The *AVPR1A* Gene and Substance Use Disorders: Association, Replication, and Functional Evidence

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Background: The liability to addiction has been shown to be highly genetically correlated across drug classes, suggesting nondrug-specific mechanisms.

Methods: In 757 subjects, we performed association analysis between 1536 single nucleotide polymorphisms (SNPs) in 106 candidate genes and a drug use disorder diagnosis (DUD).

Results: Associations ($p \leq .0008$) were detected with three SNPs in the arginine vasopressin 1A receptor gene, *AVPR1A*, with a gene-wise p value of 3×10^{-5}. Bioinformatic evidence points to a role for rs11174811 (microRNA binding site disruption) in AVPR1A function. Based on literature implicating *AVPR1A* in social bonding, we tested spousal satisfaction as a mediator of the association of rs11174811 with the DUD. Spousal satisfaction was significantly associated with DUD in males ($p < .0001$). The functional *AVPR1A* SNP, rs11174811, was associated with spousal satisfaction in males ($p = .007$). Spousal satisfaction was a significant mediator of the relationship between rs11174811 and DUD. We also present replication of the association in males between rs11174811 and substance use in one clinically ascertained ($n = 1399$) and one epidemiologic sample ($n = 2231$). The direction of the association is consistent across the clinically-ascertained samples but reversed in the epidemiologic sample. Lastly, we found a significant impact of rs11174811 genotype on AVPR1A expression in a postmortem brain sample.

Conclusions: The findings of this study call for expansion of research into the role of the arginine vasopressin and other neuropeptide system variation in DUD liability.

Key Words: Addiction, alcoholism, gene systems, genetic association, social relationships, vasopressin

Consistent with its complex etiology, the heritability of the liability to substance use disorders is determined by the additive effects of genes that are largely, if not entirely, shared between specific drug classes (1-3). The genetic correlations between these groups may be reflective of an overlap between neurobiological systems influencing etiologic pathways through drug use to dependence. The considerable shared genetic variance among the various drug use disorders (DUDs), particularly those related to illicit drugs, supports the concept of common (nondrug-specific) DUD liability (4,5), a latent trait encompassing all factors influencing the probability of developing the disorder (6).

Progress in genetic methodology and the development of a dense set of single nucleotide polymorphisms (SNPs) and linkage disequilibrium (LD)-tagging SNPs that capture a substantial proportion of common genetic variation create conditions for genetic studies of disorders of complex etiologic architecture like DUD. Genome-wide association scans have become a common approach in these studies. Nevertheless, although genome-wide association scans have proven useful in identifying some regions influencing variation in psychiatric/behavioral traits, a major drawback is that signals expected for complex diseases are unlikely, given realistic sample sizes, to meet strict thresholds for genome-wide significance, and true signals are likely to be blended with false signals (7).

Information about gene function allows the focus on variation in relevant candidate systems of genes (CSG) that influence behavior related to drug use, as well as drug response. The CSG approach provides the advantages of hypothesis-driven research, while mitigating some limitations of the candidate gene approach that usually targets only a few, sometimes functionally unrelated, loci. The CSG approach is a viable alternative to both narrowly focused candidate gene and genome-wide studies, combining the hypothesis testing of candidate gene studies and a wide systemic scope in a targeted gene search. Testing candidate systems concurrently enables examination of a reduced portion of the genome. This approach increases the prior probability of detecting true associations and places these findings in the context of neurobiological knowledge.

Data increasingly suggest that many neurobiological systems contribute to drug reward and behaviors associated with DUD risk, including, for example, social behaviors. Social behaviors, including parent-child bonding, affiliation with deviant peers, and marital

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Received May 7, 2010; revised Feb 1, 2011; accepted Feb 3, 2011

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.02.023

satisfaction, have been shown to influence the risk for a variety of psychiatric disorders including substance abuse. The biological and genetic bases for these pair-bonding behaviors have been studied extensively in model organisms. The best known example is the *AVPR1A* gene, which has been shown to have a major role explaining intraspecific and interspecific differences in the social and mating behaviors of voles (8-11). The capacity of vasopressin to modulate these behaviors is thought to depend on the species-specific distribution patterns of arginine vasopressin receptor 1A (AVPR1A) receptors in the brain, which is influenced by genetic variation near the gene promoter (12). These two homologous microsatellite polymorphisms, RS1 and RS3, exist in humans and have been linked to sibling conflict (13) and autism, the central feature of which is impaired social interactions, potentially through effects on amygdala function (14). Further links to social behavior in humans were demonstrated by association of RS1-RS3 haplotypes with the personality trait reward dependence (13), association of RS3 with altruistic behavior (15), and a recent finding of an association between *AVPR1A* and pair bonding in humans (16). Interestingly, mice lacking *AVPR1A* display increased ethanol intake (17).

Herein, we describe an application of the CSG approach in a genetic association study of 106 genes (Table S1 in Supplement 1) tagged using 1536 SNPs in a discovery sample of substance abuse cases and screened control subjects. In our discovery sample, we find compelling evidence for the association of AVPR1A with substance use. Importantly, an SNP, rs11174811, in the associated region is predicted to disrupt a microRNA (miRNA) binding site. Although the mechanisms are generally unknown, miRNAs are known regulators of gene expression. Single nucleotide polymorphisms affecting miRNA binding targets could have a significant effect on target gene expression through the creation/disruption of miRNA target sequences (18,19). Our subsequent work focused on extending and replicating the association with rs11174811. Considering that the risk for substance use disorder is associated with deviations in social behavior, including marital instability, we hypothesized that the *AVPR1A*-DUD liability association is partially explained by an *AVPR1A*-associated sociobehavioral processes. We found evidence, in our initial sample, that spousal satisfaction mediated the relationship between rs11174811 and DUD in male subjects. We also attempted replication of our findings in two male European-American (EA) data sets, a large population-based US twin sample and a large alcoholism case-control sample. While the results are equivocal, we found nominally significant associations with substance use in both samples. Lastly, we provide evidence that rs11174811 is associated with expression levels of *AVPR1A* in brain tissue.

