NIH Public Access
Author Manuscript
Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2011 April 5.

Published in final edited form as:
Am J Med Genet B Neuropsychiatr Genet. 2010 April 5; 153B(3): 745–756. doi:10.1002/ajmg.b.31043.

Association and interaction analysis of variants in *CHRNA5/CHRNA3/CHRNB4* gene cluster with nicotine dependence in African and European Americans

Ming D. Li[1)], **Qing Xu**[1)], **Xiang-Yang Lou**[1)], **Thomas J Payne**[3)], **Tianhua Niu**[1)], and **Jennie Z. Ma**[2)]

[1)]Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, VA

[2)]Department of Public Health Sciences, University of Virginia, Charlottesville, VA

[3)]Department of Otolaryngology and Communicative Sciences, University of Mississippi Medical Center, Jackson, MS

Abstract

Several previous genome-wide and targeted association studies revealed that variants in the *CHRNA5-CHRNA3-CHRNB4* (*CHRNA5/A3/B4*) gene cluster on chromosome 15 that encode the α5, α3 and β4 subunits of the nicotinic acetylcholine receptor (nAChRs) are associated with nicotine dependence (ND) in European Americans (EAs) or others of European origin. Considering the distinct linkage disequilibrium patterns in European and other ethnic populations such as African Americans (AAs), it would be interesting to determine whether such associations exist in other ethnic populations. We performed a comprehensive association and interaction analysis of the *CHRNA5/A3/B4* cluster in two ethnic samples to investigate the role of variants in the risk for ND, which was assessed by Smoking Quantity, Heaviness Smoking Index, and Fagerström test for ND. Using a family-based association test, we found a nominal association of single nucleotide polymorphisms (SNPs) rs1317286 and rs8040868 in *CHRNA3* with ND in the AA and combined AA and EA samples. Furthermore, we found that several haplotypes in *CHRNA5* and *CHRNA3* are nominally associated with ND in AA, EA, and pooled samples. However, none of these associations remained significant after correction for multiple testing. In addition, we performed interaction analysis of SNPs within the *CHRNA5/A3/B4* cluster using the pedigree-based generalized multifactor dimensionality reduction method and found significant interactions within *CHRNA3* and among the three subunit genes in the AA and pooled samples. Together, these results indicate that variants within *CHRNA3* and among *CHRNA5*, *CHRNA3*, and *CHRNB4* contribute significantly to the etiology of ND through gene-gene interactions, although the association of each subunit gene with ND is weak in both the AA and EA samples.

Keywords

Association analysis; CHRNA5; CHRNA3; CHRNB4; Interaction analysis; Nicotine dependence; Smoking

Correspondence to: Ming D. Li, Ph.D., University of Virginia, Department of Psychiatry and Neurobehavioral Sciences, Section of Neurobiology, 1670 Discovery Drive, Suite 110, Charlottesville, VA 22911, Tel: (434) 243-0566, Fax: (434) 973-7031, Ming_Li@virginia.edu.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Introduction

Cigarette smoking is the most preventable cause of morbidity and death, resulting in more than 438,000 deaths in the United States in 2000 and approximately $193 billion in health-related economic losses (Mokdad and others 2004). Over the past four decades, cigarette smoking has caused an estimated 12 million deaths, including 4.1 million from cancers, 5.5 million from cardiovascular diseases, 2.1 million from respiratory diseases, and 94,000 infant deaths attributable to maternal smoking during pregnancy (www.cdc.gov/nccdphp/publications/aag/osh.htm.). Although cigarette smoke contains many noxious compounds, including carbon monoxide, acetaldehyde, and tobacco-specific nitrosamines, it is nicotine dependence (ND) that maintains the continued use of tobacco (Stolerman and Jarvis 1995; USDHHS 2000; WHO 2002). Twin and family studies reveal that genetics contributes significantly to ND, with an estimated mean heritability of 0.56 in adult smokers (Li and others 2003).

The psychopharmacologic effects of nicotine are mediated primarily by functionally diverse neuronal nicotinic acetylcholine receptors (nAChRs), a family of ligand-gated ion channels widely distributed in the brain (Gaimarri and others 2007; Picciotto and others 2000; Watkins and others 2000). These nAChRs are involved in numerous physiological functions both in the brain and in the periphery as well (Gotti and Clementi 2004). To date, 12 neuronal nAChR subunits have been identified, consisting of nine α (α2-α10) and three β (β2-β4) subunits. The human genes for all of these subunits except α8 have been cloned (Graham and others 2002). The 11 nAChR subunit genes are located on chromosomes 1, 4, 8, 11, 15, and 20. Of these, *CHRNA5*, *CHRNA3*, and *CHRNB4* are grouped in a cluster on chromosome 15q24 (Raimondi and others 1992), in which *CHRNA3* and *CHRNA5* are located in a tail-to-tail configuration on opposite DNA strands and share some of their 3′-untranslated region (Duga and others 2001). Similarly, *CHRNB3* and *CHRNA6* are grouped in a cluster on chromosome 8p11. The clustered arrangement of *CHRNA5/A3/B4* and *CHRNB3/A6* could be related to control of the expression of these genes (Flora and others 2000; Xu and others 2006).

Several subunit genes have been investigated for association with ND and other smoking-related behaviors in human subjects [for reviews, see (Lessov-Schlaggar and others 2008; Li and Burmeister 2009)]. In a recent study, Saccone *et al.* (2007) reported associations of multiple single nucleotide polymorphisms (SNPs) in the *CHRNA5/A3/B4* cluster with ND. However, the significance of these results did not survive correction for multiple testing. More recently, several genome-wide and candidate gene-based association studies provided further evidence for the association of variants of the *CHRNA5/A3/B4* gene cluster with various nicotine-related behaviors (Berrettini and others 2008; Bierut and others 2008; Chen and others 2009; Greenbaum and others 2006; Saccone and others 2007; Schlaepfer and others 2008; Sherva and others 2008; Weiss and others 2008). In contrast, other studies have failed to reveal a significant association of this gene cluster with ND or other smoking-related phenotypes (Uhl and others 2008; Vink and others 2009).

Considering that the participants in these studies were primarily European Americans (EAs) or of European origin, and given the distinct differences in linkage disequilibrium (LD) patterns across ethnic populations, it remains unknown whether this gene cluster plays a role in the etiology of ND in other ethnic groups. The current study was designed to examine this possibility among a relatively large sample of African Americans (AAs) in comparison with EAs.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Materials and Methods

Participants and ND measures

Participants were recruited primarily from the Mid-South states of Tennessee, Mississippi, and Arkansas during 1999-2004. Extensive clinical and medical data were collected on each participant, including demographics (e.g., sex, age, race, biological relationships, weight, height, years of education, and marital status), medical history, smoking history and current smoking behavior, ND, and other relevant personal characteristics. The enrollment criteria for proband smokers were: 1) at least 21 years old, 2) smoker for at least five years, 3) consumer of an average of 20 cigarettes per day during the last 12 months, and 4) free of a self-reported history of diagnosis of any serious mental disorder; e.g., schizophrenia or other psychosis, bipolar disorder, or cognitive disorder (such as dementia or Alzheimer's disease). Once a proband was recruited, siblings and parents were invited to join the study whenever possible. All participants provided informed consent; the study protocol and forms/procedures were approved by all participating Institutional Review Boards.

The ND of each smoker was ascertained by three of the measures used most commonly in tobacco research: Smoking Quantity (SQ; defined as the number of cigarettes smoked per day), the Heaviness of Smoking Index (HSI; 0-6 scale), and the Fagerström Test for ND score (FTND; 0-10 scale) (Heatherton and others 1991). The SQ provides a simple, quantified index of consumption (using a 0–3 point compressed format), whereas HSI includes one item addressing SQ and another assessing smoking urgency on awakening. The FTND score includes the HSI plus other indicators of behavioral propensity to smoke under various circumstances. Given the presence of overlap in the content of the three ND measures, there exist fairly robust correlations among them ($r = 0.88 \sim 0.94$) in both the AA and EA samples. The primary reasons for employing all three measures were that there is a lack of consensus regarding the best approach to assess ND as a phenotype and to permit maximum cross-referencing with previous studies of ND.

