

# Expression changes in arrestin $\beta$ 1 and genetic variation in catechol-O-methyltransferase are biomarkers for the response to morphine treatment in cancer patients

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**Abstract.** Genetic differences in individuals with regard to opioid-receptor signaling create clinical difficulties for opioid treatment; consequently, useful pharmacodynamic and predictive biomarkers are needed. In this prospective study, we studied gene expression changes in peripheral blood leukocytes using a microarray and real-time RT-PCR analysis to identify pharmacodynamic biomarkers for monitoring the effect of morphine in a cohort of opioid-treatment-naïve cancer patients. We also examined genetic variations in opioid receptor mu 1 (*OPRM1*, 118A→G) and catechol-O-methyltransferase (*COMT*, 472G→A) to evaluate predictive biomarkers of the treatment outcome of morphine. The plasma concentration of morphine was measured using a liquid chromatography-tandem mass spectrometry method. Microarray analysis revealed that the mRNA expression levels of arrestin  $\beta$  1 (*ARRB1*) were significantly down-regulated by morphine treatment. Real-time RT-PCR analysis against independent samples confirmed the results ( $P=0.003$ ) and changes during treatment were negatively correlated with the plasma morphine concentration ( $R=-0.42$ ). No correlation was observed between the genotype of *OPRM1* and morphine treatment; however, the plasma concentration

of morphine and the required dose of morphine were significantly lower for the A/A genotype of *COMT* (vs. A/G+G/G,  $P=0.008$  and  $0.03$ ). We found that changes in the expression of *ARRB1* may be a novel pharmacodynamic biomarker and the *COMT* 472G→A genotype may be a predictive biomarker of the response to morphine treatment.

## Introduction

Pharmacogenetic, pharmacokinetic and pharmacodynamic variations among individuals result in a wide variety of responses to pain sensation and to analgesics; therefore, intensive investigations of biomarkers for opioid treatment have been performed to improve the effectiveness of morphine treatment (1).

The opioid receptors are G-protein coupled receptors (GPCRs), and three types of receptors  $\mu$ ,  $\delta$  and  $\kappa$ -opioid receptors (*OPRM1*, *OPRD1* and *OPRK1*) are known to serve as receptors for morphine (2). Among them, *OPRM1* generated the main analgesic effect induced by morphine in a knock-out study performed in mice (3). Agonists for opioid receptors induce the activation of GPCRs, triggering the activation of various downstream molecules (2). A regulator of the G-protein signaling (RGS)-protein family negatively regulates opioid-receptor signaling by accelerating the deactivation of G proteins, and the regulators RGS2 and RGS9 are thought to be involved in resistance to morphine (4-6). In addition, G-protein coupled receptor kinase (GRK) phosphorylates the opioid receptors, leading to the binding of arrestin  $\beta$  1 and 2 (*ARRB1* and 2) to the opioid receptors (7). Thus, GRKs and ARRBs negatively regulate opioid-receptor signaling and are thought to be involved in resistance to morphine (8,9). To identify pharmacodynamic biomarkers that are capable

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of monitoring the drug effect, we examined the gene expression changes in opioid signaling-related molecules using a microarray and real-time RT-PCR analysis in peripheral blood leucocytes (PBLs).

Meanwhile, genetic variants associated with varying pain sensitivity and responses to morphine are thought to be potential biomarkers for predicting the outcome of morphine treatment (1,10). In this study, we also evaluated two functional genetic variants, *OPRM1* 118A→G and catecholamine-O-methyltransferase gene (*COMT*) 472G→A (also known as Val158Met). The 118A→G variant of *OPRM1* leads to a change in amino acids at position 40, affecting a putative glycosylation site of the receptor and biologically altering receptor activity (1). The enzyme activity of *COMT* is genetically defined as high in G/G, intermediate in G/A and low in A/A, and its genotype is thought to be associated with the effect of opioid-signaling (11).

