

# Schizophrenia miR-137 Locus Risk Genotype Is Associated with Dorsolateral Prefrontal Cortex Hyperactivation

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**Background:** miR-137 dysregulation has been implicated in the etiology of schizophrenia, but its functional role remains to be determined.

**Methods:** Functional magnetic resonance imaging scans were acquired on 48 schizophrenia patients and 63 healthy volunteers (total sample size  $N = 111$  subjects), with similar mean age and sex distribution, while subjects performed a Sternberg Item Response Paradigm with memory loads of one, three, and five numbers. Dorsolateral prefrontal cortex (DLPFC) retrieval activation for the working memory load of three numbers, for which hyperactivation had been shown in schizophrenia patients compared with control subjects, was extracted. The genome-wide association study confirmed schizophrenia risk single nucleotide polymorphism rs1625579 (miR-137 locus) was genotyped (schizophrenia: GG  $n = 0$ , GT  $n = 9$ , TT  $n = 39$ ; healthy volunteers: GG = 2, GT  $n = 15$ , and TT  $n = 46$ ). Fisher's exact test examined the effect of diagnosis on rs1625579 allele frequency distribution ( $p =$  nonsignificant). Mixed model regression analyses examined the effects of diagnosis and genotype on working memory performance measures and DLPFC activation.

**Results:** Patients showed significantly higher left DLPFC retrieval activation on working memory load 3, lower working memory performance, and longer response times compared with controls. There was no effect of genotype on working memory performance or response times in either group. However, individuals with the rs1625579 TT genotype had significantly higher left DLPFC activation than those with the GG/GT genotypes.

**Conclusions:** Our study suggests that the rs1625579 TT (miR-137 locus) schizophrenia risk genotype is associated with the schizophrenia risk phenotype DLPFC hyperactivation commonly considered a measure of brain inefficiency.

**Key Words:** Genes, imaging, mir137, rs1625579, schizophrenia, working memory

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**A** aberrant dorsolateral prefrontal cortex (DLPFC) activation, as measured using functional magnetic resonance imaging (fMRI) during working memory processing, is a robust correlate of schizophrenia (SZ) (1,2). Consistent with DLPFC inefficiency—more activation for a similar level of performance—in schizophrenia (3), we have shown DLPFC hyperactivation during a working memory load of three items on the Sternberg Item Recognition Paradigm (SIRP) in schizophrenia patients compared with control subjects even when task performance is equivalent (4). We have identified several genetic polymorphisms that are associated with DLPFC activation in schizophrenia (5,6) and subsequently identified several other putative schizophrenia risk genes as well as microRNAs based on a gene set enrichment analysis (7). MicroRNAs are short ( $< \sim 27$  nucleotides) noncoding RNAs that regulate the expression levels of other genes (8) via messenger (m)RNA cleavage or translation inhibition (9) and are hypothesized to play a role in the genetic liability for schizophrenia (10,11). The microRNAs identified in our previous study included miR-137 (7), which was recently suggested to play an etiologic role in schizophrenia based on a large genome-wide case-control association study of 51,695 individuals that found a genome-wide significance of  $p = 1.6 \times 10^{-11}$  for the rs1625579 single nucleotide polymorphism (SNP) in linkage disequilibrium with the miR-137 primary transcript as well as significant associations with predicted miR-137 targets (12).

Apart from its observed role in cancer (13–15), miR-137 is shown to regulate neural stem cell proliferation and differentiation in mouse embryonic neural stem cells (16), neurogenesis in mouse adult neural stem cells (17), and neuronal

maturation, including regulation of dendrite length, branch points, end points and spine density in mouse adult hippocampal neuroprogenitor-derived and mouse fetal hippocampus neurons (18). Decreased spine density has also been observed in the dorsolateral cortex of patients with schizophrenia compared with control subjects consistent with poor memory performance and suggests altered synaptic connectivity (19). Combined, these studies implicate miR-137-dysregulation in the etiology of schizophrenia and suggest that miR-137 may be involved in regulating DLPFC activation by affecting neural connectivity within the DLPFC resulting in abnormal (inefficient) neural transmission. The putative miR-137 rs1625579 schizophrenia risk SNP was not part of the Illumina (San Diego, California) Infinium Human-Hap300 Bead Array used for genotyping in our previous work (5). In this study, we genotyped the rs1625579 SNP to examine directly the association between the rs1625579 (miR-137 locus) schizophrenia risk alleles (genotypes) and SIRP three-item probe DLPFC activation on the Sternberg Item Recognition Test; the SIRP contrast shown to best separate schizophrenia versus control DLPFC activation (4).

