Official Title: Neurobiology of a Mutation in Glycine Metabolism in Psychotic Disorders

NCT number: NCT01720316

Document Date: 10/17/2012

DETAILED PROTOCOL

Principal Investigator: Deborah L. Levy, Ph.D.

Protocol Title: Neurobiology of a Mutation in Glycine Metabolism in Psychotic Disorders

I. Background and Significance

a. Historical background

Multiple rare structural variants of relatively recent evolutionary origin are recognized as important risk factors for schizophrenia (SZ) and other neurodevelopmental disorders [e.g., autism spectrum disorders, mental retardation, epilepsy with odds ratios as high as 7-30 (Sebat et al. 2009; Malhotra et al. 2011; Heinzen et al. 2010; Weiss et al. 2008; McCarthy et al. 2009). We have found a *de novo* structural rearrangement on chromosome 9p24.1. In addition to other genes, the duplicated region contains the gene encoding glycine decarboxylase (*GLDC*), which affects brain glycine (Gly) metabolism. A specific focus of this project is on potential contributions of this gene to abnormal glycine homeostasis and N-methyl-D-aspartate receptor (NMDAR) dysfunction in SZ. This mutation is an obvious 'smoking gun.' *GLDC* encodes the glycine decarboxylase or glycine cleavage system P-protein, which is involved in degradation of glycine (Gly) in glia cells. Carriers of the *GLDC* triplication would be expected to have low levels of brain Gly, resulting in NMDA receptor-mediated hypofunction, which has been strongly implicated in the pathophysiology of SZ (Olney & Farber, 1995; Coyle, 2006; Javitt, 2007). Individuals with mutations that lower brain Gly or alter other aspects of glutamatergic transmission are obvious candidates for Gly augmentation or complementary NMDAR modulatory strategies, which have been used with varying degrees of success (Goff et al. 1999; Javitt et al. 2001; Lane et al. 2005; Heresco-Levy et al. 2004; Buchanan et al. 2007). Genetic risk factors, including variants that impact the synthesis and breakdown of Gly, are likely to contribute to this variability.

b. Previous pre-clinical or clinical studies leading to and supporting the proposed research

There is an extensive literature on the effects of NMDA enhancing agents on positive, negative, and depressive symptoms and on neurocognitive function (see Tsai & Lin, 2010; Lin et al. 2011 for reviews). Although many studies have reported positive results in at least one symptom domain (Heresco-Levy et al. 1996, 1999, 2004; Tsai et al. 1998, 1999, 2004a, 2006; Javitt et al. 2001; Goff et al. 1996; Lane et al. 2008),
the results of other studies have been negative or ambiguous (Goff et al. 1999; Evins et al. 2000; Duncan et al. 2004; van Berckel et al. 1999). Individual agents also differ in their impact on various domains of psychopathology and cognition. Factors likely to contribute to this variability are: mechanism of action of the agent, compliance, concurrent treatment with first- vs second generation antipsychotic drugs, baseline Gly blood levels, presence/absence of kynurenine pathway metabolic abnormalities [e.g., increased kynurenic acid) (KYNA)] (Wonodi et al. 2010; Erhardt et al. 2007) and individual differences in brain Gly uptake and metabolism (Kaufman et al. 2009; Buchanan et al. 2007). Genetic variants that impact the synthesis and breakdown of Gly, Glu, or other modulators of NMDAR function are also likely to have significant effects. Although Gly augmentation has shown variable efficacy in patients *unselected for having a mutation that would be expected to lower brain Gly levels*, the *GLDC* triplication in the two carriers would be expected to result in *unusually low* brain Gly levels, supporting its therapeutic potential as an augmentation strategy.

In previous work Kaufman and colleagues have shown that 14 days of oral Gly increases brain Gly levels (P) compared with baseline (B) levels (Kaufman et al. 2009). Oral high dose Gly also increases total brain and plasma Gly levels in mice, and the increments in brain and plasma Gly covary for several hours (Toth & Lajtha, 1986). These findings support the utility of examining how glycine uptake and degradation following a glycine loading dose using proton ^{1}H MRS.

It is possible to obtain J-resolved proton MRS provides high quality information on glutamine, glycine, and glutamate (Ongur et al. 2008; Jensen et al. 2009; Prescott et al. 2006). GABA can be quantified separately from MEGAPRESS difference spectra (Jensen et al. 2009), permitting the measurement of levels of other metabolites that may be impacted by the *GLDC* triplication.

c. Rationale behind the proposed research and potential benefits to patients and/or society

The successful discovery of genetic variants underlying adverse and optimized treatment responses underscores the high potential for genetic discoveries to impact personalized medicine (Chung et al. 2004; McCormack et al. 2011; Chen et al. 2011; Ge et al. 2009; Tanaka et al. 2009; Suppiah et al. 2009; O'Brien, 2009; Klein et al. 2009; Takeuchi et al. 2009; Cooper et al. 2008; Bollag et al 2010; Daly et al. 2009; Mallal et al. 2008; Sorlie et al. 2001; Loi et al. 2010; Bennett et al. 2012; Nathwani et al. 2011). Of particular importance, the identification of mutations in specific genes can lead to "medically actionable" treatment interventions tailored to the underlying disease biology, with positive therapeutic effects (Bainbridge et al. 2011; Worthey et al. 2011). Such individually tailored treatments may not only improve clinical response and outcome in *appropriately selected* patients, but also may reduce the heterogeneity in symptom reduction in clinical trials.

II. Specific Aims

a. Objectives/hypotheses

Ideally, the identification of mutations in specific genes would result in personalized, "medically actionable" treatment interventions (Bainbridge et al. 2011; Worthey et al. 2011; Cirak et al. 2011). We have found such a potentially informative mutation and it has high potential to impact clinical response. This project will investigate effects of a *de novo* structural rearrangement on chromosome 9p24.1 that segregates with psychosis in an extended family. In addition to other genes, the duplicated region contains the gene encoding glycine decarboxylase (*GLDC*), which affects brain glycine metabolism. A specific focus, therefore, is on potential contributions of this gene to abnormal glycine homeostasis and N-methyl-D-aspartate receptor (NMDAR) dysfunction in SZ.

We propose to (1) characterize the molecular, neurocognitive, and brain structural, functional and neurochemical properties of this mutation by carrying out targeted neurobiological follow-up of mutation carriers and non-carriers in this family and (2) to target the *GLDC* mutation by trying to normalize brain Gly levels using Gly augmentation.

Specific Aim 1: To characterize the pathophysiology of disrupted Gly homeostasis using structural magnetic resonance imaging (3.0 Tesla) and proton magnetic resonance spectroscopy (MRS; 4.0 Tesla). The MRS studies will characterize endogenous brain Gly levels as well as brain and plasma pharmacodynamics after a single oral Gly dose (30 g). MRS will also be used to measure GABA, glutamate (Glu) and glutamine (Gln) metabolite levels. *Hypothesis*: Mutation carriers will have reduced endogenous brain Gly and GABA levels and

increased brain Glu and Gln levels. Gly administration will increase brain Gly in carriers, but to a lesser extent than in non-carrier family members and controls.

Specific Aim 2: To probe the effect of dysregulated NMDA-mediated neurotransmission on visual processing. Functional MRI using behavioral paradigms that preferentially bias activity in magnocellular pathways, and visual evoked responses (ERPs) [e.g., mismatch negativity (MMN), P300] that are modulated, at least in part, by decreased NMDAR function, will be used. *Hypothesis*: reduced activation of magnocellular pathways and abnormal ERPs modulated by NMDA in carriers.

