発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hashimoto, K.	Brain-derived neurotrophic factor (BDNF) and its precursor proBDNF as a diagnosic biomarker for major depression and bipolar depression.	Eur. Arch. Psychiatry Clin. Neurosci.	265	83-84	2015
Levin, R., Abarbanel, A., Edelman, S., Durrant, A.R., Hashimoto, K., Javitt, D.C., and Heresco-Levy, U.	Behavioral and cognitive effects of the N-methyl-D-aspartate receptor co-agonist D-serine in healthy humans.	J. Psychiatry Res.	61	188-195	2015
Hashimoto, K.	Targeting the sigma-1 receptor chaperone in the treatment of perinatal brain injury.	Exp. Neurol.	265	118-121	2015
Hashimoto, K.	Activation of sigma-1 receptor chaperone in the treatment of neuropsychiatric diseases and its clinical implication.	J. Pharmacol. Sci.	127	6-9	2015
Zhang, J.C., Wu, J., Fujita, Y., Yao, W., Ren, Q., Yang, C., Li, S.X., Shirayama, Y., and Hashimoto, K.	Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in the hippocampus and nucleus accumbens after inflammation.	Int. J. Neuropsychopharmacol.		In press.	2015
Yang, C., Shirayama, Y., Zhang, J.C., Ren, Q., and Hashimoto, K.	Regional differences in brain-derived neurotrophic factor and dendritic spine density confer resilience to inescapable stress.	Int. J. Neuropsychopharmacol.		In press.	2015
Yoshimi, N., Futamura, T., and Hashimoto, K.	Improvement of dizocilpine-induced social recognition deficits in mice by brexpiprazole, a novel serotonin-dopamine activity modulator.	Eur. Neuropsychopharmacol.		In press.	2015

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ishima, T., Futamura, T., Ohgi, Y., Yoshimi, N., Kikuchi, T., and Hashimoto, K.	Potentiation of neurite outgrowth by brexpiprazole, a novel serotonin-dopamine activity modulator: a role for serotonin 5-HT1A and 5-HT2A receptors.	Eur. Neuropsychopharmacol.		In press.	2015
Yang, C., and Hashimoto, K.	Combination of nitrous oxide with isoflurane or scopolamine for treatment-resistant major depression.	Clin. Psychopharmacol. Neurosci.		In press.	2015
橋本謙二	抗炎症作用を用いた精神疾患の治療.	精神科	25 (2)	153-156	2014
橋本謙二	NMDA受容体拮抗薬ketamineの即効性の抗うつ作用.	臨床精神薬理	17	1495-1500	2014
橋本謙二	酸化的ストレス・炎症からみた精神疾患発症の予防の可能性.	日本生物学的精神医学会誌		In press.	2015
Kimura A, Hashimoto T, Niitsu T, Iyo M.	Presence of psychological distress symptoms associated with onset-related life events in patients with treatment-refractory depression.	J Affect Disord.	175C	303-309	2015
Fabbri C, Minarini A, Niitsu T, Serretti A.	Understanding the pharmacogenetics of selective serotonin reuptake inhibitors.	Expert Opin Drug Metab Toxicol.	14-Jun	1-26	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Niitsu T, Shirayama Y, Matsuzawa D, Shimizu E, Hashimoto K, Iyo M.	Association between serum levels of glial cell-line derived neurotrophic factor and attention deficits in schizophrenia.	Neurosci Lett.	575C	37-41	2014
Sasaki T, Hashimoto K, Tachibana M, Kurata T, Kimura H, Komatsu H, Ishikawa M, Hasegawa T, Shiina A, Hashimoto T, Kanahara N, Shiraishi T, Iyo M.	Tipepidine in adolescent patients with depression: a 4 week, open-label, preliminary study.	Neuropsychiatr Dis Treat.	10	719-722	2014
Niitsu T, Ishima T, Yoshida T, Hashimoto T, Matsuzawa D, Shirayama Y, Nakazato M, Shimizu E, Hashimoto K, Iyo M.	A positive correlation between serum levels of mature brain-derived neurotrophic factor and negative symptoms in schizophrenia.	Psychiatry Res.	215(2)	268-273	2014
Iguchi Y, Kosugi S, Nishikawa H, Lin Z, Minabe Y, Toda S.	Repeated exposure of adult rats to transient oxidative stress induces various long-lasting alterations in cognitive and behavioral functions.	PloS One	9	e114024	2014
Miura K, Hashimoto R, Fujimoto M, Yamamori H, Yasuda Y, Ohi K, Umeda-Yano S, Fukunaga M, Iwase M, Takeda M.	An integrated eye movement score as a neurophysiological marker of schizophrenia.	Schizophrenia Research	160(1-3)	228-9	2014
Shintani N, Onaka Y, Hashimoto R, Takamura H, Nagata T, Umeda-Yano S, Mouri A, Mamiya T, Haba R, Matsuzaki S, Katayama T, Yamamori H, Nakazawa T, Nagayasu K, Ago Y, Yagasaki Y, Nabeshima T, Takeda M, Hashimoto H.	Behavioral characterization of mice overexpressing human dysbindin-1.	Molecular Brain	9;7(1)	74	2014
Hashimoto R, Ikeda M, Yamashita F, Ohi K, Yamamori H, Yasuda Y, Fujimoto M, Fukunaga M, Nemoto K, Takahashi T, Ochigi M, Onitsuka T, Yamasue H, Matsuo K, Iidaka T, Iwata N, Suzuki M, Takeda M, Kasai K, Ozaki N.	Common variants at 1q36 are associated with superior frontal gyrus volume.	Translational Psychiatry	4	e472	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Iwata R, Ohi K, Kobayashi Y, Masuda A, Iwama M, Yasuda Y, Yamamori H, Tanaka M, Hashimoto R, Itohara S, Iwasato T.	RacGAP α2-chimaerin function in development adjusts cognitive ability in adulthood.	Cell Report	8(5)	1257-64	2014
Yamamori H, Hashimoto R, Fujita Y, Numata S, Yasuda Y, Fujimoto M, Ohi K, Umeda-Yano S, Ito A, Ohmori T, Hashimoto K, Takeda M.	Changes in plasma D-serine, L-serine, and glycine levels in treatment-resistant schizophrenia before and after clozapine treatment.	Neurosci Lett	582	93-8	2014
Yasuda Y, Hashimoto R, Fukai R, Okamoto N, Hirai Y, Yamamori H, Fujimoto M, Ohi K, Taniike M, Mohri I, Nakashima M, Tsurusaki Y, Saitsu H, Matsumoto N, Miyake N, Takeda M.	Duplication of the NPHP1 gene in patients with autism spectrum disorder and normal intellectual ability: a case series.	Annals of General Psychiatry	13	22	2014
Watanabe Y, Tanaka H, Tsukabe A, Kunitomi Y, Nishizawa M, Hashimoto R, Yamamori H, Fujimoto M, Fukunaga M, Tomiyama N.	Neuromelanin magnetic resonance imaging reveals increased dopaminergic neuron activity in the substantia nigra of patients with schizophrenia.	PLos One	11	e104619	2014
Fujino H, Sumiyoshi C, Sumiyoshi T, Yasuda Y, Yamamori H, Ohi K, Fujimoto M, Umeda-Yano S, Higuchi A, Hibi Y, Matsuura Y, Hashimoto R, Takeda M, Imura O.	Performance on the Wechsler Adult Intelligence Scale-III in Japanese patients with schizophrenia.	Psychiatry and Clinical Neurosciences	68(7)	534-541	2014
Yasuda Y, Hashimoto R, Ohi K, Yamamori H, Fujimoto M, Umeda-Yano S, Fujino H, Takeda M.	Cognitive inflexibility in Japanese adolescents and adults with autism spectrum disorders.	Workd J Psychiatr	22;4(2)	42-48	2014
Nishizawa D, Ohi K, Hashimoto R, Yamamori H, Yasuda Y, Fujimoto M, Umeda-Yano S, Takeda M, Ikeda K.	Association between genetic polymorphism rs2952768, close to the METTL21A and CREB1 genes, and intellectual ability in healthy subjects.	Journal of Addiction Research & Therapy	5(2)	1000178	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ohi K, Hashimoto R, Ikeda M, Yamashita F, Fukunaga M, Nemoto K, Ohnishi T, Yamamori H, Yasuda Y, Fujimoto M, Umeda-Yano S, Watanabe Y, Iwata N, Weinberger DR, Takeda M.	Genetic risk variants of schizophrenia associated with left superior temporal gyrus volume.	Cortex	58C	23-26	2014
Horiguchi M, Ohi K, Hashimoto R, Hao Q, Yasuda Y, Yamamori H, Fujimoto M, Umeda-Yano S, Takeda M, Ichinose H.	A functional polymorphism (C-824T) of the tyrosine hydroxylase gene affects intelligence quotient in schizophrenia.	Psychiatry and Clinical Neurosciences	68(6)	456-62	2014

Ⅳ.研究成果の刊行物・別刷

Corresponding Author: Hassan Fathallah-Shaykh, MD, PhD, University of Alabama at Birmingham, Department of Neurology, 150 3rd Ave S, FOT 1020, Birmingham, AL 35295-2410 (hfathall@uab.edu).

Conflict of Interest Disclosures: None reported.

1. Love MN, Fathallah-Shaykh H. Network for excellence in Neuroscience Clinical Trials: NeuroNEXT. *JAMA Neurol*. 2013;70(10):1227-1228. doi:10.1001/jamaneurol.2013.3663.

Serum Brain-Derived Neurotrophic Factor as a Predictor of Incident Dementia

To the Editor I read with great interest the article by Weinstein et al[1] describing the association between serum brain-derived neurotrophic factor (BDNF) levels and the risk for dementia. In this community-based cohort study of 2131 participants, with a 10-year follow-up, 140 participants developed dementia, 117 of whom had Alzheimer disease (AD). They found that dementia-free participants with higher serum BDNF levels were less likely to develop dementia and AD, after controlling for age, sex, and cohort. Interestingly, this significant association between serum BDNF and the risk for incident dementia and AD was confined to women. In contrast, *BDNF* gene variants were not associated with AD risk. Their study suggests that low serum BDNF may play a role in the development of AD, especially in older women, the group at highest risk for AD, and that serum BDNF may also serve as a novel predictor of dementia and AD in healthy adults.[1]

Brain-derived neurotrophic factor (mature BDNF) is a 13-kDa polypeptide, which is initially synthesized as a precursor protein, preproBDNF, in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF (approximately 32 kDa) is converted to mature BDNF by extracellular proteases. It was initially thought that only secreted, mature BDNF was biologically active and that proBDNF, localized intracellularly, served as an inactive precursor. However, accumulating evidence shows that both proBDNF and mature BDNF are active, eliciting opposing effects via the $p75^{NTR}$ and TrkB receptors, respectively, and that both forms play important roles in several physiological functions.[2]

The enzyme-linked immunosorbent assay kits used by Weinstein et al[1] recognize both proBDNF (precursor of BDNF) and mature BDNF owing to limited specificity of the BDNF antibody.[3] Recently, using newly available human proBDNF and mature BDNF enzyme-linked immunosorbent assay kits that differentiate between the BDNF forms, we reported high levels of both proBDNF and mature BDNF in human serum.[3,4]

Considering the high levels of both proBDNF and mature BDNF in human serum and their putative opposing functions, it would be clinically and scientifically interesting to measure the individual serum levels of precursor (proBDNF) and mature BDNF in this cohort study. In addition, orally active TrkB agonists, such as 7,8-dihydroxyflavone,[5] could serve as potential preventive or therapeutic drugs for dementia by augmenting the recorded decreases in serum BDNF levels detected in healthy people who go on to develop dementia or AD.

