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Short Report

# Association of CACNA1C polymorphisms with serum BDNF levels in bipolar disorder

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Variation in the *CACNA1C* gene has been associated with bipolar disorder in several genome-wide association studies. This gene encodes the alpha 1C subunit of L-type voltage-gated calcium channels, which play an essential role in neurons. We analysed 39 biomarkers in either cerebrospinal fluid or serum in relation to six different *CACNA1C* variants in 282 patients with bipolar disorder and 90 controls. We report associations of *CACNA1C* risk alleles with serum levels of BDNF as well as tissue plasminogen activator, which converts pro-BDNF to mature BDNF. This sheds light on links between *CACNA1C* genetic variants and pathophysiological mechanisms in bipolar disorder.

**Declaration of interest**
None.

**Keywords**
Bipolar disorder; endophenotypes; biomarkers; CACNA1C; brain-derived neurotrophic factor.



Bipolar disorder is a serious mental disorder, characterised by recurrent episodes of depression and mania, with a lifetime risk around 1%. The pathoaetiology has not been clarified but heritability estimates of up to 90% signify the importance of genetic factors. Commonly occurring single-nucleotide polymorphisms (SNPs) in the *CACNA1C* gene have been associated with bipolar disorder in several genome-wide association studies.[1] *CACNA1C* encodes the alpha 1C subunit of Lc-type voltage-gated calcium ($Ca^{2+}$) channels, which lead to an influx of extracellular $Ca^{2+}$ into a cell upon depolarisation. There are several common variants in the *CACNA1C* gene, but the strongest association with bipolar disorder has been seen for the SNP rs1006737 in the third intron.[2] Genetic association results need to be explored by studying allelic variation in relation to biomarkers and clinical phenotypes. Risk-associated *CACNA1C* SNPs predicted higher scores on depression and anxiety questionnaires in healthy individuals,[3,4] and decreased the hyperphosphorylated tau/total tau ratio in cerebral spinal fluid (CSF) from patients with bipolar disorder.[5]

Here, we investigate associations between *CACNA1C* polymorphisms and CSF or serum biomarkers in patients with bipolar disorder and controls. We report that *CACNA1C* polymorphisms are associated with serum levels of brain-derived neurotrophic factor (BDNF) and the enzyme converting pro-BDNF to mature BDNF, tissue plasminogen activator (tPA).

## Method

The sample includes 282 patients with bipolar disorder and 90 healthy controls (see Supplementary Table 1 available at https://doi.org/10.1192/bjp.2017.173 for details). Patients and controls were recruited from the St. Göran Bipolar Project, enrolling patients from the bipolar unit at the Northern Stockholm Psychiatric Clinic, Stockholm, Sweden.[6] The study was approved by the Regional Ethics Committee in Stockholm (approval number Dnr 2005/554-31/3) and conducted in accordance with the Helsinki Protocol. After a complete description of the study, all enrolled patients and controls consented orally and in writing to participate in the study. In brief, the Swedish version of the Affective Disorder Evaluation[7] was the key clinical assessment method. Bipolar diagnoses were made according to DSM-IV criteria as per the Structured Clinical Interview for DSM-IV.[8]

Participants were subjected to serum and CSF sampling. Lumbar puncture and serum sampling was performed on the same occasion when patients were in a stable mood phase, as described previously.[5] Assessment of depressive and manic symptoms are presented in Supplementary Table 7. Participants were genotyped for selected SNPs with KASP technology for Mac OS X (KBioscience, Hoddesdon, UK; see https://www.biosearchtech.com/products/pcr-kits-and-reagents/genotyping-assays/kasp-genotyping-chemistry). MATLAB (Mathworks version R2017b) and SPSS (IBM version 24), both for Mac OS X, were used for statistical calculations. The false discovery rate with threshold 0.1 was used to correct for multiple comparisons.

In total, 39 molecular species (including ratios) were measured from either CSF or serum, using a variety of analytic methods. Analytes included mature BDNF, pro-BDNF and the ratio. See Supplementary Materials and Methods for more details.

## Results

Six SNPs for *CACNA1C* were studied, but only risk alleles for SNP rs1006737 were significantly more common in patients (64 *v.* 46%, $P < 0.01$, see Supplementary Tables 2 and 3). In total, 39 molecules were measured in either CSF or serum. Here, we analyse these biomarkers in relation to *CACNA1C* SNPs in patients with bipolar disorder and controls (see Supplementary Table 4 and Supplementary File 1 for complete results). The only significant difference that withstood correction for multiple comparisons was serum levels of BDNF variants. For patients with the rs2370411 risk allele, the mature BDNF/pro-BDNF ratio was 46% higher than in patients without the risk allele (mean: 1.74 (95% CI 1.46–2.02) *v.* 1.20 (95% CI 1.00–1.39)). Although there was no significant difference in either mature BDNF or pro-BDNF itself, the former was numerically higher and the latter lower, which is consistent with the ratio being higher. For controls with risk allele rs1006737, the serum level of mature BDNF was 18% lower than in those without the risk allele (27.15 ng/mL (95% CI 25.6–28.7) *v.* 33.15 ng/mL (95% CI 30.0–36.3)). There were no significant differences in mature BDNF/pro-BDNF ratio or in pro-BDNF in controls, although the first was higher and the second lower in those with the risk allele. As the serine protease tPA is known to convert pro-BDNF to mature BDNF,[9] serum concentrations of tPA were analysed *post hoc* in



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relation to *CACNA1C* genotype. For controls with the rs2370411 risk allele (dominant model), the mature BDNF/pro-BDNF ratio was significantly correlated with tPA ($r = 0.56$, $P < 0.001$, see Supplementary Table 5 for complete information). There were no differences between risk allele carriers and those with wild type concerning age, gender, smoking status, body mass index or medication (Supplementary Tables 6 and 7).

