ORIGINAL ARTICLE

# Using Dimensional Models of Externalizing Psychopathology to Aid in Gene Identification

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**Context:** Twin studies provide compelling evidence that alcohol and drug dependence, childhood conduct disorder, adult antisocial behavior, and disinhibitory personality traits share an underlying genetic liability that contributes to a spectrum of externalizing behaviors. However, this information has not been widely used in gene identification efforts, which have focused on specific disorders diagnosed using traditional psychiatric classification systems.

**Objective:** To test the utility of using a multivariate externalizing phenotype in (1) linkage analyses and (2) association analyses to identify genes that contribute broadly to a spectrum of externalizing disorders.

**Design:** Data were analyzed from the Collaborative Study on the Genetics of Alcoholism. Linkage analyses were conducted using data from a genome-wide 10-cM microsatellite scan. Association analyses were conducted on 27 single-nucleotide polymorphisms genotyped across a candidate gene, the muscarinic acetylcholine receptor M2 gene (*CHRM2*).

**Setting:** Six centers across the United States.

**Other Participants:** Approximately 2300 individuals from 262 families.

**Main Outcome Measures:** Lifetime symptom counts of alcohol dependence, illicit drug dependence, childhood conduct disorder, and adult antisocial personality disorder and novelty seeking, sensation seeking, and general externalizing component scores consisting of a composite of the previous 6 variables.

**Results:** Principal component analyses indicated that the 6 individual variables loaded on a single externalizing factor. Linkage analyses using the resultant component scores identified a region on chromosome 7 consistent with a gene that broadly predisposes individuals to externalizing behavior. Association analyses of a candidate gene, *CHRM2*, previously of interest in the Collaborative Study on the Genetics of Alcoholism, suggest that it is involved in a general externalizing phenotype.

**Conclusions:** Broader conceptualizations of psychiatric disorders, such as studying a spectrum of externalizing psychopathology, may aid in identifying susceptibility genes and understanding the pathways through which genetic factors affect vulnerability for a variety of poor outcomes.

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EFFORTS TO IDENTIFY GENES involved in psychiatric disorders have largely used categorical models of diagnosis. Consequently, there are separate, large-scale gene identification projects under way that each focus on a particular psychiatric outcome as a qualitative phenotype. For example, these funded projects concentrate on identifying genes involved in alcohol dependence,[1] schizophrenia,[2] bipolar disorder,[3] autism,[4] major depression,[5] attention-deficit/hyperactivity disorder,[6] nicotine dependence,[7] and illicit drug dependence.[8] This likely reflects, in part, the ubiquitous influence of the *DSM* for the classification of psychiatric disorders in research as well as in practice.

While there is evidence that this strategy has been successful in identifying replicable associations between specific genes and a handful of psychiatric disorders (eg, *GABRA2* and *ADH4* associated with alcohol dependence[9-14] and dystrobrevin binding protein 1 [*DTNBP1*] and neuregulin 1 [*NRG1*] with schizophrenia[15,16]), a narrow focus on specific psychiatric outcomes in isolation may lead us to miss some important genetic variants that influence susceptibility through pathways shared across psychiatric outcomes. Twin studies have been pivotal in demonstrating shared genetic liability across a variety of psychiatric disorders. One of the largest studies of this kind, conducted by Kendler and colleagues,[17] examined the underlying structure of genetic and environmental risk factors across 10 common psychiatric disorders using data from the Virginia Twin Study. They found that genetic factors predispose individuals

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to 2 broad groups of behavior: externalizing disorders and internalizing disorders. Alcohol dependence, drug abuse/dependence, adult antisocial behavior, and childhood conduct disorder all loaded on 1 genetic factor, while major depression and anxiety disorders loaded on a second genetic factor.[17] These results indicate that some genetic factors broadly predispose individuals to a variety of externalizing disorders, with a parallel interpretation for the internalizing disorders. This does not preclude the possibility that some genetic influences are disorder specific; in particular, alcohol and drug dependence showed evidence for substantial disorder-specific genetic risk factors, which may reflect genes involved in the metabolism of drugs, such as the involvement of the *ADH* and *ALDH* genes in alcohol dependence.[12,18-20] However, most of the genetic variance for alcohol dependence, drug abuse/dependence, adult antisocial behavior, and childhood conduct disorder in the study by Kendler et al[17] was shared across the externalizing disorders, suggesting that an underlying genetic liability may contribute to a spectrum of externalizing disorders. Similarly, most of the genetic variance on major depression, generalized anxiety disorder, and phobias was shared across the disorders. These findings are consistent with the clinical observation of patterns of comorbidity across psychiatric disorders.[21,22] Furthermore, model-fitting approaches have demonstrated that phenotypic patterns of comorbidity across externalizing disorders are better represented by dimensional models of psychopathology, as opposed to categorical models, again supporting an argument for using dimensional models of externalizing in research efforts.[23] We will focus our review on externalizing disorders, as this dimension of psychiatric problems is most relevant to the analyses reported herein, though we will return to the implications of our results for a number of psychiatric outcomes in the discussion.

