Published in final edited form as:
Biol Psychiatry. 2008 December 1; 64(11): 922–929. doi:10.1016/j.biopsych.2008.04.018.

A Risk Allele for Nicotine Dependence in *CHRNA5* Is a Protective Allele for Cocaine Dependence

Richard A Grucza[1,*], **Jen C. Wang**[1], **Jerry A. Stitzel**[3], **Anthony L. Hinrichs**[1], **Scott F. Saccone**[1], **Nancy L. Saccone**[2], **Kathleen K. Bucholz**[1], **C. Robert Cloninger**[1,2], **Rosalind J. Neuman**[1], **John P. Budde**[1], **Louis Fox**[1], **Sarah Bertelsen**[1], **John Kramer**[4], **Victor Hesselbrock**[5], **Jay Tischfield**[6], **John. I. Nurnberger Jr.**[7], **Laura Almasy**[8], **Bernice Porjesz**[9], **Samuel Kuperman**[4], **Marc A. Schuckit**[11], **Howard J. Edenberg**[12], **John P. Rice**[1], **Alison M. Goate**[1,2,13], and **Laura J. Bierut**[1]

1 *Department of Psychiatry, Washington University, Saint Louis, Missouri, 63117*

2 *Department of Genetics, Washington University, Saint Louis, Missouri, 63117*

13 *Department of Neurology, Washington University, Saint Louis, Missouri, 63117*

3 *Department of Integrative Physiology, Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado, 80309*

4 *Department of Psychiatry, University of Iowa College of Medicine, Iowa City, Iowa, 52242*

5 *Department of Psychiatry, University of Connecticut Health Center, 263 Farmington Ave, Farmington, Connecticut, 06030*

6 *Department of Genetics, Rutgers, New Brunswick, New Jersey, 08901*

7 *Department of Psychiatry, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, Indiana, 46202*

8 *Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas, 78245*

9 *Department of Psychiatry, SUNY Health Science Center at Brooklyn, Brooklyn, New York, 11203*

10 *University of Iowa Hospitals, Iowa City, Iowa*

11 *Department of Psychiatry, University of California at San Diego, La Jolla, CA 92093*

12 *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, Indiana, 46202*

Abstract

Background—A non-synonymous coding polymorphism, rs16969968, of the *CHRNA5* gene which encodes the alpha-5 subunit of the nicotinic acetylcholine receptor (nAChR) has been found to be associated with nicotine dependence (20). The goal of the present study is to examine the association of this variant with cocaine dependence.

*Address for Correspondence: Richard A. Grucza, Ph.D., M.P.E., Department of Psychiatry, Washington University School of Medicine, 660 South Euclid Avenue, Box 8134, St. Louis, Missouri 63110. Phone: 314-362-7733 Fax: 314-362-5594 Rick@tci.wustl.edu.

Financial Disclosures: Authors Grucza, Stitzel, N. Saccone, Bucholz, Cloninger, Neuman, Buddde, Fox, Bertelsen, Kramer, Hesselbrock, Tischfield, Nurnberger, Almasy, Porjesz, Kuperman, Schuckit and Edenberg have no biomedical financial interests or potential conflicts of interest. Drs. Rice, Bierut, Saccone, Hinrichs, Wang, and Goate are listed as inventors on a patent (US 20070258898) held by Perlegen Sciences, Inc., covering the use of certain SNPs, including rs16969968 in diagnosing, prognosing, and treating addiction. Dr Bierut is a consultant for Pfizer, Inc.

Methods—Genetic association analysis in two, independent samples of unrelated cases and controls; 1.) 504 European-American participating in the Family Study on Cocaine Dependence (FSCD); 2.) 814 European Americans participating in the Collaborative Study on the Genetics of Alcoholsim (COGA).

Results—In the FSCD, there was a significant association between the *CHRNA5* variant and cocaine dependence (OR = 0.67 per allele, p = 0.0045, assuming an additive genetic model), but in the reverse direction compared to that previously observed for nicotine dependence. In multivariate analyses that controlled for the effects of nicotine dependence, both the protective effect for cocaine dependence and the previously documented risk effect for nicotine dependence were statistically significant. The protective effect for cocaine dependence was replicated in the COGA sample. In COGA, effect sizes for habitual smoking, a proxy phenotype for nicotine dependence, were consistent with those observed in FSCD.

Conclusion—The minor (A) allele of rs16969968, relative to the major G allele, appears to be both a risk factor for nicotine dependence and a protective factor for cocaine dependence. The biological plausibility of such a bidirectional association stems from the involvement of nAChRs with both excitatory and inhibitory modulation of dopamine-mediated reward pathways.

Keywords

Smoking; Nicotine dependence; Addiction; Substance-use disorders; Genetics; Receptors; nicotinic; Cocaine

Introduction

After marijuana, cocaine is the most frequently abused non-prescription drug in the United States and the most commonly used “hard” drug. It is estimated that about 14% of U.S. residents have used cocaine in their lifetime and over 2% have done so in the past year (1). Cocaine is highly addicting, with 25–45% of past-year users meeting DSM-IV criteria for cocaine abuse or dependence (2–4). The emergence of crack-cocaine in the late 1980s led to an increase in heavy use, and a corresponding increase in adverse health and social consequences of cocaine use, which remain at historically high levels (5–7). While the health-related sequelae of these trends are limited to drug-users, the social consequences extend to the population at large. Hence, cocaine dependence constitutes a significant public health problem, whose true costs are difficult to estimate.

Twin and family studies indicate a strong role for genetic factors in the development of drug dependence; it is estimated that 63 to 79% of the liability for the development of cocaine dependence is genetically mediated (8–12). While a number of studies show considerable overlap in genetic factors responsible for dependence on various classes of drugs, there is also evidence for drug specific effects (8,11,13). Therefore, genes encoding molecules known to interact directly with cocaine, as well as those known to be involved in reward pathways across classes of drugs, constitute logical candidates for association studies.

