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10 ORIGINAL ARTICLE

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A Common CACNA1C Gene Risk Variant has Sex-Dependent Effects on Behavioral Traits and Brain Functional Activity

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Abstract

Genome-wide association studies have suggested that allelic variations in the CACNA1C gene confer susceptibility to schizophrenia and bipolar disorder only in women. Here we investigated the sex-specific effects of the CACNA1C variant rs1024582 on psychiatry-related traits, brain activity during tasks and rest, and brain volume in 1207 normal male and female subjects. After correcting for multiple comparisons, there were significant interaction effects between sex and the minor allele of this polymorphism on the hostile behavior subscale scores of the Coronary-Prone Type Scale mediated by higher scores in female carriers of the minor allele. Imaging analyses revealed significant interaction effects between sex and the minor allele on fractional amplitude of low-frequency fluctuations in the right dorsolateral prefrontal cortex and on brain activity during the 2-back task in areas of the right posterior cingulate cortex, right thalamus, and right hippocampus, which were all mediated by reduced activity in female carriers of the minor allele. Our results demonstrated that the rs1024582 risk variant of CACNA1C is associated with reduced activity in the frontolimbic regions at rest and during a working memory task as well as with greater hostility in females in the healthy population.

Key words: CACNA1C, dorsolateral prefrontal cortex, hippocampus, imaging genetics, sex-specific effects, traits

Introduction

The alpha 1C subunit of the L-type voltage-gated calcium channel (CACNA1c) gene encodes the alpha1C subunit of the L-type voltage-gated calcium channel $Ca_v1.2$ (Striessnig et al. 2006). These channels mediate the influx of calcium for membrane polarization, thus influencing the ability of neurons to generate and transmit electrical signals (Striessnig et al. 2006). $Ca_v1.2$ is expressed in the entire nervous system, particularly in the hippocampus (Striessnig et al. 2006). In the brain, $Ca_v1.2$ mediates synaptic plasticity, learning, and memory independent of N-methyl-D-aspartate (NMDA) receptor activity by activating cAMP response element binding (CREB)-dependent transcription (Moosmang et al. 2005).

Genome-wide association studies (GWASs) have suggested that variations in single nucleotide polymorphisms (SNPs) in CACNA1C confer susceptibility to schizophrenia and bipolar disorder (Smoller et al. 2013; Zheng et al. 2014). Early GWASs mainly focused on the CACNA1C rs1006737 risk variant, but a recent genome-wide analysis involving 5 major psychiatric disorders found that another CACNA1C SNP, rs1024582, was more strongly associated with schizophrenia and bipolar disorder than other SNPs in calcium channel genes (Smoller et al. 2013). A subsequent study in a large Chinese sample showed that the rs1024582 SNP was more strongly associated with schizophrenia than the rs1006737 SNP (Zheng et al. 2014).

In efforts to elucidate mechanisms underlying susceptibility to psychiatric disorders, numerous brain imaging studies investigating effects of the CACNA1C rs1006737 risk variant have found relatively consistent changes in task-dependent brain activity in the DLPFC, medial temporal lobe, and medial parietal lobe (Heck et al. 2014; Erk et al. 2014a; 2014b).

Multiple studies have found effects of rs1006737 on psychiatric diseases only in women (Dao et al. 2010; Witt et al. 2014). Moreover, another minor variant of CACNA1C was associated with impaired recovery from schizophrenia-spectrum disorder in women but not in men (Heilbronner et al. 2015). Strohmaier et al. (2013) reported interaction effects of sex in a healthy population, but the minor risk variant was associated with traits linked to negative effects in men, including less optimism, emotional lability, depressive symptoms, trait anxiety, lower extraversion, and paranoid ideation, while the opposite pattern was observed in women.

Considering that the 2 loci, rs1024582 and rs1006737 SNP, are rather tightly linked with each other (D' : 0.9097, R^2 : 0.608, χ^2 :

126.47, P -value <0.0001 , LDlink [<http://analysistools.nci.nih.gov/LDlink/>]), both variants may have similar biological effects on susceptibility to psychiatric disorders and brain functions.

While there is considerable psychological evidence suggesting sex-specific functional differences between carriers and noncarriers of CACNA1C variant risk alleles, sex-specific differences in effects of CACNA1C risk alleles on the neural activity remain unclear.

The purpose of this study was to examine these issues in a healthy sample population. We hypothesized that the rs1024582 risk variant would impact cognitive functions such as working memory and traits related to schizophrenia and depression such as harm avoidance and paranoid ideation, as well as the neural activity of brain regions implicated in these disorders, particularly the prefrontal and medial temporal medial parietal areas in a sex-specific manner. Given the contribution of this rs1024582 polymorphism to schizophrenia and bipolar disorder risk, it is important to assess its effects in a normal population to gain further insight into disease pathogenesis.

