Effect of CYP3A4*18B polymorphisms and interactions with OPRM1 A118G on postoperative fentanyl requirements in patients undergoing radical gastrectomy

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Abstract. The present study aimed to investigate the effect of cytochrome P450 3A4 (CYP3A4)*18B polymorphisms and the interaction of the μ opioid receptor gene (OPRM1) A118G and CYP3A4*18B polymorphisms on postoperative fentanyl analgesia in Chinese Han patients undergoing radical gastrectomy. In total, 97 patients scheduled to undergo radical gastrectomy under general anesthesia were enrolled in this study. Post-operative patient-controlled intravenous analgesia of fentanyl was administered as analgesia up to 48 h following surgery. Venous blood (2 ml) was obtained from each patient to measure the OPRM1 A118G and CYP3A4*18B genotypes. The differences in fentanyl consumption and adverse effects were compared among the genotypes at 24 and 48 h following surgery. In the first 48 h following surgery, patients in the CYP3A4*18B/*18B group consumed significantly less fentanyl compared with patients in the *1/*1 group (P=0.032). With regards to the joint genetic effect, during the 48-h period, patients with AA and *1*18B polymorphisms received fewer fentanyl doses compared with those with AG and *1*1 (P=0.049), while patients with AG and *1*18B polymorphisms received significantly fewer fentanyl doses compared with those with AG and *1*1 (P=0.010), and patients with *18B*18B polymorphisms received significantly fewer fentanyl doses compared with those with AA and *1*1 (P=0.024) or those with AG and *1*1 polymorphisms (P=0.006). No correlation between OPRM1 A118G and CYP3A4*18B and postoperative nausea, vomiting and dizziness was found. Results demonstrated that 48 h following surgery, patients with the CYP3A4*18B/*18B genotype required less fentanyl than patients with the CYP3A4*1/*1 genotype to control pain. Additionally, the combined genotype of CYP3A4*18B and OPRM1 A118G may affect fentanyl doses administered for pain control, but not postoperative nausea, vomiting and dizziness.

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Introduction

Opioids, including morphine and fentanyl, have been widely used to treat various types of acute and chronic pain for a number of years. However, the analgesic efficacy of opioids is well known to vary widely among individuals (1). In recent years, with the development of pharmacogenomics, it has been hypothesized that opioids exhibit various interindividual reactions due to gene sequence differences in drug-metabolizing enzymes and drug transporters and targets (2,3). Various factors, including gender, age, weight, organ function and disease severity, are associated with differences in individual response to these drugs, however, 30-95% of drug response and disposal differences is due to genetic factors (4).

Fentanyl is mainly metabolised to norfentanyl by the cytochrome P450 3A4 (CYP3A4) enzyme and is the primary route of hepatic biotransformation (5,6). Interindividual differences in the activity and expression of the CYP3A4 enzyme contribute to its metabolising substrate pharmacokinetics (6,7). Previous studies showed that 30-90% of the interindividual variability in the activity and expression of CYP3A4 is predominantly attributed to genetic polymorphisms encoding their genes (2,4,8). Forty single nucleotide polymorphisms (SNPs) of CYP3A4 alleles have been identified. CYP3A4*18B, a SNP in intron 10 of the CYP3A4 gene, was initially identified in Japanese patients and is present at high frequency in Asian populations (9,10). This SNP creates a G→A substitution at position 82,266, which correlates with increased CYP3A4 enzymatic activity (9,10). Based on these studies, it was hypothesized that the CYP3A4*18B allele may be associated with variations in fentanyl metabolism. To date, a limited number of association studies between the CYP3A4*18B polymorphism and postoperative fentanyl requirements have been performed (11,12).

The human micro-opioid receptor gene (OPRM1) encodes the μ opioid receptor, the main target of analgesia for fentanyl. A large number of SNPs (>700) have been identified in the

OPRM1 gene (refer to the dbSNP database, the NCBI database of genetic variations). The OPRM1 A118G mutation is the most common functional variation at position 118 in exon 1. The mutation leads to an exchange of the amino acid asparagine to aspartate at position 40 (N40D) on the extracellular N-terminal domain, affecting a putative glycosylation site of the receptor (13). Studies on fentanyl and the A118G SNP remain scarce although fentanyl is one of the most commonly used opioid analgesics in clinical anesthesia and acute pain therapy. Authors have reported that subjects with the G allele of the OPRM1 A118G SNP were less sensitive to fentanyl or that the fentanyl dose was greater compared with AA subjects in patients with postoperative pain or labor analgesia (14-16). However, results of the additional three studies on this association were not consistent with these observations (17-19).