In this prospective study, we examined gene expression to explore possible pharmacodynamic biomarkers and to evaluate the use of functional genetic variants as predictive biomarkers of the response to morphine treatment in a cohort of opioid-treatment-naïve cancer patients.

## Materials and methods

**Patients and samples.** This prospective study was conducted between 2009 and 2011 at the Kinki University Faculty of Medicine and Sakai Hospital, Kinki University Faculty of Medicine. Clinicopathological features including age, gender, ECOG performance status (PS) and type of primary malignant neoplasm were recorded. Morphine treatment was performed according to the standard method including titration (NCCN Guidelines™, Adult Cancer Pain). The required doses of morphine on Day 1 and on Day 8 are thought to be associated with the results of titration and the dose in the stationary phase, respectively.

PBL samples were obtained at baseline (pretreatment) and on Day 1 for the gene expression analyses. PBL samples for DNA were obtained at baseline. To measure the plasma concentrations of morphine, blood samples were collected on Days 1 and 8. The separated plasma were stocked at -80°C until use. The present study was approved by the institutional review boards of both centers and written informed consent was obtained from all the patients.

**RNA extraction.** Each 2.5-ml whole blood sample was stored in a PAX gene Blood RNA tube (Qiagen, Hilden, Germany). RNA was extracted according to the manufacturer's protocol (Qiagen). Then, massively containing globin mRNA was removed using a biotinylated Globin-capture oligonucleotides-based method and the GLOBINclear™ kit (Ambion, Austin, TX). The quality and quantity of RNA obtained from these samples were verified using a NanoDrop2000 spectrophotometer (Cole-Parmer, Vernon Hills, IL).

**Real-time reverse transcription PCR.** The methods used in this section have been previously described (12). *GAPD* was used to normalize the expression levels in the subsequent quantitative analyses. The primers used for real-time RT-PCR were purchased from Takara (Otsu, Japan) as follows: *OPRM1*

forward, 5'-TCA ATG TCT GCA ACT GGA TCC TC-3' and reverse, 5'-CAC TGG CAT AAT GAA GGC GAA G-3'; *OPRD1* forward, 5'-CTG GGC AAC GTG CTT GTC A-3' and reverse, 5'-CAT CAG GTA CTT GGC ACT CTG GAA-3'; *OPRK1* forward, 5'-CAC TTC ACG TGC TCT TAC AGC GTT A-3' and reverse, 5'-CCC TTG TGG GCA CAT ACA GCT AC-3'; *ARRB1* forward, 5'-GAG AAC GAG ACG CCA GTA GAT ACC A-3' and reverse, 5'-GGC GAG CAA AGT CCT CAA ATA CA-3'; *ARRB2* forward, 5'-ACC AAC CTG GCT TCC AGC A-3' and reverse, 5'-AAA GGC AGC TCC ACA GAG ACA TC-3'; *GRK5* forward, 5'-GGA GCT GAA CGT GTT TGG ACC TA-3' and reverse, 5'-AGC TGG GCG AAC TCT TGG AA-3'; *RGS9* forward, 5'-GCA CAA ACC CAC ATT TAC ATG CTC-3' and reverse, 5'-GCT TTG GCC AGC ATG TCC TTA-3'; *GAPD* forward, 5'-GCA CCG TCA AGG CTG AGA AC-3' and reverse, 5'-ATG GTG GTG AAG ACG CCA GT-3'.

**Microarray analysis.** The microarray procedure was performed according to the Affymetrix protocols (Santa Clara, CA), as described previously (13). Briefly, cRNA was synthesized using the GeneChip® 3'-Amplification Reagents One-Cycle cDNA Synthesis Kit (Affymetrix). The labeled cRNAs were then purified and used for the construction of the probes. Hybridization was performed using the Affymetrix Gene Chip HG-U133 Plus 2.0 array for 16 h at 45°C. The signal intensities were measured using a GeneChip® Scanner 3000 (Affymetrix) and converted to numerical data using GeneChip Operating Software, Ver. 1 (Affymetrix).

