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Association of a Nonsynonymous Variant of DAOA with Visuospatial Ability in a Bipolar Family Sample

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Background: Bipolar disorder and schizophrenia are hypothesized to share some genetic background.

Methods: In a two-phase study, we evaluated the effect of five promising candidate genes for psychotic disorders, DAOA, COMT, DTNBP1, NRG1, and AKT1, on bipolar spectrum disorder, psychotic disorder, and related cognitive endophenotypes in a Finnish family-based sample ascertained for bipolar disorder.

Results: In initial screening of 362 individuals from 63 families, we found only marginal evidence for association with the diagnosis-based dichotomous classification. Those associations did not strengthen when we genotyped the complete sample of 723 individuals from 180 families. We observed a significant association of DAOA variants rs3916966 and rs2391191 with visuospatial ability (Quantitative Transmission Disequilibrium Test [QTDT]; \( p = 4 \times 10^{-6} \) and \( 5 \times 10^{-6} \), respectively) \((n = 159)\) with the two variants in almost complete linkage disequilibrium. The COMT variant rs165599 also associated with visuospatial ability, and in our dataset, we saw an additive effect of DAOA and COMT variants on this neuropsychological trait.

Conclusions: The ancestral allele (Arg) of the nonsynonymous common DAOA variant rs2391191 (Arg30Lys) was found to predispose to impaired performance. The DAOA gene may play a role in predisposing individuals to a mixed phenotype of psychosis and mania and to impairments in related neuropsychological traits.

Key Words: AKT1, bipolar disorder, COMT, DAOA, DTNBP1, G72, neuropsychological trait, NRG1

Bipolar disorder (BPD) is a severe mental disorder characterized by alternating episodes of depression and mania (bipolar type I [BPD-I]) or hypomania (bipolar type II [BPD-II]). Certain cognitive impairments such as poor executive functioning and verbal memory have been related to disease susceptibility (1). Despite being considered distinct clinical disorders, BPD and schizophrenia share many clinical features and treatment approaches. Sixty percent of BPD-I patients have psychotic symptoms during their lifetime (2). Bipolar disorder and schizophrenia co-segregate in many pedigrees (3), which suggest a shared genetic etiology of these two disorders at least to some extent.

In our previous study, we found evidence for contribution of distinct variants of the disrupted-in-schizophrenia 1 (DISC1) gene to features of bipolar spectrum and psychotic disorders in Finnish families ascertained for BPD (4). We screened the same set of BPD families for several promising candidate genes for psychotic disorders: d-amino acid oxidase activator (DAOA or G72) (5), catechol-O-methyl transferase (COMT) (6), dystrobrevin binding protein 1 (dysbindin or DTNBP1) (7), neuregulin 1 (NRG1) (8), and v-akt murine thymoma viral oncogene homolog 1 (AKT1) (9). Except for AKT1, these genes have been reported to associate with various neuropsychological traits within affected individuals or healthy control subjects (Supplement 1).

We evaluated the effects of variations in these genes both on clinical diagnosis of bipolar spectrum disorder or psychotic disorder, as well as on cognitive functions considered as endophenotypes (or intermediate traits) for these disorders (10).

Methods and Materials

Study Sample

The study sample includes 723 individuals from 180 families (4) (Table 1); neuropsychological test data were available altogether for 159 individuals from 65 of the families (11) (Table 1). Ascertainment strategy and sample collection are described in Supplement 2. The study was approved by the Ministry of Social Affairs and Health and the Ethical Committee of the National Public Health Institute.

Single Nucleotide Polymorphism Selection and Genotyping

We selected single nucleotide polymorphisms (SNPs) from published findings (5-9) and from the public SNP database, dbSNP (http://www.ncbi.nlm.nih.gov). Genotyping was done with homogeneous mass extension using the MassARRAY System (Sequenom, San Diego, California) in multiplexes of two to six SNPs. The COMT variants were genotyped by microarray-based allele-specific primer extension method (12). Genotyping was performed in two phases. Phase I was an initial screening involving 362 individuals from 63 families. In phase II, we examined an additional 361 individuals from 117 families.

Statistical Analysis

Association analyses using dichotomized diagnostic classes (bipolar spectrum disorder and psychotic disorder) were performed by haplotype relative risk (HRR) test of the ANALYZE package (13) for two-point analyses and by FBAT (14) for
haplotype analyses. Unaffected family members and individuals with other mental disorders were coded as unknown. Analyses were done using two different sample sets, the first including all individuals genotyped and the second including only familial cases, defined as families from two or more affected members. We used the Quantitative Transmission Disequilibrium Test (QTDT) software package (15) with the polygenic variance component option and performed association analyses of quantitative traits with the total association model assuming no population stratification. Age, gender, and presence of psychosis (16) were used as covariates. Statistical comparisons of the results in different genotyped groups and diagnostic categories were analyzed by SPSS 14.0 (SPSS Inc., Chicago, Illinois) using one-way analysis of variance (ANOVA). Interaction analysis was done by logistic regression backward stepwise model.

