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The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin

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Abstract

Interindividual variation in response to metformin, first-line therapy for type 2 diabetes, is substantial. Given that transporters are determinants of metformin pharmacokinetics, we examined the effects of promoter variants in both multidrug and toxin extrusion protein 1 (MATE1) (g.

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CONFLICT OF INTEREST
The authors declare no conflict of interest

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SLS: Development of study protocol, coordinating and conducting study, bioanalytical analysis, data analysis, manuscript preparation
KMM: Bioanalytical analysis, manuscript preparation
SWY: Data analysis, manuscript preparation
RAC: Development of study protocol, co-ordination of study, medical assistance
LX: Manuscript preparation
AD: Development of study protocol
AHR: Contributed clinical data
DMR: Contributed clinical data
RAW: Contributed clinical data
CM: Contributed clinical data
RLD: Contributed clinical data
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−66T→C, rs2252281) and MATE2 (g.−130G→A, rs12943590) on variation in metformin disposition and response. The pharmacokinetics and glucose-lowering effects of metformin were assessed in healthy volunteers (n = 57) receiving metformin. The renal and secretory clearances of metformin were higher (22% and 26%, respectively) in carriers of variant MATE2 who were also MATE1 reference ($P < 0.05$). Both MATE genotypes were associated with altered post-metformin glucose tolerance, with variant carriers of MATE1 and MATE2 having an enhanced ($P < 0.01$) and reduced ($P < 0.05$) response, respectively. Consistent with these results, patients with diabetes (n = 145) carrying the MATE1 variant showed enhanced metformin response. These findings suggest that promoter variants of MATE1 and MATE2 are important determinants of metformin disposition and response in healthy volunteers and diabetic patients.

Keywords

metformin; MATE1; MATE2; genetic polymorphism; pharmacokinetics; pharmacodynamics; glucose; healthy volunteers; type II diabetic patients; HbA1c

INTRODUCTION

As the first line therapy for the treatment of type II diabetes, metformin is the most frequently prescribed anti-diabetic drug [1]. Although controversial, studies suggest that metformin's pharmacological action is related to its activation of AMPK, which reduces hepatic glucose production, enhances glucose uptake in hepatic cells and peripheral tissues, decreases absorption of glucose from the gastrointestinal tract and increases insulin sensitivity in peripheral tissues [2, 3].

The pharmacokinetics of metformin have been studied extensively in both healthy volunteers and type II diabetic patients. About 50% of an oral dose is absorbed [4, 5] into the blood and rapidly distributed to various tissues. Metformin is not bound to plasma proteins [6] and is eliminated into urine as unchanged drug [4, 7]. The renal clearance of metformin is much greater than glomerular filtration rate, suggesting a significant contribution of tubular secretion to its elimination. Considerable inter-individual variability in the renal clearance of metformin has been observed in healthy volunteers (150–700 mL/min) [7] which includes a strong genetic component [8, 9]. In addition to pharmacokinetic inter-individual variability, the response to metformin varies substantially, with more than 30% of patients receiving metformin monotherapy classified as non-responders [10].

Metformin relies on facilitated transport for delivery to the liver, kidney and peripheral tissues. Indeed, previous studies demonstrate that membrane transporters contribute to the inter-individual variability in the pharmacokinetics and pharmacodynamics of metformin [11–15]. Metformin is transported primarily by the organic cation transporters (OCTs), particularly OCT1 and OCT2, and multi-drug and toxin extrusion proteins (MATEs), namely MATE1 and MATE2. MATE1 and OCT1 have been implicated as determinants of metformin response primarily due to their tissue distribution at major sites of metformin action. MATE1 is highly expressed in the kidney and liver (Supplemental Figure 1) with lower expression in skeletal muscle and adipose tissue [16], whereas, OCT1 is predominantly expressed in the liver. Previous reports from our laboratory and others have shown that OCT1 is the major determinant of metformin uptake into hepatocytes and polymorphisms of OCT1 are associated with reduced response to metformin in both healthy volunteers [11, 12] and diabetic patients [17, 18]. Interestingly, MATE1 and OCT1 have been shown to mediate transcellular transport of metformin *in vitro* [19, 20] and to effect metformin response in diabetic patients [14]. Other studies indicate MATE1 polymorphisms alone affect metformin response in diabetic patients [21].

In addition to effects on pharmacodynamics, transporters play a major role in metformin renal elimination [22]. In particular, OCT2 mediates the entry of metformin into the renal tubular cells, whereas MATE1 and MATE2 contribute to the efflux of metformin into the urine [19, 20] (Figure 1). Previous studies have shown that a nonsynonymous variant in OCT2 (A270S, rs316019) alters the renal clearance of metformin in healthy volunteers [15, 23, 24]. In addition, renal clearance and tissue distribution of metformin is altered in *Mate1*(−/−), but not *Mate1*(+/−) mice [25, 26]. However, the effect of genetic variation in MATE1 and MATE2 on the pharmacokinetics of metformin in humans remains unclear.

Until recently, clinical studies focused on the effects of promoter variants on drug disposition and response have been less well studied than coding and intronic region polymorphisms. Recently, our laboratory has shown that a common promoter variant, MATE2 (g.−130G>A, rs12943590), increases luciferase activity *in vitro* and associates with reduced response to metformin in diabetic patients [27]. In addition, another common promoter variant, MATE1 (g.−66T>C, rs2252281) exhibited reduced luciferase activity in reporter assays *in vitro* and was shown to associate with reduced-expression of MATE1 mRNA transcripts in the kidney [28]. In the current study, we hypothesized that these two promoter variants are determinants of metformin renal clearance and anti-diabetic response in healthy volunteers and diabetic patients. Because the two variants may have opposing effects, we also considered gene-gene interactions in our association studies. Our data demonstrate that in the absence of the MATE1 promoter variant, the MATE2 promoter variant is associated with an increased renal clearance of metformin. Interestingly, both variants associate with the glucose-lowering effects of metformin in healthy volunteers and in diabetic patents, but in opposite directions.

RESULTS

Healthy male and female Asian (n=18), African American, (n=33) and Caucasian (n=6) volunteers were genotyped for MATE1 (g.−66T>C, rs2252281), MATE2 (g.−130G>A, rs12943590), OCT1 (420del, rs72552763) and OCT2 (A270S, rs316019). All alleles were in Hardy-Weinberg equilibrium. The pharmacokinetics and pharmacodynamics of metformin were evaluated in these volunteers after oral dosing of the drug (1850 mg in total). The study design and characteristics of the type II diabetic patients have been reported previously [27]. Demographic characteristics for the healthy volunteers and a subset of patients are shown in Supplemental Table S1 and S2. Below we first discuss our analysis of the association of the MATE1 variant with the pharmacokinetics and pharmacodynamics of metformin in healthy volunteers and diabetic patients, followed by an analysis focused on the MATE2 promoter variant. For both promoter variants, we first analyzed of the effect of either variant alone, and then adjusted for each of the additional transporter variants (see Methods).

The MATE1 promoter variant, g.−66T>C, has no effect on the pharmacokinetics of metformin in healthy volunteers

The pharmacokinetic parameters obtained in the present study are similar to those previous reported in healthy volunteers [11, 15, 29–32].

