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Effect of Genetic Variation in the Organic Cation Transporter 2, OCT2, on the Renal Elimination of Metformin

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Abstract

Objective—The goal of this study was to determine the effect of a genetic variant in the organic cation transporter (OCT2), OCT2-808G/T, which results in an amino acid change, A270S, on the pharmacokinetics of the anti-diabetic drug, metformin.

Methods—The uptake of metformin was performed in stably transfected HEK-293 cells expressing the empty vector (MOCK), the reference OCT2-808G and the variant OCT2-808T. Healthy individuals with known OCT2 genotypes [fourteen homozygous for the OCT2 reference allele (808G/G) and nine heterozygous for the variant allele (808G/T, *3D)] were recruited into this study. Metformin concentrations in plasma and urine were measured by liquid chromatography-tandem mass spectrometry method. Creatinine levels were also measured in plasma and urine. Pharmacokinetic parameters were evaluated for both groups.

Results—We observed that in HEK-293 stably transfected cells, OCT2-808T had a greater capacity to transport metformin than did the reference OCT2. Metformin pharmacokinetics were characterized in twenty-three healthy volunteers of Caucasian and African American ancestries. We observed that the renal clearance (CL_R) and the net secretion ($SrCL_R$) of metformin were significantly different between the volunteers heterozygous for the variant allele (808G/T), and the volunteers homozygous for the reference allele (808G/G) ($p<0.005$). Multivariate analysis revealed that OCT2 genotype was a significant predictor of CL_R and $SrCL_R$ of metformin ($p<0.01$).

Conclusion—We conclude that genetic variation in OCT2 plays an important role in the CL_R and $SrCL_R$ of metformin in healthy volunteers.

Keywords

Organic cation transporter; Pharmacokinetics; Pharmacogenetics; Metformin; Membrane transporter; Single nucleotide polymorphism

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Introduction

Metformin is first-line drug therapy for the treatment of type II diabetes, and is the most frequently prescribed anti-diabetic drug in the United States. The mechanisms of action of metformin may include reduction of hepatic gluconeogenesis, decreased absorption of glucose from gastrointestinal tract as well as increased insulin sensitivity in peripheral tissues [1]. Metformin pharmacokinetics have been well-characterized in both healthy volunteers and diabetic patients and are unaffected by diabetes. After a single oral dose of metformin, the bioavailability of metformin is about 40 to 60%. Metformin is rapidly distributed following absorption and does not bind to plasma proteins. No metabolites or conjugates of metformin have been identified. The drug is mainly excreted into the urine in an unchanged form. The renal clearance (CL_R) of metformin is much greater than glomerular filtration rate (GFR), suggesting a significant contribution of tubular secretion in addition to GFR [2].

Primarily expressed on the basolateral membrane of polarized epithelia, organic cation transporters (OCTs) in the SLC22A family are responsible for the uptake of small organic cations, including many clinically used drugs into cells [3]. Predominantly expressed in the proximal tubule of kidney, OCT2 mediates the renal secretion of small organic cations such as metformin. Previously our laboratory identified twenty-eight single nucleotide polymorphisms (SNPs) in OCT2 (SLC22A2) from a collection of 247 ethnically diverse DNA samples by screening all 11 exons as well as 50-100 bp of flanking intronic sequence [4]. Of these, there were 12 SNPs in non-coding or intronic regions of the gene and 16 SNPs in coding regions. Seven of the 16 coding-region SNPs were synonymous, eight were non-synonymous and one was a single-nucleotide insertion that leads to a prematurely terminated region. Four polymorphic nonsynonymous variants, M165I, A270S, R400C and K432Q were functionally characterized and found to have altered transporter activity when assayed in *Xenopus laevis* oocytes [4]. The most common SNP was the non-synonymous change in nucleotide 808 (G/T), which causes the amino acid change from alanine to serine at position 270. This SNP was selected for this follow-up clinical study for the following reasons: (a) the SNP affected the function of the transporter in cellular assays; (b) the SNP had a high allele frequency (about 10% for different ethnic groups) in comparison to the other SNPs that altered function in which the allele frequencies were less than 2% in the sample population.