The participants were 1366 individuals from 402 AA families and 671 individuals from 200 EA families. The average age was 39.4 ± 14.4 (SD) years for the AA and 40.5 ± 15.5 years for the EA participants. The average nuclear family size was 3.14 ± 0.75 for AAs and 3.17 ± 0.69 for EAs. There were 1053 and 515 smokers in the AA and EA, samples, respectively, whereas all the rest had smoked fewer than 100 cigarettes in their lifetimes. The average HSI and FTND scores for smokers were 3.7 ± 1.4 and 6.26 ± 2.15, respectively for AAs and 3.9 ± 1.4 and 6.33 ± 2.22 for EAs. Age of smoking onset was 17.3 ± 4.7 (mean ± SD; years) and 15.5 ± 4.4, for the AA and EA samples, respectively. Years smoked were 20.4 ± 12.5 (mean ± SD) and 23.2 ± 13.5 for the AA and EA samples, respectively. The average number of cigarettes smoked per day was 19.4 ± 13.3 for AA smokers and 19.5 ± 13.4 for EA smokers. Detailed demographic and clinical characteristics of the two samples have been reported elsewhere (Li and others 2005; Li and others 2008b; Li and others 2006)

DNA samples, SNP selection, and SNP genotyping

DNA was isolated from the blood of each participant using a DNA Maxi kit from Qiagen, Inc. (Valencia, CA). A total of 22 SNPs, with 7 from *CHRNA5*, 11 from *CHRNA3*, and 4 from *CHRNB4*, were selected from either published papers (Berrettini and others 2008; Saccone and others 2007) or the NCBI SNP database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp) on the basis of the potential biological functions of the gene, high heterozygosity with a minor allele frequency (MAF) > 0.15, and association analysis results from other studies (Berrettini and others 2008; Saccone and others 2007), as well as an attempt to obtain uniform coverage of the gene/region.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Detailed information about these SNPs (alleles, physical position, allele frequency, and primer/probe information from ABI) is summarized in Table 1.

All SNPs were genotyped using the *Taq*Man assay in a 384-well microplate format (Applied Biosystems Inc., Foster City, CA). 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. The amplification conditions were 2 min at 50°C and 10 min at 95°C followed by 40 cycles of 95°C for 25 sec and 60°C for 1 min. Allelic discrimination analysis was performed on the ABI Prism 7900HT Sequence Detection System. To ensure the quality of the genotyping, eight positive and negative control DNA samples were added to each 384-well reaction plate.

Association analysis

The PedCheck program (O'Connell and Weeks 1998) was used to detect any genotyping inconsistencies. To avoid potential bias, the 41 inconsistencies in the AA sample and 24 in the EA sample, detected in approximately 46,200 assays (i.e., 0.14% genotyping error) for 22 SNPs across all DNA samples, were excluded from statistical analysis. Pair-wise linkage LD between all possible SNP pairs was assessed using the Haploview (v. 4.0) program (Barrett and others 2005) with the option of determining haplotype blocks according to the definitions proposed by Gabriel *et al.* (2002). Associations between individual SNPs and the three ND measures were determined with the PBAT (v. 3.5) program using generalized estimating equations (Lange and others 2003). Associations between each ND measure and haplotypes from various SNP combinations were calculated using the FBAT (v. 1.7.3) program with the option of computing the P value of the Z statistic using Monte Carlo sampling under the null distribution of no linkage and no association (Horvath and others 2004). Three genetic models (additive, dominant, and recessive) were tested for both individual and multi-locus SNPs (i.e., haplotypes). For all PBAT and FBAT association tests, sex and age were used as covariates in the EA and AA samples and sex, age, and ethnicity as covariates in the combined sample. All significant associations were corrected for multiple testing according to the SNP spectral decomposition approach (SNPSpD) (Nyholt 2004) for individual SNP analysis and using Bonferroni correction by dividing the significance level by the number of major haplotypes (> 5.0% in frequency) for haplotype-based association analysis.

Interaction analysis of variants in *CHRNA5/A3/B4* cluster

Gene–gene interactions of SNPs in the *CHRNA5/A3/B4* cluster were analyzed using a newly developed pedigree-based generalized multifactor dimensionality reduction (PGMDR) method (Lou and others 2008). As with the previous association analyses, age and sex were included as covariates for the EA and AA samples and age, sex, and ethnicity as covariates in the pooled sample. Gene–gene interactions were examined for all two- to seven-locus models. The top-ranked interaction model was chosen for a given order interaction, and its *P* value of prediction accuracy (PA) was evaluated by a permutation test based on 10,000 shuffles of the adjusted phenotypic values. Because all *P* values reported here were based on permutation tests of each given interaction model, no correction was needed. On the other hand, we considered a *P* value of 0.0083 (i.e., 0.05/6) as an experiment-wide corrected one at the 0.05 significance level given that our gene–gene interaction analyses considered six interactive models for each sample. For detailed information on the PGMDR approach, please refer to the paper by Lou *et al.* (2008).

Power analysis

Power analysis for single-locus main effects as well as two-locus interactive effects was performed using the Quanto program (version 1.2.4; http://hydra.usc.edu/gxe/).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Results

Individual SNP-based association analysis

We evaluated 22 SNPs in the *CHRNA5/A3/B4* cluster in two independent ethnic samples. Association of individual SNPs with the three ND measures was determined with the PBAT-GEE program (Lange and others 2003), and the results are shown in Table 2. For *CHRNA3*, we found marginal associations for rs1317286 with SQ and HSI (P = 0.02 and 0.05, respectively) and of rs8040868 with SQ (P = 0.019) in the combined sample. These findings were not significant after correction for multiple testing. As for *CHRNA5* and *CHRNB4*, no significant association was detected for any individual SNP.

We found substantial differences between the EA and AA samples in the allele frequencies for several SNPs (Table 2). The presence of these allele frequency discrepancies suggests genetic differences underlying ND. To eliminate any potential effect of ethnic differences on our association results, we performed independent association analyses within the individual samples. For the AA sample, rs8040868 within *CHRNA3* was significantly associated with all three adjusted ND measures (P = 0.017 to 0.039); however, no association remained significant after correction for multiple testing. No significant association with ND was detected for any individual SNP in the three genes.

Haplotype block structure and LD analysis

The pair-wise D' values of 22 SNPs for the three genes in a total of 77 kb of nucleotide sequence were determined using the Haploview program (Barrett and others 2005). On the basis of the block definition proposed by Gabriel *et al.* (2002), we found four blocks within the gene cluster in the AA, EA, and pooled samples (Figure 1). One block of 4.0 kb containing rs12441088 and rs3813567 in *CHRNB4* was observed for the three samples; three other blocks differed across the AA and EA samples, indicating the presence of different genetic architectures.

Haplotype-based association analysis

Using the FBAT program, we performed haplotype-based association analysis for all possible haplotypes in consecutive three-SNP combinations for the three ND measures. In the combined sample, we found a major haplotype, T-A-C, formed by SNPs rs17408276, rs16969968, and rs615470 in *CHRNA5* at a frequency of 14%, that was significantly associated with SQ (P = 0.018) and HSI (P = 0.019) under the additive model (Table 3). We also found three major haplotypes in *CHRNA3*, G-C-T (11%; formed by rs1317286, rs12914385, and rs2869546), C-G-C (34%; formed by rs6495308, rs3743075, and rs8040868), and A-T-T (36%; formed by rs3743075, rs8040868, and rs6495309), which showed significant association with at least two of the three ND measures (P = 0.016 ~ 0.047; Table 3). However, none of these associations remained significant after correction for multiple testing of major haplotypes.

In the AA sample, we found two major haplotypes in *CHRNA3*, with the first, G-C-T, formed by rs1317286-rs12914385-rs2869546, at a frequency of 14% showing significant associations with all the three ND measures (P = 0.016 ~ 0.043); and a second one, T-C-G, formed by rs2869546-rs6495308-rs3743075, at a frequency of 41%, revealing a significant association with SQ (P = 0.041; Table 4). Again, none of these associations remained significant after correction for multiple testing.

Regarding the EA sample, we also found two haplotypes each in *CHRNA5* and *CHRNA3* that showed significant associations with HSI (P = 0.031~0.038) or FTND (P =

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

0.035~0.041) or both. None of these associations remained significant after correction for multiple testing.

Interaction analysis of *CHRNA5/A3/B4* gene cluster

With the PGMDR method, we analyzed interactions for all possible combinations of two to seven SNPs within the *CHRNA5/A3/B4* cluster. Table 6 shows the best interactive model for three ND measures in the AA and pooled samples. In the AA sample, we found the best interaction model for three loci (i.e., rs578776, rs3743078, and rs1317286 in *CHRNA3*) with a *P* value of 0.045 for HSI and 0.003 for FTND. For the four-locus model (i.e., rs684513 and rs615470 in *CHRNA5*; rs1317286 in *CHRNA3*; rs12441088 in *CHRNB4*), *P* values of 0.002 for SQ, 0.016 for HSI, and 0.041 for FTND were identified. Finally, for the six-locus model (i.e., rs684513 and rs621849 in *CHRNA5*; rs578776, rs1317286, and rs12914385 in *CHRNA3*; rs12441088 in *CHRNB4*), *P* values of 0.005 for SQ, 0.003 for HSI, and 0.044 for FTND were noted. All three models remained significant at the experiment-wide level for at least one ND measure.

