Polymorphisms in MIR137HG and microRNA-137-regulated genes influence gray matter structure in schizophrenia

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Accessibility
Evidence suggests that microRNA-137 (miR-137) is involved in the genetic basis of schizophrenia. Risk variants within the miR-137 host gene (MIR137HG) influence structural and functional brain-imaging measures, and miR-137 itself is predicted to regulate hundreds of genes. We evaluated the influence of a MIR137HG risk variant (rs1625579) in combination with variants in miR-137-regulated genes TCF4, PTGS2, MAPK1 and MAPK3 on gray matter concentration (GMC). These genes were selected based on our previous work assessing schizophrenia risk within possible miR-137-regulated gene sets using the same cohort of subjects. A genetic risk score (GRS) was determined based on genotypes of these four schizophrenia risk-associated genes in 221 Caucasian subjects (89 schizophrenia patients and 132 controls). The effects of the rs1625579 genotype with the GRS of miR-137-regulated genes in a three-way interaction with diagnosis on GMC patterns were assessed using a multivariate analysis. We found that schizophrenia subjects homozygous for the MIR137HG risk allele show significant decreases in occipital, parietal and temporal lobe GMC with increasing miR-137-regulated GRS, whereas those carrying the protective minor allele show significant increases in GMC with GRS. No correlations of GMC and GRS were found in control subjects. Variants within or upstream of genes regulated by miR-137 in combination with the MIR137HG risk variant may influence GMC in schizophrenia-related regions in patients. Given that the genes evaluated here are involved in protein kinase A signaling, dysregulation of this pathway through alterations in miR-137 biogenesis may underlie the gray matter loss seen in the disease.

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plausible genetic factors may influence the pathophysiology of the disorder. Studies using this approach indicate that the rs1625579 SNP influences a variety of structural and functional measures. Some effects appear to be common among risk allele carriers regardless of diagnosis, whereas other effects appear to be unique to schizophrenia carriers. Patients who were homozygous rs1625579 risk allele (TT) carriers in the Lett et al. had reduced hippocampal volumes, enlarged ventricle volumes and reduced white matter integrity as compared with patients who were carriers of the protective allele (GT or GG). These protective allele carriers showed no difference from control subjects in these measures. Patel et al. found that the hippocampal and ventricle effects of rs1625579 did not replicate in a larger sample; however, there was a consistent reduction in white matter volume in the mid-posterior corpus callosum for patients but not controls who were homozygous for the risk allele. Kelly et al. also found that the genotype had no effect on white matter integrity measures in control subjects; moreover, a separate recent study showed a specific effect of risk alleles in miR-137 on orbital cortex–striatum white matter integrity in patients but not in controls, highlighting that these effects may be related to the disease. Interestingly, at-risk subjects do appear to be influenced by this genotype in functional magnetic resonance imaging measurements of task-related brain activation. Subjects at risk for schizophrenia who were homozygous TT carriers had increased activation of the amygdala and pre- and post-central gyrius compared with GT carriers, whereas the opposite genotype effect was found in controls. All of these findings together suggest that, although this variant exerts some influence across subject groups, the overall impact appears to be distinct in subjects with schizophrenia or at risk for developing the disorder. As the rs1625579 risk allele is the common allele and the disorder is thought to be polygenic in nature, we propose that the variant is not influencing brain structure and function in these patients in isolation but may act in concert with other genetic and environmental factors that may contribute to schizophrenia risk as one might predict. Given that miR-137 is known to regulate many schizophrenia-risk genes, its effects on schizophrenia-related phenotypes may be modulated by genotypic variation in its target genes. We have previously demonstrated that genes regulated by miR-137 are over-represented within many schizophrenia-relevant pathways. In evaluating the genetic risk within the psychiatric genomics consortium (PGC) data among miR-137-regulated gene sets over-represented within these pathways, we found that several pathway-specific gene sets of miR-137-regulated genes are enriched with schizophrenia-risk variants, including genes involved in protein kinase A (PKA) signaling, long-term potentiation, axonal guidance signaling, Sertoli cell junction signaling and ephrin receptor signaling. We were able to replicate the schizophrenia-risk enrichment within the miR-137-regulated gene set involved in PKA signaling identified in the PGC data within the subjects analyzed in this study. Beginning with this gene set, we have now examined the interaction of genetic variation within miR-137-regulated genes, a miR-137 risk variant and schizophrenia disease status on brain measures. To our knowledge, no studies have yet evaluated the impact of the MIR137HG risk variant in combination with subsets of target gene variants on brain imaging phenotypes in patients and controls. The genes of interest included TCF4, PTGS2, MAPK1 and MAPK3. TCF4 and PTGS2 (which encodes the COX-2 protein) are experimentally validated target genes directly regulated by this microRNA. There is substantial evidence for TCF4 (ref. 31) in schizophrenia and, more recently, for PTGS2, MAPK1 and MAPK3 are associated with schizophrenia and are indirectly regulated by miR-137. These genes group within the PKA-signaling pathway according to Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA, USA, www.ingenuity.com), and this classification is supported by experimental evidence. The PKA signaling pathway was shown previously to be over-represented with predicted miR-137 target genes. Here we evaluated the impact of the rs1625579 genotype along with a GRC combining SNPs within and upstream of miR-137-regulated genes associated with schizophrenia risk and grouped within the PKA pathway on structural patterns of gray matter concentration (GMC) loss in schizophrenia. We used independent component analyses to avoid region-by-region or voxel-by-voxel analyses, and to reduce the number of phenotypes under consideration while capturing common patterns of GMC variation throughout the brain.

