

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

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**Answer all questions accurately and completely in order to provide the McLean Hospital IRB with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.**

**PRINCIPAL/OVERALL INVESTIGATOR**

Deborah L. Levy, Ph.D.

**PROTOCOL TITLE**

Neurobiology of a Mutation in Glycine Metabolism in Psychotic Disorders

**FUNDING**

NIMH

**VERSION DATE**

11/2009

**SPECIFIC AIMS**

Concisely state the objectives of the study and the hypothesis being tested.

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.

**BACKGROUND AND SIGNIFICANCE**

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

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. Here we focus on the 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 & Farmer, 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.

## RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by McLean researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, “Enrollment at McLean will be limited to adults although the sponsor’s protocol is open to both children and adults.”

The total projected sample size is 4, consisting of two carriers of a 9p24.1 mutation and two non-carriers from the same family. 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.

Design of the study: 1) baseline procedures at McLean: physical exam (Dr. Ongur), EKG, movement disorders (extrapyramidal side effects, tardive dyskinesia - AIMS/SAS – Dr. Bodkin) (Guy, 1976; Simpson & Angus, 1970), structural MRI, Proton  $^1\text{H}$  MRS for GABA, glutamate, glutamine and GABA levels, pre-and post-glycine loading Proton  $^1\text{H}$  MRS scans and 5 cc blood samples at (pre, 20, 40, 60, 90, 120, 150, 180, 210, and 240 minutes post-Gly loading), clinical ratings (PANSS, Clinical Global Impression scale, Brief Psychiatric Rating Scale, Young Mania Rating Scale, HAM-D), neurocognitive testing (MATRICS Consensus Cognitive Battery-MCCB), and urinalysis and plasma levels [SMA-20, large neutral & excitatory amino acid, kynurenic acid (KYNA), psychotropic drug plasma levels. Total blood independent of the samples obtained for the glycine loading scan: 90 cc for non-carriers; 113 and 114 cc for the carriers

Dr. Ongur will be responsible for medical oversight of the imaging components of the study. Dr. Bodkin will be responsible for medical oversight of the glycine augmentation component of the study.

Subjects will also be asked to inform their personal physicians about their participation and their exposure to glycine to ensure that all appropriate oversight for safety has been carried out. 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 or glycine.

### 2) baseline procedures at Nathan Kline Institute (NKI): functional MRI, ERPs/EEGs

All four subjects will receive the procedures described in 1) and 2) above, including the glycine loading imaging study. At the completion of steps 1 and 2), the participation of the two non-carriers will be complete. Only the mutation carriers will participate in the procedures described below.

3) Double-blind cross-over placebo-controlled oral Glycine (Gly) (titrated to a maximum of 0.8 g/kg/d on a TID dosing schedule) (based on the subjects’ weights at date of initial screening) or Placebo (a mixture of Isomaltulose (Palatinose®), true lemon crystals, sucrose) in the two mutation carriers: An FDA application for an IND will be obtained before initiation of this part of the study and the open-label glycine arm (see below) and approval documents will be submitted to the IRB. The placebo will look and taste like glycine. Only FDA approved products will be used. The same flavorings used for the glycine powder will be used for the placebo. The McLean Pharmacy will prepare and ship the glycine and placebo mixtures. Subjects will add the flavoring and a specified amount of cold water to the glycine or placebo immediately before drinking the dose to prevent changing the stability of the glycine. 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.

a) 6 weeks on Gly or placebo with clinical ratings at end of weeks 2, 4, and 6 using skype-like video conferencing; SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels, movement disorders (113 and 114 cc) (AIMS/SAS - extrapyramidal side effects, tardive dyskinesia – using skype-like video conferencing) and MCCB, at the end of week 6 (MCCB will be administered by Dr. Levy in the subject's city of residence and the blood samples for these assays will be obtained in the clinical lab of the local medical center, which will prepare and ship the samples to the Analytical Psychopharmacology Laboratory at NKI or perform other assays in house). 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 throughout the study. 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 necessary), the subjects will also be called by a study physician (Dr. Bodkin) to assess how they are reacting to the glycine or placebo. The subjects will 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 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 if possible.

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. 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 include both the container and individual packets of lemon crystals. Each dose will be labeled by the pharmacy with the date, dose (breakfast, lunch, dinner) and instructions (e.g., Fill with cold water to the fill line, shake, pour into a glass, add lemon crystals. Drink over a 15-20 minute period after breakfast, lunch or dinner as relevant for that dose). Each dose will be in a self-contained package with instructions. Doses will be shipped in two- week supplies.

