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ORIGINAL INVESTIGATION

Significant associations of *CHRNA2* and *CHRNA6* with nicotine dependence in European American and African American populations

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Abstract The direct physiological effects that promote nicotine dependence (ND) are mediated by nicotinic acetylcholine receptors (nAChRs). In line with the genetic and pharmacological basis of addiction, many previous studies have revealed significant associations between variants in the nAChR subunit genes and various measures of ND in different ethnic samples. In this study, we first examined the association of variants in nAChR subunits α2 (*CHRNA2*) and α6 (*CHRNA6*) genes on chromosome 8 with ND using a family sample consisting of 1,730 European Americans (EAs) from 495 families and 1,892 African Americans (AAs) from 424 families (defined as the discovery family sample). ND was assessed by two standard quantitative measures: smoking quantity (SQ) and the Fagerström Test for ND (FTND). We found nominal associations for all seven tested SNPs of the genes with at least one ND measure in the EA sample and for two SNPs in *CHRNA2* in the AA sample. Of these, associations of SNPs rs3735757 with FTND ($P = 0.0068$) and rs2472553 with both ND measures (with a P value of 0.0043 and 0.00086 for SQ and FTND, respectively) continued to be significant in the EA sample even after correction for multiple tests. Further, we found several haplotypes that were significantly associated with ND in the EA sample in *CHRNA6* and in the both EA and AA samples in *CHRNA2*. To confirm the associations of the two genes with ND, we conducted a replication study with an independent case–control sample from the SAGE study, which showed a significant association of the two genes with ND, although the significantly associated SNPs were not always the same in the two samples. Together, these findings indicate that both *CHRNA2* and *CHRNA6* play a significant role in the etiology of ND in AA and EA smokers. Further replication in additional independent samples is warranted.

Introduction

Tobacco use continues to be an important worldwide health concern. According to World Health Organization, there were 1.3 billion tobacco users world wide in 2004 (World Health Organization 2012). In the USA, 46.0 million adults were cigarette smokers in 2008, and the number of deaths annually from smoking-related illnesses accounts for 30 % of deaths from cancer and nearly 80 % of deaths from chronic obstructive pulmonary disease (CDC 2008; Mokdad et al. 2004). The annual economic burden of smoking is also substantial, with a staggering $193 billion in medical costs and productivity losses (CDC 2008; Mokdad et al. 2004).

Cigarette smoking is a complex behavior, with both genetic and environmental components (Al Koudsi and Tyndale 2005; Sullivan and Kendler 1999). Many family, adoption, and twin studies of smoking addiction have

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indicated a heritability of 11–78 %, with an average heritability of 59 % for both male and female smokers (Kendler et al. 1999; Li et al. 2003; Maes et al. 2004; Vink et al. 2005).

Nicotine, the primary psychoactive ingredient in cigarette smoke, exerts its effects by readily crossing the blood–brain barrier and binding to nicotinic acetylcholine receptors (nAChRs) in various brain structures (Wonnacott 1997). Activation of nAChRs on dopaminergic terminals induces dopamine release in the mesolimbic brain reward system (Kleijn et al. 2011; Wonnacott et al. 2000). To date, 17 nAChR subunits have been identified, which are divided into muscle and neuronal types (Kalamida et al. 2007). Neuronal nAChRs are widely expressed in the nervous system in peripheral ganglia and certain areas of the brain, as well as in nonexcitable cells, such as epithelium and cells of the immune system (Cui and Li 2010). Of the neuronal nAChRs, genes for nine α (α2–α10) and three β (β2–β4) subunits have been cloned. The α7–α10 subunits are found either as homopentamers (of five α7, α8, or α9 subunits) or as heteropentamers (of α7/α8 and α9/α10) (Plazas et al. 2005). By contrast, the α2–α6 and β2–β4 subunits form heteropentameric receptors, usually with a $(\alpha_x)2(\beta_y)3$ stoichiometry.

Whereas several human neuronal nAChR subunit genes have been investigated for association with ND and other smoking-related behaviors in human subjects [for reviews, see (Berrettini and Doyle 2012; Greenbaum and Lerer 2009; Li and Burmeister 2009)], *CHRNA2* has received less attention. Early linkage analysis of the Collaborative Studies on Genetics of Alcoholism (COGA) data showed modest evidence of linkage to 8p22–23, near *CHRNA2*, using two smoking phenotypes (ever-smoked and average number of packs per year) (Bergen et al. 1999). The association of *CHRNA2* with smoking was reported in the schizophrenia families through linkage analysis and the candidate gene approach (Faraone et al. 2004). Although there is a reported association of *CHRNA2* with ND, measured by DSM-IV and FTND score, in the Iowa Adoption Studies, the results were not corrected for multiple comparisons (Philibert et al. 2009; Yates et al. 1998). In a smoking cessation trial, SNP rs2565065 in *CHRNA2* appeared to have pharmacogenetic relevance (Heitjan et al. 2008). In contrast, several other studies have failed to reveal a significant association of this gene with ND or other smoking-related phenotypes (Keskitalo-Vuokko et al. 2011).

The *CHRNA6* and *CHRNB3* genes are located contiguously in a tail-to-tail configuration on chromosome 8. Both α6- and β3-nAChRs are found in various brain regions, including the substantia nigra, ventral tegmental area (VTA), striatum, and locus coeruleus (Gotti et al. 2006a, b), which have significant roles in dopaminergic neurotransmission, thus contributing to reward and reinforcement of behavior (Cui et al. 2003). The α6β2β3- as well as α6α4β2β3-containing receptors in the striatum mediates α-conotoxin MII-sensitive dopamine release. In contrast, α6β2-containing receptors in the superior colliculus seem to be involved in GABA release (Champtiaux et al. 2003; Gotti et al. 2006a, b; Salminen et al. 2004). A recent meta-GWAS study indicated that rs2304297 in *CHRNA6* is significantly associated with ND in the European sample, but the finding did not reach genome-wide significance (Thorgeirsson et al. 2010). Candidate gene-based association studies indicated that SNPs rs2304297 in the 3′-UTR of *CHRNA6* was associated with ND in the European sample (Hoft et al. 2009b; Saccone et al. 2007), as was rs1072003 in intron 2 of *CHRNA6* with ND in an Israeli female sample (Greenbaum et al. 2006).

Considering that nearly all subjects used in these GWAS or candidate gene-based association studies were of European origin, it would be interesting to know whether *CHRNA6* and *CHRNA2* genes are also associated with ND in smokers of other ethnicities. Thus, the primary objective of this study was to determine whether significant association of variants in *CHRNA6* and *CHRNA2* with ND can be detected in independent samples, especially in African American smokers.

