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Original Article

Effects of *CYP3A4* polymorphisms on efficiency of general anesthesia combined with epidural block in patients undergoing cardiac valve replacement

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Abstract: This study aims to investigate the effects of *CYP3A4* polymorphisms (*4, *5 and *6) on efficiency of general anesthesia (GA) combined with epidural block (EB) in patients undergoing cardiac valve replacement. From January 2014 to October 2015, a total of 511 patients undergoing cardiac valve replacement (case group) and 503 healthy individuals (control group) were selected for the study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied for genotyping of *CYP3A4* gene. Central venous pressure (CVP), mean arterial pressure (MAP), heart rate (HR), pulse oximetry (SPO_2), extubation and duration of intensive care unit (ICU) stay during the surgery were observed and recorded. A nine-month follow-up was conducted. Genotype and allele frequency of *CYP3A4*4* were significantly different between the case and control groups (all $P < 0.05$). Compared with wild-type *1*1 patients with heterozygous *1*4 of *CYP3A4*4* showed significant difference in HR, MAP, SPO_2 and CVP and in the time of extubation and ICU stay. *CYP3A4*4* polymorphism may be associated with the effect of GA combined with EB in cardiac surgery. These results demonstrate that *CYP3A4*4* polymorphism is correlated with the effects of GA combined with EB in cardiac surgery. *CYP3A4* polymorphisms increase the risk of GA combined with EB among patients undergoing cardiac valve replacement.

Keywords: *CYP3A4*, *CYP3A4*4*, *CYP3A4*5*, *CYP3A4*6*, polymorphism, general anesthesia, epidural block

Introduction

General anesthesia (GA) is a drug-induced, reversible condition that includes specific behavioral and physiological characters [1]. It is reported that GA plays a crucial role in patients undergoing cardiac surgery [2]. Epidural anesthesia can relieve pain during operation by blocking the transmission of all somatosensory pain impulses to the central nerve system [3]. Thoracic epidural anesthesia (TEA) may effectively improve myocardial ischemia, reduce arrhythmia, contribute to early tracheal extubation and prevent respiratory complications during cardiac surgery [4]. Acupuncture anesthesia is one way of anesthesia in cardiac surgery but is seldom put into practice due to sophisticated preoperative preparation and inadequate muscle relaxation [5]. At present, the anesthesia type of GA combined with epidural block (EB) in abdominal aortic aneurysm surgery has gained popularity with modulating spinal sympathetic outflow with consequent vasodilatation, increased visceral perfusion as well as decreased afterload [6]. The selection of GA combined with EB may be an efficient way for patients who receive laparoscopic adrenalectomy to prevent the undulations in hormone levels [7]. A combination of GA with TEA plays a beneficial role in clinical outcomes in patients undergoing cardiac surgery; however, there exist uncertain side-effects which cannot be evaluated with the current data [8]. It is well known that the efficacy of some drugs differ among individuals due to inter- and intra-individual variability, including gene polymorphisms [9]. The diverse effects of gene factors on GA combined with EB in cardiac surgery warrant further investigation.

Ubiquitously found in animals, plants, fungi and eukaryotic organisms, cytochrome P450 (CYP)

