Genetic Polymorphisms of Cytokines Might Affect Postoperative Sufentanil Dosage for Analgesia in Patients

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Objective: To explore the effect of genetic polymorphisms of cytokines on the dosage of sufentanil for patient-controlled intravenous analgesia (PCIA) after radical lung cancer surgery.

Methods: A total of 100 patients, aged 18 years and above, with ASA grade I - II and body mass index (BMI) 18.5 to 30, who were scheduled for radical lung cancer surgery under total intravenous anaesthesia with PCIA of sufentanil from September 2015 to March 2016, were selected. DNA was collected from peripheral blood samples before surgery, and the iMLDRTM multiple single-nucleotide polymorphism typing kit was used to detect 16 related single-nucleotide polymorphism (SNP) sites of interleukin-1A (IL-1A), interleukin-1B (IL-1B), interleukin-1RN (IL-1RN), interleukin-6 (IL-6), C-X-C motif chemokine ligand 8 (CXCL8), interleukin-10 (IL-10), tumour necrosis factor (TNF), nuclear factor kappa-B1 (NFkB1), REL (REL proto-oncogene, NF-kB subunit), and nuclear factor kappa-B inhibitor alpha (NFkBIA). The general characteristics of patients, surgery and anaesthesia data, postoperative resting VAS pain scores, postoperative opioid dosages of sufentanil for PCIA and opioid-related adverse events were recorded. The effects of the examined genetic polymorphisms of the cytokines on the dosage of sufentanil were analysed.

Results: Eight of 100 patients withdrew for various reasons, and, eventually, 92 patients were included. The patients’ resting visual analogue scale (VAS) scores at 24 h, 48 h, and 72 h after surgery were 2.3 ± 1.2, 2.0 ± 0.9, and 1.9 ± 1.0, respectively. The total amounts of sufentanil used were 34.7 ± 10.5 μg, 65.2 ± 13.7 μg, and 94.7 ± 11.6 μg, respectively. We found that the TT genotype of NFkBIA rs696 had higher PCIA sufentanil dosages than the CC genotype and the CT genotype at 48–72 h postoperation (p=0.023, p=0.025, respectively).

Conclusion: The genetic polymorphisms of the cytokine NFkBIA rs696 might affect the dosage of sufentanil for PCIA after radical lung cancer surgery. The specific mechanism needs further study.

Keywords: genetic polymorphisms, single-nucleotide polymorphism, SNP, patient-controlled intravenous analgesia, PCIA, nuclear factor kappa-B inhibitor alpha, NFkBIA

Background

How to effectively alleviate postoperative pain to accelerate patients’ recovery after surgery, especially after thoracic surgery, remains a medical challenge today.1,2 These patients may endure severe postoperative acute pain due to skin and deep soft tissue damage, rib or sternal fractures, lung tissue damage and the placement of closed drainage tubes in the thoracic cavity. Poorly controlled acute postoperative
pain during the first 3 days after surgery correlates with persistent chronic post-thoracotomy pain at 6 months, which can develop to chronic pain syndrome.3

Currently, a multimodal analgesia approach consisting of non-steroidal anti-inflammatory analgesics, nerve blocks and opioids during perioperative analgesia of thoracic surgery has been highly recommended and adopted.4 The application of opioids using patient-controlled intravenous analgesia (PCIA) technology is one of the main analgesic methods after thoracic surgery.5 Sufentanil is widely used in postoperative analgesia because of its strong analgesic effects, insignificant accumulation, rapid clearance, minor effects on circulation, weak respiratory depression and low incidence of nausea and vomiting.6–8

In postoperative multimodal analgesia, opioids remain the cornerstone. An insufficient dosage of opioids may lead to a poor analgesic effect, which will then inevitably affect patients’ recovery after surgery. An overdose of opioids will lead to serious adverse reactions such as excessive sedation and respiratory depression. Therefore, individualized therapy of opioids for postoperative analgesia is the key point for patients’ safety while promoting patients’ Enhanced Recovery After Surgery (ERAS).

