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ARTICLE

Relationship of hemoglobin level and plasma coproporphyrin-I concentrations as an endogenous probe for phenotyping OATP1B

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Abstract

Plasma coproporphyrin-I (CP-I) concentration is used as a sensitive and selective endogenous probe for phenotyping organic anion transporting polypeptides 1B (OATP1B) activity in many studies. CP-I is produced in the process of heme synthesis, but the relationship between plasma CP-I concentrations and heme synthesis activity is unknown. In this study, we evaluated the relationship between plasma CP-I concentration and hemoglobin level as a biomarker of heme synthesis activity. The data of 391 subjects selected from the Japanese general population were analyzed. One hundred twenty-six participants had *OATP1B1*15* allele, 11 of whom were homozygous (*OATP1B1*15*/**15*). Multiple regression analysis identified hemoglobin level as an independent variable associated with plasma CP-I concentration ($p < 0.0001$). A significant positive correlation was observed between hemoglobin level and plasma CP-I concentration in participants without *OATP1B1*15* allele ($n = 265$; $r_s = 0.35$, $p < 0.0001$) and with *OATP1B1*15* allele ($n = 126$; $r_s = 0.27$, $p = 0.0022$). However, Kruskal–Wallis test showed no large difference in Kruskal–Wallis statistics between the distribution of plasma CP-I concentrations and that of ratio of plasma CP-I to hemoglobin among six OATP1B1 polymorphism groups. These findings suggest that the hemoglobin level seems to reflect biosynthesis of CP-I. However, correction by hemoglobin level is not required when using basal plasma CP-I concentration for phenotyping OATP1B activity.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Coproporphyrin-I (CP-I) in plasma is a sensitive and specific endogenous biomarker for phenotyping organic anion transporting polypeptides 1B (OATP1B), and has been

used for phenotyping OATP1B activity, such as in clinical drug-drug interaction studies. CP-I is produced during the process of heme synthesis, indicating that correction of plasma CP-I concentration by hemoglobin level as an indicator of heme synthesis activity may be needed.

WHAT QUESTION DID THIS STUDY ADDRESS?

Does correction by hemoglobin level improve the usefulness of CP-I as a probe for OATP1B phenotyping?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Hemoglobin level was identified as an independent variable associated with plasma CP-I concentrations. However, no large difference in Kruskal–Wallis statistics was observed between the distribution of plasma CP-I concentrations and that of CP-I/Hb ratio among six OATP1B1 polymorphism groups.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Hemoglobin level seems to reflect biosynthesis of CP-I. However, correction by hemoglobin level is not needed when using plasma CP-I concentration for phenotyping OATP1B activity.

INTRODUCTION

Pharmacokinetics of drugs show large interindividual variability, and the variability impacts drug efficacy and adverse effects.[1] Especially for drugs eliminated by hepatic metabolism, pharmacokinetic variability in individual patients is frequently difficult to predict due to the involvement of drug-metabolizing enzymes, such as cytochrome P450 (CYP) and some drug transporters.[1] For example, CYP3A is involved in the metabolism of 30%–40% of currently prescribed drugs, and the expression level and activity of CYP3A show large individual variability.[2] Regarding drug transporters, organic anion transporting polypeptide 1B (OATP1B, encoded by *SLCO1B*) is one of the hepatic uptake transporters. Hydroxymethylglutaryl-CoA reductase inhibitors and some anti-hepatitis C virus drugs are substrates of OATP1B,[3–8] and pharmacokinetics of these drugs is affected by individual variability of in vivo OATP1B activity due to environmental, physiologic, and genetic factors. As environmental factor, drug-drug interaction is important. For example, OATP inhibitors, such as rifampicin and cyclosporin A, inhibit OATP1B activity and increase plasma concentrations of OATP1B substrates.[9,10] As a physiologic factor, 3-carboxy -4-methyl-5-propyl-2-furanpropanoic acid accumulation due to chronic kidney failure has recently been reported to decrease OATP1B activity.[11–14] As genetic factors, *SLCO1B1* exhibits two major single nucleotide polymorphisms: A388G and T521C. These polymorphisms form four haplotypes: *OATP1B1*1a* (c.388A-c.521 T), *OATP1B1*1b* (c.388G-c.521 T), *OATP1B1*5* (c.388A-c.521C), and *OATP1B1*15* (c.388G-c.521C).[3,15] *OATP1B1*5* and *OATP1B1*15* are associated with decreased transporting activities of OATP1B1.[15–19] In the Japanese population, *OATP1B1*15* is the most important polymorphism that affects individual OATP1B activity in vivo, because c.521C shows strong linkage disequilibrium with c.388G ($r^2 = 0.0708$, $D' = 0.9999$).[3]

