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Targeted Treatment of Individuals with Psychosis Carrying a Copy Number Variant Containing a Genomic Triplication of the Glycine Decarboxylase Gene

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Abstract

Background: The increased mutational burden for rare structural genomic variants in schizophrenia and other neurodevelopmental disorders has thus far not yielded therapies targeting

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Disclosures:

JTC reports consulting relationships with Concert Pharm and BVF Partners. Baylor College of Medicine (BCM) and Miraca Holdings have formed a joint venture with shared ownership and governance of Baylor Genetics (BG), which performs clinical genomics studies including chromosomal microarray analysis and clinical exome sequencing. J.R.L. serves on the Scientific Advisory Board of the BG. J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, Inc. and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The other authors report no biomedical financial interests or potential conflicts of interest.

the biological effects of specific mutations. We identified two carriers (mother and son) of a triplication of the gene encoding glycine decarboxylase, *GLDC,* presumably resulting in reduced availability of the NMDA receptor (NMDAR) co-agonists glycine and D-serine and NMDAR hypofunction. Both carriers had a diagnosis of a psychotic disorder.

Methods: We carried out two double-blind placebo-controlled clinical trials of NMDAR augmentation of psychotropic drug treatment in these two individuals. Glycine was used in the first clinical trial. D-cycloserine was used in the second one.

Results: Glycine or D-cycloserine augmentation of psychotropic drug treatment each improved psychotic and mood symptoms in placebo-controlled trials.

Conclusions: These results provide two independent proof-of-principle demonstrations of symptom relief by targeting a specific genotype and explicitly link an individual mutation to the pathophysiology of psychosis and treatment response.

The trials were registered on the ClinicalTrials.gov (https://www.clinicaltrials.gov) website (and).

Keywords

NMDA receptor hypofunction; schizophrenia; bipolar disorder; copy number variant; genetics; glycine decarboxylase

Introduction

Individually rare structural variants (SVs) of relatively recent evolutionary origin such as copy number variants (CNVs) collectively account for an increased mutational burden for schizophrenia and other neurodevelopmental disorders (e.g., autism spectrum disorders, intellectual disability, epilepsy, and to a lesser extent, bipolar disorder). The most recurrent include microdeletions and microduplications with odds ratios for phenotypic expression ranging from 2 to greater than 60 and have effect sizes much larger than those associated with common genetic risk variants. (1-21) SVs and CNVs can be large, involve many genes, and their underlying structure can be complex. (22) Since pleiotropic clinical effects are the norm, discoveries based on any single mutation are potentially relevant to other individuals who carry the same mutation or mutations that impact the same biological pathways, even if the clinical phenotype differs. The fact that shared molecular mechanisms are implicated in a range of neurodevelopmental disorders (23-26) suggests that a “genotype-first” approach (27-31) may be more instructive about pathophysiology and potential treatments than a disease-oriented approach. Copy number variant loci continue to be linked to cognitive phenotypes. (32, 33) The challenge is to link mutations in specific genes to the underlying disease biology, (34) which can, in turn, be translated into targeted treatment interventions with positive therapeutic effects in appropriately selected patients. (35-39)

We identified several CNVs spanning 9p24.1 in a proband and his mother, who presented with DSM-IV (40) diagnoses of schizoaffective disorder and bipolar disorder with psychotic features, respectively (Figure 1A); this structural rearrangement seems to segregate with psychosis in this family (Figure 1B). The rearrangement was confirmed as a *de novo* event in the mother (Figure S1). (41) The complete architecture of this complex rearrangement and

the proposed DNA replicative/repair mechanism underlying its formation are described in Grochowski et al. 2018. (42) See supplemental material for details about the CNV.

Although several of the genes in the CNV may be potentially relevant to the development of neuropsychiatric disorders (see supplemental material), *GLDC* is particularly compelling, because it codes for the enzyme that catabolizes glycine, a precursor of D-serine, both of which are co-agonists at the NMDA receptor (NMDAR). (43) Triplication of *GLDC* would be expected to increase glycine catabolism, resulting in low levels of brain glycine and D-serine. Reduced availability of these two NMDAR co-agonists would result in NMDAR hypofunction, which is associated with psychotic disorders. (44-47) The *GLDC* triplication seemed fortuitously amenable to a treatment intervention tailored to normalizing the biology hypothesized to be affected by the mutation. Therefore, we undertook a proof-of-principle clinical trial to determine whether augmentation of usual psychotropic drug treatment with glycine, a full agonist at the NMDAR glycine modulatory site (GMS), reduced psychotic and mood symptoms in the two carriers of the *GLDC* triplication. Due to the encouraging results of the glycine augmentation trial, we undertook a clinical trial with D-cycloserine (DCS), a relatively selective partial agonist at the GMS at low doses. (48, 49)

Methods and Materials

Subjects

Two carriers of the 9p24.1 CNV participated in the clinical trials: the proband (subject 3363) and his mother (subject 5459). Demographic information and details of the study designs and methods are included below and in the supplemental material.

Study Design

Both studies were approved by institutional review boards at McLean Hospital and Partners Healthcare. Subjects provided written informed consent.

Glycine Augmentation Clinical Trial Methods

Procedures.—Symptom severity and treatment side effects were monitored at least weekly; formal clinical ratings were carried out every two weeks blind to drug condition using the following primary instruments: Brief Psychiatric Rating Scale (BPRS), Positive and Negative Syndrome Scale (PANSS), Young Mania Rating Scale, Hamilton Depression Scale, Columbia–Suicide Severity Rating Scale and the Clinical Global Impression (CGI) Scale. (50-55) Motor abnormalities were assessed at baseline and at the end of each treatment arm blind to condition. (56, 57) Plasma concentrations of small and large amino acids, kynurenine (KYN), kynurenic acid (KYNA), quinolinic acid, and homocysteine were obtained at baseline and during week 6 of each arm of the acute glycine trial (see supplemental material). All baseline procedures were carried out in person; some clinical assessments and movement disorder exams were carried out using a secure form of video conferencing, because the subjects were not local.

Short-term Glycine Augmentation Trial.—Both subjects were maintained on stable doses of psychotropic medications during the acute trial (see supplemental material).

