Disrupted in Schizophrenia 1 Genotype and Positive Symptoms in Schizophrenia

Pamela DeRosse, Colin A. Hodgkinson, Todd Lencz, Katherine E. Burdick, John M. Kane, David Goldman, and Anil K. Malhotra

Background: Converging evidence has demonstrated an association between the Disrupted in Schizophrenia 1 (DISC1) gene and schizophrenia (SZ). Within the DISC1 gene, a single nucleotide polymorphism (SNP), Ser704Cys, has been associated with the structure and function of the hippocampus. Because positive symptoms in SZ have also been associated with hippocampal structure and function, we hypothesized that variation in a DISC1 haplotype containing Ser704Cys would be significantly associated with positive symptomatology in SZ.

Methods: We tested for an association between variation in a haplotype block within the DISC1 gene containing Ser704Cys and lifetime history of positive symptoms in 199 Caucasian patients with SZ.

Results: We detected significant associations between a DISC1 haplotype containing Ser704Cys and Ser704Cys genotype and lifetime severity of delusions in SZ.

Conclusions: These data suggest that that the effect of DISC1 genetic variation might be associated with positive symptoms in patients with SZ.

Key Words: DISC1, haplotype, schizophrenia, SNP

The Disrupted in Schizophrenia 1 (DISC1) gene, located at the break point of a chromosomal translocation at 1q42.1, co-segregates with schizophrenia (SZ) and other psychiatric disorders. It has been suggested that this translocation, which results in a truncation of the DISC1 protein, contributes to SZ by affecting neuronal functioning in many areas of the brain including the hippocampus (Morris *et al.* 2003).

Recently, Callicott *et al.* (2005) identified a single nucleotide polymorphism (SNP) within the DISC1 gene that was significantly associated with SZ and with the structure and function of the hippocampus. Specifically, the common allele (Ser) of Ser704Cys was associated with reduced hippocampal gray matter volume and altered engagement of the hippocampus during cognitive tasks.

Dysfunction of the hippocampus, such as alterations in regional cerebral blood flow (Liddle *et al.* 1992) and volumetric reductions (Weinberger 1999), is a consistent finding in SZ and often associated with the severity of positive psychotic symptoms. For example, Jacobsen *et al.* (1998), in a longitudinal study of childhood-onset SZ, found that higher delusion ratings at a 2-year follow-up were significantly correlated with decreases in hippocampal volume. Furthermore, Strasser *et al.* (2005) found that although there were no differences in hippocampal volume between subjects with non-psychotic bipolar disorder and healthy control subjects, subjects with psychotic bipolar disorder had significantly reduced hippocampal volume relative to healthy control subjects.

On the basis of the reported relation between positive symptoms and hippocampal volume and the reported effects of DISC1 genotype on hippocampal volume, we hypothesized that DISC1 genotype would be related to lifetime severity of positive symptoms in SZ. Thus, we evaluated the effect of variation within a five-locus haplotype containing Ser704Cys on the lifetime severity of positive symptoms in SZ. Additional analyses were then conducted to assess the effects of each SNP within the haplotype on severity of positive symptoms.

From the Department of Psychiatry Research (PD, TL, KED, JMK, AKM), The Zucker Hillside Hospital, North Shore-Long Island Jewish Health System, Glen Oaks, New York; and the Laboratory of Neurogenetics (CAH, DG), National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, Maryland.

Address reprint requests to Pamela DeRosse, M.A., The Zucker Hillside Hospital, North-Shore-Long Island Jewish Health System, 75-59 263rd Street, Glen Oaks, NY 11004; E-mail: pderosse@lij.edu.

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Methods and Materials

Participants

The study sample was 199 Caucasians meeting criteria, on the basis of the Structured Clinical Interview for the DSM-IV (SCID), for SZ or schizoaffective disorder. All subjects provided informed consent to a protocol approved by the Institutional Review Board of the North Shore-Long Island Jewish Health System (NSLIJHS). Subjects were recruited from the Zucker Hillside Hospital, a division of the NSLIJHS, in Glen Oaks, New York.

