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# Impact of CYP2D6 Activity and Cachexia Progression on Enantiomeric Alteration of Plasma Tramadol and Its Demethylated Metabolites and Their Relationships with Central Nervous System Symptoms in Head and Neck Cancer Patients



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Original Article

# Impact of CYP2D6 Activity and Cachexia Progression on Enantiomeric Alteration of Plasma Tramadol and Its Demethylated Metabolites and Their Relationships with Central Nervous System Symptoms in Head and Neck Cancer Patients

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**Abstract**

This study aimed to evaluate the influence of CYP2D6 activity and cachexia progression on the enantiomeric alteration of plasma tramadol and its demethylated metabolites in head and neck cancer patients. Fifty-three head and neck cancer patients receiving oral tramadol were enrolled. The plasma concentrations of tramadol, *O*-desmethyltramadol (ODT), and *N*-desmethyltramadol (NDT) enantiomers were determined. The CYP2D6 activity score (AS) and degree of cachexia progression were assessed according to genotype and the Glasgow Prognostic Score (GPS), respectively. The enantiomeric ratio of NDT was (+)-form dominant in all patients. CYP2D6 AS had negative correlations with the plasma concentrations of (+)-NDT and (−)-NDT. The plasma concentrations of (+)-tramadol and (+)-ODT were higher in patients with GPS 1 or 2 than in those with GPS 0. Lower metabolic ratios to NDT enantiomers were observed in patients with GPS 1 or 2. In patients with GPS 1 or 2, the plasma (−)-tramadol was associated with the incidence of central nervous system symptoms. In conclusion, CYP2D6 AS partially explained the contribution of CYP2D6 activity to plasma tramadol and its demethylated metabolite enantiomers. Additionally, cachexia progression elevated the plasma (+)-tramadol and (+)-ODT levels through the reduction of *N*-demethylation of (+)-tramadol.

## INTRODUCTION AND BACKGROUND

Tramadol, a centrally acting analgesic, dually acts as a weak agonist of the mu opioid receptor (MOR) and as an inhibitor of serotonin and noradrenaline reuptakes.[1] Tramadol is commonly used for the treatment of mild to moderate cancer pain prior to strong opioid medication.[2] In head and neck cancer, chemoradiation-related oral mucositis with cancer progression complicate the pain. Pain management using the weak analgesic tramadol is required from the early stage of head and neck oncological treatments.[3] Additionally, advanced cancers tend to possess progressive cachexia, a complex syndrome related to systemic inflammation and malnutrition.[4] The inflammation and malnutrition state are potentially responsible for the large variation in pain relief using tramadol in head and neck cancer patients.

Racemic tramadol is converted to active *O*-desmethyltramadol (ODT) by cytochrome P450 (CYP) 2D6 or inactive *N*-desmethyltramadol (NDT) by CYP3A4.[5] The agonist activity for the MOR is primarily mediated by (+)-ODT, while (+)- and (−)-tramadol are mainly characterized as serotonin and noradrenaline reuptake inhibitors, respectively.[6] Advanced cancer patients with systemic inflammation showed a large variation in plasma exposures of ODT as well as tramadol.[7,8] In an *in vitro* study, CYP2D6 was found to play an important role in the stereoselective metabolisms of tramadol and its demethylated metabolites.[9] Few reports have been published on the contribution of individual CYP2D6 activity to the enantiomeric pharmacokinetics of tramadol and its demethylated metabolites in cancer patients.

The CYP2D6 gene has a large number of variants relating to its activity.[10] Although

CYP2D6 activity shows individual differences,[11] endogenous markers, which allow for a simple measure of CYP2D6 activity without exogenous probes, have not been found. CYP2D6 activity score (AS) systems are used for the quantification of individual CYP2D6 activity.[12,13] The system enables translation of genetic information into a qualitative measure of phenotype, which is employed as a surrogate measure for CYP2D6 activity. However, the utility of the AS system for prediction of the enantiomeric pharmacokinetics of tramadol and its demethylated metabolites has not been fully evaluated in cancer patients.

In advanced cancer patients with cachexia, the reductions of CYP enzymes are observed in mainly CYP3A4, as compared with CYP2D6.[14] The increases of proinflammatory cytokines such as interleukin-6 (IL-6) in the bloodstream are potentially associated with the suppression of CYP3A4-mediated *N*-demethylation of racemic tramadol.[8] The contribution of CYP2D6-mediated *O*-demethylation to tramadol metabolism is compensatorily increased in cachectic cancer patients.[8] To date, the impact of cachexia progression on the enantiomeric pharmacokinetics of tramadol and its demethylated metabolites remains to be clarified in cancer patients. Additionally, cachectic cancer patients tend to have analgesic-derived and pathophysiological condition-derived central nervous system (CNS) symptoms.[14,15]

This study aimed to evaluate the influence of CYP2D6 activity and cachexia progression on the enantiomeric pharmacokinetics of tramadol and its demethylated metabolites and their relationships with adverse events in head and neck cancer patients.

## MATERIALS AND METHODS

### Ethics

The study protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine (18-047), and was conducted in concordance with the Declaration of Helsinki and its amendments and the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. All recruited patients were fully informed of the purpose and risks of this study, and gave written informed consent before being enrolled. This study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[16]

### Patients and study schedule

This study was a single center study at Hamamatsu University Hospital (Hamamatsu, Japan). The cohort included 53 adult Japanese head and neck cancer patients treated with oral immediate release tramadol four times daily for cancer-related and treatment-related pain relief. The exclusion criteria were as follows: patients who (1) did not receive oral tramadol for more than seven days including the study period; (2) were not receiving a constant dose of oral tramadol; (3) were concomitantly receiving another MOR agonist or rescue dosing of tramadol during the observational period; (4) were being co-treated with potent CYP2D6 and CYP3A4 modifiers, such as rifampicin, paroxetine, quinidine, carbamazepine, clarithromycin, or itraconazole;[17] (5) were being co-treated with anticancer drugs or within one week; (6) had

active autoimmune disease or severe infectious disease; (7) had hepatic dysfunction (serum total bilirubin > 2.0 mg/dL), renal dysfunction (serum creatinine > 2.0 mg/dL), cerebrovascular disease, or a diagnosis of metastatic brain cancer; (8) were concomitantly receiving long-acting benzodiazepines; and (9) had poor medication adherence. Five mL of blood samples were collected just before the morning dosing on the 4th day or later after starting tramadol treatment or dose adjustment from the patients receiving a fixed dose of tramadol for at least four days. Ethylenediaminetetraacetic acid disodium salt-treated plasma was obtained using a Venoject II vacuum blood collection tube (Terumo Corporation, Tokyo). This study was registered in the University Hospital Medical Information Network (UMIN000039308).