To date, four human studies have examined the functional role of the rs1625579 (miR-137) SNP in schizophrenia (20–23). Cummings and colleagues (2012) (23) recently found in a sample of 399 patients with schizophrenia, bipolar I disorder, and schizoaffective disorder and 171 control subjects that rs1625579 TT genotype was associated with poor working memory, episodic memory, and attention. In contrast, Green and colleagues (12), who grouped schizophrenia patients into those with and without cognitive deficits, found that the rs1625579 SNP alone was not predictive of cognitive dysfunction severity or group status but that the G-allele predicted cognitive dysfunction group status when negative symptoms were included in a multinomial regression analysis. The latter finding is hard to interpret given that the common rs1625579 T allele is associated with schizophrenia risk status.

An electroencephalogram study by Decoster and colleagues (21) found that the rs1625579 SNP T allele was associated with lower P300 amplitude. This finding is in the hypothesized direction in that P300 amplitude for common rs1625579 risk allele (T) appeared to be associated with lower P300 amplitude, a well-known quantitative endophenotype for schizophrenia (24). Although the finding did not survive stringent Bonferroni correction, it nevertheless suggests a contribution of the rs1625579 (miR-137) SNP to the P300 deficit observed in schizophrenia that warrants further study.

An fMRI study by Whalley and colleagues (22) examined the effects of rs1625579 genotype on brain activation during a sentence completion task in individuals at high genetic risk for schizophrenia or bipolar disorder compared with healthy control subjects. They found a significant group by genotype interaction on left amygdala and pre-post central gyrus activation. Decomposition of the interaction effect showed that the interaction was due to higher activation in schizophrenia T allele homozygotes and lower activation in control T allele homozygotes compared with their respective G allele carriers during sentence completion versus baseline condition in the left amygdala and pre-postcentral gyrus. They also found lower right posterior medial frontal gyrus activation in rs1625579 T allele homozygotes compared with G allele carriers for a contrast sensitive to the difficulty of sentence completion irrespective of group status. These findings suggest that miR-137 gene regulation may affect brain physiology as measured with fMRI.

In this first working memory study examining the effects of rs1625579 genotypes on blood oxygen level-dependent activation

in schizophrenia, we hypothesize 1) lower SIRP load 3 working memory performance and longer response times in schizophrenia patients compared with healthy volunteers (HV) and 2) higher left DLPFC SIRP probe 3 activation in schizophrenia patients compared with healthy volunteers, consistent with findings from our previous report (4). Looking at specific effects of the rs1625579 (miR-137) SNP, our explicit directional hypothesis is 3) higher left DLPFC SIRP probe 3 activation for the rs1625579 T (schizophrenia risk) allele compared with G (nonrisk) allele carriers (TT > GT > GG genotypes) consistent with higher neural inefficiency in the schizophrenia rs1625579 risk allele carriers. Given the low frequency of the rare GG genotype, we collapsed across the GG and GT genotypes in the analyses and compared GG/GT with TT genotypes. We specifically focus on DLPFC retrieval activation on SIRP load 3 because it showed the strongest hyperactivation in patients with schizophrenia compared with control subjects even when behavioral performance was matched between the groups (4). We also explored whether the hypothesized genetic effect added to or interacted with diagnosis.

## Methods and Materials

### Participants

Forty-eight patients with schizophrenia (mean age  $\pm$  SD = 37.0  $\pm$  10.7, 35 male subjects) and 63 healthy volunteers (mean age  $\pm$  SD = 37.6  $\pm$  12.4, 39 male subjects) with similar mean age, sex, handedness, and race distributions, recruited from 9 sites part of the Function Biomedical Informatics Network Phase 2 study on multicenter functional imaging, participated in the study (Table 1). Study participants were between the ages of 18 and 70, had regular hearing levels (<25 db loss in either ear), had sufficient eyesight to see the task stimuli, were fluent in English, and were able to perform the cognitive tasks in the study. Inclusion criteria for the patients were a schizophrenia diagnosis based on the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (25). All patients were clinically stable on antipsychotic medication for at least two months. Schizophrenia patients and healthy volunteers with a history of major medical illness, a previous head injury or prolonged unconsciousness, substance and/or alcohol dependence, current migraine treatments, contraindications for MRI, and/or an IQ less than 75 were excluded. Patients with schizophreniform disorder or significant extrapyramidal symptoms/tardive dyskinesia and healthy volunteers with a current or past history of major neurologic or psychiatric illness (26) or with a first-degree relative with an Axis I psychotic disorder diagnosis were also excluded.

