


COMT gene variants and β -endorphin levels contribute to ethnic differences in experimental pain sensitivity

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Abstract

This cross-sectional study investigated whether the catechol-O-methyltransferase (COMT) gene acts as a significant regulator of pain signaling pathways, regulates β -endorphin, and contributes to ethnic differences in pain sensitivity. One-hundred-sixty healthy subjects were enrolled in this study, with Han and Uyghur groups each consisting of 80 participants. Subjects went through six pain threshold experiments. From venous blood, COMT polymorphisms were genotyped, and serum β -endorphin levels were measured. Bivariate correlation analysis and multiple linear regression were used to identify the relationships among genotypes or β -endorphin levels and different types of pain thresholds. Han and Uyghur ethnic differences were determined in terms of acute-pressure pain-perception thresholds, blunt-pressure pain-perception thresholds, blunt-pressure pain-tolerance thresholds, electric pain-tolerance thresholds, β -endorphin levels, and distributions of rs4680 and rs4633 COMT polymorphisms. β -endorphin levels did not correlate with COMT rs4680 or rs4633 genotypes in both Han and Uyghur. Statistical predictors for a lower pain-threshold performance included being young, Uyghur, female, and having a low body mass index, low β -endorphin level, and the rs4680 GA or GG allele. There is the significant difference in pain sensitivity between healthy Han and Uyghur. COMT gene variants and β -endorphin levels contribute to ethnic differences in pain sensitivity.

Keywords

COMT gene, single-nucleotide polymorphism, variants, β -endorphin, pain threshold

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Introduction

In pain genetics, many investigators have explored numerous candidate genes involved in opioid receptors, pharmacokinetics, analgesia, and neurotransmission; some noteworthy examples include opioid receptor mu (OPRM) and sodium voltage-gated channel alpha subunit 9.^{1–3} Single-nucleotide polymorphisms (SNPs) are characterized by a variation in a single nucleotide that occurs at a specific position in the genome. SNPs can be located within coding or noncoding regions of genes and can alter gene splicing, transcription factor binding, amino acid sequences, and biological functions. SNPs have been applied in genetic studies to determine whether specific genetic variants are associated with painful sensibility or clinical traits.⁴

Catechol-O-methyltransferase (COMT) metabolizes catecholamines including adrenaline, noradrenaline, and dopamine and is regulated by SNPs that induce diverse COMT activity in individuals.⁵ The COMT gene is located on the

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long arm of chromosome 22 and consists of six exons that cover over 27 kb. In this 27-kb genomic region, over 900 genetic variants have been discovered. Three COMT SNPs—rs4633, rs4680, and rs4818—are located within the central coding region and are responsible for both membranous and soluble forms of COMT (S-COMT and MB-COMT, respectively).⁶ Among several genetic variants, some COMT SNPs have been identified as biomarkers with clinical significance. A functional SNP in codon 158 (Val 158 Met or rs4680) in the COMT gene decreases COMT activity by three- to fourfold and has been reported to regulate pain perception and affect opioid demands.^{7–9} In addition, haplotypes composed of COMT alleles of rs6269, rs4633, rs4818, and rs4680 have been illustrated to influence the expression and activity of COMT and to correlate with pain responses (Figure 1(a)).^{8,10} Currently, there is a paucity of studies that have investigated the influence of COMT genotypes on ethnic differences in pain sensitivity, especially in China.¹¹

β -endorphin (β -END) is an opioid neuropeptide produced by the pituitary gland and serves various functions, ranging from cellular activity to behavioral performance, which include synaptic transmission, food intake, and pain control.¹² β -END primarily interacts with the mu-opioid peptide receptor and has the affinity to delta-opioid peptide receptors.¹³ During the 1980s, a series of published studies from clinical and animal experiments demonstrated that levels of β -END were correlated with pain responses.^{14–17} The analgesic effects of acupuncture, electrostimulation, magnetic stimulation, and physical

exercise may be attributable to β -END via increasing levels of β -END.¹² A previous clinical study in 80 patients with chronic lower back pain revealed that β -END levels were higher in controls compared to those in patients with chronic lower back pain.¹⁸ A published animal study reported that exogenous opioids, such as morphine, induced a decrease in β -END.¹⁹ These findings indicate that decreased β -END levels affect nociceptive perception, pain thresholds, and pain control.

