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Research Article

MDR1 Genotypes and Haplotypes Are Closely Associated with Postoperative Fentanyl Consumption in Patients Undergoing Radical Gastrectomy

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Fentanyl is a powerful opioid analgesic, and its analgesic effect is greatly different among individuals. This study was aimed at exploring the effects of multidrug resistance gene-1 (*MDR1*) genetic variation on postoperative fentanyl consumption. A total of 135 patients, who planned to undergo radical gastrectomy with general anesthesia, were studied. The subjects received patient-controlled analgesia (PCA) by intravenous fentanyl within 48 hours after operation and maintained a numerical rating scale (NRS) score ≤ 3. The consumption and side effects of fentanyl were recorded within 24 hours and 48 hours after the operation. Single nucleotide polymorphisms (SNPs) of all patients with *MDR1* 1236C>T, 2677G>T/A, and 3435C>T were screened by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or DNA sequence analysis after PCR. There was no difference in postoperative fentanyl consumption among patients having 2677G>T/A and 3435C>T polymorphisms (all $P > 0.05$). *MDR1* 1236C>T polymorphisms and haplotypes combined by three SNPs, however, significantly affected postoperative fentanyl consumption (all $P < 0.05$). Moreover, 1236TT genotype carriers consumed more fentanyl during 24 hours ($P = 0.038$) and 48 hours ($P = 0.003$) postoperatively. The *MDR1* TTT haplotype carriers needed more fentanyl compared with the CGC haplotype carriers during the first 48 hours after surgery ($P = 0.017$). Nausea, vomiting, and dizziness were not found to have significant differences among the above three SNPs and their haplotypes ($P > 0.05$). *MDR1* 1236C>T polymorphism and haplotypes were factors contributing to the individual variability in postoperative fentanyl consumption.

1. Introduction

Fentanyl is a widely used narcotic analgesic, but its analgesic efficacy has a large interindividual variation among patients [1, 2]. The analgesic effect of fentanyl is affected by many factors, and the gene polymorphism is one of the main factor [3–5]. Gene polymorphism plays an important role in the pharmacokinetics and pharmacodynamics of fentanyl [5]. Drug metabolizing enzymes and targets of fentanyl are both likely affected by gene polymorphisms, which in turn affect the analgesic effect of fentanyl [6, 7]. The genes contributing to drug transporters of fentanyl, however, remain largely unexplored.

P-Glycoprotein (P-gp) which is the product of the *MDR1* gene, acts as a transmembrane efflux pump in the blood-brain barrier, liver, kidney, and intestine [8]. The expression and function of P-gp are regulated by *MDR1* gene SNPs, including exon 1236C>T, 2677G>T/A, and 3435C>T SNPs [9–11]. 1236C>T and 3435C>T are synonymous mutations. They may affect the P-gp function by altering protein folding [9]. In contrast, 2677G>T/A is a nonsynonymous mutation due to the substitution of amino acid alanine with serine or threonine [9]. *MDR1* mRNA expression in heart tissue is significantly elevated in 2677AT or TT genotype carriers [10], and the homozygous T allele of 3435C>T reduces P-gp expression in the upper gastrointestinal tract [11]. On the

other hand, these three SNPs are in strong linkage disequilibrium and form haplotypes. Haplotypes containing mutant alleles have been shown to exhibit large structural modifications that result in conformational changes in the binding site and subsequent reductions in P-gp activity [12, 13].

Fentanyl is one of the substrates of P-gp [14–16]. Human and animal studies have shown that P-gp affects the absorption, distribution, excretion, and clinical effects of fentanyl [14–16]. Several authors have identified that *MDR1* 1236C>T and 3435C>T SNPs have significant effects on postoperative fentanyl consumption [7, 17]. However, other studies cannot identify such associations [18] or obtain the opposite results [19]. Overall, the effects of three *MDR1* SNPs on fentanyl efficacy are not definite. It may be the result of considering only one of the three SNPs. Since SNPs are in incomplete linkage disequilibrium, it may be necessary to consider the SNP combination to accurately assess the role of common polymorphisms in the efficacy of fentanyl. No studies, so far, have analyzed whether haplotypes of *MDR1* 1236C>T, 2677G>T/A, and 3435C>T led to variability in the dosage of fentanyl among patients. Hence, in this study, we evaluated whether genetic polymorphisms of 1236C>T, 2677G>T/A, and 3435C>T and their haplotypes affect postoperative fentanyl requirements for analgesia.

2. Materials and Methods

2.1. Ethical Statements. The research plan was reviewed and approved by the institutional ethics committee of the Third Xiangya Hospital. Each patient received information about the aim of the study and provided written informed consent. Moreover, participants were assured that the participation is completely voluntary and personal information is not disclosed to third parties.

