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RESEARCH ARTICLE

The Differential Levels of Inflammatory Cytokines and BDNF among Bipolar Spectrum Disorders

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Abstract

Objective: Emerging evidence suggests that inflammation and neurodegeneration underlies bipolar disorder. To investigate biological markers of cytokines and brain-derived neurotrophic factor between bipolar I, bipolar II, and other specified bipolar disorder with short duration hypomania may support the association with inflammatory dysregulation and bipolar disorder and, more specifically, provide evidence for other specified bipolar disorder with short duration hypomania patients were similar to bipolar II disorder patients from a biological marker perspective.

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Methods: We enrolled patients with bipolar I disorder (n = 234), bipolar II disorder (n = 260), other specified bipolar disorder with short duration hypomania (n = 243), and healthy controls (n = 140). Their clinical symptoms were rated using the Hamilton Depression Rating Scale and Young Mania Rating Scale. Inflammatory cytokine (tumor necrosis factor-α, C-reactive protein, transforming growth factor-β1, and interleukin-8) and brain-derived neurotrophic factor levels were measured in each group. Multivariate analysis of covariance and linear regression controlled for possible confounders were used to compare cytokine and brain-derived neurotrophic factor levels among the groups.
Results: Multivariate analysis of covariance adjusted for age and sex and a main effect of diagnosis was significant ($P < .001$). Three of the 5 measured biomarkers (tumor necrosis factor-α, transforming growth factor-β1, and interleukin-8) were significantly ($P = .006$, .01, and <.001) higher in all bipolar disorder patients than in controls. Moreover, covarying for multiple associated confounders showed that bipolar I disorder patients had significantly higher IL-8 levels than did bipolar II disorder and other specified bipolar disorder with short duration hypomania patients in multivariate analysis of covariance ($P = .03$) and linear regression ($P = .02$) analyses. Biomarkers differences between bipolar II disorder and other specified bipolar disorder with short duration hypomania patients were nonsignificant.
Conclusion: The immunological disturbance along the bipolar spectrum was most severe in bipolar I disorder patients. Other specified bipolar disorder with short duration hypomania patients and bipolar II disorder patients did not differ in these biological markers.

Keywords: bipolar spectrum disorder, bipolar I disorder, bipolar II disorder, subthreshold bipolarity, cytokines, BDNF

Introduction

Bipolar disorder (BP) is a severe mental illness associated with significant morbidity and mortality (Tsuang and Woolson, 1977; Coryell et al., 1993). The lifetime prevalence of BP in epidemiological surveys using the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) criteria is between 0.3% and 1.5% for bipolar I disorder (BP-I) and between 1.0% and 2.0% for bipolar II disorder (BP-II) (Weissman et al., 1996; Pini et al., 2005; Merikangas et al., 2007). However, increasing evidence supports the existence of a spectrum of BP that is far wider than recognized by current diagnostic nosology (Angst et al., 2010). A significant proportion of patients in the community (33.5–55%) have less than the DSM-IV-TR criterion of a 4-day hypomanic episode and might not be diagnosed with BP-II (Wicki and Angst, 1991; Angst et al., 2003). These patients are now classified as other specified BP (SBP) in the DSM 5. Some researchers have proposed that patients with SBP and BP-II have indistinguishable clinical characteristics: severity, functional impairment, comorbidities, and family history of BP (Benazzi and Akiskal, 2006; Angst et al., 2010, 2012). Patients with SBP who have major depressive disorder (MDD) and short-duration hypomania (2–3 days) also have a high rate of conversion to BP in longitudinal follow-ups (Axelson et al., 2011; Fiedorowicz et al., 2011), poorer response to antidepressants, and a more morbid long-term clinical course than do MDD patients without hypomanic episodes (Smith et al., 2009; Angst et al., 2012). Based on clinical presentation, some researchers (Benazzi and Akiskal, 2006; Angst et al., 2010, 2012) have even proposed the validity of a brief hypomanic episode (1, 2, or 3 days) for diagnosis of BP-II.

Most of the evidence for a spectrum of BP far wider than currently recognized comes from epidemiological studies that lack comparable biological data to explore the similarities between SBP and BP. In addition, there are few investigations of the differences in biomarker along the BP continuum of BP-I, BP-II, and SBP (Bai et al., 2014). We believe studies of the possible biological differences along the BP spectrum will provide support in addition to epidemiological data for the classification of BP-II and SBP.