Materials and methods

Subjects and ND measures

Discovery family sample

Subjects of this sample include persons of both AA and EA origin who were recruited primarily from the states of Tennessee, Mississippi, Arkansas, and Michigan. Proband smokers were required to be at least 21 years old, to have smoked for at least the last 5 years, and to have smoked at least 20 cigarettes per day during the last 12 months. Once proband smokers were identified, their biological parents and siblings were invited to participate whenever possible. Table 1 provides the detailed characteristics of the two ethnic groups. All participants provided written informed consent, and the Institutional Review Boards of each participating institution approved the study.

The ND of each smoker was assessed with the two commonly used measures of smoking quantity (SQ; the number of cigarettes smoked per day) and the Fagerström test for ND (FTND; 0–10 scale) (Fagerstrom 1978). Because of the overlap of the contents of the two measures, a fairly

Table 1 Description of discovery and replication samples

Ethnicity	Discovery family samples		Replication case–control samples	
	AA	EA	AA	EA
No. of nuclear families	424	495	–	–
Avg. members/family (SD)	4.46 (0.88)	3.49 (0.80)	–	–
No. of subjects	1,892	1,730	1,136	2,428
Female (%)	57	57	52	56
Age [years (SD)]	40.32 (14.60)	45.39 (15.77)	39.68 (6.71)	38.37 (9.65)
No. of smokers	1,013	1,088	626	1,048
CPD (SD)	21.55 (12.07)	22.07 (12.60)	24.33 (17.46)	26.16 (19.71)
FTND score (SD)	5.49 (3.60)	4.62 (3.55)	3.90 (2.88)	2.96 (3.27)

robust correlation exists among them in both populations ($r = 0.88$ for AAs and 0.89 for EAs).

Replication case–control sample

All subjects included in this sample were participants in the Study of Addiction: Genetics and Environment (SAGE) (Bierut et al. 2010) through the NCBI dbGaP database (dbGaP study accession phs000092.v1.p1). Quantitative measurements of severity of addiction to various substances, including nicotine, are provided in this dataset. For a detailed description, please see http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000092.v1.p1.

Genotyping and imputation

For the discovery family samples, genomic DNA was either extracted from cells in the peripheral venous blood of each participant using a Maxi kit (Qiagen Inc, Valencia, CA, USA) or obtained from the NIDA Genetics Repository at Rutgers University. Seven SNPs in *CHRNA2* (rs2292976, rs3735757, rs891398, and rs2472553) and *CHRNA6* (rs9298628, rs892413, and rs2217732) were selected based on results reported by other researchers (Heitjan et al. 2008; Hoft et al. 2009b; Philibert et al. 2009), the location of each SNP and a uniform coverage of the gene of interest, and allele frequency in samples with European and African origins from NCBI SNP database. All SNPs were genotyped using *Taq*Man assays in the 384-well microplate format (Applied Biosystems Inc., Foster City, CA, USA) as reported previously (Beuten et al. 2005; Li et al. 2005; Ma et al. 2005). Briefly, 15 ng of DNA was amplified in a total volume of 7 µl containing an MGB probe and 2.5 µl of *Taq*Man universal PCR master mix. Allelic discrimination analyses were performed on the ABI Prism 7900HT. To ensure the quality of genotyping, four no-template negative controls and four positive controls were added to each 384-well plate.

The SAGE samples were genotyped on commercially available platforms, including Illumina (San Diego, CA, USA). Quality control was performed in each group separately, with the goal of excluding those samples with sex or chromosomal anomalies, a low call rate, or first- or second-degree relatedness. Imputations of non-genotyped SNPs in the 1000 Genome CEU v2 (2010–11 release) and the HapMap Phase II CHB + JPT were carried out for the SAGE data using MaCH (Li et al. 2009, 2010) and IMPUTE v2 (Howie et al. 2009; Marchini et al. 2007), respectively.

Statistical analysis

To test for genotyping quality in the discovery family sample, we assessed Mendelian inconsistencies and departure from Hardy–Weinberg equilibrium (HWE) using Haploview (v. 4.0) software (Barrett et al. 2005). Subjects with any inconsistent SNP data for a given genetic variant were excluded from analysis.

Individual and haplotype-based association analysis for the discovery family sample

Associations between the seven SNPs in *CHRNA2* and *CHRNA6* and the two ND measures were determined using the pedigree-based association test (PBAT v. 3.5) based on the generalized estimating equation approach (Lange et al. 2004). Pair-wise linkage disequilibrium (LD) and haplotype blocks for the four SNPs in *CHRNA2* and three SNPs in *CHRNA6* were assessed by Haploview (v. 4.0) software (Barrett et al. 2005; Gabriel et al. 2002). Association analysis for haplotypes located in each LD block with the two ND measures was performed using the family-based association test (FBAT v.1.7.3) (Horvath et al. 2004). Three genetic models (additive, dominant, and recessive) were tested for all association analyses, with sex and age as covariates in the AA and EA samples. Statistically significant results ($P < 0.05$) for individual SNPs and major haplotypes (frequency ≥ 5 %) were adjusted for multiple testing using Bonferroni correction.

Table 2 ND-associated *P* values under the three genetic models for the first given allele of each SNP in *CHRNA2* and *CHRNA6* in the discovery family sample

Gene	dbSNP ID (Gene Location)	Alleles	African American			European American		
			Allele freq.	SQ	FTND	Allele freq.	SQ	FTND
CHRNA2	rs2292976 (Exon 8)	A/G	0.13/0.87	0.120^{a}	0.0866^{a}	0.14/0.86	-0.902^{a}	-0.664^{a}
				0.0578^{d}	0.0461^{d}		0.502^{d}	0.631^{d}
				-0.444^{r}	-0.700^{r}		-0.0378^{r}	-0.0221^{r}
	rs3735757 (Intron 5)	G/C	0.22/0.78	-0.378^{a}	-0.613^{a}	0.14/0.86	-0.589^{a}	-0.291^{a}
				-0.635^{d}	-0.810^{d}		0.750^{d}	-0.891^{d}
				-0.239^{r}	-0.464^{r}		-0.00782^{r}	-0.00675^{r}
	rs891398 (Exon 5)	T/C (T125A)	0.25/0.75	0.0201^{a}	0.00790^{a}	0.52/0.48	-0.511^{a}	-0.450^{a}
				0.026^{d}	0.0287^{d}		0.228^{d}	0.294^{d}
				0.302^{r}	0.0814^{r}		-0.0357^{r}	-0.0338^{r}
	rs2472553 (Exon 2)	T/C (T22I)	0.16/0.84	0.481^{a}	0.534^{a}	0.13/0.87	-0.595^{a}	-0.192^{a}
				0.324^{d}	0.387^{d}		0.776^{d}	-0.724^{d}
				-0.538^{r}	-0.649^{r}		-0.00429^{r}	-0.000863^{r}
CHRNA6	rs9298628 (3′-flanking)	C/T	0.25/0.75	-0.422^{a}	-0.375^{a}	0.81/0.19	-0.0455^{a}	-0.216^{a}
				-0.266^{d}	-0.232^{d}		-0.336^{d}	-0.547^{d}
				-0.913^{r}	-0.996^{r}		-0.0625^{r}	-0.251^{r}
	rs892413 (Intron 2)	C/A	0.25/0.75	-0.612^{a}	-0.397^{a}	0.80/0.20	-0.00769^{a}	-0.152^{a}
				-0.566^{d}	-0.340^{d}		-0.117^{d}	-0.189^{d}
				-0.704^{r}	-0.732^{r}		-0.0195^{r}	-0.304^{r}
	rs2217732 (Intron 2)	A/G	0.26/0.74	-0.423^{a}	-0.405^{a}	0.81/0.19	-0.0195^{a}	-0.126^{a}
				-0.247^{d}	-0.215^{d}		-0.335^{d}	-0.433^{d}
				-0.972^{r}	0.864^{r}		-0.0235^{r}	-0.163^{r}