Both patients and control subjects were assessed with demographic and other socioeconomic questionnaires, the Edinburgh Handedness Questionnaire (27), the Fagerström Test for Nicotine Dependence (28), and the North American Adult Reading Test (29). Patients were assessed with the Scales for the Assessment of Positive (30) and Negative (31) Symptoms, Schedule for the Deficit Syndrome (32), Calgary Depression Scale (33), International Suicide Prevention Trial (InterSePT) Scale for Suicidal Thinking (34), Abnormal Involuntary Movement Scale (35), Barnes Akathisia Rating Scale (36), and the Simpson-Angus Scale (37). Ratings were standardized across sites through cross-site group training sessions with experienced clinical raters and by rating videotapes from several patients for comparisons with expert assessments.

Written informed consent, including permission to share deidentified data between the centers and with the wider research community, approved by University of California Irvine,

**Table 1.** Sample Demographics

	Schizophrenia Patients ( <i>n</i> = 48)	Healthy Volunteers ( <i>n</i> = 63)	Statistic	<i>p</i> Value
Mean Age (SD)	37.0 (10.7)	37.6 (12.4)	$t_{109} = .28$	.78
Gender (Male/Female)	35/13	39/24	$\chi^2_1 = 1.49$	.22
Handedness (Bilateral/Left/Right)	1/6/41	1/5/57	FET	.77
Mean Premorbid FSIQ <sup>d</sup> Estimate (SD)	105.1 (9.5)	113.3 (7.2)	$t_{105} = -5.05$	< .0001
Subject's Mean Years of Education (SD)	13.3 (1.5)	16.0 (1.9)	$t_{100} = -7.86$	< .0001
Mother's Mean Years of Education (SD)	13.3 (3.0)	13.8 (3.0)	$t_{91} = -.83$	.41
Father's Mean Years of Education (SD)	14.6 (3.4)	14.9 (3.6)	$t_{91} = -.40$	.69
Race (% Caucasian)	66%	75.8%		
Race <sup>b</sup>			FET	.20
American Indian or Alaskan Native	0	1		
Asian	3	3		
Black or African American	10	5		
White	34	52		
SANS (Global Measures Sum)	7.49 (3.71)			
SAPS (Global Measures Sum)	6.28 (2.68)			
InterSePT Suicidality Scale (Sum Of 12 Items)	2.26 (3.18)			
Calgary Depression Scale (Total Score)	5.36 (5.04)			
rs1625579 Genotype (GG/GT/TT)	0/9/39	2/15/46	FET	.47
SIRP Load 3% Correct (SD)/Mean RT (SD) <sup>c,d</sup>				
GG		100 (0)/677 <sup>e</sup>		
GT	89.6 (12.2)/967 (235)	97.4 (3.8)/736 (110)		
TT	94.2 (8.5)/784 (130)	96.5 (4.8)/696 (91)		
Absolute Mean % (SD) Left DLPFC Activation <sup>c</sup>				
GG	–	–.023 (.242)		
GT	.195 (.380)	.075 (.340)		
TT	.298 (.323)	.216 (.250)		

DLPFC, dorsolateral prefrontal cortex; FET, Fisher's exact test; FSIQ, Full-Scale Intelligence Quotient; InterSePT, International Suicide Prevention Trial; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SIRP, Sternberg Item Recognition Paradigm.

<sup>a</sup>FSIQ derived from the North American Adult Reading Test (29).

<sup>b</sup>Race is missing for one patient and two controls.

<sup>c</sup>Absolute means are provided for all three rs1625579 genotypes (GG, GT, and TT), although the analyses combine the less frequent GG and GT genotypes.

<sup>d</sup>Response times were collected at eight of the nine sites (90 of 111 subjects).

<sup>e</sup>No SD, only a single subject with response time.

California Los Angeles, New Mexico, Iowa, Minnesota, and North Carolina as well as Brigham and Women's Hospital, Massachusetts General Hospital, and Yale and Duke University Institutional Review Boards, was obtained from all study participants.

