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Letter to the Editor

Association analysis of *DAOA* and *DAO* in bipolar disorder: results from two independent case-control studies

To the Editor:

D-amino acid oxidase activator gene [*DAOA* (previously *G72*)] is located in a region with a positive linkage peak for bipolar disorder (BD) (13q33.2) (1). The results of case-control and family-based studies of *DAOA* in BD are inconsistent, with some reporting positive single marker or haplotype association (2–8) and others reporting no association (9, 10). Two meta-analyses of *DAOA* in BD have also provided conflicting results: one reported positive association (11), while another failed to find evidence for association (12). *DAOA* is an activator of *DAO* protein (13); both proteins are involved in the metabolism of D-serine, which plays a role in glutamatergic neurotransmission (14). Thus, there is a clear biological motivation for testing epitasis between these two genes. We explored the association of *DAOA* and *DAO* with BD and their statistical interaction in two independent case-control samples from the UK and Canada.

The UK sample consisted of 515 BD patients (65% women) with a mean age (SD) of 48.0 (11.4) years. The diagnosis of BD was defined by DSM-IV operational criteria using Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (15). Patients with BD were classified as having a positive history of psychosis if they scored positive on at least one of the SCAN items of delusions or hallucinations (n = 232). The control sample consisted of 1,316 subjects (58% women) with a mean age (SD) of 41.7 (13.2) years, screened for lifetime absence of psychiatric disorder. The Canadian sample consisted of 385 patients (63% women) with a mean age (SD) of 46.0 (12.5) years and 312 controls (54% women) with a mean age (SD) of 43.7 (13.1) years. All enrolled subjects were of white European parentage. Both studies were approved by the local Research Ethics Committees and informed written consent was obtained from all participants.

Genotyping of 10 single nucleotide polymorphisms (SNPs) was performed using SNPlex Genotyping System (Applied Biosystems, Carlsbad, CA, USA). Five *DAOA* SNPs: M12, M15, M18, M23, and M24, and *DAO* SNP were chosen on the basis of previously reported association findings. Others were chosen to get better coverage of the gene area. Overall, 9 markers cover a ~95 kb region, including the 5′ and 3′ flanking regions of the *DAOA* locus, constituting the region of interest for linkage disequilibrium (LD) mapping. In total, 1,619 UK and 697 Canadian samples were genotyped. An 80% threshold was applied for the call rate across the SNP set. The call rate for each SNP varied between 94.5% and 99.7%. A total of 176 samples were regenotyped with 100% consistency. For statistical analysis, 1,408 UK and 644 Canadian samples were available. Genotype and allele frequencies were assessed for association with BD using chi-squared tests. Risk magnitudes were estimated by calculating odds ratios (OR) with 95% confidence intervals. Haplotype analysis was conducted using UNPHASED (16). Interaction analysis was performed using the Genetic Association Interaction Analysis (GAIA) application (17). We applied the Bonferroni correction for the number of genes and the number of samples investigated, and assumed that four independent tests were applied ($\alpha = 0.013$).

Allele frequencies of the SNPs are shown in Table 1. All genotype distributions were consistent with Hardy-Weinberg equilibrium ($p > 0.05$). There were no significant differences in allele or genotype frequency between cases and controls in either the UK or Canadian sample, or in the combined sample. The analysis in the subgroup with psychotic traits did not show any significant differences either (data not shown).

No significant differences in global haplotype distribution or individual haplotype frequency were revealed between cases and controls in the investigated samples (Table 2). The pattern of intermarker LD of the *DAOA* in the two samples was similar to each other and to that in a HapMap Centre d'Etude du Polymorphisme Humain (CEPH) sample (http://www.hapmap.org).

Table 1. Association analysis of *DAO* and *DAOA* and bipolar disorder in the UK and Canadian samples

				UK sample				Canadian sample			
SNP ID	Position	Location	Allele[a]	Control	Case	p-value	Control/case, n	Control	Case	p-value	Control/case, n
DAOA	13q33.2										
rs3916965 (M12)	104901361	5′UTR	A/G	0.38	0.40	0.24	940/443	0.40	0.39	0.81	284/352
rs12584489	104913592	5′UTR	A/T	0.05	0.05	0.61	918/418	0.05	0.06	0.43	269/351
rs2391191 (M15)	104917447	Exon 2	A/G	0.38	0.40	0.31	955/449	0.40	0.39	0.73	283/358
rs9558562	104922938	Exon 3	A/G	0.00	0.01	0.05	944/433	0.01	0.01	0.29	269/353
rs1935062	104926137	Intron 3	A/C	0.35	0.38	0.86	890/441	0.36	0.35	0.67	279/345
rs947267 (M18)	104937663	Intron 3	A/C	0.42	0.41	0.56	955/440	0.44	0.43	0.73	284/357
rs778292	104966952	3′UTR	C/T	0.40	0.40	0.96	955/449	0.40	0.37	0.29	285/357
rs3918342 (M23)	104983750	3′UTR	C/T	0.48	0.50	0.52	907/449	0.48	0.47	0.89	282/356
rs1421292 (M24)	104996236	3′UTR	A/T	0.46	0.47	0.66	938/450	0.49	0.49	0.89	284/355
DAO	12q24										
rs3741775 (MDAO-6)	107807732	Intron 4	G/T	0.43	0.47	0.08	946/447	0.44	0.44	0.92	279/359