2.2. Patient Selection. Since the liver and intestines affect the metabolism and absorption of fentanyl, we chose patients who were scheduled for radical gastrectomy by general anesthesia in the present study. Exclusion criteria were as follows: patient refusal; ASA classification more than III; patients with severe liver injury, renal impairment, diabetes mellitus, cardiovascular disease, psychiatric disorders, chronic pain, and pregnancy or lactation; patients taking drugs that are substrates of P-gp; long-term use of analgesic; allergy to fentanyl; or drug abuse.

2.3. Anesthesia and Postoperative Analgesia. General anesthesia was induced with 0.05 mg/kg midazolam, 0.2 mg/kg etomidate, 0.12 mg/kg vecuronium, and 4-6 μg/kg fentanyl. Anesthesia was maintained by 1%-2% sevoflurane inhalation, continuous infusion of propofol (4-12 mg·kg^{-1}·h^{-1}), and intermittent intravenous bolus of 0.05 mg/kg vecuronium. During surgery, intravenous infusion of fentanyl was started before skin incision, appended 2 μg/kg each time intraoperatively, and administration stopped once the surgeons opened the abdominal cavity. The total dose of fentanyl was less than 12 μg/kg, and the residual effect on postoperative fentanyl dose was avoided. After surgery, all patients were administered 8 mg ondansetron to prevent postoperative nausea and vomiting. Then, patients were extubated and observed in the postanesthesia care unit (PACU) for 2 hours. The antagonist of muscle relaxation was not used. The NRS score was recorded after extubation. Patients were considered to have inadequate pain control if NRS > 3, and fentanyl (20 μg per time) was intravenously given until NRS ≤ 3. Patients then received patient-controlled intravenous analgesia(PCIA) with fentanyl during the first 48 hours after surgery. In the analgesic pump, 30 μg/kg fentanyl was filled in normal saline, with a total volume of 240 mL. The pump was programmed to administer a 1.5 mL/h background dose, 20 μg bolus of fentanyl solution, and 5-minute lockout time. Postoperative pain was controlled with NRS ≤ 3 at rest. All enrolled patients used no opioid receptor antagonists and other analgesic drugs except for fentanyl after operation.

Table 1: Prime sequences of *MDR1* 1236C>T, 2677G>T/A, and 3435C>T.

Primer	Sequence
MDR1 1236C>T	F: 5′-TTCACTTCAGTTACCCATC-3′
	R: 5′-CATAGAGCCTCTGCATCA-3′
MDR1 2677G>T/A	F: 5′-GTCTGGACAAGCACTGAAAGA-3′
	R: 5′-GTGGGGAGGAAGGAAGAACA-3′
MDR1 3435C>T	F: 5′-GATCTGTGAACTCTTGTTTTCA-3′
	R: 5′-GAAGAGAGACTTACATTAGGC-3′

2.4. Genotyping. Genomic DNA from peripheral blood leukocytes was extracted using E.Z.N.A.™ SQ Blood DNA Kit (D5032-02). The primer design software Oligo 6 designed primers (Table 1). 1236C>T and 3435C>T were genotyped by the PCR-RFLP method (Figures 1(a) and 1(b)), and *MDR1* 2677G>T/A was screened by DNA sequencing (Figure 1(c)). The restriction enzyme BsuRI (Lot: 00143447, Thermo Scientific) was used to distinguish the T allele from the C allele of 1236C>T, and the restriction enzyme MboI (Lot: 00098836, Fermentas) was employed to distinguish the T allele from the C allele of 3435C>T.

2.5. Data Collection. During the first 24 hours and 48 hours, patients' age, gender, BMI, length of incision, duration of surgery, and postoperative fentanyl consumption were collected. The NRS was recorded at 4 hours, 8 hours, 12 hours, 24 hours, and 48 hours after the operation. The incidence of side effects, including nausea, vomiting, respiratory depression, and lethargy, was recorded in the first 48 hours.

2.6. Statistical Analysis. The sample size of this research was calculated based on the frequency of *MDR1* genotypes. According to the allele frequency of *MDR1* gene SNPs (1236C>T, 2677G>T/A, and 3435C>T) and their haplotypes previously reported in Chinese [7, 20], it was required that the sample size of 108 patients showed an absolute difference of 20% in the difference of fentanyl dose (power = 80%, α = 0.05). As a result of clinical experience and preliminary experiment, absolute difference of 20% was chosen as a minimum detectable difference. PASS 11 software (NCSS, Kaysville, UT) was used to calculate statistical power samples.

