Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2009 October 5; 150B(7): 960–966. doi:10.1002/ajmg.b.30925.

Common and Rare Variants of *DAOA* in Bipolar Disorder

Manjula Maheshwari^{1,†}, Jiajun Shi^{2,†}, Judith A. Badner², Andrew Skol³, Virginia L. Willour⁴, Donna M. Muzny¹, David A. Wheeler¹, Fowler R. Gerald¹, Sevilla Detera-Wadleigh⁵, Francis J. McMahon⁵, James B. Potash⁴, Elliot S. Gershon^{2,6}, Chunyu Liu^{2,*}, and Richard A. Gibbs¹

¹Human Genome Sequencing Center, Dept of Molecular & Human Genetics, Baylor College of Medicine, Houston, Texas 77030

²Department of Psychiatry, University of Chicago, Chicago, IL 60637

³Department of Medicine, University of Chicago, Chicago, IL 60637

⁶Human Genetics, University of Chicago, Chicago, IL 60637

⁴Department of Psychiatry, Johns Hopkins School of Medicine, Baltimore, MD 21287

⁵Genetic Basis of Mood and Anxiety Disorders Unit, Mood and Anxiety Program, National Institute of Mental Health, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD 20892

Abstract

The d-amino acid oxidase activator (*DAOA*, previously known as *G72*) gene, mapped on 13q33, has been reported to be genetically associated with bipolar disorder (BP) in several populations. The consistency of associated variants is unclear and rare variants in exons of the *DAOA* gene have not been investigated in psychiatric diseases. We employed a conditional linkage method - Statistical Explanation for Positional Cloning (*STEPC*) to evaluate whether any associated single nucleotide polymorphisms (SNPs) account for the evidence of linkage in a pedigree series that previously has been linked to marker D13S779 at 13q33. We also performed an association study in a sample of 376 Caucasian BP parent-proband trios by genotyping 38 common SNPs in the gene region. Besides, we resequenced coding regions and flanking intronic sequences of *DAOA* in 555 Caucasian unrelated BP patients and 564 mentally healthy controls, to identify putative functional rare variants that may contribute to disease. One SNP rs1935058 could “explain” the linkage signal in the family sample set ($P = 0.055$) using *STEPC* analysis. No significant allelic association was detected in an association study by genotyping 38 common SNPs in 376 Caucasian BP trios. Resequencing identified 53 SNPs, of which 46 were novel SNPs. There was no significant excess of rare variants in cases relative to controls. Our results suggest that *DAOA* does not have a major effect on BP susceptibility. However, *DAOA* may contribute to bipolar susceptibility in some specific families as evidenced by the *STEPC* analysis.

Keywords

G72; *DAOA*; bipolar disorder; resequencing; single nucleotide polymorphism; genetic association

*Correspondence to: Chunyu Liu, PhD, Department of Psychiatry, University of Chicago, 924 East 57th Street, Knapp Center, R012, Chicago, IL 60637, USA. Email: cliu@yoda.bsd.uchicago.edu.

[†]These authors contributed equally to the work as first authors.

INTRODUCTION

Bipolar disorder (BP) is a common neuropsychiatric disorder affecting about 1% of the adult population [Weissman et al., 1996]. Gene mapping studies using microsatellite markers have identified chromosome 13q32-33 as one of the putative loci for BP [Badner and Gershon, 2002; Detera-Wadleigh et al., 1999; Liu et al., 2001]. The *G72/G30* gene complex was identified in a 65 kb region on 13q33, on the overlapping sense and antisense DNA strands, and was reported to be associated with schizophrenia [Chumakov et al., 2002]. *G72* was officially named as d-amino acid oxidase activator (DAOA), based on the biological interaction of an artificially expressed *G72* protein with D-amino acid oxidase (DAAO) and based on its regulation of D-serine, a substrate of DAAO [Chumakov et al., 2002]. Subsequently, Hattori et al. [Hattori et al., 2003] reported significant association of *G72/G30* with BP in a sample of 22 pedigrees from the Clinical Neurogenetics (CNG) sample set [Berrettini et al., 1991], with replication of haplotypic association in a second family sample set from the National Institute of Mental Health (NIMH) Genetics Initiative for BP (waves 1-2) [Nurnberger, Jr. et al., 1997]. Since then, significant or suggestive replication of BP association with *G72/G30* has been reported in multiple candidate gene studies [Addington et al., 2004; Baum et al., 2008; Chen et al., 2004; Fallin et al., 2005; Prata et al., 2008; Schumacher et al., 2004; Sklar et al., 2008; Wellcome Trust Case Control Consortium, 2007; Williams et al., 2006]. However, significant association with BP was not found either in our meta-analysis (all $P > 0.05$ for significance testing of overall odds ratios) [Shi et al., 2008], or in recent genome-wide association studies at $P > 10^{-7}$ [Baum et al., 2008; Ferreira et al., 2008; Sklar et al., 2008; Wellcome Trust Case Control Consortium, 2007]. To investigate whether variants in the *DAOA* gene region influences BP susceptibility in European Americans, we performed an association study in a family sample overlapping with previously studied samples.