It is anticipated that all of the glycine will be used. If additional glycine is needed and there is any unused glycine, 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."

b) 2 weeks of no treatment, followed by clinical ratings at the end of week 8 using secure skype-like video conferencing; SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels (113 and 114 cc)

c) 6 weeks on Gly or placebo with clinical ratings at end of weeks 10, 12, 14 using secure skype-like video conferencing; SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels (113 and 114 cc), movement disorders (AIMS/SAS - extrapyramidal side effects, tardive dyskinesia – using skype-like video conferencing) and MCCB, at the end of week 6 (MCCB will be administered by Dr. Levy in the subject's city of residence and the blood samples for these assays will be obtained in the clinical lab of the local medical center, which will prepare and ship the samples to the Analytical Psychopharmacology Laboratory at NKI or perform other assays in house). 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. In addition, 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 also be called by a study physician to assess how they are reacting to the glycine or placebo. This schedule of side effect monitoring can be more frequent, as needed. The subjects will 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 treatment, followed by clinical ratings at the end of week 16 using secure skype-like video conferencing and SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels (113 and 114 cc).

4) Open-label glycine: 6 weeks on a maximum dose of 0.8 g/kg/d (based on the subjects' weights at date of initial screening) (titrated according to the attached titration schedule, TID dosing schedule) in a Gly powder-water mixture (dissolved as a 20% solution) prepared by the McLean pharmacy and sent by Fed Ex in 2-week supplies, which will be mixed with lemon crystals. Clinical ratings will be performed at the end weeks 2 and 4 using secure skype-like video conferencing. At the end of week 6, clinical ratings and 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 (113 and 114 cc) will be obtained at this time. An FDA IND will be obtained. 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. In addition, at the end of the first week of glycine or placebo, and at the end of weeks 3 and 5, the subjects will also 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). 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.

During the 5<sup>th</sup> week of open-glycine treatment, the procedures described in sections 1) and 2) above will be repeated, with the exception of the physical examination, EKG, and glycine-loading scan. SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels (113 and 114 cc) will also be repeated 2 weeks after the end of the 6-week treatment period (week 8).

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 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 with the results of the analyses of the blood samples 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.

Partners Collaborative Media (PCM) will provide oversight (for a fee) to ensure that the clinical assessments taking place every two weeks using skype-like video conferencing are secure (see additional details below; statement from PCM has been uploaded).

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at McLean will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

Study procedures at McLean: 3T structural MRI scan; 4T Proton <sup>1</sup>H MRS; serial 4T Proton <sup>1</sup>H MRS scans pre-glycine loading and at +20, 40, 60, 90, 120, 150, 180, 210, and 240 minutes post-Gly loading, plasma samples for glycine at the same time points, MCCB, clinical ratings, urinalysis, physical exam, EKG.

Study procedures at NKI: functional MRI, ERPs/EEGs

For studies involving treatment or diagnosis, provide information about standard of care at McLean (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

Glycine augmentation has been used to try to enhance the antipsychotic and neurocognitive efficacy of standard antipsychotic drug treatment. It is not considered part of standard care. Glycine augmentation has shown variable efficacy in patients unselected for having a mutation that would be expected to lower brain Gly levels. In contrast, the *GLDC* triplication in the two carriers would be expected to result in unusually low brain Gly levels. Both carriers of the *GLDC* mutation are currently being treated with clozapine, which is known to increase glycine. However, in order to neutralize the excess dosage effect of the *GLDC* triplication, these patients should need more glycine augmentation than clozapine alone is likely to provide. In the absence of *GLDC* inhibitors that can directly target this mutation, the real issue is whether any augmentation strategy can keep up with the excess degradation of glycine. The most logical approach to a targeted treatment intervention would be to enhance NMDAR function using glycine site agonists. The primary advantage of oral glycine powder is that the dosing has been well worked out for optimizing potential treatment response, and it can be taken indefinitely. In a recent meta-analysis, it also had the best symptom improvement profile among NMDA enhancing agents (Tsai & Lin, 2010). D-serine performed pretty comparably to Gly, but it is unlikely to receive IRB approval for more than 12 weeks, because there are no long-term toxicity data. D-cycloserine can be used indefinitely, but as a partial agonist it is less efficacious than glycine or D-serine (Heresco-Levy & Javitt, 2004b; Tsai & Lin, 2010). Sarcosine, a weak GlyT1 inhibitor, is not currently available from manufacturers for efficacy studies.

