of 1524 families. The most significant associations were found between two CHRN3 SNPs (that is, rs4950 and rs13280604) and three subjective response factors to initial tobacco use (adverse, negative physical and positive). Since then, several studies\(^36,42,52\) have examined the association between variants in the CHRN3–CHRNA6 gene cluster and dizziness at first inhalation of cigarette smoke. Although both Ehringer et al.\(^36\) and Pedneault et al.\(^42\) have detected associations with several SNPs in the putative promoter region of CHRN3 and CHRNA6, Hoft et al.\(^52\) did not, which might be attributable to the small sample and the discrepancy of the phenotypic assessment tools used in these studies.

### Table 2. Replicated SNPs in the CHRNA6 gene cluster associated with ND-related behaviors

| dbSNP ID     | Sample origin                  | Sample size | Phenotype                                          | Odds ratio or β-value | Reported P-value | Reference |
|--------------|--------------------------------|-------------|----------------------------------------------------|-----------------------|------------------|-----------|
| rs2304297    | EA and Australian              | 1929        | ND (FTND)                                         | NA                    | 0.00691          | 19        |
|              | Ethnically diverse             | 1056        | Subjective responses to tobacco (positive)        | 0.170                 | 0.003            | 31        |
|              | Caucasian, AA and Hispanic     | 1051        | Quit attempts                                     | NA                    | 0.0044           | 32        |
|              | Mixed ethnic samples           | 6178        | Response to tobacco taxation policy               | −0.032                | 0.018            | 41        |
|              | Canadian                       | 356         | Dizziness at first inhalation of cigarette smoke | 0.59                  | 0.0057           | 42        |
| rs7828365    | American                       | 2847        | ND (CPD)                                          | 0.84                  | 0.036            | 43        |
|              | Canadian                       | 356         | Dizziness at first inhalation of cigarette smoke | 0.58                  | 0.0293           | 42        |
| rs9298628    | Korean                         | 576         | NDSS (drive)                                      | NA                    | 0.02             | 37        |
|              | EA                             | 2428        | ND (FTND)                                         | NA                    | 2.18E−04         | 44        |
|              | EA and AA (meta-analysis)      | 7186        | ND (FTND)                                         | NA                    | 0.00498         | 44        |
| rs892413     | Ethnically diverse             | 935         | Smoking trajectories                              | −1.12                 | < 0.001          | 45        |
|              | EA                             | 1730        | ND (CPD)                                          | NA                    | 0.00769          | 44        |
|              | EA and AA (meta-analysis)      | 7186        | ND (FTND)                                         | NA                    | 5.30E−04         | 44        |
|              | EA and AA (meta-analysis)      | 7186        | ND (FTND)                                         | NA                    | 0.00311         | 44        |

Abbreviations: AA, African-American; CPD, cigarettes smoked per day; EA, European-American; FTND, Fagerström Test for Nicotine Dependence; NA, not available; ND, nicotine dependence; NDSS, nicotine-dependence syndrome scale; SNP, single nucleotide polymorphism.

### Figure 1. Schematic diagram of the human CHRN3–CHRNA6 gene cluster. Horizontal black arrows indicate the direction of transcription of each gene. Gray and black rectangles indicate exons and untranslated regions, respectively, while horizontal black lines represent introns (not drawn to scale). The genetic variants significantly associated with ND in EAs are shown by vertical arrows, which represent two distinct signals. EA, European-American; ND, nicotine dependence.
Apart from early-subjective responses to tobacco, there exist many other ND-related phenotypes where the CHRNA6 gene cluster may play an important role, such as smoking status (never smoking versus ever smoking),\textsuperscript{4,5} smoking trajectories from early adolescence to adulthood,\textsuperscript{4,5} and various ND endophenotypes such as ‘novelty seeking’\textsuperscript{5,3} or ‘drive.’\textsuperscript{5,3} In addition, smoking cessation is of great interest, because it is the ultimate goal of studying tobacco addiction and any other smoking-related phenotypes. Hoft et al.\textsuperscript{62} examined the association of SNPs in the CHRNA3–CHRNA6 cluster with quit attempts in a nationally representative sample of households, which revealed that three SNPs upstream of CHRNA3 (that is, rs7004381, rs4950, rs13280604) and an SNP (rs2304297) in the 3′-region of CHRNA6 were significantly associated with the number of unsuccessful quit attempts in Caucasians. Further, Fletcher et al.\textsuperscript{61} provided novel evidence of the importance of genetics in explaining different responses to tobacco taxation policy. These investigators found that individuals with the protective G/G polymorphism of rs2304297 in CHRNA6 responded to high tobacco taxation, which may help with abstinence, whereas others had no response. The inability of this tobacco control policy (high taxation) to reduce the use of cigarettes in individuals with the C/C genotype suggests that alternative methods might be needed to increase smoking cessation in this population.

**ANALYSIS OF RARE VARIANTS IN THE CHRNA3–CHRNA6 GENE CLUSTER**

Both GWAS and candidate gene-based association studies have identified multiple common variants in the CHRNA3–CHRNA6 gene cluster that contribute to ND and ND-related phenotypes. However, the role of rare variants of this cluster in ND has rarely been studied, largely because the extremely low minor allele frequency (MAF) poses great difficulties in ensuring adequate statistical power. The only study of this topic was carried out by Haller et al.,\textsuperscript{64} in which a DNA-pooling approach was used to sequence the coding and flanking regions of CHRNA6 and CHRNA3 in AA and EA ND smokers or smokers without any ND symptom (for the AAs, two case pools and two control pools; for the EAs, one case pool and one control pool). In contrast to another study performed by the same group,\textsuperscript{51} which showed that rare missense variants in CHRNA3 were associated with a risk of alcohol and cocaine dependence, there was no evidence supporting the role of the same variants in CHRNA6.\textsuperscript{54,55}

