

Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs

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Nicotine dependence is one of the world's leading causes of preventable death. To discover genetic variants that influence risk for nicotine dependence, we targeted over 300 candidate genes and analyzed 3713 single nucleotide polymorphisms (SNPs) in 1050 cases and 879 controls. The Fagerström test for nicotine dependence (FTND) was used to assess dependence, in which cases were required to have an FTND of 4 or more. The control criterion was strict: control subjects must have smoked at least 100 cigarettes in their lifetimes and had an FTND of 0 during the heaviest period of smoking. After correcting for multiple testing by controlling the false discovery rate, several cholinergic nicotinic receptor genes dominated the top signals. The strongest association was from an SNP representing *CHRN3*, the $\beta 3$ nicotinic receptor subunit gene ($P = 9.4 \times 10^{-5}$). Biologically, the most compelling evidence for a risk variant came from a non-synonymous SNP in the $\alpha 5$ nicotinic receptor subunit gene *CHRNA5* ($P = 6.4 \times 10^{-4}$). This SNP exhibited evidence of a recessive mode of inheritance, resulting in individuals having a 2-fold increase in risk of developing nicotine dependence once exposed to cigarette smoking. Other genes among the top signals were *KCNJ6* and *GABRA4*. This study represents one of the most powerful and extensive studies of nicotine dependence to date and has found novel risk loci that require confirmation by replication studies.

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INTRODUCTION

The World Health Organization estimates that if current trends continue, the annual number of deaths from tobacco-related diseases will double from five million in the year 2000 to 10 million in 2020 (1,2). Nicotine, a naturally occurring alkaloid found in tobacco, mimics acetylcholine, and nicotine's ability to bind to nicotinic cholinergic receptors (nAChRs) underlies the molecular basis of nicotine dependence [susceptibility to tobacco addiction (MIM 188890)]. Chronic nicotine exposure produces long-lasting behavioral and physiological changes that include increased synaptic strength, altered gene expression and nAChR up-regulation (3). Although nAChRs are expressed throughout the central nervous system, the addictive effects of nicotine are thought to be mediated through mesocorticolimbic dopamine (DA) pathways (4). It is believed that the interplay among glutamate, dopamine and gamma-aminobutyric acid (GABA) systems is critical for the reinforcing effects of nicotine (3,5). Cigarettes are the predominant form of tobacco used worldwide (6), and genetic factors are important to the etiology of nicotine dependence, with estimates of the heritability ranging from 44 to 60% (7).

Efforts to identify susceptibility loci influencing cigarette smoking behavior through association studies have used a candidate gene approach with both case-control and family-based designs. Several candidate genes that may influence smoking have been studied, including nicotinic receptors (8–10), nicotine metabolizing genes (11–13), dopamine system receptors (14–17), GABA receptors (18) and other neurotransmitters and receptors (19–21). There appears to be very little concordance among linkage findings and association findings in candidate genes (reviewed in 22). The only genome-wide association study (GWAS) to date is by the companion paper by Bierut *et al.* (23) which was conducted in parallel with our study and used the same case-control sample.

Our approach was to target an extensive set of candidate genes for single nucleotide polymorphism (SNP) genotyping to detect variants associated with nicotine dependence using a case-control design. We targeted over 300 genes for genotyping, with a design that allowed for approximately 4000 SNPs. These included the gene families encoding nicotinic receptors, dopaminergic receptors and GABA receptors, which are known to be part of the biological pathways involved in dependence. This was done in conjunction with a GWAS conducted in the companion paper by Bierut *et al.* (23). Both studies used a large sample of cases and controls of European descent. The 1050 nicotine dependent cases were contrasted with a unique control sample of 879 individuals who are non-dependent smokers. The size of the sample and strict control criteria should provide ample power to detect variants influencing nicotine dependence, but the depth of the coverage of known candidate genes is ambitious and requires delicate handling to deal with the complex issue of multiple testing. We used the false discovery rate (FDR) to limit the effects of multiple testing (24,25) and to report on the top FDR-controlled list of associations.

RESULTS

Our list of candidate genes initially numbered 448 and was divided into categories 'A' and 'B'. All 55 category A genes

Table 1. Results of the pooled genotyping in the candidate genes from the parallel GWAS

Category	Candidate genes	Genes tested	SNPs tested	Corrected minimum P -value (P_{corr})		
				Min	Max	$\mu \pm \sigma^a$
A	55	52	1 604	0.0017	0.94	0.33 ± 0.28
B	393	367	29 071	0.0021	0.99	0.41 ± 0.28
Total	448	419	30 675			

A total of 2 177 718 SNPs passed quality control (QC) measures and were tested for association. The results were used to rank the category B genes for SNP selection. The 'genes tested' and 'SNPs tested' columns show the number of genes and number of SNPs in those genes that passed QC and were tested for association. The minimum P -value over all SNPs tested for association in the pooled genotyping within a gene is corrected for the number of tests according to Eq. (1).

^aMean \pm standard deviation.

were targeted for SNP genotyping, but because it was beyond our resources to target all of the remaining 393 category B genes, these were prioritized for SNP genotyping according to the results of the pooled genotyping in the parallel GWAS (23). Table 1 shows a summary of the results of the pooled genotyping in the candidate genes. Out of the 393 category B genes considered for SNP selection, 296 were targeted for individual genotyping in our candidate gene study. These were chosen using the lowest corrected minimum P -values, as defined in Eq. (1), where the cutoff was approximately $P \leq 0.95$. We individually genotyped 4309 SNPs in these candidate genes, and after quality control filtering, 3713 SNPs were tested for association. There were 515 SNPs tested for 52 category A genes and 3198 SNPs tested for 296 category B genes.

In the individual genotyping for the candidate genes, the 10 smallest P -values from our primary association analysis ranged from 9.36×10^{-5} to 1.22×10^{-3} . There were 39 SNPs with an FDR <40%, indicating the presence of about 24 true signals (Tables 2 and 3; Fig. 1). These top 39 signals were dominated by nicotinic receptor genes (Figs 2 and 3). The top five FDR values corresponded to the genes *CHRNA3*, *CHRNA5* and *CHRNA3* and ranged from 0.056 to 0.166. Our best evidence was that four of these five signals were from genuine associations and were not due to random effects. The permutation FDR estimates were roughly the same as the FDR, differing by not more than 0.02, with a minimum permutation FDR of 0.07 at the SNP *rs6474413*. After selecting a single SNP from each linkage disequilibrium (LD) bin, three of these 39 SNPs showed significant evidence of a non-multiplicative model (Table 4) and several SNPs were found to have a significant gender by genotype interaction (Table 5; also see Supplementary Material, Table S1 for a list of all SNPs from Table 2 showing gender by genotype P -values and gender-specific odds ratios).

The $\beta 3$ nicotinic receptor subunit gene *CHRNA3*, located on chromosome 8, accounted for the two strongest signals from our analysis: *rs6474413* and *rs10958726* (Fig. 2A). These two SNPs effectively contributed to a single signal because they were in very high LD with an r^2 correlation ≥ 0.99 . They are in the putative 5' promoter region: the SNP *rs6474413* is within 2 kb of the first 5' promoter and the SNP *rs10958726* is

Table 2. Top associations with nicotine dependence where the weighted FDR is <40%

SNP	Gene	Function	Category	Chr	Pos (bp)	LD Bin ID	Min (r^2)	Risk allele	Primary P -value	Rank	FDR
<i>rs6474413</i>	<i>CHRNA3</i>	FP	A	8	42,670,221	8-19	0.991	T (0.81/0.76)	9.36E-05	1	0.056
<i>rs10958726</i>	<i>CHRNA3</i>	LD BIN	A	8	42,655,066	8-19	0.991	T (0.81/0.76)	1.33E-04	2	0.056
<i>rs578776</i>	<i>CHRNA3</i>	UTR	A	15	76,675,455	-	-	G (0.78/0.72)	3.08E-04	3	0.086
<i>rs6517442</i>	<i>KCNJ6</i>	FP	B	21	38,211,816	-	-	C (0.34/0.28)	5.62E-04	4	0.344
<i>rs16969968^a</i>	<i>CHRNA5</i>	NONSYN	A	15	76,669,980	15-13	0.989	A (0.38/0.32)	6.42E-04	5	0.134
<i>rs3762611</i>	<i>GABRA4</i>	FP	B	4	46,838,216	4-71	0.939	G (0.93/0.91)	9.22E-04	6	0.344
<i>rs1051730</i>	<i>CHRNA3</i>	SYNON	A	15	76,681,394	15-13	0.989	A (0.38/0.32)	9.93E-04	7	0.166
<i>rs10508649^{b,c}</i>	<i>PIP5K2A</i>	SYNON	B	10	22,902,288	-	-	T (1.00/0.99)	1.02E-03	8	0.344
<i>rs17041074^b</i>	<i>DAO</i>	INTRON	B	12	107,794,340	-	-	A (0.27/0.26)	1.12E-03	9	0.344
<i>rs3762607^b</i>	<i>GABRA4</i>	FP	B	4	46,837,266	4-71	0.939	A (0.93/0.91)	1.22E-03	10	0.344
<i>rs2767</i>	<i>CHRNA3</i>	UTR	A	2	233,225,579	2-68	0.887	G (0.39/0.34)	1.50E-03	11	0.209
<i>rs6772197^b</i>	<i>DOCK3 (GRM2)</i>	INTRON	B	3	51,126,839	3-46	0.923	A (0.84/0.83)	1.66E-03	12	0.384
<i>rs3021529^b</i>	<i>AVPR1A</i>	UTR	B	12	61,831,947	12-10	0.842	G (0.86/0.85)	1.73E-03	13	0.384
<i>rs1206549</i>	<i>CLTCL1</i>	INTRON	B	22	17,590,414	22-5	0.996	G (0.86/0.82)	1.75E-03	14	0.384
<i>rs637137</i>	<i>CHRNA5</i>	INTRON	A	15	76,661,031	15-3	0.801	T (0.81/0.76)	2.82E-03	22	0.336
<i>rs3791729</i>	<i>CHRNA3</i>	INTRON	A	2	233,220,802	2-68	0.887	A (0.36/0.32)	3.39E-03	25	0.344
<i>rs4531</i>	<i>DBH</i>	NONSYN	A	9	133,538,924	-	-	G (0.93/0.91)	5.10E-03	30	0.344
<i>rs3025382^a</i>	<i>DBH</i>	INTRON	A	9	133,531,875	-	-	G (0.90/0.88)	5.14E-03	31	0.344
<i>rs7877</i>	<i>FMO1</i>	UTR	A	1	167,986,548	1-60	0.890	C (0.74/0.70)	6.33E-03	38	0.344
<i>rs6320^b</i>	<i>HTR5A</i>	SYNON	A	7	154,300,269	-	-	T (0.72/0.71)	6.50E-03	39	0.344
<i>rs4802100^b</i>	<i>CYP2B6</i>	FP	A	19	46,187,865	19-4	0.995	G (0.10/0.08)	6.76E-03	41	0.344
<i>rs2304297</i>	<i>CHRNA6</i>	UTR	A	8	42,727,356	8-52	0.830	G (0.79/0.75)	6.91E-03	42	0.344
<i>rs3760657</i>	<i>CYP2B6</i>	FP	A	19	46,187,273	19-4	0.995	G (0.10/0.08)	6.98E-03	43	0.344
<i>rs2276560</i>	<i>CHRNA3</i>	LD BIN	A	2	233,276,424	2-63	0.931	T (0.77/0.74)	7.42E-03	44	0.344
<i>rs742350</i>	<i>FMO1</i>	SYNON	A	1	167,981,702	1-7	0.971	C (0.87/0.84)	8.45E-03	48	0.344
<i>rs684513</i>	<i>CHRNA5</i>	INTRON	A	15	76,645,455	15-3	0.801	C (0.82/0.78)	8.72E-03	49	0.344
<i>rs510769^a</i>	<i>OPRM1</i>	INTRON	A	6	154,454,133	-	-	T (0.27/0.24)	9.84E-03	58	0.344
<i>rs4245150^b</i>	<i>DRD2</i>	LD BIN	A	11	112,869,857	11-8	0.998	G (0.37/0.36)	1.08E-02	61	0.344
<i>rs3743078</i>	<i>CHRNA3</i>	INTRON	A	15	76,681,814	15-3	0.801	G (0.83/0.79)	1.10E-02	63	0.344
<i>rs1657273^b</i>	<i>HTR5A</i>	LD BIN	A	7	154,317,817	7-29	0.976	G (0.69/0.68)	1.11E-02	64	0.344
<i>rs17602038</i>	<i>DRD2</i>	LD BIN	A	11	112,869,901	11-8	0.998	C (0.37/0.36)	1.17E-02	69	0.344
<i>rs3813567</i>	<i>CHRNA3</i>	FP	A	15	76,721,606	-	-	A (0.83/0.79)	1.18E-02	70	0.344
<i>rs893109</i>	<i>HTR5A</i>	LD BIN	A	7	154,330,522	7-29	0.976	G (0.69/0.68)	1.24E-02	73	0.344
<i>rs16864387</i>	<i>FMO4</i>	UTR	A	1	168,015,501	1-7	0.971	T (0.87/0.84)	1.28E-02	74	0.344
<i>rs6045733^b</i>	<i>PDYN</i>	LD BIN	A	20	1,898,858	20-32	0.810	G (0.66/0.65)	1.55E-02	84	0.384
<i>rs4953</i>	<i>CHRNA3</i>	SYNON	A	8	42,706,816	8-13	1.000	G (0.97/0.95)	1.61E-02	85	0.384
<i>rs4952</i>	<i>CHRNA3</i>	SYNON	A	8	42,706,222	8-13	1.000	C (0.97/0.95)	1.63E-02	87	0.384
<i>rs6749955</i>	<i>CHRNA3</i>	LD BIN	A	2	233,263,422	2-63	0.931	T (0.77/0.73)	1.70E-02	91	0.384
<i>rs7517376</i>	<i>FMO1</i>	SYNON	A	1	167,983,945	1-7	0.971	A (0.87/0.84)	1.80E-02	95	0.384

