Variation at the *DAOA/G30* Locus Influences Susceptibility to Major Mood Episodes but Not Psychosis in Schizophrenia and Bipolar Disorder

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Context: Variation at the *DAOA/G30* locus has been described to be associated with both schizophrenia and bipolar disorder, but there is little consistency between studies of the tested polymorphisms or variants showing association.

Objectives: To obtain a stringent replication of association in large samples of both disorders using consistent clinical and laboratory methods, and to test the hypothesis that association at *DAOA/G30* identifies an underlying domain of psychopathological abnormalities that cuts across traditional diagnostic categories.

Design: A systematic study of polymorphisms at *DAOA/G30* using genetic case-control association analysis.

Setting: Subjects were unrelated and ascertained from general psychiatric inpatient and outpatient services.

Participants: White persons from the United Kingdom meeting criteria for *DSM-IV* schizophrenia (n=709) or bipolar I disorder (n=706) and 1416 ethnically matched controls.

Methods: Nine polymorphisms that tag common genetic variations at *DAOA/G30* were genotyped in all of the individuals, and comparisons were made between affected and unaffected individuals.

Results: We identified significant association ($P=.01$-$.047$) between 3 single-nucleotide polymorphisms and bipolar disorder but failed to find association with schizophrenia. Analyses across the traditional diagnostic categories revealed significant evidence ($P=.002$-$.02$) for association with 4 single-nucleotide polymorphisms in the subset of cases (n=818) in which episodes of major mood disorder had occurred (gene-wide $P=.009$). We found a similar pattern of association in bipolar cases and in schizophrenia cases in which individuals had experienced major mood disorder. In contrast, we found no evidence for association in the subset of cases (n=1153) in which psychotic features occurred (all $P>.08$).

Conclusions: Despite being originally described as a schizophrenia susceptibility locus, our data suggest that variation at the *DAOA/G30* locus does not primarily increase susceptibility for prototypical schizophrenia or psychosis. Instead, our results imply that variation at the *DAOA/G30* locus influences susceptibility to episodes of mood disorder across the traditional bipolar and schizophrenia categories.

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THE MAJORITY OF PSYCHIATric research on functional psychoses has proceeded under the assumption that schizophrenia and bipolar disorder are separate disease entities with different underlying etiologies and pathogenesis. However, there has been a long tradition of dissent against the validity of this view (eg, in articles by Brockington et al,[1] Crow,[2] and Taylor[3]), and the utility of the so-called Kraepelinian dichotomy has been increasingly challenged.[4-6] Arguably, the most potent challenge has come from the findings of molecular genetics research.[4] A number of linkage studies (reviewed by Berrettini[7])

and meta-analyses[8] have implicated the same or overlapping chromosomal regions in both schizophrenia and bipolar disorder, particularly in regions of chromosomes 13q, 22q, and 18.

The identification of susceptibility genes[9,10] provides a direct means to explore possible overlap between the 2 disorders. It is therefore of great interest that several recent articles[7,8,11] suggest that schizophrenia and bipolar disorder might have susceptibility genes in common. The most notable example is the locus at 13q32-q34 that harbors the gene encoding D-amino-acid oxidase (DAO) activator (*DAOA*; formerly known as *G72*) and the putative gene referred to as *G30*. These

genes physically overlap, being transcribed from opposite DNA strands, and were originally implicated in susceptibility for schizophrenia by the finding of genetic associations in 2 samples within the context of a systematic study of part of the 13q linkage region.[12] Subsequent studies have also described association between schizophrenia and markers in DAOA/G30.[13-16] In addition, studies in bipolar disorder have found evidence of association at this locus.[16-18] Indeed, it is currently the best-supported gene for bipolar disorder.[11,19] However, there has been little consistency between studies in the markers tested or in the polymorphisms or haplotypes showing association. In particular, there have been few direct comparisons of the same markers in schizophrenia and bipolar disorder. The one study to date that has directly compared the same polymorphisms suggests that the 2 disorders might show association with the same markers and haplotypes,[16] consistent with the hypothesis that susceptibility to the 2 disorders is conferred by the same genetic variants in DAOA/G30. This is of considerable interest because it potentially goes to the heart of the question of the validity of the Kraepelinian dichotomy.[4]

