Targeted Genotyping Identifies Susceptibility Locus in Brain-derived Neurotrophic Factor Gene for Chronic Postsurgical Pain

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ABSTRACT

Background: The purpose of this study was to evaluate the association between single-nucleotide polymorphisms and chronic postsurgical pain.

Methods: Using GoldenGate genotyping assays, we genotyped 638 polymorphisms within 54 pain-related genes in 1,152 surgical patients who were enrolled in our Persistent Pain after Surgery Study. Patients were contacted by phone to determine whether they had chronic postsurgical pain at 12 months. Polymorphisms identified were validated in a matched cohort of 103 patients with chronic postsurgical pain and 103 patients who were pain free. The functions of targeted polymorphisms were tested in an experimental plantar incisional nociception model using knock-in mice.

Results: At 12 months after surgery, 246 (21.4%) patients reported chronic postsurgical pain. Forty-two polymorphisms were found to be associated with chronic postsurgical pain, 19 decreased the risk of pain, and 23 increased the risk of pain. Patients carrying allele A of *rs6265* polymorphism in brain-derived neurotrophic factor (BDNF) had a lower risk of chronic postsurgical pain in the discovery and validation cohorts, with an adjusted odds ratio (95% CI) of 0.62 (0.43 to 0.90) and 0.57 (0.39 to 0.85), respectively. Age less than 65 yr, male sex, and prior history of pain syndrome were associated with an increased risk of pain. Genetic polymorphisms had higher population attributable risk (7.36 to 11.7%) compared with clinical risk factors (2.90 to 5.93%). Importantly, *rs6265* is a substitution of valine by methionine at amino acid residue 66 (Val66Met) and was associated with less mechanical allodynia in $BDNF^{Met/Met}$ mice compared with $BDNF^{Val/Val}$ group after plantar incision.

Conclusions: This study demonstrated that genetic variant of BDNF rs6265G>A is associated with decreased risk of chronic postsurgical pain. **(ANESTHESIOLOGY 2018; 128:587-97)**

POSTOPERATIVE pain is an inevitable consequence of surgery. As wound healing is completed with time, it is anticipated that pain will be reduced accompanied by a return of function. However, some patients continue to suffer persistent pain over the surgical wound, which could not be explained by disease recurrence.[1,2] This disease entity, currently known as chronic postsurgical pain, has been estimated to affect 9.2 to 80.0% of patients having a variety of surgery.[3–8] Considering that more than 230 million surgeries are performed each year worldwide,[9] the data would imply that millions of patients will continue to suffer wound pain, months to years after their surgery.

Although peripheral nerve injury, severe acute postoperative pain, and psychosocial factors are thought to increase the

What We Already Know about This Topic

- Chronic postsurgical pain affects 9 to 80% of patients, but genetic determinants are not well identified

What This Article Tells Us That Is New

- Among more than 1,000 surgical patients, 21% reported chronic postsurgical pain 12 months after surgery
- Patients were genotyped for 638 single nucleotide polymorphisms in 54 pain-related genes, with 23 and 19 polymorphisms associated with increased and decreased pain, respectively, including a polymorphism in brain-derived neurotrophic factor associated with lower risk
- Mice with the brain-derived neurotrophic factor polymorphism had a less nociceptive response to a surgical incision compared with wild-type animals

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risk for chronic postsurgical pain,[5,10] considerable efforts have been made to identify the genetic determinants of pain.[11] For instance, single-nucleotide polymorphisms (SNPs) of catecholamine-*O*-methyltransferase (*COMT*) altered pain sensitivity in experimental settings.[12] Similarly, using quantitative trait locus mapping, two variant alleles of the melanocortin-1 receptor gene (*MC1R*) were associated with greater analgesia from κ-opioid in women.[13] Other investigators have found that genetic polymorphism of interleukin-1β is involved in allodynia and possibly postsurgical neuropathic and inflammatory pain.[14] Multiple polymorphisms within GTP cyclohydrolase (*GCH1*) have also been found to predict chronic back pain syndrome.[15] In addition, our recent study found that patients with AA genotypes at polymorphisms, *rs2070697* and *rs2236742*, in cathepsin G (*CTSG*) gene significantly reduced the risk for chronic postsurgical pain.[16] We therefore hypothesized that genetic variations are associated with the development of chronic postsurgical pain. In the current study, we analyzed the association of 638 SNPs located in 54 pain-related genes and explored the risk factors associated with chronic postsurgical pain in the first 1,873 patients enrolled in our Persistent Pain after Surgery Study. We also investigated the functional role of an identified SNP (brain-derived neurotrophic factor, BDNF) in an experimental nociception model using knock-in mice.

Materials and Methods

The Persistent Pain after Surgery Study was an observational cohort study to evaluate the epidemiology and risk factors of chronic postsurgical pain. The study objectives and protocol are summarized in the Chinese Clinical Trial Registry (ChiCTR-ONC-10001099). The Clinical Research Ethics Committee approved the study, and written informed consent was obtained from all patients. Between January 2011 and July 2013, we recruited a total of 1,870 patients into the study from two tertiary referral hospitals. After 1,300 patients had completed 12-month follow-up, we noted that the rate of chronic postsurgical pain was 2.5-fold higher than we anticipated (21.4% *vs.* 8%). Recognizing that we have sufficient events, we decided to conduct an exploratory genetic association analysis in the first 1,152 patients (discovery cohort). These patients were also included in our previous report showing the association between *CTSG* gene polymorphism and risk of chronic postsurgical pain.[16] With the limited sample size, we then selected patients with and without chronic postsurgical pain in a 1:1 ratio, from the remaining patients, for a matched case-control study (validation cohort) to verify the results in the discovery cohort.

Study Participants

We enrolled patients aged 18 yr or older who had in-patient and ambulatory surgery that included a skin incision. Patients were excluded if they were scheduled for a pure endoscopic or radiologic procedure or were previously enrolled in the study. Patients who were not expected to be available for or to cooperate with interviews were also excluded.

Study Procedure and Pain Assessment

Before surgery, research staff interviewed all patients. Baseline patient characteristics, including age, sex, level of education, history of prior pain syndromes, employment status, and smoking habits were recorded. The types of surgery performed and the anesthetic techniques used were noted. After surgery, patients reported their experience of pain over the surgical wound using an 11-point pain analog scale (0 points = no pain to 10 points = worst imaginable pain). The average pain score and the total amount of opioid consumed, expressed as morphine equivalents, during the first 3 postoperative days were recorded.