Specific Aim 3: To carry out a double-blind placebo-controlled trial of Gly augmentation in the two mutation carriers. *Hypothesis*: Gly, but not placebo, will improve positive and negative symptoms as well as neurocognitive function. Specific aims 1-2 will be carried out *prior* to the Gly augmentation trial.

Specific Aim 4: To determine the effects of open-label chronic Gly treatment (6 weeks) on 1) clinical and cognitive functioning, 2) brain metabolite levels of Gly, GABA, Glu and Gln measured with proton MRS and 3) NMDAR function as reflected in visual steady state ERPs and magnocellular activation. *Hypotheses*: improved clinical and cognitive functioning; partial normalization of decreased baseline Gly and GABA and increased Glu and partially normalized magnocellular pathway activation and abnormal ERPs.

III. Subject Selection

The four subjects have already been identified and recruited by the PI and have agreed to participate. They were selected on the basis of either having a mutation involving an abnormality in glycine metabolism (N=2) that may be implicated in psychotic disorders, or being a family member who does not have this same mutation (N=2). Prior to beginning the study, all subjects will be called by the PI and all of the procedures and time frames will be re-reviewed. The subjects will be flown to Boston for the McLean procedures and will travel by train (with Dr. Levy) to NY for the NKI procedures. The procedures will be scheduled well in advance to minimize inconvenience to the subjects and to optimize successful data collection at McLean and NKI.

IV. Subject Enrollment

The total projected sample size is 4, consisting of two carriers of a 9p24.1 mutation and two individuals from the same family who are not carriers of this same mutation. The two carriers have diagnoses of bipolar disorder with psychotic features and schizo-affective disorder and will be recruited as outpatients. The two non-carriers do not have diagnoses of a psychotic disorder. The age range is 21-59 and includes two males and two females. Note that there are two consent forms, one for the two subjects who will be receiving a double-blind glycine-placebo cross-over trial and open-label glycine and one for the two subjects who will not.

V. Study Procedures

Design of the study: Overview

1) Baseline procedures at McLean: All four subjects will receive these baseline procedures.

Physical exam/EKG (by Dr. Ongur).

Movement disorders exam (extrapyramidal side effects, tardive dyskinesia - AIMS/SAS) (Guy, 1976; Simpson & Angus, 1970) (by Dr. Bodkin).

Structural MRI (3T - 15 minutes), proton ^{1}H MRS (4T) for GABA levels (MEGAPRESS - 1 hour);

Proton ^{1}H MRS (4T-J-Resolved) for glycine, glutamate, and glutamine levels, pre-glycine loading (1 hour) and post-glycine loading [serial scans after a glycine dose of 0.4g/kg (not to exceed 30 g – no IND necessary) and 5 cc blood samples at (pre, 20, 40, 60, 90, 120, 150, 180, 210, and 240 minutes post-Gly coinciding with serial MRS scans). All scan procedures have been approved by the McLean IRB as part of the following protocols: "Acute Glycine Pharmacodynamic Study" (Kaufman MJ, PI); "Proton Magnetic Resonance Imaging and Spectroscopy Studies of Brain Chemistry in Bipolar Disorder, Schizophrenia, and Related Disorders (Ongur, D, PI);" "Proton Magnetic Spectroscopy Studies of Glutathione and Gamma-butyric Acid in Bipolar Disorder, Schizophrenia and Related Disorders" ((Ongur, D, PI). Approved consent forms have been uploaded. Dr. Ongur will be responsible for medical oversight of the imaging components of the study.

Clinical ratings: PANSS (Kay et al. 1987); Clinical Global Impression Scale (Guy, 1976); Brief Psychiatric Rating Scale (Overall & Gorham, 1962); Young Mania Rating Scale (Young et al. 1978); Hamilton Depression Scale (Hamilton, 1960) (by Michael Coleman, M.A.).

Neurocognitive testing (MATRICS Consensus Cognitive Battery-MCBB (Kern et al. 2011) (by Dr. Levy).

Urinalysis and plasma levels: SMA-20, large neutral & excitatory amino acid, kynurenic acid (KYNA),
psychotropic drug plasma levels.

<u>Blood sample quantities for all subjects (two mutation carriers and two non-mutation carriers) are as follows</u>:

5 cc blood samples at (pre, 20, 40, 60, 90, 120, 150, 180, 210, and 240 minutes post-Gly loading - IV), for a
subtotal of 50cc;
5 cc – SMA-20;
6 cc - large neutral amino acid plasma levels;
6 cc - small neutral/excitatory amino acid plasma levels;
6 cc – homocysteine plasma level;
6 cc - d-serine plasma level;
4 cc –GABA plasma level;
7 cc – KYNA plasma level, KYN plasma level, and quinolinic acid plasma levels – fasting – pre-breakfast - all
subjects.
Thus, for all 4 subjects, 90 cc will be drawn for the baseline procedures.

<u>Additional blood samples for the two mutation carriers</u>:
Additional blood sample quantities for one of the mutation carriers are as follows: Note that if the subject's medications change prior to the baseline scans, plasma levels for specific dugs may change. If this results in a change in the amount of blood required, the IRB will be informed.
6 cc - cymbalta steady state plasma level;
6 cc- clozaril steady state plasma level;
5 cc - lithium steady state plasma level;
6 cc - gabapentin steady state plasma level, all prior to AM dose.
Thus, for this subject a total of 113 (including the 90 cc mentioned above) cc will be drawn at this time point.

Additional blood sample quantities for the second mutation carrier are as follows: Note that if the subject's medications change prior to the baseline scans, plasma levels for specific dugs may change. If this results in a change in the amount of blood required, the IRB will be informed.

6 cc- clozaril steady state plasma level;
6 cc – remeron steady state plasma level;
6 cc – abilify steady state plasma level;
6 cc – celexa steady state plasma level, all prior to AM dose.
Thus, for this one subject a total of 114 cc will be drawn (including the 90 cc mentioned above) at this time
point.

This amount of blood is well within safe guidelines for any healthy individual weighing at least 35 pounds according to the McLean IRB and OHRP guidelines (< 550 ml in an 8 week period).

The personal physicians of the subjects will be informed about their planned participation in a study that involves exposure to glycine and lemon juice. Each physician will be asked to provide a written statement indicating that he/she believes that there is no medical contraindication to the subject's exposure to lemon juice/crystals or glycine. The internist for both mutation carriers and one non-carrier has provided a written statement saying that she sees no problem with their exposure to glycine and lemon juice. We are still waiting to receive a statement from the physician for the second non-carrier, who recently relocated and does not yet have a physician. However, this person will receive only the single glycine loading dose in relation to the spectroscopy scan; he will not receive glycine chronically since he is not part of the double-blind placebo-controlled or open-label glycine treatment trial.

2) Baseline procedures at Nathan Kline Institute (NKI): functional MRI, ERPs/EEGs, all of which have been approved by the NKI IRB (approved consent forms have been uploaded).

At the completion of steps 1 and 2, the participation of the two non-carriers will be complete.