In the present study, we screened all of the exons of DAOA and G30 for polymorphism, and we tested selected markers for association in large, well-characterized samples of patients with schizophrenia or bipolar disorder. Our study had several aims. First, we sought to undertake a stringent test of the hypothesis that DAOA/G30 is associated with schizophrenia and bipolar disorder by conducting a systematic and well-powered study capturing most of the population variation at this locus. Second, we wished to determine whether the 2 disorders show similar patterns of associations suggesting a common genetic mechanism or whether the data are consistent with a different pattern of genetic variation underlying susceptibility to the 2 prototypical Kraepelinian psychoses. Third, we wished to test the hypothesis that association with both disorders might reflect association with an underlying domain of psychopathological abnormalities that cuts across the traditional diagnostic categories. We chose to examine psychosis and mood disorder because they can be reliably measured and are common in the patients in question. Psychotic features are almost universal in individuals with a diagnosis of schizophrenia, and episodes of major mood disorder often occur. Episodes of major mood disorder are always present in individuals with a diagnosis of bipolar disorder, and psychotic features are common.

METHODS

SAMPLE

All of the subjects were unrelated, white, and of United Kingdom origin and provided written informed consent to participate in genetic studies. Protocols and procedures were approved by relevant ethical review panels, including the United Kingdom West Midlands Multi-Center Research Ethics Committee, Birmingham, England, and the United Kingdom Wales Multi-Center Research Ethics Committee, Cardiff, Wales. Cases were recruited through mental health services in England and Wales. Diagnoses were made by the consensus lifetime best-estimate method[20] on the basis of all of the available information, including a semistructured interview (Schedules for Clinical Assessment in Neuropsychiatry)[21] and a review of psychiatric case records, and an OPCRIT checklist was completed.[22] Formative team reliability meetings took place weekly throughout recruitment.

Bipolar Cases

Bipolar cases met DSM-IV[23] criteria for bipolar I disorder (n=706; 37.4% male; mean [SD] age at interview, 47.7 [13.1] years; mean [SD] age at first impairment from major mood disorder, 26.3 [10.2] years; family history of psychiatric illness in first- or second-degree relative present in 59.6% of patients). Of the bipolar cases, 107 probands were ascertained for a sibling-pair linkage study.[24,25] Key clinical variables relating to psychosis were rated using the Bipolar Affective Disorder Dimensional Scale.[26] In this scale, a score in the range of 1 to 100 on the psychosis dimension shows the best estimate of the proportion of total episodes of illness in which psychotic features occurred. Of our bipolar sample, 62.9% had a lifetime occurrence of 1 or more psychotic features. Interrater reliability was high for the measures used in this study. This was formally assessed using 20 cases and resulted in a mean κ statistic of 0.85 for DSM-IV diagnoses and a mean intraclass correlation coefficient of 0.86 for the Bipolar Affective Disorder Dimensional Scale psychosis dimension.

Schizophrenia Cases

All of the patients in the schizophrenia group had a diagnosis of schizophrenia according to the DSM-IV. The total case-control sample used in this study comprised 709 subjects with schizophrenia from the United Kingdom and Ireland (70.9% male; mean [SD] age at first psychiatric contact for the sample, 23.6 [7.7] years; mean [SD] age at interview, 41.8 [13.5] years; 15.7% met diagnostic criteria for at least 1 episode of major affective disorder [n=82 with depression only; n=18 with mania only; n=12 with both mania and depression] during their lifetime as coded on the OPCRIT checklist; family history of psychiatric illness in a first- or second-degree relative was present in 26.6% of patients). Of the schizophrenia cases, 141 were ascertained for a sibling-pair linkage study.[27,28] High levels of reliability (κ>0.89) were achieved between raters for depressive and manic episodes.

Controls

Controls (n=1416; 51.6% male; mean [SD] age, 42.4 [11.1] years) were all white and of United Kingdom origin, and they were collected from 2 sources. One source was the British Blood Transfusion Service, Manchester, England (n=1307). The sample was not specifically screened for psychiatric illness, but individuals were not receiving regular prescribed medications. In the United Kingdom, blood donors are not remunerated, even for expenses, and are therefore not overrepresented for indigent or socially disadvantaged persons in whom the rate of psychosis might possibly rise above a threshold that would influence power.[29,30] The other source was a family practitioner clinic (n=109). Individuals were recruited from among those attending for nonpsychiatric reasons. This sample was screened to exclude a personal history of mood disorder or schizophrenia.