Methods and Materials

Because of space constraints, the sample recruitment and methods are described in detail in Supplement 1. Here, we provide a brief description of the subject recruitment and the discovery and replication samples, as well as a limited description of the statistical methods.

Subject Recruitment

Initial Discovery Sample: Center for Education and Drug Abuse Research and Substance Abuse and the Dopamine System. The Center for Education and Drug Abuse Research (CEDAR) sample was ascertained as part of a large longitudinal study of substance abuse risk. Nuclear pedigrees were ascertained through the father, who did (case; DUD+) or did not (control subject; DUD−) have a substance use disorder (abuse or dependence) related to an illicit drug (an illegal substance or nonmedical use of a prescribed psychoactive drug). A DSM-III-R diagnosis was used because DSM-IV was introduced after this study started. Control subjects had no Axis I or II psychiatric disorder. Both cases and control subjects were required to have no history of psychosis, a Wechsler Adult Intelligence Scale-Revised full-scale IQ in the normal range, and good health status and had a 10- to 12-year old child at the time of ascertainment and first assessment.

Probands in the Substance Abuse and Dopamine System (SADS) study were male subjects 12 to 18 years of age, having a DSM-IV diagnosis of substance dependence related to use of illicit drugs. Probands were recruited from substance abuse treatment programs. The CEDAR and SADS subjects were self-identified European-Americans from the same Greater Pittsburgh geographic area, and the genomic inflation factor based on all genotyped SNPs, evaluating the excess false-positive rate, was satisfactory at .98.

Virginia Twin Study of Psychiatric and Substance Use Disorders. The Virginia Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD) was ascertained through the Mid-Atlantic Twin Registry at Virginia Commonwealth University with minimal inclusion criteria: birth in the Commonwealth of Virginia within the target age cohort. In this data set, we focus on 2231 self-identified European-American male subjects. Drug use disorder is operationalized as lifetime dependence on any illicit substance (9.68%). Previous work in this sample has demonstrated a satisfactory genomic inflation factor of .99 (20).

Collaborative Studies on the Genetics of Alcoholism. The Collaborative Studies on the Genetics of Alcoholism (COGA) study's European-American male sample, ascertained for the presence/absence of alcohol dependence, was used for replication of findings. The COGA project, started in 1989, is one of the largest genetic studies of alcoholism and related traits (21,22). Recently, COGA collected a large case-control sample to complement its large multiplex pedigree sample (23). Genome-wide genotypic data are available for this sample. Alcohol-dependent probands were ascertained through alcohol treatment programs and evaluated at seven centers in the United States. The same seven centers also recruited community probands through driver's license records, random mailings to employees and students at a university, and attendees at medical and dental clinics. For the study, a sample of genetically unrelated cases and control subjects were selected from this pool of alcohol- dependent and community ascertained families. As described in Supplement 1, membership in the EA subset was verified using Multidimensional Scaling. The final EA sample included 847 alcohol-dependent cases and 552 control subjects (n = 1399 individuals).

The main criterion for system/gene selection was participation in response to more than one drug class and/or drug abuse-related behaviors. The gene set is based on and substantially overlaps with that selected for the large-scale genotyping by the National Institute on Drug Abuse Genetics Consortium (24). Genes (n = 456) were prioritized on a 1 to 5 scale and submitted for SNP selection. We iteratively adjusted our LD-tagging criteria to allow for inclusion of 106 first-priority candidate system genes in our panel for an Illumina 1536 SNP oligonucleotide pool assay.

Statistical Methods

Discovery Sample. We performed a sex-stratified (Cochran-Mantel-Haenszel test) initial association analysis on 1536 LD-tagging SNPs in 106 candidate system genes in an EA sample that included 359 male subjects affected with a DUD, 138 male control subjects, 39 female subjects affected with a DUD, and 221 female control subjects. Gene-wise significance was calculated using Fisher's approach to combining p values across the gene. To account for dependence between the SNPs, a null distribution of Fisher's com-

bined *p* values (25) was constructed by permuting case-control status 1 million times. Significance of the actual *p* values was assessed against the distribution of permuted *p* values.

Mediation/Moderation Modeling

We assessed mediation of association between an *AVPR1A* SNP (rs11174811) and DUD risk in adult male subjects (CEDAR sample) by a measure of spousal satisfaction from the Dyadic Relationship Scale of the Family Assessment Measure (FAM) (26). This relationship, with a latent FAM variable as a mediator, while modeling the latter's factor structure, was tested using Mplus (27).

Before the analyses testing that hypothesis, we examined the factor structure of the FAM. The FAM Dyadic Relationship Scale consists of 42 items organized theoretically into seven subscales (task accomplishment, role performance, communication, affective expression, involvement, control, values and norms). On this scale, one member of a spousal pair rates his or her relationship with the spouse. Examples of item text include, "This person still likes me even when I argue with him/her" and "This person often ruins things for me." The subscales are, in our data and in previous studies (28), highly correlated ($r \approx .65$–$.8$). Exploratory factor analysis showed that after dropping a single item that loaded to its own factor, a single factor had the best fit to the data. Unidimensionality was verified by confirmatory factor analysis.

