

ORIGINAL ARTICLE

# Impact of four loci on serum tamsulosin hydrochloride concentration

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Tamsulosin hydrochloride is one of the most potent drugs for treatment of benign prostatic hyperplasia (BPH), however, the efficacy of tamsulosin hydrochloride varies among individuals. In this study, we measured the maximum serum concentration (C<sub>max</sub>) of tamsulosin hydrochloride in 182 of BPH patients and found remarkable individual variability. To investigate the genetic factors that regulate pharmacokinetics of tamsulosin hydrochloride, we conducted a genome-wide association study in these 182 BPH patients. As a result, rs16902947 on chromosome 5p13.2, rs7779057 on 7q22.3, rs35681285 on 7p21.2 and rs2122469 on 8p21.3 indicated possible associations with C<sub>max</sub> of tamsulosin hydrochloride ( $P = 1.29 \times 10^{-7}$ ,  $2.15 \times 10^{-7}$ ,  $4.35 \times 10^{-7}$  and  $7.03 \times 10^{-7}$ , respectively), although these single-nucleotide polymorphisms (SNPs) did not reach the genome-wide significance threshold after Bonferroni correction. As these associated SNPs showed additive effects on serum tamsulosin hydrochloride concentration, we defined the 'C<sub>max</sub> prediction index' based on genotypes of these SNPs. This index clearly associated with C<sub>max</sub> values ( $P = 4.5 \times 10^{-6}$ ), indicating the possible roles of these four variants in tamsulosin hydrochloride pharmacokinetics. Our findings would partially explain the variability of the response to the tamsulosin hydrochloride treatment.

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**Keywords:** genome-wide association study; pharmacokinetics; prostatic hyperplasia; tamsulosin hydrochloride

## INTRODUCTION

Lower urinary tract symptoms caused by benign prostatic hyperplasia (BPH) is one of the most common problems in elderly men.<sup>1</sup> Though it is not a life-threatening disorder, BPH can affect quality of life by various symptoms such as urinary frequency, urinary urgency, nocturia and sensation of incomplete bladder emptying. In addition, the number of BPH patients has been significantly increasing in accordance with the aging of the population in developed countries.

Although the etiology of BPH is not fully understood,  $\alpha 1$  adrenergic receptors ( $\alpha 1$ ARs) were shown to have pivotal roles in the development of BPH.  $\alpha 1$ ARs belong to the G-protein-coupled receptor super family and mediate the actions of the catecholamine, norepinephrine and epinephrine. Norepinephrine released from sympathetic nerves would regulate prostatic smooth muscle tone and, subsequently, cause mechanical bladder outlet obstruction.<sup>2–5</sup>

Among three subtypes of  $\alpha 1$ ARs,  $\alpha 1A$ -AR was shown to be highly expressed in human prostate tissues.<sup>6,7</sup> Therefore, selective  $\alpha 1A$ -AR antagonists are used as the first-line medical treatment for patients with BPH-associated lower urinary tract symptoms.<sup>8,9</sup>

As tamsulosin hydrochloride exhibits some degree of specificity for the  $\alpha 1A$ -AR over  $\alpha 1B$ -AR,<sup>10</sup> tamsulosin hydrochloride is one of the most widely prescribed drugs for the treatment of BPH. In fact, tamsulosin hydrochloride could improve the clinical symptoms in two-third of BPH patients<sup>11</sup> but not in the remaining one-third. Because the efficacy of tamsulosin hydrochloride varies among individuals, prediction of treatment response would improve the overall treatment outcome. In this study, we measured the serum concentration of tamsulosin hydrochloride in 182 of BPH patients and found remarkable inter-individual variability. We also conducted genome-wide association study (GWAS) to identify the genetic factors that regulate pharmacokinetics of tamsulosin hydrochloride.

