

## Original Article

# CGRP 4218T/C polymorphism correlated with postoperative analgesic effect of fentanyl

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**Abstract:** Purpose: Our study aimed at evaluating the association between  $\alpha$ -calcitonin gene-related peptide (CGRP) 4218T/C polymorphism and the patient-controlled analgesic (PCA) effect of fentanyl on Chinese Han population. Methods: 98 patients were involved in the experiment, but only 92 patients completed the experiment. 0.1 mg/kg fentanyl was given to the patients through intravenous injection ten minutes before the ending of surgery. The patients achieved PCA by controlling the fentanyl infusion pump and a single dose was 1 mg. The CGRP 4218T/C polymorphism was genotyped with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The fentanyl consumption within the 72 hours after the surgery was recorded and the pain was assessed with numeric rating scale (NRS) method. Results: The patients were divided into three groups of wild homozygote (T/T), heterozygote (T/C), and mutant homozygote (C/C). At the 6<sup>th</sup> hour and the 12<sup>th</sup> hour after the surgery, the fentanyl consumption for PCA of the T/C group was significantly higher than the T/T group ( $P<0.05$ ). Meanwhile, the fentanyl consumption of the C/C group was much higher than the T/T group ( $P<0.05$ ) at the 12<sup>th</sup> hour and the 24<sup>th</sup> hour. Besides, the fentanyl consumption of the C/C group was more than the T/C group ( $P<0.05$ ) at the 24<sup>th</sup> hour. The differences in NRS scores, Ramsey scores, and postoperative adverse reactions between each group at all time points were not statistically significant. Conclusions: CGRP 4218T/C polymorphism may be associated with the postoperative fentanyl consumption for analgesia.

**Keywords:** CGRP, postoperative analgesia, fentanyl

## Introduction

Most of the terminal cancer patients torture severe pain. Opioid drugs are the most common and effective drugs for cancer pain treatment, but prolonged use of them is very likely to cause tolerance and even hyperalgesia [1]. Fentanyl is a representative opioid drug, and often used for analgesia before, during and after the surgery as well as the treatment of terminal cancer pain. In the past, clinicians tended to simply attribute the poor analgesic effect of opioid drugs to individual differences. However, in recent years, there were mounting evidences suggested the difference was related to certain genes [2-4]. Furthermore, significant differences in the sensitivities to fentanyl in clinical application therapy among patients also exist. Recently, many scholars suggested that the individual different response to fentanyl is closely related to the human genes [5, 6].

Calcitonin gene-related peptide (CGRP) is the most important neurotransmitter in the transmitting of pain signals. The analgesic mechanism of opioid drugs is to reduce the release of such neurotransmitters as CGRP from the pre-synaptic membrane to the synaptic cleft of A $\delta$  and C nerve fiber endings [7-9]. The effects of CGRP on opioid tolerance are still not very clear.

The present study explored the correlation of CGRP 4218T/C, a newly-found polymorphism, and postoperative fentanyl consumption for analgesia as well as adverse reactions to fentanyl so as to provide a basis for individualized pain treatment in clinic.

## Materials and methods

### General materials

The present experiment was prospective study. The patients had accepted such surgeries as

**Table 1.** General data of the patients

Items	Groups		
	T/T	T/C	C/C
Age/year	53.28±11.36	51.39±12.95	53.68±14.36
BMI/(kg m <sup>2</sup> )	22.89±3.41	23.67±4.23	23.64±4.69
Male/female	36/28	11/13	2/2
ASA (I/II)	24/40	8/16	2/2
Preoperative myotrophy urea nitrogen	66.5±23.4	53.8±16.7	63.4±18.9
MMS score	25.9±3.4	25.4±2.6	24.9±3.6
Surgical site (abdomen/spine)	26/38	11/13	2/2
Intraoperative fentanyl consumption/μg	265.4±52.5	275.0±50.0	264.5±57.5
Smoking	16/48	9/15	1/3
Drinking	23/41	10/14	2/2
Operation duration/min	128±61	117±71	134±66
Anesthesia duration/min	184±66	174±72	166±62