consists of a family of hemoprotein enzymes which answers for the biotransformation of a number of xenobiotics, including therapeutic agents, and the primary human drug-metabolizing CYPs dwells in families 1, 2 and 3 [10]. *CYP3A4* is one of the important families of CYP including 13 exons and it is located on chromosome 7q22.1 [11]. CYP3A4 enzyme is extensively found in the human liver, kidneys, lungs, gastrointestinal tract, brain, placenta, endothelium, lymphocytes and intestines and it metabolizes a variety of endobiotics, such as progesterone, testosterone, cortisol and estradiol [12]. CYP3A4 is also in charge of the metabolism of approximately 45%-60% prescribed drugs and reduced CYP3A4 production or activity is related to a functional single-nucleotide polymorphism (SNP) in intron 6 [13]. Being point mutation $A_{13989}G$, $C_{15820}G$ and A_{17776} insertion and located in exon 5, exon 7 and exon 9, respectively, *CYP3A4*4, CYP3A4*5* and *CYP3A4*6* are three mutant and variant alleles of *CYP3A4* gene and these three alleles can reduce the CYP3A4 activity in a Chinese population, although these mutations occur rarely in Chinese subjects [14]. In addition, in repaglinide's pharmacokinetics, the inter-individual variability is associated with the genetic polymorphisms of *CYP3A4* gene [12]. A new functional polymorphism in *CYP3A4* intron 6 was found to significantly affect tacrolimus pharmacokinetics in kidney transplantation [13]. However, regardless of these intriguing studies, it has not been established whether this mutation of *CYP3A4* gene has any effect on anesthesia efficacy in cardiac surgery. Therefore, we performed a study to explore the correlation of *CYP3A4* polymorphisms (*4, *5 and *6) with the efficacy of GA combined with EB in patients undergoing cardiac valve replacement.

Materials and methods

Ethical statement

The study was approved by the ethics committee of 2nd Affiliated Hospital, College of Medicine, Zhejiang University and informed consents were obtained from the subjects or legal guardians of study subjects. The study protocols followed the ethical principles and all the experimental procedures in this study were performed on the basis of Declaration of Helsinki [15].

Study subjects

From January 2014 to October 2015, a total of 511 patients (males: n = 209; females: n = 302; mean age: 51.4 ± 6.9 years; weight: 52.5 ± 2.9 kg) undergoing cardiac valve replacement with GA combined with EB in 2nd Affiliated Hospital, College of Medicine, Zhejiang University were selected as case group. Among patients in the case group, 274 cases were mitral valve disease, 133 cases were aortic valve disease and 104 cases were combined valve disease. According to the criteria of American Society of Anesthesiologists (ASA) [16], there were 149 cases in Class II: and 362 cases in Class III. The inclusion criteria: (1) patients with chronic valvular heart disease aged 30-70 years, the left ventricular ejection fraction (LVEF) ≥ 35 detected by echocardiography; (2) patients in ASA class II~III; (3) patients without complications, such as hemorrhage and embolism; (4) patients without history of cerebrovascular disease; (5) patients with clear consciousness and no difficulty in communication; (6) no consanguinity or intermarriage history among patients. The exclusion criteria: (1) patients with other cardiac procedures requiring simultaneous treatment (such as, vntricular aneurysm and congenital heart disease) and under the age of 30 years; (2) patients with 2 times aminopherase in liver than normal range; (3) patients with less than 50 mL/min creatinine clearance rate; (4) patients with allergic constitution; (5) patients with severely damaged liver or kidney functions and abnormal coagulation; (6) patients with abnormal thyroid function; (7) patients who refused to participate in the study. Besides, a total of 503 healthy members (males: n = 179; females: n = 324; mean age: 51.7 ± 7.1 years; weight: 52.8 ± 3.7 kg) who took physical examination in 2nd Affiliated Hospital, College of Medicine, Zhejiang University at the same period were classified as control group.

Sampling collection

Fasting peripheral blood (5 ml) was collected from the control group in the morning while fasting peripheral blood (5 ml) was collected from the case group the day before surgery in the morning. Ethylenediaminetetraacetic acid (EDTA) was used for anticoagulant (Shanghai Haling Biological Technology Co., Ltd, Shanghai, China). Genomic DNA was extracted with Blood

Table 1. Primer sequences for PCR-RFLP

Allele	Primer	Sequence	PCR amplicon size (bp)
CYP3A4*4	Exon5 (F)	5'-CACATTTTCTACAACCATGGAGACC-3'	249 bp
(A_{13989}→G)	Exon5 (R)	5'-TTTTATACCTCCCCACCAGATTC-3'	
CYP3A4*5	Exon7 (F)	5'-TGTTGCATGCATAGAGGAAGGATGG-3'	450 bp
(C_{15820}→G)	Exon7 (R)	5'-GATGACAGGGTTTGTGACAGGGG-3'	
CYP3A4*6	Exon9 (F)	5'-GAGCCATATTCTCAGAAGGGAGATCAAG-3'	290 bp
(A_{17776} insertion)	Exon9 (R)	5'-CAAACATGTGTCGTTCTGCTATGTGG-3'	

Note: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; F, Forward primer; R, reverse primer.

