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Association of Variants in *MANEA* With Cocaine-Related Behaviors

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Abstract

Context—Cocaine dependence (CD) and related behaviors are highly heritable, but no genetic association has been consistently demonstrated. A recent genome-wide study of drug dependence identified an association between cocaine-induced paranoia (CIP) and a single-nucleotide polymorphism (SNP) in the α-endomannosidase (*MANEA)* locus in a family-based sample of European Americans and African Americans.

Objective—To conduct a comprehensive genetic association study of the *MANEA* locus with CD and CIP.

Design—Genome-wide association study.

Setting—Four university hospitals.

Participants—A total of 3992 individuals from 2 family-based and 2 case-control samples.

Intervention—Participants were classified as having CD or CIP or as a control using the Semi-Structured Assessment for Drug Dependence and Alcoholism. They were genotyped for 11 SNPs spanning *MANEA* and its surrounding region.

Main Outcome Measure—Association of CD and CIP with individual SNPs and haplotypes.

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Results—Cocaine-induced paranoia was associated with 6 SNPs in the European American families and 9 SNPs in the African American families. The strongest evidence in the total sample of families was observed in 3 markers located in the promoter and 3′ untranslated regions ($P < .001$). The association of *MANEA* SNPs with CD in both family samples was much weaker. In the African American case-control sample, multiple markers were significantly associated with CIP and CD; CIP and CD were also significantly associated with a 2-SNP haplotype in the European American case-control sample. The *A* allele of the 3′ untranslated region SNP rs9387522 was associated with increased risk of CIP in all 4 data sets.

Conclusions—Our findings suggest that CD and associated behaviors may involve biological pathways not typically thought to be associated with brain metabolism.

COCAINE IS WIDELY USED IN the United States. The 2002 National Survey on Drug Use and Health revealed that nearly 6 million Americans aged 12 years or older used the drug during the preceding year, making cocaine second only to cannabis as the most commonly used illicit drug.[1] Compulsive use of cocaine is also common, with more than 1 million individuals meeting criteria for dependence on the drug.[1] Cocaine dependence (CD) is associated with criminal behavior and accidental injury and spans geographical region, race and ethnicity, and socioeconomic status.

Vulnerability to the development of CD varies among individuals. Adoption, twin, and family studies show a substantial genetic contribution to CD.[2-6] Identification of genes that influence CD susceptibility could help elucidate the etiology of the disorder and provide critical insight to develop efficacious treatments. Reports of genetic associations for CD derive mostly from relatively small samples,[7-9] resulting in limited capacity for replication.[10-13] Evidence for multiple subtypes of CD[14,15] makes it possible to decompose the broader set of those with CD into phenotypic subgroups, thereby reducing the genetic heterogeneity of the sample and increasing the likelihood of identifying a particular genetic factor that contributes to risk. Most (up to 60%-80%) long-term cocaine users experience transient psychotic symptoms, such as paranoia and hallucinations, that typically resolve with abstinence.[16-18] Cocaine-induced paranoia (CIP) appears to represent a reliably identifiable phenotype that reflects interindividual differences in the brain's response to cocaine.[18] Cocaine-induced paranoia has important clinical and public health significance, since, in addition to being highly prevalent, it appears to pre-dispose individuals to a number of high-risk behaviors, including accidents, self-harm, and violence toward others.[19,20]

A genome-wide linkage scan detected regions that harbor genes for CD on chromosomes 3 and 10, and for CIP on chromosome 9,[21] but no genes under these linkage peaks have yet been identified as risk loci for either condition. Moreover, studies targeting candidate genes selected because of inferred roles in cocaine metabolism or compulsive use have not yielded confirmed associations for CD or CIP, though there are several previous reports of CIP's association with dopamine-pathway genes.[22,23] Recently, we conducted a genome-wide association study using a low-density SNP array for 6 traits corresponding to 4 major substance dependence disorders (including CD) in a family-based cohort that included 2 distinct population groups. The most remarkable result was an association of a single-nucleotide polymorphism (SNP), rs1133503, in the 3′ untranslated region (UTR) of the *MANEA* gene (GenBank 79694) with CIP in European American (EA) families (P=.007), African American (AA) families (P=.002), and all families combined (P>.001).[24] Although this result was not significant after adjustment for multiple comparisons, the hypotheses that were generated prompted a more comprehensive association study of this gene with cocaine-related traits in 2 discovery data sets (EA and AA families) and 2 independent EA and AA replication data sets composed of unrelated cases and controls ascertained for studies of alcohol and drug dependence.

