A Genome-Wide Search for Quantitative Trait Loci That Influence Antisocial Drug Dependence in Adolescence

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**Background:** Among adolescents, externalizing problem behavior and substance use disorders are often comorbid. Familial influences, including shared genetic risk factors, may account for part of this comorbidity. Previously we reported 2 chromosomal regions (3q24-3q25 and 9q34) likely to contain genes that influence substance dependence vulnerability (DV) in adolescence.

**Objectives:** To identify quantitative trait loci (QTLs) that influence externalizing problem behavior in adolescence and to determine whether any identified QTL overlap chromosomal regions that influence DV.

**Design:** Regression-based QTL mapping procedures designed for selected sibling pair samples.

**Setting:** Patient probands were drawn from consecutive admissions to residential and outpatient (milieu-type) treatment facilities for substance abuse and delinquency operated by the University of Colorado; most of these patients were referred for treatment by juvenile justice or social service agencies.

**Patients:** A total of 249 proband-sibling pairs from 191 families were selected for the study. Patient probands were 13 to 19 years of age; siblings of the probands ranged in age from 12 to 25 years.

**Main Outcome Measures:** A community-based sample of 4493 adolescents and young adults was used to define clinically significant, heritable, age- and sex-normed indexes of DV, conduct disorder symptoms (CDS), and a composite index of antisocial substance dependence (DV + CDS). Siblings and parents were genotyped for 374 microsatellite markers distributed across the 22 autosomes (mean intermarker distance, 9.2 centimorgans).

**Results:** For both DV and CDS, there was evidence of linkage to the same region on chromosome 9q34, as well as to 3q24-3q25 for DV, and a novel region on chromosome 17q12 for CDS. Our composite index (DV + CDS) yielded the strongest evidence for linkage (logarithm of odds = 2.65) to the chromosome 9q34 region.

**Conclusion:** These results provide the first evidence of a potential molecular genetic basis for the comorbidity between DV and antisocial behavior.
lescent patient probands and their families (including sib-
ing pairs) from consecutive admissions to treatment
programs that specialize in the treatment of adolescents
with SUDs and comorbid antisocial behavioral prob-
lems. In a recent genome-wide search for quantitative trait
loci (QTLs) underlying substance dependence vulnerability (DV), we reported regions on chromosome 3q24-
3q25 and chromosome 9q34 that may confer risk for sub-
stance dependence. In the same adolescent sample we now extend this work, searching for chromosomal regions
that may be involved in comorbid SUDs and CD. Specif-
cally, the gene-mapping goals of the present study were
to identify QTLs that influence adolescent CD and to de-
termine whether there are overlapping chromosomal re-
gions likely to contain genes that influence comorbid risk.
Indication of shared chromosomal regions would pro-
vide the first evidence of a molecular genetic basis for
the comorbidity between these common disorders.

METHODS

ASCERTAINMENT OF CLINICAL AND
COMMUNITY SAMPLES

Details regarding sample ascertainment and inclusion and ex-
clusion criteria for the adolescent clinical and community samples have been described previously. Patient probands were drawn from consecutive admissions to residential and outpatient (milieu-
type) treatment facilities for substance abuse and delinquency ope-
rated by the University of Colorado; most of these patients were
referred for treatment by juvenile justice or social service a-

genies. The clinical sample included 249 proband-sibling pairs from
191 families. Probands were 13 to 19 years of age (mean ± SD age, 15.9 ± 1.3 years) at the time of assessment. The ethnicity distri-
bution was 7.8% African American, 36.3% Hispanic, 32.1% white, and 3.6% other. The community sample included 4493 indi-
viduals 12 to 25 years of age (mean ± SD age, 15.9 ± 2.1 years) drawn from the Colorado Twin Registry, the Colorado Adop-
tion Project, and the Colorado Adolescent Substance Abuse family
study. The ethnicity distribution of the community sample was 81.6% white, 12.1% Hispanic, 2.3% African American, and
4% of mixed or unknown ethnicity. We have previously re-
ported that substance use patterns and prevalence rates in the
community sample are comparable to those reported in na-
tional studies.

