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Mitochondrial DNA Copy Number Is Associated With Treatment Response and Cognitive Function in Euthymic Bipolar Patients Receiving Valproate

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Abstract

Background: Bipolar disorder (BD) is associated with cognitive impairment and mitochondrial dysfunction. However, the associations among mitochondrial DNA copy number (MCN), treatment response, and cognitive function remain elusive in BD patients.
Methods: Sixty euthymic BD patients receiving valproate (VPA) and 66 healthy controls from the community were recruited. The indices of metabolic syndrome (MetS) were measured. Quantitative polymerase chain reaction analysis of blood leukocytes was used to measure the MCN. Cognitive function was measured by calculating perseverative errors and completed categories on the Wisconsin Card Sorting Test (WCST). The VPA treatment response was measured using the Alda scale.
Results: BD patients had significantly higher MCN, triglyceride, and C-reactive protein (CRP) levels, waist circumference, and worse performance on the WCST than the controls. Regression models showed that BD itself and the VPA concentration exerted significant effects on increased MCN levels. Moreover, the receiver operating characteristic curve analysis showed that an MCN of 2.05 distinguished VPA responders from nonresponders, with an area under the curve of 0.705 and a sensitivity and specificity of 0.529 and 0.816, respectively. An MCN level ≥2.05 was associated with 5.39 higher odds of being a VPA responder ($P = .006$). BD patients who were stratified into the high-MCN group had a higher VPA response rate, better WCST performance, lower CRP level, and less MetS.
Conclusions: The study suggests a link between the peripheral MCN and cognitive function in BD patients. As an inflammatory status, MetS might modulate this association.

Significance Statement

This study is the first, to our knowledge, to investigate the association between mitochondrial DNA copy number (MCN) and cognitive function in euthymic bipolar disorder (BD) patients receiving valproate (VPA). In the current study, BD patients showed significantly higher MCN than the controls, even after adjustment for age, sex, and metabolic syndrome (MetS). The VPA concentration was positively correlated with the MCN in BD patients. In addition, we evaluated the stratification effect of MCN on the clinical features of BD patients. We found that the MCN significantly distinguished VPA responders from non-responders. Our results revealed a higher percentage of VPA responders and better cognitive performance of BD patients in the high-MCN group than in the low-MCN group. Taken together, the study findings provide better insights into how MCN might serve as a biomarker in BD.

Introduction

Bipolar disorder (BD) is a severe and chronic psychiatric illness characterized by recurrent episodes of mania and depression, which significantly affect the functioning and quality of life of patients. Moreover, accumulating evidence for cognitive impairments has been documented in BD patients (Green, 2006; Berk et al., 2007). Patients with BD show cognitive dysfunction in verbal memory and frontal executive tasks (Martinez-Aran et al., 2004). A more recent meta-analysis also confirmed evident deficits in verbal learning, trail-making, and verbal working tasks (Bourne et al., 2013). These cognitive impairments might be long lasting, even in stable euthymic BD patients (Mur et al., 2008). A review of meta-analyses from longitudinal studies concluded that cognitive deficits did not progress but appeared generally stable (Bora and Ozerdem, 2017). Cognitive dysfunction could partially explain compromised psychosocial and occupational functioning (Zarate et al., 2000; Mur et al., 2008).

Mitochondrial DNA (mtDNA) is a double-stranded, closed circular molecule located in the mitochondria with no introns or histones. Without histone protection, mtDNA is susceptible to oxidative injury because mitochondria can generate reactive oxygen species during ATP synthesis (Furda et al., 2012). An altered mtDNA copy number (MCN) could result from inflammation potentially playing a pathogenic role in mitochondrial dysfunction and disease (Malik and Czajka, 2013). Therefore, the MCN is a potential biomarker of mitochondrial function, and an abnormal MCN has been suggested to be correlated with various psychiatric disorders, such as schizophrenia, BD, and Alzheimer's disease (Mancuso et al., 2007; Li et al., 2015).

Recent evidence has suggested a role for mitochondrial dysfunction in the possible pathophysiology of BD (Kato and Kato, 2000). Regarding BD, several in vivo studies have examined MCNs but produced conflicting results (Chang et al., 2014; de Sousa et al., 2014; Fries et al., 2017; Rollins et al., 2018; Wang et al., 2018). Fries et al. and Rollins et al. found a significantly increased MCN in BD patients compared with controls (Fries et al., 2017; Rollins et al., 2018), while de Sousa et al. did not observe a significant difference in the MCN between depressed BD patients and healthy controls (de Sousa et al., 2014). Although a meta-analysis failed to identify any significant association between the MCN and BD, a subgroup analysis revealed a markedly lower MCN in Asian patients with BD (Yamaki et al., 2018).

