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Association of candidate genes with antisocial drug dependence in adolescents

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Abstract

The Colorado Center for Antisocial Drug Dependence (CADD) is using several research designs and strategies in its study of the genetic basis for antisocial drug dependence in adolescents. This study reports Single Nucleotide Polymorphism (SNP) association results from a Targeted Gene Assay (SNP chip) of 231 Caucasian male probands in treatment with antisocial drug dependence and a matched set of community controls. The SNP chip was designed to assay 1500 SNPs distributed across 50 candidate genes that have had associations with substance use disorders and conduct disorder. There was an average gene-wide inter-SNP interval of 3000 base pairs. After eliminating SNPs with poor signals and low minor allele frequencies, 60 nominally significant associations were found among the remaining 1073 SNPs in 18 of 49 candidate genes. Although none of the SNPs achieved genome-wide association significance levels (defined as $p < .000001$), two genes probed with multiple SNPs (OPRM1 and CHRNA2) emerged as plausible candidates for a role in antisocial drug dependence after gene-based permutation tests. The custom-designed SNP chip served as an effective and flexible platform for rapid interrogation of a large number of plausible candidate genes.

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Keywords

Antisocial drug dependence; adolescents; candidate genes; association study

1.0 Introduction

Substance abuse and dependence often co-occurs with antisocial behavior in adolescents with evidence that the genetic influences on drug abuse share common pathways with the genetic influences on antisocial behavior (Button et al., 2006; McGue et al., 2006; Stallings et al., 2005;Dick et al., 2004; Molina et al., 2002; Disney et al., 1999). The Colorado Center for Antisocial Drug Dependence (CADD), a NIDA-funded center examining the genetics of adolescent antisocial drug dependence, studies the co-morbidity of conduct disorder (CD) and substance use disorders (SUD) during adolescence. *Antisocial drug dependence* has been operationalized in this center as a composite score of age and sex normed conduct disorder symptom counts and a normalized measure of cross substance drug dependence, referred to as dependence vulnerability (Stallings et al., 2005). This has been measured with instruments (i.e. DISC-IV and CIDI-SAM) that are both valid and reliable for assessing SUD and CD in adolescents (Crowley et al., 2001). Naturally occurring genetic polymorphisms are hypothesized to contribute to the differences among adolescents on antisocial drug dependence.

The vulnerability to develop persistent, progressive, and generalized substance use, abuse, and dependence represents a heritable phenotype. In a recent study of Colorado adolescents, Young et al., (2006) found high heritability for tobacco use (0.46), marijuana use (0.44), problem alcohol use (0.70) and problem marijuana use (0.64). Other studies have also shown that genetic and environmental factors may contribute to a general predisposition to illicit drug use rather than a substance-specific effect (Kendler et al., 2003; Karkowski et al., 2000; Agrawal et al., 2004; Uhl, 2004).

Conduct disorder is a psychiatric disorder of children and adolescents who show a persistent pattern of behavior in which the basic rights of others, or societal norms or rules, are violated (APA, 1994). The clinical diagnosis requires presence of 3 of 15 symptoms clustered in one year; symptoms include: frequently initiating fights, repeatedly lying, forcing sex, intentional fire-setting, etc. Slutske et al. (1997) estimated CD's heritability at 71% in a national sample of almost 2700 twin pairs, with other estimates of CD heritability at 41% (Grove et al., 1990) and 58% (Button et al., 2006). Genome scans for quantitative trait loci (QTLs) influencing CD have suggested sites on Chromosome 19 and 2 (Dick et al., 2004), and on Chromosomes 3, 9, and 17 (Stallings et al., 2005).

The high genetic correlation observed between SUD and CD may be largely explained by a single latent factor that has been referred to as “behavioral disinhibition” (Iacono et al., 1999). A study of Colorado twins suggested that behavioral disinhibition is a highly heritable trait (0.84) (Young et al., 2000) which may be explained by the comorbidity of alcohol dependence and illicit drug dependence and is also partially explained by the genetic vulnerability they both share to conduct disorder (Button et al., 2006). The literature concerning the potential role of candidate genes that may associate with both CD and SUD has not resulted in consensus. This may be due to a lack of power, incomplete gene coverage, variable phenotype definitions, or that a candidate locus that is in linkage disequilibrium with the effective locus rather than being the actual causal locus, and that the extent of linkage disequilibrium may differ between studied samples. The use of multiple single nucleotide polymorphisms (SNPs) within candidate genes provides a more systematic approach to the detection of candidate gene effects, rather than limiting focus to single “polymorphisms of convenience” within the potential candidate. A more systematic approach might also screen

other potentially interesting genes that are part of the same metabolic pathways as previously identified candidates. A recently assembled list developed by the NIDA genetics consortium lists 348 genes identified as candidates in a study of nicotine dependence (Saccone et al., 2007). Although initially chosen as candidates for nicotine dependence, the list includes many genes potentially involved in substance dependence more generally and that may be related to more broadly defined externalizing problems.

