Published in final edited form as:
Biol Psychiatry. 2008 December 1; 64(11): 951–957. doi:10.1016/j.biopsych.2008.04.026.

Gene-gene interactions among *CHRNA4*, *CHRNB2*, *BDNF* and *NTRK2* in nicotine dependence

Ming D. Li[1], **Xiang-Yang Lou**[1], **Guobo Chen**[1,2], **Jennie Z. Ma**[3], and **Robert C Elston**[4]

1*Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, VA, USA*

2*Institute of Bioinformatics, Zhejiang University, Zhejiang, PR China*

3*Department of Public Health Sciences, University of Virginia, Charlottesville, VA, USA*

4*Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH, USA*

Abstract

Background—Extensive epidemiological data indicate that vulnerabilities to nicotine dependence (ND) are influenced by genes, environmental factors, and their interactions. Although it has been documented from molecular experiments using in vitro and animal models that brain-derived neurotrophic factor (*BDNF*) exerts its functions via neurotrophic tyrosine kinase receptor 2 (*NTRK2*) and both alpha 4 (*CHRNA4*) and beta 2 (*CHRNB2*) subunits are required to form functional α4β2-containing nicotinic receptors (nAChRs), no study is reported demonstrating that there exist gene-gene interactions among the four genes in affecting ND.

Methods—To determine if gene-gene interactions exist among the four genes, we genotyped 6 SNPs for *CHRNA4* and *BDNF*, 9 SNPs for *NTRK2*, and 4 SNPs for *CHRNB2* in a case-control sample containing 275 unrelated smokers with a FTND score of 4.0 or more and 348 unrelated nonsmokers.

Results—By using a generalized multifactor dimensionality reduction (GMDR) algorithm recently developed by us, we found highly significant gene-gene interactions for the gene pairs of *CHRNA4* and *CHRNB2*, *CHRNA4* and *NTRK2*, *CHRNB2* and *NTRK2*, and *BDNF* and *NTRK2* ($P<0.01$ for all four gene pairs), and significant gene-gene interaction between *CHRNA4* and *BDNF* ($P=0.031$) on ND. No significant interaction was detected for the gene pair *CHRNB2* and *BDNF* ($P=0.068$).

Conclusion—Our study provides first evidence on the presence of gene-gene interaction among the four genes in affecting ND. Although *CHRNB2* alone was not significantly associated with ND in several previously reported association studies on ND, we found it affects ND through interactions with *CHRNA4* and *NTRK2*.

Keywords

Interaction; tobacco smoking; nicotine dependence; epistasis; GMDR

Correspondence to: Ming D. Li, PhD, 1670 Discovery Drive, Suite 110, Charlottesville, VA 22911, Tel: 434-243-0566; Fax: 434-973-7031, Email: Ming_Li@virginia.edu.

Financial Disclosures

Dr. Lou, Mr. Chen, Dr. Ma, and Dr. Elston reported no biomedical financial interests or potential conflicts of interest. Dr. Li disclosed consulting fees from Information Management Consultants, Inc. and having received lecture fees from CME Enterprise.

Introduction

Despite increasing public awareness of the health risks of using tobacco products and legislation that reduces the availability of cigarettes and prohibits smoking in many public facilities, little reduction in smoking prevalence has occurred in this country since 1990. In 2006, an estimated 45.3 million adults in the United States were current smokers; of them, an estimated 36.3 million smoked every day, and 9.0 million smoked some days (1). Economically, smoking is responsible for about 7% of the total US health care costs, or an estimated $167 billion each year (1). It is estimated that smoking causes approximately 438,000 deaths annually in the United States or 18.1% of all deaths nationwide (2).

Smoking is a complex behavior influenced by both genetic and environmental factors. Environmental influences on the development of nicotine dependence (ND) are well documented and include peer and familial influences as the strongest contributors to how and when cigarette experimentation occurs among young people. Moreover, multiple lines of evidence indicate that genetics plays a large part in determining smoking behavior. Over the last decade, many large-sample twin studies in the United States and other countries have yielded results supporting the conclusion that genetics contribute to the risk of becoming a regular smoker (3–7). A meta-analysis of the genetic parameter estimates for ND based on 17 twin studies determined that the weighted mean polygenic heritability for ND is 0.59 in male and 0.46 in female smokers, with an average of 0.56 for all smokers (7). Thus, identifying the genes predisposing to ND and understanding their molecular mechanisms are vital to prevention and treatment.

Recent evidence supports genetic associations with ND of the nicotinic acetylcholine receptor (nAChR) α4 subunit (CHRNA4) (8–10), of the brain-derived neurotrophic factor (*BDNF*) (11,12), and of the neurotrophic tyrosine kinase receptor 2 (*NTRK2*, also known as the tyrosine kinase receptor gene, *TrkB*) (13). Biochemical studies have revealed that the α4β2-containing nAChR subtype makes up the majority of the high-affinity nicotine-binding sites in the brain (14) and that the genes for both subunits are upregulated under chronic nicotine exposure (15,16). Furthermore, it was reported that activation of *CHRNA4* is sufficient for nicotine-induced reward, tolerance, and sensitization (17). Moreover, knock-out mice for the α4 or β2 (*CHRNB2*) subunit show no high-affinity binding sites in their brains and fail to self-administer nicotine, indicating that the α4β2 subtype plays a primary role in the reinforcing effects of nicotine (17,18). However, except for *CHRNA4* that has been found to be associated with smoking in three independent samples (8–10), almost all reported studies found no association of *CHRNB2* with ND in humans (8,9,19,20).