Neuroinflammatory interactions with disturbances related to oxidative stress (Maes et al., 2011) and neurotrophic mechanisms (Monje et al., 2003) have been implicated in the neurobiological background and neuroprogressive processes of BP (Berk et al., 2011). Inflammation is thus an integrating component for the pathophysiology of BP based on evidence from shared genetic polymorphisms and gene expressions in BP and inflammation and on evidence from altered cytokine levels during symptomatic and asymptomatic intervals (Goldstein et al., 2009; Modabbernia et al., 2013; Munkholm et al., 2013a, 2013b). Changes in tumor necrosis factor-α (TNF-α) (Modabbernia et al., 2013; Munkholm et al., 2013b), C-reactive protein (CRP) (Dickerson et al., 2007), interleukin-8 (IL-8) (O'Brien et al., 2006), and transforming growth factor-β1 (TGF-β1) (Kim et al., 2004) levels were found in BP with different affective states and presented different immune pathways, from proinflammatory to regulatory action. In addition, cytokines are key signaling molecules that regulate both innate and adaptive immunological responses and have widespread effects on the neuroendocrine system, neurogenesis, neurocircuitry, and neurotransmitter metabolism (Haroon et al., 2012). Therefore, peripheral cytokine fluctuations have been proposed as biomarkers for disease activity, affective state, or the disease nature for BP (Roda et al., 2015). Most studies, however, have a mixed group of BP patients (ie, BP-I patients and BP-II patients) to compare with a control group (Modabbernia et al., 2013; Munkholm et al., 2013a, 2013b). Comparisons between all the subtypes of BP along the spectrum, especially subthreshold bipolarity, were limited. Because different subtypes of BP have variable levels of clinical outcomes, severity, and functional impairments (Akiskal et al., 2000; Angst et al., 2003, 2010), a detailed investigation of the variations of inflammatory cytokines is needed.

Neurotrophins, such as brain-derived neurotrophic factor (BDNF), are crucial for neuronal survival, growth, plasticity, and connectivity, all of which are thought to be involved in the pathophysiology of BP (Berk et al., 2011). Postmortem brains from BP patients have showed significantly lower-than-normal range levels of BDNF, which might contribute to brain atrophy and progressive cognitive changes (Kim et al., 2010). The reduction of serum BDNF levels is correlated with decreased BDNF levels in the brain (Pan et al., 1998). Studies also showed a decrease in BDNF levels in acute episodes of BP (Kapczinski et al., 2008a) and cumulative effects when the disorder progressed (Kapczinski et al., 2008b). The levels of BDNF appear to be normal in the early stages of the disorder in euthymic BP patients and to decrease in the latter stages (Kauer-Sant'Anna et al., 2009); therefore, peripheral BDNF is a BP biomarker for both state markers during acute episodes and disease progression markers that accompany a clinical course (Berk et al., 2011; Fernandes et al., 2011).

Because cytokine and BDNF are closely correlated with the underlying pathophysiological changes and clinical presentation with BP, they are candidates for the biological marker for BP. Although patients with MDD also have higher-than-normal range levels of peripheral inflammatory cytokines (Miller et al., 2009) and lower-than-normal range BDNF levels (Polyakova et al., 2015), studies have hypothesized that the disturbance of inflammation (Bai et al., 2015) and BDNF expression (Li et al., 2014) were more severe in BP than in MDD and indicated the potential differential roles of these biomarkers (Li et al., 2014).

We wanted to investigate the plasma cytokine and BDNF levels in BP-I, BP-II, SBP, and control groups to better understand the biological differences along the continuum of BP, which might also support the similarities or differences between BP-II and SBP.

Methods

Participants

The research protocol was approved by the Institutional Review Board for the Protection of Human Subjects at Tri-Service General Hospital and National Cheng Kung University Hospital. The procedures were fully explained to each participant before they were asked to sign the informed consent.

