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Prospective replication study implicates the catechol-O-methyltransferase Val^{158}Met polymorphism as a biomarker for the response to morphine in patients with cancer

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Abstract. Genetic differences in humans cause clinical difficulties in opioid treatment. Previous studies indicate that a single nucleotide polymorphism in the catechol-O-methyltransferase (*COMT*) gene (rs4680; p.Val^{158}Met) may present as a predictive biomarker for the response to morphine treatment. In our previous pilot exploratory study, patients with a G/G genotype were demonstrated to require a higher dose of morphine, compared with patients with A/A and A/G genotypes. In the present study, the aim was to replicate the findings in an independent cohort of opioid-treatment-naïve patients exhibiting various types of cancer. This prospective study was conducted from 2011 to 2012 at the Kindai University Faculty of Medicine. A total of 50 patients with opioid-treatment naïve and histologically confirmed malignant neoplasms who were scheduled to undergo opioid treatment were evaluated in the present study. Assessments were conducted pre-treatment (day 1), post-treatment (day 1), and one week after treatment (day 8). The required dose of morphine on day 1 was significantly higher for patients with the G/G genotype of COMT, compared with those with the A/A and A/G genotypes (P=0.013). The results of the present study provide additional evidence that the COMT genotype may be a predictive biomarker for the response to morphine treatment.

Introduction

Pain is a feared and common complication in patients with cancer (1,2), which increases in prevalence and intensity with disease progression (3,4), and influences multiple aspects of quality of life (5-8). Pharmacogenetic, pharmacokinetic and pharmacodynamic variations among individuals result in a wide variety of responses to pain sensation and analgesics; therefore, investigations of biomarkers for opioid treatment have been performed to improve the efficacy of morphine treatment (9). According to a review published in 2015 (10), numerous studies have proposed that the catechol-O-methyltransferase (*COMT*) 472G→A (rs4680, p.Val^{158}Met) genotype may be a predictive biomarker for the response to morphine treatment. In these studies, patients with the G/G genotype received the highest dose of morphine (11,12), while those with the A/A genotype received the lowest morphine dose (13). However, the majority of these studies had a small sample size, were retrospective, or were targeting non-cancer pain (14). A large sample study has indicated that none of the 112 single nucleotide polymorphisms (SNPs) in 25 candidate genes demonstrated significant associations with opioid dose (15); however, the quantity of concomitant analgesics [non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen] and the method for dose determination of opioids remain unclear. Furthermore, the mean Brief Pain Index (16) following treatment was relatively high (15).

An exploratory study was planned, which included 100 patients with opioid-treatment-naïve cancer, in whom the use of NSAIDs and acetaminophen, as well as the method of dose determination of morphine were kept constant. The investigation was divided into a pilot study of 50 cases, and a replication study (the current study) of 50 more cases. Expression levels of functional genetic variants as predictive biomarkers of the response to morphine were examined. The pilot study indicated that *COMT* 472G→A may be a predictive biomarker (17). The aim of the current prospective study was to replicate these findings in an independent cohort of 50 opioid-treatment-naïve patients exhibiting various types of cancer.

Materials and methods

Patients and samples. This prospective study was conducted from April 2011 to March 2012 at the Kindai University Faculty of Medicine (Osaka, Japan). A total of 50 patients with opioid-treatment naïve and histologically confirmed malignant neoplasms who were scheduled to undergo opioid treatment

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were evaluated in the study. These patients were recruited and selected by non-probabilistic convenience sampling by their physician from the outpatients and inpatients service of the Department of Medical Oncology (Kindai University Faculty of Medicine). All patients met the following inclusion criteria: Aged ≥18 years, a verified cancer diagnosis, cancer pain suitable for morphine treatment (excluding patients with neuropathic pain, predominant incidental pain, glomerular filtration rate <30 ml/min, and history of opioid/drug abuse or alcoholism from a self-reported questionnaire), daily use of NSAIDs and able to provide written informed consent. The required dose of morphine (day 1) was evaluated in 50 patients, and was assessed in 48 patients, (2 were excluded who could not continuously receive morphine due to adverse effects or mortality; Fig. 1).

