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No effect of a common allelic variant in the Reelin gene on intermediate phenotype measures of brain structure, brain function and gene expression

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Abstract

Background—A recent genome-wide association study linked a common variant in *RELN* (rs7341475G) with risk for schizophrenia in women. In the largest neuroimaging intermediate phenotype study reported so far, we evaluated the effect of rs7341475 on an extended array of different neuroscientific measures.

Methods—Brain structure was evaluated using voxel-based morphometry (VBM) and diffusion tensor imaging (DTI). Brain function during working memory was examined with functional magnetic resonance imaging (fMRI). *RELN* expression was determined in post-mortem brain tissue of the dorsolateral-prefrontal cortex and hippocampus. A total of 736 datasets were examined (VBM: n = 230, DTI: n = 93, fMRI: n = 308, *RELN* expression: n = 105).

Results—Our analyses did not provide evidence for a significant main effect of gene or gene by sex interaction effect on any of the examined measures.

Conclusions—This study does not suggest a significant impact of rs7341475 on brain structure, function and *RELN* expression, arguing that this SNP and others linked with it do not affect brain measures related to the biology of schizophrenia.

Introduction

Schizophrenia is a neurobehavioral syndrome of presumed developmental origin. Several lines of evidence have implicated the reelin gene (*RELN*, 7q22) in the neurodevelopmental deficits that are associated with increased risk for the disorder. *RELN* encodes for the glycoprotein reelin, a secretory protease that plays a pivotal role in orchestrating the molecular processes that subserve neuronal migration and synaptic plasticity (1). Evidence for a role for *RELN* in schizophrenia pathogenesis arises from genetic association (2) and from gene expression studies (3), the latter demonstrating a reduction in mRNA and protein in post-mortem brain tissue of patients with schizophrenia, although negative findings have also been reported (4).

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The role of *RELN* as a potential candidate susceptibility gene has been suggested by evidence of allelic association with illness and by studies of epigenetic mechanisms that may account for reduced expression of *RELN* in schizophrenic brain (5). Using a DNA-pooling strategy, a recent genome-wide association study (GWAS) identified a common variant in the fourth intron of *RELN* (rs7341475G) as a female-specific risk factor for schizophrenia (6), thereby sparking considerable interest in the sex-specific aspects of the genetic architecture of the disease (7). While the finding received indirect support from the association of a nearby locus with disease-related cognitive phenotypes (8), it awaits further validation.

In recent years, the intermediate phenotype concept has become a successful strategy for the biological characterization and validation of schizophrenia risk gene effects (9,10). This approach is based on the concept that susceptibility genes for psychiatric illnesses increase risk by affecting risk-associated brain biology. It predicts that at the level of neurobiology, the effect size of susceptibility genes will be greater than at the level of the complex clinical phenotype; this implicates that brain-based phenotypes are detectable in risk allele carriers even if clinical diagnostic characteristics are absent. Similar results are now well-established for other complex medical disorders (Table S3 in Supplement 1), such as genetic risk for hypertension (e.g., by impacting on the sodium ion transport in cell membranes) or risk for type 2 diabetes (e.g., by predisposing individuals to obesity). The present study used this approach to address the question of whether and how rs7341475 is associated with sex-specific neurobiological effects at the level of brain structure, brain function, and gene expression. Based on prior studies linking schizophrenia risk variants to abnormalities in frontal-temporal function (11,12), gray matter volume (13), and white matter integrity (14), as well as the presumed impact of *RELN* risk variants on working memory function (8) and protein expression (15), we examined an extended range of different neuroscientific measures tapping these dimensions. With a total of 736 analyzed data sets, this work represents the largest neuroimaging intermediate phenotype study reported so far. It is also the first study that addresses the effects of a *RELN* variant on neuroimaging and brain mRNA expression phenotypes to date. We pursued a strongly hypothesis-driven candidate gene approach focusing on rs7341475, the only *RELN* variant reaching GWAS-accepted significance levels; no other genomic loci were examined. Our results are remarkably and consistently negative.

Methods and Materials

Subjects

All participating subjects were recruited as part of the Clinical Brain Disorders Branch “Sibling Study” as previously described (16). Briefly, participants were carefully screened by a psychiatrist to ensure they were free of any lifetime history of psychiatric or neurological illness. Only Caucasians of European ancestry were included to reduce population stratification artifacts. A total of 423 healthy adult individuals were studied with MRI, 308 subjects in the functional MRI study, 230 subjects in the voxel-based morphometry (VBM) study, and 93 subjects in the diffusion tensor imaging (DTI) study. A total of 167 subjects participated in more than one neuroimaging experiment. *RELN* expression in postmortem brain was studied in 36 patients with schizophrenia, and 69 healthy controls. Subject demographics and sample overlaps are reported in Tables S1 and S2.

Genotyping Procedures

We used standard methods to extract DNA from white blood cells with the Puregene DNA purification kit (Gentra Systems). The *RELN* single nucleotide polymorphisms (rs7341475) was determined by standard allelic discrimination Taqman assay that uses the 5' exonuclease activity of Taq DNA polymerase to detect a fluorescent reporter signal generated after PCR amplification. The assay cocktail (Assays on Demand) for individual SNP was obtained from

Applied Biosystems, Foster City, CA. The genotype distribution of the examined SNP did not deviate from Hardy-Weinberg equilibrium ($P > 0.05$). Based on the low frequency of the A allele (0.183) and to increase statistical power, G/A and A/A subjects were merged together in an A-carrier group for all subsequent analyses. Genotype frequencies are reported in Tables S1.