From these, we have selected 50 high priority candidates for dense SNP genotyping using a custom designed Affymetrix GeneChip® Targeted Genotyping Assay (formerly Affymetrix MegAllele) (SNPChip)– a compromise between breadth, depth, and cost-efficiency – to investigate adolescent antisocial drug dependence systematically. These candidates were chosen for functional reasons or because they have been associated with SUD or substance-related behaviors. Genes included are involved with four major neurotransmitter systems in the brain: dopaminergic (Limosin et al., 2005; Chen et al., 2005), serotonergic (Hopfer et al., 2005; Curtis, 2004), GABAergic (Radel et al., 2004; Nishiyama et al., 2005; Parsian et al., 1998), and cholinergic (Ehringer et al., 2007; Beirut et al., 2007; Saccone et al., 2007). A matched case-control design (matched on ethnicity and birth year) was utilized to test the extent to which individual SNPs predicted case/control status using within-gene correction for multiple SNPs.

2.0 Methods

2.1 Subjects

Cases were recruited from male adolescent probands in treatment for problems of drug dependence and delinquency. In addition to their status as treatment patients, cases had to score at least one standard deviation above community norms on a composite of lifetime substance dependence symptoms from the CIDI-SAM (Robins et al., 1993) and conduct disorder symptoms from the DISC-IV (Shaffer et al., 1998) or DIS (Robins et al., 1998) (see Stallings et al., 2005 for details). The composite of substance dependence measures was the mean number of dependence symptoms averaged across substance classes subjects had tried to criterion (usually 6 or more trials), age- and sex-adjusted by community sample norms. The count of conduct disorder symptoms was similarly age- and sex-adjusted.

Controls were a mixture of epidemiologically matched males recruited simultaneously with the clinical probands (Miles et al., 1998) and subjects drawn from genetically informative community samples (Young et al., 2000). Community sample males were matched to the clinical probands for ethnicity and birth year when a prior epidemiological match was unavailable (either because no original match had been done, DNA was not available, the matched subject was under age 16 at time of initial assessment, or because the matched control subject scored in the clinical range for the composite score). Control subjects had to be at least age 16 at the time of assessment to allow for substance exposure opportunity and could not exceed one standard deviation above the mean for the composite measure of antisocial substance dependence; they averaged approximately 0.5 standard deviations below the age-adjusted mean of all community sample subjects. Characteristics of the samples are shown in Table 1 below. Buccal cell DNA samples from the subjects were collected, extracted and stored as previously described (Stallings et al., 2003).

2.2 Gene and SNP selection

The selection of candidate genes was, in part, based on published reports of association with externalizing disorders and from the consensus set of drug dependence genes developed in a large collaborative project to map genes involved in nicotine dependence (Bierut et al., 2007; Saccone et al., 2007). It includes most of the genes known to be involved in the behavioral

physiological processes of drug dependence as well as comorbid behavioral traits. To provide dense SNP coverage across the candidate genes, a computer algorithm in the Python programming language that takes as input all available SNPs in each gene acquired from the dbSNP database and then bins them in 3kb intervals using a minimum-spanning tree algorithm was developed. From each bin one SNP was selected based on its location within an annotated feature with the following priority order: validated SNP with a reported minor allele frequency (MAF) greater than 3%; exon codon changing; location within an exon; location at an intron-exon boundary; location in a regulatory element; location within 3kb of the transcriptional start site or polyadenylation site. Application of this program provided an initial pool of approximately 1600 SNPs distributed at approximately 3 kb intervals across the 50 candidate genes, as well as an additional selection of SNPs located on 9q34 to follow-up an earlier linkage result (Stallings et al., 2003, 2005). This program is freely available upon request (krauter@colorado.edu). Table 2 lists the genes and the number of SNPs per gene.

2.3 Chip development and genotyping

In developing the custom chip SNP set, each SNP was screened first on a panel of 96 individuals from the CEPH DNA collection to validate inheritance and accuracy. In an initial screen, approximately 15% of dbSNP-based markers failed and were replaced with alternative SNPs from the same genes. Overall, however, 85% of SNPs selected at all steps produced successful Affymetrix GeneChip® Targeted Genotyping Assays. Alleles are called using the GeneChip Targeted Genotyping Analysis Software supplied by Affymetrix and is based on a signal clustering algorithm that corrects for chip efficiency, background signal levels and accurate internal control calls for each chip.