Table 2. Genotyping of *CYP3A4*4*, *CYP3A4*5* and *CYP3A4*6* by gel electrophoresis

PCR product (bp)	Enzyme fragment (bp)			Gel electrophoresis (%)
	Wild-type	Heterozygous mutant	Homozygous mutant	
249	141, 94, 14	141, 94, 47, 14	94, 47, 14	Polyacrylamide gel (12)
450	450	450, 250, 200	250, 200	Agarose gel (1.5)
290	137, 129, 24	153, 137, 129, 24	153, 137	Polyacrylamide gel (20)

Note: PCR, polymerase chain reaction.

Genome DNA Extraction Kit (Tiangen Biotech Co., Ltd, Beijing, China) after blood sampling and then preserved at -20°C. And nucleic acid-protein was analyzed by nucleic acid-protein detector (Puyang Scientific Instrument Research Institute, Jiangsu, China).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

CYP3A4 polymorphisms were sequenced and analyzed by PCR-RFLP. The polymerase chain reaction (PCR) reaction system of CYP3A4 was 50 μl, including 0.2 mmol/l deoxyribonucleoside triphosphate (dNTP), 1.5 mmol/l Mg^{2+}, DNA template 100 ng, Taq DNA polymerase 1.25 u, 0.2 μmol/l of upstream and downstream primers, respectively. PCR reaction conditions: 10 min of pre-denaturation at 94°C; 40 s of denaturation at 94°C, 45 s of annealing at 55°C and 35 s of extension at 72°C for totally 35 cycles, and finally 10 min of extension at 72°C. PCR products were detected by 1% agarose gel electrophoresis with 0.5 μg/ml ethidium bromide (Beijing Ruida Henghui Science & Technology Development Co., Ltd, Beijing, China). All the primers were synthesized by TaKaRa (Dalian, China) and the primer sequences were shown in **Table 1**. PCR amplification product (5 μl) was extracted and evaluated by 1.5% agarose gel electrophoresis (70 V, 15 min) with Tris-acetate-EDTA (TAE) buffer solution (Shanghai Bioleaf Biotech Co., Ltd, Shanghai, China). The reaction product was irradiated by ultraviolet lamp.

The PCR amplification products were digested with specific restricted enzymes (BsmA I for 249 bp, Cla I for 450 bp, Hinf I for 290 bp) provided by Wu Han U-Me Biotech Company (Wuhan, China). The total reaction system was 20 μl, including 1 μl of PCR amplification product, 0.5 μl (5 U) of restricted enzymes, 2 μl of buffer solution, 0.2 μl of bovine serum albumin (Yancheng Saibao Biotechnolofy Co., Ltd, Jiangsu, China) and 16.3 μl double-distilled water, and incubated for about 3.5 h at a 37°C incubator. DNA fragment of PCR produced or lost the cleavage site of a specific enzyme because of gene mutation, which changed the DNA fragment of the enzyme product. The enzyme products were evaluated by agarose gel electrophoresis and genotypes were determined according to the electrophoresis map (**Table 2**).

Anesthesia regimens and monitoring

Patients were required to open vein access before anesthesia. All patients were injected with 20~25 ml^{-1}·kg^{-1} lactated Ringer's solution (Shandong Baisheng Biotechnology Co., Ltd, Shandong, China) at the 1st h. After that, 10~15 ml lactated Ringer's solution was injected into the patients and maintained by kg^{-1}•h^{-1}. Patients undergoing cardiac valve replacement were injected with 3~5 ml 0.5% lidocaine (Shanghai Wanjiang Biotechnology Co., Ltd, Shanghai, China) at the $T_{8\sim9}$ or $T_{9\sim10}$ intervertebral space via epidural catheter. When the anesthesia level appeared, and stabilized at $T_{5\sim10}$, GA was performed. During the surgery,

