Research Article

MOLECULAR PAIN

Molecular Pain
Volume 12: 1–9

DOI: 10.1177/1744806916646783
mpx.sagepub.com
SAGE

The brain-derived neurotrophic factor pathway, life stress, and chronic multi-site musculoskeletal pain

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Abstract

Introduction: Brain-derived neurotrophic factor (BDNF) disturbances and life stress, both independently and in interaction, have been hypothesized to induce chronic pain. We examined whether (a) the BDNF pathway (val^{66}met genotype, gene expression, and serum levels), (b) early and recent life stress, and (c) their interaction are associated with the presence and severity of chronic multi-site musculoskeletal pain.

Methods: Cross-sectional data are from 1646 subjects of the Netherlands Study of Depression and Anxiety. The presence and severity of chronic multi-site musculoskeletal pain were determined using the Chronic Pain Grade (CPG) questionnaire. The BDNF val^{66}met polymorphism, BDNF gene expression, and BDNF serum levels were measured. Early life stress before the age of 16 was assessed by calculating a childhood trauma index using the Childhood Trauma Interview. Recent life stress was assessed as the number of recent adverse life events using the List of Threatening Events Questionnaire.

Results: Compared to val^{66}val, BDNF met carriers more often had chronic pain, whereas no differences were found for BDNF gene expression and serum levels. Higher levels of early and recent stress were both associated with the presence and severity of chronic pain ($p < 0.001$). No interaction effect was found for the BDNF pathway with life stress in the associations with chronic pain presence and severity.

Conclusions: This study suggests that the BDNF gene marks vulnerability for chronic pain. Although life stress did not alter the impact of BDNF on chronic pain, it seems an independent factor in the onset and persistence of chronic pain.

Keywords

Chronic pain, nerve growth factors, child abuse, life change events

Date received: 17 December 2015; revised: 10 March 2016; accepted: 4 April 2016

Introduction

Chronic musculoskeletal pain, which may even occur without peripheral tissue damage, has been conceptualized as a central sensitization condition in which factors of the central nervous system enhance pain modulation.[1] Central sensitization may occur through disturbances of neurotrophic factors.[2] Brain-derived neurotrophic factor (BDNF) may be an important pain modulator, as it positively regulates neuronal growth, recovery and development,[3] and central and peripheral synaptic plasticity.[4,5] The influence of the BDNF pathway (BDNF genotype, gene-expression, and protein) may be especially prominent when life stress is present, as evidenced by significant BDNF-with-stress interactions found for the onset of depression.[6–8] Such an interaction may also apply to chronic pain as the condition shares similar pathophysiological mechanisms.[3]

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Some studies hypothesized that blocking BDNF in sensory neurons could represent an approach for chronic pain treatment.[2,9] This hypothesis is supported by cross-sectional studies on fibromyalgia, a syndrome with chronic multi-site pain, which consistently showed increased BDNF serum[10–12] and plasma[13] levels. One study found that increased BDNF expression in the colonic mucosa of chronic abdominal pain patients was correlated with higher pain severity scores compared to controls.[14] Other associations between variables (genetic or biomarker) of the BDNF pathway and pain are very inconsistent. A common single-nucleotide polymorphism (SNP) on the BDNF gene is val^{66}met (rs6265), which alters BDNF protein structure by a valine (val) to methionine (met) insertion at codon 66.[15] Two studies suggest that BDNF met carriers may be at higher risk for chronic pelvic or abdominal pain,[16,17] but this was not found in another study on fibromyalgia.[18] However, as the met allele has been found to decrease BDNF secretion,[3,15] it would have been expected that val carriers are at a higher risk of chronic pain. So, overall, studies on the BDNF pathway (genotype/expression/protein) in chronic pain show inconsistent results, and no earlier studies have examined the BDNF pathway in different levels covering DNA, gene expression, and protein levels.

