CACNA1C polymorphisms Impact Cognitive Recovery in Patients with Bipolar Disorder in a Six-week Open-label Trial

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Cognitive impairments in bipolar patients deteriorate as the disorder progresses. Little is known about whether genetic risks impact cognitive recovery during the course from depression to remission. In this six-week open-label trial, we shed light on the impacts of six single nucleotide polymorphisms (SNPs) in the calcium voltage-gated channel subunit alpha1 C (CACNA1C) gene on cognitive recovery in 192 bipolar patients suffering a major depressive episode (MDE). The primary outcome measures were changes in a battery of neuropsychological tests following 6-week treatment. Carriers with rs10466907 GT genotype did not significantly improve their executive function total scores on the Wisconsin Card Sorting Test after six weeks of treatment compared to the TT genotypes (β = −0.944, 95% Confidence Interval (CI) = −1.482—−0.405). Moreover, during a MDE carriers with rs58619945 GG and GA genotypes performed significantly worse than those with AA genotype on the categories completed (p = 0.013 and p = 0.001), total errors (p = 0.039 and p = 0.009), and random errors (p = 0.055 and p = 0.014, respectively). Our data suggest that the tested CACNA1C SNPs may have impacts on cognitive recovery from depression.

Cognitive impairments are core features of bipolar disorder (BD). Circumscribed deficits on domains such as visual-spatial memory and processing speed were manifested prior to the onset of BD in the relatives of BD patients, indicating inherited phenotypes. Impairments in a wide range of cognitive domains then emerge and further deteriorate as the disorder progresses, negatively affecting the quality of life in people with BD. The number of depressive episodes is positively associated with the severity of cognitive impairments. It has been suggested that cognitive impairments are, at least partly, the sequelae of disease progression, particularly major depressive episode (MDE). On average, patients with BD have medium to large effect sizes of cognitive impairments that are variable across cognitive domains. However, individuals also differed markedly in the extent of cognitive dysfunction, with some individuals being as severe as patients with schizophrenia while others retaining relatively intact cognitive functioning. Genetic factors may contribute to such variations particularly during those critical periods such as MDE and its subsequent recovery.

Genome-wide association studies (GWAS) have repeatedly reported multiple single nucleotide polymorphisms (SNPs) in the alpha1C subunit of the L-type voltage-gated calcium channel (CACNA1C) gene linked to the risk of BD onset. Despite this, it is unclear how genetic risks give rise to the psychopathology. It has been suggested that genetic vulnerabilities exert effects on brain structure and cognitive mechanisms for mental disorders such as BD and schizophrenia, rather than on specific symptoms. For instance, the minor allele (i.e. SNP rs1006737 G allele) of CACNA1C gene was found to be associated with gray matter changes in the left putamen.
and the right amygdala and hypothalamus11. It also impacted the activation levels in the prefrontal cortex and hippocampus during working memory and emotional memory tasks respectively in patients with BD12, and in the left precuneus and inferior frontal gyrus in healthy participants13. At the cognitive level, SNP rs1006737 AA reported to be associated with variations in cognitive functioning19, although inconsistent results were reported20. 

As shown in Table 2, the mixed-effect model only found that rs10466907 contributed significantly to cognitive recovery on set shifting measured by WCST test. Specifically, on the WCST test, significant differences between the genotypes (GT versus TT genotypes) were found in total scores completed (Wald \( \chi^2 = 11.801, p_{uncorrected} = 0.012, \beta = -0.944 \)), total error (Wald \( \chi^2 = 7.652, p_{uncorrected} = 0.006 (p_{corrected} = 0.006), \beta = -0.87 \)), and random error (Wald \( \chi^2 = 5.251, p_{corrected} = 0.22 \)).
(p_{uncorrected} = 0.022), \beta = -0.751), indicating carriers with GT genotype did not significantly improve their set shifting functioning after six weeks of treatment for bipolar depression compared with those carriers with TT genotypes (GG homozygotes were absent) (Fig. 1).

