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Short report

CACNA1C polymorphism and altered phosphorylation of tau in bipolar disorder

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Summary
Several genome-wide association studies and case–control studies have associated the single nucleotide polymorphism (SNP) rs1006737, situated in *CACNA1C* encoding the alpha 1C subunit of the L-type voltage-gated calcium channel, with bipolar disorder and other psychiatric disorders. However, the causal pathway linking genetic variants in *CACNA1C* with increased risk for developing brain disorders remains unclear. Here, we explored the association between the rs1006737 SNP and cerebrospinal fluid (CSF) markers. We found a significant association between the risk allele in rs1006737 and a decreased CSF hyperphosphorylated tau/total tau ratio in patients with bipolar disorder, thus linking variation in the *CACNA1C* gene to a neurochemical marker of neuroaxonal plasticity in those with this disorder.

Declaration of interest
None.

Bipolar disorder is a highly heritable psychiatric disorder but the aetiology of the disease is complex, involving multiple genetic and environmental influences. Identifying genetic variants associated with bipolar disorder will increase the understanding of disease mechanisms and may lead to development of targeted therapeutics. Several genome-wide association studies have been conducted that together suggest that the genetic susceptibility of bipolar disorder is clearly polygenic in nature.[1]

One identified single nucleotide polymorphism (SNP) that has been linked to bipolar disorder is of particular interest: rs1006737, which is situated within intron 3 of the gene *CACNA1C* at chromosome 12, with the *A* allele associated with an increased risk.[1] This gene codes the alpha 1C subunit ($Ca_v1.2$) of the L-type voltage-gated calcium channel.[2] $Ca_v1.2$ couples transient activation of inward calcium current to transcriptional regulation, which may play a role in dendritic development, neuronal survival, synaptic plasticity, memory formation, learning and behaviour.[3] The minor *A* allele has been associated with executive function deficits and has also been coupled to brain areas involved in affective regulation.[4] This allele has also been associated with schizophrenia and major depression, consistent with shared familial risks across psychiatric disorders.[5] However, the mechanisms underlying how genetic variants in *CACNA1C* may modify risk for psychiatric disorders and have an impact on cognition are unclear. One way to increase our understanding of the pathophysiology is to study the neurochemical correlates of *CACNA1C* gene variants. We have previously characterised the neurochemical profile of bipolar disorder by analyses of cerebrospinal fluid (CSF) markers of neuronal and glial function and degeneration in a large cohort of patients with bipolar disorder and healthy controls.[6,7] Here, we explored the association between the rs1006737 SNP and these CSF markers in patients with bipolar disorder and healthy controls.

Method

Our sample comprised 132 patients with bipolar disorder (type I: $n = 66$; type II: $n = 44$; other types $n = 22$) and 54 healthy controls. Patients were recruited from the St Göran Bipolar Project, enrolling patients from the bipolar unit at the Northern Stockholm Psychiatric Clinic, Stockholm, Sweden. All patients were assessed using a standardised interview protocol (the Affective Disorders Evaluation)[8] previously used in the Systematic Treatment Enhancement Program of Bipolar Disorder (STEP-BD). The study was approved by the Regional Ethics Committee in Stockholm and conducted in accordance with the latest Helsinki Protocol. After a complete description of the study, all enrolled patients and controls consented orally and in writing to participate in the study. See the online supplement DS1 for further details.

The CSF sampling (lumbar puncture) was performed when the participants were in a stable euthymic mood. Participants fasted overnight before CSF collection, which took place between 09.00 and 10.00 h. CSF samples were divided into 1.0–1.6 ml aliquots that were stored at −80°C pending analysis. The CSF concentrations of neurofilament light chain (NF-L), S100B, myelin basic protein (MBP) and heart-type fatty acid binding protein (H-FABP), hyperphosphorylated tau (P-tau), total tau (T-tau), soluble amyloid precursor protein alpha (sAPP-α), soluble amyloid precursor protein beta (sAPP-β), Aβ1-42, AβX-38, AβX-40 and AβX-42, were analysed as described by their respective manufacturers. We also included several biomarker ratios in the analysis: AβX-42/AβX-40, AβX-42/AβX-38, and P-tau/T-tau. See online supplement DS1 for further details.