Neuronal nicotinic acetylcholine receptors (nAChRs) are involved in multiple regulatory pathways within the mesolimbic dopamine system(14), and could plausibly modulate the effects of multiple drugs of abuse. A number of association studies of addiction and other psychiatric phenotypes in humans have focused on genes encoding the canonical α4 and β2 nAChR subunits(15–19). More recently, a non-synonymous coding polymorphism in *CHRNA5* on chromosome 15, which encodes the α5 nAChR subunit, has been the focus of association and functional studies. In a case-control candidate-gene study of nicotine dependence among smokers, SNP rs16969968 was associated with nicotine dependence with $p = 6.4 \times 10^{-4}$ (20). This finding was replicated in an independent case-control series derived

from a large family-based study focused on alcoholism ($p = 7.7 \times 10^{-4}$, in contrasts of heavy-smoking vs. light smoking phenotypes), and the variant protein was shown to alter receptor function in transfected cell line assays (Bierut et al, under review).[†] Most recently, a SNP that is completely correlated with rs16969968 (rs11317286) was found to be associated with cigarettes per day in a European sample ($p = 2.6 \times 10^{-6}$) (21). The minor (A) allele results in a change of a highly conserved aspartic acid residue to asparagine at position 398 (D398N) of the polypeptide chain, residing in the large intracellular domain of the α5 subunit.

The aim of the current study is to investigate the potential role of rs16969968 in cocaine dependence, a disorder that is disproportionately prevalent among persons with nicotine dependence (22). Heteromeric α4β2* (where the * denotes the presence of another subunit, frequently α5) nAChRs bind nicotine with high affinity, and therefore, as a frequent component of α4β2* heteropentamers, variation in the α5 subunit may preferentially influence nicotine dependence, rather than addiction liability in general. On the other hand, nAChRs are expressed in a variety of neurons and are involved in modulating drug related reward for numerous substances, and therefore, may have a role in modulating risk for multiple types of addiction (23–25). Hence, using data from a candidate gene study of cocaine dependence in unrelated cases and controls, we sought to determine whether SNP rs16969968 in *CHRNA5* is associated with cocaine dependence. We also sought to examine the potential contribution of comorbid nicotine dependence to the hypothesized association. Finally, as this is the first study of the association between *CHRNA5* and cocaine dependence, to our knowledge, we sought to confirm our initial findings using data on cocaine dependence from an independent sample, derived from a large, family-based study of alcoholism.

Methods

Study Overview and Sample Ascertainment

The genetic arm of the Family Study of Cocaine Dependence (FSCD) included 504 cocaine-dependent individuals and 493 unrelated control subjects. Recruitment targeted equal numbers of men and women, and equal numbers of European-Americans and African-Americans. Cocaine dependent subjects were recruited from chemical dependency treatment centers in the St. Louis area. Eligibility requirements included meeting criteria for DSM-IV cocaine dependence, being 18 years of age or older, and having a full sibling within five years of age who was willing to participate in the family-arm of the study. Control subjects were recruited through driver's license records maintained by the Missouri Family Registry, housed at Washington University School of Medicine for research purposes. Controls were matched to cocaine dependent subjects based on age, ethnicity, gender, and zip code. Exclusionary criteria for controls included dependence on alcohol or drugs, including nicotine. Controls were required to have at least used alcohol in their lifetime because substance-abstinent individuals are considered phenotypically unknown; i.e., they may carry a high genetic liability for addiction, but the absence of use would preclude their progression to dependence. Blood samples were collected from each subject for DNA analysis and submitted, together with electronic phenotypic and genetic data, to the National Institute on Drug Abuse (NIDA) Center for Genetic Studies, which manages the sharing. Procedures were approved by the Washington University Human Research Protection Office and all subjects provided informed consent.

The full FSCD sample contains approximately equal numbers of European-Americans (EA) and African-Americans (AA); current analyses focus only on the EA subsample because of

[†]Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Bertelsen S, Fox L, Grucza RA, Horton WJ, Kauwe JS, Morgan SD, Saccone NL, Saccone SF, Xuei X, Breslau N, Budde J, Cloninger CR, Dick DM, Foroud T, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Kuperman S, Madden PAF, Nurnberger J Jr, Pomerleau O, Porjesz B, Reyes O, Schuckit M, Swan G, Edenberg HJ, Rice JP, Goate AM. Missense Mutation in alpha-5 Nicotinic Receptor Increases Risk for Nicotine Dependence. *American Journal of Psychiatry*. Under Review

low allelic variation among AAs for the SNP of interest (5% frequency of the A allele among AAs compared with 33% among EAs). The EA sample comprises 504 participants, including 260 cases with DSM-IV cocaine dependence and 244 controls.

Assessment

All participants completed a modified version of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) which was designed to query alcohol and other substance dependence. The SSAGA has shown good reliability in assessing substance dependence and other psychiatric disorders (26,27). Computer assisted personal interviews were administered by trained interviewers, with quality control administered by senior project personnel. Diagnostic algorithms utilized DSM-IV criteria (28).

Strategy for Genetic Analyses

The analyses focus on a single SNP (rs16969968) that corresponds to a non-synonymous coding polymorphism of the *CHRNA5* gene (amino acid D398N). This strategy was chosen over a more exploratory analysis of multiple SNPs within *CHRNA5* because association between this SNP and nicotine dependence or smoking-related phenotypes has been previously documented in three independent samples (20,21)[†]. Additional evidence for the functional role of this particular SNP include *in vitro* molecular studies, and conservation of the ancestral allele across species (20)[†]. Hence, rs16969968 is a plausible functional candidate for any associations documented in these analyses. Because these analyses test an *a priori* hypothesis, and because independent replication data is provided herein, p-values are not adjusted for multiple testing.