To validate the SNPchip for genomic DNA from buccal cells, results were compared using buccal cell and blood-derived DNA samples from a small test set of individuals (manuscript in preparation). The average call rate for saliva and blood samples was indistinguishable at 97.74 and 97.16 percent respectively. We have assessed accuracy by: 1)Comparing duplicate samples; 2) Comparing blood DNA with buccal cell DNA from the same people; and 3) Comparing buccal cell DNA from sets of identical twins. Overall, there was greater than 99.3% concordance for blood and saliva genomic DNA samples.

2.4 Statistical analysis

Four categories of significance are relevant to the study design. Nominal significance refers to the uncorrected (for multiple testing) significance level (set at $p<0.05$) for the test of each SNP. Gene-based significance refers to the significance of the most significant SNP within a gene after correction for other tested SNPs within the gene. Experiment-wide significance refers to the significance level after controlling for the total set of assessed SNPs. Finally, genome-wide significance refers to significance after controlling for the entire potential set of SNPs across the genome (in practice defined as nominal significance with $p < .000001$). Analysis of the nominal significance of each SNP in predicting case-control status was conducted using the Cochran-Armitage trend test implemented in SAS v. 9.1 PROC FREQ. To draw inferences with respect to genes rather than to single SNPs, a gene-based approach was employed. Rather than attempting to model the SNPs within a gene via haplotype and/or regression approaches, we employed a gene-based permutation procedure (i.e., similar to the *minP* procedure from Chen et al., 2006). The first step of the permutation procedure was to reshuffle case/control status randomly within each matched-pair. Next, we calculated the Cochran-Armitage trend test for each SNP and this procedure was repeated approximately 10,000 times, each time capturing the largest chi-square test statistic (smallest p-value) across all SNPs within a gene. This provides an empirical distribution of the test statistic under the null (i.e. no association) hypothesis accounting for the linkage disequilibrium structure between SNPs. In this procedure, the strongest SNP association within a gene using the observed data is adjusted for

testing all other SNPs in that gene. Resulting p-values are considered 'gene-based' in that they reflect accurate statistical evidence for that gene. Roeder et al. (2005) have shown that this method has equivalent or better power (under a number of simulated conditions) than other available methods (Clayton et al., 2004; Fan and Knapp 2003; Zhang X., et al., 2003) proposed for conducting large-scale genetic association studies.

In this sample of mixed ethnicity, we first constructed a set of principal components capturing the genetic variation from additional SNP markers on the chip using the SMARTPCA procedure in EIGENSTRAT (Price et al., 2006; Patterson et al., 2006), then tested whether ethnic groups and cases/controls could be distinguished using discriminant analysis with the principal components as predictors. We tested whether self-defined ethnicity interacted with genotype status for the nominally-significant SNPs using logistic regression (detailed in Section 3.6).

3.0 Results

3.1 Demographics

A total of 231 matched pairs were formed (72% non-Hispanic white, 26% Hispanic). Of the 231 controls, 55 (24%) of matches were taken from the epidemiological set of controls (Miles et al., 1998) and 176 (76%) were taken from community-based samples (Young et al., 2002). Birth years are comparable in the two groups, but age at testing is higher in the controls, since controls were required to have reached their sixteenth birthday prior to assessment to allow for substance exposure. Cases are largely overlapping with the 192 probands used in the familial linkage analyses in Stallings et al., (2003; 2005), but included additional cases in treatment for adolescent antisocial drug dependence without a tested full biological sibling. The distributions of antisocial substance dependence composite scores in the case and control samples are non-overlapping with a nearly 4 S.D. difference in mean scores (3.38 - −0.42).

3.2 Gene/Marker information

Table 2 presents the 50 candidate genes, their chromosomal location, lengths, number of SNPs (both by design and realized), and measures of linkage disequilibrium between adjacent SNPs calculated by HaploView (Barrett et al., 2005). The SNP chip contained assays for 1548 unique SNPs distributed across the 50 candidate genes. Of these 1548, a small number (37) failed the reliability assessment built into the analysis software resulting in no calls, while 432 (27.9%) showed no or low (< .02 MAF) variability in this sample of 462 individuals. Six more markers were rejected for poor Hardy-Weinberg equilibrium, leaving a total of 1073 SNPs for tests of association with case-control status. For one small gene, HTR1A, the only SNP on the chip was eliminated because of low variability, reducing our number of candidate genes to 49. Average inter-SNP distance across the 45 genes with more than one useable SNP was approximately 50% larger than the originally-designed 3000 base interval, but still represents fairly saturated coverage of the genes, as indicated by the mean D' between adjacent SNPs. However, mean r^2 statistics for adjacent SNPs suggest that associations due to functional SNPs located between adjacent useable SNPs on the chip will be attenuated for most genes.