The majority of studies have analyzed the association between individual SNPs selected from a single gene region and morphine effects. Pain is a complex human experience and it is likely that the interaction of multiple genes and environmental factors affect the clinical efficacy of opioids. Therefore, a number of studies have aimed to determine the correlation between gene-gene interactions and morphine responses (20,21). To the best of our knowledge no studies have analyzed whether the combined mutation of OPRM1 and CYP3A4 polymorphisms affects fentanyl consumption and fentanyl-associated side effects. In the present study, patients undergoing radical gastrectomy were selected to evaluate whether the genetic polymorphisms of CYP3A4*18B and OPRM1 A118G affect postoperative fentanyl requirements for analgesia. In addition, the combined effects of OPRM1 and CYP3A4 polymorphisms on fentanyl analgesia and side effects in Chinese Han patients were explored.

Materials and methods

Patients. Between May 2008 and January 2010, 128 patients with an American Society of Anesthesiologists physical status of I-III, aged 20-75 years and undergoing radical gastrectomy were enrolled in the present study. Liver and renal functions were normal in all patients and no history of chronic pain, long-term application of analgesic and cortisol drugs, alcohol or drug abuse or allergies to fentanyl were found. Patients with a history of severe cardiovascular disease, diabetes mellitus, psychiatric disorders or were currently pregnant or at lactation period were excluded. Patients who had consumed drugs (1 week) or foods (3 days) known to inhibit or induce the expression of CYP3A4 enzymes prior to surgery were also excluded. The study design was approved by the Institutional Ethics Committee of the Third Xiangya Hospital of Central South University (Changsha, China). Signed informed consent was obtained from all patients.

Preoperative psychological evaluation. Evaluation of patient preoperative trait/state anxiety and depression was performed 1 night prior to surgery by two psychiatric doctors using the State-Trait Anxiety Inventory (22) and Self-Rating Depression Scale (23).

Anesthetic methods. No premedication was used. All patients received general anesthesia of 0.05 mg/kg midazolam, 0.2 mg/kg etomidate, 5 μg/kg fentanyl and 0.12 mg/kg vecuronium. End tidal CO_2 partial pressure was maintained between 35 and 40 mmHg by mechanical ventilation. Propofol and remifentanil were infused through micropumps and sevoflurane was inhaled to maintain anesthesia. Drug dosage was adjusted according to alterations in auditory-evoked potential and hemodynamics. An intermittent dose of 0.03-0.06 mg/kg vecuronium was administered to maintain adequate surgical muscle relaxation. During surgery, fentanyl infusion was discontinued following abdominal cavity opening and the total dose of fentanyl remained at 12 μg/kg to avoid a residual effect on postoperative fentanyl dose. The electrocardiogram, non-invasive blood pressure, pulse oximetry and arterial blood gas analysis were monitored.

Postoperative analgesia. Following radical gastrectomy, patients were sent to the postanesthesia care unit (PACU). The trachea was extubated and the pain severity of patients was assessed. Patients were intravenously injected with a 20-μg bolus of fentanyl until a visual analog scale (VAS: O, no pain; 10, unbearable pain) until a visual analog scale value< 3 was obtained. Patient-controlled intravenous analgesia (PCIA) commenced when patients perceived slight pain (VAS 1-3). That is, patients were administered fentanyl by PCIA when a slightly painful state (VAS 1-3) was reported. Patients were excluded if their recovery time of pain (VAS>0) exceeded 3 h or opioid receptor antagonist was provided. The electronic patient controlled analgesia (PCA) pump was filled with 30 μg/kg fentanyl with 0.9% normal saline diluted to 240 ml. The PCA was programmed to administer 1.5 ml/h background infusion with a 20-μg bolus of fentanyl solution, with a 5-min lockout time. PCA was continued for 48-h following surgery. Postoperative pain was maintained at VAS<3 at rest. When patients experienced VAS>3, despite being on PCA, the dose of fentanyl was increased by the patient by pushing the bolus button, no other rescue drugs other than intravenous fentanyl were used within the first 48 h following surgery. Nausea and vomiting following radical gastrectomy is common, therefore all patients were intravenously administered 8 mg ondansetron. Post-operative non-invasive blood pressure, heart rate, pulse oxygen saturation and VAS were documented 2, 6, 12, 24 and 48 h following surgery. The total amount of PCA fentanyl was recorded at 24 and 48 h. Adverse effects associated with fentanyl administration, including nausea, vomiting and dizziness, were assessed as events for 48 h.