We applied several approaches to modeling the factor structure of the FAM: all 41 items were specified as indicators of a single latent factor; a second order factor model with the 41 items indicating seven latent factors (with items grouped according to the theoretical scales of the original measure) loading to a single latent FAM factor; and seven FAM subscale sum scores indicating a single latent FAM variable. In each case, model fit was excellent, and the standardized coefficients for the paths describing the relationships between the measured variables (a, b, and c′ paths in classic mediation modeling [29]) across the models were nearly identical.

VATSPSUD Replication

We attempted replication of the association finding in a sample of 2231 EA male twins (9.68% substance dependent) ascertained through the Virginia Twin Study of Psychiatric and Substance Use Disorders. We used generalized linear models (nesting family ID and zygosity) to account for dependence structure induced by twin data by modeling the impact of genotype on phenotype while accounting for degree of relationship (monozygotic/dizygotic).

COGA Replication

We tested association between rs11174811 and two core COGA phenotypic measures, alcohol dependence and maximum drinks in a 24-hour period in the male European-American sample ($n = 745$). The SNP was modeled as the number of A alleles of the associated SNP using a linear model in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) (30) for the quantitative trait (maximum drinks per day).

Assessment of Impact of rs11174811 on Expression of *AVPR1A* in Postmortem Brain Tissue

The risk-associated SNP rs11174811 is predicted to disrupt a miRNA binding site. We predicted that, if this SNP is functional and impacts expression, individuals carrying the minor, binding-site disrupting allele would exhibit higher levels of *AVPR1A* expression. If the human data prove consistent with the animal data (8,11), those carrying the common allele would be at higher risk for substance abuse because of decreased *AVPR1A* expression mediated by hsa-miR-578 and hsa-miR-526b (see Bioinformatic Evidence in Supplement 1).

We assessed *AVPR1A* gene expression in RNA from the prefrontal cortex of the control group from the Stanley Medical Research Institute sample. The Stanley Foundation Brain Collection sample was genotyped for rs11174811 polymorphisms, which is located within the seed sequence of two miRNAs (hsa-miR-578 and hsa-miR-526b) predicted to bind to the 3′ untranslated region of the *AVPR1A* gene. The minor allele of this polymorphism is expected to disrupt the miRNAs binding and thus release the inhibitory effect of hsa-miR-578 and hsa-miR-526b on *AVPR1A* expression levels.

We assessed the impact of variation at rs11174811 on brain expression levels of *AVPR1A*. A sample of 91 postmortem brain samples (dorsolateral prefrontal cortex) from the Stanley Medical Research Institute was used to conduct quantitative real-time polymerase chain reaction to test the functional significance of the genetic findings. The association was tested by *t* test and linear regression to account for important covariates (pH, postmortem interval, sex, diagnosis).

Results

The Discovery Study: CEDAR-SADS Sample

Candidate System Association Analysis. Table 1 presents the top 50 results for the single SNP analyses in male and female subjects combined, as well as the results of allelic tests performed separately by gender. The genotypic odds ratios and accompanying *p* values derive from the Cochran-Mantel-Haenszel tests stratified by gender. In each of the 50 SNPs, the Breslow-Day tests indicate no heterogeneity. The allelic odds ratios are obtained in Pearson $1-\text{df}\ \chi^2$ allelic association tests on the data arranged in 2×2 contingency tables. Throughout, we present *p* values that are not corrected for multiple comparisons. If we were to treat each gene as an independent hypothesis test, we would set a Bonferroni experiment-wide corrected *p* value for gene-wise tests at a threshold of $\sim 4.7 \times 10^{-4}$ after controlling for 106 genes.

As can be seen from Table 1, three SNPs in the *AVPR1A* receptor gene, rs1587097 [$p = .0003$, odds ratio (OR) = 2.02 (1.37–3.00)], rs10784339 [$p = .0008$, OR = 1.72 (1.25–2.36)], and rs11174811 [$p = .0008$, OR = 1.72 (1.25–2.36)] generate the most significant results. In addition, these results are consistent across the genotypic and allelic tests. The results across the *AVPR1A* gene, including the allelic and sex-specific tests and the degree of LD between the SNPs, are presented in Figure 1. At the SNP level, no individual tests meet the strict Bonferroni correction threshold. The gene-wise permuted *p* value was 3×10^{-5}. Whereas male and female subgroup tests yielded similar odds ratios, the relatively small female sample lacked power to yield a significant result at that effect size.

Spousal Relationship as a Mediator of the Association of AVPR1A with DUD: To test the mediational hypothesis, we used available data on the quality of spousal relationship, a human indicator for pair bonding, measured by the Dyadic Relationship Scale of the Family Assessment Measure (26). Figure 2 illustrates the best-fitting model tested. As can be seen in Figure 2, there are significant relationships between *AVPR1A* and FAM ($p = .006$), FAM and DUD ($p < .001$), and between *AVPR1A* and DUD risk ($p = .006$) in male subjects. The latter relationship is also mediated by FAM, as indicated by the significant indirect path ($p = .013$). This mediation is not complete, suggesting the involvement of additional factors in the highly significant relationship between *AVPR1A* and DUD liability. The alternative model, assuming DUD as mediator of the association between *AVPR1A* and FAM, demonstrated an equivalent fit.