On the other hand, BDNF must act through its high-affinity receptor NTRK2 in order to support the survival and growth of diverse neuronal populations and influences the form and function of chemical synapse (21). Furthermore, it was reported that nicotine modulates expression of *BDNF* and *NTRK2* at both RNA and protein levels, indicating that nicotine regulates the BDNF/TrkB signaling pathway (22–24). Although the biological interactions of *BDNF* with *NTRK2* and *CHRNA4* with *CHRNB2* have been established experimentally using in vitro and animal models, no study is reported to demonstrate the presence of gene-gene interactions among the four genes. Thus, the primary objective of this study was to determine whether significant gene-gene interactions exist among the four genes in affecting ND, using a novel algorithm, called generalized multifactor dimensionality method (GMDR), reported recently by this group (25).

MATERIALS AND METHODS

Human study population

The subjects used in this study are of either African-American (AA) or European-American (EA) origin and were selected from a family study on ND, recruited primarily from the Mid-South states including Tennessee, Mississippi, and Arkansas, in the U. S. during 1999–2004 (8,26,27). Smokers were required to be at least 21 years of age, have smoked for at least the last five years, and have consumed an average of 20 cigarettes per day for the last 12 months. Extensive 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 personality traits assessed by various questionnaires, available at NIDA Genetics Consortium Website (http://zork.wustl.edu/nida). All participants provided informed consent. The study protocol and forms/procedures were approved by all participating Institutional Review Boards.

In the present study, ND for smokers was ascertained by the Fagerström Test for ND (FTND: 0–10 scale) (28). All smokers selected for inclusion into the study had a FTND score of 4 or above and controls are defined as those who had exposed to cigarette smoking but smoked less than 100 cigarettes in their lifetime. Given the requirement of the GMDR method for the case-control design, we selected parent(s) or only one child (either smoker or control) from each family.

DNA extraction, SNP selection, and genotyping

The DNA was extracted from peripheral blood samples of each participant using a kit from Qiagen Inc (Valencia, CA). On the basis of the high heterozygosity (minor allele frequency ≥ 0.05) and coverage of the gene of interest that was as uniform as possible, we selected 6 SNPs for *CHRNA4* and *BDNF*, 4 SNPs for *CHRNB2*, and 9 SNPs for *NTRK2* from the National Center for Biotechnology Information (NCBI) database. Table 1 provides detailed information on these SNPs, which include their location within the gene, chromosomal position, allelic variants, the minor allele frequency, and the primer and probe sequences.

All SNPs were genotyped using the *Taq*Man SNP Genotyping Assay in a 384-well microplate format (Applied Biosystems, Foster, 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. Allelic discrimination analysis was performed on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster CA). To ensure the quality of the genotyping, SNP-specific control samples were added to each 384-well plate.

Statistical analysis for gene-gene interaction

Prior to performing the statistical analysis for detecting gene-gene interaction reported here, we used the PedCheck program (29) to determine genotyping consistency for Mendelian inheritance on the basis of the genotype data from other family members. Also, we checked the SNP data for any significant departure from Hardy-Weinberg equilibrium (HWE). The HWE at each locus was assessed by the χ^2 test. The allele frequencies for each genetic marker were calculated using the FREQ program of S.A.G.E. (v. 5.0).

Statistical analyses of all SNPs between all possible pairs of the four genes were performed using a newly developed GMDR (25), as an extension of the MDR methods developed previously by other research groups (30–32) for allowing covariate adjustment. Given that both human and animal studies of nicotine administration or smoking behavior have documented that age, gender and ethnicity play significant roles in ND (7,33,34), we included age, gender

and ethnicity as covariates in all our gene-gene interaction analyses. Residual scores of all individuals were computed under a null logistic model for smoking status.

Ten-fold cross-validation was used in our GMDR analysis. That is, data were randomly split into 10 approximately equal parts; one subdivision was used as the testing set and the rest as the training set. The genotypes formed by a subset of *n* SNPs (called *n*-locus model) were classified into high-risk, low-risk, or empty cells according to whether the average score in the training set was ≥ 0, < 0, or, empty (no data). Two contrasting (i.e, high-risk and low-risk) groups were formed by pooling all high-risk and low-risk cells, respectively, and the difference between two group scores was used to measure the classification accuracy. All potential *n*-locus models were evaluated sequentially and the model with the maximum classification accuracy was selected as the best model from those with the same dimensionality. We then used the independent testing set to estimate the prediction accuracy, i.e., to calculate the score difference in the testing set between two groups formed by the same pooling pattern as the training set. Finally, the model with the maximum prediction accuracy was chose as the best model among this set of best models from different locus numbers. To reduce the fluctuations due to chance divisions of the data, each possible training set and its corresponding testing set were successively used and the results were averaged. The consistency of the model across cross-validation training sets, i.e., how many times the same MDR model is identified in all the possible training sets, is also evaluated.