Considerable inter-individual variability in CL_R of metformin has been observed. In particular, renal clearance of metformin ranges from 300 to 1000 mL/min in normal healthy subjects [5]. This variation appears to have a strong genetic component [5, 6]. In addition, the response to metformin treatment is variable. For example, 36% of patients on metformin monotherapy are considered poor responders even under approved dosage regimens [7]. Recently two studies determined that metformin renal clearance was reduced in individuals with Asian ancestries who carried the 808G/T allele [8, 9]. The goal of this study was to determine the effect of the 808G/T allele of OCT2 on renal clearance of metformin in healthy volunteers of European and African American ancestries.

Methods

Cellular uptake assays

The procedures for uptake and kinetic assays were performed as described by Chen et al. [10]. Studies of metformin transport by OCT2 reference or its variants were performed using stably transfected Flp-In-293 cells generated according to manufacture's protocol (Invitrogen, Carlsbad, CA). For uptake studies, cells were grown in monolayers on 24-well poly-D-lysine-coated plates (BD Discovery Labware, Bedford, MA). Transport assays were

initiated by adding 9.25 μM [^{14}C] metformin. The uptake was performed at room temperature and was stopped at 30 seconds by washing cells three times with ice-cold choline containing buffer. Cells were lysed and intracellular radioactivity was determined by scintillation counting and normalized to per-well protein content as measured using the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). For kinetic studies, the cells were incubated with 9.25 μM [^{14}C] metformin as well as a range of concentrations of unlabeled metformin for 30 seconds. Final uptake was calculated by subtracting the uptake in MOCK cells (cells transfected with empty vector) from that in the cells expressing OCT2-808G or OCT2-808T at each corresponding substrate concentration. The K_m and V_{max} values were obtained by fitting the Michaelis-Menten equation $V=V_{max}*[S]/(K_m + [S])$ using Graphpad Prism 4.0 (Graphpad software Inc., San Diego, CA). V refers to the rate of substrate transport, V_{max} refers to the maximum rate of substrate transport, [S] refers to the concentration of substrate, and K_m is defined as the concentration of substrate at the half-maximal transport rate.

Western blotting

Stably-transfected HEK293 cells were harvested and total protein was extracted. The extracted samples were mixed with Laemmli sample buffer (1.5% SDS/5% glycerol/65 mM Tris·HCl, pH 6.8, with or without 10 mM DTT). After boiling at 100°C for 5 min, samples were analyzed by SDS/PAGE (10% acrylamide) by using a Mini-PROTEAN II apparatus (Bio-Rad, Hercules, CA). Proteins were transferred onto poly(vinylidene difluoride) membranes for 2 h at 100 V at 4°C by using the Transblot apparatus (Bio-Rad, Hercules, CA). The membranes were blocked in Tris-buffered saline containing 2% nonfat dry milk with 0.5% Tween 20 for 1 h at room temperature and incubated with the goat anti-OCT2 or β actin (Santa Cruz Biotechnology, Santa Cruz, CA), at a dilution of 1:500, for 1 h at room temperature. After three 5-min washings in TBS with 0.5% Tween 20, the blots were incubated with anti-mouse horseradish peroxidase-linked secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:1,000 for 1 h at room temperature. After extensive washing, immunoreactivity was detected by chemiluminescence kit (GE healthcare, Pittsburgh, PA). *β*-actin (Sigma, St Louis, MO) was used to normalize for variations in loading quantities. Obtained images were analyzed with Multi Gauge v 3.0 software (Fujifilm, Rochester, NY). Data are expressed as the mean ± SD. * indicates that OCT2 level in OCT2-808T was statistically significant from that of OCT2-808G ($p < 0.05$, Student's t-test).