All patients were contacted again at 12 months after surgery with a telephone interview. Patients were asked to rate the severity of pain, and its interference with daily activities was scored using the brief pain inventory.[17] For each item, 0 points represents no pain or interference, and 10 points indicates worst imaginable pain or completely interferes with activities. The characteristic of the pain was scored using the neuropathic pain questionnaire.[18] In addition, patients reported their current health status using the EQ-5D scale.[19] Use of analgesics and other pain management therapies during the 12 months after the index surgery were also noted. The primary outcome was pain over the surgical site that has persisted for 12 months after the index surgery.[20,21]

GoldenGate Genotyping Assay for the Discovery Cohort

Genomic DNA was extracted from 5 ml of peripheral blood collected at induction of anesthesia according to a standard phenol-chloroform (Applied Biosystems by Life Technologies, USA) procedure. A total of 768 SNPs within 65 genes (Supplemental Digital Content 1, http://links.lww.com/ALN/B564) were selected based on (1) SNP tagging of Han Chinese in Beijing through the HapMap database (ftp://ftp.ncbi.nlm.nih.gov/hapmap/; accessed October 31, 2017); (2)

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the Pain Genes database (http://www.jbldesign.com/jmogil/enter.html; accessed October 31, 2017); and (3) microarray data from our previous study.[11] SNP genotyping by GoldenGate genotyping assay following the manufacturer's instructions (Beadstation 500, Illumina).

The probability of successful genotyping was predicted based on the validation status of the SNP and the minor allele frequencies from published studies using the assay design tool software (Illumina). The results were expressed as a design ability rank score, ranging from 0 to 1.1. For the 768 SNPs, the mean (± SD) design ability rank score was 0.84 ± 0.21 (range, 0.5 to 1.1). A score of 0.4 or higher was considered as having good probability for successful genotyping. We genotyped 1,140 samples with 12 samples left as blank control. To facilitate clustering of genotype, the assay algorithm was trained using one Centre de'Etude du Polymorphism Humain trio in four replicates (NA12878: daughter, NA12891: father, and NA12892: mother). We expressed the performance of the genotyping assay using the GenCall score (0 to 1), which is the ratio of separation between homozygote and heterozygote clusters for any SNP.

Validation Cohort

Patients in the validation cohort were selected from the subsequent 503 patients recruited to the study. We identified 103 patients with chronic postsurgical pain (cases). Matched controls were selected from the first patient who had surgery within the month of the case and who was in the same 5-yr age stratum, sex, and type of surgery. Genotyping of the validation cohort was performed using TaqMan assays (Life Technologies) on 384-well polymerase chain reaction plates with TaqMan genotyping master mix (Life Technologies). Based on the incidence of chronic postsurgical pain in the discovery cohort (more than 20%), we estimated that a validation cohort of 206 patients would provide 83% power to identify SNP with minor allele frequency of more than 20% for an odds ratio of more than 1.2.

Plantar Incisional Nociception Model in BDNF Knock-in Mice

This animal experiment was approved by the institutional animal care and use committee of Shandong University (Shandong, China). Adult male mice (20 to 30 g) of genotypes $BDNF^{Val/Val}$ and $BDNF^{Met/Met}$ were obtained from the Shandong Provincial Key Laboratory of Mental Disorders, Shandong University. Chronic postsurgical pain was studied using a plantar incision model as previously described.[22] In this model, a 10-mm longitudinal incision was made into the plantar surface of the hind paw. Muscle fibers under the wound were then elevated from surrounding tissues and stretched 10 times using curved forceps. The wound was closed with 6-0 nylon sutures and sterilized with iodine solution. The incision model produced consistent mechanical allodynia for more than 6 days. We studied 15 mice with each $BDNF^{Val/Val}$ and $BDNF^{Met/Met}$ genotype. Mechanical allodynia was measured with a series of 10 von Frey filaments (bending force of 0.008, 0.02, 0.07, 0.16, 0.4, 0.6, 1, 1.4, 2, and 4 g) at 6 h and then on days 1, 3, 5, and 7 after surgery. The filaments were applied to an area adjacent to the incision. A positive response was defined as withdrawal of the tested paw related to the application of stimulus. Paw withdrawal threshold was determined by Dixon's up-and-down method.[23] The threshold was calculated as the average of at least three measurements recorded 5 min apart. Investigators who performed surgery and determined the paw withdrawal threshold were blinded to the *BDNF* genotype.

Statistical Analyses

The categorical association for the disease trait (case *vs.* control) was analyzed using chi-square test or Fisher exact test, as appropriate. Student's *t* test was used for continuous variables. PLINK-1.07 (Center for Human Genetic Research, Massachusetts General Hospital Broad Institute of Harvard and MIT, Cambridge, Massachusetts) software was employed for allelic test.[24] Bonferroni correction was used to adjust the *P* values to reduce type I errors due to multiple testing. The Hardy–Weinberg equilibrium statistics and cluster plots of each significantly associated SNP were individually examined. We determined the association between SNPs by haplotype analysis using the CI method (Haploview 4.2, Daly Lab at the Broad Institute, Cambridge, Massachusetts).[25,26] We calculated the normalized coefficient of linkage disequilibrium (D′) and Pearson correlation coefficient (r^2), such that D′ and r^2 values of 1 indicate perfect linkage disequilibrium between SNPs.

We constructed a parsimonious model of independent risk factors for chronic postsurgical pain using the baseline characteristics and types of anesthetic techniques. Genetic factors (SNPs) were also included in our multivariable logistic regression model to test the predictors for chronic postoperative pain. Only factors that scored a *P* value of less than 0.05 in the univariate analysis were incorporated in the multivariable model. A forced simultaneous entry was adopted to avoid overfitting of the model.[27,28] Analysis was performed using Stata 13 (StataCorp LP, USA). We also determined the population attributable risk (PAR) for chronic postsurgical pain to indicate the proportion of outcomes that are attributable to the risk factor concerned.[29] PAR was calculated based on our multivariable regression analysis using the Interactive Risk Attributable Program (IRAP version 2.2, National Cancer Institute, Rockville, Maryland, 2002).

Factorial analysis of variance with repeated measures was used to detect any differences in paw withdrawal thresholds among groups. Significant differences at each of the time points were evaluated using a Bonferroni *post hoc* test. These analyses were performed using Stata 13.