Life stressors may add to the impact of BDNF disturbances on chronic pain. Evidence from cross sectional and longitudinal studies suggests that early and recent life stress may trigger the development of chronic pain.[19–22] Life stressors may have different impact on pain depending on an individual's genetic risk. In a previous study on depression, BDNF met carriers with a history of childhood trauma showed lower BDNF serum levels compared to met carriers without childhood trauma, whereas this pattern was reversed in the val^{66}val group.[23] A gene-environment interaction may also apply to chronic pain. However, previous studies on BDNF in chronic pain ($n < 155$) are inconsistent, and the interaction of BDNF with life stress has never been tested for chronic pain. Therefore, this cross-sectional study examines the association of (a) the BDNF pathway (val^{66}met genotype, gene expression, and serum level), (b) early and recent stress, and (c) the interaction of the BDNF pathway with early and recent life stress, with the presence and severity of chronic multi-site musculoskeletal pain. We hypothesized that an altered BDNF pathway, higher stress levels, and their combination are associated with the presence and severity of chronic pain.

Methods

Sample

The current cross-sectional study was based on data from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing cohort study conducted among 2981 adults (18–65 years at baseline) from which 94.8% were of North-European ancestry. Subjects were recruited from the general population ($n = 564$), general practices ($n = 1610$), and mental health care organizations ($n = 807$). People with current and remitted psychiatric disorders as well as controls with no psychiatric diagnosis participated. The NESDA study contains a high proportion of subjects with chronic multi-site musculoskeletal pain and provides a unique opportunity for control of relevant variables such as depressive and anxiety disorders. The research protocol was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center Amsterdam, The Netherlands. Penninx et al.[24] provided a detailed description of the NESDA study design and sampling procedures.

For this study, subjects with pain data were selected ($n = 2980$). From these subjects, 767 persons met criteria for chronic pain and 887 persons were controls (see section below for measurement criteria). Of these subjects, eight persons were excluded because no data were available on BDNF, leaving a total of 1646 subjects. All these subjects had data available on recent stress. Data on early life stress (childhood trauma) were available in 1643 subjects. Data on BDNF val^{66}met genotype were available in 1412 subjects; on BDNF gene expression in 1089 subjects; and on BDNF serum in 1608 subjects. Included subjects ($n = 1646$) had significantly lower pain scores, were older, were more often male, had less often depression or anxiety, had higher BDNF serum levels, had lower levels of early and recent stress (all $p < 0.05$), but did not differ in BDNF gene expression level and BDNF val^{66}met genotype, compared with with excluded subjects ($n = 1335$).

Chronic multi-site musculoskeletal pain

Chronic multi-site musculoskeletal pain was defined using the CPG,[25] a valid and reliable questionnaire.[26,27] The CPG first inquires about the presence of pain in the prior six months in the extremities (joints of the arms, hands, legs, or feet), back, neck, head, abdomen, chest, and the orofacial area (mouth and face).[28] The subsequent questions in the CPG refer to the most painful location and inquire (a) days in pain in the prior six months (scale 0–180); (b) pain at this moment (scale 0–10); (c) worst pain in the prior six months (scale 0–10); (d) average pain in the prior six months (scale 0–10); (e) disability days in the prior six months (scale 0–180); (f) disability in daily activities (scale 0–10); (g) disability in spare time, social life, and family activities (scale 0–10); and (h) disability in work (scale 0–10). A total pain intensity score was calculated using questions 2, 3,

and 4 of the CPG; a total pain disability score was calculated using questions 6, 7, and 8 of the CPG (average of the 0–10 ratings of the three questions multiplied by 10 resulting in a 0–100 score). Disability points (seven categories; 0–6) were calculated adding the total disability score (0–100) and the indicated points for disability days (question 5 of the CPG; see Von Korff[25] for details). Five grades were categorized: grade 0 (pain-free, no pain in the prior six months); grade I (low disability (<3 disability points), low intensity (<50)); grade II (low disability (<3 disability points), high intensity ($\geq$50)); grade III (high disability, moderately limiting (3–4 disability points)); and grade IV (high disability, severely limiting ($\geq$5 disability points)).[25] Following previous research,[22,29,30] we defined chronic multi-site musculoskeletal as grade I, II, III, or IV on the CPG and pain present in the prior six months in the extremities, the back, and the neck. The control group consisted of people with grade 0 ($n = 169$) or with grade I and pain in at most two locations ($n = 713$). The remaining subjects not meeting the criteria of the chronic multi-site musculoskeletal pain group or the control group were excluded from the present study ($n = 1327$). We also refer to the chronic multi-site musculoskeletal pain group as the chronic pain group.

