

Genetic susceptibility to nicotine addiction: Advances and shortcomings in our understanding of the CHRNA5/A3/B4 gene cluster contribution

Romain Icick, Benoît Forget, Isabelle Cloëz-Tayarani, Stéphanie Pons, Uwe Maskos, Morgane Besson

▶ **To cite this version:**

Romain Icick, Benoît Forget, Isabelle Cloëz-Tayarani, Stéphanie Pons, Uwe Maskos, et al.. Genetic susceptibility to nicotine addiction: Advances and shortcomings in our understanding of the CHRNA5/A3/B4 gene cluster contribution. Neuropharmacology, 2020, 177, pp.108234. 10.1016/j.neuropharm.2020.108234 . pasteur-02936148

HAL Id: pasteur-02936148
https://pasteur.hal.science/pasteur-02936148

Submitted on 15 Sep 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

***Highlights**

Highlights (85 characters including space each)

- *CHRNA5/A4/B4* and nicotine addiction link on SNPs in high linkage disequilibrium
- *CHRNA5/A4/B4* cross-regulatory mechanisms *should be considered in preclinical models*
- Refined neuropsychological assessment for addiction is needed in future studies

***Title page**

Genetic susceptibility to nicotine addiction: advances and shortcomings in our understanding of the *CHRNA5/A3/B4* gene cluster contribution

Romain Icick[1,2,3,4], Benoît Forget[1,5], Isabelle Tayarani[1,4], Stéphanie Pons[1,4], Uwe Maskos[1,4], Morgane Besson[1,4].

[1]Neurobiologie Intégrative des Systèmes Cholinergiques, CNRS UMR3571, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France

[2]Département de Psychiatrie et de Médecine Addictologique, Groupe Hospitalier Saint-Louis – Lariboisière – Fernand Widal, Assistance-Publique Hôpitaux de Paris

Corresponding author:

Morgane Besson (morgane.besson@pasteur.fr)

Highlights (85 characters including space each)

- *CHRNA5/A4/B4* and nicotine addiction link on SNPs in high linkage disequilibrium
- *CHRNA5/A4/B4* cross-regulatory mechanisms *should be considered in preclinical models*
- Refined neuropsychological assessment for addiction is needed in future studies

Abstract

Over the last decade, robust human genetic findings have been instrumental in elucidating the heritable basis of nicotine addiction (NA). They highlight coding and synonymous polymorphisms in a cluster on chromosome 15, encompassing the *CHRNA5, CHRNA3* and *CHRNB4* genes, coding for three subunits of the nicotinic acetylcholine receptor (nAChR). They have inspired an important number of preclinical studies, and will hopefully lead to the definition of novel drug targets for smoking cessation. Here, we review these candidate gene and genome-wide association studies (GWAS) and their direct implication in human brain function and NA-related phenotypes. We continue with a description of preclinical work in transgenic rodents that has led to a mechanistic understanding of several of the genetic hits. We also highlight important issues with regards to *CHRNA3* and *CHRNB4* where we are still lacking a dissection of their role in NA, including even in preclinical models. We further emphasize the use of human induced pluripotent stem cell-derived models for the analysis of synonymous and intronic variants on a human genomic background. Finally, we indicate potential avenues to further our understanding of the role of this human genetic variation.

Keywords

nicotine addiction, nicotinic receptor, human genetics, polymorphisms, animal models

Abstract

Over the last decade, robust human genetic findings have been instrumental in elucidating the heritable basis of nicotine addiction (NA). They highlight coding and synonymous polymorphisms in a cluster on chromosome 15, encompassing the *CHRNA5, CHRNA3* and *CHRNB4* genes, coding for three subunits of the nicotinic acetylcholine receptor (nAChR). They have inspired an important number of preclinical studies, and will hopefully lead to the definition of novel drug targets for smoking cessation. Here, we review these candidate gene and genome-wide association studies (GWAS) and their direct implication in human brain function and NA-related phenotypes. We continue with a description of preclinical work in transgenic rodents that has led to a mechanistic understanding of several of the genetic hits. We also highlight important issues with regards to *CHRNA3* and *CHRNB4* where we are still lacking a dissection of their role in NA, including even in preclinical models. We further emphasize the use of human induced pluripotent stem cell-derived models for the analysis of synonymous and intronic variants on a human genomic background. Finally, we indicate potential avenues to further our understanding of the role of this human genetic variation.

*Manuscript
Click here to view linked References

Genetic susceptibility to nicotine addiction: advances and shortcomings in our understanding of the *CHRNA5/A3/B4* gene cluster contribution

Romain Icick[1,2,3,4], Benoît Forget[1,5], Isabelle Tayarani[1,4], Stéphanie Pons[1,4], Uwe Maskos[1,4], Morgane Besson[1,4].

[1]Neurobiologie Intégrative des Systèmes Cholinergiques, CNRS UMR3571, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France

[2]Département de Psychiatrie et de Médecine Addictologique, Groupe Hospitalier Saint-Louis – Lariboisière – Fernand Widal, Assistance-Publique Hôpitaux de Paris

Corresponding author:

Morgane Besson (morgane.besson@pasteur.fr)

Highlights (85 characters including space each)

- *CHRNA5/A4/B4* and nicotine addiction link on SNPs in high linkage disequilibrium
- *CHRNA5/A4/B4* cross-regulatory mechanisms should be considered in preclinical models
- Refined neuropsychological assessment for addiction is needed in future studies

Abstract

Over the last decade, robust human genetic findings have been instrumental in elucidating the heritable basis of nicotine addiction (NA). They highlight coding and synonymous polymorphisms in a cluster on chromosome 15, encompassing the *CHRNA5, CHRNA3* and *CHRNB4* genes, coding for three subunits of the nicotinic acetylcholine receptor (nAChR). They have inspired an important number of preclinical studies, and will hopefully lead to the definition of novel drug targets for smoking cessation. Here, we review these candidate gene and genome-wide association studies (GWAS) and their direct implication in human brain function and NA-related phenotypes. We continue with a description of preclinical work in transgenic rodents that has led to a mechanistic understanding of several of the genetic hits. We also highlight important issues with regards to *CHRNA3* and *CHRNB4* where we are still lacking a dissection of their role in NA, including even in preclinical models. We further emphasize the use of human induced pluripotent stem cell-derived models for the analysis of synonymous and intronic variants on a human genomic background. Finally, we indicate potential avenues to further our understanding of the role of this human genetic variation.

Keywords

nicotine addiction, nicotinic receptor, human genetics, polymorphisms, animal models

1. Introduction

1.1. Nicotine addiction

Tobacco smoking is still responsible for more than 8 million deaths each year (WHO, https://www.who.int/news-room/fact-sheets/detail/tobacco). Tobacco-related morbidity and mortality represents one of the most dramatic, yet preventable, public health tragedies. There is a huge need for more effective therapeutic approaches considering the low success rates of the currently available psychosocial and pharmaco-therapies to assist in quitting tobacco use (Prochaska and Benowitz, 2019). Similar to other substance use disorders (SUDs), tobacco addiction is a chronic relapsing psychiatric disorder in which drug compulsive use and seeking behaviors persist despite negative consequences and a strong motivation to quit (Babb et al., 2017; Chaiton et al., 2016; Yucel et al., 2019). Among the thousands of compounds found in tobacco smoke, nicotine is the primary psychoactive substance responsible for its reinforcing and addictive properties (Caille et al., 2012; Clemens et al., 2014; Cohen et al., 2012; Dome et al., 2010; Gilpin et al., 2014). Given the inconstant efficacy of currently available treatments, and since products of tobacco combustion are the main cause of smoking-induced diseases, governmental and economical control of smoking behavior has led to the development of devices for smokeless nicotine delivery such as heated and electronic nicotine vaping products (Prochaska and Benowitz, 2019). However, the health consequences of the use, especially the chronic long-term use, of these devices are uncertain and more investigation will be required during the next decade to ascertain their safety. Moreover, while such devices are very likely to reduce the risk for lethal diseases among individuals suffering from nicotine addiction (NA), under a “harm reduction” goal, (Chatterjee et al., 2016; Etter, 2018). For recent insights on the topic, see https://www.nejm.org/vaping.

For now, there is still an urgent need to provide a better understanding of the aetiopathogeny of NA to develop alternative prevention and intervention strategies. Importantly, although the prevalence of individuals diagnosed as dependent is higher for tobacco than that for any other SUD, not all smokers become nicotine dependent (Anthony J, 1994; Birge et al., 2018; Kandel et al., 1997). Addiction is a complex disorder, and there are individual differences in the vulnerability to develop this pathology that can be conceptualized at different levels interacting with each other, including environmental and genetic components. It is of particular importance to better understand the genetic contribution of addiction vulnerability, which can account for more than 70% of an individual's risk for this pathology (Ducci and Goldman, 2012). The information obtained from research into addiction vulnerability has the potential to help preventing and treating addictive disorders in several ways (Morrow and Flagel, 2016). For instance, a better understanding of the genetic predisposition factors could lead to the discovery of biomarkers to identify individuals who are at high risk of developing addiction. Individuals who already have addiction may also benefit from knowledge of genetic vulnerability factors, notably through personalized pharmacotherapy during specific aspects of the addiction cycle, such as drug seeking reinstatement which often occurs in ‘occasion setter’ contexts (Crombag et al., 2008).

1.2. The *CHRNA5-A3-B4* gene cluster and NA vulnerability

The identification of specific genes and loci contributing to addiction vulnerability has been challenging, likely because of the genetic complexity of psychiatric diseases such as addictive disorders. Such complexity includes multiple risk alleles, gene x gene interactions and epigenetic effects (Gelernter, 2015). In this context, genome-wide association studies (GWAS) have the advantages of covering the entire genome, implementing a 'hypothesis-free' approach, and having the power for detecting relatively common alleles. The potential of GWAS in the addiction field will most likely increase in the future with more extensive data sharing, larger samples, and the optimization of phenotyping and refinement of statistical analytic methods. So far, the strongest and widely reproduced GWAS finding for addiction has been the identification of the *CHRNA5-A3-B4* gene cluster on chromosome 15q25, in association with a propensity to NA (Berrettini et al., 2008; Bierut et al., 2007; Thorgeirsson et al., 2008). This was initially discovered (Saccone et al., 2007) and further confirmed (Sherva et al., 2008; Stevens et al., 2008) by candidate gene approaches, and extensive meta-analysis (Liu et al., 2010; Tobacco and Genetics Consortium, 2010). Interestingly, the same hits were identified in GWAS for lung cancer (Amos et al., 2008; Hung et al., 2008). As for other phenotypes, human genetics of NA have mostly been studied in populations of European ancestry. However, it has been more and more acknowledged that the study of samples with diverse geographic ancestries addresses both a scientific and an ethical challenge (Peterson et al., 2019). As a result, the *CHRNA5-A3-B4* gene cluster has been increasingly studied in African and East-Asian populations. Notably, meta-analytical evidence showed that the functional *CHRNA5* rs16969968 is associated with smoking heaviness across European, African and Asian populations (Chen et al., 2012). Other variants affecting *CHRNA5* expression were also associated with heavy smoking in European and Asian, but not African samples.

The *CHRNA5-A3-B4* gene cluster encodes for the α5, α3, and β4 subunits of the nicotinic acetylcholine (ACh) receptors (nAChRs). nAChRs are homo- and hetero-pentameric cationic ligand-gated ion channels composed of α and β subunits that can co-assemble in various combinations of distinct brain distribution and functional properties. There are six α (α2-α7) and three β (β2-β4) subunits expressed in the nervous system of mammals (Changeux, 2010; Picciotto and Kenny, 2013; Taly et al., 2009). α, but not β, subunits have adjacent cysteines, analogous to cysteines 192-193 of the muscle α1 subunit (Sine and Engel, 2006). Rodent studies have established that the α3 subunit mostly combines with β4, and that α3β4* (* stands for containing) nAChRs show a discrete expression profile in the brain, mainly in the medial habenula (mHb) and the interpeduncular nucleus (IPN) (Grady et al., 2009; Salas et al., 2003b), where they play an important role for cholinergic modulation. The α5 subunit is considered as 'accessory', as it does not contribute to ACh binding sites, nor does it form functional nAChRs by itself or in combination with only one other type of subunit, though it significantly influences the receptor's functional properties (Exley et al., 2012; Kuryatov et al., 2008; Ramirez-Latorre et al., 1996; Tapia et al., 2007). The α5 subunit is expressed in

several brain areas important for addiction-like processes, including the ventral tegmental area (VTA), which is crucial for nicotine rewarding properties (Klink et al., 2001), but mostly in the IPN where it can combine with $\alpha4\beta2$* or $\alpha3\beta4$*nAChRs (Grady et al., 2009; Hsu et al., 2013). Alterations in nAChRs containing these subunits may impact the risk for NA by modifying the affinity for nicotine but also by altering the basal activity of the complex brain circuitry involved in addiction-like processes, as suggested by the implication of the *CHRNA5-A3-B4* cluster in the abuse of other substances in addition to nicotine (Besson et al., 2019; Haller et al., 2014; Sherva et al., 2010).

Importantly, the genetic risk linked to this cluster for a complex trait like NA is likely to be mediated by several sets of variant combinations. The present review focuses on how the *CHRNA5-A3-B4* genomic region has been implicated in clinical and preclinical phenotypes related to NA, and aims at examining the limitations of the studies conducted so far. It also discusses future approaches for better understanding and modelling of the interplay of the multiple variants of this cluster in NA-like processes.

2. CHRNA5

2.1. State of the art of the associations identified between *CHRNA5* variants, NA, and brain function in humans

2.1.1. General considerations for human genetic analysis

The sub-phenotypes of tobacco addiction implicate different biological and genetic correlates (Lassi et al., 2016). In the present review, we will focus on associations between NA and genetic variants in the genes coding for the α5 (*CHRNA5*), α3 (*CHRNA3*) and β4 (*CHRNB4*) nAChR subunits, considering all kinds of genetic variants: rare and frequent single nucleotide variants (SNVs), insertions and deletions (InDels) and copy number variations (CNVs). We will report data from both candidate SNV/gene association studies and GWAS. The functional impact of these variants will be discussed based on direct evidence from preclinical models or from *in silico* databases describing the impact of both exonic (synonymous or not) and intronic/intergenic variants at multiple levels, from protein structure changes to close- and long-range promoter interactions (**Table 1**).

Gene variants associated with changes in brain function measured experimentally by neuropsychological testing or brain imaging (functional and structural) and the possible relationship of these changes with NA will also be discussed. Finally, in order to provide a more global and relevant genetic architecture of NA, these results will be discussed in light of multigenic models and evidence, taking into account linkage disequilibrium (LD) and genetic correlation issues.

2.1.2. Genetic associations between CHRNA5 and NA

The *CHRNA5* gene (HGNC: 1959, ENSG00000169684) has a relative low expression in the human brain **(Fig. 1)**. It harbors ~7000 SNVs and Indels and three CNVs (one deletion, one duplication and one inversion, identified by SNV array). The gene has a pLI score of 2.5×10^{-6} (higher scores indicate poorer tolerance to mutations) with 43% haploinsufficiency, indicating an overall high tolerance to mutation. The missense mutation rs16969968 (RefSeqGene NG_023328.1:g.30064G>A), a frequent SNV (called single nucleotide polymorphism (SNP)), changing one amino acid in the α5 protein at position 398, from aspartate to asparagine, has been the focus of extensive clinical and preclinical research due to robust and repeated evidence for association of its risk allele (A in populations with Caucasian ancestry) with NA. rs16969968 has been repeatedly associated with NA, including in a first GWAS in 2008 (Thorgeirsson et al., 2008), an observation further strengthened by meta-analyses (Liu et al., 2010; Marees et al., 2018). The presence of this SNP doubles the risk to develop NA for AA compared to GG genotypes (Saccone et al., 2007). In the latest and largest GWAS to date, NA was not assessed but the proxy “cigarettes per day” (CPD) was used (Erzurumluoglu et al., 2019). It did not evidence association with rs16969968, suggesting this SNP may be rather associated with the complete NA phenotype (measured by the *Fagerström Test for Nicotine Dependence* (FTND), a stringent, yet composite measure of NA; or by DSM criteria) than with the number of CPD. This could be linked to the fact that (i) rs16969968 modulates the aversive properties of nicotine in humans (Jensen et al., 2015) (as also suggested in preclinical studies, see part 2.2.), which is not properly represented by CPD measure, (ii) this measure is highly variable as regards its association with NA, especially since the strength of smoke inhalation has high inter- and intra-individual variability (Scherer and Lee, 2014). Associations between *CHRNA5*, including with the

rs16969968, and NA are further supported by a GWAS finding regarding another important smoking phenotype, exhaled carbon monoxide level, a reliable biomarker of current cigarette exposure (Bloom et al., 2014). Other *CHRNA5* SNPs have been associated with NA in candidate-SNP studies. Intronic SNPs rs17486278 and rs569207 have been associated with NA in smokers who initiated smoking before the age of 16 (along with rs16969968) (Weiss et al., 2008). Overall, in this study, all associations between *CHRNA5-A3-B4* variants and FTND score were significant in early-onset smokers only. This might suggest that more severe smoking phenotypes such as those combining NA and early onset could have a stronger genetic basis than less severe phenotypes, as was previously tested in other psychiatric conditions such as bipolar disorder (Kennedy et al., 2015).

2.1.3. CHRNA5 and human brain function

There have been three functional neuroimaging reports published to date regarding *CHRNA5*-dependent alterations in brain circuitry in humans, all testing rs16969968. First, in a rather large functional magnetic resonance imaging (fMRI) study of 184 subjects (Hong et al., 2010), rs16969968 risk allele was associated with eight neuronal circuits originating from the dorsal anterior cingulate cortex (dACC, which was the seed for the study). This association was independent from other psychiatric diagnoses, age, gender, level of smoking exposure, and two other SNPs that modulate *CHRNA5* expression (see below). The strongest effect was a decreased resting state connectivity in a circuit including the dACC, the ventral striatum and the extended amygdala. This finding can be considered robust given the large study sample, the independence of associations from key possible confounders, and the relevance of the top circuit reported regarding smoking, affective and cognitive phenotypes. In a much smaller study (Janes et al., 2012), rs16969968 AA genotype was associated with increased reactivity of female smokers' hippocampus and dorsal striatum compared to GG genotype. Both samples were matched on age and smoking outcomes. However, another study found that chronic smokers who carried the low-risk genotype had no increase in brain activation when presented with smoking-related *vs.* neutral images during the task (Franklin et al., 2007). This discrepancy might reflect the existence of spurious associations due to the small sample sizes. Finally, the IMAGEN consortium has recently reported that both smoking tobacco and being homozygote for rs16969968 high-risk genotype were associated with a reduction in cortical thickness of the ventromedial prefrontal cortex (PFC), with additive smoking + genotype effects (Chaarani et al., 2019). Two other neuroimaging studies are worth mentioning, although they did not focus on tobacco smokers. First, an association between increased habenular connectivity and rs16969968 has been reported in opiate users (Curtis et al., 2017). Unfortunately, their clinical status was somewhat unclear, likely mixing people with light, mild, and severe opiate use disorders, making it difficult to further interpret this finding. Second, there was a negative correlation between dorsolateral PFC activation and accuracy at the 2-Back working memory test in double heterozygotes for rs16969968 and the rs1076560 from the gene encoding the dopamine (DA) receptor type 2 (*DRD2*) (Giorgio et al., 2014). Although smoking status did not interfere with the main result, the fact that significant differences were observed in risk allele heterozygotes compared to risk allele homozygotes for both SNPs is unexpected (unless one can make specific hypotheses regarding the genetic model of the risk conferred by rs16969968, which would make plausible that the risk would not necessarily increase

with the number of risk alleles). It may indicate either power issues (*i.e.* too few people homozygotes for the risk allele) or unmeasured bias.

In parallel with functional imaging studies, cognitive function in humans has also been studied as a function of the occurrence of *CHRNA5* variants. In an attempt to replicate the GWAS association between schizophrenia and rs16969968 (Schizophrenia Working Group of the Psychiatric Genomics et al., 2014), researchers reported that, in patients with early-onset schizophrenia, the minor allele was associated with poorer performance at all subscales of the Wisconsin Card Sorting Test (WCST), which assesses working memory and executive functions related to the prefrontal lobe. In tobacco smokers without schizophrenia, the effect of five tag *CHRNA5* SNPs was tested (Evans et al., 2014) after nicotine deprivation by quantification of the P300 wave (an event-related brain potential (ERP), considered a biomarker of cognitive control (Polich and Criado, 2006). Minor allele carriers of the non-coding rs588765 and rs17408276 SNPs showed reductions in P300 amplitude, suggesting weaker cognitive control, without significant association with rs16969968. This was in contrast with two previous studies reporting associations between rs16969968 minor allele and poorer cognitive function (Giorgio et al., 2014; Winterer et al., 2010). Moreover, poorer performance at the WCST was also associated with *CHRNA5* rs3841324 (an insertion) and rs615470 (Zhang et al., 2010), specifically regarding pervasive errors. Finally, in female tobacco smokers, *CHRNA5* rs680244 was further associated with reduced accuracy of familiar face recognition (Rigbi et al., 2008).

Several of the *CHRNA5* SNPs associated with altered cognitive/brain function may have functional impacts according to various mechanisms. rs615470 is associated with reduced *CHRNA5* expression in the brain. rs588765 and rs680244 are associated with reduced expression of *CHRNA3* and *CHRNA5*, but also of the *CHRNA5* antisense RNA RP11-650L12.2 in the brain (see example for rs680244 in **Fig.2**). Significantly increased cis-methylation of *CHRNA5* promoter region by these SNPs is also observed, albeit with small to very small effect sizes. Importantly, LD between all those SNPs – rs16969968 excepted – is above 0.8 (and mostly =1), meaning that all those associations indicate the same genetic information (**Fig. 3**).

