The Effect of *Yokukansan*, a Traditional Herbal Preparation Used for the Behavioral and Psychological Symptoms of Dementia, on the Drug–Metabolizing Enzyme Activities in Healthy Male Volunteers

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The concomitant use of herb and prescription medications is increasing globally. Herb–drug interactions are therefore a clinically important problem. *Yokukansan* (YKS), a Japanese traditional herbal medicine, is one of the most frequently used herbal medicines. It is effective for treating the behavioral and psychological symptoms of dementia. We investigated the potential effects of YKS on drug–metabolizing enzyme activities in humans. An open-label repeat-dose study was conducted in 26 healthy Japanese male volunteers (age: 22.7±2.3 years) with no history of smoking. An 8-h urine sample was collected after a 150-mg dose of caffeine and a 30-mg dose of dextromethorphan before and after the administration of YKS (2.5g, twice a day for 1 week). The activities of cytochrome P450 (CYP) 1A2, CYP2D6, CYP3A, xanthine oxidase (XO) and N-acetyltransferase 2 (NAT2) were assessed based on the urinary metabolic indices of caffeine and dextromethorphan, and the urinary excretion ratio of 6b-hydroxycortisol to cortisol. There were no statistically significant differences in the activities of the examined enzymes before or after the 7-d administration of YKS. Although further studies assessing the influence of YKS on the pharmacokinetics and pharmacodynamics of the substrates of the drug–metabolizing enzymes are needed to verify the present results, YKS is unlikely that a pharmacokinetic interaction will occur with concomitantly administered medications that are predominantly metabolized by the CYP1A2, CYP2D6, CYP3A, XO and NAT2.

**Key words** yokukansan (YKS); herb–drug interaction; drug–metabolizing enzyme; Kampo

Dementia is a progressive, irreversible illness and is a worldwide health problem that is associated with an increasing public health burden.1,2 Alzheimer’s disease, a neurodegenerative brain disorder, is the most common form of dementia.3,4 Fifty to ninety percent of Alzheimer’s disease patients present with behavioral and psychological symptoms of dementia (BPSD),3,5 which are associated with a rapid rate of cognitive decline, greater impairment in activities of daily living and diminished quality of life.6 Approximately 95% of Alzheimer’s disease diagnoses involve patients who are over 65 years of age, and the disease affects 4–8% of the elderly population worldwide.2 Since elderly patients are generally treated with polypharmacy regimens,4 there is a high possibility of adverse effects due to drug interactions.

Herbal medicines have been widely used as a complementary or alternative treatment for a variety of diseases.5,6 Since the concomitant use of herbal medicines and prescribed medications is increasing globally, herb–drug interactions are recognized as a clinically important problem.7–9 In Japan, a number of Japanese traditional herbal medicines (“Kampo” in Japanese), which originated from Chinese herbal medicines, are frequently used.9 *Yokukansan* (YKS; TJ-54, Tsumura & Co., Japan), one of the most frequently prescribed Kampo medicines, is derived from a Chinese herbal medicine, Yi-Gan San.10 The prescription of YKS is approved by the Ministry of Health, Labour and Welfare of Japan for the treatment of symptoms of delicate constitution and nervousness (i.e., neurosis, insomnia, and night crying and peevishness in children).10 In addition, several randomized studies have demonstrated that YKS has a therapeutic effect on BPSD,11–13 Since YKS is used for BPSD in Japan, YKS is administered frequently to elderly patients who use various medications.4 Therefore, there is a high possibility that YKS is used in combination with several drugs metabolized by drug–metabolizing enzymes, such as CYP 2D6, CYP3A and CYP1A2 (e.g. donepezil, risperidone, olanzapine, aripiprazole, paroxetine, theophylline and atorvastatin).

