RESEARCH ARTICLE

Gene Polymorphisms of OPRM1 A118G and ABCB1 C3435T May Influence Opioid Requirements in Chinese Patients with Cancer Pain

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Abstract

Backgrounds: Polymorphisms of *OPRM1 A118G* and *ABCB1 C3435T* have been suggested to contribute to inter-individual variability regarding pain sensitivity, opioid usage, tolerance and dependence and incidence of adverse effects in patients with chronic pain. This study aimed to investigate the association of both two polymorphisms with opioid requirements in Chinese patients with cancer pain. Methods: The genotypes of *rs1799971* (*OPRM1*) and *rs1045642* (*ABCB1*) were determined by PCR-RFLP and direct sequencing methods respectively in 112 patients with cancer-related pain. Comparisons between the different genotype or allele groups were performed with t-tests or one-way ANOVA tests, as appropriate. The potential relationship of allele number with opioid response was performed with a trend Jonckheere-Terpstra test. Results: In the 112 subjects, the frequencies of variant 118 G and 3435T allele were 38.4% and 37.9%, respectively. Significant higher 24h-opioid doses were observed in patients with *GG* (*P*=0.0004) and *AG + GG* (*P*=0.005) genotypes than the *AA* carriers. The dominant mutant 118G allele tended to be associated with progressively increasing 24h-opioiddoses (*P*=0.001). Compared with *CC/CT*, patients with *ABCB1 TT* genotype received higher 24h- and weight-surface area-adjusted-24h- opioids doses (*P*=0.057 and 0.028, respectively). Conclusions: The OPRM1 A118G single nucleotide polymorphism (SNP) is a key contributor for the inter-individual variability in opioidrequirements in Chinese cancer pain patients. This may possibly extend to the *ABCB1 C3435T* SNP.

Keywords: SNP - opioids requirements - cancer pain treatment - OPRM1 gene - ABCB1 gene

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Introduction

According to statistics by WHO, about 60%~90% of patients with advanced cancer are suffered from cancer pain in varying degrees, which is also a presenting symptom in 70% patients (Elliott et al., 1999). Most of the terminal cancer pain therapy finally tends to the third step-strong opioids treatment. However, inter-individual variabilities illustrated by the diversities of sensitivity of pain, bioavailability, side effects, tolerance and dependency may limit its clinical application (Reyes-Gibby et al., 2007; Smith et al., 2012; Zhou et al., 2012). Recent studies suggests that polymorphisms of mu opioid receptor gene (OPRM1) and ATP-binding cassette B1 gene (ABCB1) (multidrug resistance 1 [MDR1]) have a significant association with the analgesia effects and consumptions of opioids (Uhl et al., 1999; Campa et al., 2008; Lloret et al., 2011; Kasai et al., 2011; Haerian et al., 2013) .

The μ-opioid receptor (MOPR), the major receptor and analgesia target of most opioids, is encoded by OPRM1 gene (Ikeda et al., 2005). Recently, plenty of studies have indentified that the haplotype of OPRM1 includes more than 100 polymorphisms, however, in which only A6V, A118G (N40D), R260H, R265H, S268P are involved in analgesia effect (Hoehe et al., 2000; Befort et al., 2001; Wang et al., 2001). Particularly, A118G nonsynonymous polymorphism has been undergoing most thorough study which makes MOPR lose a glycosylation site but enhance its affinity to opioids. In human populations, the 118G variation has been considered as a contributor that closely related to pain perception and altered analgesic effects in patients administrated pain management (Campa et al., 2008; Landau et al., 2008; Sia et al., 2008; Tan et al., 2009; Klepstad et al., 2011). The 118G/Asp40-hMOPR had a lower molecular mass and also shorter half-life of the mature form than that of 118A/Asn40-hMOPR due to differential N-glycosylation (Huang et al., 2012). In addition, the 118G tended to be correlated with the reduced analgesic effects, efficiency and much less side effects induced by morphine and its active metabolite -M6G (Lötsch et al., 2006; Campa et al., 2008).