open surgeries and lumbar surgeries. Since open surgeries would cause severe pain in liver, so we precluded the patients experienced open surgeries. A total of 98 patients were finally included in the study. Among the 98 surgeries, there were 30 intestinal surgeries, 20 stomach surgeries, 17 kidney surgeries, 12 splenic surgeries, and 19 lumbar surgeries. The differences in the surgery distribution between each group were not statistically significant. The patients were all ethnic Han Chinese. 51 males and 47 females aged 29~76 years old had an average age of 52.46±16.78 years in the case group. All the patients signed an informed consent. The experiment passed audits of Medical Ethics Committee of affiliated hospital of Qingdao university for scientific clinical experiments.

#### *Inclusion criteria*

The patients included in the study showed the following characteristics: selected surgery; analgesic treatment by fentanyl during the 72 hours after general anesthesia; 29~76 years old; 42~79 kg; American Society of Anesthesiologists (ASA) grade I~II; no medical history of alcoholism, drug addiction or tolerance, epilepsy, or psychosis; class I of the liver function; and no serious cirrhosis.

#### *Exclusion criteria*

The patients with the following characteristics were precluded: postoperative analgesic treatment not by fentanyl; long-term drug history or

chronic pain history; a second surgery caused by complications within the 3 months after the first surgery; severe cardiopulmonary dysfunction; depression; or other psychonosemas.

#### *Anesthesia treatment*

2 mg midazolam, 0.2 mg remifentanyl and 70-120 mg propofol were intravenously injected. Mechanical ventilation was performed after Tracheal intubation. Anesthesia maintenance: propofol and remifentanyl were continuously pumped in by a micro pump and sevoflurane was inhaled in. The pumping speed of drugs was adjusted according to changes of haemodynamics during the surgery. Cisatracium was superadded according to specific operative needs. 0.1 mg/kg fentanyl was intravenously injected 10 minutes before the ending of surgery.

#### *Analgesic strategy*

① 20 μg/kg fentanyl and 150~250 mg of flurbiprofen axetil, ② 20 μg/kg fentanyl and 4~6 g of propacetamol. ① or ② was diluted by 0.9% sodium chloride injection to 75 ml. 2 ml of loading dose was infused. The background infusion rate was 1 ml/h. The amount for PCA was 0.5 ml each time. The lock time was 15 minutes.

#### *Infusion settings of the PCA pump*

1 mg of fentanyl and 5 mg of droperidol was diluted to 100 ml with physiological saline. The background infusion rate was 0.5 ml/h. The PCA amount was 2 ml. The lock time was 5 min-

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**Table 2.** Differences in postoperative fentanyl consumption ( $\bar{x} \pm s$ ) for PCA

Groups	n	Fentanyl consumption ( $\mu\text{g kg}^{-1}$ )				
		T <sub>0.5h</sub>	T <sub>6h</sub>	T <sub>12h</sub>	T <sub>24h</sub>	T <sub>48h</sub>
T/T	64	7.1 $\pm$ 1.2	9.9 $\pm$ 2.9	13.0 $\pm$ 4.7	14.8 $\pm$ 6.1	21.8 $\pm$ 6.7
T/C	24	7.7 $\pm$ 1.3	14.6 $\pm$ 2.9	18.4 $\pm$ 5.1	19.6 $\pm$ 6.4	22.4 $\pm$ 8.1
C/C	4	7.3 $\pm$ 1.1	12.2 $\pm$ 1.4	19.8 $\pm$ 4.5	23.4 $\pm$ 6.8	25.8 $\pm$ 7.2
T/T vs. T/C		0.784	0.042	0.037	0.052	0.184
T/T vs. C/C		0.940	0.163	0.013	0.004	0.197
T/C vs. C/C		0.813	0.384	0.674	0.021	0.216

utes. The effective pressing number per hour was restricted to be seven. The maximum limit amount of fentanyl per hour was 100  $\mu\text{g}$ .