Superscripts indicate genetic model used for analysis: *a* additive, *d* dominant, *r* recessive. For each ethnic-specific sample, age and sex were used as covariates. Negative signs indicate protective effect with the model specified in superscript letters

Individual and haplotype-based association analysis for the replication case–control SAGE sample

The association analysis was performed using a linear regression model by regressing two ND measures in PLINK (Purcell et al. 2007) on age, sex, SNP allele dosage, and other drug dependences (alcohol, cocaine, marijuana, opiates, and other drugs) covariates. Non-smokers were excluded.

Meta-analysis of individual SNP association for both the discovery and replication samples

Prior to conducting meta-analysis, we measured the heterogeneity for the SAGE case–control AA and EA samples using the program METAL (Willer et al. 2010). To combine the association analysis results from the discovery family sample and replication case–control sample, we conducted our meta-analysis of each SNP under the same genetic model used for analyzing each individual sample using Fisher's combining *P* value method (Fisher 1932). Considering that the PBAT approach used in the discovery family sample provides only *Z* score and *P* value, we used an equal weight for each studied sample.

Results

Individual SNP-based association analysis for discovery and replication samples

Results from the individual SNP-based association analyses of the discovery family sample are shown in Table 2. Of the *CHRNA2* polymorphisms, SNP rs891398 showed strong associations with FTND in the AAs ($P = 0.0079$) under an additive model and weak association in the EAs ($P = 0.0338$) under a dominant model. Also, rs373575 ($P = 0.00782$ and 0.00675) and rs2472553 ($P = 0.00429$ and 0.000863) showed strong association with SQ and FTND in the EAs under a dominant model. For the *CHRNA6* polymorphisms, the only variant significantly associated with SQ in the EAs was rs892413, with a *P* value of 0.00769 under the additive model. All these associations remained significant after Bonferroni correction for multiple testing.

The results from the replication case–control SAGE sample showed that SNP rs2292976 in *CHRNA2* had a significant association with FTND in the AAs under both an additive ($P = 0.00533$) and a dominant ($P = 0.0079$) model

Table 3 ND-associated *P* values under the three genetic models for the first given allele of each SNP in *CHRNA2* and *CHRNA6* with ND in the replication case–control sample

Gene	dbSNP ID (gene location)	Alleles	African American			European American		
			Allele freq.	SQ	FTND	Allele freq.	SQ	FTND
CHRNA2	rs2292976 (Exon 8)	A/G	0.10/0.90	-0.041^{a}	-0.00533^{a}	0.13/0.87	0.684^{a}	0.0487^{a}
				-0.058^{d}	-0.00790^{d}		0.619^{d}	0.0767^{d}
				-0.209^{r}	-0.147^{r}		-0.952^{r}	0.157^{r}
	rs3735757 (Intron 5)	G/C	0.20/0.80	-0.152^{a}	-0.267^{a}	0.13/0.87	0.982^{a}	0.114^{a}
				-0.209^{d}	-0.380^{d}		0.975^{d}	0.167^{d}
				-0.275^{r}	-0.290^{r}		-0.876^{r}	0.217^{r}
	rs891398 (Exon 5)	T/C (T125A)	0.25/0.75	-0.463^{a}	0.343^{a}	0.51/0.49	-0.172^{a}	-0.297^{a}
				-0.462^{d}	0.398^{d}		0.043^{d}	-0.124^{d}
				-0.724^{r}	0.506^{r}		-0.825^{r}	-0.864^{r}
	rs2472553 (Exon 2)	T/C (T22I)	0.16/0.84	0.185^{a}	0.102^{a}	0.13/0.87	-0.942^{a}	-0.0785^{a}
				0.192^{d}	0.122^{d}		0.812^{d}	-0.0953^{d}
				0.518^{r}	0.340^{r}		-0.635^{r}	-0.326^{r}
CHRNA6	rs9298628 (3′-flanking)	C/T	0.29/0.71	0.0323^{a}	0.056^{a}	0.79/0.21	0.104^{a}	0.000889^{a}
				0.0502^{d}	0.013^{d}		0.589^{d}	0.680^{d}
				0.143^{r}	0.941^{r}		0.036^{r}	0.000218^{r}
	rs892413 (Intron 2)	C/A	0.31/0.69	-0.119^{a}	0.208^{a}	0.80/0.20	-0.189^{a}	0.00153^{a}
				-0.0799^{d}	-0.486^{d}		-0.083^{d}	0.688^{d}
				-0.586^{r}	0.035^{r}		-0.515^{r}	0.000530^{r}
	rs2217732 (Intron 2)	A/G	0.29/0.71	0.105^{a}	0.098^{a}	0.80/0.20	0.080^{a}	0.000865^{a}
				0.125^{d}	0.026^{d}		0.828^{d}	0.513^{d}
				0.304^{r}	-0.938^{r}		0.034^{r}	0.000282^{r}

Superscripts indicate genetic model used for analysis: *a* additive, *d* dominant, *r* recessive. For each ethnic-specific sample, age, sex, and other non-nicotine drug dependences were used as covariates. Negative signs indicate protective effect with the model specified in superscript letters

(Table 3). Of the *CHRNA6* polymorphisms, rs9298628, rs892416, and rs2217732 showed a significant association with FTND in the EAs, with *P* values of 0.000889, 0.00153, and 0.000865, respectively, under the additive model and 0.000218, 0.00053, and 0.000282 under the recessive model. Again, all these associations remained significant after Bonferroni correction.

Meta-analysis associations for both discovery and replication samples

Meta-analysis was performed for the seven SNPs by combining the results from the discovery and replication samples, which included the AA sample only, EA sample only, and the AA and EA samples together (Table 4). The reason to perform meta-analysis on the AA and EA samples together was that the heterogeneity test for SAGE AA and EA case–control samples revealed no heterogeneity between the two ethnic samples on these SNPs. Of the meta-analyzed SNPs, rs2292976 in *CHRNA2* ($P = 0.0053$) and rs892413 in *CHRNA6* ($P = 0.00311$) showed the strongest association with FTND.