SNPs from category A genes were weighted 10-fold more heavily than those from category B genes when estimating FDR. The signals are sorted by the primary two-degree-of-freedom P -value of adding the genotype term and the genotype by gender interaction term to the base model in the logistic regression. SNPs with function 'FP' are within the footprint of the gene, defined for display purposes as ± 10 kb of the transcribed region. Those labeled LD BIN are outside of the footprint and were selected for genotyping for being in LD with SNPs near an exon. Genes in parentheses are the candidate genes for which the SNP was selected. The 'LD Bin ID' column identifies LD bins; SNPs with the same LD Bin ID effectively produce a single association signal. We report the minimum correlation between the tag and other SNPs in the bin in the 'Min (r^2)' column. The rank is determined by the primary P -value in all 3713 genotyped SNPs. All alleles were reported from the positive strand. The frequency of the risk allele (the allele more frequent in cases than in controls) in cases p and controls q is reported with the notation p/q .

^aThere is significant evidence for a non-multiplicative model (Table 4) (which shows one SNP per LD bin).

^bThere is significant evidence for gender-specific risk (Table 5) (which shows one SNP per LD bin).

^cVery low minor allele frequency.

an additional 15 kb upstream. Two other SNPs in *CHRNA3*, *rs4953* and *rs4952*, were also among the top signals. These are synonymous SNPs in exon 5 and are the only known coding SNPs for *CHRNA3* (dbSNP build 125, <http://www.ncbi.nlm.nih.gov/snp>). Again, these represent a single signal as their genotypes were completely correlated.

The next group of SNPs among our top signals is in the *CHRNA5-CHRNA3-CHRNA4* cluster of nicotinic receptor genes on chromosome 15 (Fig. 2B). The third most significant signal was the SNP *rs578776* in the 3'-untranslated region (UTR) of *CHRNA3*, the $\alpha 3$ nicotinic receptor subunit gene (Fig. 2B). Approximately 5 kb downstream from

CHRNA3 is our fifth strongest signal *rs16969968*, a non-synonymous coding SNP in exon 5 of *CHRNA5*, the $\alpha 5$ nicotinic receptor subunit gene. This SNP was in very strong LD with *rs1051730*, a synonymous coding SNP in *CHRNA3*, with an r^2 correlation ≥ 0.99 .

The most interesting signal appears to be the non-synonymous SNP *rs16969968* in *CHRNA5*. As discussed earlier, it is completely correlated with an SNP in the *CHRNA3* gene (Fig. 2B). Allele A of *rs16969968* has a frequency of 38% in cases and 32% in controls. There is convincing evidence for a recessive mode of inheritance for this SNP (Table 4). Compared to having no copies, the odds ratios for having one copy and two

Table 3. Details of all category A genes and any category B genes with SNPs among our top signals (i.e. SNPs that appear in Table 2)

Gene	Chr	5' position (Mb)	Size (kb)	Strand	SNPs tested	SNPs tested per kb	SNPs in top signals
Category A							
<i>ADRBK2</i>	22	24.286	159	+	5	0.0	0
<i>ANKK1</i>	11	112.764	12.6	+	23	1.8	0
<i>ARRB2</i>	17	4.561	11.0	+	3	0.3	0
<i>BDNF</i>	11	27.700	66.8	-	10	0.1	0
<i>CCK</i>	3	42.281	6.9	-	13	1.9	0
<i>CHRNA1</i>	2	175.455	16.6	-	3	0.2	0
<i>CHRNA10</i>	11	3.649	5.8	-	3	0.5	0
<i>CHRNA2</i>	8	27.393	18.5	-	17	0.9	0
<i>CHRNA3</i>	15	76.700	25.7	-	18	0.7	5
<i>CHRNA4</i>	20	61.463	16.7	-	8	0.5	0
<i>CHRNA5</i>	15	76.645	28.6	+	18	0.6	6
<i>CHRNA6</i>	8	42.743	15.8	-	4	0.3	1
<i>CHRNA7</i>	15	30.11	138.5	+	13	0.1	0
<i>CHRNA9</i>	4	40.178	19.5	+	11	0.6	0
<i>CHRNB1</i>	17	7.289	12.5	+	10	0.8	0
<i>CHRNB2</i>	1	151.353	8.8	+	4	0.5	0
<i>CHRNB3</i>	8	42.672	39.6	+	6	0.2	5
<i>CHRNB4</i>	15	76.721	17.0	-	14	0.8	5
<i>CHRNA5</i>	2	233.216	9.3	+	3	0.3	2
<i>CHRNE</i>	17	4.747	5.3	-	3	0.6	0
<i>CHRNG</i>	2	233.23	6.0	+	6	1.0	4
<i>CNR1</i>	6	88.912	5.5	-	9	1.6	0
<i>COMT</i>	22	18.304	27.2	+	13	0.5	0
<i>CYP2A6</i>	19	46.048	6.9	-	3	0.4	0
<i>CYP2B6</i>	19	46.189	27.1	+	14	0.5	2
<i>DBH</i>	9	133.531	23.0	+	10	0.4	2
<i>DDC</i>	7	50.386	85.7	-	30	0.4	0
<i>DRD1</i>	5	174.804	3.1	-	4	1.3	0
<i>DRD2</i>	11	112.851	65.6	-	29	0.4	2
<i>DRD3</i>	3	115.38	50.2	-	8	0.2	0
<i>DRD5</i>	4	9.460	2.0	+	4	2.0	0
<i>FAAH</i>	1	46.572	19.5	+	5	0.3	0
<i>FMO1</i>	1	167.949	37.5	+	14	0.4	4
<i>FMO3</i>	1	167.792	26.9	+	23	0.9	0
<i>GABRB2</i>	5	160.908	254.3	-	14	0.1	0
<i>GPR51</i>	9	98.551	421.1	-	29	0.1	0
<i>HTR1A</i>	5	63.293	1.3	-	5	3.9	0
<i>HTR2A</i>	13	46.368	62.7	-	20	0.3	0
<i>HTR5A</i>	7	154.3	13.6	+	13	1.0	3
<i>MAOA</i>	23	43.272	90.7	+	5	0.1	0
<i>MAOB</i>	23	43.498	115.8	-	10	0.1	0
<i>NPY</i>	7	24.097	7.7	+	22	2.9	0
<i>OPRD1</i>	1	28.959	51.6	+	1	0.0	0
<i>OPRK1</i>	8	54.327	22.2	-	12	0.5	0
<i>OPRM1</i>	6	154.453	80.1	+	12	0.1	1
<i>PDYN</i>	20	1.923	15.3	-	11	0.7	1
<i>PENK</i>	8	57.521	5.1	-	6	1.2	0
<i>POMC</i>	2	25.303	7.7	-	2	0.3	0
<i>SLC6A3</i>	5	1.499	52.6	-	5	0.1	0
<i>SLC6A4</i>	17	25.587	37.8	-	8	0.2	0
<i>TH</i>	11	2.150	7.9	-	6	0.8	0
<i>TPH1</i>	11	18.019	19.8	-	14	0.7	0
Category B							
<i>AVPR1A</i>	12	61.833	6.4	-	15	2.4	1
<i>CLTCL1</i>	22	17.654	112.2	-	15	0.1	1
<i>DAO</i>	12	107.776	20.8	+	7	0.3	1
<i>FMO4</i>	1	168.015	27.7	+	12	0.4	4
<i>GABRA4</i>	4	46.837	74.7	-	29	0.4	2
<i>GRM2</i>	3	51.718	9.1	+	2	0.2	1
<i>KCNJ6</i>	21	38.211	291.9	-	18	0.1	1
<i>PIP5K2A</i>	10	23.043	177.7	-	15	0.1	1

The column 'SNPs tested' refers to the number of SNPs tested for association and the column 'SNPs in top signals' refers to the SNPs that appear in Table 2. Some SNPs represent multiple genes, particularly when two genes are near each other; hence there is overlap between genes for the SNPs represented by these two columns. Genes with SNPs in our top signals are shown in boldface.