Table 1. Number of Individuals in Phase I and Phase II of the Study and the Total Number of Familial Cases

|                      | Phase I | Phase II | Familial |
|----------------------|---------|----------|----------|
|                      | Female  | Male     | Female   | Male     | Cases\a |
| Affected             | 78 (32) | 89 (30)  | 65 (4)   | 84 (14)  | 258     |
| Bipolar spectrum     | 59 (29) | 68 (24)  | 50 (4)   | 50 (9)   | 173     |
| disorder\b           |         |          |          |          |         |
| Psychotic disorder\c| 66 (27) | 65 (25)  | 51 (4)   | 69 (12)  | 212     |
| In both categories\d | 47 (24) | 44 (19)  | 36 (4)   | 35 (7)   | 127     |
| Other mental disorder\e | 22 (14) | 12 (8)   | 5 (2)    | 7 (3)    | 45      |
| Unaffected           | 92 (29) | 69 (21)  | 116 (1)  | 84 (1)   | 241     |
| Total Genotyped      | 192 (75)| 170 (59) | 186 (7)  | 175 (18) | 544     |

\aThe familial cases are from 118 families with more than one affected individual.
\bContains BPD-I (n = 214), BPD-II (n = 5), bipolar disorder NOS (n = 6), and cyclothymia (n = 2) cases.
\cContains BPD-I with intermittent psychotic features (n = 162), psychotic depression (n = 15), schizophrenia (n = 14), schizoaffective disorder (n = 51), and psychotic NOS (n = 9). Numbers are from the whole sample.
\dOverlap between bipolar spectrum and psychotic disorder categories.
\eContains depression, alcohol-related disorders and delusional, adjustment, dysthymic, and panic disorders.

Results

We initially screened 51 SNPs of the selected genes (Supplement 3) in 362 individuals from 63 families (Table 1). The most significant association of dichotomized phenotype was obtained for COMT variant rs165599 with bipolar spectrum disorder (HRR; p = .003) (Supplement 3). Adjacent 2-SNP haplotype analysis showed no evidence for association with either bipolar spectrum or psychotic disorder (Supplement 4). Several DAOA variants were associated with neuropsychological traits (Supplement 5). The strongest associations were seen between DAOA variants rs3916966 and rs2391191 (Arg30Lys) and visuospatial ability assessed with the Block Design test (p = .006 and p = .0008, respectively).

We focused our analysis on DAOA and COMT and genotyped all 723 individuals from 180 families using six DAOA variants (rs3916966, rs2391191, rs2153674, rs701567, rs778326, and rs954580) and two COMT variants (rs165599 and rs4680, also known as valine [Val]158 methionine [Met]).

In the complete sample, the COMT variant rs4680 was the only variant that associated with bipolar spectrum disorder in the complete sample (Table 2). In the familial sample, both rs4680 and rs165599 showed suggestive evidence of association with bipolar spectrum disorder. D-amino acid oxidase activator (DAOA) variants rs701567 and rs778326 were suggestively associated with psychotic disorder in the familial sample. For both genes, analysis of 2-SNP haplotypes yielded no further evidence of association (data not shown).

Analysis of the full study sample strengthened the previously observed association between DAOA variants rs3916966 and rs2391191 (Arg30Lys) and visuospatial ability (Table 3). These two variants are in almost perfect linkage disequilibrium (r^2 = .98). Visuospatial performance differed significantly between the Arg and Lys genotype groups of rs2391191, with the worst performance observed in individual homozygotes for the ancestral G (Arg) allele (Table 4, Figure 1). All study subjects performing above the average, i.e., achieving over 40 points in the Block Design test (n = 27), were either homozygous or heterozygous for the A (Lys) allele.

Catechol-O-methyl transferase (COMT) variant rs165599 associated with visuospatial ability. Individuals homozygous for the G allele had the best test performance among the rs165599 genotype groups. This difference was statistically significant in the full sample and in the psychotic subgroup but not in the nonpsychotic subgroup (Table 4).