SINGLE-NUCLEOTIDE POLYMORPHISM IDENTIFICATION

The exonic structure of DAOA was determined in silico by combining the reference exonic sequence NM_172370 with the

5 known alternatively spliced transcripts AY138547, AY223901, AY170469, AY170470, and AY170471. The exonic structure of *G30* was derived from its reference sequence NM_172368. Only 2 exons of *DAOA* and *G30* partially overlap: 111 base pairs (bp) of exon 2 *DAOA* with exon 5 *G30*, and 156 bp of exon 8 *DAOA* with exon 4 *G30*. All of the available exons were aligned according to the University of California, Santa Cruz, human genome reference sequence (May 2004 freeze). The genomic sequences were used to design primers spanning each exon using Primer3.[31] Large exons were amplified using sets of amplimers of no more than 600 bases that overlapped by no less than 50 bases. All of the polymerase chain reactions (PCRs) were performed using standard touchdown protocols previously described.[32]

The sample for mutation screening comprised 14 unrelated white subjects from the United Kingdom meeting *DSM-IV* criteria for schizophrenia, each of whom had at least 1 affected sibling. The PCR products from each were screened for sequence variation by denaturing high-performance liquid chromatography using a sensitive protocol.[32] The PCR products yielding chromatograms indicative of heteroduplex formation were sequenced in both directions using the BigDye Terminator Cycle Sequencing kit and an ABI3100 sequencer according to the manufacturer's instructions (Applied Biosystems, Foster City, Calif). All of the variants were confirmed by allele-specific primer extension using the SNaPshot kit and an ABI3100 sequencer according to the manufacturer's instructions (Applied Biosystems).

GENOTYPING

The allele frequencies for all of the polymorphisms identified by denaturing high-performance liquid chromatography were estimated in DNA pools comprising 544 blood donor controls who were white and of United Kingdom origin (388 men and 156 women). Analysis was performed on 3 different DNA pools that each contained a different set of controls. Pools were created from DNA that had been quantified using the PicoGreen dsDNA Quantitation Reagent (Molecular Probes, Eugene, Ore) and a Labsystems Fluoroskan Ascent fluorometer (LifeSciences International, Basingstoke, England). Each DNA pool was amplified in 2 separate PCR reactions, and the products were subjected to allele-specific primer extension using SNaPshot as described.[33] All of the subsequent individual genotyping was performed either by means of allele-specific PCR using the Amplifluor system (Invitrogen Ltd, Paisley, Scotland) or by single-nucleotide primer extension using either the Acycloprime (Perkin Elmer Life Science Products, Boston, Mass) or SNaPshot systems according to the manufacturers' instructions, with alleles being determined by fluorescence polarization measurement using an Analyst (LJL Biosystems Ltd, Surrey, England) or ABI3100 sequencer, respectively. All of the genotypes for each specific marker were generated by only 1 of the described methods.

LINKAGE DISEQUILIBRIUM ANALYSIS

All of the polymorphisms with an estimated minor allele frequency greater than 10% were individually genotyped in 96 individuals (48 schizophrenia cases, 48 controls) to estimate marker-marker linkage disequilibrium. The program Haploview[34] was used to examine the haplotype block structure according to the method defined by Gabriel et al[35] and to select a set of maximally informative single-nucleotide polymorphisms (SNPs) that tagged all of the haplotypes with a frequency greater than 5%, which represented 94.1% of all of the haplotypes detected in our sample. These tag SNPs were then genotyped through both schizophrenia and bipolar disorder case-control samples.

STATISTICAL ANALYSIS

Departure from Hardy-Weinberg equilibrium was tested using a χ^2 goodness-of-fit test. Tests for differences between cases and controls for allele and haplotype frequencies were performed using UNPHASED version 2.40 software.[36] The effect of haplotypes or alleles was assumed to be additive. Haplotype analysis was performed using a sliding window, excluding rare haplotypes with frequencies less than 1%. Uncertain haplotypes were estimated using the expectation maximization algorithm within the UNPHASED software. Two-tailed *P* values were noted. Nominally significant asymptotic *P* values were confirmed by permuting the case-control status over 50 000 replicates and observing the maximum test statistic in each case.