Despite the absence of genetic association data for most SNPs, functional studies conducted by us\textsuperscript{56} indicated that rare variants in the h6 subunit gene play a vital role in the etiology of ND. Although missense variations such as Asp577Asn (rs149966755) and Ser156Arg (rs373147726), Asn171Lys (rs79945499) compromises the function of h6*-nACHRs heterologously expressed in Xenopus oocytes, the nicotine sensitivity of these receptors is marginally or significantly increased by introducing Arg96His (rs188620180), Ala184Asp (rs200745568), Asp199Tyr (rs372469952) or Ser233Cys (rs369966241) variations into the h6 subunit gene. Greater sensitivity to activation by agonists (nicotine or ACh) may result in a lower risk of ND, whereas reduced sensitivity increases the risk.\textsuperscript{57} Individuals displaying altered h6*-nAChR pharmacology as a result of rare variants in CHRNA6 are expected to exhibit different responses to cigarette smoking.

Because rare variants (defined as those having an MAF of <1%), together with copy-number variants and small insertion/ deletion polymorphisms (indels) constitute the majority of human genetic variations, they might contribute, at least partly, to the missing heritability of ND. Thus, we need to take rare variants into consideration when studying ND-related phenotypes, especially rare missense functional variants.

**FUNCTIONAL STUDIES OF THE B3 AND A6 SUBUNITS BY GENETIC MANIPULATION IN RODENTS**

As described above, numerous genetic studies have revealed a highly significant association between variants in the CHRNA3–CHRNA6 gene cluster and increased vulnerability to ND,\textsuperscript{21,27,28} which generates a need to explore the underlying mechanisms. However, to date, few pharmacologic ligands have been developed that can selectively target specific nAChR subtypes. Therefore, to understand the contribution of α6 and β3 subunits to ND susceptibility \textit{in vivo}, and to circumvent the problem mentioned above, together with the difficulty associated with α6*-nACHRs in \textit{vitro} expression genetic manipulation in mice becomes highly valuable. These manipulations generally include preventing the expression of the α6 or β3-subunit (KO) and replacing it with hyperactive derivatives (KI).

More attention has been paid to α6- and β3*-nACHRs since the demonstration that these subunits exhibit an expression pattern restricted mainly to catecholaminergic and visual system neurons.\textsuperscript{58–61} By using transgenic mice expressing the α6 subunit fused to a green fluorescent protein, the α6 subunit was found to be highly and selectively expressed in the ventral tegmental area (VTA) and substantia nigra pars compacta, important regions for reinforcement of nicotine use,\textsuperscript{62,63} with functional expression also in the locus coeruleus and retinal ganglion cells.\textsuperscript{64,65} Immuno-precipitation and high-affinity \textsuperscript{125}Iα-conotoxinMII (αCtxMII)-binding studies showed that α6β2β3* and α6δ4β2β3* pentamers are the predominant α6*-nACHRs in the striatum.\textsuperscript{66,67} Furthermore, the gene encoding the β3-subunit, which is adjacent to CHRNA6 (Figure 1), usually is co-expressed with α6. Because of the accessory role of the β3-subunit, it cannot form an acetylcholine-binding site, although it has an essential role in α6*-nACHR biogenesis and function.\textsuperscript{68,69} Gotti et al.\textsuperscript{59} discovered that β3-subunit deletion dramatically reduced, but did not eliminate, α6*-nACHRs expression in the DA cell body (VTA) and terminal region (striatum), suggesting the importance of β3 for the correct assembly, stability and transport of α6-containing receptors in dopaminergic neurons. In addition, a study conducted by Cui et al.\textsuperscript{42} demonstrated that disruption of the β3 gene does not affect expression of mRNA for α6 and other subunits in the same brain areas. They also found that β3-KO mice have altered locomotor activity and prepulse inhibition of acoustic startle responses, behaviors that are regulated in part by nigrostriatal and mesolimbic dopaminergic neurotransmission. Knowledge of these alterations is supported by the evidence that a population of β3-dependent nACHRs, which are sensitive to inhibition by αCtxMII, modulate striatal dopamine release.\textsuperscript{68} In addition, Kamens et al.\textsuperscript{70} showed that the protective variant rs6474413 from human studies reduced expression of the CHRNA3 subunit, and decreased β3 gene expression resulted in reduced nicotine intake in mice.

The α6-null mice grow normally and show no obvious developmental, neurologic or behavior deficits.\textsuperscript{66,71} By using autoradiography, Champaiaux et al.\textsuperscript{71} found complete disappearance of \textsuperscript{125}IαCtxMII binding in both midbrain dopaminergic neurons and the visual system after deleting the α6 subunit, indicating that α6 is an essential component of the native-binding site of this toxin. Another study\textsuperscript{72} has shown the central role of α6 in the VTA in acute nicotine reinforcement.