Overall, there is consistent GWAS evidence supporting some direct effect of the *CHRNA5* non-synonymous SNP rs16969968 in NA. However, the strength of evidence rather comes from candidate gene studies and from the observation of a functional impact of this variant on the receptor function. Associations between other *CHRNA5* SNPs were scarce and limited to candidate SNP studies.

Overall, *CHRNA5* SNPs were rather associated with FTND scores than with the number of CPD. There were repeated associations between SNPs in *CHRNA5* and poorer performance in a range of cognitive tasks, especially those related to cognitive flexibility, with possible modulation of brain function by rs16969968, although to a lesser extent. Importantly enough, evidence from neuroimaging and cognitive studies were consistent toward altered prefrontal function depending on rs16969968 genotype.

Some limitations in the methodology implemented across the various studies that we reviewed must be acknowledged. A relative discrepancy in the outcome of the investigations is observed depending on the variables chosen to measure smoking or NA severity. Such discrepancy likely reflects methodological issues, notably as regards brain function studies which tended to rely on few (if not single) numbers of SNPs, possibly overlooking the functional and/or linkage interplay between many SNPs in the 15q25 region. Furthermore, several of these studies were performed after nicotine deprivation (minutes to weeks), limiting our ability to disentangle between vulnerability issues and the effects of alleviating withdrawal symptoms. Finally, to date, standardized methods that take into account LD to moderate the associations between genotypes and phenotypes require large GWAS datasets to run properly (Kranzler et al., 2019), leaving researchers with only assumptions or empirical/intuition-based methods to account for LD when characterizing the effects of a selected set of genetic variants in the *CHRNA5-A3-B4* cluster.

2.2. Preclinical refinement of the impact of *CHRNA5* in NA-like processes

2.2.1. NA-like phenotypes in preclinical models of CHRNA5 alterations

Most preclinical studies investigating the impact of *CHRNA5* in NA-like processes used knockout (KO) mice for this gene. α5KO mice chronically injected with nicotine exhibit reduced somatic signs of nicotine withdrawal after administration of the nicotinic antagonist mecamylamine (Jackson et al., 2008; Salas et al., 2009). They self-administer more nicotine at high doses that are considered aversive in WT mice (Fowler et al., 2011; Morel et al., 2014). They also present a conditioned place preference (CPP) for high doses of nicotine, which are not rewarding in wild-type (WT) mice, but they are able to discriminate nicotine as well as WTs (Jackson et al., 2010).

Only one polymorphism in the *CHRNA5* gene associated with tobacco dependence in humans has been preclinically studied: the non-synonymous SNP rs16969968 (hereafter called α5SNP in the context of preclinical modelling). First, a recent study took advantage of the facts that cynomolgus monkeys naturally express the α5SNP at a frequency similar to humans and that there is great homology of nAChR protein sequences between human and non-human primates (98%) (Shorey-Kendrick et al., 2015). WT (n=5) and α5SNP cynomolgus monkeys (n=2) had a choice to drink at a bottle containing a nicotine solution with sweetener or at a bottle containing water, and the concentration of nicotine was progressively increased over time (from 25 to 750 µg/ml; 6 days per concentration). Surprisingly, α5SNP monkeys drank less of the nicotine solution than WTs at low doses (100-200 µg/ml) and did not differ from WTs at high doses (400-750 µg/ml), suggesting a decreased sensitivity to nicotine. The authors also observed a difference in the pattern of nicotine consumption between genotypes, with a tendency to consume more nicotine during the nocturnal period for the α5SNP monkeys compared to WTs, and a drinking of nicotine unrelated to food consumption, contrary to WTs (Shorey-Kendrick et al., 2015). However, it is difficult to draw clear conclusions on the impact of the α5SNP on nicotine sensitivity from this study considering the low number of monkeys tested and the fact that the WT group included monkeys that were heterozygotes for the α5SNP.

Another approach is to artificially insert the α5SNP in species that do not naturally express it. It has first been done in a study where pregnant WT and homozygous α5SNP mice were forced to drink a nicotine solution and their offspring were subsequently tested for their nicotine consumption in a two-bottle choice paradigm (O'Neill et al., 2018). Interestingly, the α5SNP offspring consumed more nicotine at the highest concentration (400 µg/ml) than WT offspring, a result consistent with the increased SA of nicotine at high doses observed in α5KO mice (Fowler et al., 2011; Morel et al., 2014). Our laboratory inserted the α5SNP in the genome of the Rat using zinc finger nuclease (ZFN) - directed genome editing and tested them in long-term nicotine SA (Forget et al., 2018). We demonstrated that α5SNP rats acquire nicotine SA at a training dose as well as WT rats but self-administer more nicotine at high doses in both fixed- and progressive-ratio schedules, a result in line with the performance of α5KO mice in nicotine SA (Fowler et al., 2011;

Morel et al., 2014). In addition, while the α5SNP rats did not differ from WT rats on extinction or cue-induced reinstatement of nicotine seeking, they exhibited a strong increase in reinstatement of nicotine seeking induced by a priming dose of nicotine, a model of relapse induced by tobacco re-exposure after abstinence in humans. This last result is particularly important considering the high relapse rate in NA in humans. Taken together, these preclinical results fit well with the human situation where the α5SNP is associated with an increased risk for NA, lower ratings of aversive effect, and delayed smoking cessation (Bierut et al., 2008; Chen et al., 2015; Jensen et al., 2015; Saccone et al., 2007).

2.2.2 Brain circuits involved

The α5 nAChR subunit is expressed in discrete regions of the rodent central nervous system (CNS), including the striatum where it regulates DA transmission (Exley et al., 2012), the cerebral cortex, cerebellum, thalamus, hippocampus, substantia nigra (SN), VTA and mHb, with the highest concentration found in the IPN (Forget et al., 2018; Hsu et al., 2013;

Some studies contributed to a better understanding of the neuronal impact of the lack of α5 or the presence of the α5SNP. In particular, the re-expression of the α5 subunit in the mHb of α5KO mice restored a control-like nicotine SA behavior while a down regulation in the same structure in rats induced an increase in nicotine SA at high doses (Fowler et al., 2011), suggesting an important contribution of the α5 subunit in the mHb in nicotine consumption. However, other structures are important in the effect of the α5 subunit on behaviors related to NA. Thus, re-expression of the α5 subunit into DAergic neurons of the VTA of α5KO mice also restored nicotine SA behavior, while the expression of the α5SNP in the same VTA neurons only partially restored SA behavior, leading to the consumption of more nicotine than WT mice (Morel et al., 2014). The IPN has recently attracted a lot of attention in studies on nicotine effects due to its high concentration in α5*nAChRs. In particular, stimulation of α5 containing neurons in the IPN induced aversion after nicotine exposure (Morton et al., 2018), and the lack of α5 subunit induced a significant decrease in nicotine-induced currents in the IPN (Forget et al., 2018; Morton et al., 2018) but not in the mHb (Morton et al., 2018). Furthermore, inhibition of GABAergic neurons of the IPN projecting to the laterodorsal tegmentum (LDTg) blocked nicotine conditioned place aversion, suggesting that this pathway is a critical part of the circuitry that mediates the aversive effects of nicotine. In addition, a recent study identified a cell population containing the α5 subunit in the IPN (α5-Amigo1) that appeared to be essential for nicotine-induced CPP (Ables et al., 2017).

Finally, the α5SNP induced a reduction in nicotine-induced current in the IPN of rats and the neuronal activity in the IPN was negatively correlated with nicotine-induced reinstatement of nicotine seeking, pointing to a potentially important role of the α5 subunit of nAChRs in the IPN in this behavior (Forget et al., 2018).

In conclusion, the α5 subunit in the IPN appears essential for nicotine aversion, withdrawal and relapse while the α5 subunit in the VTA and the mHb seems important to limit nicotine SA at high doses.

2.3. Recommendation for future research

The preclinical studies described above helped to understand more specifically which behaviors related to NA are dependent on the α5 subunit of nAChRs and altered by the presence of the α5SNP rs16969968, and the neuronal pathways potentially involved. These results can thus orientate future clinical studies to specific directions, such as investigating the impact of the rs16969968 on relapse to tobacco smoking and the brain structures to image. Considering the deleterious effects of the lack of α5 subunit or the presence of the α5SNP in preclinical models related to NA, together with the restricted expression of this subunit in the brain, it would be of great interest to develop a class of therapeutic compounds designed to specifically enhance the activity of α5*nAChRs. For instance, positive α5*nAChR allosteric modulators may have clinical potential to prevent excessive tobacco smoking and relapse. However, there is more preclinical work to do to unravel the circuits underlying the role of the α5 subunit and the impact of the α5SNP on NA-related processes. For example, it would be interesting to investigate if the α5-containing neurons in the IPN projecting to the LDTg, which are key actors of nicotine aversion (Morton et al., 2018), are also involved in behaviors more directly related to NA such as nicotine-SA and relapse to nicotine seeking. More generally, the use of neuron- and pathway-specific opto- or chemo-genetic techniques would be of great help to decipher the specific neurons implicated in the phenotypes previously described. It would also be important to clarify the involvement of the mHb neurons in these phenotypes. Indeed, the fact that the lack of the α5 subunit does not modify nicotine-induced current in the ,mHb (Morton et al., 2018). Fowler et al. used a viral re-expression of the α5 subunit in the mHb that also induced α5 subunit expression in neuron terminals in the IPN via transport trough the fasciculus retroflexus (Fowler et al., 2011). Thus, the effect observed on nicotine intake might be due to the expression of α5*nAChRs on mHb axon terminals in the IPN that affect glutamatergic transmission. However, recent studies indicate that the α5 subunit within the mHb-IPN pathway are rather expressed postsynaptically in GABAergic neurons of the IPN (Hsu et al., 2013; Morton et al., 2018). Such apparent discrepancies might be reconciled if one assumes that these two circuits mediate distinct behavioral components, such as nicotine satiety for the glutamatergic neurons of the mHb, as suggested recently (Tuesta et al., 2017) and nicotine aversion for the GABAergic neurons of the IPN projecting to the LDTg (Morton et al., 2018).

The α5SNP is the only polymorphism in the *CHRNA5* gene associated with tobacco dependence in humans that was preclinically studied in details, generating important novel insights. Yet, as indicated above (see part 2.1), other *CHRNA5* SNPs have been associated with tobacco dependence in humans, such as the intronic rs17486278 and rs569207. It would thus be interesting to further study

those SNPs in preclinical models of NA to understand their mechanisms of action. As a first approach, non-human primates represent a good model organism for such investigations considering the high conservation and homology of nAChRs between humans and non-human primates and the natural presence of some SNPs homologous to humans ones (Shorey-Kendrick et al., 2015). In particular, there are ten conserved SNPs in the *CHRNA5* gene between Rhesus macaques and humans and 6 conserved SNPs in *CHRNA5* exons between cynomolgus macaques and humans, including the rs16969968 (α5SNP). However, the low number of monkeys available for such studies limits their power. It would thus be important to insert SNPs of interest into the genome of rodents as a second approach to study their impact in more details. For example, intronic *CHRNA5* SNPs have been associated with NA, alterations in the expression of several nicotinic subunits and alterations in cognitive function (see part 2.1), but the mechanisms mediating these alterations are unknown. In particular it is difficult to determine whether they have a direct functional impact or act through LD with other(s) functional SNP(s) in the cluster (McCauley et al., 2007). Recent genome editing tools, such as ZFNs and CRISPR/Cas9 techniques allow the insertion of SNPs in non-coding region, thus representing a great opportunity to study model organisms in detail without the potential influence of other SNPs in LD, as it was done recently in rats to study the α5SNP (Forget et al., 2018). However, the degree of conservation of intron sequences, structure and length between humans and rodents varies greatly among genes with an overall lower degree of homology than exonic sequences (Batzoglou et al., 2000; Roy et al., 2003). It would thus be necessary to verify the similarity of *CHRNA5* intronic sequences between humans and the organism model of choice before performing such investigations. If the degree of homology is not sufficient, an interesting alternative would be to perform the same gene editing in human to investigate the functional impact of intronic SNPs, directly in the cells or in more complex environments such as organoid culture models or cell grafts in the rodent brain for *in vivo* investigations.

3. CHRNA3

3.1. Current state of the art of the associations identified between *CHRNA3* variants, NA and brain function in humans

The *CHRNA5* has been recognized as an important candidate gene for NA as initially reported (Saccone et al., 2007), this nicotinic subunit may represent one of the best validated targets for the development of drugs which can regulate nicotine uptake (Lassi et al., 2016). Furthermore, a genomic region tagged by *CHRNA3* synonymous exonic variant, rs1051730, has consistently shown the strongest association to CPD within all polymorphisms in the cluster (Tobacco and Genetics Consortium, 2010), although this may be due to multiple haplotypic effects rather than to functional impact of this variant has also been linked to sensorimotor gating in a clinical study of schizophrenics (Petrovsky et al., 2010). Several genetic studies have then been conducted more specifically on the *CHRNA3* gene (ENSG00000080644, HGNC 1957), which has a low to moderate expression in the human brain (**Fig. 1**) *CHRNA3* harbors ~7700 SNVs and InDels and three CNVs (one deletion, one duplication and one inversion, identified by SNV array). The gene has a pLI score of 0.01 (higher scores indicate poorer tolerance to mutations) with 33% haploinsufficiency, indicating an overall high tolerance to mutation. In one recent study, the authors have evaluated the association between a *CHRNA3* (rs578776-NG_016143.1) polymorphism with head and neck cancer (HCN) development and the intensity of cigarette smoking by using a TaqMan genotyping method by real-time PCR, in a cohort of 1,067 patients from Sao Paulo Hospital (Silva et al., 2019). Results from this study did not reveal any statistically significant association between the identified SNP with both HCN and smoking risk as compared to control groups (Silva et al., 2019). The variant of *CHRNA3* (C>T) is found in the 3' untranslated region (3'-UTR) of the gene and contains regulatory sequences (Bierut et al., 2008; Conlon and Bewick, 2011). The 3'-UTR sequence is located immediately after the translation termination codon and plays an important role in gene expression through various specific sequences including microRNA elements, AU-rich elements and the poly A tail. As a consequence, one cannot exclude a role for this polymorphism in some yet undefined

functional effects (Conlon and Bewick, 2011). A previous study had suggested that the C allele of *CHRNA3* (rs 578776) may be considered as a risk allele for NA (Robinson et al., 2013). These authors used ERPs methods to evaluate reward sensitivity in smokers. Their results support the hypothesis that the T allele may have a protective effect. Importantly, they are in accordance with results from several other studies which show the association of *CHRNA3* rs578776 with reduced risk for NA (Bierut et al., 2008) and a protective effect of this SNP against the risk of heavy smoking (Conlon and Bewick, 2011; Pérez-Morales et al., 2018). Another study of Hong and collaborators identified two *CHRNA3* variants, namely the rs578776 and rs1051730 (Hong et al., 2010). The former variant corresponds to the one described above and the latter represents a synonymous variant previously associated strongly with smoking and lung cancer (Bierut et al., 2008; Saccone et al., 2007; Thorgeirsson et al., 2008). Zhang and collaborators have recently reported the existence of 69 single variants (SNP) for *CHRNA3* associated to NA (Zhang et al., 2019). A *CHRNA3*-rs2869546-T-allele was found associated to higher perseverative responses in cognitive flexibility tasks performed in two cohorts of European and Africans individuals, recruited for genetic association studies of drug or alcohol dependence (Zhang H et al, 2010). However, as discussed by the authors, since no data on nicotine exposure on the day of cognitive evaluation is available (Zhang et al., 2010).

So far, the mechanisms by which *CHRNA3* variants mediate their effects on NA within brain regions have not been fully evaluated. By using fMRI studies, Hong and *collaborators* demonstrated in their initial studies that the G risk allele of *CHRNA3* 3′UTR (rs578776) was selectively associated with an increase in the connectivity between the dorsal anterior cingulate-ventral striatal circuit and the left thalamus in humans, but did not correlate with NA severity (Hong et al., 2010). Interestingly, *CHRNA3* is particularly expressed in the thalamus in humans (https://www.proteinatlas.org/). Further studies are needed to fully understand the precise role of the different *CHRNA3* alleles.

3.2. Lack of preclinical studies for the impact of *CHRNA3* in NA-like processes

In the nervous system, the α3 subunit is highly expressed in the periphery with a more restricted expression profile in the CNS (Gotti and Clementi, 2004; Greenbaum and Lerer, 2009; Zoli et al., 2015). In the rodent brain, the α3 subunit is predominantly expressed in the IPN (Grady et al., 2009; Perry et al., 2002; Winzer-Serhan and Leslie, 1997), mHb (Grady et al., 2009; Quick et al., 1999; Zoli et al., 1995), pineal gland (Zoli et al., 1995) and olfactory bulb (Dineley-Miller and Patrick, 1992). It is also present in the SN (Azam et al., 2002) and the VTA (Greenbaum and Lerer, 2009; Perry et al., 2002). Finally, a weak expression of α3 is found in the brainstem (Morley, 1997), cerebellum (Turner and Kellar, 2005), hippocampus (Gahring et al., 2004; Winzer-Serhan and Leslie, 1997), and thalamus (Perry et al., 2002)

The *Chrna3* gene was one of the first neuronal nAChR subunits to be cloned (Boulter et al., 1986), yet the contribution of the α3 subunit in cognitive functions, in particular in processes related to NA, remains poorly understood. The role of most nicotinic subunits in NA has been studied by using various genetic tools to modify or regulate the expression of the subunit gene in animal

models. In the case of the α3 subunit, this approach has proven to be more challenging so far. Indeed, a mouse line carrying a null mutation in the α3 gene (Xu et al., 1999a) has been generated, but the homozygous KO animals are affected by multi-organ dysfunction and die before adulthood.

Salas and collaborators, using heterozygous α3 WT/KO mice, were able to show that these animals are resistant to nicotine-induced seizures, but do not show any alterations in nicotine-induced hypolocomotion, compared to their WT littermates (Salas et al., 2004a). Yet, this model did not allow further significant investigation on the role of this subunit. Interestingly, the authors demonstrated, by in situ hybridization, that the lack of β4 subunit decreases the level of expression of α3 mRNA in some specific regions of the brain like the olfactory bulb or colliculi but not in structures implied in NA like the mHb, the SN and the VTA.

No other α3 mRNA expression modification was observed in the β4 or α5 KO lines even though the *Chrna3*, *Chrnb4* and *Chrna5* genes belong to same genetic locus and share some common regulatory elements (Improgo et al., 2010).

KO animal models for *Chrna3*, the majority of studies interested in the role of this subunit in NA-related processes mostly focused on the role of α3β4*containing nAChR receptors using pharmacological approaches. As describe above, in the CNS, the mesolimbic DA pathway contains some α3β4*nAChRs but these receptors are mostly found in the mHb and IPN.

Interestingly, α3β4*nAChRs have been reported to control ACh release in the fasciculus retroflexus, the tract of cholinergic fibers projecting from the mHb to the IPN (Grady et al., 2009). In these brain regions, an important role for the α5 subunit, which can co-assemble with α3 and β4 to form functional nAChRs, has been highlighted in NA-like processes. However, in rodents, it has been shown that only a part of α3β4*nAChRs expressed in these areas include an α5 subunit in their composition (15 and 35% in the mHb and IPN of mice, respectively, and only 10% and 15% in rats). Using intracerebroventricular injections of the α3β4*nAChR selective antagonist conotoxin AuIB combined to behavioral studies on mouse models, Jackson et al (2013) showed that these receptors contribute to nicotine reward and somatic nicotine withdrawal, but do not seem to mediate the acute nicotine-induced antinociception, decrease in body temperature and hypolocomotion, nor the affective manifestation of nicotine withdrawal in mice (Jackson et al., 2013b). Toll et al (2012), using a high affinity and more selective antagonist for α3β4*nAChRs, the AT-1001 (N-(2-bromophenyl)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine), were able to demonstrate an implication of these receptors in nicotine SA in rats, a dose-dependent and reversible effect (Toll et al., 2012). This effect seems specific to nicotine since AT-1001 injections did not affect food SA in the same study. These pharmacological studies support a significant role for α3β4*nAChRs in NA but did not allow to understand the specific role of *CHRNA3* and its polymorphisms in the mechanisms of addiction. For this, novel approaches are now needed as discussed below.