Results

Patient Characteristics

Figure 1 shows the study chart, and table 1 reports patient characteristics in the discovery and validation cohorts. Overall, half of the patients were male with a mean age of 62 yr. About 11% of patients reported preexisting pain syndrome before surgery. Among these patients, 88.7% received regular nonsteroidal

antiinflammatory drugs, 74.0% had gabapentinoid, 7.8% had tramadol, and 5.9% had other opioids. The majority of patients (83.4%) received general anesthesia, whereas 5.2% had regional or neuraxial block alone. The remaining 11.4% of patients had combined general and regional anesthesia.

Incidence, Impact, and Risk Factors of Chronic Postsurgical Pain

At 1-yr follow-up, 246 patients (21.4%) in the discovery cohort (n = 1,152) reported pain over the wound site from the index surgery during the interview. Among these patients, 81 (7.0%) had severe pain with a pain score of 5 points or higher. The median (interquartile range) pain scores for patients reporting mild-to-moderate and severe pain were 2 (1 to 3) and 6 (5 to 8) points, respectively. In the validation cohort, 68 patients (of 103) had mild-to-moderate pain with a median (interquartile range) pain score of 2 (2 to 4), and the remaining 35 patients rated their pain severe with a pain score of 6 (5 to 8) points.

Chronic postsurgical pain affected all types of surgery (table 1). Tables S1, S2, and S3 in Supplemental Digital Content 2 (http://links.lww.com/ALN/B565) show the characteristics and impact of chronic postsurgical pain on daily activities and quality of life. Most patients who had chronic pain reported symptoms attributing to neuropathic pain. During the follow-up period, patients with pain reported difficulties with general activity, mood, walking, relations with others, sleep, and enjoyment of life. General health status was also adversely affected

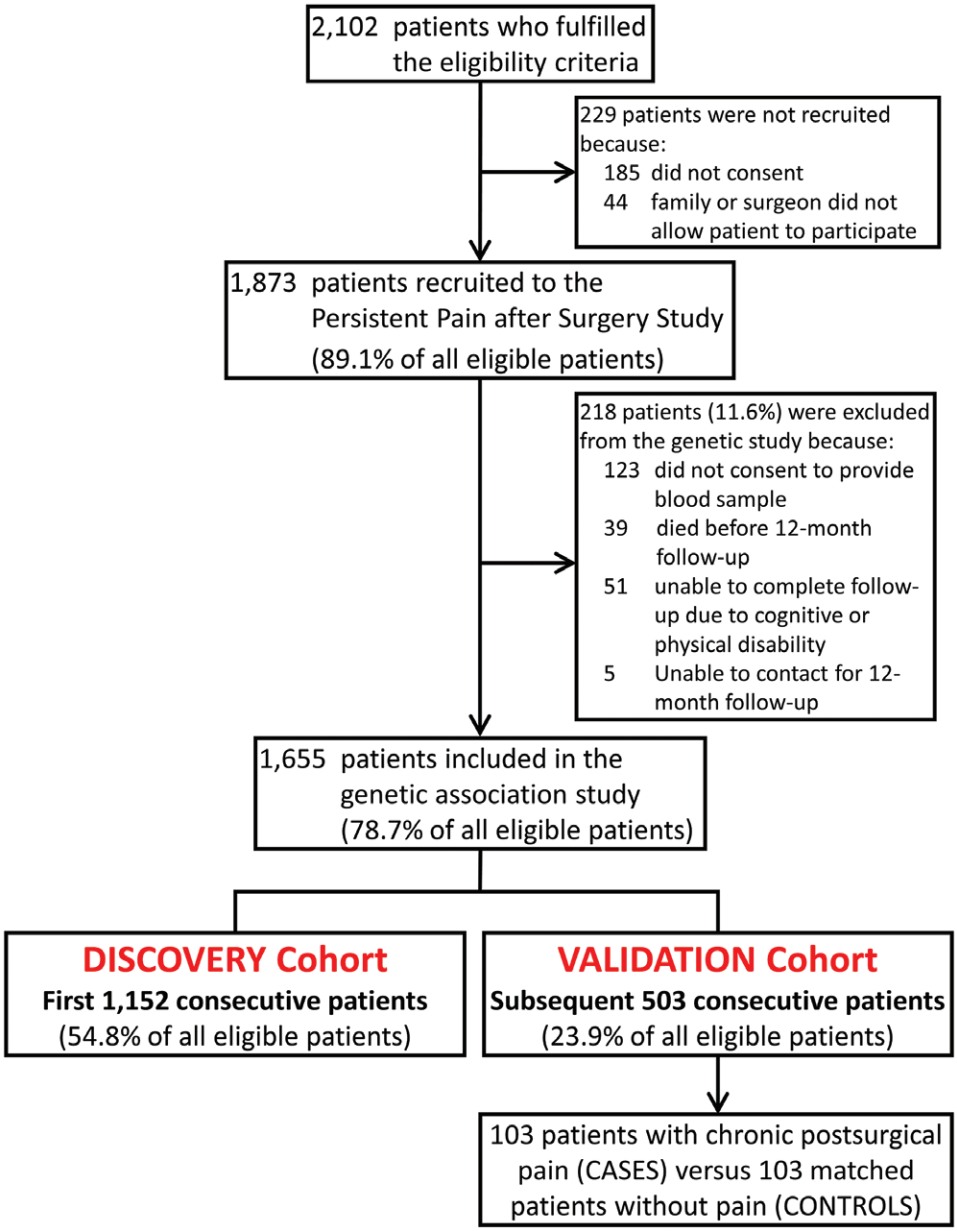

Fig. 1. Study flowchart of the discovery and validation cohorts in the Persistent Pain after Surgery Study.