For precision dosing of drugs eliminated by hepatic metabolism, direct functional analysis (phenotyping) of drug-metabolizing enzymes and hepatic uptake transporters seems to be a useful tool. For example, phenotyping of CYP3A has long been performed using CYP3A probe drugs, such as midazolam and alprazolam,[20] or endogenous probes, such as urinary 6β-hydroxycortisol to cortisol ratio,[21] formation clearance of 6β-hydroxycortisol,[22] and plasma 4β-hydroxycholesterol.[23] For phenotyping of OATP1B, several probes have been reported, such as probe drugs including Hydroxymethylglutaryl-CoA reductase inhibitors, and endogenous probes, including direct bilirubin,glycochenodeoxycholate-3-glucuronide,glycochenodeoxycholate-3-sulfate, hexadecanedioate, and coproporphyrin-I (CP-I).[24] Especially, CP-I in plasma is a sensitive and specific endogenous biomarker for phenotyping OATP1B,[24–31] and has been used for phenotyping OATP1B activity in clinical drug-drug interaction studies[32,33] as well as in model-based analysis of drug-drug interaction.[29,34–36] Furthermore, basal plasma CP-I concentration (without administration of OATP1B inhibitor) is considered to reflect basal OATP1B activity in individuals, and is utilized to evaluate the effect of disease state on OATP1B activity in vivo.[13,37]

It is important to understand the kinetic characteristics of endogenous probes to utilize them appropriately. Taking the CYP3A probe 4β-hydroxycholesterol as an example, 4β-hydroxycholesterol is produced from cholesterol by CYP3A metabolism.[23] Thus, it would be important to measure total cholesterol level when evaluating 4β-hydroxycholesterol. Indeed, it has been shown that correction by total cholesterol level (4β-hydroxycholesterol/total cholesterol) is superior to 4β-hydroxycholesterol alone for CYP3A phenotyping.[38] On the other hand, the OATP1B probe CP-I is produced

during the process of heme synthesis,[39] We hypothesize that consideration of heme synthesis activity using hemoglobin level as marker increases the reliability of CP-I for OATP1B phenotyping. In this study, we evaluated the relationship between plasma CP-I concentration and hemoglobin level in a sample of the Japanese general population. Furthermore, we evaluated whether correction by hemoglobin improves the usefulness of CP-I as a probe for OATP1B phenotyping by comparing the association with *OATP1B1* polymorphism.

METHODS

Study participants

We analyzed the data of 391 subjects from the Japanese population, as reported previously.[40] The subjects were randomly selected from individuals receiving a health check in Kyoto Prefectural University of Medicine, who met the following inclusion criteria: body mass index (BMI) lower than 30 kg/m^2, estimated glomerular filtration rate (eGFR) higher than 60 ml/min/1.73 m^2, total bilirubin lower than 1.5 mg/dl, and alanine aminotransaminase (ALT) lower than 100 IU/L. The eGFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for Japanese.[41]

This study was approved by the ethics committees of Kyoto Prefectural University of Medicine (approval number: ERB-C-1384) and Meiji Pharmaceutical University (approval number: 3023).

OATP1B1 polymorphism

Two single nucleotide polymorphisms (SNPs); rs2306283 (c. A388G, p. N130D, exon 5) and rs4149056 (c. T521C, p. V174A, exon 5), were identified from genomewide association study data in all the participants. The two SNPs form four haplotypes: *OATP1B1*1a* (c.388A-c.521 T), *OATP1B1*1b* (c.388G-c.521 T), *OATP1B1*5* (c.388A-c.521C), and *OATP1B1*15* (c.388G-c.521C).[40] No participant in the present study had *OATP1B1*5* (c.388A-c.521C), because c.521C has been reported to show strong linkage disequilibrium with c.388G in the Japanese population (r^2 = 0.0708, D' =0.9999).[3] Finally, the participants were divided into 6 polymorphism groups: *OATP1B1*1b/*1b*, *OATP1B1*1a/*1b*, *OATP1B1*1a/*1a*, *OATP1B1*1b/*15*, *OATP1B1*1a/*15*, and *OATP1B1*15/*15*.