Kenji Hashimoto, PhD

Author Affiliation: Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan.

Corresponding Author: Kenji Hashimoto, PhD, Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, 1-8-1 Inohana, Chiba 260-8670, Japan (hashimoto@faculty.chiba-u.jp).

Conflict of Interest Disclosures: Dr Hashimoto is a holder of the patents "Diagnostic and examination method for eating disorder" (US 7,754,434 B2) and "Diagnostic agent for ischemic heart disease risk group" (US 2013/0310321A1), which pertain to the measurement of brain-derived neurotrophic factor as a biomarker. In addition, Dr Hashimoto has served as a scientific consultant to Astellas and Taisho, and he has received research support from Abbvie, Dainippon Sumitomo, Otsuka, and Taisho.

1. Weinstein G, Beiser AS, Choi SH, et al. Serum brain-derived neurotrophic factor and the risk for dementia: the Framingham Heart Study. *JAMA Neurol*. 2014;71(1):55-61.

2. Hashimoto K. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. *Psychiatry Clin Neurosci*. 2010;64(4):341-357.

3. Yoshida T, Ishikawa M, Iyo M, Hashimoto K. Serum levels of mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in healthy subjects. *Open Clin Chem J*. 2012;5:7-12. doi:10.2174/1874241601205010007.

4. Yoshida T, Ishikawa M, Niitsu T, et al. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. *PLoS One*. 2012;7(8):e42676.

5. Hashimoto K. Sigma-1 receptor chaperone and brain-derived neurotrophic factor: emerging links between cardiovascular disease and depression. *Prog Neurobiol*. 2013;100:15-29.

In Reply We thank Dr Hashimoto for highlighting the presence and opposing actions of 2 circulating proteolytic isoforms of brain-derived neurotrophic factor (BDNF), proBDNF, and mature BDNF. In the context of Alzheimer disease (AD), mature BDNF, acting through the TrkB receptor, promotes cell survival and dendritic spine formation while proBDNF induces apoptosis and dendritic spine pruning in the hippocampus.[1] In animal models of learning and memory formation, the conversion of proBDNF to mature BDNF is necessary for synaptic long-term potentiation in glutamergic synapses and the acquisition and consolidation of memories, whereas elevated levels of proBDNF mediate synaptic long-term depression and memory extinction.[1] Hence, mature BDNF rather than proBDNF appears more likely to reduce the risk for AD. However, in postmortem studies of human brains, both proBDNF and mature BDNF levels were reduced in the cortex of persons with mild cognitive impairment[2] and AD.[2,3] We have previously shown in the Framingham Study cohort that TrkB levels were increased in the hippocampus of cognitively normal persons with early AD pathology compared with cognitively normal control individuals with no AD pathology; this may represent a compensatory response to the decline in BDNF levels and a biological basis for cognitive reserve.[4]

The enzyme-linked immunosorbent assay kit used in our study, while admittedly not isoform specific, is targeted toward mature BDNF with only about 13% cross-reactivity with proBDNF. In addition, the levels of proBDNF in the serum of healthy individuals are, on average, 3 times lower than mature BDNF levels.[5] Hence, it appears reasonable to assume that the inverse association between BDNF levels and incident AD risk observed in our study was largely attributable to mature BDNF. However, we concur that further studies are warranted not only to assess the role of proBDNF and mature BDNF molecules in AD, but also to investigate their respective receptors and protein-receptor pathways.

Galit Weinstein, PhD
Sudha Seshadri, MD

Author Affiliations: Department of Neurology, Boston University School of Medicine, Boston, Massachusetts (Weinstein, Seshadri); The Framingham Heart Study, Boston University School of Medicine, Boston, Massachusetts (Weinstein, Seshadri).

Corresponding Author: Sudha Seshadri, MD, Department of Neurology, Boston University School of Medicine, 72 E Concord St, B602, Boston, MA 02118 (suseshad@bu.edu).

Conflict of Interest Disclosures: None reported.

1. Carlino D, De Vanna M, Tongiorgi E. Is altered BDNF biosynthesis a general feature in patients with cognitive dysfunctions? *Neuroscientist*. 2013;19(4):345-353.

2. Peng S, Wuu J, Mufson EJ, Fahnestock M. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem*. 2005;93(6):1412-1421.

3. Michalski B, Fahnestock M. Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease. *Brain Res Mol Brain Res*. 2003;111(1-2):148-154.

4. Kao PF, Banigan MG, Vanderburg CR, et al. Increased expression of TrkB and Capzb2 accompanies preserved cognitive status in early Alzheimer disease pathology. *J Neuropathol Exp Neurol*. 2012;71(7):654-664.

5. Yoshida T, Ishikawa M, Niitsu T, et al. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. *PLoS One*. 2012;7(8):e42676.

Creating a Concussion Crisis and Chronic Traumatic Encephalopathy

To the Editor I read with skeptical interest Dr Cantu's article on sports concussion.[1] Unlike other neurology specialties, sports concussion is driven not by science but opinion in the form of the numerous consensus conferences he referenced. The mainstay of concussion treatment has been complete physical and cognitive rest based on previous conference recommendations. For the most recent international conference, a literature review was decided, which found sparse empirical evidence for this dogma.[2] Stories abound from neuropsychology colleagues of student athletes who have been granted a vacation from life for weeks on end, with no scholastic work, video games, text messaging, or similar activities that might theoretically delay recovery. In a lecture at Williams College in September 2011, Dr Cantu and his colleague, Christopher Nowinski (an ex-professional wrestler), from the Center for the Study of Traumatic Encephalopathy gave the disturbing advice that ignoring rest can lead to suicide.[3] In a number of high-profile cases, families have been led to believe the cause of the suicide was a single concussion or chronic traumatic encephalopathy (CTE).

Dr Cantu speaks of the escalating media attention. Examples abound as to how the Center for the Study of Traumatic Encephalopathy has arguably created this concussion crisis. The most disturbing for neurologists was a 2010 report suggesting concussions in football can cause amyotrophic lateral sclerosis. As is emblematic of the Center for the Study of Traumatic Encephalopathy, they fed this story to the *New York Times*, which preannounced the article and suggested that maybe Lou Gehrig did not have amyotrophic lateral sclerosis but CTE.[4] This alarmed 17 amyotrophic lateral sclerosis researchers and clinicians, causing them to send a letter to the journal editor.[5] In reference to the deleterious effects of concussion, Dr Cantu went on to discuss advanced imaging techniques, evoked potentials, computerized electroencephalography, genetics, Alzheimer disease, and the neuropathology of CTE. Accepting his assertions, including that football causes CTE, requires on the part of the clinician who is well-read on this topic to make a sizeable leap of clinical faith when you consider that just a few years ago, the rather narrow clinical world of the sports concussion doctor on the sidelines was deciding when to take a player out of a game and when to put him or her back in. Dr Cantu stated neuropsychologists have been studying the cognitive effects of concussion for nearly 40 years but the events of the last 5 years have had iatrogenic consequences. They have served only to reverse our efforts of the previous 35 years.

Jim Andrikopoulos, PhD, ABPP

Author Affiliation: Mercy Hospital Medical Center, Ruan Neurology Clinic, Des Moines, Iowa.

Corresponding Author: Jim Andrikopoulos, PhD, ABPP, Mercy Hospital Medical Center, Ruan Neurology Clinic, 1111 Sixth Ave, East Tower, Suite A100, Des Moines, IA 50314 (neuroclinic@msn.com).

Conflict of Interest Disclosures: Dr Andrikopoulos has provided expert testimony.

1. Cantu RC. The role of the neurologist in concussions: when to tell your patient to stop. *JAMA Neurol*. 2013;70(12):1481-1482.

2. McCrory P, Meeuwisse WH, Aubry M, et al. Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport, Zurich, November 2012. *J Athl Train*. 2013;48(4):554-575.

3. The impact of concussions in youth and college sports: Q and A. http://www.youtube.com/watch?v=nhoIwKhXejw. Accessed December 13, 2013.

4. Schwarz A. Study says that brain trauma can mimic ALS. *New York Times*. August 17, 2010. http://www.nytimes.com/2010/08/18/sports/18gehrig.html. Accessed December 13, 2013.

5. Bedlack RS, Genge A, Amato AA, et al. Correspondence regarding: TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy: *J Neuropathol Exp Neurol* 2010:69;918-29. *J Neuropathol Exp Neurol*. 2011;70(1):96-97, author reply 98-100.

When to Tell an Athlete to Stop Playing

To the Editor I read with interest the article by Dr Cantu[1] about when to tell your patient with concussion and postconcussion syndrome that it is time to hang up his or her gloves, football gear, hockey stick, or soccer shoes and retire. As a neurologist, I find myself increasingly struggling with this decision-making process. When it comes to the athlete who indulges in a sport primarily for recreational and exercise purposes, the decision is easy both for me to make and for my patient to accept. Better be safe than sorry! When it comes to the professional or collegiate athlete, the stakes are much higher, including the potential loss of livelihood (eg, salary, winnings, endorsement incomes, or a lucrative athletic scholarship) not just for him or her, but his or her whole family. How can I advise that when we all know of athletes who have come back from devastating knock-

Journal of Affective Disorders 160 (2014) 1–9

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

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

Kristoffer Södersten [a,*], Erik Pålsson [a], Tamaki Ishima [b], Keiko Funa [c], Mikael Landén [a,d], Kenji Hashimoto [b], Hans Ågren [a]

[a] Institute of Neuroscience and Physiology, Section of Psychiatry and Neurochemistry, University of Gothenburg, Gothenburg SE-416 85, Sweden
[b] Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan
[c] Sahlgrenska Cancer Center, University of Gothenburg, Gothenburg, Sweden
[d] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form
20 January 2014
Accepted 21 January 2014
Available online 24 February 2014

Keywords:
Bipolar disorder
Biomarker
Mature BDNF
proBDNF
Diagnosis
Serum

ABSTRACT

Background: Early detection and diagnosis of bipolar disorder can be difficult. Tools are needed to help clinicians detect bipolar disorder earlier, which would ameliorate the prognosis.
Methods: ELISA kits that distinguish between mature brain derived neurotrophic factor (BDNF) and proBDNF, we compared serum levels of mature BDNF, proBDNF, and matrix metalloproteinase-9 (MMP-9) in two independent cohorts (Sahlgrenska cohort and Karolinska cohort) of mood-stabilized bipolar patients and healthy controls. The total sample size in both cohorts consisted of 263 (48+215) bipolar patients and 155 (43+112) healthy controls.
Results: Levels of mature BDNF and the ratio mature BDNF/proBDNF were significantly higher in patients than in controls. Serum levels of proBDNF were significantly lower in patients compared to controls. Serum levels of MMP-9 did not differ between the groups but MMP-9 correlated positively and significantly with mature BDNF.