Haplotype-based association analysis

According to the haplotype block criteria defined by Gabriel et al. (2002), only one block was identified within each ethic sample in the *CHRNA2* as well as in *CHRNA6* (Fig. 1). We employed the FBAT program to perform haplotype-based association analysis for all major (defined as >5 %) haplotypes in each of the above-mentioned LD blocks with the two ND measures in *CHRNA2* (Table 5) and *CHRNA6* (Table 6) from the discovery family sample.

In *CHRNA2*, significant haplotypes in the AAs were: (1) G-C-T, formed by SNPs rs2292976, rs3735757, and rs891398 (Fig. 1), with a frequency of 22.3 %, which was associated significantly with FTND ($Z = 2.54$, $P = 0.011$); and (2) G-G-C, formed by the same SNPs, with a frequency of 9.6 %, which was associated significantly with FTND ($Z = -2.74$; $P = 0.0063$) under a dominant model. The identified haplotypes in the EAs were: (1) C-T-T, formed by SNPs rs3735757, rs891398, and rs2472553, with a frequency of 51.9 %, which was significantly associated with FTND ($Z = -2.60$; $P = 0.0093$); and (2) C-C-T, formed by SNPs rs3735757, rs891398, and rs2472553, with a

Table 4 Meta-analysis results of SNPs in *CHRNA2* and *CHRNA6* with ND in both the discovery and replication samples

Gene	dbSNP ID	Allele	African American	European American	AA + EA samples	I^2
CHRNA2	rs2292976	A	0.00325^{d}	0.195^{d}	0.00530^{d}	0
	rs3735757	G	0.404^{r}	0.011^{r}	0.0252^{r}	0
	rs891398	T	0.0187^{a}	0.402^{a}	0.0443^{a}	0
	rs2472553	T	0.554^{r}	0.00258^{r}	0.0108^{r}	0
CHRNA6	rs9298628	C	0.998^{r}	0.000592^{r}	0.00498^{r}	0
	rs892413	C	0.120^{r}	0.00290^{r}	0.00311^{r}	0
	rs2217732	A	0.981^{r}	0.000505^{r}	0.00426^{r}	0

For each SNP, meta-analysis was performed on only one genetic model, which was selected on the basis of the association analysis result for both the discovery and the replication samples; I^2 was calculated only for the replication case–control samples with the METAL program

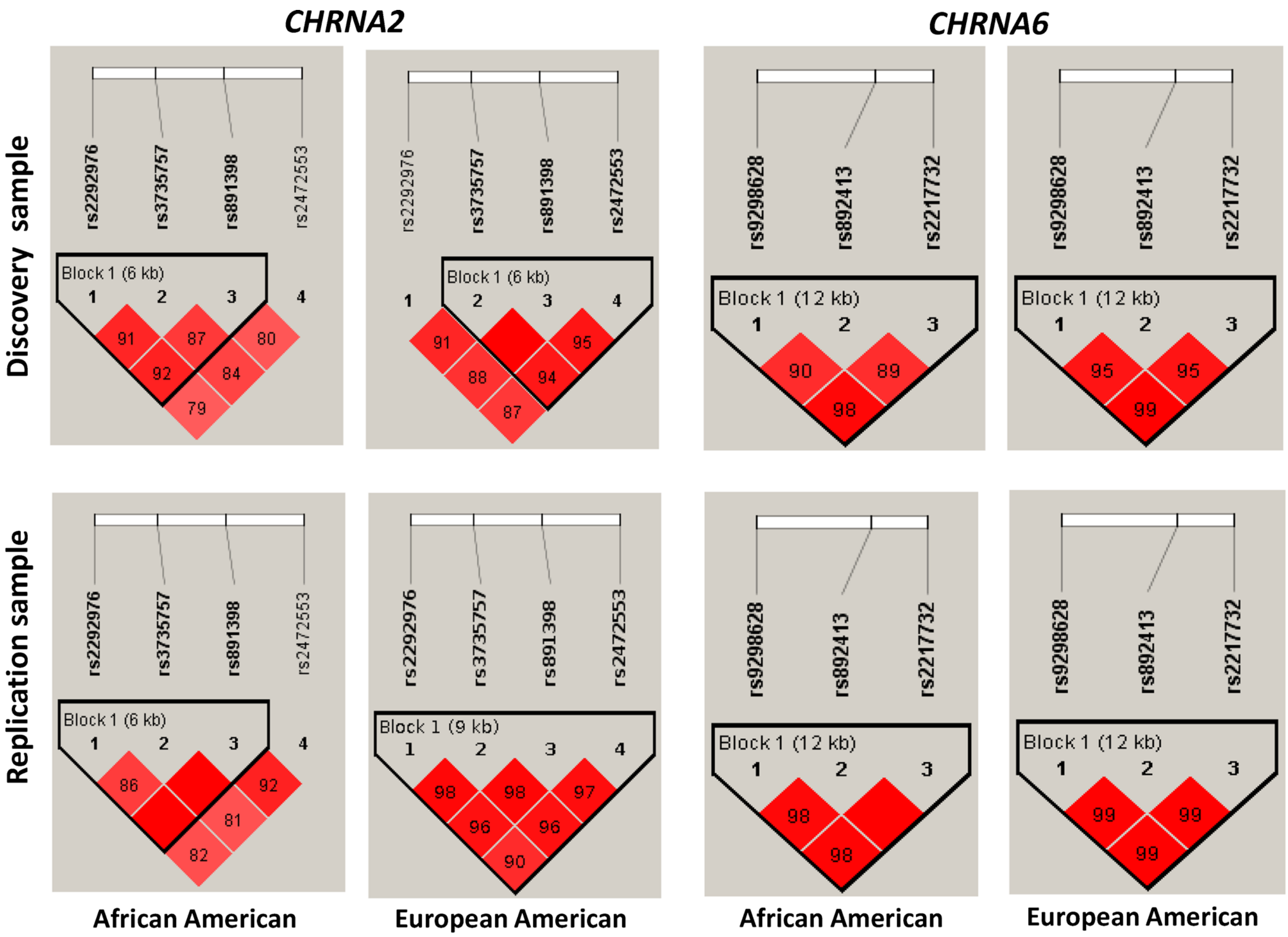

Fig. 1 LD structures for *CHRNA2* (*left*) and *CHRNA6* (*right*) SNPs in AAs and EAs from discovery and replication samples. Haploview (Barrett et al. 2005) was used to calculate all D' values, and haplotype blocks were defined according to Gabriel et al. (2002). The *number* in each *box* represents the D' value for each SNP pair surrounding that *box*

frequency of 34.4 %, which was significantly associated with FTND ($Z = 2.85$; $P = 0.0043$).

In *CHRNA6*, for the AA sample, we found no haplotypes showing significant association with ND. In the EA sample (Table 5), we found one haplotype, C-C-A, formed by SNPs rs9298628, rs892413, and rs2217732, with a frequency of 79.1 %, significantly associated with SQ ($Z = -2.71$; $P = 0.0067$). Several haplotype-based associations remained significant after Bonferroni correction for each LD block.