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## MATERIALS AND METHODS

### Subjects

In this study, newly diagnosed patients with BPH at four Japanese hospitals (Iwate Medical University, Nagoya City University, Kyoto prefectural University of Medicine and Kochi Medical School) were enrolled. All BPH patients were diagnosed by urologist on the basis of clinical features and laboratory test.<sup>12</sup> The following patients were excluded from the analysis: (1) drug-induced or neurogenic bladder dysfunction; (2) prostate cancer; and (3) urethrostenosis. All subjects were orally administered with 0.2 mg of tamsulosin hydrochloride once a day. Written informed consents had been obtained from all the subjects to participate in this study. This study was approved by the ethics committee of each Institute.

### Evaluation of serum tamsulosin hydrochloride concentration.

All study subjects were evaluated for serum tamsulosin hydrochloride concentration during the period of stable prescription at 8 weeks or later after the first administration. Each patient took 0.2 mg of tamsulosin hydrochloride (without water tablet) soon after breakfast every day. Blood samples were drawn from patients around 4–8 h after drug administration. Serum tamsulosin hydrochloride concentration was determined by high performance liquid chromatography at Mitsubishi Kagaku Co. (Tokyo, Japan). We estimated  $C_{max}$  of the serum tamsulosin based on the result in the previous pharmacokinetic study,<sup>13</sup> in which serum concentration was peaked at 8 h after oral administration of 0.2 mg tamsulosin. Estimated serum half-life was 9 h. According to these findings, we estimated the  $C_{max}$  based on the serum drug concentration at 4–8 h after drug oral administration.

### Genotyping

Genomic DNA of all the patients was extracted from whole blood by standard method. We applied 750 ng DNA of each sample to Illumina Human610-Quad Beadchip (San Diego, CA, USA) according to the manufacturer's protocols at Center for Genomic Medicine, RIKEN. Samples with call rates  $<0.98$  were excluded from the analysis. Then, we excluded single-nucleotide polymorphisms (SNPs) with call rates  $<0.99$  or ambiguous intensity plots from the following analyses. Subsequently, we excluded the SNP with minor allele frequency of  $<0.01$ , or with an exact  $P$ -value of the Hardy–Weinberg equilibrium test  $<1.0 \times 10^{-7}$ . Finally, 481 678 SNPs passed our quality control process.

### Statistical analysis

Associations of the SNPs with  $C_{max}$  of tamsulosin hydrochloride were assessed by linear regression, assuming the additive effects of the alleles on the  $C_{max}$ , using PLINK version 1.06.<sup>14</sup> Significance level after Bonferroni correction for multiple testing was  $P = 1.03 \times 10^{-7}$  ( $0.05/481\,678$ ) in this analysis. We also calculated false discovery rate-based  $q$ -values to measure the statistical significance at the genome-wide level by using R program.<sup>15</sup> Differences in  $C_{max}$  of tamsulosin hydrochloride in the different genotype groups were evaluated by Kruskal–Wallis tests using PASW statistics software (version 18.0.0; SPSS, Chicago, IL, USA).