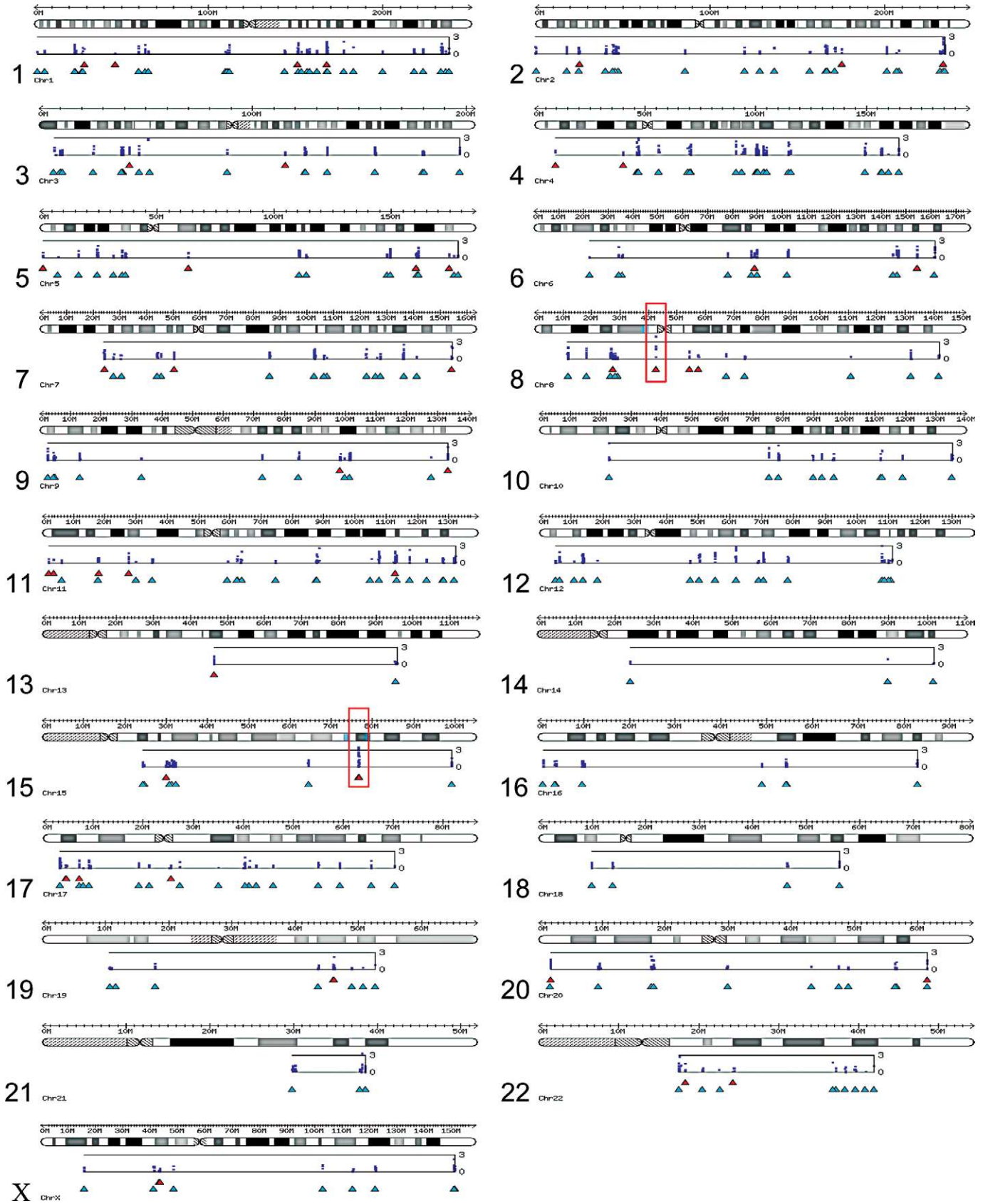


Figure 1. Results of the candidate gene association analysis. The P -values from the primary analysis are plotted for each chromosome below an ideogram using the $-\log_{10}(P)$ transformation. The bottom axis is $P = 1$ and the top axis is $P = 10^{-3}$. Category A genes are shown below the plots in red and category B genes are shown in cyan below the category A genes. Regions on chromosomes 8 and 15, which are shown in more detail in Figure 2, are highlighted in red.

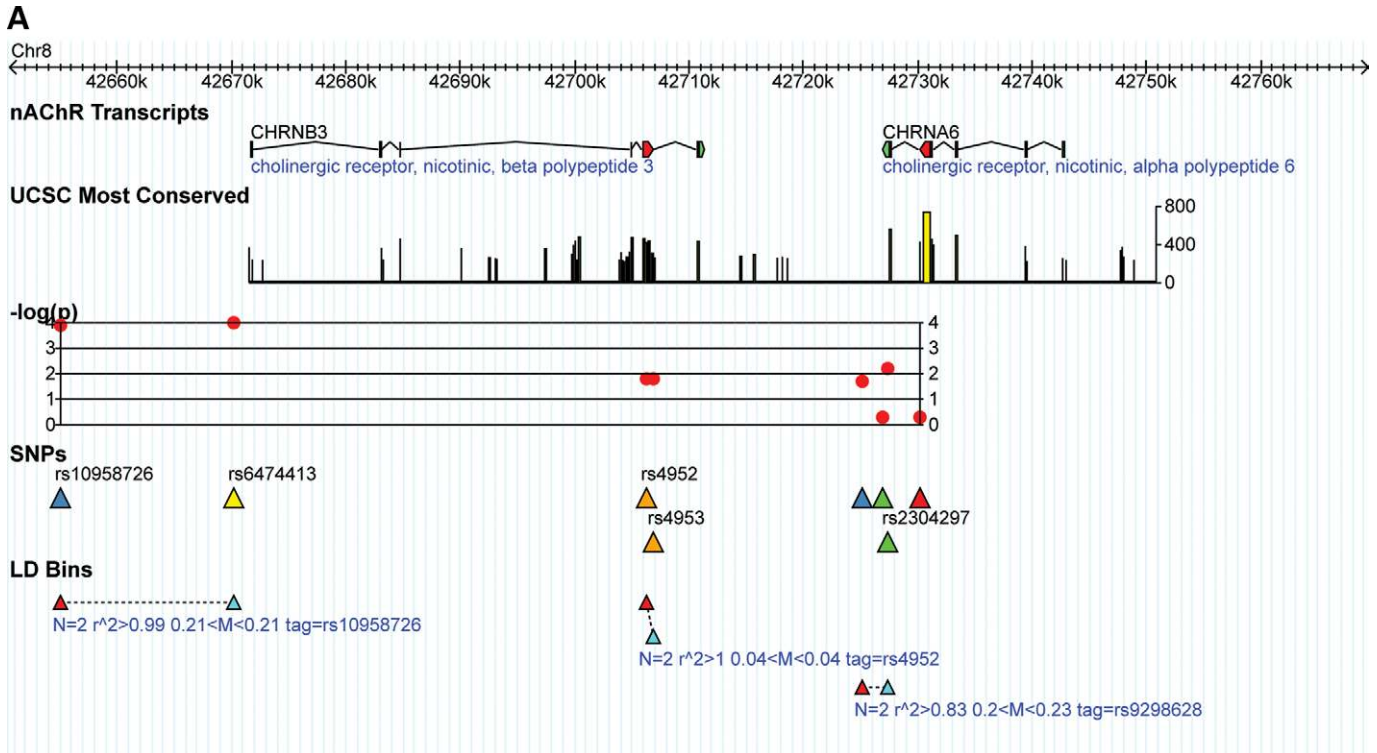


Figure 2. Detailed results for the top association signals. **(A)** The top two signals are near the *CHRN3* nicotinic receptor gene on chromosome 8. **(B)** The Non-synonymous SNP *rs16969968* and the *CHRNA5-CHRNA3-CHRN4* cluster of nicotinic receptor genes on chromosome 15. SNPs that appear in Table 2 are labeled with dbSNP rs IDs. The track 'UCSC Most Conserved' (<http://genome.ucsc.edu>, May 2004 build, table 'phastConsElements17way') highlights regions conserved between human and other species including the mouse, rat and chicken; the maximum conservation score is 1000. Primary *P*-values are plotted in red using the $-\log(P)$ transformation. The 'LD Bins' track displays the distribution of SNPs from the 'SNPs' track into LD bins where all SNPs have $r^2 \geq 0.8$ in both cases and controls with the tag SNP. Only bins with more than two SNPs are shown, and bins are annotated with number of SNPs *N*, the minimum r^2 of the tag with the other SNPs in the bin, the range of allele frequencies in the bin and the tag SNP. **(C)** A legend indicating the color scheme.

copies of the A allele were 1.1 (95% CI 0.9–1.4) and 1.9 (95% CI 1.4–2.6), respectively. That is, compared with individuals with other genotypes, individuals with the AA genotype were nearly twice as likely to have symptoms of nicotine dependence.

DISCUSSION

Nicotine addiction from tobacco smoking is responsible for over three million deaths annually, making it the leading cause of preventable mortality in the world (1). In the USA in 2003, 21.6% of adults were smokers, where 24% of men and 19% of women were smokers (26). Previous association studies have been limited to narrowly focussed candidate gene studies. Our candidate gene study was more extensive, genotyping 3713 SNPs for 348 candidates in 1050 nicotine-dependent cases and 879 non-dependent smokers, where our control group definition was particularly strict.

Our top FDR-controlled findings were dominated by nicotinic receptor genes. Our positive association findings for the $\alpha 5$ and $\beta 3$ nicotinic receptor subunits are novel. To date, most human genetic and biological studies of the nicotinic receptors and nicotine dependence have focussed on the $\alpha 4$ and $\beta 2$ subunits because they co-occur in high-affinity receptors and are widely expressed in the brain (27). However, mouse studies have demonstrated that of the

$\alpha 4\beta 2$ containing receptors that mediate dopamine release, a substantial proportion contain $\alpha 5$ as well (28). This is consistent with our evidence for an important role of $\alpha 5$ in nicotine dependence susceptibility. Furthermore, in a brain $\alpha 4\beta 2$ receptor, an $\alpha 5$ or $\beta 3$ subunit can take the fifth position in the pentamer, corresponding to $\beta 1$ of muscle. Although neither $\alpha 5$ nor $\beta 3$ is thought to participate in forming binding sites, they are able to affect channel properties and influence agonist potency because they participate in the conformational changes associated with activation and desensitization (27).

The most compelling biological evidence of a risk factor for nicotine dependence is from the non-synonymous SNP *rs16969968* in *CHRNA5*. This SNP causes a change in amino acid 398 from asparagine (encoded by the G allele) to aspartic acid (encoded by A, the risk allele), which results in a change in the charge of the amino acid in the second intracellular loop of the $\alpha 5$ subunit (29). The risk allele appeared to act in a recessive mode, in which individuals who were homozygous for the A allele are at a 2-fold risk to develop nicotine dependence. Although the $\alpha 5$ subunit has not been studied extensively and there are no reports of known functional effects of this polymorphism, it is striking that a non-synonymous charge-altering polymorphism in the corresponding intracellular loop of the $\alpha 4$ nAChR subunit has been shown to alter nAChR function

