Genetic Variation in CACNA1C Affects Brain Circuitries Related to Mental Illness

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Context: The CACNA1C gene (α-1C subunit of the L-type voltage-gated calcium channel) has been identified as a risk gene for bipolar disorder and schizophrenia, but the mechanism of association has not been explored.

Objective: To identify the neural system mechanism that explains the genetic association between the CACNA1C gene and psychiatric illness using neuroimaging and human brain expression.

Design: We used blood oxygenation level–dependent (BOLD) functional magnetic resonance imaging (fMRI) to measure brain activation in circuitries related to bipolar disorder and schizophrenia by comparing CACNA1C genotype groups among healthy subjects. We tested the effect of genotype on messenger RNA (mRNA) levels of CACNA1C in postmortem human brain. A case-control analysis was used to determine the association of CACNA1C genotype with schizophrenia.

Setting: National Institutes of Health Clinical Center.

Patients: Healthy men and women of white race/ethnicity participated in the fMRI study. Postmortem samples from normal human brains were used for the brain expression study. Patients with schizophrenia and healthy subjects were used in the case-control analysis.

Main Outcome Measures: BOLD fMRI, mRNA levels in postmortem brain samples, and genetic association with schizophrenia.

Results: The risk-associated single-nucleotide polymorphism (SNP rs1006737) in CACNA1C predicted increased hippocampal activity during emotional processing (P=.001 uncorrected, FDR=.05, z=3.20) and increased prefrontal activity during executive cognition (P=2.8e-05 uncorrected, FDR=.01, z=4.03). The risk-associated SNP also predicted increased expression of CACNA1C mRNA in human brain (P=.002). CACNA1C was associated with schizophrenia in our case-control sample (odds ratio, 1.77; P=.03).

Conclusions: The risk-associated SNP in CACNA1C maps to circuitries implicated in genetic risk for bipolar disorder and schizophrenia. Its effects in human brain expression implicate a molecular and neural system mechanism for the clinical genetic association.

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Several research groups have performed independent genome-wide association studies1-3 of bipolar disorder, with little agreement about the most associated loci. However, a comparison of the Wellcome Trust Case Control Consortium and STEP-UCL studies identified CACNA1C (α-1C subunit of the L-type voltage-gated calcium channel) as showing the strongest consistent signal.3 The most risk-associated single-nucleotide polymorphism (SNP) in this region (rs1006737) across the 2 studies showed association in a separate data set, the so-called ED-DUB-STEP2, as well as in a combined analysis of the 2 data sets (rs1006737, P = 7.0 × 10^-8),4 providing further evidence that CACNA1C is a credible susceptibility locus for bipolar disorder.

Because statistical association with clinical diagnosis does not establish biologic significance or identify a mechanism of risk, it is important to extend the statistical evidence with biological data. One approach that has become increasingly informative in translating clinical associations with psychiatric disorders into potential neural mechanisms of risk has been the use of neuroimaging to map gene effects in brain.5-7 Therefore, we used functional magnetic resonance imaging (fMRI) to test the effects of risk-associated variation in this gene on specific patterns of brain activity that have been associated with mental illness and with increased genetic risk for mental illness. Patients with bipolar disorder have previously been shown to exhibit elevated amygdala6 and hippocampal7 activity in response to emo-
tional stimuli. There also is evidence that individuals at increased genetic risk for bipolar disorder show similar patterns of brain activity, suggesting that this may reflect a neural mechanism of genetic risk.10,11 As a risk gene for bipolar disorder, we hypothesized that healthy subjects who are carriers of the risk-associated allele (A [minor allele]) of CACNA1C would have increased amygdala and hippocampal activity in response to emotional stimuli compared with carriers of the common allele (G). Because this gene has recently been associated with risk for schizophrenia, although with less statistical power,12 we further hypothesized that individuals with this risk-associated allele would show inefficient prefrontal activity during a working memory task, which has been identified as a potential biologic intermediate phenotype related to genetic risk for schizophrenia.13

**IMAGING SUBJECTS**

Healthy adults participated in an fMRI study in the Clinical Brain Disorders Branch Sibling Study at the National Institute of Mental Health, National Institutes of Health.14 The study was approved by the National Institute of Mental Health Institutional Review Board. All participants were assessed using the Structured Clinical Interview for DSM-IV. All subjects were physically and psychiatrically healthy; specific exclusion criteria have been previously reported.6 Only white subjects were physically and psychiatrically healthy; specific demographics for the imaging study are listed in Table 1.