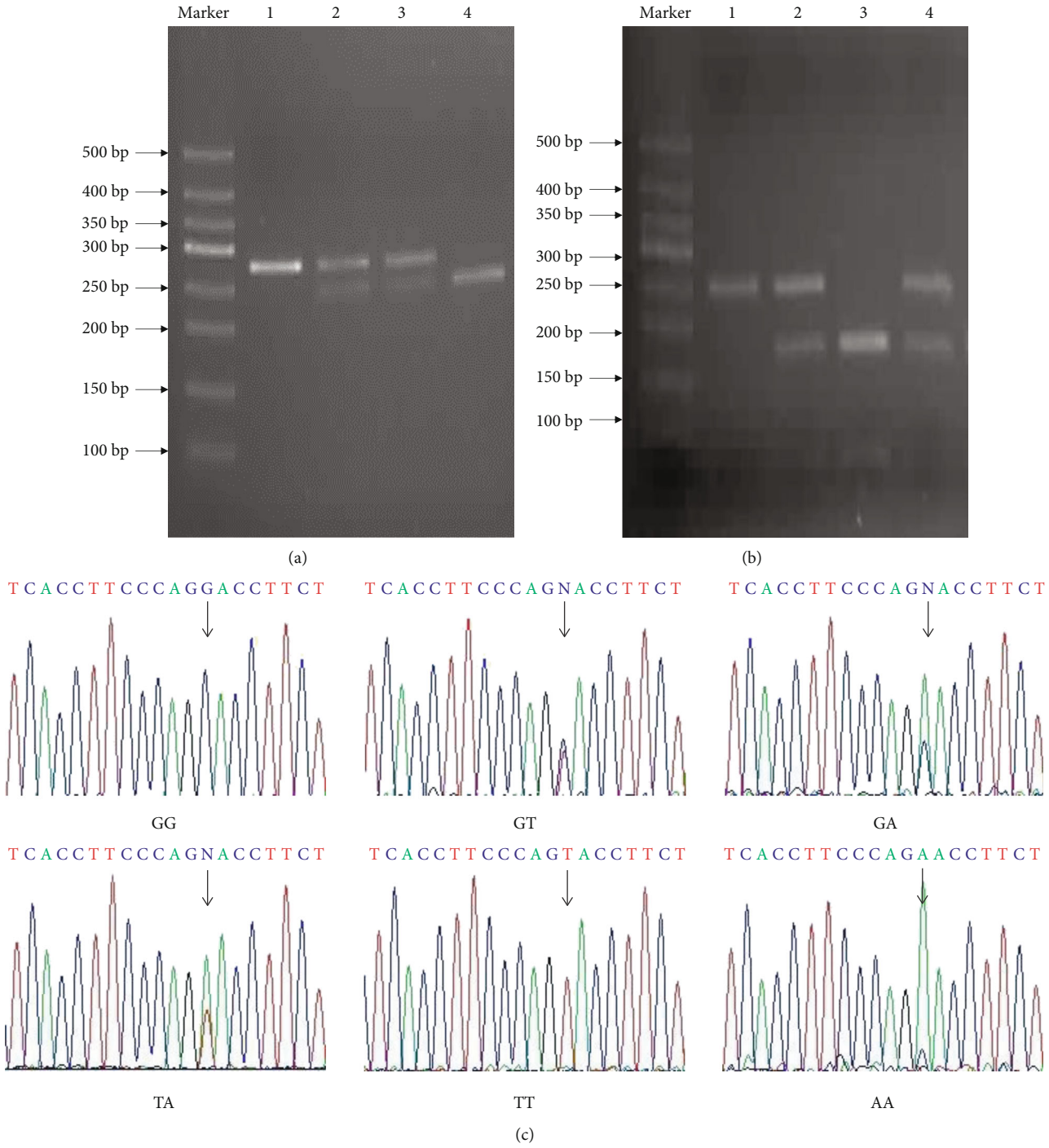

Figure 1: Genotyping of *MDR1* SNPs. (a) 1236C>T products after enzymatic degradation. Lane 1: TT, mutant homozygote; lanes 2 and 3: CT, mutant heterozygote; lane 4: CC, wild type. (b) 3435C>T products after enzymatic degradation. Lane 1: TT, mutant homozygote; lanes 2 and 4: CT, mutant heterozygote; lane 3: CC, wild type. (c) 2677G>T/A sequencing typing. GG, GT, GA, TA, TT, AA.

Clinical research and genotyping were blinded to the clinical outcome of patients. Data were analyzed using SPSS 19.0. $P < 0.05$ was statistically significant. All continuous variables were checked for normal distribution, and data were presented as means ± standard deviation or medians and range. Comparison of postoperative fentanyl consumption was performed using Kruskal-Wallis H test. Allele and genotype frequencies were estimated by gene counting. The deviation from the Hardy-Weinberg equilibrium was evaluated by chi-square tests.

3. Results

3.1. General Patient Information. In this study, 147 patients were recruited, and 12 of them were excluded because of patient quitting ($n = 5$), unavailability of blood samples

Table 2: Demographics and clinical characteristics of 1236C>T and 3435C>T.