Genotyping assays. Peripheral venous blood (2 ml) was obtained from each patient and placed in an EDTA tube. DNA was extracted from leukocytes using a standard phenol-chloroform procedure and was stored at 4-8˚C. OPRM1 A118G genotyping was performed by DNA sequence analysis of polymerase chain reaction (PCR)-amplified DNA. Primers were designed by primer design software Oligo 6.0 to cover the polymorphic site. OPRM1 A118G was amplified by PCR using the following primers: forward, GAAAAGTCTCGGTGCTCCTG and reverse, GGAGTAGAGGGCCATGATCG. DNA sequence of the fragment was determined using the automated sequencer ABI-PRISM 3730 Genetic Analyzer (Sangon Biotech Co. Ltd., Shanghai, China; Fig. 1). CYP3A4*18B was amplified by PCR using the following primers: forward,

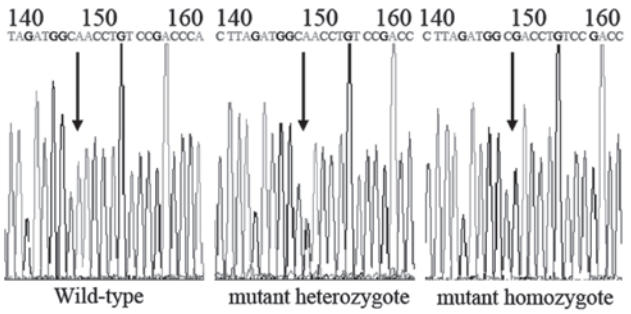

Figure 1. OPRM1:118A→G sequencing typing. OPRM1, μ opioid receptor. Wild-type, OPRM1 AA; mutant heterozygote, OPRM1 AG; mutant homozygote, OPRM1 GG.

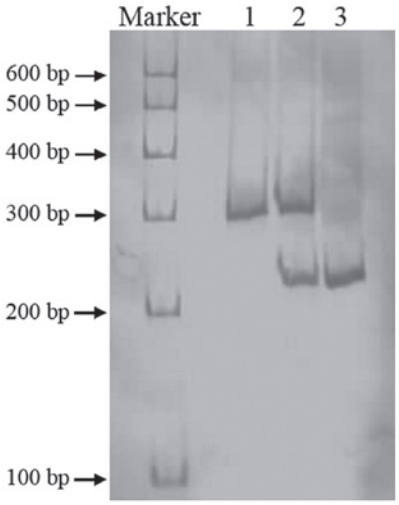

Figure 2. PCR products of CYP3A4*18B were digested using *Rsa*I, resolved by electrophoresis on non-denaturing polyacrylamide gel and stained by silver nitrate. Lanes 1, mutant homozygote CYP3A4*18B/*18B; 2, mutant heterozygote CYP3A4*1/*18B; 3, wild-type CYP3A4*1/*1. M, marker; CYP, cytochrome P450.

CACCCTGATGTCCAGCAGAAACT and reverse, AATAGAAAGCAGATGAACCAGAGCC. PCR products of CYP3A4*18B were digested by *Rsa*I, resolved by electrophoresis on a non-denaturing polyacrylamide gel and stained by silver nitrate (Fig. 2).

Statistical analysis. Clinical studies and genotyping were performed blind with regards to patient clinical outcome. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Demographic and clinical data were presented as the mean ± SD, median (interquartiles) and counts, as appropriate. P<0.05 was considered to indicate a statistically significant difference. The Chi-square test was used to detect Hardy-Weinberg equilibrium. Depending on the data form, Fisher's exact, Mann-Whitney U or t-tests were used to evaluate whether clinical parameters (including age, gender, body weight, height, surgery duration, incision length, preoperative state anxiety, trait anxiety, depression and 24 and 48 h VAS pain score) were significantly different between genotypes. In the present study, 24- and 48-h postoperative fentanyl dose was not normally distributed. Therefore, non-parametric analyses, including the Mann-Whitney U and Kruskal Wallis

Table I. Demographic and clinical data for OPRM1 A118G and CYP3A4*18B.