Mature BDNF, proBDNF, the ratio of mature BDNF/proBDNF and interactions with MMP-9 explained the diagnostic dichotomy in both cohorts with high significance, using multivariate logistic ANCOVA (gender, age, and BMI were covaried out). The model explained 41% of the diagnostic variance in the Sahlgrenska cohort ($p < 0.0001$) and 15% in the Karolinska cohort ($p < 0.0001$). In both cohorts, the equations provided good power for diagnostic classification. The diagnostic sensitivity was 89% in the Sahlgrenska and 74% in the Karolinska cohort, and specificity 77% and 64%, respectively.
Limitation: The study is cross-sectional with no longitudinal follow up. The cohorts are relatively small with no medication-free patients. There are no "ill patient controls".
Conclusion: Abnormalities in the conversion of proBDNF to mature BDNF may be associated with pathogenesis of bipolar disorder. Clinical use of these biomarkers may provide opportunities for earlier detection and correct treatment.

1. Introduction

Bipolar disorder is a debilitating mental illness with a high mortality rate (Angst et al., 2002; Belmaker, 2004). About 1–2% of the world population suffers from bipolar disorder, affecting males and females equally. Despite syndrome remission, the premorbid functional level rarely fully recovers (Goldstein et al., 2009; Tohen et al., 2000). Bipolar disorder has been shown to comprise neurodegenerative features in which relapses are toxic (Ekman et al., 2010), underlining the importance of early detection in order to prevent an otherwise negative prognosis.

The causal factors of psychiatric disorders are multifactorial, and most diagnostic phenotypes have multiple underlying etiologies with similar clinical expressions. The challenge is to define a set of biomarkers that, despite different underlying mechanisms, can be linked to a clinical phenotype, and which would allow early detection.

Brain-derived neurotrophic factor (BDNF) is a protein synthesized from a precursor, preproBDNF, which is converted to proBDNF. ProBDNF is cleaved to generate mature BDNF by extracellular proteases, and BDNF crosses the blood–brain barrier (Pan et al., 1998; Schmidt and Duman, 2010). One such extracellular

* Corresponding author. Tel.: +46 70 964 3468.
E-mail address: kristoffer.sodersten@gu.se (K. Södersten).

http://dx.doi.org/10.1016/j.jad.2014.01.009
0165-0327

protease is matrix metalloproteinase-9 (MMP-9), but there are others, *e.g.* plasmin (Park and Poo, 2013). BDNF has been proposed to be a state-related biomarker of mood disorders (Fernandes et al., 2011; Hashimoto, 2010; Lin, 2009). BDNF is widespread in the brain and abundant in the hippocampus and cerebral cortex. BDNF plays roles in mood, emotion and cognition (Ernfors et al., 1990). BDNF has also been shown to be correlated to therapeutic effects of anti-depressants (Altar, 1999; Duman et al., 1997; Hashimoto, 2010, 2013; Hashimoto et al., 2004; Martinowich et al., 2007; Nestler et al., 2002).

Accumulating evidence suggests a key role of BDNF in the pathogenesis of bipolar disorder (Hashimoto, 2010). It has been reported that blood levels of BDNF were decreased in patients with bipolar disorder during manic (Cunha et al., 2006; de Oliveira et al., 2009; Machado-Vieira et al., 2007; Palomino et al., 2006), and depressed phases (Cunha et al., 2006; de Oliveira et al., 2009; Yoshimura et al., 2006). One study also found decreased levels of BDNF during euthymia (Monteleone et al., 2008). However, these findings were not replicated in other reports (Dias et al., 2009; Huang et al., 2012; Kauer-Sant'Anna et al., 2009; Mackin et al., 2007). Meta-analyses (Fernandes et al., 2011; Huang et al., 2012; Lin, 2009) have concluded serum levels of BDNF to be significantly lower in patients with bipolar disorder, and BDNF levels were normalized after recovery from depression (Hashimoto, 2010).

One likely reason for the divergent results is that earlier ELISA kits were unable to distinguish between mature BDNF and proBDNF (Yoshida et al., 2012a). Formerly, proBDNF was argued to be biologically inactive, but subsequent studies have shown that proBDNF and mature BDNF have opposite effects via $p75^{NTR}$- and TrkB-receptors. Both play an important role in different physiological functions (Dieni et al., 2012; Hashimoto, 2007, 2010, 2013; Pang and Woo, 2005), such as synaptic plasticity, neuronal survival, and neuronal differentiation (Poo, 2001). Supporting the notion that it is important to distinguish between proBDNF and mature BDNF, Yoshida and collaborators (Yoshida et al., 2012b) reported that serum levels of mature BDNF, but not proBDNF, were significantly lower in patients with major depressive disorder (MDD) than those of healthy controls.

The aims of this study were (i) to test the hypothesis that the BDNF synthetic pathway is altered in bipolar disorder compared to normal controls, and (ii) to elucidate whether BDNF-related independent variables are useful for clinical diagnostic predictions. To these ends, we studied subcomponents in the BDNF synthetic pathway (proBDNF, mature BDNF, and MMP-9) in two independently collected case-control cohorts of bipolar patients and healthy controls.

2. Methods and materials

2.1. Participants in the Sahlgrenska cohort

Subjects were recruited primarily from an outpatient unit specialized in bipolar patients. The catchment area for the unit is socioeconomically diverse and multinational. Forty-eight mood-stabilized Caucasian patients with bipolar disorder and 43 Caucasian healthy controls were enrolled. All patients had a prior clinical diagnosis meeting the DSM-IV criteria for bipolar disorder. Healthy controls were recruited by advertisement in a newspaper. Exclusion criteria for subjects in both groups included any current or past history of metabolic disease and/or active substance abuse or dependence. Patients and controls were not matched.

Prior to commencement of the study and signing the written consent, all subjects were provided with verbal and written information about the study and about potential risks and benefits of study participation. The Regional Research Ethics Board in Gothenburg approved the study (172-08).

Serum samples were collected from fasting subjects between 9:00 and 12:00 am. The samples were centrifuged on site and stored at −20 °C until delivered by courier mail, frozen in 2 batches on dry ice, to Chiba University, Japan, for analysis.

2.2. Participants in the Karolinska cohort

The study population was recruited from the St. Göran Bipolar Project, which provides assessment, treatment, and follow-up of patients with bipolar disorder within the Northern Stockholm Mental Health Service. The project also serves as a basis for research in bipolar disorder. The methodology has previously been outlined in detail (Ekman et al., 2010; Rydén et al., 2009).

A total of 215 patients with bipolar disorder and 112 healthy controls were included. All patients had a prior clinical diagnosis meeting the DSM-IV criteria for bipolar disorder. Healthy controls were selected randomly from the national population register by Statistics Sweden (www.scb.se). These control subjects were living in the same catchment area as the patients. Exclusion criteria for controls were: (1) any on-going psychiatric or neurological disorder; (2) current treatment with any psychotropic drug; (3) past history of bipolar disorder, schizophrenia, recurrent depression or other psychiatric disorder leading to extended sick-leave; and (4) a first-degree relative with schizophrenia or bipolar disorder. Patients and controls were not matched (except for catchment area).

Prior to commencement of the study and signing the written consent, all subjects were provided with verbal and written information about the study and about potential risks and benefits of study participation. The Regional Research Ethics Board in Stockholm approved the study (2005-554-31/3).

Serum samples were collected from fasting subjects between 8:00 and 9:00 am. The samples were centrifuged on site and stored at −80 °C until delivered by courier mail, frozen on dry ice, to Chiba University, Japan, for analysis.

2.3. Assessment of clinical variables in the Sahlgrenska cohort

For patients, their diagnosis of bipolar disorder was confirmed by a thorough interview and clinical assessment of a psychiatrist (KS). To validate the diagnosis we used the structured psychiatric interview M.I.N.I., version 6 (Sheehan et al., 1998) in an authorized Swedish translation. Table 1 shows the subdiagnostic composition.

Age, height, weight, sagittal abdominal diameter, and waist circumference was measured, and the BMI was calculated. Age, age at first diagnosis, the latency before diagnosis, years with diagnosis, number of suicide attempts, and numbers of depressive, manic and mixed episodes were noted. For dimensional assessments, the Montgomery–Åsberg depression rating scale (MADRS) (Montgomery and Åsberg, 1979) and Young mania rating scale (YMRS) (Young et al., 1978) were used to assess mood states, and mini-mental state examination (MMSE) (Folstein et al., 1975) for cognitive impairment. Disability was assessed by Global Assessment of Functioning (GAF) (Hall, 1995). To assess addiction alcohol use disorder test (AUDIT) (Conigrave et al., 1995), and drug use disorder test (DUDIT) (Cassidy

Table 1
Diagnoses of bipolar patients and healthy controls in the 2 sets of data.

	Sahlgrenska set		Karolinska set	
	Males	Females	Males	Females
Bipolar I, psychotic	7	7	28	52
Bipolar I, non-psychotic	0	2	11	10
Bipolar II	4	4	31	59
Bipolar NOS	4	20	12	12
Healthy controls	19	24	48	64

Table 2
Distributions of present and past pharmacotherapy of bipolar patients and healthy controls in two independent sets

Sahlgrenska set	***Diagnosis:***			
	Bipolar I	**Bipolar II**	**Bipolar NOS**	**Healthy controls**
Pharmacotherapy:				
Antidepressants presently	7	3	10	0
Antipsychotics presently	11	4	15	0
Lithium presently	11	6	11	0
Antiepileptics presently	9	2	12	0
Antidepressants earlier	12	6	17	0
Antipsychotics earlier	11	2	13	0
Lithium earlier	5	0	3	0
Antiepileptics earlier	6	1	4	0
Karolinska set	***Diagnosis:***			
	Bipolar I	**Bipolar II**	**Bipolar NOS**	**Healthy controls**
Pharmacotherapy:				
Antidepressants presently	36	48	9	0
Antipsychotics presently	33	11	7	0
Lithium presently	70	44	13	0
Antiepileptics presently	31	38	6	0
Antidepressants earlier	76	82	20	8
Antipsychotics earlier	75	25	9	0
Lithium earlier	78	41	12	0
Antiepileptics earlier	38	36	9	0

et al., 2008) were used. When needed, supplementary information was collected from medical records. Healthy controls were assessed by the AUDIT, DUDIT, GAF, YMRS, MADRS and MMSE. All clinical measurements are listed in Table 3, split for sex.

