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Identification of Candidate Single-Nucleotide Polymorphisms in *NRXN1* Related to Antipsychotic Treatment Response in Patients with Schizophrenia

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Neurexins are presynaptic neuronal adhesion molecules that interact with postsynaptic neuroligins to form an inter-synaptic complex required for synaptic specification and efficient neurotransmission. Deletions and point mutations in the neurexin 1 (NRXN1) gene are associated with a broad spectrum of neuropsychiatric and neurodevelopmental disorders, including autism, intellectual disability, epilepsy, developmental delay, and schizophrenia. Recently, small nucleotide polymorphisms in NRXN1 have been associated with antipsychotic drug response in patients with schizophrenia. Based on previous suggestive evidence of an impact on clozapine response in patients with schizophrenia, we conducted an association study of NRXN1 polymorphisms (rs12467557 and rs10490162) with antipsychotic treatment response in 54 patients with schizophrenia in a double blind, placebo-controlled NIMH inpatient crossover trial and examined for association with risk for schizophrenia in independent case-control and family-based clinical cohorts. Pharmacogenetic analysis in the placebo controlled trial revealed significant association of rs12467557and rs10490162 with drug response, whereby individuals homozygous for the A allele, at either SNP, showed significant improvement in positive symptoms, general psychopathology, thought disturbance, and negative symptoms, whereas patients carrying the G allele showed no overall response. Although we did not find evidence of the same NRXN1 SNPs being associated with results of the NIMH sponsored CATIE trial, other SNPs showed weakly positive signals. The family and case-control analyses for schizophrenia risk were negative. Our results provide confirmatory evidence of genetically determined differences in drug response in patients with schizophrenia related to NRXN1 variation. Furthermore, these findings potentially implicate NRXN1 in the therapeutic actions of antipsychotic drugs.
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INTRODUCTION

Schizophrenia is a chronic psychiatric illness, with complex neurodevelopmental and genetic origins (Weinberger, 1987; Prathikanti and Weinberger, 2005; Sullivan *et al*, 2012; Owen, 2012). Positive symptoms (including delusions and hallucinations), negative symptoms (including lack of motivation and social withdrawal), and deficits in neurocognition represent core phenotypic features of the illness. Antipsychotic drugs, both typical and atypical, represent the mainstay of treatment for schizophrenia, but individual variability in drug efficacy, tolerability, and pharmacokinetics are major treatment challenges (Lieberman *et al*, 2005). Pharmacogenetic studies of inter-individual variation in drug response have emerged as potentially powerful tools for improving therapeutic outcomes in psychiatry (Malhotra *et al*, 2012a; Lotrich, 2012), through maximizing efficacy and minimizing antipsychotic side effects (Malhotra *et al*, 2012b). Although dopamine-serotonin receptor antagonism is the primary pharmacological target of antipsychotic drug action (Snyder, 1981; Kapur and Mamo, 2003), and polymorphisms in the dopamine D2 receptor and serotonin 5HT2C receptor have been associated with clinical response and adverse side effects (Giegling *et al*, 2013; Zhang *et al*, 2010; Ikeda *et al*, 2008), recent pharmacogenetic studies highlight targets outside of the dopaminergic/serotonergic system, including NRXN1 (Souza *et al*, 2010; Lett *et al*, 2011), KCNH2, GSK3, AKT1, and GRM8, as potential predictors of treatment response (Apud *et al*, 2012; Souza *et al*, 2008; Need *et al*, 2009).

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Pharmacogenetic identification of drug response predictors has significant utility for the identification of novel treatment targets and potential for uncovering the molecular mechanisms of antipsychotic drug action.