Genotyping

Genotyping for FSCD was conducted by the Center for Inherited Disease Research (CIDR) using a custom SNP array on an Illumina platform. Of the 1536 SNPs genotyped, 289 were dedicated to population stratification analysis, while the remaining 1247 were from selected candidate genes. Additional details of genotyping procedures are available at the CIDR website (http://www.cidr.jhmi.edu/index.html). Altogether, 1,102 samples (including EA and AA subjects) were submitted for analysis; genotyping was successful on 1,089 (98.8%). Reproducibility rate from blind-replication genotyping was 99.99%. Quality control measures included visual examination of cluster plots, call rates >99%, and Hardy-Weinberg equilibrium.

Replication Sample: The Collaborative Study on the Genetics of Alcoholism (COGA)

COGA is a multi-site family and genetic study, recruiting from six centers across the United States (29,30). Alcohol dependent probands and their family members were recruited through chemical dependency treatment programs. Institutional review boards of all participating institutions approved the study and informed consent was obtained from all participants. Diagnoses were assessed using the SSAGA. Nicotine-dependence diagnoses were not available for all subjects, hence, a “habitual smoking” phenotype was developed as a proxy. Smokers, defined as those who have smoked 100 or more cigarettes across the lifespan, were categorized as “habitual”, “light”, or “intermediate.” Habitual smoking was defined as smoking at least 20 cigarettes a day for 6 months or more, in contrast with “light smoking”, which was defined as being a smoker, but never having transitioned to smoking 10 cigarettes or more, daily (31). Smokers who did not fall into either of these categories were defined as “intermediate”, while subjects who smoked fewer than 100 cigarettes in their lifetimes were categorized as “non-smokers”. In a subset of COGA subjects who were assessed for nicotine dependence in follow-up interviews, 71% of habitual smokers met criteria for DSM-IV nicotine dependence (31).

The genetic analyses presented here utilized data from the EA subsample of the case-control phase of the COGA study, in which algorithms were derived to select the largest possible sets of unrelated alcohol-dependent cases and non-dependent controls from the broader study sample of affected families and community recruited comparison families. One subject from every set of biologically related individuals was selected for screening. Control subjects were recruited from the community-based comparison subsample or from non-biological relatives of COGA probands (e.g., relatives by marriage). Controls were required to be free of alcohol and drug abuse and dependence diagnoses, and to have no more than two symptoms of alcohol dependence; controls were not screened for nicotine dependence. In addition, they were required to have at least used alcohol in their lifetime. Cases were selected from all sets of biologically related individuals in which no person was eligible for control status, and were required to be positive for DSM-IV alcohol dependence at all assessment occasions. Among sets with multiple alcohol-dependent candidates, the proband (i.e., index case recruited through treatment) was preferentially selected.

Genotyping for the COGA was conducted using a restriction fragment length polymorphism (RFLP) assay. PCR primers were selected using the MacVector 6.5.3 program (Accelrys) to yield a 435 bp genomic fragment containing the SNP, rs16969968 (forward primer 5′-CGCCTTTGGTCCGCAAGATA-3′; reverse primer 5′-TGCTGATGGGGGAAGTGGAG-3′). Standard PCR procedures were followed to generate a product that was then digested with Taq1 restriction enzyme; fragments were separated by electrophoresis on 2% agarose gel. No deviation from Hardy-Weinberg equilibrium was detected. Call rate was 98.6%.

Population Stratification Analysis

Analyses of potential population stratification were performed using the STRUCTURE software (32). This program identifies genetically similar subpopulations through a Markov chain Monte Carlo sampling procedure using markers selected from across the genome. Genotype data for 380 unlinked marker SNPs, assessed specifically for stratification analysis, were analyzed across the 504 EA subjects in the FSCD sample using 2, 3, 4, and 5 cluster solutions. In no case was there a significant correlation between subjects' estimated cluster membership probability and case-status. Hence associations uncovered here are unlikely to be the result of confounding due to population stratification.

Genetic Association Analysis

Allelic and genotypic tests of association with cocaine-dependence status were conducted with standard χ^2 analysis. Odds ratios were estimated using logistic regression assuming an additive genetic model. Demographic covariates were not included in FSCD analyses because cases and controls were matched on gender and age, and were all European-American. COGA analyses incorporated age and sex as covariates. In order to utilize the full set genotypic data in FSCD, standard logistic regression was chosen over conditional logistic regression on matched pairs, because precise matching was available for only 226 of 260 cases (87%). Secondary analyses using conditional logistic regression on only matched pairs yielded nearly identical odds ratios and p-values.

To analyze comorbid nicotine dependence and cocaine dependence, we sought a method that could simultaneously model these disorders and their association with genotype. Hence, for multivariate analysis of comorbid phenotypes, we utilized a logistic regression method in which genotype is expressed as the left-hand side of the equation:

$$\log\left(\frac{P_1}{1-P_1}\right)=\alpha_1+\beta_1 D_1+\beta_2 D_2 \quad [1a]$$

$$\log\left(\frac{P_1+P_2}{1-P_1-P_2}\right)=\alpha_2+\beta_1 D_1+\beta_2 D_2 \quad [1b]$$

Here, P_1 and P_2 represent an individual's probability of carrying one or two copies of the risk allele, respectively, and D_1 and D_2 are diagnoses for cocaine dependence and a comorbid disorder. This model makes a "proportional odds" assumption, which, in this case, is equivalent to assuming an additive genetic model.

