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ORIGINAL ARTICLE

Association of *UGT2B7* and *ABCB1* genotypes with morphine-induced adverse drug reactions in Japanese patients with cancer

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Abstract
Purpose To investigate the effects of genetic polymorphisms on morphine-induced adverse events in cancer patients.
Methods We examined the relation of morphine-related adverse events to polymorphisms in *UDP-glucuronosyltransferase* (*UGT*) *2B7*, *ATP-binding cassette, sub-family B, number 1* (*ABCB1*), and *μ-opioid receptor 1* genes in 32 Japanese cancer patients receiving oral controlled-release morphine sulfate tablets.
Results The T/T genotype at 1236 or TT/TT diplotype at 2677 and 3435 in *ABCB1* was associated with significantly lower frequency of fatigue (grades 1–3) ($P = 0.012$ or 0.011, Fisher's exact test). The *UGT2B7*2* genotype was associated with the frequency of nausea (grades 1–3) ($P = 0.023$). The frequency of nausea was higher in patients without *UGT2B7*2* allele than others. The diplotype at 2677 and 3435 in *ABCB1* was associated with the frequency of vomiting (grades 1–3) ($P = 0.011$). No patient whose diplotype was consisted of no GC allele at 2677 and 3435 suffered from vomiting.
Conclusion Our findings suggest that pharmacogenetics can be used to predict the risk of morphine-induced adverse events.

Keywords Morphine · Cancer patients · Adverse reaction · Pharmacogenetics

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Introduction

Severe pain caused by tumors is therapeutically managed by administration of opioid analgesics. Morphine is one of the most important and widely used opioids for cancer-pain relief, but large interindividual variability in its effectiveness and adverse reactions are a major clinical disadvantage. Clinical pharmacology studies have demonstrated that wide interindividual variability in the response to a drug is associated with considerable pharmacokinetic or pharmacodynamic variability [1], which may be genetically determined [2–4].

Morphine is predominantly glucuronidated by UDP-glucuronosyltransferase (UGT) 2B7 to form morphine 6-glucuronide (M6G) and morphine 3-glucuronide (M3G) [5]. M6G has clinically been shown to be a potent analgesic, and the analgesic properties of morphine are enhanced

by the action of M6G [6]. In contrast, M3G decreases the analgesic activity of morphine and M6G [6]. Therefore, polymorphisms in the *UGT2B7* gene are associated with interindividual variability in the pharmacokinetics of morphine and its metabolites. Although the effects of genetic polymorphisms in *UGT2B7* on the pharmacokinetics of morphine and its efficacy in patients with cancer have been reported [7, 8], whether such polymorphisms influence morphine-related adverse reactions remains unclear.

Efflux transporter P-glycoprotein [ATP-binding cassette, sub-family B, member 1 (ABCB1)], coded by the *ABCB1* gene, is a major determinant of the intracellular concentration of morphine and its metabolites, M6G and M3G [9]. ABCB1 can limit the entry of morphine and its metabolites into the brain and actively pump the drug out of the central nervous system. It is thus an important component of the blood–brain barrier [10]. So far, a variety of polymorphisms in the *ABCB1* gene have been identified [11]. Recently, Campa et al. [12] have shown that pain-relief variability in patients with cancer is significantly associated with 3435C > T in the *ABCB1* gene. This finding suggests that genetic variability of *ABCB1* may affect morphine disposition in the central nervous system. However, few studies have examined the relation between *ABCB1* genetic polymorphisms and morphine-induced adverse drug reactions in patients with cancer who receive morphine.

