



Association of Brain-Derived Neurotrophic Factor Gene Val66Met Polymorphism with Primary Dysmenorrhea

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Abstract

Primary dysmenorrhea (PDM), the most prevalent menstrual cycle-related problem in women of reproductive age, is associated with negative moods. Whether the menstrual pain and negative moods have a genetic basis remains unknown. Brain-derived neurotrophic factor (BDNF) plays a key role in the production of central sensitization and contributes to chronic pain conditions. BDNF has also been implicated in stress-related mood disorders. We screened and genotyped the *BDNF* Val66Met polymorphism (rs6265) in 99 Taiwanese (Asian) PDMs (20–30 years old) and 101 age-matched healthy female controls. We found that there was a significantly higher frequency of the Met allele of the *BDNF* Val66Met polymorphism in the PDM group. Furthermore, *BDNF* Met/Met homozygosity had a significantly stronger association with PDM compared with Val carrier status. Subsequent behavioral/hormonal assessments of sub-groups (PDMs = 78, controls = 81; eligible for longitudinal multimodal neuroimaging battery studies) revealed that the *BDNF* Met/Met homozygous PDMs exhibited a higher menstrual pain score (sensory dimension) and a more anxious mood than the Val carrier PDMs during the menstrual phase. Although preliminary, our study suggests that the *BDNF* Val66Met polymorphism is associated with PDM in Taiwanese (Asian) people, and *BDNF* Met/Met homozygosity may be associated with an increased risk of PDM. Our data also suggest the *BDNF* Val66Met polymorphism as a possible regulator of menstrual pain and pain-related emotions in PDM. Absence of thermal hypersensitivity may connote an ethnic attribution. The presentation of our findings calls for further genetic and neuroscientific investigations of PDM.

Citation: Lee L-C, Tu C-H, Chen L-F, Shen H-D, Chao H-T, et al. (2014) Association of Brain-Derived Neurotrophic Factor Gene Val66Met Polymorphism with Primary Dysmenorrhea. PLoS ONE 9(11): e112766. doi:10.1371/journal.pone.0112766

Editor: Reiner A. Veitia, Institut Jacques Monod, France

Received: February 11, 2014; **Accepted:** October 15, 2014; **Published:** November 10, 2014

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Funding: This research was supported by the Taipei Veterans General Hospital (V100D-001, V100D-001-1, V100D-001-2, V100E3-002, V101E3-002, and V102E3-002), Cheng-Hsin General Hospital (101-33), National Science Council (NSC 100-2314-B-010-006-MY3, NSC 100-2629-B-010-001, NSC 101-2629-B-010-001, NSC 101-2911-I-009-101, and NSC 102-2629-B-010-001), Yen Tjing Ling Medical Foundation (CI-100-5), and The Aim for the Top University Plan of the Ministry of Education for National Yang-Ming University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Primary dysmenorrhea (PDM), defined as pain associated with menstruation in the absence of organic disease, affects 90% of adolescent girls and more than 50% of menstruating women worldwide [1–4]. The related suffering is described as severe and distressing by 10–20% of the subjects with this condition [2–4]. Despite the unclear pathogenesis of PDM, myometrial hypercontractility and vasoconstriction are the two most widely accepted mechanisms [5,6]. In addition, prostaglandins, chemokines, cytokines, growth factors, oxytocin, leukotrienes, and vasopressin, either within the uterus itself or in menstrual fluid or peripheral blood, are potential contributors to altered uterine physiology [7–10]. Furthermore, PDM can be associated with hampered daily function [11], decreased self-rated overall health [12], and negative moods (e.g., depressive and anxious symptoms) [13,14]. However, to our knowledge there is no report in the literature

discussing possible genetic causes of PDM in terms of the pain experience and associated emotions.

Brain-derived neurotrophic factor (BDNF), a member of neurotrophin family, supports neuronal growth, differentiation and survival in the peripheral and central nerve systems, and induces long-lasting changes in synaptic plasticity [15,16]. BDNF is considered a pain modulator because of its involvement in activity-dependent synaptic plasticity within the pain circuitry. With a pro-nociceptive role, BDNF generates the hyperalgesic responses in inflammatory models of pain [17]. BDNF also plays a key role in the production of central sensitization and contributes to chronic pain conditions [17,18]. Moreover, BDNF is potentially involved in stress-related mood disorders [19], e.g., major depression. Stress [19] or chronic pain [20] decreases the expression of BDNF in brain structures that control mood and contributes to depression.

Natural fluctuations of circulating sex hormones across the menstrual cycle are supposed to be associated with mood and

cognition alterations in women. BDNF serves as a mediator of sex hormone effects in the hippocampus, and interaction between estrogen and hippocampal BDNF may underlie the menstrual cycle-related problems [21,22]. Estrogen regulates *BDNF* gene expression in the brain of animal models, directly by interaction between estrogen receptor and *BDNF* [23]; and indirectly by GABAergic interneurons modulation [24]. During the estrus phase with low estrogen level, *BDNF* Met/Met homozygous mice exhibited increased anxiety-related behaviors because of decreased BDNF availability and affected BDNF signaling [22]. *BDNF* genotype may therefore alter risk for the development, and could play a role in the diagnosis and treatment of menstrual cycle-related problems, specifically PDM.