To identify the best interaction model, we did an exhaustive search for all possible two- to seven-SNP models for the SNPs between all six gene pairs. We also performed 10,000 permutations to generate the null distribution of maximum prediction accuracy for each dimensionality of the multilocus models evaluated so that the obtained empirical P values can be used as a yardstick to rank the models. Specifically, we randomly permuted the affection status for all participants to generate a set of pseudo-samples in which any potential relationship between the SNPs and the phenotype was disrupted. And then, as conducted in the above GMDR analysis for the real dataset, we used cross-validation to identify and evaluate the best interaction model. That is, we divided the pseudo-sample into two subsets of either training or testing, and then used training data to determine the best model based on the classification accuracy of the model under investigation, and testing data to evaluate the prediction accuracy for the identified model. Because of the independence of two subsets and no genetic association between the SNPs and the phenotype, the prediction accuracy from each permutation represented a sample drawn from the null distribution. The P-value was estimated by the proportion of the pseudo-samples resulting in larger prediction accuracy than the observed one in the real dataset. Based on these P values, along with the corresponding prediction accuracy and cross-validation consistency, we identified the best statistical gene-gene interaction model for all possible pairs among the four genes.

Results

To determine whether gene-gene interaction exist among the four genes in affecting ND, we studied 275 unrelated smokers with a FTND score ≥ 4.0 and 348 unrelated nonsmokers selected from the U. S. Mid-South Tobacco Family cohort (26). Of the subjects included, the average age was 46.6 ± 13.0 for smokers and 41.6 ± 17.8 for controls; 70% and 78% were females for smokers and controls; and 65% and 66% were African Americans for smokers and controls, respectively. For the smoker group, the average FTND score was 8.4 ± 1.6. Table 2 provides a summary of clinical characteristics for the cases and controls used in the current study.

As shown in Table 3, we detected highly significant interactive genetic effects on ND for the gene pairs *CHRNA4* and *CHRNB2*, *CHRNA4* and *NTRK2*, *CHRNB2* and *NTRK2*, and *BDNF* and *NTRK2* (with prediction accuracy ranging from 0.565 to 0.593; empirical P values < 0.01

for all these pairs). In addition, we found significant statistical interaction on ND between the genes *CHRNA4* and *BDNF*, with prediction accuracy 0.552 (empirical P = 0.031). For the case of *BDNF* and *CHRNB2*, we did not find any interaction model better than we show in Table 3 (empirical P = 0.068) even when we tested more than seven SNPs for the gene pair. We also examined these interactions for each ethnic sample separately. Although the detected interactions in each ethnic sample appeared to be less significant (data not shown), we did obtain similar results as reported here for the combined sample.

To determine whether interaction analysis between each gene pair yield a better model than a single gene approach, we also performed interaction analysis on the SNP(s) included in the best interaction model for each gene pair (see Table 3). A comparison of the prediction accuracy and empirical P value of each gene pair and the corresponding individual gene (with prediction accuracy from 0.494 to 0.564; empirical P value from 0.020 to 0.454; Table 3) further confirmed our finding that significant gene-gene interaction exists among these gene pairs in affecting ND.

By examining the SNPs included in the best prediction model for each gene pair, we found that SNP rs2072661 was included in all the *CHRNB2*-related gene pairs, as were SNPs rs2030324 for *BDNF* and rs4075274 for *NTRK2*. This suggests that the gene region where each SNP is located more likely harbors causative SNP(s) involved in the etiology of ND. As for *CHRNA4*, SNPs included in the three gene pairs with significant interaction are almost evenly distributed across the whole gene, with no single SNP detected in all the gene pairs, suggesting multiple causative or functional SNPs exist within the gene. We also analyzed ND as a quantitative trait and obtained almost identical results as when it was treated as a binary trait (data not shown).

Discussion

Mounting studies have pointed to the view that genes act in concert, rather than isolatedly, to affect ND. Statistical gene-gene interactions have been reported for smoking-related phenotypes for *DRD2* with *SLC6A3*, *DBH*, and *CYP2B6* (35–37). It has been long known that subunits α4 and β2 must assemble together in order to form a functional α4β2-containing heteromeric nAChR, a major highly expressed receptor type in the central nervous system. Similarly, in order for BDNF to exert its biological functions in the regulation of dopamine and serotonin neurotransmission in the brain, BDNF has to bind to its receptor, NTRK2, leading to activation of *BDNF/NTRK2* signaling pathway. However, to date, there is no human genetic epidemiological study showing evidence that *CHRNA4* indeed interacts with *CHRNB2* and so does for *BDNF* and *NTRK2* in affecting ND. Thus, this study provides first evidence that gene-gene interactions exist among the two gene pairs in influencing ND.

To gain further insight into the contributions of the four genes to ND, we propose a genetic model to explain our findings (Figure 1). Because it has been demonstrated experimentally that *CHRNA4* interacts with *CHRNB2* and *BDNF* with *NTRK2*, we conclude that these two gene pairs contribute to ND by a known biological interactive mechanism. On the other hand, since α4β2-containing nAChR and *BDNF/NTRK2* represent two independent signaling systems, we conclude that *CHRNA4* and *BDNF* or *NTRK* jointly contribute to ND in an as yet unknown indirect manner, as is also the case for *CHRNB2* and *NTRK2*.

