Data supplement to Jakobsson et al. *CACNA1C* polymorphism and altered phosphorylation of tau in bipolar disorder. Br J Psychiatry doi: 10.1192/bjp.bp.114.159806

Online supplement DS1

Method

Sample population

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 by a psychiatrist (or psychiatrist in training) using a standardized interview protocol (the Affective disorders evaluation, ADE) previously used in the Systematic Treatment Enhancement Program of Bipolar Disorder STEP-BD program.[14] The ADE guides the interviewer through a systematic assessment of the patient's current mental state, past history and diagnosis according to DSM-IV criteria as contained in the Structured Clinical Interview for DSM-IV (SCID). Co-morbid psychiatric disorders were screened for using Mini International Neuropsychiatric Interview (M.I.N.I.).[15] The full diagnostic assessment was based on all available sources of information including patient interview, case records and, if possible, interviews with the next of kin. The diagnoses were set at a diagnostic case-conference where all information at the time of admission was presented. A consensus panel of experienced board-certified psychiatrists specialized in bipolar disorder (n=2-5) made a best-estimate diagnostic decision. Using this procedure, the risk of inter-rater bias in the inclusion process was reduced. The general criteria for inclusion were patients at least 18 years old and who met the DSM-IV criteria for any bipolar disorder, i.e., type I, II, NOS (not otherwise specified), cyclothymia, or schizoaffective syndrome manic type. Information is collected about number of body mass index (BMI), depressive, manic, and mixed episodes, history of suicide attempts, family history (first or second degree relatives with bipolar disorder), history of abuse (alcohol or substances), comorbid anxiety disorders (i.e., panic disorder, social phobia, post-traumatic stress disorder, generalized anxiety disorder, obsessive-compulsive disorder, and agoraphobia), and history of psychosis. The lifetime severity of bipolar disorder was rated using the Clinical Global Impression (CGI) rating scales. This 7-point scale reflects the clinician's rate of the severity: 1=normal or not at all ill, 2=borderline mentally ill, 3=mildly ill, 4=moderately ill, 5=markedly ill, 6=severely ill, and 7=extremely ill. In order to determine euthymia, the Montgomery–Åsberg Depression Rating Scale (MADRS) and the young mania rating scale (YMRS) was used (euthymia defined as MADRS < 14 and YMRS < 14). For ethical reasons, patients continued to take their prescribed medications at the time of CSF sampling.

Age- and sex-matched healthy, population-based controls were randomly selected by Statistics Sweden (SCB) and contacted by mail. Interested persons contacted the study team that conducted a preliminary telephone screening to exclude severe mental health and neurological problems as well as substance abuse. Eligible persons were scheduled for a one-day comprehensive assessment. Of the controls that received the invitation mail, 14% contacted the research team. This is on par with other studies of similar nature according to SCB. Of those controls that volunteered, 75 were excluded at the telephone interview mainly due to drug use (N=16), changed their mind (N=14), somatic ill-health (N=12), metal objects in body excluding MRI (N=10), heredity in first degree relative of bipolar disorder or schizophrenia (N=9), ongoing mental health diagnoses (N=6), pregnancy (N=5), and moved out of area (N=2). One subject had no documented reason for exclusion. Furthermore, one

subject failed to show up for the assessments. Control subjects underwent a psychiatric interview by experienced clinicians using the M.I.N.I. to exclude psychiatric disorders.[15] Moreover the controls completed the same investigations the patients had undertaken including self-rating scales, somatic tests, blood tests and lumbar puncture. Because the assessments of controls might reveal pathological findings, case conferences were held between examining clinicians, primary investigator, and the study coordinator to decide whether or not to include such persons in the study. It was thus decided to allow past minor depressive episodes, isolated episodes of panic disorder, eating disorders or obsessive compulsive disorder that had remitted spontaneously or with brief psychotherapy counseling. Substance abuse was screened for at the telephone interview by the nurse, in the psychiatric interview, by AUDIT and DUDIT, as well as by determining serum levels of carbohydrate-deficient transferrin (CDT).[16] Overconsumption of alcohol as revealed by CDT or responses indicating large consumption (> 8 standard drinks per time more than 2 times per week), and/or amnesia and/or loss of control more than once per month resulted in the exclusion of these individuals from the study. Other exclusion criteria were neurological conditions other than mild migraines, untreated endocrinological disorders, pregnancy, dementia, recurrent depressive disorder, and suspected severe personality disorders (based on interview and SCID-II personality assessment), and a family history of schizophrenia or bipolar disorder in first-degree relatives.

The study was approved by the Regional Ethics Committee in Stockholm and conducted in accordance with the latest Helsinki Protocol. After complete description of the study, all enrolled patients and controls consented orally and in writing to participate in the study.

