

## Original Article

# The correlation between CYP450 superfamily gene polymorphisms and individualized, post-operative pain management

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**Abstract:** Pain is one of basic sensations of our body. Particularly, post-operative pain that patients suffer from poses a critical problem. Individuals have variations in their sensitivity to anesthetic drugs. Dosage differences are therefore determined to be dependent on individual patients. However, the molecular mechanisms of pain sensitivity are still elusive. This study aimed to investigate the correlation between polymorphisms of CYP450 and the MDR gene and the analgesic efficiency of anesthesia drugs. Esophageal cancer patients (n=2358) undergoing surgery from January 2012 to August 2017 were enrolled in this study, along with healthy individuals (n=1200). Blood samples were collected and the PCR-restriction fragment length polymorphism (RFLP) approach was used to analyze the genetic polymorphisms of the P450 gene. The efficiency of the anesthetic drug sodium oxybate was compared among individuals with different genotypes of P450. A higher frequency of CYP450 heterozygous, T allele, MDR homozygous and heterozygous, and G allele was found in surgical patients, compared to the frequency found in healthy individuals ( $P<0.05$ ). The genotype frequency of AG at the MDR site was significantly decreased compared to the AA genotype ( $P<0.05$ ). Logistic correlation analysis indicated the heterozygous genotype of CYP450, T allele, and MDR genotype as risk factors for anesthetic drug sensitivity on esophageal cancer patients after surgery. CYP450 and the MDR A1283G genetic polymorphism are closely correlated with anesthetic drug sensitivity in esophageal cancer patients undergoing surgery. The heterozygous genotype at the CYP450 loci, the allele at the T loci and the MDR1 genotype are risk factors for anesthetic drug sensitivity.

**Keywords:** Post-operative pain, P450, genetic polymorphism, individualized drug application, correlation analysis

## Introduction

Pain is a negative emotional phenomenon, and subjective feelings regarding pain correlate with tissue damage [1]. Pain is one of the five major biological features, with the others being body temperature, respiration, blood pressure and pulse. Post-operative pain severely affects patient recovery and prognosis. During and after surgery, cancer patients frequently receive anesthetic drugs to relieve pains [2]. Due to different sensitivities toward anesthetic drugs, dosages must be optimized for individual patients [3, 4].

Systemic anesthetic drugs are frequently administered to tumor patients during surgery. As the only non-barbital type intravenous anesthetic drug with analgesia effects, for instance,

sodium oxybate can be used for both the induction and maintenance of anesthesia [5]. Sodium oxybate functions as an NMDA receptor antagonist. It can block the transmission of the sensation of pain and activate the brain stem or limbic system. It contributes to the loss of pain sensation but leaves its recognition intact, thus achieving dissociative anesthesia. It is thus applicable for the anesthesia of elder people, children, and in neurosurgery, trauma and burn patients, on account of its wide body distribution after intravenous infusion, slow onset, and longer persistent time [6, 7].

Cytochrome P450 (CYP450) belongs to the family of heme-proteins with auto-oxidation potency, and is so named due to its specific absorbance peak at 450 nm among monooxygenase [8, 9]. CYP450 participates in the

metabolism of endogenous and exogenous substances, including drugs and environmental compounds. The human hepatocyte P450 enzyme lineage consists of at least 9 members that correlate with drug metabolism [10, 11]. The relationship between the CYP450 genetic polymorphism and anesthesia conditions by sodium oxybate is still unclear. Our study aims to analyze a possible correlation between the genetic polymorphisms of P450 members and the efficiency of sodium oxybate in analgesia based on data from esophageal carcinoma patients.

### Research objects and methods

#### *Research objects*

Esophageal carcinoma patients received systemic anesthesia by sodium oxybate. The sensitivity toward sodium oxybate was graded based on criteria stipulated by the American Anesthesia Association. This study was approved by the ethical committee of our hospital. All research objects signed informed consents. A total of 2358 esophageal carcinoma patients were recruited from Renmin Hospital of Wuhan University from January 2012 to August 2017, in parallel with 1200 healthy individuals.

**Inclusive criteria:** All research subjects had normal heart, liver and kidney function, no history of any major disease or any family history of a major disease, no history of smoking or drinking, no history of any allergy to anesthesia drugs, no cardiovascular disease or diabetes, and no neuropathic or chronic pain. The patients did not take any anti-convulsion, anti-depression, or corticosteroid drugs, nor did they have a history of taking opine analgesic drugs or non-steroid drugs, or a history of mental disorders. The Patients did not take P-glycoprotein substrate.