Design. Double-blind random-order glycine-placebo crossover followed by open-label glycine. Each of these three arms lasted six weeks, separated by two weeks to wash out treatment effects from the previous arm. (58) During each arm, each subject received pharmaceutical grade glycine (Ajinomoto) or placebo as determined by the research pharmacist's randomization. See supplemental material for details of dose preparation. Starting with a dose of 6 gm, the daily dose of glycine or placebo was titrated upward by 3 gm/d (TID dosing) until the target dose was reached or gastrointestinal (GI) side effects occurred. The target glycine dose was 0.8 gm/kg/d, based on reports that this dose yielded optimal therapeutic effects with minimal side effects. (59, 60) Subject 5459 received glycine during arm 1 and placebo during arm 2; subject 3363 received placebo during arm 1 and glycine during arm 2. See supplemental material for details regarding dosing, titration schedules, side effect management and *de facto* sustainable dosing due to GI side effects.

Chronic Glycine Augmentation Clinical Trial.—Following completion of the acute glycine study, both subjects experienced an exacerbation of clinical symptoms (Figure 2, C to D). An eight-month period elapsed between the end of the acute trial and the start of the chronic trial. The chronic glycine trial lasted 47 weeks (see supplemental material and tables). Although both subjects showed an initial reduction in total BPRS score (Figure 2, C to D), the chronic trial was temporarily suspended at 16 weeks due to the intolerability of GI side effects with chronic TID dosing. Both subjects asked to end the trial during week 47, having suffered from chronic GI side effects even at reduced doses. See supplemental material for details. The maximum sustainably tolerable doses were ~18.8-27.5% of the target doses.

DCS Augmentation Trial

The same subjects participated in the DCS study. Age and medication information are contained in supplemental material.

The first arm was an 8-week open-label trial due to reports of positive symptom exacerbation in two schizophrenia patients treated with conventional neuroleptics (61) and of a significant mean worsening of negative symptoms in patients treated with clozapine (62) during exposure to 50 mg of DCS. It therefore seemed prudent to ascertain that DCS was not negatively affecting symptom severity and that DCS plasma levels were not unusually high [see (61)] before embarking on a double-blind trial. Following a one week washout period after the open-label trial, sufficient for the 7-15 hour half-life of DCS, (63) the double-blind placebo-controlled trial started; each arm lasted 6 weeks with a washout week between arms. The dose of DCS was 50 mg (qAM), a dose at which DCS is a partial agonist of the NMDAR (48, 49, 64-66), which has been shown to reduce negative symptoms of schizophrenia in non-clozapine treated patients (61, 67) and to augment cognitive behavioral therapy for delusions. (68) The double-blind phase was followed by 24 weeks of open-label exposure. Clinical ratings (every two weeks) and movement disorder exams (at the end of each arm) were carried out blind to condition. Due to the unexpected death of an immediate family member at the end of the washout between phase 1 and phase 2 of the double-blind (week 7), the washout period was extended for an additional six weeks until the subjects' clinical states had stabilized sufficiently for the trial to resume.

Statistical Analyses.

We fit linear models with fixed effects for treatment and subject for the primary outcome, total BPRS score, from 1) the post-baseline double-blind arms; and 2) the open-label periods, which included the open-label arm of the short-term trial, the interval between the short- and long-term trials and the long-term trial. We also fit a model that included a treatment-by-subject interaction. Due to the small sample size, p-values were estimated using permutation tests and standard errors were estimated using the bootstrap method. (69) A two-tailed p-value of 0.05 was considered statistically significant.

Results

Glycine Augmentation Clinical Trial

Short-term Glycine Trial.—Both subjects showed improvement in clinical symptoms during administration of glycine. Figures 2A-B present total BPRS scores during the acute trials in each subject. During the two arms of the double-blind trial (treatment arms 1-2), the mean (SD) total BPRS score for 5459 was 30.3 (8.6) while on glycine and 35.0 (3.5) while off glycine; for 3363, the mean (SD) total BPRS score was 25.0 (6.2) while on glycine and 35.0 (6.1) while off glycine (Table 1). See Figures S2 and S3 and supplemental material for data from other salient PANSS symptom domains.

The estimated magnitude of effect, or mean (SE) decrease in total BPRS score while receiving glycine, was 7.3 (3.0) points (20%) lower than while receiving placebo, however this difference did not reach statistical significance ($p = 0.083$). The interaction between condition and subject was not statistically significant ($p=0.494$) (Table 1), indicating that the effect of glycine on total BPRS score did not significantly differ between subjects.

During the subsequent 6 weeks of open-label treatment with glycine, both subjects again showed a substantial reduction of symptoms (Figure 2, A to B, treatment arm 3). Following completion of that arm, both subjects experienced an exacerbation of clinical symptoms during the eight-month interval between the end of the short-term trial and the start of the open label chronic glycine trial (Figure 2, C to D).

Long-term Open-Label Glycine Trial.—The mean (SE) decrease in total BPRS score while receiving glycine in all open-label periods was 8.8 (1.5) ($p<0.001$), a reduction of 26%. The interaction between condition and subject was not statistically significant ($p=0.343$) (Table 1).

Plasma Levels.—At baseline, plasma glycine levels were within the normal range (Figure 3, A to B). L- serine plasma levels were at the low end of the normal range (Figure 3, C to D). During treatment with glycine, plasma levels of glycine and L-serine increased by 121-179% and 146-210%, respectively (see supplemental material). KYN levels were elevated above the normal range in both subjects independent of glycine-placebo condition and during the chronic glycine trial; the increase was particularly prominent in subject 5459 (Figure S4). KYNA levels were markedly elevated in subject 5459 at baseline, were consistently normalized during treatment with glycine acutely and during short periods when glycine was tolerated chronically; KYNA levels in subject 3363 were in the upper range of

normal at baseline, and were also reduced by glycine, but not as consistently as in subject 5459 (Figure S5).