The *BDNF* Val66Met polymorphism (rs6265) leads to the substitution of methionine (Met) for valine (Val) at codon 66 of the proBDNF protein. The Met allele leads to a reduced activity-dependent secretion of BDNF from neurons and impaired BDNF signaling [25]. The *BDNF* Val66Met polymorphism (rs6265) might be implicated in both chronic pain conditions and mood disorders. It has been reported that the Met allele carriers, compared to the Val homozygotes, displayed augmented cortical processing of experimental pain in chronic low back pain patients, whilst reduced pain processing without the co-existence of chronic pain [26]. The authors proposed a possible epigenetic modulation that enhances *BDNF* Val66Met polymorphism sensitivity in these chronic pain patients to experimentally induced mildly painful stimuli. Animal models with *BDNF* Met allele exhibited increased anxiety-related behaviors under stress, and this phenomenon implicates an effect of the gene (*BDNF* genotype) \times environment (stress) interaction on the mood [25,27]. Although the findings were controversial, previous human studies of *BDNF* Val66Met polymorphism had reported that only *BDNF* Met/Met homozygosity was associated with heightened anxiety [28,29] and an increased risk of depression [30].

In this study, we firstly set out to explore whether the *BDNF* Val66Met polymorphism (rs6265) could be associated with a higher risk of PDM among Taiwanese (Asian) people. Secondly, we reasoned that *BDNF* Met/Met homozygous PDMs might exhibit heightened menstrual pain perception and more negative emotions as compared to Val carrier PDMs. The relationships between *BDNF* Val66Met polymorphism genotypes and the pain- and emotion-related clinical manifestations of PDM, and the psychophysical assessments (pain sensitivity to experimentally induced thermal skin pain) were examined.

Methods

Participants

The inclusion criteria for the PDM group were the followings: (1) 20–30 year-old Taiwanese (Asian) females; (2) a regular menstrual cycle of approximately 27–32 days; (3) a history of PDM longer than 6 months; (4) cramping pain during the menstrual period in the last 6 months rated higher than 4 on a visual analog scale (0 = not at all, 10 = the worst pain imaginable); and (5) right-handedness, as confirmed by the Edinburgh Handedness Inventory. The inclusion criteria for the healthy control females were similar to the PDM group, except that the controls did not experience menstrual pain. The exclusion criteria for all the participants were a history of head injury, pathological pituitary gland disease, organic pelvic disease, psychiatric disorder, immediate plans for pregnancy or a positive pregnancy test, a history of childbirth, and having a metal/pacemaker implant. The participants had not used oral contraceptives within 6 months prior to the study. No analgesics had been taken within 24 hours before the

study. All the subjects in the PDM group underwent pelvic ultrasonography to exclude secondary dysmenorrhea caused by organic pelvic diseases, such as endometriosis or adenomyosis. All the participants were clinically examined and diagnosed in the Gynecology clinic by a gynecologist (HTC).

Altogether, 306 PDMs and 199 controls were originally screened between August 31, 2011 and September 30, 2013 as intended for multimodal imaging genetics studies (magnetic resonance imaging and magnetoencephalography). During the screening stage, 207 PDMs and 98 controls were excluded due to one or a combination of the following factors: an irregular menstrual cycle, a prolonged or shortened menstrual cycle, inconsistent pain intensity, pelvic abnormalities by ultrasonography, or being unwilling to complete the entire series of genetic, hormonal, behavioral, and multimodal neuroimaging studies. Eventually, a total of 99 PDMs and 101 healthy controls (matched for age and educational level) were enrolled. All these subjects also participated in multimodal imaging genetics experiments (the data will be reported elsewhere). All the experiments were completed within three menstrual cycles due to difficulties in scheduling the brain scanning within one menstrual cycle. The overall study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Taipei Veterans General Hospital. The participants provided written informed consent.

Genotyping

Blood samples for genotyping were obtained during the initial examination. Whole blood collected in 10 mL EDTA tubes was stored in 4°C fridge, and DNA extraction was subsequently performed using the Puregene kit following the manufacturer's guidelines (Genra Systems, Minneapolis, MN). Commercial TaqMan single-nucleotide polymorphism assays (Applied Biosystems, Foster City, CA) were used for genotyping. The polymerase chain reaction amplification was conducted in total volume of 1 L using the following amplification protocol: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 94°C for 15 sec and 62°C for 1 min. Fluorescence measurements were performed using the ABI HT7900 (Applied Biosystems, Foster City, CA), and allele calling was performed with the SDS 2.2 software package (Applied Biosystems). Genotypes were independently assigned to the subjects by two technicians blinded to the subjects' information.

Serum Hormone Measurements

The sera extracted from the blood samples drawn during the menstrual phase (within 1–3 days after the start of menstrual flow) and periovulatory phase (12–16 days after the start of menstrual flow) were stored for batch analysis using commercialized assays (UniCel Dx C 800 Synchron Clinical Systems, Beckman Coulter, Inc., Brea, CA). The total serum concentrations were assayed using a chemiluminescence immunoassay technique for the estradiol and progesterone, and a radioimmunoassay technique for the testosterone.

Behavioral Assessments Menstrual pain experience. The PDMs completed the McGill Pain Questionnaire during the initial examination and again during the menstrual phase to assess the recalled overall and current experiences of menstrual pain, respectively.

Personality traits and quality of life. All the participants in the two groups completed the Basic Personality Inventory [31] and the SF-36 (quality of life) [32] during the initial examination to assess personality traits and quality of life, respectively.