### Genotyping

We genotyped the rs1625579 SNP reported in the recent genome-wide association study (GWAS) study (12). Three intergenic Infinium HumanHap300 Bead Array SNPs (rs1938570, linkage disequilibrium [LD] = .828; rs1702292, LD = .806; and rs4378243, LD = .8) are in >80% LD with rs1625579 but are at greater distance to miR-137. Genotyping of rs1625579 was performed by real-time polymerase chain reaction using TaqMan probes and primers (Applied Biosystems, Grand Island, New York). We performed polymerase chain reaction on 10 ng of genomic DNA in a 5  $\mu$ L final volume using the TaqMan Genotyping Master Mix and TaqMan SNP genotyping assay on a 7900 HT real-time sequence detector (Applied Biosystems). The TaqMan assay was previously validated by Sanger sequencing with 100% concordance on 10 random samples.

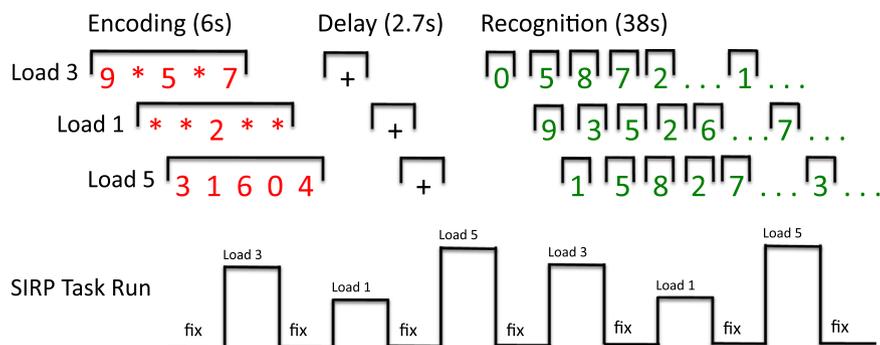
### SIRP

Participants performed the SIRP, a working memory paradigm comprising encoding, delay, and recognition phases (Figure 1). During encoding participants memorized one, three, or five red

digits that were visually presented for 6 sec. Following a 2.7 sec delay, 14 green single probe digits were presented serially (50% targets), and participants indicated, via button-box presses, whether a probe was a target or foil (member or nonmember of the memorized set, respectively). Probe digits were presented for 1.1 sec followed by a jittered delay (.6–2.486 sec). After each task block (load 1, 3, or 5) a jittered duration (mean = 12 sec, range: 4–20 sec) screen with a flickering cross was presented. Each of the three working memory loads was presented twice in pseudorandom order within a run and each participant performed three runs. Stimulus presentation and response collection was performed with E-Prime (Psychology Software Tools, Sharpsburg, Pennsylvania), and participants performed at least one practice run independently before the scan to ensure that they were able to achieve greater than 75% accuracy on the task. To sustain subject motivation throughout the imaging session, participants were able to earn 5 cents for each correct task response. Mean accuracy and mean response times were calculated for each of the working memory loads [for additional task paradigm details see Potkin *et al.* (4)].

### Image Acquisition

Imaging data were acquired at nine imaging centers (4) and included a localizer for identification of the anterior commissure–posterior commissure axis, a high-resolution sagittal T1-weighted



**Figure 1.** The Sternberg Item Response Paradigm (SIRP) is a working memory paradigm in which participants study one, three, or five digits. After a delay, they are presented with 14 probe digits. Participants are asked to indicate via button press whether the probe digits were part of the study set.

scan (General Electric: fast spoiled gradient recalled; Siemens: magnetization prepared rapid acquisition gradient echo, with field of view [FOV] = 24 cm, 1.2–1.5 mm slice thickness, 160–170 slices), a T2-weighted scan to set slice prescription for the functional imaging scans (FOV = 22 cm, matrix = 256 × 192, 27 slices [thickness/gap = 4 mm/1 mm]), b0 field maps on the Siemens scanners, and T2\*-weighted gradient echo planar imaging (EPI) scans (FOV = 22 cm, repetition time/echo time = 2 sec/30 msec, flip angle = 90°, matrix = 64 × 64, and 27 anterior commissure–posterior commissure aligned slices [thickness/gap = 4 mm/1 mm]). Participants were required to have a normal night of sleep and no more than one alcoholic drink the night before the scan. Subjects refrained from caffeinated beverages 2 hours and smoking 40 minutes before the scan.