Increased inflammation and prevalent MetS are strongly correlated with mitochondrial dysfunction (Huang et al., 2011; Malik and Czajka, 2013), and both conditions have been reported in BD patients (Kapczinski et al., 2011; Benros et al., 2013). More recently, chronic inflammation has been hypothesized to be one of the imperative underlying mechanisms of brain pathology in BD (Kapczinski et al., 2011; Benros et al., 2013; Benedetti et al., 2020; Giridharan et al., 2020). C-reactive protein (CRP), a sensitive peripheral biomarker of inflammation and metabolic disturbances (Nordestgaard and Zacho, 2009), is associated with mood status and related cognitive deficits in BD patients (Chang et al., 2012; Dickerson et al., 2013).

Although mitochondrial dysfunction and cognitive impairment have been separately observed in BD patients, the link has not been fully explored in BD. Therefore, the aims of our study were to explore whether MCN levels in euthymic BD patients receiving valproate (VPA) are associated with cognitive performance and the treatment response and to identify possible confounding factors, including medications and mood status.

METHODS

Participants

The Institutional Review Board for the Protection of Human Subjects at National Cheng Kung University Hospital approved the research protocol (IRB no. A-ER-104-031). All the participants were recruited from outpatient settings at the National Cheng Kung University Hospital and provided written informed consent regarding their willingness to participate in the research. To assess whether BD affects the MCN, we recruited healthy controls from the community after the exclusion of individuals with mental illnesses by a senior psychiatrist using the Chinese version of the Mini International Neuropsychiatry Interview. All BD patients were initially evaluated in an interview by an attending psychiatrist using the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia–Life Time, which has good interrater reliability, to determine diagnoses according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The patients also met the following inclusion criteria: (1) 20–70 years old, (2) diagnosed with BD according to the DSM-5 criteria, and (3) received VPA treatment. The mood of each patient was evaluated using the 17-item Hamilton Depression Rating Scale (HDRS) and the 11-item Young Mania Rating Scale (YMRS). All participants meeting the following criteria were excluded through chart reviews and patient-reported questionnaires to avoid confounding factors that influence mitochondrial function: (1) a serious surgical condition or physical illness, including heart disease, stroke, kidney dialysis, and transplant; (2) cerebrovascular disease; (3) neurodegenerative disorders; and (4) macrovascular disorders. In addition, all the patients with BD were treated with VPA (Depakine, Carbon Blanc, France). Concomitant fluoxetine (Prozac, Bourgoin-Jallieu,

France) (20 mg/d) was permitted to treat depressive symptoms, and lorazepam (Ativan, Munster, Germany) (<8 mg) was used for night-time sedation and to treat agitation and insomnia during the study, the dosage of which was adjusted according to the clinical manifestations and the patient's tolerance.

To collect lifestyle factors, including exercise, alcohol consumption, and smoking, we used self-report questionnaires. Regarding exercise, the categories were none (Group 1), <1 time/mo (Group 2), 1–3 times/mo (Group 3), 1–2 times/wk (Group 4), 3–5 times/wk (Group 5), and almost every day (Group 6). Since the frequency of exercise did not significantly differ between the BD patients and controls, we report the results briefly (exercise: yes [Groups 2–6] or not [Group 1]). In addition, regarding alcohol intake, participants were grouped by current alcohol use or not. They were also grouped by current smoking or not.

Assessment of the Response to VPA Treatment—The response to VPA treatment was measured using the Alda scale (Manchia et al., 2013), which consists of an A criterion and B criterion. Criterion A was used to assess the clinical improvement and indicated changes in the frequency and severity of mood symptoms. Criterion B was used to assess the causal relationship between VPA treatment and clinical improvement. The total Alda score was calculated by subtracting the total B score from the A score (A – B total). For participants with a total B score greater than the A score, the total score was set to 0. The scores ranged from 0 to 10, and a higher score indicated a greater response. We used the total Alda score as a continuous variable of the VPA treatment response and defined the dichotomous phenotype with an Alda score cutoff ≥5 as a responder and with a score <5 as a nonresponders (Ahn et al., 2017).