	OPRM1 A118G				CYP3A4*18B			
Characteristics	AA	AG	GG	P-value	*1*1	*1/*18B	*18B/*18B	P-value
Patients, n	42	41	14		48	43	6	
Age, years	51.0±12.4	53.8±13.5	51.7±13.2	0.622	53.2±12.1	52.1±13.5	45.5±15.6	0.395
Male/female	22/20	27/14	11/3	0.171	28/20	29/14	3/3	0.555
Height, cm	161.3±7.8	162.3±7.3	165.4±7.0	0.210	162.2±7.4	162.8±8.1	159.8±4.3	0.664
Weight, kg	53.9±10.8	55.5±9.9	59.7±10.7	0.195	56.7±11.1	54.8±10.0	49.7±8.2	0.271
Duration of surgery, min	211.5±60.4	190.2±74.4	181.8±67.3	0.225	194.0±73.2	205.1±62.7	182.5±66.7	0.627
Length of incision, cm	19.0±2.5	19.6±3.4	20.6±4.6	0.287	20.0±3.7	19.0±2.9	19.3±2.0	0.348
Preoperative state anxiety	41.5±7.9	42.1±7.8	40.6±6.5	0.847	42.7±7.3	41.2±7.4	34.5±8.7	0.112
Preoperative trait anxiety	43.9±8.0	45.1±9.0	45.8±5.8	0.756	45.5±7.7	44.7±7.5	38.5±11.9	0.250
Preoperative depression	37.0±7.8	35.1±6.2	37.0±6.0	0.559	35.8±6.7	37.8±6.7	30.0±5.3	0.087
VAS pain score 24 h	2.0 (4.2)	2.0 (4.3)	1.9 (4.2)	0.969	1.9 (4.3)	2.0 (4.0)	2.1 (4.5)	0.606
VAS pain score 48 h	1.9 (3.4)	2.0 (3.4)	1.9 (3.7)	0.808	1.9 (3.7)	2.0 (3.2)	2.0 (3.6)	0.733

Data are expressed as numbers, mean ± SD or median (interquartile range). VAS, visual analog scale; OPRM1, μ opioid receptor gene; CYP, cytochrome P450.

Table II. Demographic and clinical data for combined genetic variation of OPRM1 A118G and CYP3A4*18B.

Characteristics	AA + *1/*1	AG + *1/*18B	AG + *1/*1	AA + *1/*18B	*18B/*18B	P-value
Patients, n	21	17	22	18	6	-
Age, years	52.1±10.6	54.2±13.2	54.0±14.4	52.0±13.4	45.5±15.6	0.680
Male/female	11/10	11/6	14/8	11/7	3/3	0.905
Height, cm	160.2±7.8	160.1±8.3	163.7±6.5	163.3±8.2	159.8±4.3	0.363
Weight, kg	54.0±12.3	51.0±8.3	59.3±10.0	55.4±9.0	49.7±8.2	0.081
Duration of surgery, min	214.4±68.2	204.0±72.0	185.9±77.1	210.3±53.4	182.5±66.7	0.617
Length of incision, cm	19.5±2.2	19.0±2.5	20.2±4.0	18.4±3.0	19.3±2.0	0.474
Preoperative state anxiety	44.5±5.2	44.0±5.6	42.4±8.2	39.7±8.6	34.5±8.7	0.156
Preoperative trait anxiety	45.3±8.2	45.0±10.4	45.7±8.3	44.0±6.4	38.5±11.9	0.650
Preoperative depression	36.3±8.9	36.1±6.5	35.4±5.8	38.0±7.2	30.0±5.3	0.406
VAS pain score 24 h	1.9 (3.9)	2.1 (3.1)	1.9 (4.9)	1.9 (4.7)	2.1 (4.5)	0.586
VAS pain score 48 h	1.9 (3.4)	2.1 (2.5)	1.9 (3.9)	1.9 (3.5)	2.0 (3.6)	0.460

Data are expressed as numbers, mean ± SD or median (interquartile range). VAS, visual analog scale; OPRM1, μ opioid receptor gene; CYP, cytochrome P450.