METHODS

SUBJECTS

Subjects were recruited from Yale University School of Medicine (APT Foundation, New Haven, Connecticut), the University of Connecticut Health Center (Farmington), McLean Hospital (Harvard Medical School, Belmont, Massachusetts), and the Medical University of South Carolina (Charleston) into 1 of 2 study arms. Six hundred thirty-two families ascertained through affected sibling pairs that met *DSM-IV* criteria for CD or opioid dependence as previously described,[15,21,25] containing at least 1 examined sibling with CD or CIP, formed the discovery sample. Of the 632 families, 119 had at least 1 sibling pair discordant for CD and 319 had at least 1 sibling pair discordant for CIP. Of the 1612 genotyped subjects, 160 were parents (9.9%) (and the remainder were siblings) and 141 did not contribute information about substance dependence (8.7%). An independent group of 2073 unrelated subjects recruited for studies of CD (n=667), opioid dependence (n=103), or alcohol dependence (n=1303) were included in a replication sample. Genetic studies of CD and related traits in a subset of this sample have been published.[10,23]

All subjects were interviewed using the Semi-Structured Assessment for Drug Dependence and Alcoholism, which has been shown to yield reliable substance dependence diagnoses.[21,26] Subjects with a primary diagnosis of a major psychotic illness (schizophrenia or schizoaffective disorder) were excluded. A diagnosis of CD was established if the subject met 3 or more of the 7 *DSM-IV* criteria during a 12-month period. The interrater reliability of the Semi-Structured Assessment for Drug Dependence and Alcoholism diagnosis of CD was previously shown to be $\kappa=0.83$.[21] Subjects who gave an affirmative answer to the question, "Have you ever had a paranoid experience when you were using cocaine?" were diagnosed as being affected by CIP. The overall reliability of CIP diagnosis was previously shown to be excellent ($\kappa=0.87$).[21] Controls did not use cocaine, but individuals who had dependence on other substances were included. Probands were excluded from further study if they had a diagnosed major psychotic illness (eg, schizophrenia or schizoaffective disorder). Subjects who had missing responses to items on the Semi-Structured Assessment for Drug Dependence and Alcoholism that were required for the determination of CD or CIP status were also excluded.

Subjects gave informed consent as approved by the institutional review board at each clinical site. A certificate of confidentiality for the work was obtained from both the National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism. Characteristics of both samples included in this study are given in Table 1.

SNP SELECTION AND GENOTYPING

Eleven SNPs spanning 83 kilobases (kb) upstream of the *MANEA* promoter to downstream of the *MANEA* 3′ UTR were selected from the National Center for Biotechnology Information database or by the Applied Biosystems SNPbrowser, version 3.5 (Applied Biosystems, Foster City, California). Characteristics of each SNP are presented in Table 2. The average intermarker distance is 8295 base pairs (bp) for all SNPs, but only 5600 bp for the 7 SNPs in the promoter and coding regions. Most SNPs were genotyped with a fluorogenic 5′ nuclease assay method, ie, the TaqMan technique,[27] using the Applied Biosystems PRISM 7900 Sequence Detection System. One SNP (SNP 8) was genotyped in the family sample at the Center for Inherited Disease Research as part of the Illumina Linkage IVb Marker Panel (http://www.cidr.jhmi.edu).

POPULATION CLASSIFICATION

Subjects in the family-based sample were classified as AA or EA based on a Bayesian model-based clustering method as previously described[28] using approximately 400 short tandem repeat markers and more than 5000 SNP markers from the Illumina Linkage IVb Marker Panel. The race and ethnicity of subjects in the replication sample were self-identified. The genetic backgrounds of nearly two-thirds of these subjects were also analyzed with the Bayesian approach using a set of 180 ancestry informative markers. One hundred nineteen subjects in the replication sample who self-reported their ethnicity as something other than AA or EA and lacked ancestry informative marker data were excluded from further analysis. This resulted in a final replication sample of 1921 subjects. The AA and EA population groups were treated as independent samples for all primary analyses.

