LETTER TO THE EDITOR

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Replication and Refinement of the Role of rs548181 in Schizophrenia: Results From a Family Based Study

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TO THE EDITOR:

Schizophrenia is a highly heritable disorder [Sullivan et al., 2003; Lichtenstein et al., 2009], which is characterized by symptoms in multiple domains, including positive, negative, disorganization, mania, and depression symptoms [Derks et al., 2012]. The largest genome wide association study to date showed the involvement of 10 single nucleotide polymorphisms (SNPs) based on a stage one discovery sample of 21,856 individuals and a stage two replication sample of 29,839 independent subjects [Ripke et al., 2011]. The first aim of this study is to replicate the role of these 10 SNPs in schizophrenia using family based association, which has as a main advantage that it is not affected by population stratification. Secondly, we aim to increase our understanding of the functional role of these SNPs by investigating the associations with the five symptom dimensions of schizophrenia.

Symptom dimensions are phenotypically more homogeneous compared to a DSM-IV diagnosis for schizophrenia which is based on an aggregate score on diverse clinical symptoms. We expect these dimensions to be more strongly associated with biological variation. This premise is supported by recent findings of our group in which we report that high-disorganization scores are associated with a relatively large decrease in total brain volume in the brains of schizophrenia patients [Collin et al., 2012] while positive, negative, mania, and depression scores did not show such an association.

Genotypic data was collected and after extensive Quality Checks, the sample included 2,018 subjects from 894 families, including 522 schizophrenia patients and 1,496 first-degree relatives of these patients (724 parents and 772 siblings). The mean genotyping rate after QC is 0.996. The mean genotyping rates per SNP ranged between 0.992 and 0.998. The rate of missing data was not significantly different between cases and controls for any of the SNPs (all $P > 0.10$). The minor allele frequencies of the SNPs ranged between 0.026 and 0.434 and were highly similar compared to the estimates previously reported by the PGC consortium with differences ranging between 0.006 and 0.03. All 10 SNPs were in Hardy–Weinberg equilibrium (in controls, all $P > 0.10$).

Scores on five psychosis dimensions were calculated based on the comprehensive assessment of symptoms and history (CASH) lifetime rated symptoms as described previously [Derks et al., 2012].

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The correlations between the psychosis dimensions range between 0.10 and 0.49. Dimension scores were available for a subset of 1,445 subjects from 685 families. The sample included 384 schizophrenia patients and 1,061 unaffected relatives of patients (507 parents and 554 siblings). The study sample is largely independent from the PGC study; 75 of the patients and none of the relatives were included in the PGC sample. Although the primary aim was to

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conduct a family based study, genotypic data and dimension scores of 186 controls were available for posthoc analyses. Note that while genotypic data of the patient were available only for a subset of the families (371/685 = 54%), all families have at least one member who is diagnosed with non-affective psychosis. The statistical software package FBAT was used to perform family based association tests [Lake et al., 2000]. First, we tested the association of the 10 SNPs with disease status as a categorical trait. The type-I error rate was Bonferroni corrected for multiple testing and was set at 0.05/10 = 0.005. Next, for the significantly associated SNPs, we performed an analysis in which quantitative dimension scores are the dependent variables. The type-I error rate was again Bonferroni corrected for the number of phenotypes included in the analysis (N = 5) and the number of significant SNPs included in this analysis. This may be somewhat conservative given the significant correlations between the outcome measures of this study and we therefore interpret non-significant findings, which have a *P*-value below <0.05 as trend findings. In both analyses, we used an additive genetic model.

Affection status was significantly associated with rs548181. The minor allele decreased the risk for schizophrenia ($Z = -3.031$; $P = 0.002$). The remaining nine SNPs were not significantly associated with case–control status (all $P > 0.01$). In follow-up analyses, we tested the association of rs548181 with the quantitative symptom dimensions. The minor allele of rs548181 was associated with lower scores on disorganization ($Z = -2.594$; $P = 0.009$), and at a trend level with lower scores on negative symptoms ($Z = -2.458$; $P = 0.014$) and positive symptoms ($Z = -2.00$; $P = 0.045$) but was not associated with mania ($Z = -0.735$; $P = 0.463$), or depression ($Z = -0.103$; $P = 0.301$). To test whether the association with the symptom dimension scores is not merely a replication of the association with schizophrenia status, we performed a posthoc analysis in 186 non-psychiatric controls. Mean dimension scores were compared between carriers of the minor allele (AA and AG; N = 37) and homozygotes for the major allele (GG; N = 126). An association at trend level (testing at $P = 0.05/5$) was found between rs548181 and disorganization ($P = 0.022$), and positive symptoms ($P = 0.015$); the minor allele was again associated with lower dimension scores. Associations with negative ($P = 0.084$), depression ($P = 0.102$) and mania ($P = 0.058$) scores were not significant.

The aim of this study was to replicate earlier findings using a family based design and to investigate whether phenotype refinement can increase our understanding of the functional role of genetic variants. We were able to replicate only one of the ten SNPs previously shown to be associated with schizophrenia status. Taken into account the low effect sizes that were previously reported, this may be due to the relatively small sample size of this study. Interestingly though, the replicated SNP, rs548181, is significantly associated with severity of disorganization, and to a lower extent with positive and negative symptoms. It suggests that this particular SNP is associated with a functional variant that is involved in biological pathways which ultimately result in high levels of disorganization. We have previously reported on a specific subgroup of patients, the Kraepelinian subtype, which was distinguished from non-Kraepelinian patients based on high scores on disorganization and negative symptoms, and poor cognitive performance [Derks et al., 2012]. Study of progressive brain volume change further showed that disorganization scores were associated with a decrease in total brain volume after 5-year follow-up [Collin et al., 2012]. Our previous findings are supported by the present study in which we show that genetic variation is most strongly associated with the disorganization symptom dimension. Unfortunately, at this point, it remains unclear which functional variant explains the association between rs548181 and schizophrenia. Rs548181 is located at chromosome 11q24.2 within 1 kb distance from the gene *STT3A*. However, the role of this gene in schizophrenia or the Kraepelinian subtype of schizophrenia which is characterized by high levels of disorganization, relatively large cognitive impairments and progressive brain volume changes, is unknown. We can also not rule out the fact that rs548181 is associated with a functional genetic variant that is not located within this gene.

In conclusion, we have replicated the association of rs548181 with schizophrenia in a large family based sample. Analyses of refined phenotypes showed that this association is particularly strong for disorganization symptoms. Interestingly, high-disorganization scores are characteristic for the Kraepelinian subtype of schizophrenia. Future functional analyses of this genetic polymorphism should therefore focus on biological pathways involved in disorganized symptoms, brain development and cognitive performance.

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