To achieve the purpose of investigating neural mechanisms, we utilized fMRI N-back working memory task, as well as fractional amplitude of low-frequency fluctuation (fALFF).

fALFF, which is a recently developed measure for resting state fmri, is suggested to indicate the magnitude of spontaneous neural activity during rest (Yan and Zang 2010). Repeated visual stimuli consistently induce changes in fALFF in the visual cortex (Li et al. 2011), and fALFF is strongly correlated with regional metabolic rates in the brain (Tomasi et al. 2013). It is also altered in psychiatric patients (Ren et al. 2013) and is associated with higher-order cognitive functions (Takeuchi et al. 2017), suggesting the usefulness of fALFF for the purposes of this investigation.

Methods**Subjects**

The present study, which is a part of an ongoing project to investigate the association between brain imaging, cognitive function, and aging, included 1207 healthy, right-handed individuals (694 men and 513 women) from whom the data necessary for whole-brain analyses involving the CACNA1C genotype were successfully obtained. The mean age of the subjects was 20.7 years (standard deviation [SD], 1.8; age range: 18–27 years

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old). For details of subjects' information, see Supplemental Methods. Written informed consent was obtained. This study was approved by the Ethics Committee of Tohoku University.

Genotyping of Subjects

Genomic DNA was prepared from saliva samples according to standard procedures. Genotypes of the CACNA1C SNP rs1024582 were determined for the subjects using the assay-on-demand (Applied Biosystems, Foster City, CA)-based allelic discrimination method. For additional details on the genotyping procedures and checks of reproducibility and accuracy, see Supplemental Methods.

The genotypic distributions of the 1207 subjects were as follows: CACNA1C(rs1024582)A/A (male: $n = 1$, female: $n = 2$, total: $n = 3$, 0.2%), CACNA1C(rs1024582)A/G (male: $n = 70$, female: $n = 41$, total: $n = 111$, 9.2%), and CACNA1C(rs1024582)G/G (male: $n = 623$, female: $n = 470$, total: $n = 1093$, 90.6%). Allele frequencies of A and G alleles were 4.8% and 95.2% respectively, which were concordant with previous findings. A allele was called a minor allele in this study. And since there are only few subjects of A/A type, in this study, subjects of A/A type and subjects of A/G type were combined and analyzed as were the cases of previous studies of rs1006737 (Heck et al. 2014; Erk et al. 2014a; 2014b). Tests for the Hardy–Weinberg equilibrium exhibited no deviations from the expected genotype distribution ($P > 0.05$).

Psychological Measures

The following neuropsychological tests were performed according to the methods described in a previous study (Takeuchi et al. 2015). [A] A (computerized) digit span task, which is a working memory task. [B] RAPM, a nonverbal reasoning task and a representative measure of general intelligence. [C] The S-A creativity test, which is a measure for creativity measured by divergent thinking.

The following Japanese questionnaires were also administered:

[D] Harm avoidance subscore of the Temperament Character Inventory, [E] Paranoia frequency score of the Paranoia Checklist, [F] Emotion Intelligence Scale (EQS), [G] Trait anxiety score of the State–Trait Anxiety Inventory, [H] Hostile behaviors subscale of the Coronary-Prone Type Scale for the Japanese, [I] Trait anger subscale of the State–Trait Anger Expression Inventory, [J] Extraversion subscore of the NEO Five-Factor Inventory, [K] Beck Depression Inventory, [L] Life Orientation Test (as a measure of optimism), and [M] Sleep disorder subscale of the General Health Questionnaire (GHQ)-30. Details of these questionnaires are provided in Supplemental Methods along with some of the rationales for inclusion of psychological measures.

fMRI Tasks

fMRI was used to map brain activity during cognitive tasks. The N-back task is a typical task for fMRI studies with conditions of 0-back (simple cognitive processes) and 2-back (working memory). The subjects were instructed to judge if the stimuli (1 of the 4 types of Japanese vowels presented visually) appearing “N” positions previously is the same as the current stimulus by pushing a button. In the 0-back task, subjects were instructed to determine whether each presented letter was the same as the target stimulus by pushing a button. We used a simple block design. For more details, see our previous study (Takeuchi et al. 2015).

Image Acquisition

The methods for MR image acquisition were described in our previous study (Takeuchi et al. 2015) and reproduced below. All MRI data acquisition was performed using a 3-T Philips Achieva scanner.