There are two primary strategies for measuring the reinforcing effect of nicotine: one is intravenous or intracranial nicotine self-administration (SA)\textsuperscript{73,74} and the other is nicotine-induced conditioned place preference (CPP).\textsuperscript{75} The SA paradigm is usually conducted in 30 min with matched animal pairs placed in the experimental boxes, with one animal defined as active and the other as passive. Each nose-poke (NP) by the active mouse activates the computer-operated syringe pump delivering either nicotine or saline to both the active and passive animals, while NPs of the passive mouse are recoded with no scheduled
consequences. By calculating the ratio between the number of responses (NPs) of the active and passive mouse, the reinforcing effects of nicotine can be determined. When tested in this way, α6-WT mice self-administered nicotine in a unit dose of 26.3 μg kg⁻¹ per infusion (inf), whereas their α6-KO drug-naïve littermates did not. The α6-KO animals did not self-administer nicotine even in an extensive range of lower (8.7-17.5 μg kg⁻¹ per inf) and higher (35-52.6 μg kg⁻¹ per inf) doses. Importantly, when the α6 subunit was selectively re-expressed in the VTA of α6−/− mice using a lentiviral vector, the reinforcement property of nicotine was restored (Figure 2). In intracranial SA experiments where learning is required, α6-KO mice showed a trend (although it was not significant) toward reduced nicotine SA compared with wild-type (WT) control mice. These findings demonstrate that the α6 subunit in the VTA is necessary for maintaining nicotine SA. By employing the same model, Sanjakdar et al. showed that nicotine showed a typical inverted U-shaped CPP response curve in the WT mice. Although the dose of 0.5 mg kg⁻¹ nicotine led to a significant CPP in the WT mice, it failed to produce a CPP response in α6-KO mice. In contrast, the higher nicotine dose of 1.0 mg kg⁻¹ resulted in preference scores in α6-KO mice, which were significantly higher than in α6-WT littermates (Figure 3). The α6-KO mice exhibit a rightward shift in the nicotine dose–response curve compared with WT mice, indicating that the rewarding effect of nicotine is mediated by α6*-nAChRs. Pharmacologic blockade of the α6 subunit by selective antagonists (for example, a-contoxinMII) attenuates nicotine-induced CPP, further supporting the vital role of α6 in the nicotine reinforcement.

Although the KO mice model is an essential research tool for understanding the mechanisms of ND, it typically allows addressing only questions of necessity, not sufficiency. To fully understand the diverse roles of different subunits or subtypes in the process of nicotine-induced reward and withdrawal, genetic Ki strains have been developed. Replacement of 'Leu' with 'Ser' in the 9’ residue in the M2 domain of the α6 subunit produces nicotine-hypersensitive mice. These α6 L9'S strains show hyperactive locomotion and fail to habituate to a home cage, a novel environment or reduced wheel rotations, which is consistent with enhanced dopamine neuron firing and release. In addition, by crossing the α4-KO mice with α9L9’S strains, it was found that the hyperactive effects caused by the gain-of-function mutation are mediated by α64* pentamers, because α9L9’S mice lacking the α4 subunit display essentially normal behavior. Together, these studies demonstrate that α9L9’S mice are valuable in investigating the role of the α6 subunit in ND-related behaviors.

**EFFECT OF CHRONIC NICOTINE EXPOSURE ON THE EXPRESSION OF A6-CONTAINING NACHRS**

Nicotine, like other substances of abuse, enhances dopamine transmission in the mesolimbic dopamine pathway, which is thought to play a critical role in the reinforcing effects that maintain smoking behaviors. Many studies on the rewarding effects of nicotine employed an acute administration approach. However, because smoking is a chronic behavior leading to long-term adaptive changes in the brain, knowledge of these chronic changes is essential for understanding ND and implementing measures that cause smoking cessation. Therefore, if genetic manipulation of nAChR genes in mouse KO or KI models represents a powerful research tool for identification of the particular contribution of specific receptor subunits to ND susceptibility, chronic nicotine treatment in vivo or in vitro, which mimics the process of smoking in humans, is a valuable strategy. After long-term nicotine exposure, high-affinity agonist binding to nAChRs in the central nervous system increases in both animal and human brains. This process, termed 'nicotine-induced upregulation', may be involved in the pathology of nicotine addiction. An increase in [H]Ach-binding sites was reported in the brains of smokers compared with non-smokers. The essence of nAChRs upregulation is more related to greater receptor numbers than to augmentation of receptor affinity for nicotine. Furthermore, a hypothesis that nicotine acts as a pharmacologic chaperone to enhance a critical step inside the cell during the maturation of nAChRs has gained support recently. Specifically, nicotine binding to partially assembled nAChRs induces conformations that assemble more efficiently. This could be a compensatory response following desensitization of neuronal AChRs after chronic nicotine exposure.

Accumulating studies have consistently observed upregulation by radiolabeled epibatidine, which identified several nAChR subtypes in numerous brain regions after various nicotine treatments, including injection by osmotic minipumps or jugular
Table 3. Effect on the expression of α6- and β3-containing nAChRs by chronic nicotine exposure

| Change     | Species/cells | Treatment/dose | Brain region     | Subtype     | Reference |
|------------|---------------|----------------|------------------|-------------|-----------|
| Upregulation | Rat           | Injection; 6.0 mg kg⁻¹ per day; 2 weeks Injection; 1.5 mg kg⁻¹ per day; 18 days | NAcc; SC; NACc; VTA/SN; CPu; Thal | α6β2*; α6* | 97, 103   |
|            | Mouse         | Injection; 0.4 mg kg⁻¹ per h; 10 day Injection; 2 mg kg⁻¹ per h; 10 day Oral; 300 μM ml⁻¹; 2 weeks | VTA/SNC; mHB; SC | α6* | 102   |
|            | HEK tsA201 cell | Incubation; 100 μM; overnight | — | α6β2*; α6β4β3*; α6β4β3* | 100   |
|            | Neuro-2a cell | Incubation; 50 μM; 24 h | — | α6β2* | 102   |
| No change | Monkey        | Oral; 650 μg ml⁻¹; 6–8 months | NAcc | α6β2* | 109   |
|            |              | Oral; 650 μg ml⁻¹; 8 months | V Pu; DPu | α6β2* | 110   |
|            |              | Oral; 650 μg ml⁻¹; 3–6 months | NAcc | α6β2* | 111   |
|            | Rat           | Injection; 6.0 mg kg⁻¹ per day; 2 weeks | Str; SC | β3* | 105   |
|            | Neuro-2a cell | Incubation; 50 μM; 24 h | — | α6β2* | 102   |
| Downregulation | Rat           | Oral; 650 μg ml⁻¹; 6 months | CPu; Acbc; AcbSh; SNPC; VTA | α6β2* | 113   |
|            |              | Injection; 6.0 mg kg⁻¹ per day; 2 weeks Injection; 6.0 mg kg⁻¹ per day; 2 weeks Oral; 100 μg ml⁻¹; 2 weeks Oral; 25 μg ml⁻¹; 2–3 months | Str; DLG; VLG; Str | α6* | 105, 106   |
|            | Mouse         | Oral; 300 μg ml⁻¹; 1–6 weeks | NAcc | α6β2* | 107   |
|            |              | Oral; 300 μg ml⁻¹; 2 weeks Injection; 0.125–4.0 mg kg⁻¹ per h; 10 day | Str; Str; OT; VLG | α6β2* | 108   |
|            | Monkey        | Oral; 650 μg ml⁻¹; 6 months | Str | α6* | 112   |