Assessment

Each subject was assessed with a SCID administered by trained and reliable raters. The SCID contains a total of 14 positive symptom items rated on a continuous scale where 1 = absent, 2 = subthreshold, and 3 = present. Ratings on these items were recorded on the basis of the subject's report as well as medical records and other available sources. An overall delusions rating included ratings on the referential, paranoid, grandiose, somatic, control, thought broadcasting, bizarre, and other delusion items. An overall hallucinations rating included ratings on the auditory, visual, tactile, and other hallucinations items. Finally, an overall disorganization rating included the ratings on the disorganized behavior and disorganized speech items. These overall ratings, which represent lifetime presence of symptomatology, were calculated for each subject. Only those subjects who had all items rated were used in the present analyses. Thus, if any of the items in the psychosis module indicated that there was inadequate information about the symptom to allow for an accurate rating, the subject was excluded from the analysis.

Genotyping

Genotyping and haplotype construction were completed as previously described (Hodgkinson *et al.* 2004). Only two haplo-

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types with frequencies above 10% in the previously identified haploblock 6 (comprising rs821592, rs821597, rs821653, rs843979, and rs821616 [Ser704Cys]) were identified and used in these analyses (11222: frequency approximately 65%; 22111: frequency approximately 21%).

Analysis

Relationship of overall delusions, hallucinations, and disorganization ratings to each of the two haplotypes were examined with two multivariate analyses of covariance (MANCOVAs). To avoid the potential confound of an effect of genotype on overall symptom severity, Global Assessment of Functioning (GAF) score was used as a covariate in both MANCOVAs. When appropriate, post hoc analyses were used to identify the effect of genotype on individual symptoms and regression analyses were conducted to assess the relative contribution of DISC1 genotype to symptom severity. After haplotype analyses, SNPs comprising the haplotype were also assessed for their effects on overall delusions, hallucinations, and disorganization ratings.

Results

A total of 116 heterozygotes, 79 homozygotes, and 20 non-carriers of the 11222 haplotype were identified. A total of 71 carriers (61 heterozygotes, 10 homozygotes) and 124 non-carriers of the 22111 haplotype were identified. Because no differences were found between 22111 heterozygotes and homozygotes, and to increase statistical power, these groups were combined for further analyses. The MANCOVAs covarying for GAF score, comparing the haplotype groups on the overall delusions, hallucinations, and disorganization ratings, revealed an overall effect of the 22111 haplotype [$F(3,172) = 4.95$, $p = .003$] but no effect of the 11222 haplotype. Post hoc analyses revealed that carriers of the 22111 haplotype had a significantly lower overall delusions rating than non-carriers [$F(1,174) = 12.15$, $p = .001$]. This difference is shown in Figure 1. Moreover, a MANCOVA comparing carriers and non-carriers of this haplotype on individual delusion ratings including referential, paranoid, grandiose, somatic, control, thought broadcasting, bizarre, and other delusions also indicated a significant effect of haplotype [$F(8,169) = 2.28$, $p = .02$]. Post hoc analyses indicated a significant effect of haplotype on paranoid delusions [$F(1,177) = 6.55$, $p = .01$] and other delusions [$F(1,177) = 6.28$, $p = .01$].

A stepwise multiple regression including haplotype status, gender, age, duration of illness and GAF score as predictors of the severity of paranoid delusions revealed that haplotype status was the only significant predictor [$F(1,175) = 8.19$, $p = .005$], accounting for approximately 4.5% of the variance.

Because the other delusions item requires additional dichotomous rating (0 = absent, 1 = present) on religious, guilty, jealous, or erotomanic delusions, the relation between the 22111 haplotype and these delusion subtypes were analyzed with χ^2 analyses. These analyses revealed no effect of DISC1 genotype.

Exploratory analyses, MANCOVAs covarying for GAF score, assessed the effect of the individual SNPs within the haplotype on the severity of overall delusions, hallucinations, and disorganization ratings. These analyses revealed significant effects at four of the five loci: rs821592 [$F(3,190) = 3.56$, $p = .015$], rs821653 [$F(3,186) = 4.71$, $p = .003$], rs843979 [$F(3,191) = 3.88$, $p = .010$], and Ser704Cys [$F(3,189) = 5.28$, $p = .002$], and with the exception of rs821592, all of these effects remained significant after correction for multiple testing. Post hoc analyses, carried out on the three significant SNPs, indicated a significant effect of genotype on the overall delusions rating at rs821653: $p = .002$; rs843979: $p = .031$; and Ser704Cys: $p = .001$. In these analyses, however, only rs821653 and Ser704Cys remain significant after correction for multiple testing. These data are shown in Figure 1.