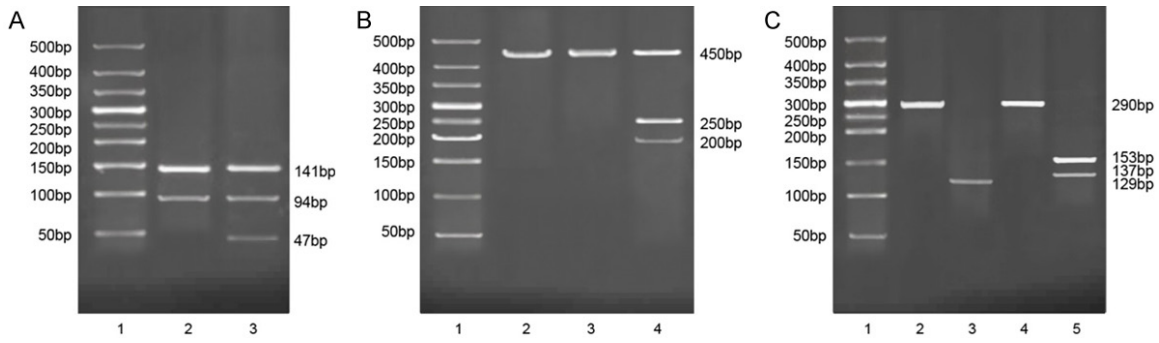

Figure 1. Genotyping of CYP3A4*4, CYP3A4*5 and CYP3A4*6 by electrophoresis map. Notes: A: Restriction digestion products for *CYP3A4*4*; B: Restriction digestion products for *CYP3A4*5*; C: Restriction digestion products for *CYP3A4*6*.

Table 3. Comparisons of genotype and allele frequency of *CYP3A4*4, CYP3A4*5* and *CYP3A4*6* between the case and control groups

SNP	Case group (n = 511)	Control group (n = 503)	X^2	*P*	OR	95% CI
CYP3A4*4						
*1*1	486 (0.951)	492 (0.978)			Ref	
*1*4	25 (0.049)	11 (0.022)	5.418	0.02	2.301	1.120-4.728
*1	997 (0.987)	995 (0.989)			Ref	
*4	25 (0.024)	11 (0.011)	5.321	0.021	2.268	1.110-4.635
CYP3A4*5						
*1*1	508 (0.994)	503 (1.0)			Ref	
*1*5	3 (0.006)	0 (0.0)	2.962	0.085	6.931	0.357-134.6
*1	1019 (0.997)	1006 (1)			Ref	
*5	3 (0.003)	0 (0.0)	2.957	0.172	6.911	0.356-134.1
CYP3A4*6						
*1*1	492 (0.963)	491 (0.976)			Ref	
*1*6	19 (0.037)	12 (0.024)	1.519	0.218	1.58	0.759-3.291
*1	1003 (0.981)	994 (0.988)			Ref	
*6	19 (0.019)	12 (0.012)	1.495	0.221	1.569	0.758-3.250

Note: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval; Ref, reference.

3~5 ml 0.5% lidocaine was injected to the patients though epidural catheter every 2 h. The effects of genotypes of *CYP3A4* on central venous pressure (VCP), mean arterial pressure (MAP), heart rate (HR), pulse oximetry (SPO_2), extubation and duration of intensive care unit (ICU) stay during the surgery were observed and recorded.

Follow-up

With approval of the patients and their families, follow-up was carried out on a regular basis by telephone and return visit of patients. Every patient was followed up every 3 months and at least once. The follow-up time ended on October 31, 2015 and the follow-up rate was 100%. The patients' conditions (limb movement, intestinal function and survival situation) were recorded regularly through follow-up feedback.