Compared with our earlier family-based individual SNP and haplotype analyses for each individual gene, we found most of the reported significant SNPs in the earlier studies yielded a stronger signal (significance) in the present study. For example, SNP rs2030324 in *BDNF* that was significant in the EA and the pooled samples (11) was identified to interact with *NTRK2* (P = 0.002) and *CHRNA4* (P = 0.031). Significant SNPs in *NTRK2*, rs1187272 and

rs1122530 in EAs, and rs993315, rs4075274, rs729560 in AAs (13), were included in the lists of the best interaction models of the current report. Significant SNPs in *CHRNA4*, rs2273504 and rs1044396 in EAs, and rs2273504, rs2273505, and rs2236196 in AAs (8) also interacted with at least one SNP of *CHRNB2*, *BDNF*, and *NTRK2*. On the other hand, we also noticed that few significant SNPs, e.g., rs1659400 of *NTRK2* significant in EAs (13) and rs3787137 of *CHRNA4* significant in AAs (8) were not included in our detected interaction models. This is more likely due to that those SNPs were in very strong LD with some SNPs included in the identified interaction models, e.g., rs1659400 with rs1187272 and rs3787137 with rs2236196, and inclusion of them in the final interaction model would be redundant and contribute no or less extra information to account for ND variation in our samples.

Also, it is worthy of mention that, although *CHRNB2* alone was not found to be associated with ND in several reported studies (8,9,19,20), it does appear to play a role in ND when analyzed jointly with *CHRNA4* or *NTRK2*. In other words, although no significant contribution of *CHRNB2* to ND could be detected in the four independent human genetics studies (8,9,19, 20), it does not exclude the possibility of involvement of the gene to ND. The reason of failing to detect significant association of *CHRNB2* with ND might be due to the strong dependency of *CHRNB2* effects on specific *CHRNA4* variants and/or small sample size for relatively small marginal effects of *CHRNB2* used in those studies. With the GMDR approach that can detect genetic contribution of multiple genes on ND, we detected significant gene-gene interaction between *CHRNB2* and *CHRNA4* in influencing ND. This indicates that it is critical to develop more powerful and sensitive methodology of detecting gene-gene and gene-environment interactions and apply them to our genetics research, which will help us to identify more genetic contributors to ND that cannot be detected at single gene level based on conventional association analysis. More importantly, detection of significant joint contribution of *CHRNB2* with *CHRNA4* in human provide a plausible explanation to well-documented experimental evidence observed in animals where knockout mice for the α4- or β2-subunit of nAChRs show no high-affinity binding site in their brains and fail to self-administer nicotine (18,38,39).

It is also interesting to notice that we detected significant interaction between *CHRNA4*/*CHRNB2* and *BDNF/NTRK2*. From our current knowledge on the biological function and signaling pathways involved by the four genes, it seems that these four genes function independently as two separate functional groups. The first group consists of *CHRNA4* and *CHRNB2* whose gene products must assemble together to form functional α4β2-containing nAChRs involved in modulation the release of dopamine and gamma-aminobutyric acid (40). Another group contains *BDNF* and *NTRK2* genes that make up *BDNF/NTRK2* signaling pathway with a critical role in regulating the survival and differentiation of neuronal populations during development and synaptic transmission and plasticity at adult synapses in many brain regions (21). Thus, to some extent, our detected interactions of *CHRNA4* and *CHRNB2* with *BDNF* and *NTRK2* is considered to be novel and intriguing from biological point of view, and such predictions deserve to be tested experimentally using in vitro and animal models.

It is agreed that ND, like any other complex trait, is controlled by multiple genetic factors, with each having a relatively small effect, as well as by environmental factors and interactions between genes or between genes and the environment. During the past decade, significant progress has been made in searching for susceptibility genes for ND through linkage and association analyses (41–44). However, these approaches are effective only for genes with moderate to major effects. The ability to identify susceptibility genes for ND and other complex traits has been improving but is still limited because of various factors such as gene interaction, modest marginal contribution, variable expressivity, small sample size, and heterogeneities (41,45,46). Of these factors, gene-gene and gene-environment interactions are of the greatest

importance. To search for determinants of gene-gene and gene-environment interactions, considerable effort has been made in the past. Several combinatorial approaches, such as the multifactor dimensionality reduction (MDR) method (30–32,47), the combinatorial partitioning method (CPM) (48), and the restricted partition method (RPM) (49), have been developed as promising tools for detecting gene-gene and gene-environment interactions (50–52). Since the original report, MDR has been applied by many research groups to detect interactions for a number of complex disorders, and the list of publications is expanding rapidly (for details, please see the Epistasis Blog Website: http://compgen.blogspot.com/2006/05/mdr-applications.html). However, these established methods have limitations that restrict their practical use. For example, MDR, CPM, and RPM do not allow adjustment for covariates; MDR is applicable only to dichotomous phenotypes, and CPM and RPM cannot handle categorical phenotypes. To overcome the limitations of these existing combinatorial approaches and meet our research needs in determining gene-gene and gene-environment interactions for ND, we recently developed a generalized approach, called generalized MDR (25) and applied this new methodology to our genetic analysis of SNP data on ND. The results reported in this communication represent a new research dimension for our ongoing efforts in searching for susceptibility genes for ND.