To evaluate the evidence for overall association in the context of testing multiple markers, we applied the product of the *P*-values method to the 9 SNP results. This is a "gene-wide" test that takes testing multiple SNPs and their linkage disequilibrium relationships into account and produces a single significance level for evidence of overall association at the gene. Following the methods used by Zaykin et al,[37] we multiplied all of the *P* values reaching a threshold of *P*<.10 to achieve a product *P* value. The significance of this product was established by permuting case-control status over 100 000 replicates and counting the number of times this product was exceeded.

In addition to undertaking comparisons against controls for the traditional diagnostic groups of schizophrenia and bipolar disorder, we also made comparisons against controls for the psychosis domain (the set of patients who had ever experienced delusions or hallucinations) and the mood episode domain (the set of patients who had experienced at least 1 episode of major mood disorder [depression or mania]).

RESULTS

We screened a total of 7315 bp of genomic sequence spanning the *DAOA/G30* locus (details are available at our Web site, http://www.cardiff.ac.uk/medicine/psychological _medicine/pub_data/daoa/) by denaturing high-performance liquid chromatography. This included exonic 5′ and 3′ untranslated region sequence as well as 3518 bp of flanking intronic sequence.

We identified 19 sequence variants spanning the *DAOA/G30* locus (11 exonic, 8 intronic). The positions of each of the polymorphisms together with their allele frequencies as estimated in DNA pools are presented at our Web site. Sequencing analysis implied that the genotypes of the 6 SNPs that we identified within the 248-bp region from chr13:103827525-103827772 cosegregated together. As all of the 6 SNPs also had similar allele frequency estimates, we interpreted this as suggesting that it was likely that 5 of the 6 SNPs were redundant. Therefore, these 5 SNPs together with an additional 8 polymorphisms with an estimated minor allele frequency of less than 10% were excluded from any subsequent analysis. The remaining 6 polymorphisms were then supplemented by an additional 3 intronic SNPs selected from the Single Nucleotide Polymorphism Database[38] so that our markers spanned the entire *DAOA/G30* locus at an average of 7.9 kilobases. All of the 9 SNPs were then genotyped in 96 unrelated individuals to establish the linkage disequilibrium structure of the *DAOA/G30* locus and a set of tag SNPs that were identified (data are available

at our Web site). The 6 tag SNPs identified by this procedure were genotyped in 709 schizophrenia cases, 706 bipolar disorder cases, and 1409 controls. The linkage disequilibrium structure observed in our data was consistent with that in data from the HapMap project.[39] In addition, 3 other SNPs (rs3916965, rs778293, and rs1421292) spanning the *DAOA/G30* locus were also genotyped through both association samples, as they had previously been described as being associated with schizophrenia.[12] The genomic structure of *DAOA* and location of SNPs typed in this study are shown in the **Figure**. A total of 559 duplicate genotypes were assayed across all of the markers, and 99.8% were concordant. Allele and genotype frequencies did not differ significantly between the blood donor and family practitioner controls, and these were therefore treated as 1 group for analyses. There were no significant deviations from Hardy-Weinberg equilibrium in either the control or case sample sets for any of the polymorphisms studied.

We found no evidence for allelic or genotypic association with any of the polymorphisms studied for schizophrenia (**Table 1**). Similarly, we found no evidence of haplotypic association for 2-locus, 3-locus, or higher-order sliding window analysis. In contrast, we observed nominally significant evidence (P=.01-.047) for allelic association with 3 of the polymorphisms for bipolar disorder and significant evidence for whole-gene association (P=.04). The results of single-marker association analysis for all of the SNPs are presented in Table 1.

When we undertook analyses across the traditional phenotype categories according to the 2 broad domains of psychopathological abnormalities, we observed no evidence for allelic or genotypic association for the subset of cases (n=1153) in which psychotic features occurred (**Table 2**) (or in parts of this subset selected according to mood congruence or incongruence of the psychotic features).[26] In contrast, when we analyzed data from the subset of cases (n=818) in which episodes of major mood disorder occurred (41.3% male; mean [SD] age at onset of impairment, 26.0 [9.7] years; *DSM-IV* diagnoses: 706 patients with bipolar I disorder and 112 patients with schizophrenia), we observed nominally significant evidence (P=.002-.02) for allelic association with 4 of the polymorphisms (Table 2). Allowing for all of the SNPs at a whole gene level, the *DAOA/G30* locus remained significant (P=.009).