Table 1. Patient Characteristics, Surgical Details, and Early Postoperative Pain

	Discovery Cohort			Validation Cohort			
Demographic Variables	Mild-to-moderate Pain	Severe Pain	No Pain	Mild-to-moderate Pain	Severe Pain	Matched Controls (No Pain)	Unselected Controls (No Pain)
Number of patients	165	81	906	68	35	103	397
Male sex	102 (61.8%)	50 (61.7%)	399 (44.0%)	51 (75.0%)	24 (68.7%)	75 (72.8%)	228 (57.4%)
Age (yr)	60 ± 13	61 ± 13	62 ± 15	62 ± 12	61 ± 12	61 ± 13	61 ± 13
Weight (kg)	66 ± 11	63 ± 11	62 ± 13	63 ± 12	61 ± 12	62 ± 13	62 ± 13
Unemployed	110 (66.7%)	55 (67.9%)	664 (73.3%)	43 (63.2%)	22 (62.5%)	69 (67.0%)	260 (65.5%)
Years of education completed							
Never went to school	21 (12.7%)	17 (21.0%)	92 (10.2%)	10 (14.7%)	4 (11.4%)	12 (11.6%)	47 (11.8%)
0–6 yr	32 (19.4%)	28 (34.6%)	224 (24.7%)	21 (30.1%)	12 (34.3%)	37 (35.9%)	137 (34.5%)
7–12 yr	47 (28.5%)	28 (34.6%)	345 (38.1%)	27 (39.7%)	14 (40.0%)	37 (35.9%)	151 (38.0%)
> 12 yr	65 (39.4%)	8 (9.8%)	245 (27.0%)	12 (17.6%)	5 (14.3%)	15 (14.6%)	62 (12.4%)
Preexisting comorbidity							
Cardiovascular diseases	11 (6.7%)	2 (2.5%)	44 (4.9%)	4 (5.9%)	2 (5.7%)	4 (3.9%)	18 (4.5%)
Current smoker	42 (25.5%)	23 (28.4%)	247 (27.3%)	26 (38.2%)	15 (42.8%)	44 (42.7%)	96 (24.2%)
Diabetes	19 (11.5%)	10 (12.3%)	130 (14.3%)	7 (10.3%)	5 (14.3%)	16 (15.5%)	56 (14.1%)
Hypertension	39 (23.6%)	17 (21.0%)	221 (24.4%)	15 (22.1%)	8 (22.8%)	26 (25.2%)	165 (41.6%)
Prior pain syndrome	17 (10.3%)	8 (9.8%)	96 (10.6%)	14 (20.6%)	5 (14.3%)	15 (14.6%)	64 (16.1%)
Types of surgery							
General surgery	26 (15.8%)	15 (18.5%)	204 (22.5%)	12 (17.6%)	7 (20.0%)	21 (20.3%)	84 (21.2%)
Neurosurgery–spine surgery	19 (11.5%)	8 (9.8%)	110 (12.1%)	6 (8.8%)	4 (11.4%)	12 (11.7%)	39 (9.8%)
Orthopedic surgery	16 (9.7%)	16 (19.8%)	146 (16.1%)	9 (13.2%)	4 (11.4%)	15 (14.6%)	55 (13.9%)
Thoracic surgery	15 (9.1%)	11 (13.6%)	79 (8.7%)	6 (8.8%)	3 (8.57%)	11 (10.7%)	83 (20.9%)
Vascular surgery	36 (21.8%)	23 (28.3%)	171 (18.9%)	12 (17.6%)	8 (22.8%)	23 (22.3%)	62 (15.6%)
Urologic or gynecologic surgery	30 (18.2%)	7 (8.6%)	141 (15.6%)	12 (17.6%)	6 (17.1%)	17 (16.5%)	48 (12.1%)
Others	18 (10.9%)	1 (1.2%)	70(7.7%)	6 (8.8%)	3 (8.57%)	7 (6.8%)	29 (7.3%)
Anesthetic techniques							
General anesthesia	133 (80.6%)	68 (83.9%)	765 (84.6%)	51 (75.0%)	28 (80.0%)	86 (83.5%)	321 (80.9%)
Neuraxial or regional blocks	9 (5.5%)	5 (6.2%)	47 (5.2%)	2 (2.9%)	2 (5.71%)	5 (4.9%)	18 (4.5%)
Combined general and regional anesthesia	23 (13.9%)	8 (9.8%)	92 (10.2%)	15 (22.1%)	5 (14.3%)	12 (11.7%)	68 (17.1%)
Cumulative opioid consumption during the first 3 days after surgery, morphine equivalent (mg)							
Median (IQR)	15 (12–17)	15 (11–19)	15 (12–18)	15 (11–16)	15 (11–17)	14 (12–19)	15 (11–18)
Mean ± SD	15 ± 14	16 ± 17	15 ± 15	14 ± 13	15 ± 17	17 ± 15	15 ± 13
Average pain score during the first 3 days after surgery							
Median (IQR)	2 (0–5)	3 (1–6)	2 (0–4)	3 (1–5)	3 (1–6)	2 (0–4)	2 (0–4)
Mean ± SD	3 ± 3	3 ± 2	2 ± 2	3 ± 3	3 ± 3	2 ± 2	2 ± 2
Severe acute postoperative pain*	41 (24.8%)	25 (30.9%)	180 (19.8)	18 (26.5%)	11 (31.4%)	21 (20.4%)	87 (21.9%)

The values are number (%), mean ± SD, or median (interquartile range [IQR]).

*Average pain score more than 5 (of 10) during the first 3 days after surgery.