In the screening of the herb–drug interactions associated with YKS, the induction or inhibition of drug–metabolizing enzymes through the use of YKS should be considered. YKS consists of seven herbs, i.e., Atractylodes lancea rhizome, Poria sclerotium, Cnidium rhizome, Uncaria hook, Japanese Angelica root, Bupleurum root and Glycyrrhiza.10 Several of the components of YKS have been reported to be associated with the activity of drug–metabolizing enzymes in vitro, including CYP2D6, CYP3A and/or CYP1A214–16 (see Supple-
YKS may therefore affect the metabolism of a number of medicines that are associated with CYP3A4 activity. However, YKS was not found to be associated with the drug disposition of triazolam or donepezil, which are mainly metabolized by CYP3A4, in rat and mouse in vivo studies. A previous in vitro study showed that YKS resulted in little inhibition of the activities of CYP3A4 and 2D6, and IC₅₀ were 0.169 mg/mL for CYP3A4 (verapamil, i.e., positive control for CYP3A4, was 0.366 µg/mL and 0.366 mg/mL for CYP2D6 (quinidine, i.e., positive control for CYP2D6, was 0.004 µg/mL). YKS consists of extract granules from the mixture of several crude drugs, and the concentration of each ingredient of YKS in humans in vivo remains to be fully elucidated. As such, the effects of YKS on the drug–metabolizing enzymes in humans must be assessed in vivo as well as in vitro.

We recently assessed the effects of the repeated dosing with several Kampo medicines, sho-saiko-to, bakumon-do-to, sho-seiryu-to, keishi-bakuryo-gan and seijo-bofu-to on the activities of drug–metabolizing enzymes, including CYP1A2, CYP3A, CYP2D6, N-acetyltransferase 2 (NAT2) and/or xanthine oxidase (XO), in healthy volunteers using caffeine and dextromethorphan (DM) as phenotyping probes and by performing urinary assays of endogenous free cortisol (FC) and 6β-hydroxycortisol (6β-HC). In this study, we attempted to evaluate the effects of YKS on the activities of drug–metabolizing enzymes (CYP1A2, CYP3A, CYP2D6, NAT2, XO) in Japanese healthy volunteers using a similar procedure. In addition, we investigated the potential effects of YKS on the activities of CYP2D6, CYP1A2 and NAT2 with these common polymorphisms. The primary objective of this clinical study was to verify the potential pharmacokinetic interaction of YKS, which is likely to be used in combination with various medicines that are mainly metabolized by the drug–metabolizing enzymes, such as antidepressant, antipsychotic and antidepressants.

MATERIALS AND METHODS

Agents Seven and a half grams of YKS (TJ-54, TSUMURA Yokukansan extract granules; Tsumura & Co.) contains 3.25 g of a dried extract of the following mixed crude drugs: *Atractylodes lancea* rhizome (4.0 g), *Poria* sclerotium (4.0 g), *Cnidium* rhizome (3.0 g), *Uncaria* hook (3.0 g), *Angelica* root (3.0 g), *Bupleurum* root (2.0 g), *Glycyrrhiza* (1.5 g) and inactive ingredients (lactose hydrate and magnesium stearate).

Study Participants The open-label study conducted in 26 healthy, Japanese male volunteers with no history of smoking (mean age, 22.7±2.3 years; mean body-weight, 65.4±7.8 kg) who were student population of Kumamoto University, Kumamoto, Japan. All of the subjects were diagnosed to be healthy by face-to-face interview. The following exclusion criteria were applied: a history of smoking; a history of the diseases that alter the metabolism and/or excretion of drugs (such as gastroenterological, cardiovascular, pulmonary and hepatic diseases); a history of allergy to YKS, any herb or non-herbal medicine, caffeine and/or DM; the use of YKS or other herbal medicines within 2 weeks before enrollment in the present study; the use of behind- or over-the-counter drugs within 1 week before enrollment in the present study; and the use of any investigational drugs within 3 months before enrollment in the present study. The written informed consent was obtained from all subjects before entry into the present study.

Genotyping Genomic DNA was extracted from whole blood using a DNA purification kit (FlexiGene DNA kit, QIAGEN, Hilden, Germany). The CYP2D6*10 (100C>T, rs1065852), CYP1A2*1C (−2964G>A, rs2695148), CYP1A2*1F (734C>A, rs762551), NAT2*6 (590G>A, rs179930) and NAT2*7 (857G>A, rs179931) genes were genotyped by a real-time TaqMan allelic discrimination assay (Applied Biosystems, CA, U.S.A.) according to the manufacturer’s protocols (assay Nos. C_3481616_20, C_1585991_30, C_8881212_40, C_572770_20 and C_1204091_10). To ensure the genotyping quality, we included DNA samples as internal controls, hidden samples of a known genotype, and negative controls (water).