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P-glycoprotein (P-gp), the product encoded by ABCB1 gene, is a member of the ATP-binding cassette (ABC) transporter superfamily (Chen et al., 1986). As an ATP-dependent transmembrane efflux pump, it has been closely linked to resistance to substrates including opioids prevented from transporting across the blood-brain barrier to decrease analgesia action in the central nervous system (CNS) (Zhou et al., 2007; Fujita et al., 2010; Fernandez et al., 2012). The pain relief of morphine and fentanyl were much better in P-gp deficient mice than wild type (WT) (Hamabe et al., 2006). Over 50 ABCB1 SNPs have been detected, which in some degree impacted on transcription and translation processing of ABCB1 gene, and subsequent pharmacokinetics and pharmacodynamics characteristics of P-gp. Accumulating evidences testified that the frequent C3435T SNP of ABCB1 gene is significantly associated with the expression of P-gp (Tang et al., 2004; Leschziner et al., 2006; Kasai et al., 2008; Baldissera et al., 2012). The plasma concentration of morphine in the C3435T variant carriers, especially the TT homozygotes, were evidently elevated (Mealey et al., 2008). Moreover, the pain duration of TT carriers was much longer than that of those with C allele (Sia et al., 2010).

To date, functional consequences of both SNPs haven't yet come to a consensus. Based on these above researches, in this study we focused on the differences in consumptions and analgesia effects of opioids across various SNPs of OPRM1 or ABCB1 gene. In addition, the association of OPRM1 A118G, ABCB1 C3435T polymorphisms with the opioids requirements was analyzed in patients with cancer pain.

Materials and Methods

Subjects

One hundred and twelve inpatients with moderate to severe cancer pain were enrolled, which were from the department of oncology of the Third People's Hospital Affiliated to shanghai jiao Tong University school of Medicine during 2007 to 2010. The subjects were all given opioids to relieve cancer pain. This study was approved by the local Ethics Committee, and signed informed consent was obtained from every patient or their families. Exclusion criteria were: history of smoking and alcohol, diabetes mellitus, cardiovascular disease, renal disease or hepatic disease; psychiatric disease or other chronic pain; or long term using chronic pain medication. According to the genotype detection results, the subjects were divided into three groups including WT homozygotes, heterozygotes and mutant homozygotes. All patients were of Chinese Han nationality.

Assessments

Visual analogue scale (VAS) was used to assess immediate pain when the patient was feeling, and then were treated with opioids titration and maintenance according to the NCCN Adult Cancer Pain Clinical Practice Guidelines. The pain intensity was evaluated and rated using a visual analog scale (VAS) prior to the administration of opioids and performed again after the first 24h administration when it come to patients' satisfaction and comfort. The well analgesia effect was set as VAS score ≤ 4. The induction and maintenance of analgesia were carried out with morphine, tramadol, sustained release morphine tablet, Oxycodone, fentanyl plaster and acetaminophen according to the variance of cancer pain intensity. The clinical application of opioids was in strict accordance with NCCN Adult Cancer Pain Clinical Practice Guidelines, all the equivalent dose and pain severity transfer factor of opioids were showed in Table 1. So far, there have been three major calculation methods of opioids requirements for patients with cancer pain including 24h-opioids doses, weight-adjusted-24h-opioids doses, weight-surface area-adjusted-24h-opioids doses. In this study, a descriptive statistical analysis with respect to general information of the subjects was conducted respectively with the three ways. For convenience, the 24h- opioids doses was mainly used to further measure the clinical application of opioids for patients with cancer pain, supplemented by another two methods.