ASSESSMENT

Participants were administered an extensive battery of cogni-
tive, psychiatric, and sociodemographic assessments that in-
cluded structured diagnostic interviews and self-report ques-
tionnaires in the treatment facilities (probands) or homes
(siblings) as described previously. All participants who were
at least 18 years of age gave informed written consent to par-
ticipate. For juveniles, a parent or legal guardian gave written consent for participation, and assent was obtained from all ju-
veniles. All research protocols and consent forms were ap-
proved by the institutional review boards of the University of
Colorado, Denver (for proband assessment), and the Univer-
sity of Colorado, Boulder (for assessments of all other re-
lates), and all subjects were paid for participation.

Substance use data were obtained using the Composite In-
ternational Diagnostic Interview-Substance Abuse Module
(CIDI-SAM). Conduct disorder was assessed using the Di-
agnostic Interview Schedule for Children (DISC) for all pa-
tient probands. Conduct disorder for siblings younger than 19
years was assessed using the DISC, whereas siblings 19 years
or older were assessed using the Diagnostic Interview Sched-
ule (DIS). Participants recruited between 1993 and 1997 were
assessed with DSM-III-R versions of the DISC and DIS. Participants recruited between 1998 and 2001 were assessed us-
ing DSM-IV versions of the DISC and DIS, modified for scor-
ing both DSM-III-R and DSM-IV symptoms and diagnoses.
Adjustments for potential instrument effects are described herein.

PHENOTYPE DEFINITIONS

We used DSM criterion (symptom) counts to create 3 quantitative
phenotypes to assess DV, CD symptoms (CDS), and a com-
posite index of antisocial substance dependence (DV + CDS). The mean number of DSM-IV dependence symptoms (ie, total symp-
tom count across all classes of substances divided by the num-
ber of substances used repeatedly) was used as our index of DV
(see Stallings et al for details regarding the validity of this in-
dex), and the total number of DSM-III-R CD symptoms was used
as our quantitative measure of CDS. (DSM-III-R criteria were used
for CDS because DSM-IV versions of the DISC and DIS assess-
ments were unavailable at the outset of the study; the version of
the CIDI-SAM that was used provided DSM-IV symptom counts for
substance dependence.)

Because of marked age trends on these indexes in our com-
community sample, the measures were age and sex corrected us-
ing standard regression procedures and then standardized within
sex groups to express scores in our selected (clinical) sibling
sample in relation to the population means and variability for
adolescents and young adults. In addition, CDS scores were ob-
tained from the DISC for probands and younger siblings but
from the DIS for older siblings, and 2 versions of the DISC and
DIS assessments were used. To account for any instrument ef-
fects that may not have been corrected for by our age adjust-
ments, interview type was dummy coded and used to obtain
residual CDS scores. (Instrument type accounted for only 1.2%
of the variance in our age- and sex-corrected CDS scores in the
community sample.)

Standardized DV and CDS scores were summed to form a com-
posite index of antisocial substance dependence (DV + CDS). This index was then subsequently restandard-
zied so that the metric of the composite (sum) index was the
same as for our individual measures of DV and CDS (ie, means
of 0 and SDs of 1 in our community sample).

DNA COLLECTION AND GENOTYPING

The methods used for preparation of genomic DNA and geno-
typing of the 374 microsatellite markers (ABI PRISM LMS2-
MD10 panels; PE Biosystems, Foster City, Calif) have been de-
scribed previously. For 47% of the families, genotypes were
available from both parents; 49% included genotypes from 1
parent, and for 4% of the sibships, no parental genotypes were
available. Sibships included 147 pairs, 35 trios, 7 sibships of
4, and 3 sibships of 5. Sex-averaged marker maps were obtained
from the Marshfield Center for Medical Genetics database (http:
//research.marshfieldclinic.org/genetics), and allele frequen-
cies were estimated from the full sample using Merlin. Table 1
gives marker information for each chromosome and means
across the 22 autosomes.