However, the dosage of opioids shows significant differences amongst individuals in PCIA. In the past, these differences were attributed to related factors such as patient’s age, gender, weight, surgical trauma, and anaesthesia. In recent years, many studies have confirmed that genetic factors such as single-nucleotide polymorphisms (SNPs) do have great impact on postoperative analgesia, especially on the absorption, transport, metabolism, elimination and efficacy of opioids.9–18 Lee et al,12 found that a genetic polymorphism at the µ-opioid receptor gene (OPRM1) A118G influenced the analgesic effect of morphine for immediate acute postoperative pain in children. Zhang et al,17 found that catechol-O-methyl transferase (COMT) gene haplotype contributed to the individual variation of postoperative analgesia with fentanyl. Yuan et al,18 found that the CYP3A4*1G genetic polymorphism decreases the metabolism of fentanyl in human liver microsomes obtained from Chinese patients. Therefore, are there any other factors that affect the dosage of opioids in genetic polymorphisms?

Thoracic surgery is often accompanied by an increased level of various immune inflammation-related cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10).19 Surgery-related stimuli will induce the release of inflammatory cytokines that have immunoregulatory functions or inflammatory or anti-inflammatory effects. Different dosages of opioids are needed for postoperative pain, which is closely related to the release of cytokines or inflammatory factors such as interleukin (IL), tumour necrosis factor-alpha (TNF-α), etc.20–22 Cytokines modulate nociceptive signalling during acute inflammation and following tissue injury, and they may cause significant inter-individual variability in postoperative pain (including development, intensity, and resolution of pain).23,24 Central cytokines and chemokines are powerful neuromodulators and play a significant role in inducing hyperalgesia and allodynia after central nervous system administration, and they drive widespread chronic pain via central sensitization.25 We speculate that the genetic polymorphisms of cytokines related to perioperative immune inflammation may affect the postoperative PCIA sufentanil dosage. For this reason, this study intends to explore the effect of genetic polymorphisms of cytokines on the dosage of PCIA sufentanil after radical lung cancer surgery to provide a theoretical basis for the development of an individual PCIA sufentanil scheme.

**Objects and Methods**

**Research Objects**

The study was conducted in accordance with the Declaration of Helsinki, and that the patient consent was written informed consent. The trial has been approved by the Ethics Committee of the First Affiliated Hospital Zhejiang University School of Medicine (2015 [254]), and consents were obtained from patients and their families.

**Inclusion Criteria**

100 patients who were diagnosed with lung cancer in the hospital from September 2015 to March 2016, were selected and received radical lung cancer surgery under total intravenous anaesthesia. They were aged ≥18 years with no gender restriction and had ASA grade I–II and BMI 18.5–30.

**Exclusion Criteria**

Patients who were unable to be treated under parecoxib sodium for postoperative analgesia for various reasons, with severe neurological and psychiatric disorders, who were on non-steroidal or opioid analgesics for a long time, or who had a total Hamilton Anxiety Scale score ≥ 7 points or Hamilton Depression Scale ≥ 8 points.
Methods

Anaesthesia and Analgesia

Patients were routinely monitored for ECG, blood pressure, and oxygen saturation after entering the operating room. Invasive arterial pressure monitoring was done in the radial artery of the non-operative side of the lung. Total intravenous anaesthesia was induced with 0.05 mg/kg midazolam, 2 mg/kg propofol, 0.6 mg/kg rocuronium and 0.3 μg/kg sufentanil. A double-lumen endotracheal tube (males #37–39, females #32–35, where the appropriate double tracheal model was selected based on the CT measurement of the tracheal diameter) was inserted 5 mins later. A fibreoptic bronchoscope was used to confirm the location of the tube. An 8 mL/kg tidal volume (VT), 60% fraction of O₂ inspiration (FiO₂), and respiratory rate (RR) of 12 times/min were used for double lung ventilation, adjusting the RR exhalation to maintain PaCO₂ at 35–45 mmHg.

A protective ventilation strategy was used for single lung ventilation with a VT of 6 mL/kg, FiO₂ of 60–100% (as low as possible), RR at 12–16 times/min, positive end-expiratory pressure ventilation (5 cm H₂O) and intermittent lung dilatation to maintain a PaCO₂ of 35–45 mmHg. Anaesthesia was maintained with 6 mg kg⁻¹ h⁻¹ propofol, 0.05 μg kg⁻¹ min⁻¹ remifentanil and 0.15 mg/kg rocuronium (every 40 mins).