Genotyping analysis

Peripheral blood samples (2 ml) were collected from all the participants. Genomic DNA from leukocytes was isolated with standard phenol /chloroform extraction protocol in this study. Genotyping of the OPRM1 A118G polymorphism was done double-blinded using polymerase chain reaction-restricted fragments length polymorphism (PCR-RELP), yet ABCB1 C3435T by direct sequencing. The primers sequences of OPRM1 and ABCB1 for PCR amplification were forward 5'-GGTCAACTTGTCCCACTTAGATCGC-3' and reverse 5'-AATCACATACATGACCAGGAAGTTT-3'; forward 5'-AGCCCATCCTGTTTGACTGC-3' and reverse 5'-TGTATGTTGGCCTCCTTTGC-3' respectively. Both sets of primers were designed by Primer Select program of DNAStar soft. The typical PCR amplification were conducted in a 50 μl reaction volume, containing 1.0 μl genomic DNA, 25 mM $MgCl_2$, 0.5 mM of each primer, 2 mM dNTP, 10×PCR buffer, 0.5μl MBI ferments Taq Polymerase and started with an initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60°C for 1 min and extension at 72 °C, followed by an 1-min terminal extension at 72°C. The PCR amplification products of OPRM1 A118G SNP were digested by Bsh1236 I (MBI ferments) for 2 h at 37 °C. And then the digested products were analyzed by electrophoresis on a 12% polyacrylamide gel dyed by 0.5 μg/ml of ethidium bromide for 5 to 10 min. Finally, the DNA bands were viewed and identified on GelDocum 2000 imaging system (Bio-Rad, USA) (Figure 1).

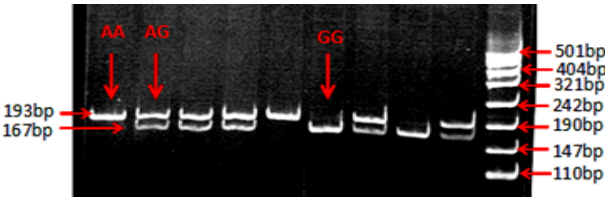

Figure 1. Electropherogram for Enzye-digested Products of OPRM1 rs1799971 Site

The PCR product of ABCB1 C3435T variant was further purified using centrifugal purification column packed with resin (SBS Gene technology Co. Ltd, Shanghai, China). Then DNA sequencing amplification was carried out in 10 μl volume comprising 1 μl purified PCR product, 2 μl of BigDye 3.1 mixture (Applied Biosystems, Foster City, CA, USA) and 1.6 ul of 1 pM primer. The sequencing primers were the same as aforementioned amplification. Amplification conditions for the sequencing were 25 cycles comprising denaturation at 95 °C for 10s, annealing at 50 °C for 5s and extension at 60 °C for 4 min on a 9600 DNA thermal cycler (Perkin-Elmer, USA). 10 μl product was purified passing through 6% Sephadex column (Sigma, USA) and loaded on an ABI 3100 automated sequencer combined with fluorescence labeling and laser measure techniques. The CHROMAS soft and MegAlign soft of DNAStar software package were used to analyzed the results of sequencing. The genotypes of all the samples were obtained proceeding with a comparative analysis between the above-mentioned results and the canonical sequence published in GenBank database (Figure 2).

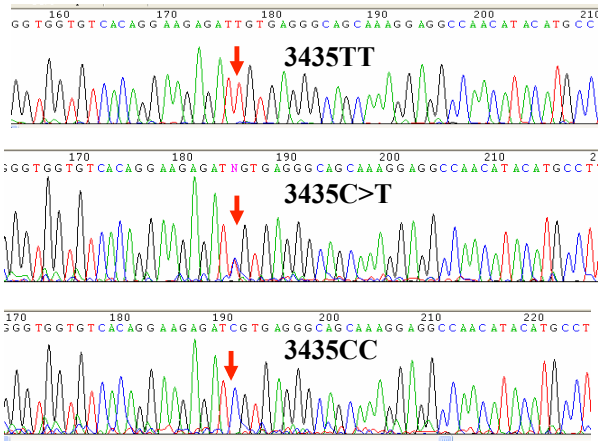

Figure 2. Sequencing Diagram for the ABCB1 SNP (rs1045642) in the ABCB1 Gene

Table 1. Conversion of Equivalent Dose and Corresponding Intensity in the Administration of Parenteral or Oral Analgesic Drugs Compared with Morphine in Single Does Research