3.3 SNP results

Of the 1073 Cochran-Armitage trend tests for individual SNPs, 10 (.93%) showed nominally significant results at a $p < .01$ level, while 60 (5.59%) were nominally significant at a $p < .05$ level. The lowest nominal significance level was shown for rs2280376 in the neuronal nicotinic receptor alpha 2 subunit gene (CHRNA2). Table 3 presents the individual SNPs of nominal significance by gene. Table 3 lists the 18 genes in which at least one nominally significant SNP was found, the proportion of significant SNPs to the number of SNPs assayed, the significance level of the most significant SNP found in the gene, and the db_SNP identifier for the significant

SNPs. Some genes, such as the GABA receptor gamma 3 gene (GABRG3) and the mu opoid receptor gene (OPRM1), showed an apparent excess number of significant SNPs for the number of assays performed, while GABRB1, where a large number of SNPs were assayed, shows only one nominally significant SNP. Using permutation tests on a SNP-by-SNP basis, none of the results for individual SNPs remained significant, which is not surprising given that the conservative Bonferroni correction significance threshold for 1000 independent tests is 0.00005.

3.4 Gene results

We used the minP (Chen et al., 2006; Roeder et al., 2005) permutation procedure to establish the gene-based significance level for the most significant SNP within a gene, controlling for potential linkage disequilibrium across SNPs. Four of the 18 genes with at least one nominally significant SNP showed a gene-based min p of less than 0.1, although only two of these genes showed more than one significant SNP: CHRNA2 & OPRM1. Table 4 presents the most significant SNP within those genes, the gene-based significance level, and the significance level and odds ratios for the identified SNP. One additional analysis used a sum of p-values approach within each gene, and identified the same subset of genes as the min p approach, with the lowest p-value (0.01) for the two-SNP-assayed HTR3A (a serotonin receptor).

3.5 Haploview results

We used HaploView to explore four additional questions about the relationships among the nominally-significant SNPs. 1) *Do haplotype blocks on the same chromosome extend across gene boundaries?* The 18 genes with nominally-significant SNPs are scattered across 13 chromosomes. Only three pairs of genes are located within the same chromosomal arms (CNR1 & OPRM1, GABRA2 & GABRB1, and HTR3A & HTR3B). Although modest LD was seen across the gene borders, no linkage blocks were identified that included SNPs from both members of these pairs. 2) *Do nominally-significant SNPs within large genes such as GABRG3 cluster within a smaller subregion of the gene?* We examined the six genes (GABRB1, GABRG3, GRIA1, HTR7, MAOB, and SLC6A11) with multiple nominally-significant SNPs, but there was no indication of overall significance within the gene using the min-P test. Although physically separated by 22 kilobases, the two SNPs in GABRB1 are in very high LD. Within GABRG3, 17 of the 24 identified SNPs are clustered in a 108 kb region at the proximal end of the gene, but are distributed into at least four distinct blocks. The two SNPs from Table 4 for GRIA1 are separated by more than 100 kb. Within HTR7, the four significant SNPs are clustered within 34 kb at the proximal end of the gene, but are distributed across four haplotype blocks. In MAOB, the two adjacent SNPs are located at the 3' end of the gene and just distal. The four SNPs within SLC6A11 are in the proximal half of the gene, but were not identified as members of a single block. 3) *What degree of association is there among the SNPs within our two most plausible candidate genes (CHRNA2 & OPRM1)?* The two SNPs from Table 4 in CHRNA2 are strongly associated (D'=.93, r^2=.75) at the distal end of the gene. SNP rs735421 and three adjacent non-significant upstream SNPs also have high D' (> .95), but r^2 is low (< .1),due to reduced heterozygosity in the adjacent SNPs. The ten SNPs from Table 4 for OPRM1 are all located within 40 kb at the proximal end of the gene and cluster into one superblock, consisting of only four haplotypes. An adjacent upstream missense mutation (rs1799971) is neither individually significant, nor in strong LD with the block. 4) *Are the haplotype blocks within OPRM1 better predictors of case-control status than the best individual SNP (rs9478503)?* Permutation testing within Haplo View indicates that this is not the case, with non-significant results for all four common haplotypes.

