Serum Brain-Derived Neurotrophic Factor Levels Are Associated with Dyssomnia in Females, but not Males, among Japanese Workers

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Study Objectives: Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors that promote the growth and survival of neurons. Recent evidence suggests that BDNF is a sleep regulatory substance that contributes to sleep behavior. However, no studies have examined the association between the serum BDNF levels and dyssomnia. The present study was conducted to clarify the association between the serum BDNF levels and dyssomnia.

Methods: A total of 344 workers (age: 40.1 ± 10.5 years, male: 204, female: 140) were included in the study. The serum BDNF levels were categorized into tertiles according to sex.

Results: The prevalence of dyssomnia was 35.1% in males and 30.0% in females. In the females, the BDNF levels were found to be negatively associated with dyssomnia after adjust-

ing for age, body mass index, hypertension, dyslipidemia, hyperglycemia, depression, smoking, alcohol intake, and regular exercise. Compared with the females in the high BDNF group, the multivariate odds ratio (95% CI) of dyssomnia was 2.08 (0.62-6.98) in females in the moderate BDNF group and 8.41 (2.05-27.14) in females in the low BDNF group. No such relationships were found in the males.

Conclusions: The serum BDNF levels are associated with dyssomnia in Japanese female, but not male, workers.

Keywords: Serum brain-derived neurotrophic factor, dyssomnia, sex, Japanese worker

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Dyssomnia is one of the most common health problems in the Japanese population. Recent surveys by the Japanese Ministry of Health Labor and Welfare have demonstrated that 21.1% of Japanese adults suffer from dyssomnia.¹ Many studies have suggested that dyssomnia is not only linked to mental disorders, including depression,² but also to endocrine disorders (e.g., obesity, diabetes mellitus) and cardiovascular disorders (e.g., hypertension, heart disease).³⁻⁶

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors. In addition to its neurotrophic and synaptotrophic actions, including the promotion of growth and survival in neurons,^{7,8} BDNF plays a role in learning and memory,⁹ the regulation of food intake,¹⁰ glucose and lipid metabolism and energy homeostasis.^{11,12} BDNF is present in the nervous system and peripheral tissues and is also found in blood.¹³⁻¹⁵ Accumulating evidence shows the serum BDNF levels to be associated with psychiatric and metabolic disorders, including depression,^{16,17} Alzheimer disease,¹⁸ and diabetes mellitus.¹⁹⁻²¹ However, no studies have examined the association between the serum BDNF levels and dyssomnia.

Recent evidence interestingly suggests that BDNF is a sleep regulatory substance.²²⁻²⁴ Faraguna et al.²⁵ showed the degree of BDNF expression during wakefulness to be causally linked to the extent of slow wave activity in the subsequent rest period. Moreover, Martinowich et al.²⁶ demonstrated that a genetic

BRIEF SUMMARY

Current Knowledge/Study Rationale: BDNF is suggested to contribute to sleep behavior. However, there is no study on the association between serum BDNF and dyssomnia.

Study Impact: The serum BDNF levels were inversely associated with dyssomnia in females, but not in males. However, further studies are needed to answer whether or not the sex differences in BDNF are related to sex differences in dyssomnia.

manipulation that leads to disruption of the activity-dependent BDNF expression results in impairments in sleep regulation. Based on this evidence showing a biological links between BDNF and sleep behavior, we hypothesized that serum BDNF levels may be associated with dyssomnia. In the present study, we examined this association in Japanese workers.

METHODS

In Japan, the Industrial Safety and Health Law requires all employers to provide annual health check-ups for their employees. The annual health check-up consists of an interview regarding lifestyle; measurement of weight, height, and blood pressure; physical examination; electrocardiogram examinations; chest x-ray; urinalysis; and blood tests. Blood samples

from the study subjects were obtained from 08:00 to 10:00 after overnight fasting. In addition to performing these routine health check-up examinations, the serum BDNF levels were measured, and sleep quality and depressive symptoms were assessed with interviews by trained nurses.

Subjects

The subjects of this study were employees of the Creative Research Community (CRC) Company (Fukuoka, Japan), which provides services such as health check-up support, genetic testing, and clinical testing. A total of 400 workers, 20 years of age or older underwent an annual health check-up at their company in 2009. Among these workers, 30 did not agree to participate and 26 who did not complete the questionnaires or biochemical tests were excluded from the study. Ultimately, a total of 344 participants (204 males and 140 females) were included in this study. Two hundred eighty-two study subjects (82%) were day workers. All participants received oral and written information about the experimental procedures before giving their written informed consent. This study was approved by the Ethics Committee of St. Mary's College and monitored by the institutional review committee.