STATISTICAL ANALYSIS

Consistency with Hardy-Weinberg equilibrium expectations for each SNP was examined with the χ^2 test in each discovery sample, using a set of unrelated subjects without CD (1 random unaffected subject from each family), and in each of the control groups from the replication sample. Two SNPs (rs9400554 and rs6937479) with significant evidence of deviation from Hardy-Weinberg equilibrium ($P>.001$) in the AA replication sample were excluded from analyses of allelic and genotypic association in that data set. In the family samples, mendelian inheritance of all genotypes was evaluated using PedCheck,[29] and pairwise marker linkage disequilibrium was examined using the Haploview program, version 4.0.[30] Association of the *MANEA* SNPs with CD and CIP in the family samples was evaluated using the FBAT program,[31] assuming an additive model under the null hypothesis of no linkage and no association. Allele frequencies were estimated by FBAT using the expectation-maximum algorithm. In the case-control samples, a χ^2 test was used to examine the association of SNP alleles and genotypes with cocaine-related traits. Odds ratios and their 95% confidence intervals for the allelic associations were computed using logistic regression analysis. Odds ratios were unaffected by adjustment for age and sex. In these analyses, controls were compared with distinct case samples of subjects with CIP and subjects with CD who did not have CIP. Haplotype association analyses were performed in the family samples using HBAT, the haplotype extension routine in the FBAT program,[32] and in the case-control samples using haplo.stats.[33]

RESULTS

FAMILY-BASED ANALYSES

In the EA family sample, 6 of the 11 markers, including rs1133503 from the genome scan,[24] showed at least a nominally significant association with CIP (Table 3). These 6 markers and 3 others were also significant in the AA family sample. The patterns of association were identical in the 2 population groups for all 9 markers (which represent all markers tested in the promoter and coding regions), evidenced by increased significance in the total sample of families. The strongest evidence for association in either population (AA, $P<.001$) and in the total sample ($P<.001$) was observed with rs6937479, which is located in the putative promoter region. The association of *MANEA* SNPs with CD was much weaker. In the EA families, nominally significant results were obtained with rs9374586 ($P = .01$), rs1133503 ($P = .04$), and rs9387522 ($P = .03$). Although no significant associations were obtained in the AA families, trends were evident in that population for several markers. Eight markers (rs9400554, rs9320497, rs6937479, rs9374586, rs9400893, rs1133503, rs9387522, and rs9387605) were nominally associated with CD in the total group of families ($.007 \leq P \leq .03$).

Haplotype analysis was conducted in the family samples to help narrow the location of a CIP susceptibility locus and to determine whether a single functional variant could explain the

pattern of association findings with individual SNPs in each population group. As a first step, we evaluated linkage distribution among the 11 SNPs to reduce the number of potentially informative markers for haplotype analysis. This analysis, shown in the Figure, revealed slightly more extensive linkage distribution in EAs than in AAs. These population-specific patterns are consistent with the linkage distribution structures reported in the HapMap database for this genetic region.[34] Taking into account the linkage distribution block structure and the association findings with individual SNPs, we selected 3 SNPs (rs9400554, rs6937479, and rs9387522) for haplotype analysis. These markers include the 2 most significant results in the combined sample and account for the potentially uniquely important information from each linkage distribution block spanning the entire region, showing significance with any marker in either population sample. The haplotype that included this SNP combination was significantly associated in AAs (global, P=.003), EAs (global, P=.02), and the combined sample of families (global, P=.001). The specific haplotype T-T-A was associated with CIP in EAs (P=.01) and AAs (P=.02), and in the pooled sample (P<.001). Haplotype C-A-C was associated with decreased risk of CIP in EAs (P=.01) and AAs (P<.001), and in the total sample (P<.001). These 2 haplotypes account for 86% and 73% of all haplotypes in the EA and AA families, respectively. A third haplotype (C-T-A), which had appreciable frequency in both EAs (6%) and AAs (23%), was also associated with increased risk of CIP. Because both rs9400554 alleles were part of different risk haplotypes, the functional variant is more likely to be closer to the other 2 SNPs. Thus, these results, showing strong evidence for association of the same haplotype to CIP in 2 distinct populations, support the existence of a single causative variant that is most likely located in the *MANEA* promoter or coding region.