## Discussion

Here, we screened for differences in biomarkers in both serum and CSF dependent on *CACNA1C* polymorphisms in patients with bipolar disorder and healthy controls. The main finding was that patients with the *CACNA1C* risk allele rs2370411 showed a 46% higher mature BDNF/pro-BDNF ratio in serum than patients without the risk allele. Further, in controls, the *CACNA1C* risk allele rs1006737 was associated with 18% lower mature BDNF serum levels.

The neurotrophic growth factor BDNF promotes the survival of existing neurons and induces differentiation and proliferation of developing neural precursors. By activating the extracellular protease plasmin, tPA regulates the cleavage of the immature form pro-BDNF to mature BDNF.[9] Importantly, pro-BDNF and mature BDNF impose opposite effects on several biological processes via different receptors.[10] Multiple studies support the role of BDNF in bipolar disorder.[11] In one study, the authors analysed protein levels of different forms of BDNF in *post mortem* material of a group of patients with major depression, schizophrenia and bipolar disorder.[12] In the parietal cortex, the concentration of the mature form was significantly lower in patients compared with healthy controls, whereas the concentration of the precursor form was higher. Also, all of tPA, mature BDNF and mature BDNF/pro-BDNF were found to be lower in patients with depression compared with controls.[13] Our results suggest that BDNF levels in serum are associated with *CACNA1C* polymorphisms, possibly via tPA. Interestingly, tPA levels were positively correlated with mature BDNF/pro-BDNF in controls, but not in patients, for those with the risk allele rs2370411. The reasons for this discrepancy is unknown. A potential confounder is that lithium has a neuroprotective function by activating BDNF signaling.[14] However, there was no difference in the proportion of patients treated with lithium in the two groups.

It remains a question for further research to unravel the mechanism by which *CACNA1C* genetic variants lead to changes in BDNF expression. It is known that BDNF expression is regulated by $Ca^{2+}$ signalling via activation of cAMP response element by CaM kinase IV.[15] The $Ca^{2+}$ channels that are encoded by *CACNA1C* are furthermore known to efficiently regulate transcription via cAMP response element.[16] One may therefore hypothesise that risk variants of *CACNA1C* lead to changes in $Ca^{2+}$ signalling, and thus altered BDNF-dependent transcription. Although the *CACNA1C* risk allele rs1006737 has gained widest interest in relation to bipolar disorder,[2] rs2370411 has also been reported to be involved in mood-related behaviour in humans and to interact with gender.[17]

To conclude, we report that different risk alleles of *CACNA1C* are associated with serum levels of BDNF in patients with bipolar disorder and controls. This sheds light on possible links between genetic variants and pathophysiological mechanisms in bipolar disorder, and opens up new lines of research.

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## Supplementary material

Supplementary material is available online at https://doi.org/10.1192/bjp.2019.173.

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EXTRA CONTENT ONLINE

## psychiatry in literature

# 'If I had not become a writer, I would probably have become a psychiatrist': on the occasion of the 160th anniversary of Anton Chekhov's birthday

**Florian Steger and Oxana Kosenko**


Anton Chekhov, photographed between 1899 and 1904. Postcard. Photographer unknown.

Last year we celebrated the 160th anniversary of Chekhov's birthday. Anton Pavlovich Chekhov (1860–1904) was a Russian physician, playwright and short-story writer. He started to write short stories to cover expenses for his medical education at Moscow University, today Sechenov University, and had become a well-known writer by the time he graduated and qualified as a physician in 1884. He described his attitude to medicine and literature in a letter to his publisher Aleksei Suvorin (1834–1912) on 11 September 1888: 'Medicine is my lawful wife, and literature is my mistress'. Both as a physician and as a writer, he was deeply interested in psychiatry and even admitted that he would probably have become a psychiatrist had he not become a writer.

In 1884, while still a student, Chekhov wrote his first clinical record of a patient with neurasthenia. Mental disorders continued to interest him while he was working as a physician in 1884–1897. Chekhov not only took care of his famous friends, such as the painter Isaak Levitan (1860–1900), who attempted suicide in 1885 and 1895, but he also treated patients with mental disorders and supported measures to combat alcoholism.

In 1890, Chekhov undertook a journey to the penal colony on the island of Sakhalin in the Russian Far East to study prisoners' daily life. He wrote a travel book *Sakhalin Island,* which showed the harsh living conditions of the convicts and the inadequacy of medical services on the island. When analysing the incidence data, he highlighted psychiatric diseases and their causes. The mentally ill convicts did not receive appropriate medical care. However, there were 'plenty of reasons for a weak person with ragged nerves to go crazy'. The book drew the Russian government's attention to the penal colony and thus contributed to alleviating the plight of the prisoners.

Chekhov's keen interest in psychiatry was reflected in many of his stories and plays, such as *Ward Number 6*, *A Nervous Breakdown*, *The Black Monk* and *Ivanov*. While working on them, he consulted the current medical literature, such as Sergei Korsakov's *Course in Psychiatry* (1893), to be precise in the description of symptoms.



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