There were no significant differences after six weeks of treatment between the genotypes in HAM-D scores (GT genotype versus TT genotype: 6.4 versus 7.7 scores, F = 0.852, p = 0.357), YMRS scores (1.3 versus 1.1 scores, F = 0.076, p = 0.783), or BPRS scores (22.1 versus 22.7, F = 0.343, p = 0.559). The remission rate for patients with GT genotype (73.2%) was slightly higher than that for patients with TT genotype (61.6%), but did not reach a significant level (X^2 = 1.879, p = 0.17).

Comparisons in baseline cognitive measures by genotypes. The MANOVA analysis revealed that only SNP rs58619945 had a main effect on baseline neurocognitive measures, including the categories completed (F = 6.969, df = 2,149, p = 0.001), total errors (F = 4.690, df = 2,149, p = 0.011), and random errors of WCST (F = 3.305, df = 2,149, p = 0.40) (Table 3). Post-hoc comparisons using the Bonferonni corrections showed that carriers with GG genotype and GA genotype performed significantly worse than those with AA genotype on the categories completed (p = 0.013 and p = 0.001, respectively), total errors (p = 0.039 and p = 0.009, respectively), and random errors (p = 0.055 and p = 0.014, respectively).

### Table 2. The impact of rs10466907 genotypes on cognitive recovery after six weeks of treatment. Note: CI: confidence interval.

| Domain                          | B    | S.E  | Lower 95%CI | Upper 95%CI | Wald Chi-Square | P     |
|---------------------------------|------|------|-------------|-------------|----------------|-------|
| Processing speed                |      |      |             |             |                | 0.001 |
| WAIS-R symbol coding           | -0.227 | 0.176 | -0.571     | 0.117       | 1.667          | 0.197 |
| Attention                       |      |      |             |             |                | 0.006 |
| WAIS-R digit Forward            | -0.421 | 0.269 | -0.948     | 0.106       | 2.450          | 0.118 |
| Memory                          |      |      |             |             |                | 0.006 |
| Working memory                  |      |      |             |             |                | 0.006 |
| WAIS-R digit Backward           | 0.170 | 0.265 | -0.350     | 0.690       | 0.412          | 0.521 |
| Visual Memory                   |      |      |             |             |                | 0.006 |
| WAIS-R visual reproduction      | -0.127 | 0.291 | -0.697     | 0.442       | 0.192          | 0.661 |
| Verbal fluency                  |      |      |             |             |                | 0.006 |
| Animal naming                   | 0.052 | 0.239 | -0.416     | 0.520       | 0.048          | 0.827 |
| Executive function              |      |      |             |             |                | 0.006 |
| Wisconsin Card Sorting Test     |      |      |             |             |                | 0.006 |
| Categories completed            | -0.944 | 0.275 | -1.482     | -0.405      | 11.801         | 0.001 |
| Total errors                    | -0.870 | 0.315 | -1.487     | -0.254      | 7.652          | 0.006 |
| Perseverative errors            | -0.753 | 0.409 | -1.554     | 0.704       | 3.388          | 0.066 |
| Random errors                   | -0.751 | 0.328 | -1.392     | -0.109      | 5.251          | 0.022 |
| Tower of Hanoi total scores     | 0.187 | 0.522 | -0.836     | 1.211       | 0.128          | 0.720 |
| Trail Marking Test B            | -0.128 | 0.958 | -2.328     | 1.406       | 0.234          | 0.628 |

### Figure 1. Cognitive changes in the Wisconsin Card Sorting Test (WCST) for rs10466907 genotypes. Note: Z scores for the different neuropsychological tests were calculated using respective mean and standard deviation (SD). WCST1: total scores completed; WCST2: total error; WCST 3: random error. Cognitive changes in the Wisconsin Card Sorting Test (WCST) for rs10466907 genotypes. Carriers with rs10466907 GT genotype did not significantly improve their cognitive functioning compared with those carriers with TT genotypes after six weeks of treatment for bipolar depression.
Other clinical considerations. To test whether baseline demographic and clinical characteristics may account for the differences in cognitive recovery, we compared the following variables across the genotype groups stratified by rs10466907 and rs58619945: gender, age, years of education, HAM-D, YMRS, and BPRS scores. As shown in Table 1, we did not find any significant effect for either genotype (all p > 0.05). The patterns of the prescribed medications across the rs10466907 genotype groups were not significantly different (data not shown).