Abbreviation: Acbc, core of nucleus accumbens; AcbSh, shell of nucleus accumbens; CPu, caudate putamen; DLG, dorsolateral geniculate; DPu, dorsal putamen; HEK, human embryonic kidney; NAcc, nucleus accumbens; Neuro, neuroblastoma; OT, olfactory tubercle; SC, superior colliculus; SN, substantia nigra; SNPC, pars compacta of substantia; Thal, thalamus; VLG, ventrolateral geniculate; VTA, ventral tegmental area; mHB.

cannula and infusion in drinking water.⁸⁵,⁹³–⁹⁶ Using [¹²⁵I]epibatidine, A-85380, and cytisine, Nguten et al.⁹⁷ demonstrated that chronic exposure to nicotine upregulates α4β2-containing receptors while having little effect on other nAChR subtypes. Nevertheless, α4β2*-nAChRs, with a wide distribution in the brain and high affinity for nicotine, clearly become desensitized at an early stage of smoking behavior and thus do not function for most of the day in smokers. Despite the clarity of α4β2*-nAChR upregulation, it is not sufficient to explain continued smoking throughout the day.¹⁶,⁹⁸ On the other hand, nAChRs with low affinity for nicotine (for example, α7, α6) are not susceptible to rapid saturation and might play an important role in continued smoking. Besides α4β2-containing receptors, other diverse populations of nAChRs, such as α6β2* and α7*, have been identified in the mesolimbic dopamine pathway. These findings shed light on the vital importance of research on the upregulation of other nAChRs.

Unlike the situation with α4β2*-nAChRs, upregulation of α6-containing receptors in response to chronic nicotine exposure is controversial.⁹⁹ There have been reports of upregulation, downregulation and no change in in vitro and in vivo experiments (Table 3). Upregulation of α6β2* or α6β2β3* nAChRs by incubation with nicotine was observed in cultured cell lines,¹⁰⁰–¹⁰² although functional expression of α6-containing receptors in a heterologous expression system was difficult until some specific strategies were used, such as chimeras, gain-of-function mutagenesis and so on. Unfortunately, in rodents, although Nguyen et al.⁹⁷ and Parker et al.¹⁰³ suggested upregulation of α6*-nAChRs in the nucleus accumbens, several other research groups¹⁰⁴–¹⁰⁸ observed downregulation in the striatum. Interestingly, Perez et al.¹⁰⁷ showed, by using the novel α-CtxMII analog E11A in α4-KO mice, that nicotine administration in the drinking water for 2 weeks increased the α6 (non-α4) β2*-nAChR population in the striatum, contrary to the reduction of total α6β2* subtypes in WT littermates. This leads us to hypothesize that α6α4β2* contributes to the downregulation in the striatum. Furthermore, in non-human primates such as the squirrel monkey, nicotine in the drinking water with a final concentration of 650 μg ml⁻¹ for >6 months did not significantly change the α6β2*-nAChR-binding site except in the study conducted by McCallum et al.¹¹² This effect might be caused by region-specific actions, because earlier studies concentrated mainly on the nucleus accumbens, whereas the later ones focused on the striatum. Analyses in other reward-related regions of the brain also were performed, but this work has yielded no clear results or conclusions.¹⁰²,¹⁰³,¹¹³

Several factors may account for these disparate findings. First, different nicotine treatment regimens with different concentrations of nicotine and exposure time were used. The importance of such changes is supported by evidence that α6β2β3* nAChR showed upregulation after 50 nM nicotine treatment but downregulation with 500 nM nicotine.¹⁰² Second, different species/cell lines, brain regions and α6-containing subtypes may play a role in the inconsistent results. Last but not least, heterogeneity of the
detection methods is an influencing factor, implying the urgency of developing more subunit-specific agonists and antibodies.

CONCLUSIONS AND FUTURE RESEARCH

In this report, we have summarized several lines of evidence that support the involvement of the *CHRNB3–CHRNA6* gene cluster in ND. A multitude of genetic studies (GWAS and candidate gene-based association studies) analyzing various ND phenotypes have implicated variants in this gene cluster in the development of ND. The most compelling evidence is for SNPs rs13277254 and rs6474413 in *CHRNB3*, as well as rs10958726 and rs1955186 within this same signal. However, not much has been found specifically for the *CHRNA6* subunit gene, contrary to its vital role in maintaining ND as demonstrated with functional studies. These findings reveal only a small fraction of variants, that is, these known polymorphisms have small effects and can explain only a small proportion of the inheritability of smoking-related behaviors. Therefore, additional loci (especially rare variants) need to be identified. Furthermore, despite the inconsistent results, it is important to study the genetics of ND in diverse populations. Differences in genetic architectures and allele frequencies in different ethnic populations can help assign statistically significant signals to potentially causal variants.