Measurement of plasma CP-I concentration

Plasma CP-I concentration was measured using ultra-high performance liquid chromatography coupled to tandem mass spectrometry according to the procedures that we reported previously.[14] Inter- and intra-assay accuracy was 92.1%–110.2% and 96.7%–100.6%, respectively, and precision was less than 7.6% and less than 6.8%.

Data analysis and statistics

Data are expressed as mean ± SD. Differences between participants with and those without *OATP1B1*15* allele were analyzed by Mann–Whitney *U* test or χ^2 test. Correlation between participant background factors was assessed by Pearson's product-moment correlation coefficient. Factors associated with plasma CP-I concentration were analyzed by multiple regression analysis by stepwise selection using Schwarz's Bayesian information criterion. Correlation between hemoglobin level and plasma CP-I concentration was assessed by Spearman's rank correlation coefficient. Plasma CP-I concentrations among six genotypes were compared using Kruskal–Wallis test with post hoc Dunn's test. A p value less than 0.05 was considered statistically significant. Statistical analyses were performed using Graph Pad Prism 7 (GraphPad Software) and EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing).[42]

RESULTS

Participant background

The background data of 391 study participants are summarized in Table 1. Mean hemoglobin level was within normal range, but the levels had large variation among participants. One hundred twenty-six participants had *OATP1B1*15* allele, 11 of whom were homozygous (*OATP1B1*15/*15*). Plasma CP-I concentrations also showed large individual variation among participants, and a significant difference was observed between participants with and those without *OATP1B1*15* ($p < 0.0001$). Significant differences were also observed in body weight and low-density lipoprotein (LDL) cholesterol between two groups, although the mean values were not markedly different.

Factors associated with plasma CP-I concentration

Regression analysis on the entire cohort was performed to identify the clinical factors related to plasma CP-I concentrations. First, we checked correlation of hemoglobin level with the other background factors by the scatter plot matrix

TABLE 1 Participant background

Characteristics	Participants without *OATP1B1*15*	Participants with *OATP1B1*15*	All participants	*p* value
No. of subjects	265	126	391	—
Males/females	81/184	31/95	112/279	NS
Age, years	55.7 ± 10.3 [39–74]	56.8 ± 9.2 [39–74]	56.1 ± 9.9 [39–74]	NS
Body weight, kg	57.4 ± 10.8 [31.6–98.6]	55.3 ± 10.1 [39.9–89.5]	56.7 ± 10.6 [31.6–98.6]	$p = 0.0458$
BMI, kg/m^2	22.1 ± 2.9 [14.5–30.0]	21.6 ± 3.1 [15.8–29.7]	21.9 ± 3.0 [14.5–30.0]	NS
Systolic blood pressure, mmHg	126.1 ± 18.9 [88–213]	123.2 ± 15.6 [88–161]	126.1 ± 18.8 [88–213]	NS
Diastolic blood pressure, mmHg	77.6 ± 11.6 [54–128]	76.5 ± 9.3 [50–104]	77.6 ± 11.6 [54–128]	NS
Hemoglobin, g/dl	13.5 ± 1.3 [9.4–16.8]	13.3 ± 1.4 [7.1–17.2]	13.5 ± 1.3 [7.1–17.2]	NS
Serum albumin, g/dl	4.4 ± 0.2 [3.7–5.0]	4.4 ± 0.2 [3.8–5.0]	4.4 ± 0.2 [3.7–5.0]	NS
Total bilirubin, mg/dl	0.79 ± 0.23 [0.4–1.4]	0.76 ± 0.22 [0.3–1.3]	0.78 ± 0.23 [0.3–1.4]	NS
ALT, IU/L	18.0 ± 9.7 [5.0–59.0]	19.1 ± 11.7 [7.0–99.0]	18.3 ± 10.4 [5.0–99.0]	NS
Serum creatinine, mg/dl	0.73 ± 0.15 [0.44–1.16]	0.69 ± 0.13 [0.48–1.09]	0.72 ± 0.14 [0.44–1.16]	NS
eGFR,[a] ml/min/1.73 m^2	78.1 ± 8.6 [60.0–96.3]	79.6 ± 7.7 [60.5–94.7]	78.6 ± 8.5 [60.0–96.3]	NS
HbA1c, %	5.5 ± 0.45 [4.6–8.3]	5.6 ± 0.42 [4.7–7.3]	5.6 ± 0.45 [4.6–8.3]	NS
LDL cholesterol, mg/dl	121.2 ± 31.8 [51–230]	127.9 ± 29.7 [64–216]	123.4 ± 31.3 [51–230]	$p = 0.0182$
Uric acid, mg/dl	4.9 ± 1.3 [0.6–9.7]	4.7 ± 1.1 [2.3–7.8]	4.8 ± 1.3 [0.6–9.7]	NS
OATP1B1 polymorphism				
*OATP1B1*1b/*1b*	103	—	103	—
*OATP1B1*1a/*1b*	122	—	122	—
*OATP1B1*1a/*1a*	40	—	40	—
*OATP1B1*1b/*15*	—	74	74	—
*OATP1B1*1a/*15*	—	41	41	—
*OATP1B1*15/*15*	—	11	11	—
Plasma CP-I concentration, ng/ml	0.46 ± 0.16 [0.13–1.41]	0.54 ± 0.18 [0.21–1.37]	0.48 ± 0.17 [0.13–1.41]	$p < 0.0001$