<u>Additional Procedures for Mutation Carriers Only</u>:

3) Double-blind placebo-controlled oral glycine (Gly) (gradual titration to a maximum daily dose of 0.8 g/kg based on the subjects' weights at date of initial screening distributed using a TID dosing schedule;) or Placebo: An FDA application for an IND will be obtained before initiation of this part of the study. A Certificate of Analysis, Material Data Safety Sheet, Sample Label, and Product Description from the manufacturer of the glycine powder, Ajinomoto, have been uploaded with the application. The titration schedule has also been uploaded. The placebo, Isomaltulose (Palatinose®), will be obtained from Beneo, Inc, which has a branch in New Jersey. A Certificate of Analysis, Material Data Safety Sheet, Sample Label, and Product Description have been uploaded. If the subjects' weights are substantially different by the time this part of the study is scheduled to take place, their revised weights will be used in the calculation of the doses. The start of this arm will be set in collaboration with the pharmacist to make sure that there is adequate time to titrate the appropriate amounts of glycine.

Dr. Bodkin will be responsible for medical oversight of the glycine augmentation component of the study. Dr. Ongur will serve as back-up medical coverage.

The patients' usual psychotropic drug regimen will not be altered as part of the study, but an attempt will be made to keep those medications unchanged throughout the study if possible. Any changes will be left up to the patients' psychiatrists.

An FDA IND will be obtained for this part of the study and for the open-label glycine arm and the IRB will sent all documentation from the FDA.

a) 6 Weeks on Gly or Placebo

The McLean Pharmacy will order pharmaceutical grade glycine powder (10kg/box) from Ajinomoto North America, Inc. The glycine will be stored in closed containers in a dry area, avoiding humidity, sunlight and high temperature.

The McLean Pharmacy will prepare the appropriate dose of glycine powder or placebo in a re-closable plastic bottle. The placebo is Isomaltulose (Palatinose®), which looks and tastes like glycine; it is a fully digestible, tooth-friendly, low glycemic sugar. Each dose will be in a self-contained package that includes an opaque plastic bottle containing glycine or placebo and individual packets of lemon crystals. Each bottle will be marked with a fill line, indicating how much cold water should be added for the glycine or placebo dose to be dissolved as a 20% solution. Each dose will be labeled by the pharmacy with the date, dose (breakfast, lunch, dinner) and instructions (e.g., "Fill the bottle with cold water to the fill line, shake, pour into a glass, add lemon crystals. Drink over a 15-20 minute period after a meal."). The McLean Pharmacy will prepare both the active and placebo mixtures. Doses will be shipped in two-week supplies. While the subjects are at McLean for the baseline scans, the pharmacist and Dr. Levy will meet with the subjects and show them what the packaging of each dose looks like and what they have to do to combine the ingredients.

It is anticipated that all of the glycine that is ordered will be used. If additional glycine is needed and there is any unused glycine powder, it will be disposed of according to the instructions on the Material Safety Data Sheet, (item 13): 'Dispose of the material as you would with a non-hazardous material in accordance with all applicable national, state and local regulations.'"

Dosage Titration (see attached Titration Schedule): Glycine will be started at a dose of 3g/d and increased by 3-6 g/d until a maximum daily dose of 0.8 g/kg (based on the subjects' weights at date of initial screening) is achieved. Dosing will be TID. This titration schedule is based on that of Heresco-Levy et al. 2004. For someone who weighs 99 kg (217.8 pounds), the estimated weight of one subject, the estimated maximum daily dose would be 79.2 g/d. For someone who weighs 85 kg (187 pounds), the estimated weight of the other subject, the estimated maximum daily dose would be 68 g/d. The maximum dosage ranges used in two previous studies, based on the same maximum dosage of 0.8g/kg/d of body weight, were 40-90 g/d (Heresco-Levy et al. 1999) and 40-80 g/d (Heresco-Levy et al. 2004). The estimated maximum doses we are proposing to use are within these ranges. If the subjects' weights are substantially different by the time this part of the study is scheduled to take place, their revised weights will be used in the calculation of the doses. The start of this arm will be set in collaboration with the pharmacist to make sure that there is adequate time to titrate the appropriate amounts of glycine.

Subjects will keep a daily log to mark the time each dose was taken (sample log attached)

At the end of weeks 2, 4, and 6:
Clinical ratings using "skype-like" video conferencing (see below and uploaded document for details on measures that will be taken to ensure confidential and secure connections under the auspices of Partners Collaborative Media);

At the end of week 6:

SMA-20, large neutral & excitatory amino acids, KYNA, and psychotropic drug plasma levels (drawn in the Clinical Laboratory of a local medical center and shipped to the Analytical Psychopharmacology Laboratory at NKI for processing (other assays may be performed in house). The same quantities described above will be drawn on the two participating subjects, for a total of 113 cc for one subject and a total of 114 cc for the other.

An assessment of movement disorders (extrapyramidal side effects, tardive dyskinesia - AIMS/SAS- using "skype-like" video conferencing);

MCCB, at the end of week 6 (administered in the subject's city of residence).

Clinical Oversight:
The subjects are well known to the PI, who will be in touch with them by phone on a weekly basis or more often as needed, including weekly during the two weeks post-drug or placebo. Both subjects' psychiatrists will also be monitoring their clinical states and side effects. Dr. Levy will inform Dr. Bodkin or Dr. Ongur immediately if any side effects are reported that warrant medical follow-up. The subjects' internist will be informed when the glycine-placebo and open-label glycine periods begin (see sample letter to physician).

At the end of the first week of glycine or placebo, and at the end of weeks 3 and 5 (more often if necessary), the subjects will be called by a study physician (Dr. Bodkin) to assess how they are reacting to the glycine or placebo. The subjects will also be given emergency contact numbers for Drs. Levy, Bodkin, Ongur, and Kaufman. The subjects will also be told to go the nearest emergency room if they experience any acute side effects (e.g., vomiting).

b) 2 Weeks of No Augmentation Treatment (weeks 7-8)
During weeks 7 and 8, the PI will contact to the subjects each week. Dr. Levy will inform Dr. Bodkin or Dr. Ongur immediately if any side effects are reported that warrant medical follow-up.
At the end of week 8:
Clinical ratings "skype-like" video conferencing;
SMA-20, large neutral & excitatory amino acids, KYNA, and psychotropic drug plasma levels (drawn in the Clinical Laboratory of a local medical center and shipped to the Analytical Psychopharmacology Laboratory at NKI for processing (other assays may be performed in house). The same quantities described above will be drawn on the two participating subjects, for a total of 113 cc for one subject and a total of 114 cc for the other.

c) 6 Weeks on Gly or Placebo (weeks 9-14; may or may not be contiguous with first glycine-placebo arm)

Subjects will keep a daily log to mark the time each dose was taken (sample log attached)

At the end of weeks 10, 12, and 14:
Clinical ratings using "skype-like" video conferencing.

At the end of week 14:
SMA-20, large neutral & excitatory amino acids, KYNA, and psychotropic drug plasma levels (drawn in the Clinical Laboratory of a local medical center and shipped to the Analytical Psychopharmacology Laboratory at NKI for processing (other assays may be performed in house). The same quantities described above will be drawn on the two participating subjects, for a total of 113 cc for one subject and a total of 114 cc for the other.

An assessment of movement disorders (extrapyramidal side effects, tardive dyskinesia - AIMS/SAS- using “skype-like” video conferencing);

MCCB, at the end of week 6 (administered in the subject’s city of residence).

Clinical Oversight:
The subjects are well known to the PI, who will be in touch with them by phone on a weekly basis or more often as needed. Both subjects’ psychiatrists will also be monitoring their clinical states and side effects. See also above.