The MATE1 g.−66T>C genotype had no significant effect on the pharmacokinetics of metformin (reference n=32, variant n=25), even after adjusting for creatinine clearance (CL_{CR}). The pharmacokinetics of metformin remained similar even after exclusion of volunteers carrying the OCT1 or OCT2 polymorphisms (Supplemental Table S3).

The MATE1 promoter variant, g.−66T>C, is associated with a greater response to metformin in healthy volunteers

Before metformin dosing, the area under the curve (AUC) of glucose (mean±SD, reference, 359±56 mg/dL/h; variant 352±77 mg/dL/h) and insulin (reference, 129±83 mU/L/h; variant 141±102 mU/L/h) were similar between MATE1 genotypes. After metformin administration, volunteers who were homozygous for the variant MATE1 allele had significantly lower glucose AUC (greater response) after the oral glucose tolerance test (OGTT) than those volunteers carrying at least one reference allele (reference, 309±39 mg/dL/h; variant, 250±37 mg/dL/h; P=0.002; Figure 3a-b). The association of the MATE1 allele with glucose AUC persisted in subsequent analysis of volunteers who were also homozygous for the reference OCT1 (MATE1 reference, n=43, 308±40 mg/dL/h; MATE1 variant, n=5, 242±41 mg/dL/h; P=0.005) or OCT2 (MATE1 reference, n=41, 306±41 mg/dL/h; MATE1 variant, n=4, 262±12 mg/dL/h; P=0.03) polymorphisms. We were unable to detect a significant effect of the MATE1 variant in healthy volunteers after removal of individuals with the MATE2 g.−130G>A variant because of a reduction in sample size, which resulted in a substantial loss of power. Insulin AUC (reference 124±74 mU/L/h; variant 109±68 mU/L/h) and concentrations 2 hours after glucose administration (reference 41±32 mU/L; variant 27±18 mU/L/h) were similar for both MATE1 reference and variant volunteers.

The MATE1 promoter variant, g.−66T>C, is associated with a greater response to metformin in type II diabetes mellitus patients

The effect of the MATE1 promoter variant on the response to metformin (relative change in HbA1c) was examined in diabetic patients from a previously described [27] cohort of Caucasian (n=185) and African American (n=64) patients receiving metformin monotherapy. Alone, the MATE1 promoter variant was not associated with the relative change in HbA1c (P>0.6). However, in our secondary analysis, in which we examined the effect of the MATE1 promoter variant together with other transporter variants, we obtained the following results. The MATE1 promoter variant was not associated with response to metformin after removal of patients who are carriers of OCT2 A270S or MATE2 g.−130G>A. In contrast, when patients carrying one or more OCT1 reduced-function variants were removed from the analysis, the MATE1 variant allele had a significant effect on response (Figure 3c, Table 1). That is, Caucasian and African American patients homozygous for the MATE1 variant allele had a significantly larger relative change in HbA1c levels (i.e. greater response to metformin) than patients carrying at least one reference MATE1 allele (Figure 3c, Table 1, P=0.01).

The MATE2 promoter variant, g.−130G>A, is associated with increased renal clearance

With the exception of half-life, the pharmacokinetics of metformin were similar for individuals with the MATE2 reference (n=27) and those with the variant allele (n=30, Figure 4a and Table 2), even after adjustment for CL_{CR}. The elimination half-life was longer in volunteers carrying at least one MATE2 (g.−130G>A) variant allele compared to those with the reference MATE2 genotype. This association remained even after adjustment for gender, body mass index (BMI) and MATE1 (g.−66T>C, rs2252281) genotype (P=0.02).

In the secondary analysis, we removed volunteers carrying at least one minor allele of the MATE1 polymorphism. In individuals who were homozygous for the reference MATE1 (n=32), the MATE2 variant was associated with lower plasma levels between 2 and 5 hours after metformin administration (P<0.05, Figure 4b). Although most of the pharmacokinetic parameters remained similar between the MATE2 variant groups, the CL_R and CL_{SR} of metformin were significantly higher in volunteers carrying at least one MATE2 variant compared to those homozygous for reference MATE2 (P<0.05, Table 2, Figure 4).

The MATE2 promoter variant, −130G>A, is associated with reduced metformin response in healthy volunteers

After metformin dosing, the glucose AUC was higher for volunteers homozygous for the MATE2 variant allele (333±37 mg/dL/h) as compared with those carrying at least one reference MATE2 allele (295±44 mg/dL/h; P=0.02; Figure 5a-b), while no effect of the MATE2 variant was observed in baseline glucose AUC before metformin treatment. Removing individuals with variants in MATE1 (MATE2 reference, 301±38 mg/dL/h; MATE2 variant, 345±36 mg/dL/h; P =0.02), OCT1 (MATE2 reference, 298±45 mg/dL/h; MATE2 variant, 333±37 mg/dL/h; P=0.04) or OCT2 (MATE2 reference, 299±41 mg/dL/h; MATE2 variant, 342±30 mg/dL/h; P =0.02) resulted in no change in the significance level of the glucose AUC between reference and variant MATE2 genotypes during metformin treatment.

Using linear regression, we determined if sex, age, BMI, fasting glucose, fasting insulin, MATE1 genotype, MATE2 genotype and metformin exposure (AUC) predicted the glucose AUC during metformin treatment. The MATE1 (P=0.02) and MATE2 genotypes (P=0.02) were the only significant predictors of metformin response, with each genotype alone explaining 7% of the variability in response to metformin. When both MATE1 and MATE2 genotype were included in a multiple linear regression model, 15% of the variance in metformin response was explained (P=0.005). The OCT2 genetic polymorphism has been previously associated with the renal clearance of metformin and nephrotoxicity of cisplatin [15, 33]. In our study, the OCT2 genotype (rs316019) alone had no effect on variation in the pharmacokinetics or pharmacodynamics of metformin.

DISCUSSION

Previous reports on genetic variants of MATE1, MATE2, OCT1 and/or OCT2 on metformin pharmacokinetics [17] and/or pharmacodynamics [11, 12, 17, 21, 26, 34] have focused on synonymous or nonsynonymous single nucleotide polymorphisms (SNPs) within non-regulatory regions of these genes. In this study, we determined the effects of two promoter variants of MATE1 (rs2252281) and MATE2 (rs12943590), discovered previously in our laboratory [27, 28], on the pharmacokinetics and pharmacological response to metformin in healthy volunteers and on the glycemic response to metformin in type II diabetic patients. In our primary analysis, we considered each variant separately. Given the co-localization of MATE1 and OCT1 in hepatocytes, and MATE1, MATE2 and OCT2 in proximal tubule cells (Figure 1), polymorphisms affecting the accumulation and/or elimination of metformin in these tissues, could confound the effects of the individual MATE1 and MATE2 promoter variants on metformin disposition and response. Therefore, whenever possible, we performed secondary analyses excluding individuals who had OCT1 (rs72552763), OCT2 (rs316019) or the other MATE genotypes that could potentially confound the measured parameters. Our major findings include: (a) The MATE1 reduced-expression promoter variant is associated with increased response to metformin in healthy volunteers and type II diabetic patients who were homozygous for the OCT1 reference allele. (b) The MATE2 enhanced-expression promoter variant is associated with reduced response to metformin in healthy volunteers. (c) The CL_R and CL_{SR} of metformin were significantly greater in volunteers carrying the promoter variant of MATE2 compared to those homozygous for reference MATE2 in a subset of healthy individuals who were homozygous for the MATE1 reference allele. To our knowledge, this is the first study to show that functional promoter polymorphisms in MATE1 and MATE2 contribute to variation in the response and disposition of metformin in both healthy volunteers and diabetic patients.