Opioid receptor agonist	Parenteral administration (mg)	Oral administration (mg)	Conversion factor (vein to oral)	Action duration (h)
CodeineS	130	200	1.5	3~4
Fentanyl	0.1	-	--	1~3
Hydrocodone	-	30~200	--	3~5
Hydromorphone	1.5	7.5	5	2~-3
Levorphane	2	4	2	3~6
Methadone	-	-	-	-
Morphine	10	30	3	3~4
Oxycodone	-	15-20	--	3~5
Oxymorphone	1	10	10	3~6
Tramdol	-	50-100 mg	--	3~7

Statistical analysis

Demographic data was given as mean ± SD. Hardy-Weinberg genetic equilibrium test was conducted on the soft provided by the Internet (http://ihg.gsf.de/ihg /snps.html). Comparisons of opioids consumptions between the different genotype or allele groups for each of the SNPs were performed with t-tests or one-way ANOVA tests, as appropriate. The potential relationship of allele number and analgesic response was performed with trend Jonckheere-Terpstra test. $P < 0.05$ was considered statistically significant. Statistical analyses were conducted using the Stata 10.1 (Stata Corporation, College Station, TX).

Results

All 112 subjects' general information is shown in Table 2. The average age, weight and Body mass index (BMI) were 63.4 ± 13.7 years (29 ~91 years); 60.4 ± 9.5 kg (40~90 kg) and 21.5 ± 2.6 kg/m^2 (15.8~30.1 kg/m^2) respectively. In addition, no significant differences were observed between gender, age, △NRS pain scores and opioids consumptions, even though all three calculation methods of drug doses were used (p>0.05).

Among the 112 subjects, 44 were AA homozygotes, 50 AG heterozygotes, and 18 GG homozygotes. The OPRM1 118 G allele frequency was 38.39% in the cancer pain patients. The distribution of the OPRM1 A118G allele was according with Hardy-Weinberg equilibrium (p>0.05). Significant statistical differences were observed according to genotypes. Analysis of trend Jonkcheere-Terpstra showed that the 24h-dose of opioids required for the AG/GG genotype carriers were 97.33 ± 69.07 mg

Table 2. Association of Opioids Doses with Gender, Age and △NRS in Cancer Pain Patients

	No.	24h-doses[a]		weight-adjusted-24h- doses[b]		weight-surface area-adjusted-24h- doses[c]	
		mean (SD)	p-value	mean (SD)	p-value	mean (SD)	p-value
Gender							
Female	37	98.05(78.00)		1.84(1.5)		1.21(1.02)	
Male	75	95.60(72.60)	0.87	1.53(1.2)	0.249	0.9(0.73)	0.066
Age (years)							
≤55	38	105.36(78.59)		1.78(1.51)		1.08(0.99)	
56~71	37	102.31(76.57)		1.74(1.3)		1.07(0.85)	
≥72	37	81.31(66.00)	0.315	1.38(1.07)	0.351	0.85(0.65)	0.398
△NRS							
≤4	57	89.22(80.24)		1.56(1.45)		0.97(0.94)	
>4	55	103.85(67.04)	0.298	1.71(1.15)	0.53	1.04(0.74)	0.666

△NRS , Numeric rating scale ; [a]mg 24h-1; [b]mg kg-124h-1; [c]mg kg-1 (m2)-124h-1 ; *p<0.05 indicated a significant association in opioids doses between groups

Table 3. Frequencies of Genotypes, Alleles of A118G Polymorphism in the OPRM1 Gene and the Association with Opioids Doses

OPRM1 A118G	No.	24h-dose (mg)	*p*-value
Genotype			
AA	44	72.48(64.05)	
AG	50	97.33(69.07)	
GG	18	152.34(83.13)	0.0004
Jonkcheere-Terpstra test: $P = 0.0004$			
AA	44	72.48(64.05)	
AG+GG	68	111.89(76.42)	0.005
Allele			
A	138	81.48(66.52)	
G	86	120.36(79.12)	0.001