At the end of the first week of glycine or placebo (week 9), and at the end of weeks 11 and 13, the subjects will be called by a study physician (Dr. Bodkin) to assess how they are reacting to the glycine or placebo. The subjects will also be given emergency contact numbers for Drs. Levy, Bodkin, Ongur, and Kaufman. The subjects will also be told to go the nearest emergency room if they experience any acute side effects (e.g., vomiting).

d) 2 weeks of No Augmentation Treatment (weeks 15-16)
During weeks 15 and 16, the PI will contact to the subjects each week. Dr. Levy will inform Dr. Bodkin or Dr. Ongur immediately if any side effects are reported that warrant medical follow-up.

At the end of week 16:

Clinical ratings at the end of week 16 using a “skype-like” video conferencing tool;
SMA-20, large neutral & excitatory amino acids, KYNA, and psychotropic drug plasma levels (drawn in the Clinical Laboratory of a local medical center and shipped to the Analytical Psychopharmacology Laboratory at NKI for processing (other assays may be performed in house). The same quantities described above will be drawn on the two participating subjects, for a total of 113 cc for one subject and a total of 114 cc for the other.

4) Open-Label Glycine: 6 weeks on a maximum daily dose of 0.8 g/kg (based on the subjects’ weights at date of initial screening) distributed using a TID dosing schedule taken after meals. The same titration schedule used in the cross-over double-blind glycine-placebo trial (described above) will be used. At least one month will pass between the end of the double-blind glycine-placebo cross-over arms and the beginning of the open-label glycine trial. The time interval will depend on subject schedules and the need for them to travel to Boston and NY at the end of week 5 for re-scanning and other procedures in week 6. If the subjects’ weights are substantially different by the time this part of the study is scheduled to take place, their revised weights will be used in the calculation of the doses. The start of this arm will be set in collaboration with the pharmacist to make sure that there is adequate time to titrate the appropriate amounts of glycine.

Subjects will keep a daily log to mark the time each dose was taken (sample log attached)

The McLean Pharmacy will prepare and label each dose as described above, which will be FedEx’ed in 2-week supplies.

Baseline: Prior to beginning the open-label glycine trial, 113 and 114 cc of blood will be drawn for baseline levels of the same amino acid and psychotropic blood levels as indicated above.

At the end weeks 2, and 4:
Clinical ratings will be performed using a “skype-like” video conferencing tool.

During week 6:
The imaging and ERP/EEG procedures at McLean and NKI described in sections 1) and 2) above will be repeated, with the exception of the physical examination, EKG, and glycine-loading scan.

At the end of week 6:

Clinical ratings, an assessment of movement disorders, and the MCCB will be performed at McLean, where the baseline procedures (structural MRI, MRS, but no glycine loading), will be repeated.

Urinalysis, SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels will be obtained at this time and at the end of week 8.

Clinical Oversight:
The PI will be in touch with the subjects by phone on a weekly basis or more often as needed. Both subjects' psychiatrists will also be monitoring their clinical states and side effects throughout the study. The subjects' internist will be informed when the glycine-placebo and open-label glycine periods begin (see sample letter to physician). In addition, at the end of the first week of glycine, and at the end of weeks 3 and 5, the subjects will also be called by a study physician to assess how they are reacting to the glycine. The subjects will also be given emergency contact numbers for Drs. Levy, Bodkin, Ongur, and Kaufman. The subjects will also be told to go the nearest emergency room if they experience any acute side effects (e.g., vomiting). The same quantities as described above will be drawn on the two participating subjects, for a total of 113 and 114 cc at the end of week 6.

During weeks 7 and 8, the PI will contact to the subjects each week. Dr. Levy will inform Dr. Bodkin or Dr. Ongur immediately if any side effects are reported that warrant medical follow-up.

The consent forms contain a provision for subjects to give permission for Dr. Javitt and the Nathan Kline Institute to provide Dr. Levy and her colleagues at McLean with the results of any tests that were performed at NKI, for Dr. Raymond Suckow and the Nathan Kline Institute to provide Dr. Levy and her colleagues at McLean with the results of any blood samples that were analyzed at NKI, and for the Clinical Laboratories at McLean Hospital and at the medical center in the subjects' home city to provide Dr. Levy and her colleagues with the results of any blood tests.

Blood samples drawn at McLean or in the subject's city of residence will be sent to the Analytical Psychopharmacology Laboratory at NKI for analysis (SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels). The urinalyses and blood samples for lithium levels will be done either by the clinical lab of the local medical center or by the clinical lab at McLean. All samples will be coded with the subject's 4-digit ID number, the date and the time of day. Dr. Suckow will not know the identities of the subjects whose blood samples he receives.

Total Amount of Blood Drawn: The total amount of blood drawn at baseline is 90cc for the two non-mutation carriers and 113 and 114 cc for the two mutation carriers. The participation of the non-carriers will be complete at this time. The baseline procedures will be carried out at least two months prior to the beginning of the glycine-placebo cross-over arms in the mutation carriers. The total amount of blood drawn from the two mutation carriers during that 16-week

period will be 113 or 114 cc at weeks 6, 8, 14, and 16, for a maximum total of 452 cc and 456 cc. Note that the two arms will not necessarily be contiguous, depending on the subjects' schedules. Even if the two arms are contiguous, the total amount of blood will not exceed 550 cc in an 8-week period. For the open-label glycine study, 113 and 114 cc will be drawn at baseline and weeks 6 and 8, again not exceeding the 550 cc limit within two months. The two carriers will be advised not to donate blood for at least one month after completing the glycine-placebo cross-over arms and the open-label glycine arm.

Neuropsychological Assessment: Cognitive functioning will be assessed using the battery developed by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative (Kern et al. 2011). The MATRICS battery (MCCB) includes 10 tasks that measure processing speed (Brief Assessment of Cognition in Schizophrenia, Symbol Coding, Animal Fluency, Trails A), attention (Continuous Performance Test), working memory (WMS-III Spatial Span, Letter-Number Span), verbal learning (Hopkins Verbal Learning Test – Revised), visual learning (Brief Visuospatial Memory Test - Revised), problem solving (Neuropsychological Assessment Battery) and social cognition (Mayer-Salovey-Caruso Emotional Intelligence Test). Total administration time is 60-90 minutes. The MATRICS is well tolerated by patients and can be administered repeatedly. Use of this battery will permit comparisons between baseline, post-glycine, and post-placebo effects on neurocognition. See attachment on the MATRICS battery.

It is anticipated that all of the glycine will be used. If there is any unused glycine powder, it will be disposed of according to the instructions on the Material Safety Data Sheet, (item 13): "Dispose of the material as you would with a non-hazardous material in accordance with all applicable national, state and local regulations."

Partners Collaborative Media (PCM) will provide oversight (for a fee) to ensure that the clinical and movement disorders assessments taking place every two weeks using skype-like video conferencing are secure. Specific web cameras recommended by PCM will be used by McLean staff and by the subjects. All calls will be initiated from McLean using a Partners computer. This computer will have the Cisco program, "blue jeans," installed. PCM will create generic credentials for the subjects such that they will not be using their own actual skype credentials. Thus, if the skype material is stolen, it cannot be linked to the subjects themselves. A McLean clinician will call the subjects by clicking on customized phone numbers and allow the McLean caller to lock the virtual meeting room.