For the pooled sample, we identified two interactive models. The first consisted of two SNPs (rs3743078 and rs1317286) in *CHRNA3*, and the second consisted of SNPs rs621849 in *CHRNA5*, rs3743078 in *CHRNA3*, and rs11637890 in *CHRNB4*. However, neither model remained significant at the experiment-wide level after correction. We performed an identical interaction analysis on the EA sample, but no significant interactive model emerged.

Discussion

In the current study, we examined the genetic associations and interactions between variations in the *CHRNA5/A3/B4* gene cluster and ND in two independent samples. Individual SNP-based association analyses revealed that rs8040868 in *CHRNA3* was significantly associated with all the ND measures in the AA sample and with SQ in the combined sample, whereas rs1317286 revealed relationships with SQ and HSI in the combined sample. We also performed haplotype-based association analyses on all SNPs using a sliding window approach. In the *CHRNA3* gene, one common haplotype (G-C-T) for SNPs rs1317286-rs12914385-rs2869546 in the combined and AA samples showed a significant positive association with ND. Moreover, we found haplotypes C-G-C/T, formed by rs6495308, rs3743075, and rs8040868, and A/G-T-T, formed by rs3743075, rs8040868, and rs6495309 in *CHRNA5*, to have a marginal association with ND in the combined and EA samples. Haplotype T-A-C, formed by rs17408276-rs16969968-rs615470 in *CHRNA5*, was significantly associated with SQ and HSI in the combined sample and only HSI in the EA sample, whereas the allelic T-G-C of this haplotype showed an inverse association with HSI in the EA sample. Although all the aforementioned haplotypes contain one or two SNPs of rs16969968, rs1317286, rs6495308, and rs8040868 that were found to have significant associations with ND in previous reports (Berrettini and others 2008; Saccone and others 2007), none of these associations remained significant in the present study after Bonferroni correction. Considering the fact that these subunit genes must assemble together in order to form functional nAChRs, we performed gene–gene interaction analysis on all SNPs in the gene cluster and found the three best interactive models in the AA sample and two in the pooled samples; only those for the AA sample remained significant at the experiment-wide level.

Recently, three independent genome-wide association studies (GWAS) provided strong evidence for associations between the variants of the *CHRNA5/A3/B4* cluster and lung cancer (Amos and others 2008; Hung and others 2008; Liu and others 2008; McKay and others 2008; Thorgeirsson and others 2008; Wang and others 2008). Regarding the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

association of this gene cluster with ND, the results are less clear. One study (Thorgeirsson and others 2008) revealed a significant association of rs1051730 within *CHRNA3* with smoking quantity ($P = 5.0 \times 10^{-16}$) in approximately 11,000 Icelandic smokers, whereas another study (Amos and others 2008) reported weak evidence of an association of SNPs rs1051730 and rs8034191 with smoking behavior in former but not current smokers. The third study (Hung and others 2008) found no association of SNPs 8034191 and rs16969968 with smoking dependence. Several other studies provide additional independent evidence pertaining to the association of variants of this gene cluster with ND. For example, Saccone *et al.* (2007) first reported significant associations of *CHRNA5/A3/B4* cluster variants with ND in a large-scale candidate gene-based case-control study of 1050 cases and 879 controls of European ancestry where cases were defined as individuals with an FTND score of ≥ 4 and controls were individuals who smoked at least 100 cigarettes over a lifetime but had an FTND score of 0. Their results indicated rs16699968 ($P = 0.0006$), a nonsynonymous coding SNP in exon 5 of *CHRNA5*, and rs578776 ($P = 0.0003$) in the 3′-UTR of *CHRNA3* showed the strongest associations. In another independent GWAS study, Berrettini *et al.* (2008) identified two other SNPs in *CHRNA3* (rs1317286 and rs6495308) associated with cigarettes per day (CPD; $P = 0.000003$ and 0.00007, respectively) in three independent European samples, although none of the individual SNPs remained statistically significant after correction for multiple testing at the genome-wide level. Most recently, a candidate gene-based study (Weiss and others 2008) revealed a significant association of the variants in this cluster with degree of ND in participants of European origin who began daily smoking at or before the age of 16, but not among those who began daily smoking later, suggesting the association between the *CHRNA5/A3/B4* region and ND is dependent on developmental stage.

There is no question that significant progress has been made in identifying the *CHRNA5/A3/B4* cluster as a susceptibility locus for smoking and lung cancer. However, several important issues remain to be addressed. First, it is important to determine which variant(s) is responsible for the associations with ND and lung cancer. Within this region, rs1051730 has been associated with both ND (Saccone and others 2007; Thorgeirsson and others 2008; Weiss and others 2008) and lung cancer (Amos and others 2008; Hung and others 2008; Thorgeirsson and others 2008) whereas rs8034191 is associated primarily with lung cancer (Amos and others 2008; Hung and others 2008). In addition, several other SNPs, such as rs16969968, rs1317286, and rs6495308, are associated with ND. Of these, rs16969968 is most noteworthy because it represents a nonsynonymous α5 coding variant (e.g., aspartic acid to asparagine at position 398 of the polypeptide chain), resulting in a substitution of a negatively charged residue within the M3–M4 intracellular loop, a region thought to be involved in receptor trafficking. A recent function study (Bierut and others 2008) showed that the variant forms of the α5 subunit alter receptor function without affecting expression. In consideration of the extensive LD within this region, a resequencing effort is needed to address this issue. Second, it is necessary to determine if the *CHRNA5/A3/B4* cluster also plays a significant role in the etiology of ND and lung cancer in other ethnic populations, such as those with Asian, African, and Hispanic origins, given that most participants to date have been of European descent, the current study being a notable exception. From information from International HapMap project and from the current study, we know LD structures differ greatly across ethnicities. Thus, it is important to determine if the association between this region and both lung cancer and ND exists, and whether the same or different functional variants are responsible (Briollais and others 2007).

More research is needed to determine which smoking-related characteristics are associated with the cluster. For example, ND was measured by SQ or SQ levels in the studies reported by Berrettini *et al.* (2008) and Thorgeirssone *et al.* (2008), whereas the study reported by Sacconne *et al.* (2007) focused on searching for susceptibility genes responsible for the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

transition from non-dependent to dependent smoking. On the other hand, Weiss *et al.* (2008) found a significant association of the same cluster with ND only in subjects who began daily smoking at or before the age of 16. Considering these findings in light of several published and unpublished studies that reveal no or weak evidence for the association of this cluster with ND (Vink and others 2009), additional research is needed to investigate the role of the cluster in a variety of smoking phenotypes. For example, it was recently reported that no association exists between this cluster and either smoking initiation or smoking cessation (Hung and others 2008; Thorgeirsson and others 2008; Uhl and others 2008).

Although the association of the *CHRNA5/A3/B4* cluster with ND has received much attention recently, other nAChR subunit genes may play significant roles in the etiology of ND as well. For example, significant associations with various SNPs in *CHRNA4* have been detected in at least three independent studies (Feng and others 2004; Hutchison and others 2007; Li and others 2005). Although no significant association of ND with *CHRNB2* has been found in four independent studies (Feng and others 2004; Li and others 2005; Lueders and others 2002; Silverman and others 2000), recent research revealed that rs2072658 and rs2072661 in the 3′-UTR of *CHRNB2* are associated with a reduced risk of smoking initiation, greater ability to quit smoking, and diminished early response to nicotine (Conti and others 2008; Ehringer and others 2007). Very recently, we showed a significant interaction between variants of *CHRNA4* and *CHRNB2* in affecting ND, concluding that *CHRNB2* has a significant effect on ND when analyzed in interaction with *CHRNA4*. A smaller study in schizophrenic smokers suggested that *CHRNA7* may be associated with smoking status (De Luca and others 2004), although the relevance of this finding to the general population of smokers is unknown. Variants in *CHRNB1*, *CHRNB3*, and *CHRM1* (the second type of acetylcholine receptor found in both the brain and the periphery) also are associated with ND (Bierut and others 2007; Lou and others 2006). Greenbaum *et al.* (2006) reported a nominally significant association of ND and related behaviors with *CHRNA7* and *CHRNB2* in a relatively small sample.

Interactions among genetic loci are being appreciated increasingly in complex human diseases (Jung and others 2009). Recent examples of diseases or conditions associated with gene–gene interaction include coronary artery disease (Tsai and others 2007), type 2 diabetes (Qi and others 2007), Alzheimer's disease (Fontalba and others 2009), schizophrenia (Gupta and others 2009), breast cancer (Briollais and others 2007), cervical cancer (Guzman and others 2008), autistic disorder (Ma and others 2009), and smoking addiction (Li and others 2008a; Tang and others 2009), to name a few. Interactive effects among genetic loci may exist without a significant main effect of any of them; in such cases, important genetic effects would have been missed if polymorphisms of such loci had not been modeled jointly (Jung and others 2009). Furthermore, in many cases, interactive effects of multiple genetic loci could be larger than the main effects at the individual loci (Robson and others 2004; Rodriguez and others 2006; Williams and others 2000).