In addition, β -END binding affinities and activity are regulated by OPRM (A118G) variants.²⁰ A joint effect and interaction between OPRM and COMT genes have been reported to influence opioid consumption and pain perception.^{21,22} Thus, we hypothesized that COMT gene variants act as significant regulators of pain signaling pathways and β -END levels and contribute to racial differences in pain sensitivity. Few studies have combined analysis of β -END levels with COMT gene polymorphisms to determine their contributions to ethnic differences in pain sensitivity between healthy individuals from Han and Uyghur lineages.²³ Therefore, we determined COMT genotypes and measured pain sensitivities and β -END levels from 80 healthy Han individuals and 80 healthy Uyghur individuals.

Materials and methods

Participants and study design

This study was the observational, cross-sectional. Healthy subjects in trial were recruited by advertisements, with

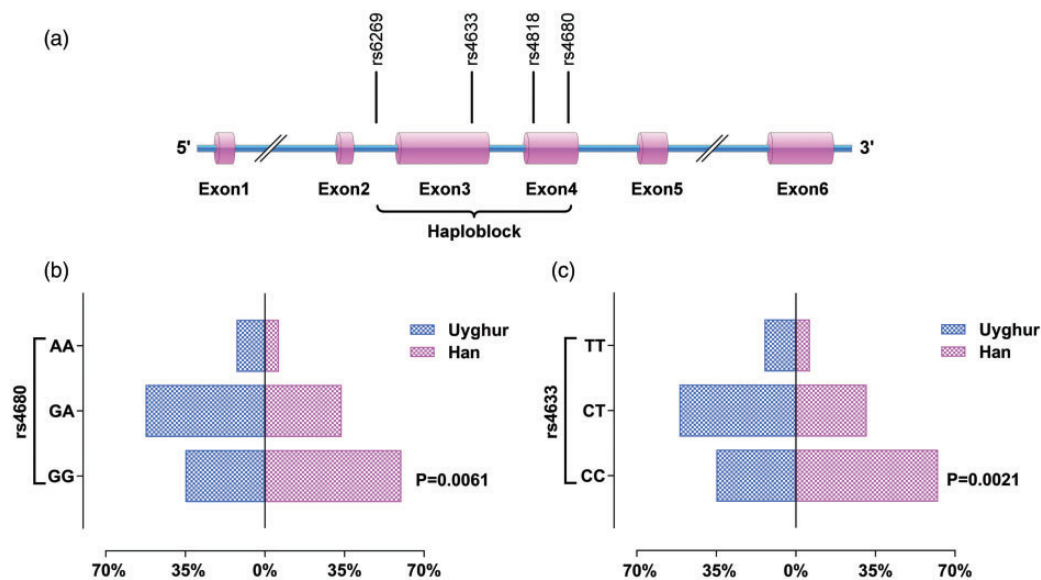


Figure 1. (a) Haploblock and SNP structure of the COMT gene. Location of four common SNPs has been shown from 5' to 3' in the COMT gene and has been demonstrated for their association with pain sensitivity. (b) COMT rs4680 genotype (AA, GA, or GG) distribution in Han and Uyghur groups. For the rs4680 genotype frequency, Han (n = 80) versus Uyghur (n = 80) yielded $\chi^2 = 10.19$ and $P = 0.0061$ via a χ^2 test. (c) COMT rs4633 genotype (TT, CT, or CC) distribution in Han and Uyghur groups. For the rs4633 genotype frequency, Han (n = 80) versus Uyghur (n = 80) yielded $\chi^2 = 12.33$ and $P = 0.0021$ via a χ^2 test.

signed informed consents. The study protocol was conducted at the First Affiliated Hospital in the School of Medicine at Shihezi University (Xinjiang) and was granted by the hospital ethics committee (approval number: 2018-099-02), in accordance with *Declaration of Helsinki*. The present study enrolled 160 healthy adults (61 males and 99 females) who were equally distributed into two ethnic groups (80 Han and 80 Uyghur). In addition, this study was also approved by the Chinese Clinical Trial Registry (register number: ChiCTR-EOC-17012968).