### Data collection

Basic physiological parameters (including blood pressure, pulse, oxygen saturation and consciousness) of the patients were assessed immediately after the patients entered in the post anesthesia care units (PACUs). The pain was evaluated with numeric rating scale (NRS) method. The mechanism of NRS was as follows: there were altogether 101 points (0~100) in a straight line; and the point 0 meant painlessness, while the point 100 meant intense pain. The mechanism of NRS was explained to the patients one day before the surgery. According to the pain degree of the patients as well as the intraoperative analgesic consumption, to use fentanyl for analgesia or not was decided. If the NRS score of a patient was higher than 30, then 25~50  $\mu\text{g}$  of fentanyl would be intravenously injected. If nausea or vomiting occurred in a patient, 3 mg of granisetron would be intravenously injected. In this case, the patient should be observed in the PACU for 30 minutes. When the NRS score of the patient was lower than or equal to 30 and there was no obvious nausea, omitting, or unconsciousness, the patient would be sent to the ward. The patients and family members thereof were guided by the medical staff to correctly use the patient-controlled analgesia pump after the surgery. The postoperative nausea was assessed with 4-point method (1: no nausea; 2: slight nausea; 3: moderate nausea; 4: severe nausea). Ramsey score was adopted to assess the sedation level of patients (1: dysphoric; 2: calm; 3: sleepy and able to follow commands; 4: quick response to external calls; 5: asleep

and slow response to external calls; 6: deep asleep; 7: no response to external calls). After the patients were back in the wards, the NRS scores and the drug side-effect (including nausea, vomiting, dyspnea, dizziness, abdominal distension, lethargy, and pruritus) scores were recorded at the 0.5<sup>th</sup> hour, the 6<sup>th</sup> hour, the 12<sup>th</sup> hour, the 24<sup>th</sup> hour and the 48<sup>th</sup> hour. Related indexes were recorded and expectant treatments were given.

### Genotyping method

5 ml of venous blood was collected with heparin anticoagulant. DNA was extracted with conventional phenol/chloroform method. CGRP 4218T/C polymorphism was analyzed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Primers were designed by Premier 5.0. The primer sequences were as follows: forward primer: 5-GGAAGAAGCAAA GACCAGGA-3; reverse primer: 5-CTGCAAGAACAATCCCACA-3. The PCR amplification product was 517 bp. PCR products were digested with Alu I enzyme. The wild homozygote (T/T) was presented with three straps of 202 bp, 169 bp and 106 bp, the heterozygote (T/C) with four bands of 371 bp, 202 bp, 169 bp, and 106 bp and the mutant homozygote (C/C) with two straps of 371 bp and 106 bp. When performing anesthesia for analgesia, the surgeons, anesthetists, or other medical staff involved in the study had no idea about the genotypes of the patients.

### Statistical analysis

The analyses were performed with SPSS 18.0. The genotype distribution was checked by Hardy-Weinberg Equilibrium (HWE) with  $\chi^2$  test. The normal measurement data was represented by  $\bar{x} \pm s$ . One-way ANOVA was adopted in the analysis of the difference on characteristics, fentanyl consumption, NPS score and Ramsey score between groups. Side effects such as nausea, vomiting and respiratory depression were checked by H test (Kruskal-Wallis test). The differences were statistically significant only when  $P < 0.05$ . The consumption of fentanyl in the remedial measure was expressed with median skew data method.

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**Table 3.** Postoperative NRS and Ramsey scores

Groups	T <sub>0.5h</sub>	T <sub>6h</sub>	T <sub>12h</sub>	T <sub>24h</sub>	T <sub>48h</sub>	Ramsey score (the 6 <sup>th</sup> hour)	Ramsey score (the 24 <sup>th</sup> hour)
T/T	34±15	19±7	19±08	18±7	15±7	2.2±0.6	3.1±1.0
T/C	29±17	22±8	21±07	21±8	18±9	2.4±0.4	2.8±0.4
C/C	31±21	23±13	20±12	18±10	14±10	2.8±0.7	2.9±0.7

### Results

#### Research subjects

Of the 98 patients, 6 failed to complete the experiment. 4 patients were precluded for being given pethidine for analgesia due to obvious postoperative pain (NRS>50) for over 30 minutes. The other 2 patients were precluded for mechanical faults in the PCA pump and severe nausea and vomiting. Then 92 patients were left for sequential studies.