VALIDATION OF GENOTYPES AND
RELATIONSHIP STATUS

Validation of genotypes and family relationships was per-
formed with the Graphical Relationship Representation pro-
gram, Pedstats, and Merlin. Details are described in our

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previous communication.\textsuperscript{34} Genetic analysis indicated 28 individuals in whom paternity and/or full-sibling status was questioned. In addition, 1 proband did not have complete CIDI-SAM and DISC assessments, and 11 individuals were genotyped for less than 50% of the markers. These individuals were removed from the sample before linkage analysis, resulting in a final sample of 249 sibling pairs. Twelve markers were omitted for low call rates; the mean call rate for the markers used was approximately 85%. Estimated marker haplotypes from Merlin indicated no evidence for excessive recombination events, and few genotypes (0.07%) were flagged as highly unlikely. Genotypes flagged as unlikely were not concentrated among certain markers, individuals, or families, and analyses that removed unlikely genotypes yielded essentially identical results to full-sample analyses, so these genotypes were retained. Identity-by-descent (IBD) sharing for all sibpairs was estimated at 1-centimorgan (cM) intervals (and also at each marker for single-point analyses) across the genome (autosomes) using Merlin.

**STATISTICAL ANALYSIS**

The regression-based sibpair linkage method that we used was proposed by Fulker et al\textsuperscript{68,69} as an extension of the methods originally developed by Fulker, DeFries, and colleagues\textsuperscript{70} for the analysis of selected twin data. The rationale of the DeFries-Fulker (DF) linkage analysis for selected sibling samples is that the siblings of selected probands should demonstrate differential regression toward the population mean on a quantitative trait commensurate with the proportion of marker alleles they share IBD with the proband, given that a QTL is linked to a particular chromosomal marker. The following regression model was fit to data from 249 proband-sibling pairs:

\[ C = \alpha + b_1 P + b_2 (\pi) = \epsilon, \]

where \(C\) is the quantitative score of the proband’s sibling, \(P\) is the proband’s score, \(\pi\) is the estimated mean proportion of alleles shared IBD for the sibling pair at location \((i)\) in the genome, and \(\alpha\) and \(\epsilon\) are the regression constant and disturbance term, respectively. A \(t\) test of the significance of the \(b_1\) regression coefficient provides a test of linkage, which can be expressed as an approximate logarithm of odds (LOD) score \(\log_{10}(2 \times \text{natural log of } 10)\). We used the DF regression-based QTL linkage analysis as operationalized in QMS2,\textsuperscript{71} a series of integrated SAS macros available at http://ibgwww.colorado.edu/~lessem/software/qms2.html.

**RESULTS**

**SAMPLE CHARACTERISTICS**

The percentages of participants who met the criteria for DSM diagnoses of substance dependence (DSM-IV) and CD (DSM-III-R) are given in Table 2. Note the greater prevalence of substance dependence in the clinical probands and their siblings compared with community adolescents. There is substantial polysubstance use, with more than half of the clinical probands (56.7%) meeting criteria for 2 or more substance dependence diagnoses. Siblings of the clinical probands also show substantially higher rates of substance dependence and dependence on multiple substances than community adolescents. Rates of CD are substantially higher among our clinical probands and their siblings compared with community adolescents.

**Table 1. Marker Information**

<table>
<thead>
<tr>
<th>Chromosome No.</th>
<th>No. of Markers</th>
<th>Mean Distance, cM*</th>
<th>Mean Heterozygosity†</th>
<th>Mean Multipoint Information‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>9.38</td>
<td>78.26</td>
<td>0.55</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>8.73</td>
<td>79.98</td>
<td>0.57</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>9.64</td>
<td>82.05</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>9.63</td>
<td>77.84</td>
<td>0.51</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>9.23</td>
<td>79.46</td>
<td>0.56</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>9.52</td>
<td>79.94</td>
<td>0.51</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>9.09</td>
<td>81.74</td>
<td>0.57</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>11.79</td>
<td>76.91</td>
<td>0.53</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>7.70</td>
<td>76.39</td>
<td>0.60</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>9.38</td>
<td>78.27</td>
<td>0.59</td>
</tr>
<tr>
<td>11</td>
<td>17</td>
<td>9.06</td>
<td>74.52</td>
<td>0.55</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>9.68</td>
<td>79.38</td>
<td>0.57</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>8.04</td>
<td>76.77</td>
<td>0.56</td>
</tr>
<tr>
<td>14</td>
<td>13</td>
<td>10.65</td>
<td>74.34</td>
<td>0.54</td>
</tr>
<tr>
<td>15</td>
<td>13</td>
<td>8.87</td>
<td>79.28</td>
<td>0.57</td>
</tr>
<tr>
<td>16</td>
<td>13</td>
<td>9.62</td>
<td>77.82</td>
<td>0.54</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>8.99</td>
<td>76.33</td>
<td>0.55</td>
</tr>
<tr>
<td>18</td>
<td>13</td>
<td>10.50</td>
<td>73.32</td>
<td>0.54</td>
</tr>
<tr>
<td>19</td>
<td>12</td>
<td>8.09</td>
<td>79.75</td>
<td>0.58</td>
</tr>
<tr>
<td>20</td>
<td>13</td>
<td>7.93</td>
<td>79.52</td>
<td>0.57</td>
</tr>
<tr>
<td>21</td>
<td>7</td>
<td>9.04</td>
<td>68.36</td>
<td>0.47</td>
</tr>
<tr>
<td>22</td>
<td>7</td>
<td>7.91</td>
<td>76.14</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Total 374 9.24 78.29 0.55