Before the finding of BP association in two family sample sets [Hattori et al., 2003], we had mapped the BP linkage region to 13q32-q33 in the CNG pedigrees, where *DAOA* resides [Detera-Wadleigh et al., 1999; Liu et al., 2001]. To evaluate whether any of the associated single nucleotide polymorphisms (SNPs) accounts for the evidence of linkage at 13q32-33 in CNG pedigrees [Liu et al., 2001], we employed a conditional linkage method introduced by Sun et al. [Sun et al., 2002]. This method is a re-linkage analysis conditional on genotypes of a SNP in the original linked region. If no evidence of linkage remains (conditional on genotype, the linkage statistic has $P > 0.05$), that SNP accounts for the linkage (see Methods, below) [Sun et al., 2002].

An alternative to the common disease-common variant hypothesis [Lander, 1996; Lohmueller et al., 2003; Reich and Lander, 2001] is the common disease-multiple rare variants (CD-MRV) hypothesis which postulates that disease is caused by many rare genetic variants each with a large effect [Pritchard, 2001; Pritchard and Cox, 2002]. The rare variants are likely to be of recent origin, highly penetrant, and may be specific to a family or individual [Gibbs, 2005; Pritchard, 2001; Pritchard and Cox, 2002]. Current association studies with limited sample size have little power to detect frequency differences between case and control groups for a specific rare variant, but may be able to detect a cumulative frequency difference over a set of rare variants (see summarized supportive evidence in [Topol and Frazer, 2007]), as well as genomic structure variants (e.g., copy number variations [Sebat et al., 2007; Walsh et al., 2008]). To identify putative functional variants that may contribute to BP, we resequenced known exons, splice sites and flanking intronic sequences in the *DAOA* gene region. We did not sequence the *G30* gene, as it has much less expression and functional evidence (Cheng L, Liu C, Gershon ES, et al, unpublished data).

Materials and Methods

Common SNP association tests

Three hundred and seventy-six parent-offspring trios (parents plus one affected child) were selected from three BP projects: the CNG project, the NIMH Genetics Initiative project, and the University of Chicago-Johns Hopkins-NIMH Intramural Program (CHIP). All participants were of European descent according to self-reported ancestry and further confirmed by a STRUCTURE [Pritchard et al., 2000] analysis using 254 unlinked SNPs (data not shown). Of 376 affected offspring, 335 met DSM-III-R or DSM-IV criteria for bipolar disorder type I (BPI) and 41 for schizoaffective disorder bipolar type (SAB); 219 were females and 157 were males. Two hundred and seventy-nine samples (24.7%) from 93 trios overlapped with the subjects tested in the original BP association study [Hattori et al., 2003].

Thirty previously tested SNPs in CNG and NIMH waves 1-2 samples ([Hattori et al., 2003] and unpublished data) were selected for the present association study. Two SNPs (rs1935057 and rs1935058, 4 base pair away from each other) were genotyped by direct sequencing of amplified fragments (primers and condition for polymerase chain reaction are available upon request). The other 28 SNPs were genotyped using Sequenom iPLEX MassArray approach (Sequenom, San Diego, CA, USA). The Assay Design 3.1 software (Sequenom, San Diego, CA, USA) was used to design multiplex PCR primers and single base extension primers. PCR amplification, dephosphorylation with shrimp alkaline, and single nucleotide extension reaction were carried out in 384-well plates. PedCheck1.1 was used to detect any Mendelian inconsistencies [O'Connell and Weeks, 1998], and Merlin to detect any unlikely recombinants [Abecasis et al., 2002]. All genotype errors were manually resolved by assigning problematic genotypes as missing prior to statistical analysis. The genotype data finally included had an average genotyping success rate of > 95.5% for 30 SNPs.

Eight additional SNPs (rs978714, rs2025522, rs3916964, rs9558551, rs7981258, rs9301029, rs1253464, and rs9519671) in the upstream region of *DAOA* were tested in the same family sample sets, but not necessary the same individuals. These 1115 individuals included six trios and seven quads (parents plus two affected children) from the CNG collections, 53 trios and 170 quads from the NIMH waves 1-4 collections, and 42 trios and 26 quads from the CHIP collections. All individuals were of European ancestry. Of the 507 affected offspring from the 101 trios and 203 quads, 481 met DSM-III-R or DSM-IV criteria for BPI and 26 for SAB. Three hundred were females and 207 were males. Three hundred and thirty-one samples (28.8%) from 31 trios and 57 quads overlapped with the subjects tested in our previous study [Hattori et al., 2003].