#### Mutation frequency

Among the 92 patients, there were 64 patients (69.6%) carrying the wild homozygote (T/T), 24 patients (26.1%) carrying the mutant heterozygote (T/C), and 4 patients (4.3%) carrying the mutant homozygote (C/C).

The differences in sex ratio, age, body mass index (BMI), ASA grade, preoperative myotrophy urea nitrogen, MMS score, surgical site (abdomen/spine), intraoperative fentanyl consumption, smoking, drinking, surgery duration, and anesthesia duration among three groups had no statistical significance ( $P>0.05$ , **Table 1**).

#### HWE test

The genotypes distribution of CGRP 4218T/C polymorphism in controls passed Hardy-Weinberg Equilibrium (HWE) test ( $P>0.05$ ), indicating that the participants of control group were representative.

#### Fentanyl consumption for PCA

At the 6<sup>th</sup> hour and the 12<sup>th</sup> hour after the surgery, the fentanyl consumption for PCA of the T/C group was significantly higher than the T/T group ( $P=0.042$ ,  $0.037$ ). At the 12<sup>th</sup> hour and the 24<sup>th</sup> hour after the surgery, the fentanyl consumption for PCA of the C/C group was significantly higher than the T/T group ( $P=0.013$ ,  $0.004$ ). At the 24<sup>th</sup> hour after the surgery, the fentanyl consumption for PCA of the C/C group

was significantly higher than the T/C group ( $P=0.021$ ) (**Table 2**).

#### NRS score, Ramsey score, and occurrence of adverse reactions

As shown in **Table 3**, for the NRS score at the 0.5<sup>th</sup> hour, the 6<sup>th</sup> hour, the 24<sup>th</sup> hour and the 48<sup>th</sup> hour after the surgery, no significant differences existed between each group ( $P>0.05$ ). For Ramsey score, there was also no statistically remarkable differences at the 6<sup>th</sup> hour and the 24<sup>th</sup> hour between each group ( $P>0.05$ ). The analysis of adverse reactions indicated that there existed no differences on nausea, vomiting and respiratory depression between each group ( $P>0.05$ ) (**Table 4**).

### Discussion

The latest statistics show that the current annual increase in global cancer cases is 12,700,000 and 7,600,000 people have died of cancers per year, among them, 56% of the terminal cancer patients are tortured by moderate or serious cancer pain for at least one month [10-12]. Fentanyl is the most common and effective drug for the treatment of cancer pain. However, long-term use or high doses of it can easily cause tolerance and even hyperalgesia [13-15]. At present, fentanyl is widely used in the treatment of acute and chronic pain, however there exists individual differences in clinical application. Cepeda et al. have revealed that respiratory depression caused by morphine is more likely to happen in native American Indians than in the White race [16]. The study of Zhou et al. suggested that sedation and respiratory depression were more likely to happen in the White race than in Asians. Additionally, men and women also react differently to opioid drugs [17]. Sear et al. have indicated that the concentration of some opioid drugs in the plasma is not closely related to the clinical effects. So it has been concluded that the differences in the analgesic efficacy of fentanyl may be associated with the modes of

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**Table 4.** Comparison of postoperative adverse reactions

		T/T	T/C	C/C
Nausea	Light	32	4	2
	Moderate	17	3	1
	Severe	4	1	0
Vomiting		6	2	1
Respiratory depression		0	0	0

gene action [18]. In recent years, the studies found that genetic variants of certain genes also could affect the efficacy of fentanyl.

The genetic polymorphisms not only affect the analgesic effect, but also influence the pain perception of people. Differences in pain perception are caused by several different genes related to pain-sensing. These genes are related to each other, and have a combined influence on pain perception. The pain perception of human beings can change due to a single mutation in a certain gene [19]. For example, if point mutations happen to *NTRK1* gene which is responsible for encoding high-affinity nerve growth factor (NGF) specific tyrosine kinase receptors, then people would become insensitive to pain from the time of birth [20]. Meanwhile, individual differences in response to pain are inflected in pain perception as well as reactions to analgesics.