All data of 24h- opioids doses are given as mean (SD); *$p<0.05$ indicated a significant association in the test

Table 4. Frequencies of Genotypes, Alleles of C3435T Polymorphism in the ABCB1 Gene and the Association with Opioids Doses

ABCB1 C3435T	No.	24h-dose*		weight-surface area-adjusted-24h- doses‡	
		mean (SD)	*p*-value	mean (SD)	*p*-value
Genotype					
CC	45	100.43 (82.24)		0.98 (0.93)	
CT	49	103.87 (71.38)		1.17 (0.83)	
TT	18	66.04 (52.57)	0.161	0.6 (0.49)	0.051
CC+CT	94	102.22 (76.37)		1.08 (0.88)	
TT	18	66.04 (52.57)	0.057	0.60 (0.49)	0.028
Allele					
C	139	101.64 (78.03)		1.05 (0.89)	
T	85	87.85 (66.21)	0.176	0.93 (0.75)	0.307

*$mg.24h^{-1}$; ‡$Mg.kg^{-1}$; $(m^2)^{-1}24h^{-1}$; **$p<0.05$ indicated significant in the test

and 152.34 ± 83.13 mg, respectively, significantly higher than that for the AA homozygous wild-type [(72.48 ± 64.05 mg), p=0.0004]. Much more opioids were required for the patients with dominant mutant AG/GG phenotype than those carrying homozygous recessive AA. In addition, significant higher daily opioids intake doses were observed in the patients with 118G allele compared with the 118A carriers (120.36 ± 79.12 mg vs. 81.48 ± 66.52 mg, p=0.001). The frequency of A118G polymorphism, all the genotype distributions and 24h-doeses of opioids consumed by patients are showed in Table 3.

Moreover, of the 112 subjects there were 45 wild-type homozygotes CC, 49 heterozygotes CT and 18 mutant homozygotes TT. The frequency of ABCB1 C3435T allele was 37.94%. The sequencing results of the ABCB1 rs1045642 were showed in Figure 1. The distribution of ABCB1 C3435T allele was in Hardy-Weinberg equilibrium (p>0.05). The following study had failed to reveal any significant difference in 24h-opioids doses among the subjects carrying various ABCB1 allele phenotype (Table 4). Compared with the CC/CT phenotype, the subjects with TT homozygotes tended to require lower opioids intake dosage, but no significant statistical differences were observed (p=0.0057). However, when we measured using weight-surface area-adjusted-24h- opioids doses instead, the dosages of opioids administrated for the TT homozygotes was 0.60 ± 0.49 mg and significantly lower than those with CC/CT phenotypes (1.08 ± 0.88 mg , p=0.028).

Discussion

The OPRM1 gene encoding MOPR is a major candidate for genetic diversities of opioids; it has been also extensively discussed in a variety of populations (Uhl et al., 1999; Kasai et al., 2011). MOPR, a member of G-protein-coupled receptor (GPCR) family, is a key target factor playing important roles in analgesia, tolerance and dependency effects interacting with G-proteins (Gi/Go). Up to now, it has been reported that there are more than 100 OPRM1 polymorphisms happened in promoter, intron and also coding region respectively, inducing the alteration of the expression level and / or activity of MOPR (Bayerer et al., 2007; Kosarac et al., 2009; Shabalina et al., 2009). Opioid drugs are binding to MOPR educe the effects of analgesia, sedation and respiratory depression. Otherwise, at the transcriptional level the OPRM1 is strictly regulated, so it stands reason that the SNPs occurred in the potential 5' regulatory region will lead to individual differences in OPRM1 expression and sensitivity to opioids (Wang et al., 1994).