Sufentanil (0.2 μg/kg) was intravenously administered 2 minutes before the incision, and the concentrations of remifentanil and propofol were adjusted according to the Bispectral Index (BIS) value and blood pressure. The BIS value was maintained at 40–60, ensuring that the blood pressure fluctuation range did not exceed ± 20% of the base value. Palonosetron hydrochloride at 0.25 mg was injected 30 mins before the end of surgery to prevent postoperative nausea and vomiting. Anaesthesia maintenance medications were discontinued 5 minutes before surgery. All of these operations were performed under a three-hole full-thoracoscopy by the same group of doctors. Patients were transferred to the postanaesthesia care unit (PACU) for anaesthesia resuscitation and extubation after the operation.

Multimodal analgesia was used during the perioperative period. Sufentanil (0.2 μg/kg) was intravenously injected 30 minutes prior to the end of the operation. Parecoxib sodium (40 mg) was intravenously injected before the operation and then twice daily for three consecutive days. When the chest was closed, a 0.50% ropivacaine 10 mL incision was used for local infiltration, and PCIA was used for postoperative analgesia. PCIA (sufentanil, 100 μg/250 mL) was used for 72 h for postoperative acute pain control. The parameters were set as follows:

- load 5 μg, background infusion rate of 3 μg/h,
- bolus 2 μg, lock time 10 min, lock dosage of 20 μg/h.

When the patient had a VAS score ≥ 4 points at the ward, a bolus of 2 μg Sufentanil was used after the patient pressed once. If the VAS score was still ≥ 4 points after two consecutive valid pressures, tramadol 100 mg hydrochloride were intramuscularly injected for analgesia. When the patient had a VAS score ≥ 7 points, Acute Pain Service was also called in to adjust the PCIA parameters to control the patient’s VAS score to ≤3 points. PCIA ceased after 3 days and if the VAS score remained ≥ 4 points, 200 mg celecoxib capsules were given.

The resting VAS pain score and the dosages of sufentanil at 24 h, 48 h, and 72 h after surgery were recorded. Tramadol was converted to equivalent sufentanil (100 mg tramadol are equivalent to 13.3 μg sufentanil) for statistical purposes. Postoperative nausea, vomiting, pruritus, sedation, respiratory depression, hypotension and other related adverse reactions were recorded.

Detection of SNPs

A total of 16 polymorphisms (SNPs) of cytokines were selected according to a literature search. Table 1 shows the primers that were selected for polymorphism genotyping. After patient entered the room, 2 mL of venous blood were drawn and injected into an ETDA anticoagulation tube and stored in a refrigerator at 4 °C. Genomic DNA was extracted from the blood using a Maxwell 16 Blood DNA purification kit (Shanghai shengzhao biotechnology co., LTD). The iMLDRTM multiple SNP typing kit (Shanghai shengzhao biotechnology co., LTD) was used to type the SNPs of the samples. Raw data collected on the sequencer were analysed by GeneMapper 4.0 (Applied Biosystems, USA).

Statistical Methods

SPSS 19.0 statistical software was used for analytical purposes. The results of the measurement data conforming to a normal distribution are expressed as the means ± standard deviation (x ± sd). To test whether alleles and genotypes meet Hardy-Weinberg equilibrium, the χ² test was used. Data between multiple groups were compared using a single factor analysis of variance (ANOVA).
count data were tested by $\chi^2$. A difference of $P < 0.05$ was statistically significant.

## Results

### General Information

Of these 100 selected patients, 8 were excluded as they failed to meet the test protocol, and 92 patients were enrolled, consisting of 58 males and 34 females. Their average age was $58.9 \pm 10.3$ years and BMI $22.2 \pm 2.6$. Details of characteristics of patients enrolled can be found in Table 2.

### Surgery and Anaesthesia Information

Surgery and anaesthesia information of patients enrolled can be found in Table 3.

### Follow-Up of Postoperative Analgesia and Adverse Reactions

The follow-up resting VAS pain scores of the 92 patients at 24 h, 48 h–72 h after operation were $2.3 \pm 1.2$, $2.0 \pm 0.9$ and $1.9 \pm 1.0$, respectively (Table 3).