Table 2. Association Between Single SNP and Disease Status in Phase I, Combined Phase I and Phase II, and Familial Cases Using HRR Analysis

|                     | Bipolar Spectrum Disorder | Psychotic Disorder |
|---------------------|---------------------------|-------------------|
|                     | Phase I (62 Families)     | Phase I + II (154 Families) | Familial Cases\a (99 Families) | Phase I (57 Families) | Phase I + II (144 Families) | Familial Cases\a (102 Families) |
| DAOA                |                           |                   |                        |                     |                   |                        |
| rs3916966           | .969                      | .966              | .906                   | .843                | .839              | .616                   |
| rs2391191           | .889                      | .913              | .735                   | .87                 | .891              | .349                   |
| rs2153674           | .072                      | .353              | .095                   | .066                | .700              | .233                   |
| rs701567            | .670                      | .593              | .407                   | .278                | .133              | .031                   |
| rs778326            | .025                      | .111              | .024                   | .010                | .186              | .018                   |
| rs954580            | .040                      | .107              | .052                   | .049                | .565              | .252                   |
| COMT                |                           |                   |                        |                     |                   |                        |
| rs4680              | .085                      | .046              | .020                   | .340                | .072              | .149                   |
| rs165599            | .003                      | .829              | .035                   | .015                | .396              | .103                   |

\aFamilial cases include only families that contain at least two affected individuals.

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As shown in Table 4, individuals homozygous for both the ancestral allele G (Arg) of DAOA variant rs2391191 and the T allele of COMT variant rs165599 performed worse in the Block Design test (mean test score = 15.6) than did subjects homozygous only for the DAOA rs2391191 G allele (mean test score = 24.8) or the COMT rs165599 T allele (mean test score = 28.6). However, backward stepwise logistic regression showed no evidence for significant interaction between the two variants (p = .5 for rs2391191*rs165599). Decreased test performance in the doubly homozygous subjects suggests a minor additive effect of DAOA and COMT variants on visuospatial ability, without evidence for epistasis.

**Discussion**

D-amino acid oxidase activator (DAOA) is a primate-specific gene encoding mitochondrial protein that promotes mitochondrial fragmentation and dendritic branching (17). D-amino acid oxidase activator (DAOA) may be involved in glutamate signaling (5), which has been shown to have multiple effects on learning and memory (18). Like some other genes encoding mitochondrial proteins in primates, the DAOA gene has evolved rapidly; the open reading frame of the human gene is twice as long as that of the chimpanzee homolog (5).

The strongest finding of our study was the association of a nonsynonymous DAOA variant, rs2391191 (Arg30Lys), with visuospatial ability, which remains significant after a conservative Bonferroni correction for multiple testing (corrected p = .005). In a recent study (19), the Arg allele associated with impairment in immediate and delayed verbal memory. In the present study, this allele showed only a trend for association with verbal memory (p < 0.1), but it predisposed to impairment with many other cognitive traits, most significantly with visuospatial ability. Thus, our data further strengthen the possibility that the Arg30Lys variation might affect cognitive functioning. Interestingly, the Lys allele that associated here with enhanced performance in the tests of general intellectual functioning is found only in humans, suggesting that DAOA might have played a part in the evolution of Homo sapiens when greater cognitive functions developed as the brain increased in size.

Catechol-O-methyl transferase (COMT) variant rs4680 (Val158Met) associated here nominally with familial bipolar spectrum disorder, but the risk allele (Met) was different from that reported by...
Shifman et al. (6). Variant rs165599 was also associated with visuospatial ability, with the best test performance seen in individuals homozygous for the C allele. An earlier study showed association of the COMT variant rs165599 with verbal memory in Caucasians (20), but better performance was associated with the T allele. The incoherence in findings on the effect of COMT on neuropsychological performance likely results from a relatively weak effect and the genetic heterogeneity behind these traits.

We found a minor additive effect of DAOA and COMT on visuospatial ability, a finding consistent with the hypothesis that interaction of glutamatergic (DAOA) and dopaminergic (COMT) neurotransmission is fundamental for many cognitive functions, particularly working memory (18). We recognize that all neuropsychological traits assessed in the present study may be state-dependent; however, many of the observed deviations are also found in euthymic BPD patients (1). Furthermore, our finding of association of DAOA with visuospatial ability did not result from an underlying effect of DAOA genotype to other illness-related parameters, such as medication or age of onset (data not shown). While there are no data for visuospatial ability being a good endophenotype for BPD, there is at least one study that showed impaired general intellectual function in relatives of psychotic patients (21). Other DAOA variants also associated weakly with psychotic disorder. The DAOA gene may play a role in predisposing individuals to a mixed phenotype of psychosis and mania and to impairments in related neuropsychological traits. However, further research is necessary to define the effect of DAOA on the complex processes of brain functions.

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**Supplementary material cited in this article is available online.**

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