学位論文

Impact of the Catechol-O-Methyltransferase Val158Met Polymorphism on the Pharmacokinetics of L-dopa and its Metabolite 3-O-Methyldopa in Combination with Entacapone
(エンタカポン併用下における L-dopa およびその代謝物である 3-O-メチルドパの体内動態に及ぼすカテコール-O-メチルトランスフェラーゼ Val158Met 遺伝子多型の影響)

山本　譲

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Impact of the Catechol-O-Methyltransferase Val158Met Polymorphism on the Pharmacokinetics of L-dopa and its Metabolite 3-O-Methyldopa in Combination with Entacapone

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ABSTRACT

In the pharmacotherapy of patients with Parkinson's disease (PD), entacapone reduces the peripheral metabolism of L-dopa to 3-O-methyldopa (3-OMD), thereby prolonging the half-life ($t_{1/2}$) of L-dopa and increasing the area under the concentration curve (AUC). The effect of entacapone on the pharmacokinetics of L-dopa differs between patients with high-activity (H/H) and low-activity (L/L) catechol-O-methyltransferase (COMT) Val158Met polymorphisms, but the effects are unclear in heterozygous (H/L) patients. 3-OMD has a detrimental effect and results in a poor response to L-dopa treatment in patients with PD; however, the influence of this polymorphism on the production of 3-OMD remains unknown. Therefore, the present study aimed to clarify the effect of the COMT Val158Met polymorphism on the concentrations of L-dopa and 3-OMD in the presence of entacapone. We performed an open-label, single-period, single-sequence crossover study at two sites in Japan. The study included 54 Japanese patients with PD, who underwent an acute L-dopa administration test with and without 100 mg entacapone on two different days. Entacapone increased L-dopa $AUC_{0\text{-infinity}}$ by 1.59 ± 0.26-fold in the H/H group, which was significantly higher than that in the H/L (1.41 ± 0.36-fold) and L/L (1.28 ± 0.21-fold) groups ($p<0.05$). The concurrent administration of L-dopa with entacapone suppressed the increase in 3-OMD levels compared with L-dopa alone in all genotypes. Our results suggest that the COMT Val158Met polymorphism may be an informative biomarker for individualized dose adjustment of COMT inhibitors in the treatment of PD.

Keywords:

L-dopa, 3-O-methyldopa, Catechol-O-methyl transferase, Entacapone, Parkinson's disease,

Polymorphism

1. Introduction

L-dopa plays a central role in the treatment of Parkinson's disease (PD). However, when L-dopa is administered for an extended period, its efficacy decreases as the disease progresses, and the “wearing-off” phenomenon occurs more frequently (Tran et al. 2018; Armstrong and Okun 2020). Monoamine oxidase (MAO)-B and catechol-O-methyl transferase (COMT) inhibitors have been developed to prolong the effects of L-dopa (Gershanik 2015; Finberg 2019). A commonly prescribed COMT inhibitor, entacapone, interferes with the O-methylation step of L-dopa metabolism. Entacapone inhibits the peripheral activity of COMT, resulting in extended half-life ($t_{1/2}$) of L-dopa, without altering the maximum plasma concentration (C_{max}) of L-dopa (Kaakkola 2000). Entacapone increases the area under the plasma concentration-time curve (AUC) of L-dopa and is useful for managing wearing-off (Gordin et al. 2004; Schrag 2005; Gershanik 2015).

In clinical practice, interindividual variability in the effectiveness of entacapone is common. The enzyme activity of COMT is affected by a genetic polymorphism (rs4608, Val158Met) (Syvänen et al. 1997) located on chromosome 22q11; rs4608 is the most commonly studied COMT polymorphism (Drożdzik et al. 2013). The Val-158 allele encodes the thermostable high-activity COMT enzyme, and the Met-158 allele encodes the thermolabile low-activity COMT enzyme (Syvänen et al. 1997). Although previous studies have investigated the effects of this genetic polymorphism on clinical efficacy of entacapone, the results are conflicting. For example, one study reported that this polymorphism is not associated with the effects of entacapone (Lee et al. 2002),

whereas another study demonstrated that entacapone is more effective in high-activity homozygous (H/H) patients than in low-activity homozygous (L/L) patients (Corvol et al. 2011). Compared to that in Caucasian populations, the proportion of individuals with the L/L genotype of the COMT gene in Mongoloids is smaller, while the proportion of those with H/H and heterozygous (H/L) genotypes is larger (Palmatier et al. 1999). Therefore, it is imperative to investigate the clinical implications of the heterozygous genotype, particularly for the treatment of PD using L-dopa.

3-O-methyldopa (3-OMD) is a major metabolite of L-dopa, formed by the action of COMT, and has an unfavorable influence on the pharmacotherapy of PD. L-dopa and 3-OMD are transported across the blood-brain barrier (BBB). Consequently, the brain permeability of L-dopa is restricted depending on the concentration of 3-OMD (Gomes and Soares-Da-Silva 1999; Nutt 2000). Moreover, the accumulation of 3-OMD in the body may adversely affect L-dopa therapy and lead to motor complications in patients with PD (Lee et al. 2008). Previous studies have demonstrated that 3-OMD impairs locomotor activity and decreases dopamine turnover rate in the rat striatum (Lee et al. 2008; Onzawa et al. 2012). In vitro, 3-OMD inhibits dopamine uptake in rat striatal membranes and PC12 cells, and induces cytotoxicity due to decreased mitochondrial membrane potential and oxidative stress (Lee et al. 2008).

Several studies have reported that 3-OMD is related to the pathogenesis of wearing-off and dyskinesia. The concentration of 3-OMD in cerebrospinal fluid is significantly elevated in PD patients with wearing-off phenomenon compared with those without (Tohgi et al. 1991). High blood

concentrations of 3-OMD have been associated with the occurrence of dyskinesia symptoms (Feuerstein et al. 1977a; Mena et al. 1987). Although such evidence supports measuring 3-OMD concentrations in patients with PD, to our knowledge, no study has analyzed the effect of the COMTVal158Met polymorphism on time-dependent 3-OMD concentration profiles following entacapone administration. Therefore, in this study, we sought to evaluate the impact of the COMT gene polymorphism on the effectiveness of entacapone by evaluating the pharmacokinetics of L-dopa and its metabolite, 3-OMD.

2. Materials and methods

2.1 Materials

Ethylenediaminetetraacetic acid disodium salt (EDTA・2Na) was obtained from Dohjindo Laboratories (Kumamoto, Japan). Sodium 1-Octanesulfonate (SOS) and 3-OMD were purchased from Nacalai Tesque (Kyoto, Japan) and Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. L-dopa, methanol, citric acid monohydrate, sodium acetate, sodium hydrogen sulfate, perchloric acid (PCA), and the other materials used for high-performance liquid chromatography (HPLC) were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan).