Results

Sample Description

Basic demographics and other characteristics of both the FSCD and COGA samples are summarized in Table 1. By design, cases and controls in the FSCD did not differ with regard to age or gender. Cases had a variety of comorbid addictions, with the most common diagnoses being alcohol and nicotine dependence. FSCD controls, by design, had no dependence on alcohol or other drugs, including nicotine. Cases and controls in COGA differed by gender and age, with males and younger subjects being over-represented among cases. COGA cases analyzed here, by design, are all affected by cocain and alcohol dependence. Comorbid drug dependence was high among COGA cases. Nicotine dependence diagnoses were not available for all COGA participants, but 67.8% of cases were positive for habitual smoking, a proxy phenotype for nicotine dependence (see Methods). COGA controls, by design, had no alcohol or other drug dependence; 20.5% were positive for habitual smoking.

Tests of Allelic and Genotypic Association in FSCD

As initial tests of association in FSCD, allele and genotype frequencies were computed in cases and controls (Table 2). Both allelic and genotypic tests for cocaine dependence were significant ($\chi^2(1) = 8.1$, $p = 0.004$; $\chi^2(2) = 12.4$, $p = 0.002$, respectively). Cases were less likely to carry the minor (A) allele than controls; the minor allele frequency (MAF) in cases was 28.7% compared with 37.1% in controls. In logistic regression analyses assuming an additive genetic model; the A allele was associated with cocaine dependence with an OR of 0.67 per allele, $p = 0.0047$ (Wald-$\chi^2(1) = 8.1$, 95% CI: 0.51, 0.88). Surprisingly, however, this association was in the *reverse* direction compared to the nicotine dependence association (20). That is, based on association results, the risk allele for nicotine dependence appears to be a protective allele for cocaine dependence. Though we excluded the African-American subsample from the primary analyses because of low minor allele frequency, and corresponding lack of statistical power, the trend in this group was consistent with that observed in the EA subsample (N = 492, OR = 0.75, 95% CI: 0.44, 1.25, $p = 0.25$).

To test whether the nicotine dependence association was evident in FSCD, genotypic and allelic tests were repeated with cocaine-dependent cases divided into those with and without nicotine dependence (Table 2). Cocaine dependent cases with nicotine dependence had *higher* minor allele frequencies (MAF = 31.1%), than those without nicotine dependence (21.1%), while both sub-sets of cases had *lower* MAF than controls (37.1%; $\chi^2(2) = 12.5$, $p = 0.002$). This pattern is consistent with the minor (A) allele being both a risk factor for nicotine dependence and a protective factor for cocaine dependence. Genotype frequencies exhibited similar patterns ($\chi^2(4) = 17.0$, $p = 0.002$).

Multivariate Analyses

In order to model both potential effects of the rs16969968 polymorphism; i.e., the putative protective effect for cocaine dependence, and the risk effect for nicotine dependence (20), a multivariate cumulative logit model (ordinal logistic regression; equation 1) was used to analyze allele count as a function of both cocaine and nicotine dependence. This approach

allows us to estimate the magnitude of the association between allele count and each phenotype, while controlling for any association between allele count and a covariate phenotype (i.e., all phenotypes are on the same side of the equation). This model assumes additive genetic effects with the further assumption of additivity among phenotypes. Because non-smokers [see *Methods* for definition] cannot be nicotine dependent, they were treated as a separate category from either nicotine dependent smokers or non-dependent smokers. Results are shown in Table 3. Inclusion of nicotine dependence in the model confirmed that nicotine dependent smokers were significantly more likely to carry the A allele than non-dependent smokers (OR = 2.14, p = 0.017), while the protective effect for cocaine dependence remained significant (OR = 0.41, p = 0.0045).

Replication in the COGA Data Set

We sought to confirm these results using COGA data. Analyses compared 290 alcohol-dependent COGA cases with comorbid cocaine dependence with 524 controls without alcohol or any drug dependence. Allelic and genotypic tests are summarized in Table 4. As with the FSCD analyses, cocaine-dependent cases had a lower MAF than controls (30.0% vs. 36.4%); both genotypic and allelic association tests were significant ($\chi^2(1) = 6.7$, p = 0.0096; $\chi^2(2) = 10.0$, p = 0.007, respectively). Logistic regression analyses assuming an additive genetic model were conducted, with age and sex included as covariates. Again, the minor (A) allele of rs16969968 was protective against cocaine dependence; the effect size was similar to that observed in FSCD: OR = 0.67 per allele (Wald-$\chi^2(1) = 8.9$, p = 0.0026, 95% CI: 0.52, 0.87). COGA subjects with alcohol dependence but not cocaine dependence (N = 530, these subjects were not included in the primary analyses), did *not* differ significantly from controls (MAF = 36.4% vs. 33.8%; $\chi^2 = 1.4$, p = 0.23). Using the primary model, adjusting for age and gender, this corresponds to an OR of 0.88 (95% CI: 0.73, 1.08; p = 0.22). Hence, the stronger association appears to be with cocaine dependence, but a modest association with alcohol dependence cannot be ruled out.

Genotypic and allelic tests were repeated with cocaine/alcohol dependent cases further subdivided by habitual smoking phenotype (proxy for nicotine dependence), and habitual smokers removed from controls (n = 109; Table 4). This analysis, parallel to that presented in the bottom of Table 2, compares subjects with cocaine dependence and habitual smoking, those with cocaine dependence but not habitual smoking, and controls with neither condition. As in the FSCD analyses, cocaine/alcohol-dependent cases with habitual smoking had higher MAF (31.3%) than cases without habitual smoking (27.2%), while both had *lower* MAF than controls (35.9%; $\chi^2(2) = 6.2$, p = 0.04). Genotype frequencies exhibited similar patterns; hence, the ordering of phenotypes with regard to allele frequencies was identical to that seen in the FSCD data set ($\chi^2(4) = 10.4$, p = 0.03).