A number of other studies lend further support to the premise that shared genetic factors influence externalizing disorders. A family study examining the transmission of alcohol dependence, drug abuse/dependence, adult antisocial personality disorder, and childhood conduct disorder suggested that a general vulnerability to externalizing disorders largely accounted for familial resemblance, with this general liability being highly heritable ($h^2 = 0.80$).[24] Several twin studies also suggest that a latent externalizing factor, including conduct disorder, adult antisocial behavior, alcohol and drug abuse/dependence, and disinhibitory personality traits, is highly heritable (80%-85%).[25,26] Thus, this latent externalizing factor appears to be more heritable than the individual disorders themselves, which show individual heritabilities of approximately 50%.[27] A final piece of evidence suggesting a shared genetic liability across externalizing psychopathology comes from the electrophysiological literature in which a number of electrophysiological endophenotypes thought to represent markers of genetic vulnerability are shared across the spectrum of externalizing disorders, including alcohol dependence, other forms of substance dependence, childhood externalizing disorders, and adult antisocial personality disorder.[28-35]

Despite overwhelming evidence for shared genetic factors that influence a spectrum of externalizing psychopathology, this information has not been widely used in gene identification efforts. Herein, we report analyses from the Collaborative Study on the Genetics of Alcoholism (COGA), in which we have examined a broad phenotype of externalizing behavior by creating component scores composed of lifetime symptoms of alcohol dependence, illicit drug dependence, adult antisocial behavior, and childhood conduct disorder, along with disinhibitory personality traits. We report results from 2 sets of analyses aimed at exploring the utility of a broad externalizing phenotype in genetic studies. First, we report genome-wide linkage analyses using these externalizing component scores. Second, we report results from association analyses with a candidate gene that has previously been associated with alcohol dependence[36,37] and electrophysiological endophenotypes[38] in the COGA sample, the muscarinic acetylcholine receptor M2 gene (*CHRM2*). The rationale for examining this gene in relation to general externalizing behavior is based on follow-up analyses of *CHRM2*, which indicated that the evidence for association with alcohol dependence in the COGA sample was driven entirely by alcohol-dependent individuals with comorbid drug dependence.[39] This subgroup was also characterized by higher rates of antisocial personality disorder and conduct disorder, suggesting that the associated subgroup may represent individuals with a predisposition toward general disinhibitory psychopathology. In addition, the gene was associated with evoked electroencephalography oscillations in the COGA sample,[38] an electrophysiological endophenotype that is shared across externalizing disorders.[32] Accordingly, we present results from both linkage and association analyses, illustrating the use of externalizing scores across genetic methodologies. We believe the results converge to illustrate that broader conceptualizations of psychiatric disorders may help us to identify genes involved in susceptibility of psychiatric problems and to understand the pathways through which genetic factors operate to influence such disorders.

## METHODS

### SAMPLE

The COGA is a project in which families were recruited from 6 centers across the United States: Indiana University, Downstate University of New York Health Science Center, University of Connecticut, University of Iowa, University of California at San Diego, and Washington University in St Louis. Probands identified through inpatient or outpatient alcohol treatment programs by each of these 6 sites were invited to participate if they had a sufficiently large family (usually sibships $>3$ with parents available) with 2 or more members in a COGA catchment area.[40] The institutional review boards of all participating centers approved the study and written consent was obtained from all participants. Additional details about the study have been published previously.[1,40] The sample available for genetic analyses consisted of 262 families with 2131 genotyped and phenotyped individuals, 1007 males and 1124 females, who were members of families in which at least 3 first-degree relatives met *DSM* criteria for alcohol dependence.

### PHENOTYPES

All individuals were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview.[41,42] Life-

time alcohol dependence was assessed using *DSM-IV* criteria.[43] Other lifetime diagnoses (drug dependence, antisocial personality disorder, and childhood conduct disorder) were assessed using *DSM-IIIR* criteria, as *DSM-IV* was under development at the time interviews were initiated; only the alcohol dependence section was adapted to emulate *DSM-IV* diagnoses. Symptom counts for each of the disorders (alcohol dependence, drug dependence, antisocial personality disorder, and childhood conduct disorder) were used in linkage and association analyses. Symptom counts were log transformed to reduce the skewness of their distributions.

In addition, 2 personality variables that were hypothesized to be related to the externalizing spectrum were included in analyses and computation of the externalizing component scores: novelty seeking scores from the Tridimensional Personality Questionnaire[44] and sensation seeking scores from the Zuckerman Sensation Seeking Scale.[45]

## STATISTICAL ANALYSES

### Computation of Component Scores

Principal component analysis was used to evaluate the factor structure of lifetime alcohol dependence symptoms, drug dependence symptoms, antisocial personality disorder symptoms, conduct disorder symptoms, and novelty and sensation seeking scores. Components with eigenvalues greater than 1 were retained. Analyses were conducted using SAS, version 8 (SAS Institute Inc, Cary, North Carolina).