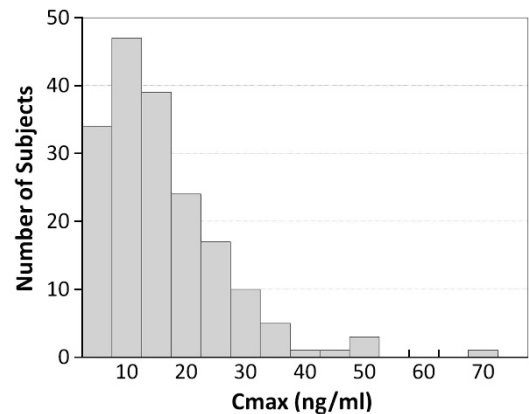
## RESULTS

This study investigated the genetic factors that influence the serum level of tamsulosin hydrochloride in patients orally taking 0.2 mg of tamsulosin hydrochloride once per day. All subjects were newly diagnosed with BPH on the basis of clinical information and laboratory test. All patients were Japanese, and median age was 71.0 ranging 55–87 years old.  $C_{max}$  was successfully determined in 182 patients by HPLC as described previously.<sup>13</sup> The result indicated that  $C_{max}$  ranged from 1.2 to 65.1  $\text{ng ml}^{-1}$  and varied enormously among individuals (Figure 1). Actually, patients who were ranked in the bottom 25% showed the average  $C_{max}$  of 3.8  $\text{ng ml}^{-1}$ , whereas those in the top 25% had about 7-fold higher  $C_{max}$  of 26.7  $\text{ng ml}^{-1}$ .

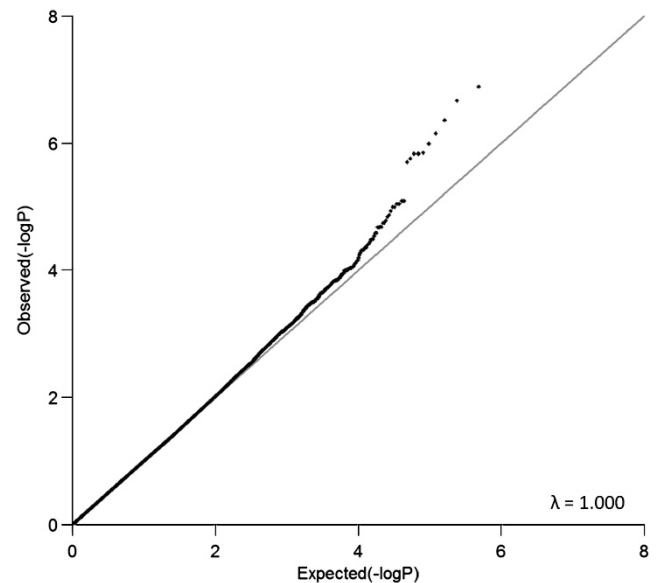
To identify genetic factors that were involved in serum level of tamsulosin hydrochloride, we performed GWAS. We genotyped 182

individuals using Illumina 610 HumanHap BeadChip. SNPs with minor allele frequency  $<1\%$  were excluded for further analysis, considering the lower statistical power. After stringent quality control filtering, we obtained the genotyping result of 481 678 SNPs in 182 patients.

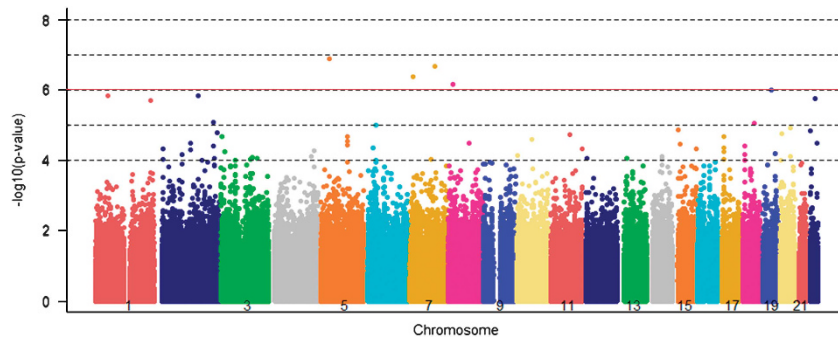
Then, we assess the association of these SNPs with  $C_{max}$  using a linear regression model. A quantile–quantile plot of  $P$ -values showed that the genomic inflation factor lambda was 1000, implying a low possibility of false-positive associations due to population substructure (Figure 2). The results of association analysis revealed that SNPs rs16902947, rs7779057, rs35681285 and rs2122469 showed  $P$ -value  $<1.0 \times 10^{-6}$  (Figure 3 and Table 1). Although these SNPs did not reach the genome-wide significance threshold after Bonferroni correction, the Bonferroni method is considered to be too conservative because many SNPs are in some degrees of linkage



**Figure 1** The frequency distribution of maximum tamsulosin serum concentration ( $C_{max}$ ) among 182 BPH patients. A total of 182 patients with BPH were analyzed for tamsulosin serum concentration at 8 weeks or later after the first administration.