**Determination of plasma tramadol and its metabolite enantiomers**

Plasma samples were pretreated as described previously.[9] Briefly, a mixture of 100 μL of plasma sample, 600 μL of acetonitrile, 100 μL of acetonitrile solution containing internal standards, and 20 μL of 28% ammonia solution was added into a low adhesion microtube. After mixing and centrifugation, the supernatant was evaporated to dryness. The residue was reconstituted and used for the following analyses. The enantiomeric plasma concentrations of tramadol and its demethylated metabolites were simultaneously measured by liquid chromatography-tandem mass spectrometry.[9] The calibration curves of tramadol, ODT, and NDT were linear over the plasma concentration ranges of 6.25–800, 1.25–160, and 3.13–400

ng/mL, for the respective enantiomers. The intra- and inter-day accuracy of all analytes had ranges of 94.2–108.3%, while the intra- and inter-day imprecisions were 0.5–6.0%. The lower limits of quantification of (+)- and (−)-tramadol, (+)- and (−)-ODT, and (+)- and (−)-NDT in human plasma were 6.25, 1.25, and 3.13 ng/mL, respectively.

**Estimation of plasma enantiomeric pharmacokinetics**

Plasma concentrations adjusted by tramadol dose and body-weight (hereinafter referred to as just plasma concentration unless otherwise stated) were used for evaluating the plasma exposure of tramadol and its demethylated metabolite enantiomers. Tramadol metabolism, which proceeds via *O*-demethylation by CYP2D6 and *N*-demethylation by CYP3A4, was assessed as the plasma concentration ratios of the demethylated metabolites to tramadol. The plasma concentration ratios of ODT and NDT to tramadol were defined as metabolic ratios to ODT and NDT, respectively. The enantiomeric ratios of (+)- to (−)-form were used for evaluating the plasma enantiomeric pharmacokinetics of tramadol and its demethylated metabolites.

**CYP2D6 activity score**

Leukocyte genomic DNA was extracted from the whole blood of each patient. The single nucleotide polymorphisms, including rs16947 (*CYP2D6*2*, *2850C>T*), rs1065852 (*CYP2D6*10* and **14A*, *100C>T*), and rs5030865 (*CYP2D6*14A* and **14B*, *1758G>A*) were

determined using a TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA, USA). The *CYP2D6*5* deletion was detected by long polymerase chain reaction assay.[18] Estimation of individual CYP2D6 activity employed the AS system based on each CYP2D6 allele.[19–21] Each allele, *CYP2D6*1, *2, *5, *10, *14A,* and **14B,* was assigned to a value of 1, 1, 0, 0.25, 0, and 0.5, respectively. The CYP2D6 activity of each patient was represented as the sum of the values assigned to both alleles.

**Evaluation of cancer cachexia progression**

The degree of cachexia progression was evaluated using the Glasgow Prognostic Score (GPS).[22] The GPS was determined using serum albumin and C-reactive protein (CRP). Patients with both hypoalbuminemia (< 35 g/L) and an increased serum CRP level (> 1.0 mg/dL) were assigned to GPS 2. Patients with only one or no abnormal values were assigned to GPS 1 or 0, respectively. Serum albumin and CRP were measured by an improved bromocresol purple method (Qualigent ALB, Sekisui Medical Co., Ltd., Tokyo) and latex agglutination immunoassay (Nanopia CRP, Sekisui Medical Co., Ltd.), respectively.

**Evaluation of adverse events**

Nausea, vomiting, drowsiness, dizziness, and delirium were assessed as adverse events under tramadol treatment. Symptoms of drowsiness, dizziness, and delirium were defined as CNS symptoms. The observation period of the adverse events was for one week beginning four

days before the blood sampling day. The incidences of these symptoms were obtained from medical records written by limited palliative care staff. The severity of the symptoms was graded according to the Common Terminology Criteria for Adverse Events, version 5.0.

**Statistical analyses**

All statistics were analyzed using R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria). The plasma parameters of tramadol and its demethylated metabolites between the enantiomers were compared using the Mann-Whitney *U* test. The correlations between the plasma parameters of tramadol and its demethylated metabolites were assessed by the Spearman rank correlation coefficient ($r_s$). Their correlations with CYP2D6 AS were also evaluated using $r_s$. The Mann-Whitney *U* test and Kruskal-Wallis test followed by the *post-hoc* Steel test were used for the comparison of plasma parameters of tramadol and its demethylated metabolite enantiomers between patients with GPS 0 (non-cachexia) and GPS 1 or 2 (pre- and progressive cachexia) and patients with GPS 0, 1, and 2, respectively. The absolute plasma concentrations of tramadol and its demethylated metabolite enantiomers between the patients with and without adverse events were compared using the Mann-Whitney *U* test. Fisher's exact test was employed to assess the incidences of nausea and CNS symptoms between patients with each GPS. The relationship between the absolute plasma concentration of (−)-tramadol and the incidence of CNS symptoms was evaluated using receiver operating characteristic (ROC) curves in patients with GPS 1 or 2. All values

are expressed as the median and interquartile range (IQR) unless otherwise stated. A two-sided *P* value of < 0.05 was considered statistically significant.

## RESULTS

### Patient characteristics

Table 1 summarizes the demographics of the study patients. The patients had a low serum albumin level (median, 35 g/L) and a high serum CRP level (median, 1.3 mg/dL). The present population included 44 patients with advanced cancer (10 with stage III and 34 with stage IV) and 31 patients with co-treatment of radiation. The patients suffered from hypopharyngeal cancer (n = 17), oral cancer (n = 14), oropharyngeal cancer (n = 6), maxillary sinus cancer (n = 5), supraglottic cancer (n = 4), and other cancers (n = 7). The median of tramadol treatment period until blood sampling was 9 days (IQR, 7–16 days).

### Plasma enantiomeric pharmacokinetics

No differences were observed in the plasma concentrations of tramadol and ODT between the enantiomers (Table 2). The plasma concentration of (+)-NDT was higher than that of (−)-NDT ($P < 0.001$). The metabolic ratio to (+)-NDT was higher than that to (−)-NDT ($P < 0.001$), while no difference was observed in the metabolic ratio to ODT between the enantiomers. The IQR of enantiomeric ratios (+/−) of tramadol, ODT, and NDT were 1.11 (1.02–1.27), 1.01 (0.85–1.21), and 4.79 (4.19–5.33), respectively. The enantiomeric ratios of NDT were

(+)-form dominant in all patients.

**Characterization of enantiomeric distribution**

The plasma concentration of (+)-NDT had no significant correlations with the enantiomeric ratios of tramadol and NDT (Figure 1). A negative correlation with the plasma concentration of (+)-NDT was observed in the enantiomeric ratio of ODT ($r_s$ = −0.598, $P$ < 0.001). The enantiomeric distributions of the plasma racemate and (−)-NDT were quite similar to that of the plasma (+)-NDT (data not shown).