Study Design and Analytical Procedures This study protocol was approved by the ethics committees of the Faculty of Life Sciences, Kumamoto University. All participants were not allowed to take behind- or over-the-counter medicines or to consume any beverages or foods containing xanthine, alcohol beverage or processed fruit products from 48 h before the drug administration until the end of the study protocol. The subjects were instructed to ingest 2.5 g of YKS twice daily for 7 d before their morning and evening meals. Compliance with YKS was assessed using a pill count and self-reporting of missed doses during the study period. The phenotyping testing was performed on the day before taking YKS (i.e., baseline) and on the 7th day after taking YKS (day 7). After bladder emptying, the volunteers received a 30 mg

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Phenotypic index</th>
<th>Baseline</th>
<th>Day 7</th>
<th>Day 7/Baseline ratio</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>AAMU/(AAMU+1U+1X)/17U</td>
<td>12.72±7.62</td>
<td>11.72±7.85</td>
<td>0.92 (0.82–1.03)</td>
<td>0.116</td>
</tr>
<tr>
<td>NAT2</td>
<td>AAMU+1U+1X+1U</td>
<td>0.74±0.13</td>
<td>0.73±0.14</td>
<td>0.99 (0.95–1.02)</td>
<td>0.499</td>
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<tr>
<td>XO</td>
<td>1U/(1U+1X)</td>
<td>0.73±0.11</td>
<td>0.73±0.12</td>
<td>0.99 (0.96–1.02)</td>
<td>0.426</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>DM/DX</td>
<td>2.21±4.06</td>
<td>1.75±4.27</td>
<td>0.79 (0.58–1.07)</td>
<td>0.237</td>
</tr>
<tr>
<td>CYP3A</td>
<td>(MM+HM)/(DM+DX)</td>
<td>0.53±0.46</td>
<td>0.50±0.51</td>
<td>0.96 (0.78–1.18)</td>
<td>0.689</td>
</tr>
<tr>
<td>CYP3A</td>
<td>6β-HC/FC</td>
<td>22.09±62.39</td>
<td>16.07±19.69</td>
<td>0.73 (0.49–1.08)</td>
<td>0.227</td>
</tr>
</tbody>
</table>

CYP, cytochrome P450; NAT2, N-acetyltransferase 2; XO, xanthine oxidase; YKS, yokukansan; AAMU, 5-acetylamino-6-amino-3-methyluracil; 1U, 1-methyl-2furanic acid; 1X, 1-methoxynanth; 17U, 1,7-dimethyluric acid; DM, dextromethorphan; DX, dextorphrin; MM, 3-methoxynorpholin; HM, 3-hydroxynorpholin; 6β-HC, 6β-hydroxycortisol; FC, free cortisol. a Data are given as geometric mean±S.D. b The ratio of the geometric mean of day 7 to that of baseline for each phenotyping index with 90% CI in parenthesis. c The log-transformed phenotypic indices of baseline and day 7 were compared using the Wilcoxon signed-rank test.
A dose of DM (MEDICON; Shionogi Ltd., Osaka, Japan) and a 150 mg dose of caffeine (Merck, Darmstadt, Germany) before bedtime. An overnight urine sample was collected from each subject (from approximately 23:00 to 7:00 h). The mean duration urine collection was 8.7 ± 0.8 h. Spot urine sample (blank controls) was also collected from each subject before the administration of caffeine and DM on the baseline and on day 7. Thereafter, a 1 mL urine sample acidulated with ascorbic acid (10 mg/mL) and a 20 mL untreated urine sample were stored until use at −30°C. The urinary concentrations of three caffeine metabolites [1,7-dimethyluric acid (17U), 1-methyluric acid (1U) and 1-methylxanthine (1X)], DM and three DM metabolites [dextrorphan (DX), 3-methoxymorphinan (MM) and 3-hydroxymorphinan (HM)], 6β-HC and FC were quantified by HPLC according to a previously described method. 5-Acetyl-amino-6-amino-3-methyluracil (AFMU) was completely converted to 5-acetyl amino-6-amino-3-methyl uracil (AAMU) under condition of a pH of 10, and then the AAMU was measured by HPLC according a previously described method.

Statistical Analysis The levels of CYP1A2, CYP2D6, CYP3A, NAT2 and XO activities were assessed according to the methods of our previous reports, using the following equations.