Clinical features, including age, sex, Eastern Cooperative Oncology Group performance status (PS) (18), and types of primary malignant neoplasm were recorded. Morphine was administered using the standard method, including titration [NCCN Guidelines (19), Adult Cancer Pain], performed by specialized palliative care doctors. Immediate release (IR) morphine (5 mg) was administered orally, and efficacy and adverse effects were reassessed at 60 min. When the pain score was not decreased, the same dose of IR morphine (5 mg) was re-administered. Dose titration was performed to decrease pain by ≥33% on the numerical rating scale (NRS) (20) pain scale at day 1 (pre-treatment), as well as to reduce the NRS to a score of ≤3.

Criteria for discontinuation of dose titration were appearance of adverse effects graded ≥3 (Common Terminology Criteria for Adverse Events, v4.0) (21), or an attending physician determining titration to be difficult to continue, even if the adverse effect was graded ≤2. Controlled release (CR) morphine, six times the dose of IR morphine, was administered orally once per day. If pain was insufficiently controlled by 10 mg IR morphine in total, 60 mg CR morphine was administered at night on day 1. The attending physician could reduce the dose of CR morphine based on the general condition of the patient. Morphine administration was discontinued following a side effect of grade ≥3. Standardized information on potential benefits and adverse effects was provided to the patients. The study was approved by the Regional Committee for Medical Research Ethics, Kindai University Faculty of Medicine. Informed consent was obtained from all participants in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Pain score. The pain score was assessed using a 0-10 NRS (0, no pain to 10, worst imaginable pain) on day 1 (pre-treatment), day 1 (post-treatment) and day 8 (one week after treatment) by asking patients the following question: "How intense was your average pain for the past 24 h?"

Genotyping. SNPs of COMT 472G→A (rs4680; p.Val[158]Met) modulate the genetic response to opioid medications (22). In an exploratory study of 50 cases, a functional genotype analysis of opioid receptor μ 1 118A→G (rs1799971; p.Asn40Asp) and COMT 472G→A (rs4680; p.Val[158]Met) was performed to establish the predictive biomarkers for the treatment outcome of morphine, based on the plasma level of morphine and the required dose according to genotype on days 1, 2 and 8. On day 1, blood was sampled for genotyping, and the pain score was recorded on days 1 (before and after treatment), 2 and 8. The required dose of morphine on day 1 was significantly higher in patients with the *COMT* G/G genotype, compared with those with A/A and A/G genotypes in the previous pilot study (P=0.03) (16). Therefore, in the current prospective study of 50 independent cases, *COMT* alone was the main focus.

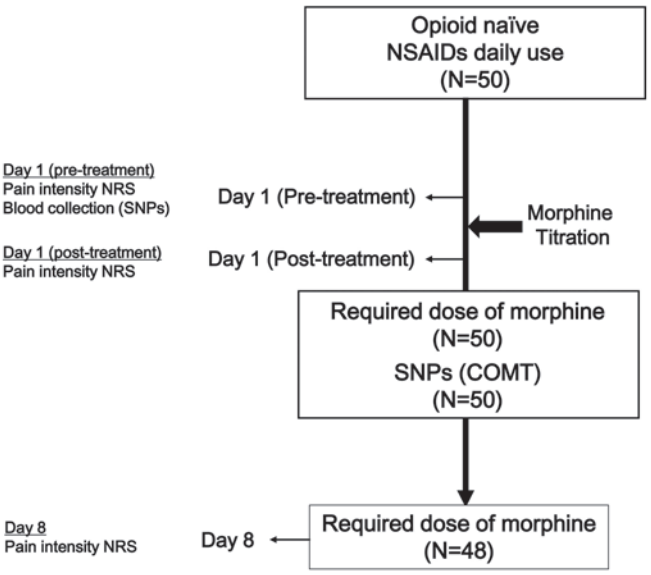

Figure 1. Schema of the study design. NSAIDs, non-steroidal anti-inflammatory drugs; NRS, numerical rating scale; SNPs, single nucleotide polymorphisms; COMT, catechol-O-methyltransferase.