Abbreviation: cM, centimorgans.

*Mean intermarker distance for each chromosome.
†Mean heterozygosity averaged across the markers on each chromosome.
‡Mean multipoint information averaged across 1-cM intervals for each chromosome from Merlin.

**Table 2. Diagnostic Prevalences and Descriptive Statistics for Quantitative Measures in the Selected Sibling and Community Samples**

<table>
<thead>
<tr>
<th></th>
<th>Probands</th>
<th>Siblings</th>
<th>Community Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prevalence by DSM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobacco dependence</td>
<td>56.6</td>
<td>28.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Alcohol dependence</td>
<td>36.1</td>
<td>9.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Marijuana dependence</td>
<td>60.7</td>
<td>17.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Other dependence†</td>
<td>26.7</td>
<td>9.2</td>
<td>1.9</td>
</tr>
<tr>
<td>≥2 Dependence diagnoses</td>
<td>56.7</td>
<td>14.4</td>
<td>5.3</td>
</tr>
<tr>
<td><strong>Quantitative measures, mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DV</td>
<td>2.27 (1.49)</td>
<td>0.53 (1.38)</td>
<td>−0.02 (0.96)</td>
</tr>
<tr>
<td>CDS</td>
<td>3.11 (1.64)</td>
<td>1.06 (1.69)</td>
<td>0 (1)</td>
</tr>
<tr>
<td>DV + CDS</td>
<td>3.15 (1.49)</td>
<td>0.97 (1.58)</td>
<td>0 (1)</td>
</tr>
<tr>
<td><strong>Phenotypic correlation (rH0)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heritability of DV</td>
<td>...</td>
<td>...</td>
<td>0.34</td>
</tr>
<tr>
<td>Heritability of CDS</td>
<td>...</td>
<td>...</td>
<td>0.38</td>
</tr>
<tr>
<td>Genetic correlation (RH0)</td>
<td>...</td>
<td>...</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Abbreviations: CD, conduct disorder; CDS, CD symptoms; DV, dependence vulnerability; DV + CDS, composite (sum) index of antisocial substance dependence.
†Illicit substance other than marijuana; means are in standard deviation units standardized for the community adolescent sample.

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On average, our clinical probands score 2 to 3 SDs higher than community adolescents on our measures of DV, CDS, and DV + CDS. Siblings of the probands show regression to the population mean but score approximately half an SD higher than community adolescents on DV and nearly 1 SD higher for CDsx and DV + CDS. Means and SDs in the community sample are 0 and 1, respectively, because scores were standardized on that sample. The mean and SD for DV differ slightly from those values because recruitment of the community sample is ongoing and the community sample is now larger (N=4493 vs 3676) than for our previous report.54 For consistency with that investigation, the standardization of DV was not recalculated for the larger sample.

Our measures of DV and CDsx correlate substantially (r = 0.49) in our community adolescent sample (Table 2). As expected, this correlation is attenuated in our highly selected clinical probands. Biometrical analyses of our community twin sample indicate that these measures are heritable ($h^2_{DV} = 0.34; h^2_{CDsx} = 0.38$), and the genetic correlation between them was estimated at $R_g = 0.47$.