CASE-CONTROL ANALYSIS

We evaluated the panel of *MANEA* SNPs in the EA and AA case-control samples in an attempt to replicate the overall association with cocaine-related traits, to determine whether or not the association is specific to CIP, and to localize the putative biological variant. In the AA replication sample, significant association at the allelic and/or genotypic level was observed between CIP and 5 markers (Table 4). These SNPs and a sixth marker were also associated with CD in the absence of CIP. The strongest and most consistent evidence for association was observed with adjacent markers rs9387522 and rs9387605. In the EA replication sample, the only significant association was found for rs4388292 with CIP, which is accounted for primarily by an underrepresentation of the TT genotype in CIP cases compared with controls. The TT genotype is interestingly also significantly lower in individuals with CD compared with controls in the AA replication sample. Although the results of analyses of individual SNPs did not show an association common to both population groups, haplotype analysis of rs900554 and rs9387522 (ie, 2 of the 3 SNPs included in the haplotype studies in the families) showed that the C-A haplotype was significantly associated with increased risk of CIP and CD in EAs and that the T-A haplotype was significantly associated with CD in AAs (Table 5). The rare T-C haplotype was also associated with increased risk of CD in EAs. Of note, when considering results from both the single SNP and haplotype analyses in the replication samples, the rs9387522 A allele is associated with CIP in all 4 data sets.

COMMENT

We observed that several polymorphic markers in the *MANEA* gene region are associated with cocaine-related traits in 2 EA and 2 AA populations, which were ascertained and analyzed in different ways. The strongest evidence was obtained for CIP with markers in the *MANEA* coding and promoter regions, spanning a distance of approximately 33.6 kb (ie, between rs9320497 and rs9387522). Haplotype analysis in the replication data sets helped confirm that the rs9387522 A allele is associated with increased risk of CIP in all 4 populations. This SNP is only 348 bp from rs1133503, the marker in the low-density genome scan that showed an

association with CIP in the EA and AA family-based samples,[24] which prompted this investigation. Our comprehensive analysis of *MANEA* SNPs and haplotypes in 4 independent data sets bolsters our initial association finding and indicates that the biologically relevant variant is most likely located in the 3′ UTR.

The results for association of *MANEA* with CD were substantially weaker in the discovery (family-based) data sets. However, these samples were ascertained through sibling pairs with CD or opioid dependence. They are, thus, much less informative for association analyses of these traits compared with those with CIP, because, in the absence of data from parents, the family-based approach requires at least 1 discordant sibling pair. To determine whether the association with *MANEA* is specific to the paranoia that often complicates CD, we compared *MANEA* SNPs and haplotypes in controls with distinct samples of subjects with CD (but no paranoia) and subjects with CIP. In the AA group, CD and CIP were significantly and comparably associated with several SNPs. Both traits showed identical patterns of association with a particular haplotype in the EA group. Thus, our study suggests that *MANEA* is associated with both CD and paranoia. It is also possible that *MANEA* is more strongly associated with CIP than CD because CIP is characteristic of a genetic subgroup of CD that is influenced by *MANEA*. Additional studies in independent samples of subjects with CD characterized for paranoia, and perhaps in subjects with other disorders involving paranoia, are necessary to determine more definitively whether the association with *MANEA* is specific for the subset of persons with CD prone to CIP.

α-Endomannosidase (MANEA), encoded by the *MANEA* gene on chromosome 6q16.1, is an enzyme that catalyzes the release of glucosyl-mannose oligosaccha-rides by cleaving the α-1,2-mannosidic bond that links them to high-mannose N-glycans.[35] Comparative genomic analysis has demonstrated high-sequence conservation in humans, rats, and mice.[36] Human MANEA is expressed in a variety of tissues including brain, though levels of MANEA in the brain are much lower than, for example, in the liver or kidney.[36] The role of MANEA is poorly understood but has been hypothesized to be involved in the quality control of N-glycosylation, [37] providing cells with the ability to recover and properly mature glucosylated structures that have bypassed glucosidase trimming by glucosidases I and II in the endoplasmic reticulum.[36]

Given MANEA's role in carbohydrate metabolism and its relatively minor expression in brain, initially it would not appear to be a good biological candidate to modulate susceptibility to CD or its associated psychotic complications. However, insight into the relationship between MANEA, paranoia, and CD can be gleaned from studies of mannosidase and other glycoproteins. α-Mannosidosis in humans is a rare autosomal recessive lysosomal storage disorder associated with decreased activity of mannosidase. Recently, α-mannosidosis was identified as the underlying cause of recurrent paranoid hallucinatory episodes in a 27-year-old woman.[38] α-Endomannosidase is 1 of several glycosidic enzymes that remove oligosaccharide chains of dopamine β-hydroxylase,[39] the enzyme that converts dopamine to norepinephrine. Low levels of β-hydroxylase in plasma or cerebrospinal fluid and polymorphisms in β-hydroxylase have been associated with greater vulnerability to psychotic symptoms in several psychiatric disorders including CD,[23,40] schizophrenia,[41] and major depression.[42] α-Endomannosidase may also influence susceptibility to CD by modifying the function of liver carboxylesterase, a glycoprotein of the high mannose type,[43] 2 forms of which hydrolyze cocaine and other drugs.[44,45]