Although the MATE1 polymorphism (g.−66T>C) did not influence metformin disposition, healthy volunteers who were homozygous for the MATE1 variant allele had a greater

glucose-lowering response to metformin (Figure 3a-b). Similarly, diabetic patients without reduced-function alleles of OCT1 who were also homozygous for the MATE1 variant allele had a 15% greater relative reduction in Hb1Ac compared to patients carrying the MATE1 reference allele (Figure 3c, Table 1). The finding that a MATE1 variant affects the pharmacodynamics, but not the pharmacokinetics of metformin, underscores the importance of transporters in tissue specific drug distribution. MATE1 is highly expressed in both the liver and kidney [16] (Supplemental Figure S1), with the liver being a major site of pharmacologic action and the kidney being predominantly a site of metformin elimination (although effects on glucose may also occur). In the kidney, MATE1 is redundantly co-expressed with MATE2 and MATE2-K, and therefore a variant in MATE1 would need to have a large effect size to have a measurable effect on the renal elimination of the drug. In contrast, in the liver, MATE1 appears to be the sole metformin transporter expressed on the bile canalicular membrane; therefore a genetic variant in MATE1 may have a more measurable effect on the pharmacodynamics compared to the pharmacokinetics of metformin. Mechanistically, our results are consistent with MATE1 acting as a proton gradient-driven efflux pump in tissues of pharmacodynamic importance, such as the liver [35, 36]. The reduced-expression promoter polymorphisms of MATE1 would presumably result in reduced transporter expression levels, leading to reduced efflux and correspondingly higher tissue levels of metformin. The higher tissue levels of metformin are predicted to associate with a greater pharmacologic response. Interestingly, when diabetic patients carrying the OCT1 reduced-function variant alleles were included in the analyses, we did not observe an effect of the MATE1 promoter variant on the response to metformin. It is possible that the reduced-function OCT1 variant masked the effects of the MATE1 variant (Figure 1). Particularly in the liver, the variant allele of OCT1 would result in lower drug levels and thus oppose the effects of the reduced-expression variant of MATE1. Our results also suggest that the MATE1 variant has a noticeable effect in patients with type II diabetes. For example, a typical diabetic patient with a baseline HbA1c of 8% (Supplemental Table S2) receiving metformin monotherapy (1000 mg/day) and reference for the OCT1 genotype would have their HbA1c decreased by an additional 1.2% if they were a carrier of the MATE1 variant allele instead of the reference MATE1. The magnitude of this effect is large and of clinical significance, given that, on average, metformin monotherapy lowers HbA1c by 1.12% within the first year of therapy [37, 38]. If our results are replicated in other cohorts, genotyping for the MATE1 polymorphism as a basis for personalizing metformin hypoglycemic therapy should be considered.

The effect of genetic variants in MATEs on metformin pharmacokinetics and pharmacologic action also suggests the potential for clinical significant drug-drug interactions (DDIs) that may "phenocopy" the effects of genetic variants. In fact, in an in vitro cell system (Chinese Hamster Ovary), inhibitors of human MATEs were identified from a diverse set of drug classes (e.g., pyrimethamine, baclofen, ketoconazole, naloxone, propranolol) [39]. In addition, recently Kusuhara et al. [40] demonstrated that pyrimethamine, an anti-protozoal drug and inhibitor of MATEs, reduced metformin renal clearance in human volunteers. This example highlights the importance of follow-up clinical studies to elucidate the clinical consequences of any in vitro DDI identified and how such a DDI may phenocopy the reduced-expression variant of MATE1.

Recent studies in our laboratory have shown that a common MATE2 promoter variant (g. −130G>A) associates with reduced response to metformin in diabetic patients [27]. In the present study we hypothesized that this variant is associated with reduced response to metformin as a result of a pharmacokinetic mechanism. Surprisingly, in healthy volunteers, MATE2 g.−130G>A did not affect the CL_R and CL_{SR} of metformin (Figure 4 and Table 2). Because MATE1 and MATE2 are expressed on the apical membrane of the proximal tubule and are likely to work together to mediate metformin renal elimination (Figure 1), we

hypothesized that the effect of the MATE2 variant may be masked by the opposing effect of the MATE1 variant. Indeed, when we removed the individuals with the reduced-expression promoter variant of MATE1 g.−66T>C, we observed that the CL_R and CL_{SR} of metformin were significantly greater in individuals carrying the MATE2 variant allele compared to the reference allele (Figure 4). Consistent with this finding, the mean metformin exposure (AUC) was lower and the mean amount of metformin excreted in urine was higher in volunteers with the variant MATE2 allele, although the difference was not statistically significant, possibly because of variability in the bioavailability of metformin among our participants (Table 2). Interestingly, metformin concentrations were significantly lower in volunteers carrying the variant MATE2 at time points after the C_{max}. Importantly, we observed a longer half-life in individuals with the MATE2 variant alleles (P=0.03). As metformin exhibits apparent flip-flop kinetics [41], early time points reflect elimination, whereas, later time points predominantly reflect drug absorption. The lower plasma levels after the C_{max} and the longer half-life of the drug in individuals homozygous for the variant MATE2 allele are consistent with a reduced rate and extent of absorption of metformin. These results suggest that expression polymorphisms of MATE2 may alter the absorption of metformin from the gastrointestinal tract, as well as, its secretion (clearance) from then kidney. Although currently not implicated in the absorption of metformin, MATE2, is expressed in the small intestine (Supplemental Figure S1) and could contribute to the interindividual variation in the bioavailability of metformin. Current immunohistochemistry data suggests that MATE2 is expressed at a moderate level in the different sections of the gastrointestinal tract (e.g. duodenum, stomach, small intestine and colon) [42]. Further studies are required to confirm the localization and determine the function of MATE2 in the gastrointestinal tract and delineate the impact of MATE2 on the absorption of metformin.

The current study in healthy volunteers is consistent with previous studies in diabetic patients [27] that associate the MATE2 promoter variant with a reduced response to metformin, and provides evidence that this effect may be the result of a pharmacokinetic mechanism. That is, plasma levels of metformin were significantly lower in the volunteers homozygous for the variant MATE2 allele (Figure 4), throughout the OGTT (2–5 hours after metformin administration), potentially decreasing glucose uptake after the OGTT. Alternatively, by modulating metformin levels in a target tissue, the MATE2 variant may directly affect the pharmacodynamics of metformin. In particular, MATE2 is predominantly expressed in the kidney (Supplemental Figure S1) [16] an organ increasingly recognized to play a significant role in both systemic glucose production (20–25%) and glucose utilization (10%) in the fasting state [43–46]. In type II diabetes, renal gluconeogenesis and glucose uptake increases [47]. Thus, individuals homozygous for the MATE2 enhanced-expression variant would achieve lower levels of metformin in the kidney, possibly translating into a reduced pharmacologic effect. A more comprehensive understanding of the peripheral targets of metformin is needed to completely interpret these results.