DCS Augmentation Trial

Both subjects showed improvement in clinical symptoms during administration of DCS. Figure 4 presents total BPRS scores during all phases of the DCS trial in each subject. During the two arms of the double-blind trial, the mean (SD) total BPRS score for 5459 was 28.3 (1.5) while on DCS and 34.3 (1.2) while off DCS; for 3363, the mean (SD) total BPRS score was 25.3 (0.6) while on DCS and 42.7 (4.0) while off DCS. Subject 3363 showed improvement in both positive psychotic and negative symptoms. Improvement in subject 5459 was restricted to positive and mood symptoms. The magnitude of the clinical improvement was statistically significant during the open-label and double-blind arms (Table 2). During the double-blind phase, the estimated magnitude of effect, or mean (SE) decrease in total BPRS score while receiving DCS, was 11.7 (1.1) points (30.3%) lower than while receiving placebo ($p=0.006$). There was a significant interaction between condition and subject ($p=0.002$). [FN1].

During the acute and chronic open-label periods, the mean (SE) decrease in total BPRS score while receiving DCS in all open-label periods was 3.8 (2.1) ($p<0.009$), a mean reduction of 12.7%. There was no significant interaction between condition and subject ($p=0.834$) (Table 2), indicating that the effect of DCS on total BPRS score did not significantly differ between subjects.

Plasma Levels.—Figures 5A-5B show increases in plasma DCS levels as a function of exposure to DCS.

In contrast to the dramatic increases in L-serine (96-99% of total serine) observed during exposure to glycine (Figure 3, C to D), total serine plasma levels increased only modestly (10.2-54%) with exposure to DCS but remained below or barely within the normal range (Figure 5, C to D). Plasma glycine level also did not change substantially as a function of treatment with DCS (Figure S6). These findings are consistent with DCS being a weak inhibitor of serine racemase. (70)

KYN levels were consistently elevated in both subjects independent of DCS-placebo condition, especially in subject 5459 (Figure S4). KYNA levels were markedly elevated in subject 5459 at baseline and were more clearly normalized during treatment with DCS than with glycine, but this normalization was not sustained during chronic exposure; KYNA levels in subject 3363 were at the upper end of the normal range at baseline, and were not altered by exposure to DCS (Figure S5).

FN1. It is likely that the 40.7% difference in severity of symptoms between the DCS-placebo conditions in 3363 exaggerates the clinical worsening associated with the placebo condition, which was confounded by the persisting impact of the unexpected death of his father. Although the study was suspended for six weeks and 3363 had returned to near his original baseline before the study was resumed, further worsening in this context is not surprising. This interpretation is strengthened by the fact that the reduction in severity of clinical symptoms during the initial open-label trial and the first arm of the double-blind, prior to this unexpected life event, was in the range of ~ 32-37%, and was in the 27-35% range for most of the chronic open-label phase, suggesting a notable reduction in symptom severity while on DCS. Similarly, the magnitude of the DCS-placebo difference in 5459 may have been attenuated by the effect of the personal loss prior to exposure to DCS during the second arm of the double-blind where the reduction in total BPRS score ranged from 12-24%, compared to the various open-label conditions when symptom reduction generally ranged from 21-35%.

Neurocognition.—No consistent changes in neurocognition were observed as a function of glycine or DCS exposure (see supplemental material, Figure S7).

Discussion

We report the results of two proof-of-principle clinical trials showing that interventions tailored to a specific genetic mutation reduced symptom severity in two individuals with psychotic disorders. Although both subjects were partially remitted at baseline, the additional 20-26% reduction in symptom severity on glycine and 13%-30% on DCS reflected substantial relief beyond that achieved by their usual psychotropic drug regimen, an effect that is considered clinically meaningful in augmentation treatment studies of schizophrenia. (71) The pleiotropic clinical effects of the *GLDC* triplication - present in schizoaffective and bipolar disorder - are consistent with the variable expressivity of rare CNVs (see supplemental material). The demonstration of tractable symptom relief by targeting a specific genotype explicitly links an individual mutation to disease biology and pathophysiology and to treatment response. Although we do not know conclusively that the *GLDC* triplication or any of the other genetic elements in the CNV region is causally implicated in the psychiatric illnesses of the carriers, (72, 73) [FN2] our data show that the severity of their symptoms was reduced by augmentation with glycine or DCS. This result underscores the importance of molecular diagnosis and targeting specific biological processes rather than clinical diagnoses *per se.* Indeed, it is not unusual for medications to be efficacious in only a subgroup of individuals treated for a particular clinical condition. (74-77) The *GLDC* gene is among the 15.4% of genes least tolerant of functional variation (see supplemental material), (78) suggesting that gain and loss of function changes in this gene may not be phenotypically neutral. Indeed, triplication of a disease gene may convey a more severe disease phenotype than does duplication of the same locus. (79-81)

The success of this "genotype first" (27) approach underscores the utility of targeting specific biological processes *in appropriately selected* individuals. NMDAR modulators have shown variable efficacy in schizophrenia patients selected on the basis of refractory negative symptoms, (82, 83) but not for having an identified disturbance in NMDAR function. Inasmuch as rare and common structural and sequence variants converge on specific biological pathways, including but not limited to the NMDAR (e.g., immune function, calcium channel signaling, etc.), (15, 25, 26, 84-89) our results are potentially relevant to a broader group than carriers of increased *GLDC* copy number *per se* (e.g., see (90)). Genes involved in glutamate neurotransmission, in particular, are over-represented among the rare variants associated with schizophrenia, autism spectrum disorders (ASD), and non-syndromic intellectual disability. (25, 26) Thus, even though the *GLDC* triplication is, so far, a private mutation, individuals with mutations in genes impacting glutamatergic and NMDAR signaling may constitute a “molecular subtype” amenable to "pathway defined treatment" (27) who would have a high prior probability of responding to treatments that normalize glutamatergic dysregulation. In keeping with the pleiotropy that is characteristic

FN2. *De novo* structural variants have been linked to sporadic psychiatric illness in some studies, (110, 111) but not in others. (41, 112) The extended family of these carriers has a history of psychotic disorders in earlier generations (Figure S9A). If some variant(s) within the 9p24.1 complex rearrangement was causal in the case of our two carriers, different genetic risk factors were likely present in cases in earlier generations.