A total of 42 transaxial gradient-echo images (echo time = 30 ms, flip angle = 90°, slice thickness = 3 mm, FOV = 192 mm, matrix = 64 × 64) covering the entire brain were acquired at a repetition time of 2.5 s, using an echo planar sequence. For the N-back session, 174 functional volumes were obtained.

For rsfMRI scans, 34 transaxial gradient-echo images (64 × 64 matrix, TR = 2000 ms, TE = 30 ms, flip angle = 70°, FOV = 24 cm, slice thickness = 3.75 mm) covering the entire brain were acquired using an echo planar sequence for resting-state fMRI analyses. For this scan, 160 functional volumes were obtained while subjects were resting (resulting in a scan length of 5 min 20 s). During the resting-state scanning, the subjects were instructed to keep still with their eyes closed, as motionless as possible, and not to sleep and not to think about anything in particular.

In addition, we performed structural analyses using the same design (analyses for regional gray matter volume and regional white matter volume using voxel-based morphometry). However, the results were not significant (for details, see Supplemental Methods, Supplemental Results, and Supplemental Discussion).

Preprocessing and Data Analysis for Functional Activation Data

Preprocessing and data analysis of functional activation data were performed using SPM8. Prior to analysis, BOLD images were realigned and resliced to the mean BOLD image of the run. They were then corrected for slice timing, coregistered, and resliced to voxel space of images of diffusion tensor imaging. Subsequently, using a previously validated 2-step new segmentation algorithm of diffusion images and the previously validated diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL)-based registration process, all images, were normalized. The voxel size of normalized BOLD images was 3 × 3 × 3 mm³ and taken to the second-level analyses of functional activities. The full description of these procedures were provided in our previous study (Takeuchi et al. 2015).

The following procedures for functional activation data analysis were reproduced from our previous study using the exact same methods (Takeuchi et al. 2015). A design matrix was fitted to each participant with one regressor for each task condition (0-, 2-back in the N-back task) using the standard hemodynamic response function. The design matrix weighted each raw image according to its overall variability to reduce the impact of movement artifacts (Diedrichsen and Shadmehr 2005). The design matrix was fit to the data for each participant individually. After estimation, beta images were smoothed (8 mm full-width half-maximum) and taken to the second level or subjected to a random effect analysis. We removed low-frequency fluctuations with a high-pass filter using a cutoff value of 128 s. The individual-level statistical analyses were performed using a general linear model.

In the individual analyses, we focused on activation related to the condition (0-back or 2-back versus rest). The resulting maps representing brain activity during the working memory condition (2-back) and simple cognitive processing condition (0-back) for each participant were then forwarded for group analysis.

Due to poor quality images, procedural errors during the acquisition of behavioral data, and motion-related problems,

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group-level analyses were performed using data from 1160 of the 1207 subjects (669 males and 491 females).

In the Supplemental Methods, we summarize the reasons for using SPM8 to analyze all EPI data and for all statistical analyses, justify our use of SPM12 for structural processing, and provide reasons for using diffusion-weighted images instead of T1-weighted structural processing images.

Preprocessing and Data Analysis for rsfMRI Data

Preprocessing and data analysis of rsfMRI data were performed using SPM8 and the SPM8 extension software DPARSF (Data Processing Assistant for Resting-state fMRI) (Yan et al. 2016). Briefly, rsfMRI BOLD images were nonlinearly registered to the BOLD images of the N-back working memory task, and were then using the abovementioned parameter for normalization of BOLD images of the N-back task, all images were normalized. The voxel size of normalized rsfMRI BOLD images was $3.75 \times 3.75 \times 3.75 \text{ mm}^3$. From these images, whole-brain, white matter, and cerebrospinal fluid (CSF) masks were created.

The normalized series of BOLD images were processed by DPARSF for individual-level analysis. First, 26 nuisance covariates (including the mean time course of signals from voxels within the white matter mask, the mean time course of signals from voxels within the CSF mask, and 24 Friston motion parameters) were regressed out. The processed images were spatially smoothed with a 7.5-mm FWHM kernel, and the resultant images were masked with the whole-brain mask.

fALFF analyses were performed over 0.01–0.08 Hz at each voxel using the DPARSF software as previously described (Takeuchi et al. 2017).

Statistical Group-Level Analysis of Imaging and Psychological Data

Behavioral data were analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL). The effects of the CACNA1C rs1024582 minor allele as revealed by differences in cognitive scores between carriers (A/A and A/G) and noncarriers (G/G) across both sexes as well as the interaction between the minor allele and sex on psychological measures were assessed by analysis of covariance (ANCOVA). In these analyses, sex was a fixed factor and both age and the presence of the minor allele (coded as 0 and 1) were covariates. In psychological analyses, results with a threshold of $P < 0.05$, corrected for false discovery rate (FDR) using the graphically sharpened method (Benjamini and Hochberg 2000), were considered statistically significant. The correction for multiple comparisons using this method was applied to the results of the abovementioned ANCOVAs. As there were 14 psychological measures conducted, analyses for the main effect and the sex interaction effect yielded 28 P-values in this correction for multiple comparisons. FDR-based methods have been shown to be more powerful and sensitive than other available approaches to multiple statistical testing (Genovese et al. 2002).

