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The putative functional rs1045881 marker of neurexin-1 in schizophrenia and clozapine response

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ABSTRACT

Neurexin-1 (NRXN1) modulates recruitment of NMDA receptors. Furthermore, clozapine reduces hyperactivity of NMDA receptors. Thus, regulation of the *NRXN1* gene may mediate the efficacy of clozapine at reducing cortical hyperactivity. We examined the putative functional SNP, rs1045881, for association with schizophrenia, and the potential role of this SNP in clozapine response. The rs1045881 variant was not significantly associated with schizophrenia (N = 302 case–control pairs), but with clozapine response (N = 163; p = 0.030). Baseline and BPRS scores after six months revealed a trend for rs1045881 genotype by treatment interaction (p = 0.079). In the *post hoc* analysis, a significant association between BPRS negative symptoms score and genotype was observed (p = 0.033). These results suggest that the rs1045881 *NRXN1* polymorphism may influence clozapine response.

1. Introduction

Deletions in the neurexin-1 (*NRXN1*; 2p16.3; gene size = 1.1 mb) gene have been strongly associated with the etiology of schizophrenia, and autism spectrum disorder (Voineskos et al., 2011). The *NRXN1* gene encodes the neurexin-1α protein that functions as a presynaptic neural adhesion molecule reported to interact with postsynaptic neuroligins mediating GABAergic and glutamatergic synapse function (Südhof, 2008). Neurexin-1α knockout mice exhibit an electrophysiological phenotype consistent with a network disruption that presents as a presynaptic loss of synaptic strength in excitatory synapses of the hippocampus (Kehrer et al., 2008; Etherton et al., 2009). Recent evidence suggests NRXN1 also binds to leucine-rich repeat transmembrane protein (LRRTM2), that modulates postsynaptic differentiation of glutamatergic synapses (de Wit et al., 2009). Therefore, *NRXN1* may, at least partially, direct excitatory synapse formation. These findings are interesting in light of reports that clozapine prevents phencyclidine-induced functional hyperactivity of *N*-methyl *D*-aspartate receptors (NMDAR) in pyramidal cells in rat medial prefrontal cortex (Arvanov and Wang, 1999; Ninan et al., 2003). Furthermore, clozapine is reported to differentially regulate dendritic spine formation and synaptogenesis in the rat hippocampal neurons (Critchlow et al., 2006). Recently, we have reported that rs1045881 is located in a putative miRNA binding site that influences frontal lobe structural white matter volume and sensorimotor function (Voineskos et al., 2011). Altogether, variation in regulation of the *NRXN1* gene may influence response to clozapine treated schizophrenia patients.

In this study, we analyzed associations of rs1045881 in schizophrenia (SCZ) matched case-controls. We have a strong a priori hypothesis to examine the association between this high-interest marker and SCZ because of our *in silico*, neuroimaging and neurobehavioral findings. Second, given the effect of clozapine on NMDAR function, and the role of *NRXN1* in NMDAR recruitment, we examine the role of the rs1045881 in prospectively assessed European-American schizophrenia patients for clozapine treatment response.

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2. Experimental/materials and methods

2.1. Participants

The case–control study was composed of 302 matched pairs that were recruited at the Centre for Addiction and Mental Health. Patients and controls were matched for sex, ethnicity, and age at recruitment (Table S1). Research interviews were conducted using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder IV (DSM-IV) Disorders. Patients with a history of major neurological disorders, major substance abuse, and head injury with significant loss of consciousness were excluded from the study. Each individual of the control group was screened for history of major psychiatric disorders using the SCID-I, and only those without major psychiatric disorders were entered as healthy controls. Our paired case–control sample had over 80% power to detect an odds ratio as low as 1.65 ($\alpha = 0.05$, minor allele frequency = 0.141, dominant model; Quanto v1.2.4 (Gauderman, 2002)).

Our clozapine response sample consisted of 169 European–American schizophrenia patients obtained from three research clinics: Case Western Reserve University in Cleveland, Ohio (HY Meltzer, N = 68); Hillside Hospital in Glen Oaks, New York (JA Lieberman, N = 73); and University of California at Irvine (SG Potkin, N = 28). These subjects had DSM-III-R or DSM-IV diagnoses of SCZ and met the criteria for treatment refractoriness or intolerance to traditional antipsychotic therapy. After a 2- to 4-week washout period, patients were treated with clozapine and evaluated prospectively for 6 months and clozapine blood levels were monitored. Treatment response was evaluated using the 18-item Brief Psychiatric Rating Scale (BPRS) at the time of enrolment into the study (baseline) and after 6 months of clozapine treatment. Differences in response rates across clinical sites were not observed ($\chi^2 = 0.901$, df = 2, $P = 0.637$, Table S2). Therefore, data from the three clinical sites were analyzed together. Our sample had 80% power to detect an odds ratio of 2.0 at a non-responder frequency of 40% (unmatched case–control design; $\alpha = 0.05$, minor allele frequency = 0.141, dominant model; Quanto v1.2.4 (Gauderman, 2002)). In our categorical response measure sample, treatment response was analyzed as a dichotomous variable. Responders were defined as a reduction >20% on the overall BPRS score after 6 months of treatment based on the criteria proposed by (Kane et al., 1988). Quantitative treatment response data was available only for a subset (total BPRS [N = 91]; positive/negative symptoms subscale [N = 87]). All experimental procedures were approved by local ethics committee and all patients signed informed consent prior to their participation, in accordance with the Declaration of Helsinki.