Presynaptic neurexins (NRXN1–3) and partners, postsynaptic neuroligins (NLGN1–5), are trans-synaptic cell-adhesion, type I membrane proteins that connect presynaptic and postsynaptic synaptic specializations and influence neural network activity via physical regulation of synaptic transmission (Sudhof, 2008; Varoqueaux *et al*, 2006; Zhang *et al*, 2010). Specifically, NRXNs are critical modulators of various neuronal processes, including the differentiation, maturation, stabilization, and plasticity of both excitatory and inhibitory synapses in the mammalian brain (Sudhof, 2008; Varoqueaux *et al*, 2006; Zhang *et al*, 2010). Copy number variation, frameshift, and missense mutations in the NRXN1 gene [2p16.3] have been consistently linked to a broad spectrum of neurocognitive disorders, including autism spectrum disorders, Alzheimer's disease, intellectual disability, and schizophrenia (Moller *et al*, 2013; Swaminathan *et al*, 2012; Duong *et al*, 2012; Bena *et al*, 2013; Dabell *et al*, 2013; Kirov *et al*, 2008; Rujescu *et al*, 2009; Kirov *et al*, 2009; Ikeda *et al*, 2010), highlighting a crucial role for NRXN1 in normal human neurodevelopment and neurocognitive disease. NRXN1 spans 1.12 Mb and encodes 23 canonical exons (Figure 1; Missler *et al*, 1998; Rowen *et al*, 2002). Two alternative promoters encode a longer α-NRXN1 transcript and a shorter *β*-NRXN1 transcript, respectively, and extensive alternative splicing of the primary transcripts results in potentially thousands of evolutionarily conserved isoforms that are differentially expressed in the brain (Figure 1; Sudhof, 2008; Missler *et al*, 1998; Rowen *et al*, 2002). Interestingly, the majority of CNVs reported in schizophrenia are nonrecurrent deletions clustering in the 5′ exonic promoter region encoding the α-NRXN1 transcript (Kirov *et al*, 2008; Rujescu *et al*, 2009; Kirov *et al*, 2009; Ikeda *et al*, 2010; Grayton *et al*, 2012).

Recently, polymorphisms in α-NRXN1 have been associated with clozapine response in schizophrenia patients (Souza *et al*, 2010; Lett *et al*, 2011) and nicotine dependence in healthy individuals (Bierut *et al*, 2007; Nussbaum *et al*, 2008). Specifically, Souza *et al* (2010) reported nominal evidence of association of polymorphisms rs12467557 and rs10490162 in intron 2 of α-NRXN1 (Figure 1) with treatment response in patients with schizophrenia, defined as a 20% reduction on the overall Brief Psychiatric Rating Scale from baseline score at enrolment after 6 months of clozapine treatment. Whereby, subjects carrying major alleles (A) at both variants were more likely to respond to clozapine treatment. Unfortunately, trend significance, lack of placebo control, and lack of examination of effects of treatment on symptom subcategories limits interpretation of these data and warrants independent replication.

In this study, we conducted a pharmacogenetic analysis of antipsychotic drug response in patients with schizophrenia ($N = 54$) in the Clinical Brain Disorders Branch/National Institute of Mental Health (NIMH) double-blind, placebo-controlled inpatient crossover trial and examined for association of the same two SNPs in NRXN1 (rs12467557 and rs10490162) with treatment response as measured by the Positive and Negative Syndrome Scale (PANSS; Kay *et al*, 1991; Apud *et al*, 2012). In a secondary analysis, we evaluated association in the NIMH-sponsored multicenter Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE) study, though the CATIE trial is a comparative efficacy trial and not a placebo-controlled therapeutic trial. Furthermore, we investigated NRXN1, rs12467557, and rs10490162 for clinical genetic association to schizophrenia. Our findings demonstrate significant association of NRXN1, rs12467557, (and rs10490162) with antipsychotic drug response in the NIMH cohort. Neither rs12467557 nor rs10490162 were significantly associated with risk for schizophrenia in the NIMH cohort ($N = 356$ families; 445 patients and 488 healthy controls) or outcome measures in the CATIE trial.

MATERIALS AND METHODS

Antipsychotic Treatment Response Study Design and Subjects

NIMH study cohort. Fifty-four patients with schizophrenia, admitted to the Clinical Brain Disorders Branch schizophrenia research inpatient unit at the NIH Clinical Center between 1998 and 2010, were included in this study (DR Weinberger, PI; see Apud *et al*, 2012). All subjects were Caucasian of European ancestry and were diagnosed with chronic schizophrenia using DSM-IV criteria. Subjects volunteered to participate in a double-blind, placebo-controlled, crossover study with atypical antipsychotics, including olanzapine, risperidone, quetiapine, ziprasidone,