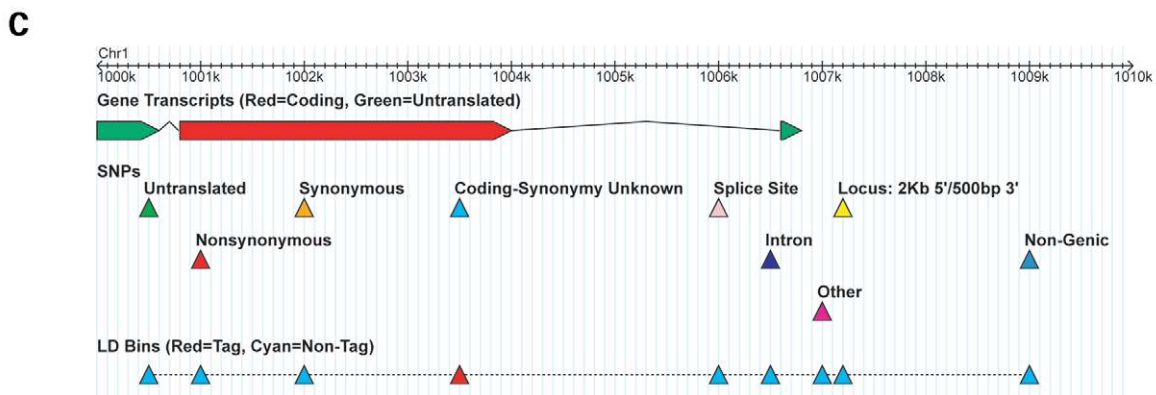
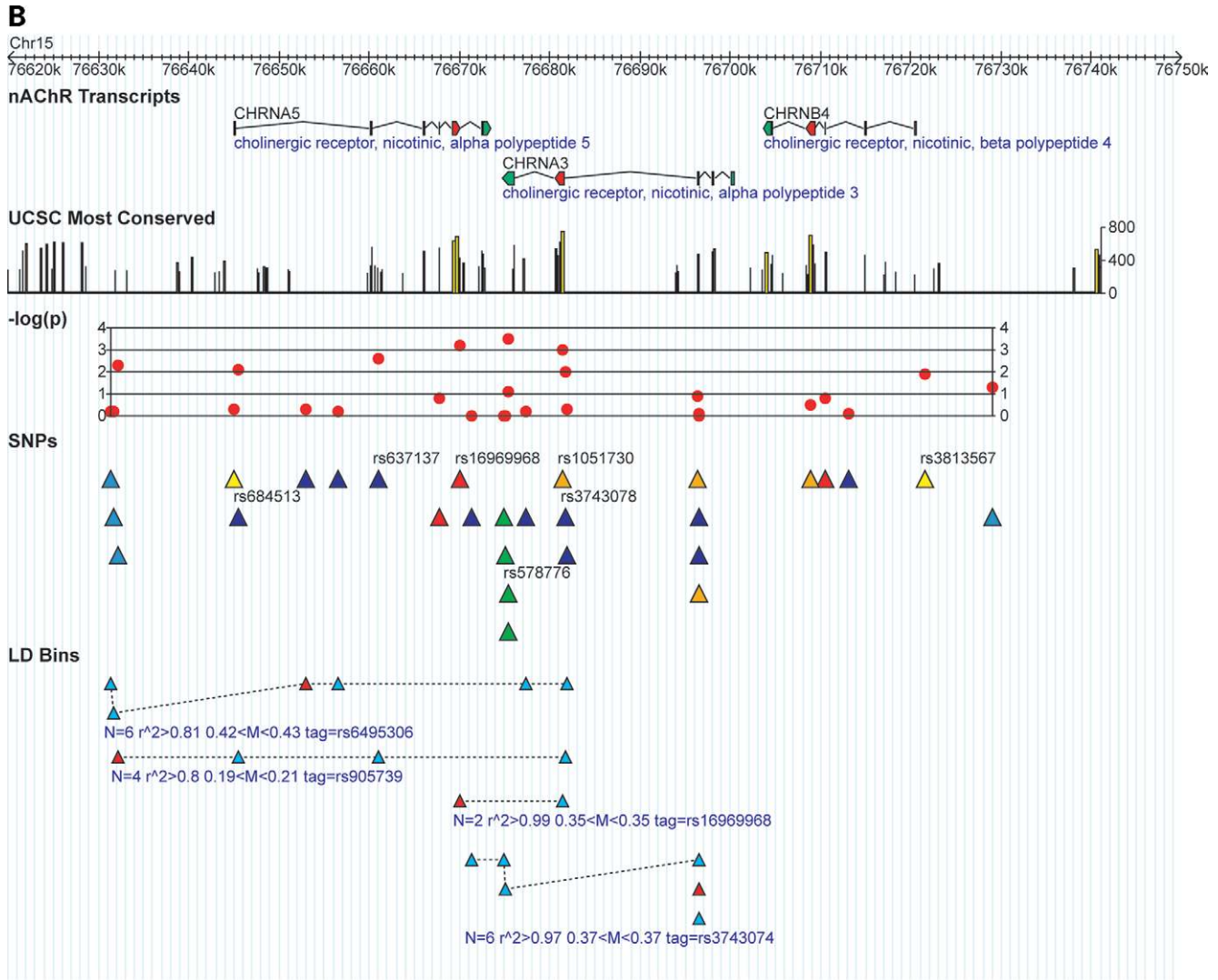


Figure 2. Continued.

in mice in response to nicotine exposure (30–33). This variant is common in the populations of European descent (allele frequency of A allele $\sim 42\%$), but uncommon in populations of Asian or African descent ($< 5\%$, data from International HapMap project, <http://www.hapmap.org>).

Also among the top 39 FDR-controlled signals were the genes *KCNJ6* (also known as *GIRK2*) and *GABRA4*. These were the only other genes besides nicotinic receptors with SNPs that had P -values less than 0.001. *KCNJ6* belongs to the inwardly rectifying potassium channel (GIRK) family of

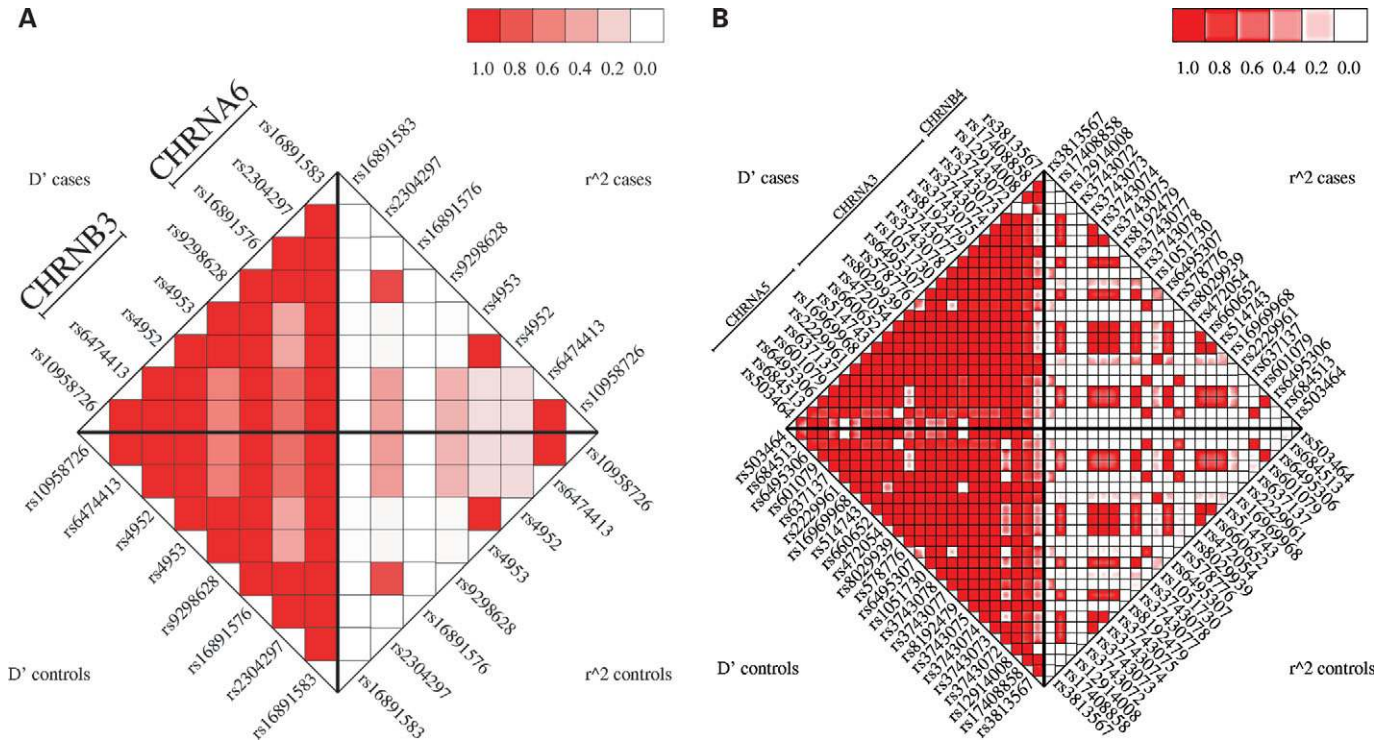


Figure 3. LD between markers in (A) the *CHRN3-CHRNA6* and (B) *CHRNA5-CHRNA3-CHRN4* clusters of nicotinic receptor genes.

Table 4. SNPs exhibiting significant deviation from a multiplicative genetic model

SNP	Gene	Non-multiplicative <i>P</i> -value	One risk allele odds ratio	Two risk alleles odds ratio
<i>rs16969968</i>	<i>CHRNA5</i>	4.04E-02	1.1 (0.9–1.4) AG/GG	1.9 (1.4–2.6) AA/GG
<i>rs3025382</i>	<i>DBH</i>	2.24E-02	0.6 (0.3–1.3) AG/AA	0.9 (0.4–2.0) GG/AA
<i>rs510769</i>	<i>OPRM1</i>	4.16E-04	1.5 (1.3–1.9) CT/CC	1.0 (0.7–1.4) TT/CC

The SNP with the smallest primary *P*-value was selected from each LD bin in Table 6. The multiplicative *P*-value is from the one degree of freedom test for the significance of the heterozygote term *H* in Eq. (3). We only show SNPs with *P* < 0.05. The last two columns show the odds ratios and 95% confidence intervals for the relative risk between genotypes. The SNP *rs16969968* clearly follows a recessive pattern where individuals carrying two copies of the A allele are nearly twice as likely to have symptoms of nicotine dependence compared with those with zero or one copy.

genes. GIRK provides a common link between numerous neurotransmitter receptors and the regulation of synaptic transmission (34). GABA is the major inhibitory neurotransmitter in the mammalian central nervous system and is critical for the reinforcing effects of nicotine (3,5). We found significant evidence that the risk due to genotype is much stronger in men than in women (Table 5), where the male odds ratio was 2.2 (95% CI 1.4–3.3).

Previously reported findings in other nicotinic receptors were not among our most significant findings. In prior

studies of *CHRNA4*, nominal association with nicotine dependence measures was reported for the SNPs *rs2236196* and *rs3787137* in African-American families and *rs2273504* and *rs1044396* in European-Americans, but only *rs2236196* in African-Americans remained after multiple testing correction (9). Also in *CHRNA4*, *rs1044396* and *rs1044397* were associated with both Fagerström test for nicotine dependence (FTND) score and qualitative nicotine dependence in a family-based sample of Asian male smokers (8). In our sample of European descent, we tested 11 SNPs for *CHRNA4* including the above-mentioned SNPs except *rs2273504*, which did not pass our stringent quality control standards. The lowest primary *P*-value across all 11 SNPs was 0.026 for *rs2236196* (study-wide rank = 132); this particular result may be considered a single test given the specific prior finding for this SNP, thus providing modest evidence for replication. The remaining four previously reported SNPs that we analyzed showed *P*-values greater than 0.8. Contrasts in these results are possibly due in part to the different ethnicities of the respective samples.

A recent study of smoking initiation and severity of nicotine dependence in Israeli women (10) analyzed 39 SNPs in 11 nicotinic receptor subunit genes. Their single SNP analyses also did not detect association with SNPs in $\alpha 4$, including *rs2236196*, *rs1044396* and *rs1044397*, although finding nominal significance in the $\alpha 7$, $\alpha 9$, $\beta 2$ and $\beta 3$ subunits. Their study did not include the same SNPs in the $\beta 3$ subunit and $\alpha 5-\alpha 3-\beta 4$ cluster comprising our four strongest associations in nicotinic receptor genes; they did analyze our fifth ranking nicotinic receptor SNP, *rs1051730*, and found a suggestive *P*-value of 0.08 when comparing ‘high’ nicotine-

Table 5. Gender-specific odds ratios and 95% confidence intervals for SNPs in Table 2

SNP	Gene	Primary <i>P</i> -value	Rank	Gender * genotype <i>P</i> -value	Male odds ratio	Female odds ratio
<i>rs10508649</i>	<i>PIP5K2A</i>	1.02E-03	8	1.09E-02	9.7 (2.1–44.2)	1.0 (0.3–3.1)
<i>rs17041074</i>	<i>DAO</i>	1.12E-03	9	3.70E-04	0.8 (0.6–1.0)	1.3 (1.1–1.6)
<i>rs3762607</i>	<i>GABRA4</i>	1.22E-03	10	3.43E-02	2.2 (1.4–3.3)	1.2 (0.9–1.6)
<i>rs6772197</i>	<i>DOCK3 (GRM2)</i>	1.66E-03	12	6.35E-04	1.6 (1.2–2.2)	0.9 (0.7–1.1)
<i>rs3021529</i>	<i>AVPR1A</i>	1.73E-03	13	8.96E-04	0.8 (0.5–1.0)	1.5 (1.1–1.9)
<i>rs6320</i>	<i>HTR5A</i>	6.50E-03	39	1.61E-03	0.7 (0.6–1.0)	1.2 (1.0–1.5)
<i>rs4802100</i>	<i>CYP2A7P1</i>	6.76E-03	41	2.82E-02	0.9 (0.6–1.4)	1.6 (1.2–2.1)
<i>rs4245150</i>	<i>DRD2</i>	1.08E-02	61	2.79E-03	0.8 (0.6–1.0)	1.2 (1.0–1.4)
<i>rs1657273</i>	<i>HTR5A</i>	1.11E-02	64	3.06E-03	0.8 (0.6–1.0)	1.2 (1.0–1.5)
<i>rs6045733</i>	<i>PDYN</i>	1.55E-02	84	4.25E-03	1.3 (1.1–1.7)	0.9 (0.7–1.0)

Only SNPs where the gender by genotype interaction was significant ($P < 0.05$) are shown, and the SNP with the most significant primary *P*-value was selected from each LD bin. The odds ratios are based on the coefficient of the genotype term *G* in Eq. (2) and represent the increase in risk for every unit increase in *G*; i.e. the risk follows a log-linear model (Tables 8 and 9).

dependent subjects with ‘low’ nicotine-dependent subjects in a much smaller sample than ours.