Brain-derived neurotrophic factor

BDNF val^{66}met genotype. For detailed description of DNA extraction, quality control, and imputation techniques, see previous studies.[31–33] BDNF genotyping was performed on multiple chip platforms (including Affymetrix-Perlegen 5.0 and Affymetrix 6.0) in (partially overlapping) different subsets of the total NESDA sample. Quality control was performed within and between chip platforms. Basic quality control steps for subjects included checks for European ancestry, sex inconsistencies, mendelian errors, missing rates, and high genome-wide homozygosity. Genotype data were further checked based on Hardy–Weinberg equilibrium ($p = 0.91$), minor allele frequencies (MAFs), and call rates. Data were imputed using the 1000 Genomes phase 1 INTEGRATED RELEASE version 3 ALL panel.[33] The Val66Met BDNF SNP (rs6265) was typed in some of the panels used for imputation and all post-imputation QC criteria were met. R^2 value for rs6265 (CHR = 11; BP = 27658369) was 0.99, indicating an almost perfect correlation between the imputed genotype and the true underlying genotype. The MAF was 0.21. The current sample ($n = 1412$) consists of 64% val^{66}val and 4% met^{66}met homozygotes, whereas 32% were val^{66}met heterozygotes. Following previous research,[23,34] we combined the low-frequency met^{66}met with val^{66}met and further refer to this group as ''met carriers'' (dominant model; val^{66}val = reference).

BDNF gene expression. To assess BDNF gene expression in whole blood, venous blood samples were drawn in the morning (7–10 AM) after an overnight fast in one 7-mL heparin-coated tube (Greiner Bio-One, Monroe, North Carolina). Heparinized whole blood samples were transferred into PAXgene Blood RNA tubes (Qiagen), incubated, and stored at −20°C before RNA isolation. Samples were hybridized to Affymetrix U219 arrays (Affymetrix, Santa Clara, CA) containing 530,467 probes summarized in 49,293 probe sets. Array hybridization, washing, staining, and scanning were carried out in an Affymetrix GeneTitan System per the manufacturer's protocol. Gene expression data were required to pass standard Affymetrix QC metrics (Affymetrix expression console) before further analysis. We excluded from further analysis probes that did not map uniquely to the hg19 (Genome Reference Consortium Human Build 37) reference genome sequence, as well as probes targeting a messenger RNA (mRNA) molecule resulting from transcription of a DNA sequence containing a single nucleotide polymorphism (based on the dbSNP137 common database). Normalized probe set expression values were obtained using Robust Multi-array Average normalization as implemented in the Affymetrix Power Tools software (version 1.12.0, Affymetrix). No additional gene expression measurement techniques were used to verify our findings. Further details on BDNF gene expression assessment and RNA processing have been described elsewhere.[35,36] In this study, we calculated the mean BDNF expression across all five probe sets targeting BDNF after adjusting for RNA per plate, position on plate, month and time of blood withdrawal, level of blood hemoglobine, and time between blood withdrawal and RNA extraction.

BDNF serum. Serum samples were kept frozen at −85°C for a period between one and four years after which BDNF concentration (ng/mL) was measured using the Emax Immuno Assay system from Promega according to the manufacturer's protocol. The undiluted serum was acid treated, which in a dilution-dependent way reliably increased the detectable BDNF. Subsequently, serum samples were diluted 100 times and stored again at −85°C for BDNF assay the next day. After dilution, the BDNF concentrations were well within the range of the standard curve. The assay sensitivity threshold was ascertained at 1.56 ng/ml reflecting the minimum level of BDNF in the serum that could be reliably determined. Three samples were below this threshold and therefore excluded from all subsequent analyses. See our previous study[37] for more details on BDNF assessment.