**Genotyping.** The genotype was evaluated for *OPRM1* 118A→G (rs1799971, p.Asn40Asp) and *COMT* 472G→A (rs4680, p.Val158Met). Genomic DNA isolated from blood samples using a QIAamp® DNA Blood Mini Kit (Qiagen) were amplified with the following primers: for *OPRM1* forward, 5'-AAG TCT CGG TGC TCC TGG CTA CC-3' and reverse, 5'-GTT TCC GAA GAG CCC CAC CAC GC-3'; and for *COMT* forward, 5'-GAT TCA GGA GCA CCA GCC CTC C-3' and reverse (intronic), 5'-CAC TGA GGG GCC TGG TGA TAG TG-3'. Each PCR reaction was performed in a 20-μl volume containing 20 ng of template, 0.5 μM of each primer, Ampdirect Plus (Shimadzu Corp., Kyoto, Japan) and 0.5 units of NovaTaq™ DNA Polymerase (Merck, Darmstadt, Germany). The amplification was performed for 35 cycles (95°C for 30 sec, 60°C for 30 sec and 72°C for 45 sec). The resulting PCR fragments consisting of 320 bp (*OPRM1*) and 210 bp (*COMT*) were directly sequenced with the corresponding forward and reverse primers, respectively.

**Measurement of plasma concentration of morphine.** The plasma concentration of morphine was measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Morphine was purchased from Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Imipramine, an internal standard (IS), was obtained from Sigma-Aldrich (St. Louis, MO, USA). Pretreatment of the plasma samples was performed using protein precipitation. Briefly, 100 μl of plasma was mixed with 250 μl of IS solution (1 ng/ml imipramine in methanol). After vortexing (30 sec) and centrifugation (13,000 rpm, 5 min), the supernatant was directly analyzed using an autosampler. An

Table I. Clinical characteristics of study patients.

Characteristics	No. of patients
Age (years)	
<65	14
≥65	34
Gender	
Male	25
Female	23
PS	
0-2	32
3-4	16
Tumor types	
Lung cancer	20
CRC	8
Gastric cancer	5
Others	15
Required dose of morphine	
Day 1	
20 mg	3
30 mg	36
60 mg	6
90 mg	2
NE	1
Day 8	
20 mg	5
30 mg	20
40 mg	1
60 mg	9
90 mg	1
120 mg	1
NE	11

PS, performance status; CRC, colorectal cancer; Others, other primary tumor types; NE, not evaluated.

The multiple reaction monitoring mode detected morphine and imipramine (IS) as follows: transitions, 286.4→152.3 and 281.2→86.0; cone voltages, 42 and 28 V; collision energies, 48 and 16 V, respectively. The chromatographic data were acquired and analyzed using MassLynx software, equipped with QuanLynx (Waters). Standard curves were prepared for a concentration range of 0.5-50 ng/ml for morphine. The inter- and intra-day variabilities in precision (expressed as the coefficient of variation) for morphine ranged from 4.2 to 7.7% and from 4.4 to 4.7%, respectively. The average accuracies for morphine were between 100.4 and 106.1%.

**Statistical analysis.** Differences between groups were analyzed using the Student's t-test or the Fisher's exact test. A P-value of <0.05 was considered statistically significant. All analyses were performed using JMP (SAS Institute, Cary, NC). A microarray analysis was performed using BRB-Array Tools software, Ver. 3.6.0 (<http://linus.nci.nih.gov/BRB-ArrayTools.html>), as described previously (13,14).

## Results

**Patient results.** A total of 48 patients with opioid-treatment naïve and histologically confirmed malignant neoplasms who were scheduled to undergo opioid treatment were evaluated in this study (Fig. 1A). All 48 patients and a total of 96 samples (baseline and Day 1) were evaluated in the gene expression analysis. Forty-one patients and samples were evaluated in the genotype analysis because the DNA samples were insufficient in 7 cases. The plasma concentration of morphine was determined for 47 samples on Day 1 and for 43 samples on Day 8.