Patients were recruited both from outpatient and inpatient settings and initially evaluated by an attending psychiatrist. They then underwent a more detailed interview by a clinical psychologist using the structured interview in the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia- Life Time (SADS-L) (Endicott and Spitzer, 1978), which has good inter-rater reliability (Huang et al., 2004), to determine the diagnosis in DSM-IV. Patients with BP-I, BP-II, or major depressive episodes and a previous subthreshold hypomania history (>2 days but <4 days) were included. Patients with other psychiatric comorbidities were evaluated using the SADS-L. Patients with an active infectious disease, autoimmune diseases, or uncontrolled clinically significant medical condition (eg, cardiac, hepatic, and renal failure) were excluded. They were then subdivided into BP-I, BP-II (hypomania lasting for more than 4 days), and SBP (short-duration hypomania: 2–3 days) groups for further analysis. The duration of hypomania was determined by "the most common duration" question according to previous suggestions (Benazzi and Akiskal, 2006). Patients who could not recall the duration of hypomania were excluded from the current analysis. The severity of mood symptoms was assessed using the Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960; Hamilton, 1967).

The healthy control group consisted of 140 volunteers recruited from the community. They were screened by telephone, and those with a sleep disorder, substance abuse disorder, mood swings, or possible symptoms of psychosis were excluded. The psychiatric conditions of the remaining volunteers were then carefully screened in face-to-face interviews with a clinical psychologist using the Chinese Version of SADS-L. All controls were free of present and past major mental illness (affective disorder, schizophrenia, anxiety disorder, personality disorder, and substance use disorders), and none had a family history of psychiatric disorder among their first-degree relatives. The inflammatory cytokine and BDNF levels were also assessed after the psychiatric screening.

Measuring Plasma Cytokine Levels

Twenty milliliters of blood was drawn from each participant. The fasting blood samples were collected between 8:00 and 10:00 AM. Plasma was isolated from the whole blood after it had been centrifuged at 3000 *g* for 15 minutes at 4°C, and then it was immediately stored at -80°C. Cytokine and BDNF levels were quantified using an antibody pair assay system (Flexia; BioSource Intl., Camarillo, CA). Sample processing and data analysis were done according to the manufacturer's instructions. The immunological parameters TNF-α, CRP, TGF-β1, IL-8, and BDNF were measured. The respective sensitivities and ranges for cytokine assays were: TNF-α (0.038–0.191 pg/mL and not detected [ND] to 2.139 pg/mL), CRP (0.005–0.022 ng/mL and 104–4185 ng/mL), TGF-β1 (1.7–15.4 pg/mL and 18289-63416 pg/mL), IL-8 (1.5–7.5 pg/mL and < 31.2 pg/mL), and BDNF(< 20 pg/mL and ND to 4137 pg/mL). All laboratory procedures were performed double-blinded and all assays were done in duplicate.

Statistical Analysis

Pearson χ^2 analysis was used to examine sex differences and other categorical variables. Fisher's exact test was substituted for the χ^2 test when values were smaller than expected (<5). We used 1-way ANOVA and the Bonferroni posthoc test to compare the differences in mean age, disease duration, and HDRS and YMRS scores in BP-I, BP-II, SBP, and controls groups. Multivariate analysis of covariance (MANCOVA) was used to compare cytokine and BDNF levels between the groups. When analyzing the BP-I, BP-II, SBP, and control groups, age and sex were controlled as covariates, and age, sex, disease duration, comorbidity, and severity (HDRS and YMRS scores) were controlled as covariates when analyzing the BP-I, BP-II, and SBP groups. One-way ANOVA was then applied to clarify the significant findings identified using MANCOVA. Linear regression models were used to determine the correlation between cytokine and BDNF levels and between bipolar spectrum disorder and other related factors. Significance was set at $P < .05$. SPSS 18.0 was used for all statistical analyses. The power estimation was calculated using G-power 3.0 (Faul et al., 2007, 2009) and SPSS 18.0.

Results

Of the 877 participants in the study, 234 had BP-I, 260 had BP-II (hypomania: ≥4 days), 243 had SBP (short-duration hypomania: 2–3 days), and 140 were controls. There were no significant differences in age and sex between bipolar spectrum groups, but there was a significantly higher percentage of men in the control group ($P = .01$) (Table 1). There were no significant differences in the disease duration and HDRS and YMRS scores between the BP-II and SBP groups (Table1), but HDRS scores were significantly lower and YMRS scores were significantly higher (both $P < .001$) in the BP-I group (Table 1). Twenty-seven (3.7%) of the bipolar spectrum patients did not complete the SADS-L interviews, which lasted for more than 4 to 6 hours, and their comorbidity data was incomplete. Although the physical comorbidity rate was not significantly different between the three BP groups, the psychiatric comorbidity rates were significantly ($P < .008$) higher for the BP-I and SBP groups than for the BP-II group (Table 2). The percentage of anxiety disorder comorbidity was significantly ($P < .001$) higher in the BP-II and SBP groups, and the percentage of substance (including smoking) use disorder was significantly ($P < .001$) higher in the BP-I group (Table 2).