2.2 Patients and L-dopa/Entacapone Administration

We performed an open-label, single-period, single-sequence crossover study at two sites in Japan. Between November 2009 and December 2019, 54 Japanese patients diagnosed with PD (25 patients at Asahikawa Medical University Hospital and 29 patients at Kyoto University Hospital) who provided written informed consent were included in the analysis. Each patient was treated according to the protocol with and without entacapone on two different days, and L-dopa and 3-OMD levels were subsequently measured. Patients were allowed to take medicines other than L-dopa and entacapone, regularly. On the mornings of the L-dopa challenge (day1 and day2), patients did not take L-dopa, entacapone, or breakfast before the test. On day 1, a combination tablet consisting of L-dopa 100 mg/carbidopa 10 mg was orally administered at 8:30 AM, and blood samples were taken 0, 0.5, 1, 2, 3, and 4 h after dosing. On day 2, a combination tablet consisting of L-dopa 100 mg/carbidopa 10 mg and a 100 mg entacapone tablet were orally administered at 8:30 AM to the same patient, and blood samples were taken 0, 0.5, 1, 2, 3, and 4 h after dosing. The collected blood samples were immediately spiked with EDTA・2Na and sodium hydrogen sulfate, and centrifuged at 1,500 × g for 10 min. Plasma was retained and stored at -20°C until HPLC analysis for determination of L-dopa and 3-OMD concentrations.

2.3 Determination of L-dopa and 3-OMD Concentrations

Plasma levels of L-dopa and 3-OMD were measured as previously described, with some modifications (Baruzzi et al. 1986). In brief, the HPLC system (LC-10AD, Shimadzu, Kyoto, Japan) was connected to an electrochemical detector (ECD-300, EICOM, Kyoto, Japan) using a C-18 reverse-phase column (5 μm, 150 × 3.0 mm, EICOM). The mobile phase consisted of 0.1 M citrate-acetate buffer, 110 mg/L SOS, and 5 mg/L EDTA・2Na, pH 2.6/13% methanol (v/v), with a pump rate of 0.5 mL/min. The detector potential was set at 750 mV.

The following PK parameters of L-dopa were determined by statistical moment analysis from the concentration–time profile in plasma (Tabata et al. 1999): area under the concentration curve from 0 to 4 h ($AUC_{0–4h}$), AUC from 0 h to infinity ($AUC_{0–inf}$), maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), and half-life ($t_{1/2}$). Differences in the level of 3-OMD before and after administration of L-dopa in each patient are expressed as $\Delta C_{3\text{-}OMD}$.

2.4 COMT Genotyping

Genomic DNA was extracted from peripheral whole venous blood of each patient using standard methods. Polymorphism of the COMT Val158Met was analyzed in each patient using a tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR)

(Ruiz-Sanz et al. 2007). Genotyping was conducted after the HPLC analyses of the PK parameters.

2.5 Statistical Analysis

Data are expressed as the mean ± standard deviation (S.D.). To compare the baseline characteristics of patients among the three genotype groups, one-way analysis of variance (ANOVA) or Fisher's test was used. The effect of genotype interaction was analyzed by repeated measures ANOVA. Data were compared according to the presence or absence of entacapone in each group using the paired Wilcoxon test. To compare the effects of entacapone on PK parameters among the three groups, the Kruskal–Wallis test was followed by the Mann–Whitney test. A probability of 0.05 was considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (Kanda 2013).

3. Results

3.1 Patients

In total, 54 Japanese patients (28 males and 26 females) were included in this study. The overall mean ± S.D. age, disease duration, daily dose of L-dopa, and L-dopa equivalent daily dose (LEDD) were 69.2 ± 9.90 years, 7.09 ± 6.06 years, 298 ± 234 mg, and 561 ± 503 mg, respectively. The baseline characteristics for patients in each COMT genotype group are described in Table 1. Overall, 24 (44.4%) patients were COMT wild type (H/H), 23 patients (42.6%) carried a heterozygous mutation (H/L), and seven patients (12.9%) had a low activity type (L/L). The observed frequencies adhered to the Hardy–Weinberg equilibrium ($p = 0.9$ in chi-square test). There were no significant differences in sex, age, disease duration, Hoehn and Yahr scale, daily L-dopa dose, LEDD, and percentage of patients currently treated with entacapone among participants with different COMT genotypes.

3.2 Effect of Entacapone on the Pharmacokinetics of L-dopa

Entacapone increased L-dopa $AUC_{0\text{-}4h}$ by 1.4-, 1.3-, and 1.1-fold in patients in the H/H, H/L, and L/L groups, respectively (Fig. 1, Table 2). The $AUC_{0\text{-}4h}$ increment ratio tended to be higher in

the H/H group compared with the L/L group, although this was not significant ($p = 0.079$). A significant increase in $AUC_{0\text{-}inf}$ was observed following entacapone administration in the H/H ($p<0.001$), H/L ($p<0.001$), and L/L ($p<0.05$) groups (Table 2). Following the simultaneous administration of L-dopa and entacapone, the plasma levels of L-dopa were elevated at 180 and 240 min in all groups; the levels were higher at 120 min in the H/H and H/L groups when L-dopa was co-administered with entacapone than when L-dopa was administered alone (Fig. 2). When the $AUC_{0\text{-}inf}$ increase ratio was compared among the three groups, it was significantly higher in the H/H group compared with the H/L and L/L groups ($p<0.05$) (Fig. 3). The $t_{1/2}$ of L-dopa was prolonged to longer duration following entacapone administration than after administration of L-dopa alone, with no significant difference among the three groups (Table 2). Furthermore, there were no significant differences among groups in terms of changes in T_{max} and C_{max} following entacapone co-administration (Table 2).

3.3 Effect of Entacapone on the Pharmacokinetics of 3-OMD

Plasma levels of 3-OMD were evaluated in all three groups following L-dopa administration. Trough 3-OMD concentrations were 2.37 ± 2.80, 1.75 ± 1.57, and 1.80 ± 1.00 μg/mL in the H/H, H/L and L/L groups, respectively, with no significant differences among the groups (Table 3). Trough plasma 3-OMD concentration/L-dopa daily dose (C/D) tended to be higher in the H/H group, but the difference among groups was not significant (Fig. 4). As shown in Figure 5, the

difference in the concentration of 3-OMD ($\Delta C_{3\text{-OMD}}$) in individual patients increased in the following order: L/L < H/L < H/H after L-dopa dosing without entacapone. Correspondingly, a comparison of the $\Delta C_{3\text{-OMD}}$ $AUC_{0\text{-}4h}$ after L-dopa dosing without entacapone revealed that the AUC of the H/H group tended to be higher than that of the other two groups; however, this difference was not significant (Fig. 6). When L-dopa and entacapone were administrated simultaneously, the increase in 3-OMD was suppressed in all genotypes compared with patients receiving L-dopa alone (Fig. 5). Finally, as shown in Table 3, this effect of entacapone ($\Delta C_{3\text{-OMD}}$) occurred to the same degree in all groups at 240 min.