These eight SNPs were genotyped using Illumina Bead Array technology [Oliphant et al., 2002]]. After data cleaning using PedCheck1.1 [O'Connell and Weeks, 1998] and Merlin [Abecasis et al., 2002]], the genotype data had an average genotyping success rate of > 99.8%.

Allelic transmission disequilibrium tests (TDTs) of 38 SNPs were carried out for the disease trait using PLINK [Purcell et al., 2007]. Haploview (version 4.0) [Barrett et al., 2005] was used to test Hardy-Weinberg Equilibrium (HWE) in the parents of affected offspring. With PBAT (www.biostat.harvard.edu/~clange/default.htm), under a multiplicative inheritance model, and at significance level of $p < 0.0013$ (for 30 SNPs tested), this sample had 80% power to detect odds ratios (ORs) of 1.9 and 1.6 for minor allele frequencies of 0.1 and 0.5, respectively.

Statistical Explanation for Positional Cloning (STEP-C) analysis for SNPs accounting for linkage

Eight SNPs in the *DAOA* gene region have shown evidence for BP association in either CNG pedigrees (rs1935058, rs1815686, rs1341402, rs12862108, rs9301030, rs1935062, rs778294)

[Hattori et al., 2003] or the combined CNG and NIMH waves 1-4 pedigrees (rs778326, unpublished data). To test whether any association underlies the previously observed linkage signals in the CNG sample set [Liu et al., 2001], we genotyped these eight SNPs in a subset of CNG pedigrees. This sample set included 19 families with 146 samples. Fifty-two individuals were diagnosed with BPI (33 females and 19 males), and 20 with BPII (16 females and 4 males). Since the previous linkage signal was for sibships with broad-defined bipolar disorder including BPII, we included BPII in *STEPC* analysis.

Genotyping was carried out using Sequenom iPLEX MassArray (Sequenom, San Diego, CA, USA). One SNP failed in genotyping (rs11815686) and was re-genotyped using a restriction fragment length polymorphism (RFLP) with enzyme BtsI. PedCheck1.1 [O'Connell and Weeks, 1998] and Merlin [Abecasis et al., 2002] were used to detect genotype errors. All genotype errors were treated as missing data. The average genotyping success rate was greater than 98.2%.

The method of Sun et al [Sun et al., 2002] as implemented in *STEPC* software (<http://hg-wen.uchicago.edu/software.html>), identifies SNPs that best explain the observed linkage to a region. The information provided by this analysis is different from that provided by tests of either linkage or association. The evidence for linkage is conditioned on the genotypes for the SNP in question. If there is no residual evidence for linkage, that is, when the P value for the conditional linkage statistic TG (Test statistic given Genotypes) is greater than 0.05, this implies that the SNP fully explains (accounts for) the evidence for linkage. Since strong linkage evidence for the 13q32-34 region (D13S779) is present in the CNG sample set [Liu et al., 2001], we carried out *STEPC* analysis on eight SNPs with association evidence (Table I), using the original microsatellite data for linkage information.

Resequencing of exons in DAOA and rare variant association test

A total of 555 unrelated bipolar patients were recruited for this study. Of them, five were from the CNG collections [Berrettini et al., 1991], 478 from the NIMH Genetics Initiative waves 1-4 collections (<http://www.nimhgenetics.org/>) [Dick et al., 2003; Nurnberger, Jr. et al., 1997], and 72 from the CHIP collection [Potash et al., 2007]. Ascertainment and diagnostic methods for participants from CNG, the NIMH waves 1-4 and the CHIP collections have been described in detail elsewhere [Berrettini et al., 1991; Nurnberger, Jr. et al., 1997; Potash et al., 2007]. All individuals were of European ancestry based on self-reported ancestry and further confirmed by a STRUCTURE [Pritchard et al., 2000] analysis using 254 unlinked SNPs (data not shown). Five hundred and thirty-one patients were diagnosed as BPI, and 24 as SAB; 327 were females and 228 were males.

Five hundred and sixty-four unrelated control subjects were selected from the NIMH Schizophrenia Genetics Initiative controls collection (<http://www.nimhgenetics.org/>, a list of the contributors to sample collection is shown in Acknowledgements). These control samples were all of European ancestry and free of major psychiatric disorders. Of these 564 subjects, 282 were females and 282 were males.