Discussions. In this six-week open-label trial for treating bipolar depression, we found preliminary evidence that CACNA1C SNP rs10466907 may have impacts on cognitive recovery. Carriers of GT genotype did not recover their executive function (i.e. set shifting) compared to TT homozogates after six weeks of treatment. The remission rate and depressive symptoms at week 6 were not significantly different between the two genotype groups, with the GT genotypes showing a non-significant higher trend in the remission rate, indicating that the differences in cognitive recovery was not driven by the clinical remission of depressive symptoms. The differential impacts by the SNP on cognitive recovery while bipolar patients were recovering from major depression might be related to the cognitive impairment heterogeneity among BD patients. Moreover, during a MDE, carrier with rs58619945 G allele (including GG homozygotes and GT heterozygotes) performed significantly poorer than those with TT genotype on set shifting, suggesting an effect of this SNP on executive function in patients with bipolar depression.

Studies demonstrated the involvement of CACNA1C gene in spatial learning by investigating cognitive deficits in CACNA1C knockout animals using a visible platform version of the Morris water maze and a spatial learning labyrinth paradigm. Moreover, patients with Timothy syndrome (TS), a disease caused by rare exonic mutations of CACNA1C gene (e.g. de novo missense mutation G406R), suffered not only cardiac arrhythmia but cognitive impairment and features of autism. Research into the physiology of TS-mutated Ca1.2 α1-subunit showed that it could lead to the upregulation of tyrosine hydroxylase expression, resulting in dysregulation of neurotransmitters norepinephrine and dopamine in depression. Furthermore, human studies support a role of voltage-gated cation channels such as CACNA1C in working memory-related learning in healthy individuals as well as in psychiatric conditions characterized by cognitive deficits. Future studies need to investigate whether the impact of rs10466907, which might act as an miRNA binding site, was mediated via regulation of CACNA1C expression.

We also studied SNP rs1006737, which might be the most-studied SNP in CACNA1C gene so far, but did not find any significant associations with cognition. Using the WCST, Sreier-de-Souza et al. found that bipolar patients with AA genotype performed significantly worse than those with GG genotype. The influences of the risk allele on gray matter (GM) changes in regions such as the prefrontal cortex (PFC), anterior cingulate cortex (ACC), and temporal cortex were also reported. Applying diffusion tensor imaging, Dietsche et al. showed an association between the risk allele and fractional anisotropy (FA) value, a measure indicative of white matter integrity, in the hippocampal formation. In the same bipolar patients, carriers with the risk allele displayed poorer