of CNVs, the pool of potential beneficiaries is likely to transcend diagnostic categories. Notably, multiple lines of evidence implicate genetic variants in *KDM4C/JMJD2C*, *GLDC* and other genes in the 9p24.1 region with schizophrenia, ASD, bipolar disorder and neurodevelopmental disorders (summarized in supplemental material). Our findings are consistent with other data illustrating the value of molecular diagnosis in clarifying the significance of newly emerging clinical symptoms (91) or guiding treatment in the context of atypical psychiatric presentations. (92)

At baseline, plasma levels of glycine were within the normal range and those of L-serine were at the low end of the normal range. Plasma levels poorly reflect brain extracellular levels of glycine and L-serine. In the brain, GLDC is expressed exclusively in astrocytes. (93) Astrocytic serine hydroxyl methyl transferase (SHMT-1) converts glycine to L-serine, which is converted to D-serine, the NMDA receptor co-agonist, by neuronal serine racemase. Thus, reduced availability of glycine and L-serine would decrease neuronal synthesis of D-serine. (94) Both glycine and L-serine plasma levels showed marked increases during glycine treatment; these increases were much greater for L-serine than for glycine. Inasmuch as glutamatergic signaling through NMDA receptors requires glycine or D-serine at the GMS, it is possible that exogenous glycine increased the conversion of glycine to L-serine, thereby increasing precursor availability for D-serine synthesis. (94) D-serine is a more potent agonist at the GMS than glycine and is the preferential agonist at forebrain NMDARs. Thus, the mechanism underlying the therapeutic effect of glycine may, at least in part, have been mediated by increased D-serine rather than by glycine *per se* (Figure S8).

We selected the particular subjects in this study to undergo NMDAR modulation based on the presence of the *GLDC* triplication, which we hypothesized would result in increased catabolism of glycine and D-serine resulting in NMDAR hypofunction. Unbeknownst to us at the start of the glycine clinical trial, both carriers had elevated baseline KYN and KYNA plasma levels (for reasons that are currently unknown). KYNA is a non-selective competitive glutamate receptor antagonist (95) with a particularly strong affinity for the GMS of the NMDAR. (96) KYNA was normalized by augmentation with glycine (although this effect was not sustained in 3363), suggesting that glycine may have partially counteracted the antagonism of KYNA *in vivo,* consistent with *in vitro* data on glycine and D-serine. (97) KYN remained elevated independent of treatment with glycine (or DCS). Conceivably, the magnitude of the glycine effect on symptom severity may have been attenuated by persistent elevations of KYN/KYNA. Thus, elevated KYN and KYNA may have potentiated the deleterious effect of increased glycine and D-serine degradation caused by the *GLDC* triplication (or vice versa). Either the *GLDC* triplication or elevated KYNA would have been sufficient to implicate NMDAR hypofunction. The possibility that two processes impairing NMDAR function were present is consistent with evidence of "multiple genetic hits" in neuropsychiatric disorders. (98, 99) Glycine's efficacy in modulating symptom severity may have been an opportunistic effect of different processes that independently contributed to NMDAR dysregulation such that glycine agonism at the GMS or antagonism of KYNA partially normalized NMDAR function, irrespective of whether this triplication or other genes in the rearranged region were causal.

DCS is a partial agonist at the GMS, with only 40-60% of the potency of glycine, with activity ranging from 65%-200% compared with glycine at saturating doses depending on NMDAR subunit composition, (100) but it crosses the blood brain barrier more readily. (63) In addition, it blocks the formation of KYNA *in vitro.* (101) These dual actions, agonism at the GMS and KYNA inhibition, suggested that DCS might produce an even greater clinical benefit than was achieved with glycine in restoring NMDA receptor function (Figure S8), and guided the rationale for undertaking the second clinical trial. Notably, DCS produced substantially greater normalization of KYNA than glycine (in 5459, although it was not sustained). These dual actions of DCS in normalizing NMDA receptor hypofunction may have contributed to the substantially greater therapeutic benefit of DCS than of glycine.

In addition to its antagonism of the GMS, KYNA is also a potent non-competitive antagonist of the alpha7 nicotinic acetylcholine receptor (α7nAChR) (102-104) (reviewed in (105)). The *CHRNA7* gene has been linked to schizophrenia. (106, 107) Both the NMDAR and the α7nAChR have important roles in cognition and synaptic plasticity. (108, 109) Notably, we observed no systematic changes in neurocognitive function in the subjects during exposure to glycine or DCS, possibly related to sustained elevations of KYN and incompletely normalized levels of KYNA. [FN3]

Other Relevant Considerations. Although side-effect-free up until the threshold for GI side effects is reached, glycine is cumbersome to use, especially over the long-term. In our experience, the "optimal"; target dose of 0.8 g/kg not only was much too high to be tolerated without prohibitive side effects, but also was above the dose needed to achieve therapeutic benefit. Indeed, we observed clinical improvement at substantially lower doses. Whether our subjects had an unusual sensitivity to glycine or DCS (related to the *GLDC* triplication or to elevated KYN/KYNA) is unclear.

Patients taking clozapine (CLZ) are generally excluded from augmentation with NMDAR modulators. [FN4] We hypothesize that the magnitude of CLZ's normalization of NMDAR receptor-dependent neurotransmission will depend on the presence and severity of a

FN3. The elevated baseline KYN and KYNA levels implicate increased activity of indoleamine 2,3-dioxygenase/tryptophan 2,3-dioxygenase and kynurenine aminotransferase (KAT-II). Normalizing KYNA through KAT-II inhibition (113, 114) has been shown to enhance cognition. (115, 116) Such a strategy alone or in combination with partial agonism of the α7nAChR (117) may have had greater benefit on cognition in our subjects.