Statistical analysis

The statistical software package SPSS 19.0 (SPSS Inc, Chicago, IL) was used for all data processing. Chi-square test was used to test the polymorphism of the case group and the control group to verify whether it fits the Hardy-Weinberg equilibrium law. Count data were showed in the form of rate or percentage and measurement data were exhibited as mean ± standard deviation ($\bar{X} \pm s$). The *t* test was used for comparison between two groups. All tests were two-sided, with $P < 0.05$ as statistically significant.

Results

*Genotyping of CYP3A4*4, CYP3A4*5 and CYP3A4*6 by electrophoresis map*

Genotyping was detected by electrophoresis. For *CYP3A4*4*, the restriction digestion products for heterozygous mutant were 141 bp, 94

Table 4. Correlation of *CYP3A4*4, CYP3A4*5* and *CYP3A4*6* polymorphisms with clinical indicators in cardiac surgery

Genotype	HR (frequency/min)	MAP (mmHg)	SPO_2 (%)	CVP (kPa)
CYP3A4*4				
*1*1	83.34 ± 17.51	59.86 ± 12.77	99.34 ± 0.06	10.98 ± 3.43
*1*4	73.85 ± 17.72	54.48 ± 12.85	99.30 ± 0.08	9.27 ± 3.05
P	0.009	0.041	0.002	0.015
CYP3A4*5				
*1*1	82.89 ± 17.65	59.65 ± 12.81	99.34 ± 0.06	10.91 ± 3.43
*1*5	81.22 ± 13.51	50.69 ± 11.65	99.30 ± 0.06	9.38 ± 3.47
P	0.87	0.228	0.25	0.442
CYP3A4*6				
*1*1	82.95 ± 17.76	59.63 ± 12.86	99.34 ± 0.06	10.94 ± 3.47
*1*6	80.98 ± 13.68	58.68 ± 11.58	99.33 ± 0.06	9.89 ± 2.14
P	0.633	0.751	0.477	0.191

Note: HR, heart rate; MAP, mean arterial pressure; SPO_2, arterial oxyhaemoglobin saturation; CVP, central venous pressure.

Table 5. Correlation of *CYP3A4*4, CYP3A4*5* and *CYP3A4*6* polymorphisms with the time of extubation and ICU stay

Genotype	Extubation (min)	ICU stay (min)
CYP3A4*4		
*1*1	88.79 ± 39.09	343.21 ± 100.03
*1*4	72.30 ± 45.07	301.64 ± 84.33
P	0.042	0.042
CYP3A4*5		
*1*1	87.99 ± 39.58	341.50 ± 99.74
*1*5	86.52 ± 31.30	286.81 ± 76.67
P	0.949	0.344
CYP3A4*6		
*1*1	88.09 ± 39.36	342.55 ± 100.03
*1*6	85.07 ± 44.22	305.76 ± 84.02
P	0.744	0.114

Note: ICU, intensive care unit.

bp and 47 bp; for wild type were 94 bp and 141 bp; and homozygous mutant showed no digestion products (had no effects on final results) (**Figure 1A**). As for *CYP3A4*5*, the digestion products for heterozygous mutant showed three fragments of 450 bp, 250 bp and 200 bp; wild type had one fragment of 450 bp; homozygous mutant exhibited no fragment (**Figure 1B**). With respect to *CYP3A4*6*, the produced fragment of 153 bp and 24 bp were for heterozygous mutant and wild type respectively; and no fragment was observed for homozygous mutant (**Figure 1C**).

*Comparisons of allele frequency and genotype distribution of CYP3A4*4, CYP3A4*5 and CYP3A4*6 between the case and control groups*

As shown in **Table 3**, allele frequency and genotype distribution of *CYP3A4*4*, *CYP3A4*5* and *CYP3A4*6* in the case and control groups reached genetic equilibrium with population representativeness according to Hardy-Weinberg equilibrium test. In the 1014 samples, no homozygous mutant was found in *CYP3A4*4*, *CYP3A4*5* and *CYP3A4*6* of *CYP3A4* gene and the mutation rate in *CYP3A4*5* alleles was zero in the healthy controls. Allele frequency and genotype distribution of *CYP3A4*4* showed significant difference between the case and control groups (all $P < 0.05$). No significant difference was observed in the genotype and allele frequency of *CYP3A4*5* and *CYP3A4*6* between the two groups (all $P > 0.05$).