4. Discussion

We present two novel findings from our study regarding the benefits of adding entacapone to L-dopa therapy: (1) the increase in L-dopa blood $AUC_{0\text{-}inf}$ is more evident for patients with the H/H genotype than for those with the H/L genotype; and (2) $\Delta C_{3\text{-OMD}}$ production is effectively suppressed in all patients, regardless of genotypes (H/H, H/L, and L/L).

Interethnic variation in terms of genetic polymorphisms in the COMT genotype is well established, and the frequency of the H/L (46%) genotype is dominant whereas that of L/L (6%) is rare, especially in Asia (Kunugi et al. 1997; Palmatier et al. 1999). In the present study, a large proportion of patients carried the H/L (43%) and H/H (44%) genotypes. We observed a significant

increase in the $AUC_{0\text{-}inf}$ of L-dopa when administered with entacapone in the H/H group compared with the H/L group. Similarly, the L-dopa $AUC_{0\text{-}4h}$ with entacapone was high, and followed the order of L/L < H/L < H/H group; however, no significant differences were observed among the groups ($p = 0.079$). These results indicate that a prolonged period of observation is required to analyze the effect of entacapone on L-dopa AUC. This notion is supported by a previous study investigating multiple doses of L-dopa and entacapone, in which the AUC of L-dopa was higher following the second administration compared with the first administration (Müller et al. 2006).

A previous study showed that the AUC of L-dopa was higher in patients with the H/H genotype than in those with the L/L genotype following the combined administration of L-dopa and entacapone (Corvol et al. 2011). However, in that study, participants were Caucasian and carried the H/H and L/L genotypes, and the results for patients with the H/L genotype were not observed (Corvol et al. 2011). Thus, our results provide new insights for determining the L-dopa dosage for patients with the H/L genotype. We also showed that de novo production of 3-OMD after L-dopa administration was almost completely suppressed by entacapone co-administration, regardless of genotype. 3-OMD production was confirmed even in patients with low COMT activity (L/L), suggesting that COMT function is not lacking completely. This is also consistent with a previous study, which reported that COMT activity in erythrocytes in the H/H genotype was approximately 1.6-fold higher than that in the L/L genotype (Corvol et al. 2011). The present results of $\Delta C_{3\text{-}OMD}$ support this previous finding.

In this study, we examined a standard low-dose entacapone (100 mg) for safety considerations. Initially, we hypothesized that this low dose would fail to inhibit the production of 3-OMD in patients with the highly active enzyme (H/H genotype). However, the results showed that 100 mg entacapone was sufficient to inhibit 3-OMD production, even in the H/H group. As the negative impact of 3-OMD on the clinical course of PD is becoming increasingly apparent (Feuerstein et al. 1977a, b; Mena et al. 1987; Lee et al. 2008), it is essential to inhibit 3-OMD accumulation in all patients, regardless of genotype. There was a concern that high baseline 3-OMD concentrations might impact the inhibitory effects of entacapone on 3-OMD formation due to saturated density; however, this was not observed in our study (data not shown). Since 3-OMD competes with L-dopa for transport to the BBB, inhibition of L-dopa metabolism to 3-OMD by entacapone would improve the ability of L-dopa to penetrate the brain. Thus, our results suggest that it may be necessary to inhibit COMT in all patients.

The present findings indicate that the Val158Met polymorphism may be a useful biomarker for dose adjustment of COMT inhibitors. Considering long-term treatment, it should be sufficient to prescribe a standard low-dose of entacapone (i.e., 100 mg) whenever patients are treated with L-dopa therapy, regardless of the COMT genotype. Dose-response studies have indicated that entacapone is maximally effective at 200 mg (Ruottinen and Rinne 1996); however, those studies did not consider the effects of genetic polymorphisms. Accordingly, a maximum dose of 200 mg should be considered in patients with H/L and L/L genotypes who present inadequate responses to

100 mg entacapone. In addition, it is recommended to adjust the dose of entacapone, based on the periodic measurement of plasma L-dopa and 3-OMD concentrations.

The current findings should be interpreted with consideration of some limitations. The sample size of patients with the L/L genotype in the current study was relatively small, and this study focused primarily on the acute effects of entacapone on the PK of L-dopa and 3-OMD. Further studies are warranted to determine the relationships among COMT polymorphisms, changes in 3-OMD, L-dopa blood levels, and motor complications after multiple administrations. The L-dopa dose in the present study was set at a relatively low level, 100 mg, based on safety considerations following consultations among neurologists and pharmacists. However, motor responses were rarely observed as the dose could have been insufficient to induce measurable motor responses. Therefore, future studies could include the assessment of motor responses at adequate L-dopa doses, such as 250 mg.

In conclusion, we demonstrated that the polymorphism of COMT Val158Met affected the plasma concentration of L-dopa administered with entacapone, particularly in individuals with the H/H genotype. Furthermore, we showed that administration of entacapone may be beneficial by suppressing the increase in blood 3-OMD concentration, regardless of the COMT genotype.

Declarations

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Conflict of interest

Ryosuke Takahashi has received research grant support from Dainippon Sumitomo Pharma Co., Ltd., Eisai, SANOFI, Pfizer Japan Inc., Novartis Pharma Co., Ltd., Takeda Pharmaceutical Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Nihon Medi-physics Co., Ltd, JB, and Medtronic Japan and has received consultation fees from Kan Institute Co., Ltd, Dainippon Sumitomo Pharma Co., Ltd., Takeda Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., and Kissei Pharmaceutical Co., Ltd.

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the ethics committee of Asahikawa Medical University (Approval no. 1431), and Kyoto University Graduate School and Faculty of Medicine (Approval no. G745).

Consent to participate

Written informed consent was obtained from all patients.

Author contributions

Conceptualization: Joe Yamamoto, Hitoshi Aizawa, and Yoshikazu Tasaki; Data curation: Joe Yamamoto, Tomohiro Omura and Yoshikazu Tasaki; Formal analysis and investigation: Joe Yamamoto, Tomohiro Omura, Shota Yamamoto, Masayoshi Kawata, Atsushi Yonezawa, Yosuke Taruno and Hisako Endo; Writing - original draft preparation: Joe Yamamoto; Writing - review and editing: Tomohiro Omura, Sachiko Kasamo, Kazuo Matsubara, and Yoshikazu Tasaki; Funding acquisition: Joe Yamamoto, Kazuo Matsubara, and Yoshikazu Tasaki; Supervision: Nobukatsu Sawamoto, Kazuo Matsubara, Ryosuke Takahashi, and Yoshikazu Tasaki.

All authors commented on the previous versions of the manuscript. All authors have read and approved the final manuscript.