The *CACNA1C* rs1006737 SNP was genotyped with the KASPar PCR SNP genotyping system (KBioscience, Hoddesdon, UK; www.lgcgenomics.com). SPSS Statistics version 20 was used for all statistical analyses. Analysis of covariance (ANCOVA) with age and gender as covariates was used to analyse effects of rs1006737 on CSF marker concentrations. All *P*-values are presented as two-tailed. Bonferroni correction was used to correct for multiple comparisons ($\alpha = 0.05/15 = 0.00333$).

Results

The *CACNA1C* rs1006737 SNP was genotyped in the 132 patients with bipolar disorder. The frequency for the *A* allele was 0.40 with the genotypes distributed according to Hardy–Weinberg equilibrium ($\chi^2 = 0.593$, $P = 0.441$). *AA* ($n = 23$) and *AG* ($n = 59$) genotype carriers were grouped together for analysis of the effects of the *A* and *G* alleles on CSF biomarker levels. Demographics of the two groups are displayed in online Table DS1. The groups did not differ significantly with regard to age, gender, smoking status,

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medications, previous episodes of psychosis, Global Assessment of Functioning[9] score, Clinical Global Investigation[10] score, diagnosis, duration of illness or number of episodes. A low P-tau/T-tau ratio was significantly associated with the *A* allele group ($F(1,128) = 13.484$, $P < 0.001$, $\alpha = 0.00333$) (online Fig. DS1), whereas the rs1006737 genotype had no effect on any of the other biomarkers (online Table DS2). We also found a significant association between the P-tau/T-tau ratio and the *A* allele under an additive model ($\beta = -0.260$, $P = 0.002$, age and gender as covariates). We next analysed whether this association was specific to bipolar disorder by analysing healthy controls ($n = 54$). The frequency for the *A* allele of rs1006737 in the control group was 0.30 with the genotypes distributed according to Hardy–Weinberg equilibrium ($\chi^2 = 0.675$, $P = 0.411$). In the control group, the rs1006737 SNP was not associated with the P-tau/T-tau ratio ($F(1,50) = 0.275$, $P = 0.602$) or with any of the other CSF biomarkers (online Table DS3).

Discussion

Variations in *CACNA1C* has previously been linked to various brain functions but it is unclear how these variations affect the brain on a chemical level. Here, we found a significant association between the rs1006737 SNP and the CSF P-tau/T-tau ratio in patients with bipolar disorder. No association was found in healthy controls, implying that the association is not a general physiological phenomenon but occurs in patients with a psychiatric illness.

$Ca_v1.2$ is primarily regulated through an interaction with Ca^{2+}-bound calmodulin (CaM), which also mediates the downstream effects of $Ca_v1.2$.[11] Downstream effectors of CaM include the CaM-dependent protein kinase (CaMK) cascade and the mitogen-activated protein kinase (MAPK) pathway.[3] Interestingly, phosphorylation of tau is regulated by a range of proline-directed and non-proline-directed kinases, including CaMK and MAPK,[12] linking calcium signalling with phosphorylation of tau. Phosphorylation of tau reduces its binding to microtubules leading to destabilisation of microtubules and promoting cytoskeletal flexibility, which have been suggested to be important for axonal and synaptic growth/development and thus neurodevelopment and synaptic plasticity.[12] In addition, tau phosphorylation is markedly increased in brain tissue in pathological conditions (i.e. tauopathies), and in CSF in Alzheimer's disease (for a review see Blennow *et al*[13]). There are, however, no differences between patients with bipolar disorder and controls in either P-tau or T-tau concentrations.[7] Thus, the difference in P-tau/T-tau between rs1006737 risk allele carriers and non-risk allele carriers probably reflects alterations in the regulation of tau phosphorylation.

Importantly, this study links variations in the *CACNA1C* gene to neuroaxonal plasticity at the neurochemical level in people with bipolar disorder. Further studies are, however, needed to sort out the biological and clinical significance of altered tau phosphorylation in relation to *CACNA1C* polymorphism in bipolar disorder and other psychiatric disorders.

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