Assessment of anxiety, depressive mood and pain catastrophizing. To evaluate the psychological status across

the menstrual cycle, all the participants in the two groups completed self-reported psychological measurements during the menstrual phase and periovulatory phase. Ovulation was confirmed using a urinary luteinizing hormone test [33–35]. The psychological battery included the Spielberger State-Trait Anxiety Inventory [36], the Beck Anxiety Inventory [37], the Beck Depression Inventory [38], and the Pain Catastrophizing Scale [39]. The order of the assessments was balanced across the subjects for the menstrual phase and periovulatory phase. Multimodal neuroimaging studies were conducted during these two phases; the results will be reported elsewhere.

Quantitative sensory testing for central sensitization. To examine central sensitization throughout the menstrual cycle, as previously reported in studies on white populations [40–44], we performed a thermal quantitative sensory testing (thermal sensation and pain thresholds) according to the established protocol of an ascending limit approach for heat pain and a descending limit approach for cold pain [45]. Heat and cold stimuli (TSA 2001-II, MEDOC, Israel) were administered to the bilateral periumbilical areas (T11-dermatome, referral area of menstrual pain) and forearm extensor areas (C7-dermatome, remote control area) during the menstrual phase and periovulatory phase. The baseline temperature was set at 32°C, and all the thresholds were obtained with a ramped stimulation method (1°C/sec). The perception thresholds for cold and warm were determined first, followed by the pain thresholds for cold and heat. The mean thresholds were calculated by averaging three consecutive measurements. The limits of the temperature range were set between 0 and 50°C.

Statistical Analysis

SPSS Statistics 20.0 (SPSS Inc., Chicago, IL) was used for all the analyses. The data are presented as the means \pm standard deviation. The results were considered significant at $P < 0.05$ (two-tailed). We did not apply multiple corrections for all these inventories since they assess different sensory, affective, cognitive, and physiological dimensions of pain, and the discrete physical and mental impacts of social life. In the first stage of the analysis, the Hardy-Weinberg equilibrium of the *BDNF* genotype distribution and the relationship between genotype and PDM were examined using the chi-square test. We then conducted the second stage of the analysis by investigating the associations between the *BDNF* Val66Met polymorphism and behavioral/hormonal manifestations. The Val/Val and Val/Met subjects were treated as a single genotype category (*BDNF* Val carriers) and compared with the *BDNF* Met/Met homozygotes in the subsequent analyses. This strategy was corroborated by our findings that there were no essential differences in any of the behavioral/hormonal assessments between the *BDNF* Val/Val and *BDNF* Val/Met subjects in the PDM and control groups (all $P > 0.05$). Between-group (PDM vs. control) and between-genotype (Met/Met homozygotes vs. Val carriers) differences in demographic characteristics and McGill Pain Questionnaire scores were examined using a two-sample t-test or the Mann-Whitney U test. A two-way analysis of variance (ANOVA) of the Basic Personality Inventory and SF-36 data was conducted for the main effects of group (PDM vs. control) and *BDNF* genotype (Met/Met homozygotes vs. Val carriers), as well as the interaction between them. For the assessments of behavior, quantitative sensory testing and serum hormone levels during the two phases, a general linear model with a repeated measures design was first applied to examine the possible effects of group (PDM vs. control), genotype (Met/Met homozygotes vs. Val carriers), menstrual cycle (menstrual phase vs. periovulatory phase) and the interactions between these factors. To further determine

whether different *BDNF* genotypes were associated with different psychological attributes of state anxiety, trait anxiety, Beck Anxiety Inventory, Beck Depression Inventory, and Pain Catastrophizing Scale scores across the menstrual cycle for the respective PDM and control group, repeated-measures ANOVAs were conducted on each psychological measurement to compare the PDM and control groups.

Results

Demographic Characteristics (Table 1)

There were no significant differences between the PDM and control groups in age, age at menarche, years of menstruating or average days of one menstrual cycle ($P > 0.05$). The PDM group had been experiencing moderate cyclic menstrual pain (recalled overall McGill Pain Questionnaire-pain rating index: 33.3 ± 12.96) for 2 (1.8 ± 0.82) days per month for approximately 10 (9.2 ± 2.70) years.

Of all the PDMs and controls who received genetic testing, 21 PDMs were excluded from the genetic-behavioral-hormonal-multimodal neuroimaging studies for the following reasons: appendicitis after recruitment ($n = 1$), fifth ventricle in the brain ($n = 2$), arteriovenous malformation in the brain ($n = 1$), MRI claustrophobia ($n = 2$), irregular menstrual cycle after recheck ($n = 1$), and withdrawal with incomplete behavioral/hormonal data ($n = 14$). Twenty controls were excluded from the genetic-behavioral-hormonal-multimodal neuroimaging studies for the following reasons: intracranial arachnoid cyst ($n = 1$), irregular menstrual cycle after recheck ($n = 1$), development of systemic lupus erythematosus after recruitment with anti-lupus medications ($n = 1$), undisclosed prior head injury ($n = 1$), and withdrawal with incomplete data ($n = 16$). The participants with the aforementioned problems and incidental MRI brain findings were excluded from further genetic-behavioral-hormonal and multimodal neuroimaging analyses. The demographic data of the remaining participants (PDM = 78, control = 81) retained similar features of the original samples ($P > 0.05$).