Genotyping of OCT2

Genomic DNA from 94 unrelated European Americans and 66 unrelated African Americans in the San Francisco Bay Area (part of the Studies of Pharmacogenetics in Ethnically Diverse Populations (SOPHIE) cohort) were obtained and genotyped for the SNP rs316019 (OCT2 808G/T). The TaqMan SNP genotyping assay (assay ID C___3111809_20) was used for genotyping on the ABI 7900 Fast HT Sequence Detection Systems (Applied Biosystems, Foster City, CA). Genomic DNA (10 ng) was amplified by TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA). The PCR reactions were as follows: 1 cycle of 95 °C for 10 min followed by 60 cycles of two-step PCR with denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1.5 min. Data were analyzed by allele discrimination.

Clinical study design

This study protocol was reviewed and approved by the Committee on Human Research at University of California, San Francisco. Healthy individuals with known OCT2 genotypes [fourteen homozygous for the OCT2 reference allele (808G/G) and nine heterozygous for the variant allele (808G/T, *3D)] were recruited into this study. Informed consent was

obtained from each subject. Prior to their enrollment in the study, subjects were screened at the General Clinical Research Center at San Francisco General. The screening included a questionnaire on health, drug use and ethnicity. Blood (20 mL) was drawn to measure a CBC and electrolyte/BUN/Creatinine to exclude subjects with anemia and insufficient renal function. On the study day, subjects reported to General Clinical Research Center at San Francisco General Hospital in a fasting state. An initial 20 ml blood was drawn to measure baseline metformin levels and urine was voided at the start of the study. Subjects were given a single oral dose of 850 mg of metformin HCl tablet with 240 mL water. A normal meal schedule was resumed 2 hr after drug administration. A series of 6 mL blood samples were drawn at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hour. At the 12-hour time point, an additional 6 mL of blood was drawn for a serum creatinine measurement to allow calculation of a 24-hour creatinine clearance (CL_{CR}) and further document the normal renal function of each volunteer. Urine samples were collected at the following time intervals: 0-2, 2-4, 4-8, 8-12, 12-24 hour. The volume and pH of urine were recorded for each interval and 20 mL of the urine was then stored at -20 °C for analysis of metformin content.

Analytical methods for metformin

Metformin concentrations in plasma and urine were assayed by a highly specific and sensitive liquid chromatography–tandem mass spectrometry method (LC/MS/MS). The method was previously described [11]. The quantification limit was 10 ng/mL for plasma and 100 ng/mL for urine. Both the intra-day and inter-day coefficients of analysis variation were less than 10%.

Clinical pharmacokinetics

The pharmacokinetic parameters of metformin were evaluated by non-compartmental analysis using WinNonlin 4.0 (Pharsight Corporation, Mountain View, CA). Samples below the lower limit of quantification were set to 0 ng/mL for analysis. Nominal sample collection times were used for the pharmacokinetic analysis.

Statistical analysis

Data are expressed as mean ± SD. Unpaired Student's t-test was used to analyze the differences of metformin pharmacokinetic parameters between the reference and variant genotype groups. The data were analyzed using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA). A p value less than 0.05 was considered statistically significant.

Results

Transport of metformin by hOCT2 and its genetic variant

Four common non-synonymous coding region variants of OCT2 (M165I, A270S, R400C and K432Q) were identified previously in our laboratory [4]. Among these variants, A270S (808G/T) is the most common variant and has about 10% frequency in different ethnic groups. To examine the interaction of genetic polymorphisms of OCT2 with metformin, we constructed stably transfected HEK-293 cells expressing the empty vector (MOCK), the reference OCT2-808G and the variant OCT2-808T. As shown in Figure 1A, the uptake of metformin in cells expressing OCT2-808G was 33-fold higher compared to that in cells expressing the empty vector, confirming that metformin is an excellent substrate of OCT2. The uptake of metformin in cells expressing the variant, OCT2-808T, was about 1.5 fold higher ($p<0.05$) than that in cells expressing OCT2-808G. To determine the mechanism for this higher uptake rate of metformin by the variant, we examined the kinetics of metformin in cells expressing the reference OCT2 and those expressing OCT2-808T (Figure 1B). The uptake of metformin by both forms of OCT2 was saturable. The K_m and V_{max} for