Within 72 h after surgery, the patients in each group had no sedation or respiratory depression. Forty-one patients had nausea with an incidence of 44.56%, 8 patients had vomiting with an incidence rate of 8.69%, 3 patients had pruritus with an incidence rate of 3.26% and 2 patients had hypotension with an incidence rate of 2.17%.

### Table 1 Selected Polymorphisms (SNPs)

| No. | SNP   | Gene | Allele | Wild-Type | Polymorphic |
|-----|-------|------|--------|-----------|-------------|
| 1   | rs17561 | IL-1A | C>A    | C         | A           |
| 2   | rs1800587 | IL-1A | G>A    | G         | A           |
| 3   | rs1143634 | IL-1β | G>A    | G         | A           |
| 4   | rs16944 | IL-1β | A>G    | A         | G           |
| 5   | rs419598 | IL-1RN | T>C    | T         | C           |
| 6   | rs1800795 | IL-6 | C>G    | C         | G           |
| 7   | rs4073 | CXCL8 | A>T    | A         | T           |
| 8   | rs1800871 | IL-10 | A>G    | A         | G           |
| 9   | rs1800872 | IL-10 | T>C    | T         | C           |
| 10  | rs1800896 | IL-10 | T>C    | T         | C           |
| 11  | rs1800629 | TNF | G>A    | G         | A           |
| 12  | rs28362491 | NFXB1 | delATTG | -         | delATTG     |
| 13  | rs374932 | NFXB1 | A>G    | A         | G           |
| 14  | rs842647 | REL | G>A    | G         | A           |
| 15  | rs89904 | NF-kBIA | G>A    | G         | A           |
| 16  | rs696 | NF-kBIA | C>T    | C         | T           |

### Table 2 Characteristics of Enrolled Patients (n=92)

| Patients' Characteristics | Value |
|--------------------------|-------|
| Age (years)              | $58.9 \pm 10.3$ |
| Gender (male/female)     | 58/34 |
| BMI (kg/m$^2$)           | $22.2 \pm 2.6$ |
| ASA classification (grade 1/1I) | 9/83 |
| Medical comorbidities    |       |
| Hypertension (yes/no)    | 31/61 |
| Diabetes mellitus (yes/no) | 7/85 |
| History of surgery (yes/no) | 32/60 |
| Occupation (worker/farmer/civil service/retirement/other) | $37/13/6/21/15$ |
| Education (college/middle school/primary school/illiterate) | $18/46/24/4$ |

### Table 3 Surgery and Anaesthesia Data, Resting VAS Pain Score, Opioid Dosage, and Opioid-Related Adverse Events Within 72 h Post-Op (n=92)

| Item                               | Value                         |
|------------------------------------|-------------------------------|
| Surgery and anaesthesia data       |                               |
| Operation time (min)               | $113.1 \pm 41.8$             |
| Volume of blood loss (mL)          | $59.2 \pm 55.9$              |
| Infusion volume (mL)               | $1275.5 \pm 366.7$           |
| Urine output (mL)                  | $231.7 \pm 185.1$            |
| Length of the incision (cm)        | $8.1 \pm 2.7$                |
| Intraoperative anaesthetics        |                               |
| Sufentanil (μg)                    | $34.7 \pm 10.5$              |
| Remifentanil (μg)                  | $741.4 \pm 321.5$            |
| Propofol (mg)                      | $902.2 \pm 274.2$            |
| Resting VAS pain score             |                               |
| 24 h post-op                       | $2.3 \pm 1.2$                |
| 48 h post-op                       | $2.0 \pm 0.9$                |
| 72 h post-op                       | $1.9 \pm 1.0$                |
| Sufentanil dosage (μg)             |                               |
| 24 h post-op                       | $34.7 \pm 10.5$              |
| 48 h post-op                       | $65.2 \pm 13.7$              |
| 72 h post-op                       | $94.7 \pm 11.6$              |

Note: Data are expressed as the mean ± SD.

Abbreviations: VAS, visual analogue scale; 24 h post-op, 24 h postoperation; 48 h post-op, 48 h postoperation; 72 h post-op, 72 h postoperation.
Preliminary Analysis of Genetic Polymorphisms of Cytokines on the Dosage of PCIA Sufentanil After Radical Lung Cancer Surgery

The detection found that the variant frequencies of rs1800795 and rs1143634 were 100% and 0.5%, respectively, which were similar to the variant frequencies in the East Asian population.