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

Glycine Augmentation Trials: Oral glycine has been widely used to augment standard psychotropic drug treatment and is well tolerated. By using a maximum daily dose of 0.8 gm/kg/d TID (based on the subjects' weights at date of initial screening) administered after meals, 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.

The glycine-loading dose in the imaging study will be 0.4g/kg of body weight on the day of the scan (not to exceed 30 g). Dr. Kaufman has had considerable experience with this dose, which is substantially lower than peak doses of glycine used in his and others' previous studies (0.8g/kg/day of body weight), was well tolerated and is designed to minimize the likelihood of side effects (e.g., nausea and vomiting).

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

The PI has talked with all of the people involved in the study (Drs. Kaufman, Ongur, Bodkin, Javitt, Visschers, Vukovic; Mr. Rosen) to ascertain that they are all in agreement that the proposed plan for medical and/or psychiatric monitoring for the patients while they are taking a novel compound is adequate.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

See below.

#### **FORESEEABLE RISKS AND DISCOMFORTS**

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/Performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

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.<sup>164</sup> 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 1 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.

The glycine-loading dose in the imaging study will be 0.4g/kg (not to exceed 30 g). Dr. Kaufman has had considerable experience with this dose, which is substantially lower than peak doses of glycine used in his and others' previous studies (0.8g/kg/day of body weight), was well tolerated and is designed to minimize the likelihood of side effects (e.g., nausea and vomiting). The maximum dose of 30g is substantially lower than the dose used in Dr. Kaufman's previous study (Kaufman et al. 2009).

Glycine Augmentation Clinical Trial: Pharmaceutical grade glycine (Ajinomoto) will be used. Glycine powder-water mixtures (dissolved in 20% solution) will be prepared by the McLean Hospital pharmacy. The dose of glycine will be slowly titrated until a maximum dose of 0.8 g/kg/d (based on the subjects' weights at date of initial screening )(administered TID 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 and at the end of each treatment arm. 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.

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. Subjects are debriefed at the end of the day by the study coordinator at which time they can ask questions and express their reactions to the study. Subjects who wish to discontinue the study may withdraw at any time.

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 random 4-digit ID number, which is used to code all material.

## **EXPECTED BENEFITS**

Describe both the expected benefits to individual subjects participating in the research and the importance of the knowledge that may reasonably be expected to result from the study. Provide a brief, realistic summary of potential benefits to subjects, for example, "It is hoped that the treatment will result in a partial reduction in tumor size in at least 25% of the enrolled subjects." Indicate how the results of the study will benefit future patients with the disease/condition being studied and/or society, e.g., through increased knowledge of human physiology or behavior, improved safety, or technological advances.

The general goal of this study is to clarify the neurobiology of a mutation in glycine metabolism 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.

## **EQUITABLE SELECTION OF SUBJECTS**

The risks and benefits of the research must be fairly distributed among the populations that stand to benefit from it. No group of persons, for example, men, women, pregnant women, children, and minorities, should be categorically excluded from the research without a good scientific or ethical reason to do so. Please provide the basis for concluding that the study population is representative of the population that stands to potentially benefit from this research.

The only scientific and ethical justification for including subjects in this study is if their participation can help to clarify the neurobiology of a mutation in glycine metabolism and if they might benefit from glycine augmentation. All other subjects are excluded. Therefore, the only eligible participants are two carriers of a specific genetic mutation who also have a diagnosis of a psychotic disorder or clinically unaffected members of the same family.

When people who do not speak English are excluded from participation in the research, provide the scientific rationale for doing so. Individuals who do not speak English should not be denied participation in research simply because it is inconvenient to translate the consent form in different languages and to have an interpreter present.

N/A

For guidance, refer to the following McLean policy:

Obtaining and Documenting Informed Consent of Subjects who do not Speak English

## RECRUITMENT PROCEDURES

Explain in detail the specific methodology that will be used to recruit subjects.

Specifically address how, when, where and by whom subjects will be identified and approached about participation. Include any specific recruitment methods used to enhance recruitment of women and minorities.

The 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 that may be implicated in psychotic disorders, or being a family member who does not have this mutation. Prior to beginning the study, 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.