Our study was unable to corroborate reported association findings of Beuten *et al.* (18) for the β_2 subunit of the GABA_B receptor *GABBR2* (also known as *GABABR2*, *GABAB2* and *GPR51*). We genotyped 32 SNPs in *GABBR2* including five SNPs reported by Beuten *et al.* (18), three of which were the most significant in European-Americans by at least one test in that study. The primary *P*-value in our study was greater than 0.07 for all 32 SNPs and greater than 0.3 for the five previously reported SNPs.

Similarly, we do not find evidence for nominal association in our primary test of the 31 SNPs we genotyped for the *DDC* gene, which includes an SNP previously reported significant in European-Americans (35). And of the 11 SNPs covering the gene *BDNF*, three (*rs6265*, *rs2030324* and *rs7934165*) were previously reported as associated in European-American males (21); these three were not significant in our sample (primary $P = 0.86$, 0.088 and 0.12, respectively), and the lowest primary *P*-value among the remaining eight SNPs was 0.02, which does not survive correction for the six LD bins covering the gene. Note that our primary test uses a log-additive model, whereas previous reports sometimes found their strongest results under other models (e.g. recessive and dominant); however, for these previously reported associations, our tests for departure from the log-additive model did not find evidence for improvement under alternative modes of inheritance.

Our primary association analysis was a two-degree-of-freedom test of the significance of adding genotype and genotype by gender interaction terms to the base predictors sex and site. This approach helps to ensure that we detect associations that are significantly influenced by gender. The disadvantage is that the extra degree of freedom makes associations with insignificant gender interaction appears to be less significant overall.

Because our controls were highly selected and could even be considered ‘protected’ against susceptibility to nicotine dependence, interpretation of our results must consider the possibility that an association signal from our study may actually represent protective rather than risk effects. We used the allele more frequent in cases for reporting these data as a convention to facilitate comparison of the odds ratios among

SNPs; this should not be viewed as a conclusion of how a particular variant influences the risk for nicotine dependence. The precise determination of the mechanism by which a variant alters risk can only come from functional studies.

We performed additional tests for association using only the individuals from the US sample to determine whether our primary conclusions still hold in this subset of 797 cases and 813 controls (the Australian sample alone is too small to test for association, with only 253 cases and 66 controls). We used the same logistic regression method as for the entire sample except for the omission of the term ‘site’. The Spearman rank-order correlation of the *P*-values between the two tests for association was 0.87. Supplementary Material, Table S2 shows the results of the US-only analysis for the 39 SNPs from our list of top associations (Table 2), with the original ordering and FDR filtering, side by side with results from the US sample. Supplementary Material, Table S3 describes the result of completely starting over and using only the US sample to order by *P*-value, filter by $FDR < 40\%$ and compute LD bins. In this case, 30 of 39 (77%) SNPs in our original set of top signals (Table 2) appeared in the list of top signals in the US-only analysis (Supplementary Material, Table S3), which includes the genes *CHRNA5* and *CHRN3*, the top genes from our initial analysis. Hence, although there were some changes in the order of the results, the primary conclusion of association with the nicotinic receptors *CHRN3* and *CHRNA5* remains valid when the analysis is performed on the US subsample.

As a companion to the candidate gene study, a GWAS was carried out in parallel (23). Approximately 2.4 million SNPs were genotyped across the human genome in a two-stage design that began with pooled genotyping in a portion of the sample and followed with individual genotyping of the entire sample for the top 40 000 signals. The 21st strongest signal from the GWAS was due to an SNP 3 kb upstream of the first 5′ promoter of *CHRN3*, the gene with the strongest signal from our candidate gene study. This signal came from the SNP *rs13277254* (genotyped only for the GWAS and not for our candidate gene study) and had a *P*-value of 6.52×10^{-5} . This convergence from two different study designs provides further support that the signals in this gene are not random effects.

In conclusion, we have identified several genetic variants as being associated with nicotine dependence in candidate genes, the majority of which are nicotinic receptor genes. One of the SNPs implicated has a number of biologically relevant consequences, making it a particularly plausible candidate for influencing smoking behavior. These variants should be considered potential sources of genetic risk. Additional research is required to establish replication and possibly its role in the pharmacogenetics of response to nicotine dosing as well as to treatments for nicotine dependence.

MATERIALS AND METHODS

Subjects

All subjects (Table 6) were selected from two ongoing studies. The Collaborative Genetic Study of Nicotine Dependence (US) recruited subjects from three urban areas in the USA and the Nicotine Addiction Genetics (Australian) study collected subjects of European ancestry from Australia. Both studies used community-based recruitment and equivalent assessments were performed. Subjects who were identified as being smokers, using the criteria that they had smoked 100 or more cigarettes in their lifetimes, were queried in more detail using the FTND questionnaire. The US samples were enrolled at sites in St Louis, Detroit and Minneapolis, where a telephone screening of community-based subjects was used to determine whether subjects met criteria for case (current FTND ≥ 4) or control status. The study participants for the Australian sample were enrolled at the Queensland Institute of Medical Research in Australia, where families were identified from two cohorts of the Australian twin panel, which included spouses of the older of these two cohorts, for a total of approximately 12 500 families with information about smoking. The ancestry of the Australian samples is predominantly Anglo-Celtic and Northern European. The Institutional Review Boards approved both studies and all subjects provided informed consent to participate. Blood samples were collected from each subject for DNA analysis and submitted, together with electronic phenotypic and genetic data for both studies, to the National Institute on Drug Abuse (NIDA) Center for Genetic Studies, which manages the sharing of research data according to the guidelines of the National Institutes of Health.

Case subjects were required to score 4 or more on the FTND (36) during the heaviest period of cigarette smoking (the largest possible score is 10). This is a common criterion for defining nicotine dependence. Control subjects must have smoked 100 or more cigarettes in their lifetimes, yet never exhibited symptoms of nicotine dependence: they were smokers who scored 0 on the FTND during the heaviest period of smoking. By selecting controls that had a significant history of smoking, the genetic effects that are specific to nicotine dependence can be examined. Additional data from the Australian twin panel support this designation of a control status (23). In the US study, using the sample of 15 086 subjects who were determined to be smokers (smoked 100 or more cigarettes in their lifetimes) during the screening process, the prevalence of 'nicotine dependence'

Table 6. A summary of covariates and FTND scores in our sample: by definition, all control subjects scored 0 on the FTND (34)

		Cases		Controls	
		USA	Australia	USA	Australia
Males	<i>N</i>	351	114	251	17
	Age				
	range	25–44	30–82	25–44	34–82
	$\mu \pm \sigma^a$	36.8 \pm 5.3	39.4 \pm 9.8	35.3 \pm 5.5	55.1 \pm 15.4
Females	FTND				
	range	4–10	4–10	–	–
	$\mu \pm \sigma$	6.4 \pm 1.7	6.1 \pm 1.6	–	–
	<i>N</i>	446	139	562	49
Combined	Age				
	range	25–45	27–79	25–44	27–78
	$\mu \pm \sigma$	37.1 \pm 5.2	40.4 \pm 10.3	35.9 \pm 5.5	46.4 \pm 14.0
	FTND				
Total	range	4–10	4–10	–	–
	$\mu \pm \sigma$	6.4 \pm 1.8	6.0 \pm 1.6	–	–
	<i>N</i>	797	253	813	66
	Total		1050		879

^aMean \pm SD.

(FTND ≥ 4) was 46.4% and the prevalence of 'smoking without nicotine dependence' (FTND = 0) was 20.1%.

Candidate gene selection

The criteria for the selection of the candidate genes were based on known biology, correlations between nicotine dependence and other phenotypes and previous reports on the genetics of nicotine dependence and related traits. Genes were nominated by an expert committee of investigators from the NIDA Genetics Consortium (<http://zork.wustl.edu/nida>), with expertise in the study of nicotine and other substance dependence. These included classic genes that respond to nicotine, such as the nicotinic receptors, and other genes involved in the addictive process.

In total, 448 genes were considered for SNP genotyping. The genes were divided into two categories: A and B. Category A genes, which included the nicotinic and dopaminergic receptors, were considered to have a higher prior probability of association and were guaranteed to be targeted for genotyping. As our study design allowed for individual genotyping of approximately 4000 SNPs, the category B genes were too numerous to receive adequate SNP coverage once the A genes had been sufficiently covered. We therefore prioritized the category B genes using the results of the pooled genotyping from the companion GWAS study (23). Genes exhibiting the most evidence for association with nicotine dependence were prioritized for coverage. Some genes are larger than others and, therefore, may receive more SNPs. These genes may therefore appear more significant because of the increased number of tests performed. Hence, we corrected for multiple testing as follows. For a given candidate gene on the B list, if P_{\min} is the minimum P -value found in the pooled genotyping of stage I of the GWAS for all the SNPs genotyped in the gene and N is the number of SNPs tested, then we computed the corrected minimum P -value

P_{corr} using the formula

$$P_{\text{corr}} = 1 - (1 - P_{\text{min}})^{(N+1)/2} \quad (1)$$

As roughly 50% of the SNPs in any chromosomal region are in high LD (37), we used $(N+1)/2$ as the exponent. The category B genes were then ranked by these corrected minimum P -values and SNPs were selected from the top of the ranked list until our resources were exhausted.

SNP selection

We chose all SNPs within exons, regardless of the allele frequency, and all SNPs within ± 2 kb of annotated gene promoters where the European-American minor allele frequency was at least 4%. We then chose tag SNPs for all European-American LD bins (38) crossing the exons of the candidate genes, with two SNPs for each bin with three or more SNPs. SNPs meeting these criteria were chosen first from those selected for individual genotyping in the companion pooled study (23) and then to cover the physical regions as uniformly as possible if there was choice available for the other SNPs. In addition, we included specific SNPs that have been reported in the literature as being associated with nicotine dependence (8,9,18,34).

Pooled genotyping

See the companion paper by Bierut *et al.* (23) for a description of the pooled genotyping.