MATERIALS AND METHODS
Subject demographics
Subject imaging and genetic data were derived from the Mind Clinical Imaging Consortium (MCIC) shared repository and Northwestern University (NU) data sets. A total of 251 Caucasian subjects were analyzed from both data sets. After removal of subjects who did not pass quality-control filters, such as relatedness (2 subjects), genetic outliers (5 subjects), missing genotypes (13 subjects) and missing image quality (10 subjects), 221 subjects remained (90 controls and 60 cases from MCIC, and 42 controls and 29 cases from NU). The schizophrenia subject cohort was identified according to the Diagnostic and Statistical Manual of Mental Disorders criteria for diagnosis of schizophrenia, schizoaffective disorder or schizoaffective disorder. See Table 1 and Supplementary Table 1 for additional subject information. All subjects provided informed consent and all subject data were collected according to the institutional review board standards and approved protocols.

Genetic data
Genotyping was performed at the Mind Research Network Neurogenetics Core lab using the Illumina Human Omni-Quad 1M BeadChip for MCIC and the Illumina Human Omni-Quad SM chip for NU. Using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/), the genotype data from MCIC and NU were merged after updating SNP locations of MCIC to match those of the more recent NU data. Quality control was performed before and after merging with the following thresholds: Hardy–Weinberg equilibrium $P \leq 10^{-5}$, minor allele frequency $< 0.05$, missing rate per SNP $< 0.02$ and missing rate per individual $< 0.02$. Caucasian subjects were identified using the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) multidimensional scaling protocol (http://enigma.ini.usc.edu/protocols/genes-protocols/). Genetic outliers and related subjects were identified with identity-by-state and identity-by-decent testing in PLINK. One individual subject was removed from each pair or group of subjects with pi-hat values of 0.2 or greater. MCIC subjects were genotyped separately for the rs1625579 SNP at the Mind Research Network Neurogenetics Core lab using a custom TaqMan assay.

miR-137-regulated gene GRS
As noted above, we have previously found enrichment of schizophrenia-risk SNPs among several biological pathways of miR-137-regulated genes and replicated enrichment of schizophrenia-risk SNPs in PKA signaling in this cohort of subjects. This pathway contains the experimentally validated miR-137-regulated genes: MAPK1, MAPK3, PTGS2 and TCF4. To assess the influence of these SNPs on GMC among these subjects, we used these SNPs to calculate a genetic risk score (GRS). Therefore, GRS was based on the variants previously identified to be the top SNPs associated with schizophrenia risk within or upstream of these miR-137-regulated genes. These SNPs were rs2276195 (TCF4), rs10489401 (PTGS2), rs9610608 (MAPK1) and rs7202714 (MAPK3). See Table 2 for additional SNP information. The GRS was calculated across these SNPs for each subject based on the number of risk alleles (0, 1 or 2) per SNP weighted by its respective natural log-normalized (In) odds ratio and summed for all four SNPs as was done in Walton et al. The risk allele and odds ratio used to create GRS were derived from the PGC recent 2014 GWAS (http://www.med.yale.edu/pgc/downloads). The MIR137HG risk SNP rs1625579 genotype was not included in the PKA-signaling-based risk score to allow for an analysis of the influence of this SNP on these miR-137-regulated gene variants.
Imaging data