Multifactor Dimensionality Reduction (MDR) is a method of detecting genetic interactions by exhaustively searching multi-locus combinations (Motsinger-Reif and others 2008; Ritchie and others 2001). In MDR, k (e.g., k=3) factors and their possible multifactor classes are represented in k-dimensional space. Each multifactor class in the space is then labeled 'high risk' if the cases-to-controls ratio meets or exceeds some threshold, or as 'low risk' if that threshold is not exceeded, thus reducing the k-dimensional space to one dimension with two levels ('low risk' and 'high risk') (Moore 2003). The best k-locus model is then selected, the model is evaluated against the testing group, and testing accuracy is calculated. The PA is then calculated for the testing set (Motsinger-Reif and others 2008). Pedigree-based generalized MDR (PGMDR), a new generalized MDR for pedigree data, is a non-parametric method based on the score of the generalized linear model, which permits

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

adjustment for covariates and handling of both dichotomous and quantitative phenotypes (Lou and others 2008). A key advantage of PGMDR is that the method can handle different pedigree structures and sizes simultaneously in the presence of various patterns of missing data. In our study, by using PGMDR, three-locus, four-locus and six-locus interaction models were detected in the AA sample, and two-locus and three-locus interaction models were detected in the pooled sample (Table 6). It is intriguing that rs1317286, an SNP in the *CHRNA3*, which was associated with both SQ and HSI in the pooled sample, was included in four of these five best interaction models. Therefore, the interactive effects detected by PGMDR are potentially valuable, such that they not only lend support to the single-locus results, but also shed significant light on the joint effects among SNPs in the *CHRNA5/A3/B4* gene cluster, which could be missed in single-locus analysis. Nevertheless, larger and more rigorous replication studies are necessary to establish such interactions. As pointed by Milne *et al.* (2008), replication in additional independent samples of observed gene–gene interaction is crucial. In particular, caution should be exercised during the replication because differences of LD between different study populations such as AA and EA may have important impacts on the detection of high-order gene–gene interactions. Even when significant interactive effects are observed in a replication study, caution is necessary in elucidating exactly what constitutes a replicated result and the biological meaning of such replication. Therefore, ideally, observed gene–gene interactions should not only be replicated from a statistical perspective but also be validated experimentally from a biological perspective.

For power calculations with Quanto for main effects, according to two recent studies (Saccone and others 2009; Schlaepfer and others 2008), the average genotypic relative risk (GRR) for the chromosome 15 *CHRNA5/A3/B4* gene cluster SNPs was about 1.3. In the current study, rs1317286 in the *CHRNA3* gene was associated only with SQ and HSI in pooled sample. Another SNP in the *CHRNA3* gene, rs8040868, is associated only with SQ in the pooled sample and with all the three adjusted ND phenotypes in the AA sample. The MAFs for rs1317286 and rs8040868 were 0.300 and 0.364, respectively, in the AA sample and 0.410 and 0.457, respectively, in the EA sample. To achieve 80% power for detecting the main effect under the assumption of GRR = 1.3, we would need 1823, 1696 and 1136 case-sibling pairs for MAF = 0.2, 0.30, and 0.40, respectively. Thus, the sample sizes of the current study, ~ 930 sibpairs in the AA sample, and ~ 330 sibpairs in the EA sample, should be sufficient for detecting a GRR of 1.3 for an SNP with an MAF of 0.40 for the pooled sample (~ 1260 sibpairs). It should be remembered that the case-sibling design is used as a reference for above mentioned power analyses. Because among the 402 AA families, there were 187, 199, and 16 families with 0, 1, and 2 parents, respectively (Li and others 2006), and among the 200 EA families, there were 46, 131, and 23 families with 0, 1, and 2 parents (Li and others 2008b), respectively, our study provides a greater statistical power than a pure case-sibling design. Given that rs1317286, a SNP in the *CHRNA3* gene with a main effect under the dominant model in the pooled sample was included in four of the five best interaction models detected by PGMDR, and another SNP, rs3743078 in the *CHRNA3* gene, without a main effect, was detected in three of the five best interaction models, power calculation with Quanto for detecting interaction between two genetic loci, *g* and *h*, was performed for three sets of parameters: (1) in the absence of main effects for *g* and *h* and in the presence of an interactive effect between *g* and *h* (assuming $R_g = 1$, $R_h = 1$, and $R_{gh} =$ 1.5, 2.0, 2.5, and 3.0); (2) in the absence of a main effect for *g* only, and in the presence of a main effect for *h* as well as an interactive effect between *g* and *h* (assuming $R_g = 1$, $R_h =$ 1.25, and $R_{gh} = 1.5$, 2.0, 2.5, and 3.0); and (3) in the presence of main effects for *g* and *h*, as well as an interactive effect between *g* and *h* (assuming $R_g = 1.25$, $R_h = 1.25$, and $R_{gh} = 1.5$, 2.0, 2.5, and 3.0) under the dominant model for both loci and the MAF for gene *h* = 0.30. The required sample sizes were 717, 754, and 795 for $R_{gh} = 2.0$, 436, 464, and 496 for $R_{gh} =$ 2.5 and 323, 347, and 375 for $R_{gh} = 3.0$ under these three respective sets of parameters when

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

the MAF of gene *g* is 0.30. Therefore, our study appears to provide sufficient sample sizes to detect an $R_{gh} \geq 2.0$ under all three sets of parameters for the AA sample (~ 930 sibpairs) and the pooled sample (~ 1260 sibpairs), using the case-sibling design as the reference.

In sum, this is the first genetic study aimed at investigating a potential association of the variants of the *CHRNA5/A3/B4* cluster with ND in a sample of AA smokers. Although our results reveal a relatively weak association of the cluster with ND, we did find significant interaction among variants of the three subunit genes in affecting ND. As for the EA population, we did not identify significant interactions of variants in this cluster, although we found a nominal association of the cluster with ND. The small size of our EA sample may have contributed, in part, to the weak association.

Acknowledgments

We are grateful for the invaluable contributions of clinical information and tissue samples by the participants in this study, as well as for the dedicated work of the research staff at different clinical sites. We also thank Dr. David L. Bronson for his excellent editing of this manuscript. This project was funded by National Institutes of Health grants DA-12844, DA-13783 and DA-025095.

References

Amos CI, Wu X, Broderick P, Gorlov IP, Gu J, Eisen T, Dong Q, Zhang Q, Gu X, Vijayakrishnan J. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nat Genet. 2008; 40(5):616–22. others. [PubMed: 18385676]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–5. [PubMed: 15297300]

Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H, Waterworth D, Muglia P, Mooser V. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. Mol Psychiatry. 2008; 13(4):368–73. [PubMed: 18227835]

Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF, Swan GE, Rutter J, Bertelsen S, Fox L. Novel genes identified in a high-density genome wide association study for nicotine dependence. Hum Mol Genet. 2007; 16(1):24–35. others. [PubMed: 17158188]

Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, Saccone NL, Saccone SF, Bertelsen S, Fox L. Variants in Nicotinic Receptors and Risk for Nicotine Dependence. Am J Psychiatry. 2008; 165(9):1163–71. others. [PubMed: 18519524]

Briollais L, Wang Y, Rajendram I, Onay V, Shi E, Knight J, Ozcelik H. Methodological issues in detecting gene-gene interactions in breast cancer susceptibility: a population-based study in Ontario. BMC Med. 2007; 5:22. [PubMed: 17683639]

Chen X, Chen J, Williamson VS, An SS, Hettema JM, Aggen SH, Neale MC, Kendler KS. Variants in nicotinic acetylcholine receptors alpha5 and alpha3 increase risks to nicotine dependence. Am J Med Genet B Neuropsychiatr Genet. 2009

Conti DV, Lee W, Li D, Liu J, Van Den Berg D, Thomas PD, Bergen AW, Swan GE, Tyndale RF, Benowitz NL. Nicotinic acetylcholine receptor beta2 subunit gene implicated in a systems-based candidate gene study of smoking cessation. Hum Mol Genet. 2008; 17(18):2834–48. others. [PubMed: 18593715]

De Luca V, Wang H, Squassina A, Wong GW, Yeomans J, Kennedy JL. Linkage of M5 muscarinic and alpha7-nicotinic receptor genes on 15q13 to schizophrenia. Neuropsychobiology. 2004; 50(2): 124–7. [PubMed: 15292665]