The distribution of allele frequencies showed a similar pattern in the bipolar cases and the subset of schizophrenia cases in which episodes of major mood disorder occurred, with a greater deviation from controls being shown by the schizophrenia subset (Table 2). A comparison between this subset of schizophrenia cases and controls demonstrated significant differences for 2 of the polymorphisms studied (Table 2) as well as significance for the whole-gene test (P=.02). Furthermore, a comparison between the subset of schizophrenia cases in which episodes of major mood disorder occurred and the remaining set of schizophrenia cases that did not meet this criterion demonstrated significant differences (P<.05) for 3 of the polymorphisms studied (data not shown). Applying the product of the *P*-values method to this comparison yielded a whole-gene *P* value of .02. In contrast, the allele distri-
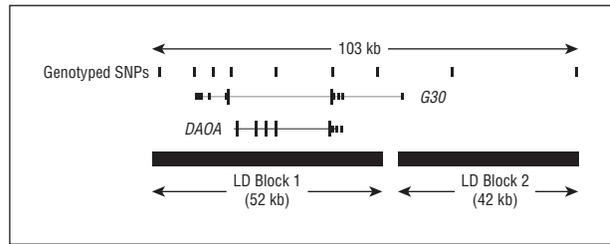

Figure. A representation of the relative genomic locations of the *DAOA* and *G30* loci together with the single-nucleotide polymorphisms (SNPs) that were genotyped in this study (left to right: rs1935058, rs1341402, rs2391191[M15], DAOA_3′UTR_SNP12, rs778294[M19], rs954581, rs778293[M22], rs1421292[M24]). The location of linkage disequilibrium (LD) blocks spanning the *DAOA/G30* locus as defined by data from the HapMap project[39] are also indicated. kb indicates kilobases.

butions were very similar in the subsets of bipolar cases with and without psychotic features (data not shown).

The genotype tests yielded *P* values similar to those in the allelic tests (data not shown). Haplotype analyses did not provide increased levels of evidence for association over those observed with individual single polymorphisms (data not shown). Taking account of age at onset of illness did not significantly influence the findings.

COMMENT

We have undertaken a systematic study of polymorphisms across the *DAOA/G30* locus with large, well-characterized samples of schizophrenia and bipolar disorder cases that were recruited from the same United Kingdom population using similar ascertainment and assessment methods. Using the traditional diagnostic categories and taking a statistical approach that allows for the examination of multiple SNPs, we have replicated prior reports of association at this gene with bipolar disorder but failed to find evidence for association with schizophrenia. However, when we undertook analyses across the traditional diagnostic groups, we found significant evidence that variation at this locus influences susceptibility to episodes of major mood disorder. Individuals with schizophrenia who had experienced at least 1 episode of major mood disorder showed a pattern of findings similar to that in the bipolar cases. In contrast, we found no evidence that variation at *DAOA/G30* influences susceptibility to psychosis.

It is unlikely that our failure to find association in schizophrenia was a type II error because our sample was large and provided power greater than 77% at *P*<.05 to detect effects of the size we observed in our bipolar sample. Indeed, we did not even observe a trend with any of the polymorphisms studied at the *P*<.10 level, where the power of our sample exceeded 85%.[40,41] We also endeavored to extract a high proportion of the genetic diversity at the locus, although we cannot exclude the possibility that we would have found associations if we had typed more SNPs. One argument that could be advanced for our failure to detect evidence of association is the lack of evidence for linkage to the 13q locus in our schizophrenia sibling-pair linkage sample[28] that was recruited from the same population as our sample of unrelated cases and that has overlapping cases.[27,28] However, most schizo-