![Fig. 1. Individual Values of the Urinary Molar Concentration Ratios of (AAMU+1U+1X) to 17U for CYP1A2 (A), AAMU to (AAMU+1X+1U) for NAT2 (B), 1U to (1U+1X) for XO (C), DM to DX for CYP2D6 (D), (MM+HM) to (DM+DX) for CYP3A (E) and 6β-HC to FC for CYP3A (F) in the 26 Healthy Male Volunteers before (Baseline) and on the Seventh Day (Day 7) after the Administration of YKS](image-url)
CYP1A2 activity = (AAMU + 1U + 1X)/17U
NAT2 activity = AAMU / (AAMU + 1X + 1U)
XO activity = 1U / (1X + 1U)
CYP2D6 activity = DM / DX
CYP3A activity = (MM + HM) / (DM + DX)
CYP3A activity = 6β – HC / FC

High index values of CYP1A2, NAT2, XO and CYP3A indicate high activity levels. Conversely, a high index value of DM/DX indicates a low CYP2D6 activity level.

The geometric mean ratios and 90% confidence intervals (CIs) were calculated for each phenotypic index as the ratio of the geometric mean on day 7 to that at baseline. We concluded that YKS had no effect if the 90% CI was within the range of 0.80–1.25. We also compared the log-transformed values of the phenotypic indices between the baseline values and the day 7 values (i.e., repeated observations of the same volunteer) by the nonparametric Wilcoxon signed-rank test.

RESULTS

All of the enrolled volunteers completed the study according to the protocol and took all of the medications that were dispensed. None of the volunteers experienced any adverse effects from YKS during the study period.

The phenotypic indices for CYP1A2, NAT2 and XO, which were calculated from the baseline caffeine metabolite (17U, 1U and 1X) levels, ranged from 4.34 to 31.25 (a 7.2-fold difference), 0.42 to 0.95 (a 2.3-fold difference) and 0.50 to 0.94 (a 1.9-fold difference), respectively. The phenotypic indices for CYP2D6 and CYP3A, which were calculated from the baseline levels of DM and its metabolites (DX, MM, HM) ranged from 0.55 to 13.54 (a 24.6-fold difference) and 0.13 to 2.42 (a 18.6-fold difference), respectively. The 6β-HC/FC indices for baseline activity of CYP3A ranged from 4.83 to 315.78 (a 65.4-fold difference). Table 1 and Fig. 1 show the phenotyping indices for CYP1A2, XO, NAT2, CYP2D6 and CYP3A activities on the day before (baseline) and on the seventh day (day 7) after the administration of YKS. There were no statistically significant differences in the baseline and day 7 geometric mean ratios (90% CI) for CYP1A2 and CYP3A.

DISCUSSION

The twice-daily administration of YKS for 7 days did not significantly affect the activities of CYP1A2, CYP3A, CYP2D6, NAT2 or XO among healthy, non-smoking male Japanese volunteers (Table 1 and Fig. 1). YKS is frequently prescribed in patients with BPSD (in the elderly population), and is therefore likely to be used in combination with various medicines (such as antidepressants, antipsychotics, antidiabetics, anxiolytics, insomnia drugs, antihypertensive and antiplatelet drugs and anti-inflammatory drugs). The results of the present study suggest that YKS may be safe to use in combination with medicines that are metabolized predominantly by CYP1A2, CYP3A, CYP2D6, NAT2 or XO.

Several components of YKS can affect CYP1A2, XO and NAT2 activity in vitro. Saffrole, isosaffrole and liquiritigenin (essential constituents of Japanese Angelica root or Glycyrrhiza, which are components of YKS) have been shown...
to reduce the CYP1A2 activity in human tissue.\textsuperscript{14–16} However, the results of the caffeine test in the present study showed that the 90\% CI ranges of the phenotypic indices of CYP1A2, NAT2 and XO were within the bioequivalence range defined by the Food and Drug Administration (FDA) (Table 1 and Fig. 1). YKS may therefore have a low potential for pharmacokinetic interaction with drugs that are metabolized by CYP1A2, NAT2 or XO (e.g. theophylline, olanzapine, clozapine). In our previous studies on the effects of Kampo medicines on the drug-metabolizing enzymes of healthy volunteers,\textsuperscript{25–27} the 7-d repeated administration of sho-saiko-to, keishi-bukuryo-gan and seijo-bofu-to inhibited CYP1A2 activity.\textsuperscript{23,26,27} Both sho-saiko-to and seijo-bofu-to contain scutellaria root bark, the components of which (baicalein, wogonin and oroxylin A) have been reported to inhibit the CYP1A2 activity in human liver microsomes.\textsuperscript{31} "Keishi-bukuryo-gan" contains cinnamon bark, the component of which (cinnamaldehyde) inhibits the CYP1A2 activity in rat liver microsomes.\textsuperscript{32} YKS does not contain either scutellaria root bark or cinnamon bark.

