

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

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Background: A nonsynonymous coding polymorphism, rs16969968, of the *CHRNA5* gene that encodes the alpha-5 subunit of the nicotinic acetylcholine receptor (nAChR) has been found to be associated with nicotine dependence. The goal of this study was to examine the association of this variant with cocaine dependence.

Methods: Genetic association analysis was performed in two independent samples of unrelated case and control subjects: 1) 504 European Americans participating in the Family Study on Cocaine Dependence (FSCD) and 2) 814 European Americans participating in the Collaborative Study on the Genetics of Alcoholism (COGA).

Results: In the FSCD, there was a significant association between the *CHRNA5* variant and cocaine dependence (odds ratio = .67 per allele, $p = .0045$, assuming an additive genetic model), but in the reverse direction compared with 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.

Conclusions: 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.

Key Words: Addiction, cocaine, genetics, nicotine dependence, nicotinic receptors, smoking, substance use disorders

After marijuana, cocaine is the most frequently abused nonprescription 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 more than 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). Although 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, the true costs of which 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%–79% of the liability for the development of cocaine dependence is genetically mediated (8–12). Although 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 $\alpha 4$ and $\beta 2$ nAChR subunits (15–19). More recently, a nonsynonymous coding polymorphism in *CHRNA5* on chromosome 15, which encodes the $\alpha 5$ nAChR subunit, has been the focus of association and functional studies. In a case-control candidate-gene study of nicotine dependence among smokers, single nucleotide polymorphism (SNP) rs16969968 was associated with nicotine dependence with $p = 6.4 \times 10^{-4}$ (20). This finding was replicated in an indepen-

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dent 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.* [21]). Most recently, an SNP that is completely correlated with rs16969968 (rs11317286) was found to be associated with cigarettes per day in a European sample ($p = 2.6 \times 10^{-6}$) (22). 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 $\alpha 5$ subunit.

The aim of this study was to investigate the potential role of rs16969968 in cocaine dependence, a disorder that is disproportionately prevalent among persons with nicotine dependence (23). Heteromeric $\alpha 4\beta 2^*$ (where the asterisk denotes the presence of another subunit, frequently $\alpha 5$) nAChRs bind nicotine with high affinity, and therefore, as a frequent component of $\alpha 4\beta 2^*$ heteropentamers, variation in the $\alpha 5$ 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 (24–26). Hence using data from a candidate gene study of cocaine dependence in unrelated case and control subjects, 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 and Materials

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, Missouri, area. Eligibility requirements included meeting criteria for DSM-IV cocaine dependence, being aged 18 years or older, and having a full sibling within 5 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. Control subjects were matched to cocaine-dependent subjects based on age, ethnicity, gender, and zip code. Exclusionary criteria for control subjects included dependence on alcohol or drugs, including nicotine. Control subjects were required to have at least used alcohol in their lifetime because substance-abstinent individuals are considered phenotypically unknown; that is, 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 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 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 case subjects with DSM-IV cocaine dependence and 244 control subjects.

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 (27,28). Computer-assisted personal interviews were administered by trained interviewers, with quality control administered by senior project personnel. Diagnostic algorithms used DSM-IV criteria (29).

Strategy for Genetic Analyses

The analyses focus on a single SNP (rs16969968) that corresponds to a nonsynonymous 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 (20–22). Additional evidence for the functional role of this particular SNP include *in vitro* molecular studies and conservation of the ancestral allele across species (20,21). 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 (Illumina, Inc., San Diego, California). Of the 1536 SNPs genotyped, 289 were dedicated to population stratification analysis, and the remaining 1247 were from selected candidate genes. Additional details of genotyping procedures are available at the CIDR Web site (<http://www.cidr.jhmi.edu/index.html>). Altogether, 1102 samples (including EA and AA subjects) were submitted for analysis; genotyping was successful on 1089 (98.8%). Reproducibility rate from blind-replication genotyping was 99.99%. Quality control measures included visual examination of cluster plots, call rates over 99%, and Hardy-Weinberg equilibrium.