| Domain and measure | GG (n=54) | GA (n=96) | AA (n=41) | MANOVA* |
|--------------------|----------|----------|----------|---------|
|                    | Mean     | SD       | Mean     | SD       | Mean     | SD       | F       | p       | Post-Hocb |
| Processing speed   |          |          |          |         |          |         |        |         |          |
| Trail Marking Test A | 55.8 ± 31.0 | 55.1 ± 25.2 | 47.7 ± 22.5 | 0.131 | 0.877 |
| WAIS-R symbol coding | 39.3 ± 17.9 | 35.8 ± 16.4 | 40.8 ± 16.3 | 0.308 | 0.736 |
| Attention          |          |          |          |         |          |         |        |         |          |
| WAIS-R digit Forward | 8.0 ± 1.3 | 7.6 ± 1.6 | 8.1 ± 1.5 | 0.742 | 0.478 |
| Memory             |          |          |          |         |          |         |        |         |          |
| Working Memory     |          |          |          |         |          |         |        |         |          |
| WAIS-R digit Backward | 4.5 ± 1.6 | 4.2 ± 1.4 | 4.5 ± 1.8 | 0.669 | 0.514 |
| Visual Memory      |          |          |          |         |          |         |        |         |          |
| WAIS-R visual reproduction | 7.1 ± 3.6 | 7.0 ± 3.2 | 8.1 ± 4.1 | 0.645 | 0.526 |
| Verbal fluency     |          |          |          |         |          |         |        |         |          |
| Animal naming      | 16.5 ± 5.2 | 15.3 ± 5.3 | 15.7 ± 6.1 | 0.659 | 0.519 |
| Executive function |          |          |          |         |          |         |        |         |          |
| Wisconsin Card Sorting Test |          |          |          |         |          |         |        |         |          |
| Categories completed | 2.7 ± 2.2 | 2.5 ± 2.1 | 2.1 ± 4.1 | 6.965 | 0.001 |
| Total errors       | 25.4 ± 12.9 | 26.1 ± 13.2 | 18.1 ± 12.7 | 4.690 | 0.011 |
| Perseverative errors | 10.6 ± 9.5 | 11.4 ± 11.4 | 7.4 ± 9.8 | 1.832 | 0.164 |
| Random error       | 14.74 ± 7.6 | 15.14 ± 8.1 | 10.7 ± 6.5 | 3.305 | 0.04 |
| Trail Marking Test B | 97.9 ± 73.5 | 97.4 ± 47.7 | 91.9 ± 93.7 | 0.413 | 0.662 |
| Tower of Hanoi     |          |          |          |         |          |         |        |         |          |
| Total scores       | 42.6 ± 18.9 | 38 ± 18.7 | 46 ± 17.8 | 0.733 | 0.482 |

Table 3. Performance on neuropsychological test by bipolar patients stratified by rs58619945 genotypes. Note: *MANOVA: Multivariate Analysis of Variance; b p < 0.05 with Bonferroni correction.
performance on the Verbal Learning and Memory Test than did those without the allele. Considering functional magnetic resonance imaging (fMRI) studies, Paulus et al. found that healthy individuals with the homozygous risk allele displayed decreased activation in the dorsolateral PFC compared to non-risk allele carriers during a working memory task. However, it is estimated that genetic risk variants could have larger effects on the manifestation of the disorder in brain structure and functions compared to cognitive performance measures. Our negative result of rs1006737 was supported by other neuropsychological studies. However, one main difference between the present and previous studies is that we specifically tested patients in major depression state. Numerous pathophysiological processes underlie this state, during which bipolar patients suffered more severe cognitive impairments compared to when being in euthymic states. As such, the effect of rs1006737, if any, could be overlaid by the influence of such a critical clinical period.

Our findings may have potential implications for understanding the genetic variants underlying the heterogeneous cognitive impairments in bipolar patients which are further deteriorated during depressive episodes. Preventing the onset of major depression may be a crucial strategy for preserving cognitive functioning for those rs10466907 G allele carriers with BD, given the very limited medications for cognitive impairments for BD. Research into the function of the SNP could help elucidate the mechanisms by which it impacts cognitive recovery, opening a possibility for developing medications targeting on cognitive impairments. Some open-label studies suggest that repetitive transcranial magnetic stimulation (rTMS) may remedy cognitive impairment in major depression. Further studies may be needed to examine whether there are interactions between the two SNPs and the intervention effects.

There were some limitations that must be stated. Firstly, this trial was a naturalistic study and medications were not controlled. As such, the effects of medications on cognitive function and their potential interactions with genetic variants could not be assessed. However, it is unlikely that the differential effects of rs10466907 on cognitive recovery were totally driven by medications as the types of medications and their doses were not significantly different across the genotype groups. Secondly, it was a convenient sample and the sample size was small. Thirdly, the trial was short-term and some patients were still not clinically remitted. As such, it was unclear whether the impacts of the rs10466907 on cognitive recovery were sustained in longer periods. Fourth, the association of SNP rs10466907 with cognitive impairments might be confounded by clinical and demographic factors.