In conclusion, this study demonstrates that promoter variants of MATE1 and MATE2 contribute to the glycemic response to metformin in healthy volunteers and in patients with type II diabetes. Further, the study provides evidence that MATE1 and MATE2 work in concert in the kidney to mediate metformin renal elimination and that genetic variants of MATEs and OCTs should be considered together when ascertaining the genetic determinants of renal elimination of metformin. Finally, the results of our study suggest an important role of MATE2 in the pharmacokinetics and pharmacodynamics of metformin.

METHODS

Healthy human volunteers

The Committee on Human Research at the University of California, San Francisco (IRB 10-03087 and 10-02578) approved this study. Healthy male and female volunteers were recruited directly from the Study of Pharmacogenetics in Ethnically Diverse Populations (IRB 10-03167) and participants were enrolled only after provided informed consent was provided. To be eligible for this study, volunteers were older than 18 years of age and not taking any medications other than vitamins and/or oral contraceptives. Screening included a comprehensive medical history, physical examination, and laboratory studies (complete blood count, electrolytes, BUN and creatinine, albumen, and liver enzymes). Volunteers with elevated liver enzymes, anemia, elevated creatinine concentrations or a positive pregnancy test were excluded.

Genotyping

MATE2 (g.−130G>A, rs12943590), OCT1 coding variants (R61C [rs12208357], G401S [rs34130495], 420del [rs72552763] and G465R [rs34059508]) and OCT2 (A270S, rs316019) were genotyped by a TaqMan assay using standard procedures. MATE1 (g. −66T>C, rs2252281) was genotyped by PCR amplification followed by sequencing of the promoter region. The OCT1 coding variants (R61C, G401S and G465R) were only genotyped in the type II diabetic cohort.

Clinical study procedures

Once enrolled, volunteers were advised to maintain stable activity levels seven days before commencing the study. After the initial 3-day carbohydrate controlled diet (200–250 g/day), volunteers were admitted to the General Clinical Research Center (GCRC) at San Francisco General Hospital and remained there for the duration of the study (72 h). After an overnight fast (10 h), each underwent a 3 h oral glucose tolerance test (OGTT, 75 g; day 1). Volunteers were dosed with 1000 mg metformin (Major Pharmaceuticals, Livonia, MI) in the evening of study day 1 followed by a dose of 850 mg early on the second study day (day 2). A second OGTT was administered 2 h after metformin administration on study day 2. Standardized meals were provided on both study days after completion of the OGTT. Following the metformin dose, volunteers were asked to drink 8 oz of water every 2 h to maintain urine flow and pH.

Timed blood samples were collected after the first (0, 0.5, 1, 2 and 11 h) and second (0, 0.5, 1, 1.5, 2, 2.25, 2.5, 3.75, 3, 3.5, 4, 6, 8, 10, 12 and 24 h) metformin dose, respectively, for the determination of plasma metformin concentrations. For metformin pharmacodynamics (glucose/insulin concentrations) blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 180 min after glucose administration. An additional blood sample was collected at 12 h after the second dose of metformin to determine serum creatinine. Urine samples were collected during the following time intervals: 0–12 h after the first dose of metformin and 0–2, 2–4, 4–6, 6–8, 8–12 and 12–24 h after the second dose of metformin.

Analytical methods

Metformin concentrations in plasma and urine were assayed by a validated liquid chromatography-tandem mass spectrometry method [11]. The quantification limit was 4 mg/L for plasma and 40 ng/mL for urine. Both the intra-day and inter-day coefficients of analysis variation were less than 5%. Glucose concentrations in plasma and creatinine concentrations in plasma and urine were determined using standard colorimetric assays. Insulin concentrations in plasma were determined using an immunoassay (Mercodia, NC) following the manufacturer’s instructions.

Clinical pharmacokinetics

The concentration-time profile of metformin was evaluated by non-compartmental analysis (WinNonlin 4.1, Pharsight Corporation, Mountain View, CA). The pharmacokinetics of metformin from both plasma and urine were calculated after the second dose as described previously [11].

Type II diabetic patients

Diabetic patients of Caucasian or African American ethnicity were recruited into a multi-center retrospective study described previously [27]. Briefly, all patients were prescribed metformin monotherapy as their initial hypoglycemic medication, had HbA1c levels measured both before and after commencement of metformin treatment and a medication possession ratio greater than 80%. The Institutional Review Boards of the Marshfield Clinic Research Foundation, Kaiser Permanente South East, Georgia and UCSF approved this study and informed consent was obtained. The review process for BioVU has been previously described [48].

Statistical Analysis

Data are presented as mean ± standard deviation unless indicated otherwise. Unpaired and paired nonparametric Student's *t*-tests were used to analyze the differences in metformin pharmacokinetic and pharmacodynamic parameters, respectively, for each genotype using GraphPad Prism 4.0 (GraphPad Software, SD). A statistically significant result was defined when $P<0.05$. The 95% confidence intervals for the relative change in HbA1c were calculated by a nonparametric bootstrap method using the R software package (www.r-project.org, version 2.12.0). Linear regression and multivariate analyses were carried out using the R software.

All analyses were conducted firstly, by assessing the effect of each variant alone and secondly, after the exclusion of individuals carrying confounding genotypes on the pharmacokinetics and pharmacodynamics of metformin. A recessive genetic model was used, unless the analyses were underpowered, whereby a dominant genetic model was used. In the secondary analyses we removed individuals who were carrying at least one minor allele for MATE1 (g.−66T>C), MATE2 (g.−130G>A), a reduced-function OCT1 coding variant (R61C, G401S, 420del and G465R) or OCT2 coding variant (A270S) that could confound the measured parameters. This secondary analysis was conducted because the MATE1 (g.−66T>C) variant opposes the effects of the MATE2 (g.−130G>A) variant (i.e. the MATE1 and MATE2 polymorphisms result in reduced and enhanced expression, respectively, in reporter assays). In addition, coding variants in OCT1 and OCT2, are known to effect metformin pharmacodynamics [11, 12, 17, 18] and pharmacokinetics [15, 23, 24], respectively, and could confound the effects of MATE variants on the disposition and response to metformin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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STUDY HIGHLIGHTS

What is the current knowledge on the topic?

Previous studies have focused on the effect of less common nonsynonymous and/or intronic variants in membrane transporters on metformin pharmacokinetics and response.

What question this study addressed?

In this study, we investigated whether two common promoter variants in multidrug and toxic extrusion 1 (MATE1) and 2 (MATE2) affect the pharmacokinetics and pharmacodynamics of metformin in healthy volunteers and in patients with type II diabetes.

What this study adds to our knowledge?

This study provides evidence that common functional promoter variants in MATE1 and MATE2 have a significant impact on the pharmacokinetics and pharmacodynamics of metformin.

How this might change clinical pharmacology and therapeutics?

This study adds to the growing body of literature demonstrating that genetic variants in membrane transporters significantly influence the pharmacokinetics and response of metformin. In future, genotyping of MATE1 and MATE2 may be used to inform metformin therapy.