**Figure 2** Quantile–quantile plot of  $P$ -values in the GWAS for  $C_{max}$  of tamsulosin. The horizontal axis indicates the expected  $-\log_{10}$  ( $P$ -values). The vertical axis indicates the observed  $-\log_{10}$  ( $P$ -values). The gray line represents  $y=x$ . The  $\lambda$  indicates the inflation factor of the test statistics. A full color version of this figure is available at the *Journal of Human Genetics* journal online.



**Figure 3** Results from a GWAS. A Manhattan plot showing the  $-\log_{10}$  ( $P$ -values) of SNPs in the first stage. Each  $P$ -value is calculated by linear regression, assuming the additive effects of the alleles on  $C_{max}$ . Red line indicates  $P$ -value  $1.0 \times 10^{-6}$ .

**Table 1** Results of QTL analysis for serum tamsulosin hydrochloride concentration

SNP ID	Chromosome Position	Region Gene	Allele	MAF (1/2) <sup>a</sup>	Beta	s.e.	P-value	q-value
rs16902947	5 36309097	5p13.2 <i>RANBP3L</i>	A/G	0.01	19.48	3.54	$1.29 \times 10^{-7}$	0.052
rs7779057	7 106594885	7q22.3 No gene	C/T	0.02	17.16	3.18	$2.15 \times 10^{-7}$	0.052
rs35681285	7 16326646	7p21.2 <i>ISPD</i>	T/C	0.02	16.75	3.19	$4.35 \times 10^{-7}$	0.07
rs2122469	8 20626321	8p21.3 No gene	C/T	0.02	18.57	3.61	$7.03 \times 10^{-7}$	0.085

Abbreviations: ID, identification number; MAF, minor allele frequency; QTL, quantitative trait locus; SNP, single-nucleotide polymorphism. QTL analysis for serum tamsulosin hydrochloride level ( $n=182$ ).  $P$ -values were obtained from logistic regression analysis.

<sup>a</sup>Allele 1: minor allele.

disequilibrium. Because all SNPs showed false discovery rate  $q$ -value  $< 0.1$ , our findings would have a low possibility of false-positive associations (Table 1). Among four SNPs, SNPs rs16902947 and rs35681285 are located within genomic regions, including RAN-binding protein 3-like (*RANBP3L*) and isoprenoid synthase domain containing (*ISPD*), respectively, whereas SNPs rs7779057 and rs2122469 are located within intergenic regions far from any annotated genes.