Eligibility criteria

Inclusion criteria as follows: 18 to 60 years old; no history of basic diseases, including hypertension, coronary heart disease, and diabetes; and subjects' parents and grandparents were all married within their respective ethnic groups. Exclusion criteria as follows: analgesics taken either orally or intravenously; long-term residents not in Xinjiang; individuals from single-parent families; orphans; women during pregnancy, lactation or menstruation; limbs with fractures, joint trauma history, or abnormal sensation; limbs with skin ulcerations, infections, abnormal feelings, or scars; history of mental illness; or an inability to communicate in basic Chinese or to cooperate with researchers.

Experimental pain measures

Subjects finished four trial sessions within a one-week period. First, subjects completed questionnaires for obtaining basic information and health status were instructed on the process of pain-threshold measurements and provided blood specimens. Then, three experimental pain-threshold tests—including electric pain threshold (EPT), blunt-pressure pain threshold (BPPT), and acute-pressure pain threshold (APPT)—were sequentially performed in the morning. Different types of pain thresholds were measured one day apart, and the same type of pain-threshold test was repeated twice, at intervals of 1 h each.

An electric pain-threshold instrument (EP601C, Shanghai, China) was used in strict accordance with the instruction manual. The primary measurement steps were as follows. The negative electrode was tied to the upper leg of the subject. Then, the positive electrode was placed 10 cm away from the right medial malleolus. The electric current was then raised from 0 to 5 mA. When subjects first felt pain, the electric current values were recorded as the electric pain-perception threshold (EPPT). In addition, when participants were no longer able to stand the electric stimulation, the electric current values were recorded as the electric pain-tolerance threshold (EPTT).

A hand-held pain meter, with measurements from 0 to 100 N, was purchased from Beijing Jinuotai Technology Development Co., Ltd. (Beijing, China). The mechanical-tenderness probe consisted of a metal cylinder with a diameter of 1 cm. During the BPPT test, the probe was placed in the middle of the right wrist joint (median nerve).²⁴ The pain instrument was pressed down, and the pressure reading on the electronic screen was observed. The pressure at the time of the first perceived pain stimulus (blunt-pressure pain-perception threshold, BPPPT) and the pressure at the time of unbearable pain (blunt-pressure pain-tolerance threshold, BPPTT) were recorded.

APPT can be measured by using an acupuncture probe with a hand-held pain meter. During APPT measurements, the probe was located in the central position of the dorsal middle finger of the right hand.²⁵ Then, the hand-held pain meter was pressed down, and the resultant pressure reading on the electronic screen was observed. We then recorded the pressure of the first felt pain stimulation (acute-pressure pain-perception threshold, APPPT) and the pressure of unbearable pain (acute-pressure pain-tolerance threshold, APPTT) of each subject.

SNP genotyping

A whole-blood genomic DNA extraction kit (Genenode, Beijing, China) was employed for total genomic DNA extraction from venous blood samples. Two COMT SNPs—rs4633 (C/T), and rs4680 (A/G)—were genotyped via real-time polymerase chain reaction (PCR) using TaqMan genotyping assays. PCR for COMT SNPs was conducted by using 2×EasyTaq PCR SuperMix (TransGen Biotech, Beijing, China). The PCR mixture was composed of 1 µl of DNA sample, 0.5 µl of forward primer (10 µM), 0.5 µl of reverse primer (10 µM), 12.5 µl of 2×EasyTaq PCR SuperMix, and 10.5 µl of double-distilled water. The settings used for PCR amplification were according to the manufacturer's instructions. The PCR primers for COMT SNP rs4633 were as follows: forward primer, 5'-GAGGCACACACCTGCTCTGTCTAC-3' and reverse primer, 5'-GTAAGGGCTTTGATGCCTGGTC-3'. The PCR primers for COMT SNP rs4680 were as follows: forward primer, 5'-CCTGCACAGGCAAGATCGTGGGA-3' and reverse primer, 5'-TTAGGGTTCTGGGATGACAAGGCC-3'.