Factors	1236C>T			*P* value	3435C>T			*P* value
	CC	CT	TT		CC	CT	TT	
Cases	27	66	42		53	62	20	
Age (year)	52.8 ± 13.9	53.7 ± 12.8	51.2 ± 13.5	0.770	52.0 ± 13.5	54.2 ± 12.6	50.1 ± 14.3	0.607
Gender (male/female)	18/9	38/28	28/14	0.553	35/18	40/22	12/8	0.891
Height (cm)	161.4 ± 7.1	161.9 ± 6.9	163.9 ± 6.9	0.422	161.9 ± 6.8	162.5 ± 7.3	163.4 ± 6.2	0.807
Weight (kg)	53.1 ± 7.9	54.2 ± 8.8	58.4 ± 11.6	0.146	54.1 ± 7.7	56.2 ± 11.0	55.6 ± 10.6	0.680
Surgery time (min)	226.9 ± 104.1	188.6 ± 51.6	203.1 ± 65.0	0.172	196.7 ± 67.9	205.2 ± 71.6	197.3 ± 71.0	0.868
Length of incision (cm)	19.2 ± 3.8	19.9 ± 3.7	19.8 ± 2.9	0.774	18.7 ± 3.0	20.6 ± 3.8	19.8 ± 3.1	0.089
NRS score 24 h postoperatively	2.1 ± 0.3	2.1 ± 0.3	2.2 ± 0.2	0.814	2.1 ± 0.2	2.1 ± 0.3	2.2 ± 0.3	0.218
NRS score 48 h postoperatively	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.3	0.337	2.0 ± 0.3	2.0 ± 0.2	2.1 ± 0.3	0.133

1236C>T: CC: wild type; CT: mutant heterozygote; TT: mutant homozygote. 3435C>T: CC: wild type; CT: mutant heterozygote; TT: mutant homozygote. NRS: numerical rating scale..

Table 3: Demographics and clinical characteristics of 2677G>T/A.

Factors	2677G>T/A						*P* value
	GG	GT	GA	TA	TT	AA	
Cases	33	37	20	13	25	7	
Age (year)	53.8 ± 13.7	54.5 ± 12.4	57.2 ± 9.4	48.6 ± 13.0	50.7 ± 14.3	39.5 ± 13.6	0.209
Gender (M/F)	24/9	23/14	12/8	7/6	17/8	2/5	0.334
Height (cm)	162.4 ± 6.9	162.2 ± 7.8	162.0 ± 6.6	160.9 ± 6.0	165.3 ± 6.8	157.2 ± 2.2	0.378
Weight (kg)	52.2 ± 8.8	55.8 ± 8.7	57.2 ± 7.5	53.7 ± 10.2	59.5 ± 13.3	49.8 ± 3.1	0.235
Surgery time (min)	191.2 ± 66.4	204.1 ± 65.6	197.0 ± 95.1	213.4 ± 51.7	196.7 ± 64.3	230.0 ± 95.1	0.920
Length of incision (cm)	18.4 ± 2.8	19.3 ± 2.8	20.9 ± 4.4	21.4 ± 4.6	20.0 ± 2.9	18.3 ± 1.9	0.150
NRS score 24 h postoperatively	2.1 ± 0.2	2.0 ± 0.3	1.9 ± 0.2	1.9 ± 0.3	2.1 ± 0.3	2.2 ± 0.2	0.246
NRS score 48 h postoperatively	2.1 ± 0.3	2.0 ± 0.2	2.0 ± 0.1	1.9 ± 0.2	2.1 ± 0.2	2.2 ± 0.3	0.371

2677G>T/A: GG: wild type; GT, GA, and TA: mutant heterozygote; TT and AA: mutant homozygote. NRS: numerical rating scale..

($n = 2$), analgesic pump failure ($n = 2$), and other conditions ($n = 3$). Finally, this study included 135 patients, all of whom were of Chinese Han ethnicity. Tables 2 and 3 show the demographics and clinical data of the patients. There was no significant difference in demographic and clinical data among different *MDR1* genotypes and haplotypes (all $P > 0.05$).

3.2. Genotypes and Allele Frequency of MDR1 SNPs. Of 135 patients, 27 cases were 1236CC genotype, 66 cases were 1236CT, and 42 cases were 1236TT. The mutation frequency of allele T was 55.6%. 2677G>T/A polymorphism developed six genotypes: GG, GT, GA, TA, TT, and AA. Among the 135 patients, 33 cases were carriers of the GG genotype, 37 cases were carriers of the GT genotype, 20 cases were carriers of the GA genotype, 13 cases were carriers of the TA genotype, 25 cases were carriers of the TT genotype, and seven cases were carriers of the AA genotype, and the mutation frequency of the allele T and allele A was 37.0% and 17.4%, respectively. There were 53 carriers of the 3435CC genotype, 62 carriers of the CT genotype, and 20 carriers of the TT genotype, and the mutation frequency of allele T was 37.8%. The allelic frequencies of 1236C>T, 2677G>T/A, and 3435C>T conformed to the Hardy-Weinberg equilibrium ($P > 0.05$).