### Image Analysis

We obtained DLPFC activation data from one of our previous studies (4). Detailed image analysis procedures are provided in Potkin *et al.* (4), and detailed multicenter fMRI quality assurance procedures have been reported on in Glover *et al.* (38). Briefly, blind to group errors in the imaging data were identified via image meta-data files, Neuroimaging Informatics Technology Initiative image headers, by visual inspection of the raw EPI data (e.g., poor fat suppression, poor slice prescription), and by AFNI's 3dToutout (39) tool (runs with more than 34 of 177 volumes with outlier spikes were removed from the analyses) (4). EPI data were corrected for head movement using FMRIB Software Library's (FSL) (40) MCFLIRT by aligning all EPI volumes to the middle volume of each run, FSL's PRELUDE and FUGUE were used to B<sub>0</sub> correct images, and slice-time correction was performed and images smoothed to 8 mm full width at half maximum (41).

First-level statistical analyses were carried out for each run using the general linear model, predicting time series functional imaging data with encoding and recognition epochs convolved with a double gamma hemodynamic response function. Temporal derivatives were included in the model to allow for a better fit of the data by accounting for temporal variation. Statistical contrasts included encoding and recognition epochs versus fixation baseline as well as between-load contrasts. First-level statistical images were registered to the Montreal Neurological Institute—152 standard space using a 12-parameter affine transformation (42) and second-level fixed effects analyses combined the three runs from each subject. Blind to group base images with obvious structural flaws or a Jaccard (overlap) index of the base image to the Montreal Neurological Institute—152 atlas larger than 1.5 interquartile range above the 75th percentile of the remaining values, and second-level statistical maps with clear

evidence of excessive noise ( $n = 6$ ) were excluded from further analyses. Third-level random effects analyses were performed to create contrast maps for patients and controls separately ( $p < .05$ , false discovery rate correction).

The left DLPFC region of interest used in this study included the union of all gray matter voxels that showed significant probe-associated increases with working memory load ( $p < .05$ , based on false discovery rate correction) in either the schizophrenia patient or healthy volunteer group analysis within Brodmann areas 9 and 46 of the WFU PickAtlas (43,44). This combined structural-functional region of interest selection method was chosen to confine the extraction of percent signal change to the DLPFC while allowing for group differences in activation patterns across subregions of the DLPFC and reducing the likelihood of washing out our hypothesized group differences due to signal averaging over a large region. DLPFC percent signal change was extracted from the second level contrast of parameter estimates (copes) images.

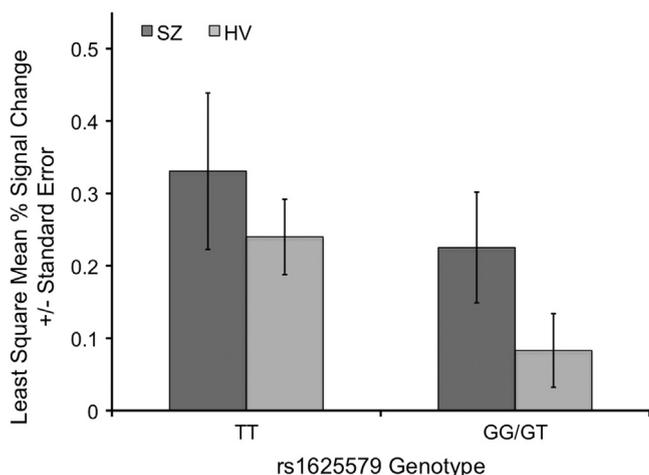
### Statistical Analyses

Given the low frequency of the rs1625579 GG genotype, the distribution of rs1625579 genotypes across diagnoses was examined using the Fisher's exact test (SAS/STAT software version 9.2, Proc Freq; SAS Institute Inc., Cary, North Carolina). The frequency distribution of rs1625579 genotypes did not differ between patients and controls (Table 1). The rs1625579 genotype frequency distributions for both patients and controls were in Hardy-Weinberg equilibrium. Given that only two control subjects had the rare GG genotype, GG and GT genotypes were collapsed in all subsequent analyses. Group (patient/control) and genotype (GG+GT/TT) effects on left DLPFC SIRP load 3 probe activation were examined using four univariate mixed model regression analyses (SAS 9.2, Proc Mixed; SAS Institute Inc.) in which left DLPFC probe 3 activation was predicted either with 1) diagnosis (patient/control), to confirm DLPFC hyperactivation in patients compared with control subjects; 2) genotype (GG+GT/TT), to test the hypothesis that the rs1625579 T allele is associated with DLPFC hyperactivation; 3) diagnosis and genotype, to examine whether group or genotype add in predicting DLPFC activation; and 4) diagnosis, genotype, and the diagnosis × genotype interaction, to examine whether group and genotype interact in predicting DLPFC activation ( $[SZ-TT \text{ vs. } HV-TT] > [SZ-GG/GT \text{ vs. } HV-GG/GT]$ ). All analyses included site, sex, and age as covariates and used planned one-tailed contrast analyses to test the specified directional hypotheses for diagnosis, genotype, and diagnosis × genotype effects. Analyses using similar models were performed to examine diagnosis and possible rs1625579 genotype effects on SIRP load 3 working memory performance and response times. Even though