There are 44 markers in *MANEA* with appreciable frequency in 1 or more populations (http://www.ncbi.nlm.nih.gov/SNP), but none are known to effect structural changes in the translated protein. Remarkably, 34 of these SNPs have minor allele frequencies of 0.24 or greater in both EAs and AAs. This excess of high-frequency polymorphisms suggests that balancing selection is occurring in this region.[46] The most robust evidence for association in

the collective data sets in this study was obtained with rs9387522, which is located in the 3′ UTR. The 3′ UTR is the major site of gene regulation by microRNA binding.[47] Polymorphic target sites for microRNA binding in the 3′ UTRs of *SLITRK1*, *FGF20*, and *HTR1B* have been identified as leading to increased risk of Tourette syndrome,[48] Parkinson disease,[49] and aggressive human behaviors,[50] respectively. The possibility that *MANEA* 3′ UTR SNPs, including rs987522, may influence risk of CD or CIP could be investigated by microRNA studies in brains of rodents exposed to cocaine or constructs transfected into cell lines to demonstrate effects on gene expression.

We acknowledge several limitations to our study. First, our discovery sample, which was ascertained through sibling pairs concordant for CD or opioid dependence, is probably enriched for genetic factors for CD and CIP compared with subjects exposed to cocaine in the general population. To overcome this issue and the problem that association findings in discovery samples tend to overestimate the effect size of the genetic risk factor,[51] we replicated our results in independent EA and AA case-control samples. Although results with individual SNPs were uneven across study samples, haplotype analysis showed significant association with the same allele of 1 SNP (SNP 9, rs9387522) in both EA and both AA data sets. Second, genetic association studies are vulnerable to false-positive results owing to population stratification and to false-negative results owing to misclassification of subjects or power. Our use of family-based controls in the discovery phase and the assignment of nearly all subjects to genetically matched groups based on analysis of many markers distributed across the genome lessened the potential for stratification. Furthermore, all of the approximately 4000 subjects included in this study were evaluated with a standardized instrument using a rigorous quality-control procedure that reliably diagnoses substance dependence and other psychiatric disorders.[21,26] In any event, it is possible that some subjects were misclassified as controls because they were not sufficiently exposed to cocaine to become dependent on the drug. This, however, would bias the results toward the null hypothesis. Because our replication samples had sufficient power to detect allele frequency differences of 7% to 10% for CIP and 8% to 15% for CD in either population, lack of significant findings with individual SNPs in the EA sample could be attributed to an inadequate sample size. However, significant haplotypic associations in this population suggest that genetic background rather than sample size was the limiting factor. Third, only one of the results from analyses of individual SNPs in the replication samples would be considered significant after adjustment for multiple comparisons using a conservative Bonferroni correction (threshold, P=.004 in EAs and P=.006 in AAs without taking intermarker correlations into account). An alternative approach to evaluating the impact of multiple testing on our results is measuring the rate of false discovery. Because the expected number of findings for a trait that surpass a nominal significance level of P=.05 in the AA sample would be less than 1 (0.05 × 9 informative SNPs × 0.5), assuming a 1-tailed test (and there were at least 3 significantly associated SNPs for each trait, taking into account the high correlation among SNPs 5 through 9 in AAs [Figure]), it is unlikely that our findings for CD and CIP in the AA replication sample are spurious. The significant global tests of association of *MANEA* haplotypes with CD and CIP in the EA replication sample take into account the comparisons of multiple haplo-types. In summary, our study shows that *MANEA* gene variants are strongly associated with CD and CIP in both EA and AA populations. This finding, which was discovered initially through a low-density genome scan, suggests that drug dependence and associated behaviors may involve biological pathways not typically associated with brain metabolism and opens a new pathway to understanding these highly prevalent disorders and their psychopathologic manifestations.