Structural T1 MRI scans were conducted across four sites (University of Minnesota, Massachusetts General Hospital, the University of Iowa and the University of New Mexico) for the MCIC data set and one site (NU) for the NU data set. MCIC scans were collected in the coronal orientation using Siemens (1.5 T), GE Signa (1.5 T) and Siemens Trio (3 T) scanners with voxel sizes of 0.625 × 0.625 × 1.5, 0.664 × 0.664 × 1.6 and 0.625 × 0.625 × 1.5, respectively. NU scans were collected in sagittal orientation with voxel sizes of 0.625 × 0.625 × 1.5, 0.664 × 0.664 × 1.6 and 0.625 × 0.625 × 1.5, respectively.

Image pre-processing

All T1 Images were co-registered to the same stereotactic space using an affine transformation, resliced to $2 \times 2 \times 2$ mm and segmented into cerebrospinal fluid and gray and white matters using the Statistical Parametric Mapping 5 software (SPMS) unified segmentation method. Images were visually inspected and evaluated for correlation to an averaged image across all subjects. Images with a correlation value $< 0.9$ to the mean image from all subjects were dropped from further analysis. Six subjects were dropped for poor image quality based on inspection and one for missing images. Three additional subjects were excluded for failing to pass the mean image correlation test. Following spatial normalization to Montreal Neurological Institute space, the images were then regressed for the effects of scanning site, gender and subject age to allow further analysis to be more sensitive to group and genotypic imaging differences. This was performed as age and gender were balanced across different sites and control/case count was balanced across sites. These regressed gray matter images were then smoothed with a full width half maximum Gaussian kernel of 10 mm. See $^{42,50,51}$ for more details on the methods used here.

Independent component analysis of imaging data

Independent component analysis (ICA) was performed on the pre-processed gray matter images. This analysis was performed using the source-based morphometry module of the GIFT toolbox (http://mialab.mrn.org/software/gift). $^{44}$ Estimation of the appropriate number of independent components to capture the variance within the subject image files was performed using a minimum description length method, and was determined to be 18. We performed an ICA using the Infomax algorithm and the ICASSO algorithm 20 times with a bootstrap and random initialization each time. The imaging data were decomposed into these 18 GMC components and loading coefficients or weights respective to the contribution of each structural component to each subject’s image. These loading coefficients were used to determine group contribution differences across these GMC components. The spatial maps for the components were all visually inspected, and no artifactual components were observed.

Imaging genetics statistical analysis

A multiple regression analysis was performed using the loading coefficients for the 18 independent components as dependent variables and diagnosis as the grouping factor to determine which components captured imaging variance because of the diagnostic group. Tests were deemed significant based on Bonferroni multiple testing correction.
(P < 0.002). The components that showed a significant difference between cases and controls were used in the following analyses (see Supplementary Table 3).

A multivariate analysis of covariance analysis was performed on the loading coefficients of the significant structural GMC components with the rs1625579 genotype, with chip type and diagnostic group included as factors, and the miR-137-regulated GRS included as a covariate (Supplementary Table 3). The following types of statistical analyses were considered: (1) The main effect of rs1625579 genotype, diagnosis, chip type and GRS, (2) the two-way interactions between genotype and GRS, genotype and diagnosis, GRS and diagnosis and (3) a three-way interaction between genotype, diagnosis and GRS. Given that only five subjects were homozygous for the G allele and that previous work found genotypic effects based on the analysis of collapsing the protective allele genotypes together, the rs1625579 genotype was analyzed in two groups, those homozygous for the T major allele and those having one or two of the minor allele G (GG/GT), as was performed previously. Power for these analyses was calculated using G*Power 3.1.53,54

RESULTS
Risk score results
We have previously identified many schizophrenia-relevant pathways that were significantly enriched with miR-137-regulated genes and associated with increased schizophrenia risk.16,29 Among these pathways, the association of schizophrenia risk with SNPs near or within miR-137-regulated genes in the PKA pathway has been replicated in the same cohort of subjects used here.29 Therefore, these SNPs were used to calculate a GRS. Before analyzing the differences in miR-137-regulated GRS between patients and controls, we examined the distribution of these scores within each group. Using the D’Agostino–Pearson normality test, which evaluates both kurtosis and skewness statistics in an omnibus test, we found that the GRS distribution for the control subjects (z = 2.36, P-value = 0.02) was not normal, whereas GRS among cases was normally distributed (z = 1.34, P-value = 0.18). Therefore, to normalize the control group scores, all scores were transformed using a negative inverse transformation (−1/(GRS+1)) in order to reduce the positive skew within the control group GRS and preserve the directionality of the risk scores. The transformed risk scores were normally distributed for both diagnostic groups (see Supplementary Figure 1 for histograms of the GRS before and after transformation). All further analyses were performed using the transformed GRS and will be further referred to simply as GRS.