Second-level statistical analyses were performed using SPM8 software. In group-level imaging analyses, we tested for the effects of the CACNA1C minor allele rs1024582 across both sexes as well as the effects of interaction between the minor allele and sex on functional activity during the working memory and simple sensorimotor tasks, and fALFF across the brain.

The analysis of fALFF was limited to the whole-brain mask created above.

In whole-brain analyses, we used voxel-wise analysis of covariance (ANCOVA), with sex difference as a grouping factor (using the full factorial option of SPM8). All analyses included age and the presence of the minor allele (coded as 0 and 1) as covariates. Additionally, fALFF analyses included volume-level mean frame-wise displacement as calculated according to a previous study (Power et al. 2012). Analyses of N-back tasks included volume-level mean frame-wise displacement, reaction time, and accuracy of the 0-back and 2-back tasks. These covariates were modeled so that each had a unique relationship with imaging measures for each sex except in the case of volume-level mean frame-wise displacement (using the interactions option in SPM8), which enabled investigation of the effects of interaction between sex and each covariate. In these analyses, the centering option was used for centering the interactions.

A multiple comparison correction was performed using threshold-free cluster enhancement (TFCE) (Smith and Nichols 2009) with randomized (5000 permutations) nonparametric testing using the TFCE toolbox (<http://dbm.neuro.uni-jena.de/tfce/>). We applied a threshold of FWE corrected at $P < 0.05$.

Results

Basic Data

The mean and standard deviation for age and covariates of functional imaging data (accuracy and reaction time in the 0-back and 2-back tasks, volume-level frame-wise displacement for the N-back fMRI task, and rsfMRI) in carriers and noncarriers of the minor allele of the CACNA1C rs1024582 polymorphism are presented in Table 1.

Main and Interaction Effects of the Minor Allele of CACNA1C Polymorphism rs1024582 on Psychological Metrics

ANCOVAs revealed no significant main effects (regardless of sex) of the minor allele of the CACNA1C polymorphism rs1024582 on psychological test scores at the uncorrected level. When the uncorrected threshold was used, however, ANCOVAs revealed significant interaction effects between sex and the presence of the minor allele of the CACNA1C polymorphism rs1024582 on the harm avoidance scale, Paranoia Checklist frequency scale, Emotional Intelligence Scale Intrapersonal factor, hostile behaviors subscale of Coronary-Prone Type Scale, and Sleep disorder subscale of the GHQ 30. After correcting for multiple comparisons (FDR), only the hostile behaviors subscale of the Coronary-Prone Type Scale remained significant (Table 2).

All results were moderated by weak minor allele associations with psychological scores that are related to negative effects (except the case of sleep disorder) in females.

Main and Interaction Effects of the Minor Allele of the CACNA1C Polymorphism rs1024582 on Intrinsic Brain Activity

Whole-brain ANCOVAs revealed no significant main effects (regardless of sex) of the minor allele of the CACNA1C polymorphism on rs1024582 on fALFF. However, whole-brain ANCOVAs revealed a significant effect of interaction between sex and the minor allele of the CACNA1C polymorphism rs1024582 on fALFF in the right DLPFC (Fig. 1, MNI coordinates: $x, y, z = 37.5, 33.75, 15$, $P = 0.040$, corrected for multiple comparison using

Table 1 Basic characteristics and covariates of imaging analyses in noncarriers and carriers of the minor allele of the CACNA1C polymorphism rs1024582

	Noncarriers of the minor allele (male)	Carriers of the minor allele (male)	Noncarriers of the minor allele (female)	Carriers of the minor allele (female)	Main effect P value (uncorrected)	Sex interaction effect P value (uncorrected)
Age	20.75 ± 1.86	21.24 ± 2.07	20.6 ± 1.61	20.56 ± 1.44	0.217	0.136
0-back accuracy	1.00 ± 0.04	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.01	0.683	0.942
0-back reaction time	4516.46 ± 746.85	4470.51 ± 666.84	4567.96 ± 778.78	4579.67 ± 853.76	0.823	0.707
2-back accuracy	0.99 ± 0.04	0.99 ± 0.02	0.99 ± 0.03	0.99 ± 0.01	0.802	0.538
2-back reaction time	6667.56 ± 1768.78	6701.15 ± 1827.1	6734.24 ± 1849.68	7020.27 ± 1774.51	0.458	0.393
Frame-wise displacement (N-back)	0.20 ± 0.05	0.20 ± 0.04	0.21 ± 0.05	0.20 ± 0.05	0.301	0.941
Frame-wise displacement (rsfMRI)	0.16 ± 0.04	0.16 ± 0.04	0.17 ± 0.04	0.17 ± 0.04	0.995	0.747