Genetic modification of the *CHRNB3* and *CHRNA6* in mice is a valuable approach to evaluate the contribution of each subunit to ND susceptibility. The use of KO mice has displayed various behavioral phenotypes related to ND. For example, α6-KO mice do not self-administer nicotine, unlike their WT counterparts. In addition, studies in α6-hypersensitive mice (KI mice) are powerful in identifying compounds that activate or antagonize α6*-nAChRs as a means to improve the development of drugs for smoking cessation. Nevertheless, this approach is limited in the *in vivo* or *in vitro* studies focusing on elucidating the functional consequences of different SNPs. This level of investigation will provide significant insights into how genetic variations in humans underlie individual differences in the reinforcement, aversion and withdrawal of nicotine. There exist significant differences in the pharmacologic properties of the α6 and β3 subunits, such as receptor upregulation after chronic nicotine treatment and differences among subtypes and brain regions. It remains to be determined how nicotine regulates the expression of α6*-nAChRs. Inconsistent results from different studies were likely a consequence of the unpredictable behavior in heterologous expression systems. Functional expression of WT α6*-nAChRs is difficult to achieve unless some modifications have been adopted, for instance, subunit chimeras, concatameric subunits and point mutagenesis of the α6 or β3 subunits. In spite of the significant progress, there still are many obstacles to be overcome. That may be why conflicting results concerning upregulation of α6-containing receptors occur in relatively few studies. Thus, advancing the heterologous expression of α6* receptors should be another focus of future research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Dr David L Bronson for excellent editing of this manuscript. This study was supported in part by the Research Center for Air Pollution and Health of Zhejiang University, Ministry of Science and Technology of China (2012AA020405), the National Natural Science Foundation of China grant 81273223, Young Scientists Fund of National Science Foundation of China (81301140) and NIH grant DA012844.

REFERENCES

1 CDC. Current cigarette smoking among adults — United States, 2011. MMWR Morb Mortal Wkly Rep 2012; 61: 889-894.
2 Li MD. Identifying susceptibility loci for nicotine dependence: 2008 update based on recent genome-wide linkage analyses. *Hum Genet* 2008; 123: 119–131.
3 Centers for Disease Control and Prevention (CDC). State-specific prevalence of current cigarette smoking among adults and secondhand smoke rules and policies in homes and workplaces. *MMWR Morb Mortal Wkly Rep* 2006; 55: 1148–1151.
4 Benowitz NL. Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. *Clin Pharmacol Ther* 2008; 83: 531–541.
5 Batra V, Patkar AA, Berrettini WH, Weinstein SP, Leone FT. The genetic determinants of smoking. *Chest* 2003; 123: 1730–1739.
6 Lessov CN, Martin NG, Statham DJ, Todorov AA, Slutske WS, Bucholz KK et al. Defining nicotine dependence for genetic research: evidence from Australian twins. *Psychol Med* 2004; 34: 865–879.
7 Lessov-Schaller CN, Pang Z, Swan GE, Guo Q, Wang S, Cao W et al. Heritability of cigarette smoking and alcohol use in Chinese male twins: the Qingdao twin registry. *Int J Epidemiol* 2006; 35: 1278–1285.
8 Maes HH, Sullivan PF, Bulik CM, Neale MC, Prescott CA, Eaves LJ et al. A twin study of genetic and environmental influences on tobacco initiation, regular tobacco use and nicotine dependence. *Psychol Med* 2004; 34: 1251–1261.
9 Sullivan PF, Kendler K. The genetic epidemiology of smoking. *Nicotine Tob Res* 1999; 1: 551–557.
10 Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. *Addiction* 2003; 98: 23–31.
11 Baker RR, Pereira da Silva JR, Smith G. The effect of tobacco ingredients on smoke chemistry. Part I: flavourings and additives. *Food Chem Toxicol* 2004; 42: 53–37.
12 Dani JA, Harris RA. Nicotine addiction and comorbidity with alcohol abuse and mental illness. *Nat Neurosci* 2005; 8: 1465–1470.
13 Le Novere N, Corringer PJ, Changeux JP. The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences. *J Neurobiol* 2002; 53: 447–456.
14 Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* 2007; 47: 699–729.
15 Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmaco Sci* 2006; 27: 482–491.
16 Rose JE. Multiple brain pathways and receptors underlying tobacco addiction. *Biochem Pharmacol* 2007; 74: 1263–1270.
17 Kalamida D, Poulas K, Avramopoulos V, Fostieri E, Lagoumintzis G, Lazaridis K et al. Muscle and neuronal nicotinic acetylcholine receptors. Structure, function and pathogenicity. *FEBS J* 2007; 274: 3799–3845.
18 Waters AJ, Shiffman S, Sayette MA, Paty JA, Gualtney CJ, Balabanis MH. Attentional bias predicts outcome in smoking cessation. *Health Psychol* 2003; 22: 378–387.
19 Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* 2007; 16: 36–49.
20 Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X et al. Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry* 2008; 165: 1163–1171.
21 Thorgersson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F et al. Sequence variants at *CHRNB3–CHRNA6* and *CYP2A6* affect smoking behavior. *Nat Genet* 2010; 42: 448–453.
22 Bierut LJ. Genetic vulnerability and susceptibility to substance dependence. *Neuron* 2011; 69: 618–627.
23 Moore C, Fattore L, Pons S, Hay YA, Marti F, Lambez B et al. Nicotine consumption is regulated by a human polymorphism in dopamine neurons. *Mol Psychiatr* 2014; 19: 930–936.
24 Wen L, Jiang K, Yuan W, Cui W, Li MD. Contribution of variants in *CHRNA5/A3/B4* gene cluster on chromosome 15 to tobacco smoking: from genetic association to mechanism. *Mol Neurobiol* 2014; 53: 472–484.
25 Berrettini WH, Doyle GA. The *CHRNA5–A3–B4* gene cluster in nicotine addiction. *Mol Psychiatry* 2012; 17: 856–866.
26 Melloy-Greif WE, Stitzel JA, Ehringer MA. Nicotinic acetylcholine receptors: upregulation, age-related effects and associations with drug use. *Genes Brain Behav* 2016; 15: 89–107.
27 Bierut LJ, Madden PA, Breslau N, Johnson EO, Hukasukami D, Pomerleau O et al. Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet* 2007; 16: 24–35.
Multiple cholinergic nicotinic receptor genes affect nicotine dependence risk in African and European Americans. Genes Brain Behav 2010; 9: 741–750.