In the replication case–control sample, only one haplotype in *CHRNA2*, A-G-C, formed by SNPs rs2292976,

Table 5 Association analysis results for haplotypes in *CHRNA2* and *CHRNA6* with ND in the discovery family sample

	rs2292976	rs3735757	rs891398	rs2472553	Freq.	SQ				FTND			
						P haplotype	*Z* score	*P* global	Family #	*P* haplotype	*Z* score	*P* global	Family #
(A) *CHRNA2*													
African American	G	C	C		54.9	0.36^{d}	−0.92		130	0.097^{a}	−1.66		221
	G	C	T		22.3	0.058^{a}	1.90	0.13	175	0.011^{a}	2.54	0.023	175
	A	G	C		10.6	0.44^{d}	0.77		105	0.28^{d}	1.09		109
	G	G	C		9.6	0.0099^{d}	−2.58		101	0.0063^{d}	−2.74		102
European American		C	C	T	34.4	0.020^{d}	2.32		193	0.0043^{d}	2.85		193
		C	T	T	51.9	0.014^{d}	−2.46	0.012	152	0.0093^{d}	−2.60	0.0039	152
		G	C	C	11.7	0.049^{d}	−1.97		10	0.015^{d}	−2.42		10

	rs9298628	rs892413	rs2217732	Freq.	SQ				FTND			
					P haplotype	*Z* score	*P* global	Family #	*P* haplotype	*Z* score	*P* global	Family #
(B) *CHRNA6*												
African American	C	C	A	22.1	0.66^{a}	−0.44	0.54	176	0.66^{d}	−0.44	0.32	157
	T	A	G	72.9	0.26^{d}	1.12		163	0.13^{d}	1.50		163
European American	C	C	A	79.1	0.0067^{a}	−2.71	0.014	176	0.12^{a}	−1.54	0.18	179
	T	A	G	18.3	0.011^{a}	2.55		171	0.094^{a}	1.67		170

Superscripts indicate genetic model used for analysis: *a* additive, *d* dominant, *r* recessive; Corrected *P* value at 0.05 is 0.0125 in AAs and 0.0167 in EAs for *CHRNA2* and 0.025 in both EAs and AAs for *CHRNA6*

Table 6 Association analysis results for SNPs in *CHRNA2* and *CHRNA6* with ND in the replication case–control sample

	rs2292976	rs3735757	rs891398	rs2472553	Freq.	SQ		FTND	
						P haplotype	*P* global	*P* haplotype	*P* global
(A) *CHRNA2*									
African American	G	C	C		54.0	0.0713^{a}		0.744^{a}	
	G	C	T		24.8	0.139^{a}	0.220	0.379^{a}	0.099
	G	G	C		10.9	0.823^{a}		0.324^{a}	
	A	G	C		9.16	0.0275^{a}		0.00649^{a}	
European American	A	G	C	T	11.4	0.769^{a}		0.0686^{a}	
	G	C	C	C	35.7	0.178^{a}	0.533	0.826^{a}	0.408
	G	C	T	C	50.1	0.158^{a}		0.240^{a}	

	rs9298628	rs892413	rs2217732	Freq.	SQ		FTND	
					P haplotype	*P* global	*P* haplotype	*P* global
(B) *CHRNA6*								
African American	T	A	G	68.4	0.0584^{a}	0.238	0.145^{a}	0.243
	C	C	A	28.9	0.105^{a}		0.0885^{a}	
European American	T	A	G	20.0	0.178^{a}	0.228	0.000947^{a}	0.00792
	C	C	A	79.2	0.122^{a}		0.00121^{a}	

Superscripts indicate genetic model used for analysis: *a* additive; Corrected *P* value at 0.05 is 0.0125 in AAs and 0.0167 in EAs for *CHRNA2* and 0.025 in both EAs and AAs for *CHRNA6*

rs3735757, and rs891398, was significantly associated with FTND in the AA sample ($P = 0.00649$) (Table 6). Two haplotypes formed by SNPs rs9298628, rs892413, and rs2217732 in *CHRNA6* (Fig. 1) showed significant associations in the EA sample: (1) T-A-G, with a frequency of 20.0 %, was significantly associated with FTND ($P = 0.000947$); and (2) C-C-A, with a frequency of 79.2 %, was significantly associated with FTND ($P = 0.00121$).

Discussion

Nicotinic acetylcholine receptors α2 and α6 play vital roles in the nervous system. To test for their association with ND, seven SNPs in *CHRNA2* and *CHRNA6* were investigated in two independent samples of either African or European origin. Association analysis revealed that multiple SNPs and haplotypes are significantly associated with ND in the both discovery and replication samples. In the discovery sample, individual SNP analysis for *CHRNA2* revealed a significant association of two SNPs in the EA sample and one SNP in the AA sample with FTND and/or SQ. In particular, we found that associations of SNPs rs3735757 and rs2472553 in *CHRNA2* with SQ and FTND remained significant after correction for multiple testing. However, such associations were not exactly the same at the SNP level in the replication sample, where only SNP rs2292976 showed significant association with FTND in the AA population. Although SNP rs892413 showed significant association with SQ in the EA discovery samples, all three *CHRNA6* SNPs exhibited significant association with FTND in the EA replication sample, even after correction for multiple testing. Further, we found a significant association between several haplotypes of *CHRNA2*/*CHRNA6* and ND in both the discovery and the replication samples, although haplotypes formed by particular SNPs were sometimes different in the two samples. Meta-analysis of the discovery and the replication samples added further support for the association of *CHRNA2* and *CHRNA6* with ND.

Compared with other nAChR subunit genes such as *CHRNA5-A3-B4*, *CHRNA4*, *CHRNB2*, and *CHRNB3* (Bierut 2010; Cui et al. 2013; Li and Burmeister 2009; Saccone et al. 2009, 2010; Thorgeirsson et al. 2010; Wang et al. 2012), *CHRNA2* has not received much attention in nicotine research. Although *CHRNA2* was one of the first nAChRs investigated as an ND candidate gene in several GWAS and candidate studies, no significant associations have been reported after correction for multiple testing or replicated in independent studies. In the present study, we demonstrated that *CHRNA2* shows a strong association with FTND after correction for multiple testing. Importantly, the SNP rs2472553, which evinced the strongest association with ND, appears to encode a functional variant in the signal peptide, causing an amino acid change from threonine to isoleucine at the 22nd residue. Our functional study with oocyte electrophysiology indicates that this mutation changes the sensitivity of functional receptors (Dash et al. in preparation).