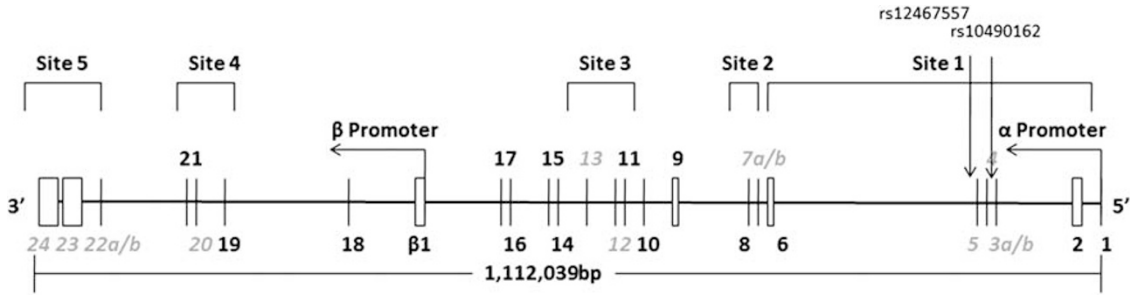

Figure 1 Organization of the human NRXN1 gene as described by Rowen *et al* (2002). The exon-intron structure is depicted (not to scale). The α-NRNX1 transcript is transcribed from the α promoter at the 5′end of the gene. Five alternative spicing sites (sites 1–5) are utilized in the gene. The approximate location of rs12467557 and rs10490162 are shown. Grey italics indicate alternative exons.

or aripiprazole. All patients, and a family member if necessary, provided informed consent for participation in the study, which had been approved by the NIH Institutional Review Board. The program typically enrolled patients who had already had adequate trials of standard antipsychotic medication interventions but were only partially effective in decreasing their symptoms. Upon admission, all patients underwent medical, neurological, and psychiatric evaluation usually lasting 4–12 weeks. If the patient had significant medical problems, a history of violence, or suicidal behavior not identified during the pre-hospitalization screening, or if that patient decided to withdraw his/her consent, he/she could leave the hospital or participate in other protocols not involving placebo studies.

All patients included in the study were on atypical antipsychotic medications before their admission. After the initial evaluation period, patients were maintained on a standard dose of one of various atypical antipsychotics (noted above) and all other medications were discontinued. The decision about which antipsychotic medication would be used in the blinded study was based on best previous response, patient preference, and side effect profile. The patients remained on the single antipsychotic medication in an open-label fashion for several weeks before transitioning to coded medication of the same specific drug and were tapered from their medication over a period of 4-7 days. Subgroup 1 ($N=35$) underwent a sequence of 4 weeks of coded placebo followed by 4 weeks of a coded standard atypical antipsychotic. Subgroup 2 ($N=19$) underwent the inverse sequence (ie, after a similar open medication and taper period, they received standard atypical antipsychotic treatment for 4 weeks followed by placebo for 4 weeks; (as described previously, Apud *et al*, 2012)). Because of the highly supervised and structured nature of the NIMH inpatient environment, most patients tolerated the 4 weeks of placebo well. At the end of the coded double-blind protocol, the patients were restarted on an active medication for 6 more weeks before starting another protocol, or until they were well enough to be discharged.

Upon admission, each patient was rated once on the 16-item PANSS scale (Kay *et al*, 1991). Thereafter, weekly PANSS rating were performed independently (by one of the four research nurses). Two weeks before starting the protocol and up to 4 weeks after completing the protocol, ratings were performed twice a week. The mean PANSS subscores before the patients entered the placebo-controlled protocol were as follows: 14.25 positive Syndrome (SD = 4.09); 15.69 for negative Syndrome (SD = 4.6); 29.58 for general psychopathology (SD = 5.81); 8.48 for anergia (SD = 2.23); 8.36 for thought disturbance (SD = 3.61); 5.08 for activation (SD = 1.31); 4.89 for paranoid/belligerence (SD = 0.85); and 8.40 for depression (SD = 2.18).

CATIE study cohort. Data from CATIE study was used to test for association of SNPs in NRXN1, although the designs of two studies are notably different as the CATIE trial is a comparative effectiveness trial, not a therapeutic or placebo-controlled trial. Only subjects involved with phase 1/1A of the CATIE study, in which patients were randomly assigned to one of the five medications (the first drug assigned), were included. To gain adequate power and to control for the genetic heterogeneity, only individuals of European ancestry were included in analysis ($N=418$). The detailed sample and approaches to analysis of these data have been described elsewhere (Apud *et al*, 2012) and more details on the CATIE study have been published (Lieberman *et al*, 2005). In brief, the primary outcome was time to discontinued use of antipsychotic medication and the secondary outcome measure was change in PANSS ratings (positive syndrome, negative syndrome, and general psychopathology and combined score) before and after initiation of the trial.