Neuroimaging

Three different neuroimaging techniques were used in this study. Brain function was studied with fMRI and a robust working memory paradigm (n -back task) as previously described (12). The impact of *RELN* genotype on global and regional gray matter volume was examined with VBM (17), using the VBM5 toolbox (<http://dbm.neuro.uni-jena.de/vbm/>) implemented in the Statistical Parametric Mapping software (SPM, <http://www.fil.ion.ucl.ac.uk/spm/>). The microstructural property of white matter tracts was examined with DTI and Tract-Based Spatial Statistics (TBSS), as implemented in the FMRIB Diffusion Toolbox (<http://www.fmrib.ox.ac.uk/fsl/>). Details on the employed MRI scanners, sequences and processing strategies are provided in the Supplementary Materials (see Supplement 1).

Statistical Analysis of Neuroimages

For all neuroimaging analyses, second-level ANCOVA models with appropriate nuisance covariates were defined to test for the main effect of genotype and the genotype by sex interaction. A hypothesis-driven region of interest (ROI) approach was used to investigate genotype-dependent alterations in brain regions that have been previously associated with schizophrenia pathophysiology (i.e., prefrontal lobe, posterior parietal lobe, basal ganglia, medial temporal lobe, and their interconnecting fiber tracts). For this purpose, masks were created using the Wake Forest University (WFU) Pickatlas (for fMRI and VBM, <http://www.fmri.wfubmc.edu>) and the John Hopkins University (JHU) white matter atlas (for DTI, <http://www.dtiatlas.org/>; details are provided in the Supplementary Materials, see Supplement 1). The significance threshold for all neuroimaging analyses was set to $P < 0.05$ false-discovery rate (FDR) corrected for multiple comparisons on the voxel level within ROIs. While this statistical procedure increased the sensitivity of our study, it provides good protection against false positives in imaging genetics analyses (18).

Gene expression study

Postmortem tissue samples of the hippocampus and the gray matter of the dorsolateral prefrontal cortex (DLPFC) were collected from 66 normal controls and 35 patients with schizophrenia described in detail in (4). Real-time PCR measurement and statistical analysis of *RELN* expression levels were performed as previously described (4).

Results

We did not detect an effect of the examined *RELN* variant on any of the examined neuroimaging parameters, neither in the whole sample, nor in the gender-stratified subsamples (Figure 1, all P values > 0.05 corrected for multiple comparisons). Also, no effect of genotype on *RELN* mRNA expression levels was observed in either schizophrenia patients or healthy controls. Finally, none of the measures examined in this study showed a significant gene by sex interaction effect. In contrast, reanalysis of the fMRI data in the very same sample provided evidence for a significant whole-brain corrected effect of the catechol-O-methyl transferase (*COMT*) Val¹⁵⁸Met polymorphism on prefrontal function (Figure S1 in Supplement 1) as previously described (19). Post hoc power analyses confirmed that thousands of subjects would have been required to show a significant effect of rs7341475 in the measured parameters (see Supplemental Materials in Supplement 1).

Discussion

In the present study, we investigated the impact of a common variant in *RELN* on the level of brain structure and function assayed with neuroimaging methods. Our results do not identify a biologic effect of this variant in *RELN* at this level of analysis, a finding that merits further comment. While the possibility exists that other populations or other measures might have yielded a different outcome, two main arguments suggest that this is a true negative result. First, we covered an extended spectrum of intermediate phenotype measures, all of which have previously been successful in characterizing the neurobiological mechanisms of schizophrenia risk gene effects (Table S1 in Supplement 1). Second, both, the sheer amount of analyzed data and the attempts to increase the sensitivity of our models make it unlikely that this result is due to a lack of statistical power; this notion was confirmed by the results of our *post hoc* power analyses. Notably, these findings do not disprove an association of *RELN* with schizophrenia in general; they implicate that a comprehensive attempt to validate the neurobiological impact of a particular allelic variant in *RELN*, that has previously been associated with risk for schizophrenia in females, was unsuccessful. In agreement with this, no association of rs7341475G with schizophrenia was observed in a recent genome-wide association study (Genetic Association Information Network, GAIN).

In recent years, a vast amount of conflicting association data has accumulated in the literature. As a result, concern has been voiced that the pathway to discovery might be obscured by false positive findings (20). Independent replication, publication of well-conducted negative studies, and cross-validation of research approaches are crucial in overcoming this issue. This work invites research on other genetic variants in *RELN* that could underlie some of the neurobiological abnormalities reported in schizophrenia.

Graphical illustration of the lack of effect of genetic variation in rs7341475 on different neurobiological phenotypes related to schizophrenia: (A) DLPFC activation during working memory performance, (B) Brain gray matter volume, (C) Fractional anisotropy of the anterior thalamic radiation, (D) *RELN* mRNA expression in the DLPFC normalized to a geometric mean of three housekeeping genes as described in (4). All plots illustrate intermediate phenotype data acquired in healthy controls stratified by genotype and gender. None of the mRNA expression analyses in the hippocampus and DLPFC provided evidence for significant effects of diagnosis, genotype, and genotype by sex interaction (all *P* values > 0.28). Bar graphs depict the mean and standard error of the measures for each genotype group (black = combined sample, males = light blue, females = dark blue).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. No effect of rs7341475 genotype on several neurobiological phenotypes