Discussion

We detected a significant association between a DISC1 haplotype and lifetime severity of delusions in SZ. Specifically, patients who were carriers of a common haplotype containing the minor allele at Ser704Cys had significantly lower ratings on paranoid delusions than non-carriers. This finding is consistent with previous reports of an association between the Ser allele and decreased hippocampal volume and between decreased hippocampal volume and severity of positive symptoms.

Although it could be argued that this locus confers risk in a subset of patients who have significant positive symptoms, it is unlikely that Ser704Cys is a susceptibility locus. In our sample, neither the individual SNP nor the haplotype carrying it was significantly associated with diagnosis (Hodgkinson *et al.* 2004), and therefore it might be more parsimonious to conclude that Ser704Cys acts as a modifier (Fanous and Kendler 2005) that influences the clinical presentation of SZ rather than increasing

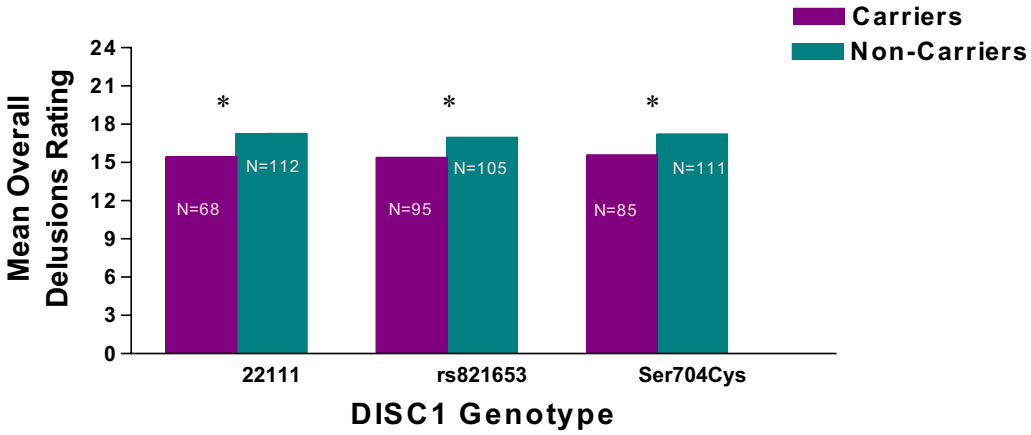

Figure 1. Overall delusions ratings in carriers and non-carriers of the 22111 disrupted in schizophrenia 1 (DISC1) haplotype and carriers and non-carriers of the minor alleles at rs821653 and Ser704Cys. Haplotype and single nucleotide polymorphisms are shown on the X-axis. The Y-axis represents the mean ratings in carriers and non-carriers. *Indicates a significant difference between carriers and non-carriers.

the risk for SZ. This type of analysis is consistent with reported relations between psychosis and 5-HTTLPR genotype (Malhotra *et al*. 1998) and contrasts with data from our group (DeRosse *et al*. 2006) and others (Fanous *et al*. 2005) in which risk haplotypes of dysbindin have been linked to negative symptoms but not positive symptoms in SZ. Recent data suggest that DISC1 might exert its influence on positive symptoms through neural circuitry within the hippocampus and prefrontal cortex (Callicott *et al*. 2005; Cannon *et al*. 2005). Gamma-aminobutyric acid (GABA)ergic neurons are located in all regions of the hippocampus, and their activity might have significant effects on neuronal activity within the prefrontal cortex and other areas associated with psychosis (Keverne 1999).

Although the specific mechanism is unknown, recent data suggest that the DISC1 protein mediates GABAergic function through its interaction with activating transcription factors (ATF) 4 and 5. Interaction of DISC1 with ATF4/5 is lost upon DISC1 truncation, and ATF4/5 regulation of GABA-B receptor function might also be lost (Morris *et al*. 2003). These data suggest that DISC1 might influence severity of psychosis by mediating GABAergic function within the hippocampal formation and related circuitry.

Several limitations of the current study should be noted. First, although the SCID assesses lifetime symptomatology, the use of more comprehensive rating scales, which were not available in the present dataset, are preferred. Second, we only analyzed a single haplotype block in our study. Although this haplotype block contains an SNP (Ser704Cys) demonstrated to relate to hippocampal volume and function, it should be noted that there are no data on the biological significance of this SNP from in vitro assays.

In summary, we have detected a significant association between a DISC1 haplotype and severity of positive symptoms in a relatively large cohort of patients with SZ. These data suggest that DISC1 genotype might modify the clinical presentation of SZ, representing another step toward a molecular classification of psychiatric illness.

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