Biochemical Pharmacology 78 (2009) 1360–1365

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Commentary: Genome-based CNS drug discovery: D-Amino acid oxidase (DAAO) as a novel target for antipsychotic medications: Progress and challenges

Michael Williams *

Department of Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

ARTICLE INFO

Article history:
Received 24 May 2009
Accepted 29 June 2009

Keywords:
Schizophrenia
Antipsychotics
Genomics
Drug targets
DAAO
G72
Drug discovery

ABSTRACT

Antipsychotics, the drugs used currently for the treatment of schizophrenia, produce their therapeutic effects via the blockade of dopamine receptors. These compounds are, however, limited in their therapeutic efficacy and have side effect liabilities that also limit their use. Agents that produce antipsychotic effects by enhancing NMDA receptor function represent a viable alternative to dopamine antagonists. D-Serine, is the prototype of this approach acting as a positive allosteric modulator of the NMDA receptor to enhance antipsychotic efficacy in the clinic. A newer approach to modulating NMDA receptor function, identified by gene association studies, is pLG72/DAOA (D-amino acid oxidase activator) a peptide that modulates D-amino acid oxidase (DAAO) activity, increasing endogenous levels of D-serine. While the initial association of DAOA with schizophrenia and its functional effects on DAAO activity have not been replicated, its identification has led to the development of several DAAO inhibitors, e.g., AS057278, CBIO and Compound 8, that are active in animal models of antipsychotic action. The complications in validating the *G72* association with schizoprenia highlight the inherent challenges in translating gene-based, disease-related associations to drug discovery targets.

1. Introduction

Completion of the draft human genome map in 2001 led to expectations that gene association studies would result in the rapid identification, validation and exploitation of novel, disease-associated drug targets [1]. This has proven to be a far from facile process with the expectation of absolutes (e.g., one gene, one disease/common disease-common variant) in genetic association studies giving way to a more pragmatic realization that multiple factors, both genetic and epigenetic, represent the rule rather than the exception [2,3]. As of April, 2007, the schizophrenia gene database, SzGene contained information on 3608 variants present in 516 genes, none with a validation equal to that of *APOE* in Alzheimer's disease but none of which had been excluded as candidate genes [4]. And while additional gene associations continue to appear on a regular basis [4–7] driven by new technologies like imaging genetics [7], the search for genetic associations has now expanded to understanding the role of rare copy number variants (CNVs) in the etiology of schizophrenia [8,9].

The diversity of information derived from gene association studies in CNS disease states, in reinforcing the complexity of CNS disorders, also underlines the challenge in developing a robust genetic basis for disease diagnosis and its potential treatment [1]. Several genetic associations in schizophrenia have supported the glutamate hypofunction hypothesis [10,11] and while no single one of these has proven causal, together they have provided a directionality that increasing NMDA receptor function is a viable alternative to the dopaminergic hypofunction hypothesis [12] in identifying improved treatment modalities for schizophrenia. To prioritize novel drug discovery approaches, new targets require, to the fullest extent possible, validation [13,14]. This may be viewed as a relatively facile process when the target(s) fit into the context of an existing hypothesis, e.g., glutamate hypofunction [10]. Accordingly, the present commentary focuses on the challenges involved in validating and "reducing to practice" a novel, gene-associated antipsychotic target, now close to a decade old, with the flavoenzyme D-amino acid oxidase (DAAO), that was at one time described as "among the most compelling in psychiatry" [15].

2. Schizophrenia genetic associations

Schizophrenia, a chronic, debilitating CNS disease affecting up to 1% of the world's population [12,16], has no consistent gender,

* Tel.: +1 484 620 6178.
E-mail address: mazarine1643@verizon.net.

0006-2952/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2009.06.108

ethnic, or social bases and is associated with mortality rates 2–3 times higher than those occurring in the general population [16]. Like autism [17], schizophrenia is considered a neurodevelopmental disorder [18] with multiple environmental and genetic associations indicating that the disease is multifactorial in its causality [1,4,17]. Schizophrenia presents as three main phenotypes: positive and negative symptoms, and cognitive dysfunction [12]. Positive symptoms reflect an excess or distortion of normal function and are manifest as auditory and visual hallucinations, disorganized thought, paranoia, bizarre behavior and other delusional states. Negative symptoms involve a decrease or loss of normal function and include affective flattening, anhedonia, social withdrawal, lack of motivation and spontaneity and decreased evidence of thought and speech. Cognitive impairment, involving deficits in executive function, processing, attention, vigilance, verbal learning and memory, verbal and spatial working memory, semantic memory and social cognition precedes the occurrence of psychotic symptoms and remains severe, with some progression, throughout the course of the disease. Alterations in cognitive function are considered to be of equal or greater importance than either positive or negative symptoms in predicting the functional outcomes of schizophrenia, including quality of life, social problem solving and work status [20].