3.6 Testing for the effect of ethnic background

Cases and controls in this study were matched on self-defined ethnicity, with two major ethnic groups represented (non-Hispanic Whites & Hispanics). Tang et al. (2005) among others have shown that self-defined ethnicity substantially overlaps with genetically-defined cluster membership, even for Hispanics, but note that regional variation exists in ancestral genetic proportions for this group. We used the SMARTPCA procedure in EIGENSTRAT (Price et al., 2006; Patterson et al., 2006) to generate ten principal components (PCs) derived from 894 SNPs located in the 9q34 chromosomal region that were also genotyped for this sample, but were not part of the candidate gene study. The PCs differed between the two major ethnic groups, with the first extracted principal component the single best predictor of group status. However, the PCs failed entirely to discriminate between cases and controls for the Hispanic subsample, and found only a suggestive difference on one PC for the non-Hispanic White sample, indicating that the case-control design for this study was not compromised by undetected population stratification. We tested whether self-defined ethnic group status (non-Hispanic White vs. other) interacted with SNP status in predicting case-control status for the 60 nominally significant SNPs identified in Table 4. For only one SNP (rs10024869 in GABRA2) out of the 60 SNPs listed in Table 4 was the interaction significant at the $p < .05$ level.

4.0 Discussion

We explored genetic differences between a sample of 231 males in treatment for adolescent antisocial substance dependence and a sample of community controls matched on the basis of sex, birth cohort, and ethnic background. The context for this study was the behavioral genetic evidence for genetic influences affecting both antisocial behavior and substance dependence and for substantial genetic overlap in these behaviors. We designed and employed a custom SNP chip to probe multiple SNPs within candidate genes nominated primarily on the basis of involvement in substance metabolism and reward pathways. Our final probe set included 1073 SNPs with useable data distributed across 49 candidate genes.

SNPs with nominal levels of significance ($p < .05$) were found in 18 of the 49 candidate genes. None of the nominally significant SNPs produced missense or nonsense mutations. A SNP (rs2280376) in the neuronal nicotinic receptor alpha 2 (CHRNA2) gene showed the lowest level of significance ($p<0.002$). In genes with a large number of probed SNPs, the mu opiod receptor gene (OPRM1) and the GABA receptor gamma 3 gene (GABRG3) had more than 10% of their SNPs reach nominal significance levels. Although none of the SNPs in any of the probed genes attained either experiment-wide or genome-wide significance after correction for multiple testing, SNPs within several genes achieved gene-based significance. Two genes, CHRNA2 and OPRM1, each probed with multiple SNPs, emerged as plausible candidates for a genetic role in antisocial drug dependence after gene-based permutation testing. Neither CHRNA2 (on Chromosome 8), nor OPRM1 (on Chromosome 6) were in regions implicated by previous linkage results. Additionally, genes in regions of interest based on linkage, such as DBH, did not survive the gene-based permutation testing.

Although CHRNA2 had been previously implicated in nicotine dependence in schizophrenic families (Faraone et al., 2004), evidence for a contribution of CHRNA2 to antisocial drug dependence is novel. However, evidence for a role of brain-expressed neuronal nicotinic receptors on substance dependence is quickly accumulating. Studies examining α4 and β2 null mutant mice have clearly demonstrated that α4 and β2 subunits are absolutely required to form the nAChR that binds nicotine with high affinity (Piciotto, 2005; Marubio et al., 1999). Two studies have reported associations between CHRNA4 haplotypes and smoking-related phenotypes (Feng et al., 2004; Li et al., 2005). Ehringer et al. (2007) found an association between SNPs in the CHRNA4 gene and current smoking and between both smoking and

alcohol subjective response phenotypes and a SNP in the CHRNB2 gene. The CHRNB3 and CHRNB5 genes have also emerged as strong candidates for nicotine dependence in two recent reports of a genome wide association and a high-throughput candidate gene survey (Bierut et al., 2007; Saccone et al., 2007). Additionally, CHRNB3 has been associated with alcohol abuse in males (Zeiger et al., 2007).