**IMRI TASKS**

**Emotional Memory Task**

The emotional memory task involved the encoding and retrieval of aversive scenes,13 which has been shown to reliably engage the hippocampus in healthy volunteers.16-18 The scenes, selected from the International Affective Picture System,19 were presented in a block-designed task with 2 blocks of aversive or neutral scenes alternating with blocks of resting state for encoding and retrieval blocks. During experimental blocks, 6 scenes of similar valence (neutral or aversive) were presented serially to subjects for 3 seconds each. During resting blocks, participants were asked to attend to a fixation cross presented in the center of the screen for 18 seconds. These fixation blocks were treated as a baseline in the fMRI analyses. During the encoding blocks, subjects were instructed to choose whether the scene depicted an indoor or outdoor scene. During the retrieval blocks, subjects were instructed to select the scenes seen during the encoding session (ie, old) or the scenes not seen during the encoding session (ie, new). In each retrieval block, half of the scenes were old (ie, presented during the encoding session). Each session (encoding or retrieval) consisted of 17 blocks (4 aversive, 4 neutral, and 9 rest conditions). Subjects completed the entire encoding session before beginning the retrieval session after a brief delay (about 2 minutes). The presentation of indoor and outdoor scenes for the encoding session, as well as the presentation of old and new scenes for the retrieval session, was counterbalanced within each block. In addition, the presentation order of aversive and neutral blocks was counterbalanced across subjects. The total imaging time was 3 minutes and 40 seconds for this task. In this study, only the aversive encoding and aversive retrieval tasks were analyzed. There were no significant differences between genotype groups during the aversive retrieval task. BOLD fMRI was performed on a 3-T imaging system (Sierra; GE Medical Systems, Milwaukee, Wisconsin) using a gradient-echo echoplanar imaging sequence for all fMRI tasks. Specific parameters for the emotional memory task are the following: 24 axial sections, 4-mm section thickness, 1-mm gap between sections, 2000-millisecond repetition time, 28-millisecond echo time, 24-cm field of view, and 64 × 64-pixel matrix.

**Emotional Faces Task**

The face-matching task is a simple perceptual task, which has previously been shown to robustly engage the amygdala.3,20,21 The block fMRI paradigm consists of the following 2 experimental conditions: an emotional face-matching condition and a sensorimotor control task. The face-matching task consisted of five 30-second blocks. Blocks 1, 3, and 5 were sensorimotor blocks, and blocks 2 and 4 were emotion blocks. Each sensorimotor and emotion block consisted of six 5-second trials. Each trial involved the presentation of 2 images in the lower panel

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**Table 1. Sample Demographics for the Imaging Studies**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Genotype</th>
<th>Emotional Memory Task</th>
<th>Emotional Faces Task</th>
<th>Working Memory (N-Back) Task</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=57)</td>
<td>GA (n=43)</td>
<td>AA (n=16)</td>
<td>All (n=116)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>29.37 (8.94)</td>
<td>29.47 (9.57)</td>
<td>27.88 (8.02)</td>
<td>29.20 (9.00)</td>
</tr>
<tr>
<td>Female sex, No. (%)</td>
<td>30 (52.6)</td>
<td>22 (51.2)</td>
<td>8 (50.0)</td>
<td>60 (51.7)</td>
</tr>
<tr>
<td>IQ, mean (SD)</td>
<td>107.8 (7.9)</td>
<td>107.7 (9.6)</td>
<td>107.2 (7.8)</td>
<td>107.6 (8.4)</td>
</tr>
</tbody>
</table>

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and 1 image in the upper panel. In the 6 trials of each sensorimotor block, the 2 lower images were shapes, and the upper panel image was identical to 1 of the shapes in the lower panel. Subjects responded using button presses (left or right) to indicate which lower panel image matches the upper panel image. In the 6 trials of each emotion block, the lower panel consisted of 2 faces, 1 angry and 1 afraid, derived from a standard set of pictures of facial affect.23 The upper panel consists of 1 of the 2 faces shown in the lower panel. Subjects responded using button presses (left or right) to indicate which lower panel face matches the upper panel face. BOLD fMRI parameters for the emotional faces task are the following: 24 axial sections, 4-mm section thickness, 1-mm gap between sections, 2000-millisecond repetition time, 28-millisecond echo time, 24-cm field of view, and 64×64-pixel matrix.