### SINGLE-POINT LINKAGE RESULTS

Table 3 gives the 29 chromosomal markers (several contiguous) where single-point DF regression analyses resulted in a significant $t$ value ($P < .05$). Reported $P$ values are actual values obtained without adjustment for multiple testing. At 3 marker locations (3q24-3q25, 9q34, and 17q12), single-point LOD scores exceeded 2.0 (corresponding to $t$ values $\geq 3.03$). Single-point analysis includes only informative sibling pairs at a given marker location, so some caution in interpretation is advised given that the number of informative pairs varies for each analysis. Despite this limitation, an advantage of single-point results is that they do not depend on a correct marker map and can be less sensitive to genotyping errors.72

### MULTIPOINT RESULTS

Figure 1 shows LOD score plots from multipoint DF regression analyses performed at 1-cM intervals across the genome (22 autosomes). The advantage of multipoint analyses compared with single-point evaluations is that they do not depend on a correct marker map and can be less sensitive to genotyping errors.
is that information from all 249 sibling pairs is used for each linkage test evaluation.

Multipoint mapping results indicated 3 regions with LOD scores greater than 1.0, generally corroborating the single-point results. These findings provide support for the hypothesis that common genes may explain part of the comorbidity between DV and CDS. The strongest evidence for linkage (LOD=2.65) was found for our composite index of antisocial substance dependence (DV/CDS), suggesting evidence for a QTL that influences both DV and CDS on chromosome 9q34 (near markers D9S1826 and D9S1838). Evidence for QTLs that primarily influence DV on chromosome 3q24-3q25 (near markers D3S1279 and D3S1614) and CDS on chromosome 17q12 (near marker D17S798) was also found. These 3 chromosomal regions are shown in greater detail in

Figure 1. Multipoint logarithm of odds (LOD) scores estimated from DeFries-Fulker regression analyses across the genome. A, Dependence vulnerability. B, Conduct disorder symptoms. C, Dependence vulnerability and conduct disorder symptoms.
Figures 2, 3, and 4. Descriptive statistics from our DF regression analyses at these locations are given in Table 4.

Table 5 gives the sibling means plotted as a function of the estimated mean number of alleles shared IBD with the proband for our maximum LOD score locations on chromosomes 3q24-3q25, 9q34, and 17q12. To compute category means, we collapsed the estimated IBD distribution into 3 categories: 0% to 25%, more than 25% to 75%, and more than 75% alleles shared IBD at each location. At our 3q24-3q25 peak location, the sibling means for DV, CDS, and DV/H11001CDS show approximately half an SD difference between sibpairs with an estimated IBD of less than 25% compared with sibling pairs with an estimated IBD of greater than 75%. For our 9q34 location, the mean difference among these groups for DV is of similar magnitude, whereas mean differences for CDS and DV+CDS are approximately 80% of an SD. As expected from our regression results, mean differences are lower in magnitude at our 17q12 location. The pattern of means at the 3q24-3q25 location suggests the possibility of a nonadditive QTL, whereas the pattern of means at the 9q34 location is consistent with an additive QTL.

CHECKS ON ANALYTICAL ASSUMPTIONS

Although the regression-based methods we used are robust to departures from normality, we performed several tests on the assumptions of our analyses. Plots of residuals by predicted values (at the 3 maximum LOD score locations) indicated no obvious heteroscedasticity problems. Consistent with expectations, approximately 5% of the absolute values of the standardized residuals exceeded 2.0 and approximately 1% exceeded 3.0 for each of these analyses. Sensitivity analyses were also conducted at these locations, systematically dropping each sibling pair from the analysis (with replacement) and recomputing regression statistics from the remaining pairs. Evaluation of regression influence statistics indicated no particularly influential cases or outliers. Nonetheless, to minimize any bias introduced by outliers, we identified the 5% most extreme individuals (2.5% in each direction), truncated their scores to the value of the most extreme cases in the remaining 95% of the sample, and then reconducted our regression analyses for each phe-
notype at the 3 maximum LOD score locations. The LOD scores for DV, CDS, and DV + CDS at our 9q34 peak location increased to 1.98, 1.84, and 2.74, respectively; LOD scores for the 3 phenotypes at our 3q24-3q25 peak location were 1.55, 0.77, and 1.57, respectively; and LOD scores at the 17q12 peak location were 0.00, 1.25, and 0.28, respectively. Comparisons between these results and those from the full sample given in Table 4 indicate that our findings are not simply artifacts that result from the undue influence of a few outliers.