Genomic DNA was isolated from blood samples using a QIAamp(r) DNA Mini kit (cat. no. 51304; Qiagen GmbH, Hilden, Germany) and was subjected to a genotype analysis using Taqman® SNP Genotyping Assay for COMT rs4680 (cat. no.4362691; Thermo Fisher Scientific Inc., Waltham, MA, USA) and a StepOnePlus™ Real-Time PCR Systems (cat no. 376600; Thermo Fisher Scientific Inc.).

Statistical analysis. All data are expressed as means ± standard deviation. Differences in the effects of morphine between gene polymorphism groups were evaluated using a Mann-Whitney U test. Two-sided P<0.05 was considered to indicate a statistically significant difference. All analyses were performed using SPSS software (v19.0; IBM SPSS, Armonk, NY, USA).

Results

Patient characteristics. The characteristics of the 50 patients (25 men and 25 women) are presented in Table I. The median age was 63 years (age, 36-80 years; 62±9.17). The primary tumors were as follows: Lung cancer, n=19 (38%); breast cancer, n=9 (18%); colorectal cancer, n=5 (10%); head and neck cancer, n=4 (8%); gastric cancer, n=3 (6%); unknown primary cause, n=3 (6%); pancreatic cancer, n=2 (4%); gallbladder cancer, n=2 (4%); and others, n=3 (6%). More than 80% of the subjects exhibited metastasis and a PS of 0 to 2. The median required doses of morphine were 31.2 mg (20-60 mg) on day 1 and

Table I. Clinical characteristics of the patients (n=50).

Characteristics	Patients (n)
Age, years	
<65	27
≥65	23
Sex	
Male	25
Female	25
Performance status	
0-2	40
3-4	10
Genotype: *COMT* 472G→A (rs4680; p.Val^{158}Met)	
G/G	29
A/G	19
A/A	2
Tumor type	
Lung	19
Breast	9
Colorectal	5
Head and neck	4
Gastric	3
Unknown primary	3
Gallbladder	2
Pancreas	2
Others	3
Required dose of morphine on day 1 (mg)	
20	2
30	43
60	5
Required dose of morphine on day 8 (mg)	
As required	8
20	3
30	30
60	6
90	1
N.E.	2
Day 1 (pre-treatment) pain NRS, mean (SD)	
All patients	6.88 (2.43)
G/G	7.07 (2.25)
Non-G/G (A/A and A/G)	6.62 (2.69)
Day 1 (post-treatment) pain NRS, mean (SD)	
All patients	2.46 (1.46)
G/G	2.38 (1.45)
Non-G/G (A/A and A/G)	2.57 (1.50)
Day 8 pain NRS, mean (SD)	
All patients	3.60 (2.73)
G/G	3.17 (2.63)
Non-G/G (A/A and A/G)	4.19 (2.80)

COMT, catechol-O-methyltransferase; N.E, not evaluated; NRS, numerical rating scale; SD, standard deviation.

Table II. Characteristics of patients with high-dose morphine-requiring cases.

Patient	Age (y)	Sex	PS	COMT genotype	Tumor type	Pain NRS (before administration)
1	63	M	1	G/G	Lung	8
2	59	M	1	G/G	Lung	7
3	70	M	2	G/G	Lung	10
4	64	F	1	G/G	Unknown primary	8
5	60	F	2	G/G	Lung	8

PS, performance status; COMT, catechol-O-methyltransferase; NRS, numerical rating scale.

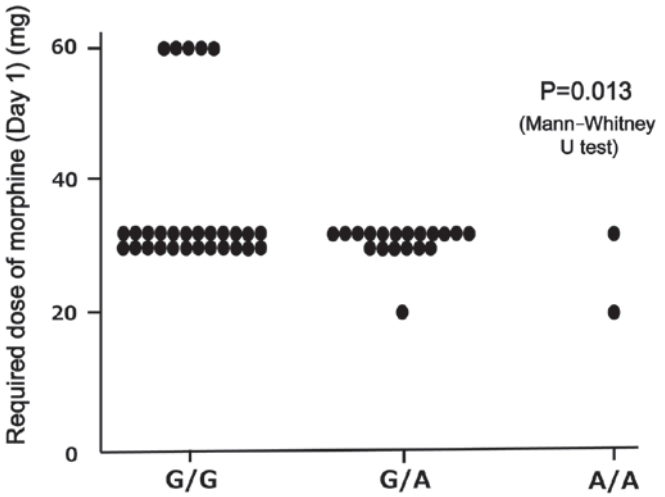

Figure 2. Genotypes were evaluated for COMT 472G→A (rs4680; p.Val^{158}Met). The treatment outcome of morphine was examined based on the required dose (day 1). Comparisons are for G/G vs. A/A and A/G of COMT. The P-value was calculated using a Mann-Whitney U test. COMT, catechol-O-methyltransferase.