Antipsychotics used to treat the symptoms of schizophrenia can be divided into two distinct classes, *typical* and *atypical* [12,19]. The former include drugs like haloperidol and chlorpromazine and the latter, compounds like clozapine, risperidone, olanzapine and aripiperazole. Typical antipsychotics are potent blockers of dopamine (DA) D2 receptors while the atypicals are generally antagonists of both D2 and $5HT_2$ receptors. A considerable body of clinical data has indicated that atypical antipsychotics are more effective in treating the negative symptoms of schizophrenia than typical antipsychotics (which act predominately on positive symptoms) and have a lower incidence of tardive dyskinesia [12]. However, two federally funded clinical trials, the NIMH-sponsored CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) [21] and the NHS-sponsored CUtLASS (Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study) [22] have reported no major differences in the clinical effectiveness of typical and atypical antipsychotics leading to considerable debate [23]. These trial outcomes, together with class-related incidents of QT prolongation [24] and metabolic syndrome, e.g., weight gain and diabetes [25], suggested that it would be unlikely that any additional drug candidates acting via blockade of the D2/$5HT_2$ receptor axis would be approved. However, the May 2009 approval of the typical antipsychotic, iloperidone, following a prior "not approvable" letter from the FDA [26] has led at least one key opinion leader to be "amazed" [27]. Nonetheless, the search continues for novel targets through which more efficacious and safer generations of antipsychotic agent can be developed.

3. The NMDA receptor hypofunction theory of schizophrenia

For more than 50 years, the demonstrated clinical effectiveness of D2 receptor antagonists in treating schizophrenia has focused research on disease etiology and treatment on dopaminergic hyperfunction as the primary cause of the disorder [12]. This hypothesis has been further supported by findings that stimulants like amphetamine and cocaine that increase brain DA levels produce symptoms reminiscent of the positive symptoms of schizophrenia [12,19]. An alternative hypothesis to schizophrenia causality is that of glutamatergic hypofunction [10]. The NMDA receptor antagonists, ketamine and phencyclidine (PCP) mimic the positive, negative, and cognitive symptoms of schizophrenia in animals and as add-on therapy to antipsychotics in humans [10]. NMDA receptor antagonists can also reinstate schizophrenia-like effects in stable patients [28]. Conversely, positive allosteric modulators of the NMDA receptor, e.g., glycine, D-serine, D-alanine, enhance antipsychotic efficacy [29,30]. These findings have prompted a search for additional targets that may address the treatment of schizophrenia by enhancing NMDA receptor function.

Gene association studies over the past decade have identified a variety of target loci [4] among which are several that have the potential to modulate glutamatergic neurotransmission [19]. These include: polymorphisms in glutamate receptor genes (*GRM3*, *GRIN1*, *GRIN2*); neuregulin (NRG1) which modulates phosphorylation of the NMDA NR2B subunit; dysbindin-1, part of the synaptic dystrophin/dystrobrevin glycoprotein complex that occurs at high levels in cells providing glutamatergic input to the hippocampal formation and; D-amino acid oxidase activator (DAOA), inhibition of which can modulate endogenous D-serine levels [31].

4. The discovery and characterization of D-amino acid oxidase activator (DAOA) as a schizophrenia target

Genotyping of a 5-Mb segment of Chr13q34 genetically linked to schizophrenia using a 191 SNP map in a Canadian cohort of schizophrenics led to the identification of two regions containing markers associated with schizophrenia, one of 65.9-kb (Bin A) and a second of 1380-Kb (Bin B) [31]. Two markers in Bin A present in the distal 3-Mb "gene desert" of the 5-Mb segment of Chr13q34 were subsequently confirmed in a Russian patient cohort leading to the annotation of three schizophrenia-related genes; *G90* present in Bin B and two overlapping genes in Bin A, *G72* and *G30*. *G72* was entirely included in *G30*. The longest open reading frame (ORF) of *G72*, *LG72* encoded a 153 amino acid protein designated as pGL72 that was primarily expressed in the brain. The longest *LG30* ORF encoded a 71mer, pLG30. Neither pLG72 nor pLG30 had homology with any known or hypothetic protein precluding any assignment of putative function. As *LG30* failed to yield a translation product, additional efforts were focused on *LG72* which was found to be a primate-specific gene. Using pLG72 as bait in yeast two-hybrid pull-down experiments involving a human brain cDNA library of half a million independent clones led to the identification of a nearly full-length clone of D-amino acid oxidase (DAAO) as a partner. This enzyme catalyzes the oxidative deamination of D-amino acids to their corresponding α-keto acids. D-Serine, an allosteric modulator of the NMDA receptor [10], is a substrate for DAAO thus providing a mechanistic link between the schizophrenia-associated gene, G72 and the NMDA hypofunction theory of schizophrenia. Additional studies [31] showed that *in vitro*, pLG72 activated pig kidney DAAO leading it to be renamed DAOA (D-amino acid oxidase activator). Disease-associated enhancement of DAAO activity by DAOA activation would thus theoretically lead to increased D-serine metabolism and a reduction in NMDA receptor function suggesting that overexpression of DAOA might be causative in schizophrenia and that an antagonist of DAOA or an inhibitor of DAAO would represent novel approaches to developing antipsychotic agents that would act by potentiating NMDA-mediated glutamatergic neurotransmission.