3.3. Linkage Disequilibrium and Haplotype Analysis. Haploview software used the existing genotype data to calculate linkage disequilibrium statistics and infer the population haplotype model. Haploview software showed that there was strong linkage disequilibrium of *MDR1* 1236C>T, 2677G>T/A, and 3435C>T. Four haplotypes (TTT, CGC, CAC, and TGC) which were constructed by three *MDR1* SNPs represented 90.7% of all haplotypes observed in this study.

3.4. Correlation between the Three SNPs of MDR1 Gene and Their Haplotypes and Postoperative Fentanyl Consumption. In this study, there was no statistically significant difference in postoperative fentanyl consumption in 2677G>T/A and 3435C>T at 24 hours and 48 hours after surgery ($P > 0.05$). However, there was a significant difference in postoperative fentanyl consumption among the polymorphisms of 1236C>T. Specifically, the 1236TT genotype carriers needed more fentanyl for postoperative analgesia within the first 24 hours and 48 hours after surgery ($P = 0.038$ and $P = 0.003$, respectively) (Table 4). Among the haplotypes of the *MDR1*

Table 4: Postoperative fentanyl consumption in different genotypes during the first 24 hours and 48 hours postoperatively.

MDR1 SNPs	Cases	Frequency (%)	Fentanyl dose for 24 hours (μg/kg)	*P* value	Fentanyl dose for 48 hours (μg/kg)	*P* value
1236C>T	135	100				
CC	27	20.0	7.4 (6.6-10.1)		14.8 (13.1-17.2)	
CT	66	48.9	8.9 (7.0-11.6)	0.038[a]	19.1 (15.6-24.7)	0.003[b]
TT	42	31.1	10.1 (7.7-15.3)		22.2 (17.6-26.4)	
2677G>T/A						
GG	33	24.5	8.7 (7.4-10.5)		18.2 (14.7-21.1)	
GT	37	27.4	8.3 (6.6-13.5)		16.8 (14.1-25.4)	
GA	20	14.8	8.4 (6.9-11.5)	0.864	16.3 (13.5-21.7)	0.128
TA	13	9.6	11.0 (7.5-15.1)		22.0 (18.8-28.3)	
TT	25	18.5	9.8 (7.6-15.0)		23.8 (17.9-26.2)	
AA	7	5.2	8.4 (6.3-19.1)		14.9 (13.7-29.9)	
3435C>T						
CC	53	39.3	8.3 (6.9-10.5)		18.0 (14.0-22.8)	
CT	62	45.9	9.0 (7.0-13.7)	0.575	19.6 (14.8-25.1)	0.176
TT	20	14.8	9.9 (7.6-15.0)		24.0 (18.7-26.2)	

1236C>T: CC: wild type; CT: mutant heterozygote; TT: mutant homozygote. 2677G>T/A: GG: wild type; GT, GA, and TA: mutant heterozygote; TT and AA: mutant homozygote. 3435C>T: CC: wild type; CT: mutant heterozygote; TT: mutant homozygote. [a,b]1236TT genotype carriers received more fentanyl than 1236CC genotype carriers at 24 hours and 48 hours postoperatively.

Table 5: Postoperative fentanyl consumption in different *MDR1* haplotypes at the first 24 hours and 48 hours postoperatively.

Haplotypes	Frequency (%)	Fentanyl dose for 24 hours (μg/kg)	*P* value	Fentanyl dose for 48 hours (μg/kg)	*P* value
TTT	32.2	10.0 (7.4-15.0)		21.7 (17.2-26.2)	
CGC	23.3	8.3 (6.7-10.0)		16.7 (14.0-20.1)	
CAC	17.8	7.8 (6.8-11.9)	0.247	17.1 (13.8-23.6)	0.017[c]
TGC	17.4	10.1 (7.5-13.7)		19.8 (15.1-25.5)	
Other	9.3	8.7 (7.2-11.9)		19.4 (14.6-21.3)	

[c]The haplotype TTT required more fentanyl than the CGC haplotype at 48 hours postoperatively.

gene, the TTT haplotype consumed more fentanyl than the CGC haplotype within the first 48 hours after surgery ($P = 0.017$) (Table 5).

3.5. Correlation between Side Effects and the Three MDR1 SNPs and Their Haplotypes. The main side effects of fentanyl were nausea, vomiting, and dizziness at postoperative 48 hours. There was no significant difference in nausea, vomiting, and dizziness within 48 hours postoperatively among 1236C>T, 2677G>T/A, 3435C>T, and their haplotypes ($P > 0.05$) (Table 6). None of the patients experienced respiratory depression or other serious side effects.

4. Discussion

In this study, we found that *MDR1* 1236C>T SNP and the *MDR1* gene haplotypes constructed from 1236C>T, 2677G>T/A, and 3435C>T were predictive of individual variations in postoperative fentanyl consumption. Moreover, 1236TT carriers and *MDR1* TTT haplotype carriers needed more postoperative fentanyl in patients undergoing radical gastrectomy.