Table III. OPRM1 A118G and CYP3A4*18B genetic variation in patients.

Genotypes/ allele	No. of patients	Frequency (%)	Fentanyl dose for 24 h (μg/kg)	P-value	Fentanyl dose for 48 h (μg/kg)	P-value
OPRM1 A118G	97	100				
AA	42	43.3	11.8±6.1	0.857	21.3±8.6	0.997
AG	41	42.3	10.6±4.5		20.8±7.5	
GG	14	14.4	10.5±3.8		20.8±6.5	
CYP3A4*18B	97	100				
*1/*1	48	49.5	11.5±5.5	0.432	22.5±7.4	0.032[a]
*1/*18B	43	44.3	11.0±5.1		20.3±8.1	
*18B/*18B	6	6.2	9.1±3.7		16.3±4.0	
Joint genotype combination	84	86.6				
AA + *1/*1	21	21.6	12.8±7.1	0.535	23.2±9.0	0.021[a]
AG + *1/*18B	17	17.5	10.5±5.0		19.4±8.2	
AG + *1/*1	22	22.7	11.2±4.1		23.5±5.9	
AA + *1/*18B	18	18.6	11.3±5.3		20.4±8.4	
*18B/*18B	6	6.2	9.1±3.7		16.3±4.0	

[a]P<0.05; OPM1, μ opioid receptor gene; CYP, cytochrome P450.

tests, were performed to detect the effect of genotype on PCA fentanyl dose. The incidence of adverse effects was analyzed using the Chi-square or Fisher's exact tests.

Results

Patient information. In the present study, 128 patients were enrolled. Of these, 31 were not included due to no available blood sample or failure to measure the blood sample (n=14), condition changes (n=6), severe dizziness or vomiting (n=3) or the patient withdrawal from the study (n=8). In total, 97 patients were analyzed in this study. All 97 patients were of Chinese Han ethnicity. Biological and clinical data of the patients are summarized in Tables I and II and no significant differences were detected among the genotype groups (P>0.05).

*Genotype and allele frequency of OPRM1 A118G, CYP3A4*18B and the genetic combination of these two SNPs*. Of the 97 patients, 42 patients were wild-type homozygotes (AA), 41 heterozygotes (AG) and 14 mutant homozygotes (GG), with the frequency of the G allele being 35.6% in the OPRM1 A118G polymorphism. There were 48 wild-type homozygotes (*1/*1), 43 heterozygotes (*1/*18B) and 6 mutant homozygotes (*18B/*18B), the frequency of the *18B allele was 28.4% in the CYP3A4*18B polymorphism. Allele frequency was found to be in Hardy-Weinberg equilibrium (P>0.05)

Table IV. Comparison of incidence of nausea, vomiting and dizziness between the genotype groups.

	OPRM1 A118G				CYP3A4*18B			
Symptoms	AA	AG	GG	P-value	*1/*1	*1/*18B	*18B/*18B	P-value
Patients, n	42	41	14		48	43	6	
Nausea, frequency (%)								
No	28 (66.7)	24 (58.5)	8 (57.1)	0.692	27 (56.3)	30 (69.8)	3 (50.0)	0.343
Yes	14 (33.3)	17 (41.5)	6 (42.9)		21 (43.7)	13 (30.2)	3 (50.0)	
Vomiting, frequency (%)								
No	34 (81.0)	33 (80.5)	12 (85.7)	0.905	39 (81.3)	36 (83.7)	4 (66.7)	0.602
Yes	8 (19.0)	8 (19.5)	2 (14.3)		9 (18.7)	7 (16.3)	2 (33.3)	
Dizziness, frequency (%)								
No	30 (71.4)	26 (63.4)	10 (71.4)	0.705	28 (58.3)	33 (76.7)	5 (83.3)	0.121
Yes	12 (28.6)	15 (36.6)	4 (28.6)		20 (41.7)	10 (23.3)	1 (16.7)	

Chi-square test. OPRM1, μ opioid receptor gene; CYP, cytochrome P450.