Provide details of remuneration, when applicable. Even when subjects may derive medical benefit from participation, it is often the case that extra hospital visits, meals at the hospital, parking fees or other inconveniences will result in additional out-of-pocket expenses related to study participation. Investigators may wish to consider providing reimbursement for such expenses when funding is available

Each of four subjects will be paid \$1,000 for completing the baseline procedures (structural and spectroscopy scans, glycine loading spectroscopy, and the MATRICS Consensus Cognitive Battery (MCCB) at McLean, followed by fMRI and ERP/EEG procedures at NKI. These procedures will involve 1.5 weeks away from work, school and other responsibilities. Two subjects will be paid an additional \$1000 after a 16-week double-blind placebo controlled trial of oral glycine, followed by 6 weeks of open-label glycine, at the end of which structural and spectroscopy scans and the MATRICS battery will be repeated at McLean and fMRI and ERP/EEG procedures will be repeated at NKI, again necessitating 1.5 weeks away from work, school and other responsibilities. All expenses incurred as part of participating (e.g., travel, hotel, local transportation, and meal costs) will be paid for by the grant supporting this study.

For guidance, refer to the following McLean IRB policies

<http://irb.mclean.harvard.edu/investigators/policy/>:

Recruitment of Research Subject

Guidelines for Advertisements for Recruiting Subjects

Remuneration for Research Subjects

## CONSENT PROCEDURES

Explain in detail how, when, where, and by whom consent is obtained, and the timing of consent (i.e., how long subjects will be given to consider participation). For most studies involving more than minimal risk and all studies involving investigational drugs/devices, a licensed physician investigator must obtain informed consent. When

subjects are to be enrolled from among the investigators' own patients, describe how the potential for coercion will be avoided.

Written informed consent will be obtained for each procedure as well as for all procedures in aggregate. Drs. Kaufman, Ongur, and Javitt already have IRB approval to perform the imaging and ERP/EEG procedures included in this study. Because the procedures included in this protocol constitute a self-contained study of this mutation, separate written informed consent will be obtained for all of the procedures involved. Drs. Levy, Kaufman, Ongur, and Javitt will obtain written informed consent after reviewing the purposes of the study, detailing the procedures and explaining the informed consent documents. The subject is given an opportunity to ask questions after which written informed consent is obtained. Competence to give informed consent will be determined by the PI and co-investigators based on the subject's understanding of the purpose of the study and procedures, what they involve, how long they take, and risk and benefits. The subject receives a copy of the Informed Consent for his/her own files.

**NOTE:** When subjects are unable to give consent due to age (minors) or impaired decision-making capacity, complete the forms for Research Involving Children as Subjects of Research and/or Research Involving Individuals with Impaired Decision-making Capacity, available on the McLean IRB website:

For guidance, refer to the following McLean policy:  
Informed Consent of Research Subjects

N/A

## **DATA AND SAFETY MONITORING**

Describe the plan for monitoring the data to ensure the safety of subjects. The plan should include a brief description of (1) the safety and/or efficacy data that will be reviewed; (2) the planned frequency of review; and (3) who will be responsible for this review and for determining whether the research should be altered or stopped. Include a brief description of any stopping rules for the study, when appropriate. Depending upon the risk, size and complexity of the study, the investigator, an expert group, an independent Data and Safety Monitoring Board (DSMB) or others might be assigned primary responsibility for this monitoring activity.

**NOTE:** Regardless of data and safety monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care.

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, Bodkin, Kaufman, and Öngür 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 imaging study (0.4g/kg) 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 0.8 g/kg based on the subjects' weights at date of initial screening administered on a TID dosing schedule after meals) is well tolerated and is a standard dose used in clinical trials with minimal, if any, side effects. Dr. Javitt, a consultant on this project, has a great deal of experience in using glycine to augment the therapeutic effects of antipsychotic medication. At least once every week, the subjects will receive a phone call from Dr. Levy to discuss how they are feeling. At the end of the first week of glycine or placebo, and every two weeks afterward, the subjects will also be called by a study physician (Dr. Bodkin) to assess how they are reacting to the glycine or placebo. Both subjects' psychiatrists will also be monitoring their clinical states and side effects throughout the study. Subjects will be told that if they experience any side effects, they must report them to Dr. Levy or any of the other co-investigators immediately (phone and page numbers through the McLean Hospital operator will be provided) so the dose can be lowered or the treatment period ended. The subjects will also be told to go the nearest emergency room if they experience any acute side effects (e.g., vomiting). 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.

Partners Collaborative Media (PCM) will provide oversight (for a fee) to ensure that the clinical 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, "movi" [mobile video], 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 about how the secure connection will be implemented will be uploaded in e-irb as soon as it is received.

In addition, to protect the study participants and prevent any potential risk to the participants' privacy, Dr. Levy will apply for a Certificate of Confidentiality from the Department of Health and Human Services. To reduce the possibility that information learned from DNA/RNA studies will affect the subject's access to health insurance or employability, no research study data will be included in the subject's medical records. Further, with the Certificate of Confidentiality from DHHS, the PI cannot be forced (e.g., by court subpoena) to disclose information that may identify a study participant in any federal, state, or local criminal, administrative, legislative, or other proceeding.