Measurements

Fasting blood samples were collected between 8:00 AM and 10:00 AM. Ten milliliters of whole blood was drawn from the antecubital vein of each patient. Plasma, which was isolated from whole blood after centrifugation at $3000 \times g$ for 15 minutes at 4°C, was immediately stored at –80°C.

Determination of the Copy Number and Oxidative Damage in Human Leukocyte mtDNA—A FlexiGene DNA Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from the buffy coat, and all the procedures followed the instructions of the FlexiGene DNA Handbook. The methods of testing leukocyte MCNs and oxidative damage were described in our previous studies (Chang et al., 2014). The leukocyte MCN was assessed using a LightCycler Instrument (Roche, Mannheim, Germany). Briefly, PCR was performed by amplifying the ND1 gene in mtDNA and the β-globin gene in nuclear DNA from sampled DNA, and the calculation of the MCN was interpolated from the linearity of the dose-dependently constructed standard plasmid of the ND1/β-globin gene.

Metabolic Index—Fasting total cholesterol, low-density lipoprotein, and triglyceride concentrations were measured using enzymatic methods with a TBA-200FR automated analyzer (Toshiba Lab Medical, Tokyo, Japan). In addition, the body mass index (BMI) of each patient was measured at each time point. BMI was calculated as weight (kg) divided by height squared (m²), and waist circumference was measured at the level midway between the lateral lower rib margin and the superior anterior iliac crest. According to the modified National Cholesterol Education Panel, Adult Treatment Panel III (NCEP ATP III) criteria (Grundy et al., 2005), MetS was defined as the presence of 3 or more of the following dysregulations of metabolic components: (1) abdominal obesity: waist circumference ≥90 cm in men or ≥80 cm in women; (2) hypertension: blood pressure ≥130/85 mmHg; (3) hyperglycemia: fasting glucose level ≥100 mg/dL or a diabetes diagnosis; (4) low high-density lipoprotein (HDL-C) concentration: HDL-C < 40 mg/dL in men and <50 mg/dL in women; and (5) hypertriglyceridemia: triglyceride level ≥150 mg/dL.

Serum VPA Concentration—The serum trough concentration of VPA was assessed using the homogeneous enzyme immunoassay method and measured at the Union Clinical Laboratory (Taipei, Taiwan). The limit of detection was 3 μg/mL.

Plasma High-Sensitivity CRP Level—Plasma CRP levels were determined using enzyme-linked immunosorbent assay with a human high sensitivity CRP Instant ELISA kit (Bender MedSystem GmbH, Vienna, Austria) according to the manufacturer's instructions. The limit of detection was 3 pg/mL, and the intra- and interassay CVs were 6.9% and 13.1%, respectively.

Cognitive Performance Task—Wisconsin Card-Sorting Test (WCST)—The WCST was administered by an experienced clinical neuropsychologist. Sixty-four cards were used for the test. All the definitions of the indices are described in the WCST manual (Heaton et al., 1993). Using a computerized version of the WCST, the patients were required to match response cards to 4 stimulus cards along 1 of 3 dimensions (color, form, or number) based on sign feedback (correct or wrong). The participants were not provided any information about the dimensions. After sorting a series of 10 cards in 1 category, the participant was asked to sort the cards again in a different category. The index of completed categories and perseverative errors were used to assess performance on the WCST.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences, version 23.0 (SPSS Inc., Chicago, IL, USA). Categorical variables are reported as numbers and percentages, and continuous variables are reported as the means ± SD unless specified otherwise. Categorical variables were assessed using chi-square tests, and continuous variables were assessed using *t* tests. Log transformation was performed to ensure that the MCN and CRP concentration data conformed to a normal distribution. Associations of MCN with sex, MetS diagnosis, and MetS components in univariate analyses were assessed using *t* tests, and correlation of MCN and age was assessed using Pearson correlation. A linear regression model was applied to examine the relationship between BD disease and MCN after adjusting for age, sex, MetS diagnosis, and MetS components. We used an ANCOVA adjusted for age, sex, and VPA concentration to compare the characteristics of BD patients. In addition, a receiver operating characteristic curve was constructed to distinguish VPA responders from nonresponders based on the MCN level. The odds ratios of the cutoff points for the MCN value and VPA responders were calculated accordingly (Parshall, 2013). The level of significance was set to .05 for 2-sided tests.