β-END enzyme-linked immunosorbent assay

A serum separator tube was used to store venous blood samples, and samples were allowed time to clot for 30 min before centrifugation for 10 min at 3000×g. Then, serum was extracted for enzyme-linked immunosorbent assay (ELISA). β-END concentrations were

measured by using ELISA. ELISA was performed according to the manufacturer's instructions (Kenuodi Biotech, Quanzhou, China).

Statistical analysis

Categorical variables were described by frequencies and percentages. Mean \pm standard deviation or median (interquartile range) was used for descriptive analysis of continuous variables. Kolmogorov–Smirnov test or Shapiro–Wilk test was performed to assess the normality. Chi-squared (χ^2) tests, independent-sample t tests, or Mann–Whitney U tests were employed for analyses of demographics. Independent-sample t tests or Mann–Whitney U tests were utilized to determine differences in pain thresholds and β -END levels between the two groups. Bivariate correlation analysis (Pearson or Spearman) was used to evaluate the correlations between phenotypic characteristics or demographic variables and pain thresholds. Only variables with a P value of <0.2 were included for further analysis in multiple linear regression, which filters and removes unrelated variables. The backward stepwise method was used in multiple linear regression to remove mixed variables, and the Durbin–Watson test was conducted to identifying independence among variables. A standardized correlation coefficient and covariance of the model (R^2) were used. SPSS 22.0 was used for data analysis, with P value <0.05 considered to be statistically significant. A Hardy–Weinberg equilibrium (HWE) test was conducted by using a χ^2 test. Two of the COMT SNPs—rs4633 (C/T) and rs4680 (A/G)—were in equilibrium after the HWE test in the data from the Han and Uyghur group. In NCBI (National Center of Biotechnology Information) SNP databases, the allele GG of rs4680 (A/G) was reported to be a common allele variant (<https://www.ncbi.nlm.nih.gov/snp/?term=rs4680>). In the present study, the percentages of rs4680 (GG) in the Han

and Uyghur groups were 0.60 and 0.35, respectively. After PASS 11.0 calculations, a sample size of 80 in the Han group and 80 in the Uyghur group was predicted to achieve 89% power in detected a difference between the two groups, via a two-sided Z test with a significance level of $P < 0.05$. Thus, this experiment has sufficient sample size to find the COMT genotype difference between the two races.

Results

Demographics

Demographics and multiple types of pain thresholds are summarized in Table 1. One-hundred-sixty healthy individuals were enrolled in the present study, with 80 subjects designated to each ethnic group. The body mass index (BMI) in the Uyghur group was greater than that in the Han group ($Z = -5.56$, $P < 0.001$). In terms of pain thresholds, in comparison to those of the Han group, the Uyghur group had lower APPPT, BPPPT, BPPTT, and EPTT ($Z = -2.89$, $P = 0.0041$; $Z = -9.40$, $P < 0.001$; $t = 9.44$, $P < 0.001$; and $Z = -3.76$, $P < 0.001$, respectively).

COMT genotype distributions in Han and Uyghur

The minor and major alleles of COMT SNPs are presented in Figure 1(b) and (c), which revealed significant differences in the genotype distributions of rs4680 and rs4633 between the two ethnic groups. For rs4680, the minor A allele frequency of the Uyghur group was greater than that of the Han group (38.8% vs. 23.1%, $\chi^2 = 9.14$, $P = 0.0025$). For rs4633, the minor T allele frequency of the Uyghur group was greater than that of the Han group (39.4% vs. 21.9%, $\chi^2 = 11.53$, $P < 0.001$).

Table 1. Demographic characteristics and multiple types of pain thresholds.