The mean ± s.d. GRS for controls was −0.99 ± 0.02 and patients was −0.97 ± 0.03. Variance of GRS did not differ between the diagnostic groups using F-tests (F(88,131) = 0.76, P = 0.15), or between rs1625579 genotypic groups (F(70,149) = 0.93, P = 0.70). GRS variance was also not different between rs15652579 genotypic groups among controls only (F(44,86) = 0.88, P = 0.61) or schizophrenia subjects (F(25,62) = 0.87, P = 0.64). A Welch two-sample t-test determined that the GRS in patients were significantly greater than those in control subjects (P = 1x10−4). GRS were not significantly different between rs1625579 genotypic groups (P = 0.37).

Imaging results
Among the components identified by ICA, three components had loading coefficients significantly associated with diagnostic group following Bonferroni correction for multiple comparisons (comp1: t = −7.85, P-value = 1.86x10−13, comp6: t = −3.53, P-value = 6x10−4 and comp 11: t = 2.735, P-value = 7x10−3). These components and labeled regions are shown in Figure 1, Supplementary Table 2 and Supplementary Figure 2. As expected, many brain regions showed decreased GMC in the patients, particularly in the medial frontal, temporal, inferior parietal and occipital lobes.

Figure 1. The spatial component showing a genetic and diagnosis-interactive effect. Regions with greater gray matter concentration (GMC) in controls than in patients include the occipital lobe (Brodmann area 19), angular gyrus (Brodmann area 39), supramarginal gyrus and the inferior parietal lobule (Brodmann area 40). The spatial map is overlaid on a template brain, thresholded with z-scores >13.51. The heat map coloring indicates z-score intensity, with red indicating findings of GMC greater in controls and blue indicating areas of GMC greater in patients. White indicates areas with greatest z-scores. z-score is indicated by the bar on the right.

Imaging genetics results
Of the three components significant for diagnosis, one (component 11) had a significant three-way interaction between the rs1625579 genotype, the miR-137-regulated GRS and diagnosis (F(1,127) = 7.03, P-value = 0.007, power = 0.88), which was significant following Bonferroni correction. In contrast to this three-way interaction, we found no significant main effects of the rs1625579 genotype, chip type or GRS on GMC. Variance of this component was not significantly different between diagnostic groups (F(74,125) = 1.19, P = 0.40), patient rs1625579 genotypic groups (F(20,53) = 1.37, P = 0.35) or control rs1625579 genotypic groups (F(41,83) = 1.01, P = 0.96), and the loading coefficients were normally distributed in both the controls (z = 1.78, P-value = 0.08) and patients (z = -0.44, P-value = 0.66) according to the D’Agostino test. See Supplementary Table 3 for the results from all effects and interactions. This three-way interaction was also significant when performing the analysis of variance and permuting the response variable (5000 iterations, P-value = 2.2x10−4).

Post hoc univariate analysis of the loadings for this component for the two diagnostic groups separately, and evaluating for two-way interactions between GRS and the rs1625579 genotype, revealed that the identified three-way interaction in the multivariate analysis was driven by a two-way interaction in the schizophrenia subjects alone. No significant main effect of GRS or rs1625579 genotype was found in either the control or patient group, and no significant interaction (F(1,127) = 0.60, P-value = 0.44) was found in the healthy control group. However, the interaction between GRS and rs1625579 genotype (F(1,84) = 8.84, P-value = 3.9x10−3, n̄ 2 = 9.52 x 10−7, power = 0.86) was significant among the schizophrenia subjects. See Figure 2 for a graphical representation of this relationship.