Working Memory (N-Back) Task
Participants also performed a working memory (N-back) task administered using a block design, with the 2-back working memory condition alternating with a no-back control condition as previously described.13 During the 0-back control task block, the subject simply responded with the current digit presented (1-4 in a diamond-shaped box). This alternated with the 2-back block in which the subject serially responded with numbers presented 2 previously (‘n’=2). BOLD fMRI parameters for the N-back task are the following: 24 axial sections, 6-mm section thickness, 2000-millisecond repetition time, 28-millisecond echo time, 24-cm field of view, and 64×64-pixel matrix.

**IMAGE ANALYSIS**
Images were processed as described previously10 using Statistical Parametric Mapping 5 (SPM5) (http://www.fil.ion.ucl.ac.uk/spm). Briefly, images were realigned to the first image of the scan run, spatially normalized into a standard stereotactic space (Montreal Neurological Institute [Quebec City, Quebec, Canada] template) using an affine and nonlinear (4×5×4 basis functions) transformation, smoothed with a full width at half maximum (FWHM) gaussian filter (8-mm FWHM for emotion tasks and 10-mm for N-back), and ratio normalized to the whole-brain global mean. In the first-level analyses, linear contrasts were produced computing t statistical parameter maps at each voxel for emotional tasks. Similarly, t statistical parameter maps were produced for the 2-back working memory condition using the 0-back condition as a baseline. These statistical images were entered in a second-level model to identify significant activations within and between genotype groups, thresholded at P < .01 uncorrected for the region of interest (ROI) using SPM5. Initially, each of the imaging studies was evaluated using an additive genetic model, with 3 levels of genotype. This was not significant for any of the studies. Therefore, a secondary model (a recessive risk-associated allele model) was tested comparing homozygotes for the risk-associated allele against other genotypes (AA vs GA + GG) using a 2-sample t test. Results are presented based on this analysis. For both emotional tasks, the ROI was defined as the amygdala, hippocampus, and parahippocampus using the Wake Forest University, Winston-Salem, North Carolina, Pickatlas.23 For the N-back task, the ROI was defined as Brodmann areas 9, 10, and 46 using the Pickatlas.23 No behavioral differences were noted among the 3 genotype groups in the accuracy and reaction times for any of the tasks.

**GENETIC ASSOCIATION COHORT**
The cohort used in the genetic association study was the Clinical Brain Disorders Branch–National Institute of Mental Health Sibling Study sample, which consists of subjects collected as part of an ongoing investigation into neurobiological traits related to genetic risk for schizophrenia.11 Participants were between the ages of 18 and 60 years. To reduce genetic heterogeneity, only white subjects of self-identified European descent were analyzed. Collection details, screening, diagnostic procedures, and exclusion criteria have been previously described.14 Sample demographics for the genetic association study are listed in Table 2.

**GENOTYPING**
The CACNA1C (OMIM *114205) SNP rs1006737 was determined in the clinical samples by standard allelic discrimination TaqMan assay that uses the 5’ nucleic activity of Taq DNA polymerase to detect a fluorescent reporter signal generated after polymerase chain reaction amplification. The assay cocktail for rs1006737 (Assays on Demand; Applied Biosystems, Foster City, California) was used. Genotype reproducibility was routinely assessed by regenotyping all samples for the selected SNP and was generally greater than 99%. The genotyping completion rate was greater than 95%. Genotypes in all groups were in Hardy-Weinberg equilibrium as determined by an exact test (P > .1 for all).

**HUMAN BRAIN EXPRESSION STUDY**
Human brain tissue was collected as part of the Clinical Brain Disorders Branch, National Institute of Mental Health brain collection. Details about the collection, screening, and dissection processes for human brain tissues have been described.24 Expression of mRNA was measured in human dorsolateral prefrontal cortex from prenatal samples (gestational weeks 14-20) and from day of birth through old age using custom microarrays (Illumina; Illumina, Inc, San Diego, California). Sample demographics for the postmortem human brain tissue study are listed in Table 3. Microarray chips were generated in the National Human Genome Research Institute Microarray Core Facility from 44 544 probes (70mer) obtained from the Illumina Oligoset Human Exonic Evidence Based Oligonucleotides (http://www.microarray.org/sfgf/heebo.do). The RNA samples (500 ng) from the Clinical Brain Disorders Branch brain series

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**Table 2. Sample Demographics for the Case-Control Genetic Association Study**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cases (n=282)</th>
<th>Controls (n=440)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex, No. (%)</td>
<td>66 (23.4)</td>
<td>236 (53.6)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>36.44 (10.54)</td>
<td>33.09 (10.09)</td>
</tr>
<tr>
<td>Age at onset, mean (SD), y</td>
<td>21.65 (5.46)</td>
<td>...</td>
</tr>
</tbody>
</table>