Table 2. Factors Associated with Severe Chronic Postsurgical Pain

Risk Factors	No. of Patients	Patients with Severe Postsurgical Pain	Univariate		Multivariable		
			OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	PAR (95% CI)*
Age (yr)							
> 65	455 (39.5%)	18 (4.0%)	Reference				
≤ 65	697 (60.5%)	63 (9.0%)	2.41 (1.41–4.13)	0.001	1.31 (1.01–1.69)	0.045	5.93% (3.46–10.1)
Sex							
Female	601 (52.2%)	31 (5.2%)	Reference				
Male	551 (47.8%)	50 (9.1%)	1.84 (1.15–2.92)	0.013	1.68 (1.21–2.33)	0.030	4.12% (3.12–5.39)
Education							
Never went to school	130 (11.3%)	17 (13.1%)	Reference				
6 yr	284 (24.7%)	28 (9.9%)	0.73 (0.38–1.38)	0.329	0.89 (0.46–1.73)	0.073	
12 yr	420 (36.5%)	28 (6.7%)	0.47 (0.25–0.90)	0.032	0.52 (0.20–1.36)	0.066	
> 12 yr	318 (27.5%)	8 (2.5%)	0.17 (0.07–0.41)	< 0.001	0.73 (0.53–1.01)	0.081	
Prior pain history							
No	1,031 (89.5%)	65 (6.3%)	Reference				
Yes	121 (10.5%)	16 (13.2%)	2.26 (1.26–4.06)	0.008	1.51 (1.05–2.16)	0.033	4.33% (3.21–5.69)
Employment							
No	829 (72.0%)	55 (6.6%)	Reference				
Yes	323 (28.0%)	26 (8.0%)	1.23 (0.76–2.00)	0.474			
Smoking history							
No	840 (72.9%)	57 (6.8%)	Reference				
Yes	312 (27.1%)	24 (7.7%)	1.14 (0.70–1.88)	0.593			
Severe acute postoperative pain							
No	906 (78.6%)	56 (6.2%)	Reference				
Yes	246 (21.4%)	25 (10.2%)	1.72 (1.05–2.81)	0.012	1.67 (1.07–2.60)	0.045	2.90% (2.06–4.05)
Remifentanil use							
No	226 (19.6%)	12 (5.3%)	Reference				
Yes	926 (80.4%)	69 (7.5%)	1.44 (0.76–2.69)	0.325			
Anesthesia technique							
General anesthesia	968 (84.0%)	68 (7.0%)	Reference				
Regional anesthesia	61 (5.3%)	5 (8.2%)	0.83 (0.32–2.14)	0.729			
Combined technique	123 (2.7%)	8 (6.5%)	1.09 (0.51–2.32)	0.831			
rs6265							
G allele	559 (47.9%)	48 (8.6%)	Reference				
A allele	593 (52.1%)	33 (5.6%)	0.51 (0.37–0.71)	3.68E-05	0.62 (0.43–0.90)	0.023	9.77% (4.06–23.5)
rs1491850							
A allele	581 (49.9%)	48 (8.3%)	Reference				
G allele	571 (50.1%)	33 (5.8%)	0.52 (0.36–0.72)	5.55E-05	0.47 (0.32–0.70)	0.035	7.36% (4.06–13.1)
rs6265–rs1491850 haplotype							
Haplotype G–A	310 (26.9%)	41 (51.2%)	Reference				
Haplotypes A–G, A–A, G–G*	842 (73.1%)	40 (48.8%)	0.33 (0.21–0.52)	1.21E-06	0.41 (0.26–0.65)	<0.001	11.7% (5.32–25.1)
Total explained			—	—	—	—	38.2% (25.3–55.8)

The values are number (%), mean ± SD, or median (range).

*Haplotype refers to patients carrying one of the two alleles from single-nucleotide polymorphisms *rs6265* and *rs1491850*, respectively.

OR = odds ratio; PAR = population attributable risk (the percentage of all outcomes attributable to the relevant risk factor from a multivariable logistic regression analysis).

Table 3. Hit Genes from GoldenGate Assay

Genes	Hit SNPs		Official Symbol	Molecular Function
	No.	NCBI_ID		
ADORA1	2	*rs7549561* *rs10920570*	Adenosine A1 receptor	Adenosine receptor
BDNF	3	*rs2030324* *rs1491850* *rs6265*	Brain-derived neurotrophic factor	Growth factor
CTSG	2	*rs2070697* *rs2236742*	Cathepsin G	Peptidase
GABRB3	3	*rs2114217* *rs4906896* *rs11632969*	$GABA_A$ receptor, β3	Ion channel ($GABA_A$ gated)
GAD1	2	*rs3791862* *rs3791853*	Glutamate decarboxylase 1	Glutamate decarboxylase (GABA production)
GRIK1	10	*rs2253443* *rs2832392* *rs1011794* *rs459249* *rs2300306* *rs363426* *rs2300318* *rs3787671* *rs2254136* *rs2255985*	Glutamate receptor, ionotropic, kainate 1	Ion channel (glutamate gated)
GRIN2A	1	*rs11646587*	Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 2A	Ion channel (glutamate gated)
GRIN2B	9	*rs219936* *rs219915* *rs1012586* *rs2284410* *rs1805476* *rs1558908* *rs7295850* *rs11055619* *rs220573*	Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 2B	Ion channel (glutamate gated)
HTR2A	5	*rs9562689* *rs2770298* *rs1328683* *rs1923886* *rs9567736*	5-Hydroxytryptamine (serotonin) receptor 2A	Amine (serotonin) receptor
NPY	1	*rs12532490*	Neuropeptide Y	Hormone
SLC18A2	2	*rs363226* *rs10082463*		Amine (serotonin) transporter
OPRK1	2	*rs6985606* *rs7836120*	Opioid receptor, κ1	Peptide receptor

GABA = γ-aminobutyric acid; $GABA_A$ = γ-aminobutyric acid type A; NCBI_ID = National Center for Biotechnology Information identifier.

by pain. Age less than or equal to 65 yr of age, male sex, receiving less than 12 yr of education, reporting prior history of pain elsewhere, and reporting severe postoperative pain during the first 3 days after surgery were associated with higher risk for chronic postsurgical pain (table 2).

Genotype Analyses

Among 768 SNPs, the minor allele frequencies of 130 SNPs were less than 5%. The remaining 638 SNPs in 54 genes had an average call rate of 99.2% (median frequency 97.8%). In 59 samples, the call rate was less than 97%. The allelic frequencies of all process controls, as integrated in the GenomeStudio software (Illumina, USA), were found to be within the reported range. In an association analysis of the discovery cohort, a total of 42 SNPs from 12 candidate genes were found to be associated with chronic postsurgical pain (table 3) with 23 SNPs (54.8%) increasing the risk of pain. After Bonferroni correction (*P* value < 0.05/638 or < 7.84E-5), two SNPs (*rs6265* and *rs1491850*) in the *BDNF* gene, and one (*rs6985606*) in the κ-opioid receptor gene (*OPRK1*)

Table 4. Allelic Frequencies of *rs6265*, *rs1491850*, and *rs6985606* of Chronic Postsurgical Pain in the Discovery and Validation Cohorts