OCT2-808G were 735 ± 104 μM and 10,900 ± 717 pmol/mg/min, respectively; the K_m and V_{max} for the variant, OCT2-808T, were 721 ± 96 μM and 14,700 ± 267 pmol/mg/min, respectively. The V_{max} for the variant was significantly higher than that of reference (p=0.02) while the K_m value was not significantly different between the reference and the variant (p=0.91). The cells stably transfected with OCT2 reference and OCT2 variant had comparable OCT2 mRNA transcript levels as determined by quantitative RT-PCR (data not shown). The OCT2 protein level in MOCK cells is negligible while much higher levels of OCT2 were observed in cells stably transfected with OCT2-808G and OCT2-808T (Figure 2A). The OCT2 protein level was 28% higher in cells transfected with OCT2-808T than that in cells transfected with OCT2-808G, which is consistent with the greater V_{max}.

Effect of OCT2 genetic variation on metformin clinical pharmacokinetics

94 unrelated European Americans and 66 unrelated African Americans in the San Francisco Bay Area (part of the Studies of Pharmacogenetics in Ethnically Diverse Populations (SOPHIE) cohort) were genotyped for the SNP rs316019 (808G/T). We found no homozygotes for the 808T/T in the 94 European samples and one homozygote for the 808T/T in the 66 African American samples. Our results were similar to the genotype frequencies shown in the HapMap. As a result, we designed our study only using healthy volunteers who are homozygous for reference and heterozygous for 808G/T. Healthy male and female subjects (fourteen with OCT2-808G/G and nine with OCT2-808G/T, *3D) were recruited and the pharmacokinetics of metformin was evaluated in these subjects after a single oral dose of 850 mg. Demographic characteristic of the study subjects are as follows. There were 64% and 44% of female subjects in reference and variant group, respectively. The majority of subjects were Caucasian (86% vs 78%). The subjects were young and healthy volunteers (27.3 ± 6.6 vs 31.2 ± 5.5 years). The mean creatinine clearance, an indicator of renal function, in these subjects was 102 ± 23 or 94 ± 26 mL/min for reference and variant group, respectively. There were no significant differences between the two genotype groups in terms of gender, race, age and creatinine clearance.

Mean metformin plasma concentration versus time curves are shown in Figure 3. Metformin concentrations were significantly higher in the individuals homozygous for the reference OCT2 than in the variant group at early times (0.5 and 1 h) after metformin administration while they were similar at other time points in these two groups. The mean C_{max} and T_{max} were not significantly different between the two genotype groups.

Metformin pharmacokinetic parameters for the two genotype groups are summarized in Table 1. The $t_{1/2}$ in the subjects homozygous for the reference OCT2 was not significantly different from that in the *3D group (3.54 ± 0.75 vs 2.95 ± 0.60 h, p=0.059) (mean ± SD). There were no significant differences in V/F or AUC_{inf} between the reference and the variant groups, respectively (V/F: 445 ± 206 vs 485 ± 232 L, p=0.667 and AUC_{inf}: 8821 ± 3489 vs 6604 ± 2195 hr*ng/ml, p=0.105).