a previous analysis showed no evidence of sample stratification (5), in the current analysis, we controlled for the possible confound of rs1625579 allele frequency differences between ethnicities by reanalysis of the data in a central European-only sample, identified based on a HAPMAP3-based multidimensional scaling and cluster analysis (<http://pngu.mgh.harvard.edu/purcell/plink>) (45). In addition to our hypothesis-driven DLPFC region of interest analysis, we performed an exploratory analysis in image space examining the effect of rs1625579 genotype on three-item SIRP probe activation ( $z > 2.3$ ,  $p < .05$ ) with a mixed effects analysis using FSL's (40) FLAME controlling for age, sex, site, and diagnosis.

## Results

Mixed-model regression analyses confirmed left DLPFC hyperactivation for SIRP load 3 probes in SZ patients compared with healthy volunteers ( $t_{100} = 1.91$ ,  $p = .03$ ). Individuals carrying the common miR-137 schizophrenia risk genotype (rs1625579 TT) showed hyperactivation compared with those carrying the less frequent GG/GT genotype ( $t_{100} = 2.17$ ,  $p = .02$ ; Figure 2). The analysis of additive effects of genotype and diagnoses showed significant effects of genotype (TT > GG/GT;  $t_{99} = 1.99$ ,  $p = .03$ ) as well as diagnosis (SZ > HV;  $t_{99} = 1.77$ ,  $p < .05$ ) on left DLPFC activation. The analysis of interaction effects of genotype and diagnosis did not show a significant interaction effect ( $t_{98} = .35$ ,  $p = .36$ ) on left DLPFC activation, whereas the main effects for diagnosis and genotype remained similar. Analysis of the CEU-only sample showed similar, but more highly significant, findings for diagnosis ( $t_{65} = 3.89$ ,  $p = .02$ ) and genotype ( $t_{65} = 4.20$ ,  $p = .01$ ) and no significant interaction. Behavioral data analysis confirmed the significantly lower working memory performance ( $t_{96} = -3.5$ ,  $p = .0007$ ) and longer response times ( $t_{76} = 40.95$ ,  $p = .0005$ ) on SIRP load 3 in SZ patients (least square mean  $\pm$  SE = 89%  $\pm$  1.3; 951  $\pm$  27 msec) compared with HV (94%  $\pm$  1.0; 837  $\pm$  20 msec) previously observed (4), but working memory performance and response times were similar for rs1625579 genotypes: 92%  $\pm$  .8% versus 92%  $\pm$  1.4% and 869  $\pm$  17 msec versus 918  $\pm$  29 msec for TT and GG/GT genotypes, respectively. Exploratory whole brain



**Figure 2.** miR-137 rs1625579 TT genotype associated with dorsolateral prefrontal cortex (DLPFC) hyperactivation during working memory. Individuals with rs1625579 TT genotypes show higher left DLPFC probe activation on Sternberg Item Response Paradigm working memory load 3 than individuals with the rs1625579 GT or GG genotypes. Schizophrenia patients (SZ) show higher DLPFC probe activation on working memory load 3 than healthy volunteers (HV).

comparisons found two significant clusters in which individuals with rs1625579 TT genotypes showed higher activations compared with rs1625579 GG/GT genotypes (Figure 3). There were no significant rs1625579 GG/GT > TT clusters. Although the DLPFC activations were not part of the significant clusters, there was a peak activation in the left DLPFC ( $z = 2.67$ ,  $p < .004$ , uncorrected; Figure 3).