Although CYP2D6 is minor abundant CYP enzyme in the liver, it is involved in the metabolism of a number of drugs and plays a role in the clearance of more than 15\% of the drugs that are in clinical use, including antidepressants (e.g. donepezil), antipsychotics (amitriptyline, paroxetine, venlafaxin), antipsychotics (aripiprazole, risperidone), codeine and many others.\textsuperscript{29} We observed no significant differences in the CYP2D6 activity before or after the administration of YKS in any of the subjects (Table 1 and Fig. 1). We therefore suggest that YKS may have a low potential for pharmacokinetic interaction with drugs that are metabolized by CYP2D6.

CYP2D6, which has more than 100 allelic variants, is highly polymorphic. The variants have been reported to be associated with the inter-individual differences in CYP2D6 activity.\textsuperscript{29} Individuals can be classified based on the presence of CYP2D6 allelic variants into the following subgroups: poor, intermediate, extensive and ultra-extensive metabolizers.\textsuperscript{29} In Asian populations, the CYP2D6*10 allele associated with intermediate enzyme activity is common, whereas the frequencies of CYP2D6 non-functional alleles are rare.\textsuperscript{29} In drug interaction studies, it is recommended that genetically-determined metabolic polymorphisms be identified when evaluating drug effects on drug-metabolizing enzymes with polymorphisms, including CYP2D6.\textsuperscript{30} We observed that the administration of YKS only induced CYP2D6 activity among CYP2D6*10 allele carriers, and that the effect disappeared following the use of Bonferroni’s correction. The CYP1A2 and NAT2 polymorphisms have also been reported to be associated with inter-individual variability in their activities.\textsuperscript{28,29} CYP1A2*IC and *IF alleles are associated with decreased or increased inducibility of CYP1A2 activity.\textsuperscript{29} NAT2*6 and *7 alleles are associated with the slow enzyme activity of NAT2.\textsuperscript{29} When the subjects were stratified according to the presence of CYP1A2*IC or *IF allele, and the NAT2 phenotypes classified according to the NAT2*6 and *7 alleles, YKS did not influence the CYP1A2 or NAT2 activities among all subject groups. Therefore, YKS may not affect the metabolism of the medicines that are mainly metabolized by CYP2D6, CYP1A2 or NAT2, regardless of the CYP2D6, CYP1A2 or NAT2 genotype. However, the sample size of each genotype group was too small to draw any definitive conclusions, and other minor functional alleles of CYP2D6, CYP1A2 and NAT2 may have been present in the study subjects and influenced their activities.\textsuperscript{28,29} Further studies are therefore needed to overcome these potential limitations associated with the present study.

CYP3A is the most abundant CYP enzyme in the human liver and gut, and the subfamily enzymes play a major role in the clearance of approximately 30\% of the drugs that are in clinical use from the human body.\textsuperscript{29} In our previous studies, we assessed the effects of sho-saiko-to, sho-seiryu-to, keishi-bukuryo-gan and seijo-bofu-to on CYP3A activity using DM as a phenotyping probe and by performing urinary assays of endogenous free cortisol and 6β-hydroxycortisol.\textsuperscript{23,25–27} Therefore, in the present study, we evaluated the CYP3A activity using a similar procedure in order to compare our findings with the results of previous studies. The findings of the present study showed that YKS did not influence the (MM+HM)/(DM+HM) and 6β-HC/FC index values (Table 1 and Fig. 1), suggesting that YKS may not have any significant effects on CYP3A activity in human beings. However, these substrates have relatively low clearance; therefore, it is not appropriate to detect the effect of YKS on CYP3A activity in the intestine. The area under the blood concentration–time curve (AUC) of oral midazolam was the most accurate marker for the in vivo assessment of CYP3A activity.\textsuperscript{30} The procedure for midazolam required multiple courses of blood sampling and oral preparation of midazolam has not been approved as a prescription drug by the Ministry of Health, Labour and Welfare of Japan. For this reason, we could not use midazolam to assess the CYP3A activity in the present study. On the other hand, the FDA guidelines list many other highly sensitive CYP3A substrates for in vivo use (e.g. lovastatin, simvastatin, nisoldipine, quetiapine, triazolam).\textsuperscript{30} Therefore, further studies using midazolam or some other highly extracted CYP3A substrates are needed to verify the present results.