**Table 3. Sample Demographics for the Postmortem Human Brain Expression Study**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>GG (n=108)</th>
<th>GA (n=116)</th>
<th>AA (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>27.35 (22.06)</td>
<td>26.11 (23.39)</td>
<td>31.59 (19.70)</td>
</tr>
<tr>
<td>Female sex, No. (%)</td>
<td>36 (33.3)</td>
<td>42 (36.2)</td>
<td>13 (35.1)</td>
</tr>
<tr>
<td>Race/ethnicity, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>46 (42.6)</td>
<td>72 (62.1)</td>
<td>30 (81.1)</td>
</tr>
<tr>
<td>White</td>
<td>62 (57.4)</td>
<td>44 (37.9)</td>
<td>7 (18.9)</td>
</tr>
</tbody>
</table>

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identity with rs1006737 (linear regression analysis to determine the effect of genotype on clinical samples, only an additive genetic model was tested using a proxy SNP was selected for genetic analysis based on linkage disequilibrium (ie, angry or fearful), which has been previously shown to robustly activate the amygdala and the hippocampus. For this emotional faces task, images from 131 subjects (64 GG genotype, 53 GA, and 14 AA) were used in an ROI analysis of the amygdala and hippocampus. There were no regions in the amygdala or hippocampus that survived a threshold of \( P < .01 \) uncorrected; however, when thresholded at \( P < .05 \), risk-associated allele homozygotes (AA) had slightly greater right amygdala activity compared with carriers of the common allele (\( P = .02 \) uncorrected, \( P_{\text{adj}} = .21 \), \( z = 2.17 \)). Although this activation was not significant by correction for multiple voxels within the region, it was similar to the pattern of hippocampal activation during encoding of aversive scenes.

**WORKING MEMORY TASK**

We also examined the effect of \( \text{CACNA1C} \) genotype on a task not related to limbic processing of emotion and not previously associated with mood disorders but linked with schizophrenia and prefrontal cognitive processing. If this gene is associated more strongly with mood disorders than with schizophrenia because of a primary effect on emotional circuitry, then a task targeting emotionally neutral cognitive processing related to prefrontal cortex should show a less robust effect. We chose the N-back, a working memory task that robustly engages prefrontal cortical circuitry and has been especially useful in characterizing neural mechanisms of genes associated with schizophrenia. Images from 316 subjects (146 GG genotype, 141 GA, and 29 AA), comparing 2-back with 0-back, were analyzed using an ROI for Brodmann areas 9, 10, and 46. Again, analysis under an additive genetic model was nonsignificant. However, testing of the recessive genetic model showed a significant effect of genotype on the efficiency of prefrontal cortex. Homozygotes for the risk-associated allele (AA) had greater activity in prefrontal cortex (\( P = 2.8e-05 \) uncorrected, \( P_{\text{adj}} = .01 \), \( z = 4.03 \) for the first cluster; \( P = 5.67e-05 \) uncorrected, \( P_{\text{adj}} = .01 \), \( z = 3.86 \) for the second cluster) (Figure 2), a pattern of inefficient engagement previously found in association with several putative schizophrenia susceptibility genotypes. Even using a whole-brain analysis, these 2 clusters survived the threshold of \( P < .001 \) and 10 contiguous voxels, which suggests that the effect of the \( \text{CACNA1C} \) gene on this working memory task is specific to prefrontal cortex. These results also remained significant after correcting for the 2

**RESULTS**

For each imaging task, subjects in each genotype group were matched for age, sex, IQ, and task performance, thus isolating the effect of genotype on brain information processing not confounded by general brain function parameters or by task performance. The first 2 tasks focused on the engagement of medial temporal lobe structures implicated in emotional processing and associated with mood disorders and increased genetic risk for mood disorders.

**EMOTIONAL IMAGING TASKS**

The first task involved the encoding and retrieval of aversive stimuli. For this emotional memory task, images from 116 subjects (57 GG genotype, 43 GA, and 16 AA) were used in an ROI analysis of the amygdala and hippocampus. Initial testing of an additive genetic model yielded nonsignificant results. Testing of the recessive genetic model showed an effect of genotype on the engagement of the hippocampus. Homozygotes for the risk-associated allele (AA) had greater bilateral hippocampal activity during the encoding of aversive images compared with carriers of the common allele (\( P = .001 \) uncorrected, \( P_{\text{adj}} = .05 \), \( z = 3.20 \) for the right hippocampus; \( P = .003 \) uncorrected, \( P_{\text{adj}} = .05 \), \( z = 2.77 \) for the left hippocampus) (Figure 1). Although the uncorrected \( P \) values were significant after correcting for the 2 different genetic models tested, the FDR-corrected results remained significant only at the trend level.