Early and recent life stress

Early life stress was assessed at baseline using the Childhood Trauma Interview (CTI) from The Netherlands Mental Health Survey and Incidence Study.[38] The CTI assesses four domains of trauma before the age of 16 by asking whether the traumatic event occurred (yes/no), and a subsequent question asking how often the event occurred. The domains included emotional neglect (lack of parental attention or support and ignorance of one's problems and experiences), psychological abuse (being verbally abused, undeserved punishment, subordinated to siblings, and being blackmailed), physical abuse (being kicked or hit with hands or an object, beaten up or physical abuse in any other way), and sexual abuse (being sexually approached against one's will, meaning being touched or having to touch someone in a sexual way). The CTI yields a score ranging 0 to 8 by adding the frequencies of occurrence (0, absent; 1, once or sometimes; 2, regularly, often or very often). Subsequently, a cumulative index called childhood trauma index was calculated as the sum of experienced number and frequency of childhood trauma for each participant, categorized into five categories (0, 1–2, 3–4, 5–6, and 7–8).

Recent life stress was assessed as the total number of recent adverse life events, which was determined at baseline using the List of Threatening Events Questionnaire .[39] The list assesses 12 life stressors in the year preceding the baseline assessment such as death of a close friend or relative or serious financial problems. We additionally inquired whether subjects experienced any other adverse life event in the past year. Following previous research,[22] the total number of adverse life events (0–13) were used in the analyses.

Statistical analyses and covariates

Descriptive baseline characteristics were reported as means or percentages in the chronic pain and control group. To examine the differences between groups, independent-sample t tests were used for continuous variables and χ^2 tests for dichotomous and categorical variables.

Logistic regression analyses were performed for each BDNF variable with the presence of chronic pain. Analyses of val^{66}met (met carriers vs. val^{66}val) were adjusted for age, sex, and three ancestry-informative principal components[40] to take possible population stratification into account. BDNF gene expression and BDNF serum analyses were adjusted for age, sex, and smoking (never/former/current), because these variables have previously been linked to BDNF.[37] For the associations of early and recent stress with chronic pain presence, we performed logistic regression analyses adjusted for age and sex. Interactions of each BDNF variable with early and recent stress in the association with chronic pain presence were examined by adding an interaction term (BDNF variable × trauma index; or BDNF variable × life events) to the logistic regression analyses also including all covariates.

For analyses of pain severity, fully adjusted linear regression analyses were performed within the chronic pain group ($n = 764$). The associations of BDNF, early and recent stress, and the interaction of BDNF with early and recent stress were tested with the total pain intensity score and the total pain disability score separately.

Depressive and anxiety disorders have previously been associated with chronic pain.[41] We also showed that BDNF was related to depression and antidepressant medication in our sample.[42] To discard these potential confounding effects, additional analyses were performed adjusting for current (past six months) depressive disorders (major depressive disorder, dysthymia) and anxiety disorders (panic disorder, agoraphobia, generalized anxiety disorder, social phobia) (established with the Composite International Diagnostic Interview: Robins et al.[43]) and frequent use of tricyclic antidepressants (ATC code: N06AA), selective serotonin reuptake inhibitors (N06AB) and other antidepressant medications (N06AF/AG/AX).

For all statistical tests, a probability level of $\leqslant 5\%$ was regarded as significant. For testing interactions, this level was set at $\leqslant 10\%$. The statistical calculations were performed using SPSS V.20 for Windows (IBM, Armonk, NY, USA).

Results

Sample characteristics

Compared to controls, chronic pain subjects had higher pain scores, were significantly older, were more often women, were more often current smokers, had more often a current depressive and/or anxiety disorder, used more frequently antidepressants, and reported more early and recent life stress (Table 1). The BDNF val^{66}met polymorphism, BDNF gene expression, and BDNF serum level showed low and non-significant intercorrelations (Pearson's r:−.03 to .02, $p > .34$; data not shown).

The BDNF pathway and the presence and severity of chronic pain

Compared to val^{66}val carriers, BDNF met carriers more often had chronic pain after adjustment for confounders (Table 2). BDNF gene expression and BDNF serum levels were not associated with the presence of chronic pain before and after adjustment for confounders ($p > .05$).