		All Patients with Chronic Postsurgical Pain								Patients with Severe Chronic Postsurgical Pain (VAS > 5).							
		Discovery Cohort				Validation Cohort				Discovery Cohort				Validation Cohort			
				Allelic Test				Allelic Test				Allelic Test				Allelic Test	
SNPs		Alleles		*P* Value	OR (95% CI)	Alleles		*P* Value	OR (95% CI)	Alleles		*P* Value	OR (95% CI)	Alleles		*P* Value	OR (95% CI)
rs6265		G*	A			G*	A			G*	A			G*	A		
	Pain	279	213	4.63E-05	0.66 (0.54–0.81)	89	117	0.005	0.57 (0.39–0.85)	96	66	3.68E-05	0.51 (0.37–0.71)	54	16	0.001	0.27 (0.12–0.60)
	No pain	840	972			117	89			912	1230			152	190		
rs1491850		G	A*			G	A*			G	A*			G	A*		
	Pain	204	288	5.05E-05	0.65 (0.53–0.80)	119	87	0.002	1.87 (1.26–2.77)	66	96	5.55E-05	0.52 (0.36–0.72)	60	10	0.002	6.67 (2.63–16.7)
	No pain	938	874			87	119			1222	920			146	196		
rs6985606		G*	A			G*	A			G*	A			G*	A		
	Pain	242	250	5.05E-05	0.71 (0.58–0.86)	99	107	0.490	0.86 (0.58–1.26)	79	83	0.008	1.54 (1.12–2.12)	34	36	0.859	1.06 (0.53–2.13)
	No pain	1076	736			107	99			1272	870			172	170		

*Reference allele.
OR = odds ratio.

Fig. 2. Haplotypic information across brain-derived neurotrophic factor (*BDNF*) gene identified by Haploview. The *triangle* shows the haploblock and the strength of linkage disequilibrium among the six single-nucleotide polymorphisms genotypes.

remained significantly associated with chronic postsurgical pain (Supplemental Digital Content 1, http://links.lww.com/ALN/B564). Association was still significant under all modes of inheritance (Supplemental Digital Content 3, http://links.lww.com/ALN/B566).

We further validated the association between the three SNPs and chronic postsurgical pain in a matched cohort of 206 surgical patients by TaqMan genotyping assay. Table 4 shows the allelic frequencies of the SNPs. The genotypic distribution of the three SNPs were in Hardy–Weinberg equilibrium. Consistent with the discovery cohort, patients carrying allele A of *rs6265* in the *BDNF* gene were associated with a lower risk for chronic postsurgical pain compared with those carrying allele G. In contrast, the effect of the SNP *rs1491850* in the *BDNF* gene was opposite in the validation cohort compared with the discovery cohort. The association of SNP (*rs6985606*) in the *OPRK1* gene on chronic postsurgical pain could not be confirmed in the validation cohort.

Based on the discovery cohort, a haploblock was identified in the *BDNF* gene (fig. 2). We found that both *rs6265* and *rs1491850* were in block with the highest strength of linkage disequilibrium ($D' = 0.899$; $r^2 = 0.74$). Similar findings were obtained with a haplotype analysis of all the SNPs in the *BDNF* gene reported in the literature (Supplemental Digital Content 4, http://links.lww.com/ALN/B567). Both SNPs in the *BDNF* gene (*rs6265* and *rs1491850*) and its haploblock remained associated with chronic postsurgical

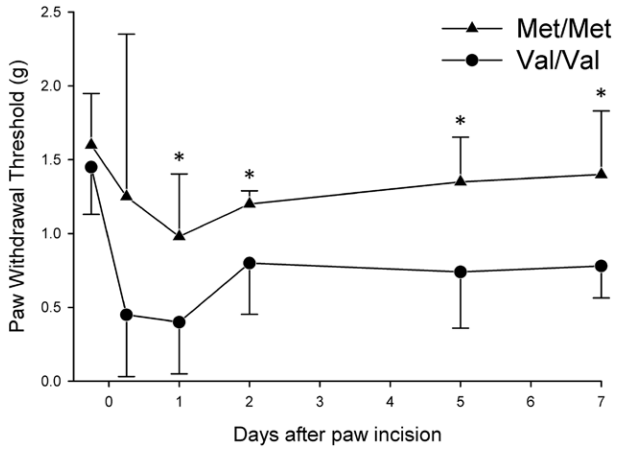

Fig. 3. Incisional nociceptive mouse model. A 10-mm longitudinal incision was made on the plantar surface of the left hind paw. Changes in paw withdrawal in response to pressure using von Frey filaments in brain-derived neurotrophic factor (*BDNF*)^{Met} knock-in (methionine/methionine [Met/Met]) and wild-type (valine/valine [Val/Val]) mice (n = 15 per group) were measured. Factorial analysis of variance with repeated measures, $P = 0.003$. $^{*}P < 0.05$ for Bonferroni *post hoc* test. The values are mean ± SD.

pain when adjusted for clinical risk factors. The genetic factors had the largest population attributable risk (table 2).

Attenuated Mechanical Allodynia after Plantar Incision in BDNF^{Met} Knock-in Mice

The data presented so far suggested that both *rs6265* and *rs1491850* in *BNDF* gene were associated with chronic postsurgical pain. Although the latter is in the promotor region, *rs6265* is a nonsynonymous SNP located in exon 2 of *BDNF* gene. To determine the functional role of *rs6265*, mechanical allodynia was measured in mice expressing the corresponding alteration of amino acid in *BDNF*. Concordant with our human data, mechanical allodynia was significantly decreased in *BDNF*$^{Met/Met}$ mice compared with the *BDNF*$^{Val/Val}$ group ($P = 0.003$, factorial analysis of variance with repeated measures; fig. 3).

Discussion

Our study showed that chronic postsurgical pain was common. In a relatively unselected group of adult patients, more than 20% of patients reported persistent pain in the wound for 1 yr after surgery. Among these patients, 32.9% rated their pain as severe. The incidence is similar to a recently reported cohort of patients having abdominal hysterectomy (25.1%) and thoracotomy (37.6%).[7] Our data also confirmed that chronic postsurgical pain affects quality of life, including social function, physical activities, emotion, and mental health.