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

COGA is a multisite family and genetic study, recruiting from six centers across the United States (30,31). 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 life span, 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 (32). Smokers who did not fall into either of these categories were defined as “intermediate,” and subjects who smoked fewer than 100 cigarettes in their lifetimes were categorized as “nonsmokers.” 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 (32).

The genetic analyses presented here used 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 case subjects and non-dependent control subjects 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 nonbiological relatives of COGA probands (e.g., relatives by marriage). Control subjects were required to be free of alcohol and drug abuse and dependence diagnoses and to have no more than two symptoms of alcohol dependence; control subjects were not screened for nicotine dependence. In addition, they were required to have at least used alcohol in their lifetime. Case subjects 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. Polymerase chain reaction (PCR) primers were selected using the MacVector 6.5.3 program (Accelrys, Inc., San Diego, California) 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 TaqI 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 (33). 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 chi-square analysis. Odds ratios (ORs) were estimated using logistic regression

assuming an additive genetic model. Demographic covariates were not included in FSCD analyses because case and control subjects were matched on sex and age and were all European American. The COGA analyses incorporated age and sex as covariates. 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 case subjects (87%). Secondary analyses using conditional logistic regression on only matched pairs yielded nearly identical ORs 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, case and control subjects in the FSCD did not differ with regard to age or sex. Case subjects had a variety of comorbid addictions, with the most common diagnoses being alcohol and nicotine dependence. FSCD control subjects, by design, had no dependence on alcohol or other drugs, including nicotine. Case and control subjects in COGA differed by sex and age, with male and younger subjects being overrepresented among case subjects. COGA case subjects analyzed here, by design, are all affected by cocaine and alcohol dependence. Comorbid drug dependence was high among COGA case subjects. Nicotine dependence diagnoses were not available for all COGA participants, but 67.8% of case subjects were positive for habitual smoking, a proxy phenotype for nicotine dependence (see Methods and Materials). COGA control subjects, 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, allelic and genotype frequencies were computed in case and control subjects (Table 2). Both allelic and genotypic tests for cocaine dependence were significant [$\chi^2(1) = 8.1, p = .004$; $\chi^2(2) = 12.4, p = .002$, respectively]. Case subjects were less likely to carry the minor (A) allele than control subjects; the minor allele frequency (MAF) in case subjects was 28.7% compared with 37.1% in control subjects. In logistic regression analyses assuming an additive genetic model, the A allele was associated with cocaine dependence with an OR of .67 per allele, $p = .0047$ [Wald- $\chi^2(1) = 8.1, 95\%$ confidence interval (CI): .51–.88]. Surprisingly, however, this

Table 1. Sample Descriptions and Demographic Characteristics

	FSCD (n = 504)		COGA (n = 814)	
	Case Subjects	Control Subjects	Case Subjects	Control Subjects
	n (% of Col)	n (% of Col)	n (% of Col)	n (% of Col)
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)
Age (Mean)	33.0 (SE = .5)	34.1 (SE = .6)	38.1 (SE = .4)	46.5 (SE = .6)
	$\chi^2(1) = .22/p = .63$ $\Delta = 1.1$ years, $t = 1.4$, $p = 0.16$		$\chi^2(1) = 144/p < .001$ $\Delta = 8.4$ years, $t = 10.5$, $p < .001$	
	Comorbid Substance Dependence (Prevalence, %)			
Nicotine Dependence	196 (75.4)	0 (0) ^a	n/a	n/a
Habitual Smoking	n/a	n/a	200 (68.9)	109 (20.8)
Alcohol Dependence	207 (79.6)	0 (0) ^a	290 (100) ^a	0 (0) ^a
Marijuana Dependence	156 (60.0)	0 (0) ^a	213 (73.5)	0 (0) ^a
Other Drug Dependence	170 (65.4)	0 (0) ^a	165 (56.9)	0 (0) ^a

COGA, Collaborative Study on the Genetics of Alcoholism; Col, column; FSCD, Family Study on Cocaine Dependence.

^aValues determined by inclusion and exclusion criteria.

association was in the *reverse* direction compared with the nicotine-dependence association (20). That is, on the basis of association results, the risk allele for nicotine dependence appears to be a protective allele for cocaine dependence. Although we excluded the AA 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 = .75, 95% CI: .44–1.25, $p = .25$).