Characteristics	Response	Han (n = 80)	Uyghur (n = 80)	Statistics
Age, years		49.50 (33.50–57.00)	45.00 (36.00–55.75)	$Z = -0.79$, $P = 0.43$
BMI/kg·m ⁻²		23.41 (21.51–25.73)	28.46 (24.71–30.46)	$Z = -5.56$, $P < 0.001$
Gender, %	Male	26 (32.50)	35 (43.75)	$\chi^2 = 2.15$, $P = 0.14$
	Female	54 (67.50)	45 (56.25)	
APPPT/N		1.27 (1.10–1.74)	1.10 (1.03–1.55)	$Z = -2.89$, $P = 0.004$
APPTT/N		4.41 (3.71–4.96)	4.16 (3.77–5.91)	$Z = -0.25$, $P = 0.80$
BPPPT/N		21.73 (20.22–22.95)	11.51 (10.84–13.26)	$Z = -9.40$, $P < 0.001$
BPPTT/N		59.46 \pm 13.81	40.49 \pm 11.52	$t = 9.44$, $P < 0.001$
EPPT/mA		1.10 (0.86–1.40)	1.15 (0.75–1.94)	$Z = -0.83$, $P = 0.41$
EPTT/mA		3.05 (2.21–4.01)	2.23 (1.45–3.23)	$Z = -3.76$, $P < 0.001$

Note: The mean \pm standard deviation or median (interquartile range) was used for descriptive analysis according to the normality or nonnormality of distributions. BMI: body mass index; APPPT: acute-pressure pain-perception threshold; APPTT: acute-pressure pain-tolerance threshold; BPPPT: blunt-pressure pain-perception threshold; BPPTT: blunt-pressure pain-tolerance threshold; EPPT: electric pain-perception threshold; EPTT: electric pain-tolerance threshold. Z value of Mann–Whitney U test; χ^2 : Chi square test; t value of independent-sample t test.

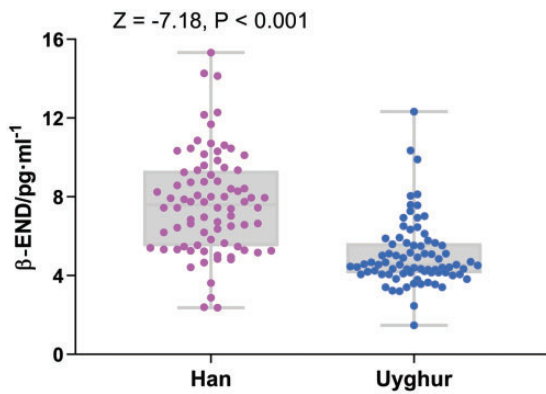


Figure 2. Serum β -END levels in Han and Uyghur groups. For serum β -END levels, Han ($n = 80$) versus Uyghur ($n = 80$) yielded $Z = -7.18$ and $P < 0.001$ via a Mann–Whitney U test.

For the COMT rs4680 genotype, when compared with those of the Uyghur group, the Han group had a lower percentage of AA and GA alleles and a higher percentage of the GG allele (6.2% of AA, 33.8% of GA, and 60% of GG in Han group vs. 12.5% of AA, 52.5% of GA, and 35% of GG in Uyghur group, $\chi^2 = 10.91$, $P = 0.0061$). For the rs4633 genotype, in comparison with those of the Uyghur group, the Han group had a lower percentage of TT and CT alleles and a higher percentage of the CC allele (6.3% of TT, 31.2% of CT, and 62.5% CC in Han group vs. 13.8% of TT, 51.2% of CT, and 35% CC in Uyghur group, $\chi^2 = 12.33$, $P = 0.0021$).

β -END levels in Han and Uyghur

β -END levels between the two groups are shown in Figure 2. Previous clinical studies have reported that β -END levels positively correlate with experimental pain thresholds.²⁶ In our present study, the Han group with higher pain thresholds had higher levels of β -END compared with those of the Uyghur group (7.59 [5.49–9.32]/Pg.ml⁻¹ vs. 4.56 [4.11–5.6]/Pg.ml⁻¹, $Z = -7.18$, $P < 0.001$).

Correlation between β -END levels and COMT genotypes in Han and Uyghur

Furthermore, to identify whether the COMT gene interacts with β -END levels to affect pain thresholds, bivariate correlation analysis was used to evaluate the potential association between these two factors. After Bonferroni correction for multiple correlation analyses, there was no significant association between β -END levels and rs4680 or rs4633 COMT genotypes, in Han or Uyghur group, as shown in Table 2.