Serum BDNF Levels

After the blood was centrifuged $2000 \times g$ for 10 min at 4°C , the serum was stored at -80°C until the analyses were performed. The serum BDNF concentrations were measured using an enzyme-linked immunoassay (ELISA) kit (Promega, Madison, WI) following the manufacturer's instructions. Briefly, 96 well plates were coated with anti-BDNF monoclonal antibodies and incubated at 4°C for 16 h. The plates were then incubated in a blocking buffer for 1 h. All of the incubation stages were conducted at room temperature. The serum samples were diluted to 1:200, and the plasma samples were diluted to 1:19 in Block & Sample 1 $\times$ Buffer. After adding the samples and the BDNF standard, the plates were incubated with shaking for 2 h, then washed in washing buffer. The plates were then incubated with anti-human BDNF polyclonal antibodies for 2 h. After being washed, the plates were incubated with anti-IgY HRP conjugate with shaking for 1 h and washed. Next, TMB One solution was added for 10 min, and the reaction was stopped with 1 M HCl. The absorbance at 450 nm was measured within 30 min after stopping the reaction.

Dyssomnia

Sleep quality was assessed according to the Pittsburgh Sleep Quality Index (PSQI). The PSQI is used worldwide as a tool for the assessment of sleep quality. The scores were obtained according to the PSQI-scoring method (0-1-2-3-4). The cutoff for the total score of the PSQI is 5.5 points, and scores above the cutoff are considered to indicate dyssomnia.²⁷

Other Variables

BMI was calculated as the weight in kilograms divided by the height in meters squared. Obesity was defined as $\text{BMI} \geq 25 \text{ kg/m}^2$.²⁸ Antihypertensive medication use, antihyperlipidemic drug use, oral hypoglycemic intake or insulin administration, and current lifestyle factors, including smoking, alcohol intake, and regular exercise were determined by interviews with trained nurses. Hypertension was defined as blood pressure $\geq 140/90$

mm Hg and/or current treatment with antihypertensive medications. Dyslipidemia was defined as LDL-cholesterol $\geq 140 \text{ mg/dL}$, triglyceride $\geq 150 \text{ mg/dL}$, HDL-cholesterol $< 40 \text{ mg/dL}$ and/or current treatment with antihyperlipidemic drugs. Hyperglycemia was defined as fasting plasma glucose concentrations $\geq 110 \text{ mg/dL}$ and/or the use of antidiabetic medications.²⁹ Depressive symptoms were evaluated using the Japanese version of the Center for Epidemiological Studies Depression Scale (CES-D). Depression was defined as a CES-D score ≥ 16 points.³⁰

Statistical Analyses

The serum BDNF levels were categorized into tertiles according to sex (males: < 10.91 , 10.92 to 13.81 , $> 13.82 \text{ ng/mL}$; females: < 9.32 , 9.33 to 12.12 , $> 12.13 \text{ ng/mL}$). The crude mean values and the frequencies of the variables were compared between the groups using the χ^2 test and one-way analysis of variance as appropriate. Dunnett test was employed for all post hoc tests. The odds ratios (OR) and 95% confidence intervals (95% CI) of dyssomnia for each BDNF tertile group were calculated by taking the highest tertile as the referent using the logistic regression models. A p-value less than 0.05 was considered to be statistically significant. All statistical analyses were performed using the SPSS software program (Statistical Package for Social Sciences, version 18.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of Participants

The prevalence of dyssomnia was 35.1% in the males and 30.0% in the females. The serum BDNF levels were significantly higher in the males ($12.72 \pm 4.08 \text{ ng/mL}$) than in the females ($11.13 \pm 3.28 \text{ ng/mL}$, $p < 0.001$).

Table 1 presents the characteristics of the male participants by tertile of the serum BDNF levels. There were no significant differences in PSQI scores or prevalence of dyssomnia among the 3 groups of males. The frequency of regular exercise was significantly higher in the low BDNF group than in the high BDNF group. There were no significant differences in any of the other parameters among the 3 groups of males. **Table 2** presents the characteristics of the female participants by tertile of the serum BDNF levels. The mean PSQI scores in the low and moderate BDNF groups were significantly higher than that in the high BDNF group among females ($p < 0.01$, $p = 0.02$, respectively). Additionally, there were significant differences in the prevalence of dyssomnia among the 3 groups ($p < 0.001$). The prevalence of dyssomnia in the low BDNF group was significantly higher than that in the high BDNF group ($p < 0.01$). There were no significant differences in any of the other parameters among the 3 groups of females.