**Table 1. Allele Distributions in Controls, Schizophrenia Cases, and Bipolar I Disorder Cases
for 9 Polymorphisms Spanning the *DAOA/G30* Locus**

| | Phenotype | | | | |
| | Controls (n = 1416) | Schizophrenia (n = 709) | | Bipolar I Disorder (n = 706) | |
Single-Nucleotide Polymorphism*	MAF†	MAF†	P Value‡	MAF†	P Value‡
rs391695 (M12)	39.4 (A)	37.6 (A)	.27	36.2 (A)	.047§
rs1935058	44.7 (G)	43.6 (G)	.52	43.0 (G)	.30
rs1341402	21.6 (C)	21.3 (C)	.82	24.8 (C)	.03§
rs2391191 (M15)	39.7 (T)	37.9 (T)	.27	36.9 (T)	.09
DAOA_3'UTR_SNP12	33.0 (A)	33.6 (A)	.73	37.1 (A)	.01§
rs778294 (M19)	28.7 (T)	28.0 (T)	.63	30.2 (T)	.35
rs954581	18.1 (G)	17.4 (G)	.67	18.3 (G)	.88
rs778293 (M22)	38.5 (G)	37.2 (G)	.44	40.0 (G)	.36
rs1421292(M24)	49.0 (A)	49.8 (A)	.66	48.1 (A)	.59
Gene-wide test	NA	NA	NS‖	NA	.045§

Abbreviations: MAF, minor allele frequency; NA, not applicable; NS, nonsignificant.
*In addition to "rs" identifying numbers, commonly used alternative names are given in parentheses. DAOA_3'UTR_SNP12 is a novel variant that was identified during this study.
†Minor allele frequencies are shown as the percentage for the specific allele in parentheses.
‡P values are for comparisons against the controls.
§P values are nominally significant at P<.05.
‖Gene-wide test was not undertaken because no individual P value was less than .10.

**Table 2. Allele Distributions in Controls and Subsets of Schizophrenia Cases
for 9 Polymorphisms Spanning the *DAOA/G30* Locus***

| | Phenotype | | | | | | | | | |
| | Controls (n = 1416) | Psychosis (n = 1153) | | Mood (n = 818) | | Schizophrenia-Mood (n = 112) | | Schizophrenia– No Mood Episodes (n = 597) | |
Single-Nucleotide Polymorphism†	MAF‡	MAF‡	P Value§	MAF‡	P Value§	MAF‡	P Value§	MAF‡	P Value§
rs391695 (M12)	39.4 (A)	37.0 (A)	.08	35.7 (A)	.02‖	32.9 (A)	.05	38.6 (A)	.27
rs1935058	44.7 (G)	43.6 (G)	.43	42.3 (G)	.14	38.5 (G)	.08	45.3 (G)	.52
rs1341402	21.6 (C)	22.7 (C)	.36	25.0 (C)	.01‖	26.6 (C)	.09	20.7 (C)	.82
rs2391191 (M15)	39.7 (T)	37.6 (T)	.14	36.1 (T)	.02‖	31.7 (T)	.02‖	39.0 (T)	.27
DAOA_3'UTR_SNP12	33.0 (A)	35.0 (A)	.16	37.8 (A)	.002‖	41.5 (A)	.01‖	32.1 (A)	.73
rs778294 (M19)	28.7 (T)	28.8 (T)	.96	30.8 (T)	.16	34.2 (T)	.08	26.3 (T)	.63
rs954581	18.1 (G)	17.8 (G)	.81	18.3 (G)	.84	18.7 (G)	.82	16.6 (G)	.61
rs778293 (M22)	38.5 (G)	38.1 (G)	.79	39.6 (G)	.49	37.1 (G)	.67	37.7 (G)	.44
rs1421292 (M24)	49.0 (A)	49.4 (A)	.78	48.6 (A)	.79	51.3 (A)	.51	50.4 (A)	.66
Gene-wide test	NA	NA	NS¶	NA	.009‖	NA	.02‖	NA	NS¶

Abbreviations: MAF, minor allele frequency; NA, not applicable; NS, nonsignificant.
*Psychosis indicates lifetime occurrence of 1 or more delusions or hallucinations; mood, lifetime occurrence of 1 or more episodes of major mood disorder (mania or depression); schizophrenia-mood, schizophrenia cases in which 1 or more episodes of major mood disorder (mania or depression) have occurred during lifetime; schizophrenia–no mood episodes, schizophrenia cases in which 1 or more episodes of major mood disorder (mania or depression) are not known to have occurred during lifetime.
†In addition to "rs" identifying numbers, commonly used alternative names are given in parentheses. DAOA_3'UTR_SNP12 is a novel variant that was identified during this study.
‡Minor allele frequencies are shown as the percentage for the specific allele in parentheses.
§P values are for comparisons against the controls.
‖P values are nominally significant at P<.05.
¶Gene-wide tests were not undertaken because at most, only 1 P value was less than .01.

phrenia samples with positive findings have similarly been unselected for concurrent linkage at this locus. Furthermore, we found no evidence for linkage at this locus in our bipolar sibling-pair study,[25] and yet we found evidence for association in our bipolar sample. (Reanalysis of our schizophrenia and bipolar disorder linkage data sets using the subset of pedigrees in which the proband carried associated alleles also failed to demonstrate significant or suggestive evidence for linkage.)