2.4. Assessment of clinical variables in the Karolinska cohort

Patients were assessed by a psychiatrist or resident in psychiatry using the affective disorders evaluation (ADE), which is a standardized protocol adapted from the systematic treatment enhancement program of bipolar disorder (STEP-BD) (Sachs et al., 2003). The ADE guides the interviewer through a systematic assessment of the patient's current and past mental state, and provides a diagnosis according to DSM-IV criteria. Table 1 shows the subdiagnostic composition.

The number of lifetime affective episodes and their characteristics were documented. Other modules assess alcohol and drug misuse, violent behavior, childhood history, family history, treatment history, reproductive history, and somatic illnesses. Interpersonal violence is defined as a violent act or serious physical threat to another person. Suicide attempt is defined as a deliberate and serious self-injury, including intoxication with medication.

The final diagnosis was established using LEAD (Longitudinal observation by Experts using All Data) (Spitzer, 1983) and confirmed by a consensus panel of 2–4 experienced clinicians. Inclusion criteria for this sub-study were diagnoses of bipolar I or II disorders. Disease severity was assessed using the clinician rated global assessment of function (GAF) and clinical global impression (CGI) scales (Guy, 1976; Luborsky, 1962). For dimensional assessments, the MADRS and YMRS were used to assess mood states, and an extensive neuropsychological test battery was used to assess cognitive impairment. To assess addiction, AUDIT and DUDIT were used. Healthy controls were assessed by the AUDIT, DUDIT, and GAF. All clinical measurements are listed in Table 4, split for sex.

2.5. Assessment of present and past pharmacotherapy

Table 2 shows distributions of present and past psychiatric drug treatment in both the Sahlgrenska and the Karolinska cohorts, split for sub diagnoses. Treatments are presented in a dichotomous way (Yes|No) for antidepressants, antipsychotics, lithium, and antiepileptics, both "presently" and "earlier". In a Supplemental table (available on the web) are given the generic names and daily dosages of the current psychopharmacological treatment for each patient in the Sahlgrenska sample.

2.6. Measurement of serum levels of proBDNF, mature BDNF, and MMP-9

Serum levels of proBDNF, mature BDNF, and MMP-9 were measured in duplicates using human proBDNF ELISA kit, mature BDNF kit (Adipo Bioscience, Santa Clara CA, USA), and the human MMP-9 ELISA Kit (R&D Systems, Minneapolis MN, USA), respectively, following manufacturers' instructions. To minimize assay variance, serum levels of proBDNF, mature BDNF and MMP-9 from all subjects in the Sahlgrenska cohort were measured on the same day. The same procedures were followed for the Karolinska cohort. A 50-fold dilution of serum was used to measure mature BDNF and MMP-9. The optical density of each well was measured using an automated microplate reader (Emax, Molecular Devices, Sunnyvale CA, USA).

Tables 3 and 4 show the results of the analysis of serum levels in the two cohorts.

2.7. Statistical analyses

The JMP 10.0.1 package from SAS, Inc., was used to analyze data. All non-dichotomous variables were inspected for skewness, and $_{10}$log transformations were applied when needed in order to normalize distributions. For ratios, the arctan transform was used, presenting angular radians in trigonometric space in order to avoid outliers. Multivariate nominal logistic fits (effect likelihood ratio tests) were applied with diagnostic categories as the predicted variables, and the serum measures and their interactions as the independent predictor variables, covarying out the influence of sex, age and body composition (BMI). Linear multiple regression models were used to investigate variation components in the serum variables measured. GraphPad Prism was used to generate graphs.

Table 3
Description and comparison of measured variables in the Sahlgrenska training cohort.

	Total	Patients				Healthy controls				All patients vs. all controls§	
	n	*Males*	*n*	*Females*	*n*	*Males*	*n*	*Females*	*n*	*t*	*p*
Arithmetic means (± SD)											
mature BDNF [ng/mL]	90	25.39 ± 9.61	15	22.86 ± 6.61	32	18.99 ± 6.55	19	15.45 ± 4.97	24	−4.42	****
proBDNF [ng/mL]	79	32.75 ± 22.44	13	23.15 ± 6.36	28	26.42 ± 9.09	17	55.65 ± 66.78	21	2.00	*
Ratio matBDNF/proBDNF	79	1.11 ± 0.72	13	1.05 ± 0.40	28	0.77 ± 0.45	17	0.57 ± 0.40	21	−4.04	****
MMP-9 [ng/mL]	90	779.6 ± 378.0	15	648.7 ± 370.5	32	559.6 ± 247.4	19	572.7 ± 382.9	24	−1.54	ns
Age [yrs]	91	36.7 ± 8.0	15	40.6 ± 11.7	33	33.8 ± 15.9	19	29.6 ± 11.2	24	−3.79	***
Height [cm]	91	181.4 ± 7.48	15	167.7 ± 7.25	33	180.1 ± 4.71	19	168.2 ± 6.83	24	0.74	ns
Weight [kg]	91	92.4 ± 22.0	15	77.2 ± 18.6	33	79.0 ± 9.14	19	63.6 ± 8.87	24	−3.20	**
Sagittal diameter [cm]	91	19.6 ± 3.8	15	19.7 ± 3.5	33	17.6 ± 2.8	19	15.4 ± 1.6	24	−5.13	****
Waist circumference [cm]	91	98.1 ± 16.7	15	90.3 ± 16.0	33	86.6 ± 10.0	19	72.2 ± 7.4	24	−4.76	****
BMI	91	27.8 ± 5.0	15	27.4 ± 6.1	33	24.4 ± 3.2	19	22.5 ± 3.0	24	−4.24	****
Age first symptoms	46	19.4 ± 7.4	14	17.5 ± 5.8	32						
Age diagnosis	45	29.8 ± 7.0	14	32.1 ± 10.8	31						
Diagnostic latency	45	10.4 ± 7.1	14	14.5 ± 10.4	31						
Yrs with diagnosis	45	7.1 ± 6.7	14	8.2 ± 10.1	31						
Suicide attempts2;#	46	0.0	14	1.75 ± 2.99	32						
Depressive episodes2;#	38	10.0 ± 7.9	13	14.0 ± 17.1	25						
Manic episodes2;#	34	6.4 ± 6.8	12	8.6 ± 8.5	22						
Mixed episodes2;#	26	0.25 ± 0.46	8	0.06 ± 0.24	18						
Medians (range)											
Age first symptoms	46	19 (6–37)	14	15.5 (7–31)	32						
Age when diagnosis	45	31.5 (20–41)	14	31.0 (18–55)	31						
Diagnostic latency	45	10.5 (0–20)	14	13.0 (0–32)	31						
Yrs with diagnosis	45	5 (0–22)	14	4 (0–38)	31						
Suicide attempts2;#	46	0	14	0.5 (0–10)	32						
Depressive episodes2;#	38	8 (1–30)	13	6 (1–60)	25						
Manic episodes2;#	34	4 (1–20)	12	5.5 (0–32)	22						
Mixed episodes2;#	26	0 (0–1)	8	0 (0–1)	18						
Fractions					χ^2						
Psychosis		57% (8/14)		56% (18/32)	ns						
Mood congruence		63% (5/8)		78% (14/18)	ns						
AD presently		29% (4/14)		50% (16/32)	ns						
AP presently		71% (10/14)		63% (20/32)	ns						
Li presently		93% (13/14)		47% (15/32)	**						
AE presently		36% (5/14)		56% (18/32)	ns						
Arithmetic means (± SD)											
AUDIT	90	5.2 ± 4.4	15	3.2 ± 2.9	32	6.1 ± 3.9	19	4.8 ± 2.6	24	2.18	*
DUDIT	90	2.3 ± 4.3	15	1.9 ± 3.8	32	0.3 ± 0.7	19	0.8 ± 1.5	24	−2.40	*
GAF	89	69.7 ± 11.2	14	66.8 ± 15.6	32	89.2 ± 4.0	19	88.0 ± 3.2	24	9.55	****
YMRS	89	1.9 ± 3.3	14	0.3 ± 0.9	32	0.2 ± 0.6	19	0.2 ± 0.7	24	−1.82	ns
MADRS	87	7.3 ± 6.7	14	10.5 ± 8.6	31	1.9 ± 5.0	19	0.7 ± 6.6	23	−6.53	****
MMSE	88	28.2 ± 2.34	13	28.5 ± 1.4	32	29.7 ± 0.6	19	29.6 ± 0.6	24	11.22	****

§ Comparisons based on log transformed values.
# Ordinal scale.

3. Results

Serum levels of mature BDNF were significantly *higher* in patients than in controls, in both cohorts ($p < 0.0001$ and $p < 0.001$), as shown in Tables 3 and 4, split for sex. Serum levels of proBDNF were significantly *lower* in patients in both cohorts ($p < 0.05$ and $p < 0.0001$). The ratio mature BDNF/proBDNF was significantly *higher* in patients in both cohorts ($p < 0.0001$ and $p < 0.0001$). There was no significant difference in MMP-9 in either cohort. Fig. 1 illustrates these differences graphically, here not split for sex.

Categorical medication status among the Sahlgrenska patients was analyzed as 4 independent variables (Yes|No for antidepressants, antipsychotics, lithium, and antiepileptics) and used as independent variables in ANCOVAs, covarying out sex, age, and BMI. Dependent variables were the serum measurements, in turns. Linear multiple regressions are shown in Table 5, demonstrating no significant overall *F* values, and no significant medication effects. The one exception was that present medication with antiepileptic drugs was linked with low MMP-9 values, but only at trend level.

Table 6 shows results from multivariate logistic analyses used to discriminate the diagnostic dichotomy (Bipolar|Control). Significant influences from sex, age, and BMI were covaried out. The results were similar in both cohorts. ProBDNF and the ratio mature BDNF/proBDNF were significant predictors in both cohorts. Mature BDNF was significant in the Sahlgrenska cohort, but only at a trend level in the Karolinska cohort. There were no significant statistical interactions between mature BDNF and proBDNF. MMP-9 was a non-significant predictor, with no detectable interactions with the BDNFs.

This discriminatory model explained 41% of the diagnostic variation ($p < 0.0001$) in the Sahlgrenska cohort and 15% in the Karolinska cohort ($p < 0.0001$). In both cohorts, the equations provided good power for diagnostic classification. Sensitivity was 89% in the Sahlgrenska cohort and 74% in the Karolinska cohort, and specificity was 77% and 64%, respectively.

ProBDNF correlated negatively with mature BDNF in the whole Sahlgrenska cohort ($r = -0.28$, $p = 0.014$; Spearman $\rho = -0.26$). This correlation strengthened when covarying out diagnosis, sex, age, and BMI ($p < 0.0020$). MMP-9 correlated positively with

Table 4
Description and comparison of measured variables in the Karolinska replication cohort.