Duga S, Solda G, Asselta R, Bonati MT, Dalpra L, Malcovati M, Tenchini ML. Characterization of the genomic structure of the human neuronal nicotinic acetylcholine receptor CHRNA5/A3/B4 gene cluster and identification of novel intragenic polymorphisms. J Hum Genet. 2001; 46(11):640–8. [PubMed: 11721883]

Ehringer MA, Clegg HV, Collins AC, Corley RP, Crowley T, Hewitt JK, Hopfer CJ, Krauter K, Lessem J, Rhee SH. Association of the neuronal nicotinic receptor beta2 subunit gene (CHRNB2)

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

with subjective responses to alcohol and nicotine. Am J Med Genet B Neuropsychiatr Genet. 2007; 144B(5):596–604. others. [PubMed: 17226798]

Feng Y, Niu T, Xing H, Xu X, Chen C, Peng S, Wang L, Laird N. A common haplotype of the nicotine acetylcholine receptor alpha 4 subunit gene is associated with vulnerability to nicotine addiction in men. Am J Hum Genet. 2004; 75(1):112–21. [PubMed: 15154117]

Flora A, Schulz R, Benfante R, Battaglioli E, Terzano S, Clementi F, Fornasari D. Transcriptional regulation of the human alpha5 nicotinic receptor subunit gene in neuronal and non-neuronal tissues. Eur J Pharmacol. 2000; 393(1-3):85–95. [PubMed: 10771001]

Fontalba A, Gutierrez O, Llorca J, Mateo I, Vazquez-Higuera JL, Berciano J, Fernandez-Luna JL, Combarros O. Gene-gene interaction between CARD8 and interleukin-6 reduces Alzheimer's disease risk. J Neurol. 2009; 256(7):1184–6. [PubMed: 19252766]

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M. The structure of haplotype blocks in the human genome. Science. 2002; 296(5576):2225–9. others. [PubMed: 12029063]

Gaimarri A, Moretti M, Riganti L, Zanardi A, Clementi F, Gotti C. Regulation of neuronal nicotinic receptor traffic and expression. Brain Res Rev. 2007; 55(1):134–43. [PubMed: 17383007]

Gotti C, Clementi F. Neuronal nicotinic receptors: from structure to pathology. Prog Neurobiol. 2004; 74(6):363–96. [PubMed: 15649582]

Graham A, Court JA, Martin-Ruiz CM, Jaros E, Perry R, Volsen SG, Bose S, Evans N, Ince P, Kuryatov A. Immunohistochemical localisation of nicotinic acetylcholine receptor subunits in human cerebellum. Neuroscience. 2002; 113(3):493–507. others. [PubMed: 12150770]

Greenbaum L, Kanyas K, Karni O, Merbl Y, Olender T, Horowitz A, Yakir A, Lancet D, Ben-Asher E, Lerer B. Why do young women smoke? I. Direct and interactive effects of environment, psychological characteristics and nicotinic cholinergic receptor genes. Mol Psychiatry. 2006; 11(3):312–22. 223. [PubMed: 16314871]

Gupta M, Chauhan C, Bhatnagar P, Gupta S, Grover S, Singh PK, Purushottam M, Mukherjee O, Jain S, Brahmachari SK. Genetic susceptibility to schizophrenia: role of dopaminergic pathway gene polymorphisms. Pharmacogenomics. 2009; 10(2):277–91. others. [PubMed: 19207030]

Guzman VB, Yambartsev A, Goncalves-Primo A, Silva ID, Carvalho CR, Ribalta JC, Goulart LR, Shulzhenko N, Gerbase-Delima M, Morgun A. New approach reveals CD28 and IFNG gene interaction in the susceptibility to cervical cancer. Hum Mol Genet. 2008; 17(12):1838–44. [PubMed: 18337305]

Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict. 1991; 86(9): 1119–27. [PubMed: 1932883]

Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. Genet Epidemiol. 2004; 26(1):61–9. [PubMed: 14691957]

Hung RJ, McKay JD, Gaborieau V, Boffetta P, Hashibe M, Zaridze D, Mukeria A, Szeszenia-Dabrowska N, Lissowska J, Rudnai P. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature. 2008; 452(7187):633–7. others. [PubMed: 18385738]

Hutchison KE, Allen DL, Filbey FM, Jepson C, Lerman C, Benowitz NL, Stitzel J, Bryan A, McGeary J, Haughey HM. CHRNA4 and tobacco dependence: from gene regulation to treatment outcome. Arch Gen Psychiatry. 2007; 64(9):1078–86. [PubMed: 17768273]

Jung J, Sun B, Kwon D, Koller DL, Foroud TM. Allelic-based gene-gene interaction associated with quantitative traits. Genet Epidemiol. 2009; 33(4):332–43. [PubMed: 19058262]

Lange C, Silverman EK, Xu X, Weiss ST, Laird NM. A multivariate family-based association test using generalized estimating equations: FBAT-GEE. Biostatistics. 2003; 4(2):195–206. [PubMed: 12925516]

Lessov-Schlaggar CN, Pergadia ML, Khroyan TV, Swan GE. Genetics of nicotine dependence and pharmacotherapy. Biochem Pharmacol. 2008; 75(1):178–95. [PubMed: 17888884]

Li MD, Beuten J, Ma JZ, Payne TJ, Lou XY, Garcia V, Duenes AS, Crews KM, Elston RC. Ethnic- and gender-specific association of the nicotinic acetylcholine receptor alpha4 subunit gene

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(CHRNA4) with nicotine dependence. Hum Mol Genet. 2005; 14(9):1211–9. [PubMed: 15790597]

Li MD, Burmeister M. New insights into the genetics of addiction. Nat Rev Genet. 2009; 10(4):225–31. [PubMed: 19238175]

Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. Addiction. 2003; 98(1):23–31. [PubMed: 12492752]

Li MD, Lou XY, Chen G, Ma JZ, Elston RC. Gene-gene interactions among CHRNA4, CHRNB2, BDNF, and NTRK2 in nicotine dependence. Biol Psychiatry. 2008a; 64(11):951–7. [PubMed: 18534558]

Li MD, Ma JZ, Payne TJ, Lou XY, Zhang D, Dupont RT, Elston RC. Genome-wide linkage scan for nicotine dependence in European Americans and its converging results with African Americans in the Mid-South Tobacco Family sample. Mol Psychiatry. 2008b; 13(4):407–16. [PubMed: 17579606]

Li MD, Payne TJ, Ma JZ, Lou XY, Zhang D, Dupont RT, Crews KM, Somes G, Williams NJ, Elston RC. A genomewide search finds major susceptibility Loci for nicotine dependence on chromosome 10 in african americans. Am J Hum Genet. 2006; 79(4):745–51. [PubMed: 16960812]

Liu P, Vikis HG, Wang D, Lu Y, Wang Y, Schwartz AG, Pinney SM, Yang P, de Andrade M, Petersen GM. Familial aggregation of common sequence variants on 15q24-25.1 in lung cancer. J Natl Cancer Inst. 2008; 100(18):1326–30. others. [PubMed: 18780872]

Lou XY, Chen GB, Yan L, Ma JZ, Mangold JE, Zhu J, Elston RC, Li MD. A combinatorial approach to detecting gene-gene and gene-environment interactions in family studies. Am J Hum Genet. 2008; 83(4):457–67. [PubMed: 18834969]

Lou XY, Ma JZ, Payne TJ, Beuten J, Crew KM, Li MD. Gene-based analysis suggests association of the nicotinic acetylcholine receptor beta1 subunit (CHRNB1) and M1 muscarinic acetylcholine receptor (CHRM1) with vulnerability for nicotine dependence. Hum Genet. 2006; 120(3):381–9. [PubMed: 16874522]

Lueders KK, Hu S, McHugh L, Myakishev MV, Sirota LA, Hamer DH. Genetic and functional analysis of single nucleotide polymorphisms in the beta2-neuronal nicotinic acetylcholine receptor gene (CHRNB2). Nicotine Tob Res. 2002; 4(1):115–25. [PubMed: 11906688]

Ma DQ, Rabionet R, Konidari I, Jaworski J, Cukier HN, Wright HH, Abramson RK, Gilbert JR, Cuccaro ML, Pericak-Vance MA. Association and gene-gene interaction of SLC6A4 and ITGB3 in autism. Am J Med Genet B Neuropsychiatr Genet. 2009 others.

McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, Byrnes G, Zaridze D, Mukeria A, Szeszenia-Dabrowska N, Lissowska J. Lung cancer susceptibility locus at 5p15.33. Nat Genet. 2008 others.