Multivariate regression analyses using the cumulative logit model (Equation 1), parallel to those conducted for FSCD (Table 3), were applied to COGA data. Case status (alcohol and cocaine dependence) along with habitual smoking as a covariate, were used to predict allele count, in order to estimate odds ratios for both habitual smoking and case-status. Results are shown in Table 5. After including habitual smoking, the OR associated with case-status remained significant (OR = 0.52, Wald-$\chi^2 = 11.0$, p = 0.0009). The OR for habitual smoking (OR = 1.37, p = 0.15), though not significant, was in the same direction that for nicotine dependence in the parallel FSCD analyses (Table 3). Hence, the protective effect for cocaine dependence uncovered in FSCD was reproduced in COGA, while the odds ratio for habitual smoking, as proxy for nicotine dependence, was consistent with effects in FSCD and with results reported elsewhere (20).

Discussion

In this work we have demonstrated an association between the rs16969968 in *CHRNA5* and cocaine dependence in two independent samples of European descent, one ascertained for cocaine dependence and the other ascertained for alcohol dependence. Most interestingly, this same variant -- which appears to be a protective factor for cocaine dependence, has previously been shown to be a risk factor for nicotine dependence, a finding also supported by the present analyses (multivariate analyses in FSCD, Table 3; see also case-only analysis of nicotine-dependence, Table 2). Specifically, nicotine dependent smokers of European descent have been shown to carry the minor (A) allele, corresponding to amino acid change D398N, with higher frequencies than non-nicotine dependent smokers (20); see also: Bierut et al under review[†]). In the FSCD sample, cocaine-dependent cases had lower frequencies of the minor allele than controls, and the protective effect for cocaine dependence appeared even stronger after controlling for the putative counterbalancing effect of nicotine dependence. These results were replicated in an independent sample ascertained for alcohol dependence (COGA); the protective effect of the minor allele of rs16969968 for cocaine dependence was significant, while the risk effect for habitual smoking (a proxy measure for nicotine dependence), though not significant, was consistent with the effects observed in FSCD. Hence, the association between rs16969968 and cocaine and dependence is clearly in the reverse direction to that between the same variant and nicotine dependence. This finding was reversed from the logical *a priori* hypothesis, that the *minor allele of* rs16969968 would be a risk factor for both nicotine and cocaine dependence, however the fact that results were consistent across two independent samples increases our confidence in its robustness.

While surprising, a dual role for the *CHRNA5* gene in modulating susceptibility to addiction is plausible from a biological perspective. The reinforcing properties of nicotine are not completely understood, but are likely to involve both direct and indirect stimulation of dopamine release in the mesolimbic dopaminergic system, which mediates the addictive properties of drugs of abuse (33,34). In this system, the α5 nAChR subunit is found as part of hetero-pentameric nAChRs (predominantly α4β2α5) on both excitatory dopaminergic and inhibitory GABAergic neurons (35,24). Relative to the major allele (G), the minor allele (A), which corresponds to an asparagine at amino acid 398, rather than an aspartic acid, results in reduced receptor function and is associated with increased risk for nicotine addiction.[†] Therefore, this polymorphism may result in reduced nicotine-stimulated GABA transmission, corresponding to disinhibited dopamine signaling. This effect may outweigh reduction in nicotine-stimulated dopamine transmission resulting from the polymorphism, with the net effect being enhanced dopamine-response and greater addiction liability for nicotine. This is consistent with the observation that enhancing GABA-ergic function results in decreased nicotine-stimulated dopamine release and reduced nicotine self-administration in rodents (36–39).

In contrast to nicotine, cocaine directly increases mesolimbic dopaminergic activity by inhibiting re-uptake though interaction with the dopamine transporter and other proteins.(40), (41) Therefore, the influence of the rs16969968 on cocaine addiction liability may be more restricted to α4β2α5 nAChRs on dopaminergic cells. In this case, reduced dopaminergic function due to diminished nAChR function would be protective against addiction. This is consistent with the observation that the administration of nicotinic antagonists results in reduced sensitivity to the reinforcing effects of cocaine in animal models (42–46,25,47).

While the above interpretation is speculative, it serves to demonstrate the biological plausibility of the genetic associations uncovered here. Although the involvement of nAChRs in mediating the rewarding effects of addictive drugs is complex, their involvement with both excitatory and inhibitory neurons that impact dopamine transmission is well established (24,14,47).

Therefore, the same genetic variant may lead to different pharmacogenetic responses to cocaine and nicotine, as a result of different mechanisms of action of these drugs in the reward system, which in turn results in different addiction liabilities.

Limitations

Most cases in both samples have a variety of comorbid addictions, with the most common being alcohol dependence. Nearly 80% of the cases in FSCD were affected by alcohol dependence, while all of the COGA cases, by design, are affected by alcohol dependence in addition to cocaine dependence. Hence, the association with cocaine dependence may be driven by comorbid dependences, or may be a non-specific association with multiple addictions, but the association with nicotine dependence is clearly in the reverse direction compared to the association uncovered using cocaine dependence as the primary phenotype. An additional limitation was that only additive genetic models were tested in regression analyses; this was done to limit the number of tests conducted when simultaneously modeling both cocaine and nicotine phenotypes.