Cui WY, Wang S, Yang J, Yi SG, Liu D, Kim YJ et al. The neuronal nicotinic subunit genes (CHRNA6 and CHRNA7) are associated with risk of nicotine dependence in the Chinese population. Drug Alcohol Depend 2008; 93: 260–270.

Hoft NR, Corley RP, McQueen MB, Schafer IR, Huizenga D, Ehringer MA. Genetic association of the CHRNA6 and CHRNA7 genes with tobacco dependence in a nationally representative sample. Neuropsychopharmacology 2009; 34: 696–706.

Saccone NL, Schwantes-An TH, Wang JC, Grucza RA, Breslau N, Hatsukami D et al. Nicotine dependence in multiple ethnic populations. Psychol Med 2010; 40: 1194–1195.

Bar-Shira A, Gana-Weisz M, Ganor-Z, Giladi E, Giladi N, Orr-Utregger A. CHRNA5 c.-57 A>G functional promoter change affects Parkinson’s disease and smoking. Neurobiol Aging 2014; 35: 2179 e2171–2176.

Ehringer MA, McQueen MB, Hoft NR, Saccone NL, Stitzel JA, Wang JC et al. Association of CHRNA5 with ‘dizziness to tobacco’. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 600–609.

Wen WY, Park B, Choi SW, Kim L, Kwon M, Kim JH et al. Genetic association of CHRNA5 and CHRNA6A gene polymorphisms with nicotine dependence symptom scale in Korean population. Psychiatric Investig 2014; 11: 307–312.

Johnson EO, Chen LS, Breslau N, Hatsukami D, Robbins T, Saccone NL et al. Cigarette smoking and the nicotinic receptor genes: an examination of genetic and environmental risks for nicotine dependence. Psychobiology 2010; 38: 2023–2029.

Cui YW, Wang S, Yang J, Yi SG, Yoon D, Kim YJ et al. Significant association of CHRNA5 variants with nicotine dependence in multiple ethnic populations. Mol Psychiatry 2013; 18: 1149–1151.

Johnson EO, Chen LS, Breslau N, Hatsukami D, Robbins T, Saccone NL et al. Multiple cholinergic nicotinic receptor genes affect nicotine dependence risk in African and European Americans. Genes Brain Behav 2010; 9: 741–750.

Cui WY, Wang S, Yang J, Yi SG, Yoon D, Kim YJ et al. Significant association of CHRNA5 variants with nicotine dependence in multiple ethnic populations. Mol Psychiatry 2013; 18: 1149–1151.

Bar-Shira A, Gana-Weisz M, Ganor-Z, Giladi E, Giladi N, Orr-Utregger A. CHRNA5 c.-57 A>G functional promoter change affects Parkinson’s disease and smoking. Neurobiol Aging 2014; 35: 2179 e2171–2176.

Ehringer MA, McQueen MB, Hoft NR, Saccone NL, Stitzel JA, Wang JC et al. Association of CHRNA5 with ‘dizziness to tobacco’. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 600–609.

Wen WY, Park B, Choi SW, Kim L, Kwon M, Kim JH et al. Genetic association of CHRNA5 and CHRNA6A gene polymorphisms with nicotine dependence symptom scale in Korean population. Psychiatric Investig 2014; 11: 307–312.

Johnson EO, Chen LS, Breslau N, Hatsukami D, Robbins T, Saccone NL et al. Cigarette smoking and the nicotinic receptor genes: an examination of genetic and environmental risks for nicotine dependence. Psychobiology 2010; 38: 2023–2029.

Cui YW, Wang S, Yang J, Yi SG, Yoon D, Kim YJ et al. Significant association of CHRNA5 variants with nicotine dependence in multiple ethnic populations. Mol Psychiatry 2013; 18: 1149–1151.

Bar-Shira A, Gana-Weisz M, Ganor-Z, Giladi E, Giladi N, Orr-Utregger A. CHRNA5 c.-57 A>G functional promoter change affects Parkinson’s disease and smoking. Neurobiol Aging 2014; 35: 2179 e2171–2176.

Ehringer MA, McQueen MB, Hoft NR, Saccone NL, Stitzel JA, Wang JC et al. Association of CHRNA5 with ‘dizziness to tobacco’. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 600–609.

Wen WY, Park B, Choi SW, Kim L, Kwon M, Kim JH et al. Genetic association of CHRNA5 and CHRNA6A gene polymorphisms with nicotine dependence symptom scale in Korean population. Psychiatric Investig 2014; 11: 307–312.

Johnson EO, Chen LS, Breslau N, Hatsukami D, Robbins T, Saccone NL et al. Multiple cholinergic nicotinic receptor genes affect nicotine dependence risk in African and European Americans. Genes Brain Behav 2010; 9: 741–750.

Cui YW, Wang S, Yang J, Yi SG, Yoon D, Kim YJ et al. Significant association of CHRNA5 variants with nicotine dependence in multiple ethnic populations. Mol Psychiatry 2013; 18: 1149–1151.

Bar-Shira A, Gana-Weisz M, Ganor-Z, Giladi E, Giladi N, Orr-Utregger A. CHRNA5 c.-57 A>G functional promoter change affects Parkinson’s disease and smoking. Neurobiol Aging 2014; 35: 2179 e2171–2176.