In the present clinical study, the subjects received 2/3 of the usual dose of YKS (7.5 g daily) for 7 d, in order to avoid adverse reactions (e.g. pseudohyperaldosteronism, hypokalemia from Glycyrrhiza).\textsuperscript{31} In Japan, patients with BPSD (depending on their symptoms) usually receive YKS for long periods of time.\textsuperscript{30} The inhibitors of drug–metabolizing enzymes usually act rapidly.\textsuperscript{33} The 7-d administration of fluvoxamine, a CYP1A2 inhibitor, prolonged the elimination half-life and impaired the oral clearance of caffeine.\textsuperscript{33} In addition, the 5-d administration of rifampicin, a CYP3A inducer, decreased the AUC of zolpidem, which is metabolized by CYP3A.\textsuperscript{33} We therefore consider the 7-d period for which YKS was administered to be appropriate. In contrast, Supplementary Table 1 shows that several ingredients of YKS herbal components were associated with drug–metabolizing enzyme activities. Therefore, the dose of ingredients of YKS components might be lower than that in the previous reports. Most of the in vitro studies used the ingredients directly, whereas YKS is a dried extract of a mixture of crude drugs, and the information regarding the dose of these ingredients in YKS is not available. We were therefore unable to compare the dose of the ingredients in YKS used in the present study with that in the previous in vitro studies. In one clinical study, the pharmacokinetic parameters of several active ingredients of YKS, such as 18β-glycyrrhetinic acid and geissoschizine methyl ether, were determined in healthy subjects;\textsuperscript{36} but there are presently no data available regarding the blood concentrations of
the ingredients listed in Supplementary Table 1. In addition, we were unable to collect enough blood samples to assess the concentration of each ingredient of these herbal components. Therefore, the exact reasons for the inconsistency between our results and previous in vitro findings remain unknown. Furthermore, the subjects of the present study were young, healthy male volunteers and YKS is frequently prescribed to elderly patients. An age-related decrease in drug–metabolizing enzyme activity can decrease drug clearance, and thus the effects of the administration of YKS on the drug–metabolizing enzymes of young and elderly individuals may be different. The present results should be verified in further studies with elderly patients who receive the standard dose of YKS after incorporating the data of adverse effects.

Our findings suggest that the effects of YKS on the activities of CYP1A2, XO, NAT2, CYP2D6 and CYP3A were not significant. Since the present study subjects were healthy volunteers, further studies, which include elderly subjects, are necessary to assess whether YKS could influence the activity levels of drug–metabolizing enzymes to a clinically significant extent. Moreover, the CYP3A phenotype was assessed according to the urinary metabolic indices of DM and the urinary excretion ratio of β6-HC to cortisol; thus, the findings should be replicated using more accurate procedures. Nevertheless, our findings suggest that YKS is unlikely to participate in pharmacokinetic drug interactions involving medicines that are predominantly metabolized by CYP1A2, CYP2D6, CYP3A, XO and NAT2, although the present results warrant further investigations with a larger number of subjects to verify the influence of YKS on the pharmacokinetics and pharmacodynamics of the substrates of drug–metabolizing enzymes.

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Conflict of Interest Dr. Kazuko Nakagawa received a Research Grant from Tsumura & Co. Dr. Norio Yasui-Furukori received grant/research support or honoraria from and spoken for Asaster, Dainippon, Eli Lilly, GSK, Janssen-Pharma, Meiji Mochida, MSD, Otsuka, Pfizer, Takada and Yoshitomi. There are no patents, products in development or marketed products to declare. The authors declare that no other competing interests exist.

Supplementary Materials The online version of this article contains supplementary materials.

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