CHRNA2 plays a vital role in other neurologic disorders such as epilepsy, with an estimated prevalence in Europeans that ranges from 3 to 8 per 1,000 individuals (Forsgren et al. 2005). The mutation I279N in the α2 subunit (i.e., rs104894063) was the first identified functional variant associated with epilepsy in *CHRNA2*; electrophysiological investigation of the I279N mutation in HEK 293 cells indicates that the $\alpha2^{I279N}/\beta4$ receptor has a significantly higher sensitivity to the natural agonist than does the wild-type α2/β4 receptor (Aridon et al. 2006). Another oocyte electrophysiology study found that $\alpha2^{I279N}$, co-expressed with the β2 subunit, causes a gain-of-function effect whose distinct biopharmacological profile includes reduced inhibition by carbamazepine and greater nicotine sensitivity (Hoda et al. 2009).

Several GWAS and candidate gene studies have revealed strong associations between *CHRNA6* and ND. However, most studies tested *CHRNB3–CHRNA6* associations as a cluster, in which most of the significant association was attributable to variants in *CHRNB3*; moreover, the association of *CHRNA6* SNPs typically did not survive correction for multiple testing (Hoft et al. 2009b; Saccone et al. 2009; Zeiger et al. 2008). In the present study, we demonstrated that *CHRNA6* is still significantly associated with FTND after correction for multiple testing. In a study by Hoft and colleagues (Hoft et al. 2009a), two SNPs from the *CHRNA6–CHRNB3* cluster were found to be associated with smoking quit attempts also: SNP rs2304297 ($P = 0.0044$) from *CHRNA6* and rs7004381 ($P = 0.0024$) from *CHRNB3*. Complementing our association study, animal self-administration studies suggest that β2*nAChRs assembled with α6 subunits would be useful pharmacological targets for smoking cessation products (Brunzell 2012): nicotine self-administration is absent in α6-knockout mice, and targeted re-expression of the α6 subunit in the VTA of α6-KO mice promptly restores nicotine self-administration (Pons et al. 2008). Further, several in vitro electrophysiological, synaptosome-release assay, and cyclic-voltammetry studies have demonstrated that nicotine-mediated elevation of dopamine release is blocked following antagonism of α6β2*nAChRs with α-CTX MII (Champtiaux et al. 2003; Drenan et al. 2008; Perez et al. 2009, 2010; Salminen et al. 2007; Zhao-Shea et al. 2011). Finally, an in vivo function study of α6*nAChRs in mesolimbic DA neurons has shown that the elevation of DA release caused by nicotine can be inhibited by intra-VTA infusion of α-CTX MII, implicating α6β2*nAChRs in the regulation of this effect (Gotti et al. 2010). Collectively, the association and functional studies of *CHRNA6* suggest that α6*nAChRs are strong candidates for drug-development research on smoking cessation.

Recently, *CHRNA6* has been found to be associated, not only with ND, but also with alcohol dependence; three SNPs (rs1072003, $P = 0.015$; rs892413, $P = 0.0033$; and rs2304297, $P = 0.012$) were associated with alcohol dependence in the National Youth Survey Family Study in a sample that was mostly EAs (Hoft et al. 2009a). Another study showed that two haplotypes of the *CHRNA6*, CCCC and TCGA, formed by SNPs rs10087172, rs10109429, rs2196129, and rs16891604, were associated with heavy alcohol consumption ($P = 0.004$ and $P = 0.035$, respectively) and with increased alcohol intake ($P = 0.004$) for the CCCC haplotype in a Spanish population (Landgren et al. 2009).

In sum, our results indicate that both *CHRNA2* and *CHRNA6* are significantly associated with ND. Such association with ND at both the individual SNP and haplotype level makes these genes good subjects for research on molecular mechanisms of dependence. A better understanding of the role of these genetic variants—especially the functional variants—may provide key insights for pharmacologic targeting to reduce or possibly eliminate some of the addictive properties of nicotine in susceptible individuals.

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Conflict of interest MDL has served as a consultant and board member of ADial Pharmaceuticals, LLC. All other authors declare that they have no conflicts of interest.

References

Al Koudsi N, Tyndale RF (2005) Genetic influences on smoking: a brief review. Ther Drug Monit 27:704–709

Aridon P, Marini C, Di Resta C, Brilli E, De Fusco M, Politi F, Parrini E, Manfredi I, Pisano T, Pruna D, Curia G, Cianchetti C, Pasqualetti M, Becchetti A, Guerrini R, Casari G (2006) Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. Am J Hum Genet 79:342–350. doi:10.1086/506459

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Bergen AW, Korczak JF, Weissbecker KA, Goldstein AM (1999) A genome-wide search for loci contributing to smoking and alcoholism. Genet Epidemiol 17:S55–S60

Berrettini WH, Doyle GA (2012) The CHRNA5-A3-B4 gene cluster in nicotine addiction. Mol Psychiatry 17:856–866. doi:10.1038/mp.2011.122

Beuten J, Ma JZ, Payne TJ, Dupont RT, Crews KM, Somes G, Williams NJ, Elston RC, Li MD (2005) Single- and multilocus allelic variants within the GABAB receptor subunit 2 (GABAB2) gene are significantly associated with nicotine dependence. Am J Hum Genet 76:859–864

Bierut LJ (2010) Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24-25. Trends Pharmacol Sci 31:46–51. doi:10.1016/j.tips.2009.10.004

Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, Fisher S, Fox L, Howells W, Bertelsen S, Hinrichs AL, Almasy L, Breslau N, Culverhouse RC, Dick DM, Edenberg HJ, Foroud T, Grucza RA, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Krueger RF, Kuperman S, Lynskey M, Mann K, Neuman RJ, Nothen MM, Nurnberger JI Jr, Porjesz B, Ridinger M, Saccone NL, Saccone SF, Schuckit MA, Tischfield JA, Wang JC, Rietschel M, Goate AM, Rice JP (2010) A genome-wide association study of alcohol dependence. Proc Natl Acad Sci USA 107:5082–5087. doi:10.1073/pnas.0911109107

Brunzell DH (2012) Preclinical evidence that activation of mesolimbic alpha 6 subunit containing nicotinic acetylcholine receptors supports nicotine addiction phenotype. Nicotine Tob Res 14:1258–1269. doi:10.1093/ntr/nts089

CDC (2008) Smoking-attributable mortality, years of potential life lost, and productivity losses—United States, 2000–2004. MMWR Morb Mortal Wkly Rep 57: 1226–1228. (pii: mm5745a3)

Champtiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Lena C, Clementi F, Moretti M, Rossi FM, Le Novere N, McIntosh JM, Gardier AM, Changeux JP (2003) Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. J Neurosci 23:7820–7829

Cui WY, Li MD (2010) Nicotinic modulation of innate immune pathways via alpha7 nicotinic acetylcholine receptor. J Neuroimmune Pharmacol 479–488. doi: 10.1007/s11481-010-9210-2

Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, Salminen O, Tritto T, Butt CM, Allen WR, Stitzel JA, McIntosh JM, Boulter J, Collins AC, Heinemann SF (2003) The beta3 nicotinic receptor subunit: a component of alpha-conotoxin MII-binding nicotinic acetylcholine receptors that modulate dopamine release and related behaviors. J Neurosci 23:11045–11053

