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# The association of genetic variation in *CACNA1C* with structure and function of a frontotemporal system

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## Abstract

**Objectives—**A single nucleotide polymorphism at the *CACNA1C* gene (rs1006737) has been reported in genome-wide association studies to be associated with bipolar disorder (BD) with genome-wide significance. However, the neural system effects of *CACNA1C* that mediate the association are not known. In this study, we assessed associations between rs1006737 variation and both morphology and functional connectivity within a corticolimbic frontotemporal neural system implicated in BD.

**Methods—**A total of 55 European Americans were divided into two groups: a GG group homozygous for the 'G' allele (n = 30) and carriers of the high risk A allele ('A-carrier' group, AA/AG genotypes; n = 25). The subjects participated in both high-resolution structural magnetic resonance imaging (MRI) scans and functional MRI scans during emotional face-processing. Voxel-based morphometry and functional connectivity analyses were performed.

**Results—**Compared to the GG group, the A-carrier group showed significantly increased gray matter volume and reduced functional connectivity within a corticolimbic frontotemporal neural system ($p < 0.05$, corrected).

**Conclusion—**The findings support effects of the rs1006737 variation on the frontotemporal neural system implicated in BD, both in gray matter morphology and functional connectivity. This suggests that influence of *CACNA1C* variation on corticolimbic structure and function may be a mechanism contributing to the neural circuitry of BD.

### Keywords

bipolar disorder; calcium channels; genetic polymorphism; magnetic resonance imaging; functional magnetic resonance imaging; prefrontal cortex

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The single nucleotide polymorphism (SNP) at the *CACNA1C* gene locus, rs1006737 (G to high risk A allele) remains one of the most compelling susceptibility loci for bipolar disorder (BD), demonstrated in independent genome-wide association studies to be

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associated with BD with genome-wide significance (1, 2). The neural system effects of *CACNA1C* that mediate the association are not known. Here, we assessed associations between rs1006737 and both morphology and functional connectivity within a corticolimbic frontotemporal neural system implicated in BD.

Converging evidence supports the involvement of morphological and functional abnormalities within a ventral prefrontal cortex (VPFC)-amygdala neural system in BD (3–16). Recent observations, using structural or functional neuroimaging approaches, suggest the presence of both morphological and functional connectivity abnormalities in the frontotemporal system in BD, implicating mechanisms that may contribute to the development of both the structural and functional system disturbances (3, 5).

The *CACNA1C* gene is implicated in the development and plasticity of frontotemporal structure and function (17). The gene codes for the major L-type voltage-dependent calcium channel, $CA_v1.2$ (alpha-1C subunit), which regulates activity-dependent influx of calcium. This in turn modulates calcium-dependent genes including *BDNF* (brain-derived neurotrophic factor) and *BCL2* (B-cell lymphoma 2), the protein products of which have been demonstrated to have neurotrophic and neuroprotective effects in corticolimbic frontotemporal structures; they have also been implicated in BD and its treatment (18). This suggests mechanisms through which the *CACNA1C* gene may influence both morphology and function within the frontotemporal system.

In healthy individuals, associations between allelic variation at rs1006737 and gray matter (GM) morphology have been reported for total GM (19), although there is a report of no difference (20), and a recent study demonstrated increases in amygdala volume (25). Functional neuroimaging studies specifically examining the amygdala (21, 22), hippocampus or prefrontal cortex (PFC) (23, 24) have detected rs1006737 associations with responses within these regions. However, these studies did not examine the functional relationships between these regions.

We investigated rs1006737 genetic association with both regional GM volumes and amygdala-associated functional connectivity during emotional processing in healthy individuals. We hypothesized that the rs1006737 variation would influence both morphology and functional connectivity within the VPFC-amygdala neural system.

## Methods

The genotypes of the SNP (rs1006737) were determined by standard Taqman methods. Subjects included 55 European Americans divided into two groups: a GG group homozygous for the 'G' allele (n = 30, mean age = 32.5 ± SD 13.4 years, 60% females) and an 'A-carrier' group (AA/AG genotypes) (n = 25, mean age = 28.8 ± SD 10.2 years, 48% females). Genotype frequencies were consistent with Hardy-Weinberg equilibrium expectations.

The subjects were recruited from the community and were without a DSM-IV Axis I Disorder themselves, confirmed by the Structured Clinical Interview for DSM-IV Axis I Disorders Version 2.0 (26), or in their first-degree family members, assessed with family history screen for epidemiologic studies (27). No subject was taking medications with potential central nervous system effects or had a history of a medical or neurological disorder, or head trauma with loss of consciousness over five minutes. Subjects did not use substances the week prior to scanning and urine toxicology screens on the day of scanning were negative. The exception was tobacco that could be used until the evening prior to scanning (five subjects were smokers at the time of study). After a complete description of the study, written informed consent was obtained from all participants in accordance with

the human investigation committees of the Yale School of Medicine and VA Connecticut Department of Veterans Affairs, New Haven, CT, USA.

Both high resolution structural magnetic resonance imaging (MRI) and functional MRI (fMRI) during emotional face-processing were performed in the same scanning session for each subject with a 3-Tesla Siemens Trio MR scanner (Siemens, Erlangen, Germany). A three-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) T1-weighted sequence was used to acquire sagittal images with parameters TR = 1500 ms, TE = 2.83 ms, FOV = 256×256 mm, matrix = 256× 256, slice thickness =1.0 mm without gap, 160 slices, 2 averages. fMRI data were acquired with a single-shot echo planar imaging (EPI) sequence in alignment with the anterior commissure-posterior commissure plane with the parameters TR = 2000 ms, TE = 25 ms, FOV = 240× 240mm$^2$, matrix = 64× 64, and 32 three-mm slices without gap. During the fMRI runs, an event-related emotional face task was completed by each participant. Participants viewed faces from the Ekman series depicting fearful, happy or neutral expressions and were instructed to press a button to make a male-female determination, as described previously (3, 28).