**Relationship with CYP2D6 activity score**

There were 15, 3, 3, 13, 1, 1, 3, and 14 patients with *CYP2D6*1/*1*, **1/*2*, **1/*5*, **1/*10*, **2/*5*, **2/*10*, **5/*10*, and **10/*10*, and 18, 14, 4, 14, and 3 patients with CYP2D6 AS 2, 1.25, 1, 0.5, and 0.25, respectively. CYP2D6 AS had no correlations with the plasma concentrations of tramadol and ODT enantiomers (Figure 2). In contrast, CYP2D6 AS had negative correlations with the plasma concentrations of (+)-NDT ($r_s$ = −0.417, $P$ = 0.002) and (−)-NDT ($r_s$ = −0.395, $P$ = 0.003). No significant correlations with CYP2D6 AS were observed in the enantiomeric ratios of tramadol and its demethylated metabolites (Figure S1).

**Relationship with cachexia progression**

There were 17, 18, and 18 patients with GPS 0, 1, and 2, respectively. The plasma

concentrations of (+)-tramadol and (+)-ODT were higher in patients with GPS 1 or 2 than in those with GPS 0 ($P$ = 0.036 and $P$ = 0.043, respectively), while no differences were observed in the plasma concentrations of (−)-tramadol and (−)-ODT ($P$ = 0.052 and $P$ = 0.176, respectively) (Table 3). GPS had no relation to the plasma concentrations of (+)- and (−)-NDT. Lower metabolic ratios to (+)- and (−)-NDT were observed in patients with GPS 2 ($P$ = 0.036 and $P$ = 0.023, respectively) and with GPS 1 or 2 ($P$ = 0.036 and $P$ = 0.030, respectively) than with GPS 0. In contrast, GPS had no association with the metabolic ratios to (+)- and (−)-ODT. There were no differences in the enantiomeric ratios of tramadol and its demethylated metabolites between patients with each GPS.

**Relationships with adverse events**

This study population had 10 patients with nausea, 13 with CNS symptoms (7 with drowsiness, 5 with dizziness, 1 with both, and 0 with delirium), and no patients with vomiting. The severity of these adverse events was grade 1 in all. The absolute plasma concentrations of tramadol and its demethylated metabolite enantiomers were not different between patients with and without these adverse events (Figure 3). The incidences in patients with GPS 0, 1, and 2 were 17.6%, 27.8%, and 11.1% for nausea, and 29.4%, 16.7%, and 27.8% for CNS symptoms, respectively, with no statistical differences ($P$ = 0.467 for nausea and $P$ = 0.723 for CNS symptoms). In patients with GPS 1 or 2, the absolute plasma concentration of (−)-tramadol was higher in those with than without CNS symptoms ($P$ = 0.040) (Figure S2).

The area under the ROC curve of the absolute plasma concentration of (−)-tramadol was 0.739 (95% confidence interval: 0.519–0.958) with a cut-off value of 111 ng/mL (sensitivity: 75.0% and specificity: 78.6%) for the incidence of CNS symptoms in patients with GPS 1 or 2.

## DISCUSSION

The present study mainly assessed the pharmacokinetic impact of CYP2D6 activity and cachexia progression on plasma tramadol and its demethylated metabolite enantiomers in a cancer population. Our findings in this study suggest that CYP2D6 AS partially explains the plasma tramadol and its demethylated metabolite enantiomers in cancer patients. Additionally, cachexia-derived metabolic reduction to NDT with (+)-form dominance contributes to the compensatory increase in plasma (+)-enantiomers of tramadol and ODT. To the best of our knowledge, this is the first report to quantitatively characterize the enantiomeric alteration of plasma tramadol and its demethylated metabolites based on scores of CYP2D6 activity and cachexia progression.

Plasma NDT had an enantiomeric property of (+)-form dominance in the study population, while the plasma tramadol and ODT did not. Healthy subjects receiving oral tramadol also possessed (+)-form dominant plasma NDT and little enantiomeric differences in tramadol and ODT.[23] Similar enantiomeric allocations were observed in neuropathic pain patients receiving oral tramadol.[24] These data indicate that cancer-related disease conditions

do not strongly alter the enantiomeric allocations of tramadol and its demethylated metabolites. The (+)-form dominant demethylation was observed in CYP3A4-mediated conversion to NDT. In contrast, CYP2D6-mediated production of ODT showed no enantiomeric difference. The degree of (+)-form dominant production of NDT may have determined the enantiomeric plasma concentrations of tramadol and its demethylated metabolites in our cancer patients.

Plasma NDT exposure altered the enantiomeric allocation of ODT in this study. Since the *O*- and *N*-demethylation of tramadol share the same substrate, (+)-form dominance of *N*-demethylation leads to a decrease in plasma (+)-tramadol. A high conversion to (+)-NDT from (+)-tramadol potentially results in the compensatory decrease in (+)-ODT production. Haage et al. demonstrated that little (+)-ODT was detected in CYP2D6-deficient subjects due to the compensatory increased *N*-demethylation of tramadol, although (−)-ODT was detected to some extent.[25] The individual metabolic balance between *O*- and *N*-demethylation of (+)-tramadol may be responsible for the enantiomeric allocation of ODT.

A lower CYP2D6 AS was associated with a compensatory increase in CYP3A4-mediated *N*-demethylation of tramadol in this study. The CYP2D6 AS system for prediction of the plasma enantiomers of tramadol and its demethylated metabolites has not been evaluated in cancer patients. A previous study in cancer patients also revealed that CYP2D6 intermediate metabolizers (IM) and poor metabolizers (PM) had a compensatory increase in plasma NDT.[8] Inversely, co-administration of fluconazole, a CYP3A4 inhibitor,

with oral tramadol caused a decrease in plasma NDT and a compensatory increase in plasma tramadol and ODT.[26] CYP3A4-related dysfunctional genotypes such as **1B* allele are not found in Asians.[27] These data indicate that individual CYP3A4 activity based on genetic variants has little influence on tramadol metabolism. Thus, the reduction of CYP3A4 activity by non-genetic factors including its inhibitors and cancer-related inflammation may alter tramadol metabolism.