No significant difference was detected in the dosage of sufentanil at 72 h postoperative in many SNPs (ie, rs17561 C> A, rs1800587 G> A, rs16944 G> A, rs419598 T> C, rs4073 A> T, rs1800871 A> G, rs1800872 T> G, rs1800896 T> C, rs1800629 G> A, rs28362491 delATTG, rs3774932 A> G, rs842647 G> A, and rs8904 G> A) (p>0.05). The dosage of sufentanil between different genotype groups of rs696 C> T (p<0.05) was statistically significant at 72 h after operation, as shown in Table 4.

The Rs696 Polymorphism Might Affect Postoperative Sufentanil Dosage for Analgesia in Patients After Radical Lung Cancer Surgery

**Rs696 Genotype and Allele Frequency Analysis**

The analysis showed that the variant frequency of the rs696 C> T allele in patients with lung cancer surgery was 35.3%. The distribution of alleles and genotypes were in accordance with Hardy-Weinberg equilibrium (p>0.05). Patients were divided into wild-type homozygous (CC group), heterozygous (CT group) and homozygous (TT group) according to their genotypes. The rs696 C> T genotype and allele frequencies are shown in Table 5.

**Analysis of the General Characteristics, Surgery and Anaesthesia Data, and Opioid-Related Adverse Events**

Patients were grouped according to the direct sequencing results of the rs696 C> T genotype. There was no significant difference in age, gender, BMI, ASA classification, medical comorbidities, occupation or education in each group (p>0.05) (Table 6). There was no statistically significant difference in the surgical time, volume of blood loss, infusion volume, urine volume, length of incision, or intraoperative dosages of sufentanil, remifentanil and propofol in each group (p> 0.05) (Table 6).

Within 72 h postoperation, the patients in each group were followed-up without hypersedation or respiratory depression. There were no significant differences in nausea, vomiting, pruritus or hypotension in the three groups (p>0.05) (Table 6).

**Comparison of the Resting VAS Pain Scores and PCIA Sufentanil Dosages Within 72 h of Each Group**

The resting VAS pain scores of the three groups were not significantly different (p>0.05) (Figure 1A). The dosages of PCIA sufentanil of three groups were not significantly different at 24 h postoperation (p>0.05) (Figure 1B). The dosage of PCIA sufentanil in the TT group was higher than that in the CC group and the CT group at 48–72 h postoperation (p<0.05) (Figure 1B). There was no significant difference in the dosage of PCIA sufentanil between the CC group and the CT group at 48–72 h postoperation (p>0.05) (Figure 1B).

**Discussion**

The present study aims to explore the effect of genetic polymorphisms of cytokines on the dosage of sufentanil for PCIA after radical lung cancer surgery. This study demonstrated that the dosage of PCIA sufentanil in the homozygous TT genotype of rs696 was higher than that in the wild-type homozygous CC and the heterozygous CT genotypes at 48–72 h postoperation, suggesting that the rs696 genetic polymorphism may cause individual differences in the analgesic effect of PCIA sufentanil after radical lung cancer surgery.

Postoperative pain is closely related to inflammatory cytokines. Inflammation gene polymorphisms have significant importance in modulating pain severity.28 We hypothesized that genetic polymorphisms of cytokines might affect the dosage of postoperative opioids. To this end, we wanted to explore whether or not the genetic polymorphisms of cytokines affect the dosage of PCIA sufentanil after radical lung cancer surgery. After reviewing the available studies, we found that IL-1A,24,29–31 IL-1B,24,29,31,32 IL-1RN,24,29,33 IL-6,24,29,34,35 CXCL8,24,29,35,36 IL-10,24,29 TNF,20,24,28 NFκB1,24,29,37 REL and NFκBIA are closely related to pain. After searching the NCBI SNP database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=Snp) and inputting related genes, 16 related genetic polymorphism sites were selected with a variant frequency> 5%. We did not find that these SNPs...
Table 4 Preliminary Analysis of the Effect of Genetic Polymorphisms of Cytokines on the Dosage of PCIA Sufentanil After Radical Lung Cancer Surgery