The mu opiod receptor is widely expressed in the brain, has a high affinity for β-endorphin and enkephalin and binds drugs such as heroin, morphine and methadone (Zhang H et al., 2006). Haplotypes in OPRM1 have been associated with drug dependence (Zhang H et al., 2006). Polymorphisms in OPRM1 have been associated with increased craving for alcohol (van den Wildenberg et al., 2007), positive subjective responses to first use of heroin (Zhang D et al., 2006) and smoking initiation and nicotine dependence (Zhang L et al., 2006). In a recent survey of SNP differences between heroin-dependent cases and controls, a polymorphism upstream of OPRM1 was among the most significant of those located in candidate genes (Nielsen et al., 2008).

In this study we have attempted a systematic investigation of the association between variation in 50 plausible candidate genes and antisocial substance dependence during adolescence using a single custom-designed genotyping platform. Although our gene list was certainly not exhaustive, candidates were chosen carefully from a larger list developed by the NIDA genetics consortium for a study of nicotine dependence (Saccone et al., 2007; Bierut et al., 2007), focusing on genes potentially related to substance dependence in general, as well as other externalizing disorders. Multiple SNPs within or proximate to those genes were selected for inclusion on the SNPChip on the bases of spacing, location (preferentially at interesting regions within the gene), and minor allele frequency. Average achieved inter-SNP intervals within the genes was 4600 base pairs.

Another strength of this study was the clear identification of cases in this case-control design. Cases were drawn from consecutive admissions of adolescent males to treatment programs for antisocial substance dependence who agreed to participate in our research center. Beyond their status as patients, cases averaged nearly four standard deviations more extreme on a composite of age- and sex-normed substance dependence and conduct disorder symptom measures than matched controls.

Only two major ethnic groups represented 98% of the cases and controls in this sample, Anglo and Hispanic (primarily of Mexican-American descent), the two groups that make up the largest proportions of patients admitted for treatment in the CADD's recruitment facilities. Populations with different ancestral SNP contributions, e.g., African-American subjects, may show different patterns of association between genetic polymorphisms and antisocial substance dependence; thus, it is unknown whether the findings from this study can be generalized across different ethnic groups.

The primary limitation of this study was its lack of power to cleanly identify any SNPs with genome-wide significance, despite the magnitude of their estimated effects. The top ten SNPs identified on the basis of nominal significance yielded odds-ratios between 1.5 and 1.7 when logistic regressions of case status on allele frequency were conducted. We estimate that a three-fold sample size increase would yield genome-wide significance with those estimated odds ratios, while a two-fold sample size increase would yield experiment-wide significance levels.

In this study, we chose SNPs within our candidates on the basis of physical spacing. There is no consensus on the best strategy for the selection of SNPs. While selection of SNPs based on HapMap data to maximize coverage of all genetic diversity in selected populations can be effective, recent work has also shown that "pseudo random" selection of SNPs, particularly at high density, can be more effective at uncovering diversity in discreet populations (Barrett and

Cardon, 2006; Carlson, 2006). It can be reasonably argued, given a very large set of SNPs in a region, that haplotypes defined within populations other than those in the current study may exclude the most important polymorphisms if a selected population, e.g., Hispanics, differs from that used to develop the HapMap.

Another choice made in this study was to limit our investigation to a small subset of the genome, represented by the selected 50 candidate genes, rather than use commercially available chips, such as the Affymetrix 500K chip set, to probe a much wider selection of the genome. Although cost played a role in the adoption of the custom SNP chip strategy, our primary concern was the decreased confidence in any observed positive statistical association in a case-control sample of this size when SNPs to be tested are essentially chosen at random. A recent simulation study by Sullivan (2007) suggests that when large numbers of SNPs are assayed, true associations will likely be swamped by associations due to chance in smaller association studies such as ours.

The true test for association findings such as ours comes from replication. Our group in Colorado, in collaboration with researchers in San Diego [Sandra Brown, PI] has obtained a replication sample of probands that will triple our sample size of cases, and collected comparable phenotypic data from siblings and other family members as well. We are currently seeking funding to assess the persistence of antisocial substance dependence into adulthood, and to genotype those families in which these behaviors persist.

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Table 1

Study sample characteristics

	Controls (n=231)				Cases (n=231)			
	Mean	**Std. Deviation**	**Min**	**Max**	**Mean**	**Std. Deviation**	**Min**	**Max**
Birth year	1981.95	2.51	1975	1989	1981.62	2.38	1974	1986
Age at test	17.95	1.55	16.01	26.93	16.36	1.18	13.27	19.71
Antisocial substance depdendence	−0.42	0.64	−1.63	0.97	3.38	1.24	1.05	6.80