CYP2D6 AS was used for the quantification of individual CYP2D6 activity in this study. *CYP2D6*10* is a major dysfunctional allele in Japanese.[28] The conventional classifications such as CYP2D6 extensive metabolizers (EM) and IMs did not precisely reflect the genetic influence of **10* allele.[29] Although *CYP2D6*1/*1* and **1/*10* show different phenotypes, both genotypes are classified as EMs.[30] CYP2D6 AS enables semi-quantitative prediction with multiple genetic impacts including **10* allele in Japanese. CYP2D6 AS did not alter the enantiomeric allocations of tramadol and its demethylated metabolites in the present study. Enantiomeric allocation of flecainide, a CYP2D6 substrate, was altered by its inhibitors.[31] In tramadol metabolism, a lowered CYP2D6 activity resulted in the compensatory increased *N*-demethylation by CYP3A4. The strong metabolic contribution of CYP3A4 with a higher (+)-form dominance may be responsible for the little impact of CYP2D6 on the enantiomeric allocations in humans.

Cancer patients with a higher degree of cachexia progression had higher plasma (+)-enantiomers of tramadol and ODT probably owing to metabolic reduction to (+)-NDT.

Cachectic cancer patients have higher serum IL-6 levels which is related to the down-regulation of CYPs.[4,32] CYP3A4 was sensitive to suppression by IL-6 with a 50% inhibitory concentration ($IC_{50}$) of 3.2 pg/mL, while CYP2D6 was not sensitive with an $IC_{50}$ of 151 pg/mL in human hepatocytes.[33] Most cancer patients requiring tramadol treatment have a serum IL-6 level exceeding the $IC_{50}$ for suppression of CYP3A4 but not CYP2D6.[8] Cachexia can alter CYP3A4 activity, while cachectic cancer patients conserved inherent CYP2D6 activity based on individual genotypes.

The present study could not evaluate the activity of organic cation transporter 1 (OCT1) which is involved in the uptake of ODT into hepatic cells.[34] Chiral drugs including verapamil and propranolol have different affinities to OCT1 between enantiomers.[35] The affinities of ODT enantiomers to OCT1 remains to be clarified in humans. Dysfunctional alleles of OCT1 raised plasma ODT in healthy subjects receiving oral tramadol, while the enantiomeric ratio of ODT was unchanged.[34] Cachectic cancer patients potentially have a lower expression of OCT1 owing to the down-regulation by increased serum IL-6.[36] A high degree of cachexia progression did not alter the enantiomeric ratio of ODT in the present study. These data suggest that OCT1 activity does not affect the enantiomeric allocation of ODT.

The patients with a higher degree of cachexia progression had higher absolute plasma (−)-tramadol relating to the incidence of CNS symptoms in this study. The relationship between plasma (+)-tramadol which was highly correlated with plasma (−)-tramadol ($r$ =

0.97) and the incidence of CNS symptoms was close to significance ($P < 0.1$). These data suggest that plasma (+)-tramadol is also associated with CNS symptoms. CYP2D6 IMs and PMs with high plasma tramadol had higher incidences of CNS symptoms.[37,38] CYP2D6 is also highly expressed in brain in addition to liver.[39] Tramadol with a high permeability to the blood-brain barrier (BBB) may be converted to ODT by cerebral CYP2D6.[34,39] Cachexia progression with high serum IL-6 also enhances the permeability of the BBB.[14,15] The high permeability of the BBB in patients with a higher degree of cachexia progression and increased plasma tramadol may trigger the CNS symptoms in cancer patients.

Our study has several limitations. First, it partially enrolled head and neck cancer patients with early cancer stages and with radiation co-treatment. Some of the cancer patients used tramadol for cancer treatment-related pain. Careful application of our data to other cancer populations or more advanced cancer populations may be needed. Second, this study could not evaluate CYP2D6 ultrarapid metabolizers (UM) and PMs. In the Japanese population, CYP2D6 UMs and PMs are rare phenotypes (< 1%).[28] If the present population included CYP2D6 UMs or PMs, the predictability of plasma tramadol and its demethylated metabolites using CYP2D6 AS may improve due to extension of the applicable score range. Third, the present study employed a GPS for the evaluation of cachexia progression. Cancer cachexia is commonly assessed by symptoms such as the loss of body weight or body mass index.[22] The GPS explains the pharmacokinetic alteration of a strong opioid.[14] The present population includes non-cachexia and pre-cachexia in addition to cachexia patients. Cancer

patients with progressive cachexia may have a higher incidence of CNS symptoms based on analgesics and their pathophysiological condition. Fourth, this study did not evaluate the serum level of proinflammatory cytokines that potentially support our findings. In particular, serum IL-6 level associated with the cancer cachexia stages.[14] In cancer patients, serum CRP level had a strong correlation with serum IL-6 level.[8] These reports suggest that the elevation of serum CRP reflects the progression of IL-6 associated cancer cachexia. Determination of relevant cytokines in relation to evaluation with CYP2D6 AS and GPS would add the values to our findings.

Our observational study investigated the clinical implications of scoring of CYP2D6 activity and cachexia progression on pain management using tramadol in cancer patients. CYP2D6 AS partially clarified the metabolic contribution of CYP2D6 to tramadol metabolism. However, sufficient predictions of plasma tramadol and ODT using CYP2D6 AS were difficult in this study population. In contrast, cachexia progression raised the plasma (+)-tramadol and (+)-ODT levels via the reduction of CYP3A4 activity. For cancer patients with a higher score of cachexia progression, careful attention to excessive stimulation of MOR by increased plasma (+)-ODT and the triggering of CNS symptoms by increased plasma (+)-tramadol are needed. Furthermore, the reduction of CYP3A4 activity caused by cachexia progression potentially affects the pharmacokinetics of and clinical responses to some drugs that are predominantly metabolized by CYP3A4. However, the reduction of CYP3A4 activity may have different clinical implications depending on drug characteristics

including the presence of alternative metabolic pathways and active metabolites. The impact of cachexia progression on treatment with CYP3A4 substrate needs to be considered individually based on drug characteristics.

## CONCLUSION

CYP2D6 AS partially explained the contribution of CYP2D6 activity to plasma tramadol and its demethylated metabolite enantiomers. Cancer cachexia progression elevated the plasma (+)-tramadol and (+)-ODT levels through the reduction of *N*-demethylation of (+)-tramadol. Additionally, cachectic cancer patients with increased plasma tramadol experienced the higher incidence of CNS symptoms.

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## CONFLICT OF INTEREST

None.

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Table 1. Patient characteristics

| | |
|---|---|
| Gender, male/female | 47/6 |
| Age, years | 67 (62–71) |
| Body weight, kg | 52.9 (46.1–60.1) |
| Body mass index, kg/m$^2$ | 20.0 (17.5–21.9) |
| Serum total protein, g/L | 63 (59–67) |
| Serum albumin, g/L | 35 (30–38) |
| Serum creatinine, mg/dL | 0.84 (0.68–0.95) |
| Blood urea nitrogen, mg/dL | 18 (14–21) |
| Aspartate aminotransferase, U/L | 21 (17–28) |
| Alanine aminotransferase, U/L | 20 (14–35) |
| Alkaline phosphatase, U/L | 236 (202–312) |
| Total bilirubin, mg/dL | 0.4 (0.3–0.5) |
| C-reactive protein, mg/dL | 1.3 (0.3–2.6) |

Data are expressed as the median and interquartile range in parentheses.