### Linkage Analyses

Genotyping for the microsatellite linkage scan was carried out in laboratories at Indiana University and Washington University in St Louis using radioactive- and fluorescence-based detection systems, as described previously.[46] The current analyses are based on a map of 315 autosomal microsatellite markers with a mean intermarker distance of 11.5 cM. Pedigrees were checked for nonmendelian inheritance using the GeneMaster database and the programs CRIMAP, version 2.4 (Phil Green, Washington University, St Louis, Missouri), and USERM13 (University of Michigan, Ann Arbor).[47] Recombination-based marker maps were generated from the sample using CRIMAP. Maximum likelihood estimates of marker allele frequencies were computed from the data using USERM13. Multipoint linkage analyses were carried out using MERLIN Regress software (University of Michigan, Ann Arbor).[48] This regression-based approach to quantitative trait linkage, based on the work of Sham and colleagues,[49] can handle nonrandomly ascertained samples and deviations from multivariate normality of the observed data but retains the statistical power of variance-components linkage methods. The complexity of several large, multigenerational COGA families required that familial structures be simplified to make data analysis feasible and efficient with available hardware; 7 families were divided into separate branches. The mean family size in the analysis data file was 8.13 members, and the mean number of generations per family was 2.89 (range, 2-5). There were 2386 sibling, 152 half-sibling, 1971 parent-child, 249 grandparent-grandchild, 1509 avuncular, and 707 cousin pairs used in analyses. We used a heritability estimate of 80% in our analyses for the externalizing factor scores and 50% for other phenotypes based on existing twin literature. Multipoint lod scores were estimated. Evidence for a chromosomal region containing a gene that broadly predisposes individuals to externalizing behaviors would be suggested by a pattern of results in which the strongest evidence for linkage was found using the component scores, with the individual phenotypes showing more modest evidence of linkage to the same region (since quantitative symptom counts were analyzed for the individual disorders, sample size is virtually identical across phenotypes allowing for direct comparison of lod scores). Chromosomal regions that yielded results following this pattern with an overall externalizing lod score of 1.5 or higher are presented here.

### Association Analyses

Publicly available databases, dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and the International HapMap Project (http://www.hapmap.org), were used to identify single-nucleotide polymorphisms (SNPs) within and flanking *CHRM2*. In addition, a number of novel SNPs were identified by DNA sequencing. We genotyped 27 SNPs within and flanking *CHRM2*.[50] Single nucleotide polymorphisms were selected to cover the single coding exon as well as all 5 exons in the promoter region and a region in intron 3 that is conserved across multiple species. The minor allele frequency was greater than 0.10 in all cases (mean, 0.45). Genotyping was done with a modified single nucleotide extension reaction, with allele detection by mass spectroscopy (Sequenom MassARRAY System; Sequenom, San Diego, California). All genotypic data were checked for mendelian inheritance of marker alleles with the USERM13[47] option of the MENDEL linkage computer programs (University of Michigan, Ann Arbor), which was then used to estimate marker allele frequencies. Trio data from white individuals genotyped in the COGA data set were entered into the Haploview program[51] to examine the linkage disequilibrium structure of the genotyped SNPs. Six linkage disequilibrium blocks were identified in our data set, with several SNPs located in interblock regions. Information about the linkage disequilibrium block structure of the SNPs is included in **Table 1**.

Association was evaluated with the Quantitative Pedigree Disequilibrium Test using the QPDTPHASE program contained in the UNPHASED software suite (MRC Human Genome Mapping Project Resource Centre, Cambridge, England).[52] QPDTPHASE implements the quantitative trait in the Pedigree Disequilibrium Test described by Monks and Kaplan,[53] with extensions to deal with haplotypes and missing data. The null hypothesis is no linkage or no association, in which the trait and genotypes are uncorrelated. The covariance is estimated within each family and the estimates combined across the data set by the central limit theorem. We tested for association with each of the SNPs genotyped in *CHRM2* and the externalizing component scores, as well as each of the component phenotypes (eg, log-transformed symptom counts for each of the 4 externalizing disorders and the 2 personality scales) that composed the component scores.