The second task involved matching of emotional faces (ie, angry or fearful), which has been previously shown to robustly activate the amygdala and the hippocampus. For this emotional faces task, images from 131 subjects (64 GG genotype, 53 GA, and 14 AA) were used in an ROI analysis of the amygdala and hippocampus. There were no regions in the amygdala or hippocampus that survived a threshold of \( P < .01 \) uncorrected; however, when thresholded at \( P < .05 \), risk-associated allele homozygotes (AA) had slightly greater right amygdala activity compared with carriers of the common allele (\( P = .02 \) uncorrected, \( P_{\text{adj}} = .21 \), \( z = 2.17 \)). Although this activation was not significant by correction for multiple voxels within the region, it was similar to the pattern of hippocampal activation during encoding of aversive scenes.
different genetic models tested. Although the significance level seemed greater in prefrontal cortex during this working memory task compared with the hippocampal response in the emotional memory task, this was likely due to a difference in sample size, as the effect sizes are similar (0.76 for emotional memory and 0.78 for working memory).

CLINICAL GENETIC ASSOCIATION WITH SCHIZOPHRENIA

The finding of an intermediate brain phenotype associated with genetic risk for schizophrenia suggests that CACNA1C might also show an association with the clinical diagnosis of schizophrenia, and indeed this has recently been reported, including a study with the same SNP as that reported herein (rs1006737, P = .03). Genomewide association results of the International Schizophrenia Consortium showed an association of schizophrenia with CACNA1C, although not to the same SNP (rs2238090, P = 7.7 × 10−6). We examined CACNA1C rs1006737 in a case-control analysis (282 cases and 440 controls) and found a nominal association (P = .03) with schizophrenia (odds ratio for risk-associated allele homozygotes, 1.77; 95% confidence interval, 1.07-2.91).

ASSOCIATION WITH EXPRESSION OF CACNA1C mRNA IN HUMAN BRAIN

CACNA1C is highly expressed in heart and in brain. To understand the molecular mechanism underlying the clinical association and the functional differences in brain circuitry, we tested the effect of genetic variation in CACNA1C on mRNA expression in a large cohort of human postmortem brain samples. The proxy SNP (rs2159100) for rs1006737 (R² = 1.0) showed a significant effect on CACNA1C expression; carriers of the risk-associated genotype (AA) had highest expression, with heterozygotes (GA) having intermediate expression and the common allele carriers (GG) having the lowest expression (P = .002 for linear regression analysis) (Figure 3 and eTable; available at http://www.archgenpsychiatry.com). Because of the small size of the postmortem sample, we also generated an empirical P value by permutation analysis, which involved randomizing the genotypes and level of expression and 100 000 repetitions of the regression analysis, yielding an empirical P = .002. Expression of the probe did not significantly change across age, and genotype groups did not differ in mean age; therefore, age was not used as a covariate. There was a difference in expression between persons of white vs African American race/ethnicity, which is likely due to a difference in minor allele frequency (26% vs 45%, respectively). However, there was not a race/ethnicity × genotype interaction, implicating genotype as an independent predictor of expression. An analysis using race/ethnicity as a covariate showed a significant effect of genotype on expression (F = 3.275, P = .04).

We found that genetic variation, previously implicated as a risk factor for bipolar disorder and for schizophrenia, shows effects on brain functions related to mediobasal temporal emotional processing and prefrontal cortical working memory processing that have been associated with risk for bipolar disorder and for schizophrenia, respectively. Our results suggest that the pleotropic effects of the risk-associated genotype on these diverse brain circuits parallel the diagnostic nonspecificity of the clinical associations and may reflect the underlying neural system mechanisms involved. Genetic variation in voltage-gated calcium channel genes has been associated with several other complex multigenic neuropsychiatric disorders, including autism, epilepsy and migraine, and schizophrenia. In addition, a missense mutation in CACNA1C results in Timothy syndrome, which is characterized by multiorgan dysfunction, including cardiac arrhythmias and cognitive abnormalities.