The primary site of action of morphine is the μ-opioid receptor, which is encoded by the *opioid receptor μ1* (*OPRM1*) gene. *OPRM1* is thus an initial candidate gene for studies evaluating the role of polymorphisms in the clinical response to morphine. A variety of polymorphisms have been identified in the *OPRM1* gene [13, 14]. The most prevalent polymorphism in the *OPRM1* gene is a nucleotide substitution 118A > G, causing amino acid change N40D at a putative *N*-glycosylation site in the extracellular region of the receptor. Recently, 118A > G was demonstrated to lower mRNA and functional protein expression in human brain tissue and in transfected cells [15]. To date, the association between single nucleotide polymorphism (SNP) and the efficacy of morphine has been relatively well investigated. Cancer patients homozygous for the G allele were found to be poor responders to morphine [12] and to require higher doses of morphine to relieve pain [16]. However, the association between 118A > G in *OPRM1* and morphine-induced adverse reactions in patients with cancer remains unclear.

We examined the effects of polymorphisms in the *UGT2B7*, *ABCB1*, and *OPRM1* genes on morphine-related adverse reactions in patients with cancer who received morphine therapy.

Methods

Materials

Morphine hydrochloride was kindly provided by Shionogi (Osaka, Japan). M3G, M6G, and naloxone hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents were of the highest grade commercially available.

Patients

Japanese patients with cancer who were receiving controlled-release morphine sulfate tablets (MS Contin, Shionogi, Osaka, Japan) to relieve cancer pain were enrolled. The protocols for the pharmacokinetic study of morphine and its metabolites and the pharmacogenetic study were approved by the Institutional Review Board of Saitama Medical University. All patients gave written informed consent for their peripheral blood samples and medical information to be used for research purposes.

Treatments

Controlled-release morphine sulfate tablets were orally administered to patients according to the standard protocol described in the package insert. When the initial dose was appropriate to relive pain, the dose was determine as maintenance dose. If necessary, the initial morphine dose was modified to achieve the pain relief. When the pain was relieved enough by the modification of dose, the dose was determined as the maintenance dose. If patients did not tolerate with morphine treatment because of adverse events, the dose was adopted as maintenance dose. Thus, maintenance dose depended on the pain intensity of the patients and susceptibility of them to morphine. When the maintenance dose was obtained, morphine-induced adverse events were evaluated.

Morphine-induced adverse reactions

Morphine-induced adverse events, including constipation, nausea, vomiting, drowsiness/confusion, and fatigue, were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTCAE), Version 3.0.

Genotyping

Genomic DNA was extracted from 200 μl of peripheral blood, which had been stored at −80°C until analysis, with the use of a QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany).

UGT2B7 gene fragments containing 211G > T [rs12233719, A71S] and 802C > T [rs7439366, H268Y,

*UGT2B7*2*] were amplified by polymerase chain reaction (PCR). Genomic DNA samples (100 ng) were added to the PCR mixtures (50 μl), consisting of 1× PCR buffer, 3 mM $MgCl_2$ for 211G > T or 4 mM $MgCl_2$ for *2, 0.25 μM of each primer, 200 μM dNTPs, and 1.25 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA). The PCR primers used to amplify the *UGT2B7* gene fragments containing the respective polymorphisms were 2B7A71S-F (5′-TTAGTTTTGTGTCAATGGACTGCAGAAAC-3′) and 2B7A71S-R (5′-AAATAAGTTAGAGCTTCATGTTACT GATTG-3′) for 211G > T, and 2B7*2-F (5′-CTGTCAG GAAGACCCACTAC-3′) and 2B7*2-R (5′-TTTACCTTA GGCAGGGGTTT-3′) for 802C > T. All amplifications included a 15-min initial denaturation at 94°C. PCR was performed under the following conditions: 30 s at 94°C (40 s for *2), 40 s at 57°C (30 s at 56°C for *2), and 1 min at 72°C for 211G > T (30 s for *2) for 30 cycles, followed by a final extension at 72°C for 3 min. After the purification of the PCR products with QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany), direct sequencing was performed with BigDye terminator var. 3.1 cycle sequencing kit and on a 3130 genetic analyzer (Applied Biosystems, CA, USA).