Individual genotyping

For individual genotyping, we designed custom high-density oligonucleotide arrays to interrogate SNPs selected from candidate genes, as well as quality control SNPs. Each SNP was interrogated by 24 25mer oligonucleotide probes synthesized on a glass substrate. The 24 features comprise four sets of six features interrogating the neighborhoods of SNP reference and alternate alleles on forward and reference strands. Each allele and strand is represented by five offsets: -2 , -1 , 0 , 1 and 2 , indicating the position of the SNP within the 25mer, with 0 being at the 13th base. At offset 0 , a quartet was tiled, which includes the perfect match to reference and alternate SNP alleles and the two remaining nucleotides as mismatch probes. When possible, the mismatch features were selected as purine nucleotide substitution for a purine perfect match nucleotide and as a pyrimidine nucleotide substitution for a pyrimidine perfect match nucleotide. Thus, each strand and allele tiling consisted of six features comprising five perfect match probes and one mismatch.

Individual genotype cleaning

Individual genotypes were cleaned using a supervised prediction algorithm for the genotyping quality, compiled from 15 input metrics that describe the quality of the SNP and the genotype. The genotyping quality metric correlates with a probability of having a discordant call between the Perlegen platform and outside genotyping platforms (i.e. non-Perlegen

HapMap project genotypes). A system of 10 bootstrap aggregated regression trees was trained using an independent data set of concordance data between Perlegen genotypes and HapMap project genotypes. The trained predictor was then used to predict the genotyping quality for each of the genotypes in this data set (see Supplementary Material for more information regarding cleaning).

Population stratification analysis

In order to avoid false positives due to population stratification, we performed an analysis using the STRUCTURE software (39). This program identifies subpopulations of individuals who are genetically similar through a Markov chain Monte Carlo sampling procedure using markers selected across the genome. Genotype data for 289 high performance SNPs were analyzed across all 1929 samples. This analysis revealed no evidence for population admixture.

Genetic association analysis

An ANOVA analysis testing the predictive power of various phenotypes indicated that gender and site (USA or Australia) were the most informative and that age and other demographic variables did not account for significant additional trait variance (Table 7). Our primary method of analysis was based on a logistic regression: if P is the probability of being a case, then our linear logistic model has the form

$$\log\left(\frac{P}{1-P}\right) = \alpha + \beta_1 g + \beta_2 s + \beta_3 G + \beta_4 gG \quad (2)$$

where α is the intercept, g the gender coded 0 or 1 for males or females, respectively, and s the site coded as 0 or 1 for USA or Australia, respectively. The variable G represents genotype and is coded as the number of copies of the risk allele, defined as the allele more common in cases than in controls. It follows from Eq. (2) that the risk due to genotype is being modeled using a log-linear (i.e. multiplicative) scale rather than an additive scale. Maximum likelihood estimates for the coefficients and confidence intervals for odds ratios were computed using the SAS software package (40).

The predictors of our base model were gender and site. We then tested whether the addition of genotype and gender by genotype interaction to the base model significantly increased the predictive power and used the resulting two-degree-of-freedom χ^2 statistic to rank the SNPs by the corresponding P -values. Table 8 shows the formulas for the odds ratios in terms of the coefficients.

Following these primary analyses, we further analyzed the top ranked SNPs for significant evidence of dominant or recessive mode of inheritance. This was done using a logistic regression of the form

$$\log\left(\frac{P}{1-P}\right) = \alpha + \beta_1 g + \beta_2 s + \beta_3 G + \beta_4 H \quad (3)$$

where H is 1 for heterozygotes and 0 otherwise. When H is significant, the interpretation is that the genetic effect deviates signifi-

Table 7. ANOVA analysis of covariates

Model	ANOVA evaluated covariate	χ^2 (1 df)	P-value
Gender	Gender	40.0	4.2×10^{-10}
Gender + age	Age	10.3	1.3×10^{-03}
Gender + site	Site	100.4	1.2×10^{-23}
Gender + site + age	Age	0.25	0.62
Gender + site + gender*site	Gender*site	0.84	0.36

Logistic regression, modeling the probability of being a case, was performed for the indicated covariates. The χ^2 statistic is from the formula $-2(\Delta \log L)$, where $\Delta \log L$ is the change in likelihood in the logistic regression. The variable site has two levels: USA and Australia.

Table 8. Coding of the gender term g and the genotype term G used in the primary logistic regression model

Genotype	g	G	Odds ratio
AA	0	0	–
aA	0	1	e^{β_3}
aa	0	2	$e^{2\beta_3}$
AA	1	0	–
aA	1	1	$e^{\beta_3} e^{\beta_4}$
aa	1	2	$e^{2\beta_3} e^{2\beta_4}$

The allele a is the risk allele, the allele more common in cases than in controls. The variable G is defined as the number of copies of the risk allele, and g is 0 or 1 for male or female, respectively. The last column shows the expression for the gender-specific odds ratio for a given genotype compared with the AA genotype, which follows directly from the logistic regression model in Eq. (2).

cantly from the log-linear model. We then compute odds ratios for dominant and recessive models, as described in Table 9.

Linkage disequilibrium

We estimated r^2 correlation separately in cases and controls for all pairs of SNPs within 1 Mb windows using an EM algorithm as implemented in the computer program Haploview (version 3.2, <http://www.broad.mit.edu/mpg/haploview>) (41). Our final measure of LD is the minimum r^2 from the two samples. Following the algorithm in Hinds *et al.* (38) and Carlson *et al.* (42), SNPs were grouped into bins, where every bin contains at least one ‘tag SNP’ satisfying $\min(r^2) \geq 0.8$ with every SNP in the bin. The group of association signals from such an LD bin can be viewed essentially as a single signal.

Correcting for multiple testing

To account for multiple testing, we estimated the FDR (24,25) to control the proportion of false positives among our reported signals. As category A genes were considered to have a higher prior probability of association, we followed the recommendations of Roeder *et al.* (43) and weighted category A gene SNPs a moderate 10-fold more heavily. Therefore, the category B genes must have stronger association signals for inclusion in our list of FDR-filtered top signals. For each

Table 9. Codings used for the secondary logistic regression model

Genotype	G	H	Odds ratio
AA	0	0	–
aA	1	1	$e^{\beta_3} e^{\beta_4}$
aa	2	0	$e^{2\beta_3}$

The odds ratios follow directly from Eq. (3). Note that for a dominant model, the two odds ratios are equal, and for a recessive model, the odds ratio for aA is 1.

P -value, we computed a weighted P -value P_w using the formula

$$P_w = \begin{cases} wP & \text{category A genes} \\ 10wP & \text{category B genes} \end{cases}$$

where w was defined so that the average of the weights is 1 (this depends on the number of SNPs selected for A and B genes). For every weighted P -value P_w , we computed a q -value q_{w0} that has the property that the FDR is no greater than q_{w0} among all SNPs with $q_w < q_{w0}$ (25,44). This was done using the computer program QVALUE (version 1.1, <http://faculty.washington.edu/jstorey/qvalue>) (45). Our estimates of the FDR are based on the q -values.

This method of estimating the FDR does not take into account LD. Therefore, as an additional measure to correct for multiple testing and to assess statistical significance, we estimated the FDR using permutations and P -values weighted for A and B genes, which preserves the LD structure. This was done by performing 1000 random permutations of the case–control status and testing the permuted data for association. The significance of a P -value from the original data was assessed by counting the number of times a more significant weighted P -value occurs in the random permutations, where the weights were the same as those used for the FDR estimates.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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SUPPLEMENTARY MATERIAL [ONLINE ONLY]

Cholinergic Nicotinic Receptor Genes Implicated in a Nicotine Dependence Association Study Targeting 348 Candidate Genes with 3,713 SNPs

DNA Preparation. DNA was extracted from whole blood and EBV transformed cell lines on an AutoPure LS automated DNA extractor using the PuraGene Reagent System (GENTRA Systems). RNase was added to the WBC lysis stage with isopropanol precipitation of the DNA and resuspension in 1X TE Buffer (pH 8.0). DNA was quantified by optical density (OD) at 260nm on a DU-640 spectrophotometer (Beckman) and OD 260/280 absorbance ratios were between 1.8-2.0. DNA was aliquoted and stored frozen at -80°C until distributed to the genotyping labs.

Individual Genotype Cleaning. Concordance is computed independently for both reference and alternate allele feature sets, then a maximum is taken of the two values. For each allele at each offset for both the forward and reverse strand feature sets the identity of the brightest feature is noted. The concordance for a particular allele is computed as a ratio of the number of times the perfect match feature is the brightest to the total number of offsets over the forward and reverse strands. In the 24 feature SNP tiling each allele is represented by 6 features, distributed along 5 offsets and forward and reverse strands, with five perfect match probes and one mismatch. If N_{PM}^X is the number of times for allele X when the perfect match feature was brighter than the mismatch feature over all offsets and both strands, then:

$$\text{concordance} = \max\left(\frac{N_{PM}^{\text{Ref}}}{10}, \frac{N_{PM}^{\text{Alt}}}{10}\right)$$

SNP feature sets with concordance < 0.9 were discarded from further evaluation.

Let I^{TM} be the trimmed mean of perfect match intensities for a given allele and strand denoted by the subscript. The trimmed mean disregards the highest and the lowest intensity from the 5 perfect match intensities in the 24-feature tilings before computing the arithmetic mean. Let I^M be the mean of the mismatch intensity; since there is only one mismatch for each allele and strand no trimming is performed. We then define signal to background ratio (signal/background) to be the ratio between the amplitude of signal, computed from trimmed means of perfect match feature intensities, and amplitude of background, computed from means of mismatch feature intensities. The signal and background are computed as follows:

$$signal = \sqrt{\left(\left(I_{PM,Ref,Fwd}^{TM} + I_{PM,Ref,Rev}^{TM}\right)/2\right)^2 + \left(\left(I_{PM,Alt,Fwd}^{TM} + I_{PM,Alt,Rev}^{TM}\right)/2\right)^2}$$

$$background = \sqrt{\left(\left(I_{MM,Ref,Fwd}^M + I_{MM,Ref,Rev}^M\right)/2\right)^2 + \left(\left(I_{MM,Alt,Fwd}^M + I_{MM,Alt,Rev}^M\right)/2\right)^2}$$

SNP feature sets with signal/background < 1.5 were discarded from further evaluations. The number of saturated features was computed as the number of features that reached the highest intensity possible for the digitized numeric intensity value. SNPs with a nonzero number of saturated features were discarded from further evaluations.

As a final test, SNPs were tested for Hardy-Weinberg equilibrium (HWE). Those SNPs with an exact HWE p -value of less than 10^{-15} in either the cases or controls were discarded. SNPs with a HWE p -value between 10^{-15} and 10^{-4} were visually inspected and were discarded when problems with clustering were detected.

Table S1. Gender-specific odds ratios and 95% confidence intervals for SNPs in table 2. The odds ratios are based on the coefficient of the genotype term G in equation (2) and represent the increase in risk for every unit increase in G ; i.e., the risk follows a log-linear model (see table 8).