Clinical cohorts. Family-based and case-control samples were used for clinical genetic investigation of NRXN1 polymorphisms. The samples were ascertained as part of the Clinical Brain Disorders/National Institute of Mental Health (NIMH) Sibling Study (CBDB/SS, DR Weinberger, PI). DNA was available from 445 probands, 400 siblings of probands, 612 parents, and 488 unrelated controls (as previously described in detail, Law *et al*, 2012). All probands met DSM-IV criteria for a broad diagnosis category consisting of schizophrenia, schizoaffective disorder, simple schizophrenia, psychosis NOS, delusional disorder, schizotypal, schizoid, or paranoid personality disorder. Control subjects were ascertained from the NIMH normal volunteer office and required absence of diagnosis of a psychiatric disorder, extended to include first-degree relatives. All subjects were interviewed with the structured diagnostic evaluation for DSM-IV diagnoses (SCID interview) and were examined for medical and psychiatric exclusions as described elsewhere (Law *et al*, 2012). For family-based association analysis, 356 families with a single affected proband were available. A partially independent case-control analysis was used comprising 445 unrelated probands and 488 unrelated healthy controls. Inclusion criteria for all participants were: self-identification as Caucasian (mostly European ancestry), aged between 18 and 60 years and IQ scores >70 (for probands, premorbid IQ). All subjects gave written informed consent. Patients were also interviewed by a clinical psychiatrist or psychologist for evaluation of ongoing symptoms using the PANSS scale.

Genotyping and Statistical Analysis

In the NIMH inpatient cohort, we genotyped two polymorphisms in NRXN1 (rs10490162 and rs12467557), which are nominally associated with clozapine response in patients with schizophrenia (Souza *et al*, 2010) and nicotine dependence (Bierut *et al*, 2007; Nussbaum *et al*, 2008). rs10490162 and rs12467557 are located in intron 2 of the NRXN1 gene and are 5.3 kb apart and in weak-to-moderate LD ($r^2=0.52$; $D'=1$). Genotypes were determined using the Taqman 5′-exonuclease fluorescent assay (details available upon request) and the allelic discrimination was read on ABI 7900 SDS systems (Applied Biosystems, Foster City, CA). SNP genotypes from the CATIE study that mapped to the NRXN1 genic region ($N=269$) were acquired from a genome-wide association study of schizophrenia (Sullivan *et al*, 2008). Because rs124677557 was not genotyped in the

original study, we used a proxy SNP rs17041184, which was in complete linkage disequilibrium ($D' = 1$ and $r^2 = 1$). Of the 269 SNPs in the GWAS data set mapping to the NRXN1 locus, 73 can be considered independent variants ($r^2 < 0.2$).

Statistical analyses were performed based on the specific study and measurement outcomes. In the NIMH inpatient study, which is a within-subject crossover design with repeated measures, we used a general linear mixed model to examine the effects of treatment, genotype and their interaction on PANSS rating. CATIE data were analyzed using the Cox proportional hazard model and general linear mixed model, respectively, to examine the association of NRXN1 genotype with time to discontinued use of medication and PANSS ratings. Analysis was performed while controlling for age, years of treatment, and CPZEQ. Drug clearance was important in evaluating the antipsychotic drug response, and clearance data were obtained through an ancillary project to the CATIE study (Bigos *et al*, 2008, 2011). For clinical genetic association, main effect analyses of single SNPs were conducted using unconditional logistic regression models controlling for sex and age in the case-control sample and using the family-based association test (FBAT, www.biostat.harvard.edu/fbat/fbat.htm) in families with permutation testing for significance assessment.

RESULTS

Antipsychotic Treatment Response in the CBDB/NIMH Cohort

Treatment effects were observed on several neuropsychiatric symptoms and syndromal clusters ascertained from the PANSS ratings (Supplementary Table S1 and Apud *et al*, 2012). Overall, medications significantly improved symptoms, including those comprising the positive syndrome (Estimate $= -1.3565$, $p = 0.0007$), general psychopathology (Estimate $= -2.2851$, $p = 0.0093$), thought disturbance (Estimate $= -0.8928$, $p = 0.0004$), and activation (Estimate $= -0.6841$, $p = 0.0018$). A suggestive effect on the negative syndrome was also observed (Estimate $= -1.0836$, $p = 0.0124$) but was not significant after correction for multiple testing.

NRXN1 Polymorphisms and Antipsychotic Treatment Response in the CBDB/NIMH Cohort

We observed a significant interaction between rs12467557 genotypes and antipsychotic treatment response on improvement of PANSS ratings of the negative syndrome ($F = 4.27$; $p = 0.044$), positive syndrome ($F = 4.89$; $p = 0.032$); general psychopathology ($F = 9.96$; $p = 0.0029$); anergia ($F = 5.6$; $p = 0.022$), and thought disturbance ($F = 7.74$; $p = 0.008$). These effects are particularly noteworthy considering the relatively small sample size in our study. Similar but less-significant interactions were observed for rs10490162 (Table 1). Genotypic groups did not significantly differ on any demographic variable, including sex, age, IQ, and antipsychotic drug dose, in the treatment arm of the trial (Table 3). In addition, the genotypic groups did not differ with regards to smoking status (Table 2), importantly controlling for previously identified associations of these polymorphisms to smoking dependence in normal individuals (Bierut *et al*, 2007; Nussbaum *et al*, 2008).