To test whether the nicotine-dependence association was evident in FSCD, genotypic and allelic tests were repeated with cocaine-dependent case subjects divided into those with and without nicotine dependence (Table 2). Cocaine dependent case subjects with nicotine dependence had *higher* minor allele frequencies (MAF = 31.1%) than those without nicotine dependence (21.1%), whereas both subsets of case subjects had *lower* MAF than control subjects [37.1%; $\chi^2(2) = 12.5$, $p = .002$]. 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 [$\chi^2(4) = 17.0$, $p = .002$].

Multivariate Analyses

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; Equations 1a and 1b) was used to analyze allele count as a function of both cocaine and nicotine dependence. This approach allowed 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 nonsmokers (see Methods and Materials for definition) cannot be nicotine dependent, they were treated as a separate category from either nicotine-dependent smokers or nondependent 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 nondependent smokers (OR = 2.14, $p = .017$), whereas the protective effect for cocaine dependence remained significant (OR = .41, $p = .0045$).

Replication in the COGA Data Set

We sought to confirm these results using COGA data. Analyses compared 290 alcohol-dependent COGA case subjects with comorbid cocaine dependence with 524 control subjects without alcohol or any drug dependence. Allelic and genotypic tests are summarized in Table 4. As with the FSCD analyses, cocaine-

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	(% of Row)	N	(% of Row)	N	(% of Row)	N	(% of Row)	N	(% of Row)
Case Subjects (n = 260)	371	(71.4)	149	(28.6)	135	(51.9)	101	(38.9)	24	(9.2)
Control Subjects (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)
	$\chi^2(1) = 8.1/p = .004$				$\chi^2(2) = 12.4/p = .002$					
Nicotine-Dependent Case Subjects (n = 196)	270	(68.9)	122	(31.1)	95	(48.5)	80	(40.8)	21	(10.7)
Non-Nicotine-Dependent Case Subjects (N = 64)	101	(78.9)	27	(21.1)	40	(62.5)	21	(32.8)	3	(4.7)
Control Subjects (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)
	$\chi^2(2) = 12.5/p = .002$				$\chi^2(4) = 17.0/p = .002$					

FSCD, Family Study on Cocaine Dependence.

Table 3. Multivariate Allelic Association Among rs16969968 in *CHRNA5*, Cocaine Dependence, and Nicotine Dependence

	<i>n</i>	OR	(95% CI)	<i>p</i>
Cocaine Dependence (Case)	260	.41	(.22, .76)	.0045
No Cocaine Dependence (Control)	244	1.00		
Nicotine Dependence	196	2.14	(1.15, 4.01)	.0171
Nonsmoker	237	1.37	(.77, 2.44)	.28
Non-Nicotine-Dependent Smoker	71	1.00		

CI, confidence interval; OR, odds ratio.

dependent case subjects had a lower MAF than control subjects (30.0% vs. 36.4%); both genotypic and allelic association tests were significant [$\chi^2(1) = 6.7, p = .0096$; $\chi^2(2) = 10.0, p = .007$, 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 = .67 per allele [Wald- $\chi^2(1) = 8.9, p = .0026$, 95% CI: .52-.87]. The 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 control subjects (MAF = 36.4% vs. 33.8%; $\chi^2 = 1.4, p = .23$). Using the primary model, adjusting for age and sex, this corresponds to an OR of .88 (95% CI: .73-1.08; $p = .22$). 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 case subjects further subdivided by habitual smoking phenotype (proxy for nicotine dependence), and habitual smokers removed from control subjects ($n = 109$; 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 control subjects with neither condition. As in the FSCD analyses, cocaine/alcohol-dependent case subjects with habitual smoking had higher MAF (31.3%) than case subjects without habitual smoking (27.2%), whereas both had lower MAF than control subjects [35.9%; $\chi^2(2) = 6.2, p = .04$]. 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 [$\chi^2(4) = 10.4, p = .03$].