Genotypes and Allele Distributions (Table 2)

Participants in the two groups were all successfully genotyped. The genotype distributions of the *BDNF* Val66Met (rs6265) for both groups were in Hardy-Weinberg equilibrium ($P > 0.05$; Table 2). A sample size of 200 (99 PDMs, 101 controls) achieved 64% statistical power to detect an effect size of 0.184 using a 2-degree-of-freedom chi-square test with a significance level of 0.05. The PDM group differed significantly from the control group in *BDNF* genotypes ($P = 0.034$) and alleles ($P = 0.013$) distributions. There was a significant excess of the Met allele in the PDM group compared with the control group (Table 2). The PDM prevalence difference in all *BDNF* Met/Met homozygotes (63.8%) vs. all Val carriers (43.7%) attained high significance ($P = 0.012$). Treating the *BDNF* Val allele carriers as the baseline, the odds ratio for *BDNF* Met/Met homozygosity was 2.27 (95% confidence interval = 1.21–4.27) ((PDM-Met/Met \times control-Val-carriers)/(PDM-Val-carriers \times control-Met/Met)). It implied that those carrying the *BDNF* Met/Met genotype had an increased risk of PDM. The *BDNF* Val66Met polymorphism had no association with the age of onset (Val carriers, 14.2 ± 1.76 years of age; Met homozygotes, 13.9 ± 1.56) or duration of menstrual pain per cycle (Val carriers, 1.8 ± 0.86 days; Met/Met homozygotes, 1.8 ± 0.75) in the PDM group.

For the behavioral/hormonal assessments (PDM = 78, control = 81; eligible for multimodal neuroimaging studies), the subgroups retained the original group features in terms of the

Table 1. Demographic data of the original genotyping groups and the behavioral/hormonal sub-groups.

	PDM		Control	
	Genotyping	Behavioral/hormonal	Genotyping	Behavioral/hormonal
Subject number	99	78	101	81
Age	23.3 (2.33)	23.4 (2.41)	23.8 (2.35)	23.9 (2.38)
Age at menarche	12.0 (1.27)	12.0 (1.25)	12.2 (1.05)	12.3 (1.08)
Years of menstruating	11.3 (2.57)	11.4 (2.66)	11.5 (2.46)	11.6 (2.56)
Days of one menstrual cycle	29.5 (1.12)	29.4 (1.18)	29.6 (1.20)	29.6 (1.23)
Years of dysmenorrhea history	9.2 (2.70)	9.3 (2.79)		
Days of menstrual pain per cycle	1.8 (0.82)	1.9 (0.83)		
MPQ: recalled pain rating index	33.3 (12.96)	34.5 (13.17)		
MPQ: recalled pain intensity	3.1 (1.21)	3.1 (1.17)		

Abbreviations: MPQ, McGill Pain Questionnaire; PDM, primary dysmenorrhea. The data are presented as the means (SD).

doi:10.1371/journal.pone.0112766.t001

genotype and allele distributions (Table S1). No differences in demographic data were noted between the *BDNF* Met/Met homozygotes and the Val carriers in the sub-groups (all $P>0.05$).

Differences in Behavioral Assessments

Menstrual pain experience (Table 3). During the menstrual phase, differences in pain rating index ($P=0.131$) and present pain intensity ($P=0.925$) of McGill Pain Questionnaire were not demonstrated between the *BDNF* Met/Met homozygous PDMs and the Val carrier PDMs. However, the *BDNF* Met/Met homozygous PDMs scored significantly higher on the sensory subscale of McGill Pain Questionnaire compared with the Val carriers ($P=0.017$).

Personality traits and quality of life (Table 4). There was a significant main effect of group (but no significant main effect of *BDNF* genotype or interaction between group and *BDNF* genotype) on the anxiety ($F=10.975$, $R^2=0.068$, $P=0.001$), depression ($F=8.875$, $R^2=0.056$, $P=0.003$), and hypochondriasis ($F=27.314$, $R^2=0.154$, $P<0.001$) dimensions of Basic Personality Inventory. Significant group effect was noted also on physical ($F=31.554$, $R^2=0.173$, $P<0.001$) and mental ($F=40.455$, $R^2=0.211$, $P<0.001$) components of SF-36. Regardless of genotype, the PDMs (compared with the controls) scored significantly higher on the anxiety, depression, and hypochondriasis dimensions of Basic Personality Inventory and significantly lower on the physical and mental components of SF-36.

Anxiety, depressive mood and pain catastrophizing (Table 5). For the analyses of the entire cohort, there was a significant main effect of group on the state anxiety ($F=27.089$, $R^2=0.152$, $P<0.001$), trait anxiety ($F=17.593$, $R^2=0.106$, $P<0.001$), Beck Anxiety Inventory ($F=59.864$, $R^2=0.284$, $P<0.001$), Beck Depression Inventory ($F=17.810$, $R^2=0.106$, $P<0.001$), and Pain Catastrophizing Scale ($F=72.171$, $R^2=0.334$, $P<0.001$) scores, with a significant interaction between menstrual cycle and group on the state anxiety ($F=24.269$, $R^2=0.138$, $P<0.001$), Beck Anxiety Inventory ($F=70.943$, $R^2=0.320$, $P<0.001$), Beck Depression Inventory ($F=32.477$, $R^2=0.177$, $P<0.001$), and Pain Catastrophizing Scale ($F=4.412$, $R^2=0.030$, $P=0.037$) scores. There was also a significant main effect of menstrual cycle on the state anxiety ($F=16.459$, $R^2=0.098$, $P<0.001$), Beck Anxiety Inventory ($F=60.690$, $R^2=0.287$, $P<0.001$), Beck Depression Inventory ($F=28.713$, $R^2=0.160$, $P<0.001$), and Pain Catastrophizing Scale ($F=4.514$, $R^2=0.030$, $P=0.035$) scores and a significant main effect of *BDNF* genotype