Table 1. Baseline characteristics.[a]

	N#	Controls ($n = 882$)	Chronic pain ($n = 764$)	p[b]
Pain scores				
Days of pain in the prior six months	1645	27.7 (48.7)	108.0 (69.5)	<0.001
Pain intensity		21.9 (14.7)	52.9 (17.9)	<0.001
Pain disability		9.8 (13.5)	39.7 (25.9)	<0.001
Sociodemographic factors				
Age, years		42.0 (13.6)	44.7 (12.2)	<0.001
Women, %		56.7	73.6	<0.001
Lifestyle				
Smoking, %				0.02
Never smoker		31.5	26.0	
Former smoker		35.7	31.3	
Current smoker		32.8	42.7	
Current affective disorders				
Depression and/or anxiety, %		37.6	72.6	<0.001
Antidepressant medication, %		17.5	31.0	<0.001
BDNF				
BDNF $Val^{66}Met$ genotype	1412			0.07
Val/Val, %		66.5	61.9	
Val/Met, %		29.0	34.7	
Met/Met, %		4.5	3.4	
BDNF gene expression	1089	2.18 (0.1)	2.17 (0.1)	0.11
BDNF serum level (ng/ml)	1608	9.1 (3.2)	9.3 (3.7)	0.26
Early life stress	1643			<0.001
Childhood trauma index [0], %		64.7	42.8	
Childhood trauma index [1–2], %		29.3	39.8	
Childhood trauma index [3–4], %		6.0	17.3	
Recent life stress				<0.001
Recent adverse life events [0], %		61.8	53.4	
Recent adverse life events [1–2], %		33.7	38.5	
Recent adverse life events [3–6], %		4.5	8.1	

BDNF: Brain-derived neurotrophic factor.

[a]Values are mean ± SD unless otherwise indicated. *N#* sample size is $n = 1646$, unless otherwise indicated because of missings on individual measures.

[b]Based on independent-sample t test for continuous variables and χ^2 test for dichotomous and categorical variables. Early and recent life stress are presented as categories for illustrative purposes; continuous variables were used in subsequent analyses.

No significant associations were found for the BDNF variables with neither pain intensity nor pain disability among chronic pain subjects (Table 3). When BDNF expression and serum analyses were additionally adjusted for depression, anxiety, and antidepressants, similar non-significant associations were found with chronic pain presence and severity, with the exception of a significant association for higher BDNF expression with lower pain disability ($\beta = -0.09$, $p = .05$; data not shown).

Previous research has indicated that other confounders may also influence BDNF serum levels.[37] Therefore, we additionally adjusted our fully adjusted model for time of blood withdrawal, time of sample storage, alcohol and food intake, and urbanicity (five categories: <500, 500–1000, 1000–1500, 1500–2500, and >2500 persons/m^2). This did not affect our findings for BDNF serum with the presence of chronic pain (OR [95%CI] = 1.00[0.97–1.03], $p = .91$).

According to a previous study,[44] findings for BDNF levels might differ between men and women, and therefore sex-specific associations with chronic pain may exist. To test this, we repeated analyses of the BDNF pathway with the presence of chronic pain while adding BDNF variable × sex interaction terms to the model. No significant interactions were found for any of the variables of the BDNF pathway (all $p > 0.10$).

Table 2. The associations of the BDNF pathway and life stress with the presence of chronic multi-site musculoskeletal pain.

	N	OR [95%CI]	*P*
BDNF			
BDNF met carrier genotype[a]	1412	1.26 [1.01–1.58]	0.04
BDNF gene expression[b]	1089	0.53 [0.21–1.31]	0.17
BDNF serum levels[b]	1608	1.00 [0.97–1.03]	0.91
Early life stress			
Childhood trauma index [c]	1643	1.56 [1.42–1.72]	<0.001
Recent life stress			
Recent adverse life events [c]	1646	1.30 [1.17–1.44]	<0.001

BDNF: Brain-derived neurotrophic factor.
Note: BDNF genotype: reference group consists of subjects with the Val[66]Val genotype.
Gene expression: OR are per 1 unit increase (relative measure); BDNF serum: OR are per 1 unit (ng/mL) increase; Childhood trauma index: OR are per 1 unit increase in the index; Recent adverse life events: OR are per 1 additional event.
Based on logistic regression analyses, [a]adjusted for age, sex, and population structure; [b]adjusted for age, sex, and smoking; [c]adjusted for age and sex.