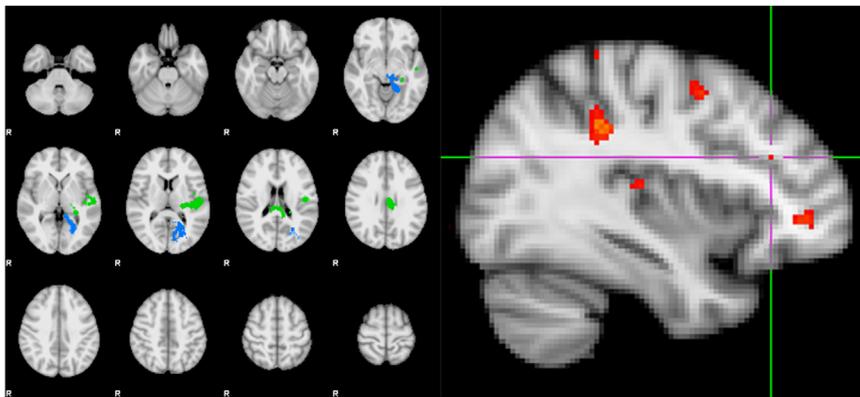
## Discussion

The main finding of our study is that the rs1625579 TT (miR-137) schizophrenia risk genotype is associated with left DLPFC hyperactivation compared with the combined GG/GT genotype irrespective of diagnosis. Given equivalent working memory performance between rs1625579 TT and GG/GT carriers, we interpret the observed DLPFC hyperactivation in individuals with the rs1625579 TT genotype compared with those with the GG/GT genotypes as being consistent with neural inefficiency in rs1625579 schizophrenia risk allele carriers; similar to DLPFC inefficiency observed in patients with schizophrenia (3,4). To our knowledge, this is the first report of a link between the rs1625579 SNP (miR-137) and working memory-associated brain activation as measured with fMRI.

The biology underlying the observed miR-137 TT genotype associated neural inefficiency is as of yet unknown though studies of miR-137 expression in mice have shown that miR-137 regulates neural stem cell proliferation and differentiation (16), neurogenesis (17), and neuronal maturation (18). Together with evidence that miR-137 is highly expressed in the rat neuron synaptosome compared with the cytosome (46) and evidence of reduced spine density in DLPFC cortical pyramidal neurons in schizophrenia, these findings suggest that miR-137 may be involved in the development of dysfunctional (neural, synaptic) connectivity within the DLPFC (19) as well as possibly between brain regions (47,48).

To date, no human postmortem studies have shown aberrant expression of miR-137 in brains of adult patients with schizophrenia compared with control subjects; for review see Beveridge *et al.* (49). However, recent *in vitro* data indicating that five schizophrenia candidate genes are regulated by miR-137 (50,51) strongly suggests a role for miR-137 in schizophrenia. Moreover, it is possible that other mechanisms such as differential expression miR-137 across the life span or epigenetic mechanisms regulating miR-137 target genes may account for the lack of observed effects in published postmortem work. In fact, miR-137 does not function in isolation but is indirectly regulated by miR-132 (17), the expression of which has been found to differ between patients and control subjects in several studies (52) and may also be influenced by more general perturbations in the microRNA biogenesis pathway (49).

With regard to the functional phenotype associated with the miR-137 risk SNP in living humans, there is some convincing evidence of an impact of miR-137 on cognition (23,46), some evidence of an impact on the P300 event-related potential component (21) and on the fMRI-based blood oxygen level-dependent signal (22), including that reported in the current study. Of interest is also that some cases diagnosed with autism spectrum disorder with hemizygous deletions on chromosome 1p21.3 have been reported (53). Autism has established clinical and genetic overlap with schizophrenia (54–56), and the possible involvement of miR-137 in chromosome 1p21.3 deletion syndrome was recently investigated (46).



**Figure 3.** Exploratory whole brain comparison of rs1625579 TT vs. GG/TG genotypes showed two significant clusters ( $z = 2.3$ ,  $p < .05$ ). Left: the cluster in green included peak activations in the left cingulate gyrus—posterior division ( $z = 4.11$ ; Montreal Neurological Institute [MNI] coordinates:  $-6, 22, 28$ ), left Heschl's gyrus ( $z = 3.72$ ; MNI coordinates  $48, 22, 6$ ) extending into the left planum temporale ( $z = 3.46$ ; MNI coordinates  $50, 24, 2$ ), and the left central opercular cortex ( $z = 3.45$ ; MNI coordinates  $46, 14, 18$ ). The cluster in blue included the left parahippocampal gyrus—posterior division, extending in the left lingual gyrus ( $z = 4.25$ ; MNI coordinates  $12, 40, -4$ ), left intracalcarine cortex ( $z = 3.98$ ; MNI coordinates  $20, 72, 10$ ), the left lingual gyrus ( $z = 3.84$ ; MNI coordinates  $18, 48, -2$ ), and the left intracalcarine cortex ( $z = 3.8$ ; MNI coordinates  $22, 68, 8$ ). Right: Exploratory examination beyond the significant clusters, for the rs1625579 TT > rs1625579 GG/TG genotype contrast at  $z = 2.67$ ,  $p < .004$  (uncorrected) showed significant voxels in the left middle frontal gyrus activation ( $z = 2.67$ ; MNI coordinates  $36, 30, 24$ ). L, left; R, right.