| SNP Site | Genotype | Quantity | p value* | Sufentanil Dosage (μg) | p value<sup>b</sup> |
|----------|----------|----------|----------|------------------------|-----------------|
| IL-1α    | rs17561  |          |          |                        |                 |
|          | CC       | 79       | 0.77     | 95.2 ± 11.3            | 0.179           |
|          | CA       | 13       |          | 90.6 ± 11.3            |                 |
|          | AA       | 0        |          |                        |                 |
| rs1800587| GG       | 79       | 0.77     | 95.2 ± 11.3            | 0.179           |
|          | GA       | 13       |          | 90.6 ± 11.3            |                 |
|          | AA       | 0        |          |                        |                 |
| IL-1β    | rs16944  |          |          |                        |                 |
|          | AA       | 17       | 0.87     | 92.2 ± 10.0            | 0.372           |
|          | GA       | 48       |          | 96.1 ± 12.0            |                 |
|          | GG       | 27       |          | 93.3 ± 10.8            |                 |
| IL-1RN   | rs419598 |          |          |                        |                 |
|          | TT       | 85       | 0.93     | 94.5 ± 11.5            | 0.945           |
|          | CT       | 7        |          | 94.8 ± 9.8             |                 |
|          | CC       | 0        |          |                        |                 |
| CXCL8    | rs4073   |          |          |                        |                 |
|          | AA       | 8        | 0.70     | 95.6 ± 9.0             | 0.802           |
|          | TA       | 44       |          | 93.7 ± 9.8             |                 |
|          | TT       | 40       |          | 95.2 ± 13.2            |                 |
| IL-10    | rs1800871|          |          |                        |                 |
|          | AA       | 45       | 0.67     | 93.2 ± 9.8             | 0.416           |
|          | GA       | 36       |          | 96.5 ± 13.7            |                 |
|          | GG       | 11       |          | 93.8 ± 7.6             |                 |
| rs1800872| TT       | 45       | 0.67     | 93.2 ± 9.8             | 0.416           |
|          | GT       | 36       |          | 96.5 ± 13.7            |                 |
|          | GG       | 11       |          | 93.8 ± 7.6             |                 |
| rs1800896| TT       | 74       | 0.62     | 93.7 ± 11.0            | 0.141           |
|          | CT       | 16       |          | 99.4 ± 12.3            |                 |
|          | CC       | 2        |          | 88.8 ± 3.3             |                 |
| TNF      | rs1800629|          |          |                        |                 |
|          | GG       | 81       | 0.83     | 94.6 ± 11.5            | 0.968           |
|          | GA       | 11       |          | 94.4 ± 10.5            |                 |
|          | AA       | 0        |          |                        |                 |
| NFκB1    | rs28362491|        |          |                        |                 |
|          | ins/ins  | 74       | 0.62     | 93.7 ± 11.0            | 0.141           |
|          | ins/del  | 16       |          | 99.4 ± 12.3            |                 |
|          | del/del  | 2        |          | 88.8 ± 3.3             |                 |
| rs3774932| AA       | 32       | 0.82     | 95.1 ± 10.3            | 0.361           |
|          | GA       | 42       |          | 95.6 ± 10.0            |                 |
|          | GG       | 18       |          | 91.1 ± 15.3            |                 |
| REL      | rs842647 |          |          |                        |                 |
|          | GG       | 66       | 0.72     | 95.4 ± 12.1            | 0.456           |
|          | GA       | 25       |          | 92.6 ± 9.1             |                 |
|          | AA       | 1        |          | 86.4                  |                 |

(Continued)
(ie, rs17561 C> A, rs1800587 G> A, rs16944 G>A, rs419598 T> C, rs1800629 G> A, rs28362491 delATTG, rs3774932 A> G, and rs842647 G> A) were associated with dosage of PCIA sufentanil, which may be related to factors such as ethnic differences and population conditions. However, we found for the first time that the NfκB1a rs696 genetic polymorphism might have an impact on the dosage of PCIA sufentanil after radical lung cancer surgery.

Rs696 is located in the 3 prime UTR variants of the NfκB1a gene. Studies have shown that the frequency of rs696 C>T variants is related to differences in population and ethnicity. Americans have a variant frequency of 30% compared to Africans, who have a variant frequency of 65% and East Asians, who have a variant frequency of 39% (https://www.ncbi.nlm.nih.gov/snp/rs696 # frequency_ tab). In this study, the rs696 C>T variant frequency of patients with lung cancer was 35.3%, which was similar to that of the East Asian population, suggesting certain population representativeness.