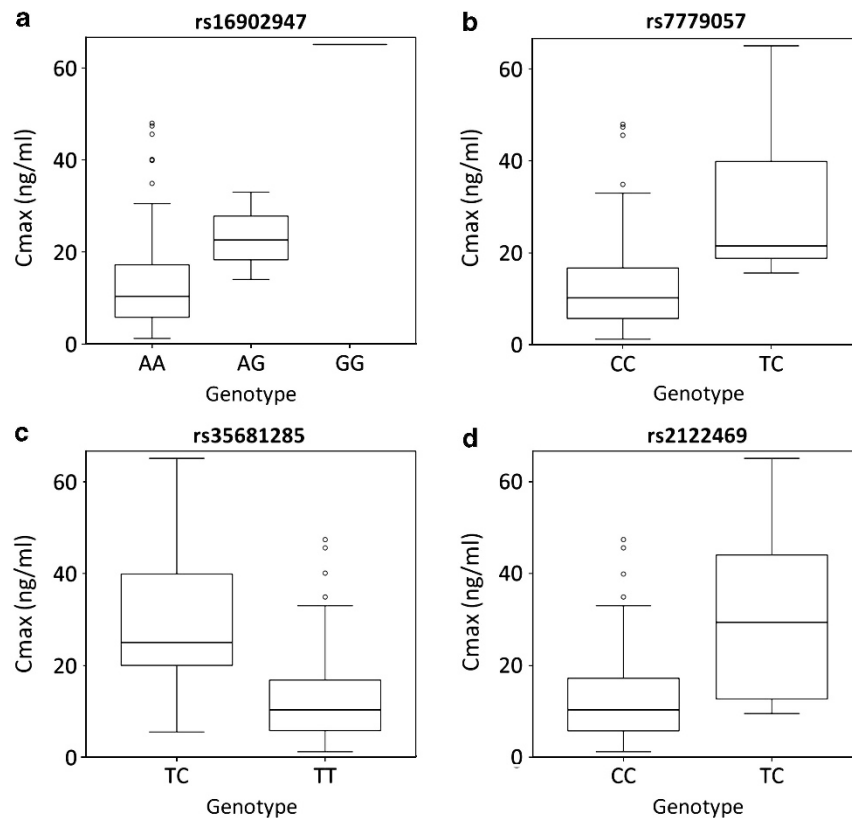
Then, we also evaluated  $C_{max}$  level stratified by SNP genotype.  $C_{max}$  levels were correlated with the number of G allele at SNP rs16902947 (median 10.4, 22.6 and 65.1  $\text{ng ml}^{-1}$  for AA, AG and GG genotypes, respectively, Figure 4a). Median  $C_{max}$  in the patients with CC genotypes at SNP rs7779057 (10.2  $\text{ng ml}^{-1}$ ) was less than those among TC carriers (21.5  $\text{ng ml}^{-1}$ , Figure 4b). Median  $C_{max}$  was also lower in the patients with TT genotypes than TC genotypes at rs35681285 (10.3 and 25.0  $\text{ng ml}^{-1}$ , Figure 4c), and in the patients with CC genotypes than TC genotypes at rs2122469 (10.3 and 29.4  $\text{ng ml}^{-1}$ , Figure 4d). To assess the combined impact of these four SNPs, we defined  $C_{max}$  prediction index as the number of alleles associated with higher  $C_{max}$  level (G at rs16902947, T at rs7779057, C at rs35681285 and T at rs2122469). Patients were classified into three groups based on  $C_{max}$  prediction index (0, 1 and  $\geq 2$ ). As shown in Figure 5,  $C_{max}$  prediction index was significantly associated with  $C_{max}$  values ( $P = 4.5 \times 10^{-6}$ , Kruskal–Wallis analysis), indicating the possible roles of these four variants in pharmacokinetics of tamsulosin hydrochloride.

*CYP2D6* variations were shown to be associated with various drug metabolism.<sup>16</sup> In addition, treatment with *CYP2D6* or *CYP3A4* inhibitor, such as cimetidine hydrochloride, was shown to increase serum tamsulosin concentration,<sup>17,18</sup> indicating the important roles of *CYP2D6* and *CYP3A4* in tamsulosin metabolism. In our GWAS,

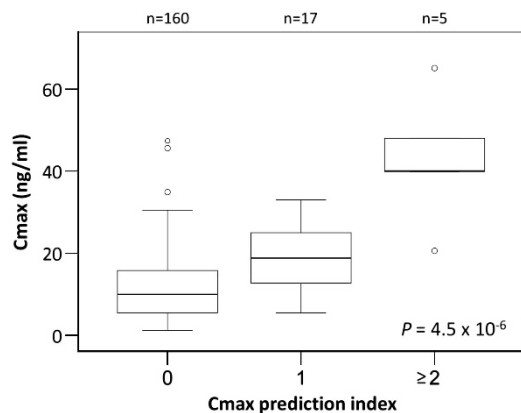
we analyzed five SNPs in *CYP2D6* and *CYP3A4* loci and found suggestive association between SNP rs28371759 ( $P = 0.044$ ) and  $C_{max}$  (Table 2), although the association was not statistically significant after correction for multiple testing. *CYP2D6* was also involved in the metabolism of tamoxifen, and its genetic variations were shown to be associated with treatment outcome of adjuvant tamoxifen therapy for estrogen receptor-positive breast cancer patients.<sup>19–22</sup> However, in our previous GWAS of adjuvant tamoxifen therapy for breast cancer patients, SNPs in *CYP2D6* locus did not significantly associate with treatment response,<sup>23</sup> probably due to the complicated haplotype structure of this gene locus. To further elucidate the role of *CYP2D6* and *CYP3A4* variations, comprehensive genotyping of CYP polymorphisms would be necessary.