Prior to this, PCM will run tests on the subjects' computers to determine if they have enough bandwidth, are hooked into a land line or use a provider (i.e., Comcast) and if their computers have a web camera built in. The systems at the subjects' homes will be customized to be secure and compatible with the system in place at McLean.

A technical statement from PCM about how the secure connection will be implemented has been uploaded in e-irb.

VI. Biostatistical Analysis

All data acquisition and processing will be carried out blind to mutation status. The McLean investigators and Dr. Javitt have extensive imaging and ERP databases involving controls and patients with psychotic disorders. Thus, we are well positioned to compare fMRI activation, ERPs and MRS spectra between carriers and non-carriers even if formal statistical testing is not always possible. At a minimum, using data from groups scanned on the same scanners under the same conditions, we can establish where our subjects fall in the range of values compared

with other subject groups using z-scores or % difference scores (e.g., Glu, GABA, Gln metabolite levels; Gly and other metabolite concentrations; Gln:Glu ratios). Pharmacokinetic curves of brain and plasma Gly levels (pre-/post-bolus administration) will be generated and tested for correlation, which can be compared with controls who undergo the same protocol. In the carriers, it will be possible to assess the magnitude of change in clinical and neurocognitive function and to correlate change in amino acid levels with clinical and neurocognitive change scores during Gly vs placebo. This is not a population study; it is designed to provide mechanistic insights about the effects of this mutation in the brain.

VII. Risk and Discomforts

The imaging procedures have been used extensively at McLean and NKI without complications and subjects will be carefully screened.

The procedures described above pose no serious physical risk to subjects and no psychological, social, or legal risks are anticipated.

Although there are no known general risks associated with MRI scans, there are risks to individual subjects who have contraindications to MRI scanning, including those with metal implants in their body (pacemaker, aneurysm clips, metal screws and plates for orthopedic purposes, hearing implants, certain kinds of tattoos, sheetmetal workers with lodged metal fragments in the eyes). Subjects are screened carefully and excluded if there are even suspected contraindications to scanning. All subjects are asked to remove jewelry, belts and other metal-containing objects. Surgical records will be retrieved prior to scan for subjects who have had metal placed in their body intra-operatively to ensure the hardware is MRI safe, even if the subject has been told the hardware is MRI-safe or if they have had MRI scans since the operation. As an additional precaution, subjects are screened with a handheld metal-detection wand prior to the scan to ensure that no unidentified metal objects remain on the subject. The noise generated by the pulsing of the gradients can lead to temporary decrease in hearing. The use of disposable earplugs is an easy and reliable means of preventing hearing loss. The risks associated with Specific Absorption Rate (SAR) are related to the fact that given a large enough SAR, heating of the tissue may occur. These experiments will comply with all FDA guidelines with regard to RF power deposition. There is also the potential risk of injury from a projectile (i.e., ferromagnetic objects being attracted into the magnet); and of asphyxiation due to large amounts of cryogenic gases generated during a quench (i.e., the event which occurs when a magnet makes the sudden transition from superconducting to resistively conducting). Routine safety procedures are in place at both scanning centers to screen subjects prior to scanning, maintain security of the restricted access areas, and ensure that system security features are in good working order. Both imaging centers associated with this study (McLean, NKI) are very experienced with MRI scanning and have impeccable safety records. The effects of MRI on the fetus are not well characterized. Therefore, females of childbearing age must be sexually inactive or be using a contraceptive measure for three months prior to being scanned. A urine test will be used to establish that the one female of childbearing age is not pregnant.

The scans involve use of a standard clinical MRI scanner (3T) as well as a high field (4T) MRI scanner. The 4T scanner is not used for routine clinical studies in children or adults, but the FDA has determined (July 14, 2003) that scanners with magnetic field strengths of less than 8 Tesla do not represent a significant risk to adults, children, or infants older than 1 month. [164] Most people experience no ill effects from 3T or 4 T scans, but some do report claustrophobia, dizziness, mild nausea, headaches, a metallic taste in their mouth, back tingling, double vision, or sensation of flashing lights. These symptoms, if present, disappear shortly after leaving the

scanner. During the scan, the examiner can see and hear the subjects and will ask them to report any problems so the scan can be stopped if necessary. A magnetic resonance scan may be uncomfortable due to claustrophobia, lying still for an hour, or loud sounds. Subjects who express serious concern about these will not be included. The scan will be stopped if the subject expresses discomfort. Total time in the scanner for the structural scan and 2 MRS scans at McLean is 150 minutes, with breaks occurring between scans (structural: 15 minutes; MEGAPRESS: 60 minutes; J-PRESS: 75 minutes). Total time for the structural and fMRI scan at NKI is I hour. The glycine-loading scan requires multiple scans over the 4 hour period in the scanner. Subjects will be allowed to leave the scanner between scans and can be re-positioned for the next scan, as described in the application. All four subjects have successfully completed imaging procedures.

Oral glycine has been widely used to augment standard psychotropic drug treatment and is well tolerated. By using a weight-adjusted maximum dose of 0.8 g/kg (based on the subjects' weights at date of initial screening)
administered on a TID schedule, the dose will be well within the range of a glycine dose that is tolerated without undue adverse effects. If necessary, the dose can be lowered to minimize GI side effects. The subjects will be monitored closely by the research team and by their own psychiatrists. Blood chemistries, including liver/kidney function tests, will be monitored at baseline and at the end of each treatment arm.

Glycine Augmentation Clinical Trial: Pharmaceutical grade glycine (Ajinomoto) will be used. Glycine powder-water mixtures (dissolved as a 20% solution) will be prepared by the McLean Hospital pharmacy. The dose of glycine will be slowly titrated until a TID maximum dose of 0.8 g/kg of body weight (based on the subjects' weights at date of initial screening) (administered after meals) is reached. This dose is well within the range of doses that were well tolerated in previous patient studies (Goff et al. 1999; Heresco-Levy et al. 1996, 1999, 2004). The most common side effect is mild gastrointestinal distress, which is reversible upon discontinuation, should that be necessary. At a mean dose similar to that proposed here, 82% and 95% of patients, respectively, completed the clinical trial (Heresco-Levy et al. 1999, 2004). No adverse effects on kidney, liver, hematology or blood chemistry values were observed over a 6-week period. If necessary, the dose can be lowered to minimize GI side effects. The subjects will be monitored closely by the research team and by their own psychiatrists. Blood chemistries, including liver/kidney function tests, will be monitored at baseline.

The titration schedule is based on that of Heresco-Levy et al. 2004. For someone who weighs 99 kg (217.8 pounds), the approximate expected weight of one subject, the expected maximum daily dose would be 79.2 g/d. For someone who weighs 85 kg (187 pounds), the approximate expected weight of the other subject, the expected maximum daily dose would be 68 g/d. The maximum dosage ranges used in two previous studies, based on the same maximum dosage of 0.8g/kg/d of body weight, were 40-90 g/d (Heresco-Levy et al. 1999) and 40-80 g/d (Heresco-Levy et al. 2004). The estimated maximum doses we are proposing to use are within these ranges.

Periodic blood samples and the IV involve the slight discomfort of a needle stick and the small risk of a bruise. Every attempt is made to have the subject feel comfortable and at ease with the environment and the staff.

Other than the paste used to adhere ERPs/EEGs electrodes to the scalp, no discomforts or risks should be incurred from these procedures, which are quite routine.

Risks to privacy and confidentiality are minimal. All subjects are assigned a random4-digit ID number, which is used to code all material.