3.3. Key possible approaches to model *CHRNA3* alterations in future studies

A comprehensive analysis of the role of each nAChR subtype in addictive behaviors has been initiated in animal models. As stated above, transgenic rodent models for several nAChR subunits have been developed. Such models mostly reside in the deletion of selective nAChR genes but also on targeted

knock-in (KI) gene mutations. Further experiments were carried out either by re-expressing a deleted gene into the mouse brain using stereotaxic injection of a lentiviral vector which carries the missing gene, or by injecting a short hairpin RNA (shRNA) to repress a selective gene expression (Avale et al., 2008; Changeux, 2010; Fowler et al., 2011; Picciotto and Kenny, 2013). In this context, several approaches to better understand the role of the specific role of the α3 subunit in NA-like processes could now be implemented. One possible approach would be to use the Gensat's transgenic mice Tg(Chrna3-cre)NO196Gsat, generated with the BAC clone RP23-336O6, where the expression of the Cre-recombinase is driven by the regulatory sequences of the α3 promoter, to over-express this subunit in brain regions where it is constitutively present by injecting viral vectors expressing a floxed copy of *Chrna3* and evaluate the consequences of such over-expression on NA phenotypes and mechanisms. Another possibility would be to combine the injections of viral vectors (lentiviruses or AAVs) expressing the Cre-recombinase in target brain areas of conditional mice developed by the European Conditional Mouse Mutagenesis (EUCOMM) program, wherein the *Chrna3* sequence is flanked by lox sites (Smedley et al., 2011). This would result in the local inactivation of α3 expression in specific brain areas or pathways of interest, such as the Hb-IPN system. Then, the expression of specific *CHRNA3* variant(s), identified in the human genetic studies, could be locally targeted through lentiviral gene delivery within these pathways on top on the inactivation of native α3 subunit expression, as it has been done for the α5SNP rs16969968 in α5KO mice (Morel et al., 2014). Another way to achieve a comprehensive study of the role of the α3 subunit could be to generate local silencing of *CHRNA3*, using lentival vectors expressing either shRNA or, as previously shown (Koukouli et al., 2017), specific CRISPR against *CHRNA3* (Peng et al., 2019).

However, as the *CHRNA3* SNP most robustly associated to CPD is a synonymous exonic variant (Tobacco and Genetics Consortium, 2010), a KI mouse or rat model could potentially provide important clues.

To our knowledge, studies with human cells have been so far exclusively focused on the *CHRNA5* polymorphism. Modeling *CHRNA3* alterations would be possible by using the same experimental approaches. Some authors have even been able to develop a kind of transchromosomic mice expressing selective gene clusters (Abe et al., 2017). Such humanized models have been obtained by performing SNP modification using genome editing technology in mouse ES cells and fertilized eggs (Abe et al., 2017). A similar approach could be applied to model the *CHRNA5-A3-B4* gene cluster. Gene editing in iPSC-derived cells enhances their potential both for basic research and render in vivo studies possible. The

CRISPR/Cas9 genome engineering has been recently demonstrated in vivo through the use of engraftable human brain-derived neural stem cells (Dever et al., 2019). The CRISPR/Cas9-genome engineering has also been applied to three-dimensional organoid culture models (Bian et al., 2018). This approach offers new technical options to model *CHRNA3* polymorphism in human neurons in near future. Furthermore, it could provide a unique opportunity to investigate the possibilities of cross-regulation between *CHRNA3* and *CHRNA5*, which have been shown to interact at the mRNA level through the formation of RNA-RNA duplexes in humans but not in rodents (Solda et al., 2005).

4. CHRNB4

4.1. State of the art of the associations identified between *CHRNB4* variants, NA and brain function in humans

Unlike the α5 subunit, the nAChR β4 subunit participates in forming ACh binding sites in nAChR pentamers (Changeux, 2010). *CHRNB4*, the gene encoding the β4 subunit (HGNC 1964, ENSG00000117971), is also located at 15q25. It harbors ~23400 SNVs, including ~2600 InDels, and is encompassed by 54 CNVs ranging from short tandem repeats to large mosaic rearrangements (see **Table 1** for database references). Those larger CNVs have been associated with severe neurodevelopmental delay, although the *CHRNB4* gene itself is tolerant to mutations (pLI =0.019). Several *CHRNB4* SNPs have been associated with NA. Recently, it was made clear that some of these variants act as enhancers toward other genes, notably through increased methylation of *cis* CpG (cytosine nucleotide followed by a guanine nucleotide) sites, rather than directly affecting β4*nAChR function. In particular, the *CHRNB4* variant rs1948, which was associated with NA, showed one of the largest effect on *CHRNA5* expression, and the effect of *CHRNA5* rs16969968 was found strongly dependent on rs1948. In that study, the *CHRNB4* SNP rs7178270 exhibited similar associations and properties as rs1948. Strikingly, another *CHRNB4* variant, rs11636753, was the most significantly associated SNP with NA across five independent cohorts (Hancock et al., 2015). Haplotype analyses showed that the simultaneous presence of rs11636753-G and rs16969968-A alleles together increased the risk for NA more than each variant alone [$p = 3.1 \times 10^{-12}$, OR (95% CI) = 1.32 (1.22–1.43)]. rs11636753, rs7178270 and rs1948 are in moderate LD (r^2 =0.56), suggesting that they are inherited quite independently. rs4887074, which is associated with reduced *CHRNB4* expression, has been associated with a reduced NA risk, both alone and in the context of diplotype/haplotype analysis of the cluster (Lee et al., 2018). Using the TAD browser ((http://dna.cs.miami.edu/TADKB/) applied to the 15q25 region, we evidenced a close-range loop within *CHRNB4*. Interestingly, this loop spatially bonds positions 78920000-78930000, which harbor two SNPs associated with NA, and 79000000-79010000, which contain three transcription factor binding sites, one open chromatin region, and one enhancer. These are very likely to further regulate *CHRNB4* expression, although the precise effects of these elements on β4*nAChRs remain unclear.

As regards brain function, *CHRNB4* rs11637890 has been associated with perseverative responses in general, and errors in particular, in the WCST (Zhang et al., 2010). Unexpectedly, the major allele was responsible for this association. Elements regulating gene expression in the cluster are also found in intergenic regions. In 403 patients with ADHD, vocabulary and planning abilities were tested using the relevant subscales from the Wechsler Adult Intelligence Scale–Revised (WAIS-R) (Schuch et al., 2016). Among eight SNPs from the *CHRNA5-A3-B4* cluster analyzed individually and as possible haplotypes, stratified by current smoking status, only rs8023462 (an intergenic SNP between *CHRNA3* and *CHRNB4*) was associated with poorer vocabulary abilities, solely in current tobacco smokers. In line with this finding, *CHRNB4* rs12914008 was associated with intra-individual variability in attention (*i.e.* "unstable attention during the test") in a GWAS of 479 adults suffering from ADHD (Alemany et al., 2015). We could not find any neuroimaging study in humans involving *CHRNB4* SNPs. *CHRNB4* variants that were associated with either NA or cognitive function were all intron variants, which elicit major changes in gene expression in the 15q25 region. The minor allele of rs11637890 and rs7178270 are both associated with increased *CHRNA5* and *CHRNA3* expression in the brain. These SNPs have been further associated with methylation changes at trans CpG sites between positions 78856949 and 79053858, thus encompassing the entire *CHRNA5-A3-B4* cluster. Methylation was decreased for both *CHRNA5* and *CHRNA3* CpG sites, in line with the observation of enhanced gene expression, and increased for intergenic CpG sites located downwards the cluster (reverse strand), the impact of which remaining to be elucidated. Conversely, rs1948 and rs8023462 were associated with reduced brain expression of *CHRNA3* and *CHRNA5*, which is consistent with their association to a mostly increased methylation of their CpG sites. rs12914008 is also associated with increased methylation of a *CHRNA3* CpG site.

Taken altogether, association studies and GWAS evidence followed by assessment of gene expression and methylation uncovered a key functional role of several *CHRNB4* SNPs in the relationship between the *CHRNA5-A3-B4* cluster and NA. Along with *CHRNA3* (see above) and intergenic variants, the cluster has been legitimately described as a regulome containing multigenic haplotypes, including rs16969968 or not, showing complex associations with NA (Barrie et al., 2017). Two sets of *CHRNB4* SNPs showing opposite regulatory features can be identified from gene expression (**Fig. 2** for rs8023462) and methylation databases, some data being redundant due to high LD (**Fig. 3**). All *CHRNB4* SNPs reviewed here form a haplotype block (**Fig.3**, bottom-right) and, thus, carry the same statistical information although they show distinct regulatory characteristics. This recent line of evidence should be more taken into account in future clinical and preclinical studies, in order to better characterize the pathophysiology of NA and the neurobiology of the nicotinic system.

4.2. Preclinical studies of the role of *CHRNB4* in NA-like processes

The β4 nAChR subunit can combine with several other subunits to form functional nAChRs but mostly assembles with the α3 subunit. Co-expression of β4 and α3 has been observed for many brain areas where β4 is strongly expressed, notably the mHb and the IPN (Gotti et al., 2009; Grady et al., 2009). β4*nAChRs display specific properties such as slow-rate desensitization and low affinity for nicotine (Cachelin and Jaggi, 1991; Parker et al., 1998). Several studies have attempted to study the role of α3β4*nAChRs in NA-like behaviors through pharmacological agonism or antagonism of these

receptors (as discussed above, see part 3.2). Notably, the 18- Methoxycoronaridine (18-MC), a potent antagonist which is relatively selective of α3β4*nAChRs, has been proposed as a promising anti-addictive compound as it was shown to decrease SA of several drugs including nicotine in rodents (Maisonneuve and Glick, 2003).

These studies do not bring insights into the discrete role of the β4 subunit in the phenotypes observed. In this way, the use of mice KO for the β4 subunit gene has provided a good opportunity to better characterize the role of this subunit in nicotine properties.

Amongst several processes related to NA, rodent nicotine withdrawal is associated with the occurrence of somatic signs and hyperalgesia, together with affective manifestations such as elevations in brain reward thresholds measured through intracranial self-stimulation, considered as a form of anhedonia. Interestingly, somatic symptoms, hyperalgesia and reward threshold elevation were found decreased in β4KO mice during spontaneous or mecamylamine-induced nicotine withdrawal (Salas et al., 2004b; Stoker et al., 2012), indicating an important role of β4*nAChRs in the several aspects of nicotine withdrawal.

The fact that similar phenotypes were observed in α5KO mice (see part 2.2) suggests that nAChRs containing both α5 and β4 subunits in the mHb-IPN axis are key modulators of nicotine aversion signalling. Yet, a subtler role of these receptors in the balance between rewarding versus aversive nicotine signalling is supported by studies that examined nicotine rewarding effects in several models of transgenic mice for the β4 subunit. First, increased nicotine- evoked mHb currents, stronger aversive responses to nicotine and reduced nicotine intake were observed in transgenic mice overexpressing the β4 subunit (‘Tabac’ mice) (Frahm et al., 2011). Interestingly, the latter phenotype was reversed upon viral-mediated expression, in the mHb, of the α5SNP previously associated with heavy smoking in humans, further supporting the interplay of α5 and β4 subunits in mediating nicotine signalling and intake. Moreover, lentiviral-mediated overexpression of β4 or expression of the β4 T374I gain of function variant (associated with decreased risk for smoking (Haller et al., 2012)), in the mHb, was found sufficient to produce nicotine aversion (Slimak et al., 2014). Alterations in the balance between the rewarding and the aversive effects of nicotine were also observed in another model of transgenic mice overexpressing the human *CHRNA5/A3/B4* cluster with increased functional α3β4*nAChRs (Gallego et al., 2012). These mice displayed an increased sensitivity to the pharmacological effects of nicotine including higher nicotine-

induced hypolocomotion, seizures and mHb activation, but reduced nicotine-induced activation of VTA DA neurons, together with increased nicotine intravenous SA under a progressive ratio schedule. Thereafter, the role of β4*nAChRs in nicotine signalling and intake was further elucidated in β4KO mice (Harrington et al., 2016). In these mice, nicotine intravenous SA was found decreased at low and high doses, and DA neurons of the mesolimbic system showed enhanced sensitivity to nicotine while NAcb basal extracellular levels of DA were increased. Lentivirus-mediated re-expression of β4*nAChRs in the IPN, but not in the mHb, rescued nicotine SA and VTA DA neuron sensitivity to nicotine. These observations suggest a role for IPN β4*nAChRs in regulating the response to nicotine of the reward system despite a lack of direct efferent connection of the IPN with the VTA (Antolin-Fontes et al., 2015). They further indicate a distinct contribution of mHb and IPN β4*nAChRs to nicotine signalling. Such a dichotomy between mHb and IPN β4*nAChRs had previously been suggested in rats, with the observation of decreased and increased nicotine intravenous SA following the local blockade of *α*3β4*nAChRs by the antagonist 18-MC in the mHb and the IPN, respectively (Glick et al., 2011). Recently, β4KO mice were found to show decreased and increased intra-VTA nicotine SA at low and high doses, respectively, in a paradigm particularly suitable for the assessment of both reinforcing and aversive of nicotine. By contrast, Tabac mice displayed increased aversion for high doses of nicotine in the same paradigm. These data further support an important role for β4*nAChRs in the modulation of nicotine sensitivity, and confirm that the strength of nicotine aversive effects varies with β4*nAChR availability. The same study also reports that lentivirus-mediated re-expression of β4*nAChRs in the VTA of β4KO mice enhanced intra- VTA nicotine SA at all doses, while β4*nAChR re-expression in either the MH or the IPN limited intra- VTA nicotine SA at high doses.

Taken together with the data obtained from studies examining the effects of systemic nicotine (Frahm et al., 2011; Glick et al., 2011; Harrington et al., 2016; Slimak et al., 2014), these recent observations indicate that MHb and IPN β4*nAChRs play distinct roles in controlling nicotine intake when directly mediating nicotine signalling, but drive a similar control of the VTA response to nicotine when mediating endogenous ACh signalling. Such a comprehensive and subtle role of β4*nAChRs in both ACh and nicotine signalling may explain the complexity of the preclinical information gathered so far about the involvement of these receptors in NA-like behaviors, and may also substantially contribute to the strong clinical implication of the *CHRNA5-A3-B4* gene cluster in tobacco smoking.

4.3. Recommendation for future research

As described above, preclinical data strongly suggest that the β4 nAChR subunit plays a central role in the nicotine reward/aversion balance, which likely represents a key element for better understanding

inter-individual variability in the risk to develop NA (De Biasi and Dani, 2011). Surprisingly, there is only a very limited number of studies specifically investigating the role of this subunit in NA-related processes, despite the fact that β4 KO mice have been available for many years (Xu et al., 1999b). The novel insights obtained from recent preclinical work highlight the importance of further deciphering the role of the β4 nAChR subunit in the circuits directly and indirectly connecting the VTA, mHb and IPN. It will be also important to further investigate the role of β4 in the mediation of nicotine action on the brain beyond its rewarding and aversive properties. For this, behavioral models capturing features of NA with stronger face and construct validities should be implemented. A crucial role of the α5 subunit has notably been established in the mediation of nicotine seeking relapse after abstinence in transgenic rats (Forget et al., 2018). It would be of interest to generate a line of rats in order to assess how the β4 nAChR subunit may also significantly influence different steps implicated in long-term nicotine SA. In particular, β4 may impact the levels of perseverative nicotine seeking during extinction since human genetic studies have linked *CHRNB4* to perseverative behavior (Zhang et al., 2010).

Additional studies at the cellular level of investigation will also be helpful to better characterize the interplay between the β4 subunit and both the α3 and the α5 subunits in modulating the functional properties of nAChR, especially in the different cell populations of the IPN, where α5 is mostly expressed and where α3 is the main partner of β4 (Grady et al., 2009). This could be achieved by the combination of several technologies, including synaptosome analysis, single cell RT PCR, slice patch clamp and in vivo electrophysiological recording, both in drug naïve conditions, i.e. in response to endogenous cholinergic stimulation, and in response to nicotine.

To our knowledge, there is no preclinical model attempting to model *CHRNB4* genetic variation so far. Considering that all the *CHRNB4* variants which have been associated with NA in human studies concern non-exonic regions, it seems complicated to propose straightforward animal models to further investigate their impact on NA. The use of viral vectors for targeted expression of mutated nAChR subunit gene, in specific brain areas of KO mice, has proven to be very efficient to unravel key mechanisms of action of the α5SNP rs16969968 on NA-like behaviors (Besson et al., 2016; Frahm et al., 2011; Morel et al., 2014). However, implementing a similar approach may be compromised in the case of non-exonic SNPs which would require to encompass the gene in its entirety. Other methodologies are conceivable, like the use of efficient genome editing tools in rodents, including in rats. Yet, several limitations, such as a possible lack of similarity in the *CHRNA5, CHRNA3*, and *CHRNB4* gene cross-regulation mechanisms between humans and rodents, will first need to be assessed and overcome whenever it will be feasible, as discussed above for the modelling of *CHRNA5* intronic variants (see part 2.3).

5. General discussion and conclusion

5.1. General considerations

We are providing here a comprehensive summary of the human genetic and preclinical findings linking polymorphisms in a cluster on chromosome 15q to NA. The genetic architecture of this cluster, encompassing multiple SNPs in the *CHRNA5, CHRNA3* and *CHRNB4* genes, is complex, and includes substantial LD. This leads to the presence of haplotypes in many human populations. Recent advances in human genomics indicate that the *CHRNA5-A3-B4* cluster lies in a particularly rich regulatory region. Most SNPs associated with NA are synonymous and/or non-exonic variants. The role of the coding polymorphism rs16969968 has received a lot of attention, and is reviewed (Maskos, 2020) . The magnitude of association between this SNP and NA has been shown to depend strongly on other variants reviewed here, in line with the fact that SNPs showing significant eQTLs in the brain mostly modulate *CHRNA5* expression, whichever their harboring gene is. Thus, in the cluster, sources of variability include haplotypes, enhancers/repressors of gene expression acting across genes, and changes in chromatin states (which can either facilitate or limit gene transcription). Using the TAD browser (http://dna.cs.miami.edu/TADKB/) applied to the 15q25 region (based on very recent evidence about the 3D organizational structure of DNA, at the kilobasepair level (Liu et al., 2019)), we observed that the *CHRNA5, CHRNA3 and CHRNB4* are clustered in a region accounting for 25 possible variations of chromatin states and expressing over 50 long non-coding RNAs, which are important regulators of gene expression. There was no evidence of long-range DNA interactions in this region, but a close-range loop within *CHRNB4*, this gene appearing to fulfil the strongest regulatory role in the cluster according to the information available so far. Interestingly enough, SNPs that alter *CHRNA5* expression also commonly alter that of the mRNA antisense gene *RP11-650L12.2* (which represses *CHRNA5* expression), in the same direction, evoking a negative feedback loop. Repeated associations between rs16969968 and NA may imply several parameters of the NA phenotype, which is often measured by the FTND. The FTND notably measures the “delay between wake-up and first cigarettes”, a proxy for withdrawal avoidance, and CPD, along with other variables reflecting other aspects of NA including psychosocial ones. Such repeated associations between rs16969968 and NA could also reflect alterations in the aversive effects of nicotine (Jensen et al., 2015), which may impair the ability to self-regulate/titrate nicotine intake (Macqueen et al., 2014).

Overall, the wide range of genetic associations between the *CHRNA5-B4-A3* cluster and NA suggests that different SNPs and SNP combinations are associated with different components of the NA syndrome. Component of the different stages of the NA cycle is only partially overlapping (Sharp and Chen, 2019). A similar complexity has been observed in the preclinical literature, where rs16969968 has been associated with various functional consequences at the molecular and behavioral levels. The current review focused on frequent genetic polymorphisms. However, rare variants (i.e. with minor allele frequencies <5%), such as the missense mutations *CHRNB4* T375I and T91I (Haller et al., 2012) and *CHRNA3* RH7 (Clark et al., 2016), have been associated with reduced NA risk. Since part of these associations were found at the gene set level, meaning that these variants were involved in statistically significant association in combination with other variants in the same gene, and since they are rare, they likely mediate only a low fraction of NA variability in human populations, even though they may leverage significant functional consequences (see part 4.2).

Refinement and broadening of the neuropsychological phenotyping implemented to assess NA is now needed to better characterize the impact of *CHRNA5, CHRNA3 and CHRNB4* polymorphisms on constructs even more relevant to addiction (Yucel et al., 2019), especially since the most robust genetic associations with NA only explain ~1% variance of the phenotype – leaving much NA heritability missing (Sharp and Chen, 2019). Along with the use of polygenic risk scoring (Belsky et al., 2013) and data mining (Barrie et al., 2017; Civelek and Lusis, 2014), future human genetic studies should notably feed on preclinical insights and, for instance, assess the associations between the cluster and relapse-related parameters more carefully. In addition, various functions strongly linked to the development of addiction should deserve more attention, including reward-processing, stress sensitivity, self- and cognitive-control.

There is also a strong need to develop novel preclinical models. First, the cluster should be considered as a whole to evaluate its impact with greater face validity, finally taking into consideration the cross-regulation between the three nicotinic genes. For this, a combination of several approaches can be envisaged, as discussed throughout the review, including the use of iPSC. Such novel approaches should also be considered to finally provide a model for *CHRNA3* alterations, which has been crucially lacking so far. Finally, as for clinical studies, future animal investigations should consider implementing behavioral models with stronger translational value for NA, as it has started to be developed in a few studies which have made efforts to assess behaviors reflecting a loss of control over nicotine intake (Caille et al., 2012; Clemens et al., 2014; Cohen et al., 2012; Gilpin et al., 2014).

5.2. Gender background considerations

Gender effects have consistently been evidenced regarding NA, with men exhibiting more persistent and heavier smoking than women, although the sex ratio in the prevalence of current smoking has been closing to one in the recent years (Peters et al., 2014). Such gender effects are especially important in terms of mental health comorbidity usually associated with NA, gender, or both. Furthermore, men and women do not show the same sensitivity to the toxic effects of tobacco. Yet, a few studies have specifically examined the gender impact on the influence of nAChR genetics on

smoking outcomes. First evidence came from a twin study, showing that the genetic vulnerability in smoking initiation was higher for women than men, with an opposite pattern regarding smoking persistence; along with further gender differences in environmental influences on smoking (Li et al., 2003). In line with these findings, *CHRNA5* rs16969968 has been shown to interact with childhood adversity in risk to develop NA in males only, even if both genders had increased NA prevalence in the case of childhood adversity (Xie et al., 2012). Finally, using recent polygenic risk scoring and genetic correlation methods, a significant interaction between *CHRNA5* and *NR3C2*, a gene encoding glucocorticoid receptors (which play a key role in the stress response mediated by the hypothalamo-pituitary axis) has become evident (Schmitz et al., 2019). Overall, it seems that the gender variable, in genetic studies on NA, has been more considered as a cofactor rather than a significant parameter for refining genetic associations in specific subpopulations, despite the clinical and biological relevance to do so. Further research, both in humans and in preclinical models, should consider analysing males and females separately.