Exons of all 14 splicing forms of *DAOA* reported and/or identified so far [Cheng et al., 2007; Chumakov et al., 2002; Hattori et al., 2003], splice sites, and flanking intronic sequences (with reference to NM_172370), were sequenced in all the selected subjects. The target sequences were amplified in ten fragments. Exon specific primer pairs (Supplementary Table I) were designed using the program Primer 3 (<http://frodo.wi.mit.edu/>). Universal M13 forward or reverse primer tails were attached to each amplicon-specific primer except for exon 2. For exon 2, internal forward and reverse sequencing primers were designed separately. PCR reactions were established using the Qiagen PCR kit, with an individual amplicon size of 270-550 base pairs. PCR products were analyzed on 2% agarose gels and purified using ExoSap (USB

Corporation, Cleveland, Ohio). Bidirectional sequencing of each fragment was performed separately using big dye terminator sequencing chemistry on 3730XL DNA Sequencers (Applied Biosystems, Foster City, CA). The resulting sequences were analyzed using the program SNPdetector [Zhang et al., 2005], and SNPs were confirmed manually with assistance from the program Sequencher (Gene Codes Corporation, Ann Arbor, MI).

All sequencing-derived variants were tested for departures from HWE. The genotype data, excluding 27 samples with more than five missing genotypes, had an average genotyping success rate of > 99%. A total of 543 cases and 549 controls were used for further analysis. Allele frequency in cases and controls were calculated using Helix Tree version 6.1 (Golden Helix Inc., Bozeman, MT, USA).

We tested for an excess of rare variants using two ad hoc methods. First, we restricted the analysis to those variants that were observed either only in patients or only in controls, and then conditional on the number of alleles observed at each SNP, determined if significantly more alleles were observed in cases than in controls. The second method is identical to the first but the analysis is restricted to only SNPs with $MAF \leq 0.01$ in the combined sample of cases and controls. Significance was determined in two ways: by permuting case and control status, which preserves the correlation between variants due to linkage disequilibrium, and by permuting alleles between cases and controls one SNP at a time, which ignores linkage disequilibrium between variants.

RESULTS

No significant allelic association with disease was found for 38 common SNPs tested in the family samples (Supplementary Table II). The genotype distribution of *bcm_35726* in parents was not in HWE ($P = 0.0001$).

For the CNG dataset, which is a subset of the originally reported linkage data [Detera-Wadleigh et al., 1999; Liu et al., 2001], we had an unconditional linkage statistic (TG) of 2.72 (LOD score of 1.61, $P < 0.003$). Results for eight SNPs are displayed in Table I. The results showed that the best weighted linkage statistic is T_{w3} with $P = 0.0032$. Using this statistic, the significant SNP that best explains the linkage result is *rs1935058* with $P = 0.055$ (Table I).

A total of 53 SNPs were detected in our resequencing effort of exons of the *DAOA* gene in 543 cases and 549 controls (Supplementary Table III). Of them, 46 were novel and seven had been previously reported in NCBI dbSNP. Thirteen SNPs were unique to cases, 17 unique to controls, 11 common to both with $MAF \leq 0.01$ and 12 common to both with $MAF > 0.01$. Supplementary Table III summarizes the allele frequencies of major and minor alleles of all the SNPs detected in cases and controls.

Six samples were heterozygous for two SNPs (*bcm_104927620* and *bcm_104940194*) and three of these six samples were also heterozygous for the SNP *bcm_104917996* (Supplementary Table IV). Interestingly, all three SNPs were present in cases only. However, we did not detect a significant excess of rare variants in cases relative to controls (case only/control only: $P = 0.088$; all variants considered: $P = 0.131$).

DISCUSSION

DAOA has been reviewed as one of the best supported bipolar disorder candidate genes [Craddock and Forty, 2006; Farmer et al., 2007; Maier et al., 2005]. However, the associated SNPs or haplotypes have not been consistent across studies [Detera-Wadleigh and McMahon, 2006; Shi et al., 2008]. Recent genome-wide association studies [Baum et al., 2008; Ferreira et al., 2008; Sklar et al., 2008; Wellcome Trust Case Control Consortium, 2007] have not

identified any single SNP association with BP across any ethnic group studied. These results, together with results of this study, suggest that DAOA does not have a major effect on BP susceptibility. However, it is possible that DAOA may contribute to disease risk in a specific population or in specific families, as shown in our STEPC analysis in the CNG families (Table I) as well as in previous association analyses [Addington et al., 2004; Baum et al., 2008; Chen et al., 2004; Fallin et al., 2005; Hattori et al., 2003; Prata et al., 2008; Schumacher et al., 2004; Sklar et al., 2008; Wellcome Trust Case Control Consortium, 2007; Williams et al., 2006]. Moreover, the DAOA gene has shown to a link to BP with persecutory delusions [Schulze et al., 2005] and major depression with trait anxiety [Rietschel et al., 2008]. Therefore DAOA may be genetically linked to subgroup of BP (i.e., endophenotype) [Bearden and Freimer, 2006; Cannon and Keller, 2006; Gottesman and Gould, 2003]. With limited sample size, we did not perform further subtype analysis, leaving that to several better powered ongoing large case-control BP studies, including the Genetic Association Information Network (GAIN) BP project, which has significant overlap of samples with this study.