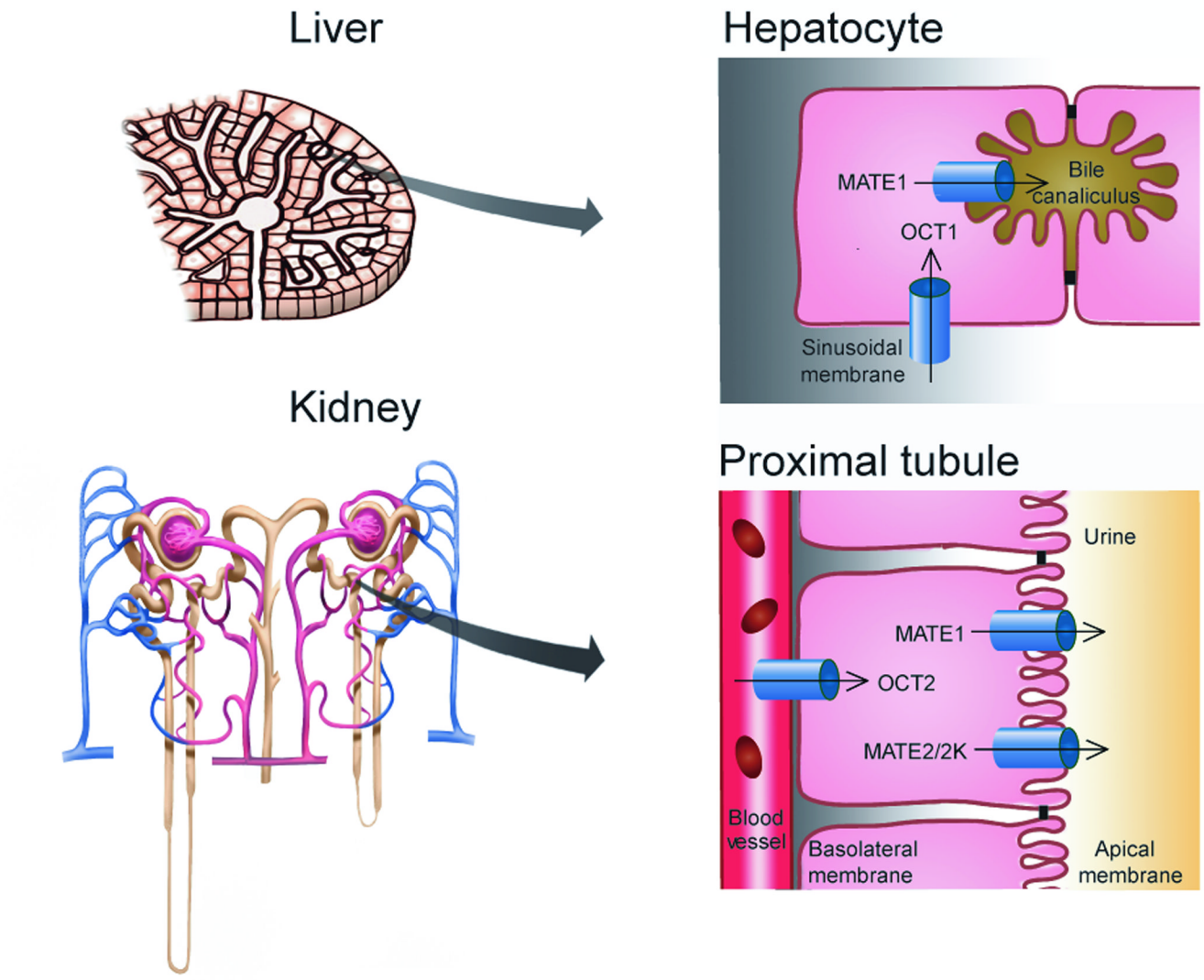

Figure 1. Representative cartoon of metformin transporters in the hepatocyte of the liver and nephron of the kidney

This cartoon shows the liver (top left) and nephron (bottom left) and the cation transporters in the hepatocyte (top right) and proximal tubule cell (bottom right) that have been identified as important determinants of the pharmacokinetics and response to metformin. MATE2 in the kidney has two functional isoforms, MATE2 and MATE2-K.

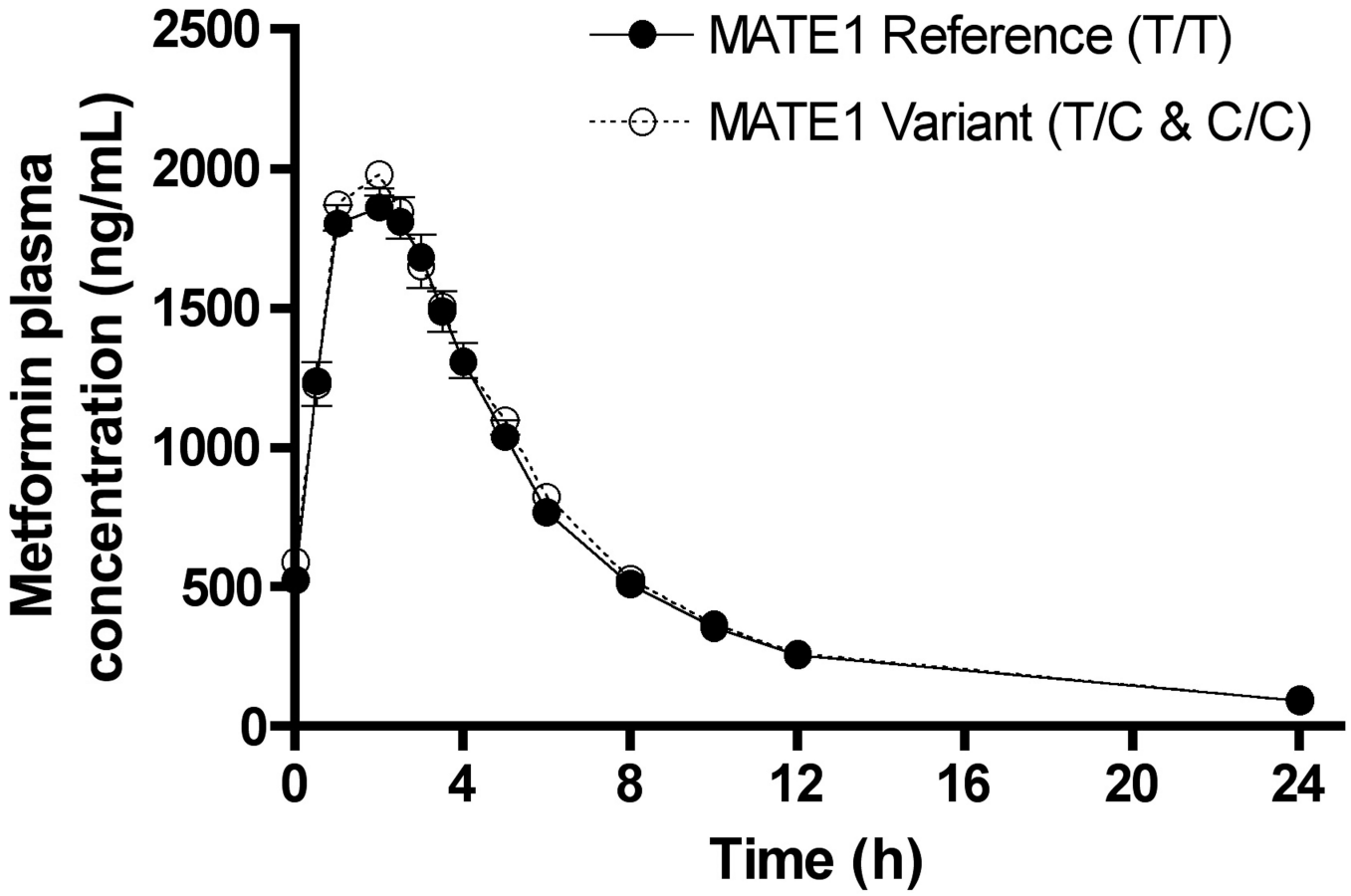

Figure 2. The effect of MATE1 (g.−66T>C) on the pharmacokinetics of metformin in 57 healthy volunteers

Shown is the mean plasma concentration-time curve of metformin after oral administration to healthy volunteers who carry at least one MATE1 (g.−66T>C) variant allele (n=25, open circles) or those who are homozygous for the reference MATE1 alleles (n=32, closed circles). The volunteers were given two doses of metformin (1850 mg in total). The plasma metformin concentration-time curves after the second dose are shown. Data represent mean ± SEM.