In summary, we have determined not only that *CHRNA4* interacts with *CHRNB2* and *BDNF* with *NTRK2* to contribute to ND by known biological interactive mechanisms that have been demonstrated experimentally, but also that *CHRNA4* interacts with *BDNF* or *NTRK*, and *CHRNB2* with *NTRK2*, contributing to ND in an as yet unknown manner, as the gene pairs represent two independent signaling systems. Further replication of these findings in independent samples is thus needed in future study. Moreover, our interaction investigation suggests that *CHRNB2* is involved in the etiology of ND when analyzed jointly with *CHRNA4* or *NTRK2*. This provides an example of how traditional analysis may fail to identify important risk genes and thus that the use of a validated detection strategy for interactions is warranted.

Acknowledgements

We acknowledge the invaluable contributions of personal information and blood samples by all participants in the study. This project was funded by National Institutes of Health Grant DA-12844 to MDL.

References

1. CDC. Cigarette smoking among adults--United States, 2006. MMWR Morb Mortal Wkly Rep 2007;56:1157–1161. [PubMed: 17989644]
2. CDC. Annual smoking-attributable mortality, years of potential life lost, and productivity losses--United States, 1997–2001. MMWR Morb Mortal Wkly Rep 2005;54:625–628. [PubMed: 15988406]
3. Carmelli D, Swan GE, Robinette D, Fabsitz R. Genetic influence on smoking--a study of male twins. N Engl J Med 1992;327:829–833. [PubMed: 1508241]
4. Heath AC, Madden PA, Slutske WS, Martin NG. Personality and the inheritance of smoking behavior: a genetic perspective. Behav Genet 1995;25:103–117. [PubMed: 7733853]
5. Sullivan PF, Kendler KS. The genetic epidemiology of smoking. Nicotine Tob. Res 1999;1:S51–S57. [PubMed: 11768187]discussion S69–70.
6. Tyndale RF. Genetics of alcohol and tobacco use in humans. Ann. Med 2003;35:94–121. [PubMed: 12795339]
7. Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. Addiction 2003;98:23–31. [PubMed: 12492752]
8. Li MD, Beuten J, Ma JZ, Payne TJ, Lou XY, Garcia V, et al. Ethnic- and gender-specific association of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) with nicotine dependence. Hum Mol Genet 2005;14:1211–1219. [PubMed: 15790597]

9. Feng Y, Niu T, Xing H, Xu X, Chen C, Peng S, et al. 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;75:112–121. [PubMed: 15154117]
10. Hutchison KE, Allen DL, Filbey FM, Jepson C, Lerman C, Benowitz NL, et al. CHRNA4 and tobacco dependence: from gene regulation to treatment outcome. Arch Gen Psychiatry 2007;64:1078–1086. [PubMed: 17768273]
11. Beuten J, Ma JZ, Payne TJ, Dupont RT, Quezada P, Huang W, et al. Significant association of BDNF haplotypes in European-American male smokers but not in European-American female or African-American smokers. Am J Med Genet B Neuropsychiatr Genet 2005;139:73–80. [PubMed: 16152573]
12. Lang UE, Sander T, Lohoff FW, Hellweg R, Bajbouj M, Winterer G, Gallinat J. Association of the met66 allele of brain-derived neurotrophic factor (BDNF) with smoking. Psychopharmacology (Berl) 2007;190:433–439. [PubMed: 17186223]
13. Beuten J, Ma JZ, Payne TJ, Dupont RT, Lou XY, Crews KM, et al. Association of Specific Haplotypes of Neurotrophic Tyrosine Kinase Receptor 2 Gene (NTRK2) with Vulnerability to Nicotine Dependence in African-Americans and European-Americans. Biol Psychiatry 2007;61:48–55. [PubMed: 16713586]
14. Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regulated by chronic nicotine treatment. Mol Pharmacol 1992;41:31–37. [PubMed: 1732720]
15. Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, Collins AC. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. J Neurosci 1992;12:2765–2784. [PubMed: 1613557]
16. Whiteaker P, Sharples CG, Wonnacott S. Agonist-induced up-regulation of alpha4beta2 nicotinic acetylcholine receptors in M10 cells: pharmacological and spatial definition. Mol Pharmacol 1998;53:950–962. [PubMed: 9584223]
17. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, et al. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science 2004;306:1029–1032. [PubMed: 15528443]
18. Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, et al. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 1998;391:173–177. [PubMed: 9428762]
19. Silverman MA, Neale MC, Sullivan PF, Harris-Kerr C, Wormley B, Sadek H, et al. 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;96:646–653. [PubMed: 11054772]
20. 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;4:115–125. [PubMed: 11906688]
21. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog Neurobiol 2005;76:99–125. [PubMed: 16099088]
22. Yamada K, Nabeshima T. Brain-derived neurotrophic factor/TrkB signaling in memory processes. J Pharmacol Sci 2003;91:267–270. [PubMed: 12719654]
23. Sun D, Huang W, Hwang YY, Zhang Y, Zhang Q, Li MD. Regulation by nicotine of Gpr51 and Ntrk2 expression in various rat brain regions. Neuropsychopharmacology 2007;32:110–116. [PubMed: 16794563]
24. Serres F, Carney SL. Nicotine regulates SH-SY5Y neuroblastoma cell proliferation through the release of brain-derived neurotrophic factor. Brain Res 2006;1101:36–42. [PubMed: 16790237]
25. Lou XY, Chen GB, Yan L, Ma JZ, Zhu J, Elston RC, Li MD. A Generalized Combinatorial Approach for Detecting Gene-by-Gene and Gene-by-Environment Interactions with Application to Nicotine Dependence. Am J Hum Genet 2007;80:1125–1137. [PubMed: 17503330]
26. Li MD, Payne TJ, Ma JZ, Lou XY, Zhang D, Dupont RT, et al. A genomewide search finds major susceptibility Loci for nicotine dependence on chromosome 10 in african americans. Am J Hum Genet 2006;79:745–751. [PubMed: 16960812]