#### *Collection of blood samples from esophageal carcinoma surgery patients and healthy individuals*

Using previously reported approaches [23], 5 ml venous blood samples were collected from esophageal carcinoma surgery patients and healthy individuals, and the samples were treated with heparin for anti-coagulation. The venous blood samples were collected from fasting patients and healthy individuals using negative pressure blood collecting tubes. The

blood samples were mixed with anti-coagulant at a 1:7 ratio. 2 mL venous blood was gently mixed at room temperature for measuring serum factors and for extracting genomic DNA.

#### *Genomic DNA extraction*

Based on a previously reported method [8], genomic DNA was extracted from the blood samples using a genomic DNA extraction kit (Tiangen Bio, China). Specifically, 20 ml extracting buffer I was pre-heated at 60°C. 5-10 g tissue samples were homogenized into powder in liquid nitrogen and were immediately added into pre-warmed tubes for vigorous shaking, followed by 60°C incubation for 30-60 min. A 20 mL chloroform/ethanol/pentanol solution was added for mixing at room temperature for 5-10 min. If necessary, the mixture was centrifuged at 5000 rpm for 5 min to separate the aqueous and organic phases. The supernatant was carefully saved and mixed with an equal volume of isopropanol. After mixing and gentle incubation at room temperature, a silk-like DNA precipitation was observed. The DNA pellet was then collected, dried using clean filter paper, and transferred to a TE buffer in a tube for dissolving. If the DNA was still not transformed into a precipitate, a further 5000 rpm centrifugation for 5 min was performed, and the precipitate was transferred. DNA resolving can be accelerated using a 60°C water-bath for 15 min. The DNA solution was centrifuged at 12000 rpm for 5 min, and the supernatant was transferred, and then 5 µl of RNaseA (10 µg/µl) was added to it and then incubated at 37°C for 10 min. 1/10 mol/L NAAC and 2X volume of iced ethanol were added for 20 min of mixing at -20°C. When the DNA was formed into a precipitate, 70% ethanol was used to rinse the pellets, which were dried using clean filter paper.

#### *PCR*

Using genomic DNA as the template, PCR amplification was performed to measure the P450 member genes, using the following sequences: CYP4A-P1, 5'-TTACT GTCGG AATCC TGCTC-3'; CYP4A-P2, 5'-CAGCT GACCT ATCCA TACAG-3'; MDR-P3, 5'-GGAAT CCTAC CTTTC AAGCA-3'; MDR-P4, 5'-AAGGA AGGCT GGAAG AGTGC-3'; Actin-P5, 5'-GGAAT CTTGT CCTGC ACTTC-3'; Actin-P6, 5'-CAATG CTGTC CACCT AACAG-3'. The primers were synthesized by Yuanzhi Bio (China), as the sequences shown in **Table 1**.

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**Table 1.** Primer sequences

| Primer | Sequence                   |
|--------|----------------------------|
| P1     | 5'TTACTGTCTCGGAATCCTGCTC3' |
| P2     | 5'CAGCTGACCTATCCATACAG3'   |
| P3     | 5'GGAATCCTACCTTTCAAGCA3'   |
| P4     | 5'AAGGAAGGCTGGAAGAGTGC3'   |
| P5     | 5' GGAATCTTGTCTGCACTTC3'   |
| P6     | 5'CAATGCTGTCCACCTAACAG3'   |

The PCR system consisted of a 0.5 µl genomic DNA template, 1.0 µl 10XPCR buffer, 1.0 µl dNTP mixture (1 mM), 0.5 µl of primer 1 and primer 2 (20 µM), 0.05 µl Taq DNA Polymerase, 0.75 µl MgCl<sub>2</sub> (10 mM), 3 µl H<sub>2</sub>O. The PCR conditions were: 95°C pre-denature for 5 min, followed by 30 cycles of 95°C 40 s, 56°C 30 s and 72°C 40 s, finally 72°C for 8 min.

### *Enzymatic digestion*

PCR amplification and PCR-restriction fragment length polymorphisms (RFLP) were used to analyze the P450 member gene polymorphisms from the blood samples, in order to compare differential responses to sodium oxybate among patients with different P450 member genotypes.