Gene fragments of *ABCB1* that included 1236C > T [rs1128503, G412G], 2677G > T, A [rs2032582, A893T, S], and 3435C > T [rs1045642, I1145I] were amplified by PCR. Forward and reverse primers used for PCR to amplify *ABCB1* gene fragments containing these polymorphic sites were ABCB1-1236F (5′-TGAATGAAGAGTTTCTGATGT TTTCTTG-3′) and ABCB1-1236R (5′-ATACATTTGTAAT TGAAAGGGCAACAT-3′), ABCB1-2677F (5′-AATGAAT ATAGTCTCATGAAGGTGAGTTTT-3′) and ABCB1-2 677R (5′-CATTCTTAGAGCATAGTAAGCAGTAGGG-3′), and ABCB1-3435F (5′-TGGCAGTTTCAGTGTAAGAAA TAATGA-3′) and ABCB1-3435R (5′-TAATTTCTCTTCA CTTCTGGGAGACC-3′), respectively. PCR was carried out in a total volume of 50 μl in the presence of 100 ng of genomic DNA, 0.25 μM each primer, 1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, and 1.25 U of AmpliTaq Gold DNA polymerase. An initial denaturation at 94°C for 15 min was followed by 30 cycles of 0.5 min at 94°C, 40 s at 62°C, and 40 s at 72°C, as well as a final extension period of 3 min at 72°C. PCR products were sequenced directly as described above.

OPRM1 gene fragment containing 118A > G [rs17999 71, N40D] was amplified by means of PCR. The forward and reverse primers used were 5′-TTTCCCTCCTCCCTCC CTTC-3′ and 5′-GCCTTGGGAGTTAGGTGTCTCTTT-3′, respectively. Genomic DNA samples (100 ng) were added to the PCR mixtures (50 μl) consisting of 1× PCR buffer, 4 mM $MgCl_2$, 0.25 μM of each primer, 200 μM dNTPs, and 1.25 U of AmpliTaq Gold DNA polymerase. Amplification was performed by denaturation at 95°C for 30 s, annealing at 61°C for 40 s, and extension at 72°C for 1 min for 30 cycles, followed by a final extension at 72°C for 3 min. PCR products were subsequently directly sequenced as mentioned above.

Determination of morphine, M6G, and M3G

Blood samples for pharmacokinetic analysis were obtained after oral administration of morphine. A blood sample was arbitrarily obtained during the period between one dose of morphine and the next dose. The samples were centrifuged immediately, and resulting plasma samples were stored at −80°C until analysis.

Plasma concentrations of morphine, M6G, and M3G were analyzed by reverse-phase high-performance liquid chromatography (HPLC). The HPLC system consisted of an EP-300 pump, ATC-300 column thermostat, EP-300 electron chemical detector (ECD), DG-300 degasser (Eicom, Kyoto, Japan), SIL-20A auto-sampler, SPD-10AVVP ultraviolet (UV) detector, and C-R6A Chromatopac (Shimadzu, Kyoto, Japan).

Morphine and M6G were analyzed with the use of an ECD and a SuperODS column (4.6 × 100 mm, 2.3 μm; Tosoh, Tokyo, Japan). The oxidation potential was 750 mV. The mobile phase was a mixture of 0.1 M phosphate buffer (pH 2.1) containing 30 μM EDTA and 10 mM sodium dodecyl sulfate, acetonitrile, and methanol at a ratio of 90:8:2 (v/v). The column temperature was 40°C and the flow rate was 1.0 ml/min.

M3G was analyzed with the use of a UV detector and an L-column ODS (4.6 × 250 mm, 5 μm; Chemicals Evaluation and Research, Saitama, Japan). The wavelength of the UV detector was 210 nm, and the column temperature was 40°C. The mobile phase consisted of 0.1 M phosphate buffer (pH 2.1) containing 30 μM EDTA and 10 mM sodium dodecyl sulfate, acetonitrile, and methanol at a ratio of 74:24:2 (v/v) and was delivered at a flow rate of 1.0 ml/min.

The lower limits of quantification were 67.2 pg/ml (236 pM) for morphine, 380 pg/ml (0.823 nM) for M6G, and 496 pg/ml (1.08 nM) for M3G.