32.6 mg (20-60 mg) on day 8. The pain NRS before and after treatment did not differ between the G/G and non-G/G groups (P=0.177). A dose of 60 mg morphine was required in 4 cases of lung cancer and 1 case of unknown primary cancer. The causes of pain were lymph node metastases and bone metastases in 4 cases each, and pleural seeding in 1 case. Features of 5 high-dose morphine-requiring cases, defined as requiring 2 or more cycles of 5 mg IR morphine, are presented in Table II.

Pain titration. Morphine at the required dose was administered to patients as a controlled-release agent. On day 1, dose titration was performed to reach NRS ≤3 and pain control ≥33%. Titration was successful in 76% of all cases, but unsuccessful in 60% of G/G genotype cases.

Genotypes of COMT and treatment outcomes of morphine. A functional genotype analysis of *COMT* 472G→A (rs4680; p.Val^{158}Met) was performed to establish the predictive biomarkers for the outcome of morphine treatment (Fig. 2). The

outcome based on the required morphine dose on days 1 and 8 was examined according to the genotype. The required dose of morphine (mg) on day 1 in GG, AG, and AA were 35.2±11.5, 29.5±2.3, and 25.0±7.1, respectively, and was significantly higher for patients with the *COMT* G/G genotype, compared with those with non-G/G (A/A and A/G) genotypes (P=0.013).

Discussion

To the best of our knowledge, the present study is the first to demonstrate the reproducibility of differences between required doses of morphine among individuals, due to genetic polymorphisms, in an independent cohort of opioid-treatment-naïve patients exhibiting various types of cancer. The most important finding is that patients with the *COMT* G/G genotype require a higher dose of morphine (day 1), compared with patients with A/A and A/G genotypes.

The morphine effect varies due to genetic differences; therefore, genetic polymorphisms are considered to be predictive markers. Various clinical studies have demonstrated that the required dose of morphine is higher in subjects with a *COMT* G/G genotype, compared with other genotypes, in cancer and non-cancer pain (11-14). The current findings are consistent with these results. A large sample study has indicated that none of 112 SNPs in the 25 candidate genes showed significant associations with opioid dose (15); however, this study lacked clarity regarding the doses of concomitant analgesics, the dose determination of opioids, and the high average total dose of morphine of 90 mg/day. Despite this dose, residual pain was relatively high (pain NRS in the past 24 h, 3.20±2.10 subsequent to analgesic use and no data for pain NRS prior to analgesic use) (15). This suggests a high proportion of refractory pain. By contrast, in the current study, the dose of concomitant analgesics was controlled (all patients received daily regular doses of NSAIDs and acetaminophen), the method of dose titration was consistent, and pain was relatively well-controlled (pain NRS following treatment, 2.46±1.46). Patients who report a higher NRS for baseline pain generally exhibit a larger change in raw pain intensity (23). The patients in the present study had relatively strong pain (pain NRS in the past 24 h, 6.88±2.43) that was relatively well-controlled, and this may have made it easier to identify a difference due to genetic polymorphism.

Notably, the required dose of morphine on day 1 was significantly higher in patients with the *COMT* G/G genotype. The enzyme activity of COMT is defined as high in G/G, intermediate in G/A and low in A/A. Impairment of the COMT enzyme suppresses the production of enkephalin, which causes subsequent opioid receptor expression upregulation (10). In the A/A genotype, the function of the μ receptor is weak and patients tend to be sensitive to pain. However, the density of μ receptors is proposed to be increased in this genotype, which may be the reason for the low quantity of morphine required (24,25).

The nature of the pain NRS questionnaire may also be relevant. As patients with the *COMT* A/A genotype are more sensitive to pain, the reported NRS may be higher than that for the quantity of morphine required (26). A meta-analysis by Lee *et al* (27) revealed an association between fibromyalgia and the *COMT* A/A genotype.