Ehringer MA, McQueen MB, Hoft NR, Saccone NL, Stitzel JA, Wang JC et al. Association of CHRNA5 with ‘dizziness to tobacco’. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 600–609.

Wen WY, Park B, Choi SW, Kim L, Kwon M, Kim JH et al. Genetic association of CHRNA5 and CHRNA6A gene polymorphisms with nicotine dependence symptom scale in Korean population. Psychiatric Investig 2014; 11: 307–312.

Johnson EO, Chen LS, Breslau N, Hatsukami D, Robbins T, Saccone NL et al. Multiple cholinergic nicotinic receptor genes affect nicotine dependence risk in African and European Americans. Genes Brain Behav 2010; 9: 741–750.

Cui YW, Wang S, Yang J, Yi SG, Yoon D, Kim YJ et al. Significant association of CHRNA5 variants with nicotine dependence in multiple ethnic populations. Mol Psychiatry 2013; 18: 1149–1151.

Bar-Shira A, Gana-Weisz M, Ganor-Z, Giladi E, Giladi N, Orr-Utregger A. CHRNA5 c.-57 A>G functional promoter change affects Parkinson’s disease and smoking. Neurobiol Aging 2014; 35: 2179 e2171–2176.

Ehringer MA, McQueen MB, Hoft NR, Saccone NL, Stitzel JA, Wang JC et al. Association of CHRNA5 with ‘dizziness to tobacco’. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 600–609.

Wen WY, Park B, Choi SW, Kim L, Kwon M, Kim JH et al. Genetic association of CHRNA5 and CHRNA6A gene polymorphisms with nicotine dependence symptom scale in Korean population. Psychiatric Investig 2014; 11: 307–312.

Johnson EO, Chen LS, Breslau N, Hatsukami D, Robbins T, Saccone NL et al. Multiple cholinergic nicotinic receptor genes affect nicotine dependence risk in African and European Americans. Genes Brain Behav 2010; 9: 741–750.

Cui YW, Wang S, Yang J, Yi SG, Yoon D, Kim YJ et al. Significant association of CHRNA5 variants with nicotine dependence in multiple ethnic populations. Mol Psychiatry 2013; 18: 1149–1151.

Bar-Shira A, Gana-Weisz M, Ganor-Z, Giladi E, Giladi N, Orr-Utregger A. CHRNA5 c.-57 A>G functional promoter change affects Parkinson’s disease and smoking. Neurobiol Aging 2014; 35: 2179 e2171–2176.

Ehringer MA, McQueen MB, Hoft NR, Saccone NL, Stitzel JA, Wang JC et al. Association of CHRNA5 with ‘dizziness to tobacco’. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 600–609.
75 Gruber SD, Martin BR, Brown SE, Damaj MI. Nicotine place preference in the mouse: influences of prior handling, dose and strain and attenuation by nicotine receptor antagonists. *Psychopharmacology* 2006; 184: 456–463.

76 Exley R, Maubourguet N, David V, Edinne R, Evard A, Pons S et al. Distinct contributions of nicotinic acetylcholine receptor subunit alpha 4 and subunit alpha 6 to the reinforcing effects of nicotine. *Proc Natl Acad Sci USA* 2011; 108: 7577–7582.

77 Sanjakdar SS, Maldoon PP, Marks MJ, Brunzell DH, Maskos U, McIntosh JM et al. Differential roles of alpha6beta2* and alpha4beta2* neuronal nicotinic receptors in nicotine- and cocaine-conditioned reward in mice. *Neuropsychopharmacology* 2015; 40: 350–360.

78 Jackson KJ, McIntosh JM, Brunzell DH, Sanjakdar SS, Damaj MI. The role of alpha6-containing nicotinic acetylcholine receptors in nicotine reward and withdrawal. *J Pharmacol Exp Ther* 2009; 331: 547–554.

79 Drenan RM, Grady SR, Whiteaker P, McClure-Begley T, McKinney S, Miwa JM et al. In vivo activation of midbrain dopamine neurons via sensitized, high-affinity alpha 6 nicotinic acetylcholine receptors. *Neuron* 2008; 60: 123–136.

80 Drenan RM, Grady SR, Steele AD, McKinney S, Patzlaff NE, McIntosh JM et al. Cholinergic modulation of locomotion and striatal dopamine release is mediated by alpha6alpha4* nicotinic acetylcholine receptors. *J Neurosci* 2010; 30: 9877–9889.

81 Grady SR, Drenan RM, Breining SR, Yohannes D, Wageman CR, Fedorov NB et al. Structural differences determine the relative selectivity of nicotinic compounds for native alpha 4 beta 2**, alpha 6 beta 2**, alpha 3 beta 4** and alpha 7 nicotinic acetylcholine receptors. *Neuropharmacology* 2010; 58: 1054–1066.

82 Cohen BN, Mackey ED, Grady SR, McKinney S, Patzlaff NE, Wageman CR et al. Nicotinic cholinergic mechanisms causing elevated dopamine release and abnormal locomotor behavior. *Neuroscience* 2012; 200: 31–41.

83 Wang Y, Lee JW, Oh G, Grady SR, McIntosh JM, Brunzell DH et al. Enhanced synthesis and release of dopamine in transgenic mice with gain-of-function alpha6* nACHRs. *J Neurochem* 2014; 129: 315–327.

84 Webster JC, Francis MM, Porter JK, Robinson G, Stokes H, Horenstein B et al. Antagonist activities of mecamylamine and nicotine show reciprocal dependence on beta subunit sequence in the second transmembrane domain. *Br J Pharmacol* 1999; 127: 1337–1348.

85 Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF et al. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J Pharmacol Exp Ther* 1999; 289: 1545–1552.