The *MDR1* 1236C>T mutation is more commonly detected in Chinese Han. In our study, the frequency of the 1236T variant was 55.6%, and Xiao et al. [20] report that the frequency of the 1236T variant is 67.8% in Chinese Han. The *MDR1* 1236C>T mutation may induce a change in the substrate binding site and reduce the P-gp activity [9, 21]. Zhang et al. [7] has found that patients with the TT genotype of 1236C>T consume significantly more fentanyl than those with the CC and CT genotypes within 24 hours and 48 hours after lower segment caesarean section surgery, and we got the same results within 24 hours and 48 hours after radical gastrectomy. Elkiweri et al. [22] have indicated that fentanyl concentration in the rat's brain is decreased in the presence of the P-gp inhibitor verapamil. P-gp is a transmembrane efflux pump from inside the cell [8]. These results could not be explained by P-gp distributed in the blood-brain barrier but by P-gp in the liver. The decrease of P-gp activity in hepatocytes of the TT genotype groups means that fentanyl is accumulated in the liver and metabolized by hepatic microsomal enzymes, resulting in increased consumption of fentanyl [7].

In addition, we also found that there was no significant difference in postoperative fentanyl consumption among the 2677G>T/A and 3435C>T genotypes, which was consistent with Kim et al.'s study [18]. In another study [21], 2677G>T/A and 3435C>T are not associated with fentanyl's pharmacokinetics and pharmacodynamics. The above

Table 6: Incidence of nausea, vomiting, and dizziness of 1236C>T, 2677G>T/A, and 3435C>T genotypes and haplotypes.

MDR1	Nausea		*P* value	Vomiting		*P* value	Dizziness		*P* value
	Yes	No		Yes	No		Yes	No	
1236C>T									
CC	6 (22.2%)	21 (77.8%)		7 (25.9%)	20 (74.1%)		7 (25.9%)	20 (74.1%)	
CT	23 (34.8%)	43 (65.2%)	0.214	10 (15.2%)	56 (84.8%)	0.455	17 (25.8%)	49 (74.2%)	0.825
TT	18 (42.9%)	24 (57.1%)		7 (16.7%)	35 (83.3%)		13 (31.0%)	29 (69.0%)	
2677G>T/A									
GG	10 (30.3%)	23 (69.7%)		5 (15.2%)	28 (84.8%)		7 (21.2%)	26 (78.8%)	
GT	13 (35.1%)	24 (64.9%)		7 (18.9%)	30 (81.1%)		10 (27.0%)	27 (73.0%)	
GA	8 (40.0%)	12 (60.0%)	0.449	5 (25.0%)	15 (75.0%)	0.789	7 (35.0%)	13 (65.0%)	0.446
TA	2 (15.4%)	11 (84.6%)		1 (7.7%)	12 (92.3%)		2 (15.4%)	11 (84.6%)	
TT	12 (48.0%)	13 (52.0%)		4 (16.0%)	21 (84.0%)		10 (40.0%)	15 (60.0%)	
AA	2 (28.6%)	5 (71.4%)		2 (28.6%)	5 (71.4%)		1 (14.3%)	6 (85.7%)	
3435C>T									
CC	20 (37.7%)	33 (62.3%)		11 (20.8%)	42 (79.2%)		11 (20.8%)	42 (79.2%)	
CT	17 (27.4%)	45 (72.6%)	0.155	10 (16.1%)	52 (83.9%)	0.763	18 (29.0%)	44 (71.0%)	0.240
TT	10 (50.0%)	10 (50.0%)		3 (15.0%)	17 (85.0%)		8 (40.0%)	12 (60.0%)	
Haplotypes									
TTT	42.5%	57.5%		16.1%	83.9%		34.5%	65.5%	
CGC	30.2%	69.8%		15.9%	84.1%		31.7%	68.3%	
CAC	29.2%	70.8%	0.128	27.1%	72.9%	0.302	18.8%	81.2%	0.178
TGC	42.6%	57.4%		19.1%	80.9%		19.1%	80.9%	
Other	20.0%	80.0%		8.0%	92.0%		32.0%	68.0%	

researches demonstrate that *MDR1* 2677G>T/A and 3435C>T mutations do not affect the analgesic effect of fentanyl.