5.3. Conclusion

Associations between the chromosome 15 *CHRNA5-A3-B4* cluster and NA are robust. However, the current review emphasizes recent evidence which highlighted that these associations rely on a more complex, interdependent pattern of SNPs in high LD, mostly affecting gene expression levels, especially those of *CHRNA5*. In our opinion, methodological limitations of the studies conducted so far, at both the preclinical and clinical level, reflect the need to better define and model the genetic context in which the role of nAChR subunit genes and their variants are studied. Refined neuropsychological assessment for the different stages of the NA cycle should also be considered in future studies, both in patient cohorts and animal models.

Author contribution

All authors listed have made substantial, direct and intellectual contribution to the work, and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Romain ICICK has given a lecture for Pierre Fabre Inc. in 2019.

References

Abe, S., Kobayashi, K., Oji, A., Sakuma, T., Kazuki, K., Takehara, S., Nakamura, K., Okada, A., Tsukazaki, Y., Senda, N., Honma, K., Yamamoto, T., Ikawa, M., Chiba, K., Oshimura, M., Kazuki, Y., 2017.

Modification of single-nucleotide polymorphism in a fully humanized CYP3A mouse by genome editing technology. Scientific Reports 7, 15189.
Ables, J. L., Gorlich, A., Antolin-Fontes, B., Wang, C., Lipford, S. M., Riad, M. H., Ren, J., Hu, F., Luo, M., Kenny, P. J., Heintz, N., Ibanez-Tallon, I., 2017. Retrograde inhibition by a specific subset of interpeduncular alpha5 nicotinic neurons regulates nicotine preference. Proc Natl Acad Sci U S A 114, 13012-13017.
Ahn, S., Kim, T.-G., Kim, K.-S., Chung, S., 2016. Differentiation of human pluripotent stem cells into Medial Ganglionic Eminence vs. Caudal Ganglionic Eminence cells. Methods 101, 103-112.
Aitman, T. J., Critser, J. K., Cuppen, E., Dominiczak, A., Fernandez-Suarez, X. M., Flint, J., Gauguier, D., Geurts, A. M., Gould, M., Harris, P. C., Holmdahl, R., Hubner, N., Izsvak, Z., Jacob, H. J., Kuramoto, T., Kwitek, A. E., Marrone, A., Mashimo, T., Moreno, C., Mullins, J., Mullins, L., Olsson, T., Pravenec, M., Riley, L., Saar, K., Serikawa, T., Shull, J. D., Szpirer, C., Twigger, S. N., Voigt, B., Worley, K., 2008. Progress and prospects in rat genetics: a community view. Nature genetics 40, 516-522.
Alemany, S., Ribasés, M., Vilor-Tejedor, N., Bustamante, M., Sánchez-Mora, C., Bosch, R., Richarte, V., Cormand, B., Casas, M., Ramos-Quiroga, J. A., Sunyer, J., 2015. New suggestive genetic loci and biological pathways for attention function in adult attention-deficit/hyperactivity disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 168, 459-470.
Amos, C. I., Wu, X., Broderick, P., Gorlov, I. P., Gu, J., Eisen, T., Dong, Q., Zhang, Q., Gu, X., Vijayakrishnan, J., Sullivan, K., Matakidou, A., Wang, Y., Mills, G., Doheny, K., Tsai, Y. Y., Chen, W. V., Shete, S., Spitz, M. R., Houlston, R. S., 2008. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nature genetics 40, 616-622.
Anthony J, C., Lynn A. Warner, and Ronald C. Kessler, 1994. Comparative Epidemiology of Dependence on Tobacco, Alcohol, Controlled Substances, and Inhalants: Basic Findings From the National Comorbidity Survey. Experimental and Clinical Psycho pharmacology 2, 244-268.
Antolin-Fontes, B., Ables, J. L., Gorlich, A., Ibanez-Tallon, I., 2015. The habenulo-interpeduncular pathway in nicotine aversion and withdrawal. Neuropharmacology 96, 213-222.
Avale, M. E., Faure, P., Pons, S., Robledo, P., Deltheil, T., David, D. J., Gardier, A. M., Maldonado, R., Granon, S., Changeux, J. P., Maskos, U., 2008. Interplay of beta2* nicotinic receptors and dopamine pathways in the control of spontaneous locomotion. Proc Natl Acad Sci U S A 105, 15991-15996.
Azam, L., Winzer-Serhan, U. H., Chen, Y., Leslie, F. M., 2002. Expression of neuronal nicotinic acetylcholine receptor subunit mRNAs within midbrain dopamine neurons. Journal of Comparative Neurology 444, 260-274.
Babb, S., Malarcher, A., Schauer, G., Asman, K., Jamal, A., 2017. Quitting Smoking Among Adults - United States, 2000-2015. MMWR Morb Mortal Wkly Rep 65, 1457-1464.
Baddick, C. G., Marks, M. J., 2011. An autoradiographic survey of mouse brain nicotinic acetylcholine receptors defined by null mutants. Biochem Pharmacol 82, 828-841.
Bailey, C. D. C., De Biasi, M., Fletcher, P. J., Lambe, E. K., 2010. The Nicotinic Acetylcholine Receptor 5 Subunit Plays a Key Role in Attention Circuitry and Accuracy. Journal of Neuroscience 30, 9241-9252.
Baran, Y., Subramaniam, M., Biton, A., Tukiainen, T., Tsang, E. K., Rivas, M. A., Pirinen, M., Gutierrez-Arcelus, M., Smith, K. S., Kukurba, K. R., Zhang, R., Eng, C., Torgerson, D. G., Urbanek, C., Consortium, t. G., Li, J. B., Rodriguez-Santana, J. R., Burchard, E. G., Seibold, M. A., MacArthur, D. G., Montgomery, S. B., Zaitlen, N. A., Lappalainen, T., 2015. The landscape of genomic imprinting across diverse adult human tissues. Genome Research 25, 927-936.
Barrie, E. S., Hartmann, K., Lee, S.-H., Frater, J. T., Seweryn, M., Wang, D., Sadee, W., 2017. The *CHRNA5/CHRNA3/CHRNB4* Nicotinic Receptor Regulome: Genomic Architecture, Regulatory Variants, and Clinical Associations. Human Mutation 38, 112-119.
Batzoglou, S., Pachter, L., Mesirov, J. P., Berger, B., Lander, E. S., 2000. Human and mouse gene structure: comparative analysis and application to exon prediction. Genome Res 10, 950-958.
Beiranvand, F., Zlabinger, C., Orr-Urtreger, A., Ristl, R., Huck, S., Scholze, P., 2014. Nicotinic acetylcholine receptors control acetylcholine and noradrenaline release in the rodent habenulo-interpeduncular complex. Br J Pharmacol 171, 5209-5224.

Belsky, D. W., Moffitt, T. E., Baker, T. B., Biddle, A. K., Evans, J. P., Harrington, H., Houts, R., Meier, M., Sugden, K., Williams, B., Poulton, R., Caspi, A., 2013. Polygenic risk and the developmental progression to heavy, persistent smoking and nicotine dependence: evidence from a 4-decade longitudinal study. JAMA Psychiatry 70, 534-542.
Berrettini, W., Yuan, X., Tozzi, F., Song, K., Francks, C., Chilcoat, H., Waterworth, D., Muglia, P., Mooser, V., 2008. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. Mol Psychiatry 13, 368-373.
Besson, M., Forget, B., 2016. Cognitive Dysfunction, Affective States, and Vulnerability to Nicotine Addiction: A Multifactorial Perspective. Frontiers in Psychiatry 7, 160.
Besson, M., Forget, B., Correia, C., Blanco, R., Maskos, U., 2019. Profound alteration in reward processing due to a human polymorphism in CHRNA5: a role in alcohol dependence and feeding behavior. Neuropsychopharmacology 44, 1906-1916.
Besson, M., Guiducci, S., Granon, S., Guilloux, J. P., Guiard, B., Reperant, C., Faure, P., Pons, S., Cannazza, G., Zoli, M., Gardier, A. M., Maskos, U., 2016. Alterations in alpha5* nicotinic acetylcholine receptors result in midbrain- and hippocampus-dependent behavioural and neural impairments. Psychopharmacology (Berl) 233, 3297-3314.
Bian, S., Repic, M., Guo, Z., Kavirayani, A., Burkard, T., Bagley, J. A., Krauditsch, C., Knoblich, J. A., 2018. Genetically engineered cerebral organoids model brain tumor formation. Nature Methods 15, 631-639.
Bierut, L. J., Madden, P. A., Breslau, N., Johnson, E. O., Hatsukami, D., Pomerleau, O. F., Swan, G. E., Rutter, J., Bertelsen, S., Fox, L., Fugman, D., Goate, A. M., Hinrichs, A. L., Konvicka, K., Martin, N. G., Montgomery, G. W., Saccone, N. L., Saccone, S. F., Wang, J. C., Chase, G. A., Rice, J. P., Ballinger, D. G., 2007. Novel genes identified in a high-density genome wide association study for nicotine dependence. Hum Mol Genet 16, 24-35.
Bierut, L. J., Stitzel, J. A., Wang, J. C., Hinrichs, A. L., Grucza, R. A., Xuei, X., Saccone, N. L., Saccone, S. F., Bertelsen, S., Fox, L., Horton, W. J., Breslau, N., Budde, J., Cloninger, C. R., Dick, D. M., Foroud, T., Hatsukami, D., Hesselbrock, V., Johnson, E. O., Kramer, J., Kuperman, S., Madden, P. A. F., Mayo, K., Nurnberger, J., Pomerleau, O., Porjesz, B., Reyes, O., Schuckit, M., Swan, G., Tischfield, J. A., Edenberg, H. J., Rice, J. P., Goate, A. M., 2008. Variants in Nicotinic Receptors and Risk for Nicotine Dependence. American Journal of Psychiatry 165, 1163-1171.
Birge, M., Duffy, S., Miler, J. A., Hajek, P., 2018. What Proportion of People Who Try One Cigarette Become Daily Smokers? A Meta-Analysis of Representative Surveys. Nicotine Tob Res 20, 1427-1433.
Bloom, A. J., Hartz, S. M., Baker, T. B., Chen, L. S., Piper, M. E., Fox, L., Martinez, M., Hatsukami, D., Johnson, E. O., Laurie, C. C., Saccone, N. L., Goate, A., Bierut, L. J., 2014. Beyond cigarettes per day. A genome-wide association study of the biomarker carbon monoxide. Annals of the American Thoracic Society 11, 1003-1010.
Boissart, C., Poulet, A., Georges, P., Darville, H., Julita, E., Delorme, R., Bourgeron, T., Peschanski, M., Benchoua, A., 2013. Differentiation from human pluripotent stem cells of cortical neurons of the superficial layers amenable to psychiatric disease modeling and high-throughput drug screening. Translational Psychiatry 3, e294-e294.
Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., Heinemann, S., Patrick, J., 1986. Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor alpha-subunit. Nature 319, 368-374.
Boyle, A. P., Hong, E. L., Hariharan, M., Cheng, Y., Schaub, M. A., Kasowski, M., Karczewski, K. J., Park, J., Hitz, B. C., Weng, S., Cherry, J. M., Snyder, M., 2012. Annotation of functional variation in personal genomes using RegulomeDB. Genome Research 22, 1790-1797.
Bueno-Junior, L. S., Simon, N. W., Wegener, M. A., Moghaddam, B., 2017. Repeated Nicotine Strengthens Gamma Oscillations in the Prefrontal Cortex and Improves Visual Attention. Neuropsychopharmacology 42, 1590-1598.
Bult, C. J., Blake, J. A., Smith, C. L., Kadin, J. A., Richardson, J. E., Mouse Genome Database, G., 2019. Mouse Genome Database (MGD) 2019. Nucleic Acids Res 47, D801-D806.

Cachelin, A. B., Jaggi, R., 1991. Beta subunits determine the time course of desensitization in rat alpha 3 neuronal nicotinic acetylcholine receptors. Pflugers Arch 419, 579-582.
Caille, S., Clemens, K., Stinus, L., Cador, M., 2012. Modeling nicotine addiction in rats. Methods Mol Biol 829, 243-256.
Campos, M. W., Serebrisky, D., Castaldelli-Maia, J. M., 2017. Smoking and Cognition. Current Drug Abuse Reviews 9, 76-79.
Chaarani, B., Kan, K.-J., Mackey, S., Spechler, P. A., Potter, A., Orr, C., D'Alberto, N., Hudson, K. E., Banaschewski, T., Bokde, A. L. W., Bromberg, U., Büchel, C., Cattrell, A., Conrod, P. J., Desrivières, S., Flor, H., Frouin, V., Gallinat, J., Gowland, P., Heinz, A., Ittermann, B., Martinot, J.-L., Nees, F., Papadopoulos-Orfanos, D., Paus, T., Poustka, L., Smolka, M. N., Walter, H., Whelan, R., Higgins, S. T., Schumann, G., Althoff, R. R., Stein, E. A., Garavan, H., Mann, K., Struve, M., Rietschel, M., Spanagel, R., Fauth-Bühler, M., Millenet, S., Grimmer, Y., Ivanov, N., Strache, N., Rapp, M., Ströhle, A., Reuter, J., Barbot, A., Thyreau, B., Schwartz, Y., Lalanne, C., Bricaud, Z., Briand, F. G., Lemaitre, H., Massicotte, J., Vulser, H., Pentillä, J., Galinowski, A., Jia, T., Werts, H., Topper, L., Reed, L., Andrew, C., Mallik, C., Ruggeri, B., Nymberg, C., Smith, L., Loth, E., Havatzias, S., Stueber, K., Stringaris, A., Constant, P., Brühl, R., Ihlenfeld, A., Walaszek, B., Hübner, T., Müller, K., Ripke, S., Rodehacke, S., Mennigen, E., Schmidt, D., Vetter, N., Ziesch, V., Jones, J., Poline, J.-B., Fadai, T., Yacubian, J., Schneider, S., Lawrence, C., Newman, C., Head, K., Heym, N., Pausova, Z., Tahmasebi, A., 2019. Low Smoking Exposure, the Adolescent Brain, and the Modulating Role of CHRNA5 Polymorphisms. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 4, 672-679.
Chaiton, M., Diemert, L., Cohen, J. E., Bondy, S. J., Selby, P., Philipneri, A., Schwartz, R., 2016. Estimating the number of quit attempts it takes to quit smoking successfully in a longitudinal cohort of smokers. BMJ Open 6, e011045.
Changeux, J.-P., 2010. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. Nature Reviews Neuroscience 11, 389-401.
Chatterjee, K., Alzghoul, B., Innabi, A., Meena, N., 2016. Is vaping a gateway to smoking: a review of the longitudinal studies. Int J Adolesc Med Health 30.
Chen, L. S., Hung, R. J., Baker, T., Horton, A., Culverhouse, R., Saccone, N., Cheng, I., Deng, B., Han, Y., Hansen, H. M., Horsman, J., Kim, C., Lutz, S., Rosenberger, A., Aben, K. K., Andrew, A. S., Breslau, N., Chang, S. C., Dieffenbach, A. K., Dienemann, H., Frederiksen, B., Han, J., Hatsukami, D. K., Johnson, E. O., Pande, M., Wrensch, M. R., McLaughlin, J., Skaug, V., van der Heijden, H. F., Wampfler, J., Wenzlaff, A., Woll, P., Zienolddiny, S., Bickeboller, H., Brenner, H., Duell, E. J., Haugen, A., Heinrich, J., Hokanson, J. E., Hunter, D. J., Kiemeney, L. A., Lazarus, P., Le Marchand, L., Liu, G., Mayordomo, J., Risch, A., Schwartz, A. G., Teare, D., Wu, X., Wiencke, J. K., Yang, P., Zhang, Z. F., Spitz, M. R., Kraft, P., Amos, C. I., Bierut, L. J., 2015. CHRNA5 risk variant predicts delayed smoking cessation and earlier lung cancer diagnosis--a meta-analysis. J Natl Cancer Inst 107, djv100, https://doi.org/110.1093/jnci/djv1100.
Chen, L. S., Saccone, N. L., Culverhouse, R. C., Bracci, P. M., Chen, C. H., Dueker, N., Han, Y., Huang, H., Jin, G., Kohno, T., Ma, J. Z., Przybeck, T. R., Sanders, A. R., Smith, J. A., Sung, Y. J., Wenzlaff, A. S., Wu, C., Yoon, D., Chen, Y. T., Cheng, Y. C., Cho, Y. S., David, S. P., Duan, J., Eaton, C. B., Furberg, H., Goate, A. M., Gu, D., Hansen, H. M., Hartz, S., Hu, Z., Kim, Y. J., Kittner, S. J., Levinson, D. F., Mosley, T. H., Payne, T. J., Rao, D. C., Rice, J. P., Rice, T. K., Schwantes-An, T. H., Shete, S. S., Shi, J., Spitz, M. R., Sun, Y. V., Tsai, F. J., Wang, J. C., Wrensch, M. R., Xian, H., Gejman, P. V., He, J., Hunt, S. C., Kardia, S. L., Li, M. D., Lin, D., Mitchell, B. D., Park, T., Schwartz, A. G., Shen, H., Wiencke, J. K., Wu, J. Y., Yokota, J., Amos, C. I., Bierut, L. J., 2012. Smoking and genetic risk variation across populations of European, Asian, and African American ancestry--a meta-analysis of chromosome 15q25. Genet Epidemiol 36, 340-351.
Civelek, M., Lusis, A. J., 2014. Systems genetics approaches to understand complex traits. Nat Rev Genet 15, 34-48.
Clark, S. L., McClay, J. L., Adkins, D. E., Aberg, K. A., Kumar, G., Nerella, S., Xie, L., Collins, A. L., Crowley, J. J., Quakenbush, C. R., Hillard, C. E., Gao, G., Shabalin, A. A., Peterson, R. E., Copeland, W. E., Silberg, J. L., Maes, H., Sullivan, P. F., Costello, E. J., van den Oord, E. J., 2016. Deep Sequencing of

Three Loci Implicated in Large-Scale Genome-Wide Association Study Smoking Meta-Analyses. Nicotine Tob Res 18, 626-631.
Clemens, K. J., Castino, M. R., Cornish, J. L., Goodchild, A. K., Holmes, N. M., 2014. Behavioral and neural substrates of habit formation in rats intravenously self-administering nicotine. Neuropsychopharmacology 39, 2584-2593.
Cohen, A., Koob, G. F., George, O., 2012. Robust escalation of nicotine intake with extended access to nicotine self-administration and intermittent periods of abstinence. Neuropsychopharmacology 37, 2153-2160.
Collo, G., Cavalleri, L., Bono, F., Mora, C., Fedele, S., Invernizzi, R. W., Gennarelli, M., Piovani, G., Kunath, T., Millan, M. J., Merlo Pich, E., Spano, P., 2018. Ropinirole and Pramipexole Promote Structural Plasticity in Human iPSC-Derived Dopaminergic Neurons via BDNF and mTOR Signaling. Neural Plasticity 2018, 1-15.
Conlon, M. S., Bewick, M. A., 2011. Single Nucleotide Polymorphisms in CHRNA5 rs16969968, CHRNA3 rs578776, and LOC123688 rs8034191 Are Associated With Heaviness of Smoking in Women in Northeastern Ontario, Canada. Nicotine & Tobacco Research 13, 1076-1083.
Crespi, A., Colombo, S. F., Gotti, C., 2018. Proteins and chemical chaperones involved in neuronal nicotinic receptor expression and function: an update: Chaperones for nAChRs. British Journal of Pharmacology 175, 1869-1879.
Crombag, H. S., Bossert, J. M., Koya, E., Shaham, Y., 2008. Review. Context-induced relapse to drug seeking: a review. Philos Trans R Soc Lond B Biol Sci 363, 3233-3243.
Curtis, K., Viswanath, H., Velasquez, K. M., Molfese, D. L., Harding, M. J., Aramayo, E., Baldwin, P. R., Ambrosi, E., Madan, A., Patriquin, M., Frueh, B. C., Fowler, J. C., Kosten, T. R., Nielsen, D. A., Salas, R., 2017. Increased habenular connectivity in opioid users is associated with an alpha5 subunit nicotinic receptor genetic variant. Am J Addict 26, 751-759.
David, R., Ciuraszkiewicz, A., Simeone, X., Orr-Urtreger, A., Papke, R. L., McIntosh, J. M., Huck, S., Scholze, P., 2010. Biochemical and functional properties of distinct nicotinic acetylcholine receptors in the superior cervical ganglion of mice with targeted deletions of nAChR subunit genes. Eur J Neurosci 31, 978-993.
De Biasi, M., Dani, J. A., 2011. Reward, addiction, withdrawal to nicotine. Annu Rev Neurosci 34, 105-130.
Deflorio, C., Blanchard, S., Carla Carisì, M., Bohl, D., Maskos, U., 2017. Human polymorphisms in nicotinic receptors: a functional analysis in iPS-derived dopaminergic neurons. The FASEB Journal 31, 828-839.
Dever, D. P., Scharenberg, S. G., Camarena, J., Kildebeck, E. J., Clark, J. T., Martin, R. M., Bak, R. O., Tang, Y., Dohse, M., Birgmeier, J. A., Jagadeesh, K. A., Bejerano, G., Tsukamoto, A., Gomez-Ospina, N., Uchida, N., Porteus, M. H., 2019. CRISPR/Cas9 Genome Engineering in Engraftable Human Brain-Derived Neural Stem Cells. iScience 15, 524-535.
Dineley-Miller, K., Patrick, J., 1992. Gene transcripts for the nicotinic acetylcholine receptor subunit, beta4, are distributed in multiple areas of the rat central nervous system. Molecular Brain Research 16, 339-344.
Dome, P., Lazary, J., Kalapos, M. P., Rihmer, Z., 2010. Smoking, nicotine and neuropsychiatric disorders. Neurosci Biobehav Rev 34, 295-342.
Ducci, F., Goldman, D., 2012. The genetic basis of addictive disorders. Psychiatr Clin North Am 35, 495-519.
Ellenbroek, B., Youn, J., 2016. Rodent models in neuroscience research: is it a rat race? Dis Model Mech 9, 1079-1087.
Erzurumluoglu, A. M., Liu, M., Jackson, V. E., Barnes, D. R., Datta, G., Melbourne, C. A., Young, R., Batini, C., Surendran, P., Jiang, T., Adnan, S. D., Afaq, S., Agrawal, A., Altmaier, E., Antoniou, A. C., Asselbergs, F. W., Baumbach, C., Bierut, L., Bertelsen, S., Boehnke, M., Bots, M. L., Brazel, D. M., Chambers, J. C., Chang-Claude, J., Chen, C., Corley, J., Chou, Y. L., David, S. P., de Boer, R. A., de Leeuw, C. A., Dennis, J. G., Dominiczak, A. F., Dunning, A. M., Easton, D. F., Eaton, C., Elliott, P., Evangelou, E., Faul, J. D., Foroud, T., Goate, A., Gong, J., Grabe, H. J., Haessler, J., Haiman, C.,