Table 3 shows the inflammatory cytokines and BDNF levels in the BP-I, BP-II, SBP, and control groups. Among the three patient groups and the control group, MANCOVA showed that there was an overall multivariate effect of the diagnostic group for cytokines and BDNF levels (Pillai's $F = 4.09$, $df = [15, 2607]$, $P < .001$). Significant univariate effects were found in TNF-α ($P = .006$), TGF-β1 ($P = .01$),

Table 1. Demographic Data and HDRS and YMRS Scores between BP Patients and Controls

	BP-I	BP-II	SBP	Controls	Statistics	P	post hoc
Patients (n)	234	260	243	140			
Age (y)[a]	33.6±11.7	31.6±12.3	33.0±12.6	31.9±8.17	F=1.46	.23	
Sex (male/female)	115/119	135/125	100/143	81/59	χ^2=11.27	.01*	
Disease duration (y)[a]	13.6±9.9	15.4±11.6	16.1±11.8		F=2.43	.09	
HDRS scores[a]	14.3±7.3	16.8±5.4	16.6±5.3		F=11.90	<.001*	B=C>A
YMRS scores[a]	12.8±5.9	10.9±4.3	10.4±4.1		F=15.47	<.001*	A>B=C

Abbreviations: A, BP-I; B, BP-II; C, SBP; D, controls.
a(mean± SD).
*P<.05.

Table 2. Comorbidities in Bipolar Spectrum Disorders

	BP-I (n=211)	BP-II (n=258)	SBP (n=241)	χ2	P
Physical comorbidities	44 (20.9%)	59 (22.9%)	59 (24.5%)	0.84	.66
Cardiovascular disease	5 (2.4%)	4 (1.6%)	3 (1.2%)	0.90	.64
Hypertension	13 (6.2%)	17 (6.6%)	11 (4.6%)	1.02	.60
Diabetes mellitus	5 (2.4%)	7 (2.7%)	4 (1.7%)	0.65	.73
Hyperlipidemia	5 (2.4%)	6 (2.3%)	7 (2.9%)	0.20	.90
Liver disease	13 (6.2%)	21 (8.1%)	9 (3.7%)	4.26	.12
Renal disease	1 (0.5%)	5 (1.9%)	6 (2.5%)	2.90	.23
Thyroid disease	7 (3.3%)	7 (2.7%)	10 (4.1%)	0.79	.67
Lung disease	7 (3.3%)	19 (7.4%)	18 (7.5%)	4.29	.12
Cancer	1 (0.5%)	3 (1.2%)	3 (1.2%)	0.82	.67
Psychiatric Comorbidities	117 (55.5%)	134 (43.4%)	112 (55.6%)	9.66	.008*
Anxiety disorder	49 (23.2%)	121 (46.9%)	83 (34.4%)	28.60	<.001*
Substance use disorder (other than smoking)	22 (10.4%)	8 (3.1%)	10 (4.1%)	13.23	.001*
Smoking	34 (16.1%)	12 (4.7%)	18 (7.5%)	19.66	<.001*
Personality disorder	7 (3.3%)	0	2 (0.8%)	10.77	.005*
Impulse control disorder	5 (2.4%)	8 (3.1%)	7 (2.9%)	0.24	.89
Eating disorder	5 (2.4%)	6 (2.3%)	3 (1.2%)	1.00	.61
Neurodevelopmental disorder	4 (1.9%)	10 (3.9%)	7 (2.9%)	1.61	.45

*P<.05.

Table 3. Cytokines and BDNF Levels in BP-I, BP-II, and SBP Patients and Controls

	BP-I	BP-II	SBP	Controls	Model 1			Model 2		
Cytokines	(n=234)	(n=260)	(n=243)	(n=140)	F	P	post hoc	F	P	post hoc
TNF-α (pg/mL)	2.15±1.77	2.41±2.24	2.27±2.17	1.68±1.59	4.13	.006*	A=B=C>D	1.21	.30	
CRP (μg/mL)	1.81±1.94	1.61±1.57	1.59±1.66	1.55±1.36	0.88	.45		1.01	.37	
TGF-β1 (ng/mL)	32.20±17.31	30.23±15.65	30.04±18.24	27.25±15.73	3.58	.01*	A>D,B=C=D	2.85	.06	
IL-8 (pg/mL)	4.51±6.32	3.05±4.89	3.09±5.11	1.59±3.31	9.87	<.001*	A>B=C>D	3.44	.03*	A>B=C
BDNF (ng/mL)	15.83±10.23	17.32±14.45	14.79±8.36	16.74±9.09	1.73	.16		2.00	.14	