Table 2. Bivariate correlation analysis between β -END levels and rs4633 or 4680 COMT SNPs.

Race	Independent variables	Responses, N = 160 (%)	β -END levels	
			R	P
Han	rs4680	1-AA 5 (6.25)	0.034	0.77
		2-GG 48 (60)		
		3-AG 27 (33.75)		
Uyghur	rs4633	1-CC 50 (62.5)	-0.15	0.18
		2-TT 5 (6.25)		
		3-CT 25 (31.25)		
Han	rs4680	1-AA 10 (12.5)	0.014	0.90
		2-GG 28 (35)		
		3-AG 42 (52.5)		
Uyghur	rs4633	1-CC 28 (35)	0.089	0.43
		2-TT 11 (13.75)		
		3-CT 41 (51.25)		

Note: β -END: β -endorphin; R: correlation coefficient. Spearman correlation used for bivariate correlation analysis. Bonferroni's correction was used for multiple correlation analyses: $P < 0.025$.

Bivariate correlation analysis in pain thresholds

In order to further explore the underlying factors related to some types of pain thresholds, bivariate correlation analyses—including Spearman and Pearson correlation tests—were used to investigate the relationship between different kinds of pain thresholds and demographics or SNP variants. Table 3 summarizes some factors associated with pain thresholds. With a significance level of $P < 0.2$, BPPPT, BPPTT, and EPTT were associated with demographics, SNP variants, and β -END levels, which effectively removes unrelated variables in further analysis. Thus, BPPPT, BPPTT, EPTT, and relevant factors were included in multiple linear regression.

Multiple linear regression in pain thresholds

Multiple linear regression was fitted to examine the effects of COMT SNP phenotypes/genotypes, β -END levels, and demographics on pain sensitivities. Age, BMI, ethnicity, and the rs4680 COMT variant had statistically significant effects on EPTT ($F = 12.49$, $P < 0.001$, adjusted $R^2 = 0.22$). Gender, ethnicity, and β -END levels had statistically significant effects on BPPTT ($F = 46.24$, $P < 0.001$, adjusted $R^2 = 0.46$). Finally, age, ethnicity and gender had statistically significant effects on BPPPT ($F = 116.83$, $P < 0.001$, adjusted $R^2 = 0.69$). Statistical predictors for a lower pain-threshold performance included being young, Uyghur, female, and having a low BMI, low β -END level, and rs4680 GA allele or GG allele (Table 4).

Discussion

The present study investigated the association between COMT variants—responsible for metabolizing

Table 3. Bivariate correlation analysis of factors associated with different kinds of pain thresholds.

Independent variables	Response, N = 160 (%)	Dependent variables											
		APPPT		APPTT		BPPPT		BPPTT		EPPT		EPTT	
		R	P	R	P	R	P	R	P	R	P	R	P
Age		-0.051	0.53	-0.081	0.31	0.27	0.0010	-0.0060	0.94	0.17	0.034	0.27	<0.001
BMI		-0.035	0.67	0.038	0.64	-0.24	0.0022	-0.18	0.027	0.25	0.0013	0.14	0.088
Ethnicity	1-H 80 (50)	-0.23	0.0037	0.020	0.80	-0.75	<0.001	-0.62	<0.001	0.067	0.40	-0.30	<0.001
	2-U 80 (50)												
Gender	1-M 61 (38)	-0.27	<0.001	-0.36	<0.001	-0.081	0.44	-0.27	<0.001	-0.30	<0.001	-0.12	0.12
2-F 99 (62)													
rs4680 variant	1-AA 15 (9)	-0.064	0.42	-0.055	0.49	-0.14	0.086	-0.14	0.077	-0.10	0.19	-0.25	0.0015
	2-GG 76 (48)												
	3-AG 69 (43)												
rs4633 variant	1-CC 78 (49)	-0.072	0.37	-0.048	0.55	-0.22	0.0049	-0.17	0.031	-0.026	0.74	-0.22	<0.001
	2-TT 16 (10)												
	3-CT 66 (41)												
β -END		0.035	0.66	-0.088	0.91	0.43	<0.001	0.42	<0.001	-0.10	0.20	0.078	0.33