Cui WY, Wang S, Yang J, Yi SG, Yoon D, Kim YJ, Payne TJ, Ma JZ, Park T, Li MD (2013) Significant association of CHRNB3 variants with nicotine dependence in multiple ethnic populations. Mol Psychiatry. doi:10.1038/mp.2012.190

Drenan RM, Grady SR, Whiteaker P, McClure-Begley T, McKinney S, Miwa JM, Bupp S, Heintz N, McIntosh JM, Bencherif M, Marks MJ, Lester HA (2008) In vivo activation of midbrain dopamine neurons via sensitized, high-affinity alpha 6 nicotinic acetylcholine receptors. Neuron 60:123–136. doi:10.1016/j.neuron.2008.09.009

Fagerstrom KO (1978) Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addict Behav 3:235–241

Faraone SV, Su J, Taylor L, Wilcox M, Van Eerdewegh P, Tsuang MT (2004) A novel permutation testing method implicates sixteen nicotinic acetylcholine receptor genes as risk factors for smoking in schizophrenia families. Hum Hered 57:59–68. doi:10.1159/000077543

Fisher RA (1932) Statistical methods for research workers, 4th edn. Oliver and Boyd, Edinburgh

Forsgren L, Beghi E, Oun A, Sillanpaa M (2005) The epidemiology of epilepsy in Europe—a systematic review. Eur J Neurol 12:245–253. doi:10.1111/j.1468-1331.2004.00992.x

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225–2229

Gotti C, Riganti L, Vailati S, Clementi F (2006a) Brain neuronal nicotinic receptors as new targets for drug discovery. Curr Pharm Des 12:407–428

Gotti C, Zoli M, Clementi F (2006b) Brain nicotinic acetylcholine receptors: native subtypes and their relevance. Trends Pharmacol Sci 27:482–491. doi:10.1016/j.tips.2006.07.004

Gotti C, Guiducci S, Tedesco V, Corbioli S, Zanetti L, Moretti M, Zanardi A, Rimondini R, Mugnaini M, Clementi F, Chiamulera C, Zoli M (2010) Nicotinic acetylcholine receptors in the mesolimbic pathway: primary role of ventral tegmental area alpha6beta2* receptors in mediating systemic nicotine effects on dopamine release, locomotion, and reinforcement. J Neurosci 30:5311–5325. doi:10.1523/JNEUROSCI.5095-09.2010

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. doi:10.1038/mp.2009.59

Greenbaum L, Kanyas K, Karni O, Merbl Y, Olender T, Horowitz A, Yakir A, Lancet D, Ben-Asher E, Lerer B (2006) Why do young women smoke? I. Direct and interactive effects of environment, psychological characteristics and nicotinic cholinergic receptor genes. Mol Psychiatry 11(312–22):223

Heitjan DF, Guo M, Ray R, Wileyto EP, Epstein LH, Lerman C (2008) Identification of pharmacogenetic markers in smoking cessation therapy. Am J Med Genet B Neuropsychiatr Genet 147B:712–719. doi:10.1002/ajmg.b.30669

Hoda JC, Wanischeck M, Bertrand D, Steinlein OK (2009) Pleiotropic functional effects of the first epilepsy-associated mutation in the human CHRNA2 gene. FEBS Lett 583:1599–1604. doi:10.1016/j.febslet.2009.04.024

Hoft NR, Corley RP, McQueen MB, Huizinga D, Menard S, Ehringer MA (2009a) SNPs in CHRNA6 and CHRNB3 are associated with alcohol consumption in a nationally representative sample. Genes Brain Behav 8:631–637. doi:10.1111/j.1601-183X.2009.00495.x

Hoft NR, Corley RP, McQueen MB, Schlaepfer IR, Huizinga D, Ehringer MA (2009b) Genetic association of the CHRNA6 and CHRNB3 genes with tobacco dependence in a nationally representative sample. Neuropsychopharmacology 34:698–706. doi:10.1038/npp.2008.122

Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM (2004) Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. Genet Epidemiol 26:61–69. doi:10.1002/gepi.10295

Howie BN, Donnelly P, Marchini J (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 5:e1000529. doi:10.1371/journal.pgen.1000529

Kalamida D, Poulas K, Avramopoulou V, Fostieri E, Lagoumintzis G, Lazaridis K, Sideri A, Zouridakis M, Tzartos SJ (2007) Muscle and neuronal nicotinic acetylcholine receptors. Structure, function and pathogenicity. FEBS J 274:3799–3845. doi:10.1111/j.1742-4658.2007.05935.x

Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA (1999) A population-based twin study in women of smoking initiation and nicotine dependence. Psychol Med 29:299–308

Keskitalo-Vuokko K, Pitkaniemi J, Broms U, Heliovaara M, Aromaa A, Perola M, Ripatti S, Salminen O, Salomaa V, Loukola A, Kaprio J (2011) Associations of nicotine intake measures with CHRN genes in Finnish smokers. Nicotine Tob Res 13:686–690. doi:10.1093/ntr/ntr059

Kleijn J, Folgering JH, van der Hart MC, Rollema H, Cremers TI, Westerink BH (2011) Direct effect of nicotine on mesolimbic dopamine release in rat nucleus accumbens shell. Neurosci Lett 493:55–58. doi:10.1016/j.neulet.2011.02.035

Landgren S, Engel JA, Andersson ME, Gonzalez-Quintela A, Campos J, Nilsson S, Zetterberg H, Blennow K, Jerlhag E (2009) Association of nAChR gene haplotypes with heavy alcohol use and body mass. Brain Res 1305(Suppl):S72–S79. doi:10.1016/j.brainres.2009.08.026

Lange C, DeMeo D, Silverman EK, Weiss ST, Laird NM (2004) PBAT: tools for family-based association studies. Am J Hum Genet 74:367–369

Li MD, Burmeister M (2009) New insights into the genetics of addiction. Nat Rev Genet 10: 225–231. doi:10.1038/nrg2536

Li MD, Cheng R, Ma JZ, Swan GE (2003) A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. Addiction 98:23–31

Li MD, Beuten J, Ma JZ, Payne TJ, Lou XY, Garcia V, Duenes AS, Crews KM, Elston RC (2005) Ethnic- and gender-specific association of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) with nicotine dependence. Hum Mol Genet 14:1211–1219

Li Y, Willer C, Sanna S, Abecasis G (2009) Genotype imputation. Annu Rev Genomics Hum Genet 10:387–406. doi:10.1146/annurev.genom.9.081307.164242

Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol 34:816–834. doi:10.1002/gepi.20533

Ma JZ, Beuten J, Payne TJ, Dupont RT, Elston RC, Li MD (2005) Haplotype analysis indicates an association between the DOPA decarboxylase (DDC) gene and nicotine dependence. Hum Mol Genet 14:1691–1698