Family Assessment Measure score was associated with DUD in male subjects [$p < .0001$; OR (95% confidence interval) = 2.51 (1.80–3.50)] but not female subjects [$p = .09$; OR (95% confidence

Table 1. Results of Top 50 SNPs Ranked by Combined (Cochran-Mantel-Haenszel) *p* Value

CHR	Gene	SNP	Alleles	Case MAF	Control MAF	Genotypic OR	*p*	Allelic OR	*p*	Female Allelic OR	*p*	Male Allelic OR	*p*
12	AVPR1A	rs1587097	A/G	.15	.09	2.02 (1.37–3)	3.30E-04	1.75 (1.27–2.42)	5.915E-04	2.21 (1.16–4.21)	.014	1.95 (1.2–3.17)	5.968E-03
12	AVPR1A	rs10784339	C/G	.22	.15	1.72 (1.25–2.36)	7.76E-04	1.59 (1.22–2.07)	5.242E-04	1.6 (.9–2.84)	.106	1.77 (1.21–2.59)	3.197E-03
12	AVPR1A	rs11174811	A/C	.22	.15	1.72 (1.25–2.36)	7.76E-04	1.59 (1.22–2.07)	5.242E-04	1.6 (.9–2.84)	.106	1.77 (1.21–2.59)	3.197E-03
23	GRIA3	rs557762	G/A	.16	.24	0.52 (.35–.77)	9.40E-04	.61 (.44–.84)	2.513E-03	.47 (.23–.95)	.031	.55 (.34–.88)	1.194E-02
9	SLC1A1	rs12553697	G/A	.11	.16	.56 (.39–.79)	1.03E-03	.66 (.49–.88)	5.322E-03	.48 (.2–1.15)	.092	.58 (.4–.85)	4.800E-03
5	GABRG2	rs2268582	A/G	.12	.17	.57 (.41–.8)	1.14E-03	.65 (.48–.87)	3.401E-03	.61 (.28–1.32)	.204	.56 (.39–.82)	2.425E-03
5	HTR4	rs4597955	G/A	.4	.48	.68 (.53–.87)	1.65E-03	.75 (.61–.92)	4.963E-03	.87 (.53–1.4)	.557	.63 (.47–.83)	9.555E-04
6	OPRM1	rs483481	A/G	.44	.39	1.47 (1.14–1.88)	2.60E-03	1.2 (.98–1.48)	.077	1.17 (.72–1.88)	.522	1.59 (1.19–2.13)	1.798E-03
5	SLC1A3	rs891189	G/A	.47	.51	.69 (.54–.88)	2.71E-03	.86 (.7–1.05)	.140	.81 (.5–1.31)	.379	1.53 (1.15–2.02)	3.077E-03
4	TACR3	rs3796958	G/A	.18	.12	1.61 (1.15–2.26)	4.12E-03	1.58 (1.19–2.11)	.002	2.7 (1.5–4.86)	.001	1.33 (.9–1.99)	.155
9	SLC1A1	rs2039216	G/A	.06	.03	2.74 (1.36–5.52)	.01	2.43 (1.43–4.12)	.001	.73 (.16–3.26)	.679	4.09 (1.61–10.37)	.001
4	TACR3	rs11733295	G/A	.18	.12	1.56 (1.12–2.17)	.01	1.55 (1.17–2.07)	.002	2.64 (1.47–4.75)	.001	1.29 (.87–1.91)	.199
7	TAS2R38	rs1726866	G/A	.43	.49	.72 (.57–.91)	.01	.78 (.64–.96)	.019	.88 (.54–1.41)	.585	.67 (.51–.89)	.005
5	SLC1A3	rs7734056	A/T	.19	.14	1.57 (1.13–2.2)	.01	1.41 (1.07–1.85)	.015	1.48 (.82–2.69)	.191	1.61 (1.08–2.41)	.019
23	GRIA3	rs551166	G/A	.25	.18	1.67 (1.14–2.43)	.01	1.47 (1.08–2.01)	.014	1.73 (1.01–2.96)	.046	1.62 (.96–2.74)	.068
4	GABRA4	rs13139021	A/G	.33	.28	1.43 (1.1–1.86)	.01	1.31 (1.05–1.64)	.015	1.76 (1.07–2.9)	.024	1.32 (.97–1.8)	.074
5	HTR4	rs3995090	C/A	.43	.35	1.4 (1.09–1.79)	.01	1.36 (1.11–1.68)	.004	1.09 (.67–1.78)	.721	1.52 (1.14–2.03)	.004
4	NPY1R	rs4234955	G/A	.23	.27	.69 (.53–.91)	.01	.81 (.64–1.02)	.070	.57 (.3–1.08)	.080	.73 (.54–1)	.047
15	CHRM5	rs554303	A/G	.21	.25	.69 (.52–.92)	.01	.81 (.63–1.03)	.079	.8 (.44–1.46)	.467	.66 (.49–.91)	.011
6	ESR1	rs7757956	A/T	.16	.13	1.54 (1.09–2.18)	.01	1.28 (.96–1.71)	.088	1.86 (1.04–3.33)	.033	1.42 (.93–2.16)	.102
12	AVPR1A	rs11836346	G/A	.16	.12	1.58 (1.1–2.25)	.01	1.39 (1.04–1.86)	.026	1.34 (.71–2.54)	.363	1.68 (1.09–2.59)	.019
4	GABRA2	rs16859227	A/G	.23	.27	.7 (.53–.93)	.01	.82 (.64–1.03)	.090	.49 (.25–.96)	.034	.77 (.56–1.05)	.100
23	GRIA3	rs5956542	C/A	.54	.46	1.47 (1.08–1.99)	.01	1.39 (1.08–1.79)	.009	1.5 (.93–2.42)	.096	.69 (.47–1.03)	.067
7	TAS2R38	rs10246939	G/A	.38	.43	.72 (.55–.94)	.01	.82 (.66–1.03)	.081	.91 (.54–1.55)	.740	.67 (.49–.9)	.008
10	HTR7	rs2226116	A/C	.18	.14	1.52 (1.08–2.13)	.01	1.34 (1.01–1.77)	.042	1.54 (.85–2.79)	.155	1.51 (1.01–2.27)	.046
11	DRD2	rs2234689	G/C	.16	.2	.68 (.5–.92)	.02	.79 (.6–1.02)	.071	.48 (.22–1.04)	.057	.74 (.52–1.05)	.086
5	DRD1	rs265974	G/A	.35	.31	1.38 (1.06–1.79)	.02	1.2 (.97–1.49)	.091	1.05 (.64–1.73)	.854	1.53 (1.12–2.08)	.007
5	GABRA6	rs11959228	A/G	.19	.15	1.52 (1.09–2.12)	.02	1.29 (.98–1.69)	.066	1.11 (.6–2.05)	.736	1.72 (1.14–2.58)	.009
6	OPRM1	rs569284	C/A	.05	.04	2.19 (1.16–4.13)	.02	1.55 (.94–2.54)	.085	1.71 (.66–4.4)	.260	2.52 (1.05–6.03)	.032
6	ESR1	rs3020383	C/G	.1	.08	1.66 (1.08–2.55)	.02	1.35 (.94–1.94)	.100	2.39 (1.22–4.68)	.009	1.39 (.82–2.36)	.224
11	DRD2	rs12422191	A/G	.09	.11	.61 (.41–.91)	.02	.76 (.55–1.07)	.114	.22 (.05–.93)	.024	.72 (.47–1.11)	.137
5	SLC1A3	rs6451304	G/A	.09	.06	1.73 (1.08–2.76)	.02	1.55 (1.04–2.31)	.030	2.55 (1.21–5.4)	.012	1.46 (.82–2.59)	.191
5	HTR1A	rs1364043	C/A	.2	.23	.7 (.53–.94)	.02	.82 (.64–1.05)	.112	.67 (.35–1.3)	.235	.71 (.52–.98)	.040
5	HTR4	rs9325102	G/A	.04	.06	.53 (.31–.89)	.02	.79 (.5–1.26)	.326	.32 (.04–2.41)	.242	.56 (.33–.98)	.038
7	GRM3	rs2299227	A/G	.06	.03	2.08 (1.11–3.89)	.02	1.88 (1.12–3.17)	.016	2.48 (.92–6.66)	.063	1.93 (.89–4.19)	.092
5	HTR4	rs7721661	A/G	.17	.11	1.53 (1.07–2.18)	.02	1.57 (1.17–2.11)	.003	1.38 (.7–2.73)	.348	1.58 (1.04–2.4)	.030
23	GRIA3	rs5911556	A/C	.03	.02	4 (1.33–11.99)	.02	1.76 (.76–4.05)	.179	2.25 (.69–7.37)	.168		
11	DRD2	rs4648317	A/G	.16	.14	1.52 (1.07–2.16)	.02	1.19 (.9–1.58)	.222	1.17 (.63–2.16)	.623	1.71 (1.1–2.65)	.016
8	ADRA1A	rs10503800	A/C	.32	.29	1.37 (1.05–1.78)	.02	1.17 (.94–1.46)	.163	1.14 (.69–1.88)	.614	1.47 (1.07–2.01)	.017
9	SLC1A1	rs301434	A/G	.45	.5	.75 (.59–.96)	.02	.8 (.66–.98)	.033	.82 (.51–1.33)	.421	.73 (.55–.97)	.028
13	HTR2A	rs9567731	A/G	.3	.25	1.36 (1.04–1.79)	.02	1.26 (1.01–1.59)	.043	1.57 (.94–2.6)	.080	1.3 (.94–1.78)	.111
23	GABRA3	rs5970269	A/G	.17	.12	1.7 (1.08–2.65)	.02	1.49 (1.05–2.12)	.025	1.13 (.58–2.22)	.715	2.31 (1.2–4.43)	.010
6	GRM4	rs6901097	C/G	.41	.36	1.33 (1.04–1.7)	.03	1.22 (.99–1.51)	.057	1.23 (.76–2)	.397	1.36 (1.02–1.82)	.036
11	SLC1A2	rs4755409	A/T	.11	.14	.68 (.48–.96)	.03	.8 (.59–1.08)	.148	.99 (.47–2.11)	.989	.61 (.41–.9)	.011
5	ADRA1B	rs10053468	C/A	.09	.06	1.71 (1.06–2.77)	.03	1.43 (.97–2.12)	.073	1.74 (.79–3.82)	.162	1.7 (.93–3.09)	.082