Note: Pearson or Spearman correlations were used for bivariate correlation analysis, according to classified data or continuous data. BMI: body mass index; H: Han; U: Uyghur; M: male; F: female; β -END: β -endorphin; APPPT: acute-pressure pain-perception threshold; APPTT: acute-pressure pain-tolerance threshold; BPPPT: blunt-pressure pain-perception threshold; BPPTT: blunt-pressure pain-tolerance threshold; EPPT: electric pain-perception threshold; EPTT: electric pain-tolerance threshold. R: correlation coefficient.

Table 4. Multiple linear regression analysis in COMT variants, demographic variables, EPTT, and BPPTT.

Independent variable	Unstandardized coefficients	SE	Standardized coefficients	t	P
Dependent variable: EPTT					
Constant	2.31	0.60		3.85	<0.001
Age	0.018	0.0070	0.20	2.70	0.0078
BMI	0.065	0.021	0.25	3.06	0.0026
Race	-0.83	0.18	-0.37	-4.70	<0.001
rs4680	-0.35	0.12	-0.20	-2.84	0.0051
Model fit: R = 0.49, R ² = 0.24, adjusted R ² = 0.22, DW = 1.74, F = 12.49, P < 0.001					
Dependent variable: BPPTT					
Constant	87.74	6.36		13.79	<0.001
Gender	-10.04	1.91	-0.31	-5.26	<0.001
Race	-17.90	2.17	-0.57	-8.26	<0.001
β -END	0.84	0.42	0.14	1.98	0.049
Model fit: R = 0.69, R ² = 0.47, adjusted R ² = 0.46, DW = 1.53, F = 46.24, P < 0.001					
Dependent variable: BPPPT					
Constant	26.75	1.45		18.49	<0.001
Race	-8.37	0.47	-0.79	-17.71	<0.001
Age	0.10	0.020	0.23	5.20	<0.001
Gender	-1.51	0.49	-0.14	-3.11	0.0023
Model fit: R = 0.83, R ² = 0.69, adjusted R ² = 0.69, DW = 2.06, F = 116.83, P < 0.001					

Note: The backward method used in multiple linear regression and F value of analysis of variance used for detecting the statistical significance of model fitting. BPPPT: blunt-pressure pain-perception threshold; BPPTT: blunt-pressure pain-tolerance threshold; EPTT: electric pain-tolerance threshold. R: correlation coefficient; β -END: β -endorphin; DW: Durbin-Watson test; SE: standard error; BMI: body mass index. Bonferroni's correction was used for multiple analyses: P < 0.05/3 (0.017).

catecholamines and mediating nociceptor signaling pathways—and β -END levels, which are involved in pain perception and pain thresholds, in healthy Han and Uyghur individuals. Differences in APPPT, BPPPT, BPPTT, EPTT, β -END levels, and COMT rs4680 and rs4633 genotype distributions were found between the Han and Uyghur groups. After multiple linear regression analyses, age, BMI, ethnicity, and the COMT rs4680 variant correlated with EPTT; gender, race, and β -END levels correlated with BPPTT; finally, age, race, and gender correlated with BPPPT. In addition, Spearman correlation analysis failed to reveal a relationship between β -END levels and COMT rs4680 and rs4633 genotypes. Thus, the present study represents the first report describing COMT rs4680 variants and β -END levels contributing to differences in pain thresholds between healthy Han and Uyghur individuals in China.

Among numerous clinical observational studies of COMT SNPs, many investigators have focused on the effect of rs4680 on clinical conditions, including experimental pain sensitivity, dosage/efficiency of opioids, and pain occurrence in several diseases (e.g., cancer).^{27–29} A polymorphism of rs4680 in exon 4 changes the amino acid sequence, transforming valine (Val) to methionine (Met) at a location of 108 in S-COMT and a location of 158 in MB-COMT, both of which are associated with enzymatic activity.^{2,6} According to the rs4680 genotype, COMT activity is classified into either a low level (AA), median level (AG), or high level (GG).³⁰ Previous studies that have recruited healthy individuals have reported that participants with the GG genotype of rs4680 have higher pain sensitivity to pain stimuli.³¹ Similarly, in our present study, after multiple-factor analysis, the rs4680 genotype was associated with EPTT, and carriers of the AG or GG allele also had lower pain thresholds in comparison with those of carriers of the AA allele. In addition, the Uyghur group had a greater percentage of the AG allele compared to that in the Han group. Therefore, an imbalance in the rs4680 genotype distribution contributed to a difference in experimental pain thresholds between the two ethnic groups assessed in the present study.