The patient characteristics are summarized in Table I. The median age was 69 years (40-85 years); 25 patients were men and 23 patients were women. Sixty-seven percent of the patients had a PS of 0-2 and 42% had advanced lung cancer. The other primary tumors were 8 colorectal cancers, 5 gastric cancers, 4 unknown primary cancers, 2 pancreas cancers, 2 breast cancers, 2 gallbladder cancers, 1 renal cell carcinoma, 1 bladder cancer, 1 malignant lymphoma, 1 malignant pheochromocytoma and 1 skin cancer. The median required dose of morphine on Day 1 was 30 mg (20-90 mg), while that on Day 8 was 30 mg (20-120 mg).

LC-MS/MS device was equipped with an Acquity UPLC (Ultra Performance LC) system and a Xevo TQ MS (Waters, Milford, MA, USA). Chromatographic separations were obtained under gradient conditions using an ACQUITY UPLC BEH C18 Column (100 mm x 2.1 mm ID, 1.7- $\mu$ m particle size; Waters). The mobile phase consisted of eluent A (10 mmol/l ammonium formate) and eluent B (methanol). The flow rate was 0.5 ml/min and the gradient was from 2 to 60% B in 3.5 min, then an increase to 98% B in 0.5 min, holding at 98% B for 1 min and resetting to the initial conditions. The total run time was 8.5 min per sample. The column temperature was 45°C, the sample temperature was 10°C and the injection volume was 5  $\mu$ l. The retention times of morphine and imipramine (IS) were 1.69 and 4.31 min, respectively. The mass spectrometer was operated in a positive electrospray mode. The capillary voltage was 0.5 kV and the desolvation temperature was 500°C.

**Down-regulation of *ARRB1* mRNA expression and morphine treatment.** To identify pharmacodynamic biomarkers for monitoring the effect of morphine, we examined changes in gene expression during morphine treatment (baseline vs. Day 1) using a microarray analysis for 20 samples from 10 cases and validated the results using real-time RT-PCR for 76 samples from 38 cases, focusing on opioid receptor signaling. A schema for opioid receptor signaling is shown (Fig. 1B). The microarray analysis revealed that the mRNA expression levels of *ARRB1* and *GRK5* were significantly down-regulated by morphine treatment ( $P=0.01$  and  $0.001$ , Table II). Interestingly, down-regulated genes including *ARRB1*, *GRK5* and *RGS9* ( $P=0.054$ ) are known as negative regulators of opioid receptor signaling. The gene expressions of the opioid receptors were not changed. To confirm these results, we examined the gene expressions of these genes including *OPRM1*, *OPRD1*,