Table 2 Psychometric scale scores in noncarriers and carriers of the minor allele of the CACNA1C polymorphism rs1024582

	Noncarriers of minor allele (male)	Carriers of minor allele (male)	Noncarriers of minor allele (female)	Carriers of minor allele (female)	Main effect P value ^a (uncorrected, FDR)	Sex interaction effect ^b (uncorrected, FDR)
Digit span (N = 1201)	28.84 ± 3.84	28.34 ± 4.07	28.05 ± 3.83	28.58 ± 3.62	0.588, 0.670	0.892, 0.826
RAPM (N = 1207)	28.84 ± 3.84	28.34 ± 4.07	28.05 ± 3.83	28.58 ± 3.62	0.960, 0.857	0.189, 0.464
Total score of TBIT (N = 1084)	114.36 ± 12	113.57 ± 10.65	110.38 ± 11.68	106.68 ± 7.21	0.067, 0.279	0.260, 0.464
S-A creativity test (N = 1207)	37.63 ± 10.44	37.07 ± 11.48	39.26 ± 10.05	39.65 ± 9.12	0.877, 0.826	0.590, 0.670
Harm avoidance (N = 1204)	18.83 ± 7.34	17.34 ± 7.07	18.96 ± 7.11	21.43 ± 5.88	0.401, 0.557	0.013, 0.117
Frequency-Paranoia Checklist (N = 985)	29.09 ± 8.04	27.71 ± 7.1	26.97 ± 7.03	30.06 ± 8.67	0.255, 0.464	0.014, 0.117
Intrapersonal factor-EIS (N = 1204)	46.04 ± 12.03	47.34 ± 11.3	44.19 ± 12.01	39.51 ± 12.56	0.133, 0.416	0.021, 0.131
Trait anxiety-STAI (N = 1117)	44.26 ± 9.34	43.71 ± 9.33	44.61 ± 9.97	45.97 ± 9.52	0.629, 0.684	0.431, 0.558
Hostile behaviors-CTS (N = 1204)	22.87 ± 8.31	20.94 ± 6.98	21.08 ± 8.05	24.4 ± 8.69	0.358, 0.557	0.002, 0.050
Trait anger-STAXI (N = 1204)	19.59 ± 5.42	18.54 ± 4.57	20.13 ± 5.85	20.74 ± 6.02	0.774, 0.806	0.176, 0.464
Extraversion-NEOFFI (N = 1205)	25.89 ± 7.1	25.58 ± 6.39	26.95 ± 6.64	26.19 ± 5.97	0.372, 0.557	0.854, 0.826
Beck Depression Inventory (N = 1117)	7.77 ± 6.12	9.16 ± 7.01	8.54 ± 6.62	8.82 ± 6.77	0.221, 0.464	0.400, 0.557
Optimism-LOT (N = 1204)	9.48 ± 2.65	9.64 ± 2.52	9.54 ± 2.71	9 ± 2.5	0.446, 0.558	0.229, 0.464
Sleep disorder-GHQ 30 (N = 1202)	1.26 ± 1.16	1.30 ± 1.23	1.21 ± 1.2	0.77 ± 0.8	0.104, 0.371	0.039, 0.195

CTS = Coronary-Prone Type Scale; EIS = Emotional Intelligence Scale; FDR = false discovery rate; LOT = Life Orientation Test; STAXI = State-Trait Anger eXpression Inventory; RAPM = Raven's Advanced Progressive Matrix; STAI = State-Trait Anxiety Inventory; TBIT = Tanaka B-type Intelligence Test.

^aP value for main effects (regardless of sex) of the minor allele of the CACNA1C polymorphism rs1024582.

^bP value for the interaction between sex and the minor allele of the CACNA1C polymorphism rs1024582.