Pharmacokinetic parameters

Individual oral clearances (l/h) of morphine were estimated by empirical Bayes estimates, based on a prior non-linear mixed effect analysis fit, using a 1-compartment model. Non-linear mixed effect analysis was performed with NONMEM program version VI (Globemax LLC, Hanover, MD, USA) to develop a population pharmacokinetic model.

Statistical analysis

Genotype and allele frequencies for each polymorphic allele in the respective genes were determined by using

SNPAlyze 5.1 (Dynacom, Yokohama, Japan). The significance of deviations from Hardy–Weinberg equilibrium was also tested with the program SNPAlyze 5.1. Linkage disequilibrium analysis to create a pairwise two-dimensional map of correlation coefficients r^2 and D' among SNPs in the *ABCB1* gene was performed with SNPAlyze 5.1. Relations between the morphine maintenance dose and morphine-induced adverse reactions were evaluated with the use of Spearman's rank correlation coefficient. The Fisher's exact test were employed to analyze the association of *UGT2B7*, *ABCB1*, and *OPRM1* diplotypes or genotypes with morphine-related adverse events as graded by NCI-CTCAE, ver. 3.0 (grade 0 versus other) (JMP version 6 software, SAS Institute, Inc., Cary, NC, USA). A *P*-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics and morphine-induced adverse reactions

A total of 32 Japanese patients with cancer who received controlled-release morphine sulfate were enrolled in this study from July 2006 through February 2007. The patient characteristics are summarized in Table 1. The most frequent tumor was breast cancer, followed by colorectal and pancreatic cancers. Most patients (29/32) had metastases to various organ(s). Renal function evaluated on the basis of the serum creatinine level was normal in all patients.

Table 1 Patient characteristics

Characteristics	Values	Number of patients
Age (years)[a]	64.5 (38–77)	32
Sex (male/female)		15/17
Serum creatinine (mg/dl)[a]	0.7 (0.5–1.2)	32
Total bilirubin (mg/dl)[a]	0.4 (0.2–14.4)	32
Tumor type		
Breast		8
Colorectal		7
Pancreas		4
Stomach		3
Esophagus		3
Others		7
Maintenance dose (mg/day)	20/30/40/60/80	21/3/3/4/1
Metastasis		
	Yes/no	29/3

[a] Values are expressed as medians, with ranges in parentheses

Hepatic function estimated on the basis of the total bilirubin level was normal in all but one patient, who had a value of 14.4 mg/dl. Morphine-induced adverse reactions are shown in Table 2. There was no relation between the maintenance dose of morphine and the respective morphine-induced adverse reactions (Spearman's rank correlation coefficient).

Genotype and allele frequencies of polymorphisms in *UGT2B7*, *ABCB1*, and *OPRM1* genes

The genotype and allele frequencies of polymorphisms in the *UGT2B7*, *ABCB1*, and *OPRM1* genes are shown in Table 3. Allele frequencies of polymorphisms in the *UGT2B7* and *ABCB1* genes were roughly equal to those previously reported [7, 11]. The genotype and allele frequencies of 118A > G in the *OPRM1* gene were consistent with the HapMap data reported for Japanese (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1799971). All polymorphisms were in Hardy–Weinberg equilibrium ($P > 0.05$). We found that 3435C > T in the *ABCB1* gene was strongly linked to 2677G > T ($r^2 = 0.711$ and $D' = 0.927$), but not to 1236C > T, although Sai et al. [17] demonstrated linkage among 1236C > T, 2677G > T, and 3435C > T.

UGT2B7, *ABCB1*, and *OPRM1* genotypes and morphine-induced adverse reactions

The *UGT2B7*2* genotype was significantly associated with the frequency of nausea (grades 1–3; $P = 0.023$; Fig. 1). The frequency of nausea was higher in patients without *UGT2B7*2* allele than others. However, the systemic oral clearance of morphine did not differ significantly between the two groups of genotype, without *UGT2B7*2* and at least one *UGT2B7*2* allele. The frequency of other adverse reactions were also slightly higher in patients without *UGT2B7*2* than in those with at least one *UGT2B7*2* allele. A71S mutation was not related to any type of morphine-induced adverse events or to morphine clearance.