on the state anxiety ($F=6.207$, $R^2=0.039$, $P=0.014$) and Beck Anxiety Inventory ($F=4.974$, $R^2=0.032$, $P=0.027$) scores. Significant interactions between menstrual cycle and *BDNF* genotype ($F=7.595$, $R^2=0.048$, $P=0.007$), as well as among menstrual cycle, group and *BDNF* genotype ($F=4.509$, $R^2=0.029$, $P=0.035$), on the Beck Anxiety Inventory score were noted.

In the individual group analyses, the PDM group exhibited a significant main effect of menstrual cycle on state anxiety ($F=33.144$, $R^2=0.309$, $P<0.001$), Beck Anxiety Inventory ($F=85.498$, $R^2=0.536$, $P<0.001$), Beck Depression Inventory ($F=45.579$, $R^2=0.381$, $P<0.001$), and Pain Catastrophizing Scale ($F=7.556$, $R^2=0.095$, $P=0.008$) scores, as well as a significant interaction between menstrual cycle and *BDNF* genotype on the Beck Anxiety Inventory score ($F=7.743$, $R^2=0.095$, $P=0.007$) (Table 5). In view of R^2 statistics, the significant interaction between menstrual cycle and *BDNF* genotype should mainly be attributed to the main effect of menstrual cycle in the PDM group. By contrast, the control group exhibited neither significant main effects nor interactions (Table S2). Compared with the controls, the PDMs reported significantly higher state anxiety, trait anxiety, Beck Anxiety Inventory, Beck Depression Inventory, and Pain Catastrophizing Scale scores during either the menstrual phase or periovulatory phase. The PDMs had significantly higher state anxiety, Beck Anxiety Inventory, Beck Depression Inventory, and Pain Catastrophizing Scale scores, but not trait anxiety score, during the menstrual phase than the periovulatory phase. The controls demonstrated no significant between-phase differences in any of the psychological measurements. The Met/Met homozygous PDMs, compared with the Val carrier PDMs, manifested higher Beck Anxiety Inventory scores during the menstrual phase, but not during the periovulatory phase. In the controls, an absence of between-genotype differences was noted in all the psychological measurements.

Quantitative sensory testing results (Table S3). Because there was no right-left difference in any of the measured thresholds in each subject (all $P>0.05$), we averaged the bilateral values for the corresponding dermatome areas for the group comparisons. No main effect of menstrual cycle, group, *BDNF* genotype or interactions between them was noted for any of the measured thresholds. In brief, we detected no regional or generalized central sensitization of superficial (skin) pain in the Taiwanese (Asian) participants.

Table 2. *BDNF* rs6265 genotype distributions and allele frequency.

	Genotype (n, %)		Allele frequency		P
	G/G (Val/Val)	G/A (Val/Met)	Val	Met	
PDM (n = 99)	24, 24.2%	38, 38.4%	43.4%	56.6%	0.013
Control (n = 101)	33, 32.7%	47, 46.5%	55.9%	44.1%	0.034
			A/A (Met/Met)		
			37, 37.4%		
			21, 20.8%		

Abbreviations: *BDNF*, brain-derived neurotrophic factor; PDM, primary dysmenorrhea; A, adenine; G, guanine; Val, Valine; Met, Methionine.
doi:10.1371/journal.pone.0112766.t002

Serum Hormone Levels (Table S4)

There was a significant main effect of menstrual cycle, but no main effect of group or *BDNF* genotype, on the serum estradiol ($F = 85.604$, $R^2 = 0.393$, $P < 0.001$), progesterone ($F = 8.937$, $R^2 = 0.063$, $P = 0.003$), and testosterone ($F = 31.150$, $R^2 = 0.190$, $P < 0.001$) levels. The serum gonadal hormone levels were significantly higher during the periovulatory phase than during the menstrual phase, regardless of group or *BDNF* genotype.

Discussion

The Association of *BDNF* Val66Met Polymorphism with PDM

To our knowledge, this is the first study to report a significant association between the *BDNF* Val66Met polymorphism and PDM. The genotype distributions and allele frequencies of the *BDNF* Val66Met polymorphism exhibit substantial ethnic differences, which may consequently affect the results of genetic association studies. The Met allele frequency in Asian populations (40–50%) is significantly higher than that of white populations (25–32%) [15,46]. The *BDNF* genotype distribution and allele frequency of the overall sample (Met allele frequency: 50.25%) in our study concur with previous reports on Asian populations [47,48]. The finding that Taiwanese PDMs exhibit a significantly higher *BDNF* Met/Met prevalence and *BDNF* Met allele frequency implies that young *BDNF* Met/Met homozygous Taiwanese females may have an increased risk of PDM (Table 2).