27. 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. 2007
28. 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;86:1119–1127. [PubMed: 1932883]
29. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am. J. Hum. Genet 1998;63:259–266. [PubMed: 9634505]
30. Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, Moore JH. Multifactor-dimensionality reduction reveals high-order interactions among estrogenmetabolism genes in sporadic breast cancer. Am J Hum Genet 2001;69:138–147. [PubMed: 11404819]
31. Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 2003;19:376–382. [PubMed: 12584123]
32. Moore JH, Gilbert JC, Tsai CT, Chiang FT, Holden T, Barney N, White BC. A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. J Theor Biol 2006;241:252–261. [PubMed: 16457852]
33. Picciotto MR. Nicotine as a modulator of behavior: beyond the inverted U. Trends Pharmacol. Sci 2003;24:493–499. [PubMed: 12967775]
34. Madden PA, Heath AC, Pedersen NL, Kaprio J, Koskenvuo MJ, Martin NG. The genetics of smoking persistence in men and women: a multicultural study. Behav Genet 1999;29:423–431. [PubMed: 10857247]
35. Lerman C, Caporaso NE, Audrain J, Main D, Bowman ED, Lockshin B, et al. Evidence suggesting the role of specific genetic factors in cigarette smoking. Health Psychol 1999;18:14–20. [PubMed: 9925041]
36. Johnstone EC, Yudkin PL, Hey K, Roberts SJ, Welch SJ, Murphy MF, et al. Genetic variation in dopaminergic pathways and short-term effectiveness of the nicotine patch. Pharmacogenetics 2004;14:83–90. [PubMed: 15077009]
37. David SP, Brown RA, Papandonatos GD, Kahler CW, Lloyd-Richardson EE, Munafo MR, et al. Pharmacogenetic clinical trial of sustained-release bupropion for smoking cessation. Nicotine Tob Res 2007;9:821–833. [PubMed: 17654295]
38. Picciotto MR, Zoli M, Lena C, Bessis A, Lallemand Y, Le Novere N, et al. Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. Nature 1995;374:65–67. [PubMed: 7870173]
39. Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Lena C, Le Novere N, de Kerchove d'Exaerde A, et al. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. Nature 1999;398:805–810. [PubMed: 10235262]
40. Lindstrom JM. Nicotinic acetylcholine receptors of muscles and nerves: comparison of their structures, functional roles, and vulnerability to pathology. Ann N Y Acad Sci 2003;998:41–52. [PubMed: 14592862]
41. Li MD. Identifying susceptibility loci for nicotine dependence: 2008 update based on recent genome-wide linkage analyses. Hum Genet 2008;123:119–131. [PubMed: 18205015]
42. Li MD. The genetics of nicotine dependence. Curr Psychiatry Rep 2006;8:158–164. [PubMed: 16539894]
43. Lessov-Schlaggar CN, Pergadia ML, Khroyan TV, Swan GE. Genetics of nicotine dependence and pharmacotherapy. Biochem Pharmacol 2008;75:178–195. [PubMed: 17888884]
44. Ho MK, Tyndale RF. Overview of the pharmacogenomics of cigarette smoking. Pharmacogenomics J 2007;7:81–98. [PubMed: 17224913]
45. Schnoll RA, Johnson TA, Lerman C. Genetics and smoking behavior. Curr Psychiatry Rep 2007;9:349–357. [PubMed: 17915073]
46. Swan GE, Lessov CN. Gene-environment interaction in nicotine addiction: the need for a large-scale, collaborative effort. Subst Use Misuse 2004;39:2083–2085. [PubMed: 15587964]
47. Martin ER, Ritchie MD, Hahn L, Kang S, Moore JH. A novel method to identify gene-gene effects in nuclear families: the MDR-PDT. Genet Epidemiol 2006;30:111–123. [PubMed: 16374833]

48. Nelson MR, Kardia SL, Ferrell RE, Sing CF. A combinatorial partitioning method to identify multilocus genotypic partitions that predict quantitative trait variation. Genome Res 2001;11:458–470. [PubMed: 11230170]

49. Culverhouse R, Klein T, Shannon W. Detecting epistatic interactions contributing to quantitative traits. Genet Epidemiol 2004;27:141–152. [PubMed: 15305330]

50. Hoh J, Ott J. Mathematical multi-locus approaches to localizing complex human trait genes. Nat Rev Genet 2003;4:701–709. [PubMed: 12951571]

51. Thornton-Wells TA, Moore JH, Haines JL. Genetics, statistics and human disease: analytical retooling for complexity. Trends Genet 2004;20:640–647. [PubMed: 15522460]