SNP	Gene	Primary p-value	Rank	Gender * Genotype p-value	Male OR	Female OR
<i>rs6474413</i>	<i>CHRN3</i>	9.36E-05	1	1.12E-01	1.2 (0.9-1.5)	1.5 (1.3-1.9)
<i>rs10958726</i>	<i>CHRN3</i>	1.33E-04	2	1.04E-01	1.2 (0.9-1.5)	1.5 (1.2-1.9)
<i>rs578776</i>	<i>CHRNA3</i>	3.08E-04	3	4.12E-01	1.5 (1.2-1.9)	1.3 (1.1-1.6)
<i>rs6517442</i>	<i>KCNJ6</i>	5.62E-04	4	6.17E-01	1.4 (1.1-1.7)	1.3 (1.1-1.5)
<i>rs16969968</i>	<i>CHRNA5</i>	6.42E-04	5	8.13E-01	1.3 (1.1-1.7)	1.3 (1.1-1.5)
<i>rs3762611</i>	<i>GABRA4</i>	9.22E-04	6	7.50E-02	2.1 (1.4-3.2)	1.3 (0.9-1.8)
<i>rs1051730</i>	<i>CHRNA3</i>	9.93E-04	7	1.00E+00	1.3 (1.0-1.6)	1.3 (1.1-1.5)
<i>rs10508649</i>	<i>PIP5K2A</i>	1.02E-03	8	1.09E-02	9.7 (2.1-44.2)	1.0 (0.3-3.1)
<i>rs17041074</i>	<i>DAO</i>	1.12E-03	9	3.70E-04	0.8 (0.6-1.0)	1.3 (1.1-1.6)
<i>rs3762607</i>	<i>GABRA4</i>	1.22E-03	10	3.43E-02	2.2 (1.4-3.3)	1.2 (0.9-1.6)
<i>rs2767</i>	<i>CHRND</i>	1.50E-03	11	1.08E-01	1.5 (1.2-1.8)	1.1 (1.0-1.4)
<i>rs6772197</i>	<i>DOCK3 (GRM2)</i>	1.66E-03	12	6.35E-04	1.6 (1.2-2.2)	0.9 (0.7-1.1)
<i>rs3021529</i>	<i>AVPRIA</i>	1.73E-03	13	8.96E-04	0.8 (0.5-1.0)	1.5 (1.1-1.9)
<i>rs1206549</i>	<i>CLTCL1</i>	1.75E-03	14	9.11E-01	1.4 (1.1-1.9)	1.4 (1.1-1.7)
<i>rs637137</i>	<i>CHRNA5</i>	2.82E-03	22	3.18E-01	1.5 (1.1-1.9)	1.2 (1.0-1.5)
<i>rs3791729</i>	<i>CHRND</i>	3.39E-03	25	3.10E-01	1.4 (1.1-1.7)	1.2 (1.0-1.4)
<i>rs4531</i>	<i>DBH</i>	5.10E-03	30	9.11E-01	1.5 (1.0-2.1)	1.5 (1.1-2.0)
<i>rs3025382</i>	<i>DBH</i>	5.14E-03	31	1.82E-01	1.6 (1.2-2.3)	1.2 (0.9-1.6)
<i>rs7877</i>	<i>FMO1</i>	6.33E-03	38	8.81E-01	1.3 (1.0-1.6)	1.3 (1.1-1.6)
<i>rs6320</i>	<i>HTR5A</i>	6.50E-03	39	1.61E-03	0.7 (0.6-1.0)	1.2 (1.0-1.5)
<i>rs4802100</i>	<i>CYP2B6</i>	6.76E-03	41	2.82E-02	0.9 (0.6-1.4)	1.6 (1.2-2.1)
<i>rs2304297</i>	<i>CHRNA6</i>	6.91E-03	42	1.59E-01	1.1 (0.8-1.4)	1.4 (1.1-1.7)
<i>rs3760657</i>	<i>CYP2B6</i>	6.98E-03	43	3.38E-02	0.9 (0.7-1.4)	1.6 (1.2-2.1)
<i>rs2276560</i>	<i>CHRNA5</i>	7.42E-03	44	8.58E-02	1.5 (1.1-1.9)	1.1 (0.9-1.3)
<i>rs742350</i>	<i>FMO1</i>	8.45E-03	48	2.67E-01	1.2 (0.9-1.6)	1.5 (1.1-1.9)
<i>rs684513</i>	<i>CHRNA5</i>	8.72E-03	49	1.72E-01	1.5 (1.1-1.9)	1.2 (0.9-1.4)
<i>rs510769</i>	<i>OPRM1</i>	9.84E-03	58	1.38E-01	1.1 (0.8-1.4)	1.3 (1.1-1.6)
<i>rs4245150</i>	<i>DRD2</i>	1.08E-02	61	2.79E-03	0.8 (0.6-1.0)	1.2 (1.0-1.4)
<i>rs3743078</i>	<i>CHRNA3</i>	1.10E-02	63	1.54E-01	1.5 (1.1-2.0)	1.2 (0.9-1.4)
<i>rs1657273</i>	<i>HTR5A</i>	1.11E-02	64	3.06E-03	0.8 (0.6-1.0)	1.2 (1.0-1.5)
<i>rs17602038</i>	<i>DRD2</i>	1.17E-02	69	3.13E-03	0.8 (0.6-1.0)	1.2 (1.0-1.4)
<i>rs3813567</i>	<i>CHRN4</i>	1.18E-02	70	9.10E-02	1.5 (1.1-2.0)	1.1 (0.9-1.4)

<i>rs893109</i>	<i>HTR5A</i>	1.24E-02	73	3.46E-03	0.8 (0.6-1.0)	1.2 (1.0-1.5)
<i>rs16864387</i>	<i>FMO4</i>	1.28E-02	74	3.82E-01	1.2 (0.9-1.7)	1.4 (1.1-1.9)
<i>rs6045733</i>	<i>PDYN</i>	1.55E-02	84	4.25E-03	1.3 (1.1-1.7)	0.9 (0.7-1.0)
<i>rs4953</i>	<i>CHRNA3</i>	1.61E-02	85	1.00E+00	1.6 (0.9-2.8)	1.7 (1.1-2.5)
<i>rs4952</i>	<i>CHRNA3</i>	1.63E-02	87	1.00E+00	1.6 (0.9-2.8)	1.7 (1.1-2.5)
<i>rs6749955</i>	<i>CHRNA3</i>	1.70E-02	91	1.67E-01	1.4 (1.1-1.8)	1.1 (0.9-1.4)
<i>rs7517376</i>	<i>FMO1</i>	1.80E-02	95	3.78E-01	1.2 (0.9-1.6)	1.4 (1.1-1.8)

Table S2. Top associations with nicotine dependence showing results from the primary analysis side by side with results based on the U.S. sample only. The conventions are the same as for table 2.

SNP	Gene	Function	Cat ^a	Risk Allele	US-only Risk Allele	Primary p-value	US-only p-value	Primary Rank	US-only Rank	Primary FDR	US-only FDR
rs6474413	CHRNA3	FP	A	T (0.81/0.76)	T (0.81/0.76)	9.36E-05	3.23E-03	1	19	0.056	0.228
rs10958726	CHRNA3	LD BIN	A	T (0.81/0.76)	T (0.81/0.77)	1.33E-04	4.69E-03	2	27	0.056	0.228
rs578776	CHRNA3	UTR	A	G (0.78/0.72)	G (0.78/0.71)	3.08E-04	8.48E-05	3	1	0.086	0.071
rs6517442	KCNJ6	FP	B	C (0.34/0.28)	C (0.35/0.28)	5.62E-04	6.93E-04	4	5	0.344	0.228
rs16969968	CHRNA5	NONSYN	A	A (0.38/0.32)	A (0.38/0.32)	6.42E-04	7.32E-04	5	7	0.134	0.176
rs3762611	GABRA4	FP	B	G (0.93/0.91)	G (0.94/0.91)	9.22E-04	5.29E-03	6	31	0.344	0.533
rs1051730	CHRNA3	SYNON	A	A (0.38/0.32)	A (0.38/0.32)	9.93E-04	8.41E-04	7	10	0.166	0.176
rs10508649	PIP5K2A	SYNON	B	T (1.00/0.99)	T (1.00/0.99)	1.02E-03	3.44E-04	8	2	0.344	0.228
rs17041074	DAO	INTRON	B	A (0.27/0.26)	A (0.27/0.27)	1.12E-03	1.90E-03	9	13	0.344	0.349
rs3762607	GABRA4	FP	B	A (0.93/0.91)	A (0.94/0.91)	1.22E-03	6.16E-03	10	40	0.344	0.565
rs2767	CHRNA3	UTR	A	G (0.39/0.34)	G (0.39/0.34)	1.50E-03	4.87E-03	11	28	0.209	0.228
rs6772197	(GRM2)	INTRON	B	A (0.84/0.83)	A (0.85/0.83)	1.66E-03	7.39E-03	12	47	0.384	0.599
rs3021529	AVPR1A	UTR	B	G (0.86/0.85)	G (0.87/0.86)	1.73E-03	5.96E-02	13	298	0.384	0.867
rs1206549	CLTCL1	INTRON	B	G (0.86/0.82)	G (0.87/0.82)	1.75E-03	4.35E-04	14	3	0.384	0.228
rs637137	CHRNA5	INTRON	A	T (0.81/0.76)	T (0.80/0.75)	2.82E-03	2.80E-03	22	16	0.336	0.228
rs3791729	CHRNA3	INTRON	A	A (0.36/0.32)	A (0.37/0.32)	3.39E-03	1.70E-02	25	113	0.344	0.325
rs4531	DBH	NONSYN	A	G (0.93/0.91)	G (0.93/0.91)	5.10E-03	2.34E-02	30	143	0.344	0.383
rs3025382	DBH	INTRON	A	G (0.9/0.88)	G (0.92/0.88)	5.14E-03	8.17E-04	31	9	0.344	0.176
rs7877	FMO1	UTR	A	C (0.74/0.70)	C (0.74/0.70)	6.33E-03	8.46E-03	38	59	0.344	0.228
rs6320	HTR5A	SYNON	A	T (0.72/0.71)	T (0.72/0.71)	6.50E-03	6.04E-03	39	38	0.344	0.228
rs4802100	CYP2A7P1	FP	A	G (0.10/0.08)	G (0.10/0.09)	6.76E-03	5.28E-02	41	263	0.344	0.533
rs2304297	CHRNA6	UTR	A	G (0.79/0.75)	G (0.79/0.75)	6.91E-03	1.38E-02	42	95	0.344	0.295
rs3760657	CYP2A7P1	FP	A	G (0.10/0.08)	G (0.10/0.09)	6.98E-03	5.50E-02	43	277	0.344	0.540
rs2276560	CHRNA3	LD BIN	A	T (0.77/0.74)	T (0.77/0.74)	7.42E-03	1.04E-02	44	72	0.344	0.256
rs742350	FMO1	SYNON	A	C (0.87/0.84)	C (0.87/0.84)	8.45E-03	5.51E-03	48	33	0.344	0.228
rs684513	CHRNA5	INTRON	A	C (0.82/0.78)	C (0.81/0.77)	8.72E-03	8.15E-03	49	54	0.344	0.228
rs510769	OPRM1	INTRON	A	T (0.27/0.24)	T (0.27/0.24)	9.84E-03	2.84E-02	58	167	0.344	0.410
rs4245150	DRD2	LD BIN	A	G (0.37/0.36)	G (0.37/0.36)	1.08E-02	1.29E-02	61	87	0.344	0.284
rs3743078	CHRNA3	INTRON	A	G (0.83/0.79)	G (0.82/0.79)	1.10E-02	1.98E-02	63	128	0.344	0.349
rs1657273	HTR5A	LD BIN	A	G (0.69/0.68)	G (0.69/0.68)	1.11E-02	7.74E-03	64	50	0.344	0.228
rs17602038	DRD2	LD BIN	A	C (0.37/0.36)	C (0.37/0.36)	1.17E-02	1.43E-02	69	98	0.344	0.298
rs3813567	CHRNA3	FP	A	A (0.83/0.79)	A (0.83/0.79)	1.18E-02	1.18E-02	70	81	0.344	0.274
rs893109	HTR5A	LD BIN	A	G (0.69/0.68)	G (0.69/0.68)	1.24E-02	7.84E-03	73	52	0.344	0.228

<i>rs16864387</i>	<i>FMO4</i>	<i>UTR</i>	A	T (0.87/0.84)	T (0.88/0.84)	1.28E-02	7.58E-03	74	48	0.344	0.228
<i>rs6045733</i>	<i>PDYN</i>	<i>LD BIN</i>	A	G (0.66/0.65)	G (0.66/0.65)	1.55E-02	1.56E-02	84	108	0.384	0.318
<i>rs4953</i>	<i>CHRN3</i>	<i>SYNON</i>	A	G (0.97/0.95)	G (0.97/0.95)	1.61E-02	2.67E-02	85	160	0.384	0.410
<i>rs4952</i>	<i>CHRN3</i>	<i>SYNON</i>	A	C (0.97/0.95)	C (0.97/0.95)	1.63E-02	2.71E-02	87	163	0.384	0.410
<i>rs6749955</i>	<i>CHRN3</i>	<i>LD BIN</i>	A	T (0.77/0.73)	T (0.77/0.73)	1.70E-02	2.09E-02	91	135	0.384	0.349
<i>rs7517376</i>	<i>FMO1</i>	<i>SYNON</i>	A	A (0.87/0.84)	A (0.88/0.84)	1.80E-02	7.74E-03	95	51	0.384	0.228

^a Category.