	Total	Patients					Healthy controls				*All patients vs. all controls*[§]	
	n	*Males*	*n*	*Females*	*n*		*Males*	*n*	*Females*	*n*	*t*	*p*
Arithmetic means (± SD)												
mature BDNF [ng/mL]	327	30.6 ± 7.3	82	31.5 ± 7.4	133		30.3 ± 7.2	48	27.2 ± 7.0	64	−3.24	***
proBDNF [ng/mL]	327	36.3 ± 62.7	82	58.0 ± 97.2	133		47.5 ± 58.2	48	161.2 ± 400.4	64	4.81	****
Ratio matBDNF/proBDNF	327	1.57 ± 1.10	82	1.37 ± 1.16	133		0.97 ± 0.56	48	0.70 ± 0.52	64	−6.10	****
MMP-9 [ng/mL]	327	533.2 ± 242.2	82	523.7 ± 223.3	133		501.7 ± 192.7	48	559.1 ± 271.3	64	0.00	ns
Age [yrs]	327	41.7 ± 12.8	82	37.3 ± 12.9	133		40.2 ± 14.4	48	36.3 ± 12.9	64	−0.73	ns
Height [cm]	322	181.0 ± 6.5	82	167.0 ± 5.9	133		183.0 ± 5.7	47	167.4 ± 6.3	60	1.76	ns
Weight [kg]	322	85.3 ± 12.2	82	68.8 ± 13.2	133		82.1 ± 11.1	47	66.2 ± 11.5	60	−1.10	ns
BMI	322	26.0 ± 3.2	82	24.7 ± 4.8	133		24.5 ± 2.8	47	23.6 ± 3.9	60	−2.54	*
Age first symptoms	212	21.2 ± 12.0	82	18.6 ± 10.4	130							
Suicide attempts2;[#]	212	0.9 ± 2.6	82	1.9 ± 5.2	130							
Depressive episodes2;[#]	212	19.1 ± 24.8	81	18.8 ± 25.4	131							
Manic episodes2;[#]	215	1.4 ± 2.1	82	1.5 ± 2.5	133							
Hypomanic episodes2;[#]	215	6.1 ± 11.9	82	7.7 ± 15.4	133							
Mixed episodes2;[#]	213	1.6 ± 5.5	82	1.1 ± 4.2	131							
Medians (range)												
Age first symptoms	213	19 (3-19)	82	16 (2-64)	131							
Suicide attempts2;[#]	212	0 (0-20)	82	0 (0-30)	130							
Depressive episodes2;[#]	212	10 (0-160)	81	9 (1-150)	131							
Manic episodes2;[#]	215	1 (0-10)	82	0 (0-15)	133							
Hypomanic episodes2;[#]	215	2 (0-80)	82	2 (0-99)	133							
Mixed episodes2;[#]	213	0 (0-30)	82	0 (0-30)	131							
Fractions						χ^2						
Psychosis		43.9% (36/46)	82	47.4% (63/70)	133	ns						
AD presently		35.4% (29/53)	82	48.1% (64/69)	133	ns						
AP presently		30.5% (25/57)	82	19.5% (26/107)	133	ns						
Li presently		63.4% (52/30)	82	56.4% (75/58)	133	ns						
AE presently		32.9% (27/55)	82	36.1% (48/85)	133	ns						
Arithmetic means (± SD)												
$GAF_{function}$	215	68.3 ± 11.9	82	66.3 ± 9.5	133							
$GAF_{symptom}$	215	67.2 ± 13.5	82	66.5 ± 9.5	133							
YMRS	172	1.3 ± 2.7	69	1.1 ± 2.0	103							
MADRS	327	4.2 ± 6.2	69	2.8 ± 4.5	103							

[§] Comparisons based on log transformed values.
[#] Ordinal scale.

mature BDNF (r=0.37, n=90, p=0.0003), but not with proBDNF (r=−0.01, n=79, n.s.). Fig. 1 shows the slopes of the two regression lines compared in the Sahlgrenska cohort. There were no significant intercorrelations between these variables in the Karolinska cohort.

For the Sahlgrenska cohort only, Table 7, Column A shows global assessment of function (GAF) scores to be highly discriminatory for the Bipolar|Control dependent variable, which is to be expected (variance explained 54%, sensitivity 88%, specificity 78%, χ^2=32.26). In Column B, sex, age, and BMI have been added (variance explained 67%, sensitivity 95%, specificity 85%, χ^2=54.26). Column C shows that the predictive power increased even more by adding mature BDNF, proBDNF, their ratio, and their interactive product (variance now explained was 85%, sensitivity 100%, specificity 95%, χ^2=65.65). The strongest predictor was mature BDNF. Similarly highly significant results, although somewhat weaker, were obtained when mini mental state examination (MMSE) scores were used instead of GAF (results not shown). This subanalysis could only be done on the Sahlgrenska cohort, since GAF data had not been routinely collected in the Karolinska healthy controls.

Subdiagnostic categorical divisions (between bipolar I, bipolar II, and bipolar NOS) and the controls were compared with the plasma variables in focus (proBDNF, mature BDNF, their ratio, and MMP-9), using the All Pairs Tukey–Kramer test (a post-hoc conservative test which protects significance tests of all combinations of pairs) from the JMP package. There were no significant or even trend differences between any of the three possible bipolar subcategories (all $p > 0.20$).

Current MDRS and YMRS scores ($_{10}$log transformed after adding unity) were compared between the above categorical divisions. The 3 bipolar subgroups all had significantly higher MADRS score than controls, but they did not differ significantly between themselves. As for the YMRS score, no significant differences were seen even *versus* the control group. The MDRS and YMRS scores and the subdiagnostic 4-pronged category were then compared (as independent variables) in turns with all plasma data (as dependent variables). No significant or trend correlation was discovered.

4. Discussion

We examined two independently collected cohorts of mood-stabilized bipolar patients and healthy controls and found that serum levels of mature BDNF were significant higher in patients compared to controls, in both cohorts. Moreover, the ratio of mature BDNF/proBDNF was significantly higher in patients than controls. In logistic ANCOVAs, the consistently significant independent variables predicting the diagnostic dichotomy were proBDNF and the ratio mature BDNF/proBDNF. Using the serum-only information for diagnostic predictions, it was possible to classify bipolar disorder patients *versus* healthy controls with

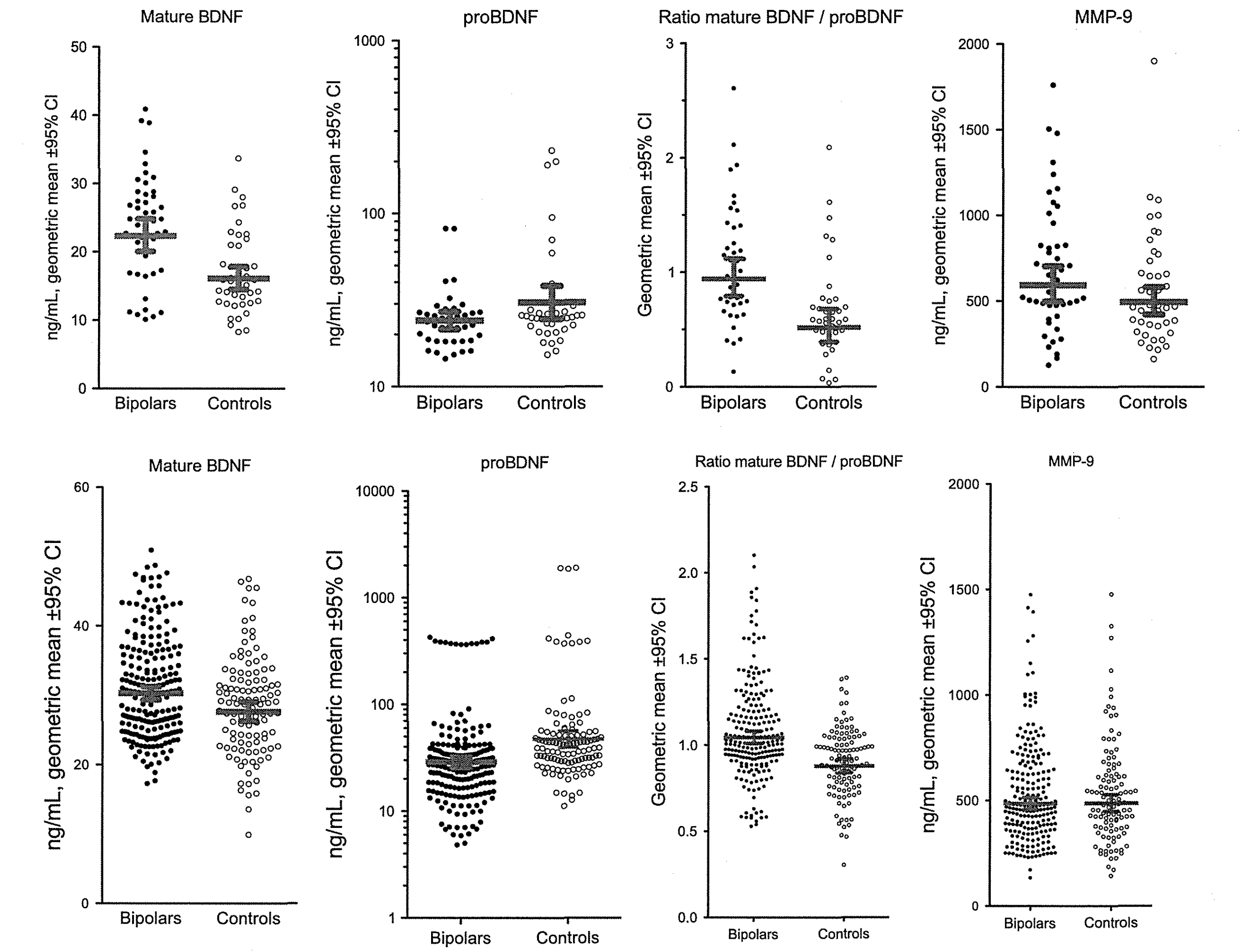

Fig. 1. Distributions and compared geometric means of serum measures.

Table 5
Linear regressions in Sahlgrenska bipolar patients, predicting serum values from clinical information and current medication. Sahlgrenska training cohort.