Summary

The current study provides evidence of a protective association between cocaine dependence and the minor allele of rs16969968 in both the FSCD study and an independent replication sample (COGA). Evidence that the same variant is a risk factor for nicotine dependence includes association in three independent samples, functional data in transfected cells, and association among cocaine-dependent cases in the FSCD sample (presented here) (20,21)[†]. To our knowledge, no other studies have provided strong evidence of bidirectional association for a single genetic variant with two different addictive disorders. These findings support a "common and specific" effects model for liability to addiction, which invokes drug-specific effects in addition to common genetic contributions to genetic liability for addiction (8,11, 48,31), over a general-liability model (12,49). While these results demonstrate that a single molecule is associated with different addictive disorders, the variant that protects against one is a risk factor for the other, and *vice-versa.* As new pharmacological treatments for addictions emerge, it will be essential to consider such phenomena as potential contributors to unintended side effects.

Acknowledgements

The FSCD has been supported by NIH grants R01 DA19963 and R01 DA013423. Analyses partially supported by K01 DA16618 (RAG). Genotyping services for FSCD were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C. The Collaborative Study on the Genetics of Alcoholism (COGA), Co-Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes nine different centers where data collection, analysis, and storage take place. The nine sites and Principal Investigators and Co Investigators are: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenberg, J. Nurnberger Jr., P.M. Conneally, T. Foroud); University of Iowa (S. Kuperman, R. Crowe); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, A. Goate, J. Rice); University of California at San Diego (M. Schuckit); Howard University (R. Taylor); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy). Zhaoxia Ren serves as the NIAAA Staff Collaborator. This national collaborative study is supported by the NIH Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA).

In memory of Henri Begleiter and Theodore Reich, Principal and Co-Principal Investigators of COGA since its inception; we are indebted to their leadership in the establishment and nurturing of COGA, and acknowledge with great admiration their seminal scientific contributions to the field.

References

1. Substance Abuse and Mental Health Services Administration. Results from the 2005 National Survey on Drug Use and Health: National Findings. Rockville, MD: Office of Applied Studies, NHSDA Series H-30, DHHS Publication No. SMA 06–4194; 2006.

2. Substance Abuse and Mental Health Services Administration. Results from the 2002 National Survey on Drug Use and Health: National Findings. Rockville, MD: Office of Applied Studies, NHSDA Series H-22, DHHS Publication No. SMA 03–3836; 2003.

3. Compton WM, Conway KP, Stinson FS, Colliver JD, Grant BF. Prevalence, correlates, and comorbidity of DSM-IV antisocial personality syndromes and alcohol and specific drug use disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. J Clin Psychiatry 2005;66:677–685. [PubMed: 15960559]

4. Grucza RA, Abbacchi AM, Przybeck TR, Gfroerer JC. Discrepancies in estimates of prevalence and correlates of substance use and disorders between two national surveys. Addiction 2007;102:623–629. [PubMed: 17309538]

5. Everingham, S.; Rydell, C. Controlling Cocaine: Supply vs. Demand Programs. RAND Corporation; Santa Monica, CA: 1994.

6. Everingham, S.; Rydell, C. Modeling the Demand for Cocaine. RAND Corporation; Santa Monica, CA: 1994.

7. Lloyd, J. ONDCP Drug Policy Information Clearinhouse Fact Sheet. Office of National Drug Control Policy; Rockville, MD: 2002.

8. Bierut LJ, Dinwiddie SH, Begleiter H, Crowe RR, Hesselbrock V, Nurnberger JI Jr, et al. Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. Arch Gen Psychiatry 1998;55:982–988. [PubMed: 9819066]

9. Kendler KS, Prescott CA. Cannabis use, abuse, and dependence in a population-based sample of female twins. Am J Psychiatry 1998;155:1016–1022. [PubMed: 9699687]

10. Kendler KS, Prescott CA. Cocaine use, abuse and dependence in a population-based sample of female twins. Br J Psychiatry 1998;173:345–350. [PubMed: 9926041]

11. Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, et al. Familial transmission of substance use disorders. Arch Gen Psychiatry 1998;55:973–979. [PubMed: 9819065]

12. Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. Am J Psychiatry 2003;160:687–695. [PubMed: 12668357]

13. Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, et al. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. Arch Gen Psychiatry 1998;55:967–972. [PubMed: 9819064]

14. Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 2007;47:699–729. [PubMed: 17009926]

15. Kim SA, Kim JW, Song JY, Park S, Lee HJ, Chung JH. Association of polymorphisms in nicotinic acetylcholine receptor alpha 4 subunit gene (*CHRNA4*), mu-opioid receptor gene (OPRM1), and ethanol-metabolizing enzyme genes with alcoholism in Korean patients. Alcohol 2004;34:115–120. [PubMed: 15902904]

16. 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]

17. De Luca V, Voineskos S, Wong G, Kennedy JL. Genetic interaction between alpha4 and beta2 subunits of high affinity nicotinic receptor: analysis in schizophrenia. Exp Brain Res 2006;174:292–296. [PubMed: 16636791]

18. Ehringer MA, Clegg HV, Collins AC, Corley RP, Crowley T, Hewitt JK, et al. Association of the neuronal nicotinic receptor beta2 subunit gene (CHRNB2) with subjective responses to alcohol and nicotine. Am J Med Genet B Neuropsychiatr Genet 2007;144:596–604. [PubMed: 17226798]

19. Lee J, Laurin N, Crosbie J, Ickowicz A, Pathare T, Malone M, et al. Association study of the nicotinic acetylcholine receptor alpha4 subunit gene, *CHRNA4*, in attention-deficit hyperactivity disorder. Genes Brain Behav. 2007

20. Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum Mol Genet 2007;16:36–49. [PubMed: 17135278]

21. Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H, et al. alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. Mol Psychiatry. 2008
23. Breslau N. Psychiatric comorbidity of smoking and nicotine dependence. Behav Genet 1995;25:95–101. [PubMed: 7733862]
24. Davis TJ, de Fiebre CM. Alcohol's actions on neuronal nicotinic acetylcholine receptors. Alcohol Res Health 2006;29:179–185. [PubMed: 17373406]
25. Hansen ST, Mark GP. The nicotinic acetylcholine receptor antagonist mecamylamine prevents escalation of cocaine self-administration in rats with extended daily access. Psychopharmacology (Berl) 2007;194:53–61. [PubMed: 17530475]
26. Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI Jr, et al. A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. J Stud Alcohol 1994;55:149–158. [PubMed: 8189735]
27. Hesselbrock M, Easton C, Bucholz KK, Schuckit M, Hesselbrock V. A validity study of the SSAGA--a comparison with the SCAN. Addiction 1999;94:1361–1370. [PubMed: 10615721]
28. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4. Washington, D.C: American Psychiatric Association; 2000. Fourth Edition Text Revision
29. Bierut LJ, Schuckit MA, Hesselbrock V, Reich T. Co-occurring risk factors for alcohol dependence and habitual smoking: results from the collaborative study on the genetics of alcoholism. Alcohol Res Health 2000;24:233–241. [PubMed: 15986718]
30. Grucza RA, Bierut LJ. Update on COGA findings: Co-occurring risk factors for alcohol dependence and habitual smoking. Alcohol Res Health. 2006In Press
31. Bierut LJ, Rice JP, Goate A, Hinrichs AL, Saccone NL, Foroud T, et al. A genomic scan for habitual smoking in families of alcoholics: common and specific genetic factors in substance dependence. Am J Med Genet A 2004;124:19–27. [PubMed: 14679582]
32. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000;155:945–959. [PubMed: 10835412]
33. Mansvelder HD, McGehee DS. Cellular and synaptic mechanisms of nicotine addiction. J Neurobiol 2002;53:606–617. [PubMed: 12436424]
34. Di Matteo V, Pierucci M, Di Giovanni G, Benigno A, Esposito E. The neurobiological bases for the pharmacotherapy of nicotine addiction. Curr Pharm Des 2007;13:1269–1284. [PubMed: 17504235]
35. Gotti C, Riganti L, Vailati S, Clementi F. Brain neuronal nicotinic receptors as new targets for drug discovery. Curr Pharm Des 2006;12:407–428. [PubMed: 16472136]
36. Dewey SL, Brodie JD, Gerasimov M, Horan B, Gardner EL, Ashby CR Jr. A pharmacologic strategy for the treatment of nicotine addiction. Synapse 1999;31:76–86. [PubMed: 10025686]
37. Schiffer WK, Gerasimov MR, Bermel RA, Brodie JD, Dewey SL. Stereoselective inhibition of dopaminergic activity by gamma vinyl-GABA following a nicotine or cocaine challenge: a PET/microdialysis study. Life Sci 2000;66:PL169–173. [PubMed: 10737423]
38. Corrigall WA, Coen KM, Zhang J, Adamson KL. GABA mechanisms in the pedunculopontine tegmental nucleus influence particular aspects of nicotine self-administration selectively in the rat. Psychopharmacology (Berl) 2001;158:190–197. [PubMed: 11702093]
39. Ashby CR Jr, Paul M, Gardner EL, Gerasimov MR, Dewey SL, Lennon IC, Taylor SJ. Systemic administration of 1R,4S-4-amino-cyclopent-2-ene-carboxylic acid, a reversible inhibitor of GABA transaminase, blocks expression of conditioned place preference to cocaine and nicotine in rats. Synapse 2002;44:61–63. [PubMed: 11891877]
40. Nestler EJ. Is there a common molecular pathway for addiction? Nat Neurosci 2005;8:1445–1449. [PubMed: 16251986]
41. Hummel M, Unterwald EM. D1 dopamine receptor: a putative neurochemical and behavioral link to cocaine action. J Cell Physiol 2002;191:17–27. [PubMed: 11920678]
42. Reid MS, Mickalian JD, Delucchi KL, Berger SP. A nicotine antagonist, mecamylamine, reduces cue-induced cocaine craving in cocaine-dependent subjects. Neuropsychopharmacology 1999;20:297–307. [PubMed: 10063490]
43. Levin ED, Mead T, Rezvani AH, Rose JE, Gallivan C, Gross R. The nicotinic antagonist mecamylamine preferentially inhibits cocaine vs. food self-administration in rats. Physiol Behav 2000;71:565–570. [PubMed: 11239676]

44. Zachariou V, Caldarone BJ, Weathers-Lowin A, George TP, Elsworth JD, Roth RH, et al. Nicotine receptor inactivation decreases sensitivity to cocaine. Neuropsychopharmacology 2001;24:576–589. [PubMed: 11282258]
45. Schoffelmeer AN, De Vries TJ, Wardeh G, van de Ven HW, Vanderschuren LJ. Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. J Neurosci 2002;22:3269–3276. [PubMed: 11943828]
46. Champtiaux N, Kalivas PW, Bardo MT. Contribution of dihydro-beta-erythroidine sensitive nicotinic acetylcholine receptors in the ventral tegmental area to cocaine-induced behavioral sensitization in rats. Behav Brain Res 2006;168:120–126. [PubMed: 16313978]
47. Zanetti L, Picciotto MR, Zoli M. Differential effects of nicotinic antagonists perfused into the nucleus accumbens or the ventral tegmental area on cocaine-induced dopamine release in the nucleus accumbens of mice. Psychopharmacology (Berl) 2007;190:189–199. [PubMed: 17061109]
48. Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Arch Gen Psychiatry 2003;60:929–937. [PubMed: 12963675]
49. Hicks BM, Krueger RF, Iacono WG, McGue M, Patrick CJ. Family transmission and heritability of externalizing disorders: a twin-family study. Arch Gen Psychiatry 2004;61:922–928. [PubMed: 15351771]