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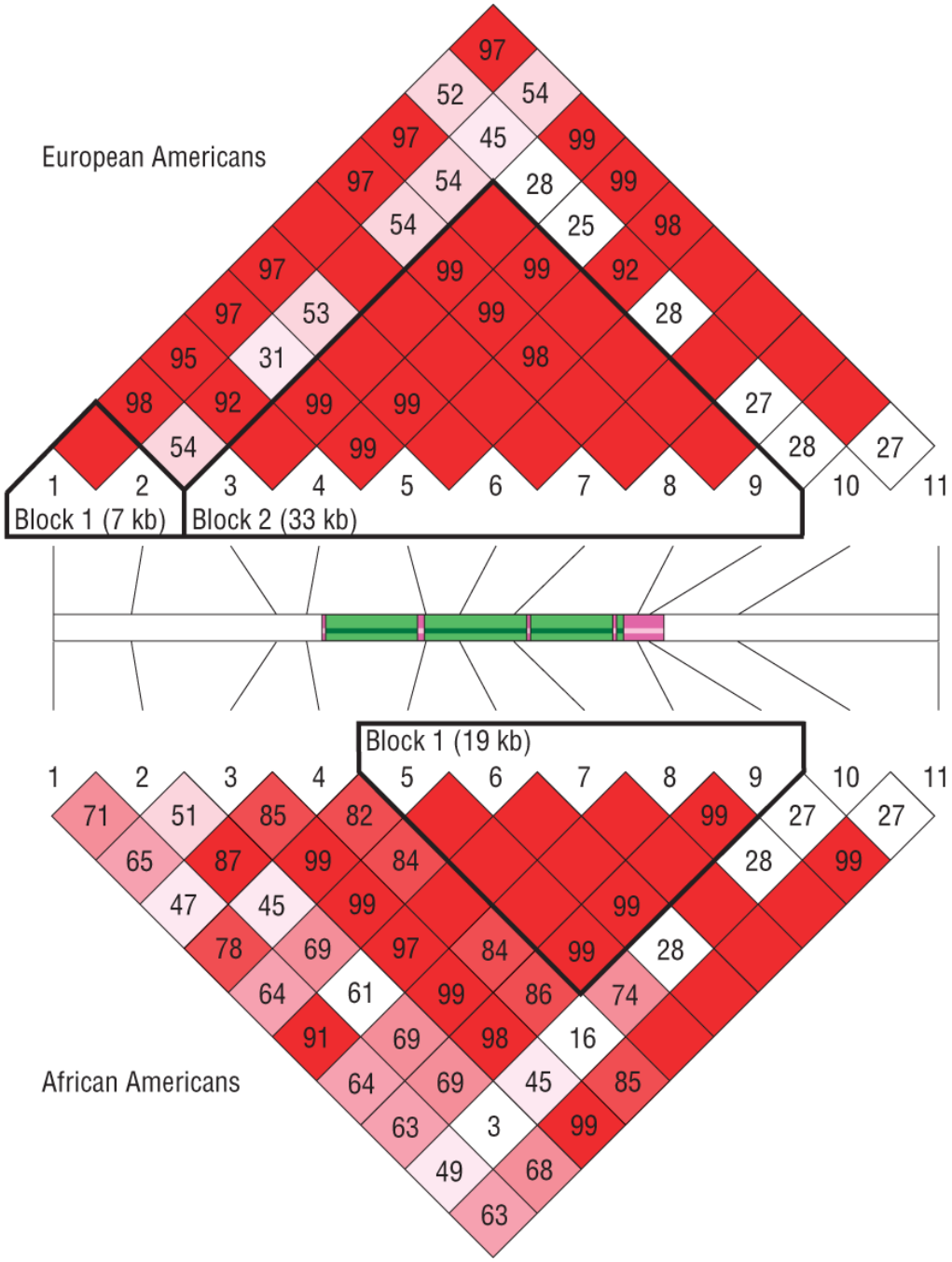

Figure.

Locations and linkage disequilibrium map structure of single-nucleotide polymorphisms in the *MANEA* gene region. Measures of linkage disequilibrium among all possible pairs of single-nucleotide polymorphisms (identified by marker number) are shown (white represents very low D′ and dark red represents very high D′) and are numerically denoted by the r^2 values within each square. The *MANEA* gene structure, including intergenic regions (white), introns (green), and exons (pink), is shown starting from the 5′ upstream region on the left. kb indicates kilobases.