Table 2. Differences in the plasma parameters of tramadol and its demethylated metabolites between the enantiomers in head and neck cancer patients

| | (+)-form | (−)-form | *P* value |
|---|---|---|---|
| Plasma tramadol, ng/mL per mg/kg | 131 (66.7–191) | 122 (56.7–175) | 0.326 |
| Plasma ODT, ng/mL per mg/kg | 27.4 (17.2–41.1) | 29.7 (17.5–37.7) | 0.992 |
| Plasma NDT, ng/mL per mg/kg | 65.1 (29.7–112) | 11.8 (4.65–24.1) | < 0.001 |
| Metabolic ratio to ODT | 0.211 (0.166–0.352) | 0.263 (0.196–0.385) | 0.138 |
| Metabolic ratio to NDT | 0.537 (0.260–0.933) | 0.109 (0.061–0.210) | < 0.001 |

Data are expressed as median and interquartile range in parentheses. ODT, *O*-desmethyltramadol; and NDT, *N*-desmethyltramadol. *P* values between (+)- and (−)-form were determined by the Mann-Whitney *U* test.

Table 3. Relationships between the degree of cachexia progression and plasma parameters of tramadol and its demethylated metabolite enantiomers in head and neck cancer patients

| Progression degree of cachexia | GPS 0 (n = 17) | GPS 1 (n = 18) | GPS 2 (n = 18) | GPS 1 or 2 (n = 36) |
|---|---|---|---|---|
| Tramadol dose, mg per dosing | 25 (25–25) | 25 (25–38) | 25 (25–38) | 25 (25–38) |
| Plasma (+)-tramadol, ng/mL per mg/kg | 75.7 (61.3–150) | 133 (88.0–204) | 149 (124–192) | 144 (92.1–199)† |
| Plasma (−)-tramadol, ng/mL per mg/kg | 62.3 (50.7–135) | 133 (78.9–188) | 124 (98.8–177) | 128 (85.7–182) |
| Plasma (+)-ODT, ng/mL per mg/kg | 20.8 (13.3–28.2) | 32.9 (19.8–40.0) | 33.5 (22.2–47.4) | 33.5 (21.0–44.0)† |
| Plasma (−)-ODT, ng/mL per mg/kg | 23.1 (14.8–31.7) | 30.6 (17.4–34.2) | 32.7 (24.7–44.2) | 31.6 (21.0–41.1) |
| Plasma (+)-NDT, ng/mL per mg/kg | 66.1 (31.6–94.2) | 74.3 (35.8–123) | 38.1 (27.4–76.1) | 57.3 (29.0–113) |
| Plasma (−)-NDT, ng/mL per mg/kg | 14.9 (4.65–17.9) | 20.2 (7.54–26.3) | 8.30 (4.93–12.6) | 11.2 (5.73–24.3) |
| Metabolic ratio to (+)-ODT | 0.207 (0.173–0.362) | 0.209 (0.140–0.268) | 0.266 (0.165–0.352) | 0.221 (0.164–0.352) |
| Metabolic ratio to (−)-ODT | 0.273 (0.207–0.477) | 0.250 (0.155–0.367) | 0.279 (0.231–0.351) | 0.257 (0.177–0.363) |
| Metabolic ratio to (+)-NDT | 0.933 (0.379–1.23) | 0.536 (0.293–0.740) | 0.354 (0.177–0.673)* | 0.408 (0.208–0.711)† |
| Metabolic ratio to (−)-NDT | 0.203 (0.089–0.238) | 0.113 (0.069–0.169) | 0.086 (0.042–0.125)* | 0.105 (0.056–0.147)† |
| Enantiomeric ratio of tramadol (+/−) | 1.11 (1.05–1.22) | 1.08 (1.02–1.20) | 1.26 (1.07–1.34) | 1.11 (1.02–1.32) |
| Enantiomeric ratio of ODT (+/−) | 0.949 (0.757–1.05) | 1.07 (0.848–1.25) | 1.13 (0.959–1.38) | 1.11 (0.901–1.30) |
| Enantiomeric ratio of NDT (+/−) | 4.79 (4.16–5.62) | 4.60 (4.19–4.91) | 4.85 (4.54–6.11) | 4.76 (4.19–5.03) |

Data are expressed as median and interquartile range in parentheses. GPS, Glasgow Prognostic Score; ODT, *O*-desmethyltramadol; and NDT, *N*-desmethyltramadol. *P* values between GPS 0 and 1 and between GPS 0 and 2 were determined by the Kruskal-Wallis test and *post-hoc* Steel test (*$P < 0.05$, different from GPS 0). *P* values between GPS 0 and 1 or 2 were determined by the Mann-Whitney *U* test (†$P < 0.05$).

**FIGURE LEGENDS**

Figure 1. Relationship between plasma (+)-*N*-desmethyltramadol (NDT) and enantiomeric ratios of tramadol and its demethylated metabolite enantiomers in head and neck cancer patients.

The correlations between the plasma concentration of (+)-NDT and enantiomeric ratio of (A) tramadol, (B) *O*-desmethyltramadol (ODT), and (C) NDT were evaluated using the Spearman rank correlation test.

Figure 2. Relationship between the CYP2D6 activity score (AS) and plasma tramadol and its demethylated metabolite enantiomers in head and neck cancer patients.

The correlations between the CYP2D6 AS and plasma concentrations of (A) (+)-tramadol, (B) (+)-*O*-desmethyltramadol (ODT), (C) (+)-*N*-desmethyltramadol (NDT), (D) (−)-tramadol, (E) (−)-ODT, and (F) (−)-NDT were evaluated using the Spearman rank correlation test.

Figure 3. Comparisons of the absolute plasma tramadol and its demethylated metabolite enantiomers between the patients with and without adverse events.

The effects of the absolute plasma concentrations of (A) tramadol, (B) *O*-desmethyltramadol (ODT), and (C) *N*-desmethyltramadol (NDT) enantiomers on the incidence of nausea and of the absolute plasma concentrations of (D) tramadol, (E) ODT, and (F) NDT enantiomers on the incidence of central nervous system (CNS) symptoms were evaluated using the

Mann-Whitney *U* test. The present population included patients with (n = 10) and without (n = 43) nausea and patients with (n = 13) and without (n = 40) CNS symptoms.