Cerebrospinal fluid sampling

CSF sampling (lumbar puncture) was performed when the participants were in a stable euthymic mood. Patients fasted overnight before the CSF collection that occurred between 9.00 and 10.00 a.m. The spinal needle was inserted into the L3/L4 or L4/L5 interspace and a total volume of 12 ml of the CSF was collected, gently inverted to avoid gradient effects, and divided into 1.0-1.6 ml aliquots that were stored at -80˚C pending analysis. An identical procedure was performed for the controls. All samples in this study were thawed and refrozen once before analysis.

Biomarker analysis

All biochemical analyses were performed at the Clinical Neurochemistry Laboratory in Mölndal, Sweden, by experienced laboratory technicians who were blinded to clinical information. The CSF concentrations of sAPP-α and sAPP-β, and Aβ38, Aβ40, and Aβ42 were determined using the MSD® sAPP-α/sAPP-β Multiplex Assay and MSD®Human/Rodent (4G8) Abeta-Triplex Assay, respectively, as described by the manufacturer (Meso Scale Discovery, Gaithersburg, MD, USA). CSF concentrations of hyperphosphorylated-Tau (P-tau), total-tau (T-tau), and Aβ1-42 were measured simultaneously by the Luminex xMAP technology using the Inno-Bia AlzBio3 kit (Innogenetics, Zwijndrecht, Belgium). NF-L was analyzed as previously described with a commercial ELISA assay (NF-light®, UmanDiagnostis AB, Umeå, Sweden). S100B was determined by an electrochemoluminescence immunoassay using the Modular system and the S100 reagent kit (Elecsys S100®, Roche Diagnostics, Penzberg, Germany). MBP (MBP ELISA, Beckman Coulter Inc., Brea, USA) and H-FABP (Human H-FABP, Hycult Biotechnology, Uden, The Netherlands) were measured using commercially available ELISA assays.

Additional references

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Table DS1. Demographics of the two *CACNA1C* genotype groups (N=132 bipolar disorder patients).

	G:G (n=50)	**G:A/A:A (n=82)**	
	N (%)	*N (%)*	*p*
Males	21 (42)	33(40)	0.857
Smoking [a]	15 (35)	20 (27)	0.407
Diagnosis			
BD type I	23 (46)	43 (52)	0.591
BD type II	18 (36)	26 (32)	0.165
BD others	9 (18)	13 (16)	0.812
Previous psychotic episodes [b]	24 (50)	42 (53)	0.855
Medications			
Lithium	29 (58)	47 (57)	1.000
Lamotrigine	7 (14)	22 (27)	0.431
Valproate	8 (16)	9 (11)	0.128
Antidepressants	23 (46)	36 (44)	0.858
Benzodiazepines	12 (24)	14 (17)	0.371
Antipsychotics	16 (32)	20 (24)	0.421
	Median (IQR)	*Median (IQR)*	*p*
Age (years)	34 (29-47)	37 (28-51)	0.769
BMI (kg/m^2)	25.2 (22.3-27.5)	12.6/87.4	0.647
Duration of illness (years)	12 (5-17)	11 (4-20)	0.765
Number of episodes			
Manic	0 (0-2)	1 (0-3)	0.204
Hypomanic	2 (0.5-4)	2 (0-8)	0.867
Depressive	6 (3-10)	4 (3-10)	0.204
Total	10 (6.5-18.5)	8 (5-20)	0.618
MADRS	4 (1-13)	3 (0-10)	0.284
YMRS	0 (0-1)	0 (0-2)	0.943
GAF	65 (60-70)	70 (60-75)	0.120
CGI	4 (4-5)	4 (4-5)	0.573

IQR = interquartile range, BMI = body mass index, CGI = clinical global impression, GAF = global assessment of functioning
[a] = data missing in 15 cases, [b] = data missing in 15 cases,

Table DS2. Effect of *CACNA1C* genotype on cerebrospinal fluid marker levels in bipolar disorder patients.

Biomarker	***GG* (n=50)**	***GA* or *AA* (n=82)**			
	Median (IQR)	*Median (IQR)*	*F*	*df*	*p*
S100B[1,2] (pg/ml)	809 (696-1020)	837 (692-1020)	0.159	1	0.690
h-FABP[1] (pg/ml)	399 (270-549)	418 (317-574)	0.376	1	0.541
NF-L[1,2] (pg/ml)	340 (230-610)	395 (240-560)	0.016	1	0.899
MBP[1,2] (pg/ml)	48 (36-64)	56 (36-72)	1.139	1	0.288
T-tau (pg/ml)	30 (23-41)	35 (27-42)	2.832	1	0.095
P-tau (pg/ml)	27 (21-32)	25 (22-29)	1.279	1	0.26
P-tau/T-tau[1]	0.84 (0.76-0.96)	0.75 (0.63-0.88)	13.484	1	<0.001*
sAPP-α[2] (ng/ml)	671 (482-878)	696 (535-928)	0.765	1	0.383
sAPP-β[2] (ng/ml)	281 (185-357)	271 (207-398)	0.305	1	0.582
Aβ1-42 (pg/ml)	250 (215-300)	260 (226-298)	1.212	1	0.273
AβX-38 (pg/ml)	1014 (785-1453)	1226 (935-1452)	2.088	1	0.151
AβX-40 (pg/ml)	7039 (5795-9076)	7961 (6681-9236)	2.245	1	0.137
AβX-42 (pg/ml)	831 (588-1166)	950 (701-1123)	0.647	1	0.423
AβX-42/AβX-40	0.115 (0.103-0.129)	0.117 (0.106-0.128)	0.038	1	0.846
AβX-42/AβX-38	0.812 (0.753-0.884)	0.775 (0.724-0.846)	3.123	1	0.080