The Role of *BDNF* Val66Met Polymorphism as a Regulator of Menstrual Pain

In animal studies, *BDNF* functions either as a pro-nociceptive or an anti-nociceptive role in the brainstem pain modulatory system (midbrain periaqueductal gray and rostral ventromedial medulla). On one hand, *BDNF* mRNA and protein levels are upregulated in the dorsal root ganglion, spinal cord and brainstem pain modulatory system under peripheral noxious stimulation. *BDNF* herein exerts pro-nociceptive effects and contributes to descending pain facilitation and chronic pain hypersensitivity [49,50]. On the other hand, *BDNF* can manifest protective anti-nociceptive effects under repeated painful conditions as a result of multiple mechanisms, including a direct neurotransmitter-like effect or processes of neural plasticity in the anti-nociceptive systems, induction of serotonin-related analgesia, and a modulatory effect at the supraspinal level [51]. *BDNF* can therefore be consociated with both an enhancement of nociceptive impact, and a dampened response to protect the brain from pain overstimulation [51]. Thus, the overall effect of *BDNF* on pain perception and experience depends on the net interaction output of these apparently contrasting activities at different neural structures in which *BDNF* is produced, secreted, and left to act [17,50,51]. The *BDNF* Val66Met polymorphism can lead to impaired *BDNF* signaling in the brainstem pain modulatory system and it may be associated with altered menstrual pain modulation in PDMs. The aforementioned dual effects of *BDNF* on pain may explain in part the diversity of the results of behavioral and psychological assessments, as demonstrated that *BDNF* genotype expressed an effect on the sensory dimension scores but not on the other dimension scores and the total scores (pain rating index) of McGill Pain Questionnaire reported during the menstrual phase.

PDM is not only associated with irritable bowel syndrome and fibromyalgia but is also viewed as a harbinger of these conditions [52,53]. Central sensitization-induced viscerovisceral [44] and viscerosomatic hyperalgesia [40] outside the uterine referral area during early life can be a mechanism contributing to these chronic

Table 3. *BDNF* rs6265 genotype effect on McGill Pain Questionnaire scores of PDMs during the menstrual phase.

	Met/Met	Val carrier	<i>P</i>
Subject number	29	49	
Pain rating index	32.3 (13.47)	26.7 (12.02)	0.131
Sensory	18.5 (6.21)	14.7 (6.13)	0.017
Affective	4.2 (3.57)	3.4 (2.29)	0.554
Evaluative	2.4 (2.02)	2.1 (2.01)	0.613
Miscellaneous	7.2 (3.72)	6.5 (3.81)	0.418
Present pain intensity	2.7 (1.02)	2.6 (1.01)	0.925

Abbreviations: *BDNF*, brain-derived neurotrophic factor; PDM, primary dysmenorrhea; Val, valine; Met, methionine. The data are presented as the means (SD). doi:10.1371/journal.pone.0112766.t003

pain conditions later in life. In PDMs with comorbid irritable bowel syndrome, treating menstrual pain helps to relieve the pain associated with irritable bowel syndrome [54]. A role for BDNF protein in chronic pain conditions such as irritable bowel syndrome [55] and fibromyalgia [56–58] has been suggested. It has been postulated that BDNF protein strengthens the synaptic plasticity within the pain modulatory circuitry and contributes to descending pain facilitation and the subsequent visceral pain and hypersensitivity [50]. It has been reported that irritable bowel syndrome was associated with the up-regulation of BDNF in the intestinal mucosa and that colonic BDNF expression correlated with the severity of abdominal pain [55]. Fibromyalgia has been reported to be associated with the up-regulation of BDNF in the cerebrospinal fluid [56], and the extent of this up-regulation was correlated with the duration of chronic pain. However, it remains to be explored whether *BDNF* Val66Met polymorphism substantially modulates the process of central sensitization and whether a specific genotype(s) renders PDMs more vulnerable to the development of irritable bowel syndrome and fibromyalgia later in life.

Personality Traits and Quality of Life of PDMs

Compared with the controls, the PDMs manifested significantly higher levels of depressive mood, anxiety, and hypochondriasis as their personality traits (Table 4). These findings are in line with previous studies [59,60]. However, these levels did not reach the degree of clinical severity, such as an anxiety disorder, that is mandatory for psychiatric intervention. The PDMs reported a significantly lower quality of life, encompassing both physical and mental components, compared with the controls (Table 4). The absence of main effects of genotype or the interaction between group and genotype in both the Basic Personality Inventory and SF-36 assessments connotes that the *BDNF* Val66Met polymorphism does not directly influence personality traits and quality of life. Rather, menstrual pain per se is the dominant factor influencing these two life facets. Such dysmenorrhea-associated dysfunction, in its severe and protracted form, may through chronification eventually impact women's long-term health as profoundly as chronic diseases [12].

The Possible Role of *BDNF* Val66Met Polymorphism as a Regulator of Emotions in PDM

The PDMs exhibited more prominent negative moods (anxiety and depression) and pain catastrophizing compared with the controls (Table 5 and Table S2). Women with high pain catastrophizing scores may have altered cognitive-affective processing of menstrual pain-laden emotional orientations, which may

precipitate depression, anxiety and altered pain experience [61]. Although the interaction between the *BDNF* genotype and menstrual cycle for Beck Anxiety Inventory scores in the PDM group was not substantial, we found an effect of the gene (*BDNF* genotype) × environment (menstrual pain) interaction on the Beck Anxiety Inventory scores when considering menstrual pain as an environmental stressor. Additionally, the *BDNF* Met/Met homozygous PDMs displayed higher anxiety during the menstrual phase compared with the Val carrier PDMs (Table 5). It is noteworthy that the *BDNF* Val66Met polymorphism produced no significant effects on mood across the menstrual cycle in the control group. These findings collectively connote that the *BDNF* Val66Met polymorphism is modestly associated with the supraspinal modulation of menstrual pain-laden emotional processing. Although *BDNF* Val66Met polymorphism alone may not effectuate depressive and anxious moods, it may represent a predisposing factor, and chronic stress like cyclic menstrual pain is necessary for the detection [62]. Our arguments are in agreement with a meta-analysis that concluded that *BDNF* Met/Met homozygous Asians are predisposed to a sub-significantly higher risk of anxiety disorders [28].