RESULTS

We recruited 66 healthy controls and 60 BD patients receiving VPA. The demographic data and clinical characteristics are shown in Table 1. Age and sex did not differ between the healthy controls and BD patients. BD patients had significantly higher HDRS and YMRS scores, MCN (Fig. 1), and CRP levels than the controls. The indices of MetS also significantly differed between the groups. Regarding cognitive tests, BD patients had worse performance on the WCST cognitive test than the controls. BD patients had more perseverative errors (14.14±11.07

Table 1. Demographic Characteristics and Measurements of the Healthy Controls and BD Patients

Demographic data	BD Mean ± SD	Control Mean ± SD	t/χ^2	Comparison 95% CI	P value
Age, y	37.9 ± 12.98	35.45 ± 10.99	1.144	(−1.78, 6.68)	.255
Sex, female, n (%)	36 (60%)	39 (59.1%)	0.011	—	1.000
HDRS score	4.03 ± 5.04	1.15 ± 1.96	4.155	(1.50, 4.26)	<.001*
YMRS score	1.68 ± 3.19	0.14 ± 0.58	3.686	(0.70, 2.37)	<.001*
Exercise, n (%)	37 (61.7%)	44 (66.7%)	0.342	—	.581
Smoker, n (%)	10 (16.9%)	9 (13.6%)	0.265	—	.627
Alcohol intake, n (%)	8 (13.3%)	14 (21.2%)	1.354	—	.348
MCN	1.97 ± 0.16	1.91 ± 0.17	2.108	(0.01, 0.12)	.037*
CRP, ng/mL	5.22 ± 0.56	4.93 ± 0.66	2.424	(0.05, 0.53)	.017*
VPA concentration, μg/mL	62.42 ± 26.51	—	—	—	—
Indices of MetS					
Waist circumference, cm	87.13 ± 13.42	80.08 ± 13.3	2.959	(2.33, 11.77)	.004*
TG, mg/dL	126.58 ± 81.36	94.68 ± 60.03	2.484	(6.45, 57.36)	.015*
HDL-C, mg/dL	53.83 ± 19.62	59.64 ± 13.92	−1.928	(−11.76, 0.15)	.056
Fasting glucose, mg/dL	93.09 ± 23.43	90.83 ± 10.5	0.706	(−4.06, 8.57)	.482
HbA1c, %	5.50 ± 0.97	5.39 ± 0.36	0.786	(−0.15, 0.36)	.433
SBP, mm Hg	120.18 ± 15.95	120.56 ± 25.05	−0.100	(−7.87, 7.11)	.921
DBP, mm Hg	76.15 ± 11.05	78.52 ± 15.94	−0.959	(−7.25, 2.52)	.340
MetS diagnosis, n (%)	16 (26.7%)	10 (15.2%)	2.545	—	.127
MetS components	1.62 ± 1.46	0.91 ± 1.17	3.008	—	.004*
Abdominal obesity, n (%)	36 (60.0%)	19 (28.8%)	12.448	—	.001*
Hypertriglyceridemia, n (%)	15 (25.0%)	9 (13.6%)	2.632	—	.117
Low HDL-C, n (%)	21 (35.0%)	9 (13.6%)	7.907	—	.006*
Hyperglycemia, n (%)	10 (16.7%)	10 (15.2%)	0.054	—	1.000
Hypertension, n (%)	15 (25.0%)	20 (30.3%)	0.441	—	.554
Cognitive function in the WCST					
Perseverative errors	14.14 ± 11.07	8.97 ± 4.84	3.305	(2.05, 8.28)	.001*
Categories completed	2.24 ± 1.69	3.38 ± 1.37	−4.117	(−1.68, −0.59)	<.001*

Abbreviations: BD, bipolar disorder; 95% CI, 95% confidence interval; CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HDRS, 17-item Hamilton Depression Rating Scale; MetS, metabolic syndrome; MCN, mitochondrial DNA copy number; SBP, systolic blood pressure; TG, triglyceride; VPA, valproate; WCST, Wisconsin Card Sorting Test; YMRS, Young Mania Rating Scale.
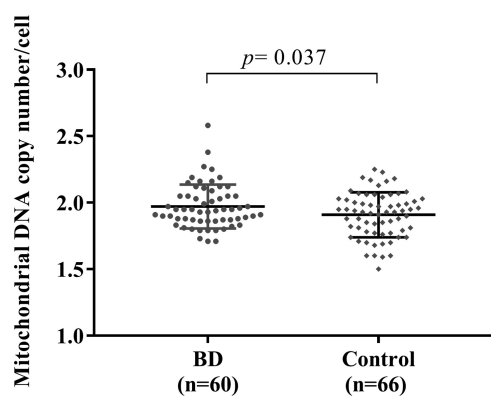*P < .05.