# RESULTS

## PRINCIPAL COMPONENT ANALYSES

**Table 2** presents the correlations across the externalizing variables used in the principal component analyses. Only 1 component was extracted with an eigenvalue greater than 1.0. The first component had an eigenvalue of 3.20 and accounted for 53% of the variance. There was an eigenvalue difference of 2.30 between the first and second factors, and all other factors accounted for less than 15% of the variance. All variables loaded onto the first factor. The component loadings for each of the variables were as follows: alcohol dependence symptoms, 0.74; antisocial personality disorder symptoms, 0.83; conduct disorder symptoms, 0.69; drug dependence symptoms, 0.76; novelty seeking scores, 0.65;

**Table 1. Family-Based Association Analyses of Externalizing Psychopathology Symptoms and Scores**

| SNP | NCBI Position | Gene Location | LD Block Location[a] | *P* Value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AD | ASPD | CD | DD | NS | SS | Component Score |
| rs1424558 | 135988926 | Upstream of exon 1 | Block 1 | .62 | .71 | .93 | .22 | .86 | .87 | .73 |
| rs1424574 | 136006288 | | Block 1 | .91 | .98 | .76 | .46 | .45 | .42 | .59 |
| rs13247260 | 136010518 | | | .39 | .65 | .69 | .72 | .81 | .45 | .98 |
| rs1424569 | 136026671 | Intron 3-4 | | .91 | .71 | .96 | .86 | .23 | .49 | .58 |
| rs1424387 | 136046865 | | Block 2 | .15 | .17 | .96 | .48 | .17 | .36 | .19 |
| rs2350780 | 136050224 | | Block 2 | .36 | .4 | .89 | .06 | .82 | .28 | .16 |
| rs978437 | 136071433 | | | .02 | .06 | .26 | .02 | .03 | .09 | .006[b] |
| rs36210734 | 136080468 | | Block 3 | .57 | .46 | .84 | .37 | .22 | .07 | .15 |
| rs36210735 | 136080901 | | Block 3 | .02 | .03 | .36 | .21 | .047 | .008[b] | .006[b] |
| rs7782965 | 136081388 | | Block 3 | .04 | .06 | .16 | .01 | .09 | .01 | .004[b] |
| rs7800170 | 136081575 | | Block 3 | .08 | .08 | .73 | .34 | .04 | .004[b] | .01 |
| rs1455858 | 136088958 | | Block 3 | .04 | .07 | .02 | .01 | .04 | .03 | .005[b] |
| rs1378646 | 136092256 | | Block 3 | .01 | .02 | .06 | .01 | .007[b] | .02 | .001[b] |
| rs1824024 | 136100949 | Intron 4-5 | Block 3 | .01 | .046 | .18 | .007[b] | .008[b] | .02 | .002[b] |
| rs2061174 | 136118655 | | Block 4 | .10 | .06 | .51 | .09 | .09 | .13 | .04 |
| rs7799047 | 136128813 | | Block 4 | .29 | .18 | .79 | .12 | .14 | .08 | .07 |
| rs2350786 | 136133825 | | Block 4 | .35 | .21 | .81 | .14 | .14 | .51 | .15 |
| rs36210736 | 136134189 | Exon 5 | Block 5 | .42 | .62 | .91 | >.99 | .68 | .11 | .3 |
| rs6948054 | 136138056 | Intron 5-6 | Block 5 | .15 | .07 | .37 | .10 | .17 | .12 | .04 |
| rs324640 | 136146251 | | | .02 | .15 | .30 | .42 | .1 | .08 | .19 |
| rs324650 | 136150916 | | Block 6 | .008[b] | .06 | .72 | .54 | .02 | .03 | .05 |
| rs324651 | 136156516 | | Block 6 | .56 | .73 | .19 | .1 | .94 | .25 | .38 |
| rs8191992 | 136158563 | 3′UTR | | .03 | .1 | .88 | .46 | .05 | .04 | .04 |
| rs8191993 | 136158818 | | | .73 | .37 | >.99 | .11 | .53 | .24 | .24 |
| rs1378650 | 136162406 | Downstream of exon 6 | | .03 | .19 | .75 | .27 | .09 | .08 | .09 |
| rs1424548 | 136167015 | | | .01 | .25 | .72 | .19 | .01 | .08 | .26 |
| rs324656 | 136171367 | | | .33 | .59 | .85 | .97 | .38 | .85 | .48 |

Abbreviations: AD, alcohol dependence symptoms; ASPD, antisocial personality disorder symptoms; CD, conduct disorder symptoms; DD, illicit drug dependence symptoms; LD, linkage disequilibrium; NCBI, National Center for Biotechnology Information; NS, novelty seeking score; SNP, single-nucleotide polymorphism; SS, sensation seeking scale score; UTR, untranslated region.

[a] Single-nucleotide polymorphisms without a block number were located between blocks.

[b] $P < .01$.