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Table 1. Baseline characteristics of patients by catechol-O-methyltransferase (COMT) genotype

Characteristic	COMT genotype			*p*
	H/H (n = 24)	H/L (n = 23)	L/L (n = 7)	
Sex ratio (Male: Female)	1.00 (12:12)	0.77 (10:13)	1.33 (4:3)	0.806
Age, years	71.9 ± 7.55	67.4 ± 10.5	65.4 ± 13.6	0.170
Disease duration, years	6.92 ± 6.61	7.30 ± 6.26	8.43 ± 4.58	0.854
Hoehn and Yahr scale	3.40 ± 1.25	3.20 ± 1.21	2.71 ± 0.49	0.401
L-dopa (mg/day)	290 ± 277	272 ± 207	414 ± 125	0.367
L-dopa equivalent daily dose (mg)	454 ± 438	583 ± 580	852 ± 352	0.178
No. of patients currently treated with entacapone (%)	6 (25%)	8 (35%)	4 (57%)	0.291

Values are means ±S.D.

Table 2. The pharmacokinetics of L-dopa for group comparison

COMT genotype	H/H			H/L			L/L		
	ET (-)	ET (+)	Delta Ratio	ET (-)	ET (+)	Delta Ratio	ET (-)	ET (+)	Delta Ratio
L-dopa $AUC_{0\text{-}4h}$[a] (μg/mL・h)	2.52 ± 0.91	3.50 ± 1.59***	0.97 ± 0.91	2.19 ± 0.97	2.81 ± 1.13***	0.62 ± 0.41	2.70 ± 1.01	3.10 ± 1.20	0.41 ± 0.50
			1.39 ± 0.30			1.31 ± 0.19			1.13 ± 0.22
L-dopa $AUC_{0\text{-}inf}$[b] (μg/mL・h)	2.83 ± 1.11	4.48 ± 1.79***	1.65 ± 0.89†	2.44 ± 1.06	3.31 ± 1.32***	0.87 ± 0.75	3.09 ± 1.08	3.90 ± 1.51*	0.81 ± 0.76
			1.59 ± 0.26§			1.41 ± 0.36			1.28 ± 0.21
$t_{1/2}$[b] (h)	1.13 ± 0.32	1.55 ± 0.71***	0.42 ± 0.62	1.12 ± 0.21	1.37 ± 0.27***	0.25 ± 0.25	1.20 ± 0.18	1.96 ± 1.46*	0.87 ± 1.80
			n/a			n/a			n/a
C_{max} (μg/mL)	1.59 ± 0.66	1.85 ± 0.97	0.26 ± 0.85	1.51 ± 0.86	1.52 ± 0.70	0.01 ± 0.52	2.08 ± 1.20	1.58 ± 0.65	-0.50 ± 0.91
			n/a			n/a			n/a
T_{max} (h)	0.82 ± 0.59	1.06 ± 0.78	0.24 ± 0.95	0.90 ± 0.57	0.82 ± 0.45	-0.09 ± 0.51	0.82 ± 0.59	0.79 ± 0.57	-0.04 ± 0.62
			n/a			n/a			n/a

Values are means ± S.D. ET = entacapone; AUC = area under the concentration curve; Delta = ET (+)-ET (-); Ratio=ET (+)/ET (-); n/a = Not applicable.

a: One patient with the H/L genotype was not included in the calculation of $AUC_{0\text{-}4h}$ due to a patient to stop sampling.

b: Three patients with the H/H genotype were excluded because the $AUC_{0\text{-}inf}$ and $t_{1/2}$ could not be calculated due to atypical time-concentration curves.

Significant difference between ET (-) and ET (+): *p<0.05, ***p<0.001.

Significant difference between the H/H and H/L groups: †p<0.05.

Significant difference compared with the other groups: §p<0.05.

Table 3. Comparison of trough $C_{3\text{-OMD}}$ and $\Delta C_{3\text{-OMD}}$ at the final sampling time (4 hours) in the three groups

COMT genotype	H/H			H/L[a]			L/L		
	ET (-)	ET (+)	Difference	ET (-)	ET (+)	Difference	ET (-)	ET (+)	Difference
Trough $C_{3\text{-OMD}}$ (μg/mL)	2.37 ± 2.80	2.50 ± 2.73		1.75 ± 1.57	1.76 ± 1.44		1.80 ± 1.00	1.85 ± 1.02	
$\Delta C_{3\text{-OMD}}$ (μg/mL)	0.55 ± 0.62	-0.06 ± 0.38***	0.61 ± 0.66	0.36 ± 0.18	-0.06 ± 0.18***	0.42 ± 0.35	0.34 ± 0.24	-0.05 ± 0.21*	0.39 ± 0.29

Values are means ± S.D. ET = entacapone.

The difference in the level of 3-OMD before and after administration of L-dopa in each patient is expressed as $\Delta C_{3\text{-OMD}}$.

a: One patient with the H/L genotype was not included in the calculation due to a request to stop sampling.

Significant difference between ET (-) and ET (+): *p<0.05, ***p<0.001.

There was no significant difference between the three groups.

Figure legends

Fig. 1. Comparisons of the L-dopa $AUC_{0\text{-}4h}$ ratio according to the COMT Val158Met polymorphism

The effect of concomitant entacapone on the $AUC_{0\text{-}4h}$ of L-dopa in each genotype group. The boxplot represents the 25th percentile, median, and 75th percentile, with the whiskers representing the minimum (25th – 1.5*interquartile range) and maximum (75th + 1.5*interquartile range). Unfilled circles represent outliers.

Fig. 2. Plasma concentration-time profile of L-dopa

Plasma concentration-time curve of L-dopa in patients following administration of L-dopa/carbidopa 100/10 mg only (open circles, dotted line) or entacapone 100 mg (closed circles, solid line). Panels (A), (B), and (C) denote patients in the H/H (n = 24), H/L (n = 23), and L/L (n = 7) genotype groups, respectively. The plots represent the mean ± SD. A significant difference for the effect of entacapone co-administration is indicated as *$p<0.05$, ***$p<0.001$.

Fig. 3. Comparisons of the L-dopa $AUC_{0\text{-}inf}$ ratio according to the COMT Val158Met polymorphism

The effect of combined entacapone administration on the L-dopa $AUC_{0\text{-}inf}$ was compared between each genotype group. $AUC_{0\text{-}inf}$ was calculated using the moment analysis method. The boxplot

represents the 25th percentile, median, and 75th percentile, with whiskers representing the minimum (25th – 1.5*interquartile range) and maximum (75th + 1.5*interquartile range), and unfilled circles representing outliers. *p<0.05

Fig. 4. Comparison of the trough 3-OMD concentration/L-dopa daily dose (C/D) ratio

The C/D ratio of 3-OMD was calculated for patients who had received L-dopa before the study (n = 16 in the H/H group, n = 18 in the H/L group, and n = 7 in the L/L group). The boxplot represents the 25th percentile, median, and 75th percentile, with whiskers representing the minimum (25th − 1.5*interquartile range) and maximum (75th + 1.5*interquartile range), and unfilled circles representing outliers.