Multivariate regression analyses using the cumulative logit

Table 5. Multivariate Allelic Association Among rs16969968 in *CHRNA5*, Alcohol Dependence, and Habitual Smoking in the COGA Sample

	<i>n</i>	OR	(95% CI)	<i>p</i>
Alcohol Dependence + Cocaine Dependence (Case)	290	.52	(.36, .77)	.0009
No Alcohol Dependence or Cocaine Dependence (Control)	524	1.00		
Habitual Smoker	309	1.37	(.89, 2.12)	.15
Moderate Smoker	58	1.40	(.76, 2.59)	.28
Nonsmoker	338	1.14	(.74, 1.75)	.55
Light Smoker	109	1.00		

CI, confidence interval; COGA, Collaborative Study on the Genetics of Alcoholism; OR, odds ratio.

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 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 = .52, Wald- $\chi^2 = 11.0, p = .0009$). The OR for habitual smoking (OR = 1.37, $p = .15$), although 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, whereas 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 our 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

Table 4. Association Between rs16969968 in *CHRNA5* and Cocaine Dependence in the COGA Sample and the Role of Comorbid Habitual Smoking

	Allele Distribution (<i>n</i> = 1628)				Genotype Distribution (<i>n</i> = 814)					
	G		A		GG		GA		AA	
	N	(% of Row)	N	(% of Row)	N	(% of Row)	N	(% of Row)	N	(% of Row)
Case subjects (<i>n</i> = 290)	406	(63.7)	174	(36.4)	206	(50.7)	112	(38.6)	31	(10.7)
Control subjects (<i>n</i> = 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)
Habitual-Smoking Case Subjects (<i>n</i> = 200)	275	(68.8)	125	(31.3)	97	(48.5)	81	(40.5)	22	(11.0)
Non-Habitual Smoking Case Subjects (<i>n</i> = 90)	131	(72.8)	49	(27.2)	50	(55.6)	31	(34.4)	9	(10.0)
Control Subjects, Excluding Habitual Smokers (<i>n</i> = 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)

$\chi^2(1) = 6.7/p = .0096$

$\chi^2(2) = 10.0/p = .007$

$\chi^2(2) = 6.2/p = .04$

$\chi^2(4) = 10.4/p = .03$

COGA, Collaborative Study on the Genetics of Alcoholism.

than non-nicotine-dependent smokers (20); see also (21). In the FSCD sample, cocaine-dependent case subjects had lower frequencies of the minor allele than control subjects, 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, whereas the risk effect for habitual smoking (a proxy measure for nicotine dependence), although 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.

Although 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 (34,35). In this system, the $\alpha 5$ nAChR subunit is found as part of heteropentameric nAChRs (predominantly $\alpha 4\beta 2\alpha 5$) on both excitatory dopaminergic and inhibitory gamma-aminobutyric acid (GABA)ergic neurons (25,36). 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 (21). 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 (37–40).

In contrast to nicotine, cocaine directly increases mesolimbic dopaminergic activity by inhibiting reuptake through interaction with the dopamine transporter and other proteins (41,42). Therefore the influence of the rs16969968 on cocaine addiction liability may be more restricted to $\alpha 4\beta 2\alpha 5$ 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 (26,43–48).

Although this 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 (14,25,48). 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 case subjects in both samples had a variety of comorbid addictions, with the most common being alcohol dependence. Nearly 80% of the case subjects in FSCD were affected by alcohol dependence, and all of the COGA case subjects, 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 nonspecific association with multiple addictions, but the association with nicotine dependence is clearly in the reverse direction compared with 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

This 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 case subjects in the FSCD sample (presented here) (20–22). To our knowledge, no other studies have provided strong evidence of bidirectional association for a single genetic variant with two 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 (8,11,32,49) over a general-liability model (12,50). Although 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 pharmacologic treatments for addictions emerge, it will be essential to consider such phenomena as potential contributors to unintended side effects.

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Authors Grucza, Stitzel, Bucholz, Cloninger, Neuman, Budde, 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, S. Saccone, Hinrichs, Wang, and Goate are listed as inventors on a patent (US 20070258898) held by Perlegen Sciences, Inc., covering the use of certain single nucleotide polymorphisms, including rs16969968, in diagnosing, prognosing, and treating addiction. Dr Bierut is a consultant for Pfizer, Inc. Dr. N. Saccone is the spouse of Dr. S. Saccone, who is listed on the above-named patent.

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