5. Validation of DAOA/DAAO as novel schizophrenia drug targets

As with the identification of many novel gene-derived CNS disease targets, it was necessary to both replicate the initial association at the genetic level using additional patient cohorts [1,2] and explore the functional role of the target in traditional biological systems [14]. While several studies confirmed the association of DAOA/G72 with schizophrenia [32–37], others demonstrated an association with bipolar disorder [32,38–40] and autism [41]. Additionally, morphine altered DAAO mRNA

expression in rat brain [42], suggesting a role of the enzyme in nociception. A number of studies also failed to replicate the schizophrenia association of DAOA/G72 [43–47] which were explained by the associated prevalence of mood disorders with schizophrenia and differences in allelic variants [45]. These conflicting/"failure-to-replicate" findings, although not unusual in the field of genetic associations for multifactorial CNS diseases [1,2], led to the DAOA/G72 gene association with schizophrenia, initially described as "among the most compelling in psychiatry" [15], segueing into being considered as "enigmatic" [46] or "weak" [48,49]. While DAOA was initially reported as an activator of porcine DAAO *in vitro* [31], additional studies [50] while confirming an interaction between DAOA and human DAAO, showed that the peptide was a negative effector of the human enzyme rather than an activator confounding the precise role of DAOA in modulating DAAO activity, at least *in vitro*, as the activator effect had not been demonstrated *in vivo* [31].

Nonetheless, additional work demonstrated: i) an inverse relationship between D-serine levels and DAAO expression in development [51]; ii) high levels of D-serine in DAAO$^{-/-}$ mice that showed reduced stereotypic and rotational activity in response to NMDA receptor antagonists compared to wild-type mice, improved Morris Water Maze performance, improved long term potentiation in the hippocampus and enhanced NMDA receptor function [52,53]; DAAO activity two-fold higher in the brains of schizophrenics than controls [54]; increased transcript levels of *G72* but not *G30* in the dorsolateral prefrontal cortex of schizophrenics [32]; and reduced D-serine levels in the serum and CSF of schizophrenics [54,55]. Thus, irrespective of the inconsistencies surrounding both the psychiatric disease association(s) of *G72* and the molecular action of DAOA, *G72* has provided additional support for a relationship between D-serine metabolism and schizophrenia [56], supporting DAAO as a potentially novel antipsychotic target.

6. DAAO inhibitors as novel treatments for schizophrenia

DAAO has been extensively studied since the 1930s [57] and was implicated in the metabolism of endogenous D-serine in the early 1990s [51]. Interest in its role as a target for CNS drug discovery was however, overshadowed by a presumed beneficial role of the kidney form of the enzyme in destroying the D-amino acids produced by gastrointestinal bacteria [58] that are potentially nephrotoxic [59]. Nonetheless, the relationship between DAAO and schizophrenia has prompted efforts to identify small molecule DAAO inhibitors, a weak prototype of which was benzoic acid ([60]; Fig. 1).

6.1. AS057278

AS057278 (5-methylpyrazole-3-carboxylic acid; Fig. 1) inhibited DAAO with an IC_{50} value of 901 nM. It had IC_{50} values of greater than 10 μM at the glycine site on the NMDA receptor, rat D-aspartate oxidase and serine racemase [61]. The cellular activity of AS057278, in terms of protecting cell viability in the presence of 50 mM D-serine, occurred with IC_{50} values of 2.2–3.95 μM. AS057278 was orally bioavailable (%F = 40) and at 2 h (10 mg/kg i.v.) showed modest increases in rat cortex and midbrain D-serine levels. In the mouse PCP-induced prepulse inhibition (PPI) startle inhibition response, an animal model that is used to evaluate antipsychotic agents [19], AS057278 given acutely (80 mg/kg p.o.) and chronically (28 days, bid, 10 mg/kg i.v.) normalized the PPI in a manner similar to the atypical antipsychotic, clozapine (3 mg/kg p.o.). Chronic, but not acute, treatment with AS057278 also normalized PCP-induced hyperlocomotion.