Post-hoc analyses revealed that individuals homozygous for the major allele (A) at rs12467557 (or rs10490162) showed a superior response to antipsychotic treatment (Table 1). Specifically, AA genotype individuals had significant reductions in ratings of positive syndrome (Estimate $= -1.45$, $p = 0.002$), negative syndrome (Estimate $= -1.45$, $p = 0.005$), general psychopathology (Estimate $= -3.01$, $p = 0.005$), thought disturbance (Estimate $= -0.95$, $p = 0.001$), and activation (Estimate $= -0.73$, $p = 0.005$) when in the medicated phase of the trial. It is noteworthy that the magnitude of the genotype effect on the change in each of these syndrome scores was greater than the overall effect of medication. Subjects carrying minor alleles (GG + AG) at the same variants did not exhibit any significant improvement based on measures of treatment response (Table 1). Importantly, PANSS ratings were not different for the AA *vs* AG + GG genotypic groups in the placebo *vs* placebo phase, demonstrating that the AA individuals were not significantly differentially more symptomatic compared with the other genotypes. It is important to note that because in these analyses we treat genotype as categorical variables, the regression coefficient estimate actually denotes the mean chance in PANSS rating after adjusting for covariates. Finally, we also performed haplotype analyses of two SNPs, which did not increase the strength of the association (data not shown), likely because these SNPs are in weak LD in this sample ($r^2 = 0.4$).

NRXN1 Polymorphisms and Antipsychotic Treatment Response in the CATIE Study

We failed to replicate association of either rs17041184 or rs10490162 with PANSS ratings (general psychopathology, negative, and positive) in the CATIE data set. In the analysis of three PANSS ratings using the same coding as the placebo controlled data (Table 3), we did not found any significant difference in the PANSS scores before and after treatment by genotype group, except that rs10490162 was trend associated with negative PANSS rating ($p = 0.088$). Analysis of time to discontinued use of medication showed that minor allele carriers of rs17041184 (OR $= 0.526$, $P = 0.0418$) were less likely to discontinue before the end of the phase 1/1A trial (Supplementary Table S2).

In a subsidiary analysis, we explored additional SNPs in the genic region of NRXN1 and found nominal association with both PANSS ratings and time to discontinued use of medication for several SNPs. Eleven SNPs were associated with time to discontinuation ($p < 0.1$; Table 4), which also showed association signals in the combined p values of three PANSS syndromes ($p < 0.05$), and association with time to discontinued use of medication was observed at three intronic SNPs rs7568888 ($p = 0.00574$), rs10176824 ($p = 0.00173$), and rs17496470 ($p = 0.01395$); the association of these three SNPs with the change in PANSS ratings between before and after treatment were also significant. Two other SNPs, rs6545111 ($p = 0.005209$) and rs1718049 ($p = 0.00848$), also showed association with negative symptoms (Table 4). We note, however, that none of these

Table 1 Antipsychotic Treatment Response by Genotype as Assessed by the Positive and Negative Syndrome Scale (PANSS)

	NRXN1_rs12467557				NRXN1_rs10490162			
	Estimate[a]	**t**	***p***	***p****	**Estimate[a]**	**t**	***p***	***p****
Negative syndrome				0.0447				0.0863
AA	−1.45	−2.94	0.0051		−1.57	−2.98	0.0047	
AG + GG	1.80	1.19	0.2399		0.54	0.5	0.6219	
Positive syndrome				0.0324				0.2319
AA	−1.35	−3.29	0.002		−1.34	−2.99	0.0045	
AG + GG	1.55	1.23	0.2265		−0.10	−0.11	0.9139	
General psychopathology				0.0029				0.1397
AA	−3.01	−2.96	0.005		−2.94	−2.54	0.0146	
AG + GG	7.30	2.32	0.025		1.01	0.43	0.6728	
Anergia				0.0223				0.0225
AA	−0.58	−2.01	0.0505		−0.70	−2.29	0.0266	
AG + GG	1.60	1.81	0.0772		0.95	1.5	0.1409	
Thought disturbance				0.0080				0.1187
AA	−0.95	−3.51	0.001		−0.95	−3.16	0.0029	
AG + GG	1.45	1.75	0.0873		0.14	0.23	0.8186	
Activation				0.1600				0.4347
AA	−0.73	−2.98	0.0046		−0.73	−2.78	0.008	
AG + GG	0.39	0.52	0.6082		−0.26	−0.47	0.6372	
Paranoid belligerence				0.0459				0.2725
AA	−0.26	−1.58	0.1212		−0.25	−1.43	0.1598	
AG + GG	0.81	1.63	0.1115		0.19	0.53	0.596	
Depression				0.1302				0.6567
AA	−0.62	−1.59	0.1183		−0.56	−1.34	0.1869	
AG + GG	1.30	1.09	0.2832		−0.14	−0.16	0.8747	