Table 1

Characteristics of Subjects in a Genetic Study of Cocaine Dependence (CD) and Cocaine-Induced Paranoia (CIP)

	No. of Subjects			
	Family Data Set		Case-Control Data Set	
Characteristic	**European American**	**African American**	**European American**	**African American**
Total families	313	319		
Total subjects (parents)	685 (38)	786 (26)	876	1045
Additional genotyped subjects (parents)	75 (50)	66 (46)		
Female sex, %	47.0	57.5	41.8	48.9
Subjects with CIP	403	443	309	430
Subjects with CD, without CIP	176	251	136	199
Subjects without CIP or CD, controls	106	92	431	416
Age of subjects with CIP, mean (SD), y	36.6 (8.7)	40.4 (6.3)	38.8 (8.9)	41.2 (7.5)
Age of subjects with CD, without CIP, mean (SD), y	37.4 (8.3)	40.6 (6.2)	38.3 (9.8)	42.7 (7.4)
Age of controls, mean (SD), y	43.5 (15.3)	43.7 (12.2)	38.1 (14.6)	35.8 (12.8)

Table 2

SNP Characteristics

Marker No.	dbSNP rs No.	Alleles	Map Location, bp	Distance From Previous Marker, bp	SNP Type
1	rs9400554	C/T	96 106 842		Upstream of 5′ UTR
2	rs10782175	C/T	96 114 037	7195	Upstream of 5′ UTR
3	rs9320497	A/T	96 128 061	14 024	Promoter
4	rs6937479	A/T	96 130 476	2415	Promoter
5	rs9374586	C/T	96 141 896	11 420	Intron
6	rs9400893	A/G	96 144 620	2724	Intron
7	rs7757276	G/T	96 149 401	4781	Intron
8	rs1133503[a]	C/T	96 161 308	11 907	3′ UTR
9	rs9387522	A/C	96 161 656	348	3′ UTR
10	rs9387605	A/G	96 170 200	8544	Downstream of 3′ UTR
11	rs4388292	G/T	96 189 788	19 588	Downstream of 3′ UTR

Abbreviations: bp, base pairs; dbSNP, National Center for Biotechnology Information SNP database; SNP, single nucleotide polymorphism; UTR, untranslated region.

[a]Included in Illumina SNP linkage panel.

Table 3

Association of *MANEA* SHPs With CIP in Discovery Samples

		European American Families				African American Families				All Families			
SNP	**Minor Allele**	**MAF**	**Families, [a] No.**	***p* Value**	**Risk Allele**	**MAF**	**Families,[a] No.**	***P* Value**	**Risk Allele**	**MAF**	**Families,[a] No.**	***P* Value**	**Risk Allele**
rs9400554	T	0.442	82	.16		0.467	67	.02[b]	T	0.452	149	.009[b]	T
rs10782175	T	0.344	59	.64		0.160	40	.28		0.264	99	.3	
rs9320497	A	0.441	70	.02[b]	T	0.359	58	.003[b]	T	0.405	128	<.001[b]	T
rs6937479	A	0.482[c]	77	.02[b]	T	0.398	63	<.001[b]	T	0.465	140	<.001[b]	T
rs9374586	T	0.385	76	.07	C	0.324	59	<.001[b]	C	0.358	135	<.001[b]	C
rs9400893	A	0.440	74	.01[b]	G	0.378	63	.002[b]	G	0.412	137	<.001[b]	G
rs7757276	G	0.130	31	.46		0.128	39	.01[b]	T	0.129	70	.02[b]	T
rs1133503	C	0.439	80	.007[b]	T	0.373	68	.002[b]	T	0.410	148	<.001[b]	T
rs9387522	C	0.437	71	.003[b]	A	0.378	64	.001[b]	A	0.411	135	<.001[b]	A
rs9387605	A	0.445	70	.03[b]	G	0.377	62	.007[b]	G	0.415	132	<.001[b]	G
rs4388292	T	0.200	45	.45		0.283	54	.77		0.237	99	.79	

Abbreviations: CIP, cocaine-induced paranoia; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

[a] Informative families.

[b] Significant result.

[c] T allele.