We observed a significant difference in metformin CL_R between the reference and the variant groups. The mean CL_R in subjects homozygous for reference OCT2 was 441 ± 108 mL/min whereas in variant subjects, the mean CL_R was 614 ± 158 mL/min. The mean CL_R was significantly different between the reference and the variant groups [p=0.005, 95% CI (57, 287)]. This difference could not be explained by CL_{CR}, which was similar between the two groups (102 ± 23 vs 94 ± 26 mL/min, p=0.468). When the genotype groups were compared in terms of $SrCL_R$ of metformin (CL_R - CL_{CR}), the difference was also significant (340 ± 97 mL/min in the subjects homozygous for the reference OCT2 vs 519 ± 148 mL/min in variant subjects, p=0.002). The effect of genotype on CL_R and $SrCL_R$ are shown graphically for each individual subject (Figure 4). Both CL_R and $SrCL_R$ were significantly greater in variant subjects than in the subjects homozygous for the reference OCT2. Using

multi-variant analysis, we tested the following variables (OCT2 genotype, gender, age, race, CL_{CR}) as predictors of metformin CL_R. Among these variables, only genotype (p=0.009) and CL_{CR} (p=0.023) were significant predictors of metformin CL_R. With genotype alone explaining slightly more of total variance in metformin CL_R than CL_{CR} (28% vs 22%). OCT2 genotype was the only significant predictor of metformin $SrCL_R$ (p=0.003).

Discussion

The importance of drug transporters in drug disposition and clinical drug response is increasingly being recognized. Recent pharmacogenetic studies demonstrated that genetic variations in drug transporters such as OCT1 and OCTN1 [11, 12] had significant effects on pharmacokinetics or pharmacodynamics of substrate drugs. These studies provided good examples to understand the contribution of genetic variation in drug transporters on inter-individual variation in drug disposition and response. Leabman et al. [4] identified four polymorphic nonsynonymous variants of OCT2 (M165I, A270S, R400C and K432Q) from a collection of 247 DNA samples from ethnically diverse populations. Among these, the A270S is the most common variant in individuals from all ancestries studied. In cellular studies, the variant A270S has been shown to variably alter the transport function of OCT2. Leabman et al. [4] showed that A270S variant had a K_m value for 1-methyl-4-phenylpyridinium (MPP^+) uptake which was not significantly different from that of the OCT2 reference in oocytes whereas Kang et al. [13] reported that the uptakes of MPP^+ and tetraethylammonium (TEA) were about 40% lower in MDCK cells transiently transfected with OCT2-A270S (808 G/T) variant than that in MDCK cells transiently transfected with reference OCT2. In contrast, recent studies in this laboratory indicated K_m and V_{max} values for the herbicide, paraquat, were increased in HEK-293 cells stably transfected with the OCT2-808T variant compared with that in cells expressing OCT2 reference [10]. In this study, we found that metformin exhibited an increased V_{max} without a change in K_m in cells expressing OCT2-808T in comparison to cells expressing the reference OCT2. A higher protein level for the variant was observed on Western analysis consistent with the greater V_{max}. A substition of a serine, which is capable of hydrogen bonding, for an alanine may alter the stability or post-translational processing of the protein resulting in higher expression levels. However increased expression level of OCT2 may not be the only reason for increased uptake. V_{max} depends on both turnover rate and expression level. OCT2-808T also exhibited an increased capacity to transport paraquat although the intrinsic clearance (ratio of V_{max}/K_m) stayed the same [10]. Metformin and paraquat are chemically different with paraquat having two positive charges and metformin having only a single charge. It is likely that different amino acid residues affect the substrate recognition and translocation by OCT2.

To determine whether metformin disposition, particularly renal clearance, was altered in individuals carrying the OCT2-808G/T variant, we studied healthy volunteers who were homozygous for reference and heterozygous for the 808G/T variant. Homozygotes of 808T/T were not recruited due to very low frequency in our study populations. Metformin is mainly excreted into urine in an unchanged form. The renal clearance of metformin is much greater than glomerular filtration rate, suggesting that active tubular secretion plays an important role in renal elimination of metformin [2]. OCT2 appears to be the major transporter for metformin on the renal basolateral membrane [14]. Recent studies in Asian populations have demonstrated that 808G/T is associated with reduced metformin CL_R [8, 9]. In contrast, in this study, we demonstrated that 808G/T was associated with a greater renal clearance of metformin in healthy volunteers of European and African ancestries. In particular, we observed that CL_R and $SrCL_R$ of metformin were significantly greater in subjects heterozygous for the variant allele (808G/T) of OCT2 than those homozygous for the reference allele (808G/G). Our cellular assays supported these findings (see Figure 2).