Hallmans, G., Hammerschlag, A. R., Harris, S. E., Hattersley, A., Heath, A., Hsu, C., Iacono, W. G., Kanoni, S., Kapoor, M., Kaprio, J., Kardia, S. L., Karpe, F., Kontto, J., Kooner, J. S., Kooperberg, C., Kuulasmaa, K., Laakso, M., Lai, D., Langenberg, C., Le, N., Lettre, G., Loukola, A., Luan, J., Madden, P. A. F., Mangino, M., Marioni, R. E., Marouli, E., Marten, J., Martin, N. G., McGue, M., Michailidou, K., Mihailov, E., Moayyeri, A., Moitry, M., Muller-Nurasyid, M., Naheed, A., Nauck, M., Neville, M. J., Nielsen, S. F., North, K., Perola, M., Pharoah, P. D. P., Pistis, G., Polderman, T. J., Posthuma, D., Poulter, N., Qaiser, B., Rasheed, A., Reiner, A., Renstrom, F., Rice, J., Rohde, R., Rolandsson, O., Samani, N. J., Samuel, M., Schlessinger, D., Scholte, S. H., Scott, R. A., Sever, P., Shao, Y., Shrine, N., Smith, J. A., Starr, J. M., Stirrups, K., Stram, D., Stringham, H. M., Tachmazidou, I., Tardif, J. C., Thompson, D. J., Tindle, H. A., Tragante, V., Trompet, S., Turcot, V., Tyrrell, J., Vaartjes, I., van der Leij, A. R., van der Meer, P., Varga, T. V., Verweij, N., Volzke, H., Wareham, N. J., Warren, H. R., Weir, D. R., Weiss, S., Wetherill, L., Yaghootkar, H., Yavas, E., Jiang, Y., Chen, F., Zhan, X., Zhang, W., Zhao, W., Zhao, W., Zhou, K., Amouyel, P., Blankenberg, S., Caulfield, M. J., Chowdhury, R., Cucca, F., Deary, I. J., Deloukas, P., Di Angelantonio, E., Ferrario, M., Ferrieres, J., Franks, P. W., Frayling, T. M., Frossard, P., Hall, I. P., Hayward, C., Jansson, J. H., Jukema, J. W., Kee, F., Mannisto, S., Metspalu, A., Munroe, P. B., Nordestgaard, B. G., Palmer, C. N. A., Salomaa, V., Sattar, N., Spector, T., Strachan, D. P., Understanding Society Scientific Group, E.-C. V. D. G. C. f. G. o. S. B. C. H. D. E. c., van der Harst, P., Zeggini, E., Saleheen, D., Butterworth, A. S., Wain, L. V., Abecasis, G. R., Danesh, J., Tobin, M. D., Vrieze, S., Liu, D. J., Howson, J. M. M., 2019. Meta-analysis of up to 622,409 individuals identifies 40 novel smoking behaviour associated genetic loci. Mol Psychiatry.

Etter, J. F., 2018. Gateway effects and electronic cigarettes. Addiction 113, 1776-1783.

Evans, D. E., MacQueen, D. A., Jentink, K. G., Park, J. Y., Lin, H. Y., Drobes, D. J., 2014. CHRNA5 variants moderate the effect of nicotine deprivation on a neural index of cognitive control. Genes, Brain, and Behavior 13, 626-632.

Exley, R., McIntosh, J. M., Marks, M. J., Maskos, U., Cragg, S. J., 2012. Striatal α5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 32, 2352-2356.

Forget, B., Scholze, P., Langa, F., Morel, C., Pons, S., Mondoloni, S., Besson, M., Durand-de Cuttoli, R., Hay, A., Tricoire, L., Lambolez, B., Mourot, A., Faure, P., Maskos, U., 2018. A Human Polymorphism in CHRNA5 Is Linked to Relapse to Nicotine Seeking in Transgenic Rats. Current Biology 28, 3244-3253.e3247.

Fowler, C. D., Lu, Q., Johnson, P. M., Marks, M. J., Kenny, P. J., 2011. Habenular alpha5 nicotinic receptor subunit signalling controls nicotine intake. Nature 471, 597-601.

Frahm, S., Slimak, M. A., Ferrarese, L., Santos-Torres, J., Antolin-Fontes, B., Auer, S., Filkin, S., Pons, S., Fontaine, J. F., Tsetlin, V., Maskos, U., Ibanez-Tallon, I., 2011. Aversion to nicotine is regulated by the balanced activity of beta4 and alpha5 nicotinic receptor subunits in the medial habenula. Neuron 70, 522-535.

Franklin, T. R., Wang, Z., Wang, J., Sciortino, N., Harper, D., Li, Y., Ehrman, R., Kampman, K., O'Brien, C. P., Detre, J. A., Childress, A. R., 2007. Limbic Activation to Cigarette Smoking Cues Independent of Nicotine Withdrawal: A Perfusion fMRI Study. Neuropsychopharmacology 32, 2301-2309.

Fürth, D., Vaissière, T., Tzortzi, O., Xuan, Y., Märtin, A., Lazaridis, I., Spigolon, G., Fisone, G., Tomer, R., Deisseroth, K., Carlén, M., Miller, C. A., Rumbaugh, G., Meletis, K., 2018. An interactive framework for whole-brain maps at cellular resolution. Nature Neuroscience 21, 139-149.

Gahring, L. C., Persiyanov, K., Dunn, D., Weiss, R., Meyer, E. L., Rogers, S. W., 2004. Mouse Strain-Specific Nicotinic Acetylcholine Receptor Expression by Inhibitory Interneurons and Astrocytes in the Dorsal Hippocampus. Journal of Comparative Neurology 468, 334-346.

Gallego, X., Molas, S., Amador-Arjona, A., Marks, M. J., Robles, N., Murtra, P., Armengol, L., Fernandez-Montes, R. D., Gratacos, M., Pumarola, M., Cabrera, R., Maldonado, R., Sabria, J., Estivill, X., Dierssen, M., 2012. Overexpression of the CHRNA5/A3/B4 genomic cluster in mice increases the sensitivity to nicotine and modifies its reinforcing effects. Amino Acids 43, 897-909.

Gaunt, T. R., Shihab, H. A., Hemani, G., Min, J. L., Woodward, G., Lyttleton, O., Zheng, J., Duggirala, A., McArdle, W. L., Ho, K., Ring, S. M., Evans, D. M., Davey Smith, G., Relton, C. L., 2016. Systematic

identification of genetic influences on methylation across the human life course. Genome Biology 17, 61-74.
Gelernter, J., 2015. Genetics of complex traits in psychiatry. Biol Psychiatry 77, 36-42.
Gilpin, N. W., Whitaker, A. M., Baynes, B., Abdel, A. Y., Weil, M. T., George, O., 2014. Nicotine vapor inhalation escalates nicotine self-administration. Addict Biol 19, 587-592.
Giorgio, A. D., Smith, R. M., Fazio, L., D'Ambrosio, E., Gelao, B., Tomasicchio, A., Selvaggi, P., Taurisano, P., Quarto, T., Masellis, R., Rampino, A., Caforio, G., Popolizio, T., Blasi, G., Sadee, W., Bertolino, A., 2014. DRD2/CHRNA5 Interaction on Prefrontal Biology and Physiology during Working Memory. PLOS ONE 9, e95997.
Glick, S. D., Sell, E. M., McCallum, S. E., Maisonneuve, I. M., 2011. Brain regions mediating alpha3beta4 nicotinic antagonist effects of 18-MC on nicotine self-administration. Eur J Pharmacol 669, 71-75.
Gotti, C., Clementi, F., 2004. Neuronal nicotinic receptors: From structure to pathology. Progress in Neurobiology, pp. 363-396.
Gotti, C., Clementi, F., Fornari, A., Gaimarri, A., Guiducci, S., Manfredi, I., Moretti, M., Pedrazzi, P., Pucci, L., Zoli, M., 2009. Structural and functional diversity of native brain neuronal nicotinic receptors. Biochem Pharmacol 78, 703-711.
Goulburn, A. L., Stanley, E. G., Elefanty, A. G., Anderson, S. A., 2012. Generating GABAergic cerebral cortical interneurons from mouse and human embryonic stem cells. Stem Cell Research 8, 416-426.
Grady, S. R., Moretti, M., Zoli, M., Marks, M. J., Zanardi, A., Pucci, L., Clementi, F., Gotti, C., 2009. Rodent habenulo-interpeduncular pathway expresses a large variety of uncommon nAChR subtypes, but only the αβ4* and αβ3β4* subtypes mediate acetylcholine release. Journal of Neuroscience 29, 2272-2282.
Greenbaum, L., Lerer, B., 2009. Differential contribution of genetic variation in multiple brain nicotinic cholinergic receptors to nicotine dependence: Recent progress and emerging open questions. Mol Psychiatry 14, 912-945.
Grucza, R. A., Wang, J. C., Stitzel, J. A., Hinrichs, A. L., Saccone, S. F., Saccone, N. L., Bucholz, K. K., Cloninger, C. R., Neuman, R. J., Budde, J. P., Fox, L., Bertelsen, S., Kramer, J., Hesselbrock, V., Tischfield, J., Nurnberger, J. I., Jr., Almasy, L., Porjesz, B., Kuperman, S., Schuckit, M. A., Edenberg, H. J., Rice, J. P., Goate, A. M., Bierut, L. J., 2008. A risk allele for nicotine dependence in CHRNA5 is a protective allele for cocaine dependence. Biol Psychiatry 64, 922-929.
Hahn, A., Breakspear, M., Rischka, L., Wadsak, W., Godbersen, G. M., Pichler, V., Michenthaler, P., Vanicek, T., Hacker, M., Kasper, S., Lanzenberger, R., Cocchi, L., 2020. Reconfiguration of functional brain networks and metabolic cost converge during task performance. eLife 9, e52443.
Hahn, B., 2015. Nicotinic Receptors and Attention. In: Balfour, D. J. K., Munafò, M. R., (Eds), The Neurobiology and Genetics of Nicotine and Tobacco. Springer International Publishing, Cham, pp. 103-135.
Haller, G., Druley, T., Vallania, F. L., Mitra, R. D., Li, P., Akk, G., Steinbach, J. H., Breslau, N., Johnson, E., Hatsukami, D., Stitzel, J., Bierut, L. J., Goate, A. M., 2012. Rare missense variants in CHRNB4 are associated with reduced risk of nicotine dependence. Hum Mol Genet 21, 647-655.
Haller, G., Kapoor, M., Budde, J., Xuei, X., Edenberg, H., Nurnberger, J., Kramer, J., Brooks, A., Tischfield, J., Almasy, L., Agrawal, A., Bucholz, K., Rice, J., Saccone, N., Bierut, L., Goate, A., 2014. Rare missense variants in CHRNB3 and CHRNA3 are associated with risk of alcohol and cocaine dependence. Hum Mol Genet 23, 810-819.
Han, W., Zhang, T., Ni, T., Zhu, L., Liu, D., Chen, G., Lin, H., Chen, T., Guan, F., 2019. Relationship of common variants in CHRNA5 with early-onset schizophrenia and executive function. Schizophrenia Research 206, 407-412.
Harrington, L., Vinals, X., Herrera-Solis, A., Flores, A., Morel, C., Tolu, S., Faure, P., Maldonado, R., Maskos, U., Robledo, P., 2016. Role of beta4* Nicotinic Acetylcholine Receptors in the Habenulo-Interpeduncular Pathway in Nicotine Reinforcement in Mice. Neuropsychopharmacology 41, 1790-1802.

Hashimoto, K., 2015. Targeting of α7 Nicotinic Acetylcholine Receptors in the Treatment of Schizophrenia and the Use of Auditory Sensory Gating as a Translational Biomarker. Current Pharmaceutical Design 21, 3797-3806.
Hawrylycz, M. J., Lein, E. S., Guillozet-Bongaarts, A. L., Shen, E. H., Ng, L., Miller, J. A., van de Lagemaat, L. N., Smith, K. A., Ebbert, A., Riley, Z. L., Abajian, C., Beckmann, C. F., Bernard, A., Bertagnolli, D., Boe, A. F., Cartagena, P. M., Chakravarty, M. M., Chapin, M., Chong, J., Dalley, R. A., David Daly, B., Dang, C., Datta, S., Dee, N., Dolbeare, T. A., Faber, V., Feng, D., Fowler, D. R., Goldy, J., Gregor, B. W., Haradon, Z., Haynor, D. R., Hohmann, J. G., Horvath, S., Howard, R. E., Jeromin, A., Jochim, J. M., Kinnunen, M., Lau, C., Lazarz, E. T., Lee, C., Lemon, T. A., Li, L., Li, Y., Morris, J. A., Overly, C. C., Parker, P. D., Parry, S. E., Reding, M., Royall, J. J., Schulkin, J., Sequeira, P. A., Slaughterbeck, C. R., Smith, S. C., Sodt, A. J., Sunkin, S. M., Swanson, B. E., Vawter, M. P., Williams, D., Wohnoutka, P., Zielke, H. R., Geschwind, D. H., Hof, P. R., Smith, S. M., Koch, C., Grant, S. G. N., Jones, A. R., 2012. An anatomically comprehensive atlas of the adult human brain transcriptome. Nature 489, 391-399.
Hellwig, S., Domschke, K., 2019. Update on PET imaging biomarkers in the diagnosis of neuropsychiatric disorders:. Current Opinion in Neurology 32, 539-547.
Hernandez, S. C., Vicini, S., Xiao, Y., Davila-Garcia, M. I., Yasuda, R. P., Wolfe, B. B., Kellar, K. J., 2004. The nicotinic receptor in the rat pineal gland is an alpha3beta4 subtype. Mol Pharmacol 66, 978-987.
Hong, L. E., Hodgkinson, C. A., Yang, Y., Sampath, H., Ross, T. J., Buchholz, B., Salmeron, B. J., Srivastava, V., Thaker, G. K., Goldman, D., Stein, E. A., 2010. A genetically modulated, intrinsic cingulate circuit supports human nicotine addiction. Proceedings of the National Academy of Sciences 107, 13509-13514.
Hsu, Y. W., Tempest, L., Quina, L. A., Wei, A. D., Zeng, H., Turner, E. E., 2013. Medial habenula output circuit mediated by alpha5 nicotinic receptor-expressing GABAergic neurons in the interpeduncular nucleus. J Neurosci 33, 18022-18035.
Hung, R. J., McKay, J. D., Gaborieau, V., Boffetta, P., Hashibe, M., Zaridze, D., Mukeria, A., Szeszenia-Dabrowska, N., Lissowska, J., Rudnai, P., Fabianova, E., Mates, D., Bencko, V., Foretova, L., Janout, V., Chen, C., Goodman, G., Field, J. K., Liloglou, T., Xinarianos, G., Cassidy, A., McLaughlin, J., Liu, G., Narod, S., Krokan, H. E., Skorpen, F., Elvestad, M. B., Hveem, K., Vatten, L., Linseisen, J., Clavel-Chapelon, F., Vineis, P., Bueno-de-Mesquita, H. B., Lund, E., Martinez, C., Bingham, S., Rasmuson, T., Hainaut, P., Riboli, E., Ahrens, W., Benhamou, S., Lagiou, P., Trichopoulos, D., Holcatova, I., Merletti, F., Kjaerheim, K., Agudo, A., Macfarlane, G., Talamini, R., Simonato, L., Lowry, R., Conway, D. I., Znaor, A., Healy, C., Zelenika, D., Boland, A., Delepine, M., Foglio, M., Lechner, D., Matsuda, F., Blanche, H., Gut, I., Heath, S., Lathrop, M., Brennan, P., 2008. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 452, 633-637.
Husson, M., Harrington, L., Tochon, L., Cho, Y., Ibanez-Tallon, I., Maskos, U., David, V., 2020. beta4-Nicotinic Receptors Are Critically Involved in Reward-Related Behaviors and Self-Regulation of Nicotine Reinforcement. J Neurosci 40, 3465-3477.
Improgo, M. R. D., Scofield, M. D., Tapper, A. R., Gardner, P. D., 2010. The nicotinic acetylcholine receptor CHRNA5/A3/B4 gene cluster: dual role in nicotine addiction and lung cancer. Progress in Neurobiology 92, 212-226.
Jackson, K. J., Fanous, A. H., Chen, J., Kendler, K. S., Chen, X., 2013a. Variants in the 15q25 gene cluster are associated with risk for schizophrenia and bipolar disorder:. Psychiatric Genetics 23, 20-28.
Jackson, K. J., Marks, M. J., Vann, R. E., Chen, X., Gamage, T. F., Warner, J. A., Damaj, M. I., 2010. Role of alpha5 nicotinic acetylcholine receptors in pharmacological and behavioral effects of nicotine in mice. J Pharmacol Exp Ther 334, 137-146.
Jackson, K. J., Martin, B. R., Changeux, J. P., Damaj, M. I., 2008. Differential role of nicotinic acetylcholine receptor subunits in physical and affective nicotine withdrawal signs. J Pharmacol Exp Ther 325, 302-312.

Jackson, K. J., Sanjakdar, S. S., Muldoon, P. P., McIntosh, J. M., Damaj, M. I., 2013b. The α3β4* nicotinic acetylcholine receptor subtype mediates nicotine reward and physical nicotine withdrawal signs independently of the α5 subunit in the mouse. Neuropharmacology 70, 228-235.
Janes, A. C., Smoller, J. W., David, S. P., Frederick, B., Haddad, S., Basu, A., Fava, M., Evins, A. E., Kaufman, M. J., 2012. Association between CHRNA5 genetic variation at rs16969968 and brain reactivity to smoking images in nicotine dependent women. Drug Alcohol Depend 120, 7-13.
Jensen, K. P., DeVito, E. E., Herman, A. I., Valentine, G. W., Gelernter, J., Sofuoglu, M., 2015. A CHRNA5 Smoking Risk Variant Decreases the Aversive Effects of Nicotine in Humans. Neuropsychopharmacology 40, 2813-2821.
Jones, E. G., 2009. The Origins of Cortical Interneurons: Mouse versus Monkey and Human. Cerebral Cortex 19, 1953-1956.
Kandel, D., Chen, K., Warner, L. A., Kessler, R. C., Grant, B., 1997. Prevalence and demographic correlates of symptoms of last year dependence on alcohol, nicotine, marijuana and cocaine in the U.S. population. Drug Alcohol Depend 44, 11-29.
Kassel, J. D., 1997. Smoking and attention: a review and reformulation of the stimulus-filter hypothesis. Clinical Psychology Review 17, 451-478.
Kennedy, K. P., Cullen, K. R., DeYoung, C. G., Klimes-Dougan, B., 2015. The genetics of early-onset bipolar disorder: A systematic review. Journal of Affective Disorders 184, 1-12.
Klink, R., de Kerchove d'Exaerde, A., Zoli, M., Changeux, J. P., 2001. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J Neurosci 21, 1452-1463.
Koukouli, F., Rooy, M., Tziotis, D., Sailor, K. A., O'Neill, H. C., Levenga, J., Witte, M., Nilges, M., Changeux, J.-P., Hoeffer, C. A., Stitzel, J. A., Gutkin, B. S., DiGregorio, D. A., Maskos, U., 2017. Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. Nature Medicine 23, 347-354.
Kranzler, H. R., Zhou, H., Kember, R. L., Smith, R. V., Justice, A. C., Damrauer, S., Tsao, P. S., Klarin, D., Baras, A., Reid, J., Overton, J., Rader, D. J., Cheng, Z., Tate, J. P., Becker, W. C., Concato, J., Xu, K., Polimanti, R., Zhao, H., Gelernter, J., 2019. Genome-wide association study of alcohol consumption and use disorder in 274,424 individuals from multiple populations. Nature Communications 10, 1-11.
Krishnaswamy, A., Cooper, E., 2009. An activity-dependent retrograde signal induces the expression of the high-affinity choline transporter in cholinergic neurons. Neuron 61, 272-286.
Kuryatov, A., Onksen, J., Lindstrom, J., 2008. Roles of accessory subunits in alpha4beta2(*) nicotinic receptors. Mol Pharmacol 74, 132-143.
Lassi, G., Taylor, A. E., Timpson, N. J., Kenny, P. J., Mather, R. J., Eisen, T., Munafò, M. R., 2016. The CHRNA5-A3-B4 Gene Cluster and Smoking: From Discovery to Therapeutics. Trends in Neurosciences 39, 851-861.
Le, B. D., Stein, J. L., 2019. Mapping causal pathways from genetics to neuropsychiatric disorders using genome-wide imaging genetics: Current status and future directions. Psychiatry and Clinical Neurosciences 73, 357-369.
Lee, S. H., Ahn, W. Y., Seweryn, M., Sadee, W., 2018. Combined genetic influence of the nicotinic receptor gene cluster CHRNA5/A3/B4 on nicotine dependence. BMC Genomics 19, 826.
Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A. F., Boguski, M. S., Brockway, K. S., Byrnes, E. J., Chen, L., Chen, L., Chen, T. M., Chin, M. C., Chong, J., Crook, B. E., Czaplinska, A., Dang, C. N., Datta, S., Dee, N. R., Desaki, A. L., Desta, T., Diep, E., Dolbeare, T. A., Donelan, M. J., Dong, H. W., Dougherty, J. G., Duncan, B. J., Ebbert, A. J., Eichele, G., Estin, L. K., Faber, C., Facer, B. A., Fields, R., Fischer, S. R., Fliss, T. P., Frensley, C., Gates, S. N., Glattfelder, K. J., Halverson, K. R., Hart, M. R., Hohmann, J. G., Howell, M. P., Jeung, D. P., Johnson, R. A., Karr, P. T., Kawal, R., Kidney, J. M., Knapik, R. H., Kuan, C. L., Lake, J. H., Laramee, A. R., Larsen, K. D., Lau, C., Lemon, T. A., Liang, A. J., Liu, Y., Luong, L. T., Michaels, J., Morgan, J. J., Morgan, R. J., Mortrud, M. T., Mosqueda, N. F., Ng, L. L., Ng, R., Orta, G. J., Overly, C. C., Pak, T. H., Parry, S. E., Pathak, S. D., Pearson, O. C., Puchalski, R. B., Riley, Z. L., Rockett, H. R., Rowland, S. A., Royall, J. J., Ruiz, M. J., Sarno, N. R., Schaffnit, K., Shapovalova, N. V., Sivisay, T., Slaughterbeck, C. R., Smith, S. C., Smith, K.