In an attempt to investigate the genetic factors for chronic postsurgical pain, we evaluated a total of 638 SNPs from 54 pain-related genes. After rigorous screening, we found that SNP *rs6265* in *BDNF* gene was associated with chronic postsurgical pain in two independent cohorts. BDNF is a secretory protein in the nervous system that binds to tyrosine-related kinase B (TrkB) and regulates synaptic plasticity by activating several signaling cascades.[30] In an experimental nociception model, *de novo* synthesis of BDNF protein activates postsynaptic excitatory receptor (*e.g.*, *N*-methyl-D-aspartate receptor) and suppresses inhibitory receptors (*e.g.*, γ-aminobutyric acid receptors).[31] Each of these processes contributes to central sensitization in different parts of nociceptive pathways. Interestingly, antagonism of BDNF or TrkB alleviated nociceptive response in animals.[32,33] Recently, it has been shown that BDNF levels correlate with pain intensity. For instance, an elevated BDNF level was found in patients with frequent and severe abdominal pain because of chronic pancreatitis and irritable bowel syndrome.[34]

The SNP *rs6265* is located in the 5′-prodomain of immature BDNF protein, leading to a methionine (Met) substitution for the normal valine (Val) at codon 66 and hence also known as Val66Met.[34,35] In our study, patients who carried the G allele (Val/Met and Val/Val) were associated with higher risk of chronic postsurgical pain. In this regard, *rs6265* (Val66Met) is one of the most widely studied SNPs in the *BDNF* gene. A number of genetic association studies have evaluated the role of Val66Met variants in nervous system function or disorders, such as memory formation, anxiety, and substance abuse. Recently, it was reported that the Val66Met variant may also affect cortical pain processing during experimental pain stimuli.[36] In that study, the Met allele augmented cortical pain processing in chronic pain patients and reduced pain processing in the pain-free controls. Similarly, in cultured hippocampal neurons, BDNF protein carrying the Met variant exhibited lower depolarization-induced secretion.[37] In Met variant *BDNF*-transgenic mice, secretion of BDNF from neurons was abolished, accompanied by an increased stress-related anxiety.[38,39] These data suggested Val66Met is a functional variant that reduces BDNF-dependent activity. In the nociceptive pathway, activity-dependent release of endogenous BDNF has been reported in nociceptors[40] and can be enhanced during inflammation.[41] These studies confirmed the hypothesis that the Met allele impairs the BDNF release from nociceptors and thus reduces pain. Our findings from the clinical cohort study and transgenic *BDNF*^{Met} knock-in mice are consistent with this hypothesis. In mice, Met carriers were less likely to develop mechanical allodynia after plantar incision surgery. Previous studies suggested that BDNF affected chronic pain development *via* modulating ion channel activity and gene expression.[31–37] BDNF induces long-term potentiation in *N*-methyl-D-aspartate receptor, and this may last for more than 8 h in the central nociceptive neurons, such as those in the spinal dorsal horn lamina II neurons and therefore contributes to the production of neuropathic pain. On the other hand, it has been reported that the insufficient BDNF release and therefore the lack of late long-term potentiation in postsynaptic neurons prevented neuropathic pain. BDNF also causes transcriptional changes in spinothalamic neurons and contributes to the long-lasting central sensitization in the nociceptive pathway.[40,41]

Previous studies have shown that between 30 and 76% of pain behaviors in animals are due to heritability factors.[32–41] In our study, we found that 9.77 and 7.36% of chronic postsurgical pain could be attributed to genetic variants at *rs6265* and *rs1491850* in *BNDF*, respectively. Aside from genetic factors, other risk factors have been proposed to increase the risk of chronic postsurgical pain. In our study, we found that younger patients (65 yr of age or less) were associated with increased risk of chronic pain after surgery, which is consistent with a previous study.[4] Our study also found that male patients are at higher risk for chronic postsurgical pain than females. Importantly, we found the SNPs and its haplotype (PAR: 7.36 to 11.7%) had larger contributions than other clinical factors (PAR: 2.90 to 5.93%) for the development of chronic postsurgical pain.

Taken together, our data suggest that the variant of BDNF rs6265G>A was associated with lower risk of chronic postsurgical pain. It is propitious that routine genotyping of the SNP may help to identify high-risk surgical patients for more intense management of postsurgical pain.

Appendix. Persistent Pain after Surgery Study Group

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Competing Interests

The authors declare no competing interests.

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References

1. Macrae WA, Davies HT: Chronic postsurgical pain. Epidemiology of Pain: A Report of the Task Force on Epidemiology of the International Association for the Study of Pain. Edited by Crombie IK, Croft PR, Linton SJ, LeResche L, Von Korff M. Seattle, IASP Press, 1999, pp. 125–42
2. Werner MU, Kongsgaard UE: I. Defining persistent post-surgical pain: Is an update required? Br J Anaesth 2014; 113:1–4
3. Bruce J, Krukowski ZH: Quality of life and chronic pain four years after gastrointestinal surgery. Dis Colon Rectum 2006; 49:1362–70
4. Gärtner R, Jensen MB, Nielsen J, Ewertz M, Kroman N, Kehlet H: Prevalence of and factors associated with persistent pain following breast cancer surgery. JAMA 2009; 302:1985–92
5. Niraj G, Rowbotham DJ: Persistent postoperative pain: Where are we now? Br J Anaesth 2011; 107:25–9
6. Johansen A, Romundstad L, Nielsen CS, Schirmer H, Stubhaug A: Persistent postsurgical pain in a general population: Prevalence and predictors in the Tromsø study. Pain 2012; 153:1390–6
7. Montes A, Roca G, Sabate S, Lao JI, Navarro A, Cantillo J, Canet J; GENDOLCAT Study Group: Genetic and clinical factors associated with chronic postsurgical pain after hernia repair, hysterectomy, and thoracotomy: A two-year multicenter cohort study. Anesthesiology 2015; 122:1123–41
8. Chan MT, Peyton PJ, Myles PS, Leslie K, Buckley N, Kasza J, Paech MJ, Beattie WS, Sessler DI, Forbes A, Wallace S, Chen Y, Tian Y, Wu WK; and the Australian and New Zealand College of Anaesthetists Clinical Trials Network for the ENIGMA-II investigators: Chronic postsurgical pain in the Evaluation of Nitrous Oxide in the Gas Mixture for Anaesthesia (ENIGMA)-II trial. Br J Anaesth 2016; 117:801–11
9. Weiser TG, Regenbogen SE, Thompson KD, Haynes AB, Lipsitz SR, Berry WR, Gawande AA: An estimation of the

global volume of surgery: A modelling strategy based on available data. Lancet 2008; 372:139–44