2.2. Genetics

Genomic data was extracted from venous blood (Lahiri and Nurnberger, 1991). The rs1045881 variant was genotyped, using Taqman 5′ nuclease assay (Applied Biosystems; Foster City, CA, USA). Genotyping accuracy was assessed by repeating 10% of the sample, and results showed 100% concordance.

2.3. Statistical analysis

Analysis of SCZ cases versus matched controls was done using log-likelihood χ^2 ratio test both in terms of allele frequencies and genotype frequencies in UNPHASED 3.1 (Dudbridge, 2008). Haploview 4.2 was used to determine Hardy Weinberg equilibrium (HWE) (Barrett et al., 2005).

To test the effect of *NRXN1* genotype on quantitative treatment response, a repeated measure analysis of variance (RM ANOVA) tests were performed. *NRXN1* genotype was the between-group factor, and BPRS scores at baseline and 6 months were the within-group factor. These analyses were performed using Statistical Package for the Social Sciences 15.0.0 (Chicago, IL, USA).

3. Results

3.1. Association with Schizophrenia

The rs1045881 polymorphism was in HWE in both cases and controls ($p > 0.05$). No significant allelic or genotypic associations between rs1045881 and SCZ in our matched case–control samples were detected ($p = 0.37$; $p = 0.27$, respectively; Table 1). Furthermore, we found no significant associations in European–Americans alone suggesting that our results are not masked by population stratification (Table S3).

3.2. Influence of NRXN1 on clozapine response

The rs1045881 variant did not deviate from HWE in responders or non-responders groups ($p > 0.05$). Furthermore, there was no significant difference in gender, age at onset, and treatment duration based on rs1045881 genotype (Table S4). Our categorical analysis found the rs1045881C allele of *NRXN1* to be associated with clozapine response ($p = 0.012$, OR = 2.199 [1.185–4.080]; Table 2). Additionally, the C/C genotype showed association with treatment response under a dominant model ($p = 0.030$, OR = 2.153 [1.077–4.304]; Table 2). This was further supported by the trend observed in our quantitative measure of treatment response (RM ANOVA: $F_{1,87}$ Within-subject = 3.151 $p = 0.079$; Fig. 1; Table S4).

In *post hoc* analysis, we examined positive and negative symptom subscales of the BPRS. RM ANOVA of negative symptoms showed a significant genotype association ($F_{1,85}$ Between-subject = 4.686, $p = 0.033$) and a trend for genotype by treatment response ($F_{1,85}$ Within-subject = 3.293, $p = 0.075$; Fig. 2; Table S5). In contrast, there was no genotype or genotype by treatment response effect for positive symptoms (Fig. 2; Table S5).

4. Discussion

Our results show an association between the rs1045881 and clozapine response. The rs1045881T allele was over-represented in the non-responder group, suggesting that the rs1045881 variant of *NRXN1* may influence clozapine response. This is consistent with recent findings by our group that found two other markers of *NRXN1* to be nominally associated with clozapine response (Souza et al., 2010) and our observed trend of association between rs1045881 and quantitative total BPRS treatment response. There was no observable effect of rs1045881T on change in negative symptoms; although overall, T-allele carriers had lower negative symptom scores. The trend seen in the C allele homozygotes suggests their responsiveness to clozapine treatment.

The core clinical symptoms of schizophrenia are negative symptoms (Andreasen, 1982) which do not respond well to existing treatment (Murphy et al., 2006). Previous structural and diffusion tension imaging MRI brain imaging finding have reported that a generalized reduction in frontal lobe white matter correlate with greater severity of negative symptoms (Sanfilipo et al., 2000; Wolkin et al., 2003). This suggests that the increased frontal lobe white matter we previously reported in T-allele carriers could be related to the

Table 1
The association between schizophrenia diagnosis and genotypic and allele frequencies of rs1045881.

		Case	Control		
Schizophrenia versus control	T	88	99	χ^2	Allelic *p*-value
	C	514	501	0.811	0.368
	T/T + T/C	82	94	χ^2	Genotypic *p*-value
	C/C	219	206	1.214	0.270

T-allele carriers versus C/C homozygotes were compared in genotypic test because cells with less than five T/T homozygotes were observed. χ^2 = Likelihood ratio chi squared.