(Table III). To analyze gene-gene interactions, SNPs were combined in pairs in 9 possible combinations in 97 patients, including 21 with AA + *1/*1 (21.6%), 18 with AA + *1/*18B (18.6%), 3 with AA + *18B/*18B (3.1%), 22 with AG + *1/*1 (22.7%), 17 with AG + *1/*18B (17.5%), 2 with AG + *18B/*18B (2.1%), 5 with GG + *1/*1 (5.2%), 8 with GG + *1/*18B (8.2%) and 1 with GG + *18B/*18B (1.0%). Since only 6 subjects were identified with *18B/*18B, they were combined into a single group, thus 7 groups of combinations were compared in total.

*Postoperative fentanyl dose and polymorphisms of OPRM1 A118G and CYP3A4*18B*. Fentanyl dose was 5.2-17.8 and 12.2-30.7 μg/kg at postoperative 24 and 48 h, respectively. No correlation was observed in the fentanyl analgesic dose at postoperative 24 and 48 h between the genotype groups of OPRM1 A118G (P>0.05). In addition, the fentanyl analgesic dose at postoperative 24 h was not found to be significantly different between the genotype groups of CYP3A4*18B (P>0.05). However, 48 h following surgery, patients with CYP3A4*18B/*18B required less fentanyl than patients with CYP3A4*1/*1 (P=0.032; Table III), but no significant difference in fentanyl dose was noted between CYP3A4*18B/*18B and CYP3A4*1/*18B or between CYP3A4*1/*18B and CYP3A4*1/*1 (P>0.05). In the joint genetic effect, there were four statistically different groups: 1, patients with OPRM1 AA and CYP3A4*1*18B polymorphisms received fewer fentanyl doses during the 48-h period compared with those with OPRM1 AG and CYP3A4*1*1 (P=0.049); 2, patients with OPRM1 AG and CYP3A4*1*18B polymorphisms received significantly fewer fentanyl doses during the 48-h period compared with those with OPRM1 AG and CYP3A4*1*1 (P=0.010); 3 and 4, patients with CYP3A4*18B*18B polymorphisms received significantly fewer fentanyl doses during the 48-h period compared with those with OPRM1 AA and CYP3A4*1*1 (P=0.024) or those with OPRM1 AG and CYP3A4*1*1 polymorphisms (P=0.006). No significant differences were detected in the demographics, including gender ratio, preoperative anxiety, depression and postoperative VAS pain scores, across genotypes and joint genotype combination (P>0.05; Tables I-III).

*Side effects and polymorphisms of OPRM1 A118G and CYP3A4*18B*. At 48 h following surgery, the main side effects of fentanyl were nausea, vomiting and dizziness in 38.1, 18.6 and 32.0% of patients, respectively. No significant differences were found in nausea, vomiting and dizziness among the genotype groups of OPRM1 A118G and CYP3A4*18B (P>0.05) or the groups of joint genotype combination (P>0.05; Tables IV and V). Only 2/97 patients (2.1%) developed mild pruritus. None of the patients developed respiratory depression or any other serious side effects.

Discussion

In the present study, fentanyl dose in 97 patients was 5.2-17.8 and 12.2-30.7 μg/kg 24 and 48 h following surgery, respectively, indicating that postoperative fentanyl doses vary greatly among individuals. A number of factors, including the character and intensity of external pain stimuli, age, gender, weight and genetic variation, may affect analgesic requirements. Among these, the contribution of variations in the CYP3A4 and OPRM1 genes has attracted considerable attention (10-21,24).

The CYP3A4*18B polymorphism was identified to be associated with significant variability in fentanyl consumption 48 h following radical gastrectomy in Chinese Han patients. This polymorphism was previously found at high frequencies in Asian populations (9,10). The CYP3A4*18B mutant allele frequency is 28.4% in patients of this study, consistent with previous reports of 24.9% in 416 Japanese individuals (9), 22.7% in 176 Chinese patients undergoing lower abdominal surgery (11) and 26.9% in 143 Chinese gynecological patients (12). Results indicate that patients with *18B/*18B consumed significantly less fentanyl than patients with *1/*1, consistent with previous studies (11,12). Authors of those studies (11,12) found that the CYP3A4*18B (CYP3A4*1G) genetic polymorphism decreased CYP3A4

Table V. Incidence of nausea, vomiting and dizziness among genetic combination groups.