Figure 1. Comparison of the mitochondrial DNA copy number (MCN) between bipolar disorder (BD) patients receiving valproate (VPA) and controls. The MCN in BD patients treated with VPA was higher than that in controls.

vs 8.97 ± 4.84, t = 3.305, df = 124, P = .001) and fewer completed categories (2.24 ± 1.69 vs 3.38 ± 1.37, t = −4.117, df = 124, P < .001).

To investigate whether age, sex, MetS diagnosis, and MetS components were associated with the MCN, univariate analyses were performed in BD and controls, respectively. The MCN level was significantly associated with MetS diagnosis and hypertriglyceridemia in BD patients, whereas it was associated with abdominal obesity and hyperglycemia in the control (supplementary Tables 1 and 2). Age was not correlated with

MCN in BD patients and the controls (r = −0.088 and P = .502 vs r = 0.200 and P = .108, respectively). To investigate whether the fluoxetine treatment variable was associated with the MCN, univariate analysis was performed. We found that the MCN level did not significantly differ between fluoxetine users (n = 7) and nonusers (n = 53) (1.93 ± 0.06 vs 1.98 ± 0.17, t = −1.478, P = .152). Furthermore, to clarify the association between BD and MCN, regression models predicting MCN among all of the participants were performed. The regression model (Table 2) showed that BD itself exerted a significant effect on increased MCN even after adjusting for age, sex, and MetS. In addition, we identified a significant positive correlation between the VPA concentration and MCN (r = 0.264, P = .041), and the result was consistent even after adjustment for age and sex (partial correlation r = 0.333, P = .011) (Fig. 2).

Moreover, the MCN was significantly different between VPA responders and nonresponders (2.06 ± 0.18 vs 1.94 ± 0.14, t = 2.667, df = 53, P = .010). To investigate whether the MCN could be a differential biomarker for VPA responders and nonresponders, we further estimated the cutoff value of the MCN. Receiver operating characteristic curve analysis (Fig. 3) was performed in the 2 groups, and the results showed that an MCN of 2.05 distinguished VPA responders from nonresponders (P = .012), with an area under the curve of 0.705. The sensitivity and specificity were 0.529 and 0.816, respectively.

Furthermore, we stratified the BD patients into high (≥2.05) and low (<2.05) MCN groups using this cutoff point for the MCN

Table 2. Linear Regression Models Predicting the Mitochondrial Copy Number Among All Participants (n = 126)

	Model 1		Model 2	
	Beta	P value	Beta	P value
Participant group (patients vs controls)	0.194	.031*	0.188	.047*
Age	0.108	.249	0.119	.218
Sex	0.181	.048*	0.220	.022*
MetS (yes vs no)	−0.119	.209	N/A	N/A
Abdominal obesity (yes vs no)	N/A	N/A	0.098	.366
Hypertriglyceridemia (yes vs no)	N/A	N/A	−0.176	.100
Low HDL-C level (yes vs no)	N/A	N/A	−0.112	.271
Hyperglycemia (yes vs no)	N/A	N/A	0.096	.337
Hypertension (yes vs no)	N/A	N/A	−0.147	.129

Abbreviations: HDL, high-density lipoprotein; MetS, metabolic syndrome; N/A, not applicable. Model 1 was adjusted for demographic characteristics and metabolic syndrome. Model 2 was adjusted for demographic characteristics and 5 components of metabolic syndrome. Beta: standardized coefficients calculated using the linear regression model.
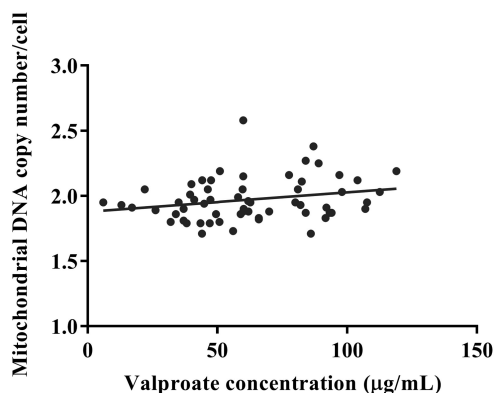*P < .05.