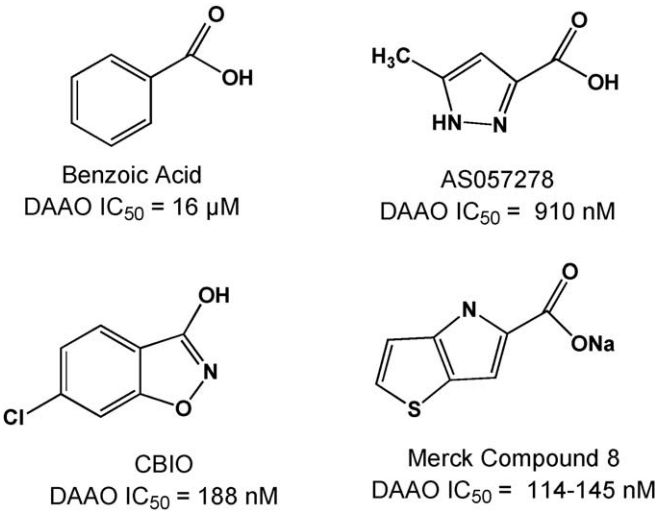

Fig. 1. Structures of DAAO inhibitors.

6.2. CBIO

CBIO (6-chlorobenzo[d]isoxazol-3-ol; Fig. 1) was identified by compound screening. It is a competitive inhibitor of DAAO activity with an IC_{50} value of 188 nM [62]. *In vivo*, CBIO (30 mg/kg p.o.) increased both plasma and brain levels of co-administered D-serine having no effect on brain D-serine levels on its own, possibly due to an inability to cross the blood brain barrier. CBIO however potentiated the effects of both D-serine [63] and D-alanine [64] in attenuating MK-801-induced PPI deficits.

6.3. Merck Compound 8

Compound 8 (4*H*-thieno[3,2-*b*] pyrrole-5-carboxylic acid; Fig. 1] inhibited recombinant human (IC_{50} = 145 nM) and rat (IC_{50} = 114 nM) DAAO [65]. At 30 μM, it had no measurable activity at 150 other receptors, ion channels or enzymes thus demonstrating a greater than 200-fold selectivity in its DAAO interaction. *In vivo*, Compound 8 (200 mg/kg, i.p.) inhibited rat kidney and cerebellar DAAO by approximately 96% (kidney) and 80% (cerebellar) at 1 h that was sustained (~87%, kidney; ~60%, cerebellum) for up to 8 h. At this time point, D-serine levels in plasma, CSF and brain cortex were elevated to 220% (plasma), 175% (CSF) and 133% (cortex) of controls. The effects of Compound 8 on D-serine levels were however less pronounced than that of systemically administered D-serine. At a behaviorally effective dose of D-serine (1280 mg/kg s.c.), plasma D-serine levels were elevated 500-fold over control. Compound 8 had no effect on amphetamine-induced psychomotor activity, nucleus accumbens dopamine (DA) release or a MK-801-induced deficit in a novel object recognition (NOR) paradigm. In contrast, D-serine (1280 mg/kg s.c.) attenuated amphetamine-induced psychomotor activity and DA release and improved NOR performance. The behaviorally effective dose of D-serine increased CSF levels of D-serine to approximately 40-fold above those achieved by the maximal dose of Compound 8 (200 mg/kg i.p) suggesting that while acute DAAO inhibition increased D-serine levels in the periphery and CNS, the levels achieved with Compound 8 were insufficient to produce behavioral effects similar to those seen with high dose D-serine. Compound 8 however, decreased spontaneous locomotor activity indicating some effect on behavior, albeit distinct from that of D-serine. The authors suggested that Compound 8 dosing might achieve behavioral efficacy similar to D-serine if administered chronically.

6.4. Miscellaneous

A series of patent applications [66–69] on other small molecule DAAO inhibitors, e.g., benzisoxazoles have been published. From these, one compound of unknown structure, SEP-227900, is reportedly in early stage clinical trials for neuropathic pain, an indication based on the effects of morphine on DAAO expression in rat brain [42], the reduction in nociception in DAAO knockout mice [70], and the well established role of glutamate in nociception [71,72].

7. A null hypothesis?

For nearly two decades, research in schizophrenia has focused on the identification of gene associations to provide new targets for drug discovery [4,19]. A large number of associations have been identified [4] that have often proven difficult to replicate or have shown additional, promiscuous associations with a diversity of diseases [19]. The present commentary has focused on the evolution of the schizophrenia-associated gene, *G72* and its expression product, DAOA/PLG72 towards a validated drug target.

While the initial association has not withstood replication and similarly, the mechanism of action of DAOA is controversial [31,50], this genetic association has helped focus attention on DAAO as a potentially novel target for antipsychotic drug discovery. It is noteworthy however, that in the absence of earlier data [51] it is quite possible that the identification of DAAO as a 'partner" for DAOA in the yeast two-hybrid studies may have been overlooked or considered a false positive.