Association between Serum BDNF Levels and Dyssomnia by Sex

Table 3 shows the association between the serum BDNF levels and dyssomnia. Compared with the females in the high BDNF group, the age-adjusted OR (95% CI) of dyssomnia was 2.04 (0.68-6.09) in females in the moderate BDNF group and 8.18 (2.89-23.13) in females in the low BDNF group. These associations remained statistically significant even after ad-

Table 1—Characteristics of participants by tertile of serum BDNF levels in men (n = 204)

	Serum BDNF level ng/mL			p value
	High (≥ 13.82)	Middle (10.92-13.81)	Low (≤ 10.91)	
Number of subjects	69	69	69	
Age (years)	43.1 $\pm$ 10.0	41.8 $\pm$ 10.9	42.6 $\pm$ 11.6	0.78
BMI (kg/m ²)	24.1 $\pm$ 4.0	23.0 $\pm$ 2.7	24.0 $\pm$ 3.2	0.11
Obesity	26 (37.7%)	16 (23.2%)	22 (33.2%)	0.17
SBP (mm Hg)	135.2 $\pm$ 18.8	133.6 $\pm$ 16.9	136.4 $\pm$ 16.4	0.65
DBP (mm Hg)	84.0 $\pm$ 16.6	82.8 $\pm$ 12.3	81.9 $\pm$ 12.4	0.69
Hypertension	29 (42.0%)	30 (43.5%)	30 (45.5%)	0.92
TC (mg/dL)	218.7 $\pm$ 33.4	216.7 $\pm$ 31.9	207.2 $\pm$ 33.4	0.10
HDL-C (mg/dL)	59.2 $\pm$ 14.2	61.7 $\pm$ 15.3	60.4 $\pm$ 11.4	0.56
LDL-C (mg/dL)	126.5 $\pm$ 30.2	126.5 $\pm$ 28.9	120.7 $\pm$ 27.4	0.41
TG (mg/dL)	147.2 $\pm$ 110.6	123.4 $\pm$ 67.6	11.2 $\pm$ 62.7	0.05
Dyslipidemia	33 (47.8%)	34 (49.3%)	26 (39.4%)	0.46
FBG (mg/dL)	102.9 $\pm$ 26.3	98.1 $\pm$ 15.5	101.4 $\pm$ 19.6	0.40
HbA1C (%)	5.0 $\pm$ 0.8	4.8 $\pm$ 0.5	4.9 $\pm$ 0.7	0.32
Hyperglycemia	11 (15.9%)	6 (8.7%)	16 (24.2%)	0.05
PSQIG score	5.4 $\pm$ 2.4	4.8 $\pm$ 2.3	5.0 $\pm$ 2.4	0.32
Dyssomnia	29 (43.3%)	19 (27.5%)	23 (34.8%)	0.73
CESD score	12.0 $\pm$ 6.5	10.1 $\pm$ 6.3	11.1 $\pm$ 7.1	0.25
Depression	16 (23.5%)	11 (15.9%)	15 (22.7%)	0.49
Smoking	27 (39.7%)	30 (43.5%)	28 (42.4%)	0.90
Alcohol drinking	41 (60.2%)	43 (62.3%)	50 (75.7%)	0.12
Regular exercise	25 (36.8%)	22 (32.4%)	37 (55.4%)	0.02
Service form				
Day worker	52 (77.6%)	55 (80.9%)	55 (84.6%)	0.59
Two shift worker (the day and night)	15 (22.4%)	13 (19.1%)	10 (15.4%)	

Data presented are number (row percentages) or mean value $\pm$ standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, Fasting blood glucose; BDNF, brain-derived neurotrophic factor.

justing for age, BMI, dyslipidemia, diabetes mellitus, depression, regular exercise, and so on (moderate: OR 1.73, 95% CI 0.51-5.90; low: OR 8.77, 95% CI 2.71-28.38). In contrast, compared with the males in the high BDNF group, the males in the low BDNF group showed a decreased age-adjusted OR for dyssomnia (OR 0.47, 95% CI 0.23-0.97). However, this association disappeared after adjusting for confounding factors (OR 0.58, 95% CI 0.23-1.28).

Association between Serum BDNF Levels and Patterns of Dyssomnia

Table 4 shows the association between serum BDNF levels and the scores of 7 components of PSQIG. Serum BDNF levels in females were significantly inversely correlated with the score of sleep duration ($r = -0.191$, $p < 0.05$), sleep disturbance ($r = -0.179$, $p < 0.05$), daytime dysfunction ($r = -0.270$, $p < 0.01$), and global ($r = -0.295$, $p < 0.001$). No such correlations were found in males.