Abbreviations: A, BP-I; B, BP-II; BDNF, brain-derived neurotrophic factor; C, SBP; CRP, C-reactive protein; D, controls; IL-8, interleukin-8; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α.
Model 1: MANCOVA and Bonferroni post hoc comparisons; Group: BP-I, BP-II, SBP, and controls; covarying for age and sex.
Model 2: MANCOVA and Bonferroni post hoc comparisons; Group: BP-I, BP-II and SBP; covarying for age, sex, disease duration, comorbidity, and HDRS and YMRS scores.
*P<.05.

and IL-8 (P<.001) (Table 3). Post hoc comparisons showed that the BP-I group had higher levels of TNF-α, TGF-β1, and IL-8 than did the control group (P < .05) (Table 3). BP-II and SBP patients had higher levels of TNF-α and IL-8 than did the controls but lower levels of IL-8 than did BP-I patients (P<.05) (Table 3). No significant differences were found between TNF-α, TGF-β1, and IL-8 levels in BP-II and SBP patients (P > .05) (Table 3).

After covarying for disease duration, comorbidity, and HDRS and YMRS scores, MANCOVA analysis still showed a main multivariate effect for all studied groups (Pillai's F = 2.18, df = [10,1200], P = .02). There was a significant univariate effect for IL-8 (P=.03) (Table 3). The level of IL-8 was significantly higher in BP-I patients (P<.05), but there was no difference between BP-II and SBP patients (Table 3).

The linear regression model also showed that BP-I patients had significantly (t=-2.40, P=.02) higher plasma IL-8 levels than did those with BP-II and SBP patients (Figure 1). Other cytokine and BDNF levels were not significantly associated with diagnosis of bipolar spectrum disorders in regression model. However, the associations between TNF-α levels and HDRS (t=-2.74, P=.006),

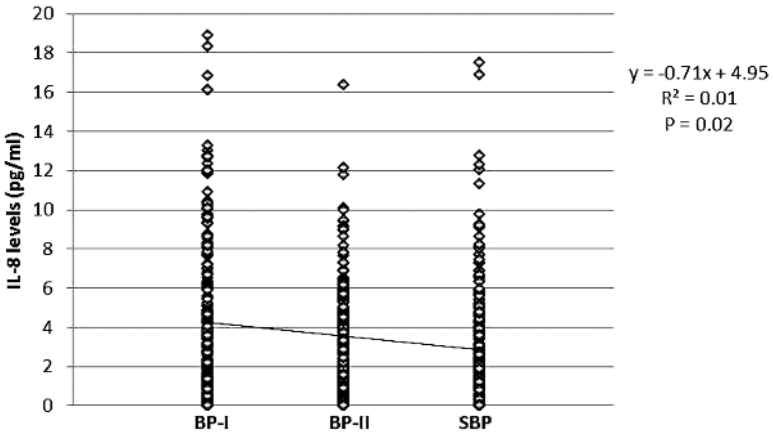

Figure 1. Scatter plot of plasma interleukin-8 (IL-8) levels with representative linear regression of plasma IL-8 levels in the bipolar spectrum disorders (covarying for age, sex, disease duration, comorbidity, Hamilton Depression Rating Scale [HDRS], and Young Mania Rating Scale [YMRS] scores).

and between TGF-β1 levels and disease duration (t = -2.47, *P* = .01), comorbidities (t = -2.17, *P* = .03), HDRS (t = 2.11, *P* = .04), and YMRS (t = -3.49, *P* = .01) were significant.

The power estimation in MANCOVA was approximately 1.00 for global effects and 0.45 to 0.99 for specific effects in different cytokines and BDNF in comparisons between the BP-I, BP-II, SBP, and control groups. In a comparison between the BP-I, BP-II, and SBP groups, the study had a power of 0.92 for global effects and 0.23 to 0.65 for specific effects for different cytokine and BDNF levels. For linear regression analysis, the study had a power of 0.81 to detect a small effect and of 1.00 to detect a medium effect and a large effect. In this power analysis, the effect-size conventions were determined based on Faul et al. (2007, 2009)as follows: small effect size = 0.02, medium effect size = 0.15, and large effect size = 0.35 for the linear regression model (alpha = 0.05).