VIII. Potential Benefits

The general goal of this study is to clarify the neurobiology of a mutation in glycine mutation and to determine whether carriers of this mutation may preferentially benefit from glycine augmentation of their medication regimen. Although subjects receive no immediate benefit from the brain imaging and ERP/EEG procedures beyond contributing to important research and reasonable monetary compensation for the time commitment, the potential scientific yield could have a major impact on identifying causal mechanisms in psychotic disorders. If the glycine augmentation is beneficial, these two subjects may experience a significant reduction in psychotic symptoms and improvement in neurocognition, which may also help other subjects with mutations impacting the glycine metabolic pathway.

IX. Monitoring and Quality Assurance

Data safety monitoring is carried out to ensure and maintain the scientific integrity of research projects involving human participants and to protect the safety of the participants. Safety monitoring is any process during a study that involves the review of accumulated outcome data for groups of participants to determine if any of the study procedures should be altered or stopped. The plan includes details of how the data collections are monitored and how adverse events are detected and reported. The safety data include all imaging procedures, ERPs/EEGs, then neurocognitive function battery, as well as the double-blind placebo-controlled and open-label glycine augmentation trials. Efficacy data includes all glycine-related procedures.

Drs. Levy, Kaufman, Öngür, and Bodkin will be responsible for monitoring the safety of this study, executing the DSMP, and complying with the reporting requirements to the McLean Hospital Institutional Review Board (IRB). The investigators are very familiar with these responsibilities and Dr. Kaufman serves as Vice-Chair of the McLean Hospital IRB. All adverse events will be promptly reported to the McLean IRB, NIMH, and the FDA as appropriate.

The research will be altered or stopped if subjects have adverse reactions to any of the procedures (e.g., claustrophobia in the scanners) or significant side effects to glycine. The dose of glycine in the glycine loading study (0.4g/kg of body weight on the day of the scan) will not exceed 30 grams, a dose that is unlikely to cause significant side effects in our experience.

The dose of glycine used in the augmentation trials (a maximum of 0.8 g/kg/d (based on the subjects' weights at date of initial screening) using a TID dosing schedule after meals) is well tolerated and is a standard dose used in clinical trials with minimal, if any, side effects. The PI will be in touch with the subjects by phone on a weekly basis or more often as needed. Both subject's psychiatrists will also be monitoring their clinical states and side effects throughout the study. Their local internist will be informed when each arm of the glycine-placebo and open-label glycine begins (sample letter informing the internist has been uploaded). In addition, at the end of the first week of glycine or placebo, and at the end of weeks 3, 5, 9, 11, and 13 (more often if needed) the subjects will also be called by a study physician (Dr. Bodkin) to assess how they are reacting to the glycine or placebo. During the open-label glycine trial, side effects will also be assessed at the end of weeks 1, 3, and 5, or more often as needed. The subjects will also be monitored weekly during the two weeks post-drug.

The subjects will also be given emergency contact numbers for Drs. Levy, Bodkin, Ongur, and Kaufman. The subjects will also be told to go the nearest emergency room if they experience any acute side effects (e.g., vomiting). Dr. Javitt, a consultant on this project, has a great deal of

experience in using glycine to augment the therapeutic effects of antipsychotic medication and will be available to advise the study team about any needed changes (i.e., slower titration, dose reduction, discontinuation) based on side effects. It is conceivable that subjects may experience more side effects as the dose is increased, requiring either temporary discontinuation and/or dose reduction and/or slower titration. It may also be necessary to lower the maximum daily dose or to extend each arm of the study for several weeks in order to accommodate a longer titration period up to the therapeutically optimal dose. Decisions about how best to proceed will be made in consultation among Drs. Levy, Bodkin, (Ongur if Dr. Bodkin is not available), Javitt, and the McLean pharmacist. Based on Dr. Javitt's extensive experience using doses of glycine comparable to those proposed here and the fact that the dose is being titrated upward more slowly in this study, it is very unlikely that it will be necessary to discontinue the trial altogether. Should there be changes in any arm of the study, such as temporary discontinuation, a need for slower titration, extending the time period, the IRB will be notified. If subjects develop side effects that make them too uncomfortable or that make it medically necessary to discontinue the study, the study will be stopped. This level of oversight for subjects who are not local seems reasonable for monitoring subjects taking a dietary supplement that has been widely and safely used as an augmentation strategy. As stated above, we have purposefully included the flexibility to slow the titration schedule if needed and to lengthen each treatment arm to accommodate individual differences in metabolism.

The PI has talked all of the people involved in the study (Drs. Kaufman, Ongur, Bodkin, Javitt, Visschers, and Vukovic; Mr. Rosen) about the adequacy of the plan to provide medical and/or psychiatric monitoring of these patients while they are taking a novel compound. With the exception of Mr. Rosen, who has been on medical leave, they have agreed that the proposed plan is acceptable.

The PI will monitor the validity and integrity of the data and ensure that all appropriate forms (e.g., consent forms) have been thoroughly completed and that all blood samples are collected and shipped in accordance with the approved protocol. Monitoring will be done on an ongoing basis in close collaboration with the co-investigators.

X. References

Bainbridge MN, Wiszniewski W, Murdock DR, Friedman J, Gonzaga-Jauregui C, Newsham I, Reid JG, Fink JK, Morgan MB, Gingras M-C, Muzny DM, Hoang LD, Yousaf S, Lupski JR, Gibbs RA. Whole-genome sequencing for optimized patient management. *Sci Translational Med* 2011;3:87re3.

Bennett J, Ashtari M, Wellman J, Marshall KA, Cyckowski LL, Chung DC, McCague S, Pierce EA, Chen Y, Bennicelli JL, Zhu X, Ying G-S, Sun J, Wright JF, Auricchio A, Simonelli F, Shindler KS, Mingozzi F, High KA, Maguire AM. AAV2 gene therapy readministration in three adults with congenital blindness. *Sci Translational Med* 2012;4:120ra15.

Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, Burton EA, Wong B, Tsang G, West BL, Powell B, Shellooe R, Marimuthu A, Nguyen H, Zhang KYJ, Artis DR, Schlessinger J, Su F, Higgins B, Iyer R, D'Andrea D, Koehler A, Stumm M, Lin PS, Lee RJ, Grippo J, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, Chapman PB, Flaherty KT, Xu X, Nathanson KL, Nolop K. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* 2010;467:596-599.

Buchanan RW, Javitt DC, Marder SR, Schooler NR, Gold JM, McMahon RP, Heresco-Levy U, Carpenter WT. The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): The

efficacy of glutamatergic agents for negative symptoms and cognitive impairments. *Am J Psychiatry* 2007;164:1593-1602.

Chen P, Lin J-J, Lu C-S, Ong C-T, Hsieh PF, Chih-Chao Yang C-C, Tai C-T, Wu S-L, Lu C-H, Hsu Y-C, Yu H-Y, Ro L-S, Lu C-T, Chu C-C, Tsai J-J, Su Y-H, Lan S-H, Sung S-F, Lin S-Y, Chuang H-P, Huang L-C, Chen Y-J, Tsai P-J, Liao H-T, Lin Y-H, Chen C-H, Chung W-H, Hung S-L, Wu J-Y, Chang C-F, Chen L, Chen Y-T, Shen C-Y. Carbamazepine-induced toxic effects and HLA-B*1502 screening in Taiwan. *N Engl J Med* 2011;364:1126-1133.