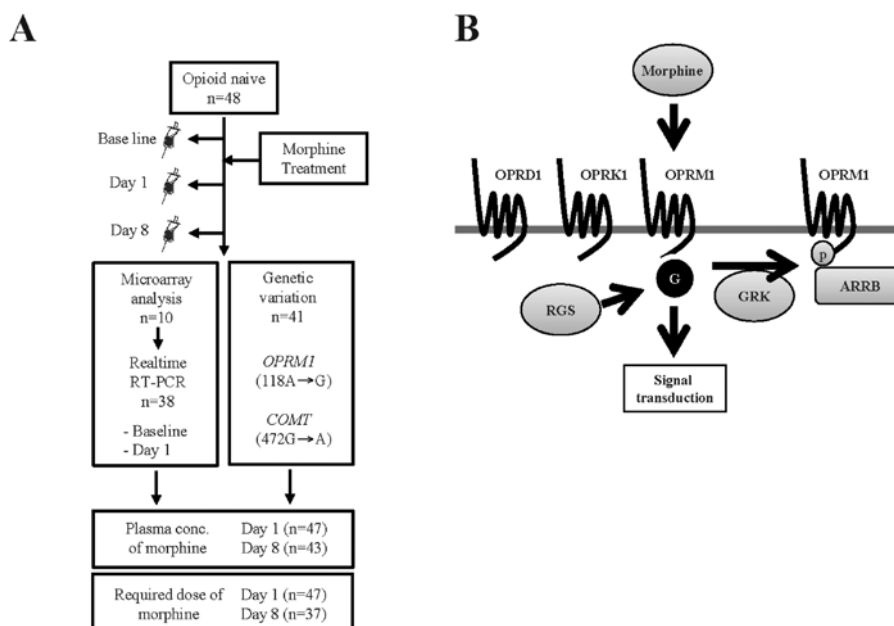


Figure 1. Schemas of study design and opioid receptor signaling. (A) Flow diagram of study. (B) Schema of opioid receptor signaling. OPRM1, D1 and K1 represent opioid receptor  $\mu$  1,  $\delta$  1 and  $\kappa$  1, respectively; ARRB1 and 2 represent arrestin  $\beta$  1 and 2; GRK, G protein-coupled receptor kinase; RGS, regulator of G-protein signaling; G, G protein; p, phosphorylation.

Table II. Gene expression changes in opioid signal-related molecules during morphine treatment.

Symbol	Name	Microarray (n=10)			Real-time RT-PCR (n=38)		
		Base line	Day 1	P-value <sup>a</sup>	Base line	Day 1	P-value <sup>a</sup>
<i>OPRM1</i>	Opioid receptor, $\mu$ 1	10.8 $\pm$ 1.6	10.9 $\pm$ 1.4	0.83	0.3 $\pm$ 0.9	1.4 $\pm$ 7.9	0.40
<i>OPRD1</i>	Opioid receptor, $\delta$ 1	11.3 $\pm$ 4.2	10.0 $\pm$ 0.0	0.37	0.2 $\pm$ 0.4	0.3 $\pm$ 1.4	0.46
<i>OPRK1</i>	Opioid receptor, $\kappa$ 1	13.7 $\pm$ 4.4	12.6 $\pm$ 3.5	0.31	0.2 $\pm$ 0.5	0.2 $\pm$ 0.3	0.68
<i>ARRB1</i>	Arrestin $\beta$ 1	123.7 $\pm$ 40.2	101.0 $\pm$ 30.9	0.01 <sup>b</sup>	48.6 $\pm$ 18.1	41.8 $\pm$ 17.0	0.003 <sup>b</sup>
<i>ARRB2</i>	Arrestin $\beta$ 2	1193.5 $\pm$ 476.5	1158.3 $\pm$ 317.6	0.77	416.6 $\pm$ 177.4	430.9 $\pm$ 164.3	0.56
<i>GRK1</i>	G protein-coupled receptor kinase 1	10.3 $\pm$ 1.3	11.3 $\pm$ 2.7	0.36	ND	ND	ND
<i>GRK4</i>	G protein-coupled receptor kinase 4	11.2 $\pm$ 3.6	10.9 $\pm$ 2.5	0.82	ND	ND	ND
<i>GRK5</i>	G protein-coupled receptor kinase 5	419.6 $\pm$ 121.1	346.7 $\pm$ 137.4	0.001 <sup>b</sup>	14.9 $\pm$ 6.9	13.8 $\pm$ 6.1	0.12
<i>GRK6</i>	G protein-coupled receptor kinase 6	464.4 $\pm$ 87.5	457.3 $\pm$ 120.8	0.87	ND	ND	ND
<i>RGS2</i>	Regulator of G-protein signaling 2	5776.5 $\pm$ 1845.2	5872.8 $\pm$ 1847.0	0.83	ND	ND	ND
<i>RGS9</i>	Regulator of G-protein signaling 9	26.7 $\pm$ 16.5	17.6 $\pm$ 7.9	0.05	2.8 $\pm$ 2.4	2.5 $\pm$ 1.7	0.17

Gene expression changes were examined using microarray and real-time RT-PCR. Peripheral blood leukocytes sampled during morphine treatment at baseline (pretreatment) and Day 1 (after treatment) were used for the analysis. <sup>a</sup>Comparisons between baseline vs. Day 1. The P-values were calculated using a t-test. <sup>b</sup>P<0.05. Data are shown as the average  $\pm$  standard deviation.