A., Smith, B. I., Sodt, A. J., Stewart, N. N., Stumpf, K. R., Sunkin, S. M., Sutram, M., Tam, A., Teemer, C. D., Thaller, C., Thompson, C. L., Varnam, L. R., Visel, A., Whitlock, R. M., Wohnoutka, P. E., Wolkey, C. K., Wong, V. Y., Wood, M., Yaylaoglu, M. B., Young, R. C., Youngstrom, B. L., Yuan, X. F., Zhang, B., Zwingman, T. A., Jones, A. R., 2007. Genome-wide atlas of gene expression in the adult mouse brain. Nature 445, 168-176.

Li, M. D., Cheng, R., Ma, J. Z., Swan, G. E., 2003. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. Addiction 98, 23-31.

Lim, L., Mi, D., Llorca, A., Marín, O., 2018. Development and Functional Diversification of Cortical Interneurons. Neuron 100, 294-313.

Liu, J. Z., Tozzi, F., Waterworth, D. M., Pillai, S. G., Muglia, P., Middleton, L., Berrettini, W., Knouff, C. W., Yuan, X., Waeber, G., Vollenweider, P., Preisig, M., Wareham, N. J., Zhao, J. H., Loos, R. J., Barroso, I., Khaw, K. T., Grundy, S., Barter, P., Mahley, R., Kesaniemi, A., McPherson, R., Vincent, J. B., Strauss, J., Kennedy, J. L., Farmer, A., McGuffin, P., Day, R., Matthews, K., Bakke, P., Gulsvik, A., Lucae, S., Ising, M., Brueckl, T., Horstmann, S., Wichmann, H. E., Rawal, R., Dahmen, N., Lamina, C., Polasek, O., Zgaga, L., Huffman, J., Campbell, S., Kooner, J., Chambers, J. C., Burnett, M. S., Devaney, J. M., Pichard, A. D., Kent, K. M., Satler, L., Lindsay, J. M., Waksman, R., Epstein, S., Wilson, J. F., Wild, S. H., Campbell, H., Vitart, V., Reilly, M. P., Li, M., Qu, L., Wilensky, R., Matthai, W., Hakonarson, H. H., Rader, D. J., Franke, A., Wittig, M., Schäfer, A., Uda, M., Terracciano, A., Xiao, X., Busonero, F., Scheet, P., Schlessinger, D., St, D. C., Rujescu, D., Abecasis, G. R., Grabe, H. J., Teumer, A., Völzke, H., Petersmann, A., John, U., Rudan, I., Hayward, C., Wright, A. F., Kolcic, I., Wright, B. J., Thompson, J. R., Balmforth, A. J., Hall, A. S., Samani, N. J., Anderson, C. A., Ahmad, T., Mathew, C. G., Parkes, M., Satsangi, J., Caulfield, M., Munroe, P. B., Farrall, M., Dominiczak, A., Worthington, J., Thomson, W., Eyre, S., Barton, A., Mooser, V., Francks, C., Marchini, J., 2010. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. Nature Genetics 42, 436-440.

Liu, Q., Han, H., Wang, M., Yao, Y., Wen, L., Jiang, K., Ma, Y., Fan, R., Chen, J., Su, K., Yang, Z., Cui, W., Yuan, W., Jiang, X., Li, J., Payne, T. J., Wang, J., Li, M. D., 2018. Association and cis-mQTL analysis of variants in CHRNA3-A5, CHRNA7, CHRNB2, and CHRNB4 in relation to nicotine dependence in a Chinese Han population. Translational Psychiatry 8, 83.

Liu, T., Porter, J., Zhao, C., Zhu, H., Wang, N., Sun, Z., Mo, Y.-Y., Wang, Z., 2019. TADKB: Family classification and a knowledge base of topologically associating domains. BMC Genomics 20, 217.

Liu, Y., Liu, H., Sauvey, C., Yao, L., Zarnowska, E. D., Zhang, S.-C., 2013. Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. Nature Protocols 8, 1670-1679.

Livint Popa, L., Dragos, H., Pantelemon, C., Verisezan Rosu, O., Strilciuc, S., 2020. The Role of Quantitative EEG in the Diagnosis of Neuropsychiatric Disorders. Journal of Medicine and Life 13, 8-15.

Macqueen, D. A., Heckman, B. W., Blank, M. D., Janse Van Rensburg, K., Park, J. Y., Drobes, D. J., Evans, D. E., 2014. Variation in the α 5 nicotinic acetylcholine receptor subunit gene predicts cigarette smoking intensity as a function of nicotine content. The Pharmacogenomics Journal 14, 70-76.

Maisonneuve, I. M., Glick, S. D., 2003. Anti-addictive actions of an iboga alkaloid congener: a novel mechanism for a novel treatment. Pharmacol Biochem Behav 75, 607-618.

Mansvelder, H. D., van Aerde, K. I., Couey, J. J., Brussaard, A. B., 2006. Nicotinic modulation of neuronal networks: from receptors to cognition. Psychopharmacology (Berl) 184, 292-305.

Mar, A. C., Horner, A. E., Nilsson, S. R., Alsio, J., Kent, B. A., Kim, C. H., Holmes, A., Saksida, L. M., Bussey, T. J., 2013. The touchscreen operant platform for assessing executive function in rats and mice. Nat Protoc 8, 1985-2005.

Marees, A. T., Hammerschlag, A. R., Bastarache, L., de Kluiver, H., Vorspan, F., van den Brink, W., Smit, D. J., Denys, D., Gamazon, E. R., Li-Gao, R., Breetvelt, E. J., de Groot, M. C. H., Galesloot, T. E., Vermeulen, S. H., Poppelaars, J. L., Souverein, P. C., Keeman, R., de Mutsert, R., Noordam, R., Rosendaal, F. R., Stringa, N., Mook-Kanamori, D. O., Vaartjes, I., Kiemeney, L. A., den Heijer, M., van Schoor, N. M., Klungel, O. H., Maitland-Van der Zee, A. H., Schmidt, M. K., Polderman, T. J. C., van der

Leij, A. R., Posthuma, D., Derks, E. M., 2018. Exploring the role of low-frequency and rare exonic variants in alcohol and tobacco use. Drug Alcohol Depend 188, 94-101.
Marks, M. J., Pauly, J. R., Gross, S. D., Deneris, E. S., Hermans-Borgmeyer, I., Heinemann, S. F., Collins, A. C., 1992. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. J Neurosci 12, 2765-2784.
Maskos, U., 2020. The nicotinic receptor alpha5 coding polymorphism rs16969968 as a major target in disease: Functional dissection and remaining challenges. J Neurochem.
McCauley, J. L., Kenealy, S. J., Margulies, E. H., Schnetz-Boutaud, N., Gregory, S. G., Hauser, S. L., Oksenberg, J. R., Pericak-Vance, M. A., Haines, J. L., Mortlock, D. P., 2007. SNPs in Multi-species Conserved Sequences (MCS) as useful markers in association studies: a practical approach. BMC Genomics 8, 266.
McKay, B. E., Placzek, A. N., Dani, J. A., 2007. Regulation of synaptic transmission and plasticity by neuronal nicotinic acetylcholine receptors. Biochem Pharmacol 74, 1120-1133.
Mechawar, N., Saghatelyan, A., Grailhe, R., Scoriels, L., Gheusi, G., Gabellec, M. M., Lledo, P. M., Changeux, J. P., 2004. Nicotinic receptors regulate the survival of newborn neurons in the adult olfactory bulb. Proc Natl Acad Sci U S A 101, 9822-9826.
Mineur, Y. S., Abizaid, A., Rao, Y., Salas, R., DiLeone, R. J., Gundisch, D., Diano, S., De Biasi, M., Horvath, T. L., Gao, X. B., Picciotto, M. R., 2011. Nicotine decreases food intake through activation of POMC neurons. Science 332, 1330-1332.
Miranda, K., Girard-Dias, W., Attias, M., de Souza, W., Ramos, I., 2015. Three dimensional reconstruction by electron microscopy in the life sciences: An introduction for cell and tissue biologists: T HREE D IMENSIONAL R ECONSTRUCTION B Y E LECTRON M ICROSCOPY. Molecular Reproduction and Development 82, 530-547.
Morel, C., Fattore, L., Pons, S., Hay, Y. A., Marti, F., Lambolez, B., De Biasi, M., Lathrop, M., Fratta, W., Maskos, U., Faure, P., 2014. Nicotine consumption is regulated by a human polymorphism in dopamine neurons. Mol Psychiatry 19, 930-936.
Morley, B. J., 1997. The embryonic and post-natal expression of the nicotinic receptor α3-subunit in rat lower brainstem. Molecular Brain Research 48, 407-412.
Morrow, J. D., Flagel, S. B., 2016. Neuroscience of resilience and vulnerability for addiction medicine: From genes to behavior. Prog Brain Res 223, 3-18.
Morton, G., Nasirova, N., Sparks, D. W., Brodsky, M., Sivakumaran, S., Lambe, E. K., Turner, E. E., 2018. Chrna5-Expressing Neurons in the Interpeduncular Nucleus Mediate Aversion Primed by Prior Stimulation or Nicotine Exposure. J Neurosci 38, 6900-6920.
Nicholas, Cory R., Chen, J., Tang, Y., Southwell, Derek G., Chalmers, N., Vogt, D., Arnold, Christine M., Chen, Y.-Jiun J., Stanley, Edouard G., Elefanty, Andrew G., Sasai, Y., Alvarez-Buylla, A., Rubenstein, John L. R., Kriegstein, Arnold R., 2013. Functional Maturation of hPSC-Derived Forebrain Interneurons Requires an Extended Timeline and Mimics Human Neural Development. Cell Stem Cell 12, 573-586.
O'Neill, H. C., Wageman, C. R., Sherman, S. E., Grady, S. R., Marks, M. J., Stitzel, J. A., 2018. The interaction of the Chrna5 D398N variant with developmental nicotine exposure. Genes Brain Behav 17, e12474.
Oni, E. N., Halikere, A., Li, G., Toro-Ramos, A. J., Swerdel, M. R., Verpeut, J. L., Moore, J. C., Bello, N. T., Bierut, L. J., Goate, A., Tischfield, J. A., Pang, Z. P., Hart, R. P., 2016. Increased nicotine response in iPSC-derived human neurons carrying the CHRNA5 N398 allele. Scientific Reports 6, 34341.
Parker, C. C., Chen, H., Flagel, S. B., Geurts, A. M., Richards, J. B., Robinson, T. E., Solberg Woods, L. C., Palmer, A. A., 2014. Rats are the smart choice: Rationale for a renewed focus on rats in behavioral genetics. Neuropharmacology 76 Pt B, 250-258.
Parker, M. J., Beck, A., Luetje, C. W., 1998. Neuronal nicotinic receptor beta2 and beta4 subunits confer large differences in agonist binding affinity. Mol Pharmacol 54, 1132-1139.
Peng, C., Yan, Y., Kim, V. J., Engle, S. E., Berry, J. N., McIntosh, J. M., Neve, R. L., Drenan, R. M., 2019. Gene editing vectors for studying nicotinic acetylcholine receptors in cholinergic transmission. Eur J Neurosci 50, 2224-2238.

Pérez-Morales, R., González-Zamora, A., González-Delgado, M. F., Calleros Rincón, E. Y., Olivas Calderón, E. H., Martínez-Ramírez, O. C., Rubio, J., 2018. *CHRNA3* rs1051730 and *CHRNA5* rs16969968 polymorphisms are associated with heavy smoking, lung cancer, and chronic obstructive pulmonary disease in a mexican population. Annals of Human Genetics 82, 415-424.
Perry, D. C., Xiao, Y., Nguyen, H. N., Musachio, J. L., Dávila-Garcí, M. I., Kellar, K. J., 2002. Measuring nicotinic receptors with characteristics of α4β2, α3β2 and α3β4 subtypes in rat tissues by autoradiography. Journal of Neurochemistry 82, 468-481.
Peters, S. A., Huxley, R. R., Woodward, M., 2014. Do smoking habits differ between women and men in contemporary Western populations? Evidence from half a million people in the UK Biobank study. BMJ Open 4, e005663.
Peterson, R. E., Kuchenbaecker, K., Walters, R. K., Chen, C. Y., Popejoy, A. B., Periyasamy, S., Lam, M., Iyegbe, C., Strawbridge, R. J., Brick, L., Carey, C. E., Martin, A. R., Meyers, J. L., Su, J., Chen, J., Edwards, A. C., Kalungi, A., Koen, N., Majara, L., Schwarz, E., Smoller, J. W., Stahl, E. A., Sullivan, P. F., Vassos, E., Mowry, B., Prieto, M. L., Cuellar-Barboza, A., Bigdeli, T. B., Edenberg, H. J., Huang, H., Duncan, L. E., 2019. Genome-wide Association Studies in Ancestrally Diverse Populations: Opportunities, Methods, Pitfalls, and Recommendations. Cell 179, 589-603.
Petrovsky, N., Quednow, B. B., Ettinger, U., Schmechtig, A., Mossner, R., Collier, D. A., Kuhn, K. U., Maier, W., Wagner, M., Kumari, V., 2010. Sensorimotor gating is associated with CHRNA3 polymorphisms in schizophrenia and healthy volunteers. Neuropsychopharmacology 35, 1429-1439.
Picciotto, M. R., Kenny, P. J., 2013. Molecular mechanisms underlying behaviors related to nicotine addiction. Cold Spring Harb Perspect Med 3, a012112.
Polich, J., Criado, J. R., 2006. Neuropsychology and neuropharmacology of P3a and P3b. International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology 60, 172-185.
Poorthuis, R. B., Mansvelder, H. D., 2013. Nicotinic acetylcholine receptors controlling attention: behavior, circuits and sensitivity to disruption by nicotine. Biochem Pharmacol 86, 1089-1098.
Prochaska, J. J., Benowitz, N. L., 2019. Current advances in research in treatment and recovery: Nicotine addiction. Sci Adv 5, eaay9763.
Proulx, E., Piva, M., Tian, M. K., Bailey, C. D., Lambe, E. K., 2014. Nicotinic acetylcholine receptors in attention circuitry: the role of layer VI neurons of prefrontal cortex. Cell Mol Life Sci 71, 1225-1244.
Quick, M. W., Ceballos, R. M., Kasten, M., McIntosh, J. M., Lester, R. A., 1999. Alpha3beta4 subunit-containing nicotinic receptors dominate function in rat medial habenula neurons. Neuropharmacology 38, 769-783.
Radonjić, Nevena V., Ayoub, Albert E., Memi, F., Yu, X., Maroof, A., Jakovcevski, I., Anderson, Stewart A., Rakic, P., Zecevic, N., 2014. Diversity of Cortical Interneurons in Primates: The Role of the Dorsal Proliferative Niche. Cell Reports 9, 2139-2151.
Ramirez-Latorre, J., Yu, C. R., Qu, X., Perin, F., Karlin, A., Role, L., 1996. Functional contributions of alpha5 subunit to neuronal acetylcholine receptor channels. Nature 380, 347-351.
Rassadi, S., Krishnaswamy, A., Pie, B., McConnell, R., Jacob, M. H., Cooper, E., 2005. A null mutation for the alpha3 nicotinic acetylcholine (ACh) receptor gene abolishes fast synaptic activity in sympathetic ganglia and reveals that ACh output from developing preganglionic terminals is regulated in an activity-dependent retrograde manner. J Neurosci 25, 8555-8566.
Rigbi, A., Kanyas, K., Yakir, A., Greenbaum, L., Pollak, Y., Ben-Asher, E., Lancet, D., Kertzman, S., Lerer, B., 2008. Why do young women smoke? V. Role of direct and interactive effects of nicotinic cholinergic receptor gene variation on neurocognitive function. Genes, Brain and Behavior 7, 164-172.
Robinson, J. D., Versace, F., Lam, C. Y., Minnix, J. A., Engelmann, J. M., Cui, Y., Karam-Hage, M., Shete, S. S., Tomlinson, G. E., Chen, T. T.-L., Wetter, D. W., Green, C. E., Cinciripini, P. M., 2013. The CHRNA3 rs578776 Variant is Associated with an Intrinsic Reward Sensitivity Deficit in Smokers. Frontiers in Psychiatry 4, 114.
Rojewski, A. M., Baldassarri, S., Cooperman, N. A., Gritz, E. R., Leone, F. T., Piper, M. E., Toll, B. A., Warren, G. W., Network, C. W. o. t. S. f. R. o. N. a. T. S. T., 2016. Exploring Issues of Comorbid