Several studies have described associations between *DAOA* and schizophrenia,[12-16] although some studies have found negative results.[42] Our findings imply that whether

significant associations are seen will depend on the proportion of cases that have had episodes of mood disorder. Only 16% of our subjects with schizophrenia had episodes of mood disorder. Given our observed effect sizes, the proportion of cases with mood episodes would need to be approximately 65% for us to have identified an overall effect with schizophrenia. It is, of course, to be expected that the proportion of cases with mood disorder will vary according to the method of ascertainment and the research context. Diagnostic categorization requires judgments about the balance of mood and schizophrenic psychopathological abnormalities. Consequently, it is possible that more cases with prominent mood episodes will be included within schizophrenia samples collected by groups studying only schizophrenia than by groups studying both schizophrenia and mood disorder because in the latter, the researchers may attach more diagnostic weight to mood episodes.

We also need to consider the possibility that the positive findings we obtained were spurious or the results of type I error. Differences between cases and controls that are unrelated to disease status can be caused by the presence of so-called population structure, which may result in differential sampling of cases and controls from genetically distinct subpopulations. However, this is unlikely to be the cause of our findings for the following reasons: (1) case and control samples were both sampled from the white United Kingdom population; (2) genotype distributions were consistent with Hardy-Weinberg equilibrium for the groups separately and pooled together, suggesting absence of substantial variation in genotype frequency across the population; (3) we previously found no evidence for the existence of hidden population stratification within the samples[43,44] through formal testing of stratification using the STRUCTURE software[45]; and (4) allele and genotype distributions within our schizophrenia cases were similar to those in controls, as significant differences were observed only within the subset in which episodes of major mood disorder had occurred, suggesting that the effect is phenotype-driven rather than the result of population stratification.

Type I errors can occur as a consequence of multiple statistical comparisons. However, the use of whole-gene tests allowed for the study of multiple SNPs and provided P values that do not require correction for the number of SNPs studied. We have examined 2 disease categories and 2 domains of psychopathological abnormalities, and this requires correction. We found nominally significant evidence on whole-gene tests for 2 of these 4 tests. Our most significant finding was the comparison of the set of patients with major mood episodes against controls ($P = .009$). Applying a very conservative Bonferroni correction for 4 tests, we still found a nominally significant whole-study P value of .03. Further confidence that our positive findings are not type I errors is provided by the prior positive findings at this gene in bipolar disorder and schizophrenia. However, one of the difficulties in making direct comparisons between this study and previous studies is that there has been substantial variation between studies in the number and location of the typed polymorphisms and resulting variation in the

polymorphisms and haplotypes showing evidence for association. Nonetheless, of the polymorphisms showing significant association in our study, rs391695 (also called M12) showed significant evidence of association in one of the schizophrenia samples described in the original study by Chumakov et al[12] and in the schizophrenia samples described by Schumacher et al[16] and Zou et al.[14] In addition, significant association was shown by rs131402 in one of the bipolar samples described by Hattori et al[17] and by rs2391191 in the schizophrenia samples described by Chumakov and colleagues, Zou and colleagues, and Addington et al.[15]

We note the preponderance of men in our schizophrenia sample and women in our bipolar sample; this is consistent with sex biases described in epidemiological studies.[46,47] However, there was no difference in the distributions of alleles between men and women for any of the polymorphisms studied, and there was no evidence in our data that associations differed according to sex (data not shown).

Clearly, it is important that our findings are replicated within independent samples. This will require appropriately large data sets that have detailed phenotypic characterization of mood features. The ideal samples will comprise bipolar and schizophrenia cases recruited from a single geographical, ethnic, and clinical population using consistent assessment methods.