The current small molecule inhibitors of DAAO, AS057278 [61], CBIO [62] and Compound 8 [65] have limited efficacy in animal models in which antipsychotics are robustly active [19], not achieving the magnitude of effect seen with the prototypic allosteric NMDA receptor modulator, D-serine [65]. This may reflect the potency of these compounds, all of which have IC_{50} values of greater than 100 nM, limitations in their drug-like characteristics that appear to limit their dosing or lack of access to the brain. That one DAAO inhibitor, SEP-227900, has advanced to early stage clinical trials, albeit in neuropathic pain, suggests that further optimization of current compounds is both possible and required. Additionally, DAAO appears to modulate the metabolism of kyneurenic acid, a dual NMDA/nicotinic α7 receptor antagonist [73].

DAAO knockout mice demonstrate anxiogenic activity [74] suggesting that the development of more efficacious DAAO inhibitors as potential antipsychotic agents might be confounded by side effects. In this context, an alternative approach to the allosteric enhancement of NMDA receptor function, inhibition of the glycine transporter-1 (GlyT-1) to increase extrasynaptic glycine, was associated preclinically with impaired rotorod performance, labored breathing, tremors, bloody tears, hypothermia, tachycardia and body weight loss in rodents [75], underlining the challenges in drug discovery that are not always obvious at the gene level. Thus despite the heuristic logic of some of the novel gene-associated targets in antipsychotic drug research, their reduction to chemistry-driven, lead optimization efforts is not necessarily a predictable path as evidenced by current progress on DAOA and DAAO inhibitors.

8. Summary

To a skeptic, the ever-expanding repertoire of gene-associated, schizophrenia-related targets raises the question as to whether continued studies will eventually link all known genes to the disease. Additionally, when novel targets are identified that lie outside the currently accepted concepts of disease causality and indeed, of CNS drug targets, e.g., *IL3RA* (interleukin 3 receptor alpha), *CSF2RA* (colony stimulating factor 2 receptor alpha) [4], *ZNF804a* (putative zinc finger binding protein) [5], *RSRC1* (arginine/serine-rich coiled coil 1) and *ARHGAP18* (Rho GTPase-activating protein 18) [7], it is an imperative to reassess what is currently understood about the disease and how current antipsychotics are thought to work to see whether the newer information might represent the elusive paradigm shift in the understanding of disease causality and its treatment that could lead to innovative medications. With such "out of the box" associations, the task of the drug hunter becomes infinitely more complex and challenging especially if the neurodevelopmental foundation [18] and potential immunological origins [76–78] of the disease at these new targets requires prophylactic rather than palliative therapy to truly improve disease treatment. These findings also question whether the continued search for gene-associated targets for schizophrenia is a Sisyphean approach where the technical ability to identify gene loci far exceeds the capacity, ability (and perhaps willingness) to reduce these to practice adds minimal value and insight to the search for the elusive NEW target for effective disease treatment and instead merely adds additional archival data to SzGene [4]. Such considerations echo Horrabin's concerns regarding the Castalian nature of current biomedical research and its contribution to improved patient health [79] and also underpin the European Comission's NEWMEDS (Novel Methods Leading to New Medications in Depression and Schizophrenia) inititative in schizophrenia [80] to "develop better animal models... generate translational technology that could help provide early indicators of efficacy... and.to develop tools to improve patient stratification" to focus on the complexity and heterogeneity of the disease.

Acknowledgements

The author would like to thank Jim Barrett and Mike Marino for helpful discussions.

References

[1] Williams M. Genome-based drug discovery: prioritizing disease-susceptibility/disease-associated genes as novel drug targets for schizophrenia. Curr Opin Invest Drugs 2003;4:31–6.
[2] Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med 2009;360:1759–68.
[3] Vogelstein B, quoted in Hayden EC. Cancer complexity slows quest for cure. Nature 2008;455:148.
[4] Williams HJ, Owen MJ, O'Donovan MC. New findings from genetic association studies of schizophrenia. J Hum Genet 2009;54:9–14.
[5] O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, et al. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 2008;40:1053–5.
[6] Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V, et al. A primate-specific, brain isoform of *KCNH2* affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. Nat Med 2009;15:509–18.
[7] Potkin SG, Turner JA, Fallon JA, Kaetor DB, Guffanti G, Macciardi F. Gene discovery through imaging genetics: identification of two novel genes associated with schizophrenia. Mol Psychiatr 2009;14:416–28.
[8] Cantor RM, Geschwind DH. Schizophrenia: genome, interrupted. Neuron 2008;58:165–7.
[9] Owen MJ, Williams HJ, O'Donovan MC. Schizophrenia genetics: advancing on two fronts. Curr Opin Genetics Develop 2009. doi: 10.1016/j.gde.2009.02.008.
[10] Coyle JT. Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell Mol Neurobiol 2006;26:365–84.
[11] Millan MJ. N-Methyl-D-aspartate receptors as a target for improved antipsychotic agents: novel insights and clinical perspectives. Psychopharmacology (Berlin) 2005;179:30–53.
[12] Carpenter Jr WT, Conley RR, Buchanan RW. Schizophrenia. In: Enna SJ, Coyle JT, editors. Pharmacological Management of Neurological and Psychiatric Disorders. New York: McGraw-Hill; 1998. p. 27–51.
[13] Lindsay M. Target discovery. Nat Rev Drug Discov 2003;2:831–8.
[14] Kopec K, Bozyczko-Coyne DB, Williams M. Target identification and validation in drug discovery: the role of proteomics. Biochem Pharmacol 2005;69:1133–9.
[15] Detera-Wadleigh SD, McMahon FJ. G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. Biol Psychiatry 2006;60:106–14.