*Relationship between CYP3A4*4, CYP3A4*5 and CYP3A4*6 polymorphisms and effects of GA combined with EB in cardiac surgery*

As shown in **Table 4**, compared with wild-type *1*1, the HR, MAP, SPO_2 and CVP were significantly different in patients with mutant heterozygous *CYP3A4*4* *1*4 (all $P < 0.05$), indicating that *CYP3A4*4* polymorphism might be associated with the effect of GA combined with EB in cardiac surgery. While the HR, MAP, SPO_2 and CVP in patients with different genotypes of *CYP3A4*5* and *CYP3A4*6* showed no significant difference (all $P > 0.05$). As showed in **Table 5**, compared with wild-type *1*1, the time of extubation and ICU stay in patients with mutant heterozygous *1*4 of *CYP3A4*4* gene were significantly different (all $P < 0.05$), indicating that *CYP3A4*4* polymorphism may be associated with the effect of GA combined with EB in cardiac surgery. However, there was no significant difference in those of patients with different genotypes of *CYP3A4*5* and *CYP3A4*6* (all $P > 0.05$).

Table 6. Relationship between the *CYP3A4* polymorphisms and prognosis of patients undergoing cardiac valve replacement with GA combined with EB

Genotype	n	Motor dysfunction	Bowel dysfunction	Backache	Survival rate
CYP3A4*4					
*1*1	486	33	10	32	0.938
*1*4	25	1	2	1	0.92
P		0.585	0.056	0.608	0.608
CYP3A4*5					
*1*1	508	33	12	32	0.939
*1*5	3	1	0	1	0.667
P		0.063	0.788	0.058	0.052
CYP3A4*6					
*1*1	492	34	11	33	0.939
*1*6	19	0	1	0	0.895
P		0.236	0.393	0.242	0.462

Note: GA, general anesthesia; EB, epidural block.

Effect of CYP3A4 polymorphism on the prognosis of patients undergoing cardiac valve replacement with GA combined with EB

After 9 months of follow-up, motor dysfunction, bowel dysfunction and backache occurred in patients with heterozygous *1*4 of *CYP3A4*4* mutation were 1 case, 2 cases and 1 case, respectively. And the survival rate was up to 92.0%. No significant difference was found between the wild-type *1*1 and heterozygous *1*4 (all $P > 0.05$). No bowel dysfunction, 1 case of motor dysfunction and 1 case of backache occurred in patients with mutant heterozygous *1*5 of *CYP3A4*5*, and the survival rate was up to 66.7%. Compared with wild-type *1*1, there was no significant difference (all $P > 0.05$). One case of bowel dysfunction, no motor dysfunction or backache occurred in patients with mutant heterozygous *1*6 of *CYP3A4*6*. The survival rate was up to 89.5%. Compared with wild-type *1*1, there was no significant difference (all $P > 0.05$) (**Table 6**).

Discussion

In this investigation, we tried to explore the delicate relationship between *CYP3A4* polymorphisms and the efficacy of GA combined with EB in cardiac surgery. We focused on the three variant alleles (*CYP3A4*4, CYP3A4*5* and *CYP3A4*6*) of *CYP3A4* gene and compared their differences in some indexes related to our study. All these data demonstrated that *CYP3A4*4* polymorphism was associated with the risk of GA combined with EB among patients undergoing cardiac valve replacement.