In this study, rs1935058, accounting for the linkage signal on 13q32-34 in a small-size family sample, is 7.2 kb upstream of the transcript start site of *DAOA*, and possibly affects gene expression and function. Further functional annotation of this SNP or proxy SNP(s) in LD with it is warranted. However, it cannot be excluded that other variants on this chromosome region (harboring at least 59 known genes according to data in the UCSC Genome Browser and NCBI Mapview) contribute to the linkage signals and/or increase risk of bipolar disorder [Detera-Wadleigh et al., 2007].

Although the CD-MRV hypothesis has received some experimental support [Sebat et al., 2007; Topol and Frazer, 2007; Walsh et al., 2008], our analysis of rare variants in *DAOA* suggest that it is unlikely that multiple rare variants from this gene contribute significantly to a polygenic phenotype. On the other hand, if this gene with multiple rare variants is associated with disease, a large sample may be needed to reach statistical significance and statistical power. Moreover, it is possible that *G72* may underlie disease risk through interactions with other genes in the same neurobiological pathway (i.e., N-methyl D-aspartate receptor-mediated glutamatergic signaling) or related neuronal systems. Identifying rare functional variants in such genes from pathway(s) or the whole genome requires high-throughput resequencing technologies as well as complex approaches to functional validation.

To summarize, we failed to detect significant disease association of common or rare variants in the *DAOA* gene region in relatively large sample sets, but found that rs1935058 best explained previously identified linkage signal in a specific family sample set. It remains possible that *DAOA* may contribute to bipolar susceptibility in some specific families.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Control subjects from the National Institute of Mental Health Schizophrenia Genetics Initiative (NIMH-GI), data and biomaterials are being collected by the "Molecular Genetics of Schizophrenia II" (MGS-2) collaboration. The investigators and co-investigators are: ENH/Northwestern University, Evanston, IL, MH059571, Pablo V. Gejman, M.D. (Collaboration Coordinator; PI), Alan R. Sanders, M.D.; Emory University School of Medicine, Atlanta, GA, MH59587, Farooq Amin, M.D. (PI); Louisiana State University Health Sciences Center, New Orleans, Louisiana, MH067257, Nancy Buccola APRN, BC, MSN (PI); University of California-Irvine, Irvine, CA, MH60870, William Byerley, M.D. (PI); Washington University, St. Louis, MO, U01, MH060879, C. Robert Cloninger, M.D. (PI); University of Iowa, Iowa, IA, MH59566, Raymond Crowe, M.D. (PI), Donald Black, M.D.; University of Colorado, Denver, CO, MH059565, Robert Freedman, M.D. (PI); University of Pennsylvania, Philadelphia, PA, MH061675, Douglas Levinson M.D. (PI); University of Queensland, Queensland, Australia, MH059588, Bryan Mowry, M.D.

(PI); Mt. Sinai School of Medicine, New York, NY, MH59586, Jeremy Silverman, Ph.D. (PI). The samples were collected by V L Nimgaonkar's group at the University of Pittsburgh, as part of a multi-institutional collaborative research project with J Smoller, MD DSc and P Sklar, MD PhD (Massachusetts General Hospital) (grant MH 63420). This study was also supported by the National Institute of Mental Health (NIMH) awards R01MH061613 (to ES Gershon) and R01 MH042243 (to JB Potash), National Alliance for Research on Schizophrenia and Depression (NARSAD) Young Investigator Awards (to J Shi), NIMH award 1R21MH083521 and Brain Research Foundation at the University of Chicago (to C Liu), the Gerald Norton Foundation and the Eklund Family.

