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Original Article

Association of *ADRB1* gene polymorphisms with pain sensitivity in a Chinese population

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Abstract: Objective: The present study aims to observe the influence of single-nucleotide polymorphisms (SNPs) in the ADRB1 gene on individual differences in pain sensitivity. Methods: We analyzed the associations between pain sensitivity and ADRB1 gene SNPs (A145 G and G1165 C) in 324 Chinese patients who underwent surgery. The genotyping was performed using PCR-RFLP technique. Results: The patients who carried the A-allele of the A145 G SNP were more sensitive to cold pressor-induced pain than those who did not carry this allele ($P < 0.05$). We did not found G1165 C polymorphism associated with pain sensitive in the present study. The haplotype analysis revealed A-C haplotype carriers have reduced fentanyl use in 24-h postoperative ($P < 0.05$). Conclusion: *ADRB1* gene polymorphisms are associated with pain and analgesic sensitivity.

Keywords: β1-adrenergic receptor, gene polymorphism, opioid, pain, individual difference

Introduction

Excessive pain will increase psychological health problems markedly, therefore, analgesics was commonly used in the management of pain. However, proper analgesic doses can markedly differ between individuals. These differences may result from environmental and genetic factors [1, 2]. Several candidate genes involving in the pain analgesic sensitivity have been reported previously [3-5], however, the underlying mechanisms of interindividual differences in pain and analgesic sensitivity have not yet been sufficiently elucidated.

Recent study indicated that the human *â1*-adrenergic receptor gene (*ADRB1*) was associated with pain and analgesic sensitivity [6]. *ADRB1* gene consists of only one exon that contains short untranslated regions [6]. There are several SNPs in *ADRB1* gene have been reported to be associated with clinical traits [7-9] including A145 G SNP (rs1801252) and G1165 C SNP (rs1801253). Rs1801252 causes an amino acid substitution from serine to glycine at amino acid position 49 and the rs1801253 causes a Gly389 Arg amino acid substitution at the intracellular *C*-terminus of the β1-adrenergic receptor. Although these two SNPs has been reported to be associated with hypertension [10], stroke [7], and heart disease [11], only one study reported the relation between *ADRB1* genetic polymorphisms and pain and analgesic sensitivity [6]. However, the association between *ADRB1* and pain sensitivity in Chinese population remains unclear.

In the present study, we focused on the *ADRB1* gene and analyzed the influence of the A145 G and G1165 C SNPs in the *ADRB1* gene on individual differences in pain and analgesic sensitivity in a Chinese population.

Subjects and methods

Subjects

324 patients who were scheduled to undergo surgery at Eastern Hepatobiliary Surgery Hospital of Second Military Medical University from February 2010 to December 2014 were selected. Patients with chronic pain, who took pain medication, or had experienced Raynaud's phenomenon were excluded.

The study protocol was approved by the Institutional Review Board, Eastern Hepatobiliary

Table 1. Primer sequences and PCR products

SNPs	Primer sequence	PCR product	Annealing temperature	Endonuclease
rs1801252	upstream:5'-GACCTCCCTCTGCGCACCAC-3'	389	57	Sau96
	downstream:5'-CTGAGGTCCACAGCTCGCAGA-3'			
rs1801253	upstream:5'-ACGCTGGGCATCATCATGGGC-3'	332	56	Mva I
	downstream:5'-ACATCGTCGTCGTCGTCGTCC-3'			

Table 2. Genotype and allele frequency of these two SNPs

SNPs	Genotype			Allele	
rs1801252	AA	AG	GG	A	G
	230 (71.0%)	84 (25.9%)	10 (3.1%)	0.84	0.16
rs1801253	CC	CG	GG	C	G
	190 (58.6%)	106 (32.7%)	28 (8.7%)	0.75	0.25

Table 3. General comparison of different genotypes

Genotype	N	Sex (M/F)	Age (Year)	BMI (Kg/m^2)
rs1801252				
AA	230	148/82	42.5±12.4	19.3±2.6
AG	84	55/29	41.8±12.1	18.9±2.5
GG	10	6/4	42.0±12.1	19.0±2.8
P value		0.146	0.871	0.456
rs1801253				
CC	190	101/69	41.9±11.5	19.1±2.8
CG	106	85/34	42.7±12.3	18.8±2.6
GG	28	32/13	42.1±12.7	19.0±2.4
P value		0.641	0.476	0.552

Surgery Hospital of Second Military Medical University. Written informed consent was obtained from all of the patients and parents if required, and the study was conducted in accordance with the Declaration of Helsinki.