It is reported that many genetic variants of *CYP3A4* gene have been investigated to describe the biological function in pharmacogenetics and toxicogenetics [17]. *CYP3A4* is closely related to the metabolism of endocannabinoid anandamide, which plays an essential role in multiple physiological processes including pain-sensation [18]. The polymorphism of *CYP3A4* gene which participates in hepatic metabolism of taxanes can increase the concentration of drugs for lack of elimination, which leads to an incredible toxicity level [19]. Firstly, we found three heterozygous mutants and two wild type in the restriction digestion products of *CYP3A4*4*, three heterozygous mutants, one wild type in *CYP3A4*5*, as well as one band each for heterozygous mutant and wild type in *CYP3A4*6,* but no homozygous mutant in the three alleles. Meanwhile, allele frequency and genotype distribution of *CYP3A4*4* showed significant difference between the case and control groups. In consistent with our study, Hsieh *et al*. also detected three heterozygous subjects and no homozygous in *CYP3A4*4*, and in their study, *CYP3A4*4* was found in 2.9% of the Chinese subjects for the first time [14]. The study conducted by Xin *et al*. also showed that there was no homozygous mutant subject in *CYP3A4*4, CYP3A4*5* and *CYP3A4*6* alleles in a case-control study of 368 cases of Chinese girls [20]. In the six well conserved *CYP3A* residues in the substrate recognition site 1, Ser119 played an important role in the *CYP3A4* specificity and was found to be a key determinant in the active site topology of steroid 6b-hydroxylation [21]. Hsieh *et al*. revealed that subjects carrying the *CYPA4*4*, *CYPA4*5* or *CYPA4*6* alleles showed below average 6β-hydroxycortisol, implying decreased catalytic activity for its variant proteins [14].

Another important result showed that compared with wild-type *1*1, the HR, MAP, SPO_2 and CVP were significantly different in patients with heterozygous *1*4 of *CYP3A4*4* undergo-

ing cardiac valve replacement with GA combined with EB. However, the HR, MAP, SPO_2 and CVP in patients with different genotypes of *CYP3A4*5* and *CYP3A4*6* showed no significant difference. *CYP3A4* played a significant role in drug metabolism and its different activities were suggested to be accountable for individual variability in response to many drugs [22]. *CYP3A4* polymorphisms was reported to be associated with blood pressure response to amlodipine among high-risk African-Americans and *CYP3A4* genotype testing could be a clinical prediction for blood pressure response to amlodipine [23]. CVP, also known as right atrial pressure, can be used to detect adequacy of cardiac preload and circulating blood volume [24]. According to Tan *et al*., *CYP3A4*4* was an allelic variant associated with a functional reduction of CYP3A4 activity; for instance, *CYP3A4*4* carriers have exhibited a higher decline of triglycerids and cholesterol levels with abundant simvastatin than non-carriers [25]. We also found that compared with wild-type *1*1, the time of extubation and ICU stay of patients with heterozygous *1*4 of *CYP3A4*4* gene were significantly different while there was no significant difference in those of patients with different genotypes of *CYP3A4*5*. Taken together, our study suggests that heterozygous mutation in *CYP3A4*4* gene serves as a risk factor for patients receiving cardiac valve replacement under GA combined with EB. Kadlubar *et al*. reported that the mutational allele *CYP3A4*1B* of *CYP3A4* gene was related to incremental testosterone availability and the early onset of puberty in young girls [20]. CYP3A4 enzyme is accountable for the metabolism of tacrolimus and *CYP3A4* polymorphism is associated with pharmacokinetic variability [26]. CYP3A4 genetic variation had a confusing effect on evaluating the predictive role of *CYP3A4* polymorphisms for reacting to risperidone and the functional variation in *CYP3A4* gene was related to patients' poor metabolisers of CYP2D6 [27].

In conclusion, our study supported a significant role of the *CYP3A4* polymorphisms in cardiac surgery under GA combined with EB. It is a novel contribution for our study to investigate the relationship between the mutation allele *CYP3A4*4* of *CYP3A4* gene and the risk factors in cardiac surgery under GA combined with EB for the first time, which helps to provide a reference for anesthetic dosage in cardiac surgery. However, it must be noted that our findings need to be further validated by additional functional studies and larger well-designed molecular epidemiological studies that include diverse ethnic populations. In addition, data should be collected to enable inclusion of relevant confounding factors in the analysis.

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Disclosure of conflict of interest

None.

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