Conditions in People Who Smoke. Nicotine & Tobacco Research: Official Journal of the Society for Research on Nicotine and Tobacco 18, 1684-1696.
Roy, S. W., Fedorov, A., Gilbert, W., 2003. Large-scale comparison of intron positions in mammalian genes shows intron loss but no gain. Proc Natl Acad Sci U S A 100, 7158-7162.
Saccone, S. F., Hinrichs, A. L., Saccone, N. L., Chase, G. A., Konvicka, K., Madden, P. A., Breslau, N., Johnson, E. O., Hatsukami, D., Pomerleau, O., Swan, G. E., Goate, A. M., Rutter, J., Bertelsen, S., Fox, L., Fugman, D., Martin, N. G., Montgomery, G. W., Wang, J. C., Ballinger, D. G., Rice, J. P., Bierut, L. J., 2007. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum Mol Genet 16, 36-49.
Salas, R., Cook, K. D., Bassetto, L., De Biasi, M., 2004a. The alpha3 and beta4 nicotinic acetylcholine receptor subunits are necessary for nicotine-induced seizures and hypolocomotion in mice. Neuropharmacology 47, 401-407.
Salas, R., Orr-Urtreger, A., Broide, R. S., Beaudet, A., Paylor, R., De Biasi, M., 2003a. The nicotinic acetylcholine receptor subunit alpha 5 mediates short-term effects of nicotine in vivo. Mol Pharmacol 63, 1059-1066.
Salas, R., Pieri, F., De Biasi, M., 2004b. Decreased signs of nicotine withdrawal in mice null for the beta4 nicotinic acetylcholine receptor subunit. J Neurosci 24, 10035-10039.
Salas, R., Pieri, F., Fung, B., Dani, J. A., De Biasi, M., 2003b. Altered anxiety-related responses in mutant mice lacking the beta4 subunit of the nicotinic receptor. J Neurosci 23, 6255-6263.
Salas, R., Sturm, R., Boulter, J., De Biasi, M., 2009. Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. J Neurosci 29, 3014-3018.
Scherer, G., Lee, P. N., 2014. Smoking behaviour and compensation: a review of the literature with meta-analysis. Regul Toxicol Pharmacol 70, 615-628.
Schizophrenia Working Group of the Psychiatric Genomics, C., Ripke, S., Neale, B. M., Corvin, A., Walters, J. T. R., Farh, K.-H., Holmans, P. A., Lee, P., Bulik-Sullivan, B., Collier, D. A., Huang, H., Pers, T. H., Agartz, I., Agerbo, E., Albus, M., Alexander, M., Amin, F., Bacanu, S. A., Begemann, M., Belliveau Jr, R. A., Bene, J., Bergen, S. E., Bevilacqua, E., Bigdeli, T. B., Black, D. W., Bruggeman, R., Buccola, N. G., Buckner, R. L., Byerley, W., Cahn, W., Cai, G., Campion, D., Cantor, R. M., Carr, V. J., Carrera, N., Catts, S. V., Chambert, K. D., Chan, R. C. K., Chen, R. Y. L., Chen, E. Y. H., Cheng, W., Cheung, E. F. C., Ann Chong, S., Robert Cloninger, C., Cohen, D., Cohen, N., Cormican, P., Craddock, N., Crowley, J. J., Curtis, D., Davidson, M., Davis, K. L., Degenhardt, F., Del Favero, J., Demontis, D., Dikeos, D., Dinan, T., Djurovic, S., Donohoe, G., Drapeau, E., Duan, J., Dudbridge, F., Durmishi, N., Eichhammer, P., Eriksson, J., Escott-Price, V., Essioux, L., Fanous, A. H., Farrell, M. S., Frank, J., Franke, L., Freedman, R., Freimer, N. B., Friedl, M., Friedman, J. I., Fromer, M., Genovese, G., Georgieva, L., Giegling, I., Giusti-Rodríguez, P., Godard, S., Goldstein, J. I., Golimbet, V., Gopal, S., Gratten, J., de Haan, L., Hammer, C., Hamshere, M. L., Hansen, M., Hansen, T., Haroutunian, V., Hartmann, A. M., Henskens, F. A., Herms, S., Hirschhorn, J. N., Hoffmann, P., Hofman, A., Hollegaard, M. V., Hougaard, D. M., Ikeda, M., Joa, I., Julià, A., Kahn, R. S., Kalaydjieva, L., Karachanak-Yankova, S., Karjalainen, J., Kavanagh, D., Keller, M. C., Kennedy, J. L., Khrunin, A., Kim, Y., Klovins, J., Knowles, J. A., Konte, B., Kucinskas, V., Ausrele Kucinskiene, Z., Kuzelova-Ptackova, H., Kähler, A. K., Laurent, C., Lee Chee Keong, J., Hong Lee, S., Legge, S. E., Lerer, B., Li, M., Li, T., Liang, K.-Y., Lieberman, J., Limborska, S., Loughland, C. M., Lubinski, J., Lönnqvist, J., Macek Jr, M., Magnusson, P. K. E., Maher, B. S., Maier, W., Mallet, J., Marsal, S., Mattheisen, M., Mattingsdal, M., McCarley, R. W., McDonald, C., McIntosh, A. M., Meier, S., Meijer, C. J., Melegh, B., Melle, I., Mesholam-Gately, R. I., Metspalu, A., Michie, P. T., Milani, L., Milanova, V., Mokrab, Y., Morris, D. W., Mors, O., Murphy, K. C., Murray, R. M., Myin-Germeys, I., Müller-Myhsok, B., Nelis, M., Nenadic, I., Nertney, D. A., Nestadt, G., Nicodemus, K. K., Nikitina-Zake, L., Nisenbaum, L., Nordin, A., O'Callaghan, E., O'Dushlaine, C., O'Neill, F. A., Oh, S.-Y., Olincy, A., Olsen, L., Van Os, J., Pantelis, C., Papadimitriou, G. N., Papiol, S., Parkhomenko, E., Pato, M. T., Paunio, T., Pejovic-Milovancevic, M., Perkins, D. O., Pietiläinen, O., Pimm, J., Pocklington, A. J., Powell, J., Price, A., Pulver, A. E., Purcell, S. M., Quested, D., Rasmussen, H. B., Reichenberg, A., Reimers, M. A., Richards, A. L., Roffman, J. L., Roussos, P., Ruderfer, D. M., Salomaa, V., Sanders, A. R., Schall, U., Schubert, C. R., Schulze, T. G., Schwab, S. G., Scolnick, E. M., Scott, R. J., Seidman, L. J.,

Shi, J., Sigurdsson, E., Silagadze, T., Silverman, J. M., Sim, K., Slominsky, P., Smoller, J. W., So, H.-C., Spencer, C. A., Stahl, E. A., Stefansson, H., Steinberg, S., Stogmann, E., Straub, R. E., Strengman, E., Strohmaier, J., Scott Stroup, T., Subramaniam, M., Suvisaari, J., Svrakic, D. M., Szatkiewicz, J. P., Söderman, E., Thirumalai, S., Toncheva, D., Tosato, S., Veijola, J., Waddington, J., Walsh, D., Wang, D., Wang, Q., Webb, B. T., Weiser, M., Wildenauer, D. B., Williams, N. M., Williams, S., Witt, S. H., Wolen, A. R., Wong, E. H. M., Wormley, B. K., Simon Xi, H., Zai, C. C., Zheng, X., Zimprich, F., Wray, N. R., Stefansson, K., Visscher, P. M., Trust Case-Control Consortium, W., Adolfsson, R., Andreassen, O. A., Blackwood, D. H. R., Bramon, E., Buxbaum, J. D., Børglum, A. D., Cichon, S., Darvasi, A., Domenici, E., Ehrenreich, H., Esko, T., Gejman, P. V., Gill, M., Gurling, H., Hultman, C. M., Iwata, N., Jablensky, A. V., Jönsson, E. G., Kendler, K. S., Kirov, G., Knight, J., Lencz, T., Levinson, D. F., Li, Q. S., Liu, J., Malhotra, A. K., McCarroll, S. A., McQuillin, A., Moran, J. L., Mortensen, P. B., Mowry, B. J., Nöthen, M. M., Ophoff, R. A., Owen, M. J., Palotie, A., Pato, C. N., Petryshen, T. L., Posthuma, D., Rietschel, M., Riley, B. P., Rujescu, D., Sham, P. C., Sklar, P., St Clair, D., Weinberger, D. R., Wendland, J. R., Werge, T., Daly, M. J., Sullivan, P. F., O'Donovan, M. C., 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511, 421-427.

Schmitz, L. L., Gard, A. M., Ware, E. B., 2019. Examining sex differences in pleiotropic effects for depression and smoking using polygenic and gene-region aggregation techniques. Am J Med Genet B Neuropsychiatr Genet 180, 448-468.

Scholze, P., Koth, G., Orr-Urtreger, A., Huck, S., 2012. Subunit composition of alpha5-containing nicotinic receptors in the rodent habenula. J Neurochem 121, 551-560.

Schote, A. B., C, A. L. S., Pabst, K., Meier, J. K., Frings, C., Meyer, J., 2019. Sex, ADHD symptoms, and CHRNA5 genotype influence reaction time but not response inhibition. J Neurosci Res 97, 215-224.

Schuch, J. B., Polina, E. R., Rovaris, D. L., Kappel, D. B., Mota, N. R., Cupertino, R. B., Silva, K. L., Guimarães-da-Silva, P. O., Karam, R. G., Salgado, C. A. I., White, M. J., Rohde, L. A., Grevet, E. H., Bau, C. H. D., 2016. Pleiotropic effects of Chr15q25 nicotinic gene cluster and the relationship between smoking, cognition and ADHD. Journal of Psychiatric Research 80, 73-78.

Sharp, B. M., Chen, H., 2019. Neurogenetic determinants and mechanisms of addiction to nicotine and smoked tobacco. Eur J Neurosci 50, 2164-2179.

Sheffield, E. B., Quick, M. W., Lester, R. A., 2000. Nicotinic acetylcholine receptor subunit mRNA expression and channel function in medial habenula neurons. Neuropharmacology 39, 2591-2603.

Sherva, R., Kranzler, H. R., Yu, Y., Logue, M. W., Poling, J., Arias, A. J., Anton, R. F., Oslin, D., Farrer, L. A., Gelernter, J., 2010. Variation in nicotinic acetylcholine receptor genes is associated with multiple substance dependence phenotypes. Neuropsychopharmacology 35, 1921-1931.

Sherva, R., Wilhelmsen, K., Pomerleau, C. S., Chasse, S. A., Rice, J. P., Snedecor, S. M., Bierut, L. J., Neuman, R. J., Pomerleau, O. F., 2008. Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with 'pleasurable buzz' during early experimentation with smoking. Addiction 103, 1544-1552.

Shorey-Kendrick, L. E., Ford, M. M., Allen, D. C., Kuryatov, A., Lindstrom, J., Wilhelm, L., Grant, K. A., Spindel, E. R., 2015. Nicotinic receptors in non-human primates: Analysis of genetic and functional conservation with humans. Neuropharmacology 96, 263-273.

Silva, M. R., Gattás, G. J. F., Antonio, J., Firigato, I., Curioni, O. A., Gonçalves, F. d. T., 2019. Polymorphisms of *CHRNA3* and *CHRNA5* : Head and neck cancer and cigarette consumption intensity in a Brazilian population. Molecular Genetics & Genomic Medicine 7, doi: 10.1002/mgg1003.1998. Epub 2019 Oct 1009.

Sine, S. M., Engel, A. G., 2006. Recent advances in Cys-loop receptor structure and function. Nature 440, 448-455.

Slimak, M. A., Ables, J. L., Frahm, S., Antolin-Fontes, B., Santos-Torres, J., Moretti, M., Gotti, C., Ibanez-Tallon, I., 2014. Habenular expression of rare missense variants of the beta4 nicotinic receptor subunit alters nicotine consumption. Front Hum Neurosci 8, 12.

Smedley, D., Salimova, E., Rosenthal, N., 2011. Cre recombinase resources for conditional mouse mutagenesis. Methods 53, 411-416.

Smith, J. R., Hayman, G. T., Wang, S. J., Laulederkind, S. J. F., Hoffman, M. J., Kaldunski, M. L., Tutaj, M., Thota, J., Nalabolu, H. S., Ellanki, S. L. R., Tutaj, M. A., De Pons, J. L., Kwitek, A. E., Dwinell, M. R., Shimoyama, M. E., 2020. The Year of the Rat: The Rat Genome Database at 20: a multi-species knowledgebase and analysis platform. Nucleic Acids Res 48, D731-D742.
Solda, G., Boi, S., Duga, S., Fornasari, D., Benfante, R., Malcovati, M., Tenchini, M. L., 2005. In vivo RNA-RNA duplexes from human alpha3 and alpha5 nicotinic receptor subunit mRNAs. Gene 345, 155-164.
Spanagel, R., 2017. Animal models of addiction. Dialogues Clin Neurosci 19, 247-258.
Stevens, V. L., Bierut, L. J., Talbot, J. T., Wang, J. C., Sun, J., Hinrichs, A. L., Thun, M. J., Goate, A., Calle, E. E., 2008. Nicotinic receptor gene variants influence susceptibility to heavy smoking. Cancer Epidemiol Biomarkers Prev 17, 3517-3525.
Stoker, A. K., Olivier, B., Markou, A., 2012. Role of alpha7- and beta4-containing nicotinic acetylcholine receptors in the affective and somatic aspects of nicotine withdrawal: studies in knockout mice. Behav Genet 42, 423-436.
Stout, J., Hozer, F., Coste, A., Mauconduit, F., Djebrani-Oussedik, N., Sarrazin, S., Poupon, J., Meyrel, M., Romanzetti, S., Etain, B., Rabrait-Lerman, C., Houenou, J., Bellivier, F., Duchesnay, E., Boumezbeur, F., 2020. Accumulation of Lithium in the Hippocampus of Patients With Bipolar Disorder: A Lithium-7 Magnetic Resonance Imaging Study at 7 Tesla. Biol Psychiatry, S0006322320312932.
Sun, Q., Li, X., Ren, M., Zhao, M., Zhong, Q., Ren, Y., Luo, P., Ni, H., Zhang, X., Zhang, C., Yuan, J., Li, A., Luo, M., Gong, H., Luo, Q., 2019. A whole-brain map of long-range inputs to GABAergic interneurons in the mouse medial prefrontal cortex. Nature Neuroscience 22, 1357-1370.
Taly, A., Corringer, P. J., Guedin, D., Lestage, P., Changeux, J. P., 2009. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat Rev Drug Discov 8, 733-750.
Tapia, L., Kuryatov, A., Lindstrom, J., 2007. Ca2+ permeability of the (alpha4)3(beta2)2 stoichiometry greatly exceeds that of (alpha4)2(beta2)3 human acetylcholine receptors. Mol Pharmacol 71, 769-776.
Thorgeirsson, T. E., Geller, F., Sulem, P., Rafnar, T., Wiste, A., Magnusson, K. P., Manolescu, A., Thorleifsson, G., Stefansson, H., Ingason, A., Stacey, S. N., Bergthorsson, J. T., Thorlacius, S., Gudmundsson, J., Jonsson, T., Jakobsdottir, M., Saemundsdottir, J., Olafsdottir, O., Gudmundsson, L. J., Bjornsdottir, G., Kristjansson, K., Skuladottir, H., Isaksson, H. J., Gudbjartsson, T., Jones, G. T., Mueller, T., Gottsater, A., Flex, A., Aben, K. K. H., de Vegt, F., Mulders, P. F. A., Isla, D., Vidal, M. J., Asin, L., Saez, B., Murillo, L., Blondal, T., Kolbeinsson, H., Stefansson, J. G., Hansdottir, I., Runarsdottir, V., Pola, R., Lindblad, B., van Rij, A. M., Dieplinger, B., Haltmayer, M., Mayordomo, J. I., Kiemeney, L. A., Matthiasson, S. E., Oskarsson, H., Tyrfingsson, T., Gudbjartsson, D. F., Gulcher, J. R., Jonsson, S., Thorsteinsdottir, U., Kong, A., Stefansson, K., 2008. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature 452, 638-642.
Tian, M. K., Bailey, C. D. C., De Biasi, M., Picciotto, M. R., Lambe, E. K., 2011. Plasticity of Prefrontal Attention Circuitry: Upregulated Muscarinic Excitability in Response to Decreased Nicotinic Signaling Following Deletion of 5 or 2 Subunits. Journal of Neuroscience 31, 16458-16463.
Tobacco and Genetics Consortium, 2010. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nature Genetics 42, 441-447.
Toll, L., Zaveri, N. T., Polgar, W. E., Jiang, F., Khroyan, T. V., Zhou, W., Xie, X. S., Stauber, G. B., Costello, M. R., Leslie, F. M., 2012. AT-1001: A high affinity and selective α3B4 nicotinic acetylcholine receptor antagonist blocks nicotine self-administration in rats. Neuropsychopharmacology 37, 1367-1376.
Tuesta, L. M., Chen, Z., Duncan, A., Fowler, C. D., Ishikawa, M., Lee, B. R., Liu, X. A., Lu, Q., Cameron, M., Hayes, M. R., Kamenecka, T. M., Pletcher, M., Kenny, P. J., 2017. GLP-1 acts on habenular avoidance circuits to control nicotine intake. Nat Neurosci 20, 708-716.
Turner, J. R., Kellar, K. J., 2005. Nicotinic cholinergic receptors in the rat cerebellum: Multiple heteromeric subtypes. Journal of Neuroscience 25, 9258-9265.

Vazin, T., Ashton, R. S., Conway, A., Rode, N. A., Lee, S. M., Bravo, V., Healy, K. E., Kane, R. S., Schaffer, D. V., 2014. The effect of multivalent Sonic hedgehog on differentiation of human embryonic stem cells into dopaminergic and GABAergic neurons. Biomaterials 35, 941-948.
Vermeulen, J. M., Wootton, R. E., Treur, J. L., Sallis, H. M., Jones, H. J., Zammit, S., van den Brink, W., Goodwin, G. M., de Haan, L., Munafò, M. R., 2019. Smoking and the risk for bipolar disorder: evidence from a bidirectional Mendelian randomisation study. The British Journal of Psychiatry, 1-7.
Wada, E., McKinnon, D., Heinemann, S., Patrick, J., Swanson, L. W., 1990. The distribution of mRNA encoded by a new member of the neuronal nicotinic acetylcholine receptor gene family (alpha 5) in the rat central nervous system. Brain Res 526, 45-53.
Wang, J. C., Grucza, R., Cruchaga, C., Hinrichs, A. L., Bertelsen, S., Budde, J. P., Fox, L., Goldstein, E., Reyes, O., Saccone, N., Saccone, S., Xuei, X., Bucholz, K., Kuperman, S., Nurnberger, J., Jr., Rice, J. P., Schuckit, M., Tischfield, J., Hesselbrock, V., Porjesz, B., Edenberg, H. J., Bierut, L. J., Goate, A. M., 2009. Genetic variation in the CHRNA5 gene affects mRNA levels and is associated with risk for alcohol dependence. Mol Psychiatry 14, 501-510.
Weiss, R. B., Baker, T. B., Cannon, D. S., von Niederhausern, A., Dunn, D. M., Matsunami, N., Singh, N. A., Baird, L., Coon, H., McMahon, W. M., Piper, M. E., Fiore, M. C., Scholand, M. B., Connett, J. E., Kanner, R. E., Gahring, L. C., Rogers, S. W., Hoidal, J. R., Leppert, M. F., 2008. A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. PLoS Genet 4, e1000125.
WHO, https://www.who.int/news-room/fact-sheets/detail/tobacco.
Winterer, G., Mittelstrass, K., Giegling, I., Lamina, C., Fehr, C., Brenner, H., Breitling, L. P., Nitz, B., Raum, E., Müller, H., Gallinat, J., Gal, A., Heim, K., Prokisch, H., Meitinger, T., Hartmann, A. M., Möller, H.-J., Gieger, C., Wichmann, H. E., Illig, T., Dahmen, N., Rujescu, D., 2010. Risk gene variants for nicotine dependence in the CHRNA5-CHRNA3-CHRNB4 cluster are associated with cognitive performance. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics 153B, 1448-1458.
Winzer-Serhan, U. H., Leslie, F. M., 1997. Codistribution of nicotinic acetylcholine receptor subunit α3 and β4 mRNAs during rat brain development. Journal of Comparative Neurology 386, 540-554.
Wu, J., Lukas, R. J., 2011. Naturally-expressed nicotinic acetylcholine receptor subtypes. Biochem Pharmacol 82, 800-807.
Xie, P., Kranzler, H. R., Zhang, H., Oslin, D., Anton, R. F., Farrer, L. A., Gelernter, J., 2012. Childhood adversity increases risk for nicotine dependence and interacts with alpha5 nicotinic acetylcholine receptor genotype specifically in males. Neuropsychopharmacology 37, 669-676.
Xu, W., Gelber, S., Orr-Urtreger, A., Armstrong, D., Lewis, R. A., Ou, C. N., Patrick, J., Role, L., De Biasi, M., Beaudet, A. L., 1999a. Megacystis, mydriasis, and ion channel defect in mice lacking the alpha3 neuronal nicotinic acetylcholine receptor. Proc Natl Acad Sci U S A 96, 5746-5751.
Xu, W., Orr-Urtreger, A., Nigro, F., Gelber, S., Sutcliffe, C. B., Armstrong, D., Patrick, J. W., Role, L. W., Beaudet, A. L., De Biasi, M., 1999b. Multiorgan Autonomic Dysfunction in Mice Lacking the β2 and the β4 Subunits of Neuronal Nicotinic Acetylcholine Receptors. The Journal of Neuroscience 19, 9298-9305.
Yoo, P. E., John, S. E., Farquharson, S., Cleary, J. O., Wong, Y. T., Ng, A., Mulcahy, C. B., Grayden, D. B., Ordidge, R. J., Opie, N. L., O'Brien, T. J., Oxley, T. J., Moffat, B. A., 2018. 7T-fMRI: Faster temporal resolution yields optimal BOLD sensitivity for functional network imaging specifically at high spatial resolution. NeuroImage 164, 214-229.
Yucel, M., Oldenhof, E., Ahmed, S. H., Belin, D., Billieux, J., Bowden-Jones, H., Carter, A., Chamberlain, S. R., Clark, L., Connor, J., Daglish, M., Dom, G., Dannon, P., Duka, T., Fernandez-Serrano, M. J., Field, M., Franken, I., Goldstein, R. Z., Gonzalez, R., Goudriaan, A. E., Grant, J. E., Gullo, M. J., Hester, R., Hodgins, D. C., Le Foll, B., Lee, R. S. C., Lingford-Hughes, A., Lorenzetti, V., Moeller, S. J., Munafo, M. R., Odlaug, B., Potenza, M. N., Segrave, R., Sjoerds, Z., Solowij, N., van den Brink, W., van Holst, R. J., Voon, V., Wiers, R., Fontenelle, L. F., Verdejo-Garcia, A., 2019. A transdiagnostic dimensional approach towards a neuropsychological assessment for addiction: an international Delphi consensus study. Addiction 114, 1095-1109.

Zhang, H., Kranzler, H. R., Poling, J., Gelernter, J., 2010. Variation in the Nicotinic Acetylcholine Receptor Gene Cluster CHRNA5–CHRNA3–CHRNB4 and Its Interaction with Recent Tobacco Use Influence Cognitive Flexibility. Neuropsychopharmacology 35, 2211-2224.
Zhang, X., Lan, T., Wang, T., Xue, W., Tong, X., Ma, T., Liu, G., Lu, Q., 2019. Considering Genetic Heterogeneity in the Association Analysis Finds Genes Associated With Nicotine Dependence. Frontiers in Genetics 10, 448.
Zhao-Shea, R., Liu, L., Pang, X., Gardner, P. D., Tapper, A. R., 2013. Activation of GABAergic neurons in the interpeduncular nucleus triggers physical nicotine withdrawal symptoms. Curr Biol 23, 2327-2335.
Zoli, M., Le Novere, N., Hill, J. A., Changeux, J. P., 1995. Developmental regulation of nicotinic ACh receptor subunit mRNAs in the rat central and peripheral nervous systems. Journal of Neuroscience 15, 1912-1939.
Zoli, M., Lena, C., Picciotto, M. R., Changeux, J. P., 1998. Identification of four classes of brain nicotinic receptors using beta2 mutant mice. J Neurosci 18, 4461-4472.
Zoli, M., Pistillo, F., Gotti, C., 2015. Diversity of native nicotinic receptor subtypes in mammalian brain. Neuropharmacology 96, 302-311.