Figure 2. The correlation between the valproate (VPA) concentration and mitochondrial DNA copy number (MCN). There was a positive correlation between the MCN and VPA concentration in bipolar patients (correlation coefficients $r = 0.264$, $P = .041$; $r = 0.333$, $P = .011$ adjusted for age and sex).
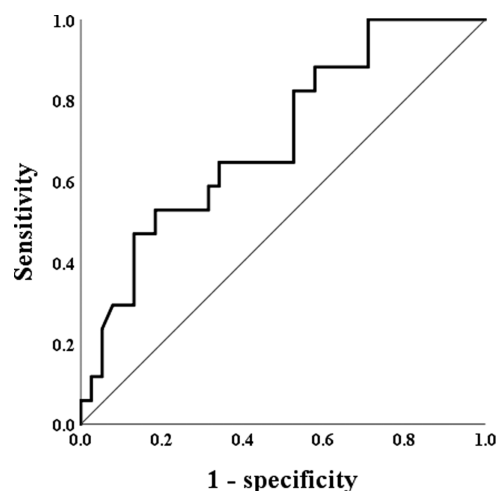

Figure 3. Receiver operating characteristic curves for mitochondrial DNA copy number (MCN) cutoff points to determine valproate (VPA) responders and nonresponders in bipolar disorder (BD) patients. A receiver operating characteristic curve analysis showed that an MCN of 2.05 distinguished VPA responders from non-responders, with an area under the curve (AUC) of 0.705 (95% confidence interval = 0.560 to 0.851, $P = .012$). The sensitivity and specificity were 0.529 and 0.816, respectively. An MCN ≥ 2.05 was associated with 5.39 higher odds of being a VPA responder ($P = .006$).

10 patients were VPA responders in the high-MCN group, and 9 patients were VPA responders in the low-MCN group. We found that BD patients with MCN ≥ 2.05 had 5.39 higher odds of being VPA responders ($z = 2.723$, $P = .006$, 95% CI = 1.60 to 18.16). In terms of cognitive function, BD patients in the high-MCN group exhibited better cognitive performance. The perseverative errors on the WCST were significantly lower in the high-MCN group than in the low-MCN group (9.77 ± 5.23 vs 15.91 ± 12.31, $t = −2.688$, df = 58, $P = .009$), while the number of categories completed did not significantly differ (2.77 ± 1.64 vs 2.02 ± 1.67, $t = 1.548$, df = 58, $P = .127$).

Discussion

To the best of our knowledge, this study is the first to investigate the association between the MCN and cognitive function in euthymic BD patients receiving VPA. BD patients showed significantly worse cognitive performance and a higher MCN than the controls, and the results remained consistent even after adjustment for important clinical confounders, such as age, sex, and MetS. The VPA concentration was positively correlated with the MCN in BD patients. In addition, we evaluated the stratification effect of MCN on the clinical features of BD patients. We found that the MCN significantly distinguished VPA responders from nonresponders. Our results also revealed a higher percentage of VPA responders and better cognitive performance of BD patients in the high-MCN group than those in the low-MCN group. Regarding metabolic effects, BD patients in the high-MCN group also had lower plasma CRP levels and fewer MetS components than in the low-MCN group. The strengths of the study are that we adjusted for potential confounding factors related to MCN, such as age, sex, MetS, and components of MetS, and considered their effects. In addition, to control the influence of mood status and medication, we recruited euthymic BD patients treated with VPA. Taken together, the inclusion of controls provides better insights into how MCN might serve as a disease biomarker in BD, and our study is the first to suggest positive associations among

value in BD patients to compare the clinical features (Table 3). Demographic characteristics, such as age, sex, and illness duration, did not differ between the groups (supplementary Table 3). The CRP level was significantly lower in the high-MCN group than in the low-MCN group (5.02 ± 0.55 vs 5.43 ± 0.50 ng/mL, $t = −3.006$, df = 58, $P = .004$), even after adjustment for age, sex, and VPA concentration ($P = .010$). In addition, the percentage of patients with a MetS diagnosis and the number of MetS components were significantly lower in the high-MCN group than in the low-MCN group (5.9 vs 34.9%, $\chi^2 = 5.240$, df = 58, $P = .025$; 0.94 ± 1.14 vs 1.88 ± 1.50, $t = −2.333$, df = 58, $P = .023$, respectively). In terms of the treatment response, more BD patients in the high-MCN group were VAP responders than in the low-MCN group (58.8 vs 20.9%, $\chi^2 = 6.785$, df = 58, $P = .022$). To evaluate the odds ratio of the cutoff point for the MCN value and VPA response,

Table 3. Characteristics and Measurements of BD Patients Receiving VPA Sub-grouped by the Cut-off Point of MCN