NfκB1a genetic polymorphisms are related to a variety of inflammatory, autoimmune and tumour diseases. Many of the same inflammatory factors that promote tumour growth are also hypothesized to function as pain modulators. Reyes-Gibby et al found that an additive model for NfκB1a Ex6+50 C>T (rs8904) was predictive of severe pain in lung cancer patients. However, no relevant study has been conducted to examine NfκB1a genetic polymorphism and dosages of postoperative opioids.

The NfκB1a gene mainly encodes IκB, which inhibits the NfκB signalling pathway under several physiological processes, such as by biasing NfκB nucleocytoplasmic dynamics and blocking its DNA binding. Inhibiting the NfκB signalling pathway can effectively decrease levels of inflammatory cytokines and reduce the severity of pain. Zhao et al found that the NfκB1a rs1957106 polymorphism is associated with lower mRNA and NFKBIA protein levels. We speculate that the homozygous TT genotype of rs696 might cause lower mRNA levels and protein expression of IκB, decreasing the inhibitory function of the NfκB signalling pathway and increasing the levels of perioperative inflammatory cytokines, which leads to increased postoperative pain and opioid dosage.

### Table 4 (Continued).

| SNP Site  | Genotype | Quantity | p value<sup>a</sup> | Sufentanil Dosage (μg) | p value<sup>b</sup> |
|-----------|----------|----------|----------------------|------------------------|---------------------|
| NfκB1a rs8904 | GG | 37 | 0.80 | 93.0 ± 12.1 | 0.052 |
|           | GA | 45 | | 94.3 ± 9.5 | |
|           | AA | 10 | | 103.0 ± 15.6 | |
| rs696     | CC | 36 | 0.53 | 93.2 ± 12.2 | 0.025* |
|           | CT | 47 | | 93.9 ± 9.5 | |
|           | TT | 9 | | 104.6 ± 15.6 | |

Notes: Data are expressed as the frequencies or mean ± SD, as appropriate. p value<sup>a</sup> for Hardy–Weinberg analyses of different genotypes; p value<sup>b</sup> for PCIA sufentanil dosage of different genotypes; *The difference in the dosage of PCIA sufentanil between three groups was significant, p < 0.05.

Abbreviations: SNP, single-nucleotide polymorphism; IL-1A, interleukin-1A; IL-1β, interleukin-1β; IL-1RN, interleukin-1RN; CXCL8, C-X-C motif chemokine ligand 8; IL-10, interleukin-10; NfκB1a, nuclear factor kappa-B1; REL, a member of the nuclear factor xB gene family; TNF, tumour necrosis factor-alpha; NfκB1a, nuclear factor kappa-B inhibitor alpha.

### Table 5 NfκB1a Rs696 C>T Genotypes and Allele Frequency

| Allele | C | T | Total | Genotypes | CC | CT | TT | Total |
|--------|---|---|-------|-----------|----|----|----|-------|
| Quantity | 119 | 65 | 184 | 36 | 47 | 9 | 92 |
| Frequency (%) | 64.67% | 35.33% | 100% | 100% | 100% | 100% | 100% |
Table 6 Patients’ Characteristics, Surgery and Anaesthesia Data, and Opioid-Related Adverse Events Based on the Different Genotypes of NFκBIA Rs696