Chung W-H, Hung S, Hong H-S, Hsih M-S, Yang Y-C, Ho H-C, Wu J-Y, Chen Y-T. A marker for Stevens-Johnson syndrome. *Nature* 2004;428:486.

Cirak S, Arechavala-Gomeza V, Guglieri M, Feng L, Torelli S, Anthony K, Abbs S, Garralda ME, Bourke J, Wells DJ, Dickson G, Wood MJA, Wilton SD, Straub V, Kole R, Shrewsbury SB, Sewry C, Morgan JE, Bushby K, Muntoni F. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: An open-label, phase 2, dose-escalation study. *Lancet* 2011;378:595-605.

Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI, Ritchie MD, Stein CM, Roden DM, Smith JD, Veenstra DL, Rettie AE, Rieder MJ. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. *Blood* 2008;112:1022-1027.

Coyle JT. Glutamate and schizophrenia: Beyond the dopamine hypothesis. *Cell Mol Neurobiol* 2006;26:365-384.

Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, Daly MJ, Goldstein DB, John S, Nelson MR, Graham J, Park BK, Dillon JF, Bernal W, Cordell HJ, Pirmohamed M, Aithal GP, Day CP, DILIGEN Study,

Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* 1960;23:56–62.

International SAE Consortium. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet* 2009;41:816-819.

Duncan EJ, Szilagyi S, Schwartz MP, Bugarski-Kirola D, Kunzova A, Negi S, Stephanides M, Efferen TR, Angrist B, Peselow E, Corwin J, Gonzenbach S, Rotrosen JP. Effects of D-cycloserine on negative symptoms in schizophrenia. *Schiz Res* 2004;71:239-248.

Erhardt S, Schwieler L, Nilsson L, Linderholm K, Engberg G. The kynurenic acid hypothesis of schizophrenia. *Physiol Behav* 2007;92: 203-209.

Evins AE, Fitzgerald SM, Wine L, Rosselli R, Goff DC. Placebo-controlled trial of glycine added to clozapine in schizophrenia. *Am J Psychiatry* 2000;157:826-828.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Mark Sulkowski4, McHutchison2 JG, Goldstein1 DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;461:399-401.

Goff DC, Tsai G, Manoach DS, Coyle JT. A dose-finding trial of D-cycloserine added to neuroleptics for native symptoms in schizophrenia. *Am J Psychiatry* 1995;152:1213-1215.

Goff DC, Tsai G, Manoach DS, Flood J, Darby DG, Coyle JT. D-cycloserine added to clozapine for patients with schizophrenia. *Am J Psychiatry* 1996;153:1628-1630.

Goff DC, Henderson DC, Evins AE, Amico E. A placebo-controlled crossover trial of D-cycloserine added to clozapine in patients with schizophrenia. *Biol Psychiatry* 1999;45:512-524.

Guy W. Abnormal involuntary movement scale (AIMS). In: Guy W, ed. *ECDEU assessment Manual for Psychopharmacology: Revised*. Rockville, MD: US Department of Health, Education and Welfare; 1976:118-119.

Guy W. Clinical global impression (CGI) Scale. In: Guy W, ed. *ECDEU Assessment Manual for Psychopharmacology*. Rockville, MD: US Department of Health, Education, and Welfare; 1976:125-126.

Heinzen EL, Radtke RA, Urban TJ, Cavalleri GL, Depondt C, Need AC, Walley NM, Nicoletti P, Ge D, Catarino CB, Duncan JS, Kasperaviciutey D, Tate SK, Caboclo LO, Sander JW, Clayton L, Linney KN, Shianna KV, Gumbs CE, Smith J, Cronin KD, Maia JM, Doherty CP, Pandolfo M, Duncan JS, Sander JW, Delanty N, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Kalviainen R, Eriksson K, Kantanen A-M, Dorn T, Hansen J, Kramer G, Steinhoff BJ, Wieser H-G, Steinhoff BJ, Kramer G, Hansen J, Dorn T, Zumsteg D, Ortega M, Wood NW, Huxley-Jones J, Mikati M, Gallentine WB, Husain AM, Buckley PG, Stallings RL, Podgoreanu MV, Delanty N, Sisodiya SM, Goldstein DB. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet* 2010;86:707-718.

Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Horowitz A, Kelly D. Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. *Br J Psychiatry* 1996;169:610-617.

Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. *Arch Gen Psychiatry* 1999;56:29-36.

Heresco-Levy U, Ermilov M, Lichtenberg P, Bar G, Javitt DC. High-dose glycine added to olanzapine and risperidone for the treatment of schizophrenia. *Biol Psychiatry* 2004;55:165-171.

Heresco-Levy U, Javitt DC. Comparative effects of glycine and D-cycloserine on persistent negative symptoms in schizophrenia: A retrospective analysis. *Schizophr Res* 2004b;66:89-96.

Javitt DC. Glutamate and schizophrenia: Phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. *Int Rev Neurobiol* 2007;78:69-108.

Javitt DC, Silipo G, Cienfuegos A, Shelley AM, Bark N, Park M, Lindenmayer JP, Suckow R, Zukin SR. Adjunctive high-dose glycine in the treatment of schizophrenia. *Int J Neuropsychopharmacol* 2001;4:385-391.

Jensen JE, Licata SC, Ongur D, Friedman SD, Prescot AP, Henry ME, Renshaw PF. Quantification of J-resolved proton spectra in two-dimensions with LCModel using GAMMA-simulated basis sets at 4 Tesla. *NMR Biomed* 2009;22:762-769.

Kaufman MJ, Prescot AP, Ongur D, Evins AE, Barros TL, Medeiros CL, Covell J, Wang L, Fava M, Renshaw PF. Oral glycine administration increases brain glycine/creatine ratios in men: a proton magnetic resonance spectroscopy study. *Psychiatry Res* 2009;173:143-149.

Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. 1987;13:261-276.

Kern RS, Gold JM, Dickinson D, Green MF, Nuechterlein KH, Baade LE, Keefe RS, Mesholam-Gately RI, Seidman LJ, Lee C, Sugar CA, Marder SR. The MCCB impairment profile for schizophrenia outpatients: Results from the MATRICS psychometric and standardization study. *Schiz Res* 2011;126:124-131.

Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* 2009;360:753-764.

Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. *Arch Gen Psychiatry* 2005;62:1196-1204.

Lane HY, Liu YC, Huang CL, Chang YC, Liau CH, Perng CH, Tsai GE. Sarcosine (N-methylglycine) treatment for acute schizophrenia: a randomized, double-blind study. *Biol Psychiatry* 2008;63:9-12.

Lin CH, Lane HY, Tsai GE. Glutamate signaling in the pathophysiology and therapy of schizophrenia. *Pharmacol Biochem Behav.* 2011;100:665-677.

Loi S, Haibe-Kains B, Majjaj S, Lallemand F, Durbecq V, Larsimont D, Gonzalez-Angulo AM, Pusztai L, Symmans WF, Bardelli A, Ellis P, Tutt AN, Gillett CE, Hennessy BT, Mills GB, Phillips WA, Piccart MJ, Speed TP, McArthur GA, Sotiriou C. *PIK3CA* mutations associated with gene signature of low mTORC1 signaling and better outcomes in estrogen receptor-positive breast cancer. *Proc Nat'l Acad Sci* 2010;107:10208–10213.