Among those polymorphisms, the SNP (rs1799971) A118G occurred in exon 1 of OPRM1 gene, in which replacing an adenine with guanine substitutes an aspartic acid for an asparagine at putative N-glycosylation site (N40D) so that a glycosylation site was lost. Genetic association studies for A118G SNP between pain thresholds, analgesic effects and requirements of opioids have been well deliberated (Campa et al., 2008; Landau et al., 2008; Sia et al., 2008; Tan et al., 2009; Klepstad et al., 2011). During the chronic cancer pain management, it had been observed that two cases were distinct-different in response to morphine. One was given sustained-release morphine only 10 mg/d, 2 days later clinical manifestation of severe drowsiness, alienation emerged and withdraw symptom disappeared; yet another one had been administrated 200 mg just control cancer pain. Finally, it turned out that the former was carrying AA phenotype, the other with AG (Hirota et al., 2003).

Previous studies had showed that there was a significant racial diversity for the A118G polymorphism. It is low in African American (1.6% ~2.8%) and Caucasian (10.5%~16.4%), with higher prevalence in Asian ancestry populations, especially in Chinese (~30%) (Tan et al., 2003; Campa et al., 2008; Tan et al., 2009). So it will be of obvious clinical significance to conduct an association study between OPRM1 A118G and the consumptions of opioids for Chinese cancer pain patients receiving management. In our study, 112 Chinese patients with cancer pain were enrolled as the objects, affording standard opioids titration and maintenance. When it come to patients' satisfaction and comfort after the first 24h administration, a significant individual difference of 24h doses of opioids across OPRM1 A118G phenotypes was detected. In the case of achieving effective analgesia, the consumptions of opioids were significantly increased for the subjects with GG homozygotes than those in AA/

AG groups. In other words, the 118G variant may be a lose-of-function mutation and the requirements of drugs has a trend of positive relationship with the number of G118 allele, suggesting it is a more safety and effective analgesia to administrate higher doses of opioids for patients with GG phenotype, which was identical to the previous researches (Oertel et al., 2006; Campa et al., 2008; Landau et al., 2008; Sia et al., 2008; Tan et al., 2009; Klepstad et al., 2011).

To the date, the mechanism underlying the correlation between OPRM1 A118G SNP and requirements of opioids is still unclear. A human autopsy study had reported allele-specific mRNA expression from AG heterozygotes; the 118G mRNA was obviously lower than the 118A allele. The unbalance of allele-specific mRNA expression induced by A118G polymorphism could be accounted for the altered transcription or heterogeneous nuclear RNA (hnRNA) transition into mature mRNA, and also explained by mRNA decay in the processing of hnRNA maturation and precursor protein synthesis. Additionally, there was a decreased expression of MOPR in the 118G variant to 7-fold lower than that in the 118A using HEK 293 cells (Beyer et al., 2004). However, previous studies had indentified that OPRM1- 118G acquired 3-fold higher binding affinity for β-endorphin than that WT in transfected AV-12 cells expressing the MOPR variants (Bond et al., 1998), implying a gain-of-function. In vitro studies, when it took comprehensive factors into account, the functional consequences of the A118G SNP had been proved to be associated with the alteration in receptor expression and signal transduction but not binding affinity (Landau et al., 2008). In a word, the existing evidences are in support of our study finding: more doses of opioids were required to analgesia for patients with the OPRM1 A118G variant, suggesting a lose-of-function mutation. Additionally, other SNPs of OPRM1 and possible linkage disequilibrium with A118G polymorphism will bring into the subsequent discussion.

P-gp is extensively expressed in intestine, liver, gall bladder, kidney, which possess excretory function and also distributes in brain epithelia which is an essential component of blood-brain barrier (Zhou et al., 2007; Fernandez et al., 2012). Previous investigations have observed that opoids are the substrates for P-gp involved in drugs' cellular membrane permeability, disposition, and therefore analgesia effect in CNS (Ambudkar et al., 2003).