**Table 2. Pearson Correlations Between Externalizing Variables**

| Externalizing Variables | Alcohol Dependence | Antisocial Personality Disorder | Conduct Disorder | Drug Dependence | Novelty Seeking | Sensation Seeking |
|---|---|---|---|---|---|---|
| **Alcohol Dependence** | 1.00 | | | | | |
| **Antisocial Personality Disorder** | 0.59 | 1.00 | | | | |
| **Conduct Disorder** | 0.39 | 0.57 | 1.00 | | | |
| **Drug Dependence** | 0.50 | 0.55 | 0.44 | 1.00 | | |
| **Novelty Seeking** | 0.32 | 0.41 | 0.30 | 0.36 | 1.00 | |
| **Sensation Seeking** | 0.39 | 0.44 | 0.32 | 0.43 | 0.55 | 1.00 |

and sensation seeking scores, 0.71. Component scores for this first latent component, which were composed of the 6 externalizing variables, were computed for all individuals and used in linkage and association analyses.

## LINKAGE ANALYSES

We found 1 region of the genome that yielded the hypothesized pattern of results, with the strongest evidence of linkage associated with the externalizing component score and more modest evidence with the individual phenotypes. This region was on chromosome 7, with a peak lod score for the component score of 1.57 at 101.9 cM at the marker D7S1797 (**Figure 1**). We have previously reported linkage using affected sibling pair analyses with COGA alcohol dependence diagnoses (defined by a *DSM-IIIR* diagnosis of alcohol dependence and Feighner definite criteria) in this region on chromosome 7.[36] The peak lod score with COGA alcohol dependence was approximately 20 cM more distal. Those analyses used a dichotomous affection status method of analysis. Accordingly, they differed from our report both in the breadth of the phenotype (alcohol dependence vs a broader externalizing phenotype) and the methodology. To further explore the possibility that a gene in this region more generally predisposes individuals to

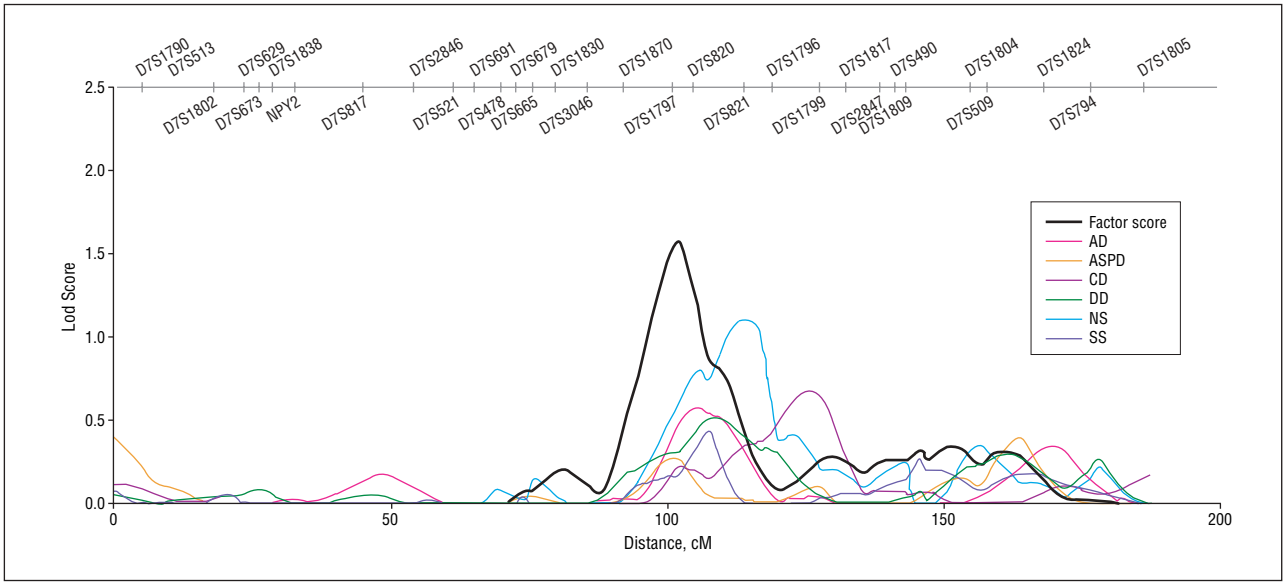


**Figure 1.** Linkage analyses from chromosome 7 using MERLIN Regress software (University of Michigan, Ann Arbor)[48] for externalizing component scores and component phenotypes. AD indicates alcohol dependence symptoms; ASPD, antisocial personality disorder symptoms; CD, conduct disorder symptoms; DD, drug dependence symptoms; NS, novelty seeking score; SS, sensation seeking score.