FN4. The reason is that some of CLZ's neurochemical actions affect synaptic glycine levels, either by inhibiting GlyT1 activity (118) or by inhibiting System A mediated transport of glycine and other amino acids.(124) Consistent with these mechanisms for neutralizing the effects of NMDAR modulators, CLZ-induced increase in extracellular D-serine leads to subsequent NMDAR-dependent release of L-glutamate in rats. (119) This is likely the reason that augmentation with NMDAR modulators tends to show clinical efficacy primarily in schizophrenia patients taking antipsychotics other than CLZ, (60, 61, 120, 121) and often not in patients taking CLZ. (82, 122-125) However, no clinical benefit (67) or an equivocal benefit (61) of DCS has also been observed in patients not taking CLZ, small groups of CLZ- and non-CLZ treated patients experienced similar clinical benefit from glycine, (126) and CLZ-resistant patients experienced significant improvement in negative and overall symptom severity with sodium benzoate augmentation of CLZ. (127) Two studies even reported statistically significant (but clinically modest) worsening of negative symptoms with DCS and CLZ (62, 124) compared with CLZ alone. Notably, clinical benefit and worsening with DCS and glycine have been almost entirely limited to negative symptoms (82, 83, 128) (except for enhancing the effect of cognitive behavioral treatment of delusions), (68) whereas the effects in our subjects were primarily on positive symptoms. The most parsimonious explanation for this pattern of findings in non-genotyped individuals is that schizophrenia patients are heterogeneous with respect to NMDAR hypofunction. In the subgroup of patients with NMDAR hypofunction, augmentation is most likely to provide clinical benefit in those patients whose NMDAR function has not been normalized (i.e., non-CLZ antipsychotic medication); in this same subgroup CLZ may generally be sufficient to normalize NMDAR function, resulting in no further benefit from NMDAR modulators. When CLZ is not sufficient to normalize NMDAR function, clinical benefit may occur. In the subgroup of patients who do not have NMDAR hypofunction, augmentation with NMDAR modulators would not be expected to have a clinical benefit.

glutamatergic deficit. In carriers of mutations that compromise NMDAR-dependent glutamatergic function, CLZ may provide only partial neurochemical remission, leaving room for additional normalization with NMDAR modulators. It thus seems reasonable to propose that patients with mutations in NMDAR and glutamate related genes may benefit from NMDAR modulators even when taking CLZ.

Several limitations should be considered. First, the optimal compound to antagonize an excess of GLDC is a GLDC inhibitor. Since no such compound is available, we used proxies to try to normalize the effects of the *GLDC* triplication. Ideally, FDA approved compounds found to have GLDC inhibitory activity could be repurposed to more directly target increased degradation. Second, the small sample size and limited number of observations during the double-blind arms reduced power to detect a statistically significant effect of glycine in this short-term trial, and precluded use of more standard analytic techniques (e.g., modeling subject as a fixed, rather than random, effect, modeling trajectory of response or baseline to endpoint change rather than comparing observations across all post-baseline time points). Notably, the magnitude of the reduction in symptom severity in the double-blind phase of the glycine trial was consistent with the statistically significant treatment effect observed during the open-label phases and with the significant reduction in symptom severity in the double-blind and open-label arms of the DCS study. Third, carryover effects from prior glycine exposure may have reduced the estimate of the magnitude of the treatment effect (Figure 2). Given the small sample size, it is not possible to formally evaluate these potential effects. [FN5] Similarly, subject 3363 had a partial response to placebo during the first arm of the double blind in the glycine study, attenuating the magnitude of the difference between placebo and glycine. Fourth, although the blind was not broken until after the short-term glycine study ended, both staff and the subjects correctly guessed when they received glycine on the basis of side effects. Although this recognition may have favorably influenced the subjects' clinical states, they were so much less symptomatic on glycine that they were willing to tolerate the side effects and dosing for extended periods, suggesting that their substantial clinical improvements are unlikely to reflect placebo effects alone. Notably, there were no side effects during exposure to DCS, making it unlikely that the clinical improvements observed reflected placebo effects. Finally, it would have been ideal had the subjects not suffered a personal loss during the DCS trial and had it been feasible to undertake multiple crossovers between drug and placebo conditions in both studies.

In summary, we report two individuals with psychotic disorders in whom identification of a specific genomic variation resulted in improved clinical symptoms during two proof-of-principle trials targeting a similar mechanism implicated by the mutation. This study has important implications for the treatment of other patients with alterations of the same or overlapping biochemical pathways.

FN5. Two early studies of glycine augmentation in treatment-resistant chronic schizophrenia, one involving seven patients and the other involving nine patients, reported that the significant improvement in negative symptoms observed during six weeks of treatment with glycine was sustained for the next eight weeks (two-week washout and six weeks of placebo). (59, 126) A third study by the same group did not report a significant carryover effect in seven patients. (60) The persisting effects of glycine on positive symptoms that we observed, however, did not last longer than two weeks (Figure 2), but may have reduced the magnitude of the difference in symptom severity between on vs off glycine assessments. Given the half-life of glycine (26-245 minutes depending on dose), (58) a two-week washout would generally be expected to be long enough to eliminate persisting effects of the drug.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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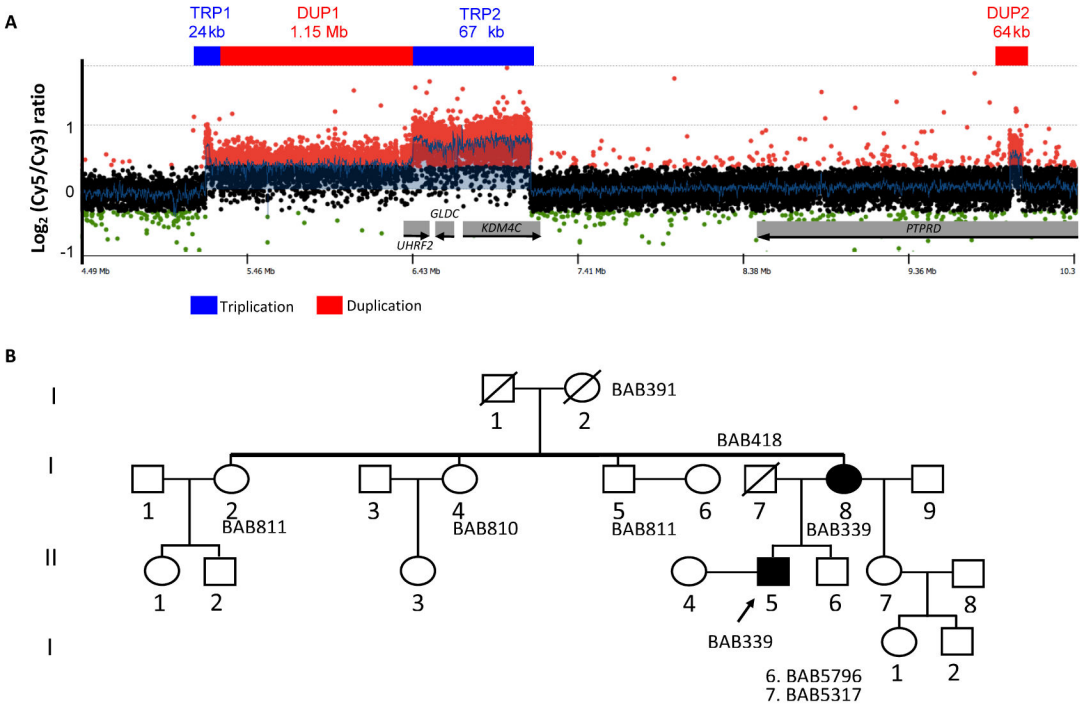