[16] McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 2008;30:67–76.
[17] Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 2009. doi: 10.1038/nature07999.
[18] Lewis DA, Levitt P. Schizophrenia as a disorder of neurodevelopment. Annu Rev Neurosci 2003;25:409–32.
[19] Marino MJ, Knutsen LJS, Williams M. Emerging opportunities for antipsychotic drug discovery in the postgenomic era. J Med Chem 2008;51:1077–107.
[20] Green MF. Stimulating the development of drug treatments to improve cognition in schizophrenia. Annu Rev Clin Psychol 2007;3:159–80.
[21] Lieberman JA, Stroup S, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. NEngl J Med 2005;353:1209–23.
[22] Jones PB, Barnes TR, Davies L, Dunn G, Lloyd H, Hayhurst KP, et al. Randomized controlled trial of the effect on Quality of Life of second- vs first-generation antipsychotic drugs in schizophrenia: Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1). Arch Gen Psychiatry 2006; 63:1079–87.
[23] Lewis S, Liberman J. CATIE and CUtLASS: can we handle the truth? Br J Psychiatry 2008;192:161–3.
[24] Taylor DM. Antipsychotics and QT prolongation. Acta Psychiatr Scand 2003; 107:85–95.
[25] McEvoy J, Meyer J, Goff D, Nasrallah H, Davis S, Sullivan L, et al. Prevalence of the metabolic syndrome in patients with schizophrenia: baseline results from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia trial and comparison with national estimates from NHANES III. Schizophr Res 2005;80:19–32.
[26] Hollingsworth C. Vanda shares surge 626% on FDA approval of Fanapt. BioWorld Today, 2009, 20 (88) May, 8, 2009, 1/3.
[27] Herper M. Is The FDA Easing Up? Forbes.com. http://www.forbes.com/2009/05/07/fda-vanda-trials-business-health-care-antipsychotics.html; 2009.
[28] Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, Breier A. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology 1997;17: 141–50.
[29] Heresco-Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, et al. D-Serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. Biol Psychiatry 2005;57:577–85.
[30] Yang CR, Svennson KA. Allosteric modulation of NMDA receptor via elevation of brain glycine and D-serine: the therapeutic potential for schizophrenia. Pharmacol Ther 2008;120:317–32.
[31] Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci USA 2002;99:13675–80.
[32] Korostishevsky M, Kaganovich M, Cholostoy A, Ashkenazi M, Ratner Y, Dahary D, et al. Is the G72/G30 locus associated with schizophrenia? Single nucleotide polymorphisms, haplotypes, and gene expression analysis. Biol Psychiatry 2004;56:169–76.
[33] Schumacher J, Jamra RA, Freudenberg J, Becker T, Ohlraun S, Otte AC, et al. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. Mol Psychiatry 2004;9:203–7.
[34] Goldberg TE, Straub RE, Callicott JH, Hariri A, Mattay VS, Bigelow L. The G72/G30 gene complex and cognitive abnormalities in schizophrenia. Neuropsychopharmacology 2006;31:2022–32.
[35] Liu X, He G, Wang X, Chen Q, Qian X, Lin W. Association of DAAO with schizophrenia in the Chinese population. Neurosci Lett 2004;369:228–33.
[36] Shinkai T, De Luca V, Hwang R, Muller DJ, Lanktree M, Zai G, et al. Association analyses of the DAOA/G30 and D-amino-acid oxidase genes in schizophrenia: further evidence for a role in schizophrenia. Neuromol Med 2007;9:169–77.
[37] Ohnuma T, Shibata N, Maeshima H, Baba H, Hatanao T, Hanzawa R, Arai H, et al. Association analysis of glycine- and serine-related genes in a Japanese population of patients with schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry 2009;33:511–8.
[38] Hattori E, Liu C, Badner JA, Bonner TI, Christian SL, Maheshwari M, et al. Polymorphisms at the G72/G30 gene locus, on 13q33, are associated with bipolar disorder in two independent pedigree series. Am J Hum Genet 2003; 72:1131–40.
[39] Chen YS, Akula N, Detera-Wadleigh SD, Schulze TG, Thomas J, Potash JB, et al. 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.
[40] Zhang Z, Li Y, Zhao Q, Huang K, Wang P, Li S, et al. First evidence of association between G72 and bipolar disorder in the Chinese Han population. Psychiatry Genet 2009;19:151–3.
[41] Jeon JWJ, Chung SHC, Hong JPH, Yoo HIY. Association of DAO and DAOA genes with autism spectrum disorders in Korean boys. Eur Neuropsychopharmacol 2006;16(Suppl. 4):S526.
[42] Yoshikawa M, Andoh H, Ito K, Suzuki T, Kawaguchi M, Kobayashi H, Oka T, Hashimoto A. Acute treatment with morphine augments the expression of serine racemase and D-amino acid oxidase mRNAs in rat brain. Eur J Pharmacol 2005;525:94–7.
[43] Yue W, Liu Z, Kang G, Yan J, Tang F, Ruan Y, et al. Association of G72/G30 polymorphisms with early-onset and male schizophrenia. Neuroreport 2006; 17:1899–902.
[44] Liu YL, Fann CS, Liu CM, Chang CC, Wu JY, Hung SI, et al. No association of G72 and D-amino acid oxidase genes with schizophrenia. Schizophr Res 2006;87: 15–20.
[45] Williams NM, Green EK, Macgregor S, Dwyer S, Norton N, Williams H, et al. Variation at the DAOA/G30 locus influences susceptibility to major mood episodes but not psychosis in schizophrenia and bipolar disorder. Arch Gen Psychiatry 2006;63:366–73.