52. Williams SM, Haines JL, Moore JH. The use of animal models in the study of complex disease: all else is never equal or why do so many human studies fail to replicate animal findings? Bioessays 2004;26:170–179. [PubMed: 14745835]

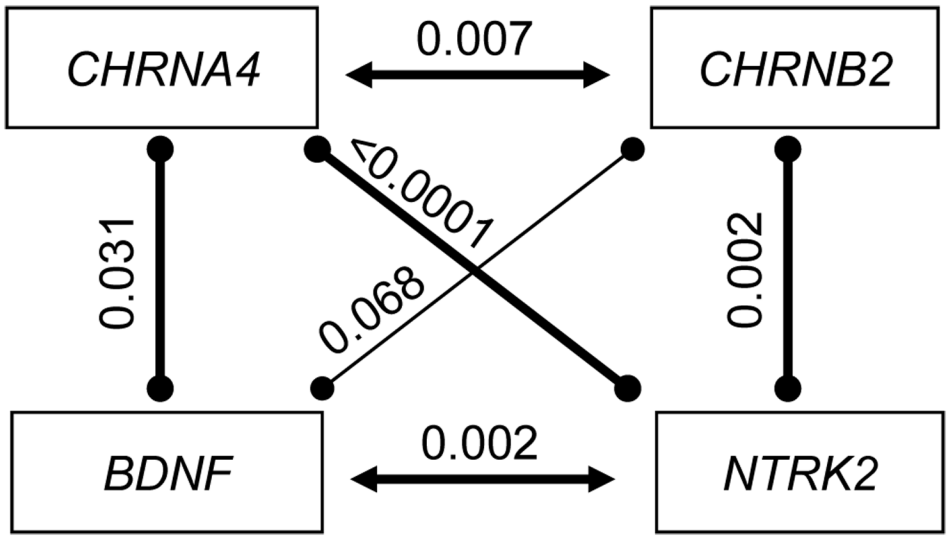

Figure 1.
Illustration of detected genetic effects of *CHRNA4*, *CHRNB2*, *BDNF*, and *NTRK2* on ND. Two types of genetics effects are shown in the figure: known biological interaction (shown in horizontal line) and joint action (shown in vertical line). Value shown on the top of each line represents the empirical *p*-value from 10,000 replicates.

Table 1

Position, nucleotide variation, minor allele frequency and primer/probe sequences of 25 SNPs within *CHRNA4, CHRNB2, BDNF, and NTRK2*

Gene	SNP	Domain	Physical position	Alleles [a]	Reported MAF [b]	Forward (F) and Reverse ® Primer and Probe Sequences (5'-3")
CHRNA4	rs2273505	Intron 2	61461322	C/**T**	0.119	F: CCCCTGTGCTCCTTGCA R: CAGCTCATTGACGTGGTAGGT P: CCATCG/ATGCACTGTGA
	rs2273504	Intron 2	61458505	**A**/G	0.470	F: AGGCTTCCCCAGCTAAGGA R: GCCGGGAGGGAGCAA P: AAGGAGGCTCT/CCCATGC
	rs2229959	Exon 5	61451998	**G**/T	0.179	F: AGCGGCACCCAGAGC R: CTCAGCCGGCACATCCA P: TGCACCCT/GCCCTCAC
	rs1044396	Exon 5	61451578	**C**/T	0.389	F: GCAAATGCACATGCAAGAAGGA R: GTGCTGCGGGTCTTGAC P: TCCCCGAGC/TGCCACG
	rs3787137	Intron 5	61449544	**A**/G	0.261	F: ACAACCCCCAAGCTGATGAG R: GGGACCCAGGACACCCT P: CTGGGCCCA/GGCCGT
	rs2236196	Intron 6	61448000	A/**G**	0.266	F: ACCCTCTCCTAGCGAAGCA R: CTCTCGGGCCCCATGAG P: ATTGGAGCA/GCTGCTGG
CHRNB2	rs2072658	5'-flanking	152806849	**A**/G	0.074	F: CCCCGGAGGCGGAAAC R: GGCAGAACCAATCGAAGACTATCC P: CTTTTTTTTCCTG/AGGACCC
	rs2072660	Exon 6	152815345	C/**T**	0.258	F:GCTGCTAAGTGGAAGACAGAGATG R: GGAGGCAGCAGACAATCCT P: CCTTGCCCG/ATCACTC
	rs2072661	Exon 6	152815504	**A**/G	0.261	F:CCTGACACAATGGTAGCTCTGAAG R: CCAGCTGCTGTCCACTCAAG P: CCTGGGTA/GTGACCTG
	rs3811450	3'-flanking	152817656	C/**T**	0.132	F: GCCCTCACCTCTTCCTTATTGTG R: GCAGTGTCATTCCCTCCATCT P: CAAAGGGCTGCG/ATACAG
BDNF	rs6265	Exon 2	27636492	**A**/G	0.269	F: CTTGACATCATTGGCTGACACTTT R: TTCTTCATTGGGCCGAACTTT P: CGAACACG/ATGATAGAA
	rs2049045	Intron 1	27650817	**C**/G	0.053	F: CCCTCTCCAACCAAAATCTCTCTT R: CACCAACCTAGAAATTGGGTTACCT P: CTTCGATAAAC/GTTCC
	rs6484320	Intron 1	27659764	A/**T**	0.310	F: AACACATGAGACTCAGAGAATTACAACAA R: GCATGCTTCATACCTAAATATGCTTCAC P: AACAGTTTTAGTCA/TTGTAAAC
	rs988748	Intron 1	27681321	C/**G**	0.340	F: ATTCATCTTACAACCTGGGAACCAA R: GAGGGCATGAAGCTGGATACC P: TACCCCAG/CAGACCCT
	rs2030324	Intron 1	27683491	**C**/T	0.431	F: CACAGCCTAAATAGGTGAGTCTCAA R: ACCAAAGGGTTTCAGGACATTGA P: ATGAAAGATGAACG/ATTAGCT
	rs7934165	Intron 1	27688559	**A**/G	0.438	F: CCCTTACCCAGAATATATCCCAGCTT R: CTGGGTCCTTTGTGTCTTTGC P: CAGAGTTCTGAA/GTTGGT
NTRK2	rs993315	Intron 2	86477541	**C**/T	0.495	F: TCCTGATCTTAGCTGCCTGATGTAT R: GCAACAACAATTGTGAGGACAGAT P: CTGCTGAAATCAG/ATTGATT
	rs1659400	Intron 6	86515814	C/**T**	0.353	F: TGTACCGTGGACTGGCTACT R: TGCCATACTCACCTTTACTTTGTTCT

