Title:

Association of CACNA1Cpolymorphisms with serum BDNF levels in bipolar disorder

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# Materials and Methods

*Genotyping*

Genotypes for the selected SNPs were determined by Kompetitive Allele Specific PCR (KASP) technology (LGC, Queens Road, UK). Standard PCR cycling conditions was used according to the manufacturer’s instructions. The samples were analyzed on a 7900HT Fast Real-Time PCR System (Applied Biosystems). All cluster plots were manually inspected, and ambiguous results were excluded.

*Chemical analyses*

The number of patients and controls, respectively, with data on the corresponding biomarker denoted is outlined below. Analytes comprise serum BDNF including mature BDNF, pro BDNF, and the mature/pro ratio (N=220/90 [1]), serum matrix metallopeptidase 9 (MMP9, N=220/90 [1]), CSF S100 calcium-binding protein B (S100 B, N=136/88 [2]), CSF interleukin 5 (IL5, N=139/73 [3]), CSF monocyte chemoattractant protein 1 (MCP1, N=138/73 [4]), CSF kynurenic acid (KYNA, N=102/90 [5]), CSF neuropeptide Y (NPY, N=129/0 [6] ), CSF TIMP metallopeptidase inhibitor 1 and 2 (TIMP1/2, N=136/89 [4]), CSF cluster of differentiation 14 (CD14, N=137/89 [4]), CSF chitinase-3-like protein 1 (YKL40, N=137/89 [4]), CSF soluble amyloid precursor protein-α/β (sAPP-α/β, N=139/73 [7]), CSF amyloid beta 38/40/42 and isoform ratios (Aβ38/40/42, N=139/73 [7]), CSF hyperphosphorylated tau (H-tau, N=139/73 [7]), CSF phosphorylated tau (P-tau, N=139/73 [7]), CSF chromogranin B (CgB, N=139/73 [8]), CSF secretogranin II (SgII, N=139/89 [8]), CSF neurofilament light (NF-L, N=139/73 [2]), serum/CSF albumin ratio (N=139/89), CSF homovanillic acid (HVA, N=134/89 [9]), CSF 5-hydroxyindoleacetic acid (5-HIAA, N=134/89 [9]) and finally CSF 3-methoxy-4-hydroxyphenylglycol (MHPG, N=132/88 [9]). Given the results that *CACNA1C* risk alleles were associated with the mature BDNF / pro BDNF ratio, we post hoc acquired data on serum concentrations of tissue plasminogen activator that convert pro-BDNF to mature BDNF (tPA, N=197/89 [10]).

## *Statistical calculations*

Most analyses were performed using custom made scripts in MATLAB (MathWorks R2017b). Hypothesis testing was done using unpaired Student´s t test for numerical data and Fisher’s exact test or Chi-square test for dichotomous data. Chi-square tests were performed in SPSS (IBM Version 25). Numeric values are presented as means and standard deviation. For each biomarker and each SNP, the value for those with the risk allele was compared with the value for those without (patients and controls treated separately). The problem with multiple comparisons was addressed by controlling the false discovery rate (FDR). For biomarker analyses, a threshold FDR value of 0.10 was used, meaning that 10% of all significant values might be false positives. This rather liberal value was used in order to avoid false negatives. FDR correction was done separately for all SNPs.

**Results**

*Background characteristics*

Background data for patients and controls are shown in **Supplementary** **Table 1**. Among the patients, the mean (SD) GAF-F score was 66.9 (10.2), GAF-S 66.7 (10.9), and the mean CGI score 1.5 (0.57).

## *CACNA1C polymorphisms*

From the 1000 Genomes project, the following empirical minor allele frequencies are known: 19% (rs2370411), 30% (rs4765905), 14% (rs4765913), 19% (rs7297582), 30% (rs1006737), and 14% (rs1062577). In our cohort, the minor alleles were more common than in the 1000 Genomes project. The only significant difference between patients and controls was seen for SNP rs1006737 (**Supplementary Table 2-3**).

## *Biomarkers associated with genotype*

Case-control comparisons with respect to the biomarkers mentioned above have been presented previously ([1-10]).

# Tables

Supplementary Table 1 Characteristics of patients and controls.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Patients | Controls | Statistics |
| N | 282 | 90 |  |
| Age  | 37.9 (12.8) | 38.4 (14.3) | ns1 |
| Females (%) | 62.1 | 55.6 | ns2 |
| Smoking (%) | 31.2 | 12.2 | p < 0.012 |
| BMI | 25.0 (4.1) | 23.7 (2.9) | p < 0.011 |
| Post-secondary education (%) | 54.6 | 60.0 | ns2 |

BMI=Body mass index. Mean (SD) for age and BMI. 1 Unpaired Student’s t-test and 2 Fisher’s exact test.

Supplementary Table 2. Distribution of genotypes for patients and controls.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Patients | Controls | Statistics |
| rs2370411 AA/AC/CC (%) | 57/34/9 | 50/38/12 | ns |
| rs4765905 GG/GC/CC (%) | 36/47/16 | 44/43/14 | ns |
| rs4765913 TT/TA/AA (%) | 54/37/9 | 56/38/6 | ns |
| rs7297582 CC/CT/TT (%) | 32/50/18 | 37/49/14 | ns |
| rs1006737 GG/GA/AA (%) | 36/48/16 | 54/38/9 | p < 0.05 |
| rs1062577 TT/TA/AA (%) | 84/16/0 | 93/7/0 | ns |

Chi-square test.