The genotype at 1236 in *ABCB1* gene was associated with the frequency of fatigue (grades 1–3; $P = 0.012$;

Table 2 Morphine-induced adverse reactions

Adverse reactions	Grade	Numbers of patients
Constipation	0/1/2/3	15/12/4/1
Nausea	0/1/2/3	21/7/3/1
Vomiting	0/1/2/3	25/3/1/1
Drowsiness/confusion	0/1/2/3	22/1/9/0
Fatigue	0/1/2/3	18/7/6/1

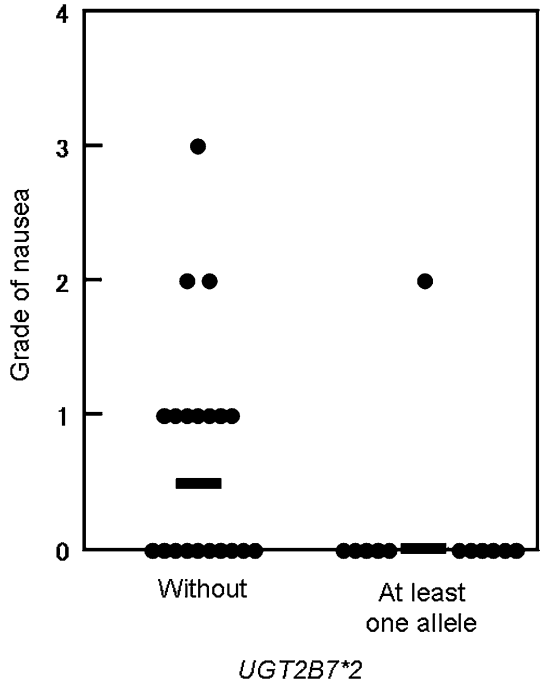

Fig. 1 *UGT2B7*2* genotype and morphine-induced nausea. Bars indicate medians

Fig. 2). The frequency of fatigue in patients with the T/T genotype at 1236 was significantly lower than that with other genotypes. The frequency of fatigue (grades 1–3) was also significantly lower in patients with TT/TT diplotype at 2677 and 3435 in *ABCB1* gene than in other patients ($P = 0.011$; Fig. 2). Morphine oral clearance was slightly but not significantly higher in patients with T/T genotype at 1236 or TT/TT diplotype at 2677 and 3435 than in other patients ($P = 0.103$ and 0.116, Mann–Whitney *U*-test). The diplotype at 2677 and 3435 in *ABCB1* was associated with the frequency of vomiting (grades 1–3; $P = 0.011$; Fig. 3). No patient without GC allele at 2677 and 3435 suffered from vomiting. The frequency of nausea (grades 1–3) in patients without GC allele at 2677 and 3435 in *ABCB1* was tended to be lower than others ($P = 0.061$). The oral clearance of morphine, M6G, and M3G did not differ significantly between these groups.

Morphine-induced adverse reactions were not associated with the polymorphism of 118A > G in the *OPRM1* gene.

The maintenance dose of morphine did not differ significantly between any two groups divided according to genotypes or diplotype for any of the adverse reactions described above.

Discussion

Our study showed that the grade of morphine-related adverse reactions was associated with genetic polymorphisms in genes that encode factors related to morphine pharmacokinetics and pharmacodynamics, including *UGT2B7*, *ABCB1*, and *OPRM1*.