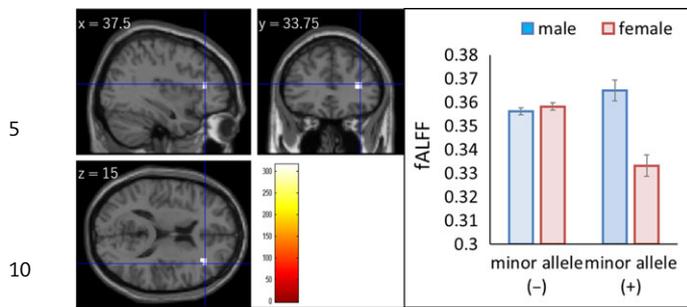


Figure 1. Interaction effects between sex and the presence of the minor allele of the CACNA1C polymorphism rs1024582 on fALFF. *Left panel:* The results were obtained using a threshold for TFCE of $P < 0.05$ based on 5000 permutations. Findings are overlaid on a “single-subject T1” image from SPM8. The color bar represents the TFCE value. The effect was seen in the right DLPFC. *Right panel:* Profiles of fALFF in the right DLPFC of male and female carriers and noncarriers of the minor allele.

permutation based on TFCE [FWE], TFCE value = 315.12, 264 mm³). This effect appeared to be moderated by the lower fALFF in female carriers of the minor allele.

Main and Interaction Effects of the Minor Allele of the CACNA1C Polymorphism rs1024582 on Task-Related Brain Activity

Whole-brain ANCOVAs revealed no significant main effects (regardless of sex) of the minor allele of the CACNA1C polymorphism rs1024582 on brain activity during the 0-back and 2-back tasks. Conversely, analysis revealed a significant effect of the interaction between sex and the minor allele of the CACNA1C polymorphism rs1024582 on brain activity during the 2-back task in the anatomical cluster within and adjacent to the right posterior cingulate cortex and right thalamus (Fig. 2, MNI coordinates: $x, y, z = 30, -36, 15, P = 0.023$, corrected, TFCE value = 525.71, 7263 mm³) and in the anatomical cluster within the right parahippocampal gyrus and the right hippocampus (Fig. 2, MNI coordinates: $x, y, z = 36, -36, -9, P = 0.048$, corrected, TFCE value = 423.97, 405 mm³). These effects appeared to be moderated by the lower activity in female carriers of the minor allele.

Discussion

The present study demonstrated that the minor allele of the CACNA1C polymorphism rs1024582, which has been shown to be strongly associated with schizophrenia and bipolar disorder risk, influences psychological traits and brain activity in a large sample of normal young adults. In addition, there were significant effects of interaction between sex and the risk variant on psychological traits and regional brain activity patterns. Psychological analyses corrected for multiple comparisons revealed that female carriers of the minor allele of rs1024582 showed greater hostility. Further, at the uncorrected level, female carriers of the minor allele of rs1024582 showed greater harm avoidance, increased frequency of paranoia, lower intrapersonal emotional intelligence, and better sleep quality. Neuroimaging analyses revealed that female carriers of the minor allele of rs1024582 exhibited decreased brain activity amplitude during rest in the right DLPFC and decreased brain activity during working memory performance in the right posterior cingulate cortex, right thalamus, right parahippocampal gyrus, and right hippocampus. These results are partly consistent with our hypothesis that similar to the CACNA1C

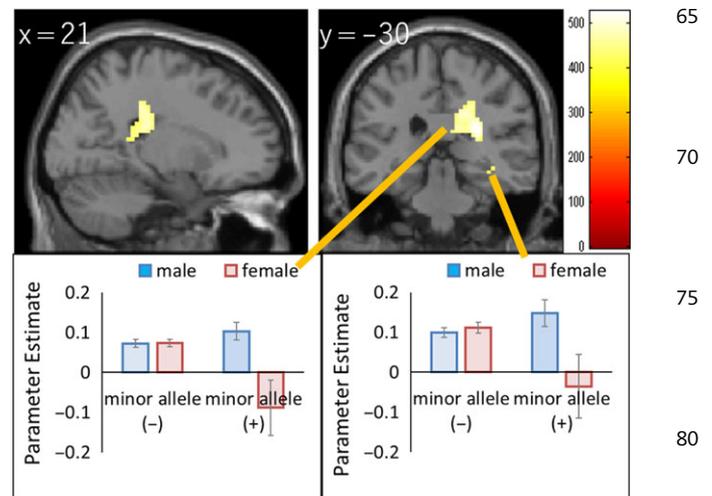


Figure 2. Interaction effects between sex and presence of the minor allele of the CACNA1C polymorphism rs1024582 on brain activity related to working memory. *Upper panel:* Results were obtained using a threshold for TFCE of $P < 0.05$ based on 5000 permutations. Findings are overlaid on a “single-subject T1” image from SPM8. Effects were observed in and adjacent to the right thalamus and right posterior cingulate cortex and in the right hippocampus and right parahippocampal gyrus. *Lower left panel:* Brain activity profiles for the significant cluster (in and adjacent to the right thalamus and right posterior cingulate cortex) during the 2-back task in male and female carriers and noncarriers of the minor allele. *Lower right panel:* Brain activity profiles for the significant clusters (in and adjacent to the right hippocampus and right parahippocampal gyrus) during the 2-back task in male and female carriers and noncarriers of the minor allele.

polymorphism rs1006737 risk variant, the CACNA1C polymorphism rs1024582 risk variant is associated with stronger expression of traits related to schizophrenia and affective disorders as well as altered brain activity during rest and during working memory in the prefrontal, medial temporal, and medial parietal areas in a gender specific manner.