In sum, several lines of converging evidence indicate that miR-137 may play a role in schizophrenia. The GWAS finding by Ripke and colleagues (12) in conjunction with the Gene Set Enrichment Analysis finding by Potkin and colleagues (7), miR-137 involvement in developmental brain processes (16–18,46), the confirmed regulation of schizophrenia candidate genes by miR-137 (50,51), some recent observations of rs1625579 associations with cognition (23,46), electroencephalographic (21) and fMRI (22) measures as well as the therapeutic promise of microRNA related treatments provide impetus for future studies of the role of miR-137 in schizophrenia. The findings from this study suggest that miR-137 may regulate neural efficiency shown to be abnormal in schizophrenia.

Our findings lend additional support to the use of fMRI-based physiologic activations to understand the brain effects of schizophrenia liability genes (57) including those that regulate other genes (e.g., microRNAs). They encourage further investigation of miR-137 microstructure, expression of microRNA in different cell types and compartments, and its interactions with other non-coding elements (e.g., miR-132) and its target genes (50,51). It must be noted that DLPFC inefficiency is influenced by other gene variations, including ones that affect neurotransmitter levels such as D-amino acid oxidase and catechol-*O*-methyltransferase (58) and spine density such as DISC1 (59), corroborating the interpretation that genes contributing to altered synaptic connectivity may influence DLPFC inefficiency.

Strengths of the study are that 1) it used a well-established Sternberg item response functional imaging paradigm that shows robust hyperactivation in schizophrenia patients compared with controls (for retrieval at load 3) when using data collected from multiple scanners even when behavioral performance is matched between the groups (4); 2) it examined a gene identified by a large GWAS study with independent sample replication (12) as well as implicated by an independent group (7); 3) activation differences were observed between genotypes despite similar SIRP load 3 working memory performance and response times; 4) the rs1625579 genotypes were based on a validated genotyping assay, which is considered less ambiguous than based on imputation (60); 5) the strength of the findings increased in a more homogeneous, CEU-only sample that controls for possible

stratification confounds; and 6) a voxelwise comparison, although not cluster-level significant, showed a peak activation (rs1625579 TT > GG/TG) in the left DLPFC. Some limitations must also be noted. The sample size is relatively small for a genetic study without a replication cohort. However, concerns about type I error are minimized given that 1) the study examines the functional role of the rs1625579 risk SNP, which was identified as a schizophrenia risk allele based on a GWA study with a cohort of 51,695 individuals that included a replication sample; 2) the significant rs1625579 risk SNP effects on DLPFC activation were in the a priori-predicted direction (DLPFC hyperactivation in patients with schizophrenia as well as in individuals with the schizophrenia risk allele [T]); and 3) the effects were in the same direction for both patients and controls. The G allele is rare, and our sample included only two control subjects with the GG genotype and therefore did not allow for a more parametric analysis of the genotype effect (TT > GT > GG). The rs1625579 SNP maps about 9 kb from the pre-miR137 sequence within an intron of the MIR137HG locus telomeric to the pre-miR137 sequence that maps within the third exon of the MIR137HG. The high LD of the region extends to include the gene DPYD (61). A clump LD analysis cannot exclude that rs1625579 may map to DPYD which is also a predicted miR-137 target (62).

MiR-137 was previously implicated in a gene set enrichment analysis (7), an SNP (rs1625579) recently showed the strongest effect in a GWAS with a discovery sample of 21,856 and a replication sample of 29,839 independent individuals (12) and shows an association with a schizophrenia phenotype that has been well characterized in a large functional imaging study (4). This phenotype that has been shown to be highly heritable (63) in a region shown to be highly correlated with genetic liability for schizophrenia (64,65), lending significant weight to the finding and encouraging further exploration of the functional role of miR-137 in schizophrenia etiology.

Our study suggests that the putative miR-137 locus rs1627759 TT schizophrenia risk genotype (12) is associated with DLPFC hyperactivation, a known schizophrenia risk phenotype and a measure of brain inefficiency. These findings suggest that the functional implications of miR-137 gene-regulation are measurable with functional magnetic resonance imaging even at relatively

modest sample sizes and that the miR-137 gene regulatory network merits further investigation with regard to its etiologic role in schizophrenia.

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