Fig. 5. Plasma concentration-time profile of $\Delta C_{3\text{-OMD}}$

Plasma concentration-time curve of increasing concentrations of 3-OMD($\Delta C_{3\text{-OMD}}$) in patients following administration of L-dopa/carbidopa 100/10 mg only (open circles, dotted line) or entacapone 100 mg (closed circles, solid line). The difference in 3-OMD pre- and post-administration of L-dopa in each patient is expressed as $\Delta C_{3\text{-OMD}}$. Panels (A), (B), and (C) represent the H/H (n = 24), H/L (n = 23), and L/L (n = 7) genotype groups, respectively. The plots represent the mean ± SD. A significant difference for the effect of entacapone co-administration is indicated as *p<0.05, **p<0.01, ***p<0.001.

Fig. 6. Comparisons of the $\Delta C_{3\text{-}OMD}$ AUC $_{0\text{-}4h}$ (L-dopa without entacapone) according to the COMT Val158Met polymorphism

The effect of genotype on the $\Delta C_{3\text{-}OMD}$ AUC $_{0\text{-}4h}$ following the administration of L-dopa only without entacapone was compared (n = 23 in the H/H group, n = 22 in the H/L group, and n = 7 in the L/L group). The results of one patient with the H/H genotype were not included in the calculation due to atypical time–concentration curves, and those of one patient with the H/L genotype were not included due to a request to stop sampling. $AUC_{0\text{-}4h}$ was calculated using the moment analysis method. The boxplot represents the 25th percentile, median, and 75th percentile, with whiskers representing the minimum (25th – 1.5*interquartile range) and maximum (75th + 1.5*interquartile range), and unfilled circles representing outliers.

Fig. 1

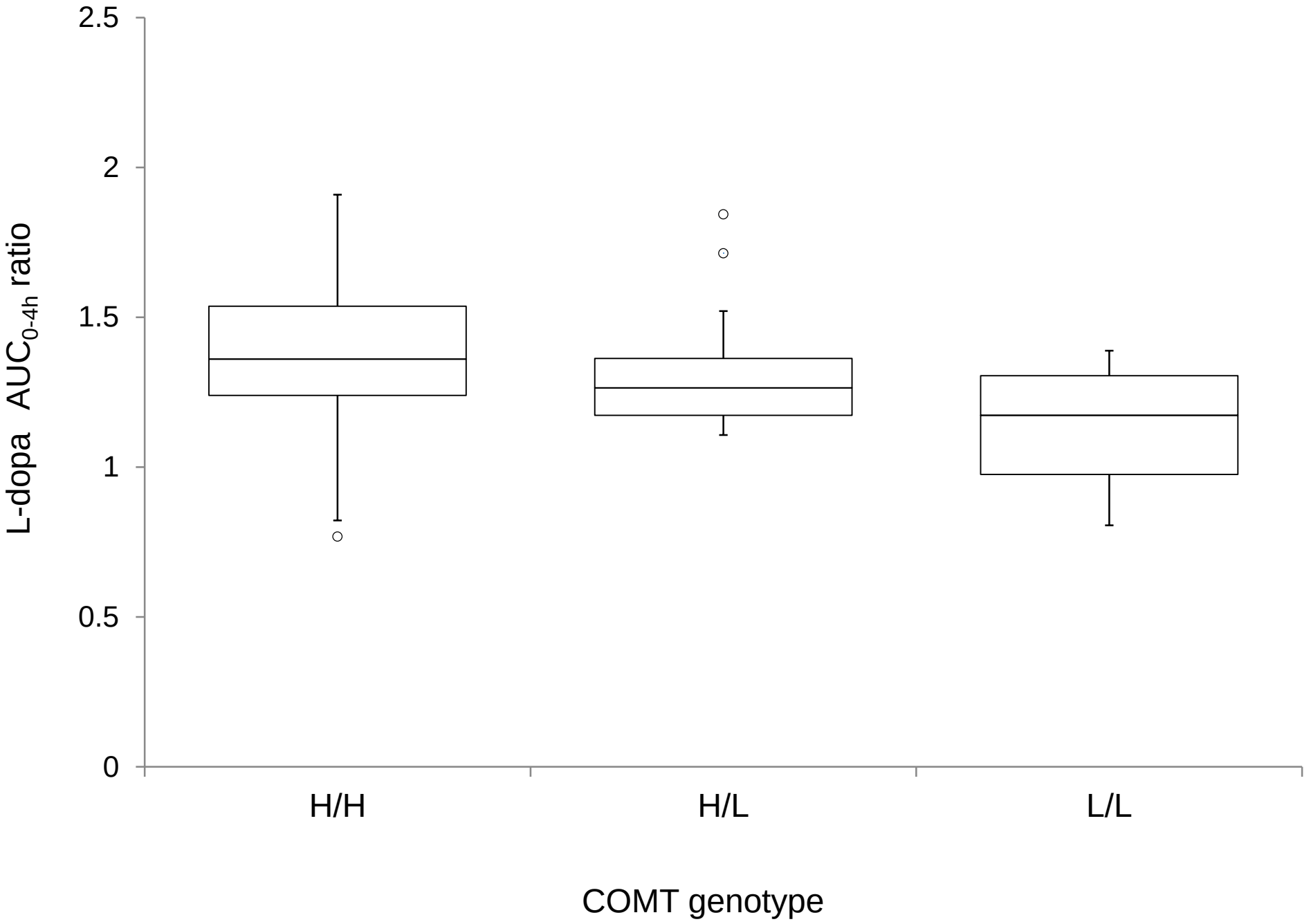
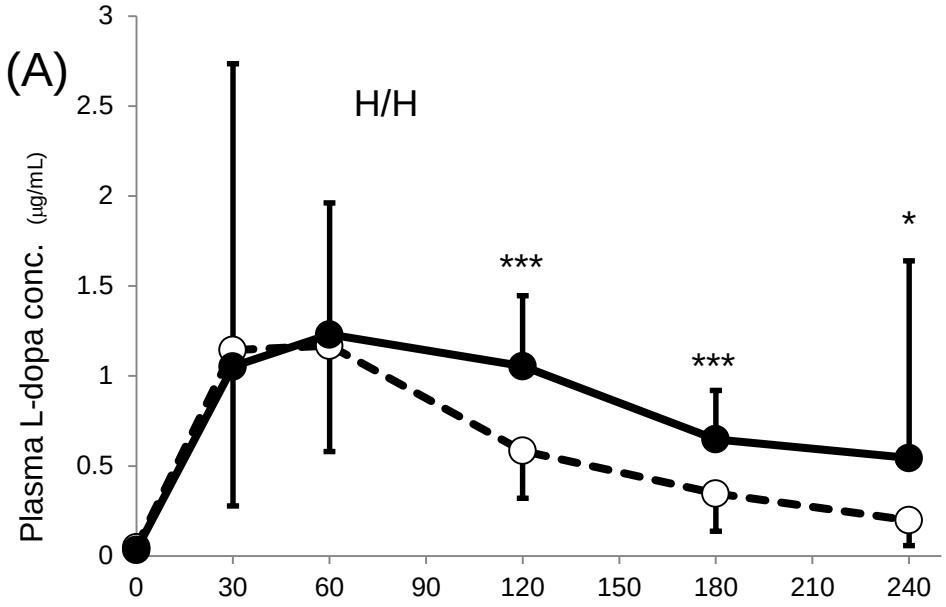
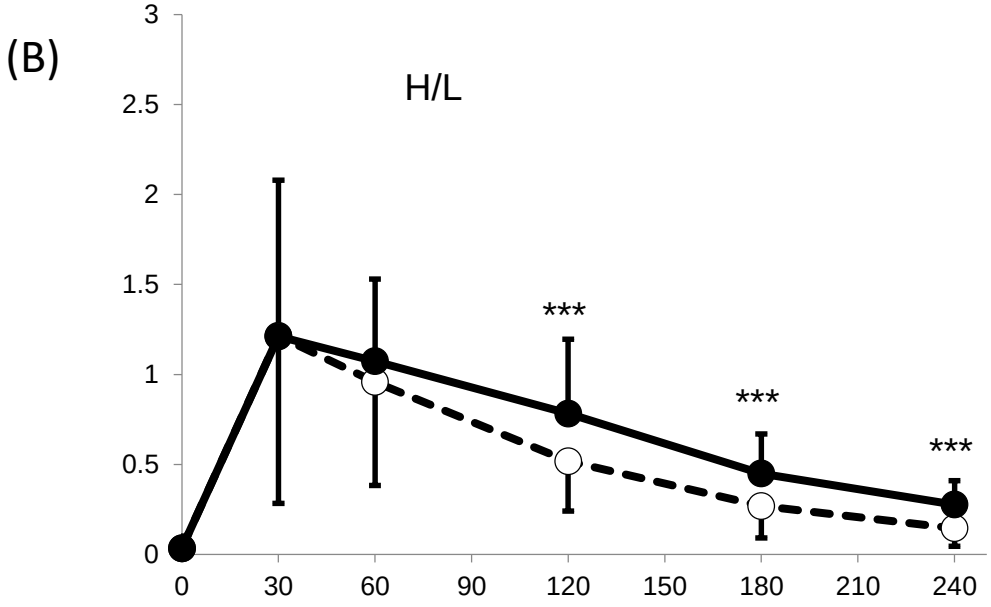