Table 1. (continued)

CHR	Gene	SNP	Alleles	Case MAF	Control MAF	Genotypic OR	*p*	Allelic OR	*p*	Female Allelic OR	*p*	Male Allelic OR	*p*
23	AR	rs2361634	G/A	.06	.09	.5 (.27–.92)	.03	.64 (.39–1.05)	.072	.28 (.07–1.19)	.066	.63 (.31–1.25)	.183
8	CRH	rs6999100	G/A	.17	.16	1.44 (1.03–2.01)	.03	1.09 (.83–1.43)	.545	1.59 (.91–2.76)	.100	1.38 (.91–2.08)	.127
1	RGS1	rs1923949	G/A	.24	.29	.74 (.56–.97)	.03	.77 (.62–.97)	.029	.57 (.31–1.03)	.060	.8 (.59–1.09)	.159
17	CRHR1	rs4792825	G/A	.11	.09	1.63 (1.06–2.53)	.03	1.21 (.86–1.71)	.275	1.02 (.48–2.16)	.966	2.06 (1.16–3.66)	.012
8	ADRA1A	rs488323	C/A	.47	.44	1.31 (1.02–1.66)	.03	1.14 (.93–1.4)	.196	.9 (.56–1.45)	.652	1.49 (1.12–1.98)	.006