[46] Kvajo M, Dhilla A, Swor DE, Karayiorgou M, Gogos JA. Evidence implicating the candidate schizophrenia/bipolar disorder susceptibility gene G72 in mitochondrial function. Mol Psychiatr 2008;13:685–96.
[47] Ohi K, Hashimoto R, Yasuda Y, Yoshida T, Takahashi T, Iike N, et al. Association study of the G72 gene with schizophrenia in a Japanese population: a multicenter study. Schizophr Res 2009;109:80–5.
[48] Li D, He L. G72/G30 genes and schizophrenia: a systematic meta-analysis of association studies. Genetics 2007;175:917–22.
[49] 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;9:89–97.
[50] Saachi S, Bernasconi M, Martineau M, Mothet J-P, Ruzzene M, Pilone MS, et al. pLG72 modulates intracellular D-serine levels through its interaction with D-amino acid oxidase. Effect on schizophrenia susceptibility. J Biol Chem 2008;283:22244–56.
[51] Schell MJ, Molliver ME, Snyder SH. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc Natl Acad Sci USA 1995;92:3948–52.
[52] Almond SL, Fradley RL, Armstrong EJ, Heavens RB, Rutter AR, Newman RJ, et al. Behavioral and biochemical characterization of a mutant mouse strain lacking D-amino acid oxidase activity and its implications for schizophrenia. Mol Cell Neurosci 2006;32:324–34.
[53] Maekawa M, Watanabe M, Yamaguchi S, Konno R, Hori Y. Spatial learning and long-term potentiation of mutant mice lacking D-amino-acid oxidase. Neurosci Res 2005;53:34–8.
[54] Madeira C, Freitas ME, Vargas-Lopes C, Wolosker H, Panizzutti R. Increased brain D-amino acid oxidase (DAAO) activity in schizophrenia. Schizophr Res 2008;101:76–83.
[55] Hashimoto K, Fukushima T, Shimizu E, Komatzu N, Watanabe H, Shinoda N, et al. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. Arch Gen Psychiatry 2003;60:572–6.
[56] Boks MP, Rietkerk T, van de Beek MH, Sommer IE, de Koning TJ, Kahn RS. Reviewing the role of the genes G72 and DAAO in glutamate neurotransmission in schizophrenia. Eur Neuropsychopharmacol 2007;17:567–72.
[57] Pollegioni L, Piubelli K, Sacchi S, Pilone MS, Molla G. Physiological functions of D-amino acid oxidases: from yeast to humans. Cell Mol Life Sci 2007;64:1373–94.
[58] Konno R, Yasumura Y. Mouse mutant deficient in D-amino acid oxidase activity. Genetics 1983;103:277–85.
[59] Krug AW, Volker K, Dantzler WH, Silbernagl S. Why is D-serine nephrotoxic and AIB (α-amino-iso-butyric acid) protective? Am J Physiol Renal Physiol 2007; 293:F382–90.
[60] Vanconi MA, Cosma A, Mazzeo D, Mattevi A, Todone F, Curti B, et al. Limited proteolysis and X-ray crystallography reveal the origin of substrate specificity and of the rate-limiting product release during oxidation of D-amino acids by mammalian D-amino acid oxidase. Biochemistry 1997;36:5624–32.
[61] Adage T, Trillat A-C, Quattropani A, Perrin D, Cavarec L, Shaw J, et al. In vitro and in vivo pharmacological profile of AS057278, a selective D-amino acid oxidase inhibitor with potential anti-psychotic properties. Eur Neuropsychopharmacol 2008;18:200–14.
[62] Ferraris D, Duvall B, Ko Y-S, Thomas AG, Rojas C, Majer P, et al. Synthesis and biological evaluation of D-amino acid oxidase inhibitors. J Med Chem 2008; 51:3357–9.
[63] Hashimoto K, Fujita Y, Horio M, Kunitachi S, Iyo M, Ferraris D, Tsukamoto T. Co-administration of a D-amino acid oxidase inhibitor potentiates the efficacy of D-serine in attenuating prepulse inhibition deficits after administration of dizocilpine. Biol Psychiatry 2009. PMID 19217074.
[64] Horio M, Fujita Y, Ishima T, Iyo M, Ferraris D, Tsukamoto T, Hashimoto K. Effects of D-amino acid oxidase inhibitor on the extracellular D-alanine levels and the efficacy of D-alanine on dizocilpine-induced prepulse inhibition deficits in mice. Open Clin Chem J 2009;2:16–21.
[65] Smith SM, Uslaner JM, Yao L, Mullins CM, Surles NO, Huszar SI, et al. The behavioral and neurochemical effects of a novel D-amino acid oxidase inhibitor, 4H-thieno [3,2-b] pyrrole-5-caroboxylic acid (Compound 8) and D-serine. J Pharmacol Exp Ther 2009;328:921–30.
[66] Fang QK, Hopkins S, Jones S. Benzo[d]isoxazol-3-ol DAAO inhibitors. US 2005/0143434 A1.
[67] Heffernan MLR, Foglesong RJ, Hopkins SC, Soukri M, Jones SW, Spear KL, Varney MA. Fluoro-substituted inhibitors of D-amino acid oxidase. US 2008/0004327 A1.
[68] Dorsey JM, Heffernan MLR, Fang QK, Foglesong RJ, Hopkins SC, Ogbu CO, Soukri M, Spear KL. Fused heterocycles. US 2008/0004328 A1.
[69] Kennis LEJ, Vanhoof GCP, Bongartz J-PAM, Luyckx MGM, Minke WE. DAAO inhibiting benzisoxazoles and their use for the treatment of mental disorders. WO/2005/089753.
[70] Zhao W, Konno R, Zhou Z-J, Yin M, Wang Y-X. Inhibition of D-amino acid oxidase activity induces pain relief in mice. Cell Mol Neurobiol 2008;28:581–91.
[71] Goudet C, Magnaghi V, Landry M, Nagy F, Gereau RW, Pin IV J-.. Metabotropic receptors for glutamate and GABA in pain. Brain Res Rev 2009;60:43–56.