The structural and functional MRI data were processed and analyzed with Statistical Parametric Mapping (SPM5) (http://www.fil.ion.ucl.ac.uk/spm), by previously described methods (3, 5, 29). Briefly, the SPM5 segmentation function was used for structural data processing. Structural images were segmented, spatially normalized and smoothed by an 8-mm full width at half maximum (FWHM) Gaussian kernel. fMRI data were realigned, spatially normalized and smoothed by an 8-mm FWHM. The amygdala seed region of interest (ROI) was defined with the WFU Pick Atlas Tool (http://www.fmri.wfubmc.edu/download.htm). For each subject, a mean time series for the amygdala seed ROI was calculated by averaging the time series for all voxels within the amygdala ROI. Correlational analyses were then performed between the amygdala time series and the time series for each brain voxel (30, 31), resulting in a correlation map for each subject that contained the correlation coefficient for each voxel with that of the amygdala ROI. For further statistical analysis, the correlation coefficients were transformed to *Z*-values using Fisher *r*-to-*z* transformation (32).

Whole brain voxel-based two-sample *t*-tests were used to compare group differences in GM volume and functional connectivity (correlation coefficients) from an amygdala ROI during fear, happy and neutral face-processing. Consistent with our previous study (3), findings were considered significant for $p < 0.005$ (uncorrected) and for clusters > 640 mm$^3$. Analyses were also performed with small volume correction (SVC) for multiple comparisons ($p < 0.05$, corrected) to further confirm the findings for the hypothesized frontotemporal regions. Functional connectivity data were also inspected for survival for Bonferroni correction for study of three types of face processing which required a p-value of 0.017.

## Results

The GG and A-carrier groups did not differ significantly in age or sex (all $p > 0.2$). Compared with GG subjects, A-carriers had increased GM in bilateral ventral, rostral and dorsolateral PFC, anterior cingulate and temporal cortices, insular, parietal and occipital cortices (Fig. 1). The findings in the bilateral ventral, rostral and dorsolateral PFC, and anterior cingulate and temporal cortices remained significant with SVC. A-carriers showed functional connectivity decreases from amygdala to VPFC during fear and happy conditions (Fig. 1). Functional connectivity decreases were also observed in parietal cortex during happy face-processing. The VPFC findings remained significant with SVC and Bonferroni

adjustment for multiple testing (all $p < 0.017$). There were 5 subjects with current nicotine use. The results remained significant if the analyses were performed without these subjects.

## Discussion

*CACNA1C* rs1006737 was associated with both frontotemporal gray matter morphology and functional connectivity implicated in BD, suggesting that influence of *CACNA1C* variation on corticolimbic structure and function may be a mechanism contributing to the neural circuitry of the disorder.

The findings are partially consistent with previous neuroimaging studies of effects of this SNP. The GM increases detected herein are consistent with a previous report of larger total GM (19), and suggest that these may have derived particularly from frontotemporal differences. However, increases in volume specifically in amygdala were not detected as in a previous study that utilized region-of-interest methods (25). Although previous functional connectivity findings were not reported for rs1006737, previous findings of activation differences within mesial temporal and frontal regions (21–24) could result from frontotemporal dysconnectivity observed.

A causal relationship between the increased frontotemporal volumes and decreased functional connectivity cannot be concluded from this study. Possibilities include abnormalities in morphology, such as could result from impaired pruning or abnormal cell type distribution, which could disrupt functional connections. Alternatively, abnormal functional connections could disrupt morphological development. However, it is also possible that volume increases represent a compensatory effect in healthy participants who carry risk allele.

A recent demonstration that this SNP is associated with changes in PFC *CACNA1C* expression (23) suggests that the neural effects of the SNP may be attributable to alterations in gene expression. Effects detected may derive from some other variant in linkage disequilibrium with this SNP; further studies of *CACNA1C* are needed to identify the underlying functional variants. The relative developmental timing of, and interactions between, the structural and functional effects cannot be determined from this study. Future studies are needed to address these issues and to directly assess effects within individuals with BD.

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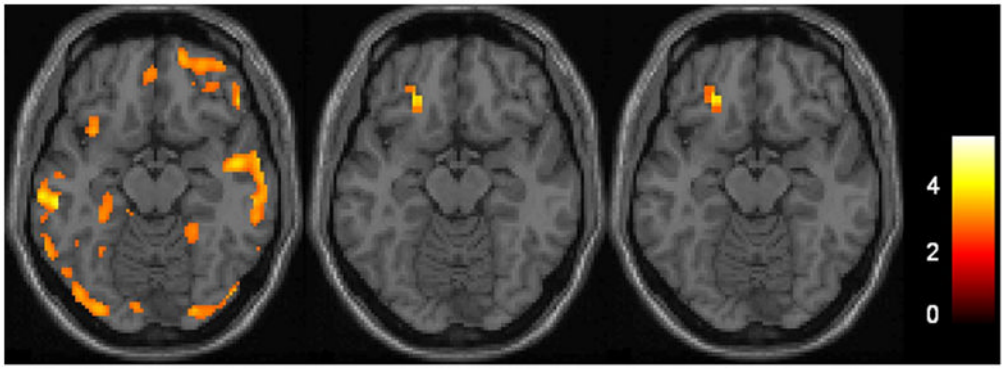


**Fig. 1.**

Increased gray matter volume (left) and decreased functional connectivity to amygdala (middle: fear; right: happy) in rs1006737 A-carriers ($p < 0.005$, cluster > 640mm$^3$). The color bar represents the range of *t*-values. Montreal Neurological Institute z-plane = −14mm.