*OPRK1*, *ARRB1*, *ARRB2*, *GRK5* and *RGS9* using real-time RT-PCR in 38 independent cases. The mRNA expression level of *ARRB1* was significantly and reproducibly down-regulated by morphine treatment (P=0.003, Table II and Fig. 2A). This result strongly suggests that *ARRB1* may be a promising and pharmacodynamic biomarker of morphine.

Next, we evaluated whether the down-regulation of *ARRB1* was correlated with the plasma concentration of morphine or the required dose. A moderate and weak inverted correlation was observed between the down-regulation of *ARRB1* and

the plasma concentration of morphine (R=-0.42, Fig. 2B) or the required dose of morphine (R=-0.19, Fig. 2C). The results suggest that a higher plasma concentration or a higher dose of morphine induces the significant down-regulation of *ARRB1* and the change in *ARRB1* expression may be useful as a monitoring marker for morphine, although further studies are necessary.

*COMT* genotype is involved in outcome of morphine treatment. To find predictive biomarkers of the treatment outcome of morphine, we performed a functional genotype analysis

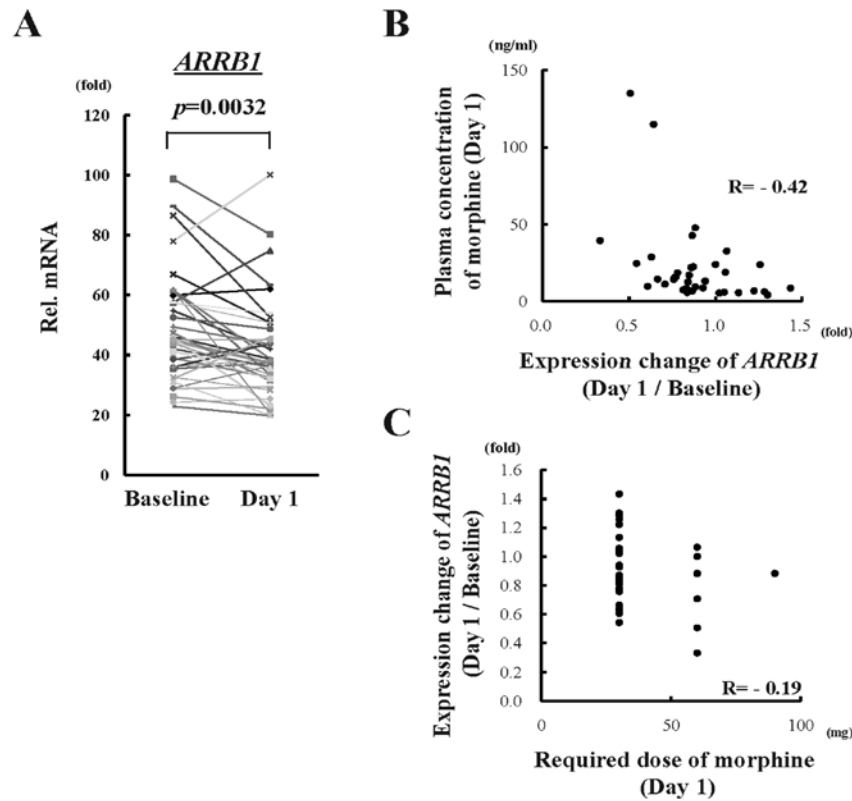


Figure 2. Down-regulation of arrestin  $\beta$  1 (*ARRB1*) and outcome of morphine treatment. (A) A real-time RT-PCR analysis of peripheral blood leukocytes obtained at baseline (pretreatment) and on Day 1 showed that the mRNA level of *ARRB1* was significantly down-regulated during morphine treatment. Rel mRNA, normalized mRNA expression levels (*ARRB1*/*GAPD* $\times 10^6$ ). (B) Expression changes in *ARRB1* and plasma concentration of morphine treatment on Day 1. R, correlation coefficient. (C) Expression changes of *ARRB1* and required dose of morphine on Day 1.