Note: The *p* value: participants without *OATP1B1*15* vs. those with *OATP1B1*15*. Data are expressed as number of participants (*n*) or mean ± SD [range].

Abbreviations: ALT, alanine aminotransaminase; BMI, body mass index; CP-I, coproporphyrin-I; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; NS, not significant; OATP1B1, organic anion transporting polypeptides 1B1.

[a]eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation for Japanese.

(Figure 1). Hemoglobin level correlated with body weight ($r = 0.44$), sex ($r = -0.55$), and uric acid ($r = 0.43$). These variables were excluded from multivariate analysis due to multicollinearity. Multiple regression analysis using the remaining factors (age, BMI, systolic blood pressure, diastolic blood pressure, hemoglobin, serum albumin, total bilirubin, ALT, eGFR, HbA1c, and LDL cholesterol) as independent variables identified *OATP1B1*15* allele, hemoglobin, LDL cholesterol, BMI, total bilirubin, ALT, and HbA1c as significant independent variables associated with plasma CP-I concentration (Table 2).

Figure 2 shows the relationship between hemoglobin levels and plasma CP-I concentrations. A significant positive correlation was observed between them in participants without *OATP1B1*15* allele (Figure 2a; $r_s = 0.35$, $p < 0.0001$) and with *OATP1B1*15* allele (Figure 2b; $r_s = 0.27$, $p = 0.0022$).

Comparison of plasma CP-I concentration and ratio of plasma CP-I to hemoglobin level in association with OATP1B1 polymorphism

We compared the distribution of plasma CP-I concentrations and that of ratio of plasma CP-I to hemoglobin level (CP-I/Hb ratio) among 6 OATP1B1 polymorphism groups: *OATP1B1*1b/*1b*, *OATP1B1*1a/*1b*, *OATP1B1*1a/*1a*, *OATP1B1*1b/*15*, *OATP1B1*1a/*15*, and *OATP1B1*15/*15*. As shown in Figure 3a, there was a significant difference in plasma CP-I concentrations among the six groups, with significant increases in *OATP1B1*1b/*15*, **1a/*15*, and **15/*15* groups compared with the *OATP1B1*1b/*1b* group by post hoc analysis. Similarly, a significant difference was observed in CP-I/Hb ratio among the 6 groups, with significant increases in *OATP1B1*1b/*15*, **1a/*15*, and **15/*15* groups compared