The present study has various limitations. First, it is not possible to rule out that differences in patients' background between the *COMT* G/G genotype and *COMT* non-G/G genotype groups may be responsible for the higher required dose of morphine in the G/G group. Although there was no significant difference in age between the G/G genotype and non-G/G genotype groups (P=0.658), the PS tended to be poor in the G/G genotype group (P=0.053). However, there were no patients with poor PS (3 and 4) in the high-dose morphine group. The *COMT* G/G genotype group has a marginally higher NRS score (prior to treatment) than the non-G/G genotype group. Although there was no significant difference in the NRS score before treatment between these groups (P=0.177), NRS scores tended to be higher in high-dose morphine-requiring cases compared with other cases. Thus, patient backgrounds require evaluation in future studies. In addition, the patients were included in this study based on the decision of attending physicians in a single hospital; consequently, the results may not be generalizable to other centers. Furthermore, disease duration, comorbidity, use of drugs other than opioids, NSAIDs, acetaminophen, and psychosocial factors, including education level and occupation, were not examined. Finally, the sample size is not considered to be large enough for a genetic study, which increases the rate of false positives. Therefore, the current results should be treated with caution until the present study is repeated using a larger population sample.

In conclusion, within these limitations, these results indicate that the *COMT* genotype influences the outcome of morphine treatment; therefore, it may be useful as a predictive biomarker for morphine treatment. The frequency of the GG allele has been found to be around 20% in previous studies (11,12,25), but close to 50% in a study targeting Japanese patients (17). This demonstrates ethnic differences in allele frequencies. This observation supports that it is clinically meaningful not to choose morphine as the first opioid in G/G patients with cancer pain, as higher doses may be required. However, these findings are from a prospective observational study, and an interventional study is required for further evaluation.

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References

1. Cleeland CS: Undertreatment of cancer pain in elderly patients. JAMA 279: 1914-1915, 1998.
2. Patrick DL, Ferketich SL, Frame PS, Harris JJ, Hendricks CB, Levin B, Link MP, Lustig C, McLaughlin J, Reid LD, *et al*; National Institutes of Health State-of-the-Science Panel: National Institutes of Health State-of-the-Science Conference Statement: Symptom management in cancer: pain, depression, and fatigue, July 15-17, 2002. J Natl Cancer Inst Monogr 32: 9-16, 2004.
3. Potter J and Higginson IJ: Pain experienced by lung cancer patients: A review of prevalence, causes and pathophysiology. Lung Cancer 43: 247-257, 2004.