Fig. 2





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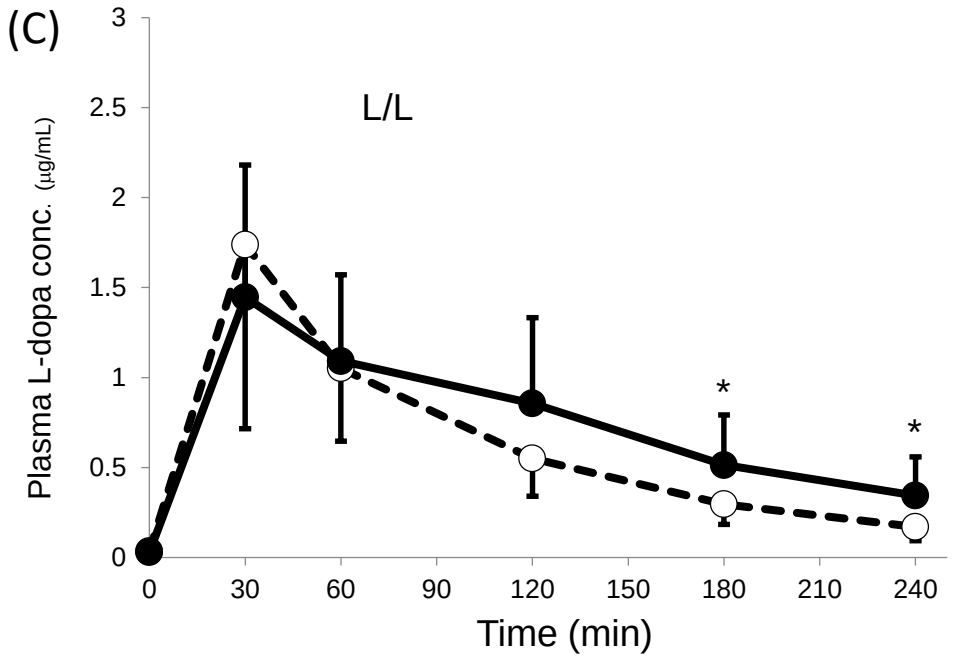
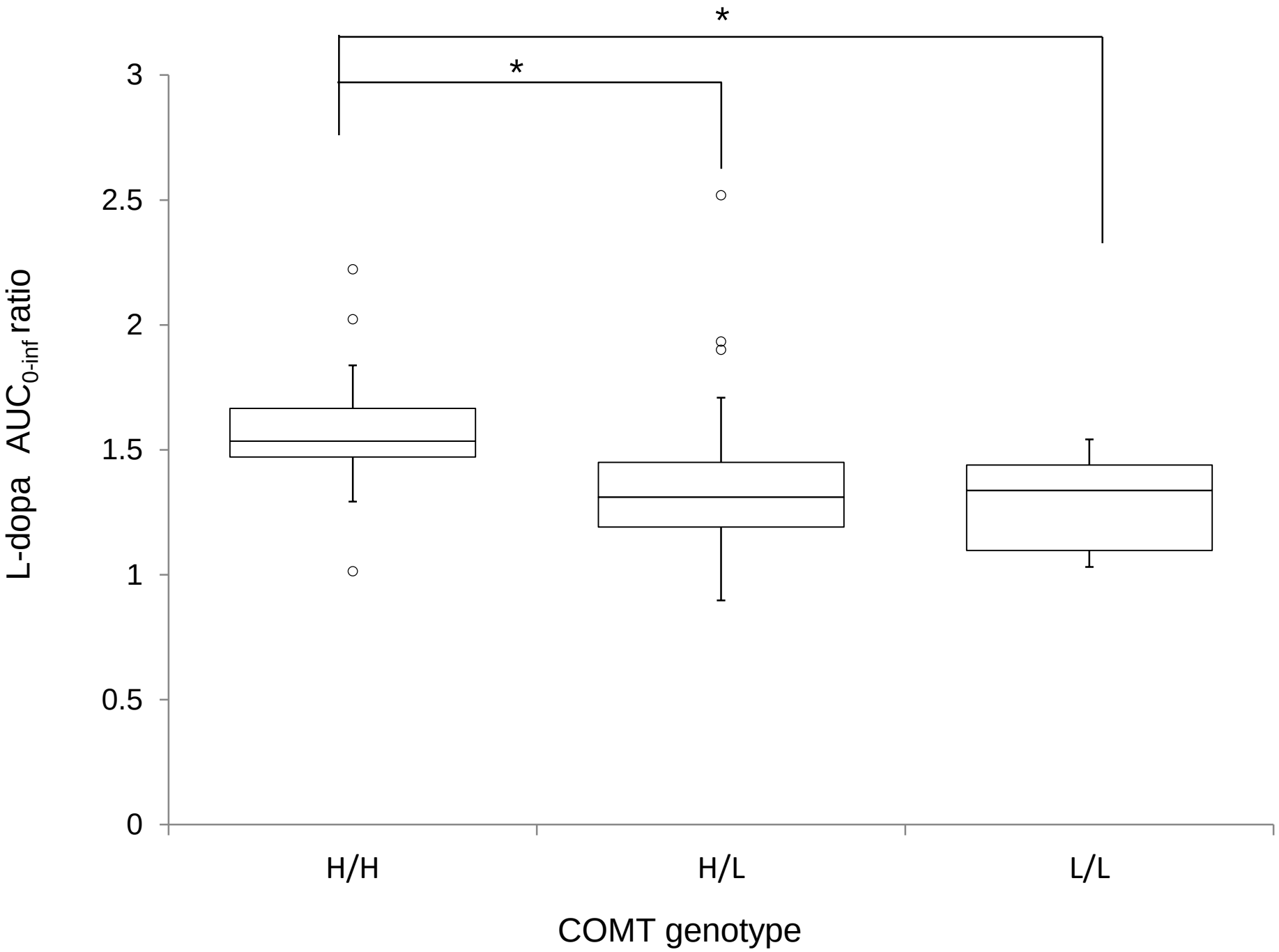