Figure 1.
Structure of the 9p24.1 duplication-triplication (A) and pedigree (B).

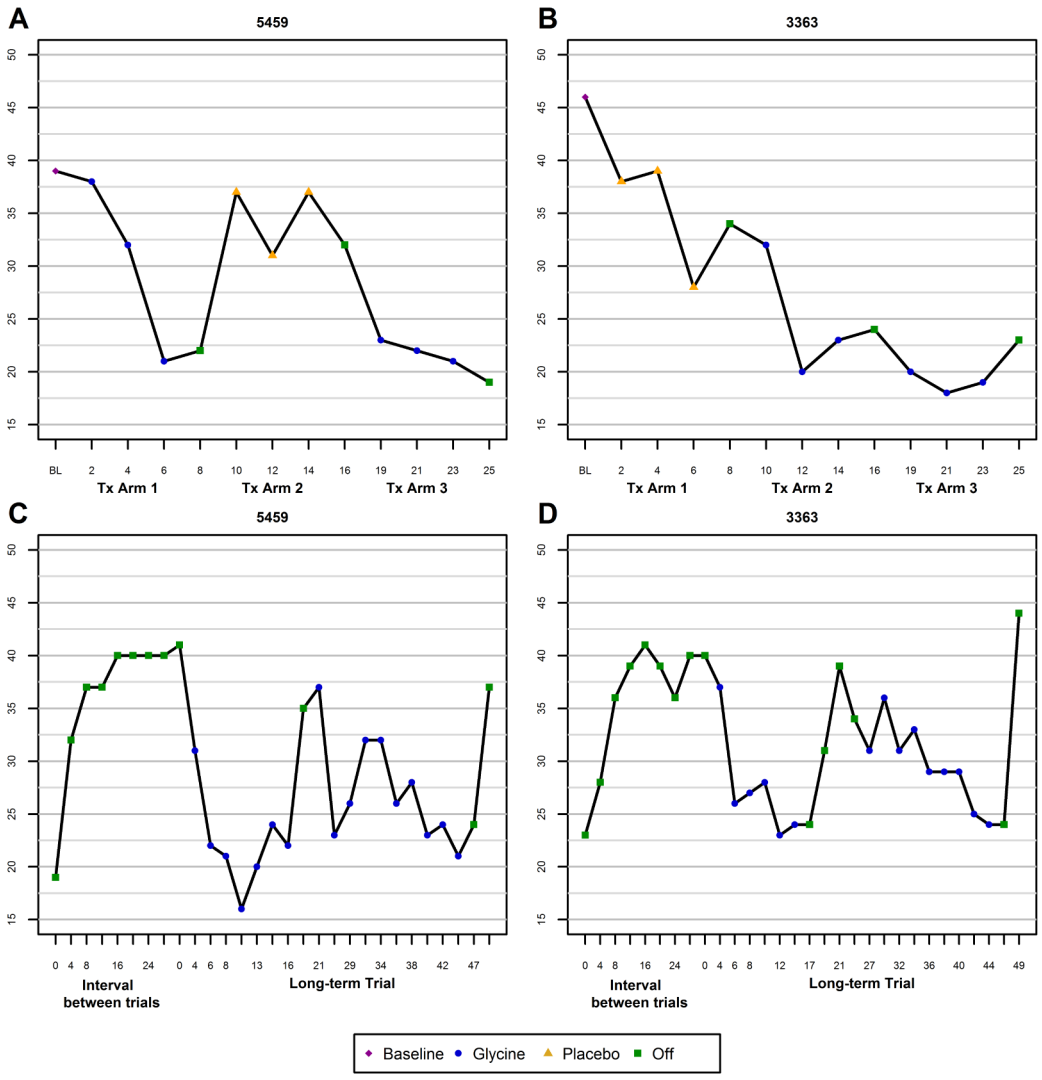

Figure 2.

Changes in total Brief Psychiatric Rating Scale (BPRS) score as a function of treatment with glycine or placebo in subject 5459 (A) and subject 3363 (B) during the short-term trials. Changes in total BPRS score during the interval between the short-term and long-term glycine trials and during long-term glycine treatment in subject 5459 (C) and subject 3363 (D).