Our findings add to the weight of evidence challenging current psychiatric nosology.[1-6] In a recent twin study, Cardno et al[48] used an analysis unconstrained by the diagnostic hierarchy inherent in current classification systems (ie, the principle that schizophrenia "trumps" mood disorder in diagnosis). This demonstrated an overlap in the genetic susceptibility to mania and schizophrenia, suggesting the existence of genes that confer risk across the Kraepelinian divide. However, it also provided evidence to support the traditional notion that there are susceptibility genes that are relatively specific to schizophrenia and others that are specific to bipolar disorder.[48] In other words, there appear to be at least 3 classes of susceptibility genes that contribute susceptibility to the mood-psychosis spectrum.[4] Undoubtedly, this represents only a crude model of the full complexity of psychosis, but it provides a simple framework in which to interpret our findings. The absence of evidence for association within our schizophrenia sample suggests that variation at the *DAOA/G30* locus does not primarily increase susceptibility for prototypical schizophrenia—despite the fact that it was originally identified in studies of schizophrenia samples.[12] Indeed, the subset of schizophrenia cases in which episodes of major mood disorder had occurred significantly differed from the remaining schizophrenia cases (Table 2), even though the cases in both groups satisfied *DSM-IV* criteria for schizophrenia and *not* for schizoaffective disorder. In contrast, we found no evidence that there was an enhanced association signal in psychotic bipolar disorder (Table 2). Further, the allele frequencies were all closely similar between this subset and the remaining bipolar cases. This suggests that variation at the *DAOA/G30* locus does not confer specific susceptibility to a set of cases that represent the middle ground of the mood-psychosis spectrum, where cases simulta-

neously have marked schizophrenia and bipolar features. Rather, our results are consistent with the notion that variation at *DAOA/G30* influences susceptibility to episodes of mood disorder across the traditional bipolar and schizophrenia categories. Within this simple framework, the *DAOA/G30* locus can therefore be thought of best as a locus conferring risk to episodes of mood disorder. This locus has not been implicated in linkage studies of unipolar depression to date. This suggests either that the locus does not influence unipolar depressive illness or that the effect size is not great enough for reliable detection. Recent support for involvement of *DAOA/G30* in susceptibility to affective disturbance comes from the description of associations with panic disorder,[49] a finding of particular interest given the suggestion that comorbid panic disorder identifies a subtype of bipolar disorder.[50] These findings suggest that, ultimately, systems of classification might need to address etiological overlap across a wide range of syndromes and severity.

DAOA is a primate-specific gene expressed in the caudate and amygdala. It was originally identified by Chumakov et al[12] by experimental annotation of a region containing SNPs associated with schizophrenia and was called *G72*. Using yeast 2-hybrid analysis, evidence for physical interaction was found between the G72 protein and DAO. D-Amino-acid oxidase is expressed in the human brain where it oxidizes D-serine, a potent activator of N-methyl-D-aspartate (NMDA) glutamate receptors. Coincubation of the G72 protein with DAO in vitro revealed a functional interaction between the two, with G72 enhancing the activity of DAO. Consequently, G72 has now been named DAO activator. Chumakov et al[12] suggested that genetic variation in *DAOA* might influence the risk of schizophrenia through altered NMDA receptor function since D-serine, which is oxidized by DAO, is a known activator of NMDA receptors via the glycine modulatory site.[51]

It has been pointed out that the association between *DAOA* and schizophrenia is consistent with hypotheses of deficient glutamatergic transmission in the pathophysiology of schizophrenia,[52,53] as are other genetic findings in schizophrenia.[9] The present findings, however, again raise the important question of whether NMDA receptors play a role in the pathophysiological abnormalities of affective disorders.[54] The NMDA receptor antagonists have been shown to be effective in animal models of depression.[55-60] Conversely, antidepressant administration has been shown to affect NMDA receptor function[61] and receptor binding profiles.[62] Finally, ketamine infusion leads to significant improvement in depressive symptoms within 72 hours in patients with depression.[63] These findings suggest that depression is associated with enhanced glutamatergic function. It therefore follows that, if we are correct that *DAOA* is associated with affective symptoms and not schizophrenia per se, susceptibility variants should be associated with reduced rather than increased activity or expression of DAO activator. This hypothesis will most readily be tested once specific susceptibility or protective variants have been identified, but this will require further, more detailed genetic studies of this locus.

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