Table 3. The associations of the BDNF pathway and life stress with pain severity in subjects with chronic multi-site musculoskeletal pain.

		Pain intensity		Pain disability	
	N	Beta	*p*	Beta	*p*
BDNF					
BDNF met carrier genotype[a]	654	0.04	0.36	−0.03	0.50
BDNF gene expression[b]	534	−0.03	0.44	−0.08	0.06
BDNF serum levels[b]	740	0.04	0.31	−0.03	0.40
Early life stress					
Childhood trauma index[c]	761	0.13	<0.001	0.19	<0.001
Recent life stress					
Recent adverse life events[c]	764	0.15	<0.001	0.14	<0.001

BDNF: Brain-derived neurotrophic factor.
Note: BDNF genotype: reference group consists of subjects with the Val[66]Val genotype.
Based on linear regression analyses, [a]adjusted for age, sex, and population structure; [b]adjusted for age, sex, and smoking; [c]adjusted for age and sex.

Early and recent life stress and the presence and severity of chronic pain

Early and recent life stress were significantly associated with both the presence and the severity (intensity and disability) of chronic pain (see Tables 2 and 3; all $p < 0.001$). Additionally adjusting for depression, anxiety, and antidepressants showed somewhat lower, however significant, odds ratios for both early stress (OR [95%CI] = 1.37[1.24–1.52], $p < 0.001$) and recent stress (OR [95%CI] = 1.20[1.07–1.34], $p = 0.001$) with chronic pain presence. Similarly, the associations with pain severity were somewhat weaker, however, remained statistically significant (early stress: $\beta = 0.10$, $p = 0.004$ with intensity and $\beta = 0.17$, $p < 0.001$ with disability; recent stress: $\beta = 0.13$, $p < 0.001$ with intensity and $\beta = 0.12$, $p = 0.001$ with disability).

Table 4. The interaction of the BDNF pathway with life stress in the association with the presence of chronic multi-site musculoskeletal pain.

	N	P
BDNF × early life stress		
BDNF genotype × trauma[a]	1409	0.53
BDNF expression × trauma[b]	1087	0.74
BDNF serum × trauma[b]	1605	0.24
BDNF × recent life stress		
BDNF genotype × events[a]	1412	0.11
BDNF expression × events[b]	1089	0.19
BDNF serum × events[b]	1608	0.64

BDNF: Brain-derived neurotrophic factor.
Note: BDNF genotype: met carriers vs. Val[66]Val (reference group); trauma = childhood trauma index; events = total number of recent adverse life events.
Based on logistic regression analyses, [a]adjusted for age, sex, and population structure; [b]adjusted for age, sex, and smoking.

The interaction of BDNF with life stress and the presence and severity of chronic pain

No significant interaction effect of the BDNF pathway with life stress was found in the associations with chronic pain presence and severity (see Tables 4 and 5). Two from a total of 18 tested interaction terms appeared statistically significant ($p < 0.10$; Table 5). Additionally adjusting the analyses for depression, anxiety, and antidepressants did not affect these findings.

Discussion

This study showed that BDNF met carriers more often had chronic multi-site musculoskeletal pain compared to val[66]val carriers. BDNF gene expression and serum levels were not associated with chronic pain. Higher early and recent stress levels were independently associated with chronic pain presence and severity but did not alter the impact of BDNF on pain.

Our finding that BDNF met carriers are more likely to have chronic pain is in line with previous studies on pain

Table 5. The interaction of the BDNF pathway with life stress in the association with the severity of chronic multi-site musculoskeletal pain.

		Pain intensity	Pain disability
	N	*p*	*p*
BDNF × early life stress			
BDNF genotype × trauma[a]	651	0.24	0.92
BDNF expression × trauma[b]	532	0.82	0.37
BDNF serum × trauma[b]	737	0.36	0.82
BDNF × recent life stress			
BDNF genotype × events[a]	654	0.19	0.05
BDNF expression × events[b]	534	0.73	0.49
BDNF serum × events[b]	740	0.06	0.16

BDNF: Brain-derived neurotrophic factor.