Fig. 3



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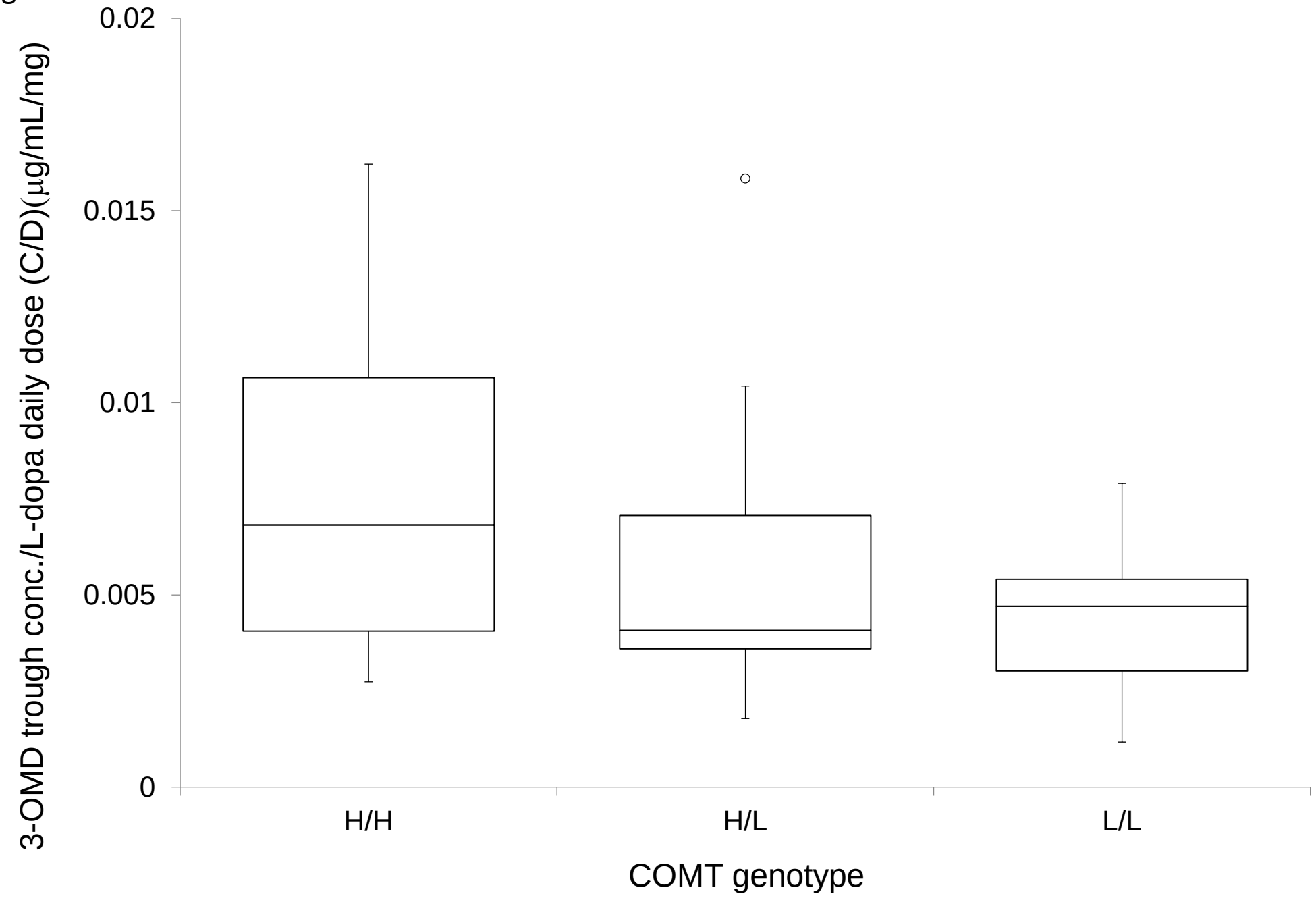

0.02
0.015
0.01
0.005
0
3-OMD trough conc./L-dopa daily dose (C/D)(μg/mL/mg)
H/H
H/L
L/L
COMT genotype