Females carriers of the minor allele of the CACNA1C polymorphism rs1024582 showed lower fALFF amplitude during rest in the right DLPFC, consistent with previous studies on the CACNA1C rs1006737 variant. For example, a previous study found that carriers of the rs1006737 risk allele showed significantly decreased brain activity in the DLPFC during an episodic memory task (Erk et al. 2014a). In addition, physiological studies showed an association of the rs1006737 risk variant with greater mRNA expression in the DLPFC, which was suggested to disrupt DLPFC function (Bigos et al. 2010). The right DLPFC plays a key role in emotional control, and activity in this area during rest is associated with better emotional intelligence (Takeuchi et al. 2013). The smaller fALFF in this area may reflect the functional disruption of the DLPFC in carriers of the CACNA1C rs1024582 risk allele as well. In turn, this dysfunction may be associated with negative influences on certain psychological traits in normal adults, thereby increasing the risk of psychiatric disorders.

Females carriers of the minor allele of the CACNA1C polymorphism rs1024582 also exhibited decreased brain activity during the 2-back working memory in areas of the right posterior cingulate gyrus, right thalamus, right parahippocampal gyrus, and right hippocampus, which was partly consistent with previous studies on the CACNA1C rs1006737 allele. Previous studies have found the effects of the CACNA1C gene in these areas. For example, a previous study found that carriers of the

rs1006737 risk allele showed significantly decreased brain activity in the hippocampus during an episodic memory task (Erk et al. 2014a) and impaired spatial working memory among schizophrenia patients and healthy controls (Zhang et al. 2012). A previous study also found multiple SNPs in multiple genes, including CACNA1C, that altered WM performance and WM-related brain activity in medial parietal areas (Heck et al. 2014). While the CACNA1C gene encodes the alpha 1C subunit of the L-type voltage-gated calcium channel Cav1.2, for memory formation, Cav1.2 plays a role in NMDA receptor-independent memory formation by activating CREB-dependent protein synthesis. The selective inactivation of CACNA1C in the hippocampus and neocortex has been suggested to lead to a disturbance of long-term potentiation and to impaired memory formation (Krug et al. 2014). However, we found no significant association of the rs10254582 risk variant and working memory performance, which was in contrast to the results of a previous study (Heck et al. 2014) and our hypothesis. The exact reason for this discrepancy is unknown but may stem from an insufficient sample size or the exclusive enrollment of college students who may lack substantial variability in higher-order cognitive functions.

The traits associated with the CACNA1C risk variant in the female subgroup were mostly related to negative effects. The associations of the CACNA1C rs10254582 risk variant with traits of negative effects are consistent with the results of previous studies described in the Introduction section. In addition to the DLPFC, the hippocampus is linked to traits associated with negative effects through strong interactions with the amygdala for the mediation of emotional memory (LeDoux 2000) and motivational aspects of personality (Hahn et al. 2010). It has been suggested that hippocampal processing of threat and stress signals could be part of the causal pathway linking genetic risk to affective disorders (Roussos et al. 2011). The present study found hippocampal hypoactivation in female carriers of the CACNA1C rs1024582 minor allele in the absence of performance deficits, while a previous study showed that carriers of another CACNA1C risk allele presented with profound hippocampal hypoactivation during an episodic memory task without performance deficits (Erk et al. 2010). Hippocampal hypofunction may be linked to enhanced dendritic arborization in the amygdala, which in turn may lead to amygdalar overactivity and enhanced emotional arousal (McLaughlin et al. 2009). This amygdalar overactivity may lead to negative effects and enhanced risk of psychiatric disorders (Roussos et al. 2011).

However, previous functional neuroimaging studies on effects of the CACNA1C rs1006737 risk variant on functional activities have demonstrated its effect in both sexes, while in this study, the CACNA1C rs100254582 risk variant showed significant effects of interaction with sex in functional activity analyses. Reasons for this discrepancy are not clear, but we cite a few speculations. First, although the 2 SNPs show strong correlation as described ($R^2 = 0.608$), the 2 SNPs are different; therefore, observed differences in the results may be attributed to such differences in SNPs themselves. Second, in this study, we recruited a larger sample size than that recruited in previous studies; therefore, it may have been easier to statistically differentiate the effects in males and females. Finally, in some of these previous studies, the sex ratio was biased toward females in all samples or in the sample of carriers with 2 risk alleles, and such sample characteristics may have led to significant effects across sexes even when there are female-specific effects.