Reference List

- Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 2002;30:97–101. [PubMed: 11731797]
- Addington AM, Gornick M, Sporn AL, Gogtay N, Greenstein D, Lenane M, Gochman P, Baker N, Balkissoon R, Vakkalanka RK, Weinberger DR, Straub RE, Rapoport JL. Polymorphisms in the 13q33.2 gene G72/G30 are associated with childhood-onset schizophrenia and psychosis not otherwise specified. *Biol Psychiatry* 2004;55:976–980. [PubMed: 15121480]
- Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 2002;7:405–411. [PubMed: 11986984]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265. [PubMed: 15297300]
- Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze TG, Cichon S, Rietschel M, Nothen MM, Georgi A, Schumacher J, Schwarz M, Abou JR, Hofels S, Propping P, Satagopan J, tera-Wadleigh SD, Hardy J, McMahon FJ. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. *Mol Psychiatry* 2008;13:197–207. [PubMed: 17486107]
- Bearden CE, Freimer NB. Endophenotypes for psychiatric disorders: ready for primetime? *Trends Genet* 2006;22:306–313. [PubMed: 16697071]
- Berrettini WH, Goldin LR, Martinez MM, Maxwell ME, Smith AL, Guroff JJ, Kazuba DM, Nurnberger JI Jr. Hamovit J, Simmons-Allings S, Muniec D, Choi H, York C, Robb AS, Gershon ES. A bipolar pedigree series for genomic mapping of disease genes: Diagnostic and analytic considerations. *Psychiatric Genetics* 1991;2:125–160.
- Cannon TD, Keller MC. Endophenotypes in the genetic analyses of mental disorders. *Annu Rev Clin Psychol* 2006;2:267–290. [PubMed: 17716071]
- Chen YS, Akula N, tera-Wadleigh SD, Schulze TG, Thomas J, Potash JB, DePaulo JR, McInnis MG, Cox NJ, McMahon FJ. Findings in an independent sample support an association between bipolar affective disorder and the G72/G30 locus on chromosome 13q33. *Mol Psychiatry* 2004;9:87–92. [PubMed: 14699445]
- Cheng LJ, Hattori E, Nakajima M, Tang YP, Gershon ES, Liu C. G72/G30 transgenic mice and brain expression profiling. *Biol Psychiatry* 2007;61:35S.
- Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, Bougueleret L, Barry C, Tanaka H, La Rosa P, Puech A, Tahri N, Cohen-Akenine A, Delabrosse S, Lissarrague S, Picard FP, Maurice K, Essioux L, Millasseau P, Grel P, Debailleul V, Simon AM, Caterina D, Dufaure I, Malekzadeh K, Belova M, Luan JJ, Bouillot M, Sambucy JL, Primas G, Saumier M, Boubkiri N, Martin-Saumier S, Nasroune M, Peixoto H, Delaye A, Pinchot V, Bastucci M, Guillou S, Chevillon M, Sainz-Fuertes R, Meguenni S, urich-Costa J, Cherif D, Gimalac A, Van Duijn C, Gauvreau D, Ouellette G, Fortier I, Raelson J, Sherbatich T, Riazanskaia N, Rogaev E, Raeymaekers P, Aerssens J, Konings F, Luyten W, Macciardi F, Sham PC, Straub RE, Weinberger DR, Cohen N, Cohen D. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc Natl Acad Sci U S A* 2002;99:13675–13680. [PubMed: 12364586]
- Craddock N, Forty L. Genetics of affective (mood) disorders. *Eur J Hum Genet* 2006;14:660–668. [PubMed: 16721402]
- Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G, Rollins DY, Moses T, Sanders AR, Karkera JD, Esterling LE, Zeng J, Ferraro TN, Guroff JJ, Kazuba D, Maxwell ME, Nurnberger JI Jr. Gershon ES. A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci U S A* 1999;96:5604–5609. [PubMed: 10318931]