*p**, *p* values for interaction between SNPs and treatment on PANSS ratings adjusted for duration of illness.
A = common allele, G = minor allele.
[a]Estimates reflects mean difference in PANSS ratings between placebo and treatment arms by genotype based on *post-hoc* least-square estimation from the general linear mixed model.

Table 2 Demographic Characteristics of Schizophrenia Patients by *NRXN1* Genotype rs10490162

Characteristic	**AA**			**AG/GG**		
	N	**Mean**	**SD**	***N***	**Mean**	**SD**
Sex (female %)	42	24%	0.43%	12	0.33%	0.49%
Age (years)	42	28.07	7	12	27.75	6.426
Age at onset (years)	42	20.64	4.51	12	21.75	4.957
Education (years)	42	13.64	2.03	12	13.83	1.85
Antipsychotic dose (CPZ equivalents; treatment arm)	37	605.67	296.02	12	558.64	276.78
Smoker (%)	40	0.48	0.51	12	0.58	0.515
Past smoker (%)	40	0.63	0.49	12	0.67	0.492
Duration of illness (years)	42	7.9	6.44	12	6.5	6.882
Full-scale IQ	42	90.45	13.6	12	99	11.747

Note: No statistical significant difference in demographic characteristics were observed ($p > 0.05$).

associations would survive correction for multiple testing. The detailed estimates can be found in Supplementary Table S3. Among these 11 SNPs, 2 were in strong LD and 2 were in moderate LD (see Table 4). The other seven SNPs, including those showing the most consistent association, were relatively independent ($r^2 < 0.2$).

Table 3 Least Square Mean Estimates of Three PANSS Ratings by Genotype Groups in the European Ancestry Sample of CATIE Study

Genotype	General psychopathology				Negative PANSS				Positive			
	Estimate	SE	*p*	*p**	Estimate	SE	*p*	*p**	Estimate	SE	*p*	*p**
rs17041184[a]												
TT	2.5426	0.477	<0.0001	0.6116	0.944	0.3135	0.0028	0.3621	1.4958	0.3056	<0.0001	0.5685
CT + CC	1.8205	1.3385	0.1745		1.797	0.8808	0.042		0.9758	0.8582	0.2562	
rs10490162												
AA	2.1518	0.501	<0.0001	0.1835	0.8123	0.3276	0.0136	0.0888	1.2985	0.32	<0.0001	0.3871
AG + GG	3.6357	0.9947	0.0003		2.0560	0.6513	0.0017		1.9148	0.636	0.0028	

Note: *p*, *p* value for treatment effect in each genotype group from the *post-hoc* analysis based on general linear mixed model; *p**, *p* value for genotype and treatment interaction to test whether or not antipsychotic response was different by genotype group.
[a]rs17041184 is a proxy SNP of rs12467557 ($r^2 = 1$ and $D' = 1$), which was not genotyped in CATIE data.

Table 4 Association of NRXN1 SNPs with Time to Discontinuation ($p < 0.1$) and PANSS Rating (Comb $p < 0.05$) in the CATIE Study

Chr	Gene	Location	BP(HG18)	SNP	distance	Discontinuation	PANSS ratings			comb3_p
						*P**	p_gen	p_neg	p_pos	
2			49553295	rs6545111		0.07746	0.194454	0.005209	0.187465	0.001789
2	—	Upstream	49768414	rs17180439	453872	0.06720	0.102235	0.008480	0.118872	0.000769
2	—	Upstream	49780074	rs1518826	465532	0.02267	0.159074	0.045965	0.098444	0.003508
2			49845664	rs1914791		0.04683	0.163218	0.047506	0.405138	0.015106
2	—	Upstream	49810789	rs10211040	496247	0.04346	0.126967	0.036632	0.011133	0.000380
2	NRXN1	Intron	50099333	rs7568888	0	0.00574	0.118042	0.188359	0.105221	0.009398
2	NRXN1	Intron	50115890	rs10176824	0	0.00173	0.164830	0.092664	0.014243	0.001433
2	NRXN1	Intron	50150244	rs17496470	0	0.01395	0.102509	0.489385	0.180992	0.034503
2	NRXN1	Intron	50547858	rs6726487	0	0.02236	0.127715	0.562803	0.187808	0.048390
2	—	Upstream	51420208	rs17524147	311299	0.03482	0.253534	0.841519	0.029657	0.042337
2	—	Upstream	51435011	rs17590125	326102	0.04651	0.126366	0.576434	0.049158	0.019242