Preoperative cold pressor-induced pain test

Preoperative cold pressor-induced pain test were performed according to the previous literatures [6]. Briefly, the dominant hand was immersed up to the wrist in the ice-cold water. The patients were instructed to keep their hand calm in the ice-cold water and withdraw it as soon as they perceived any pain. All of the patients had the test conducted by the same investigator. The baseline latency to pain perception (PPLpre) was defined as the time of immersion of the hand in the ice water before fentanyl injection. A cut-off time of 150 s was set to avoid tissue damage. The hand was warmed until the sensation of cold was completely abolished. Three minutes after the injection, the pain perception latency of the dominant hand (PPLpost) was measured again. The analgesic effect of fentanyl in the preoperative cold pressor-induced pain test was evaluated simply as the difference between PPLpost and PPLpre (PPLpost-PPLpre).

Postoperative pain management

Intraoperative fentanyl use and postoperative PCA fentanyl use during the first 24-h postoperative period were recorded. Doses of fentanyl administered intraoperatively and postoperatively were normalized with body weight. Additionally, perioperative fentanyl use was calculated as the sum of intraoperative fentanyl use and postoperative fentanyl use because the analgesic effect of the intermediate-acting opioid fentanyl, administered pre- and intraoperatively, could outlast the duration of surgery and thus affect postoperative fentanyl use, especially in patients who received a large dose of fentanyl intraoperatively. Therefore, in the present study, we considered perioperative fentanyl use an appropriate indicator of fentanyl analgesia in addition to postoperative fentanyl use.

Genotyping procedures

5 ml peripheral blood was collected before surgery and anticoagulated by EDTA; phenol - chloroform extraction method was used to extract DNA. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) was used for the genotyping of A145 G and G1165 C SNP in *ADRB1* gene. PCR primer sequences [6] and PCR-RFLP conditions were shown in **Table 1**.

Statistical analyses

Measurement data were presented as mean ± standard deviation (Mean ± SD); factorial analysis of two factors and three levels was per-

Table 4. Comparison of pain threshold and pain tolerance among different genotypes

Genotype	N	PPLpre (s)	Analgesic effect (PPLpost-PPLpre) (s)	24-h postoperative fentanyl use (μg/kg)	VAS pain score at 24 h (mm)
rs1801252					
AA	230	17.4 [9.5, 33.5]	13.1 [4.7, 38.0]	2.5 [1.5, 4.8]	24.4 [9.3, 43.3]
AG	84	18.7 [9.0, 34.8]	14.1 [6.4, 41.1]	3.4 [1.1, 4.9]	26.2 [16.1, 53.4]
GG	10	37.3 [18.5, 90.4]	40.4 [10.7, 94.6]	1.6 [0.6, 8.8]	20.4 [3.9, 34.0]
P value		0.032	0.021	0.543	
rs1801253					
CC	190	17.1 [9.5, 25.0]	14.4 [5.6, 51.3]	2.7 [1.5, 4.8]	24.8 [10.6, 42.6]
CG	106	17.5 [8.1, 24.7]	10.3 [5.3, 30.5]	2.8 [1.1, 4.9]	25.6 [12.4, 44.2]
GG	28	19.1 [9.3, 23.3]	15.4 [9.4, 39.4]	2.6 [0.5, 6.3]	21.6[3.9, 36.5]
P value		0.419	0.129	0.376	0.449