Note: BDNF genotype: met carriers vs. Val[66]Val (reference group); trauma = childhood trauma index; events = total number of recent adverse life events.

Based on linear regression analyses, [a]adjusted for age, sex, and population structure; [b]adjusted for age, sex, and smoking.

disorders,[16,17] but in contrast to the hypothesis of increased neuroplasticity with upregulated BDNF in pain.[9] Possibly, the met allele might play a role in the brain morphology underlying chronic pain. However, a previous meta-analyses examining val[66]met and hippocampal changes in depression pointed out deleterious effects of underpowered studies and overestimations of effect sizes.[45] Such potential methodological issues should be considered when further exploring the met risk allele as an underlying mechanism of chronic pain.

The met allele has previously been thought to induce lower BDNF secretion.[3,15] However, this study found no direct associations of BDNF gene expression and serum levels with chronic pain, unlike previous studies.[10–13] We also found that val[66]met was uncorrelated with BDNF expression and serum levels. This supports the idea that there is no one-to-one mapping of genotype to gene expression and serum levels.[36,46,47] An explanation may be that peripheral BDNF levels are uncorrelated with whole blood BDNF.[48] Differences in examined body tissues may also explain why previous studies showed inconsistent results for peripheral BDNF levels. On the other hand, previous research has indicated that whole blood does seem to share significant gene expression similarities with multiple brain tissues.[49] Nevertheless, peripheral BDNF levels are likely determined by other factors than the BDNF gene, such as environmental factors,[50] immune[51] or neuroendocrine factors,[52] other genes, and epigenetics.[53]

Life stress did not seem to alter the impact of the BDNF pathway on pain, contrary to our previous NESDA study on depression.[23] Instead, higher levels of early and recent life stress were directly associated with the presence and severity of chronic pain. This finding does support several previous studies.[19,20] Although recall bias cannot be excluded in our cross-sectional study, our results are in line with the notion that psychosocial factors trigger the development of chronic pain.[21,22] The process underlying the link between life stressors and chronic pain should be further examined. The etiology of chronic pain is likely multifactorial including sociodemographic, lifestyle, somatic, and several psychosocial factors.

There are some methodological considerations to our study. First, we only assessed the val[66]met variant, whereas the genetic risk of chronic pain may be increased by polygenetic variation. Studies examining genome-wide associations or a polygenetic risk score for chronic multi-site pain may be useful. Nonetheless, this study was driven by a well-founded hypothesis that BDNF plays a crucial role in pain modulation and neural plasticity.[4,5] Second, we measured BDNF on one day, whereas future research could examine BDNF levels based on several measurements. Nevertheless, we have likely compensated the potentially limited reliability of our BDNF assessment with our large sample size. Third, our classification of chronic pain was pain in the extremities, the back, and the neck in the prior six months. Previous studies mostly examined chronic widespread pain, axial and bilateral pain above and below the waist according to the American College of Rheumatology criteria.[54] We may have included some patients with milder pain due to our ''less stringent'' definition. However, the majority of multi-site pain patients which do not fulfil the ''widespread'' criteria still suffer from severe limitations, high pain intensity, and significant psychological distress.[55] It might hence be useful to set broader parameters for studying mechanisms underlying chronic pain. Fourth, we did not take subjects' appraisals of the life events into account. Future studies could examine whether findings are different when including appraisal of the past experiences. Fifth, our sample contains a high proportion of subjects with depression and anxiety. Nonetheless, these disorders co-occur frequently with chronic pain, and our design did allow us to adjust for their potential confounding effects. This large-scale study was the first to examine the complete BDNF pathway in relation to chronic pain, while testing potential interactions with life stress.

Our data provide evidence that the BDNF val[66]met polymorphism marks vulnerability for chronic pain. Life stress does not seem to increase the impact of BDNF on pain. Instead, life stress may directly trigger the onset or persistence chronic pain.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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