DISCUSSION

We found the serum BDNF levels to be negatively associated with dyssomnia in females. Because the serum BDNF levels have been reported to change according to age,³¹ body weight, BMI,³² depression,^{16,17} metabolic disorders, including diabetes

mellitus,^{19,21} and regular exercise,^{33,34} we adjusted the model for these potential confounding factors. The association between the serum BDNF levels and dyssomnia remained statistically significant even after adjusting for these confounders. Among females, the multivariable-adjusted odds ratio of dyssomnia in the low BDNF group was eight times higher than that in the high BDNF group. However, these associations were not observed in the male subjects. To our knowledge, this is the first study to demonstrate an association between the serum BDNF levels and dyssomnia.

There are many kinds of dyssomnia, and it is an important issue to determine what types of dyssomnia correlate with the serum BDNF levels. Low level of serum BDNF is considered to associate with intrinsic circadian rhythm disorder, since the majority of study subjects were day workers. Therefore, the association between serum BDNF levels and extrinsic circadian rhythm disorder should be investigated in the future. The results of this study showed that serum BDNF levels were negatively associated with sleep duration, sleep disturbance, and daytime dysfunction in the female, although the degrees of these associations seem to be weak. Thus, further large-scale studies are recommended to confirm how serum the BDNF level correlates with the occurrence of dyssomnia.

An association between the serum BDNF levels and dyssomnia is biologically plausible. Since BDNF can cross the blood-

Table 2—Characteristics of participants by tertile of serum BDNF levels in women (n = 140)

	Serum BDNF level ng/mL			p value
	High (≥ 12.13)	Middle (9.33-12.12)	Low (≤ 9.32)	
Number of subjects	47	47	46	
Age (years)	37.1 $\pm$ 8.8	36.0 $\pm$ 8.5	36.6 $\pm$ 9.6	0.82
BMI (kg/m ²)	20.6 $\pm$ 2.8	20.9 $\pm$ 3.8	21.2 $\pm$ 2.5	0.69
Obesity	2 (4.3%)	4 (8.5%)	4 (8.7%)	0.64
SBP (mm Hg)	122.3 $\pm$ 15.5	118.1 $\pm$ 11.4	118.1 $\pm$ 12.8	0.21
DBP (mm Hg)	75.3 $\pm$ 11.1	74.8 $\pm$ 8.6	74.0 $\pm$ 9.1	0.74
Hypertension	7 (14.9%)	4 (8.5%)	6 (13.0%)	0.62
TC (mg/dL)	218.7 $\pm$ 33.8	206.2 $\pm$ 39.5	203.4 $\pm$ 26.0	0.49
HDL-C (mg/dL)	75.4 $\pm$ 15.5	77.0 $\pm$ 14.8	75.1 $\pm$ 13.6	0.37
LDL-C (mg/dL)	114.3 $\pm$ 29.2	111.9 $\pm$ 26.8	110.3 $\pm$ 22.6	0.76
TG (mg/dL)	77.1 $\pm$ 50.0	64.0 $\pm$ 27.0	75.3 $\pm$ 44.9	0.26
Dyslipidemia	10 (21.3%)	6 (13.0%)	11 (23.4%)	0.41
FBG (mg/dL)	93.1 $\pm$ 15.1	91.4 $\pm$ 14.4	90.2 $\pm$ 7.2	0.54
HbA1C (%)	4.7 $\pm$ 0.3	4.7 $\pm$ 0.6	4.6 $\pm$ 0.2	0.66
Hyperglycemia	3 (6.4%)	4 (8.5%)	1 (2.2%)	0.41
PSQIG score	4.3 $\pm$ 2.2	3.9 $\pm$ 2.2	5.6 $\pm$ 2.4	< 0.01
Dyssomnia	6 (12.8%)	11 (23.4%)	25 (54.3%)	< 0.001
CESD score	9.7 $\pm$ 7.7	9.6 $\pm$ 7.2	11.5 $\pm$ 8.2	0.41
Depression	6 (12.8%)	10 (21.3%)	13 (28.3%)	0.18
Smoking	8 (17.0%)	6 (13.0%)	4 (8.7%)	0.49
Alcohol drinking	21 (44.7%)	22 (46.8%)	28 (60.9%)	0.24
Regular exercise	16 (34.0%)	15 (32.6%)	16 (36.4%)	0.93
Service form				
Day worker	39 (83.0%)	42 (91.3%)	39 (84.8%)	0.47
Two shift worker (the day and night)	8 (17.0%)	4 (8.7%)	7 (15.2%)	

Data presented are number (row percentages) or mean value $\pm$ standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, Fasting blood glucose; BDNF, brain-derived neurotrophic factor.