**EMPIRICAL SIGNIFICANCE LEVELS**

We used permutation simulations to derive empirical P values for our obtained results at each of our peak locations. Empirical null distributions were simulated by randomly permuting IBD estimates with phenotypic scores for a given sibling pair (maintaining the family structure). We used permutation simulations to derive empirical P values for the regression test statistics obtained at each of our maximum LOD score locations (ie, reported P values are the proportion of times of 5000 simulations that we observed t values greater than or equal to the maximum value we obtained at 3q24-3q25, 9q34, and 17q12). Those P values, given in Table 4, are not substantially different from those assuming a t distribution, again suggesting that our results are not unduly biased by violations of the assumptions of our analyses.

**QUALITATIVE ANALYSES**

To explore these data further, we used a variant of the affected sibling pair method. We defined affected status as scoring above a z-score cutoff of 1.96 for each of our 3 phenotypic measures. Sibling pairs were considered concordant extreme-scoring pairs if they both scored above the respective z-score cutoff. When only the proband exceeded the threshold and the sibling did not, the pair was considered a discordant pair. A z score greater than 1.96 would include approximately 2.5% of the general population. By defining affection status this way, we are not suggesting that many of our sibling pairs are unaffected. This analysis was used to determine whether sibling pairs concordant for extreme scores on our quantitative phenotypes also showed increased allele sharing IBD at the locations suggested by our regression analyses.

**Table 5. Sibling Means by IBD at Peak LOD Score Locations**

<table>
<thead>
<tr>
<th>Peak Location</th>
<th>No. of Sibling Pairs</th>
<th>Phenotypes, Mean (SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3q24-3q25</td>
<td></td>
<td>DV (n = 51) CDS (n = 129) DV + CDS (n = 0.129)</td>
</tr>
<tr>
<td>&lt;25%</td>
<td>51</td>
<td>-0.20 (0.15) -0.07 (0.23) -0.16 (0.19)</td>
</tr>
<tr>
<td>25%-75%</td>
<td>129</td>
<td>-0.11 (0.12) -0.17 (0.14) -0.16 (0.13)</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>69</td>
<td>0.35 (0.19) 0.36 (0.21) 0.41 (0.20)</td>
</tr>
<tr>
<td>9q34</td>
<td></td>
<td>DV (n = 51) CDS (n = 110) DV + CDS (n = 0.110)</td>
</tr>
<tr>
<td>&lt;25%</td>
<td>74</td>
<td>-0.29 (0.14) -0.42 (0.16) -0.42 (0.15)</td>
</tr>
<tr>
<td>25%-75%</td>
<td>110</td>
<td>-0.00 (0.13) 0.04 (0.15) 0.02 (0.14)</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>65</td>
<td>0.34 (0.19) 0.41 (0.25) 0.43 (0.22)</td>
</tr>
<tr>
<td>17q12</td>
<td></td>
<td>DV (n = 51) CDS (n = 139) DV + CDS (n = 0.139)</td>
</tr>
<tr>
<td>&lt;25%</td>
<td>59</td>
<td>0.08 (0.20) -0.26 (0.17) -0.12 (0.18)</td>
</tr>
<tr>
<td>25%-75%</td>
<td>139</td>
<td>-0.04 (0.11) -0.06 (0.14) -0.06 (0.13)</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>51</td>
<td>0.08 (0.20) 0.48 (0.28) 0.30 (0.25)</td>
</tr>
</tbody>
</table>

*Means can be interpreted in standard deviation units.