Table III. Genotypes and treatment outcome of morphine.

Symbol	Days	<i>OPRM1</i> 118A→G				<i>COMT</i> 472G→A			
		A/A	A/G	G/G	P-value <sup>a</sup>	G/G	G/A	A/A	P-value <sup>b</sup>
Plasma concentration of morphine (ng/ml)	D1	26.1 $\pm$ 36.1	21.0 $\pm$ 23.2	15.1 $\pm$ 18.3	0.45	34.1 $\pm$ 35.7	11.9 $\pm$ 6.2	8.7 $\pm$ 4.0	0.008 <sup>c</sup>
	D8	28.0 $\pm$ 20.0	29.6 $\pm$ 25.2	28.1 $\pm$ 22.2	0.94	33.0 $\pm$ 21.2	23.1 $\pm$ 23.2	36.7 $\pm$ 26.3	0.56
Required dose of morphine (mg)	D1	43.1 $\pm$ 23.9	32.2 $\pm$ 9.0	34.0 $\pm$ 15.2	0.78	43.7 $\pm$ 21.4	28.9 $\pm$ 3.2	30.0 $\pm$ 0.0	0.03 <sup>c</sup>
	D8	38.9 $\pm$ 16.2	37.1 $\pm$ 15.4	36.0 $\pm$ 15.2	0.82	40.0 $\pm$ 15.4	34.6 $\pm$ 15.1	40.0 $\pm$ 17.3	0.81

Genotypes were evaluated for *OPRM1* 118A→G (rs1799971, p.Asn40Asp) and *COMT* 472G→A (rs4680, p.Val158Met). The treatment outcome of morphine was examined using the plasma concentration of morphine (Days 1 and 8) and the required dose of morphine (Days 1 and 8).

<sup>a</sup>Comparisons between G/G vs. A/A+A/G of the *OPRM1*. <sup>b</sup>Comparisons between A/A vs. G/G+G/A of *COMT*. The P-values were calculated using a t-test. <sup>c</sup>P<0.05. Data are shown as the average  $\pm$  standard deviation.

of *OPRM1* 118A→G (rs1799971, p.Asn40Asp) and *COMT* 472G→A (rs4680, p.Val158Met). The treatment outcome of morphine was examined based on the plasma concentration of morphine (Days 1 and 8) and the required dose (Days 1 and 8) according to genotype. No correlation was observed between the *OPRM1* 118A→G genotype and the plasma concentration or the required dose of morphine (Table III). However, the plasma morphine concentration on Day 1 was significantly lower in patients with the A/A genotype of *COMT*, compared with those with the A/G+G/G genotypes (A/A: n=4, 8.7 $\pm$ 4.0 ng/ml; G/A:

n=18, 11.9 $\pm$ 6.2 ng/ml; G/G: n=19, 34.1 $\pm$ 35.7 ng/ml; P=0.008, Fig. 3A). In addition, the required dose of morphine on Day 1 was also significantly lower for the A/A genotype of *COMT*, compared with the A/G+G/G genotypes (A/A, 30.0 $\pm$ 0.0 mg; G/A, 28.9 $\pm$ 3.2 mg; G/G, 43.7 $\pm$ 21.4 mg; P=0.03, Fig. 3B). On the other hand, the genotype was not correlated with the treatment outcome of morphine on Day 8. Collectively, our results indicate that the *COMT* genotype is involved in the outcome of morphine treatment, suggesting that it may be useful as a predictive biomarker for morphine treatment.