MDR1 1236C>T, 2677G>T/A, and 3435C>T constructed four main haplotypes. In this study, the proportions of TTT, CGC, CAC, and TGC haplotypes were 32.2%, 23.3%, 17.8%, and 17.4%, respectively, which were consistent with previous studies [23]. These data showed that the TTT haplotype was the predominant genotype of Chinese Han populations. No studies have analyzed whether *MDR1* haplotypes contribute to the individual variability in postoperative fentanyl consumption. We found that the *MDR1* gene haplotypes constructed from the SNPs, 1236C>T, 2677G>T/A, and 3435C>T, could predict the individual variability in postoperative fentanyl consumption. TTT haplotype carriers needed more fentanyl within the first 48 hours after surgery, while CGC haplotype carriers needed less fentanyl. It has been reported that the efflux ability of P-gp in cells transfected with *MDR1* CGC is higher than that in cells overexpressing the *MDR1* TTT variant haplotype [12]. Thus, our results could also be explained by the transport activity of P-gp in hepatocytes, which were decreased in the TTT haplotype and increased in the CGC haplotype, leading to differences in fentanyl metabolism. Overall, using the commonly detected haplotypes (1236C>T, 2677G>T/A, and 3435C>T) could be more efficient compared to just studying only one polymorphism. Our study provided preliminary evidence that the *MDR1* haplotype could influence fentanyl response.

Postoperative nausea, vomiting, and dizziness are common side effects of fentanyl. Our data showed that the frequency of nausea, vomiting, and dizziness was 34.8%, 17.8%, and 27.4%, respectively. No significant differences in the aforementioned side effects were found among 1236C>T, 2677G>T/A, 3435C>T, and their haplotypes within 24 hours and 48 hours postoperatively. Severe side effects, such as respiratory depression, were not detected in this study.

There were some limitations in our study. First, the 2677G>T/A allele G could mutate to allele A or T, and the frequency of the allele A variant was low. We only observed seven AA carriers in this study, while limited sample sizes might affect the objectivity of this research to a certain extent. Second, whether the *MDR1* 1236C>T SNP and haplotypes affect the efflux ability of P-gp-mediated fentanyl in hepatocytes is only speculation and needs further verification. However, our findings provided preliminary evidence that the *MDR1* 1236C>T polymorphism and haplotypes composed of 1236C>T, 2677G>T/A, and 3435C>T greatly contributed to the individual variability in postoperative analgesia with fentanyl. As far as we know, this is the first study to explore the effect of *MDR1* gene haplotypes on fentanyl analgesia. It is possible to improve the perioperative pain management by preoperative *MDR1* genotyping.

5. Conclusion

The *MDR1* gene 1236C>T polymorphism and haplotypes composed of 1236C>T, 2677G>T/A, and 3435C>T greatly contributed to the individual variability of postoperative fentanyl consumption in patients undergoing radical gastrectomy.

Data Availability

The data used to support the findings of this study may be released upon application to the Department of Anesthesiology, Third Xiangya Hospital, Central South University, which can be contacted at xy3irb@163.com.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Fan Zhang performed the conceptualization; Fan Zhang, Jie Hu, and Qin Liao performed the data curation; Fan Zhang and Jie Hu performed the formal analysis; Fan Zhang and Wenxiang Qing performed the investigation; Jianbin Tong, Zhonghua Hu, and Qin Liao performed the methodology; Fan Zhang and Jie Hu gathered the resources; Fan Zhang wrote the original draft; Jianbin Tong, Jie Hu, and Qin Liao wrote, reviewed, and edited the manuscript.

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References

[1] K. Ikeda, S. Ide, W. Han, M. Hayashida, G. R. Uhl, and I. Sora, "How individual sensitivity to opiates can be predicted by gene analyses," *Trends in Pharmacological Sciences*, vol. 26, no. 6, pp. 311–317, 2005.

[2] T. Naito and J. Kawakami, "Interindividual variation of pharmacokinetic disposition of and clinical responses to opioid analgesics in cancer pain patients," *Yakugaku Zasshi*, vol. 135, no. 5, pp. 709–715, 2015.

[3] E. Lucenteforte, L. Vagnoli, A. Pugi et al., "A systematic review of the risk factors for clinical response to opioids for all-age patients with cancer-related pain and presentation of the paediatric STOP pain study," *BMC Cancer*, vol. 18, no. 1, 2018.

[4] E. J. M. Kuip, W. H. Oldenmenger, M. F. Thijs—Visser et al., "Effects of smoking and body mass index on the exposure of fentanyl in patients with cancer," *PLoS One*, vol. 13, no. 6, article e0198289, 2018.

[5] G. S. Gerhard, S. Kaniper, and B. Paynton, "Fentanyl overdoses and pharmacogenetics," *Pharmacogenetics and Genomics*, vol. 30, no. 1, pp. 5–8, 2020.

[6] Q. Yan, Y. Su, L. Gao et al., "Impact of CYP3A4 * 1G polymorphism on fentanyl analgesia assessed by analgesia nociception index in Chinese patients undergoing hysteroscopy," *Chinese Medical Journal*, vol. 131, no. 22, pp. 2693–2698, 2018.

[7] J. Zhang, L. Zhang, X. Zhao, S. Shen, X. Luo, and Y. Zhang, "Association between MDR_1/CYP3A4/$OPRM_1$ gene polymorphisms and the post-caesarean fentanyl analgesic effect on Chinese women," *Gene*, vol. 661, pp. 78–84, 2018.