Supplementary Table 3 Distribution of genotypes, dominant model. For complete results, see supplement.

|  |  |  |  |
| --- | --- | --- | --- |
| Major allele/minor allele | Patients | Controls | Statistics |
| rs2370411 AA/AC or CC (%) | 57/43 | 50/50 | ns |
| rs4765905 GG/GC or CC (%) | 36/64 | 44/56 | ns |
| rs4765913 TT/TA or AA (%) | 54/46 | 56/44 | ns |
| rs7297582 CC/CT or TT (%) | 32/68 | 37/63 | ns |
| rs1006737 GG/GA or AA (%) | 36/64 | 54/46 | p < 0.05 |
| rs1062577 TT/TA or AA (%) | 84/16 | 93/7 | ns |

Fisher’s exact test.

Supplementary Table 4. The table outlines the significant differences in serum levels of BDNF dependent on genotype.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Patient? | SNP | p value | FDR | Fold difference |
| Mature BDNF / pro BDNF | Yes | rs2370411 | 5.75e-04 | 0.022 | 1.46 |
| Mature BDNF  | No | rs1006737 | 0.0023 | 0.044 | 0.82 |

Unpaired Student’s t-test.

Supplementary Table 5. Relation between tPA and BDNF ratio.

|  |  |  |
| --- | --- | --- |
|  | Patients | Controls |
| rs2370411 AA | r=-0.003, p=0.98 (N=114) | r=-0.02, p=0.92 (N=47) |
| rs2370411 AC | r=0.03, p=0.85 (N=62) | r=0.41, p=0.021 (N=32) |
| rs2370411 CC | r=-0.25, p=0.027 (N=21) | r=0.80, p=0.005 (N=10) |

 Pearson correlation.

Supplementary Table 6. Characteristics of controls with either risk or wild type allele for rs1006737.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Risk allele | Wild type | Statistics |
| N | 26 | 30 |  |
| Age  | 39.4 (14.6) | 38.6 (14.7) | ns1 |
| Females (%) | 57.7 | 66.7 | ns2 |
| Smoking (%) | 11.5 | 13.3 | ns2 |
| BMI | 24.1 (3.6) | 24.2 (3.9) | ns1 |

Mean (SD). 1 Unpaired Student’s t-test and 2 Fisher’s exact test.

Supplementary Table 7. Characteristics of patients with either risk or wild type allele for rs2370411.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Risk allele | Wild type | Statistics |
| N | 92 | 127 |  |
| Age  | 36.4 (12.5) | 40.2 (13.3) | ns1 |
| Females (%) | 65.2 | 60.6 | ns2 |
| Smoking (%) | 40.2 | 35.4 | ns2 |
| BMI | 25.2 (5.1) | 25.1 (4.0) | ns1 |
| MADRS | 3.5 (5.5) | 3.6 (5.2) | ns1 |
| YMRS | 1.5 (2.8) | 1.1 (2.0) | ns1 |
| GAF-F | 68.7 (9.9) | 66.5 (10.5) | ns1 |
| GAF-S | 68.2 (10.4) | 66.4 (11.5) | ns1 |
| Years of illness | 18.4 (12.1) | 19.5 (12.8) | ns1 |
| Suicide attempt (%) | 38 | 37.8 | ns2 |
| Mood stabilizer (%) | 79.4 | 74.8 | ns2 |
| Lithium (%) | 51.1 | 56.7 | ns2 |
| Antipsychotics (%) | 25 | 22.1 | ns2 |
| Antidepressants (%) | 30.4 | 43.3 | ns2 |
| Anxiolytics (%) | 17.4 | 17.3 | ns2 |

Mean (SD). 1 Unpaired Student’s t-test and 2 Fisher’s exact test. MADRS: Montgomery-Åsberg Depression Rating Scale. YMRS: Young Ziegler Mania Rating Scale. GAF-F/S: Global Assessment of Functioning-Function/Symptom.

# References

1. Sodersten, K., et al., *Abnormality in serum levels of mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in mood-stabilized patients with bipolar disorder: a study of two independent cohorts.* J Affect Disord, 2014. **160**: p. 1-9.

2. Jakobsson, J., et al., *Elevated concentrations of neurofilament light chain in the cerebrospinal fluid of bipolar disorder patients.* Neuropsychopharmacology, 2014. **39**(10): p. 2349-56.

3. Isgren, A., et al., *Increased cerebrospinal fluid interleukin-8 in bipolar disorder patients associated with lithium and antipsychotic treatment.* Brain Behav Immun, 2015. **43**: p. 198-204.

4. Jakobsson, J., et al., *Monocyte and microglial activation in patients with mood-stabilized bipolar disorder.* J Psychiatry Neurosci, 2015. **40**(4): p. 250-8.

5. Olsson, S.K., et al., *Elevated levels of kynurenic acid in the cerebrospinal fluid of patients with bipolar disorder.* J Psychiatry Neurosci, 2010. **35**(3): p. 195-9.

6. Sandberg, J.V., et al., *Low neuropeptide Y in cerebrospinal fluid in bipolar patients is associated with previous and prospective suicide attempts.* Eur Neuropsychopharmacol, 2014. **24**(12): p. 1907-15.

7. Jakobsson, J., et al., *CACNA1C polymorphism and altered phosphorylation of tau in bipolar disorder.* Br J Psychiatry, 2016. **208**(2): p. 195-6.

8. Jakobsson, J., et al., *Decreased cerebrospinal fluid secretogranin II concentrations in severe forms of bipolar disorder.* J Psychiatry Neurosci, 2013. **38**(4): p. E21-6.

9. Palsson, E., et al., *Cerebrospinal fluid monoamine metabolite profiles in bipolar disorder, ADHD, and controls.* J Neural Transm (Vienna), 2017.

10. Lundberg, M., et al., *Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood.* Nucleic Acids Res, 2011. **39**(15): p. e102.