*CGRP*, a kind of neuropeptide widely distributed in the nervous system, takes part in the nociceptive information transmission as well as the production of hyperalgesia in peripheral nerves and the spinal cord, and interacts with such bioactive substances or receptors as substance P, excitatory amino acid (EAA), capsaicine type I receptor, chemokine, activin, cannabinoid receptor, opiate receptor, NGF, vasoactive intestinal peptide (VIP), 5-hydroxytryptamine, and glucocorticoid in the pain modulation process [21, 22]. Recently, a study concerning the relationship between *CGRP* and parkinsonism and major depression performed by Buervenich et al. suggested that a new single nucleotide polymorphism (SNP) (4128T/C) was found in exon 3 of the *CGRP* gene [23]. Studies have shown that *CGRP* can be released to ventral segmental area, amygdala and ventral straitum as a kind of central neuropeptide via nerve fibers, and can adjust the transmission of dopamine (DA) by influencing the limbic

axis and corpus striatum axis of the midbrain of the dopamine system in these regions [24, 25]. No studies have reported the effects of *CGRP* 4218T/C polymorphism on the analgesic effect of fentanyl yet, so we performed a study to investigate the relationship between *CGRP* 4218T/C polymorphism and the analgesic effect of fentanyl.

The results of our study indicated that the *CGRP* 4218T/C polymorphism had a significant influence on postoperative fentanyl consumption for analgesia. For the analysis of T/T vs. T/C, the fentanyl consumption for PCA had a significant increase at the 6<sup>th</sup> hour and the 12<sup>th</sup> hour. At the 12<sup>th</sup> hour and the 24<sup>th</sup> hour, the fentanyl consumption of TT group was much more than C/C. Meanwhile, the fentanyl consumption of T/C group at the 24<sup>th</sup> hour was significantly higher compared with C/C.

It is undeniable that that there are many factors that may impact the postoperative analgesic effect of patients clinically, such as individual differences in the sensitivity to pain, the metabolism of analgesics, and target proteins of the drug action. Furthermore, genetic factors, environmental factors, and the physiological and pathological conditions of the human body all have influences on the metabolic process of drugs in the human body in different degrees. The design of our experiment has prevented the interference of other factors, such as the physiological and pathological conditions of patients, combined medication and anesthetics used, on the analgesic effect of fentanyl as much as possible, which indicated that our results were reliable.

Besides, this study investigated the association between *CGRP* 4218T/C polymorphism and the postoperative adverse reactions caused by fentanyl. The results showed that *CGRP* 4218T/C polymorphism had no significant influence on the incidence of adverse reactions. Due to the limited sample size, we failed to observe any difference in the incidence of adverse reactions between different genotype groups. Furthermore, the mechanism of such postoperative adverse reactions as nausea and vomiting is very complex. The drugs can penetrate through the blood barrier to stimulate such locations as chemical triggers and nucleus tractus solitarius, thus leading to nausea or vomiting; and the endocrine regula-

tion and gastrointestinal irritation of the human body are also related to these adverse reactions. At present, in order to clinically reduce the incidence of adverse reactions caused by opioid drugs and achieve a good analgesic effect, the dosage of opioid drugs for postoperative analgesia is often reduced and other analgesics are also used in combination. Nevertheless, due to complex clinical situation and various influencing factors, potential influence of unknown causes also exists that can impact the pharmacogenomics studies of fentanyl. Therefore, further studies exploring the relationship of *CGRP* 4218T/C polymorphism with the pharmacokinetics of fentanyl needs to be performed in the future.

In conclusion, our results indicated that the *CGRP* 4218T/C polymorphism was associated with the postoperative analgesic effect of fentanyl after general anesthesia. However, this conclusion needs to be verified in the future. Also, more serious surgery requirements should be adopted and the concentration of fentanyl and metabolites in the serum should be observed in further studies. With the pain treatment becoming more and more individualized, more studies investigating the association of *CGRP* 4218T/C polymorphism with the metabolism of fentanyl and pain treatment should be carried out.

#### Disclosure of conflict of interest

None.

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