Discussion

This is the first study that compares the inflammatory cytokines and BDNF levels in BP patients with different bipolar spectrum disorders and especially in bipolar patients with short-duration hypomania. We found that the levels of TNF-α, TGF-β1, and IL-8 were significantly higher in BP spectrum patients than in controls. The levels of TNF-α and TGF-β1 were sensitive to several confounding factors. In addition, the plasma IL-8 levels were significantly higher in BP-I than in BP-II and SBP patients after controlling for multiple possible confounding factors. There were no significant differences between BP-II and SBP patients for any of our measured potential biomarkers.

Epidemiological studies (Angst et al., 2003; Benazzi and Akiskal, 2006; Angst et al., 2010) have suggested that there was a gradient distribution from the most severe group, BP-I, to the least severe group, subthreshold BP in the disease severities and functional impairment levels. Neuropsychological data (Lin et al., 2015) also showed that patients with SBP performed significantly better than did patients with BP-I in set shifting and visual-spatial memory and that patients with SBP performed similarly to those with BP-II in all of the cognitive domains. Bai et al. (2014) compared inflammatory cytokine levels in BP-I and-II and reported that patients with BP-II had significantly lower levels of soluble (s)TNF-R1 than did patients with BP-I, but there were no data on SBP patients. Our study provided comparable evidence supporting their claim that along the BD spectrum, BP-I is strongly associated with inflammatory cytokines dysregulation and that SBP and BP-II have no significant differences in levels of inflammatory changes, which was consistent with other studies (Angst et al., 2003, 2010; Benazzi and Akiskal, 2006; Lin et al., 2015).

The diagnostic boundaries for BP are controversial, especially the required duration for hypomania, because the 4-day minimum is not evidence based (Benazzi, 2001), and ideas about shorter durations have changed significantly over the years (Nusslock and Frank, 2011). This definition is derived from averaging the 2-day (probable) and 7-day (definite) threshold for hypomania in the Research Diagnostic Criteria (Spitzer et al., 1979). It is suggested that the current 4-day threshold may unnecessarily narrow the range of bipolar spectrum disorders and a cut-off of 2 days should be used instead (Akiskal et al., 1977, 1979, 2000; Cassano et al., 1992; Coryell et al., 1995; Angst, 1998; Benazzi, 2001; Akiskal and Benazzi, 2003; Angst et al., 2003, 2010; Benazzi and Akiskal, 2006). However, concerns remain for broadening the diagnostic criteria based only on clinical characteristics and symptomatology data (Zimmerman, 2012), even in the DSM-5. The current diagnostic criteria are descriptions of syndromes and represent an underlying group of psychological, behavioral, and biological dysfunction; they might be imperfect representation of unknown core pathophysiological changes (Zimmerman, 2012). Considerations and comparisons using biological markers might provide data that are more convincing and might improve the sensitivity and specificity of diagnoses (Zimmerman, 2012). The most recognized biological evidence that supports the hypothesis that SBP and BP-II are identical is from family studies, which report that family histories of bipolarity in individuals displaying shorter duration of hypomania are more in line with BP-II than unipolar depression (Cassano et al., 1992; Angst, 1998; Angst et al., 2003; Benazzi and Akiskal, 2006). Neuropsychological tests also offer evidence that patients with SBP performed similarly to patients with BP-II in all of the cognitive domains (Lin et al., 2015). Our study, which showed no significant difference in the immunological aspects of SBP and BP-II, might provide biological evidence that supports the notion that BP-II and SBP are indistinguishable. This finding might be an important addition as a biological reference that supports past studies (Angst et al., 2012), which calls for further revision of the hypomania duration criterion to improve recognition of bipolarity and the choice of appropriate treatment regimen (Angst et al., 2011).