Note: SNPs rs1518826 and rs10211040 are in moderate LD ($r^2 = 0.79$) and rs17524147 and rs17590125 are in strong LD ($r^2 = 0.93$). Thus, there are nine relatively independent signals in the analysis. *p**, *p* value for testing for genetic variation with time to discontinuation; p_gen, p_neg and p_pos are *p* values for genotype × treatment interactions on PANSS general psychopathology, negative and positive symptom, respectively, using general linear mixed model; comb3_p, combined *p* values of three PANSS symptoms using Stouffer's *Z*-score method.

Association of NRXN1 Polymorphisms with Schizophrenia

Single marker analysis in the family-based sample revealed no evidence of association with rs12467557 or rs10490162 ($p = 0.33$; $p = 0.54$, respectively). Likewise, we failed to observe evidence of association of these SNPs with schizophrenia in our case-control sample (rs12467557, $p = 0.74$; rs10490162, $p = 0.959$). Both SNPS were in Hardy-Weinberg equilibrium in both cases and controls ($p > 0.7$) and were in moderate linkage disequilibrium ($r^2 = 0.522$), consistent with that observed in the NIMH treatment response cohort.

DISCUSSION

Our study provides statistically significant evidence of association of NRXN1 rs12467557 and rs10490162 with atypical antipsychotic treatment response in a placebo-controlled, in-patient treatment response study. Schizophrenia patients homozygous for major alleles at either variant (rs10490162 and rs12467557) were more likely to show favorable treatment response as opposed to those carrying minor alleles, including improvement of negative symptoms. Notably, the effect size of the pharmacogenetic association is greater than the effect of antipsychotic treatment, *per se*. These results are of particular interest given the relatively small sample size and tightly controlled nature of this study and are consistent with previous data suggestive of an impact of these SNPs on clozapine response in an uncontrolled trial (Souza *et al*, 2010).

Our failure to find association with risk for schizophrenia in our family and case-control data sets is consistent with the negative signals for common variant associations in much larger studies (Ripke *et al*, 2013). Given the recent findings of association of rare, highly penetrant NRXN1

CNVs with schizophrenia (Kirov *et al*, 2008; Rujescu *et al*, 2009; Kirov *et al*, 2009; Ikeda *et al*, 2010), it is perhaps not surprising that we fail to find significant association of either common variant with risk for illness. Most of the recurrent CNVs that have been associated with schizophrenia appear to represent biological risk that is not represented by common variants at the same loci.

Over the past several years, evidence has emerged suggesting that CNVs have a role in the etiology of neurodevelopmental disorders, such as schizophrenia, autism, and intellectual disability (Kirov *et al*, 2008; Rujescu *et al*, 2009; Kirov *et al*, 2009; Ikeda *et al*, 2010; Muhleisen *et al*, 2011; Stefansson *et al*, 2008; Bassett *et al*, 2008; International Schizophrenia Consortium, 2008). Deletions within NRXN1 were first reported in schizophrenia by Kirov *et al* in 2008, whereby a deletion spanning the proximal promoter and first exon of α-NRXN1 was observed in a single proband and an affected sibling with schizophrenia. Subsequently, chromosomal deletions disrupting NRXN1 have been replicated by a number of groups (Rujescu *et al*, 2009; Kirov *et al*, 2009; Ikeda *et al*, 2010; Muhleisen *et al*, 2011; Stefansson *et al*, 2008; Bassett *et al*, 2008; International Schizophrenia Consortium, 2008). A meta-analysis of these studies revealed strong evidence that deletions of the NRXN1 gene that impact the proximal region of α-NRXN1 confer a substantial increase in risk of schizophrenia, which further increases when restricted to functional deletions > 100 kb (Kirov *et al*, 2009).