	Predicted variables:											
	log proBDNF			**log mature BDNF**			**arctan(log proBDNF/log matBDNF)**			**log MMP-9**		
	β	***t***	***p***	***β***	***t***	***p***	***β***	***t***	***p***	***β***	***t***	***p***
Predictor variables:												
Intercept	1.54	4.04	**0.0003**	1.10	4.60	**0.0001**	0.70	3.99	**0.0003**	2.42	5.60	**0.018**
Sex	−0.03	−0.69	>0.10	−0.02	−0.83	>0.10	−0.03	−0.16	>0.10	−0.03	−0.69	>0.10
logAge	−0.02	−0.07	>0.10	0.25	1.61	>0.10	0.06	0.49	>0.10	0.16	0.55	>0.10
BMI	−0.00	−0.78	>0.10	0.00	−1.28	>0.10	0.00	0.03	>0.10	0.00	0.59	>0.10
Antidepressant tx presently	−0.01	−0.22	>0.10	0.01	0.35	>0.10	0.00	0.27	>0.10	0.01	0.24	>0.10
Antipsychotic tx presently	−0.01	−0.30	>0.10	−0.01	−0.33	>0.10	0.00	−0.20	>0.10	0.01	0.28	>0.10
Lithium tx presently	0.02	0.53	>0.10	0.03	1.27	>0.10	0.00	−0.06	>0.10	0.06	1.55	>0.10
Antiepileptics tx presently	−0.03	−1.00	>0.10	−0.03	−1.61	>0.10	0.00	0.13	>0.10	−0.07	−1.95	0.060
Overall ANOVA	F=0.68		>0.10	F=1.34		>0.10	F=0.06		>0.10	F=1.51		>0.10
df	7,38			7,46			7,38			7,46		
n	46			54			46			54		
R^2	0.112			0.170			0.012			0.187		

sensitivities of 89% for the Sahlgrenska cohort and 74% for the Karolinska cohort, and specificities of 77% and 64%, respectively; all highly significant. Significant influences from sex and BMI (as well as a non-significant age influence) were covaried out. Our results indicate that BDNF measurements have a potential for usage as clinical biomarkers by differentiating bipolar patients

Table 6
Nominal logistic fits predicting diagnosis with serum measures as predictors, covarying out sex, age, and BMI. Sahlgrenska training cohort and Karolinska replication cohort compared.

	Predicted variable: all bipolars vs. controls			
	Sahlgrenska cohort		Karolinska cohort	
	χ^2	*p*	χ^2	*p*
Predictor variables:				
Sex	7.65	**0.0057**	4.34	**0.037**
log Age	0.62	> 0.10	0.49	> 0.10
BMI	9.66	**0.0019**	5.52	**0.019**
log mature BDNF	6.19	**0.013**	3.31	0.069
log proBDNF	4.10	**0.043**	8.46	**0.0036**
arctan(matBDNF/proBDNF)	4.28	**0.039**	14.80	**0.0001**
log matBDNF × log proBDNF	1.10	> 0.10	2.53	> 0.10
log MMP-9	0.01	> 0.10	0.18	> 0.10
log MMP-9 × log matBDNF	2.07	> 0.10	0.23	> 0.10
log MMP-9 × log proBDNF	0.71	> 0.10	2.86	0.091
Whole model test	44.34	< **0.0001**	61.64	< **0.0001**
df	10		10	
n	79		321	
R^2	0.405		0.151	
Classification sensitivity	89%		74%	
Classification specificity	77%		64%	
Classification matrix, df=1, χ^2	31.27	< **0.0000**	30.84	< **0.0000**
ROC AUC	0.88		0.75	

Table 7
Nominal logistic fits predicting diagnosis with serum measures as predictors, covarying out GAF scores, sex, age, and BMI. Sahlgrenska cohort only.

Effect likelihood ratio tests	*Predicted variable: all bipolars vs. controls*					
	A		B		C	
Sahlgrenska cohort	χ^2	*p*	χ^2	*p*	χ^2	*p*
Predictor variables:						
GAF	66.81	< **0.0001**	58.20	< **0.0001**	46.28	< **0.0001**
Sex			2.72	0.099	8.53	**0.0035**
log Age			8.98	**0.0027**	6.87	**0.0088**
BMI			1.48	> 0.10	0.03	> 0.10
log mature BDNF					7.16	**0.0075**
log proBDNF					5.65	**0.017**
arctan(matBDNF/proBDNF)					5.83	**0.016**
log matBDNF × log proBDNF					2.15	> 0.10
Whole model test	66.81	< **0.0001**	82.35	< **0.0001**	90.88	< **0.0001**
df	1		4		8	
n	89		89		77	
R^2	0.542		0.668		0.852	
Classification sensitivity	88%		95%		100%	
Classification specificity	78%		85%		95%	
Classification matrix, df=1, χ^2	32.26	< **0.0000**	54.26	< **0.0000**	65.65	< **0.0000**
ROC AUC	0.92		0.97		0.99	

from healthy control individuals. Future studies should explore whether this usefulness extends to differentiating bipolar disorder from MDD and schizophrenia.

Our study is the first to investigate proBDNF and mature BDNF in bipolar disorder. BDNF in mood disorders has primarily been studied in unipolar depression. BDNF has been argued to be state-related, but previous results have been divergent. One reason is that earlier commercially available human BDNF ELISA kits were unable to distinguish between proBDNF and mature BDNF (Yoshida et al., 2012a). Consequently, earlier studies have reported combined levels of proBDNF and mature BDNF.

Our results are cross-sectional and can therefore neither support nor contradict whether BDNF levels are mainly related to state or trait. An abnormal conversion of proBDNF → mature BDNF, leading to increased levels of mature BDNF and reduced levels of proBDNF, may nevertheless play a role in the pathophysiology of bipolar disorder. There is a large preclinical literature on links between BDNF and neurogenesis within the brain, being relevant for human mood disorders (Bath et al., 2012; Bekinschtein et al., 2011; Marlatt et al., 2012; Peng et al., 2008; Rossi et al., 2006), but we cannot at this stage predict which BDNF component would be pivotal. Somewhat unexpectedly, we found no difference in levels between the three subdiagnostic categories. Current depressive symptoms (MADRS scores) or (hypo)manic symptoms (YMRS scores) had no influence. This would offer some indirect evidence favouring differences in plasma BDNF levels as to represent trait phenomena.

We found no difference in serum MMP-9 levels between patients with bipolar disorder and healthy controls, consistent with previous reports that serum MMP-9 levels were not altered in patients with major MDD (Yoshida et al., 2012b). A positive correlation has been reported between serum MMP-9 levels and the severity of depression in MDD patients, although the role of MMP-9 in the pathophysiology of MDD is currently unknown (Yoshida et al., 2012b). We did not find any correlation with diagnosis—except an interaction at trend level between MMP-9 and proBDNF (but only in the Karolinska cohort). Further studies are necessary to examine the role of MMP-9 in the pathophysiology of bipolar disorder. Nevertheless, MMP-9 has been shown to play a role in synaptic plasticity of the brain as well as in mood disorders (Ethell and Ethell, 2007; Hashimoto, 2013; Yoshida et al., 2012b).

The main strength of this study is that significant findings have been replicated in two independent cohorts. A limitation is that all patients in the two cohorts were on psychoactive medication. Previous studies show that blood BDNF (sum of proBDNF and mature BDNF) levels were significantly increased after the pharmacological treatment of manic state (Fernandes et al., 2011; Hashimoto, 2010; Lin, 2009), indicating that the medication might "restitute" serum levels of proBDNF+mature BDNF. Yet, in the analyzable Sahlgrenska cohort, we found no correlation between medication with any drug (antidepressants, antipsychotics, lithium, and antiepileptics), and any measured serum variable. Thus, the dynamics of pharmacological influence is not well understood, and there is a need to analyze medication-free patients, even though they may be hard to find.

5. Conclusion

Using serum-only information on proBDNF and mature BDNF to predict diagnosis, it was possible to correctly classify bipolar disorder patients *versus* healthy controls with sensitivities of 89% for the Sahlgrenska cohort and 74% for the Karolinska cohort, and with specificities of 77% and 64%, respectively, all highly significant. Adding a clinical assessment scale strengthened both sensitivity and specificity to over 90%, which would be strong enough to work as a clinical biomarker predicting the diagnostic dichotomy.

6. Limitations

Further longitudinal studies will be needed – measuring serum levels of proBDNF, mature BDNF, the ratio mature BDNF/proBDNF, and MMP-9 – using larger cohorts, if possible with medication-free patients, and "ill patient controls" with MDD and schizophrenia. A next step would then be to investigate if this type of algorithm could be used as a clinical aid to differentiate between a bipolar depressive disorder and, *e.g.*, MDD.

Role of funding source
Funding/support

This study is partly supported by a grant to K.H. from Comprehensive Research on Disability, Health and Welfare, Health and Labor Sciences Research Grants, Japan. The Sahlgrenska project is supported by regional agreement grants from the Västra Götaland County (ALF 76410, 76420, and 76160). The St. Göran bipolar project is funded by grants from the Swedish Foundation for Strategic Research, the Swedish Medical Research Council (K2011-61X-14647-09-3, K2010-61X-21569-01-1 and K2010-61P-21568-01-4), the Swedish Brain Foundation, and the regional agreement on medical training and clinical research between Stockholm County Council and the Karolinska Institutet (ALF 20100305). K.F. is funded by grants from the Swedish Medical Research Council (K2011-62X-21856-01-6), ALF Västra Götaland regionen (ALFGBG-140001), BioCARE, a national strategic research program at University of Gothenburg, the Åhlén Foundation, and the Lundgren Foundation.

Financial disclosure

Reported.

Data access and responsibility

H.Å. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest
None.

Acknowledgements
For expert help in administration and tests we thank Richard Brodd, R.N., for the Sahlgrenska patients, and Martina Wennberg, Agneta Carlswärd-Kjellin, and data manager Haydeh Olofsson for the St. Göran patients.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2014.01.009.

References

Altar, C.A., 1999. Neurotrophins and depression. Trends Pharmacol. Sci. 20, 59–62.
Angst, F., Angst, J., Stassen, H.H., Clayton, P.J., 2002. Mortality of patients with mood disorders: follow-up over 34–38 years. J. Affective Dis. 68, 167–181.
Bath, K.G., Akins, M.R., Lee, F.S., 2012. BDNF control of adult SVZ neurogenesis. Dev. Psychobiol. 54, 578–589.
Bekinschtein, P., Oomen, C.A., Saksida, L.M., Bussey, T.J., 2011. Effects of environmental enrichment and voluntary exercise on neurogenesis, learning and memory, and pattern separation: BDNF as a critical variable? Semin. Cell Dev. Biol. 22, 536–542.
Belmaker, R.H., 2004. Medical progress: bipolar disorder. N. Engl. J. Med. 351, 476–486.
Cassidy, C.M., Schmitz, N., Malla, A., 2008. Validation of the alcohol use disorders identification test and the drug abuse screening test in first episode psychosis. Can. J. Psychiatry 53, 26–33.
Conigrave, K.M., Hall, W.D., Saunders, J.B., 1995. The AUDIT questionnaire: choosing a cut-off score. Alcohol use disorder identification test. Addiction 90, 1349–1356.
Cunha, A.B., Frey, B.N., Andreazza, A.C., Goi, J.D., Rosa, A.R., Goncalves, C.A., Santin, A., Kapczinski, F., 2006. Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. Neurosci. Lett. 398, 215–219.
de Oliveira, G.S., Cereser, K.M., Fernandes, B.S., Kauer-Sant'Anna, M., Fries, G.R., Stertz, L., Aguiar, B., Pfaffenseller, B., Kapczinski, F., 2009. Decreased brain-derived neurotrophic factor in medicated and drug-free bipolar patients. J. Psychiatric Res. 43, 1171–1174.
Dias, V.V., Brissos, S., Frey, B.N., Andreazza, A.C., Cardoso, C., Kapczinski, F., 2009. Cognitive function and serum levels of brain-derived neurotrophic factor in patients with bipolar disorder. Bipolar Disorders 11, 663–671.
Dieni, S., Frotscher, M., Barde, Y.-A., Matsumoto, T., Dekkers, M., Rauskolb, S., Ionescu, M.S., Deogracias, R., Gundelfinger, E.D., Kojima, M., Nestel, S., 2012. BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. J. Cell Biol. 196, 775–788.
Duman, R.S., Heninger, G.R., Nestler, E.J., 1997. A molecular and cellular theory of depression. Arch. Gen. Psychiatry 54, 597–606.
Ekman, C.J., Lind, J., Rydén, E., Ingvar, M., Landén, M., 2010. Manic episodes are associated with grey matter volume reduction—a voxel-based morphometry brain analysis. Acta Psychiatrica Scand. 122, 507–515.
Ernfors, P., Wetmore, C., Olson, L., Persson, H., 1990. Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron 5, 511–526.
Ethell, I.M., Ethell, D.W., 2007. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J. Neurosci. Res. 85, 2813–2823.
Fernandes, B.S., Kapczinski, F., Gama, C.S., Maria Ceresér, K., Yatham, L.N., Fries, G.R., Colpo, G., de Lucena, D., Kunz, M., Gomes, F.A., 2011. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. J. Psychiatric Res. 45, 995–1004.
Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatric Res. 12, 189–198.
Goldstein, T.R., Keller, M., Birmaher, B., Axelson, D., Goldstein, B.I., Gill, M.K., Esposito-Smythers, C., Ryan, N.D., Strober, M.A., Hunt, J., 2009. Psychosocial functioning among bipolar youth. J. Affective Dis. 114, 174–183.