externalizing disorders, we conducted secondary linkage analyses on chromosome 7 using an affected sibling pair method to more closely mimic analyses used in the study of linkage to alcohol dependence by Wang et al.[36] We used the sib_ibd option in the program ASPEX,[54] which limits the analyses to only affected sibling pairs with genotyped parents, allowing for unambiguous estimation of identity by descent. We ran analyses separately using sibling pairs affected with each disorder: *DSM-IV* alcohol dependence (557 pairs from 231 families), adult antisocial personality disorder (471 pairs from 191 families), childhood conduct disorder (113 pairs from 67 families), and illicit drug dependence (405 pairs from 175 families). Finally, we analyzed a combined, dichotomous externalizing disorder phenotype, whereby individuals were considered affected if they met criteria for any of the 4 aforementioned disorders (948 pairs from 290 families). Thus, for this externalizing disorder phenotype, an affected sibling pair could consist of a pair in which, for example, 1 sibling was affected with alcohol dependence and the other sibling met criteria for antisocial behavior. These analyses differed in that they used binary diagnoses (affected/unaffected) rather than quantitative symptom counts. In addition, we could not include the quantitative personality scores in the analyses. However, performing linkage analysis with this externalizing phenotype again tests the hypothesis that these disorders are alternative manifestations of a shared underlying vulnerability, the specific expression of which may depend on additional genes and/or the environment. Although the results from the affected sibling pair analyses should be interpreted while keeping in mind that the sample size changed across phenotypes and that lod scores are influenced by sample size, the pattern of results replicates that seen with the quantitative externalizing component score analyses: modest evidence of allele sharing with each of the individual disorders, with the strongest evidence for linkage in the region found with the broad externalizing disorder phenotype (**Figure 2**). The peak lod score was 2.11 at 122.9 cM near the marker D7S1796.

## ASSOCIATION ANALYSES

The results from association analyses between SNPs genotyped across *CHRM2* and each of the quantitative phenotypes (symptom counts of alcohol dependence, drug dependence, antisocial personality disorder, and childhood conduct disorder; novelty seeking and sensation seeking scores; and general externalizing component scores) are presented in Table 1. Although all phenotypes show some SNPs to be significant ($P < .05$), the association is strongest with the general externalizing component score, with 6 of the 27 SNPs yielding $P < .01$ compared with 1 SNP significant at this level with alcohol dependence symptoms, 0 with antisocial personality disorder or conduct disorder symptoms, 2 with drug dependence symptoms, 2 with novelty seeking scores, and 2 with sensation seeking scores. Furthermore, the component score appears to concentrate the evidence for association to the third linkage disequilibrium block, located on intron 3 to 4, with 5 of 7 SNPs in this block significant ($P < .01$) and the other 2 SNPs in the block yielding $P$ values of .15 and .01 with general externalizing component scores. **Table 3** presents the linkage disequilibrium across the SNPs located in block 3 and also includes the SNP located between blocks 2 and 3, which shows evidence of an association with the component scores. Although the D′ is very high across the SNPs, the $r^2$ values are less than 1.0, indicating that these SNPs are not purely redundant and do yield some independent evidence for association.

## COMMENT

Gene identification studies in psychiatry have traditionally focused on clinically diagnosed psychiatric disor-

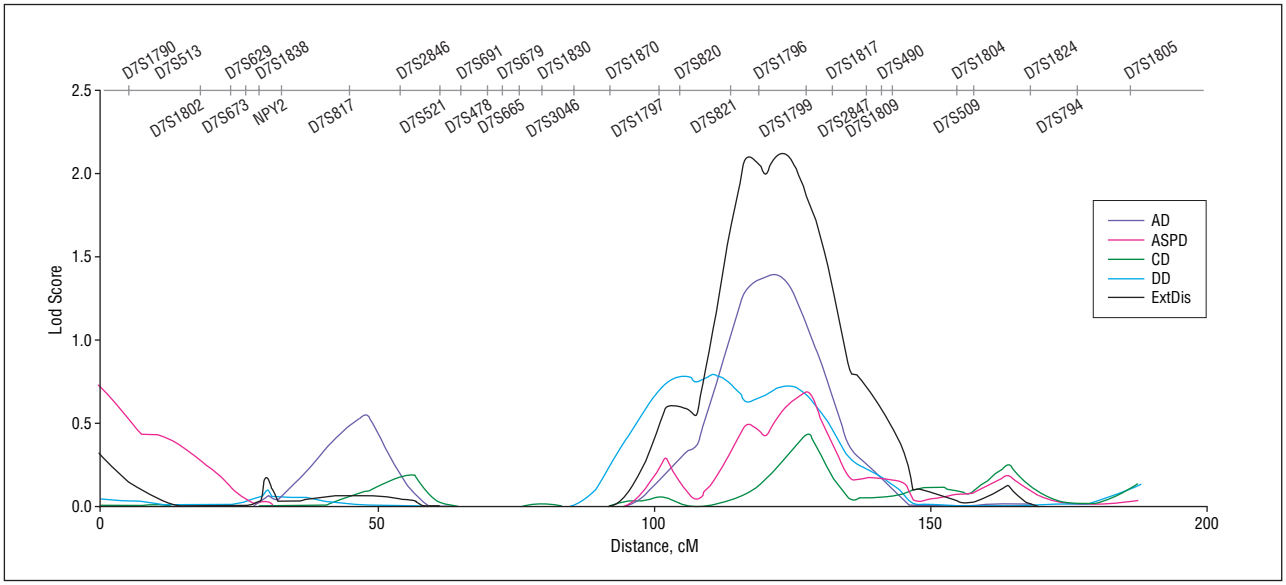


**Figure 2.** Linkage analyses from chromosome 7 using affected sibling pair methods in ASPEX.[54] AD indicates alcohol dependence diagnosis; ASPD, antisocial personality disorder diagnosis; CD, conduct disorder diagnosis; DD, drug dependence diagnosis; ExtDis, any externalizing disorder.