The BDNF levels in our study were not significantly different between the patient and control groups or between the 3 different bipolar spectrum patients. The results of peripheral

BDNF levels in BP were mixed in past studies, partly related to different mood episodes, disease states, or the disease progression process (Fernandes et al., 2011). Although studies that compared the BDNF levels in patients with different types of mood disorders suggested that BP-I patients had lower levels of BDNF than did MDD patients (Fernandes et al., 2009), the comparisons between BDNF levels in BP-I, BP-II, and SBP were limited. D'Addario et al. (2012) reported a significant downregulation of the BDNF gene expression and hypermethylation of the BDNF promoter region in BD-II than controls, but not in BD-I. However, the BDNF levels in our BP-II patients were not significantly lower than those in BP-I patients, and only a trend suggested the possibility of lower BDNF levels in SBP patients than in BP-I, BP-II patients, or controls. The plasma BDNF level is sensitive to multiple confounders, such as the metabolic profile (Chaldakov et al., 2004) and medications (Fernandes et al., 2011); thus, future investigations should control more possible confounders and longitudinal follow-ups, which might provide more information.

We also found a significant correlation between the TNF-α level and HDRS, which is consistent with reports that TNF-α levels in depressed BP patients were higher than in controls (O'Brien et al., 2006; Ortiz-Dominguez et al., 2007). We found no association between TNF-α level and YMRS (Munkholm et al., 2013b); rather, we found associations between TGF-β1 levels and disease duration, comorbidities, HDRS, and YMRS, which was rarely reported before (Munkholm et al., 2013b). The TGF-β1 is an important modulator of inflammatory cytokines (Letterio and Roberts, 1997; Strober et al., 1997). Lower levels of TGF-β1 were found in BP patients and higher levels after treatment (Kim et al., 2004). The high initial plasma level of TGF-β1 was also associated with a better prognosis during pharmacological treatment (Li et al., 2015). The negative correlations between the levels of TGF-β1 and BP disease duration, comorbidities, and YMRS might partially support and explain previous findings (Kim et al., 2004; Li et al., 2015). In addition, the difference between TNF-α and TGF-β1 levels between the 3 bipolar spectrum disorders became nonsignificant after controlling for the associated factors of disease duration, comorbidities, and symptom severity. The differences between our findings and those of prior studies (Modabbernia et al., 2013; Munkholm et al., 2013a, 2013b) might be because these factors were not controlled for in prior analyses.

Our study has some limitations. Our sample size is relative larger than those in past studies; however, a still larger sample might provide more compelling results. Although we tried to control for factors that might affect the changes of cytokine and BDNF levels, other possible factors, such as metabolic profiles, medications, and relapsing and remitting states, might also have affected our findings and should be controlled in future research. Additionally, we included only patients with short-duration hypomania, which constituted only part of subthreshold bipolar patients. To generalize our results to all subthreshold BP patients might require supplementary data. A lack of comparison between BP and MDD is another limitation. However, it took more than 3 years to identify and enroll such a large cohort of BP. It would have been difficult for us to recruit a large enough cohort of MDD patients in a short period. Additional analyses of cytokine and BDNF levels in MDD patients might be needed and offer more solid evidence to classify SBP patients. Finally, about 7.5% of the patients diagnosed with BP-II became manic in a 10-year follow-up study (Coryell et al., 1995). A misdiagnosis with current subtypes is possible, and long-term follow-ups for patients with BP-II or SBP to confirm these findings might be needed. Therefore, our findings should be interpreted with caution.

Conclusion

Despite these limitations, our data show a significant immunological dysregulation in all BP spectrum patients based on analyses of their peripheral inflammatory cytokines. The deviance was more apparent in BP-I patients than in BP-II and SBP patients. BP-II and SBP patients shared similar features among several measured biological markers in our study. The present study supports the similarity between BP-II and SBP from the immunological aspects, which are also associated with clinical symptoms. It also provides an initial understanding of the biological difference of the BP spectrum. Our findings might be a significant biological addition to current efforts that support recognizing short-duration hypomania as an important indicator for the diagnosis of BP.

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Statement of Interest

None.

R.-B.L. designed the study and wrote the protocol. S.-L.C., Y.-H.C., S.-H.C., and C.-H.C. managed lab work and the data analyses. S.-Y.H., N.-S.T., S.-Y.L., P.S.C., I.H.L., K.-C.C., Y.K.Y., and R.-B.L. managed to recruit participants. T.-Y.W. wrote the first draft of the manuscript. S.-Y.L. made contributions to literature review and discussion. All authors contributed to and reviewed the final manuscript.

The research protocol was approved by the Institutional Review Board for the Protection of Human Subjects at National Cheng Kung University Hospital and Tri-Service General Hospital.

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