Database	*URL*	*Information provided*
ENSEMBL v. 98	http://grch37.ensembl.org/Homo_sapiens	National Institutes of Health (NIH) information hub for gene structure, mapping, function and variants
Single Nucleotide Polymorphism Database (dbSNP) v. 151	https://www.ncbi.nlm.nih.gov › snp	ENSEMBL database for SNPs
Genotype-Tissue Expression (GTEx) portal v. 8 (Baran et al., 2015)	https://gtexportal.org/	Tissue- and variant- specific gene expression data
Methylation quantitative trait loci database (mQTLdb) (Gaunt et al., 2016)	http://www.mqtldb.org	Genome-wide DNA methylation analysis of 1,000 mother-child pairs at serial time points across the life-course (ARIES)
Regulome database (regulomeDB) (Boyle et al., 2012)	http://www.regulomedb.org	DNA features and regulatory elements in non-coding regions
Topologically Associating Domain Knowledge Base (TADKB) (Liu et al., 2019)	http://dna.cs.miami.edu/TADKB/	Exploring topologically associating domains (TADs)
DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources (DECIPHER) v. 9.30 (Lek et al., 2016)	https://decipher.sanger.ac.uk	Gene tolerance to mutations [score of tolerance to loss-of-function mutations (pLI), level of haploinsufficiency]

Table 1: Main databases used in the present review for gene function and properties and variant frequency and impact.

SNP id and risk allele	Gene	Position on chr15 (base pairs)	REF Allele	ALT Allele	MAF in European populations	MAF in African-American populations	MAF in East Asian populations	Number of significant eQTLs for the cluster in the brain[a]	Number of significant mQTLs in the brain[b]	RegulomeDB score
rs588765	***CHRNA5***	78865424	T	C	0.6123	0.7511	0.8284	19	9	N/A
rs17486278		78867481	A	C	0.3658	0.2973	0.2887	10	6	2b
rs680244		78871287	T	C	0.6103	0.6036	0.7698	18	8	N/A
rs569207		78873118	C	T	0.2445	0.3162	0.4812	5	7	N/A
rs17408276		78881617	T	C	0.3429	0.0651	0.1726	17	7	N/A
rs16969968		78882924	G	A	0.3658	0.0234	0.0268	13	6	5
rs615470		78885987	T	C	0.6561	0.6316	0.8264	17	7	N/A
rs578776	***CHRNA3***	78888399	G	A	0.2813	0.6036	0.8006	3	6	6
rs1051730		78894338	G	A	0.3688	0.087	0.0268	14	6	6
rs8023462	***CHRNB4***	78914733	C	T	0.664	0.8374	0.7877	17	7	4
rs1948		78917398	A	G	0.6809	0.7814	0.5188	16	4	4
rs7178270[c]		78921076	C	G	0.3638	0.32	0.3978	18	8	5
rs11636753		78928945	G	T	0.3797	0.2988	0.1667	N/A	10	2b
rs11637890[c]		78935418	C	G	0.3678	0.1324	0.1002	18	10	1f

Table 2: Single Nucleotide Polymorphisms (SNPs) reliably associated with nicotine addiction and the width of their functional impact on gene expression, known modulation of DNA transcription. SNP id, SNP name according to the dbSNP database v.153 ; eQTL, expression quantitative trait loci (i.e. associations with altered gene expression) ; mQTL, methylation quantitative trait loci (i.e. associations with altered gene methylation) in samples from middle-aged individuals. All mQTL sites were cis, meaning that increased methylation is associated with decreased DNA transcription. RegulomeDB scores read as listed. N/A, not available.[a]cluster refers to *CHRNA5-A3-B4* genes and *RP11-650L12.2* and brain to brain regions as defined by GTEx portal (https://gtexportal.org/home/), except for the spinal chord. [b]CpG islands located 50 kbp up- or downstream *CHRNA5*, *CHRNA3* or *CHRNB4* were considered.

All were trans methylation sites. [c]rs11637890 and rs7178270 were the only ones to be associated with upregulation of *CHRNA3*, *CHRNA5* and *RP11-650L12.2*.

Score	Supporting data
1a	eQTL + TF binding + matched TF motif + matched DNase Footprint + DNase peak
1b	eQTL + TF binding + any motif + DNase Footprint + DNase peak
1c	eQTL + TF binding + matched TF motif + DNase peak
1d	eQTL + TF binding + any motif + DNase peak
1e	eQTL + TF binding + matched TF motif
1f	eQTL + TF binding / DNase peak
2a	TF binding + matched TF motif + matched DNase Footprint + DNase peak
2b	TF binding + any motif + DNase Footprint + DNase peak
2c	TF binding + matched TF motif + DNase peak
3a	TF binding + any motif + DNase peak
3b	TF binding + matched TF motif
4	TF binding + DNase peak
5	TF binding or DNase peak
6	other

Table 3: RegulomeDB score classification (from http://www.regulomedb.org). eQTL, expression quantitative trait loci; TF, transcription factor.

Figure legends

Figure 1: Scheme representing the expression of *CHRNA5-B4-A3* cluster genes in the brain (GTEx database V. 8)

Figure 2: Scheme representing the brain eQTLs for rs680244, rs1051730 and rs8023462 Single Nucleotide Polymorphisms (SNPs) from the cluster. All normalized effect sizes (NES) are below zero, indicating downregulation of gene expression. RP11-650L12.2 encodes an antisense RNA that represses *CHRNA5* expression. Only significant associations are displayed.

Figure 3: Linkage Disequilibrium (LD) map in European populations of Single Nucleotide Polymorphisms associated with Nicotine Addiction in the cluster. LD is expressed as minor alleles correlation (r^2). The more red the color, the higher the correlation.

Table(s)

Database	***URL***	***Information provided***
ENSEMBL v. 98	http://grch37.ensembl.org/Homo_sapiens	National Institutes of Health (NIH) information hub for gene structure, mapping, function and variants
Single Nucleotide Polymorphism Database (dbSNP) v. 151	https://www.ncbi.nlm.nih.gov › snp	ENSEMBL database for SNPs
Genotype-Tissue Expression (GTEx) portal v. 8 (Baran et al., 2015)	https://gtexportal.org/	Tissue- and variant- specific gene expression data
Methylation quantitative trait loci database (mQTLdb) (Gaunt et al., 2016)	http://www.mqtldb.org	Genome-wide DNA methylation analysis of 1,000 mother-child pairs at serial time points across the life-course (ARIES)
Regulome database (regulomeDB) (Boyle et al., 2012)	http://www.regulomedb.org	DNA features and regulatory elements in non-coding regions
Topologically Associating Domain Knowledge Base (TADKB) (Liu et al., 2019)	http://dna.cs.miami.edu/TADKB/	Exploring topologically associating domains (TADs)
DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources (DECIPHER) v. 9.30 (Lek et al., 2016)	https://decipher.sanger.ac.uk	Gene tolerance to mutations [score of tolerance to loss-of-function mutations (pLI), level of haploinsufficiency]

Table 1: Main databases used in the present review for gene function and properties and variant frequency and impact.

SNP id and risk allele	Gene	Position on chr15 (base pairs)	REF Allele	ALT Allele	MAF in European populations	MAF in African-American populations	MAF in East Asian populations	Number of significant eQTLs for the cluster in the brain[a]	Number of significant mQTLs in the brain[b]	RegulomeDB score
rs588765	***CHRNA5***	78865424	T	C	0.6123	0.7511	0.8284	19	9	N/A
rs17486278		78867481	A	C	0.3658	0.2973	0.2887	10	6	2b
rs680244		78871287	T	C	0.6103	0.6036	0.7698	18	8	N/A
rs569207		78873118	C	T	0.2445	0.3162	0.4812	5	7	N/A
rs17408276		78881617	T	C	0.3429	0.0651	0.1726	17	7	N/A
rs16969968		78882924	G	A	0.3658	0.0234	0.0268	13	6	5
rs615470		78885987	T	C	0.6561	0.6316	0.8264	17	7	N/A
rs578776	***CHRNA3***	78888399	G	A	0.2813	0.6036	0.8006	3	6	6
rs1051730		78894338	G	A	0.3688	0.087	0.0268	14	6	6
rs8023462	***CHRNB4***	78914733	C	T	0.664	0.8374	0.7877	17	7	4
rs1948		78917398	A	G	0.6809	0.7814	0.5188	16	4	4
rs7178270[c]		78921076	C	G	0.3638	0.32	0.3978	18	8	5
rs11636753		78928945	G	T	0.3797	0.2988	0.1667	N/A	10	2b
rs11637890[c]		78935418	C	G	0.3678	0.1324	0.1002	18	10	1f

Table 2: Single Nucleotide Polymorphisms (SNPs) reliably associated with nicotine addiction and the width of their functional impact on gene expression, known and modulation of DNA transcription. SNP id, SNP name according to the dbSNP database v.153 ; eQTL, expression quantitative trait loci (i.e. associations with altered gene expression) ; mQTL, methylation quantitative trait loci (i.e. associations with altered gene methylation) in samples from middle-aged individuals. All mQTL sites were cis, meaning that increased methylation is associated with decreased DNA transcription. RegulomeDB scores read as listed. N/A, not available.[a]cluster refers to *CHRNA5-A3-B4* genes and *RP11-650L12.2* and brain to brain regions as defined by GTEx portal (https://gtexportal.org/home/), except for the spinal chord. [b]CpG islands located 50 kbp up- or downstream *CHRNA5*, *CHRNA3* or *CHRNB4* were considered.

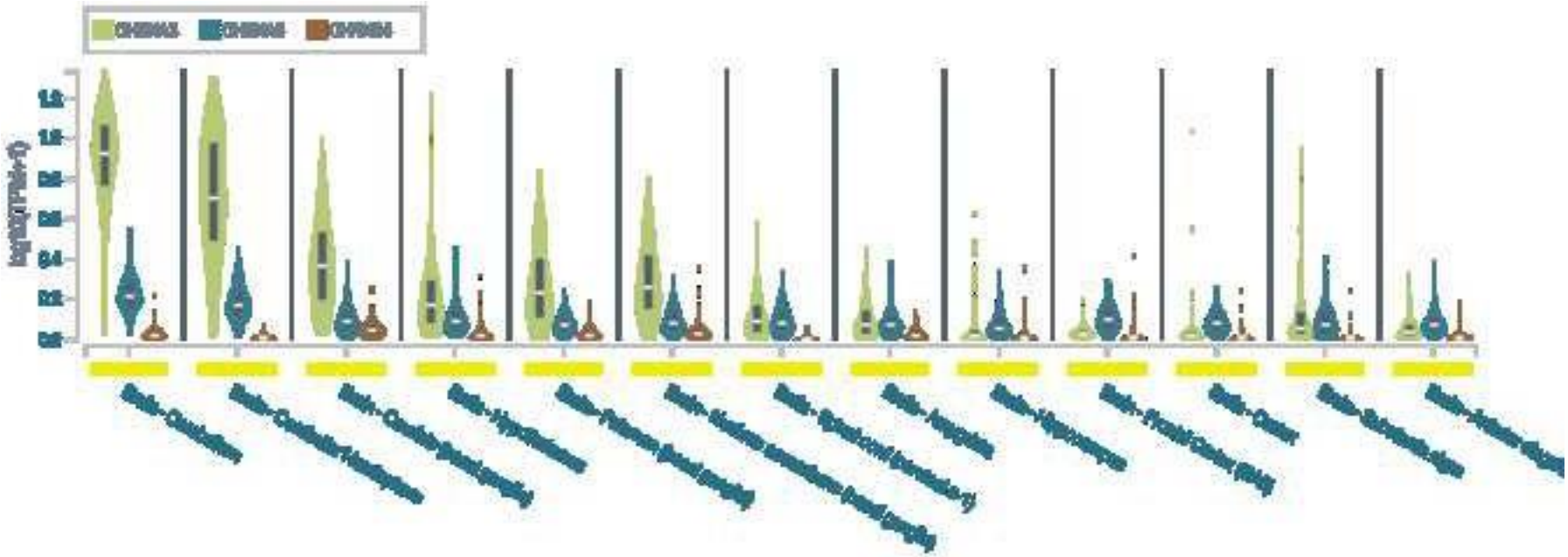
All were trans methylation sites. [c]rs11637890 and rs7178270 were the only ones to be associated with upregulation of *CHRNA3*, *CHRNA5* and *RP11-650L12.2*.

Score	Supporting data
1a	eQTL + TF binding + matched TF motif + matched DNase Footprint + DNase peak
1b	eQTL + TF binding + any motif + DNase Footprint + DNase peak
1c	eQTL + TF binding + matched TF motif + DNase peak
1d	eQTL + TF binding + any motif + DNase peak
1e	eQTL + TF binding + matched TF motif
1f	eQTL + TF binding / DNase peak
2a	TF binding + matched TF motif + matched DNase Footprint + DNase peak
2b	TF binding + any motif + DNase Footprint + DNase peak
2c	TF binding + matched TF motif + DNase peak
3a	TF binding + any motif + DNase peak
3b	TF binding + matched TF motif
4	TF binding + DNase peak
5	TF binding or DNase peak
6	other

Table 3: RegulomeDB score classification (from http://www.regulomedb.org). eQTL, expression quantitative trait loci; TF, transcription factor.

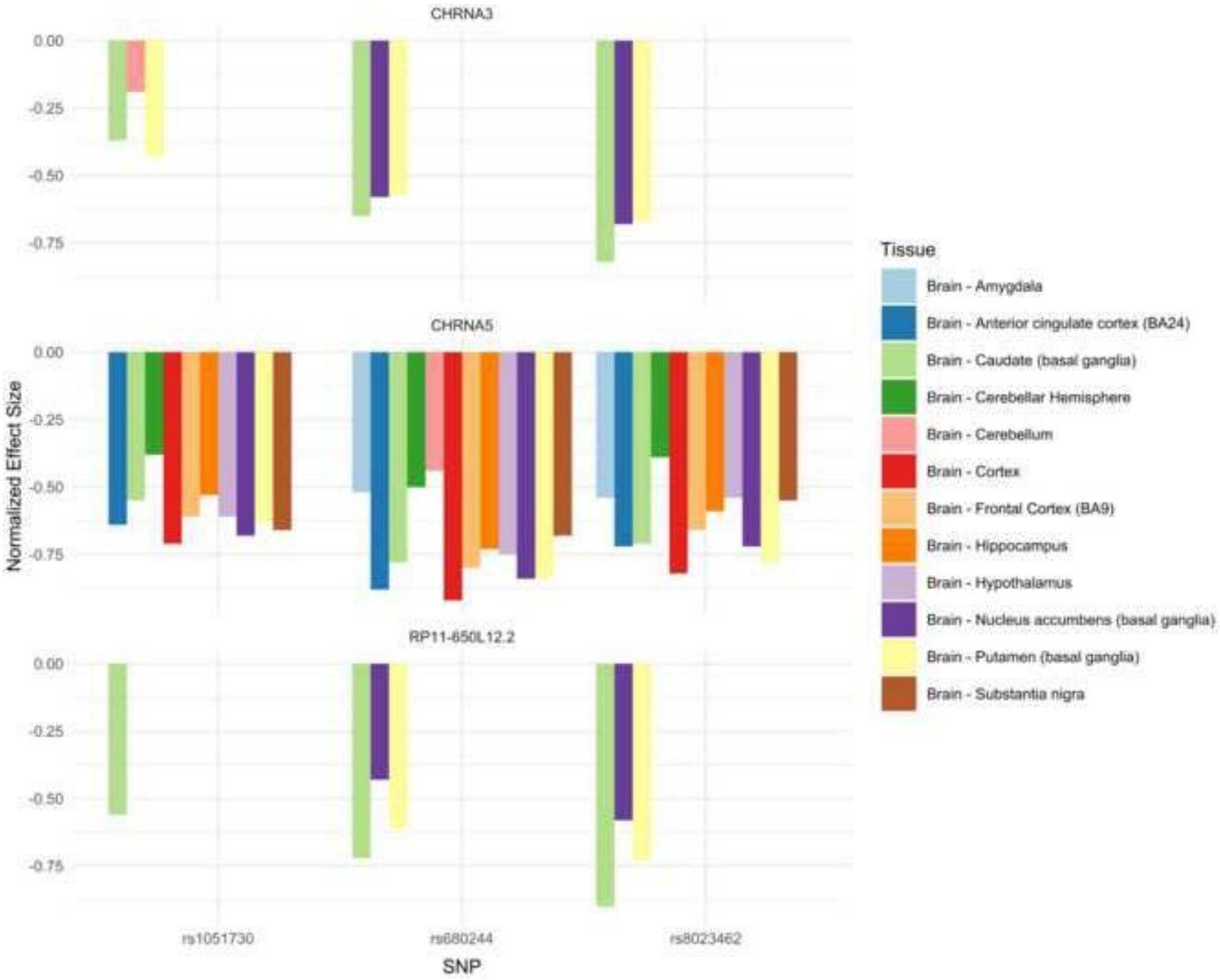
Figure(s) 1

Click here to download high resolution image



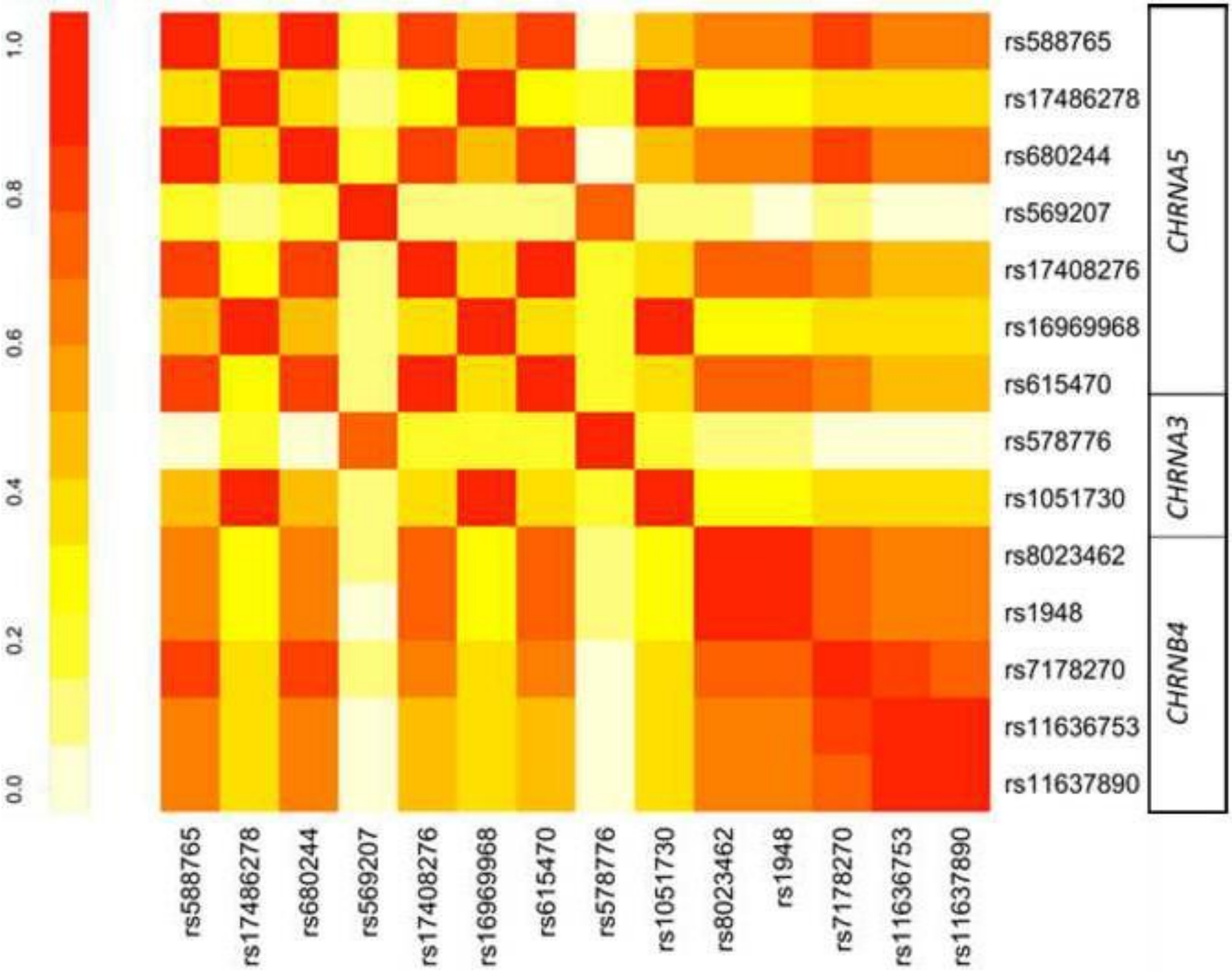
Figure(s) 2

Click here to download high resolution image



Figure(s) 3

Click here to download high resolution image



***Credit Author Statement**

Credit author statement

All authors listed have made substantial, direct and intellectual contribution to the work, and approved it for publication.