Table 3 Genotype and allele frequencies of polymorphisms in the *UGT2B7*, *ABCB1*, and *OPRM1* genes

	Polymorphism	Genotype	Number (frequency)	Allele	Frequency
UGT2B7	−327G > A	G/G	17 (0.53)	G	0.70
		G/A	11 (0.34	A	0.30
		A/A	4 (0.13)		
	211G > T	G/G	29 (0.91)	G	0.95
		G/T	3 (0.09)	T	0.05
		T/T	0 (0)		
	802C/T	C/C	20 (0.62)	C	0.75
		C/T	8 (0.25)	T	0.25
		T/T	4 (0.13)		
ABCB1	1236C > T	C/C	5 (0.16)	C	0.38
		C/T	14 (0.43)	T	0.62
		T/T	13 (0.41)		
	2677G > T, A	G/G	6 (0.19)	G	0.39
		G/T	9 (0.28)	T	0.45
		G/A	4 (0.13)	A	0.16
		T/A	4 (0.13)		
		T/T	8 (0.24)		
		A/A	1 (0.03)		
	3435C > T	C/C	7 (0.22)	C	0.5
		C/T	18 (0.56)	T	0.5
		T/T	7 (0.22)		
OPRM1	118A > G	A/A	7 (0.22)	A	0.48
		A/G	17 (0.53)	G	0.52
		G/G	8 (0.25)		

The frequency of nausea was higher in patients without *UGT2B7*2* allele than others (Fig. 1); the systemic oral clearance of morphine did not differ significantly between the two groups. Coffman et al. [18] have demonstrated that UGT2B7.2 is capable of catalyzing morphine to inactive M3G more efficiently than to active M6G. Therefore, morphine might be detoxified more rapidly in patients with *UGT2B7*2* than in other patients, supporting our results that the incidence of morphine-induced adverse events was lower in patients with *UGT2B7*2*.

In our study, the T/T genotype at 1236 or TT/TT diplotype at 2677 and 3435 in *ABCB1* was associated with significantly lower frequency of fatigue (Fig. 2). This difference might be attributed to lower systemic exposure to morphine in patients homozygous for T allele at 1236 or TT/TT diplotype at 2677 and 3435. This notion is supported by the fact that morphine oral clearance in patients

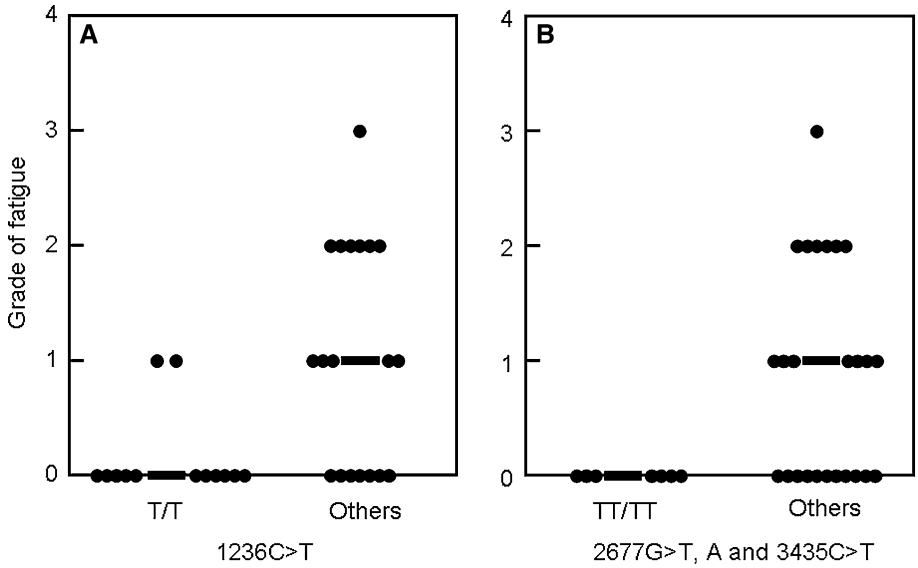

Fig. 2 *ABCB1* genotype or diplotype and morphine-related fatigue **a** 1236C > T, **b** 2677C > T, A and 3435C > T. Bars represent medians

with T/T genotype at 1236 or TT/TT diplotype at 2677 and 3435 tended to be higher than that in other patients. However, Meineke et al. [19] showed that the TT genotype of 3435C > T is associated with lower ABCB1 expression. To date, the functional effects of 1236C > T, 2677G > T, A, and 3435C > T in the *ABCB1* gene on the pharmacokinetics, efficacy, and adverse events of drugs remain controversial [20–32]. Further studies are necessary to elucidate the roles of these polymorphisms on the functions of ABCB1.