To further assess this two-way interaction, a one-sided Pearson’s correlation analysis was performed for both genotype groups separately. This correlation analysis demonstrated a significant negative correlation (r(61) = −0.24, t = −1.9, P-value = 0.03) between miR-137-regulated GRS and GMC loading coefficients for this component among schizophrenia patients with the TT MiR137HG high-risk genotype. In contrast, a significant positive correlation (r(24) = 0.42, t = 2.25, P-value = 0.02) was identified between miR-137-regulated GRS and loading coefficients among patients with the GG/GT MiR137HG low-risk or protective genotype. No significant correlation was identified within the
Among these patients the rs1625579 genotype appears to indicate that the increased genetic risk by the MIR137HG genotype with cumulative risk summed across a subset of miR-137-regulated risk gene SNPs. The interaction of this genotype with the miR-137-regulated GRS is associated with reduced GMC within the occipital, parietal and temporal lobes. Within patients with schizophrenia, those with two risk alleles (TT) showed decreasing GMC in these regions with increasing GRS, whereas those with only a single or no risk allele showed increasing GMC with increasing GRS. This suggests that the miR-137-regulated gene risk may actually be protective in the absence of the miR-137-associated risk genotype, further emphasizing the importance of evaluating the combined influence of risk genotypes. This pattern was not present in healthy controls, and if anything was reversed (although not significantly). This is in agreement with previous findings22–25 demonstrating that this variant may exert some influence on brain structure and function in a manner unique to schizophrenia subjects, and provides further evidence that some effects of the variant may be dependent or modulated by other genetic factors such as the risk SNPs within these miR-137-regulated genes studied here. This finding may be indicative of a complex and interactive genetic architecture where the same networks of genes influence endophenotypes in patients and controls in distinct ways. Supporting this idea, a recent study suggests that the hidden heritability underlying the polygenic risk of schizophrenia may be mediated by complex gene–gene interactive networks that are associated with distinct endophenotypes.56 Genes that show limited significance for genetic risk in GWAS may have a robust cumulative influence on endophenotypes, as has been demonstrated for dorsolateral prefrontal cortex inefficiency, a well-established schizophrenia-associated intermediate phenotype.57 Recent research suggests that the combined methylation status of hsa-miR219a-5p target genes is correlated with hippocampal volume, another schizophrenia-related endophenotype, further implicating a role of miRNA regulation in choreographing complex genetic influence on schizophrenia-associated endophenotypes.58

**DISCUSSION**

Our results indicate a schizophrenia-specific interaction of the rs1625579 genotype with cumulative risk summed across a subset of miR-137-regulated risk gene SNPs. The interaction of this genotype with the miR-137-regulated GRS is associated with reduced GMC within the occipital, parietal and temporal lobes. Within patients with schizophrenia, those with two risk alleles (TT) showed decreasing GMC in these regions with increasing GRS, whereas those with only a single or no risk allele showed increasing GMC with increasing GRS. This suggests that the miR-137-regulated gene risk may actually be protective in the absence of the miR-137-associated risk genotype, further emphasizing the importance of evaluating the combined influence of risk genotypes. This pattern was not present in healthy controls, and if anything was reversed (although not significantly). This is in agreement with previous findings22–25 demonstrating that this variant may exert some influence on brain structure and function in a manner unique to schizophrenia subjects, and provides further evidence that some effects of the variant may be dependent or modulated by other genetic factors such as the risk SNPs within these miR-137-regulated genes studied here. This finding may be indicative of a complex and interactive genetic architecture where the same networks of genes influence endophenotypes in patients and controls in distinct ways. Supporting this idea, a recent study suggests that the hidden heritability underlying the polygenic risk of schizophrenia may be mediated by complex gene–gene interactive networks that are associated with distinct endophenotypes.56 Genes that show limited significance for genetic risk in GWAS may have a robust cumulative influence on endophenotypes, as has been demonstrated for dorsolateral prefrontal cortex inefficiency, a well-established schizophrenia-associated intermediate phenotype.57 Recent research suggests that the combined methylation status of hsa-miR219a-5p target genes is correlated with hippocampal volume, another schizophrenia-related endophenotype, further implicating a role of miRNA regulation in choreographing complex genetic influence on schizophrenia-associated endophenotypes.58