Guy, W., 1976. Early Clinical Drug Evaluation (ECDEU) Assessment Manual for PsychopharmacologyDepartment of Health, Education and Welfare
Hall, R.C., 1995. Global assessment of functioning. A modified scale. Psychosomatics 36, 267–275.
Hashimoto, K., 2007. BDNF variant linked to anxiety-related behaviors. Bioessays 29, 116–119.
Hashimoto, K., 2010. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions: BDNF as a biomarker of mood disorders. Psychiatry Clin. Neurosci. 64, 341–357.
Hashimoto, K., 2013. Sigma-1 receptor chaperone and brain-derived neurotrophic factor: emerging links between cardiovascular disease and depression. Prog. Neurobiol. 100, 15–29.
Hashimoto, K., Shimizu, E., Iyo, M., 2004. Critical role of brain-derived neurotrophic factor in mood disorders. Brain Res. Rev. 45, 104–114.
Huang, T.L., Hung, Y.Y., Lee, C.T., Chen, R.F., 2012. Serum protein levels of brain-derived neurotrophic factor and tropomyosin-related kinase B in bipolar disorder: effects of mood stabilizers. Neuropsychobiology 65, 65–69.
Kauer-Sant'Anna, M., Kapczinski, F., Andreazza, A.C., Bond, D.J., Lam, R.W., Young, L.T., Yatham, L.N., 2009. Brain-derived neurotrophic factor and inflammatory markers in patients with early- *vs.* late-stage bipolar disorder. Int. J. Neuropsychopharmacol. 12, 447–458.
Lin, P.Y., 2009. State-dependent decrease in levels of brain-derived neurotrophic factor in bipolar disorder: a meta-analytic study. Neurosci. Lett. 466, 139–143.
Luborsky, L., 1962. Clinician's judgments of mental health. Arch. Gen. Psychiatry 7, 407–417.
Machado-Vieira, R., Dietrich, M.O., Leke, R., Cereser, V.H., Zanatto, V., Kapczinski, F., Souza, D.O., Portela, L.V., Gentil, V., 2007. Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. Biol. Psychiatry 61, 142–144.
Mackin, P., Gallagher, P., Watson, S., Young, A.H., Ferrier, I.N., 2007. Changes in brain-derived neurotrophic factor following treatment with mifepristone in bipolar disorder and schizophrenia. Aust. N. Z. J. Psychiatry 41, 321–326.
Marlatt, M.W., Potter, M.C., Lucassen, P.J., van Praag, H., 2012. Running throughout middle-age improves memory function, hippocampal neurogenesis, and BDNF levels in female C57BL/6J mice. Dev. Neurobiol. 72, 943–952.
Martinowich, K., Manji, H., Lu, B., 2007. New insights into BDNF function in depression and anxiety. Nat. Neurosci. 10, 1089–1093.
Monteleone, P., Serritella, C., Martiadis, V., Maj, M., 2008. Decreased levels of serum brain-derived neurotrophic factor in both depressed and euthymic patients with unipolar depression and in euthymic patients with bipolar I and II disorders. Bipolar Disorders 10, 95–100.
Montgomery, S.A., Åsberg, M., 1979. A new depression scale designed to be sensitive to change. Br. J. Psychiatry 134, 322–389.
Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., Monteggia, L.M., 2002. Neurobiology of depression. Neuron 34, 13–25.
Palomino, A., Vallejo-Illarramendi, A., Gonzalez-Pinto, A., Aldama, A., Gonzalez-Gomez, C., Mosquera, F., Gonzalez-Garcia, G., Matute, C., 2006. Decreased levels of plasma BDNF in first-episode schizophrenia and bipolar disorder patients. Schizophrenia Res. 86, 321–322.
Pan, W., Banks, W.A., Fasold, M.B., Bluth, J., Kastin, A.J., 1998. Transport of brain-derived neurotrophic factor across the blood–brain barrier. Neuropharmacology 37, 1553–1561.
Pang, P.T., Woo, N.H., 2005. The yin and yang of neurotrophin action. Nat. Rev. Neurosci. 6, 603–614.
Park, H., Poo, M.M., 2013. Neurotrophin regulation of neural circuit development and function. Nat. Rev. Neurosci. 14, 7–23.
Peng, Q., Masuda, N., Jiang, M., Li, Q., Zhao, M., Ross, C.A., Duan, W., 2008. The antidepressant sertraline improves the phenotype, promotes neurogenesis and increases BDNF levels in the R6/2 Huntington's disease mouse model. Exp. Neurol. 210, 154–163.
Poo, M.-m., 2001. Neurotrophins as synaptic modulators. Nat. Rev. Neurosci. 2, 24–32.
Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., Fabbri, M.E., Tessarollo, L., Maffei, L., Berardi, N., Caleo, M., 2006. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur. J. Neurosci. 24, 1850–1856.
Rydén, E., Johansson, C., Blennow, K., Landén, M., 2009. Lower CSF HVA and 5-HIAA in bipolar disorder type 1 with a history of childhood ADHD. J. Neural Transm. 116, 1667–1674.
Sachs, G.S., Thase, M.E., Otto, M.W., Bauer, M., Miklowitz, D., Wisniewski, S.R., Lavori, P., Lebowitz, B., Rudorfer, M., Frank, E., Nierenberg, A.A., Fava, M., Bowden, C., Ketter, T., Marangell, L., Calabrese, J., Kupfer, D., Rosenbaum, J.F., 2003. Rationale, design, and methods of the systematic treatment enhancement program for bipolar disorder (STEP-BD). Biol. Psychiatry 53, 1028–1042.
Schmidt, H.D., Duman, R.S., 2010. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 35, 2378–2391.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl 20), 22–33 (quiz 34–57).
Spitzer, R.L., 1983. Psychiatric diagnosis: are clinicians still necessary? Compr. Psychiatry 24, 399–411.
Tohen, M., Cohen, B.M., Hennen, J., Zarate, J.C.M., Baldessarini, R.J., Strakowski, S.M., Stoll, A.L., Faedda, G.L., Suppes, T., Gebre-Medhin, P., 2000. Two-year syndromal and functional recovery in 219 cases of first-episode major affective disorder with psychotic features. Am. J. Psychiatry 157, 220–228.
Yoshida, T., Ishikawa, M., Iyo, M., Hashimoto, K., 2012a. Serum levels of mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in healthy subjects. Open Clin. Chem. J. 5, 7–12.
Yoshida, T., Ishikawa, M., Niitsu, T., Nakazato, M., Watanabe, H., Shiraishi, T., Shiina, A., Hashimoto, T., Kanahara, N., Hasegawa, T., Enohara, M., Kimura, A., Iyo, M., Hashimoto, K., 2012b. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. PLoS One 7, e42676.
Yoshimura, R., Nakano, Y., Hori, H., Ikenouchi, A., Ueda, N., Nakamura, J., 2006. Effect of risperidone on plasma catecholamine metabolites and brain-derived neurotrophic factor in patients with bipolar disorders. Hum. Psychopharmacol. 21, 433–438.
Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. Br. J. Psychiatry 133, 429–435.

BDNF and proBDNF as biomarkers for bipolar disorder

Kenji Hashimoto

BJP 2014, 205:410.
Access the most recent version at DOI: 10.1192/bjp.205.5.410

BJPsych | The British Journal of Psychiatry (2014) 205, 409–412

Correspondence

Edited by Kiriakos Xenitidis and Colin Campbell

Contents

Use of the first-person pronoun in schizophrenia

In their recent publication, Fineberg *et al* examined word use in first-person accounts of schizophrenia in comparison with word use in first-person accounts of mood and anxiety disorders.[1] One of their hypotheses concerned the use of the first-person singular pronoun 'I'. On the basis of research showing patients with mood disorders to be particularly self-focused, as well as phenomenological reports by patients suffering from schizophrenia describing a disrupted sense of self, they predicted that 'writers with schizophrenia would use "I" less often than persons with mood disorder'. They found this hypothesis to be supported by their data.

One obvious limitation of this study, admitted by the authors, is the lack of a healthy control group. Data from two such control groups, however, are readily at hand. First, one can compare the word frequencies found in their first-person accounts with their frequency in general language, as represented in reference corpora such as the Corpus of Contemporary American English.[2] Second, in order to compare a text format that is as similar as possible to first-person accounts of mental illness, one can make use of articles published in the *Schizophrenia Bulletin* under the rubric 'First-person account' that are not written by sufferers of schizophrenia, but by (supposedly) healthy family and friends of someone with schizophrenia (I will refer to those as 'second- person' accounts). Such comparison, based on analyses of a corpus of the *Schizophrenia Bulletin* using CQP software,[3] yields results that markedly differ from Fineberg *et al*'s findings (for a general introduction to corpus linguistics, see Lüdeling & Kytö[4]).

Since 1979, the *Schizophrenia Bulletin* has published 98 first-person accounts and 30 second-person accounts of schizophrenia. The frequency of 'I' in the first-person accounts is 34 621.67/106 words and 20 804.18/106 words in the second-person accounts. The authors of the first-person accounts use 'I' 3.34 times more often than it is used in general American English and 1.90 times more often than it occurs in general spoken American English. Comparing first- and second-person accounts, 'I' is used 1.66 times more often by people identifying as having schizophrenia spectrum disorders than by their mentally healthy friends and family members. The log likelihood test shows this difference to be significant ($P<0.01$).