The frequency of vomiting was significantly higher in patients with one GC allele at 2677 and 3435 than in other patients (Fig. 3). In contrast, Coulbaut et al. [33] demonstrated that the GC/GC diplotype at 2677 and 3435 in the *ABCB1* gene was significantly associated with lower incidences of morphine-related nausea and vomiting as evaluated by the use of ondansetron. The results of these studies do not agree with our findings. One reason for the discrepancy may the difference in the administration route of morphine. In our study, morphine was administered orally, whereas in the study by Coulbaut et al. [33], morphine was administered intravenously to control postoperative pain. Since orally administered morphine is subject to the actions of ABCB1 expressed in the small intestine, the effects of polymorphisms in the *ABCB1* gene on pharmacokinetics might differ between intravenously and orally administered morphine.

Although Campa et al. [12] have shown that pain-relief variability in patients with cancer is significantly associated with 3435C > T in the *ABCB1* gene, they have not found any relations between morphine-induced adverse events and *ABCB1* polymorphisms, which was inconsistent with our present results.

Morphine-induced adverse reactions were not associated with the polymorphism of 118A > G in the *OPRM1* gene. As reported previously, cancer patients homozygous for the

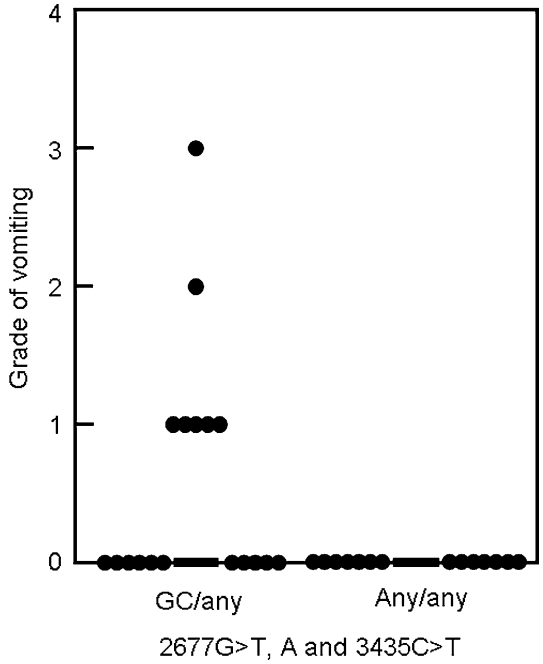

Fig. 3 *ABCB1* diplotype and morphine-induced vomiting. Bars represent medians

G allele were found to be poor responders to morphine [12] and to require higher doses of morphine to relieve pain [16]. These findings suggest that morphine-related adverse reactions are likely to occur in patients with G allele at 118, given that the mechanism of morphine-induced adverse events involves OPRM1. However, our results do not support this notion. Further analysis is necessary to confirm whether morphine-induced fatigue is directly related to the function of OPRM1.

It has been reported that V158M in catechol-*O*-methyl transferase (COMT) is associated with the response of morphine and further with morphine dose requirement [34]. We

are now investigating the association of morphine-related adverse events with polymorphisms in *COMT*, together with polymorphisms in *UGT2B7*, *ABCB1*, and *OPRM1* which we have not yet examined.

Large prospective studies are needed to determine whether genetic testing for *UGT2B7*, *ABCB1*, and *OPRM1* helps to predict the risk of morphine-induced adverse reactions and to elucidate the detailed mechanisms of morphine-related adverse events, taking into account medical aspects as well as cost effectiveness.

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Conflict of interest statement None.

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