Each CYPA gene fragment has about 170 bp length after PCR amplification. The enzymatic reaction was performed after PCR, using a system that included a 4 µL 10X reaction buffer, 0.4 µL acetylated BSA (5 µg/µL), 1 µL Hsp92 II restriction endonuclease (10 U/µL), 14.6 µL water, for an 8h incubation at 37°C.

After PCR amplification, the MDR gene fragment was 153 bp. Enzymatic digestion was performed in a system containing 4 µL 10X reaction buffer, 0.4 µL acetylated BSA (5 µg/µL), 1 µL Hsp92 II restriction endonuclease (10 U/µL), 14.6 µL water, for 37°C incubation for 8 h.

### *Agarose gel electrophoresis for PCR products and digestion products*

Agarose gel electrophoresis was used to detect the PCR products. Specifically, PCR products were mixed with a 5X loading buffer and were loaded into an agarose gel well for 1% gel electrophoresis under 100 mV for 16 min. After the reaction ended, images of the gel were taken in a gel imaging system for analysis.

### *Sequence of PCR bands*

Using P450 member gene primers, the PCR products were sequenced by Dingguo Biotech (China). The sequence of the P450 member gene was aligned with sequences from the NCBI database.

### *Data statistics and analysis*

In this study, all data were analyzed by SPSS 17.0 software and were presented as the mean ± the standard deviation (SD). The enumeration data were tested by chi-square analysis, and the measurement data were processed by an analysis of variance (ANOVA) with Tukey's post hoc test. Statistical significance was defined when P<0.05.

## **Results**

### *General conditions of esophageal carcinoma patients and healthy individuals*

The analysis of the general conditions of the esophageal carcinoma patients and healthy individuals is shown in **Table 2**. No significant differences were found regarding sex, age, BMI, and glucose indicators between the two groups.

### *Genotyping of P450 member genes between esophageal carcinoma surgery patients and healthy individuals*

The expression levels of the P450 member genes in those esophageal carcinoma patients receiving surgery and who were sensitive to anesthesia drugs are shown in **Figure 1**. No significant differences were identified.

The differential genotyping of the P450 members between the esophageal carcinoma surgery patients and the healthy individuals is shown as in **Figure 2**.

### *Gene polymorphisms of CYPA between the esophageal carcinoma surgery patients and the healthy individuals*

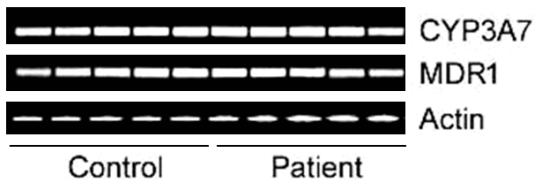
The distribution of genotypes of the CYPA gene in esophageal carcinoma patients and healthy controls all fit the Hardy-Weinberg equilibrium (CC, 75.2%; TT, 0%, CT, 24.8%).

We further analyzed the allele frequency of the CYPA gene between the esophageal carcinoma surgery patients and the healthy control group

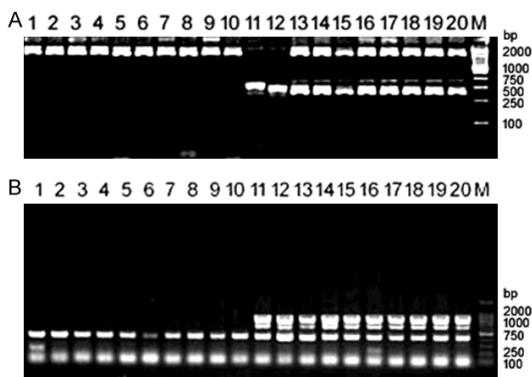
## CYP3A gene and pain relief

**Table 2.** Analysis of the general conditions between esophageal carcinoma surgery patients and healthy individuals

| Group                        | N    | Sex (male/female) | Age        | BMI (Kg/m <sup>2</sup> ) | Glucose (mm Hg) |
|------------------------------|------|-------------------|------------|--------------------------|-----------------|
| Esophageal carcinoma surgery | 2358 | 1297/1061         | 67.9 ± 2.5 | 24.3 ± 4.6               | 6.2 ± 2.5       |
| Healthy individuals          | 1200 | 624/576           | 68.0 ± 0.9 | 24.6 ± 3.7               | 6.3 ± 2.1       |
| <i>p</i> value               |      | 0.089             | 0.180      | 0.192                    | 0.245           |



**Figure 1.** Analysis of P450 member gene expression between esophageal carcinoma surgery patients and healthy individuals.