[*] *= significant after correction for multiple comparisons (Bonferroni)*
[1] *= age is significantly ($p<0.05$) associated with biomarker levels*
[2] *= gender is significantly ($p<0.05$) associated with biomarker levels*
IQR = interquartile range, S100B = calcium-binding protein S100 B, h-FABP = heart-fatty acid binding protein, NF-L = Neurofilament light chain, MBP = myelin basic protein, T-tau = total tau, P-tau = phosphorylated tau, sAPP = soluble amyloid precursor protein, Aβ = amyloid beta,

Table DS3. Effect of *CACNA1C* genotype on cerebrospinal fluid marker levels in healthy controls.

Biomarker	***GG* (n=28)**	***GA* or *AA* (n=26)**			
	Median (IQR)	*Median (IQR)*	*F*	*df*	*p*
S100B[1,2] (pg/ml)	791 (692-963)	856 (761-1000)	0.103	1	0.751
h-FABP[1] (pg/ml)	367 (262-528)	372 (268-547)	0.371	1	0.545
NF-L[1,2] (pg/ml)	270 (215-440)	285 (230-450)	0.188	1	0.666
MBP[1,2] (pg/ml)	460 (350-621)	502 (396-640)	0.018	1	0.895
T-tau (pg/ml)	34 (27-41)	40 (27-49)	0.857	1	0.359
P-tau (pg/ml)	27 (23-28)	30 (26-33)	4.117	1	0.048
P-tau/T-tau[1]	0.80 (0.66-0.96)	0.78 (0.67-0.92)	0.275	1	0.602
sAPP-α[2] (ng/ml)	816 (664-949)	833 (721-1090)	1.206	1	0.277
sAPP-β[2] (ng/ml)	347 (249-431)	341 (280-435)	0.231	1	0.633
Aβ1-42 (pg/ml)	248 (200-295)	274 (240-306)	2.661	1	0.109
AβX-38 (pg/ml)	1105 (910-1472)	1277 (1053-1490)	0.767	1	0.385
AβX-40(pg/ml)	6942 (6267-9672)	8474 (7153-9635)	1.097	1	0.300
AβX-42 (pg/ml)	791 (631-1105)	977 (706-1149)	1.807	1	0.185
AβX-42/AβX-40	0.110 (0.096-0.115)	0.109 (0.100-0.121)	1.451	1	0.234
AβX-42/AβX-38	0.695 (0.650-0.768)	0.728 (0.667-0.822)	3.442	1	0.069

[1] *= age is significantly ($p<0.05$) associated with biomarker levels*
[2] *= gender is significantly ($p<0.05$) associated with biomarker levels*
IQR = interquartile range, S100B = calcium-binding protein S100 B, h-FABP = heart-fatty acid binding protein, NF-L = Neurofilament light chain, MBP = myelin basic protein, T-tau = total tau, P-tau = phosphorylated tau, sAPP = soluble amyloid precursor protein, Aβ = amyloid beta

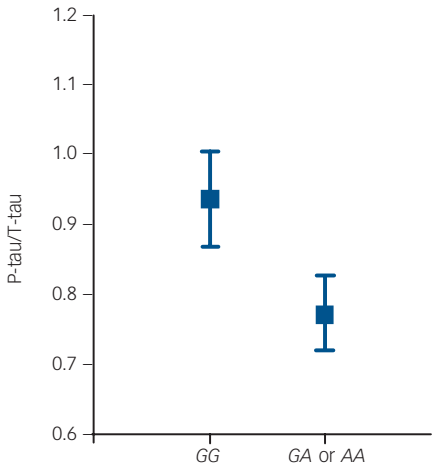

Fig. DS1 Hyperphosphorylated tau (P-tau)/total tau (T-tau) ratios in patients with bipolar disorder with (*GA* or *AA*) or without (*GG*) the rs1006737 risk allele.

Means adjusted for age and gender; error bars represent the 95% confidence interval of adjusted mean.