Data in this study suggest that calcium channel dysfunction may contribute in part to the genetic etiology of bipolar disorder and schizophrenia through alterations in the functional activity of brain circuits implicated in both conditions. This is analogous to other genes (eg, COMT).
GRM3, BDNF, DISC1) that have been associated with both diagnoses and both patterns of neural circuitry effects. Investigations of the N-back working memory task have shown that patients with schizophrenia and their healthy siblings have increased prefrontal cortical activity for a given level of performance, suggesting that inefficiency in this circuitry is heritable and a good intermediate phenotype related to genetic risk for schizophrenia. Analogous studies have been performed among patients with bipolar disorder using neuroimgaging tasks that target mood circuitry in the temporal lobe. Other studies have targeted serotonin signaling genes and have found that healthy subjects who are carriers of the short allele of the serotonin transporter, the target of drugs that treat the mood symptoms of bipolar disorder, have higher ratings of anxiety and depression and have greater activation of the limbic circuitry, similar to these data. Although we found stronger statistical evidence of association with prefrontal processing, this was likely an artifact of the reduced power of the smaller sample in the emotional processing tasks, as the effect sizes were similar.

The molecular mechanism of genetic risk seems to relate at least in part to regulation of gene expression. Carriers of the risk-associated allele had increased levels of CACNA1C mRNA. It may be that a specific transcript of CACNA1C that is measured by the oligonucleotide probe is involved in the function of the brain regions measured by fMRI in this study. Chemical channels are involved in various aspects of neuronal development and in the establishment of maintenance of connectivity during development and throughout adulthood; therefore, alterations in gene expression may affect brain structure, which could also affect brain function. A recent study reported that variation in CACNA1C results in alterations in cerebral gray matter. Total gray matter was highest in AA carriers of the risk-associated SNP (rs1006737) compared with GA and GA carriers. However, it is unclear whether and how this finding relates to the genetic association with psychiatric illness.

Our data add to the growing literature that genes weakly associated with psychiatric diseases show stronger effects at the level of brain processing of emotional and cognitive information. This has been shown for several other genes that have been found to be positively but weakly associated with clinical diagnosis in large population studies, only to show strong effects in imaging among much smaller samples. For example, a recent study of the ZNF804A gene, which showed significant genome-wide association with schizophrenia (P < 10⁻⁸) in a sample of more than 23,000, also showed strong association (P = 0.006) with an imaging phenotype in a much smaller sample (n = 115) of healthy subjects. We can explore this question of relative statistical power directly in our data compared with the results of the large-scale clinical study of CACNA1C that showed genome-wide significant association (P < 10⁻⁸) in a sample of 10,960 total subjects. Using the imaging data from our N-back study, in which P = 2.8⁻⁵⁵ for a sample size of 316 subjects, if we increase the sample size to 10,000 to approximate the sample size in the previous genome-wide association studies and hold the effect size constant, our P-value drops to P = 4.87e⁻¹⁰⁹. By the same token, using our postmortem expression data, in which P = 0.002 for a sample size of 261, if the sample size was increased to 10,000, the P-value would be P = 1.24e⁻⁷⁰. It should come as no surprise that in principle our findings of associations with quantitative biological measures related to clinical illness and to genetic risk for illness show greater effect sizes than association with clinical diagnosis for the following 2 reasons: (1) a quantitative trait phenotype has greater statistical power in general compared with a categorical variable and (2) the measures we studied in brain are likely closer to the neurobiological function of the gene and its effect on illness than is the clinical diagnosis. Analogous findings have been reported in other areas of complex medical genetics; for example, genes that show weak association with complex syndromes such as hypertension and cardiovascular disease show much stronger statistical association with quantitative biological traits related to these syndromes (ie, sodium homeostasis and lipid metabolism, respectively).

The association of CACNA1C with brain-related quantitative phenotypes has potential clinical implications. Our demonstration of increased expression of the CACNA1C transcript suggests that, if this translates into increased calcium channel activity, calcium channel inhibitors may have clinical value in treating these disorders. Indeed, anecdotal reports and results of small clinical trials suggest benefits of these agents for some patients, but the data have been inconsistent and limited. Genotype or brain imaging–based phenotypes might be considered as individual predictors of response to these agents in future trials. Although further studies are necessary to fully characterize the mechanism by which alterations in CACNA1C expression result in brain function changes, this study identifies a potential mechanism of risk for bipolar disorder and schizophrenia.

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Online-Only Material: The eTable is available at http://www.archgenpsychiatry.com.

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