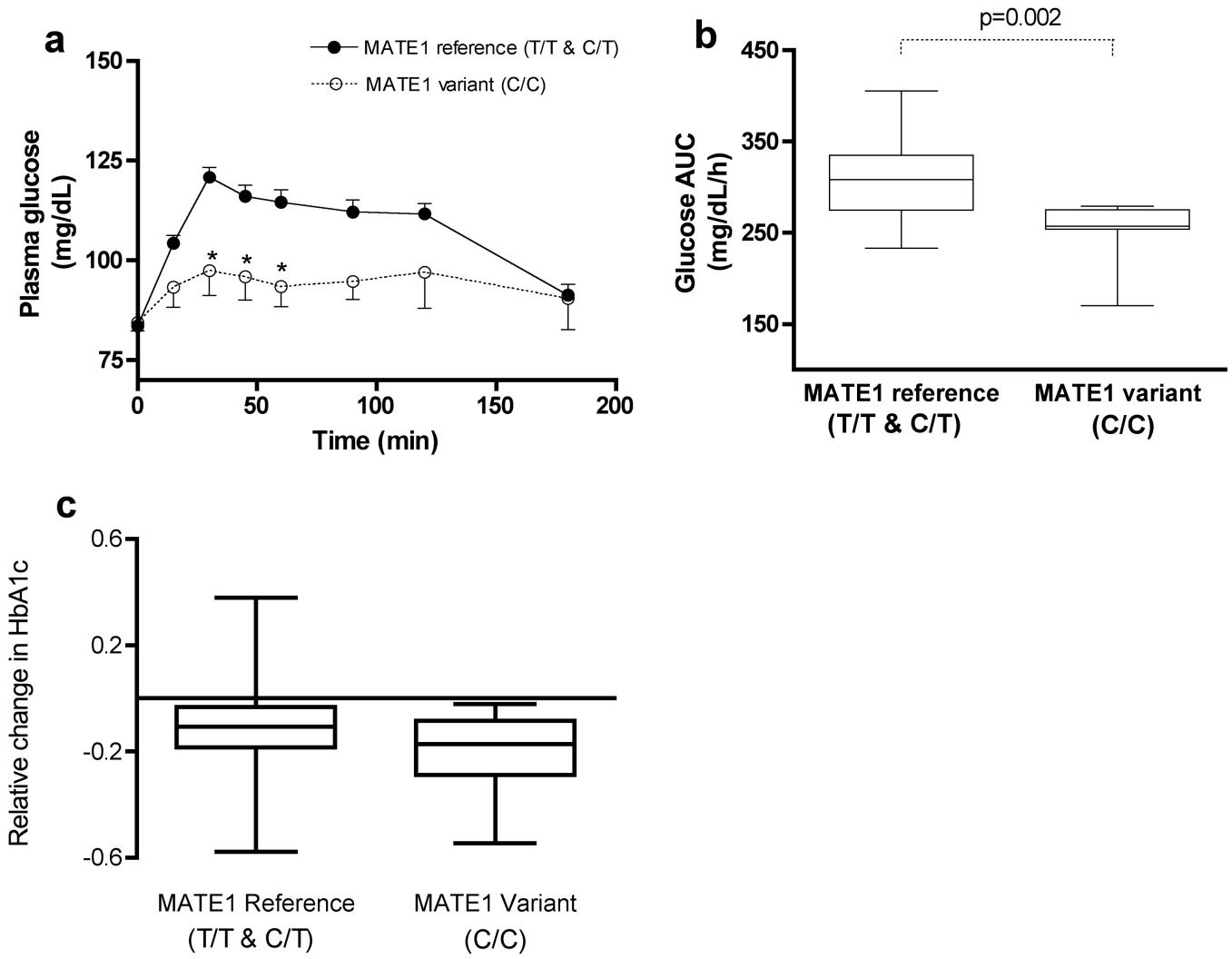

Figure 3. The MATE1 promoter variant (g.−66T>C) is associated with different response to metformin in healthy volunteers and type II diabetic patients

(**a**) The time course of plasma glucose concentrations after an oral glucose tolerance test (OGTT) during metformin treatment in healthy volunteers carrying at least one reference MATE1 (n=49, closed circles) and those carrying both MATE1 variant (n=8, open circles) alleles. The data are expressed as mean ± SEM; **P*<0.05 compared with volunteers with at least one reference allele. (**b**) The glucose exposure with OGTT (AUC) after metformin treatment in the same healthy volunteers represented in (a). (**c**) The relative change in glycosylated hemoglobin (HbA1c) in diabetes patients (n=145) receiving metformin monotherapy who are homozygous for the major alleles of OCT1 and carrying at least one reference MATE1 allele (n=122) or homozygous for the MATE1 variant allele (n=23). Relative change in HbA1c was calculated as follows: (treatment minus baseline HbA1c)/ baseline HbA1c. Relative change of −0.15 is interpreted as a decrease in HbA1c level by 15% from baseline. The box plots (b and c) display the median and interquartile range (the 25th–75th percentile). The whiskers display lower and upper values within 1.5 times the interquartile range beyond the 25th and 75th percentile.