CHR, chromosome; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism.

interval) = 1.37 (.96–1.95)]. In a linear regression model, with the genotype indexed in an additive fashion, the *AVPR1A* SNPs found above to be associated with DUD liability were also associated with FAM factor score in male subjects ($p = .007$) but not female subjects ($p = .74$). Given that this finding was obtained in an EA male sample, we decided to focus replication efforts in male EA samples.

Replication Studies of Association Between *AVPR1A* and Substance Use Disorder

Replication in the VATSPSUD. The DUD liability-associated SNP rs11174811 was genotyped and the association with dependence on any illicit drug was tested. A male-specific significant relationship ($p = .007$) between dependence on any illicit substance and rs11174811 was detected. The frequency of the A allele was 14.9% in unaffected male subjects and 12.5% in affected male subjects. This association is thus in the direction opposite to that observed in the discovery study, where the frequency of the A allele was 15% and 22% in respective samples (Table 1). There was also a modest ($p = .036$) male-specific relationship between rs11174811 and perceived marital warmth.

Replication in the Collaborative Studies on the Genetics of Alcoholism Case-Control Sample. While alcohol dependence was not associated with rs11174811 ($p = .13$), modest association was detected for maximum drinks per day ($p = .03$; PLINK $\beta = 3.02$). This relationship was in the same direction as in the discovery sample.

AVPR1A Expression Studies

The expression levels in samples from 26 individuals heterozygous and homozygous for the minor (T) allele of rs11174811 were higher than in 76 individuals homozygous for the major allele (unpaired *t* test with Welch's correction, two-tailed $p = .01$; Figure 3). After controlling for relevant covariates, the significance of the association increased (Table S2 in Supplement 1, $p = .0003$).

Discussion

We report an association between *AVPR1A* and the risk for DUD. Consistent with the hypothesis that vasopressin (AVP) may influence the risk for dysregulated behavioral outcomes through social/affiliative behaviors, this association is mediated in the discovery sample, in part, by a measure of spousal relationship quality. While association or even immediate involvement of sociobehavioral mechanisms in the risk for DUD would not be surprising, the finding suggests a specific genetic foundation for this relationship and opens avenues for future research. It should be noted that the mediation relationship suggested by our findings does not necessarily identify the actual mediator of the association. The *AVPR1A* gene may have pleiotropic effects on affiliative and social behaviors, and spousal relationship quality may be an indicator of a more general socialization characteristic (e.g., attachment, peer selection, and bonding). Particularly germane, affiliation with deviant peers is among the social behaviors with consistent evidence of a role in substance abuse risk (31-36). In addition, parent-child bonding significantly reduces the risk for a substance abuse outcome (31,32,37-41). Social support or social network formation has also been related to substance abuse patterns (42-45).

While a genetic overlap between social behaviors and addictive processes may explain the phenotypic overlap, specific biological mechanisms have not been elucidated. However, many intriguing lines of evidence exist. For example, it is possible that "narcotic addiction operates partially through mechanisms which ensured mammalian social bonding over the course of evolution" (46), as supported, for instance, by involvement of the opioid system in