In summary, CACNA1C SNP rs10466907 may have impact on the cognitive recovery after six weeks of treatment for bipolar depression. Carriers of SNP rs10466907 G allele did not significantly improve their cognitive function. For SNP rs58619945, carriers with G allele performed significantly poorer on set shifting compared with AA homozygotes during an MDE. Our data suggest the involvement of CACNA1C SNP rs10466907 in cognitive recovery during the treatment of depression and an effect of rs5861995 on cognitive impairment during major depression. These findings may have potential implications for understanding the genetic variants underlying the heterogeneous cognitive impairments in bipolar patients which are further deteriorated during depressive episodes.
Immediate Visual Reproduction subtest of the Wechsler Memory Scale-Revised by China (WMS-RC) \(^4\). Patients were free of medications when being administered the cognitive assessments at baseline as they were either newly diagnosed cases or had discontinued psychiatric medications for at least two weeks. The primary outcome measures used in this trial were changes in the neurocognitive scores from baseline to week 6 of treatment.

**Genotyping.** We genotyped six SNPs in or near the CACNA1C gene which were chosen based on their previously reported potentials to affect gene expression/regulation as mentioned earlier. These SNPs were rs1006737, rs1051375, rs10466907, rs11062319, and rs58619945. The LD matrix between these SNPs and their positions on chromosome are shown Fig. 2.

Genomic DNA was extracted from whole blood according to standard procedures. Investigation of the SNPs was performed on the commercially available Sequenom MassARRAY platform. The six SNPs were simultaneously identified by a genotyping technology called iPLEX Gold (Sequenom) followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis\(^5,6\).

**Statistical analysis.** Plink 1.07 was used to establish a Hardy-Weinberg test statistics for each SNP (alpha = 10e-5) and to calculate pairwise LD between the SNPs. Neurocognitive measures with inverse scale properties were back-inverted so that higher scores indicated better performance. Demographic and clinical variables were compared among genotype groups using Chi-square test or one-way analyses of variance (ANOVA) where appropriate.

To test the effect of CACNA1C SNPs on changes in cognitive function, we used a mixed-effect regression model with unstructured covariance to model a 6-week change in cognitive performance by the CACNA1C SNPs tested. Each of the twelve neurocognitive measures was z-transformed using respective mean and standard deviation (SD) for the ease of comparisons. The model adjusted for the following variables: gender, age, years of education, depressive symptoms at baseline, psychotic symptoms at baseline, and medication (types of
antidepressant and antipsychotics). We excluded rs1006737 and rs11062319 in the following analysis because the frequency of minor allele (A allele = 2.9%; C allele = 2.7%, respectively) was less than 5%. As such, four SNPs in total were included in the analyses. Wald statistic (W) was applied to determine the significance of each predictor. To account for the multiple statistical testing, we set the significance level of p < 0.0125 for the four SNPs (Bonferroni corrections, 0.05/4 = 0.0125). Holm-Bonferroni method was further performed for the multiple-testing of the 12 neurocognitive measures with a significance level of p < 0.0125. As it is possible that such stringent significance level may produce unwanted false negatives, we also reported uncorrected significant effects (p < 0.05) for completeness.

To test the association of CACNA1C polymorphisms with cognitive function during a MDE, multivariate analysis of variance (MANOVA) was applied to compare neuropsychological measures at baseline among genotype groups, adjusting for gender, age, years of education, depressive symptoms at baseline, and psychotic symptoms at baseline. In order to reduce multiple statistical testing, all tested SNPs were simultaneously entered into the MANOVA model as fixed factors. Bonferroni corrections for multiple comparisons were applied in post-hoc testing with a significance level of p < 0.05.

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**Author Contributions**

The authors’ responsibilities were as follows; K.L.: performed the statistical analyses, interpreted the data, and wrote the manuscript; G.X.: designed and conducted the trial, interpreted the data; L.S.: did the genetic analysis and identified the genotypes; W.L.: drew the tables and figure; L.G.: did the statistical analysis; H.O., K.C., and Y.D.: conducted the trial; L.Z.; gave critical comments; and K.F.S.: interpreted the data, had overall responsibility for the study, and revised the manuscript. All authors read and approved the final manuscript.

**Additional Information**

**Competing Interests:** The authors declare that they have no competing interests.

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