**Figure 2.** Genotyping results of P450 member genes between esophageal carcinoma surgery patients and healthy individuals. A. Gene polymorphisms of the CYP3A gene. M, molecular marker; Lane 1-10, normal people; Lane 11-20, surgery patients. B. MDR gene polymorphism assay. M, molecular marker; Lane 1-10, normal people; Lane 11-20, surgery patients.

and found there was a significant difference between the two groups ( $P < 0.05$ , **Tables 3 and 4**).

### *MDR gene polymorphisms between esophageal carcinoma surgery patients and healthy volunteers*

The distribution of genotypes of the MDR gene in the esophageal carcinoma patients and the healthy controls all accorded with the Hardy-Weinberg equilibrium (AA, 71.0%; GG, 11.8%; AG, 48.1%).

We further analyzed the allele frequency of the MDR gene and found that in the esophageal carcinoma surgery patients it was significantly

different from the frequency in the healthy control group ( $P < 0.01$ , **Tables 5 and 6**).

### *Correlation analysis between the P450 gene polymorphisms and anesthetic drug sensitivity in esophageal carcinoma surgery patients*

A logistic correlation analysis (**Figure 3**) showed that the heterozygous genotype at the CYP3A loci, the T allele, and the MDR genotype are risk factors for the anesthetic drug sensitivity of esophageal carcinoma surgery patients (OR=0.12 and 0.29). These results indicated a close correlation between the P450 gene polymorphism and anesthesia drug sensitivity in esophageal carcinoma patients during surgery. We further analyzed the risk factors for anesthesia drug sensitivity in esophageal carcinoma patients, including the heterozygous genotype at CYP3A, the T allele, and the MDR genotype (CT vs CC: OR=0.42, 95% CI: 0.27-0.66; TT vs CC: OR=0.46, 95% CI: 0.26-0.81; CT + TT vs CC: OR=0.52, 95% CI: 0.39-0.70).

## Discussion

Anesthesia during surgery is often accompanied by a sensation of pain. Due to individual differences, the same dosage of an anesthesia drug may lead to different levels of pain in individual patients [1]. In recent years, the idea of individualized drug delivery has emerged. The strategy of individualized drug delivery represents an indispensable stage of anesthesia development and a future direction [6]. However, due to the existence of gene polymorphisms, the effective evaluation and prediction based on pain-related gene polymorphisms in different individuals requires further investigation in clinical practice [12, 13]. For instance, the optimization of anesthesia drugs shows promise in the improvement of peri-operative efficiency and safety [14].

The P450 CYP3A4 family consists of multiple genotypes that can fulfil the requirement of individualized drug administration, and in the meantime brings better anesthesia levels [15,



## CYPA gene and pain relief

**Table 3.** CYPA gene polymorphisms and genotype frequencies between esophageal carcinoma surgery patient and healthy volunteers

| Group                        | N    | CC (%) | TT (%) | CT (%) |
|------------------------------|------|--------|--------|--------|
| Esophageal carcinoma surgery | 2358 | 75.2   | 0      | 24.8   |
| Healthy individuals          | 1200 | 99     | 0      | 100    |
| <i>p</i> value               |      | 0.000  | 1      | 0.000  |

**Table 4.** CYPA gene polymorphisms and allele frequency analysis between esophageal carcinoma surgery patients and healthy volunteers

| Group                        | N    | C (%) | T (%) |
|------------------------------|------|-------|-------|
| Esophageal carcinoma surgery | 2358 | 87.6  | 12.4  |
| Healthy individuals          | 1200 | 99.5  | 0.5   |
| <i>p</i> value               |      | 0.000 | 0.000 |

**Table 5.** MDR gene polymorphisms and genotype frequencies between esophageal carcinoma surgery patients and healthy volunteers

| Group                        | N    | AA (%) | GG (%) | AG (%) |
|------------------------------|------|--------|--------|--------|
| Esophageal carcinoma surgery | 2358 | 71.0   | 11.8   | 48.1   |
| Healthy individuals          | 1200 | 40.1   | 5.0    | 24.0   |
| <i>p</i> value               |      | 0.000  | 0.000  | 0.000  |

**Table 6.** MDR gene polymorphisms and allele frequency analysis between esophageal carcinoma surgery patients and healthy volunteers

| Group                        | N    | A (%) | G (%) |
|------------------------------|------|-------|-------|
| Esophageal carcinoma surgery | 2358 | 64    | 36    |
| Healthy individuals          | 1200 | 83    | 17    |
| <i>p</i> value               |      | 0.000 | 0.000 |