β -END levels have been demonstrated to correlate with pain sensitivity.^{12,32,33} Previous studies have reported that SNPs of the ATP-binding cassette B1 and OPRM genes affect β -END binding affinities, activities, and/or concentrations.^{18,34} Hence, we hypothesized that some pain-related candidate genes interacting with β -END levels modulate pain sensitivities. However, we failed to discover an interaction between COMT SNPs and β -END levels in terms of impacting differences in pain thresholds between the Han and Uyghur groups. A plausible explanation for this result is that COMT is primarily responsible for metabolizing adrenaline,

noradrenaline, and dopamine, whereas it rarely affects binding affinities of opioid receptors or endogenous opioid peptides in healthy individuals. After univariate and multivariate correlation analysis, we found that β -END levels were positively associated with pain thresholds. In addition, ethnic differences in β -END levels were also discovered between Han and Uyghur cohorts, which suggests that β -END levels contribute to ethnic differences in experimental pain sensitivities.

Differences in demographic characteristics—such as age, gender, BMI, and race—have been shown to lead to variability of pain sensibility.^{35–38} In our present work, we also demonstrated that demographic characteristics were indispensable predictors of pain thresholds. EPT and PPT are often used in quantitative assessment of pain sensitivity in clinical studies.^{39–41} In this work, EPT and PPT, as the reliable tool of pain sensitivity measurement, were also employed to identify the race difference in mechanical pain threshold, by compression and electric current stimulation, which efficiently prevented pain stimulus error caused by the single repetitive stimulus.

We applied three types of experimental pain-threshold tests, which effectively avoided biases caused by a single measurement method. In addition, bivariable correlation analysis was harnessed to eliminate unrelated variables and to increase the accuracy and efficiency of multiple linear regression analysis. However, our present study had some limitations. The average age of our study was 45.11 years, which may affect the sensitivity of pain stimulus. Older age, to some extent, reduces or disturbs the effect COMT gene variants on pain threshold. After all, our paper has shown that pain sensitivity declines with aging. Furthermore, pain sensitivity is influenced by multiple biomedical patterns including physiology, psychology, society, and so on. Many factors such as education, salary, marriage status, culture, life style, and so on, are not all included in study. In terms of multiple biomedical mode, gene-by-environment interaction may be a determinant of some epigenetic phenomena or clinical conditions.⁴² Hence, the exploration of pain epigenetic phenotype is multi-dimensional and diversified. Our future studies will be designed to examine the potential role of common COMT haplotypes interacting with environments on pain sensitivities in healthy Chinese individuals.

The present study described the ethnic differences in APPPT, BPPPT, BPPTT, EPTT, β -END levels, and COMT rs4680 and rs4633 genotype distributions between Han and Uyghur group. β -END levels and COMT rs4680 variants, as the valuable bio-marker of pain sensitivity, are helpful to hold the explanation of the diversity of pain sensitivity in multiple ethnic groups. The interaction between β -END levels and COMT SNPs on pain sensitivity has not been found in our work. Multiple-factor analysis reported that demographic

characteristics—such as age, gender, BMI, and race, β -END levels or COMT SNPs, were associated with pain threshold, which suggests gene-by-environment interaction pattern may be as the determinant in diversity of pain feeling.

Authors' Contributions

Study design and plan: FX, JY, and EX; study performing: FX, JY, EX, RW, JZ, LX, YL, XQ, EW, QZ, YZ, SF, and SW; statistical analysis: FX and JY; drafting paper: FX; revising paper: FX, JY, EX, and SW.

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Declaration of Conflicting Interests

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