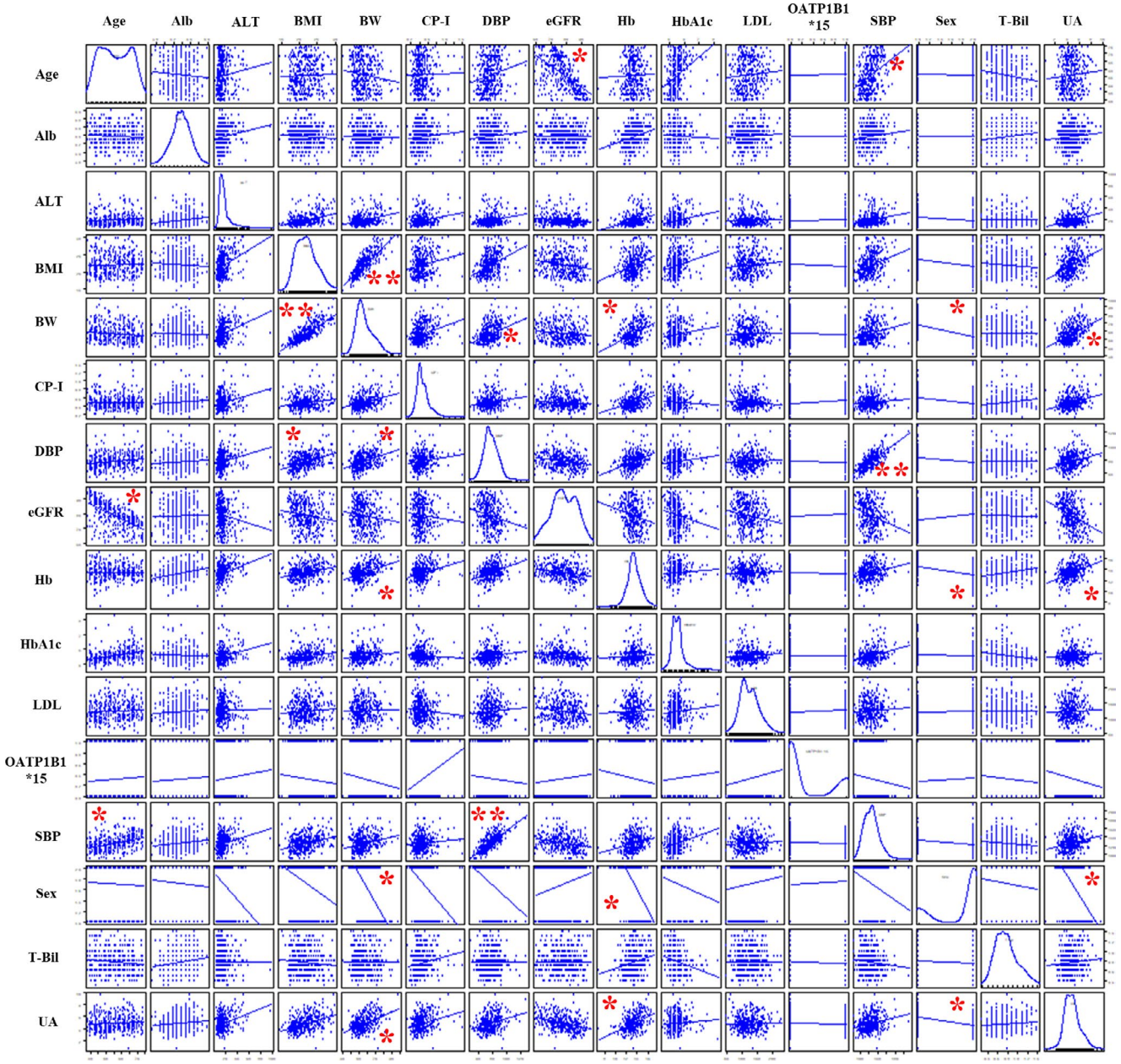

FIGURE 1 Scatter plot matrix for background factors. *$r > 0.4$ and **$r > 0.7$ by Pearson's product-moment correlation coefficient. Alb, albumin; ALT, alanine aminotransaminase; BMI, body mass index; BW, body weight; CP-I, coproporphyrin-I; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; SBP, systolic blood pressure; T-Bil, total bilirubin; UA, uric acid

with the *OATP1B1*1b/*1b* group (Figure 3b). Kruskal–Wallis statistics were higher for CP-I/Hb ratio than for plasma CP-I concentration (39.5 vs. 34.0), but the difference was not so large.