Describe the plan to be followed by the Principal Investigator/study staff for review of adverse events experienced by subjects under his/her care, and when applicable, for review of sponsor safety reports and DSMB reports. Describe the plan for reporting adverse events to the sponsor and the McLean IRB and, when applicable, for submitting sponsor safety reports and DSMB reports to the McLean IRB. When the investigator is also the sponsor of the IND/IDE, include the plan for reporting of adverse events to the FDA and, when applicable, to investigators at other sites.

NOTE: In addition to the adverse event reporting requirements of the sponsor, the principal investigator must follow the McLean Hospital IRB guidelines for Adverse Event Reporting

See above

## **MONITORING AND QUALITY ASSURANCE**

Describe the plan to be followed by the principal investigator/study staff to monitor and assure the validity and integrity of the data and adherence to the IRB-approved protocol. Specify who will be responsible for monitoring, and the planned frequency of monitoring. For example, specify who will review the accuracy and completeness of case report form entries, source documents, and informed consent.

NOTE: Regardless of monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for ensuring that the study is conducted at his/her investigative site in accordance with the IRB-approved protocol, and applicable regulations and requirements of the IRB.

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.

For guidance, refer to the following McLean policies:  
Data and Safety Monitoring Plans and Quality Assurance

Adverse Event Reporting Guidelines

## **PRIVACY AND CONFIDENTIALITY**

Describe methods used to protect the privacy of subjects and maintain confidentiality of data collected. This typically includes such practices as substituting codes for names and/or medical record numbers; removing face sheets or other identifiers from completed surveys/questionnaires; proper disposal of printed computer data; limited access to study data; use of password-protected computer databases; training for research staff on the importance of confidentiality of data, and storing research records in a secure location.

NOTE: Additional measures, such as obtaining a Certificate of Confidentiality, should be considered and are strongly encouraged when the research involves the collection of sensitive data, such as sexual, criminal or illegal behaviors.

Each subject will be assigned a 4-digit randomly generated ID number. All scans and blood samples will be labeled with this ID number. Signed consent forms and demographics forms with identifying information will be kept in locked file cabinets stored in a secure location with access limited to Drs. Levy, Kaufman, Ongur, Bodkin, or Javitt. Computer databases are password protected.

### **SENDING SPECIMENS/DATA TO RESEARCH COLLABORATORS OUTSIDE MCLEAN**

Specimens or data collected by McLean investigators will be sent to research collaborators outside McLean, indicate to whom specimens/data will be sent, what information will be sent, and whether the specimens/data will contain identifiers that could be used by the outside collaborators to link the specimens/data to individual subjects.

Blood samples will be sent to Dr. Raymond Suckow, Analytical Psychopharmacology Laboratory at the Nathan Kline Institute, for amino acid and psychotropic drug levels. Samples will be labeled with a 4-digit randomly generated ID number for each subject, a date and a time of day. The identity of the subjects will not be known to Dr. Sukow.

Specifically address whether specimens/data will be stored at collaborating sites outside McLean for future use not described in the protocol. Include whether subjects can withdraw their specimens/data, and how they would do so. When appropriate, submit documentation of IRB approval from the recipient institution.

Dr. Javitt has agreed to make the results of the ERP/EEG and fMRI studies performed at NKI available to McLean investigators, but the original data will remain at NKI and will not be used for any purpose other than this protocol. Subjects can withdraw their fMRI and EEG/ERP data from NKI by written request. The fMRI and EEG/ERP protocols and consent forms at NKI are IRB approved. Attached with this application are copies of the approved consent forms and the protocol approval.

Dr. Suckow will make the results of the analytical chemistry results available to McLean investigators. Specimens will be used up as they are assayed, so there will be no specimens stored at NKI.

### **RECEIVING SPECIMENS/DATA FROM RESEARCH COLLABORATORS OUTSIDE MCLEAN**

When specimens or data collected by research collaborators outside McLean will be sent to McLean investigators, indicate from where the specimens/data will be obtained and whether the specimens/data will contain identifiers that could be used by McLean investigators to link the specimens/data to individual subjects. When appropriate, submit documentation of IRB approval and a copy of the IRB-approved consent form from the institution where the specimens/data were collected.

N/A

#### **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.

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.

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.

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

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.

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, 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.

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

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.

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.

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, Rocanova 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, Chittkara 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.

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

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.

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.

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.

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.