The possible mechanisms underlying the modulatory function of the *BDNF* Val66Met polymorphism on mood may be manifold. According to the neurotrophic model for stress-related mood disorders [19], Met/Met homozygotes may exhibit a deficient expression of BDNF in the mood control areas of the brain during stress (e.g., chronic pain [20]), which may predispose vulnerable subjects to mood disorders [63]. This hypothesis is corroborated by animal studies in which Met/Met homozygotes exhibited defective activity-dependent BDNF secretion from neurons and manifested anxiety-related behaviors in stressful settings [25]. Furthermore, declines in estrogen (as a positive regulator that enhances BDNF expression) during the menstrual phase may lead to a decreased expression of BDNF in the brain, which in turn could hamper the BDNF signaling in the mood-controlling networks of susceptible subjects [21,22]. This study did not reveal a group or genotype main effect (or the interaction between them) on gonadal hormones (Table S4). Thus, the role of estrogen as a regulator of PDM-related emotions remains elusive.

Absence of Central Sensitization to Experimental Skin Pain

Having a sample size many times greater than those reported in the literature [40–43], we found no group (PDM vs. control), menstrual cycle (menstrual phase vs. periovulatory phase), or genotype (*BDNF* Met/Met homozygotes vs. Val carriers) effects on pain sensitivity to experimental skin thermal stimuli (Table S3).

Table 4. ANOVA results of Basic Personality Inventory and SF-36 assessments, stratified by group and BDNF genotype.

	PDM		Control		Main effect		Interaction	
	Met/Met	Val carrier	Met/Met	Val carrier	Group (P)	Genotype (P)	Group*Genotype (P)	
Subject number	29	49	17	64				
Basic Personality Inventory								
Depression	3.2 (2.72)	3.7 (3.44)	2.4 (2.47)	1.7 (1.87)	0.003	0.887	0.240	
Anxiety	6.1 (3.25)	4.9 (3.42)	3.4 (3.10)	3.9 (2.70)	0.001	0.480	0.135	
Social introversion	3.5 (2.49)	3.4 (2.86)	4.1 (2.28)	3.7 (2.57)	0.357	0.688	0.776	
Self depreciation	2.1 (2.14)	2.5 (2.70)	2.1 (1.64)	2.6 (2.40)	0.929	0.283	0.835	
Interpersonal problems	4.8 (2.43)	4.2 (2.22)	4.5 (3.06)	3.5 (2.71)	0.269	0.082	0.709	
Impulse expression	4.6 (3.29)	4.4 (3.03)	4.2 (3.55)	4.5 (2.97)	0.878	0.945	0.681	
Deviation	2.4 (1.93)	2.5 (2.34)	2.3 (1.49)	2.1 (1.95)	0.393	0.830	0.653	
Hypochondriasis	4.6 (3.25)	4.7 (2.84)	2.4 (1.41)	2.2 (1.92)	<0.001	0.986	0.757	
Persecutory ideas	2.7 (2.11)	2.8 (2.03)	2.8 (1.74)	2.0 (2.15)	0.393	0.342	0.205	
Thinking disorder	1.8 (1.94)	1.9 (1.63)	1.6 (1.62)	1.5 (1.80)	0.363	0.926	0.837	
SF-36								
Physical component	46.3 (10.10)	45.2 (10.12)	52.6 (5.64)	54.7 (4.08)	<0.001	0.727	0.243	
Mental component	46.2 (7.51)	46.5 (7.19)	53.1 (4.95)	53.7 (4.69)	<0.001	0.668	0.915	

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; SF-36, 36-Item Short-Form Health Survey; PDM, primary dysmenorrhea; Val, valine; Met, methionine. The data are presented as the means (SD). doi:10.1371/journal.pone.0112766.t004

Table 5. Results of repeated-measures ANOVA of psychological measurements: effects of menstrual cycle and *BDNF* genotype in the PDM group.

	Met/Met	Val carrier	Main effect		Interaction
			Phase (<i>P</i>)	Genotype (<i>P</i>)	Phase*Genotype (<i>P</i>)
Subject number	29	49			
State anxiety					
MENS	45.5 (9.68)	41.1 (8.61)	<0.001	0.052	0.495
POV	38.9 (7.84)	36.0 (5.60)			
Trait anxiety					
MENS	45.6 (10.10)	44.0 (8.21)	0.056	0.386	0.886
POV	44.3 (9.37)	42.4 (8.55)			
Beck anxiety					
MENS	14.9 (6.95)	10.9 (6.82)	<0.001	0.138	0.007
POV	6.3 (5.96)	6.3 (5.78)			
Beck depression					
MENS	13.8 (10.52)	11.3 (9.73)	<0.001	0.429	0.295
POV	6.1 (7.27)	5.7 (6.94)			
Pain catastrophizing					
MENS	22.6 (11.05)	19.6 (12.58)	0.008	0.383	0.512
POV	18.8 (9.27)	17.3 (12.35)			