DISCUSSION

Recently, plasma CP-I concentration is utilized as a sensitive endogenous OATP1B probe for phenotyping OATP1B activity in many studies.[29,32–36] Plasma CP-I concentration is considered to increase when hepatic uptake of CP-I is reduced due to decreased OATP1B activity. On the other hand, there is also a possibility that plasma CP-I concentration may increase due to excessive production of CP-I from augmented heme synthesis activity. Thus, correction of plasma CP-1 concentration by heme biosynthesis activity may improve the usefulness of CP-I as a probe for OATP1B phenotyping. Hemoglobin level has been shown to be an appropriate biomarker to assess the activity of heme synthesis both in vivo[43] and in vitro[44]; however, the relationship between plasma CP-I concentration and hemoglobin level is

TABLE 2 Multiple regression analysis of independent factors associated with plasma CP-I concentration

Dependent/explanatory variable	Adjusted r^2	*p* value	Regression coefficient
Plasma CP-I concentrations	0.22		
*OATP1B1*15 allele*		<0.0001	0.094
Hemoglobin		<0.0001	0.030
LDL cholesterol		0.0022	−0.00075
BMI		0.0032	0.0084
Total bilirubin		0.0070	0.094
ALT		0.0087	0.0021
HbA1c		0.0100	−0.0046

Abbreviations: ALT, alanine aminotransaminase; BMI, body mass index; CP-I, coproporphyrin-I; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein.

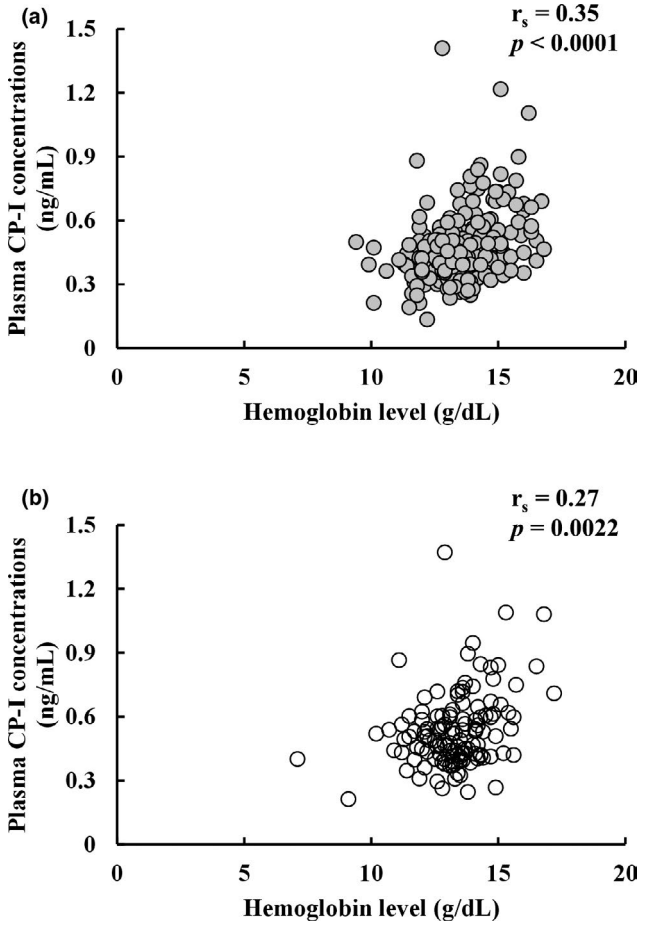

FIGURE 2 Correlation between hemoglobin level and plasma coproporphyrin-I (CP-I) concentration in participants with *OATP1B1*15* allele ($n = 126$) (a) and without *OATP1B1*15* allele ($n = 265$) (b)

unknown. In this study, we revealed a correlation between plasma CP-I concentration and hemoglobin level, indicating the need to correct plasma CP-I concentration by hemoglobin level for OATP1B phenotyping.