Table 2

SNP distribution on the SNPChip

Gene	Location	Function	Total length	SNPs on chip	Useable SNPs	Inter-SNP distance	Mean adj_D'	Mean adj_r^2	MAF mean (sd)
CHRNA2	8p21.1	Nicotinic receptor alpha 2	18,493	9	7	3,338	0.84	0.46	0.19 (0.10)
CHRNA5	15q24	Nicotinic receptor alpha 5	28,532	11	9	4,250	0.98	0.56	0.31 (0.10)
CHRNB2	1q21	Nicotinic receptor beta 2	12,245	6	3	6,749	0.86	0.03	0.18 (0.14)
CHRNB3	8p11.2	Nicotinic receptor beta 3	39,647	10	7	6,083	0.99	0.84	0.17 (0.11)
CNR1	6q14-15	Cannabinoid Receptor 1	26,083	13	9	3,005	0.82	0.48	0.28 (0.10)
COMT	22q11.2	Catechol O-methyl transferase	28,116	12	7	4,969	0.95	0.19	0.28 (0.20)
CREB1	2q32.3-34	cAMP resp-element bind protein	68,875	22	15	4,775	0.99	0.59	0.24 (0.11)
CYP2B6	19q13.2	Cytochrome P450-2B6	27,097	13	11	2,643	0.98	0.26	0.21 (0.14)
CYP2E1	10q24.3	Cytochrome P450-E	33,857	7	4	4,751	0.96	0.51	0.16 (0.08)
DBH	9q34	Synthesize norepinephrine	22,981	9	4	5,939	0.65	0.19	0.37 (0.20)
DRD1	5q35.2	D(1A) dopamine receptor	3,126	6	5	1,981	0.99	0.33	0.25 (0.15)
DRD2	11q23	Dopamine D_2 receptor	65,575	28	19	3,793	0.92	0.47	0.23 (0.13)
DRD3	3q13.3	Dopamine D_3 receptor	50,199	19	7	8,794	0.93	0.19	0.26 (0.17)
DRD4	11p15	Dopamine D_4 receptor	3,398	7	5	5,755	0.94	0.44	0.28 (0.10)
GABRA2	4p13	GABA receptor alpha 2	140,361	57	46	3,203	0.98	0.84	0.40 (0.09)
GABRA4	4p14	GABA receptor alpha 4	75,507	29	26	2,801	0.97	0.51	0.31 (0.15)
GABRA5	15q11.2	GABA receptor alpha 5	113,598	15	11	11,528	0.81	0.53	0.35 (0.14)
GABRA6	5q31.1	GABA receptor alpha 6	16,141	8	7	3,110	1.00	0.21	0.27 (0.12)
GABRB1	4p13	GABA receptor beta 1	395,084	156	117	3,401	0.95	0.45	0.26 (0.13)
GABRG1	4p14	GABA receptor gamma 1	88,312	34	28	3,116	0.97	0.78	0.43 (0.11)
GABRG2	5q31.1	GABA receptor gamma 2	87,827	38	23	3,997	0.86	0.31	0.28 (0.12)
GABRG3	15q11.2	GABA receptor gamma 3	652,193	226	178	3,682	0.85	0.46	0.28 (0.15)
GRIA1	5q33.2	Glutamate receptor 1 precursor	320,855	144	108	3,039	0.96	0.48	0.29 (0.14)
HTR1A	5q11.2	Serotonin receptor 1A	1,268	1	0	---	---	---	
HTR1B	6q13	Serotonin receptor 1B	1,259	2	1	---	---	---	0.40
HTR1D	1p36.12	5-hydroxytryptamine 1D receptor	4,229	5	4	2,670	1.00	0.94	0.33 (0.02)
HTR1E	6q15	5-hydroxytryptamine 1E receptor	79,145	27	20	4,242	0.92	0.31	0.12 (0.10)
HTR1F	3p11.2	5-hydroxytryptamine 1F receptor	2,127	3	1	---	---	---	0.29
HTR2A	13q14	Serotonin receptor 2A	62,665	30	21	3,045	0.86	0.28	0.27 (0.16)
HTR2B	2q36.3	Serotonin receptor 2B	16,869	8	4	4,191	1.00	0.27	0.23 (0.16)
HTR2C	Xq23	5-hydroxytryptamine 2C receptor	326,073	128	67	4,938	??	??	0.15 (0.08)
HTR3A	11q23	Serotonin receptor 3A	15,201	8	2	6,249	0.96	0.45	0.41 (0.06)
HTR3B	11q23	Serotonin receptor 3B	41,763	17	10	4,501	0.93	0.19	0.19 (0.14)
HTR4	5q32	5-hydroxytryptamine 4 receptor	203,037	85	57	3,707	0.95	0.49	0.31 (0.15)
HTR5A	7q36.2	5-hydroxytryptamine 5A receptor	15,425	11	10	2,400	0.97	0.6	0.32 (0.04)
HTR6	1p36.13	5-hydroxytryptamine 6 receptor	14,275	8	5	5,211	0.97	0.48	0.17 (0.06)
HTR7	10q23.31	5-hydroxytryptamine 7 receptor	117,093	46	34	3,720	0.92	0.24	0.23 (0.14)
MAOA	Xp11.23	Monoamine oxidase A	90,659	32	18	5,622	??	??	0.27 (0.10)
MAOB	Xp11.23	Monoamine oxidase B	115,835	40	7	16,843	??	??	0.20 (0.15)
NPY	7p15.1	Neuropeptide Y	7,670	5	4	3,324	0.96	0.35	0.16 (0.19)
NPY1R	4q36	Neuropeptide Y receptor	8,631	4	3	5,323	0.77	0.48	0.06 (0.04)
OPRD1	1p36.1	Opiate receptor delta 1	51,554	16	10	6,028	0.92	0.35	0.27 (0.17)
OPRK1	8q11.2	Opiate receptor kappa 1	25,918	14	11	2,594	0.89	0.44	0.12 (0.07)
OPRM1	6q24	Opiate receptor mu 1	207,557	32	28	3,086	0.98	0.45	0.20 (0.10)
SLC2A1	3p25	GABA transporter type 1	46,493	21	17	2,976	0.75	0.25	0.25 (0.12)
SLC6A11	3p23.3	GABA transporter type 3	122,229	53	41	---	0.94	0.32	0.26 (0.14)
SLC6A2	16q12.2	Norepinephrine transporter	48,628	24	18	3,045	0.89	0.28	0.29 (0.14)
SLC6A3	5p15.3	Dopamine transporter	52,631	28	15	4,056	0.79	0.23	0.29 (0.13)
SLC6A4	17q11.1	Serotonin transporter	37,799	17	8	5,279	0.93	0.54	0.34 (0.17)
TH	11p.15.5	Tyrosine hydroxylase	7,877	4	1	---	---	---	0.36