Abbreviations: ANOVA, analysis of variance;
BDNF, brain-derived neurotrophic factor;
 PDM, primary dysmenorrhea; MENS, menstrual phase;
 POV, periovulatory phase;
 Val, valine; Met, methionine.
 The data are presented as the means (SD).
 doi:10.1371/journal.pone.0112766.t005

Our findings are at odds with published studies (based on very small samples of 10–22 PDMs and 10–31 controls; most studies had 10 of each) that dysmenorrheic white women, compared with non-dysmenorrheic ones, exhibit heightened pain sensitivity or central sensitization (either regional or generalized) to experimental somatic [40–43] and visceral stimuli [44]. Notwithstanding, other studies on dysmenorrheic white women have also yielded conflicting results [43,64]. Our results indicate that additional factors contributing to the diversity of the clinical manifestations of PDM may include different genetic constitutions (in the context of ethnicity) and the diverse nature and quality of experimental pain caused by different stimuli [65]. Future studies employing imaging genetics [66] in combination with more comprehensively designed psychophysical experiments (including deep somatic pain and visceral pain assessments) on a larger sample may be helpful to elucidate the supraspinal (brain) mechanisms that underlie the presence or absence of central sensitization in the context of ethnic differences. It should be born in mind that such mechanisms of presence or absence of central sensitization may occur at different level of the neuraxis of the central nervous system.

Limitations and Further Considerations

Our study indicates an association of the *BDNF* Val66Met polymorphism with PDM, and our data suggest, yet not strongly affirm, a possible role of *BDNF* Val66Met polymorphism as a regulator of menstrual pain and pain-related emotions in PDM. The discordance between genetic association and clinical manifestations in our study has been corroborated by a previous study on Taiwanese that reported the genetic association of the *BDNF* Val66Met polymorphism with a higher risk of geriatric depression,

but not with the disease severity (depression and cognitive deficit) [30]. Some possible explanations are suggested below. Firstly, we speculate that the overall manifestations of pain experience of PDM may be determined in part by the net output of contrasting pro- and anti-nociceptive effect of *BDNF* in the pain circuitry, that may result in the discordance between the genetic risk predisposition and pain-emotional expressions. This can be better appreciated in the context that PDM, dissimilar with major affective disorders and neurological diseases, usually does not manifest intense mood liability and functional disability except the severe form that leads to absenteeism from school and work. Secondly, the *BDNF* Val66Met polymorphism, as an individual gene, may not play a dominant role in the overall expressions of menstrual pain and pain-related emotions, in which polygenic interactions and regulations (e.g., genes related to the serotonergic and dopaminergic systems) are most likely involved [67–69]. Thirdly, the behavioral assessments we used may not perfectly reflect the pain- and emotion-related experiential facets of PDM. Fourthly, functional variants within the coding or regulatory regions of the *BDNF*, other than the *BDNF* Val66Met polymorphism, might also contribute to the clinical manifestations of PDM. The *BDNF* G-712A polymorphism, a genetic variant located in the putative promoter region [70], has been reported to be associated with major depression in Chinese [71]. Lastly, the *BDNF* Val66Met polymorphism may not be directly involved in the pathogenesis of PDM, and the association between the *BDNF* Val66Met polymorphism and PDM may be through linkage disequilibrium with other genetic variants of the *BDNF* or nearby genes.

The sample sizes in the current report are relatively small due to the rigorous inclusion/exclusion criteria and the demanding processes of associated longitudinal multimodal neuroimaging studies for each participant. We consider the novel findings reported in this study as preliminary. However, our observations do echo the recent mandate for a thorough revisit of PDM [4,52], and invite further studies of larger sample size for the genetic and neuroscientific underpinnings of the clinical (pain and related emotions) and psychophysical (pain sensitivity) manifestations of PDM females.

Conclusions

Our data suggest that the *BDNF* Val66Met polymorphism is associated with PDM in Taiwanese (Asian) people. *BDNF* Met/Met homozygous young Taiwanese females may have an increased risk of PDM. The putative role of *BDNF* Val66Met polymorphism as a supraspinal regulator of menstrual pain and pain-laden emotions remains to be further verified. Absence of thermal hypersensitivity may connote an ethnic attribution. Ethnicity of different genetic constitutions should be taken into consideration in future PDM research.

Supporting Information

Table S1 *BDNF* rs6265 genotype distributions and allele frequency in the behavioral/hormonal sub-groups.
(DOC)

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Table S2 Results of repeated-measures ANOVA of psychological measurements: effects of menstrual cycle and *BDNF* genotype in the control group.

(DOC)

Table S3 Results of repeated-measures ANOVA of quantitative sensory testing: effects of group, *BDNF* genotype and menstrual cycle.

(DOC)

Table S4 Results of repeated-measures ANOVA of gonadal hormone levels: effects of group, *BDNF* genotype and menstrual cycle.

(DOC)

Acknowledgments

We extend particular appreciation to Ian-Ting Chu, Intan Low, Wei-Chi Li, Feng-Xuan Jian, and Shin-Hua Lin for their technical and experimental help.

Author Contributions

Conceived and designed the experiments: LCL CHT LFC JCH. Performed the experiments: LCL CHT. Analyzed the data: LCL CHT MWL JCH. Contributed reagents/materials/analysis tools: HDS MWL. Wrote the paper: LCL CHT MWL JCH. Clinical Assessment: HTC. Final approval: JCH.

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