Genetic variation in the ABCB1 has been demonstrated to be associated with the expression and function of P-gp and subsequent analgesia activity in CNS. So far, it has been found that more than 50 SNPs in the coding region of ABCB1 translated to both synonymous and nonsynonymous but not nonsense mutation. The association of ABCB1 SNP with drug response, clinical outcome, and susceptibility to some diseases has been well characterized. Especially, in human populations, a frequently studied SNP (rs1045642) occurred in exon 26 of ABCB1, in which a cytosine to thymine substitution leads to a C3435T synonymous polymorphism (Ile1145Ile). The C3435T was first reported to be linked to the expression and function of MDR1 in the duodenum (Hoffmeyer et al., 2000). Moreover, significant distinctions in morphine requirements across ABCB1 genotypes in respect of C3435T SNP were detected in this study. Many additional studies have subsequently verified this association in 117 cancer pain patients. Recent studies have testified that women with the 3435T allele tended to have a higher risk of development of pain in post-caesarean patients (Sia et al., 2010). In patients receiving pain management, significantly decreased daily doses of opioids were administrated for the 3435T allele carriers (Lötsch et al., 2006; Oertel et al., 2010). Moreover, homozygotes but not heterozygotes for 3435T were found significantly higher pain relief than those homozygotes CC in the morphine therapy for cancer pain (Campa et al., 2008). In addition, loperamide, an opioid antidiarrheal medicine without morphine-like central inhibition effects, can be rapidly pumped out of CNS by P-gp; it has reported that the C3435T SNP was significantly related to its CNS opioid effects such as miosis (Lötsch et al., 2006). However, in vitro studies there are still no sufficient evidences to demonstrate the association of C3435T SNP with the expression of MDR1 and its transport function (Salama et al., 2006; Kimchi-Sarfaty et al., 2007). One reasonable explanation is the interaction between the C3435T SNP and undetected SNPs in the non-coding region, however, this possibility has been denied (Sia AT et al., 2008). Altered mRNA stability is also responsible for the contradiction, but it remains controversial (Kimchi-Sarfaty et al., 2007; Wang et al., 2005; Hung et al., 2008).

Clearly, several investigations using linkage analysis have indicated that the C3435T SNP with other SNPs of ABCB1 formed a series of haplotypes, in which an strong linkage disequilibrium has been normally founded among the C1236T, G2677 T/A, C3435T polymorphisms (Tang et al., 2004; Leschziner et al., 2006; Sia et al., 2008). Otherwise, since the allele and genotype frequencies of the C3435T polymorphism varies duo to ethnicity, Asians and Indians which have obviously higher TTT haplotype frequency than the other groups were susceptible chosen for the objects (Tang et al., 2004; Li et al., 2007). So we selected the Chinese cancer pain patients. A study in Korean undergoing spinal anesthesia with intravenous fentanyl showed that patients with TTT haplotype had a trend of more early and serious suppression of respiration than those carrying the WT (CGC) allele. The ABCB1 haplotype including A61G, G1199A, C1236T, G2677T and C3435T has an impact on daily methadone consumptions. Alternatively, in opioid-dependent or -non-dependent patients, no significant differences in the frequencies of the C3435T SNP and haplotypes appeared to be indentified (Oertel et al., 2006). Furthermore, no significant association of ABCB1 haplotypes based upon only G2677T (A) and also C3435T SNPs with the miotic effects of levomethadone has been revealed (Lötsch et al., 2006).

In our study, we had failed to reveal any significant difference in 24 h opioids doses among the subjects carrying various ABCB1 C3435T phenotypes. However, when we measured using weight-surface area-adjusted-24h-opioids doses instead, TT homozygotes tended to require significantly lower opioids intake dosage than the CC/CT carriers. It suggests that diversities of drug

metabolism way and detection method may conduce to the differences. Nonetheless, compared with only using the C3435T SNP of ABCB1, the frequent haplotypes (C1236T, G2677 T/A, C3435T) may be more prevalent in the following studies. Further discussions are required to certify the concrete mechanisms of the observed discrepancies of the functional consequence in ABCB1 C3435T SNPs and haplotypes.

In conclusion, the OPRM1 A118G polymorphism is one of genetic factors inducing the variability of opioids requirement for cancer pain relief, and maybe the ABCB1 C3435T SNP also is a candidate gene. So more in-depth studies based on gene polymorphism will be beneficial to individuation management for cancer pain and direct the reasonable clinical application of opioids.

Acknowledgements

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