10. Kehlet H, Jensen TS, Woolf CJ: Persistent postsurgical pain: Risk factors and prevention. Lancet 2006; 367:1618–25
11. Hoofwijk DM, van Reij RR, Rutten BP, Kenis G, Buhre WF, Joosten EA: Genetic polymorphisms and their association with the prevalence and severity of chronic postsurgical pain: A systematic review. Br J Anaesth 2016; 117:708–19
12. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D: COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science 2003; 299:1240–3
13. Mogil JS, Wilson SG, Chesler EJ, Rankin AL, Nemmani KV, Lariviere WR, Groce MK, Wallace MR, Kaplan L, Staud R, Ness TJ, Glover TL, Stankova M, Mayorov A, Hruby VJ, Grisel JE, Fillingim RB: The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. Proc Natl Acad Sci USA 2003; 100:4867–72
14. Danis VA, Millington M, Hyland VJ, Grennan D: Cytokine production by normal human monocytes: Inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol 1995; 99:303–10
15. Tegeder I, Costigan M, Griffin RS, Abele A, Belfer I, Schmidt H, Ehnert C, Nejim J, Marian C, Scholz J, Wu T, Allchorne A, Diatchenko L, Binshtok AM, Goldman D, Adolph J, Sama S, Atlas SJ, Carlezon WA, Parsegian A, Lötsch J, Fillingim RB, Maixner W, Geisslinger G, Max MB, Woolf CJ: GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. Nat Med 2006; 12:1269–77
16. Liu X, Tian Y, Meng Z, Chen Y, Ho IH, Choy KW, Lichtner P, Wong SH, Yu J, Gin T, Wu WK, Cheng CH, Chan MT: Up-regulation of cathepsin G in the development of chronic postsurgical pain: An experimental and clinical genetic study. Anesthesiology 2015; 123:838–50
17. Wang XS, Mendoza TR, Gao SZ, Cleeland CS: The Chinese version of the Brief Pain Inventory (BPI-C): Its development and use in a study of cancer pain. Pain 1996; 67:407–16
18. Backonja MM, Krause SJ: Neuropathic pain questionnaire: Short form. Clin J Pain 2003; 19:315–6
19. Rabin R, de Charro F: EQ-5D: A measure of health status from the EuroQol Group. Ann Med 2001; 33:337–43
20. Turk DC, Dworkin RH, Burke LB, Gershon R, Rothman M, Scott J, Allen RR, Atkinson JH, Chandler J, Cleeland C, Cowan P, Dimitrova R, Dionne R, Farrar JT, Haythornthwaite JA, Hertz S, Jadad AR, Jensen MP, Kellstein D, Kerns RD, Manning DC, Martin S, Max MB, McDermott MP, McGrath P, Moulin DE, Nurmikko T, Quessy S, Raja S, Rappaport BA, Rauschkolb C, Robinson JP, Royal MA, Simon L, Stauffer JW, Stucki G, Tollett J, von Stein T, Wallace MS, Wernicke J, White RE, Williams AC, Witter J, Wyrwich KW; Initiative on Methods, Measurement and Pain Assessment in Clinical Trials: Developing patient-reported outcome measures for pain clinical trials: IMMPACT recommendations. Pain 2006; 125:208–15
21. Dworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP, Kerns RD, Stucki G, Allen RR, Bellamy N, Carr DB, Chandler J, Cowan P, Dionne R, Galer BS, Hertz S, Jadad AR, Kramer LD, Manning DC, Martin S, McCormick CG, McDermott MP, McGrath P, Quessy S, Rappaport BA, Robbins W, Robinson JP, Rothman M, Royal MA, Simon L, Stauffer JW, Stein W, Tollett J, Wernicke J, Witter J; IMMPACT: Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain 2005; 113:9–19
22. Pogatzki EM, Raja SN: A mouse model of incisional pain. Anesthesiology 2003; 99:1023–7
23. Dixon WJ: The up-and-down method for small samples. J Am Stat Assoc 1965; 60: 967–78
24. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559–75
25. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. Science 2002; 296:2225–9
26. Barrett JC, Fry B, Maller J, Daly MJ: Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263–5
27. Steyerberg EW, Eijkemans MJ, Harrell FE Jr, Habbema JD: Prognostic modeling with logistic regression analysis: In search of a sensible strategy in small data sets. Med Decis Making 2001; 21:45–56
28. Derksen S, Keselman H: Backward, forward and stepwise automated subset selection algorithms: Frequency of obtaining authentic and noise variables. Br J Math Stat Psychol 1992; 45: 265–82
29. Cox C: Model-based estimation of the attributable risk in case-control and cohort studies. Stat Methods Med Res 2006; 15:611–25
30. Berglind WJ, Whitfield TW Jr, LaLumiere RT, Kalivas PW, McGinty JF: A single intra-PFC infusion of BDNF prevents cocaine-induced alterations in extracellular glutamate within the nucleus accumbens. J Neurosci 2009; 29:3715–9
31. Metsis M, Timmusk T, Arenas E, Persson H: Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. Proc Natl Acad Sci USA 1993; 90:8802–6
32. Zafra F, Castrén E, Thoenen H, Lindholm D: Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. Proc Natl Acad Sci USA 1991; 88:10037–41
33. Ramer LM, McPhail LT, Borisoff JF, Soril LJ, Kaan TK, Lee JH, Saunders JW, Hwi LP, Ramer MS: Endogenous TrkB ligands suppress functional mechanosensory plasticity in the deafferented spinal cord. J Neurosci 2007; 27:5812–22
34. Yu YB, Zuo XL, Zhao QJ, Chen FX, Yang J, Dong YY, Wang P, Li YQ: Brain-derived neurotrophic factor contributes to abdominal pain in irritable bowel syndrome. Gut 2012; 61:685–94
35. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR: The BDNF Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003; 112:257–69
36. Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS: Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 2006; 314:140–3
37. Vossen H, Kenis G, Rutten B, van Os J, Hermens H, Lousberg R: The genetic influence on the cortical processing of experimental pain and the moderating effect of pain status. PloS One 2010; 5:e13641
38. Malcangio M, Ramer MS, Jones MG, McMahon SB: Abnormal substance P release from the spinal cord following injury to primary sensory neurons. Eur J Neurosci 2000; 12:397–9
39. Lever IJ, Bradbury EJ, Cunningham JR, Adelson DW, Jones MG, McMahon SB, Marvizón JC, Malcangio M: Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation. J Neurosci 2001; 21:4469–77
40. Lariviere WR, Wilson SG, Laughlin TM, Kokayeff A, West EE, Adhikari SM, Wan Y, Mogil JS: Heritability of nociception: III. Genetic relationships among commonly used assays of nociception and hypersensitivity. Pain 2002; 97:75–86
41. Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, Pieper JO, Hain HS, Belknap JK, Hubert L, Elmer GI, Chung JM, Devor M: Heritability of nociception: II. "Types" of nociception revealed by genetic correlation analysis. Pain 1999; 80:83–93