Minor allele frequency and p-values for allele distribution are shown. SNP = single nucleotide polymorphism.
[a]Underlined allele represents the minor allele.

Table 2. Haplotype analysis of *DAOA* in the UK and Canadian samples

	UK sample		Canadian sample	
Haplotypes	χ^2 (df)	Global p	χ^2 (df)	Global p
1-2-3	3.08 (2)	0.21	0.45 (2)	0.80
2-3-4	1.22 (2)	0.57	0.74 (2)	0.69
3-4-5	2.55 (3)	0.47	1.63 (3)	0.65
4-5-6	0.89 (2)	0.64	1.65 (2)	0.44
5-6-7	3.21 (5)	0.67	3.88 (5)	0.57
6-7-8	3.44 (6)	0.75	4.21 (7)	0.76
7-8-9	2.87 (3)	0.41	2.87 (3)	0.41

Global p-values for the differences of haplotype distribution between cases and controls are shown. 1 = rs3916965; 2 = rs12584489; 3 = rs2391191; 4 = rs9558562; 5 = rs1935062; 6 = rs947267; 7 = rs778292; 8 = rs3918342; 9 = rs1421292.

The LD structure was characterized by two blocks of high LD: rs3916965–rs947267 and rs778292–rs1421292.

No significant interaction between *DAOA* and *DAO* was shown in the Canadian sample. The best interaction p-value in the UK sample was 0.048 (*DAOA* rs3916965–*DAO* rs3741775), while the best overall p-value was 0.027 (*DAOA* rs1935062–*DAO* rs3741775), neither of which withstand Bonferroni correction.

Our results are consistent with the findings from previous studies of European origin and a meta-analysis, which did not confirm a single marker association (5, 7, 9, 12). Two studies reported positive association of rs3918342 (M23) with BD, but the direction of association was opposite (2, 6). M23 was genotyped in our sample, allowing direct comparison with previous studies. If rs3918342 is associated with BD due to LD with the true risk variant(s), then LD structure, which is known to be highly population dependent, could explain the inconsistency of the results of these association studies. It is possible that the relevant functional variants affect the pattern of splicing or protein expression levels. In this respect, further functional annotation of rs391834, which is in the 3′-UTR region, or proxy SNP(s) in LD with it, is needed.

The main limitation of our study is power. For detection of the allelic association with a minor allele frequency (MAF) of 0.48–0.50, with a nominal significant p-value ($p = 0.05$) and an odds ratio equal to 1.5, we had sufficient power to detect effect (94%); however, power decreased to 35% when a more realistic and smaller OR of 1.2 was considered.

In conclusion, our results in two independent Caucasian samples did not provide evidence for association of *DAOA* or *DAO* with BD, nor did we find convincing evidence of statistical epitasis.

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Darya Gaysina[a,b], Sarah Cohen-Woods[a], Philip C. Chow[a], Livia Martucci[a], Alexandra Schosser[a,c], Harriet A. Ball[a], Federica Tozzi[d], Julia Perry[d], Pierandrea Muglia[d], James L. Kennedy[e], Nicole King[e], John B. Vincent[e], Sagar V. Parikh[e], John Strauss[e], Ian W. Craig[a], Peter McGuffin[a] and Anne Farmer[a]

[a]Medical Research Council, Social, Genetic and Developmental Psychiatry Centre, King's College London, Institute of Psychiatry, [b]Medical Research Council, Unit for Lifelong Health and Aging, University College London, London, UK, [c]Division of Biological Psychiatry, Department of Psychiatry and Psychotherapy, Medical University Vienna, Vienna, Austria, [d]GlaxoSmithKline Research and Development, Medical Genetics, Clinical Pharmacology and Discovery Medicine, Verona, Italy, and Middlesex, UK, [e]Neurogenetics Section, Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Toronto, ON, Canada

Corresponding author:
Darya Gaysina, Ph.D.
MRC Unit for Lifelong Health and Ageing
33 Bedford Place
London, WC1B 5JU, UK
Fax: +442075801501
E-mail: d.gaysina@nshd.mrc.ac.uk

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