Maes HH, Sullivan PF, Bulik CM, Neale MC, Prescott CA, Eaves LJ, Kendler KS (2004) A twin study of genetic and environmental influences on tobacco initiation, regular tobacco use and nicotine dependence. Psychol Med 34:1251–1261

Marchini J, Howie B, Myers S, McVean G, Donnelly P (2007) A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 39:906–913. doi:10.1038/ng2088

Mokdad AH, Marks JS, Stroup DF, Gerberding JL (2004) Actual causes of death in the United States, 2000. JAMA 291:1238–1245

Perez XA, O'Leary KT, Parameswaran N, McIntosh JM, Quik M (2009) Prominent role of alpha3/alpha6beta2*nAChRs in regulating evoked dopamine release in primate putamen: effect of long-term nicotine treatment. Mol Pharmacol 75:938–946. doi:10.1124/mol.108.053801

Perez XA, Bordia T, McIntosh JM, Quik M (2010) alpha6ss2* and alpha4ss2* nicotinic receptors both regulate dopamine signaling with increased nigrostriatal damage: relevance to Parkinson's disease. Mol Pharmacol 78:971–980. doi:10.1124/mol.110.067561

Philibert RA, Todorov A, Andersen A, Hollenbeck N, Gunter T, Heath A, Madden P (2009) Examination of the nicotine dependence (NICSNP) consortium findings in the Iowa adoption studies population. Nicotine Tob Res 11:286–292. doi:10.1093/ntr/ntn034

Plazas PV, Katz E, Gomez-Casati ME, Bouzat C, Elgoyhen AB (2005) Stoichiometry of the alpha9alpha10 nicotinic cholinergic receptor. J Neurosci 25:10905–10912. doi:10.1523/JNEUROSCI.3805-05.2005

Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, Changeux JP, Maskos U, Fratta W (2008) Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. J Neurosci 28:12318–12327. doi:10.1523/JNEUROSCI.3918-08.2008

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575. doi:10.1086/519795

Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, Swan GE, Goate AM, Rutter J, Bertelsen S, Fox L, Fugman D, Martin NG, Montgomery GW, Wang JC, Ballinger DG, Rice JP, Bierut LJ (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

Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Grucza RA, Hatsukami D, Johnson EO, Madden PA, Swan GE, Wang JC, Goate AM, Rice JP, Bierut LJ (2009) Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. Am J Med Genet B Neuropsychiatr Genet 150B:453–466. doi:10.1002/ajmg.b.30828

Saccone NL, Schwantes-An TH, Wang JC, Grucza RA, Breslau N, Hatsukami D, Johnson EO, Rice JP, Goate AM, Bierut LJ (2010) Multiple cholinergic nicotinic receptor genes affect nicotine dependence risk in African and European Americans. Genes Brain Behav 9:741–750. doi:10.1111/j.1601-183X.2010.00608.x

Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, Grady SR (2004) Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. Mol Pharmacol 65:1526–1535. doi:10.1124/mol.65.6.152665/6/1526

Salminen O, Drapeau JA, McIntosh JM, Collins AC, Marks MJ, Grady SR (2007) Pharmacology of alpha-conotoxin MII-sensitive subtypes of nicotinic acetylcholine receptors isolated by breeding of null mutant mice. Mol Pharmacol 71:1563–1571. doi:10.1124/mol.106.031492

Sullivan PF, Kendler KS (1999) The genetic epidemiology of smoking. Nicotine Tob Res 1(Suppl 2):S51–S57 (discussion S69–S70)

Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, Gieger C, Rawal R, Mangino M, Prokopenko I, Magi R, Keskitalo K, Gudjonsdottir IH, Gretarsdottir S, Stefansson H, Thompson JR, Aulchenko YS, Nelis M, Aben KK, den Heijer M, Dirksen A, Ashraf H, Soranzo N, Valdes AM, Steves C, Uitterlinden AG, Hofman A, Tonjes A, Kovacs P, Hottenga JJ, Willemsen G, Vogelzangs N, Doring A, Dahmen N, Nitz B, Pergadia ML, Saez B, De Diego V, Lezcano V, Garcia-Prats MD, Ripatti S, Perola M, Kettunen J, Hartikainen AL, Pouta A, Laitinen J, Isohanni M, Huei-Yi S, Allen M, Krestyaninova M, Hall AS, Jones GT, van Rij AM, Mueller T, Dieplinger B, Haltmayer M, Jonsson S, Matthiasson SE, Oskarsson H, Tyrfingsson T, Kiemeney LA, Mayordomo JI, Lindholt JS, Pedersen JH, Franklin WA, Wolf H, Montgomery GW, Heath AC, Martin NG, Madden PA, Giegling I, Rujescu D, Jarvelin MR, Salomaa V, Stumvoll M, Spector TD, Wichmann HE, Metspalu A, Samani NJ, Penninx BW, Oostra BA, Boomsma DI, Tiemeier H, van Duijn CM, Kaprio J, Gulcher JR, McCarthy MI, Peltonen L, Thorsteinsdottir U, Stefansson K (2010) Sequence variants at

CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. Nat Genet 42: 448–453. doi:10.1038/ng.573

Vink JM, Willemsen G, Boomsma DI (2005) Heritability of smoking initiation and nicotine dependence. Behav Genet 35:397–406

Wang JC, Kapoor M, Goate AM (2012) The genetics of substance dependence. Annu Rev Genomics Hum Genet 13:241–261. doi:10.1146/annurev-genom-090711-163844

Willer CJ, Li Y, Abecasis GR (2010) METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26:2190–2191. doi:10.1093/bioinformatics/btq340

Wonnacott S (1997) Presynaptic nicotinic ACh receptors. Trends Neurosci 20:92–98

Wonnacott S, Kaiser S, Mogg A, Soliakov L, Jones IW (2000) Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. Eur J Pharmacol 393:51–58

World Health Organization (2012) Drug report 2012. World Health Organization

Yates W, Cadoret R, Troughton E (1998) The Iowa adoption studies methods and results. In: LaBuda M, Grigorenko E (eds) On the way to individuality: methodological issues in behavioral genetics. Nova Science Publishers, Hauppauge, pp 95–125

Zeiger JS, Haberstick BC, Schlaepfer I, Collins AC, Corley RP, Crowley TJ, Hewitt JK, Hopfer CJ, Lessem J, McQueen MB, Rhee SH, Ehringer MA (2008) The neuronal nicotinic receptor subunit genes (CHRNA6 and CHRNB3) are associated with subjective responses to tobacco. Hum Mol Genet 17:724–734. doi:10.1093/hmg/ddm344

Zhao-Shea R, Liu L, Soll LG, Improgo MR, Meyers EE, McIntosh JM, Grady SR, Marks MJ, Gardner PD, Tapper AR (2011) Nicotine-mediated activation of dopaminergic neurons in distinct regions of the ventral tegmental area. Neuropsychopharmacology 36:1021–1032. doi:10.1038/npp.2010.240