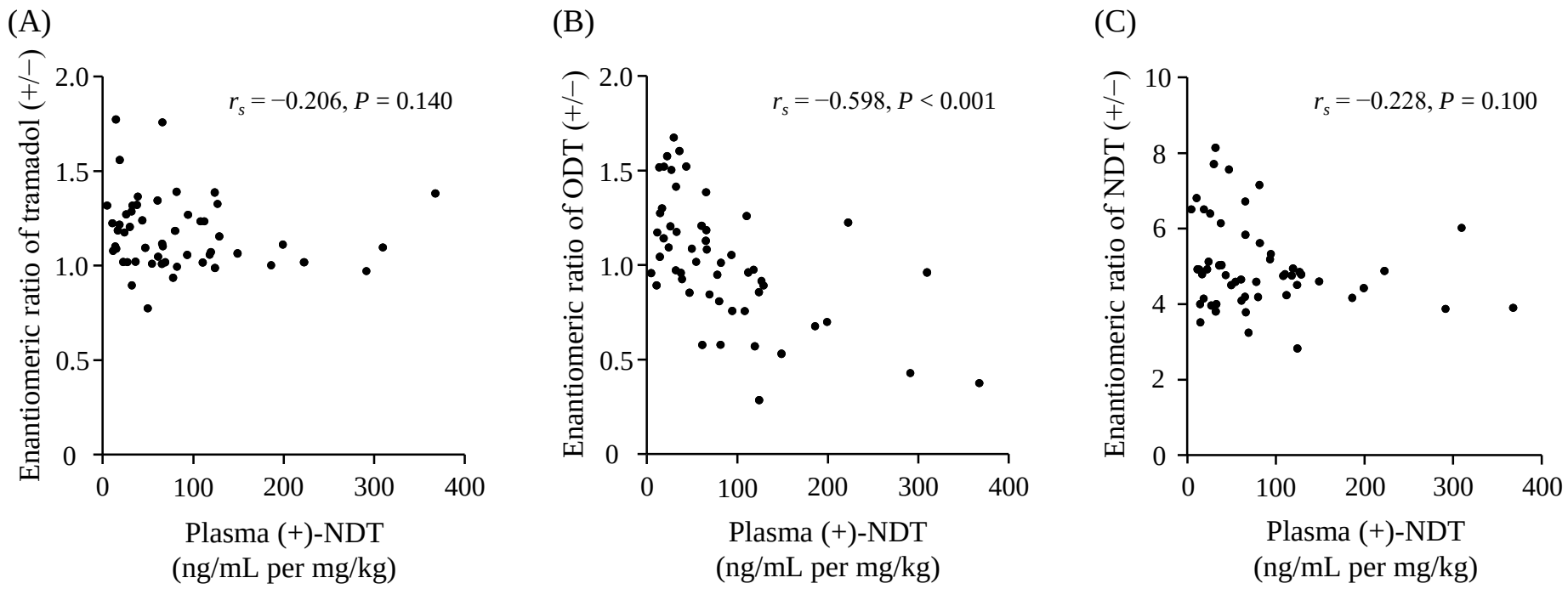
(A)
Enantiomeric ratio of tramadol (+/−)
$r_s = -0.206$, $P = 0.140$
Plasma (+)-NDT
(ng/mL per mg/kg)
(B)
Enantiomeric ratio of ODT (+/−)
$r_s = -0.598$, $P < 0.001$
Plasma (+)-NDT
(ng/mL per mg/kg)
(C)
Enantiomeric ratio of NDT (+/−)
$r_s = -0.228$, $P = 0.100$
Plasma (+)-NDT
(ng/mL per mg/kg)

Figure 1

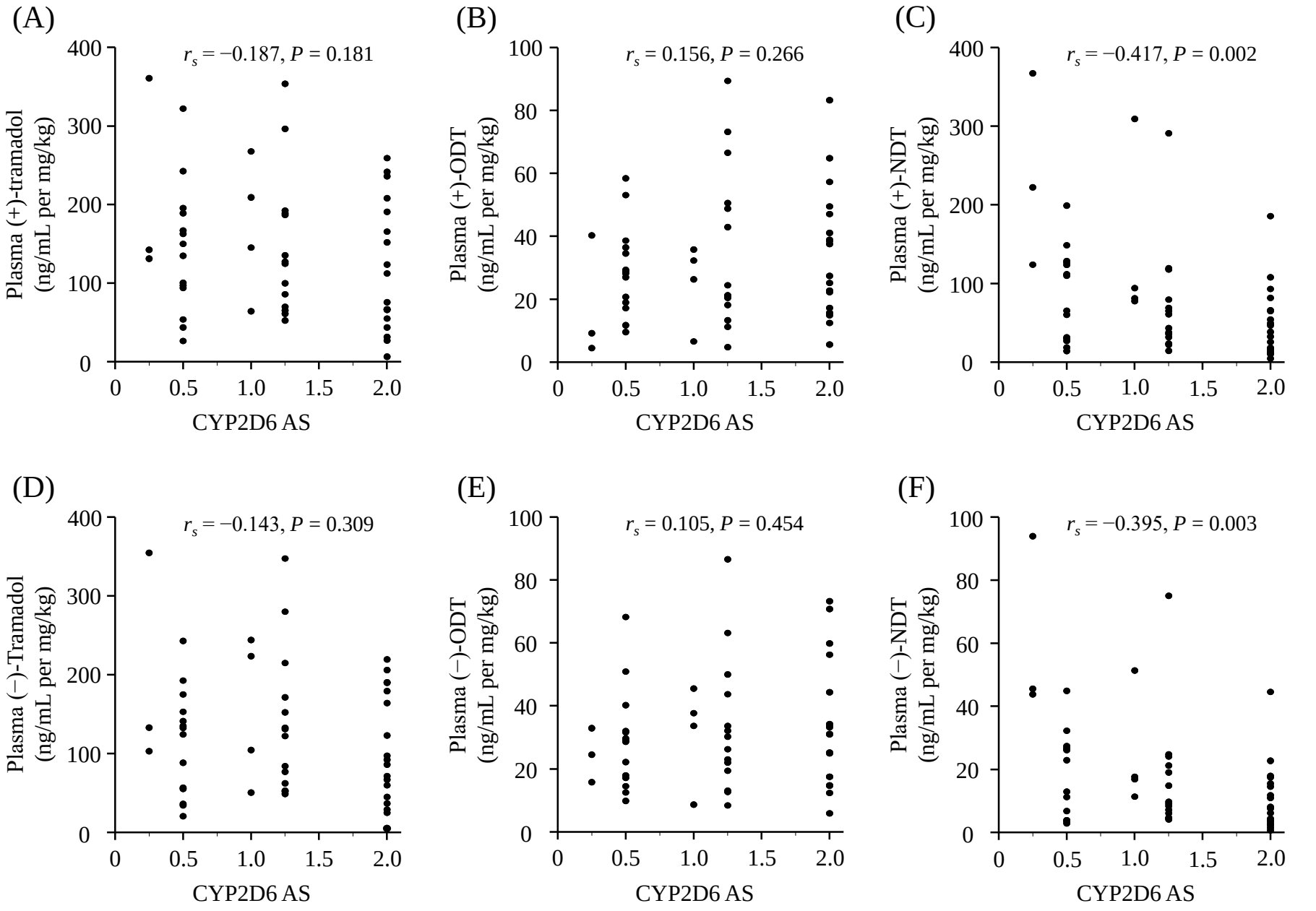
(A)
$r_s$ = −0.187, $P$ = 0.181
Plasma (+)-tramadol (ng/mL per mg/kg)
CYP2D6 AS
(B)
$r_s$ = 0.156, $P$ = 0.266
Plasma (+)-ODT (ng/mL per mg/kg)
CYP2D6 AS
(C)
$r_s$ = −0.417, $P$ = 0.002
Plasma (+)-NDT (ng/mL per mg/kg)
CYP2D6 AS
(D)
$r_s$ = −0.143, $P$ = 0.309
Plasma (−)-Tramadol (ng/mL per mg/kg)
CYP2D6 AS
(E)
$r_s$ = 0.105, $P$ = 0.454
Plasma (−)-ODT (ng/mL per mg/kg)
CYP2D6 AS
(F)
$r_s$ = −0.395, $P$ = 0.003
Plasma (−)-NDT (ng/mL per mg/kg)
CYP2D6 AS