Consistent with this finding, we observed that the total clearance (CL/F) was higher in subjects heterozygous for the variant allele of OCT2, although the difference was not statistically significant possibly due to variability in bioavailability among our subjects. Interestingly, we observed that metformin levels were significantly higher in the reference group at earlier times after metformin administration. Since metformin exhibits apparent flip-flop kinetics [2], early time points reflect elimination; therefore lower plasma concentrations would be expected in individuals with the variant OCT2, who eliminate the drug more quickly, in comparison to individuals who have the reference alleles. At later time points, concentrations may predominantly reflect drug absorption and differences in elimination may be obscured.

In addition to genetic factors, multiple factors such as age, gender, race, renal function, and drug-drug interactions may affect metformin renal elimination. It has been shown that in patients with decreased renal function (based on measured CL_{CR}), the CL_R is decreased in proportion to the decrease in CL_{CR} [15, 16]. The study by Sambol et al. further indicated that both renal function (as measured by CL_{CR}) and age were predictors of metformin CL_R. CL_{CR} was a significant predictor as a single covariate while age was only significant when CL_{CR} was considered [17]. Consistent with this finding, we also found that CL_{CR} was a significant predictor of metformin CL_R, which explained 22% of the variance in metformin CL_R. In contrast, age was not found to be significant predictor of metformin CL_R probably because subjects included in this study were all young and healthy (18 to 40 years old). We also found that metformin CL_R or $SrCL_R$ did not differ significantly between males and females ($p=0.23$ and $p=0.30$, respectively), consistent with previous studies [18]. The effect of race on metformin pharmacokinetic parameters has not been well characterized. Karim et al reported that race (Caucasians, Hispanics, and African Americans) has minimal effect on metformin drug exposure [18]. In our study, about 80% of subjects were Caucasians and 20% were African Americans. In our multivariate analysis race was not a significant predictor of metformin CL_R ($p=0.632$). Further pharmacokinetic studies including individuals from different races will be necessary to determine whether race has effects on metformin CL_R.

We found that OCT2 genotype was the best predictor of metformin CL_R and was responsible for a greater fraction (28%) of the variance in metformin CL_R in our healthy, young volunteers than CL_{CR}. OCT2 genotype was the only significant predictor of metformin $SrCL_R$. We also genotyped for variants in the OCT2 paralog, OCT1, which has previously been associated with variation in metformin pharmacokinetics, though not renal clearance [11]. Among the 23 subjects, 7 subjects carried a reduced function OCT1 allele. We observed that OCT1 genotype had no significant effects on metformin pharmacokinetics. We had powered the current study based on the potential difference for the OCT2 variant, not the OCT1 variant. Testing the effect of OCT1 variants on metformin pharmacokinetics was not our primary objective. With only 7 subjects carrying reduced function variants, the power to detect a difference of (7700 ± 970 vs 9200 ± 1200 ng.hr/ml from Shu et al. [11]) in AUC is 0.65 for the current study. Therefore the current study was underpowered to detect such differences. A multivariate analysis found that OCT1 genotype had no effect on metformin CL_R and $SrCL_R$ either on its own or in a model that included OCT2 genotype ($p>0.05$). Differences in metformin pharmacokinetics between the current study and the previous study of Shu et al. [11] are likely to be due to multiple factors. First, metformin pharmacokinetics is known to be highly variable. For example, across multiple studies, the mean half-life has been reported to range from 2 to 8 hr after an oral dose. In the study of Shu et al. [11], the mean half-life of metformin was 7.3 hr whereas the mean half life in the current study was 3.54 hr. Second, the study design by Shu et al [11] differed from the study. In this study, single doses of 850 mg metformin-HCl were given to subjects, whereas, in the study of Shu et al. [11], two doses (850 mg and 1000 mg) were given to the