Symptoms	AA + *1/*1	AG + *1/*18B	AG + *1/*1	AA + *1/*18B	*18B/*18B	P-value
Patients, n	21	17	22	18	6	
Nausea, frequency (%)						
No	13 (61.9)	11 (64.7)	12 (54.5)	13 (72.2)	3 (50)	0.784
Yes	8 (38.1)	6 (35.3)	10 (45.5)	5 (27.8)	3 (50)	
Vomiting, frequency (%)						
No	18 (85.7)	15 (88.2)	17 (77.3)	14 (77.8)	4 (66.7)	0.736
Yes	3 (14.3)	2 (11.8)	5 (22.7)	4 (22.2)	2 (33.3)	
Dizziness, frequency (%)						
No	13 (61.9)	12 (70.6)	12 (54.5)	14 (77.8)	5 (83.3)	0.472
Yes	8 (38.1)	5 (29.4)	10 (45.5)	4 (22.2)	1 (16.7)	

Chi-square test.

activity or fentanyl pharmacokinetics and therefore, patients with the CYP3A4*18B/*18B genotype required significantly less fentanyl for postoperative pain control than patients with the wild-type or CYP3A4 *1/*18B genotype. However, there were two differences in the present study. Firstly, in the discussed previous studies, variations in fentanyl dose were identified at postoperative 24 h, while in the present study, fentanyl dose was different at postoperative 48 h only. A recent study by Dong *et al* (24) reported that patients with the *18B allele consumed less fentanyl than the *1/*1 group at postoperative 2 and 4 h, but not at 24 and 48 h, among the three genotype groups. Second, although our results indicate that patients with the CYP3A4*1/*18B required less fentanyl than those with CYP3A4*1/*1, the difference was not found to be statistically significant (P>0.05). This discrepancy may, in part, be explained by variations in surgery types (upper vs. lower abdominal surgery), small sample size [97 (present study) and 79 (22) vs. 176 (11) and 143 (12) patients], analgesic formula [fentanyl only vs. fentanyl + droperidol (11,12)] and additional unknown factors. By contrast, Hu *et al* (10) reported that the CYP3A4*18B genotype significantly affects cyclosporine pharmacokinetics and is likely to result from higher enzymatic activity associated with this mutation. Results of both previous studies and the present study indicate that the CYP3A4*18B variant allele may be critical for polymorphic CYP3A4 enzymatic activities and thus variations in the consumption of fentanyl. The exact functional significance of the CYP3A4*18B polymorphism requires additional studies using different drugs in large sample sizes.

In the present study, no correlation was detected between OPRM1 A118G and fentanyl dose at postoperative 24 and 48 h, consistent with studies on labor analgesia and cancer pain (17,18). However, results of this study are inconsistent with findings of other studies (14-16) which found that fentanyl was less effective in subjects with the G allele of the OPRM1 A118G and these individuals required more fentanyl for adequate postoperative pain control compared with those with the A allele. These inconsistencies may be due to variations in the analgesia method and the analgesic used for postoperative pain. In this study, fentanyl only was administered by PCIA and no rescue analgesics were used. In Hayashida *et al* (16), 138 patients received continous epidural analgesia with fentanyl or morphine with rescue analgesics, including buprenorphine, pentazocine and pethidine, as well as non-steroidal anti-inflamatory drugs (NSAIDs). Analgesic requirement was determined as the sum of systemic fentanyl equivalent doses of all opioids and NSAIDs used for analgesia during the first 24 h following surgery. This conversion may not reflect precise fentanyl doses due to variations in properties and routes of different analgesics. In Fukuda *et al* (15), although the analgesic effects of fentanyl were evaluated using a cold pressor prior to surgery and found to be reduced in subjects carrying the G allele compared with subjects not carrying this allele, the A118G SNP revealed no significant association with postoperative 24 h fentanyl, perioperative fentanyl or total perioperative analgesic use in patients undergoing orofacial cosmetic surgery. This result was consistent with present observations only with regards to the association between the OPRM1 A118G polymorphism and postoperative dose of fentanyl. It is likely that gene-gene interactions, not single genes, in addition to the effect of environmental factors, affect the clinical efficacy of opioids. Therefore, the joint effect of OPRM1 A118G and CYP3A4*18B in predicting clinical efficacy of fentanyl for postoperative pain control was determined in the present study.