Table S3. Top associations with nicotine dependence based on the U.S. sample only. The p -value for the U.S. sample uses the same logistic regression model as for the primary analysis with the “site” term omitted. Only results where the weighted FDR in the U.S. sample is less than 40% are shown. LD estimates used for bins are from the U.S. sample. The conventions are the same as for table 2.

SNP	Gene	Function	Cat ^a	LD Bin ID	Min r^2	U.S.-only Risk Allele	U.S.-only p-value	Primary p-value	U.S.-only Rank	Primary Rank	U.S.-only FDR	Primary FDR
<i>rs578776</i>	<i>CHRNA3</i>	<i>UTR</i>	A	.	.	G (0.78/0.71)	8.48E-05	3.08E-04	1	3	0.071	0.086
<i>rs10508649</i>	<i>PIP5K2A</i>	<i>SYNON</i>	B	.	.	T (1.00/0.99)	3.44E-04	1.02E-03	2	8	0.228	0.344
<i>rs1206549</i>	<i>CLTCL1</i>	<i>INTRON</i>	B	22-5	0.994	G (0.87/0.82)	4.35E-04	1.75E-03	3	14	0.228	0.384
<i>rs807429</i>	<i>CLTCL1</i>	<i>INTRON</i>	B	22-5	0.994	A (0.87/0.82)	4.89E-04	1.93E-03	4	15	0.228	0.402
<i>rs6517442</i>	<i>KCNJ6</i>	<i>FP</i>	B	.	.	C (0.35/0.28)	6.93E-04	5.62E-04	5	4	0.228	0.344
<i>rs2180529</i>	<i>SNX5</i>	<i>LD BIN</i>	B	20-6	0.920	T (0.30/0.27)	7.28E-04	4.87E-03	6	28	0.228	0.505
<i>rs16969968</i>	<i>CHRNA5</i>	<i>NONSYN</i>	A	15-12	0.989	A (0.38/0.32)	7.32E-04	6.42E-04	7	5	0.176	0.134
<i>rs10246819</i>	<i>CHRM2</i>	<i>LD BIN</i>	B	7-49	0.867	C (0.56/0.54)	7.99E-04	3.33E-03	8	24	0.228	0.471
<i>rs3025382</i>	<i>DBH</i>	<i>INTRON</i>	A	.	.	G (0.92/0.88)	8.17E-04	5.14E-03	9	31	0.176	0.344
<i>rs1051730</i>	<i>CHRNA3</i>	<i>SYNON</i>	A	15-12	0.989	A (0.38/0.32)	8.41E-04	9.93E-04	10	7	0.176	0.166
<i>rs1061418</i>	<i>GABRE</i>	<i>UTR</i>	B	.	.	A (0.14/0.12)	8.43E-04	6.15E-03	11	36	0.228	0.570
<i>rs1378650</i>	<i>CHRM2</i>	<i>LD BIN</i>	B	.	.	G (0.56/0.51)	1.67E-03	1.78E-02	12	93	0.325	0.744
<i>rs17041074</i>	<i>DAO</i>	<i>INTRON</i>	B	.	.	A (0.27/0.27)	1.90E-03	1.12E-03	13	9	0.349	0.344
<i>rs17636651</i>	<i>CAMK2D</i>	<i>FP</i>	B	.	.	G (0.95/0.93)	2.02E-03	1.39E-02	14	79	0.349	0.693
<i>rs3803431</i>	<i>ALDH1A3</i>	<i>SYNON</i>	B	.	.	C (0.97/0.95)	2.51E-03	2.78E-02	15	137	0.398	0.783
<i>rs637137</i>	<i>CHRNA5</i>	<i>INTRON</i>	A	15-3	0.805	T (0.80/0.75)	2.80E-03	2.82E-03	16	22	0.228	0.336
<i>rs16143</i>	<i>NPY</i>	<i>UTR</i>	A	7-1	0.803	T (0.28/0.26)	3.21E-03	2.49E-02	18	126	0.228	0.446
<i>rs6474413</i>	<i>CHRN3</i>	<i>FP</i>	A	8-21	0.988	T (0.81/0.76)	3.23E-03	9.36E-05	19	1	0.228	0.056
<i>rs16142</i>	<i>NPY</i>	<i>UTR</i>	A	7-1	0.803	G (0.28/0.26)	4.46E-03	3.31E-02	26	173	0.228	0.471
<i>rs10958726</i>	<i>CHRN3</i>	<i>LD BIN</i>	A	8-21	0.988	T (0.81/0.77)	4.69E-03	1.33E-04	27	2	0.228	0.056
<i>rs2767</i>	<i>CHRNA4</i>	<i>UTR</i>	A	2-68	0.877	G (0.39/0.34)	4.87E-03	1.50E-03	28	11	0.228	0.209
<i>rs16478</i>	<i>NPY</i>	<i>UTR</i>	A	7-1	0.803	A (0.28/0.26)	5.31E-03	3.80E-02	32	194	0.228	0.495
<i>rs742350</i>	<i>FMO1</i>	<i>SYNON</i>	A	1-7	0.974	C (0.87/0.84)	5.51E-03	8.45E-03	33	48	0.228	0.344
<i>rs2302761</i>	<i>CHRN1</i>	<i>INTRON</i>	A	17-8	0.933	C (0.83/0.78)	5.64E-03	4.61E-02	34	238	0.228	0.504
<i>rs7210231</i>	<i>CHRN1</i>	<i>INTRON</i>	A	17-8	0.933	C (0.82/0.77)	5.74E-03	4.18E-02	35	218	0.228	0.498
<i>rs6320</i>	<i>HTR5A</i>	<i>SYNON</i>	A	.	.	T (0.72/0.71)	6.04E-03	6.50E-03	38	39	0.228	0.344
<i>rs16149</i>	<i>NPY</i>	<i>FP</i>	A	7-1	0.803	A (0.28/0.26)	6.12E-03	3.51E-02	39	183	0.228	0.480
<i>rs16138</i>	<i>NPY</i>	<i>INTRON</i>	A	.	.	C (0.28/0.26)	6.19E-03	4.42E-02	41	227	0.228	0.504
<i>rs2236196</i>	<i>CHRNA4</i>	<i>UTR</i>	A	.	.	G (0.28/0.23)	6.68E-03	2.63E-02	42	132	0.228	0.446
<i>rs16864387</i>	<i>FMO4</i>	<i>UTR</i>	A	1-7	0.974	T (0.88/0.84)	7.58E-03	1.28E-02	48	74	0.228	0.344

<i>rs1657273</i>	<i>HTR5A</i>	<i>LD BIN</i>	A	7-29	0.974 G (0.69/0.68)	7.74E-03	1.11E-02	50	64	0.228	0.344
<i>rs7517376</i>	<i>FMO1</i>	<i>SYNON</i>	A	1-7	0.974 A (0.88/0.84)	7.74E-03	1.80E-02	51	95	0.228	0.384
<i>rs893109</i>	<i>HTR5A</i>	<i>LD BIN</i>	A	7-29	0.974 G (0.69/0.68)	7.84E-03	1.24E-02	52	73	0.228	0.344
<i>rs684513</i>	<i>CHRNA5</i>	<i>INTRON</i>	A	15-3	0.805 C (0.81/0.77)	8.15E-03	8.72E-03	54	49	0.228	0.344
<i>rs7877</i>	<i>FMO1</i>	<i>UTR</i>	A	1-62	0.887 C (0.74/0.70)	8.46E-03	6.33E-03	59	38	0.228	0.344
<i>rs740602</i>	<i>COMT</i>	<i>SYNON</i>	A		G (1.00/0.99)	9.53E-03	3.43E-02	62	180	0.249	0.477
<i>rs16159</i>	<i>NPY</i>	<i>LD BIN</i>	A	7-1	0.803 T (0.31/0.29)	9.83E-03	7.43E-02	63	373	0.249	0.614
<i>rs2276560</i>	<i>CHRNA6</i>	<i>LD BIN</i>	A	2-63	0.931 T (0.77/0.74)	1.04E-02	7.42E-03	72	44	0.256	0.344
<i>rs7215056</i>	<i>CHRNA1</i>	<i>INTRON</i>	A	17-8	0.933 C (0.82/0.78)	1.07E-02	6.09E-02	73	295	0.256	0.570
<i>rs3813567</i>	<i>CHRNA4</i>	<i>FP</i>	A		A (0.83/0.79)	1.18E-02	1.18E-02	81	70	0.274	0.344
<i>rs17149039</i>	<i>NPY</i>	<i>LD BIN</i>	A	7-1	0.803 G (0.33/0.31)	1.27E-02	8.45E-02	85	428	0.284	0.632
<i>rs4245150</i>	<i>DRD2</i>	<i>LD BIN</i>	A	11-8	0.997 G (0.37/0.36)	1.29E-02	1.08E-02	87	61	0.284	0.344
<i>rs2304297</i>	<i>CHRNA6</i>	<i>UTR</i>	A	8-52	0.830 G (0.79/0.75)	1.38E-02	6.91E-03	95	42	0.295	0.344
<i>rs17602038</i>	<i>DRD2</i>	<i>LD BIN</i>	A	11-8	0.997 C (0.37/0.36)	1.43E-02	1.17E-02	98	69	0.298	0.344
<i>rs6045733</i>	<i>PDYN</i>	<i>LD BIN</i>	A	20-34	0.803 G (0.66/0.65)	1.56E-02	1.55E-02	108	84	0.318	0.384
<i>rs3791729</i>	<i>CHRNA1</i>	<i>INTRON</i>	A	2-68	0.877 A (0.37/0.32)	1.70E-02	3.39E-03	113	25	0.325	0.344
<i>rs12056414</i>	<i>OPRK1</i>	<i>INTRON</i>	A	8-14	1.000 A (0.09/0.07)	1.71E-02	3.71E-02	116	191	0.325	0.492
<i>rs3743078</i>	<i>CHRNA3</i>	<i>INTRON</i>	A	15-3	0.805 G (0.82/0.79)	1.98E-02	1.10E-02	128	63	0.349	0.344
<i>rs16148</i>	<i>NPY</i>	<i>FP</i>	A	7-1	0.803 C (0.35/0.33)	2.01E-02	1.14E-01	130	556	0.349	0.676
<i>rs6045819</i>	<i>PDYN</i>	<i>SYNON</i>	A	20-29	0.859 A (0.90/0.88)	2.05E-02	2.98E-02	133	159	0.349	0.470
<i>rs6749955</i>	<i>CHRNA6</i>	<i>LD BIN</i>	A	2-63	0.931 T (0.77/0.73)	2.09E-02	1.70E-02	135	91	0.349	0.384
<i>rs4531</i>	<i>DBH</i>	<i>NONSYN</i>	A		G (0.93/0.91)	2.34E-02	5.10E-03	143	30	0.383	0.344
<i>rs12056411</i>	<i>OPRK1</i>	<i>INTRON</i>	A	8-14	1.000 A (0.09/0.07)	2.53E-02	5.21E-02	154	263	0.398	0.522

^aCategory.