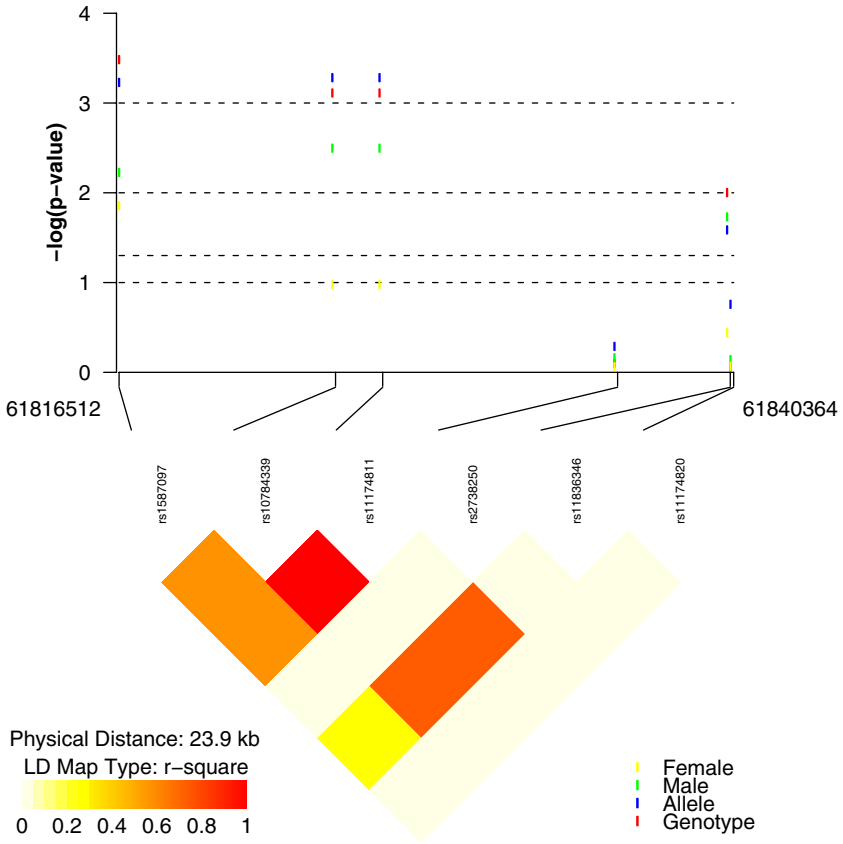

Figure 1. Association results across the *AVPR1A* region for the genotypic (Cochran-Mantel-Haenszel), allelic, and sex-limited allelic tests. –Log (*p*) for each single nucleotide polymorphism and association test plotted across the *AVPR1A* gene. The bottom panel displays linkage disequilibrium measures between the single nucleotide polymorphisms across the *AVPR1A* region. LD, linkage disequilibrium.

separation distress. It has also been noted (4) that illicit drug abuse, a common behavioral attribute in deviant social groups, may be part of the repertoire of affiliative and reproductive behavior consistent with Zahavi's handicap principle (47), where potentially socially disabling (e.g., resulting in imprisonment) behavioral/personality characteristics convey a fitness benefit in a deviant group and provide group protection. Drug abuse is also associated with insecure attachment (48,49) and can be a key for transition from parental to (delinquent) peer affiliation influence.

The primary neuropeptides linked to social behaviors are AVP and oxytocin (OXT), which play critical roles in pair bond formation, one of the most important relationships for human mental health (50). Centrally released OXT facilitates social motivations and approach behavior, including maternal nurturing behaviors (51). Vasopressin regulates several male-typical social behaviors, including scent marking, aggression (52), and paternal care (53). The AVP and OXT neural systems are also involved in other social behaviors, including anxiety (54), processing of social cues (55), and social recognition (56). Many of these effects are sex-specific. For instance, AVP has been shown to differentially influence social communication, decreasing it in male subjects and increasing it in female subjects (57). Vasopressin is more influential in male subjects and OXT is more influential in female subjects (58). It is important to consider our finding in the context of the existing literature. The finding most germane to our study supports the notion that alleles increasing *AVPR1A* expression would decrease marital stability in male subjects (16). It is thus not surprising that the effect sizes for the tests in male and female subjects in our discovery sample differ and do not reach significance in female subjects. It is important to caution that this may also be due to the smaller female sample and thus lower statistical power.

The finding that the association of the rs11174811 with DUD liability had opposite directions in the studies based on clinical samples (CEDAR/SADS and COGA) and in the population-based VATSPSUD study requires explanation. Although largely speculative at this stage, several possibilities can be contemplated. The association may be a spurious finding. This possibility conflicts, however, with the fact that the allelic effect of the original finding is supported by the association in another clinical sample, by the mediational effects on the family functioning indicator consistent with the hypothesized gene effect, as well as by the functional data. This "flip-flop" (59) finding may also be possible in the case of true allelic heterogeneity between the studies (60). Elucidation of the source of this heterogeneity might be impossible even with dense genotype data from each of the studies. The difference in the direction of the association may also be related to the differences in the sample ascertainment. In both CEDAR/SADS and COGA samples, the affected individuals, ascertained clinically, have a relatively severe DUD, frequently related to polysubstance abuse, whereas in the population-based Virginia sample, the drug abuse is less severe. It is conceivable that recreational drug use that does not involve/reach clinical strength drug abuse/dependence may be itself an attribute of affiliative behavior and thus related to the prosocial function of the *AVPR1A*, whereas the other allele may be associated with elevated antisociality, a frequent attribute of and precursor to a clinical DUD that requires stepping over a higher liability thresh-

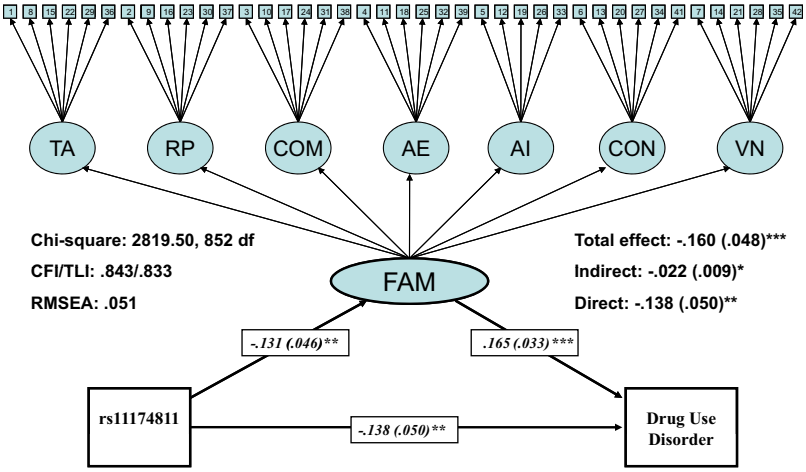

Figure 2. Path model of mediation of the relationship between rs11174811 and drug use disorder by spousal satisfaction measured by the Dyadic Relationship Scale of the Family Assessment Measure in male subjects. The complete factor structure of the Family Assessment Measure and its seven subscales is maintained. Significance of standardized path coefficients and effects is indicated by * < .05, ** < .01, and *** < .001. AE, affective expression; AI, involvement; CFI/TLI, Comparative Fit Index/Tucker-Lewis Index; COM, communication; CON, control; FAM, Family Assessment Measure; RMSEA, root mean square error of approximation; RP, role performance; TA, task accomplishment; VN, values and norms.

old. The potential explanations are probably not restricted to these possibilities and are not mutually exclusive. Regardless of its source, however, the flip-flop suggests caution in assigning risk or protection modalities to allelic effects in variation in complex traits.