- Detera-Wadleigh SD, Liu CY, Maheshwari M, Cardona I, Corona W, Akula N, Steele CJ, Badner JA, Kundu M, Kassem L, Potash JB, Gibbs R, Gershon ES, McMahon FJ. Sequence variation in DOCK9 and heterogeneity in bipolar disorder. *Psychiatr Genet* 2007;17:274–286. [PubMed: 17728666]
- Detera-Wadleigh SD, McMahon FJ. G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. *Biol Psychiatry* 2006;60:106–114. [PubMed: 16581030]
- Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL, Moe PR, Samavedy N, El-Mallakh R, Manji H, Glitz DA, Meyer ET, Smiley C, Hahn R, Widmark C, McKinney R, Sutton L, Ballas C, Grice D, Berrettini W, Byerley W, Coryell W, DePaulo R, MacKinnon DF, Gershon ES, Kelsoe JR, McMahon FJ, McInnis M, Murphy DL, Reich T, Scheftner W, Nurnberger JI Jr. Genomewide linkage analyses of bipolar disorder: a new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. *Am J Hum Genet* 2003;73:107–114. [PubMed: 12772088]
- Fallin MD, Lasseter VK, Avramopoulos D, Nicodemus KK, Wolyniec PS, McGrath JA, Steel G, Nestadt G, Liang KY, Hagan RL, Valle D, Pulver AE. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. *Am J Hum Genet* 2005;77:918–936. [PubMed: 16380905]
- Farmer A, Elkin A, McGuffin P. The genetics of bipolar affective disorder. *Curr Opin Psychiatry* 2007;20:8–12. [PubMed: 17143075]
- Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, Fan J, Kirov G, Perlis RH, Green EK, Smoller JW, Grozeva D, Stone J, Nikolov I, Chambert K, Hamshere ML, Nimgaonkar VL, Moskvina V, Thase ME, Caesar S, Sachs GS, Franklin J, Gordon-Smith K, Ardlie KG, Gabriel SB, Fraser C, Blumenstiel B, DeFelice M, Breen G, Gill M, Morris DW, Elkin A, Muir WJ, McGhee KA, Williamson R, Macintyre DJ, Maclean AW, St CD, Robinson M, Van BM, Pereira AC, Kandaswamy R, McQuillin A, Collier DA, Bass NJ, Young AH, Lawrence J, Nicol FI, Anjorin A, Farmer A, Curtis D, Scolnick EM, McGuffin P, Daly MJ, Corvin AP, Holmans PA, Blackwood DH, Gurling HM, Owen MJ, Purcell SM, Sklar P, Craddock N. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet* 2008;40:1056–1058. [PubMed: 18711365]
- Gibbs R. Deeper into the genome. *Nature* 2005;437:1233–1234. [PubMed: 16251930]
- Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 2003;160:636–645. [PubMed: 12668349]
- Hattori E, Liu C, Badner JA, Bonner TI, Christian SL, Maheshwari M, Detera-Wadleigh SD, Gibbs RA, Gershon ES. Polymorphisms at the G72/G30 gene locus, on 13q33, are associated with bipolar disorder in two independent pedigree series. *Am J Hum Genet* 2003;72:1131–1140. [PubMed: 12647258]
- Lander ES. The new genomics: global views of biology. *Science* 1996;274:536–539. [PubMed: 8928008]
- Liu C, Badner JA, Christian SL, Guroff JJ, Detera-Wadleigh SD, Gershon ES. Fine mapping supports previous linkage evidence for a bipolar disorder susceptibility locus on 13q32. *Am J Med Genet* 2001;105:375–380. [PubMed: 11378853]
- Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33:177–182. [PubMed: 12524541]
- Maier W, Hofgen B, Zobel A, Rietschel M. Genetic models of schizophrenia and bipolar disorder: overlapping inheritance or discrete genotypes? *Eur Arch Psychiatry Clin Neurosci* 2005;255:159–166. [PubMed: 15995899]
- Nurnberger JI Jr, DePaulo JR, Gershon ES, Reich T, Blehar MC, Edenberg HJ, Foroud T, Miller M, Bowman E, Mayeda A, Rao NL, Smiley C, Conneally PM, McMahon F, Meyers D, Simpson S, McInnis M, Stine OC, Detera-Wadleigh SD, Goldin L, Gurroff J, Maxwell E, Kazuba D, Gejman PV, Badner J, Sanders A, Rice J, Bierut L, Goate A. Genomic survey of bipolar illness in the NIMH genetics initiative pedigrees: a preliminary report. *Am J Med Genet* 1997;74:227–237. [PubMed: 9184304]
- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998;63:259–266. [PubMed: 9634505]
- Oliphant A, Barker DL, Stuelpnagel JR, Chee MS. BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. *Biotechniques* 2002;32(Suppl):56–58. 60–61. [PubMed: 11808699]