Milne RL, Fagerholm R, Nevanlinna H, Benitez J. The importance of replication in gene-gene interaction studies: multifactor dimensionality reduction applied to a two-stage breast cancer case-control study. Carcinogenesis. 2008; 29(6):1215–8. [PubMed: 18482998]

Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. JAMA. 2004; 291(10):1238–45. [PubMed: 15010446]

Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. Hum Hered. 2003; 56(1-3):73–82. [PubMed: 14614241]

Motsinger-Reif AA, Reif DM, Fanelli TJ, Ritchie MD. A comparison of analytical methods for genetic association studies. Genet Epidemiol. 2008; 32(8):767–78. [PubMed: 18561203]

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004; 74(4):765–9. [PubMed: 14997420]

O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet. 1998; 63(1):259–66. [PubMed: 9634505]

Picciotto MR, Caldarone BJ, King SL, Zachariou V. Nicotinic receptors in the brain. Links between molecular biology and behavior. Neuropsychopharmacology. 2000; 22(5):451–65. [PubMed: 10731620]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Qi L, van Dam RM, Asselbergs FW, Hu FB. Gene-gene interactions between HNF4A and KCNJ11 in predicting Type 2 diabetes in women. Diabet Med. 2007; 24(11):1187–91. [PubMed: 17894829]

Raimondi E, Rubboli F, Moralli D, Chini B, Fornasari D, Tarroni P, De Carli L, Clementi F. Chromosomal localization and physical linkage of the genes encoding the human alpha 3, alpha 5, and beta 4 neuronal nicotinic receptor subunits. Genomics. 1992; 12(4):849–50. [PubMed: 1572664]

Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, Moore JH. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet. 2001; 69(1):138–47. [PubMed: 11404819]

Robson KJ, Lehmann DJ, Wimhurst VL, Livesey KJ, Combrinck M, Merryweather-Clarke AT, Warden DR, Smith AD. Synergy between the C2 allele of transferrin and the C282Y allele of the haemochromatosis gene (HFE) as risk factors for developing Alzheimer's disease. J Med Genet. 2004; 41(4):261–5. [PubMed: 15060098]

Rodriguez E, Mateo I, Llorca J, Sanchez-Quintana C, Infante J, Berciano J, Combarros O. Genetic interaction between two apolipoprotein E receptors increases Alzheimer's disease risk. J Neurol. 2006; 253(6):801–3. [PubMed: 16311892]

Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Grucza RA, Hatsukami D. 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. 2009; 150B(4):453–66. others. [PubMed: 19259974]

Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum Mol Genet. 2007; 16(1):36–49. others. [PubMed: 17135278]

Schlaepfer IR, Hoft NR, Collins AC, Corley RP, Hewitt JK, Hopfer CJ, Lessem JM, McQueen MB, Rhee SH, Ehringer MA. The CHRNA5/A3/B4 gene cluster variability as an important determinant of early alcohol and tobacco initiation in young adults. Biol Psychiatry. 2008; 63(11):1039–46. [PubMed: 18163978]

Sherva R, Wilhelmsen K, Pomerleau CS, Chasse SA, Rice JP, Snedecor SM, Bierut LJ, Neuman RJ, Pomerleau OF. Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with 'pleasurable buzz' during early experimentation with smoking. Addiction. 2008; 103(9):1544–52. [PubMed: 18783506]

Silverman MA, Neale MC, Sullivan PF, Harris-Kerr C, Wormley B, Sadek H, Ma Y, Kendler KS, Straub RE. Haplotypes of four novel single nucleotide polymorphisms in the nicotinic acetylcholine receptor beta2-subunit (CHRNB2) gene show no association with smoking initiation or nicotine dependence. Am J Med Genet. 2000; 96(5):646–53. [PubMed: 11054772]

Stolerman IP, Jarvis MJ. The scientific case that nicotine is addictive. Psychopharmacology (Berl). 1995; 117(1):2–10. discussion 14-20. [PubMed: 7724697]

Tang X, Guo S, Sun H, Song X, Jiang Z, Sheng L, Zhou D, Hu Y, Chen D. Gene-gene interactions of CYP2A6 and MAOA polymorphisms on smoking behavior in Chinese male population. Pharmacogenet Genomics. 2009; 19(5):345–52. [PubMed: 19415821]

Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, Magnusson KP, Manolescu A, Thorleifsson G, Stefansson H, Ingason A. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature. 2008; 452(7187):638–42. others. [PubMed: 18385739]

Tsai CT, Hwang JJ, Ritchie MD, Moore JH, Chiang FT, Lai LP, Hsu KL, Tseng CD, Lin JL, Tseng YZ. Renin-angiotensin system gene polymorphisms and coronary artery disease in a large angiographic cohort: detection of high order gene-gene interaction. Atherosclerosis. 2007; 195(1): 172–80. [PubMed: 17118372]

Uhl GR, Liu QR, Drgon T, Johnson C, Walther D, Rose JE, David SP, Niaura R, Lerman C. Molecular genetics of successful smoking cessation: convergent genome-wide association study results. Arch Gen Psychiatry. 2008; 65(6):683–93. [PubMed: 18519826]

USDHHS. Reducing tobacco use: A report of the Surgeon General. US Department of Health & Human Services, Center for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promortion; Atlanta, Georgia: 2000.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Vink JM, Smit AB, de Geus EJ, Sullivan P, Willemsen G, Hottenga JJ, Smit JH, Hoogendijk WJ, Zitman FG, Peltonen L. Genome-wide association study of smoking initiation and current smoking. Am J Hum Genet. 2009; 84(3):367–79. others. [PubMed: 19268276]

Wang Y, Broderick P, Webb E, Wu X, Vijayakrishnan J, Matakidou A, Qureshi M, Dong Q, Gu X, Chen WV. Common 5p15.33 and 6p21.33 variants influence lung cancer risk. Nat Genet. 2008 others.

Watkins SS, Koob GF, Markou A. Neural mechanisms underlying nicotine addiction: acute positive reinforcement and withdrawal. Nicotine Tob Res. 2000; 2(1):19–37. [PubMed: 11072438]

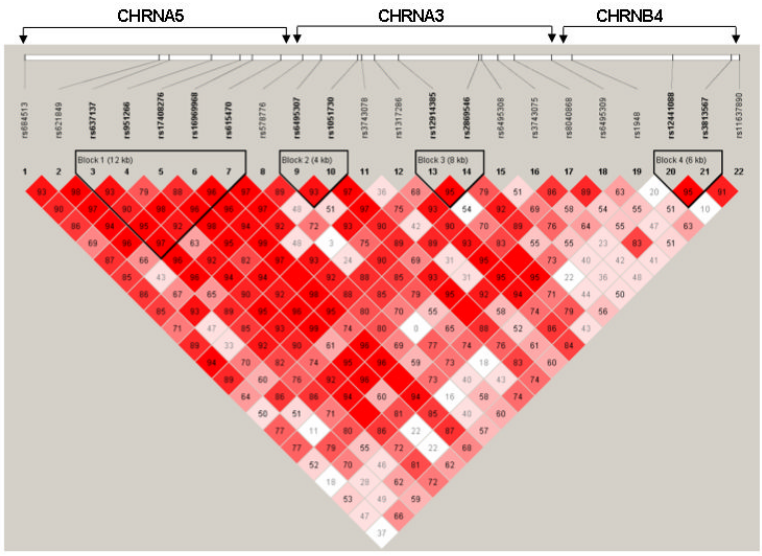
Weiss RB, Baker TB, Cannon DS, von Niederhausern A, Dunn DM, Matsunami N, Singh NA, Baird L, Coon H, McMahon WM. A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. PLoS Genet. 2008; 4(7):e1000125. others. [PubMed: 18618000]

WHO. The World Health Report 2002. World Health Organization; 2002.

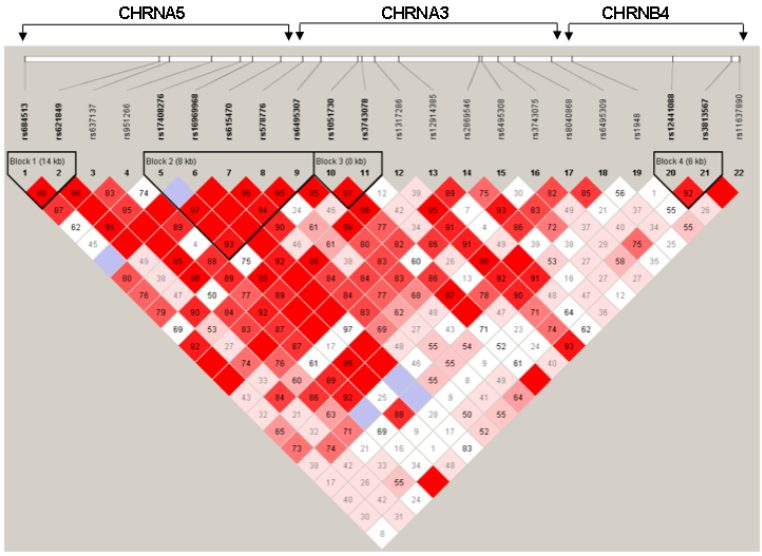
Williams SM, Addy JH, Phillips JA 3rd, Dai M, Kpodonu J, Afful J, Jackson H, Joseph K, Eason F, Murray MM. Combinations of variations in multiple genes are associated with hypertension. Hypertension. 2000; 36(1):2–6. others. [PubMed: 10904004]