## DISCUSSION

With a better understanding of the mechanisms underlying the heterogeneity in the response to a variety of therapeutic agents, future research will focus on the establishment of individualized treatment based on physiological variables, genetic characteristics and environmental factors. Recent advances in pharmacogenetic research revealed a number of genetic variants that affect the efficacy and safety of drugs.<sup>16,24–28</sup> For example, analysis of *VKORC1* and *CYP2C9* genotypes can predict warfarin sensitivity and maintenance dosage.<sup>26,27</sup> The new clinical trial, Clarification of Optimal Anticoagulation through Genetics, will test this approaches to determining the initial dose of warfarin in patients who are expected to need therapy for 3 months or longer.<sup>27</sup> Variations in *CYP2D6* and *ABCC2* were also shown to be significantly associated with the prognosis of breast cancer patients receiving tamoxifen monotherapy.<sup>16</sup> In addition, three *ABCB1* variants were associated



**Figure 4** Association between genotype and Cmax of tamsulosin. Box plot showing maximum tamsulosin serum concentration (Cmax) according to genotype with rs16902947 (a), rs7779057 (b), rs35681285 (c) and rs2122469 (d). The horizontal lines indicate the median values, and the boxes cover 25–75 percentiles. Points outside them show up as outliers.



**Figure 5** Cumulative effects of four SNPs on Cmax of tamsulosin. Patients were classified into three groups according to genotypes of four predictive SNPs. Box plot indicated Cmax of tamsulosin for each group. Kruskal–Wallis test was used for statistical analysis.

**Table 2** Variations in CYP2D6 and CYP3A4 loci

SNP ID	Chromosome Position	Region Gene	Allele (1/2) <sup>a</sup>	MAF	Beta	s.e.	P-value
rs4646440	7	7p22.1					
	99198806	CYP3A4	T/C	0.20	0.014	1.33	0.992
rs28371759	7	7p22.1					
	99199562	CYP3A4	C/T	0.01	9.110	4.50	0.044
rs4646437	7	7p22.1					
	99203019	CYP3A4	T/C	0.07	1.978	2.19	0.368
rs2246709	7	7p22.1					
	99203655	CYP3A4	G/A	0.33	1.261	1.10	0.252
rs764481	22	22q13.2					
	40848370	CYP2D6	A/G	0.13	−0.315	1.58	0.842

Abbreviations: ID, identification number; MAF, minor allele frequency; QTL, quantitative trait locus; SNP, single-nucleotide polymorphism.

QTL analysis for serum tamsulosin hydrochloride level ( $n = 182$ ).  $P$ -values were obtained from logistic regression analysis.

<sup>a</sup>Allele 1: minor allele.

with higher digoxin serum concentrations in a cohort of European digoxin users.<sup>28</sup>

As tamsulosin hydrochloride is widely used for the treatment of BPH because of its better tolerability than other type of  $\alpha$ 1AR antagonists,<sup>10,29</sup> prediction of drug efficacy and toxicity would contribute to better treatment of BPH patients. In general, serum drug concentration is presumably correlated with the efficacy of

drugs. Therefore, we conducted GWAS to elucidate the genetic factors that associate with serum tamsulosin hydrochloride concentration. Recently, GWAS have enabled us to detect genetic variations those are associated with the risk of diseases,<sup>30</sup> laboratory test values,<sup>31</sup> and drug adverse event<sup>32</sup> without prior knowledge of position or function. To our knowledge, this is the first GWAS for serum drug concentration.