Table 5. Interaction analysis between OPRM1 and COMT genes

Haplotype	PPLpre (s)	Analgesic effect (PPLpost-PPLpre) (s)	24-h postoperative fentanyl use (μg/kg)	VAS pain score at 24 h (mm)
A-C	15.1 [9.1, 34.2]	11.2 [4.4, 38.9]	1.6 [1.1, 4.0]	20.1 [9.0, 43.0]
A-G	20.6 [9.3, 38.3]	14.2 [4.5, 38.7]	2.8 [1.6, 4.9]	24.5 [9.1, 46.6]
G-C	30.3 [9.1, 88.1]	22.3 [8.4, 41.5]	3.6 [1.0, 5.8]	26.0 [16.1, 48.4]
G-G	38.0 [20.2, 93.4]	40.1 [10.5, 94.0]	2.8 [1.6, 8.4]	22.3 [4.1, 39.0]
P value	0.014	0.024	0.048	0.201

formed; a weighted analysis of variance was performed between groups. Count data were compared using the chi-square test. Hardy-Weinberg equilibrium was tested by chi-square test. $P < 0.05$ was considered statistically significant.

Results

Hardy-Weinberg equilibrium test

Genotyping results of A145G and G1165 C SNP in ADRB1 gene were shown in **Table 2**; the distribution of genotypes was consistent with Hardy-Weinberg equilibrium (all $P > 0.05$).

Comparison of the characteristic of patients between different genotypes

According to different genotypes, the age, gender, BMI and other indicators were compared, and there were no significant differences between genotypes (**Table 3**).

Associations between genotypes of the ADRB1 SNP and sensitivity to pain and analgesics

PPLpre was significantly less in patients with the A-allele of the A145G SNP compared with patients without this allele in all of the patients and males, respectively ($P = 0.032$ and 0.036, respectively; **Table 4**), indicating that the patients who carried the A-allele of A145G SNP were more sensitive to cold pressor-induced pain than those who did not carry this allele, especially in male patients. However, we did not found G1165C SNP associated with the pain sensitivity.

In the analysis of PPLpost-PPLpre, which reflects the preoperative analgesic effect, although we did not find significant differences among A145 G or G1165 C genotype in fentanyl use in 24-h postoperative, we found A-C haplotype carriers have reduced fentanyl use in 24-h postoperative (**Table 5**).

Discussion

In the present study, we found the *ARDB1* genetic polymorphisms were associated with pain and fentanyl sensitivity in Chinese population. To the best of our knowledge, this is the first study to reveal the relation between *ARDB1* polymorphism and pain and fentanyl sensitivity in Chinese population.

PPLpre in patients who carried the A-allele of A145 G (rs1801252) in the β1-adrenergic

receptor gene was significantly lower than in patients who did not carry the A-allele, suggesting that the A-allele is associated with high sensitivity to pain. The A145 G SNP (rs1801252) causes an amino acid substitution. β1-adrenergic receptors with 49 Gly reportedly have high agonist affinity, cyclic adenosine monophosphate (cAMP) activity, and avidity to metoprolol, an inverse agonist [12].

The G1165 C SNP induces an amino acid substitution from glycine to arginine at position 389 of the β1 - adrenergic receptor. The 389 Arg variant of the β1-adrenergic receptor showed higher cAMP activation and [^{35}S] guanosine triphosphate-γ S binding affinity induced by isoproterenol than the 389 Gly variant [13].

Although we did not found association of G1165 C with pain sensitivity, the haplotype analysis revealed that carrying the A-C haplotype was significantly associated with fewer doses of postoperative analgesic requirements and pain sensitivity. Considering these results, one may suggest the possibility that carrying both the A-allele in the A145 G SNP and the C-allele in the G1165 C SNP (i.e., the AC haplotype) might have led to a significant decrease in postoperative analgesic requirements.

In conclusion, the results of the present study suggested that the *ADRB1* gene was associated with the sensitivity to pain and fentanyl in Chinese population.

Disclosure of conflict of interest

None.

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