86 Hogg RC, Raggenbass M, Bertrand D. Nicotinic acetylcholine receptors: from structure to brain function. *Rev Physiol Biochem Pharmacol* 2003; 147: 1–46.

87 Breeze CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC et al. Effect of smoking history on [3H]nicotine binding in human postmortem brain. *J Pharmacol Exp Ther* 1997; 282: 7–13.

88 Buisson B, Bertrand D. Chronic exposure to nicotine upregulates the human (alpha)4[(beta)2] nicotinic acetylcholine receptor function. *J Neurosci* 2001; 21: 1819–1829.

89 Lester HA, Xiao C, Srinivasan R, Son CD, Miwa J, Pantoja R et al. Differential contributions of alpha6- and beta3-containing nicotinic cholinergic receptors in rat during long-term self-administration of nicotine: disproportionate increase of the alpha6 subunit. *Mol Pharmacol* 2004; 65: 611–622.

90 Nguyen HN, Rasmussen BA, Perry DC. Subtype-selective up-regulation by chronic nicotine of high-affinity nicotinic receptors in rat brain demonstrated by receptor autoradiography. *J Pharmacol Exp Ther* 2003; 307: 1090–1097.

91 Wootorton JR, Pidoplichko VI, Broide RS, Dani JA. Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci* 2003; 23: 3176–3189.

92 Srinivasan R, Henderson BJ, Lester HA, Richards CI. Pharmacological chaperoning of alpha6 nAChRs: a therapeutic target for Parkinson’s disease. *Pharmacol Res* 2014; 83: 20–29.

93 Tumkosit P, Kuryatov A, Luo J, Lindstrom J. Beta3 subunits promote expression and nicotine-induced up-regulation of human nicotinic alpha6* nicotinic acetylcholine receptors expressed in transfected cell lines. *Mol Pharmacol* 2006; 70: 1358–1368.

94 Walsh H, Govind AP, Mastro R, Hoda JC, Bertrand D, Vallejo Y et al. Up-regulation of nicotinic receptors by nicotine varies with receptor subtype. *J Biol Chem* 2008; 283: 6022–6032.

95 Henderson BJ, Srinivasan R, Nichols WA, Dilworth CN, Gutierrez DF, Mackey ED et al. Nicotine exploits a COPII-mediated process for chaperone-mediated up-regulation of its receptors. *J Gen Physiol* 2014; 143: 51–66.

96 Parker SL, Fu Y, McAleney K, Luo J, McIntosh JM, Lindstrom JM et al. Up-regulation of brain nicotinic acetylcholine receptors in the rat during long-term self-administration of nicotine: disproportionate increase of the alpha6 subunit. *Mol Pharmacol* 2004; 65: 611–622.

97 Lac I, Paremeswaran N, Khwaja M, Whiteaker P, Lindstrom JM, Fan H et al. Long-term nicotine treatment decreases striatal alpha 6 nicotinic acetylcholine receptor sites and function in mice. *Mol Pharmacol* 2005; 67: 1639–1647.

98 Perry DC, Mao D, Gold AB, McIntosh JM, Pezzullo JC, Kellar KJ. Chronic nicotine differentially regulates alpha6– and beta3-containing nicotinic cholinergic receptors in rat brain. *J Pharmacol Exp Ther* 2007; 322: 306–315.

99 Doura MB, Gold AB, Keller AB, Perry DC. Adult and periadolescent rats differ in expression of nicotinic cholinergic receptor subtypes and in the response of these subtypes to chronic nicotine exposure. *Brain Res* 2008; 1215: 40–52.

100 Perez XA, Bordia T, McIntosh JM, Grady SR, Quik M. Long-term nicotine treatment differentially regulates striatal alpha6alpha6beta2* and alpha6(nona4alpha4) beta2* nAChR expression and function. *Mol Pharmacol* 2008; 74: 844–853.

101 Perez XA, Bordia T, McIntosh JM, Quik M. Differential regulation of mesolimbic alpha 3/alpha 6 beta 2 and alpha 4 beta 2 nicotinic acetylcholine receptor sites and function after long-term oral nicotine to monkeys. *J Pharmacol Exp Ther* 2006; 318: 381–388.

102 Perez XA, O’Leary KT, Paremeswaran N, McIntosh JM, Quik M. Prominent role of alpha3(alpha6alpha6beta2*) nAChRs in regulating evoked dopamine release in primate putamen: effect of long-term nicotine treatment. *Mol Pharmacol* 2009; 75: 938–946.

103 Perez XA, Ly J, McIntosh JM, Quik M. Long-term nicotine exposure depresses dopamine release in nonhuman primate nucleus accumbens. *J Pharmacol Exp Ther* 2012; 342: 335–344.

104 McCallum SE, Paremeswaran N, Bordia T, Fan H, McIntosh JM, Quik M. Differential regulation of mesolimbic alpha 3/alpha 6 beta 2 and alpha 4 beta 2 nicotinic acetylcholine receptor sites and function after long-term oral nicotine to monkeys. *J Pharmacol Exp Ther* 2006; 318: 381–388.

105 Perez XA, O’Leary KT, Paremeswaran N, McIntosh JM, Quik M. Prominent role of alpha3(alpha6alpha6beta2*) nAChRs in regulating evoked dopamine release in primate putamen: effect of long-term nicotine treatment. *Mol Pharmacol* 2009; 75: 938–946.

106 Perez XA, Ly J, McIntosh JM, Quik M. Long-term nicotine exposure depresses dopamine release in nonhuman primate nucleus accumbens. *J Pharmacol Exp Ther* 2012; 342: 335–344.

107 Perez XA, McIntosh JM, Quik M. Long-term nicotine treatment down-regulates alpha6beta2* nicotinic receptor expression and function in nucleus accumbens. *J Neurochem* 2013; 127: 762–771.

© The Author(s) 2016

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/