16]. It is worth noting that in clinical practice, anesthesia levels may be affected by smoking, surgery duration, and patient age [17]. The relationship between CYP3A4 gene polymorphisms and anesthesia affects analgesic sensitivity and has direct effects on treatment efficiency, which can fully reflect the coming need for patient-centered medicine, and can help obtain satisfactory clinical efficiency [18]. Meanwhile, the MDR gene has significant polymorphisms, which determine that the MDR gene in individualized surgeries could achieve a satisfactory level of anesthesia [19]. The results of this study show that the P450 member gene polymorphisms are closely correlated with anesthesia drug sensitivity in esophageal carcinoma patients. The heterozygous genotype at

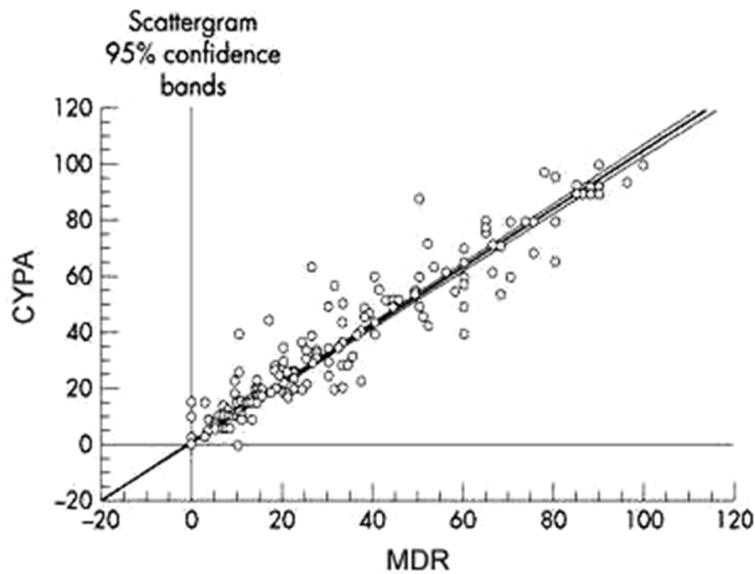
CYPA loci, the allele at T loci, and the MDR genotype are risk factors for anesthesia the drug sensitivity of esophageal carcinoma patients after surgery. This is consistent with previous results [6].

The effect of individual inheritance on drug metabolism and efficiency and single nucleotide polymorphisms (SNP) of the drug metabolic enzyme gene CYP3A4 can affect the efficiency of many pain killer medicines [20]. Some genetic studies have suggested that intron polymorphisms affect product activity [21]. Of note, we speculated that besides the SNPs of the CYP3A4 exons, its intron SNPs are also significantly correlated with the post-op anesthesia of cancer patients. To substantiate this hypothesis, we investigated esophageal carcinoma patients in our hospital and tested differences likely caused by diverse post-op PCA and environmental factors. Our data indicated a higher frequency of CYPA heterozygous, T alleles, MDR homozygous and heterozygous, and G alleles in surgical patients, compared to that the frequency in healthy individuals. The genotype frequency of AG at the MDR site was significantly decreased compared to the AA genotype. Heterozygous genotypes of CYPA, T alleles, and MDR genotypes are risk factors for anesthetic drug sensitivity in esophageal cancer patients after surgery. However, the limitations in this study still exist, namely, that external factors including age or surgical duration,

smoking on analgesic, were not included in the analysis. Further study may focus on exploring the molecular mechanisms between the P450 member gene polymorphisms and pain. After individualized surgery, the use of the MDR1 gene and 4P50 (CY3PA4) should be promoted in clinics, in order to improve analgesic effects.

### Conclusion

This study suggests that the P450 member gene polymorphisms are closely related to the anesthesia drug sensitivity of esophageal carcinoma patients after surgery. The heterozygous genotype at the CYPA loci, the allele at M loci, and the MDR genotype are risk factors govern-



**Figure 3.** Logistic correlation analysis result. CYPA loci heterozygous genotype, T allele and MDR genotype are risk factors for anesthesia drug sensitivity in esophageal carcinoma patients (OR=0.12, 0.29).

ing anesthesia drug sensitivity in esophageal carcinoma patients after surgery.

**Disclosure of conflict of interest**

None.

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