- Potash JB, Toolan J, Steele J, Miller EB, Pearl J, Zandi PP, Schulze TG, Kassem L, Simpson SG, Lopez V, MacKinnon DF, McMahon FJ. The bipolar disorder phenome database: a resource for genetic studies. *Am J Psychiatry* 2007;164:1229–1237. [PubMed: 17671286]
- Prata D, Breen G, Osborne S, Munro J, St CD, Collier D. Association of DAO and G72(DAOA)/G30 genes with bipolar affective disorder. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:914–917. [PubMed: 18165970]
- Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? *Am J Hum Genet* 2001;69:124–137. [PubMed: 11404818]
- Pritchard JK, Cox NJ. The allelic architecture of human disease genes: common disease-common variant...or not? *Hum Mol Genet* 2002;11:2417–2423. [PubMed: 12351577]
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;155:945–959. [PubMed: 10835412]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am J Hum Genet* 2007;81:559–575. [PubMed: 17701901]
- Reich DE, Lander ES. On the allelic spectrum of human disease. *Trends Genet* 2001;17:502–510. [PubMed: 11525833]
- Rietschel M, Beckmann L, Strohmaier J, Georgi A, Karpushova A, Schirmbeck F, Boesshenz KV, Schmal C, Burger C, Jamra RA, Schumacher J, Hofels S, Kumsta R, Entringer S, Krug A, Markov V, Maier W, Propping P, Wust S, Kircher T, Nothen MM, Cichon S, Schulze TG. G72 and its association with major depression and neuroticism in large population-based groups from Germany. *Am J Psychiatry* 2008;165:753–762. [PubMed: 18346999]
- Schulze TG, Ohlraun S, Czerski PM, Schumacher J, Kassem L, Deschner M, Gross M, Tullius M, Heidmann V, Kovalenko S, Jamra RA, Becker T, Leszczynska-Rodziewicz A, Hauser J, Illig T, Klopp N, Wellek S, Cichon S, Henn FA, McMahon FJ, Maier W, Propping P, Nothen MM, Rietschel M. Genotype-phenotype studies in bipolar disorder showing association between the DAOA/G30 locus and persecutory delusions: a first step toward a molecular genetic classification of psychiatric phenotypes. *Am J Psychiatry* 2005;162:2101–2108. [PubMed: 16263850]
- Schumacher J, Jamra RA, Freudenberg J, Becker T, Ohlraun S, Otte AC, Tullius M, Kovalenko S, Bogaert AV, Maier W, Rietschel M, Propping P, Nothen MM, Cichon S. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. *Mol Psychiatry* 2004;9:203–207. [PubMed: 14966479]
- Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimäki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M. Strong association of de novo copy number mutations with autism. *Science* 2007;316:445–449. [PubMed: 17363630]
- Shi J, Badner JA, Gershon ES, Liu C. Allelic association of G72/G30 with schizophrenia and bipolar disorder: a comprehensive meta-analysis. *Schizophr Res* 2008;98:89–97. [PubMed: 18023149]
- Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K, Nimgaonkar VL, McQueen MB, Faraone SV, Kirby A, de Bakker PI, Ogdie MN, Thase ME, Sachs GS, Todd-Brown K, Gabriel SB, Sougnez C, Gates C, Blumenstiel B, DeFelice M, Ardlie KG, Franklin J, Muir WJ, McGhee KA, Macintyre DJ, McLean A, VanBeck M, McQuillin A, Bass NJ, Robinson M, Lawrence J, Anjorin A, Curtis D, Scolnick EM, Daly MJ, Blackwood DH, Gurling HM, Purcell SM. Whole-genome association study of bipolar disorder. *Mol Psychiatry* 2008;13:558–569. [PubMed: 18317468]
- Sun L, Cox NJ, McPeck MS. A statistical method for identification of polymorphisms that explain a linkage result. *Am J Hum Genet* 2002;70:399–411. [PubMed: 11791210]
- Topol EJ, Frazer KA. The resequencing imperative. *Nat Genet* 2007;39:439–440. [PubMed: 17392801]
- Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, Stray SM, Rippey CF, Roccanova P, Makarov V, Lakshmi B, Findling RL, Sikich L, Stromberg T, Merriman B, Gogtay N, Butler P, Eckstrand K, Noory L, Gochman P, Long R, Chen Z, Davis S, Baker C, Eichler EE, Meltzer PS, Nelson SF, Singleton AB, Lee MK, Rapoport JL, King MC, Sebat J. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 2008;320:539–543. [PubMed: 18369103]

- Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, Joyce PR, Karam EG, Lee CK, Lellouch J, Lepine JP, Newman SC, Rubio-Stipec M, Wells JE, Wickramaratne PJ, Wittchen H, Yeh EK. Cross-national epidemiology of major depression and bipolar disorder. *JAMA* 1996;276:293–299. [PubMed: 8656541]
- Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–678. [PubMed: 17554300]
- Williams NM, Green EK, MacGregor S, Dwyer S, Norton N, Williams H, Raybould R, Grozeva D, Hamshere M, Zammit S, Jones L, Cardno A, Kirov G, Jones I, O'Donovan MC, Owen MJ, Craddock N. Variation at the DAOA/G30 locus influences susceptibility to major mood episodes but not psychosis in schizophrenia and bipolar disorder. *Arch Gen Psychiatry* 2006;63:366–373. [PubMed: 16585465]
- Zhang J, Wheeler DA, Yakub I, Wei S, Sood R, Rowe W, Liu PP, Gibbs RA, Buetow KH. SNPdetector: a software tool for sensitive and accurate SNP detection. *PLoS Comput Biol* 2005;1:e53. [PubMed: 16261194]

Table 1
*STEP*C results of eight single nucleotide polymorphisms in the *DAOA* gene region

Marker	Position on 13q ^a	Alleles (1/2)	Allele 1 frequency ^b	sib_tdt <i>P</i> -value	Conditional TG ^c	TG <i>P</i> -value ^c
rs1935058	104909351	C/T	0.47	0.0009	1.60	0.055
rs1341402	104913510	T/C	0.81	0.0083	1.77	0.038
rs9301030	104924309	G/A	0.71	0.031	1.78	0.038
rs1815686	104907485	C/G	0.55	0.039	1.88	0.030
rs12862108	104924209	G/T	0.80	0.020	1.97	0.024
rs1935062	104926137	A/C	0.61	0.0074	1.98	0.024
rs778294	104940236	A/G	0.21	0.017	1.99	0.023
rs778326	104948042	A/T	0.80	0.026	2.00	0.023

TG: Test statistic given Genotypes.

^aBased on human genome sequence (NCBI Build 35, May 2004, hg17).

^bAllele 1 frequencies based on unrelated parents.

^cTest statistics conditional on genotypes of corresponding single nucleotide polymorphism.