4. Butler LD, Koopman C, Cordova MJ, Garlan RW, DiMiceli S and Spiegel D: Psychological distress and pain significantly increase before death in metastatic breast cancer patients. Psychosom Med 65: 416-426, 2003.
5. Bischoff K, Weinberg V and Rabow MW: Palliative and oncologic co-management: Symptom management for outpatients with cancer. Support Care Cancer 21: 3031-3037, 2013.
6. Chase DM, Huang HQ, Wenzel L, Cella D, McQuellon R, Long HJ, Moore DH and Monk BJ: Quality of life and survival in advanced cervical cancer: A Gynecologic Oncology Group study. Gynecol Oncol 125: 315-319, 2012.
7. Kelsen DP, Portenoy RK, Thaler HT, Niedzwiecki D, Passik SD, Tao Y, Banks W, Brennan MF and Foley KM: Pain and depression in patients with newly diagnosed pancreas cancer. J Clin Oncol 13: 748-755, 1995.
8. Turk DC, Sist TC, Okifuji A, Miner MF, Florio G, Harrison P, Massey J, Lema ML and Zevon MA: Adaptation to metastatic cancer pain, regional/local cancer pain and non-cancer pain: Role of psychological and behavioral factors. Pain 74: 247-256, 1998.
9. Lötsch J, Geisslinger G and Tegeder I: Genetic modulation of the pharmacological treatment of pain. Pharmacol Ther 124: 168-184, 2009.
10. Bell GC, Donovan KA and McLeod HL: Clinical implications of opioid pharmacogenomics in patients with cancer. Cancer Control 22: 426-432, 2015.
11. Rakvåg TT, Klepstad P, Baar C, Kvam TM, Dale O, Kaasa S, Krokan HE and Skorpen F: The Val^{158}Met polymorphism of the human catechol-O-methyltransferase (*COMT*) gene may influence morphine requirements in cancer pain patients. Pain 116: 73-78, 2005.
12. Rakvåg TT, Ross JR, Sato H, Skorpen F, Kaasa S and Klepstad P: Genetic variation in the catechol-O-methyltransferase (*COMT*) gene and morphine requirements in cancer patients with pain. Mol Pain 4: 64, 2008.
13. Reyes-Gibby CC, Shete S, Rakvåg T, Bhat SV, Skorpen F, Bruera E, Kaasa S and Klepstad P: Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and *COMT* gene. Pain 130: 25-30, 2007.
14. Candiotti KA, Yang Z, Buric D, Arheart K, Zhang Y, Rodriguez Y, Gitlin MC, Carvalho E, Jaraba I and Wang L: Catechol-o-methyltransferase polymorphisms predict opioid consumption in postoperative pain. Anesth Analg 119: 1194-1200, 2014.
15. Klepstad P, Fladvad T, Skorpen F, Bjordal K, Caraceni A, Dale O, Davies A, Kloke M, Lundström S, Maltoni M, *et al*: European Association for Palliative Care Research Network: Influence from genetic variability on opioid use for cancer pain: A European genetic association study of 2294 cancer pain patients. Pain 152: 1139-1145, 2011.
16. Atkinson TM, Mendoza TR, Sit L, Passik S, Scher HI, Cleeland C and Basch E: The Brief Pain Inventory and its 'pain at its worst in the last 24 hours' item: Clinical trial endpoint considerations. Pain Med 11: 337-346, 2010.
17. Matsuoka H, Arao T, Makimura C, Takeda M, Kiyota H, Tsurutani J, Fujita Y, Matsumoto K, Kimura H, Otsuka M, *et al*: Expression changes in arrestin β 1 and genetic variation in catechol-O-methyltransferase are biomarkers for the response to morphine treatment in cancer patients. Oncol Rep 27: 1393-1399, 2012.
18. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET and Carbone PP: Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5: 649-655, 1982.
19. Swarm R, Abernethy AP, Anghelescu DL, Benedetti C, Blinderman CD, Boston B, Cleeland C, Coyle N, Deleon-Casasola OA, Eilers JG, *et al*; NCCN Adult Cancer Pain: Adult cancer pain. J Natl Compr Canc Netw 8: 1046-1086, 2010.
20. Caraceni A, Cherny N, Fainsinger R, Kaasa S, Poulain P, Radbruch L and De Conno F: Pain measurement tools and methods in clinical research in palliative care: Recommendations of an expert working group of the European Association of Palliative Care. J Pain Symptom Manage 23: 239-255, 2002.
21. National Cancer Institute: Common Terminology Criteria for Adverse Events (CTCAE) v4.03. https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Accessed June 14, 2010.
22. Montagna P: Recent advances in the pharmacogenomics of pain and headache. Neurol Sci 28 (Suppl 2): S208-S212, 2007.
23. Farrar JT, Polomano RC, Berlin JA and Strom BL: A comparison of change in the 0-10 numeric rating scale to a pain relief scale and global medication performance scale in a short-term clinical trial of breakthrough pain intensity. Anesthesiology 112: 1464-1472, 2010.
24. Tunbridge EM, Harrison PJ and Weinberger DR: Catechol-o-methyltransferase, cognition, and psychosis: Val^{158}Met and beyond. Biol Psychiatry 60: 141-151, 2006.
25. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS and Goldman D: *COMT* val^{158}Met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science 299: 1240-1243, 2003.
26. Arnold BS, Alpers GW, Süss H, Friedel E, Kosmützky G, Geier A and Pauli P: Affective pain modulation in fibromyalgia, somatoform pain disorder, back pain, and healthy controls. Eur J Pain 12: 329-338, 2008.
27. Lee YH, Kim JH and Song GG: Association between the *COMT* Val^{158}Met polymorphism and fibromyalgia susceptibility and fibromyalgia impact questionnaire score: A meta-analysis. Rheumatol Int 35: 159-166, 2015.