**Table 6. Mean Allele Sharing IBD at Peak LOD Locations on C3, C9, and C17 for Concordant High-Scoring Pairs and Discordant Sibling Pairs**

<table>
<thead>
<tr>
<th>Location</th>
<th>Concordant High-Scoring Pairs (z&gt;1.96), Mean (SD)*</th>
<th>Discordant Pairs, Mean (SD)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DV (n = 30) CDS (n = 51) DV + CDS (n = 44)</td>
<td>DV (n = 124) CDS (n = 143) DV + CDS (n = 156)</td>
</tr>
<tr>
<td>Chromosome 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3S1279</td>
<td>0.61 (0.05) 0.62 (0.05) 0.59 (0.05)</td>
<td>0.50 (0.03) 0.46 (0.03) 0.49 (0.03)</td>
</tr>
<tr>
<td>D3S1614</td>
<td>0.57 (0.06) 0.63 (0.05) 0.60 (0.06)</td>
<td>0.54 (0.03) 0.50 (0.03) 0.53 (0.03)</td>
</tr>
<tr>
<td>Peak LOD score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D9S1826</td>
<td>0.58 (0.07) 0.60 (0.05) 0.57 (0.05)</td>
<td>0.45 (0.03) 0.45 (0.03) 0.46 (0.03)</td>
</tr>
<tr>
<td>D9S1838</td>
<td>0.59 (0.07) 0.61 (0.05) 0.58 (0.05)</td>
<td>0.44 (0.03) 0.45 (0.03) 0.46 (0.03)</td>
</tr>
<tr>
<td>Peak LOD score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D17S798</td>
<td>0.46 (0.05) 0.55 (0.04) 0.53 (0.04)</td>
<td>0.50 (0.03) 0.45 (0.02) 0.46 (0.02)</td>
</tr>
<tr>
<td>Peak LOD score</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: CDS, conduct disorder symptoms; DV, dependence vulnerability; DV + CDS, composite (sum) index of antisocial substance dependence; IBD, identity by descent; LOD, logarithm of odds.

†Sibling pairs in which both the proband and sibling both score above the z-score cutoff.

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scoring sibling pairs for our 3q24-3q25 and 9q34 peak LOD score regions. As expected, this trend is less evident for our 17q12 location, where only our CDS phenotype yielded an LOD score greater than 1.0. A trend for decreased allele sharing among discordant sibling pairs was found for all phenotypes at our 9q34 region. This was not found for our 3q24-3q25 region, again suggesting the possibility of a nonadditive QTL in this region.

**COMMENT**

This study extends our initial findings of putative QTLs underlying DV in adolescence by performing additional genome scans for CDS and a composite phenotype reflecting antisocial substance dependence (DV + CDS) on the same adolescent sample. Our findings provide preliminary support for the hypothesis that common genes may influence CD and substance dependence vulnerability in adolescence. Single-point analyses indicated 29 markers with nominally significant (P < .05) test results for linkage for 1 or more of our measures. Multipoint mapping analyses corroborated 3 locations, with the strongest evidence for QTLs influencing CD and DV detected on chromosome 9q34 (near markers D9S1826 and D9S1838). A QTL more strongly associated with DV was detected on chromosome 3q24-3q25 (near markers D3S1279 and D3S1614), and a putative QTL unique to CDS was reported on chromosome 17q12 (near marker D17S198).

Our maximum LOD score in the region of 9q34 meets criteria for suggestive linkage (LOD = 2.65). The evidence for linkage to 3q24-3q25 and 17q12 is less strong but of sufficient magnitude to warrant further study of these regions. Although these locations do not meet the criteria of Lander and Kruglyak for significant linkage, few studies of comparable sample size will individually have sufficient power to identify QTLS at this level of significance. A strength of our design was the use of a sample of 249 selected sibling pairs, which provides roughly the same power as 1000 to 2000 unselected sibling pairs, using our analytical methods. Even so, the power to detect QTLS of low effect size (<15% heritability) is limited. Clearly, these findings require replication in independent adolescent samples. However, a series of checks on our current analyses indicate that our linkage results are robust to outliers and the phenotypic distributional properties of our sample.