Authors identifying as having schizophrenia thus use the first-person singular pronoun more often than healthy controls. Therefore, Fineberg *et al*'s finding that authors with schizophrenia use 'I' less often than authors with mood disorders does not warrant any inferences regarding pathologies of the self in schizophrenia. To further investigate the relationship between language and self-disturbances, it would be desirable to analyse linguistic data from people undergoing an acute psychotic episode as well as to consider pronouns in their wider grammatical context rather than looking at mere word frequencies.

1 Fineberg SK, Deutsch-Link S, Ichinose M, McGuiness T, Bessette AJ, Chung CK, et al. Word use in first-person accounts of schizophrenia. *Br J Psychiatry* 2014; doi: 10.1192/bjp.bp.113.140046.

2 Brigham Young University. *The Corpus of Contemporary American English*. Brigham Young University (http://corpus2.byu.edu/coca).

3 Hardie A. CQPweb – combining power, flexibility and usability in a corpus analysis tool. *Int J Corpus Linguist* 2012; **17**: 380–409.

4 Lüdeling A, Kytö M. *Corpus Linguistics – An International Handbook*. De Gruyter, 2009.

Anke Maatz, speciality trainee in psychiatry and postdoctoral research fellow, University Hospital of Psychiatry Zurich, Switzerland. Email: anke.maatz@puk.zh.ch

doi: 10.1192/bjp.205.5.409

Authors' reply: We very much appreciate the concerns Dr Maatz raises. Indeed, we raised many of them in our discussion. Here we'll take the opportunity to elaborate on our decision-making process with regard to the analyses we reported.

As Dr Maatz and we ourselves point out, we did not include a non-psychiatric control group in our analysis. We found it difficult to identify an appropriate control for our particular corpus. Writing about illness in a journal for medical professionals is a rather particular kind of enterprise that commands specific language. We considered the caregiver and family-member accounts in the *Schizophrenia Bulletin* (which Dr Maatz called 'second-person accounts'). However, we were concerned about comparing samples with different themes (writing about oneself in the first group, writing about other people in the proposed control group). That would almost certainly change pronoun use. Furthermore, family members can sometimes present with attenuated, subclinical versions of the experiences, behaviours and deficits observed in psychotic illness.[2] We thought these might detract from our original objective, which was to analyse word use by people with schizophrenia compared with that by individuals with another mental illness.

We agree with Dr Maatz that this comparison between two illness groups limits the conclusions we can draw. We felt we were suitably circumspect but we are happy to rehearse the point. We are gathering new data, in which process we ask standard questions of participants (including questions that engage discussion of self, others, and impersonal topics). Furthermore we are gathering those data from participants at various illness phases (prodrome, acute psychosis, chronic illness) in order to examine the hypotheses suggested by our initial study of the *Schizophrenia Bulletin* corpus.

With respect to context analysis (how words co-occur), we agree that this is an interesting and important issue. We do not think that our word-counting approach is the final word on meaning in computational linguistics (no pun intended). We are eager to analyse larger meaning structures in our corpus using the new computational techniques Dr Maatz suggests,[3] among others.[4] We look forward to reading more about the analyses of the *Schizophrenia Bulletin* corpus she mentions in the peer-reviewed literature.

Indeed, we hope that this approach, analysing the writing and speech of patients with mental illness using computational linguistics, becomes another tool employed by those committed to understanding and treating mental illness. We are glad that Dr Maatz is interested in joining us in this venture.

1 Callicott JH, Egan MF, Mattay VS, Bertolino A, Bone AD, Verchinksi B, et al. Abnormal fMRI response of the dorsolateral prefrontal cortex in cognitively intact siblings of patients with schizophrenia. *Am J Psychiatry* 2003; **160**: 709–19.

2 Hardie A. CQPweb – combining power, flexibility and usability in a corpus analysis tool. *Int J Corpus Linguistics* 2012; **17**: 380–409.

3 Brown C, Snodgrass T, Kemper SJ, Herman R, Covington MA. Automatic measurement of propositional idea density from part-of-speech tagging. *Behav Res Methods* 2008; **40**: 540–5.

4 Mota NB, Vasconcelos NA, Lemos N, Pieretti AC, Kinouchi O, Cecchi GA, et al. Speech graphs provide a quantitative measure of thought disorder in psychosis. *PLoS One* 2012; **7**: e34928.

Philip Corlett, **Sarah Fineberg**, Yale University Department of Psychiatry, Connecticut Mental Health Center, New Haven, CT, USA. Email: philip.corlett@yale.edu

doi: 10.1192/bjp.205.5.409a

BDNF and proBDNF as biomarkers for bipolar disorder

I read with great interest the recent article by Li *et al*, describing plasma levels of brain-derived neurotrophic factor (BDNF) in patients with bipolar disorder in their first depressive episode.[1] A total of 203 patients with a first major depressive episode, as well as 167 healthy controls, were enrolled. After 3 years of bi-annual follow-up, 164 patients with a major depressive episode completed, and of these, 21 patients were diagnosed as having bipolar disorder and 143 patients were diagnosed as having major depressive disorder. At baseline, patients with bipolar disorder and depression showed significantly lower BDNF mRNA levels ($P<0.001$ and $P=0.02$, respectively) and plasma BDNF levels ($P=0.002$ and $P=0.01$, respectively) compared with healthy controls. Interestingly, plasma BDNF levels in patients with bipolar disorder were lower than those in patients with depression.

This study suggests that the model for predicting bipolar disorder during a first depressive episode is a combination of BDNF mRNA with plasma BDNF levels.[1] BDNF (mature BDNF) is a 13 kDa polypeptide, which is initially synthesised as a precursor protein, preproBDNF, in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF (~32 kDa) is converted to mature BDNF by extracellular proteases. It was initially thought that only secreted, mature BDNF was biologically active, and that proBDNF, localised intracellularly, served as an inactive precursor. However, accumulating evidence shows that both proBDNF and mature BDNF are active, eliciting opposing effects via the p75NTR and TrkB receptors, respectively, and that both forms play important roles in several physiological functions.[2]

The enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) used by Li *et al* recognise both proBDNF (precursor of BDNF) and mature BDNF, because of the limited specificity of the BDNF antibody.[3] Using newly available human proBDNF and mature BDNF ELISA kits, which differentiate between the BDNF forms, we have reported high levels of both proBDNF and mature BDNF in human serum.[3] We reported that serum levels of mature BDNF, but not proBDNF, in patients with major depressive disorder were significantly lower than those in healthy controls.[4] And we recently found that serum levels of mature BDNF and the ratio of mature BDNF to proBDNF in mood-stabilised patients with bipolar disorder were significantly higher than in healthy controls.[4] Interestingly, serum levels of proBDNF in mood-stabilised patients with bipolar disorder were significantly lower than those in healthy controls.[5] These findings were confirmed in two independent cohorts (Sahlgrenska set and Karolinska set in Sweden).[5] Considering the high levels of both proBDNF and mature BDNF in human serum, and their putative opposing functions, it would be clinically and scientifically interesting to measure the individual serum levels of proBDNF and mature BDNF in this cohort study.

1 Li Z, Zhang C, Fan J, Yuan C, Huang J, Chen J, et al. Brain-derived neurotrophic factor levels and bipolar disorder in patients in their first depressive episode: 3-year prospective longitudinal study. *Br J Psychiatry* 2014; **205**: 29–35.

2 Hashimoto K. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. *Psychiatry Clin Neurosci* 2010; **64**: 341–57.

3 Yoshida T, Ishikawa M, Iyo M, Hashimoto K. Serum levels of mature brain-derived neurotrophic factor and its precursor proBDNF in healthy subjects. *Open Clin Chem J* 2012; **5**: 7–12.

4 Yoshida T, Ishikawa M, Niitsu T, Nakazato M, Watanabe H, Shiraishi T, et al. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. *PLoS One* 2012; **7**: e42676.

5 Södersten K, Pĺlsson E, Ishima T, Funa K, Landén M, Hashimoto 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 Dis* 2014; **160**: 1–9.

Kenji Hashimoto, Professor, Chiba University Center for Forensic Mental Health. Email: hashimoto@faculty.chiba-u.jp

doi: 10.1192/bjp.205.5.410

Declaration of interest

K.H. is a holder of the patents 'Diagnostic and examination method for eating disorder' (US 7,754,434 B2) and 'Diagnostic agent for ischemic heart disease risk group' (US 2013/0310321A1), which pertain to the measurement of BDNF as a biomarker. In addition, He has served as a scientific consultant to Astellas and Taisho and he has received research support from Abbvie, Dainippon Sumitomo, Otsuka and Taisho.

Authors' reply: While we agree with Professor Hashimoto's comments regarding the predictive role of mature brain-derived neurotrophic factor (mBDNF) and its precursor, proBDNF, in bipolar disorder, several points merit further discussion.

First, we presented preliminary data describing a potential role for BDNF as a biomarker for predicting bipolar disorder in major depressive disorder, although we detected the serum BDNF level using commercial kits that do not differentiate between mBDNF and proBDNF. When we reviewed the literature regarding mBDNF and proBDNF in bipolar disorder and major depressive disorder, we noticed that lower serum levels of mBDNF and higher serum levels of proBDNF were found among patients with major depressive disorder.[1,2] Södersten *et al* also reported that higher serum levels of mBDNF and lower proBDNF were observed among patients with bipolar disorder.[3] These disparate results suggest that levels of mBDNF and proBDNF, as well as the ratio of mBDNF to proBDNF, might be sensitive enough to help differentiate bipolar disorder from major depressive disorder.

Second, our previous studies indicated that BDNF probably has some sex-specific characteristics. Tang *et al*[4] reported that the ratio of mBDNF to proBDNF differs in a sex-specific manner in zebra finches. These findings suggest that mBDNF and proBDNF are different in males and females and should be further investigated.

Third, the findings of one of our previous studies implied that genetic interactions between genes encoding BDNF and its receptor enhance the risk of treatment-resistant depression.[5] Recent studies have found that mBDNF and proBDNF elicit biological effects via interaction with their respective receptors, p75NTR and TrkB. Accordingly, we concluded that evaluations of mBDNF and proBDNF should also consider their receptors. On the whole, we appreciate Professor Hashimoto's insightful comments in directing our future work.

1 Yoshida T, Ishikawa M, Niitsu T, Nakazato M, Watanabe H, Shiraishi T, et al. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. *Plos One* 2012; **7**: e42676.

2 Zhou L, Xiong J, Lim Y, Ruan Y, Huang C, Zhu Y, et al. Upregulation of blood proBDNF and its receptors in major depression. *J Affect Disord* 2013; **150**: 776–84.

3 Södersten K, Palsson E, Ishima T, Funa K, Landen M, Hashimoto K, et al. Abnormality in serum levels of mature brain-derived neurotrophic factor (BDNF)