Figure 2

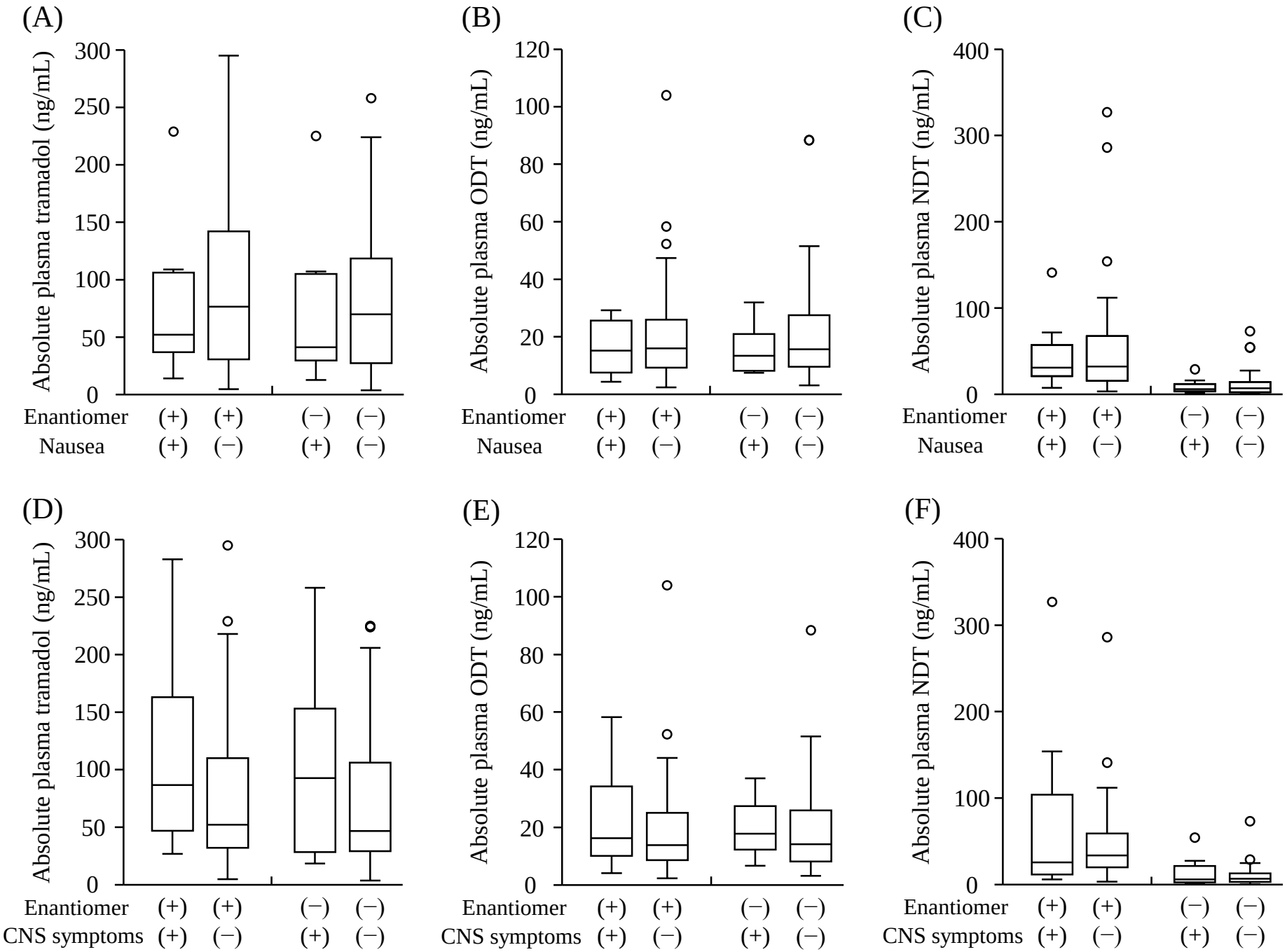
(A)
Absolute plasma tramadol (ng/mL)
300
250
200
150
100
50
0
Enantiomer (+) (+) (−) (−)
Nausea (+) (−) (+) (−)
(B)
Absolute plasma ODT (ng/mL)
120
100
80
60
40
20
0
Enantiomer (+) (+) (−) (−)
Nausea (+) (−) (+) (−)
(C)
Absolute plasma NDT (ng/mL)
400
300
200
100
0
Enantiomer (+) (+) (−) (−)
Nausea (+) (−) (+) (−)
(D)
Absolute plasma tramadol (ng/mL)
300
250
200
150
100
50
0
Enantiomer (+) (+) (−) (−)
CNS symptoms (+) (−) (+) (−)
(E)
Absolute plasma ODT (ng/mL)
120
100
80
60
40
20
0
Enantiomer (+) (+) (−) (−)
CNS symptoms (+) (−) (+) (−)
(F)
Absolute plasma NDT (ng/mL)
400
300
200
100
0
Enantiomer (+) (+) (−) (−)
CNS symptoms (+) (−) (+) (−)