Gene	SNP	Domain	Physical position	Alleles [a]	Reported MAF [b]	Forward (F) and Reverse ® Primer and Probe Sequences (5'-3")
						P: CACTTTCGGTAATTC/TTTGAC
	rs1187272	Intron 12	86593906	**C**/T	0.443	F: GTCCCTGTTAGCCTCACTGTT R: TTTGCCAAGAGCTGAGGTATCTG P: CTTCTCTCCCC/TCTGCGTG
	rs1122530	Intron 12	86654172	**C**/T	0.218	F: GTCCAATTATCTTCTGGGCAAGGAA R: GCACGGATCCATGATTACGTTAGAT P: CTCTTCTT/CGCCTCTCC
	rs736744	Intron 14	86704227	**A**/G	0.221	F: CCAGACTGGGAAGTAGGTCATG R: TCTCTCTCTCTGGAAAGTCAGGAG P: TGGGAGGGCAA/GGTG
	rs920776	Intron 14	86728156	C/**T**	0.217	F: CTGACCTGTATCTTAATGAATGAACATTTTATTTTTACAT R: CCACATTCTGATTGTGTTATTGTCATTGATATATT P: CCATCATTAGAGTATG/ATGCACA
	rs1078947	Intron 15	86753072	C/**T**	0.262	F: GCAAAAGATGAGAAAACAGCCTAGT R: AGCTTTGCATATGCCTAAGGAGTT P: CTAAGGAAATAACA/GTTTGTG
	rs4075274	Intron 17	86786382	A/**G**	0.332	F: TGTGGACATAATAGCCAAAGTAAAATGCT R: AAAGCTGATGTGTTTTTTCATTGTCTTCT P: AACATGTAAGTATCA/GTACCTT
	rs729560	Intron 17	86824125	A/**G**	0.387	F: GGACTACTGTGCAGAAATCTGTTCA R: CATGGAGGAAAGTTAGGAAGCTCAT P: TTTGAAATCA/GGGCATCC

[a] The nucleotide of each SNP shown in bold font represents the minor allele as given in NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/).

[b] Based on the allele frequency presented in the NCBI dbSNP database (Build 123).

Table 2

Clinical characteristics for the cases and controls used in the study

Characteristics	Cases	Controls
Sample size	275	348
Age (± SD; years)	46.6 ± 13.0	41.6 ± 17.8
Females (%)	70%	78%
African Americans (%)	65%	66%
FTND (± SD)	8.4 ± 1.6	NA

Table 3

The best interaction model detected for each gene pair on the basis of prediction accuracy, cross-validation consistency, and empirical P value from 10,000 permutations (Columns 5–7), and the prediction ability of the SNPs included in the identified interaction model for each corresponding gene (Columns 3 and 4)

Gene pair	SNP(s) included in each interaction model	Prediction by the SNP(s) of each gene in the identified model		Prediction by interaction model		
		Prediction accuracy	Empirical P value	Prediction accuracy	Cross-validation consistency	Empirical P value
CHRNA4 *CHRNB2*	rs2273504, rs2229959, rs2236196 rs2072661, rs2072660	0.508 0.507	0.348 0.345	0.565	6	0.007
CHRNA4 *BDNF*	rs2229959, rs1044396 rs2030324	0.552 0.535	0.024 0.094	0.552	4	0.031
CHRNA4 *NTRK2*	rs2273505 rs4075274	0.534 0.533	0.076 0.107	0.578	9	<0.0001
CHRNB2 *BDNF*	rs3811450, rs2072661 rs2030324	0.508 0.535	0.322 0.079	0.541	6	0.068
CHRNB2 *NTRK2*	rs2072661 rs993315, rs729560, rs1187272, rs1122530, rs1078947, rs4075274	0.494 0.564	0.454 0.020	0.593	6	0.002
BDNF *NTRK2*	rs2030324 rs4075274	0.534 0.533	0.090 0.106	0.578	9	0.002