Table 1

Sample Descriptions and Demographic Characteristics

	FSCD (N = 504)		COGA (N = 814)	
	Cases N (Col %)	**Controls N (Col %)**	**Cases N (Row %)**	**Controls N (Row %)**
Men	128 (49.2%)	115 (47.1%)	210 (72.2%)	151 (28.4%)
Women	132 (50.8%)	129 (52.9%)	80 (27.8%)	373 (71.6%)
		$\chi^2(1) = 0.22/p = 0.63$		$\chi^2(1) = 144/p<0.001$
Age (Mean)	33.0 (SE = 0.5)	34.1 (SE = 0.6)	38.1 (SE = 0.4)	46.5 (SE = 0.6)
		$\Delta = 1.1$ years, $t = 1.4$, $p = 0.16$		$\Delta = 8.4$ years, $t = 10.5$, $p<0.001$
Comorbid Substance Dependence (Prevalence, %)				
Nicotine Dependence	196 (75.4%)	0 (0%)*	n/a	n/a
Habitual Smoking	n/a	n/a	200 (68.9%)	109 (20.8%)
Alcohol Dependence	207 (79.6%)	0 (0%)*	290 (100%)*	0 (0%)*
Marijuana Dependence	156 (60.0%)	0 (0%)*	213 (73.5%)	0 (0%)*
Other Drug Dependence	170 (65.4%)	0 (0%)*	165 (56.9%)	0 (0%)*

*Values determined by inclusion and exclusion criteria.

Table 2

Association Between rs16969968 in *CHRNA5* and Cocaine Dependence in the FSCD Sample, and the Role of Comorbid Nicotine Dependence.

	Allele Distribution (N = 1008)		**Genotype Distribution (N = 504)**		
	G	**A**	**GG**	**GA**	**AA**
	N (%)[1]	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Cases (N = 260)	371 (71.4)	149 (28.6)	135 (51.9)	101 (38.9)	24 (9.2)
Controls (N = 244)	307 (62.9)	181 (37.1)	89 (36.5)	129 (52.9)	26 (10.6)
Total	*678 (67.3)*	*330 (32.7)*	*224 (44.2)*	*230 (45.9)*	*50 (9.9)*
		$\chi^2(1) = 8.1/p = 0.004$		$\chi^2(2) = 12.4/p = 0.002$	
Nicotine-Dependent Cases (N = 196)	270 (68.9)	122 (31.1)	95 (48.5)	80 (40.8)	21 (10.7)
Non-Nicotine Dependent Cases (N = 64)	101 (78.9)	27 (21.1)	40 (62.5)	21 (32.8)	3 (4.7)
Controls (N = 244)	307 (62.9)	181 (37.1)	89 (36.5)	129 (52.9)	26 (10.7)
Total	*678 (67.3)*	*330 (32.7)*	*224 (44.2)*	*230 (45.9)*	*50 (9.9)*
	$\chi^2(2) = 12.5/p = 0.002$			$\chi^2(4) = 17.0/p = 0.002$	

[1] Row Percentage

Table 3

Multivariate Allelic Association Between rs16969968 in *CHRNA5*, Cocaine Dependence and Nicotine Dependence

	N	*OR* (95% CI)	*P*
Cocaine Dependence (Case)	260	0.41 (0.22, 0.76)	0.0045
No Cocaine Dependence (Control)	244	1.00	
Nicotine Dependence	196	2.14 (1.15, 4.01)	0.0171
Non-Smoker	237	1.37 (0.77, 2.44)	0.28
Non-Nicotine Dependent Smoker	71	1.00	

Table 4

Association Between rs16969968 in *CHRNA5* and Cocaine Dependence in the COGA Sample, and the Role of Comorbid Habitual Smoking.

	Allele Distribution (N = 1628)		Genotype Distribution (N = 814)		
	G N (%)[1]	**A N (%)**	**GG N (%)**	**GA N (%)**	**AA N (%)**
Cases (N = 290)	406 (63.7)	174 (36.4)	206 (50.7)	112 (38.6)	31 (10.7)
Controls (N = 524)	667 (70.0)	381 (30.0)	147 (39.3)	255 (48.7)	63 (12.0)
Total	*1073 (65.9)*	*555 (34.1)*	*353 (43.4)*	*367 (45.1)*	*94 (11.6)*
	$\chi^2(1) = 6.7/p = 0.0096$			$\chi^2(2) = 10.0/p = 0.007$	
Habitual-Smoking Cases (N = 200)	275 (68.8)	125 (31.3)	97 (48.5)	81 (40.5)	22 (11.0)
Non-Habitual Smoking Cases (N = 90)	131 (72.8)	49 (27.2)	50 (55.6)	31 (34.4)	9 (10.0)
Controls, excluding Habitual Smokers (N = 415)	532 (64.1)	298 (35.9)	164 (39.5)	204 (49.2)	47 (11.3)
Total	*938 (66.5)*	*472 (33.5)*	*311 (44.1)*	*316 (44.8)*	*78 (11.1)*
		$\chi^2(2) = 6.2/p = 0.04$		$\chi^2(4) = 10.4/p = 0.03$	

[1] Row Percentage

Table 5

Multivariate Allelic Association Between rs16969968 in *CHRNA5*, Alcohol Dependence and Habitual Smoking in the COGA sample.

	N	*OR* (95% CI)	*P*
Alcohol Dependence + Cocaine Dependence (Case)	290	0.52 (0.36, 0.77)	0.0009
No Alcohol Dependence or Cocaine Dependence (Control)	524	1.00	
Habitual Smoker	309	1.37 (0.89, 2.12)	0.15
Moderate-Smoker	58	1.40 (0.76, 2.59)	0.28
Non-Smoker	338	1.14 (0.74, 1.75)	0.55
Light Smoker	109	1.00	