Figure 3

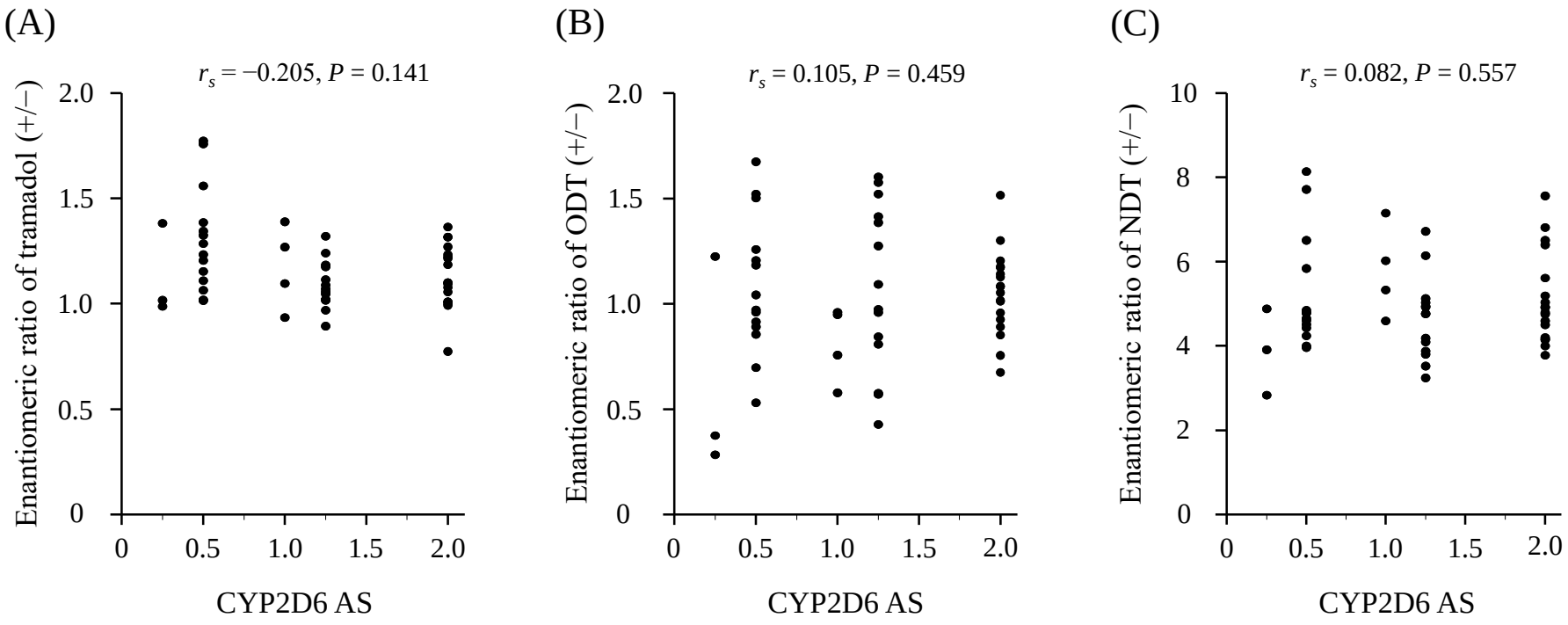


**Figure S1. Relationships between the CYP2D6 activity score (AS) and enantiomeric ratios of tramadol and its demethylated metabolites in head and neck cancer patients.**

The correlations between CYP2D6 AS and enantiomeric ratios of (A) tramadol, (B) *O*-desmethyltramadol (ODT), and (C) *N*-desmethyltramadol (NDT) were evaluated using the Spearman rank correlation test.

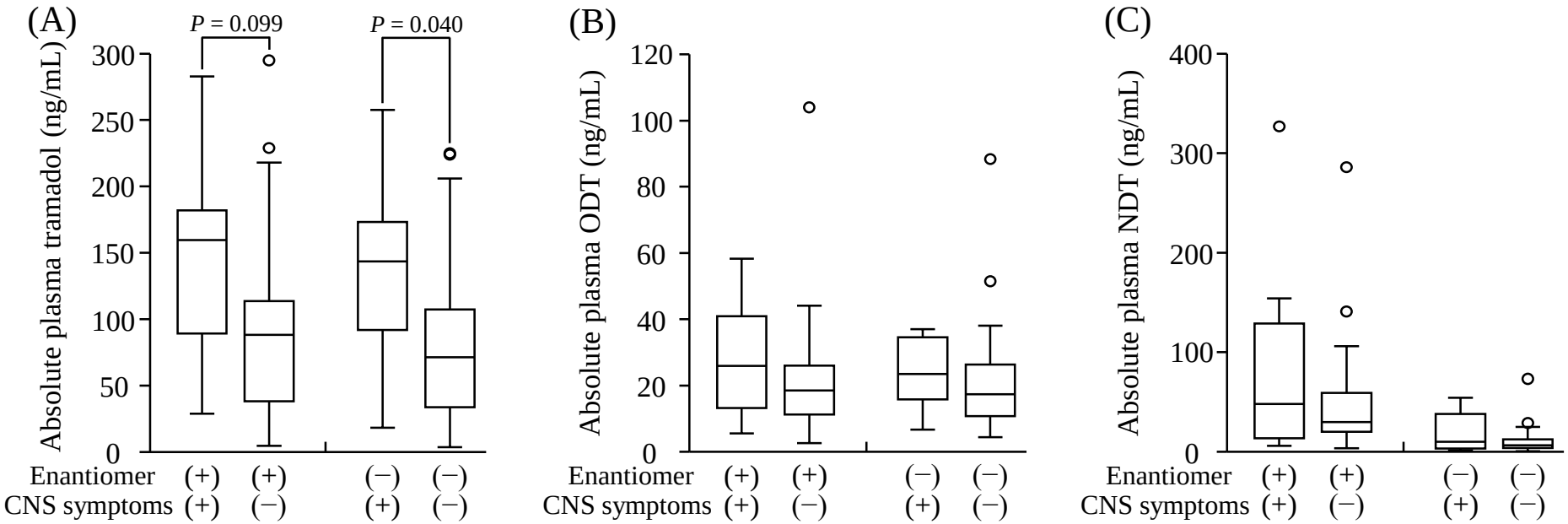


**Figure S2. Relationships between the symptoms of central nervous system (CNS) and absolute plasma concentrations of tramadol and its demethylated metabolite enantiomers in head and neck cancer patients with Glasgow Prognostic Score (GPS) 1 or 2.**

The effects of the absolute plasma concentrations of (A) tramadol, (B) *O*-desmethyltramadol (ODT), and (C) *N*-desmethyltramadol (NDT) enantiomers on the incidence of CNS symptoms were evaluated using the Mann-Whitney *U* test. The patients with GPS 1 or 2 (n = 36) included 8 patients with CNS symptoms and 28 patients without.