Fig. 5

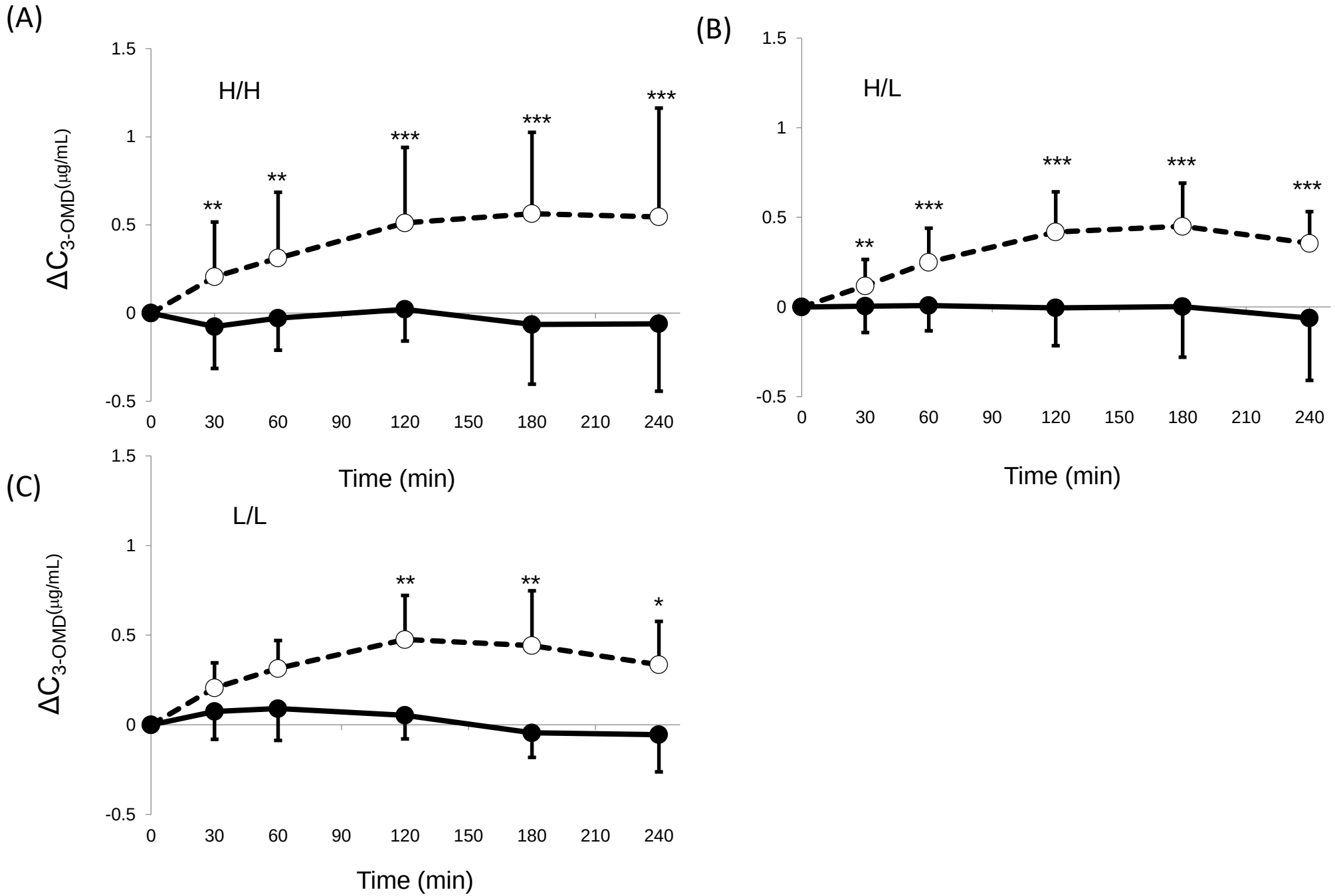
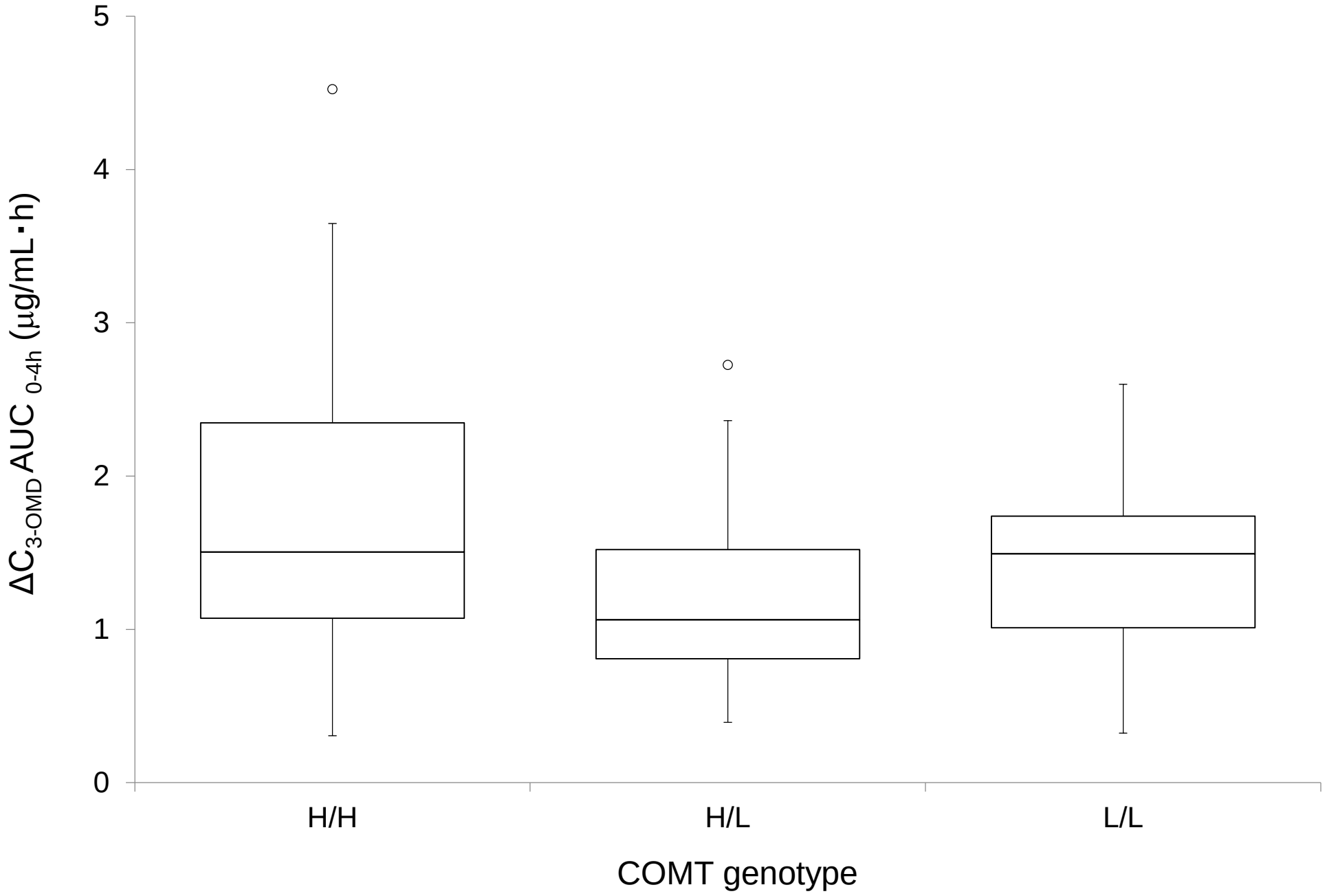

(A)
H/H
$\Delta C_{3\text{-}OMD}$(μg/mL)
1.5
1
0.5
0
-0.5
0
30
60
90
120
150
180
210
240
Time (min)
**
**
***
***
***
(B)
H/L
1.5
1
0.5
0
-0.5
0
30
60
90
120
150
180
210
240
Time (min)
**
***
***
***
***
(C)
L/L
$\Delta C_{3\text{-}OMD}$(μg/mL)
1.5
1
0.5
0
-0.5
0
30
60
90
120
150
180
210
240
Time (min)
**
**
*

Fig. 6


5
4
3
2
1
0
$\Delta C_{3\text{-OMD}}$ AUC $_{0\text{-}4h}$ (μg/mL·h)
H/H
H/L
L/L
COMT genotype