Another caveat of this analysis is that, unsurprisingly, the model assuming the DUD diagnosis as the mediator has an identical fit and thus cannot be rejected statistically. Nevertheless, we focused this study a priori on the hypothesis informed by the previous literature on the role of variation in the AVPR1A gene in social behavior and the role of social bonding in subsequent drug use behavior. Further longitudinal research is certainly needed to definitively support this hypothesis.

The findings of this study, if confirmed, call for expansion of research into the role of the AVP-OXT system variation in other social behaviors as mediators of DUD liability. Clearly, this variation is also potentially influenced by other genetic (as well epigenetic and nongenetic) mechanisms, some of which have already been detected. These include, for instance, the contribution of the *MAOA* gene and its interaction with parenting style to liability to DUD (61,62). It should be noted that even a nominally highly significant association finding may be difficult to replicate using purely genetic methods. Establishing mediation of a detected genetic association with the trait of interest (e.g., DUD risk) at a phenotypically intermediate level (e.g., gene expression; premorbid trait) would provide strong validity support, serving as both internal control and a mechanistic model test.

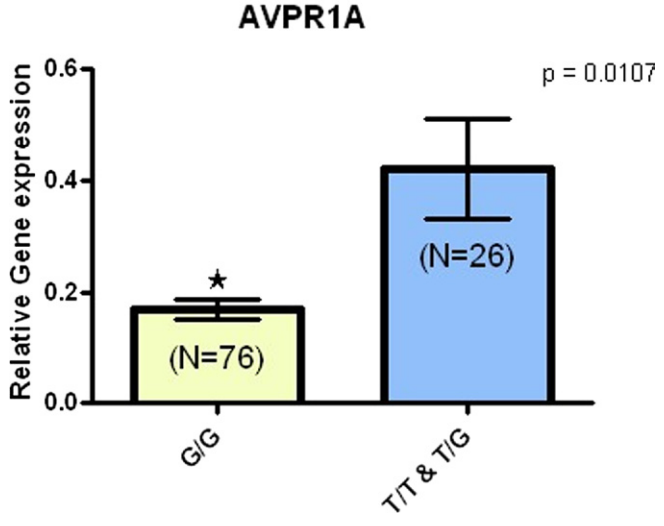

Figure 3. *AVPR1A* expression levels in samples from 26 individuals heterozygous and homozygous for the minor (T) allele of rs11174811 were higher than in 76 individuals homozygous for the major allele (unpaired *t* test with Welch's correction, two-tailed $p = .01$). *$p < .05$.

This study was supported by the National Institute on Drug Abuse (Grant P50DA005605 to RET; Grants R01DA011922, R01DA019157, and K02DA018701 to MMV). The Collaborative Study on the Genetics of Alcoholism (COGA) is supported by National Institutes of Health Grant U10AA08401. GlaxoSmithKline partially funded genotyping costs in the Center for Education and Drug Abuse Research/Substance Abuse and the Dopamine System samples.

The Collaborative Study on the Genetics of Alcoholism, Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenburg, and L. Bierut, includes 10 different centers: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenburg, J. Nurnberger Jr., T. Foroud); University of Iowa (S. Kuperman, J. Kramer); State University of New York Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, A. Goate, J. Rice, K. Bucholz); University of California at San Diego (M. Schuckit); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy), Howard University (R. Taylor); and Virginia Commonwealth University (D. Dick). Other COGA collaborators contributing to the genome-wide association studies data set include: L. Bauer (University of Connecticut); D. Koller, S. O'Connor, L. Wetherill, X. Xuei (Indiana University); Grace Chan (University of Iowa); N. Manz, M. Rangaswamy (State University of New York Downstate); A. Hinrichs, J. Rohrbaugh, J-C Wang (Washington University in St. Louis); A. Brooks (Rutgers University); and F. Aliev (Virginia Commonwealth University). A. Parsian and M. Reilly are the National Institute on Alcohol Abuse and Alcoholism Staff Collaborators. We continue to be inspired by our memories of Henri Begleiter and Theodore Reich, founding Principal Investigator and Co-Principal Investigator of COGA, and also owe a debt of gratitude to other past organizers of COGA, including Ting-Kai Li, currently a consultant with COGA, P. Michael Conneally, Raymond Crowe, and Wendy Reich, for their critical contributions. This national collaborative study is supported by National Institutes of Health Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism and the National Institute on Drug Abuse.

The authors reported no biomedical financial interests or potential conflicts of interest.

We also acknowledge the National Institute on Drug Abuse Genetics Consortium, whose members contributed to the selection of candidate system genes for this study, including Andrew Bergen, Joseph Cubells, Ken Krauter, Mary Jeanne Kreek, Sharon Murphy, Huijin Ring,

Ming Tsuang, Kirk Wilhelmsen, Pamela A.F. Madden, Naomi Breslau, Eric O. Johnson, Dorothy Hatsukami, Ovide Pomerleau, Gary E. Swan, Alison M. Goate, Joni Rutter, Sarah Bertelsen, Louis Fox, Douglas Fugman, Nicholas G. Martin, Grant W. Montgomery, Jen C. Wang, Dennis G. Ballinger, John P. Rice, and Laura Jean Bierut. Dr. Maher is currently affiliated with the Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

Supplementary material cited in this article is available online.

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