In the present study, the CACNA1C rs10254582 risk allele was also associated with better sleep quality in females. This

association of CACNA1C with better sleep quality is consistent with the results of a previous study demonstrating a robust association between sleep quality and another minor allele of CACNA1C (Parsons et al. 2013). In addition, a previous genome-wide study showed that the loci of this gene were associated with narcolepsy (Shimada et al. 2010). The exact mechanisms of these associations with sleep are unclear and cannot be elucidated by macrolevel brain imaging studies. However, one possible explanation is that the lower resting and task-dependent activity and excitability associated with the minor allele leads to better sleep quality.

The exact mechanism for the interaction effects of the CACNA1C polymorphism on psychological and brain imaging measures is unclear. However, SNPs in CACNA1C have been suggested to affect gene expression. For instance, SNPs may increase CACNA1C mRNA expression in the DLPFC (Bigos et al. 2010) and lead to mRNA up- or downregulation in other regions (Eckart et al. 2016). Concomitant upregulation of calcium channel current density (Quinn et al. 2010) leads to greater intracellular calcium levels (Yoshimizu et al. 2015), which in turn causes aberrant activation of calcium-dependent pathways that are normally latent or activated only at low levels, with ensuing metabolic imbalance, increased free radical generation, and possible cell death (Sattler and Tymianski 2000). These conditions may lead to increased risk of psychiatric disorders. And it has been shown that estrogens directly potentiate neuronal L-type Ca^{2+} channels (Sarkar et al. 2008), and therefore, the biological cascade caused by the risk allele may be accelerated in females.

The minor allele of the CACNA1C polymorphism rs1006737 increased the risk of psychiatric disease only in females (Dao et al. 2010; Witt et al. 2014), while others found that the same polymorphism leads to traits associated with negative effects in men (Strohmaier et al. 2013). The latter finding appears incongruent with the present results. The exact reasons for this discrepancy are currently unknown and beyond the scope of this study. However, Strohmaier et al. (2013) suggested that the use of a super-normal population as a control in the study by Dao et al. (2010) underlies this discrepancy. The sample population had a higher educational level in the present study than in the general population; therefore, it may have characteristics similar to the control in Dao et al. (2010). Assuming that the accumulation of genetic and environmental risks leads to psychiatric disorders or preclinical conditions, the presence of other risk factors in the population may determine the ultimate effects of the CACNA1C rs1024582 risk allele.

There is at least one limitation to the study. In this study, we did not perform a replication analysis. However, we focused on a candidate SNP whose effects on phenotypes have been confirmed by a genome-wide analysis (Smoller et al. 2013) and we performed permutation-based statistical tests, which are known to be robust (Silver et al. 2012). Still, the genetic basis of psychiatry disorders is polygenic (Smoller et al. 2013). Thus, the findings on the candidate genes without a replication analysis should be interpreted with caution.

The present study newly describes the psychological and neural effects of the CACNA1C rs1024582 risk allele, which is robustly associated with the risk of major psychiatric disorders as well as their sex differences in a normal population. The present study reveals that the CACNA1C rs1024582 minor allele is associated with greater hostility in females at the corrected level as well as with other traits of negative effects in females at the uncorrected level, including greater harm avoidance, increased frequency of paranoia, and lower intrapersonal

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emotional intelligence and better sleep quality in females at the uncorrected level. These latter findings at the uncorrected level, require replication. Further, the CACNA1C rs1024582 minor (risk) allele is associated with reduced resting brain activity in the right DLPFC as well as reduced brain activity during working memory performance in the right hippocampus and parahippocampal gyrus, right medial parietal cortex, and right thalamus in females. Collectively, these abnormalities may increase the risk of major psychiatric disorders in females.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

Funding

JST/RISTEX, JST/CREST, a Grant-in-Aid for Young Scientists (B) (KAKENHI 23700306) and a Grant-in-Aid for Young Scientists (A) (KAKENHI 25700012) from the Ministry of Education, Culture, Sports, Science, and Technology.

Notes

We thank Yuki Yamada for operating the MRI scanner, Haruka Nouchi for conducting the psychological tests, all other assistants for helping with the experiments and the study, and the study participants and all our other colleagues at IDAC, Tohoku University for their support. The authors would like to thank Enago (www.enago.jp) for the English language review. *Conflict of Interest*: Authors declare no conflict of interest.

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