Significant variability among the combined genetic groups was observed. First, patients with OPRM1 AA and CYP3A4*1*18B polymorphisms received fewer fentanyl doses during the 48-h period compared with those with OPRM1 AG and CYP3A4*1*1 (P=0.049). With respect to a single gene, no significant difference in fentanyl dose was found between patients with AA or AG in OPRM1 A118G or between patients with *1*1 and those with *1*18B in CYP3A4*18B. However, the association between these polymorphisms and fentanyl consumption became significant when combined respectively. This observation indicates that variability in fentanyl consumption is not associated with genetic variation in a single gene, but in two or more genes and this was demonstrated further in the second group, in which although the fentanyl dose was not different between patients with *1*18B

and those with *1*1, combination with OPRM1 AG led to a significant difference (P=0.010). These results are similar to a previous study in which Kolesnikov *et al* (21) reported that when 48-h PCA morphine consumption was analyzed in 102 patients who underwent abdominal surgery, no significant statistical difference among the catechol-O-methyltransferase (COMT) G1947A or OPRM1 A118G groups was detected, however, patients with OPRM1 A118G and COMT G1947A polymorphisms received significantly fewer morphine doses compared with patients with OPRM1 wild-type homozygous. These results indicate that genetic effects on opioid analgesic dose are under the control of multiple, not single genes and may account for discrepancies between genetic associations with pain modulatory effects in clinical studies. In addition, patients with CYP3A4*18B*18B polymorphisms (groups 3 and 4) received significantly fewer fentanyl doses during the 48-h period compared, not only with those with OPRM1 A118 and CYP3A4*1*1 (P=0.024), but also with those with OPRM1 A118G and CYP3A4*1*1 polymorphisms (P=0.006). Moreover, of the 7 combinations, patients with CYP3A4*18B*18B consumed the lowest fentanyl dose, demonstrating that 48 h following surgery, variations in fentanyl consumption were largely determined by the CYP3A4*18B*18B genotype in the patient population. The CYP3A4*18B polymorphism was therefore an important determinant of fentanyl variation for postoperative pain control.

The correlation between fentanyl-associated side effects and CYP3A4 and OPRM1 genes in the patient cohort was also investigated. Incidence of nausea, vomiting and dizziness was 38.1, 18.6 and 32.0%, respectively. Analysis revealed no significant association between side effects (nausea, vomiting and dizziness) and genetic variation in CYP3A4*18B and OPRM1 genes, including their joint genetic variation. In the present study, the adverse effects were assessed by events, not rating scale, therefore, it was not a standard and precise assessment. However, results were consistent with previous studies (14,25), in which A118G and CYP3A4*18B were found to have no effect on nausea or vomiting, did not reduce frequency of these side effects and had no effect on sedation scores. This indicated that the OPRM1 A118G and CYP3A4*18B genetic polymorphisms are not the main factors affecting nausea, vomiting and dizziness during PCIA with fentanyl.

Findings of this study provide preliminary insight into the effect of the CYP3A4*18B polymorphisms and its joint effects with OPRM1 A118G in the clinical efficacy of fentanyl. To the best of our knowledge, this is the first study to have explored the joint effects of OPRM1 and CYP3A4*18B in fentanyl analgesia. However, the present study is associated with a number of limitations. Firstly, the activity of CYP3A4 and plasma fentanyl concentration in these patients was not detected and the mechanisms associated with differences noted between various CYP3A4 phenotypes have yet to be elucidated. Secondly, the mixed-gender study population may have increased variability in postoperative fentanyl requirement, although no statistically significant differences in gender were found between the different genotypes. In addition, only two SNPs of two genes associated with pain treatment, OPRM1 A118G and CYP3A4*18B, were analyzed, leaving a number of genes with functional significance to be assessed in future studies. Moreover, the sample size was too small, thus a decisive conclusion could not be drawn from the results. Future studies with larger cohorts are required to further characterize the joint effects of the OPRM1 and CYP3A4 mutations, in addition to environmental factors.

In the present study, patients with the CYP3A4*18B/*18B polymorphism required a reduced dose of fentanyl for pain relief compared with patients with other polymorphisms. Although postoperative fentanyl analgesic dose, nausea, vomiting and dizziness were not found to be significantly different between OPRM1 A118G polymorphisms, the combination of OPRM1 A118G and CYP3A4*18B affected the fentanyl requirements of Chinese Han patients following radical gastrectomy.

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