**Table 3. Linkage Disequilibrium (LD) Across the SNPs Located in *CHRM2* LD Block 3[a]**

| SNPs | rs978437[b] | rs36210734 | rs36210735 | rs7782965 | rs7800170 | rs1455858 | rs1378646 | rs1824024 |
|---|---|---|---|---|---|---|---|---|
| **rs978437[b]** | | 0.980 | 0.944 | 0.943 | 0.944 | 0.892 | 0.936 | 0.890 |
| **rs36210734** | 0.052 | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **rs36210735** | 0.478 | 0.098 | | 1.000 | 0.998 | 0.949 | 0.960 | 0.931 |
| **rs7782965** | 0.843 | 0.051 | 0.509 | | 1.000 | 0.986 | 0.997 | 0.992 |
| **rs7800170** | 0.473 | 0.099 | 0.988 | 0.506 | | 0.945 | 0.964 | 0.941 |
| **rs1455858** | 0.785 | 0.055 | 0.482 | 0.935 | 0.476 | | 0.979 | 0.974 |
| **rs1378646** | 0.859 | 0.055 | 0.504 | 0.925 | 0.504 | 0.926 | | 0.978 |
| **rs1824024** | 0.787 | 0.053 | 0.462 | 0.941 | 0.472 | 0.939 | 0.931 | |

Abbreviations: *CHRM2*, muscarinic acetylcholine receptor M2 gene; SNP, single-nucleotide polymorphism.
[a] $D'$ is shown above the diagonal; $r^2$ is shown below the diagonal. Because quantitative symptom counts were analyzed for the individual disorders, sample size is virtually identical across phenotypes allowing for direct comparison of lod scores.
[b] Located between LD blocks 2 and 3.

ders. Recognition of the limitations of this approach has led to increasing interest in using endophenotypes in gene identification projects, based on the hypothesis that these may represent simpler phenotypes more proximal to the underlying genetic effects.[55-57] In this article, we argue that another strategy to advance our understanding of genetic contributions to psychiatric outcome is to use information from twin and family studies about the underlying structure of genetic influences on behavior to define relevant phenotypes for genetic analysis.

There is a large body of evidence suggesting that shared genetic factors influence a spectrum of externalizing disorders, including alcohol dependence, illicit drug dependence, conduct disorder, antisocial behavior, and disinhibitory personality traits. However, up to this point, this information has not been integrated largely into efforts to identify genes involved in the externalizing spectrum as a whole, with a recent exception in a study of adolescents.[58] Here, we analyzed multivariate externalizing phenotypes that encompassed information across alcohol dependence symptoms, illicit drug dependence symptoms, conduct disorder symptoms, antisocial personality disorder symptoms, and disinhibitory personality traits. We found evidence of linkage to a region of chromosome 7, which appears to contain a gene (or genes) conferring risk to the spectrum of externalizing psychopathology. Although the linkage is modest in this region (maximum lod score, 1.6), our confidence in this finding is bolstered by the parallel pattern of results yielded by affected sibling pair analyses. In addition, we ascertained the sample through alcohol-dependent probands at treatment centers, which can lead to skewed estimates of the mean and variance of the quantitative traits and weaken the power to detect linkage (though there is no evidence that this ascertainment scheme would inflate a type 1 error).[49] A genome-wide linkage scan of the P3 event-related potential in a sample of 647 twins/siblings from 311 families in Australia also found significant evidence of linkage (lod score, 3.88) to the same region of chromosome 7q.[59] To the extent that the P3 event-related potential reflects a predisposition toward general externalizing disorders,[33,60] these results further support our conclusion of genetic factors in this region predisposing individuals to a constellation of disinhibitory behaviors.