Table 3—Distribution of Japanese workers with and without dyssomnia according to serum BDNF levels, with corresponding OR and 95%CI

Serum BDNF level	Number of participants	Dyssomnia case	Age- and Sex-adjusted OR (95%CI)	p value	Multivariable-adjusted OR (95%CI)	p value
Male						
High	69	29	1		1	
Middle	69	19	0.67 (0.33-1.36)	0.32	0.51 (0.23-1.12)	0.09
Low	66	23	0.47 (0.23-0.97)	0.05	0.58 (0.23-1.28)	0.18
Female						
High	47	6	1		1	
Middle	47	11	2.04 (0.68-6.09)	0.02	1.73 (0.51-5.90)	0.38
Low	46	25	8.18 (2.89-23.13)	< 0.001	8.77 (2.71-28.38)	< 0.001

Multivariable-adjusted OR: The odds ratios (OR) and 95% confidence intervals (95% CI) of dyssomnia for each BDNF tertile group were calculated by taking the highest tertile as the referent using the logistic regression models, adjusted for age, BMI, dyslipidemia, hyperglycemia, depression, smoking, alcohol drinking, and regular exercise, service form.

brain barrier in both directions³⁵ and brain tissue is the main contributor to circulating BDNF,³⁶ low serum BDNF levels may reflect decreased BDNF levels in the brain. An experimental animal study suggested that BDNF in the brain contributes to the regulation of sleep behavior and promotes NREM sleep.²² Hence, decreased levels of brain BDNF may be related to poor control of sleep behavior.

On the other hand, decreased serum BDNF levels may be caused by dyssomnia. Recent studies in humans suggest that acute or chronic sleep deprivation affects the hypothalamic-pituitary-adrenal (HPA) system and changes the secretion of cortisol.^{37,38} Vgontzas et al.³⁸ demonstrated that 24-h mean cortisol secretion in chronic insomniacs is higher than that in normal controls.²⁴ Intriguingly, glucocorticoids have been reported

Table 4—The association between serum BDNF level and PSQIG subscores (n = 344)

	All n = 344	Male n = 204	Female n = 140
Sleep quality	0.038	0.111	-0.138
Sleep latency	-0.043	-0.014	-0.106
Sleep duration	-0.019	0.072	-0.191*
Sleep efficiency	0.000	0.005	-0.049
Sleep disturbance	-0.044	0.063	-0.179*
Hypnotic medication	-0.031	-0.030	-0.089
Daytime dysfunction	0.001	0.125	-0.270**
Global	-0.044	0.079	-0.295***

*p < 0.05, **p < 0.01, ***p < 0.001.

to suppress the BDNF expression in the hippocampus.³⁹ Additionally, a human study demonstrated a negative association between the cortisol levels and the BDNF levels in the blood.⁴⁰ Therefore, dyssomnia may reduce the BDNF levels in the brain and the blood by altering the activity of the HPA system to increase the secretion of cortisol.

Many epidemiological studies have suggested gender differences are associated with dyssomnia.⁵ However, it remains unclear as to whether or not the sex differences in BDNF observed in the results of the present study are related to sex differences in dyssomnia.

Several limitations should be noted. First, the cross-sectional design of the study limits the interpretation of causality between the serum BDNF levels and dyssomnia. Second, since the sample size was relatively small and the subjects were workers, the subjects may not be representative of the entire Japanese population. Third, we obtained only one serum sample at morning for measurement of serum BDNF level from study subjects. Therefore, we could not investigate the association between the circadian change of serum BDNF levels and dyssomnia in this study. This association should be investigated in future study, since the serum level of BDNF has been demonstrated to be influenced by several conditions, such as meal intake and level of activity.¹⁰⁻¹² Finally, we did not measure any other hormones or mediators which were reported to correlate with dyssomnia. Thus, further study is needed to clarify how serum BDNF levels associate with those hormones and mediators, such as cortisol, growth hormone, sex hormones, and melatonin.

CONCLUSION

In this study, serum BDNF levels were associated with dyssomnia in females but not in males. The association observed in the female subjects remained statistically significant even after adjusting for possible confounding factors, including age, BMI, hypertension, dyslipidemia, diabetes mellitus, depression, smoking, drinking, and regular exercise. Our results support the emerging concept that BDNF is a sleep regulatory substance and may contribute to improving understanding of the pathogenic mechanisms of dyssomnia. Further longitudinal studies of large populations are required to elucidate the precise relationship between the serum BDNF levels and dyssomnia.

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DISCLOSURE STATEMENT

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