[72] Morita K, Motoyama N, Kitayama T, Morioka N, Kifune K, Dohi T. Spinal antiallodynia action of glycine transporter inhibitors in neuropathic pain models in mice. J Pharmacol Exp Ther 2008;326:633–45.
[73] Fukushima T, Sone Y, Mitsuhashi S, Tomiya M, Toyo'oka T. Alteration of kynurenic acid concentration in rat plasma following optically pure kynurenine administration: a comparative study between enantiomers. Chirality 2008;21:468–72.
[74] Labrie V, Clapcote SJ, Roder JC. Mutant mice with reduced NMDA-NR1 glycine affinity or lack of D-amino acid oxidase function exhibit altered anxiety-like behaviors. Pharmacol Biochem Behav 2009;91:610–20.
[75] Perry KW, Falcone JF, Fel MJ, Ryder JW, Yu H, Love PL, et al. Neurochemical and behavioral profiling of the selective GlyT1 inhibitors ALX5407 and LY2365109 indicate a preferential action in caudal vs. cortical brain areas. Neuropharmacology 2008;55:743–54.
[76] Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I. et al. Common variants on chromosome 6p22.1 are associated with schizophrenia Nature 2009 doi:10.1038/nature08192.
[77] International Schizophrenia Consortium. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 2009 doi:10.1038/nature08185.
[78] Stefansson H, Ophoff RA, Steinberg S. Andreassen OA Cichon S, Rujescu D et al. Common variants conferring risk of schizophrenia. Nature 2009 doi:10.1038/nature08186.
[79] Horrobin DF. Modern biomedical research: an internally self-consistent universe with little contact with medical reality? Nat Rev Drug Discov 2003; 2:151–4.
[80] Hughes B. Novel consortium to address shortfall in innovative medicines for psychaitric disorders. Nature Rev Drug Discov 2009;8:523–4.