	MCN ≥ 2.05(n = 17)	MCN < 2.05(n = 43)		95% CI	Comparison	
	Mean ± SD	Mean ± SD	t/χ²	(Lower, Upper)	P value	P1 value
mtDNA copy no.	2.18 ± 0.13	1.89 ± 0.08	10.226	(0.23, 0.35)	<.001*	<.001*
CRP, ng/mL	5.02 ± 0.55	5.43 ± 0.5	−3.006	(−0.69, −0.14)	.004*	.010*
MetS diagnosis, n (%)	1 (5.9)	15 (34.9)	5.240	—	.025*	—
MetS components, n	0.94 ± 1.14	1.88 ± 1.50	−2.333	(−1.75, −0.13)	.023*	—
Alda scale (A-B total)	4.31 ± 3.09	2.74 ± 2.05	0.677	(−1.18, 2.32)	.506	.392
Responder, n (%)	10 (58.8)	9 (20.9)	6.785	—	.022*	—
Cognitive function WCST						
Perseverative errors	9.77 ± 5.23	15.91 ± 12.31	−2.688	(−10.71, −1.57)	.009*	.020*
Completed categories	2.77 ± 1.64	2.02 ± 1.67	1.548	(−0.22, 1.7)	.127	.175

Abbreviations: 95% CI, 95% confidence interval; CRP, C-reactive protein; MCN, mitochondrial DNA copy number; MetS, metabolic syndrome; VPA, valproate; WCST, Wisconsin card sorting test.

[1]The *P* value was obtained using ANCOVA after adjustment for age, sex and VPA concentration. In ANCOVA analysis, age and VPA concentration were continuous variables, and sex and groups (using the cutoff point of 2.05 for the MCN value: MCN ≥ 2.05 or MCN < 2.05) were categorical variables. Clinical measurements, including mtDNA copy number, CRP, Alda scale, and cognitive function, were dependent variables (outcome variables).

*$P < .05$.

MCN, the VPA treatment response, and cognitive function in euthymic BD patients treated with VPA.

CRP, a biomarker of systemic inflammation and the risk of inflammatory disease, was negatively correlated with the MCN in our study. Previous studies have reported that BD is associated with severe inflammatory dysregulation and that CRP potentially represents a marker of mood state (Bai et al., 2015). This finding is consistent with our study; BD patients had higher CRP levels than the controls. The association between MetS and inflammation has been emphasized, and chronic inflammation is an integral process in MetS (Lopez-Candales et al., 2017). This result might partially explain why several MetS components, such as abdominal obesity and low HDL-C levels, were significantly increased in our BD patients.

MCN alterations are considered indicators of mitochondrial dysfunction; however, only a few studies have examined whether changes in the MCN occur in BD patients, and the results have been inconclusive (Chang et al., 2014; de Sousa et al., 2014; Fries et al., 2017; Rollins et al., 2018; Wang et al., 2018). These inconsistencies in previous studies might be attributed to differences in age, clinical features, tissue samples, medication use, or ethnicity. Factors such as age (Hartmann et al., 2011), smoking (Wu et al., 2019), alcohol use, and MetS (Fazzini et al., 2021) affect the MCN to a certain degree. For example, aging was reported to be associated with the downregulation of mtDNA-associated genes and a decrease in the MCN (Hartmann et al., 2011). However, previous studies of BD have rarely simultaneously elucidated these factors. These studies investigated patients with euthymic (Chang et al., 2014), depressive (de Sousa et al., 2014), three-mood (Wang et al., 2018), and nonclassified states (Fries et al., 2017; Yamaki et al., 2018). BD has been proposed to be caused by aberrant mitochondrial bioenergetics, and mitochondrial respiration and ATP production have been reported to be increased in the manic state and decreased in the euthymic/depressive state (Bai et al., 2015; Morris et al., 2017). Therefore, further investigations are needed to specify each mood state and to evaluate mitochondrial function.