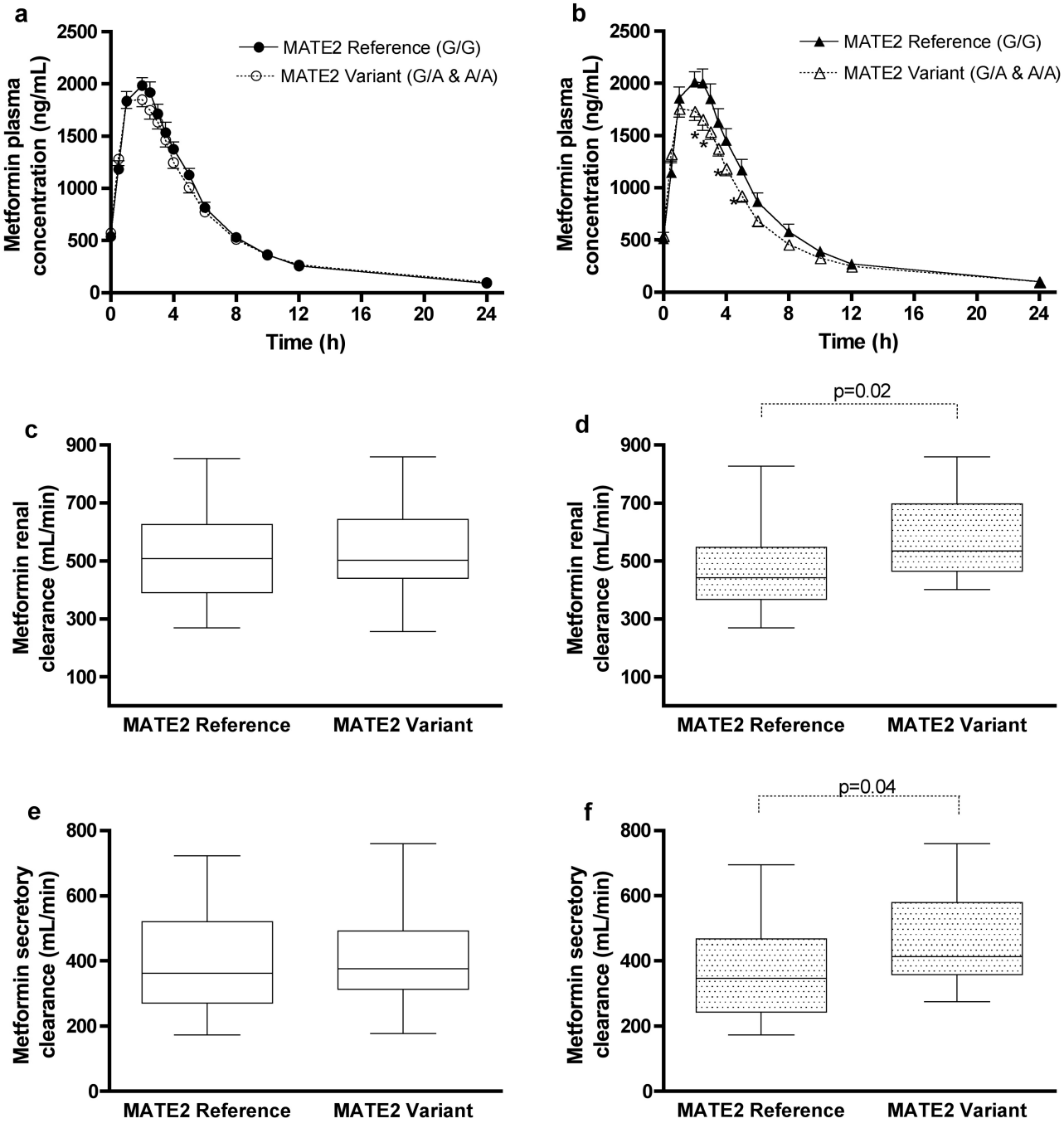

Figure 4. The effect of MATE2 (g.−130G>A) on the pharmacokinetics of metformin in 57 healthy volunteers

The plasma concentration-time curves of metformin after oral administration to healthy volunteers (**a**) who carry at least one MATE2 variant allele (n=30, open circles) or those who carry only MATE2 reference alleles (n=27, closed circles) and carry either the reference or variant alleles of MATE1 or (**b**) who carry at least one MATE2 variant allele (n=17, open triangles) or those who carry only MATE2 reference alleles (n=15, closed triangles) and only carry both reference alleles for MATE1. Data represent mean ± SEM; **P*<0.05 compared with volunteers with at least one reference allele. The renal clearance (**c**) and net tubular secretion (**e**) of the same volunteers (open boxes) depicted in (a). The renal

clearance (**d**) and net tubular secretion (**f**) of the same volunteers (shaded boxes) depicted in (b). The box plots (c-f) display the median and interquartile range (the 25^{th}–75^{th} percentile). The whiskers display lower and upper values within 1.5 times the interquartile range beyond the 25^{th} and 75^{th} percentile. The renal secretion of metformin was calculated by subtracting the clearance of creatinine from the renal clearance of metformin. The volunteers were given two doses of metformin (1850 mg in total). The plasma metformin concentration-time curves after the second dose are shown.

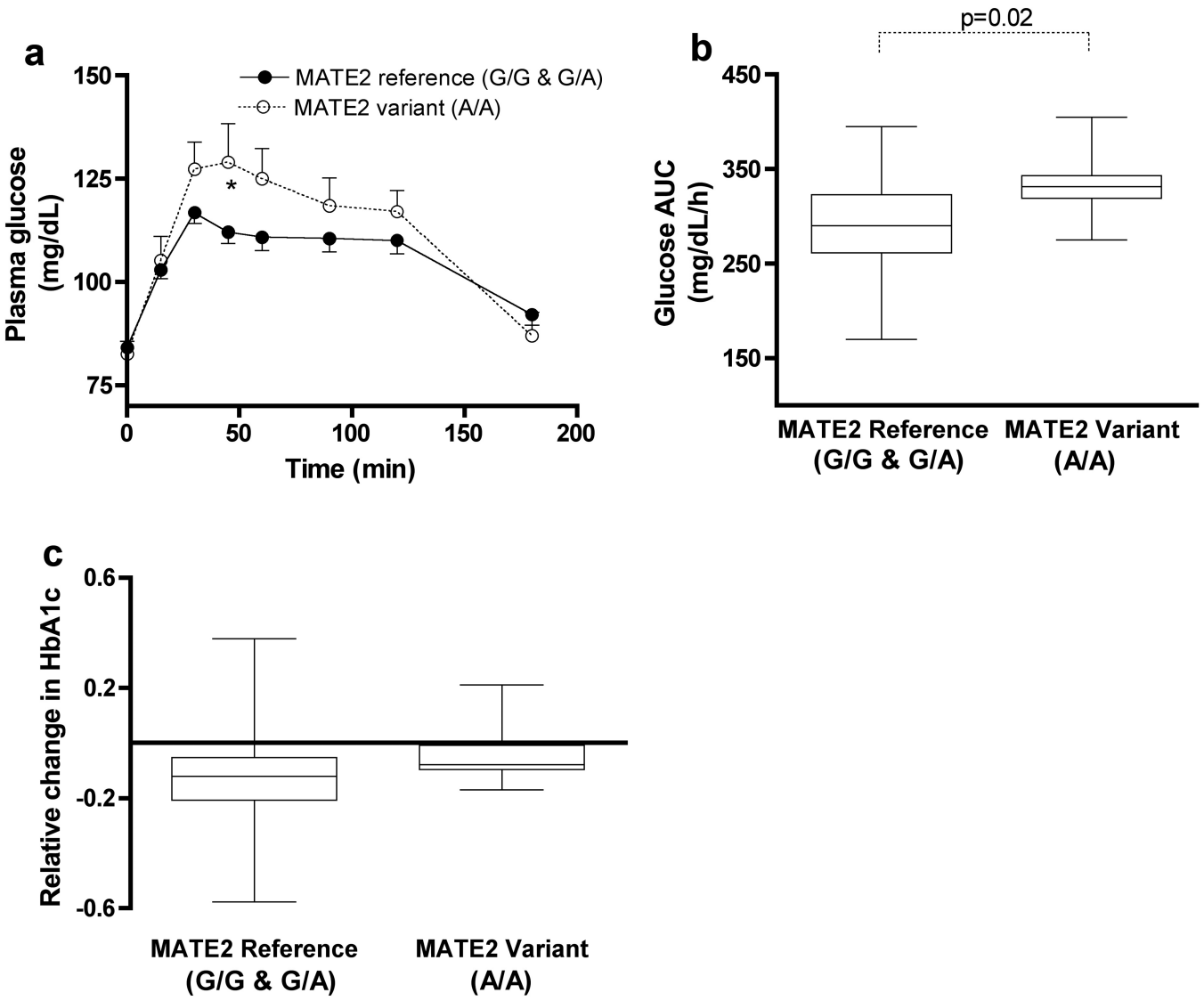

Figure 5. MATE2 genetic variants are associated with different response to metformin in healthy volunteers and type II diabetic patients