Intron 2 of NRXN1-α, which contains rs12467557 and rs10490162 (Figure 1), is in the region most frequently disrupted by CNVs in schizophrenia (Kirov *et al*, 2009; Muhleisen *et al*, 2011; Stefansson *et al*, 2008; Chen *et al*, 2013). The intron spans 101 kb, making it one of the largest segments within the gene (Rowen *et al*, 2002). Its large size, taken together with its high level of sequence conservation throughout vertebrates, suggests that it has played an important role in the evolution of the NRXN1 gene. Introns are ubiquitous throughout the human genome and have been postulated to fulfill a variety of cellular functions, including carriers of transcriptional regulatory elements as well as key players in alternative splicing (Fedorova and Fedorov, 2003). Large introns (> 50 kb) show an abundance of interspersed repetitive elements (SINEs and LINEs) that form stable structures, which have been hypothesized to bring donor and splicing junctions closer together, thus enhancing splicing efficiency (Shepard *et al*, 2009). Consistent with this, examination of the UCSC genome bioinformatics suite incorporating the ENCODE Regulation track reveals that rs10490162 lies 226 bp from a LINE (L1ME3) element (chr2:51247202-51247431; GRCh37/hg19). Recent evidence also demonstrates that small-size inverted repeats are overrepresented in the 5′ portion of NRXN1 and its immediate upstream region, indicative of a region of genomic instability and a mechanism underlying the region as a deletion hotspot in neurocognitive and psychiatric disease (Chen *et al*, 2013).

At present the mechanism of association of NRXN1 polymorphisms with antipsychotic drug response is unclear. *In-silico* evidence suggests that the region monitored by these SNPs has a role in splicing or regulation of expression of the gene. Indeed, primary NRXN transcripts undergo complex alternative splicing that is predicted to give rise to > 1000 distinct isoforms (Missler *et al*, 1998; Rowen *et al*, 2002). α-NRXNs contain five canonical splice sites, referred to as 1–5, with only 4 and 5 shared by β-NRXNs (Figure 1; Rowen *et al*, 2002). Splice site 1 in NRXN1-α involves splicing of exons 3, 4, and 5, which reside in intron 2, placing the splice site in direct proximity to rs12467557 and rs10490162 (Figure 1). Interestingly, rs10490162 lies within a spliced EST cloned from cerebellum, DA128541 (chr2:51247164-51247752). The general significance of these observations is at present unclear given that association was suggested to polymorphisms in the 3′ intronic region of NRXN1 in the CATIE data set, a region common to both NRXN1-α and NRXN1-β. Deep resequencing of the NRXN1 association regions, combined with experimental verification of functionality, is required to determine whether genotyped SNPs are in linkage disequilibrium with unknown functional/causal variants or are themselves functional.

Although there is no direct evidence that NRXN signaling is a molecular regulator of antipsychotic drug activity, NRXNs are expressed in brain areas that control the activity of dopaminergic signaling (Hishimoto *et al*, 2007), the primary mechanism of antipsychotic drug action and a signaling mechanism, which may explain association of NRXN1 and NRXN3 with nicotine and substance dependence (Bierut *et al*, 2007; Nussbaum *et al*, 2008; Hishimoto *et al*, 2007; Docampo *et al*, 2012). Furthermore, NRXN1-α has been shown to be critical for calcium-mediated neurotransmitter release and the regulation of voltage-dependent Ca^{2+} channel activity, via coupling of Ca^{2+} channels to synaptic vesicle release (Zhang *et al*, 2010; Missler *et al*, 2003; Dudanova *et al*, 2006); thus it is plausible that NRXN1 may directly impact dopaminergic signaling in brain.

Finally, although we failed to replicate direct association of rs10490162 and rs12467557 with outcome measures in the CATIE data set, we present nominal evidence of association with other common variants in the NRXN1 gene with both time to discontinuation of medication and PANSS scores, providing additional support for association of the NRXN1 gene with antipsychotic treatment response in patients with schizophrenia. We note that failure to replicate the exact SNP association (observed herein in the CBDB/NIMH study and in the study of Souza *et al*, 2010) in the CATIE data set should not be overinterpreted. The two trials used in this study were performed using vastly different clinical and experimental models and with different outcome measures, ie, CATIE, an outpatient parallel, comparative cohort, drug-only design and the other a double-blind, placebo-controlled inpatient crossover study, which ultimately makes direct comparison between the studies difficult.

Future studies examining the association of NRXN1 polymorphisms with antipsychotic treatment response are warranted by our results. The elucidation of the functional significance of variants within the NRXN1 gene may reveal new pharmacological targets for the development of novel therapeutic strategies for schizophrenia. Such discoveries may be vitally important given the limited efficacy of current antipsychotics to ameliorate symptoms associated with the disease and the need for a new generation of psychiatric drugs.

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The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)