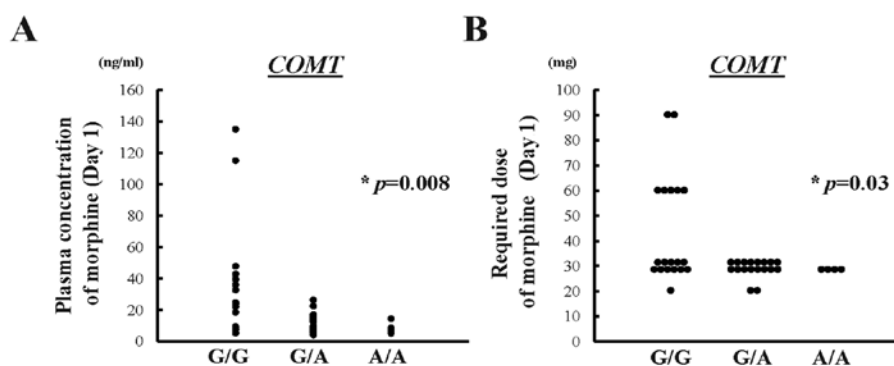


Figure 3. Catechol-O-methyltransferase genotypes (*COMT* 472G→A, rs4680, p.Val158Met) and outcome of morphine treatment. (A) Genotypes and plasma concentration of morphine treatment on Day 1. \*Comparisons between A/A vs. G/G+G/A of *COMT*. (B) Genotypes and required dose of morphine on Day 1.

## Discussion

Morphine activates opioid receptor signaling in the cells of the central nervous system (CNS). Unlike easily available tissues, such as blood cells, these CNS cells cannot be sampled and used for analysis; therefore, clinically useful pharmacodynamic biomarkers of morphine have remained largely unclear to date. We recently described an approach examining PBLs as surrogate tissues to evaluate drug response and found that it is a feasible, non-invasive and repeatable pharmacodynamic approach in clinical settings (15). In this study, we found that *ARRB1* mRNA expression is a reproducible and useful biomarker for monitoring the effects of morphine treatment using PBLs as surrogate tissues.

*ARRB1* regulates the desensitization of numerous GPCRs including OPRM1, D1 and D2 dopamine receptors and emerging evidence has demonstrated that *ARRB1* functions as a scaffold protein that links GPCRs to intracellular signaling, such as MAPK and as a transcription factor that translocates to the nucleus (16,17). A recent study showed that chronic morphine treatment blocked the agonist-induced redistribution of *ARRB1* in stably *OPRM1*-transfected HEK293 cells through the persistent stimulation of MAPK activity and the authors concluded that chronic morphine treatment produces adaptational changes at the *ARRB1* level (18). These observations and our findings suggest that the drug response of PBLs to morphine mediates the down-regulation of *ARRB1* expression during morphine treatment and reflects the overall cellular response to opioid signaling in an individual.

*COMT* is one of the enzymes that inactivate catecholamines; therefore, it is regarded as key regulator of adrenergic, noradrenergic and dopaminergic signaling (19). Various diseases are thought to be involved in *COMT* function including mental disorders, suicidal behavior and personality traits, and tardive dyskinesia (11,21-23). Regarding the *COMT* genotype as it relates to cancer pain, individuals with a A/A (Met/Met) genotype had a lower regional opioid signal response to pain and a higher sensitivity to pain, compared with heterozygous individuals (24). On the other hand, several clinical studies have demonstrated that the required dose of morphine was lower in subjects with an A/A genotype of *COMT*, compared with others (25-27). These results are consistent with our

result. The question why the required dose of morphine is lower in patients with an A/A genotype, even though they are more sensitive to pain, can be explained by a possibly elevated density of OPRM1 in patients with the A/A genotype (28). Our results indicate that *COMT* 472G→A may be a predictive biomarker, although further studies are necessary.

Taken together, our results may provide novel insights into the relations between morphine treatment and *ARRB1* expression and the *COMT* 472G→A genotype.

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