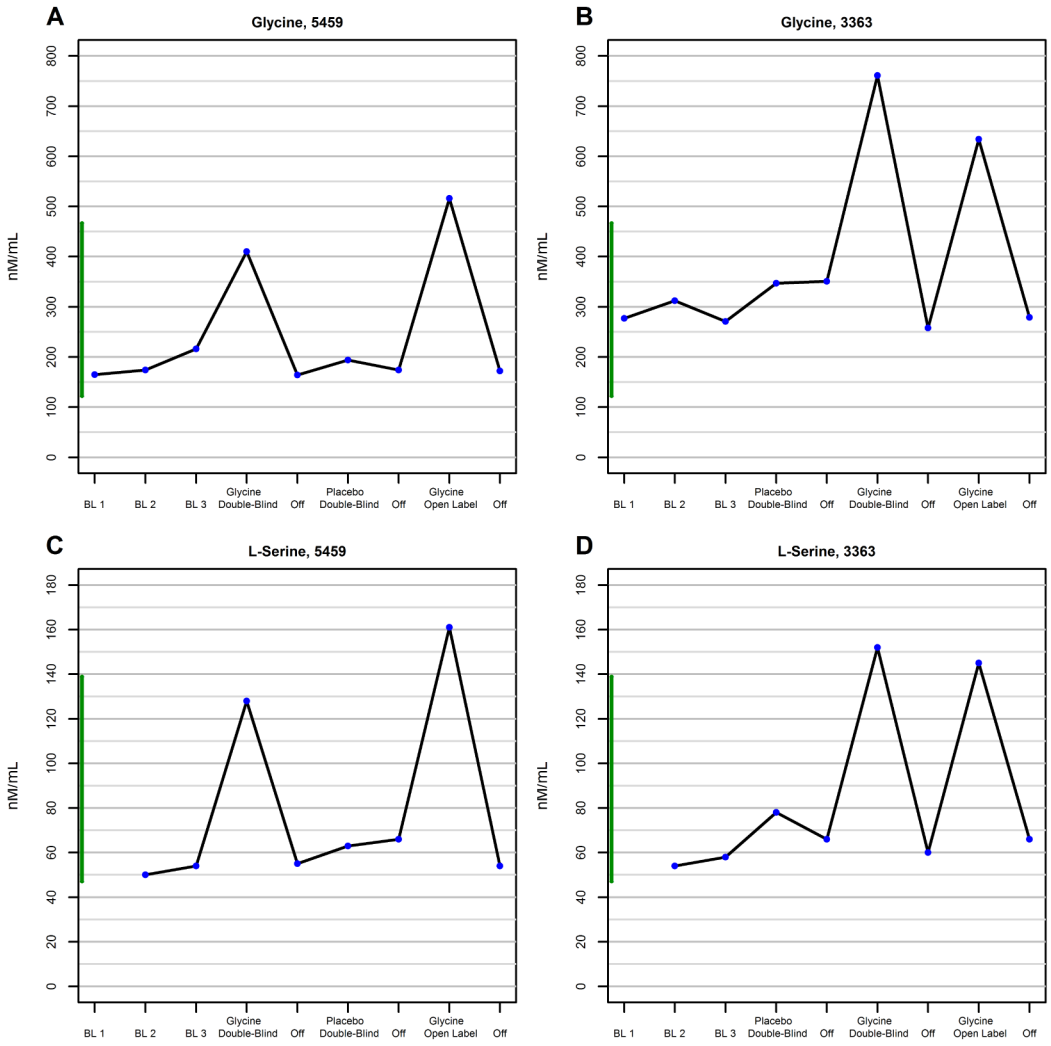

Figure 3.

Changes in plasma glycine level as a function of short-term treatment with glycine or placebo in subject 5459 (A) and subject 3363 (B). Changes in plasma L-serine level as a function of short-term treatment with glycine or placebo in subject 5459 (C) and subject 3363 (D). All data are from the acute trials.

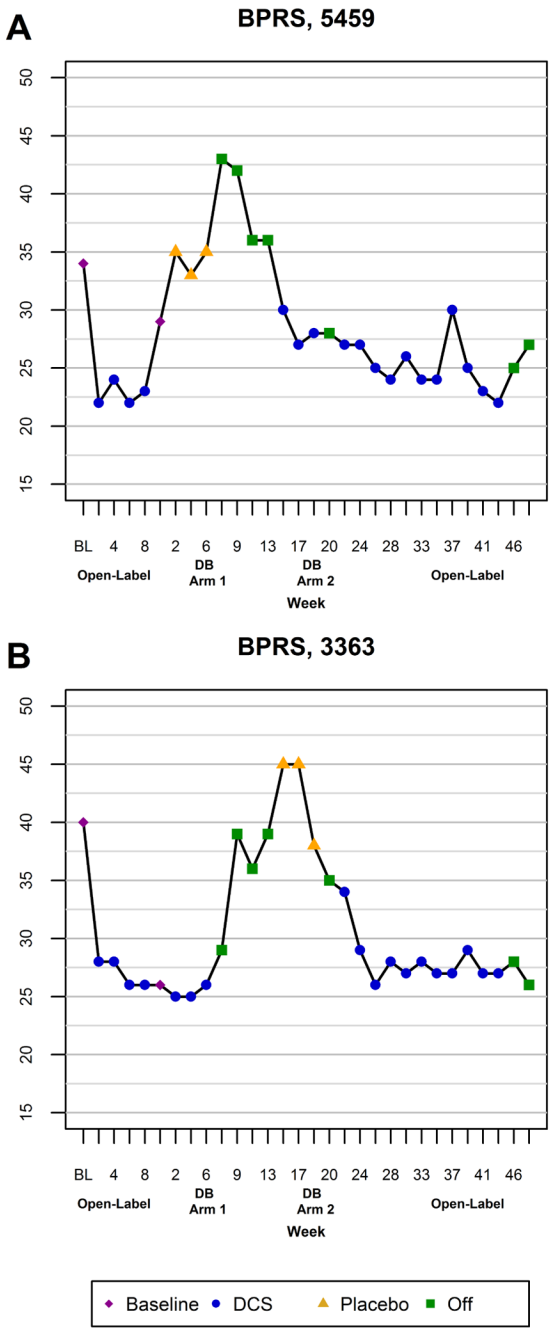

Figure 4.

Changes in total Brief Psychiatric Rating Scale (BPRS) score as a function of treatment with DCS or placebo in subject 5459 (A) and subject 3363 (B).