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Table 4

Association of *MANEA* SNPs With CIP and CD in Replication Samples

					CIP vs Control			CD vs Control			
		MAF				*P* Value			*P* Value		
SNP	Minor Allele	CIP Cases	CD Cases	Controls	OR (95% CI)	Allele	Genotype	OR (95% CI)	Allele	Genotype	Risk Allele
European American											
rs9400554	T	0.471	0.459	0.480	1.04 (0.84-1.28)	.73	.8	1.09 (0.83-1.44)	.54	.32	T
rs10782175	T	0.348	0.329	0.341	1.03 (0.83-1.29)	.76	.37	1.05 (0.78-1.42)	.73	.26	C
rs9320497	A	0.393	0.410	0.418	1.10 (0.90-1.37)	.34	.58	1.04 (0.78-1.37)	.80	.94	A
rs6937479	A	0.495	0.496[a]	0.498	1.01 (0.82-1.25)	.92	.70	1.03 (0.78-1.35)	.86	.27	A
rs9374586	T	0.324	0.353	0.354	1.14 (0.92-1.43)	.24	.42	1.01 (0.75-1.35)	.96	.96	T
rs9400893	A	0.386	0.398	0.416	1.13 (0.91-1.40)	.26	.42	1.08 (0.81-1.43)	.60	.88	A
rs7757276	G	0.086	0.094	0.097	1.15 (0.80-1.65)	.46	.61	1.03 (0.65-1.65)	.89	.96	G
rs1133503	C	0.384	0.392	0.416	1.14 (0.92-1.42)	.22	.35	1.10 (0.83-1.47)	.48	.79	C
rs9387522	C	0.384	0.393	0.410	1.11 (0.90-1.39)	.33	.52	1.07 (0.81-1.43)	.63	.90	C
rs9387605	A	0.396	0.412	0.421	1.11 (0.89-1.38)	.35	.58	1.04 (0.78-1.38)	.79	.97	A
rs4388292	T	0.191	0.170	0.141	1.44 (1.09-1.92)[b]	.01[b]	.04[b]	1.25 (0.86-1.83)	.24	.47	G
African American											
rs9400554	T	0.460	0.500	0.458	ND	ND	ND	ND	ND	ND	
rs10782175	T	0.154	0.145	0.156	1.01 (0.78-1.32)	.92	.05	1.09 (0.78-1.52)	.62	.24	T
rs9320497	A	0.344	0.322	0.389	1.22 (0.99-1.49)	.06	.046[b]	1.35 (1.04-1.73)[b]	.02[b]	.07	T
rs6937479	A	0.408	0.365	0.429	ND	ND	ND	ND	ND	ND	
rs9374586	T	0.291	0.276	0.320	1.14 (0.93-1.41)	.22	.093	1.24 (0.94-1.62)	.12	.32	T
rs9400893	A	0.354	0.329	0.404	1.24 (1.01-1.51)	.04[b]	.02[b]	1.38 (1.07-1.78)[b]	.01[b]	.05	G
rs7757276	G	0.087	0.076	0.100	1.16 (0.83-1.61)	.37	.63	1.35 (0.87-2.09)	.18	.39	G
rs1133503	C	0.362	0.330	0.401	1.18 (0.96-1.44)	.11	.02[b]	1.36 (1.06-1.76)[b]	.02[b]	.07	T
rs9387522	C	0.345	0.319	0.401	1.27 (1.03-1.56)	.02[b]	.007[b]	1.43 (1.10-1.85)[b]	.007[b]	.04[b]	A
rs9387605	A	0.371	0.325	0.417	1.21 (0.99-1.49)	.05	.02[b]	1.48 (1.15-1.92)[b]	.003[b]	.02[b]	G
rs4388292	T	0.308	0.289	0.310	1.01 (0.82-1.25)	.92	.88	1.10 (0.85-1.45)	.46	.046[b]	G

Abbreviations: CD, cocaine dependence; CI, confidence interval; CIP, cocaine-induced paranoia; MAF, minor allele frequency; ND, test not done because controls were not in Hardy-Weinberg equilibrium; OR, odds ratio; SNP, single nucleotide polymorphism.

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[a] T allele.

[b] Significant result.

Table 5

Haplotype Association of *MANEA* With Cocaine-Induced Paranoia in the Replication Samples

Allele by SNP		European Americans			African Americans		
rs9400554	**rs9387522**	**Haplotypic Frequency**	***Z* Score**	***P* Value**	**Haplotypic Frequency**	***Z* Score**	***P* Value**
			Cocaine-Induced Paranoia[a]				
T	A	0.474	-0.64	.52	0.4	0.7	.48
C	C	0.4	-1	.32	0.31	-1.32	.19
C	A	0.12	2.25	.02[b]	0.23	1.55	.12
T	C	0			0.06	-1.7	.09
			Cocaine Dependence[c]				
T	A	0.47	-1.08	.28	0.41	1 99	.046[b]
C	C	0.4	-0.81	.42	0.31	-1.92	.06
C	A	0.12	2.25	.02[b]	0.22	0.74	.46
T	C	0.006	2.86	.004[b]	0.06	-1.67	.09

[a] In European Americans: global, P=.04; in African Americans: global, P=.14.

[b] Significant result.

[c] In European Americans: global, P=.003; in African Americans: global, P=.06.