All of the 391 participants had no hepatic and renal failure in this study, suggesting that plasma CP-I concentrations were not affected by disease state. Scatter plot matrix identified body weight, sex, and uric acid as factors associated with hemoglobin level. Previous meta-analyses have reported female sex and low body weight as factors of low hemoglobin level,[45,46] supporting our results. Uric acid also correlated with body weight ($r = 0.48$) and sex ($r = -0.51$) in our study, suggesting that uric acid was a confounding factor of body weight and sex. Thus, multiple regression analysis was performed excluding body weight, sex, and uric acid due to multicollinearity. Analyses using the remaining factors as independent variables identified *OATP1B1*15* allele, hemoglobin, LDL cholesterol, BMI, total bilirubin, ALT, and HbA1c as significant independent variables associated with plasma CP-I concentration. *OATP1B1*15* allele had the strongest association with plasma CP-I concentration, supporting our previous study.[40] Hemoglobin level had the second strongest association with plasma CP-I concentration, indicating that hemoglobin level is closely involved in plasma CP-I concentration, and is further confirmed by a significant positive correlation between hemoglobin level and plasma CP-I concentration. These findings suggest that augmented heme synthesis may increase plasma CP-I concentration regardless of OATP1B activity. LDL cholesterol was also identified as a significant independent factor associated with plasma CP-I concentration. The mechanism is unknown, but OATP1B activity may be partially associated with LDL cholesterol level because OATP1B-mediated hepatic thyroid hormone entry has been reported to be a key determinant of cholesterol homeostasis.[47] It was unclear why the other variables, including BMI, total bilirubin, ALT, and HbA1c, were associated with plasma CP-I concentrations, although these variables may have slight association with hepatic and other physiological functions. Incidentally, when we performed multiple regression analysis using all the factors, including body weight, sex, and uric acid, that showed multicollinearity in the scatter plot matrix, as independent variables, *OATP1B1*15* allele, sex, total bilirubin, ALT, HbA1c, and body weight were identified as significant independent variables (Table S1). Adjusted r^2 was almost the

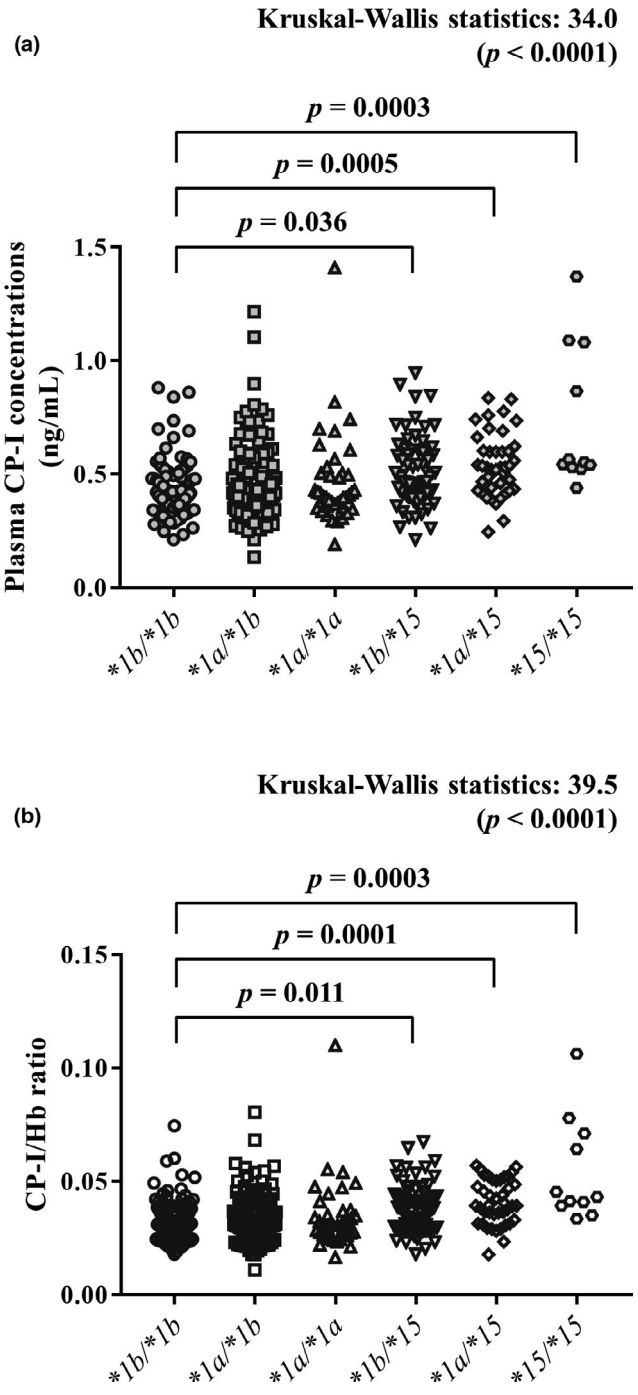

FIGURE 3 Comparison of distribution of plasma coproporphyrin-I (CP-I) concentration (a) and distribution of ratio of plasma CP-I to hemoglobin level (CP-I/Hb ratio) (b) among six OATP1B1 polymorphism groups by Kruskal–Wallis test