Regarding the effects of VPA on the MCN, possible underlying mechanisms have been suggested in previous studies (Bachmann et al., 2009; Sitarz et al., 2014; Beltrán-Sarmiento et al., 2018). In a cell line study, VPA administration increased the MCN by regulating mitochondrial biogenesis in BD patients (Sitarz et al., 2014). In cell and animal studies, Bcl-2 was the target of long-term VPA use, providing protection against methamphetamine-induced neurotoxicity and mitochondrial damage in the brain (Bachmann et al., 2009). VPA could enhance mitochondrial function and protect against mitochondrially mediated toxicity (Bachmann et al., 2009). This result is consistent with our study showing that the VPA concentration is positively correlated with the MCN ($r = 0.264$, $P = .041$) in BD patients. In addition, 12 months of VPA treatment in epileptic children showed a significant antioxidant effect of decreasing seizure activity by increasing the activities of plasmatic antioxidant enzymes (such as glutathione reductase, superoxide dismutase, and catalase) and decreasing oxidant markers (such as hydrogen peroxide) (Beltrán-Sarmiento et al., 2018). Although the mechanism underlying the association between MCN and VPA is unclear, the anti-inflammatory and antioxidant effects of VPA in the brain might play an important role (Ximenes et al., 2013).

Considering BD patients alone, after adjusting for age, sex, and VPA use, the high-MCN group had significantly lower CRP levels and fewer MetS components and perseverative errors than the low-MCN group (Table 3). In a population-based longitudinal study, the MCN level was negatively correlated with CRP levels in older adults (Wu et al., 2017). The leukocyte MCN level was lower among postmenopausal women with MetS than among those without MetS (Kim et al., 2012). However, in contrast to our findings, increased oxidative stress or inflammation led to enhanced mitochondrial biogenesis and increased MCN (Malik and Czajka, 2013; Morris et al., 2017). Obesity-related MetS is possibly associated with deregulated lipid and carbohydrate metabolism. An increase in either of these substrates will increase the demand on the mitochondria and the utilization of the electron transport chain (Rudich et al., 2007).

The broad consensus is that even euthymic BD patients present cognitive deficits compared with controls, such as deficits in attention, verbal and nonverbal memory, and executive function (Zarate et al., 2000; Lima et al., 2018). Regarding the association between cognitive performance and the MCN, BD patients who had a higher MCN performed better regarding perseverative errors on the WCST. In a general population, elderly Korean women with higher MCNs performed better on cognitive tests after adjusting for age and education level (Lee et al., 2010). In addition, the MCN in peripheral blood cells is associated with cognition in patients with mild cognitive impairment

or dementia (Lee et al., 2010; Delbarba et al., 2016), and a reduction in the mtDNA amount is a feature of the early pathogenesis occurring in patients with Alzheimer's disease. Until now, it has not been totally clear how changes in mitochondrial dysfunction could cause cognitive impairment in BD. In an animal study, antioxidant treatment resolved mitochondrial integrity, cognitive behavior, and cortical connectivity (Fernandez et al., 2019). mtDNA mutations or polymorphisms result in altered mitochondrial calcium regulation (Kato, 2007), and the therapeutic mechanism of lithium is involved in intracellular calcium signaling (Vawter et al., 2000). In addition, cognitive impairment in BD patients might reflect the disease process, and vice versa. Therefore, this fact implicates mitochondria-related oxidative stress as a therapeutic target in BD or in cognitive impairment due to BD. Moreover, BD patients with impaired insulin sensitivity perform worse on neurocognitive tests (Chang et al., 2021); although the underlying mechanism remains unclear, insulin resistance might lead to neuroprogression through blood-brain barrier leakage and proinflammatory cytokine activation in the brain (Craft and Watson, 2004). Further studies are necessary to investigate the mechanism underlying the association between the MCN and cognitive function and to provide possible therapeutic targets in BD.

Our study has some limitations, including those inherent to a case-control, observational analysis in which no causal relationship could be deduced. The mean HDRS and YMRS scores of our patients with BD were within the normal range, indicating that our patients were clinically euthymic; therefore, the results of our study should not be generalized to other disease states. Furthermore, the MCN was measured in peripheral leukocytes instead of brain tissues, but Feng and colleagues reported a positive association between the MCN in peripheral leukocytes and brain tissues (Feng et al., 2013). Using peripheral blood as a proxy for brain tissues to measure MCN levels is more feasible and less invasive.

In summary, euthymic BD patients receiving VPA had significantly higher MCN levels than controls, even after adjustment for confounding factors, and the VPA concentration was positively correlated with the MCN. In addition, the MCN distinguished VPA responders from nonresponders. Furthermore, we identified a positive correlation among the leukocyte MCN treatment response and cognitive function in euthymic BD patients. More studies are needed to assess longitudinal changes in the MCN and cognition in BD patients and to explore the mechanisms underlying the association between the MCN and cognition.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

Acknowledgments

Interest Statement

None.

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