Table 3

Nominally significant SNPs by gene

Gene	Proportion Significant	Minimum Significance	Nominally significant SNPs, from most significant to least within gene						
CHRNA2	2:7	p < .002	rs2280376	rs735421					
CNR1	1:9	p < .02	rs806375						
CYP2B6	1:12	p < .02	rs2306606						
DBH	1:4	p < .05	rs739398						
DRD3	1:8	p < .03	rs7619130						
GABRA2	1:46	p < .03	rs10024869						
GABRB1	2:119	p < .03	rs4376121	rs6289					
GABRG3	24:177	p < .005	rs9744111	rs7171856	rs4555109	rs12148267	rs2889395	rs4887549	rs7179236
			rs6606858	rs1432127	rs12591360	rs4132750	rs12372959	rs4887551	rs724456
			rs2195817	rs8041947	rs9672931	rs1864797	rs4555125	rs891802	rs8042799
			rs11635092	rs2116526	rs1432129				
GRIA1	2:106	p < .02	rs11167640	rs10040991					
HTR3A	1:2	p < .03	rs10160548						
HTR3B	1:9	p < .03	rs12288145						
HTR7	4:34	p < .02	rs12249377	rs11597471	rs7087400	rs6583737			
MAOB	2:7	p < .05	rs1040400	rs2239449					
NPY1R	1:3	p < .05	rs13306006						
OPRM1	10:28	p < .005	rs9478503	rs3778156	rs495491	rs499796	rs511435	rs3823010	rs9478501
			rs524731	rs557748	rs3778148				
SLC6A11	4:43	p < .03	rs901026	rs971930	rs3774118	rs11715462			
SLC6A2	1:18	p < .02	rs10521329						
SLC6A3	1:14	p < .05	rs3776513						

Table 4

Gene-based tests for SNPs using minP permutation procedure (10,000 replicates).

GENE	SNP	p-value (gene)	p-value (snp)	Odds Ratio
CHRNA2	rs2280376	0.007	0.002	1.60 (1.19, 2.15)
HTR3A	rs10160548	0.033	0.021	1.37 (1.05, 1.81)
OPRM1	rs495491	0.060	0.006	1.47 (1.11, 1.96)
CYP2B6	rs2306606	0.082	0.018	0.69 (0.52, 0.94)

Note: Odds ratios are allelic odds ratios with 95% confidence intervals.