same in the two analyses (0.22 vs. 0.24). Sex was selected instead of hemoglobin level probably because of its strong association with hemoglobin level (Figure 1). Although it is unknown whether hemoglobin or sex is more associated with plasma CP-I concentration, hemoglobin level seems to be more appropriate for analysis of the mechanism associated with heme synthesis.

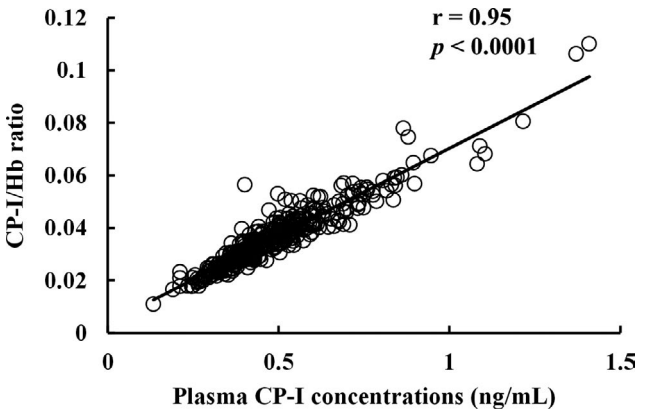

FIGURE 4 Correlation of plasma coproporphyrin-I (CP-I) concentrations and ratio of plasma CP-I to hemoglobin level (CP-I/Hb ratio)

To examine whether correction by hemoglobin level improves the usefulness of plasma CP-I concentration as a probe for OATP1B phenotyping, we compared the results of Kruskal–Wallis test on the distribution of the two measures among six OATP1B1 polymorphism groups. As shown in Figure 3, no large difference was observed in Kruskal–Wallis statistics between the result for plasma CP-I concentrations and that for CP-I/Hb ratio. This finding suggests that hemoglobin level has less clinical significance than *OATP1B1*15* allele on plasma CP-I concentration, and that correction by hemoglobin level is not needed when using plasma CP-I concentration for phenotyping OATP1B activity. Indeed, CP-I/Hb correlated strongly with plasma CP-I concentration in this study (Figure 4), suggesting that the two measures are probably equivalent. The results of multiple regression analysis may suggest that hemoglobin level reflects the biosynthesis of CP-I. The results of Kruskal–Wallis test may imply that the clearance of CP-I has bigger impact than biosynthesis of CP-I on plasma CP-I concentration, as was previously observed in a model-based study.[29] As an endogenous probe for phenotyping OATP1B activity in vivo, plasma CP-I concentration should be used without correction by hemoglobin level.

There is are limitations in this study. The participants were selected from the general population, and there was no information on whether some participants had anemia. Thus, it is unknown whether correction of plasma CP-I concentration by hemoglobin level is needed in patients with low hemoglobin level due to anemia. Further studies are required to elucidate the need of correction by hemoglobin level when plasma CP-I concentration is used for phenotyping OATP1B in patients with anemia.

In conclusion, hemoglobin level is an independent factor associated with basal plasma CP-I concentration in general population subjects, but no large difference in Kruskal–Wallis statistics was observed between the distribution of plasma CP-I concentration and that of CP-I/Hb ratio among six OATP1B1 polymorphism groups. These

findings suggest that correction by hemoglobin level is not needed when using plasma CP-I concentration for phenotyping OATP1B activity.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

Yo.S., T.K., M.N., and K.O. wrote the manuscript. Y.S., T.K., R.U., and K.O. designed the research. Yo.S., Yu. S., T.K., C.Y., A.O., M.N., M.K., and Y.M. performed the research. Yo.S., Yu.S., and C.Y. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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