Other investigators have suggested QTLS in the vicinity of ours for substance-related behaviors. Uhl et al. investigating QTLS for polysubstance abuse vulnerability in an adult sample, reported a significant association with a region on chromosome 3 within 10 cM of our peak location (173-175 cM). Long et al also reported suggestive evidence for linkage to 3 markers on chromosome 3, spanning the region 165.3 to 181.1 cM in a genome scan for genetic linkage to alcohol dependence. Bergen et al reported a similar region associated with smoking status on chromosome 3 (171.7 cM) and a region close to our peak location on chromosome 9 (161 cM). A recent genome scan for QTLS associated with CD did not find the same locations as we did for our CDS measure. The authors did not report a QTL on chromosome 3 (134 cM) near a secondary peak (LOD = 0.80) evident from our genome scan for our DV measure (Figure 2). However, their adult sample was selected for high-density alcoholism, and CD symptoms were retrospectively reported. In addition, their sample was not highly selected for CD (approximately 13% would meet criteria for a diagnosis), so our samples are not directly comparable.

There are several limitations of our study. First, endophenotypes that uniquely represent specific genetic contributions to CDS and DV are unknown. Our phenotypes were constructed from standard psychiatric symptom clusters (DSM criteria). These clusters, although useful for guiding currently available treatments, generally do not consider differences in etiologies.

Second, because CD and SUDs develop over time, we used age- and sex-normed quantitative traits to express CD and DV in our selected sibling sample in relation to the population means and variability for community adolescents. Although CD and polysubstance use are characteristic of our high-risk probands and their siblings, not all of our study participants have fully passed through the age of risk, and this may limit the validity of our phenotypes.

Finally, our clinical adolescent sample was selected for both problem substance use and CD; thus, our findings may not generalize to broader substance-abusing populations without comorbid antisocial behavior. However, joint selection for both of these phenotypes would not be expected to lead to the finding of a common QTL in the absence of pleiotropy or strong linkage disequilibrium.

To explore our results further, we conducted a search for candidate genes within ±5 megabases (Mb) of each of our peak locations (http://genome.ucsc.edu). These searches yielded approximately 100 genes within each region. We highlight herein some candidates of interest (a full listing of identified genes in these regions can be found on our Web site at http://ibgwww.colorado.edu/cadd/a_center/research). Potential candidates in the region of 3q24-3q25 include the phospholipase D1 gene (PLD1); a serine proteinase inhibitor gene (SERPIN1); claudin 11, an oligodendrocyte transmembrane protein gene (CLDN11); and neuroligin 1 (NLG1). A notable candidate near our 9q34 region is the dopamine β-hydroxylase gene (DBH). However, when we added a SNP marker within the DBH gene (DBH-1021C → T) to our chromosome 9 map, there was no evidence of linkage near this marker, suggesting that our putative QTL is distal to the DBH locus. Other potential candidates in the region include GRIN1 (NMDARI), a gene that codes for a subunit of the N-methyl-d-aspartate receptor; a neuroblastoma protein gene (AMY); a chloride intracellular channel gene (CLIC3); and a calcium channel gene (CACNA1B). Candidates within ±5 Mb of our 17q12 peak location include the serotonin transporter gene (SLC6A4); a cyclin-dependent kinase 5 regulatory subunit (CDK5R1) that codes for a protein that is a neuron-specific activator of CDK5; and OMG, a gene that codes for an oligodendrocyte myelin glycoprotein. Although we restricted our systematic search to ±5 Mb, we note with interest that DARPP-32, a dopamine- and cyclic adenosine monophosphate–regulated phosphoprotein that ap-
pears to be essential for dopaminergic and serotonergic neurotransmission, is located 6.3 Mb from our chromosome 17 peak. Putative gene function was ascertained from the GeneCards database (http://bioinfo1.weizmann.ac.il/gene_cards/).

In summary, CD and SUDs are frequently comorbid in adolescence. Although the precise etiologic mechanisms that underlie the frequent co-occurrence of these problem behaviors remain unclear, the current investigation was motivated by increasing evidence that common genetic risks may explain part of the comorbidity. The goal of our current program of research is to extend this biometrical evidence to localize chromosomal regions that contain genes that influence both these disorders. Results of this study provide the first evidence of a potential molecular genetic basis for the comorbidity between drug dependence vulnerability and antisocial behavior in adolescents.

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