In our GWAS, four SNPs indicated suggestive association on C<sub>max</sub> of tamsulosin hydrochloride. However, these SNPs did not reach genome-wide significance based on Bonferroni correction. Although Bonferroni correction is the best-known approach, GWAS may fall short of discovering any true positive gene, particularly when it has a relatively small sample size. On the other hand, false discovery rate is another statistical method in multiple testing that offers a more liberal criterion.<sup>15</sup> In the current analysis, four SNPs showed q-value < 0.1, indicating the strong association of these SNPs with C<sub>max</sub> of tamsulosin.

Multicenter randomized studies have shown the correlation between  $\alpha$ 1-blocker dosage and their effects.<sup>9,26</sup> In addition, the mean changes in the American Urological Association symptom score were significantly greater in the 0.8 mg per day tamsulosin hydrochloride group compared with the 0.4 mg per day group.<sup>33</sup> Therefore, higher serum tamsulosin hydrochloride concentration is very likely to improve drug efficacy. The individuals with prediction index of 0 indicate nearly one-fourth C<sub>max</sub> level compared with the subjects with index score of  $\geq 2$ . Thus 'C<sub>max</sub> prediction index' would be inversely related with the required dosage.

SNP rs16902947 is located at 7 kb upstream of RANBP3L, which encodes a paralog of RANBP3. RanBP3 functions as a negative regulator of transforming growth factor-beta signaling through the interaction with the R-SMAD proteins.<sup>34</sup> In addition, transforming growth factor-beta was shown to induce uridine diphosphate glucose dehydrogenase,<sup>35</sup> which acts as a substrate for glucuronosyl transferase and regulates glucuronidation. Because glucuronidation is a key step of tamsulosin metabolism, RanBP3L may also regulate glucuronidation and, subsequently, alter tamsulosin metabolism. SNP rs35681285 is located within an intron 5 of ISPD. Mutations of ISPD were shown to be the cause of Walker-Warburg syndrome, a rare form of autosomal-recessive congenital muscular dystrophy.<sup>36,37</sup> ISPD belongs to the IspD family and is required for protein O-linked mannosylation. However, there is no report that mannosylation affects the drug metabolism so far. SNPs rs7779057 and rs2122469 are at more than 40 kb away from any known gene or microRNA. Further functional analysis is necessary to elucidate the molecular mechanism, whereby these variations exert their effects on the serum tamsulosin hydrochloride concentration.

Although no previous pharmacokinetic and pharmacodynamic studies demonstrated the correlation between C<sub>max</sub> and the drug efficacy, the drug dosage was shown to be correlated with the drug response,<sup>38</sup> and drug dosage is reflected by C<sub>max</sub>.<sup>13</sup> Thus, our findings would partially explain the diversity of response to tamsulosin hydrochloride treatment. Pharmacokinetics of tamsulosin was shown to be influenced by various factors, including food intake,  $\alpha$ 1-acid glycoprotein levels, hepatic and renal impairment, and comedication with CYP2A6 and/or 3A4 inhibitors.<sup>18</sup> In addition, genetic variations in ARs might also affect the drug efficacy even if the serum drug concentrations are same. We would like to conduct the association analysis of C<sub>max</sub> and drug efficacy with genetic variations in the future study. For the Japanese patients, 0.2 mg of tamsulosin hydrochloride is commonly used for BPH patients as the initial dose, and the dosage is adjusted in accordance with the treatment efficacy. We hope that our study would contribute to estimate the required dosage of tamsulosin hydrochloride and, subsequently, establish the personalized medical treatment of BPH patients in the future.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ACKNOWLEDGEMENTS

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