Malhotra D, McCarthy S, Michaelson JJ, Vacic V, Burdick KE, Yoon S, Cichon S, Corvin A, Gary S, Gershon ES, Gill M, Karayiorgou M, Kelsoe JR, Krastoshevsky O, Krause V, Leibenluft E, Levy DL, Makarov V, Bhandari A, Malhotra AK, McMahon FJ, Nothen MM, Potash JB, Rietschel M, Schulze TG, Sebat J. High frequencies of *de novo* CNVs in bipolar disorder and schizophrenia. *Neuron* 2011;72:951-963.

Malhotra D, McCarthy S, Michaelson JJ, Vacic V, Burdick KE, Yoon S, Cichon S, Corvin A, Gary S, Gershon ES, Gill M, Karayiorgou M, Kelsoe JR, Krastoshevsky O, Krause V, Leibenluft E, Levy DL, Makarov V, Bhandari A, Malhotra AK, McMahon FJ, Nothen MM, Potash JB, Rietschel M, Schulze TG, Sebat J. High frequencies of *de novo* CNVs in bipolar disorder and schizophrenia. *Neuron* 2011;72:951-963.

Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, Jägel-Guedes E, Rugina S, Kozyrev O, Cid JF, Hay P, Nolan D, Hughes S, Hughes A, Ryan S, Fitch N, Thorborn D, Team BAftP-S. HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008;358:568-579.

McCarthy S, Makarov V, Kirov G, Addington A, McClellan J, Yoon S, Perkins D, Dickel DE, Kusenda M, Krastoshevsky O, Krause V, Kumar RA, Grozeva D, Malhotra D, Walsh T, Zackai

EH, Kaplan P, Ganesh J, Krantz ID, Spinner NB, Roccanova P, Bhandari A, Pavon K, Lakshmi B, Leotta A, Kendall J, Lee Y, Vacic V, Gary S, Iakoucheva L, Crow TJ, Christian SL, Lieberman J, Stroup S, Lehtimaki T, Puura K, Haldeman-Englert C, Pearl J, Goodell M, Willour VL, DeRosse P, Steele J, Kassem L, Wolff J, Chitkara N, McMahon F, Malhotra AK, Potash JB, Schulze T, Nothen MM, Cichon S, Rietschel M, Leibenluft E, Sutcliffe JS, Skuse D, Gill M, Gallagher L, Mendell NR, Wellcome Trust Case Control Consortium, Craddock N, Owen MJ, O'Donovan MC, Shaikh TH, Susser E, DeLisi LE, Sullivan PF, Deutsch CK, Rapoport J, Levy DL, King M-C, Sebat J. Microduplications of 16p11.2 are associated with schizophrenia. *Nat Genet* 2009;41:1223-1227.

McCormack M, Alfirevic A, Bourgeois S, Farrell JJ, Kasperavièiute D, Carrington M, Sills GJ, Marson T, Jia X, de Bakker PIW, Chinthapalli K, Molokhia M, Johnson MR, O'Connor GD, Chaila E, Alhusaini S, Shianna KV, Radtke RA, Heinzen EL, Walley N, Pandolfo M, Pichler W, Park BK, Depondt C, Sisodiya SM, Goldstein DB, Deloukas P, Delanty N, Cavalleri GL, Pirmohamed M. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. *N Engl J Med* 2011;364:1134-1143.
Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 1995;52:998-1007.

Nathwani AC, Tuddenham EGD, Rangarajan S, Rosales C, McIntosh J, Linch DC, Chowdary P, Riddell A, Pie AJ, Harrington C, O'Beirne J, Smith K, Pasi J, Glader B, Rustagi P, Ng CYC, Kay MA, Zhou J, Spence Y, Morton CL, Allay J, Coleman J, Sleep S, Cunningham JM, Srivastava D, Basner-Tschakarjan E, Mingozzi F, High KA, Gray JT, Reiss UM, Nienhuis AW, Davidoff AM. Adenovirus-associated virus vector– mediated gene transfer in Hemophilia B. *N Engl J Med* 2011;365:2357-2365.

O'Brien TR. Interferon-α, interferon-λ and hepatitis C. *Nat Genet* 2009;41:1048-1050.

Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 1995;52:998-1007.

Öngür D, Jensen JE, Prescot AP, Stork C, Lundy M, Cohen BM, Renshaw PF. Abnormal glutamatergic neurotransmission and neuronal-glial interactions in acute mania. *Biol Psychiatry* 2008;64:718-726.

Overall J, Gorham D. The Brief Psychiatric Rating Scale. *Psychol Rep* 1962;10:799-812.

Prescot AP, Frederick B, Wang L, Brown J, Jensen JE, Kaufman MJ, Renshaw PF. *In vivo* detection of brain glycine with echo-time- averaged ^{1}H magnetic resonance spectroscopy at 4.0 T. *Magn Reson Med* 2006;55:681-686.

Sebat J, Levy DL, McCarthy SE. Rare structural variants in schizophrenia. *Trends in Genetics* 2009;25:528-535.

Simpson GM, Angus JW. A rating scale for extrapyramidal side effects. *Acta Psychiatr Scand Suppl* 1970;212:11-19.

Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lønning P, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Nat'l Acad Sci* 2001;100:8418-8423.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J, Study HC. IL28B is associated with response to chronic hepatitis C interferon-□ and ribavirin therapy. *Nat Genet* 2009;41:1100-1104.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-á and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;41:1105-1111.

Takeuchi F, McGinnis R, Bourgeois S, Barnes C, Eriksson N, Soranzo N, Whittaker P, Ranganath V, Kumanduri V, McLaren W, Holm L, Lindh J, Rane A, Wadelius M, Deloukas P. A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. *PLoS Genet* 2009;5:e1000433.

Toth E, Lajtha A. Antagonism of phencyclidine-induced hyperactivity by glycine in mice. *Neurochem Res* 1986;11:393-400.

Tsai GE, Lin PY. Strategies to enhance N-methyl-D-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Curr Pharm Des* 2010;16:522-537.

Tsai G, Yang P, Chung LC, Lange N, Coyle JT. D-serine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 1998;44:1081-1089.

Tsai G, Yang P, Chung LC, Tsai CW, Coyle JT. D-serine added to clozapine for the treatment of schizophrenia. *Am J Psychiatry* 1999;156:1822-1825.

Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 2004a;55:452-456.

Tsai GE, Yang P, Chang YC, Chong MY. D-Alanine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 2006;39:230-234.

van Berckel BN, Evenblij CN, van Loon BJ, Maas MF, van der Geld MA, Wynne HJ, van Ree JM, Kahn RS. D-cycloserine increases positive symptoms in chronic schizophrenic patients when administered in addition to antipsychotics: a double-blind, parallel, placebo controlled study. *Neuropsychopharmacol* 1999;21:203-210.

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MAR, Green T, Platt OS, Ruderfer DM, Walsh CA, Altschuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu B-L, Daly MJ. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* 2008;358:667-675.

Wonodi I, Schwarcz R. Cortical kynurenine pathway metabolism: A novel target for cognitive enhancement in schizophrenia. *Schiz Bull* 2010;36:211-218.

Worthey EA, Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B, Serpe JM, Dasu T, Tschannen MR, Veith RL, Basehore MJ, Broeckel U, Tomita-Mitchell A, Arca MJ, Casper JT, Margolis DA, Bick DP, Hessner MJ, Routes JM, Verbsky JW, Jacob HJ, Dimmock DP. Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genetics in Medicine* 2011;13:255-262.

Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: Reliability, validity and sensitivity. *Br J Psychiatry* 1978;133:429-435.