|                        | CC Group (n=36) | CT Group (n=47) | TT Group (n=9) | p value |
|------------------------|----------------|-----------------|---------------|---------|
| Patients’ characteristics |                |                 |               |         |
| Age (years)            | 58.1 ± 10.9    | 58.9 ± 10.2     | 61.7 ± 8.8    | 0.646   |
| Gender (male/female)   | 22/14          | 32/15           | 4/5           | 0.394   |
| BMI (kg/m²)            | 21.7 ± 2.1     | 22.7 ± 2.7      | 21.5 ± 3.3    | 0.176   |
| ASA classification (grade Ⅰ/Ⅱ) | 4/42          | 5/42            | 0/9           | 0.375   |
| Medical comorbidities  |                |                 |               |         |
| Hypertension (yes/no)  | 4/32           | 3/44            | 0/9           | 0.352   |
| Diabetes mellitus (yes/no) | 14/22         | 15/32           | 2/7           | 0.588   |
| History of surgery (yes/no) | 9/27          | 19/28           | 4/5           | 0.272   |
| Occupation             | 13/5/2/9/7     | 20/7/4/10/6     | 4/1/0/2/2     | 0.951   |
| (worker/farmer/civil service/retirement/other) | 8/17/8/3     | 13/35/8/1      | 3/4/2/0      | 0.754   |
| Education              |               |                 |               |         |
| (primary/secondary/university/illiterate) | 58.1 ± 10.9    | 58.9 ± 10.2     | 61.7 ± 8.8    | 0.646   |
| Surgery and anaesthesia data | |                 |               |         |
| Operation time (min)   | 118.8 ± 50.0   | 106.3 ± 33.6    | 125.5 ± 43.3  | 0.263   |
| Volume of blood loss (mL) | 58.4 ± 52.9   | 60.6 ± 62.3     | 55.5 ± 30.8   | 0.964   |
| Infusion volume (mL)   | 1333.3 ± 348.4 | 1241.4 ± 378.0 | 1222.2 ± 384.1 | 0.480   |
| Urine output (mL)      | 280.0 ± 243.0  | 197.4 ± 130.9   | 217.7 ± 123.4 | 0.128   |
| Length of incision (cm) | 8.0 ± 3.0      | 8.2 ± 2.6       | 8.2 ± 1.5     | 0.919   |
| Intraoperative anaesthetics |               |                 |               |         |
| Sufentanil (μg)        | 55.0 ± 9.3     | 57.0 ± 9.4      | 52.9 ± 9.7    | 0.395   |
| Remifentanil (μg)      | 758.0 ± 393.4  | 721.2 ± 259.6   | 779.8 ± 325.2 | 0.818   |
| Propofol (mg)          | 894.9 ± 307.1  | 900.1 ± 249.3   | 942.5 ± 288.6 | 0.897   |
| Opioid-related adverse events |               |                 |               |         |
| Hypersedation (yes/no) | 0/36           | 0/47            | 0/9           |         |
| Respiratory depression (yes/no) | 0/36         | 0/47            | 0/9           |         |
| Nausea (yes/no)        | 13/23          | 23/23           | 3/6           | 0.230   |
| Vomiting (yes/no)      | 3/33           | 4/43            | 1/8           | 0.966   |
| Pruritus (yes/no)      | 2/34           | 1/46            | 0/9           | 0.519   |
| Hypotension (yes/no)   | 1/35           | 0/47            | 1/8           | 0.146   |

Note: Data are expressed as the frequencies or mean ± SD, as appropriate.

Abbreviations: CC group, the wild-type homozygotes of NFκBIA rs696 CC group; CT group, the heterozygotes of NFκBIA rs696 CT group; TT group, the homozygotes of NFκBIA rs696 TT group; BMI, body mass index; ASA, American Society of Anesthesiologists.

Our study has some limitations. First, the preoperative pain state and use of steroids have not been considered in this study, but they may play a big role in determining inflammation and pain perception, and indirectly, the need for opioids. Second, we did not examine the levels of the corresponding cytokines and their changes in the blood. Third, only partial gene fragments, not complete genes, were detected in this study. The differences in the selected gene fragments may have a certain effect on the results, while the selected gene segments were highly representative with a variant frequency> 5%. Finally, the selected group of lung cancer patients in East Asia may have a certain effect on the results, and more studies might be needed to confirm our findings.

In summary, genetic polymorphisms of cytokines, specifically NFκBIA rs696, may be one of the individual genetic factors that causes a difference in the analgesic effect of PCIA sufentanil after radical lung cancer surgery.
Disclosure

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Figure 1 (A and B) Comparison of the resting VAS scores and PCA sufentanil dosages within 72 h by the different genotypes of NFkBIA rs696. "The difference of the dosage of PCA sufentanil between groups was significant, p < 0.05. Abbreviations: CC group, the wild-type homozygotes of NFkBIA rs696; CT group, the heterozygotes of NFkBIA rs696; TT group, the homozygotes of NFkBIA rs696; VAS, visual analogue scale; 24 h post-op, 24 h postoperative; 48 h post-op, 48 h postoperation; 72 h post-op, 72 h postoperation.”
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