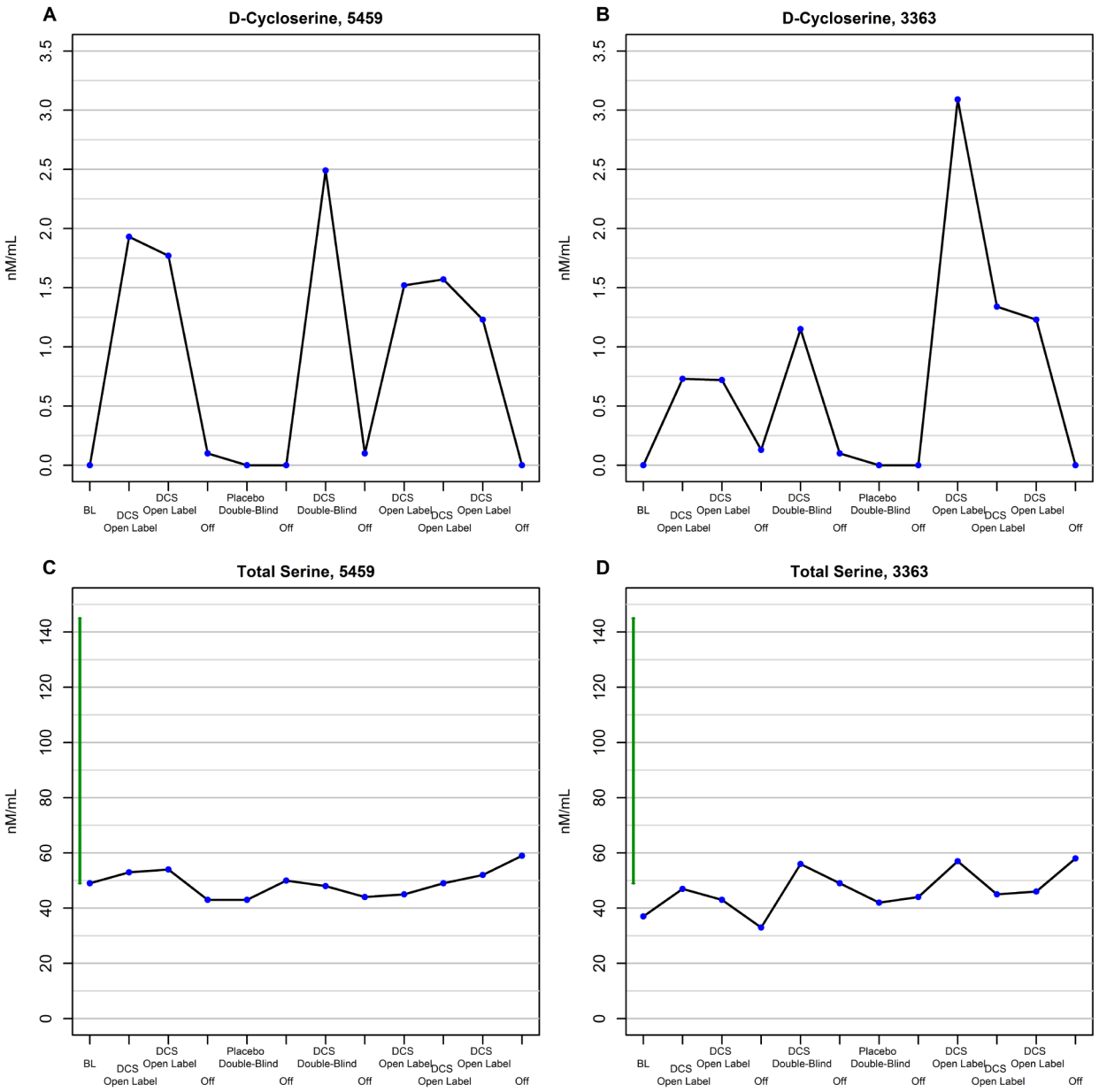

Figure 5.

Changes in plasma DCS level as a function of treatment with DCS or placebo in subject 5459 (A) and subject 3363 (B). Changes in plasma total serine level as a function of short-term treatment with DCS or placebo in subject 5459 (C) and subject 3363 (D).

Table 1.

Changes in mean (SD) BPRS as a function of glycine or placebo.

		Condition		
Trial	**Subject**	**Off Glycine**	**On Glycine**	**%Δ**
Double-Blind[a]	5459	35.0 (3.5)	30.3 (8.6)	13.4
	3363	35.0 (6.1)	25.0 (6.2)	28.6
Open Label[b]	5459	35.2 (7.0)	24.7 (5.0)	29.8
	3363	34.5 (6.9)	27.2 (5.4)	21.2

[a] Average decrease: 7.3 (3.0) (20.0%)

[b] Average decrease: 8.8 (1.5) (25.9%)

Table 2.

Changes in mean (SD) BPRS as a function of DCS or placebo.

		Condition		
Trial	**Subject**	**Off DCS**	**On DCS**	**%Δ**
Double-Blind[a]	5459	34.3 (1.2)	28.3 (1.5)	17.5
	3363	42.7 (4.0)	25.3 (0.6)	40.7
Open Label[b]	5459	28.7 (4.7)	24.5 (2.2)	14.6
	3363	313 (7.6)	27.8 (2.0)	11.2

[a] Average decrease: 11.7 (1.1) (30.3%)

[b] Average decrease: 3.8 (2.1) (12.7%)

Key Resource Table

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use “this paper” if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Other	Plasma Excitatory Amino Acids Assay	Nathan Kline Institute	N/A	
Other	Plasma Large Neutral Amino Acids Assay	Nathan Kline Institute	N/A	
Other	Plasma d-Serine Assay	Nathan Kline Institute	N/A	
Other	Plasma Tryptophan Assay	Nathan Kline Institute	N/A	
Other	Plasma GABA Assay	Nathan Kline Institute	N/A	
Other	Plasma Citalopram + Metabolites Assay	Nathan Kline Institute	N/A	
Other	Plasma Kynurenic Acid Assay	Nathan Kline Institute	N/A	
Other	Plasma Quinolinic Acid Assay	Nathan Kline Institute	N/A	
Other	Plasma Kynurenine Assay	Nathan Kline Institute	N/A	
Other	Plasma Total Homocysteine Assay	Nathan Kline Institute	N/A	
Other	Plasma Clozapine & Norclozapine Assay	Nathan Kline Institute	N/A	
Other	Plasma Aripiprazole & Dehydroaripraprazole Assay	Nathan Kline Institute	N/A	
Other	Plasma Duloxetine Assay	Nathan Kline Institute	N/A	
Other	Plasma Gabapentin Assay	Nathan Kline Institute	N/A	