(**a**) The time course of plasma glucose concentrations after an OGTT during metformin treatment in healthy volunteers carrying at least one reference MATE2 (n=49, closed circles) and those carrying both MATE2 variant (n=8, open circles) alleles. The data are expressed as mean ± SEM; **P*<0.05 compared with volunteers with at least one reference allele. (**b**) The glucose exposure with OGTT (AUC) after metformin treatment for healthy volunteers represented in (b). (**c**) The relative change in glycosylated hemoglobin (HbA1c) in Caucasian (n=189) and African American (n=64) type II diabetes patients receiving metformin monotherapy who are reference for the MATE2 reduced-function coding variant (c.485C>T) and carrying at least one reference MATE2 allele (n=232) or homozygous for the MATE1 variant allele (n=16). Relative change in HbA1c was calculated as follows: (treatment minus baseline HbA1c)/baseline HbA1c. Relative change of −0.15 is interpreted as a decrease in HbA1c level by 15% from baseline. The box plots (b, c) display the median and interquartile range (the 25th–75th percentile). The whiskers display lower and upper values within 1.5 times the interquartile range beyond the 25th and 75th percentile.

Table 1

Association analyses of MATE1 g.−66T>C with metformin response (relative change in HbA1c) in patients with type II diabetes.

(i) Caucasians (n=90)		
Genotype group g.−66T>C	Number	Mean Relative Change (95% Confidence Interval)
TT	37	−0.15 (−0.20, −0.10)
TC	42	−0.088 (−0.12, −0.057)
CC	11	−0.27 (−0.36, −0.19)
TT/TC	79	−0.12 (−0.15, −0.088)
Statistical Analyses		
Kruskal-Wallis test		P=0.0015
Mann-Whitney test (TT/TC vs CC)		P=0.0011
Linear regression model (Recessive): Relative Change = variant allele + dose		Coefficient = −0.14 (−0.23, −0.051); P=0.0022
(ii) Caucasians and African Americans (n=145)		
Genotype group g.-66T>C	Number	Mean Relative Change (95% Confidence Interval)
TT	59	−0.16 (−0.20, −0.12)
TC	63	−0.078 (−0.11, −0.048)
CC	23	−0.21 (−0.27, −0.016)
TT/TC	122	−0.12 (−0.14, −0.09)
Statistical Analyses		
Kruskal-Wallis test		P=0.0008
Mann-Whitney test (TT/TC vs CC)		P=0.0064
Linear regression model (Recessive): Relative Change = variant allele + dose + ethnicity		Coefficient = −0.087 (−0.15 −0.021); P = 0.011

Mean relative change of HbA1c levels were calculated for each MATE1 g.-66T>C genotype group in the Caucasians (n=90), or the combined Caucasian and African Americans (n=145). All patients were homozygous for the OCT1 major alleles. The 95% confidence intervals were calculated by non-parametric bootstrap estimates of the 95% confidence interval. Relative change = (treatment HbA1c minus baseline HbA1c)/ baseline HbA1c.

Note: relative change of −0.15 is interpreted as decreased in HbA1c level by 15% from baseline. In the linear regression model, the coefficient represents the increase (positive value) or decrease (negative value) in relative change in HbA1c for those patients who carry homozygous variant g.-66T>C allele.

Table 2

Summary of the metformin pharmacokinetic parameters in healthy volunteers with known MATE1, OCT1 and OCT2 genotype and homozygous for the MATE2 reference allele (−130G/G) and heterozygous or homozygous for the MATE2 variant allele (−130G/A or −130A/A)

	All		MATE1 reference (TT)		OCT1 reference (AA)		OCT2 reference (CC)	
	MATE2 reference (GG)	MATE2 variant (GA & AA)	MATE2 reference (GG)	MATE2 variant (GA & AA)	MATE2 reference (GG)	MATE2 variant (GA & AA)	MATE2 reference (GG)	MATE2 variant (GA & AA)
N	27	30	15	17	41	8	38	7
T_{max} (h)	1.73 ± 0.66	1.79 ± 0.64	1.95 ± 0.66	1.69 ± 0.69	1.67 ± 0.68	1.97 ± 0.43	1.72 ± 0.65	1.96 ± 0.47
C_{max} (ng/mL)	2187 ± 490	2182 ± 601	2277 ± 501	2087 ± 598	2205 ± 470	2088 ± 883	2161 ± 509	2195 ± 897
AUC_{0-24} (ng.h/mL)	13400 ± 3200	13500 ± 2800	14000 ± 3700	12700 ± 2400	13700 ± 3000	12400 ± 3400	13300 ± 2900	12900 ± 3300
AUC_{inf}(ng.h/mL)	14100 ± 3300	14600 ± 2900	1500 ± 3700	13800 ± 2500	14600 ± 3000	13200 ± 3600	14300 ± 3000	13700 ± 3600
V/F (L)	538 ± 152	598 ± 205	515 ± 172	648 ± 202	556 ± 184	642 ± 208	581 ± 185	585 ± 142
CL/F (mL/min)	1056 ± 256	1015 ± 224	1033 ± 304	1063 ± 205	1020 ± 238	1138 ± 283	1091 ± 270	1040 ± 263
$T_{1/2}$(h)	5.87 ± 1.03	6.77 ± 1.71*	5.71 ± 0.86	7.05 ± 1.93*	6.29 ± 1.63	6.52 ± 1.22	6.45 ± 1.61	6.27 ± 1.09
Amount in $urine_{0-24}$(mg)	375 ± 88	377 ± 101	350 ± 56	387 ± 117	376 ± 99	368 ± 80	375 ± 86	372 ± 85
CL_R (mL/min)	527 ± 162	533 ± 143	473 ± 145	579 ± 142*	518 ± 146	582 ± 133	533 ± 149	550 ± 128
CL_{CR} (mL/min)	119 ± 32	122 ± 41	108 ± 28	118 ± 34	117 ± 28	145 ± 58	120 ± 30	134 ± 52
CL_{SR} (mL/min)	407 ± 158	410 ± 137	365 ± 145	461 ± 144*	401 ± 146	437 ± 106	413 ± 149	427 ± 110

Data were obtained from healthy volunteers given two doses of metformin. The first dose (1000 mg) was given at 1800 hours on study day 1 and the second dose (850 mg) was given at 0700 hours on study day 2. Blood and urine samples for the pharmacokinetic analysis were collected 0–24 h after the second dose. T_{max}, time to the maximal plasma concentration; C_{max}, maximal plasma concentration; AUC_{0-24}, area under the concentration-time curve from 0–24 h time point; AUC_{inf}, area under the plasma concentration versus time curve from 0 to infinity; V/F, apparent volume of distribution; CL/F, apparent oral clearance; $T_{1/2}$, plasma terminal elimination half-life; CL_R, renal clearance; CL_{CR}, creatinine clearance; CL_{SR}, renal clearance by secretion;

*$P<0.05$ compared to reference.