In addition, we found evidence from association analyses that *CHRM2* predisposes individuals to a spectrum of externalizing behaviors. We note that although *CHRM2* is located on chromosome 7, it is not located under the linkage peak but is rather approximately 55 cM distal to the externalizing component scores peak. It was originally genotyped and analyzed in relation to alcohol dependence in the COGA sample[36] owing to its proximity to a linkage peak observed with the visual oddball paradigm theta frequency band–evoked oscillation, which is the primary constituent of the P3-evoked component.[38] To the extent that the linkage at *CHRM2* was observed with an electrophysiological endophenotype known to index general risk to a spectrum of externalizing disorders, it is encouraging that we found evidence that the association observed with *CHRM2* similarly fits this pattern. Although *CHRM2* is not directly under the linkage peak identified here, simulations have demonstrated that the exact location of linkage peaks can be imprecise in relation to the underlying gene(s) involved[61]; accordingly, to test whether the association of *CHRM2* was related to the linkage signal, we performed the linkage analyses again with the externalizing component scores using the most significantly associated SNP in *CHRM2* (rs1378646) as a covariate. The evidence for linkage was virtually unchanged (maximum lod at peak, 1.49, compared with 1.57 without covariate), suggesting that *CHRM2* had an independent influence on general externalizing behavior and that the linkage signal identified here on chromosome 7 reflects additional genetic variants in the region that influences general externalizing behavior.

We believe these analyses demonstrate that the strategy of using multivariate externalizing phenotypes can be useful in both linkage and association analyses in identifying genes that broadly predispose individuals to a spectrum of risk that spans traditional psychiatric classification systems. Groups studying other psychiatric conditions have also adopted this approach. For example, ongoing gene identification projects in both Virginia and the Netherlands are capitalizing on the literature that demonstrates that depression and anxiety share a common genetic etiology[62] and are using multivariate phenotypes consisting of symptoms of depressive and anxious disorders, as well as the related personality trait, neuroticism,[63] in genetic studies.[64,65] Furthermore, genetic findings of schizophrenia overlap with those of bipolar disorder, suggesting that there may be shared susceptibility across these disorders, perhaps through involvement of psychosis.[66]

Identification of genes that confer vulnerability across psychiatric conditions may provide insight into the pathways through which specific genes are involved in outcome. For example, although we previously reported an association between *CHRM2* and alcohol dependence,[36] these analyses, demonstrating involvement in a broader spectrum of externalizing, suggest that *CHRM2* is involved in risk for alcohol dependence via more general disinhibitory pathways. The means through which genetic variants in *CHRM2* contribute to functional differences remain unknown. However, clues about the mechanism through which *CHRM2* may be involved in a variety of behavioral outcomes can be found in the electrophysiological literature. Our COGA colleagues have previously proposed a model in which they suggest that evoked response potential abnormalities, evident in individuals at risk for a number of forms of externalizing psychopathology, represent a deficit of central nervous system inhibition and/or an excess of central nervous system excitation.[67] This central nervous system hyperexcitability reflects a disequilibrium in the homeostatic mechanisms that are responsible for maintaining a balance between excitation and inhibition. The inverse relationship between severity of alcohol dependence and P3 amplitude supports the idea that an imbalance in central nervous system excitation and inhibition has implications for behavior. Variations in *CHRM2* may be involved in creating this homeostatic imbalance, which may in turn increase risk for a number of outcomes. We note that in addition to the association with externalizing disorders reported here, *CHRM2* has also been associated with performance on IQ tests,[50,68,69] heart rate recovery after exercise,[70] and depression.[36] Accordingly, this muscarinic receptor appears to have broad-based effects on behavior, and there may be multiple variants in the gene affecting different outcomes.[50] Better understanding of the biologic alterations associated with genetic variation in *CHRM2* will be necessary to understand exactly how this gene affects outcome.

In this article, we have compared the results from genetic analyses that separately examined clinical phenotypes that fall under the spectrum of externalizing psychopathology (ie, alcohol dependence, antisocial personality disorder, conduct disorder, and illicit drug dependence) with results from analyses of a composite phenotype that combined information across forms of externalizing psychopathology. As previously stated, twin studies have suggested that much of the genetic variation is shared across these disorders by a general externalizing factor, with some evidence for residual disorder-specific variance. Analyses of the separate psychiatric disorders essentially combine both the general (common) and specific genetic components. In our analyses reported here, we focused on the general component by analyzing the latent externalizing composite scores. We also conducted secondary analyses examining the residual scores for each of the clinical phenotypes (eg, the residual variance in alcohol dependence symptom scores after the externalizing scores were regressed out). There was no longer evidence for linkage on chromosome 7 with any of the residual scores (lod scores $<0.1$). Similarly, there was little evidence of association of SNPs in *CHRM2* with the residual scores. These analyses suggest that the strongest contribution to the linkage peak on chromosome 7 and, separately, the association observed with *CHRM2* are due to a general influence on externalizing psychopathology.

With the development of the *DSM-V* under way, there has been much discussion about the use of dimensional models of psychiatric diagnoses, with a suggestion that augmenting traditional categorical diagnoses with dimensional information will be critical for the future of psychiatric research.[23,71] Here, we demonstrate that gene identification in psychiatry is one area that could benefit from dimensional models of psychopathology.

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