Xu X, Scott MM, Deneris ES. Shared long-range regulatory elements coordinate expression of a gene cluster encoding nicotinic receptor heteromeric subtypes. Mol Cell Biol. 2006; 26(15):5636–49. [PubMed: 16847319]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript



Combined Sample



AA Sample

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

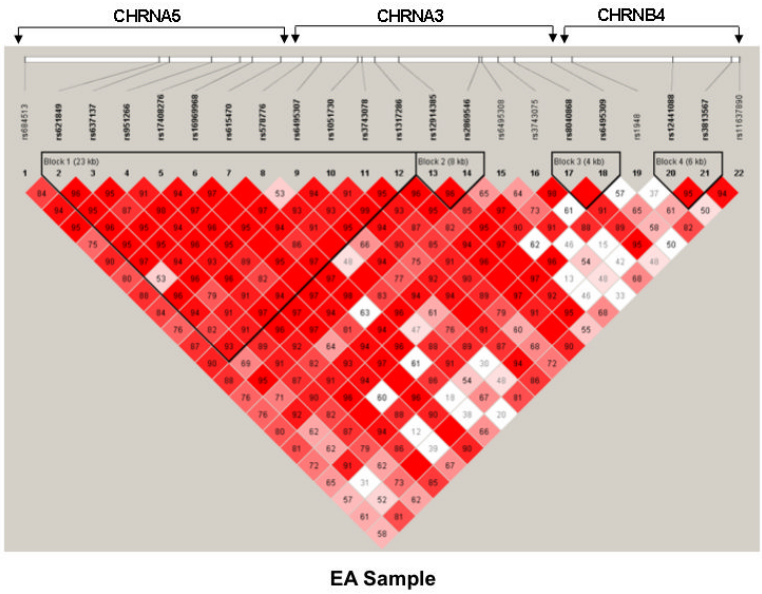


Figure 1.

Haploview-generated LD patterns for 22 SNPs within the *CHRNA5/A3/B4* cluster in the pooled (A), AA (B), and EA (C) samples. Pair-wise LD between all SNPs was evaluated using the Haploview program (Barrett and others 2005) with the option of determining haplotype blocks according to the criteria defined by Gabriel *et al.* (2002). The number in each box represents the *D*' value for each SNP pair.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Positions, nucleotide variation, allele frequency, and primer/probe sequences

Gene	Marker number	dbSNP ID	Chrom. pos.	Alleles	SNP location	MAF (EA)*	MAF (AA)*	Primer and probe#
CHRNA5	1	rs684513	76645455	C/G	Intron1	0.217	0.158	C_7051_10
	2	rs621849	76659916	A/G	Intron1	0.375	0.425	C_5864_10
	3	rs637137	76661031	A/T	Intron1	0.202	0.259	C_5866_10
	4	rs951266	76665596	C/T	Intron2	0.417	0.117	C_9510534_10
	5	rs17408276	76668673	C/T	Intron4	0.396	0.325	C_34624626_10
	6	rs16969968	76669980	A/G	Exon5	0.424	0.205	C_26000428_20
	7	rs615470	76673043	C/T	3′ UTR	0.325	0.342	C_18757_10
CHRNA3	8	rs578776	76675455	C/T	3′ UTR	0.242	0.342	C_721253_10
	9	rs6495307	76677376	C/T	Intron5	0.345	0.307	C_1713433_10
	10	rs1051730	76681394	C/T	Exon5	0.4	0.11	C_9510307_20
	11	rs3743078	76681814	C/G	Intron4	0.203	0.333	C_27472090_10
	12	rs1317286	76683184	A/G	Intron4	0.41	0.3	C_9510308_10
	13	rs12914385	76685778	C/T	Intron4	0.43	0.198	C_12106059_10
	14	rs2869546	76694400	C/T	Intron4	0.333	0.322	C_15836855_10
	15	rs6495308	76694711	C/T	Intron4	0.23	0.34	C_222570_10
	16	rs3743075	76696507	A/G	Exon4	0.317	0.442	C_27472089_10
	17	rs8040868	76698236	C/T	Exon2	0.457	0.364	C_261698_10
	18	rs6495309	76702300	C/T	Promoter	0.2	0.25	C_30730895_10
CHRNB4	19	rs1948	76704454	C/T	3′ UTR	0.267	0.117	C_11941837_10
	20	rs12441088	76715319	G/T	Intron1	0.25	0.3	C_30730878_10
	21	rs3813567	76721606	C/T	Promoter	N/F	N/F	C_27488001_10
	22	rs11637890	76722474	C/G	Promoter	0.347	0.067	C_31121610_10

Notes:

*Based on the allele frequency from the NCBI SNP database

#ID of primer/probe provided by Applied Biosystems Inc.

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2011 April 5.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

Allele frequencies and P values for associations of individual SNPs with three ND measures in the EA, AA, and combined samples

			European-American sample				African-American sample				Combined sample			
Gene	**SNP ID**	**Alleles**	**Freq.**	**SQ**	**HSI**	**FTND**	**Freq.**	**SQ**	**HSI**	**FTND**	**Freq.**	**SQ**	**HSI**	**FTND**
	rs684513	C/G	0.81/0.19	0.16^{r}	0.16^{d}	0.10^{d}	0.80/0.20	0.23^{d}	0.37^{d}	0.47^{a}	0.81/0.19	0.29^{a}	0.39^{a}	0.36^{a}
	rs621849	A/G	0.59/0.41	0.18^{a}	0.28^{r}	0.38^{r}	0.56/0.44	0.20^{d}	0.094^{d}	0.061^{d}	0.57/0.43	0.38^{a}	0.13^{d}	0.063^{d}
	rs637137	A/T	0.22/0.78	0.57^{d}	0.12^{a}	0.20^{a}	0.29/0.71	0.22^{d}	0.24^{d}	0.20^{d}	0.27/0.73	0.19^{d}	0.19^{d}	0.19^{d}
CHRNA5	rs951266	C/T	0.34/0.63	0.17^{a}	0.14^{a}	0.44^{a}	0.10/0.90	0.76^{d}	0.89^{d}	0.81^{a}	0.18/0.82	0.35^{a}	0.29^{a}	0.48^{a}
	rs17408276	C/T	0.35/0.65	0.16^{a}	0.40^{a}	0.71^{d}	0.13/0.87	0.61^{r}	0.51^{d}	0.58^{d}	0.19/0.81	0.23^{a}	0.65^{d}	0.69^{r}
	rs16969968	A/G	0.37/0.63	0.17^{a}	0.088^{a}	0.26^{a}	0.04/0.96	0.62^{a}	0.51^{d}	0.61^{a}	0.14/0.86	0.15^{a}	0.11^{a}	0.22^{a}
	rs615470	C/T	0.65/0.35	0.085^{a}	0.21^{a}	0.54^{a}	0.58/0.42	0.77^{d}	0.60^{d}	0.47^{d}	0.61/0.39	0.45^{a}	0.76^{d}	0.65^{d}
	rs578776	C/T	0.27/0.73	0.78^{d}	0.38^{a}	0.64^{a}	0.54/0.46	0.61^{d}	0.51^{a}	0.39^{a}	0.46/0.54	0.61^{d}	0.37^{a}	0.36^{a}
	rs6495307	C/T	0.59/0.41	0.23^{d}	0.48^{d}	0.69^{d}	0.58/0.42	0.79^{d}	0.62^{a}	0.61^{a}	0.58/0.42	0.46^{d}	0.88^{d}	0.87^{r}
	rs1051730	C/T	0.37/0.63	0.18^{d}	0.11^{d}	0.29^{d}	0.11/0.89	0.38^{d}	0.58^{d}	0.43^{d}	0.19/0.81	0.14^{d}	0.25^{a}	0.33^{d}
	rs3743078	C/G	0.23/0.77	0.46^{d}	0.29^{a}	0.34^{a}	0.59/0.41	0.59^{d}	0.37^{r}	0.33^{d}	0.48/0.52	0.59^{a}	0.25^{d}	0.22^{d}
	rs1317286	A/G	0.62/0.38	0.23^{d}	0.21^{d}	0.54^{d}	0.75/0.25	0.07^{d}	0.15^{d}	0.10^{d}	0.71/0.29	**0.02** ^{d}	**0.05** ^{d}	0.07^{d}
CHRNA3	rs12914385	C/T	0.58/0.42	0.23^{d}	0.21^{d}	0.44^{d}	0.84/0.16	0.12^{d}	0.14^{d}	0.14^{d}	0.76/0.24	0.55^{d}	0.79^{r}	0.52^{d}
	rs2869546	C/T	0.37/0.63	0.17^{d}	0.49^{d}	0.70^{d}	0.39/0.61	0.58^{r}	0.56^{d}	0.36^{r}	0.38/0.62	0.28^{d}	0.62^{d}	0.48^{d}
	rs6495308	C/T	0.84/0.16	0.36^{d}	0.15^{d}	0.14^{d}	0.80/0.20	0.43^{a}	0.39^{d}	0.41^{d}	0.81/0.19	0.68^{d}	0.77^{d}	0.66^{d}
	rs3743075	A/G	0.64/0.36	0.12^{a}	0.24^{a}	0.51^{a}	0.56/0.44	0.37^{d}	0.52^{d}	0.36^{r}	0.59/0.41	0.18^{a}	0.32^{a}	0.32^{d}
	rs8040868	C/T	0.43/0.57	0.30^{a}	0.27^{d}	0.49^{d}	0.35/0.65	**0.02** ^{d}	**0.039** ^{d}	**0.017** ^{d}	0.37/0.63	**0.019** ^{d}	0.07^{a}	0.08^{a}
	rs6495309	C/T	0.16/0.84	0.79^{d}	0.29^{d}	0.34^{a}	0.18/0.82	0.27^{d}	0.25^{d}	0.28^{d}	0.17/0.83	0.40^{d}	0.39^{d}	0.38^{d}
	rs1948	C/T	0.31/0.69	0.15^{d}	0.25^{d}	0.42^{d}	0.22/0.78	0.79^{a}	0.43^{d}	0.34^{a}	0.25/0.75	0.29^{d}	0.42^{d}	0.31^{d}
CHRNB4	rs12441088	G/T	0.23/0.77	0.23^{a}	0.23^{a}	0.51^{a}	0.32/0.68	0.57^{d}	0.23^{d}	0.15^{d}	0.29/0.71	0.58^{d}	0.19^{d}	0.13^{d}
	rs3813567	C/T	0.82/0.18	0.38^{d}	0.11^{a}	0.22^{a}	0.81/0.19	0.40^{d}	0.66^{d}	0.86^{d}	0.81/0.19	0.52^{d}	0.56^{r}	0.52^{a}
	rs11637890	C/G	0.63/0.37	0.35^{d}	0.36^{d}	0.43^{d}	0.83/0.17	0.76^{d}	0.47^{d}	0.47^{a}	0.77/0.23	0.47^{d}	0.49^{d}	0.55^{d}