**Figure 2.** Relationship between gray matter concentration (GMC), diagnosis, rs1625579 genotype and miR-137-regulated genetic risk score (GRS). These graphs depict the interaction between the rs1625579 genotype and the miR-137-regulated GRS on structural loading coefficients of component 11, and the directionality of the relationship across diagnostic groups. The solid red lines indicate the best-fit trendline for subjects with the low-risk GG/GT genotype, whereas the solid blue line indicates that of the subjects with the TT or high-risk rs1625579 genotype. The area colored around the respective trend line s.e. The formula for each trendline is indicated on the figure nearest to the respective line. The difference in slope among the healthy control trendlines was nonsignificant (F(1,173) = 0.19, P-value = 0.7), whereas the difference among the schizophrenia trendlines was found to be significant (F(1,85) = 8.95, P-value = 3.6x10^-3).
Given the role of the genes studied here in the PKA pathway, our results suggest that, within schizophrenia, the rs1625579 MIR137HG SNP in conjunction with specific genotypes within the PKA pathway may cause altered regulation of the genes within our risk score, leading to decreased GMC within Brodmann area 19 of the occipital lobe, Brodmann area 39 of the angular gyrus and Brodmann area 40 of the supramarginal gyrus and inferior parietal lobe. These regions have all previously been implicated in schizophrenia and are associated with exploratory eye movement dysfunction,\textsuperscript{59} theory of mind or self-sensing deficits,\textsuperscript{60} hallucinations,\textsuperscript{61-64} adaptive control deficits\textsuperscript{65} and attention deficits.\textsuperscript{66,67} In addition, these regions are involved in visual processing, which is disrupted in schizophrenia.\textsuperscript{68} The evaluated miR-137-regulated genes and PKA signaling pathway appear to be critical for proper maturation of visual processing systems.\textsuperscript{69} This raises promising possibilities that these genetic variations within people with schizophrenia could be related to GMC and functional loss within these networks, leading to previously unexplained variation in clinical symptoms and capabilities.

Interestingly, a risk genotype for a variant within another miR-137 experimentally validated target gene, CACNA1C, was associated with reduced activity in Brodmann area 40 during orientation of attention in a functional magnetic resonance imaging study.\textsuperscript{66} Gray matter volume decrease of the supramarginal gyrus was also associated with poor cognitive test scores in unaffected relatives of schizophrenia patients,\textsuperscript{66} further suggesting genetic liability of alterations in this region.\textsuperscript{70} The 22q11.2 deletion, one of the highest known risk factors for schizophrenia, is associated with altered miRNA biogenesis\textsuperscript{71} and cortical morphological reduction of this region.\textsuperscript{72} The effect of the MIR137HG risk SNPs on the biogenesis of the miRNA has not been determined; however, a recent study indicates that major alleles in these SNPs including the risk T allele of rs1625579 results in lower levels of mature miR-137 than those with the minor and protective G allele.\textsuperscript{73} Furthermore, a post-mortem tissue analysis\textsuperscript{74} also suggests that the biogenesis of this miRNA may be disrupted in carriers of the rs1625579 risk genotype. Therefore, other findings implicating this region in schizophrenia may be caused in part by a reduction in miR-137.

Very few studies have evaluated the role of PKA signaling on GMC in the brain regions implicated here. However, it is clear that this signaling pathway\textsuperscript{74} and each of the investigated genes are associated with schizophrenia.\textsuperscript{31-34} Further research will be required to determine the exact mechanism of the association presented in this study, evaluate the replicability of these findings in other cohorts and determine whether these genetic factors also influence exploratory eye movement, self-sensing, hallucinations, adaptive control or attention characteristics. One important limitation of our study is the fact that subjects were scanned at multiple sites with different scanners, increasing intersubject variability and possibly lowering the power. However, our research suggests that our method of regressing site and scanner effects from the images during pre-processing can lead to reproducible results across multiple sites of data found within well-powered single sites alone.\textsuperscript{51} In addition, we cannot decipher the influence of antipsychotic medication usage and disease status on the GMC measures in the schizophrenia subjects, as medication information was not available for every subject. Indeed, it is possible that these genetic variants may influence the severity of such medication influence on gray matter. More research is required to tease apart the influence of the variants and medication use on GMC and to investigate the influence of other variants in miR-137-regulated genes. Nonetheless, our analysis is a first step into determining the combined effect of MIR137HG risk SNPs with miR-137-regulated gene variants on gray matter structure. Our results suggest that key schizophrenia regions, previously implicated in both structural and functional brain-imaging studies, are affected by rs1625579 vulnerability.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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