[8] F. J. Sharom, "The P-glycoprotein multidrug transporter," *Essays in Biochemistry*, vol. 50, no. 1, pp. 161–178, 2011.

[9] C. H. Hsin, M. S. Stoffel, M. Gazzaz et al., "Combinations of common SNPs of the transporter gene ABCB1 influence apparent bioavailability, but not renal elimination of oral digoxin," *Scientific Reports*, vol. 10, no. 1, 2020.

[10] K. Meissner, G. Jedlitschky, H. Meyer zu Schwabedissen et al., "Modulation of multidrug resistance P-glycoprotein 1 (ABCB1) expression in human heart by hereditary polymorphisms," *Pharmacogenetics*, vol. 14, no. 6, pp. 381–385, 2004.

[11] M. Omar, A. Crowe, R. Parsons, H. Ee, C. Y. Tay, and J. Hughes, "P-glycoprotein expression in Helicobacter pylori-positive patients: the influence of MDR1 C3435T polymorphism," *Journal of Digestive Diseases*, vol. 13, no. 8, pp. 414–420, 2012.

[12] R. Wang, X. Sun, Y. S. Deng, and X. W. Qiu, "Effects of MDR1 1236C > T-2677G > T-3435C > T polymorphisms on the intracellular accumulation of tacrolimus, cyclosporine A, sirolimus and everolimus," *Xenobiotica*, vol. 49, no. 11, pp. 1373–1378, 2019.

[13] D. Vivona, L. T. Lima, A. C. Rodrigues et al., "ABCB1 haplotypes are associated with P-gp activity and affect a major molecular response in chronic myeloid leukemia patients treated with a standard dose of imatinib," *Oncology Letters*, vol. 7, no. 4, pp. 1313–1319, 2014.

[14] E. D. Kharasch, C. Hoffer, T. G. Altuntas, and D. Whittington, "Quinidine as a probe for the role of p-glycoprotein in the intestinal absorption and clinical effects of fentanyl," *Journal of Clinical Pharmacology*, vol. 44, no. 3, pp. 224–233, 2004.

[15] C. Yu, M. Yuan, H. Yang, X. Zhuang, and H. Li, "P-Glycoprotein on blood-brain barrier plays a vital role in fentanyl brain exposure and respiratory toxicity in rats," *Toxicological Sciences*, vol. 164, no. 1, pp. 353–362, 2018.

[16] W. Hamabe, T. Maeda, Y. Fukazawa et al., "P-Glycoprotein ATPase activating effect of opioid analgesics and their P-glycoprotein-dependent antinociception in mice," *Pharmacology, Biochemistry, and Behavior*, vol. 85, no. 3, pp. 629–636, 2006.

[17] V. Dzambazovska-Trajkovska, J. Nojkov, A. Kartalov et al., "Association of single-nucleotide polymorhism C3435T in the ABCB1 gene with opioid sensitivity in treatment of postoperative pain," *Prilozi*, vol. 37, no. 2-3, pp. 73–80, 2016.

[18] K. M. Kim, H. S. Kim, S. H. Lim et al., "Effects of genetic polymorphisms of OPRM1, ABCB1, CYP3A4/5 on postoperative fentanyl consumption in Korean gynecologic patients," *International Journal of Clinical Pharmacology and Therapeutics*, vol. 51, no. 5, pp. 383–392, 2013.

[19] X. D. Gong, J. Y. Wang, F. Liu et al., "Gene polymorphisms of OPRM1 A118G and ABCB1 C3435T may influence opioid requirements in Chinese patients with cancer pain," *Asian Pacific Journal of Cancer Prevention*, vol. 14, no. 5, pp. 2937–2943, 2013.

[20] Z. Xiao, G. Yin, Y. Ni et al., "MDR1 polymorphisms affect the outcome of Chinese multiple myeloma patients," *Biomedicine & Pharmacotherapy*, vol. 95, pp. 743–748, 2017.

[21] M. Saiz-Rodríguez, D. Ochoa, C. Herrador et al., "Polymorphisms associated with fentanyl pharmacokinetics, pharmacodynamics and adverse effects," *Basic & Clinical Pharmacology & Toxicology*, vol. 124, no. 3, pp. 321–329, 2019.

[22] I. A. Elkiweri, Y. L. Zhang, U. Christians, K. Y. Ng, M. C. Tissot van Patot, and T. K. Henthorn, "Competitive substrates for P-glycoprotein and organic anion protein transporters differentially reduce blood organ transport of fentanyl and

loperamide: pharmacokinetics and pharmacodynamics in Sprague-Dawley rats," *Anesthesia and Analgesia*, vol. 108, no. 1, pp. 149–159, 2009.

[23] G. Yin, Z. Xiao, Y. Ni et al., "Association of MDR1 single-nucleotide polymorphisms and haplotype variants with multiple myeloma in Chinese Jiangsu Han population," *Tumour Biology*, vol. 37, no. 7, pp. 9549–9554, 2016.