Notes:

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2011 April 5.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

1) Significant associations at the 0.05 level before correction for multiple testing are given in bold.

2) Superscripts indicate genetic model used for analysis:

3) For each ethnic-specific sample, age and sex were used as covariates; for the Combined sample, age, sex, and ethnicity were used as covariates.

a = additive

d = dominant

r = recessive.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 3

Haplotypes within *CHRNA5* and *CHRNA3* associated with three ND measures in the combined sample

					SQ				HSI				FTND			
CHRNA5	**5**	**6**	**7**	**Freq**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**
	T	A	C	0.14	**0.019** [a]	2.33	0.073	121	**0.018** [a]	2.357	0.064	121	0.065	1.849	0.15	126
	T	G	C	0.47	0.33[a]	−0 .968		285	0.11[a]	−1.58		286	0.14	−1.481		298
CHRNA3																
12	**13**	**14**	**15**	**16**	**17**	**18**										
G	C	T					0.11	**0.016** [a]	2.4	0.064	125	**0.041** [a]	2.047	0.09	123	**0.035** [a]	2.108	0.24	132
A	C	C					0.38	0.81[a]	−0.236		321	0.82[a]	0.233		320	0.86[a]	0.18		334
			C	G	C		0.34	**0.016** [d]	2.402	0.16	200	**0.038** [d]	2.074	0.34	205	**0.029** [d]	2.171	0.31	218
			C	C	T		0.35	0.092[a]	−1.684		278	0.12[a]	−1.56		276	0.16[a]	−1.422		290
				A	T	T	0.36	**0.027** [a]	−2.2	0.17	278	**0.047** [a]	−1.984	0.21	276	0.057[r]	−1.902	0.21	101
				G	C	T	0.34	0.083[d]	1.729		196	0.18[d]	1.353		208	0.19[d]	1.314		217

Notes:

1) Only major haplotypes are shown; the one with a P value <0.05 is given in bold.

2) For the 0.05 significance level, significant P value after Bonferroni correction for four major haplotypes within both CHRNA5 and CHRNA3 is 0.0125.

3) Superscripts indicate the genetic model used in the analysis

4) The ND measures were corrected for age, sex, and ethnicity.

[a]= additive

[d]= dominant

[r]= recessive.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 4

Haplotypes within *CHRNA3* associated with three ND measures in the AA sample

						SQ				HSI				FTND			
12	**13**	**14**	**15**	**16**	**Freq**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**
G	C	T			0.14	**0.016** [a]	2.397	0.12	126	**0.043** [a]	2.023	0.29	126	**0.036** [a]	2.09	0.35	128
A	C	C			0.37	0.91[a]	−0.114		242	0.83[a]	0.217		243	0.91[a]	−0.114		245
		T	C	G	0.41	**0.041** [r]	−2.046	0.06	100	0.10[r]	−1.63	0.18	100	0.18[r]	−1.355	0.19	100
		C	C	C	0.33	0.34	−0.963		70	0.45	−0.76		70	0.29	−1.069		70

Notes:

1) Only major haplotypes are shown; the one with a P value <0.05 is given in bold.

2) For the 0.05 significance level, significant P value after Bonferroni correction for four major haplotypes is 0.0125.

3) Superscripts indicate the genetic model used in the analysis

4) The ND measures were corrected for age and sex.

[a] = additive

[d] = dominant.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 5

Haplotypes within *CHRNA5* and *CHRNA3* associated with three ND measures in the EA sample

					SQ				HSI				FTND			
CHRNA5																
5	**6**	**7**	**Freq**		**P-haplotype**	**Z-score**	**P-global**	**No. of families**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**	**P-haplotype**	**Z-score**	**P-global**	**No. of families**
T	A	C	0.36		0.053[a]	1.933	0.27	83	**0.035** [a]	2.111	0.12	83	0.16[a]	1.394	0.32	83
T	G	C	0.28		0.21[a]	−1.273		55	**0.031** [a]	−2.157		55	0.073[a]	−1.795		55
CHRNA3																
15	**16**	**17**	**18**													
C	G	T		0.083	0.092[a]	1.661	0.19	12	**0.038** [a]	2.074	0.096	12	**0.041** [a]	2.038	0.17	12
C	G	C		0.42	0.078[a]	1.76		75	0.097[a]	1.658		76	0.27[a]	1.097		76
	G	T	T	0.077	0.24[a]	1.175	0.45	13	**0.033** [a]	2.13	0.12	13	**0.035** [a]	2.105	0.19	13
	A	C	T	0.43	0.19[a]	1.266		79	0.31[a]	1.021		76	0.72[a]	0.353		76

Notes:

1) Only major haplotypes are shown; the one with a P value <0.05 is given in bold.

2) For the 0.05 significance level, significant P value after Bonferroni correction for three major haplotypes within CHRNA5 is 0.0167; value for four major haplotypes within CHRNA3 is 0.0125.

3) Superscripts indicate genetic model used in the analysis

4) The ND measures were corrected for age and sex.

[a]= additive.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 6

Best interactive models for *CHRNA5/A3/B4* cluster with ND in the AA and pooled sample on the basis of prediction accuracy and empirical *P* value from 10,000 permutations

Sample	No. of loci	Best Model	ND Measure	Prediction Accuracy	*P* value
AA	3	*CHRNA3*: rs578776; rs3743078; rs1317286	HSI	0.532	0.045
			FTND	0.5482	0.003
	4	*CHRNA5*: rs684513; rs615470 *CHRNA3*: rs1317286 *CHRNB4*: rs12441088	SQ	0.553	0.002
			HSI	0.543	0.016
			FTND	0.531	0.041
	6	*CHRNA5*: rs684513, rs621849 *CHRNA3*: rs578776; rs1317286; rs12914385 *CHRNB4*: r12441088	SQ	0.546	0.005
			HSI	0.549	0.003
			FTND	0.529	0.044
Pooled sample	2	*CHRNA3*: rs3743078; rs1317286	SQ	0.511	0.165
			HSI	0.512	0.141
			FTND	0.525	0.031
	3	*CHRNA5*: rs621849 *CHRNA3*: rs3743078 *CHRNB4*: rs11637890	SQ	0.530	0.011
			HSI	0.520	0.061
			FTND	0.516	0.106