subjects and pharmacokinetic parameters were calculated from data obtained after the second dose. The residual contribution of the first metformin dose was considered during the calculation of the pharmacokinetic parameters in the study of Shu et al. [11]. Metformin is known to exhibit dose dependent absorption, with a lower bioavailability at higher doses. Thus CL/F and V/F would be expected to be greater in the study of Shu et al. [11], in which higher doses of metformin were used, in comparison to the current study, and indeed, that is the case. Other PK parameters such as T_{max}, C_{max}, AUC and CL_R were similar between these two studies.

It is important to note that our studies in healthy, young volunteers cannot be extrapolated to older patients who may have renal dysfunction. In these individuals, contribution of variation in GFR to variation in metformin renal clearance may be greater than in our younger subjects. However, since metformin is contraindicated in individuals with GFR values less than 50 mL/min, OCT2 genotype may remain a major determinant of the renal clearance of metformin in the individuals who are prescribed the drug.

As noted above, our results are in contrast to those obtained in two recent studies of metformin pharmacokinetics in healthy volunteers from Asian populations [8, 9]. In particular, these two studies reported a reduced renal clearance of metformin in individual with the 808G/T whereas we observed a greater renal clearance in individuals carrying this variant. In order to explain these differences, we conducted a linkage analysis. We identified the SNPs that are in linkage with the 808G/T SNP (r^2>0.8) in various ethnic populations in HapMap (Release 21). Using Haploview, the SNPs that are in linkage to the 808G/T SNP in the CEU (CEPH, Utah residents with ancestry from Northern and Western Europe) and CHB (Han Chinese) populations were identified and a total of 3 haplotypes were designated (Haplotype A to C) (Table 2). Haplotype C with the 808G/T is only found in the CHB population and is the most common haplotype in that population that includes the 808G/T (A270S). The 808G/T SNP in CHB population is linked to 4 intronic SNPs (rs3912162, rs9346814, rs9364551 and rs16891232). These four intronic SNPs are not found in the CEU and YRI (Yoruban) populations (data from dbSNP Build 129 and HapMap Build 36). One of the 4 intronic SNPs or another SNP in linkage with these 4 SNPs may have resulted in changes in OCT2 expression level in the CHB population and thus differences in results between our studies. We observed that a recently discovered variant -578_-576delAAG (ss94002365) that was associated with reduced expression levels of OCT2 in the kidney of Japanese subjects [19] was not in linkage with 808T in our Chinese subjects (unpublished data) suggesting that this variant cannot explain the differences between our studies and those in Chinese and Korean subjects [8, 9].

Renal secretion of organic cations is a process that involves an entry step at the basolateral membrane and an exit step at the apical membrane of renal tubule cells. While OCT2 appears responsible for the entry of metformin into the renal tubular cells [3], recently identified transporters, multidrug and toxic compound extrusion proteins (MATEs) [20], may contributes to the flux of metformin from the tubule cells to the tubule lumen. Metformin was shown to be a good substrate of both MATE1 and MATE2-K [21, 22]. It is possible that genetic variation in MATE1 or MATE-2K also contributes to the interindividual variation in metformin CL_R and response.

In summary, we demonstrated that the CL_R and $SrCL_R$ of metformin were significantly affected by OCT2 genotype in healthy Caucasian and African American volunteers. Our clinical findings are supported by our cellular studies demonstrating an enhanced V_{max} and expression level of the variant transporter. Studies are ongoing to determine whether 808G/T variant is an important determinant of response to metformin in diabetic patients.

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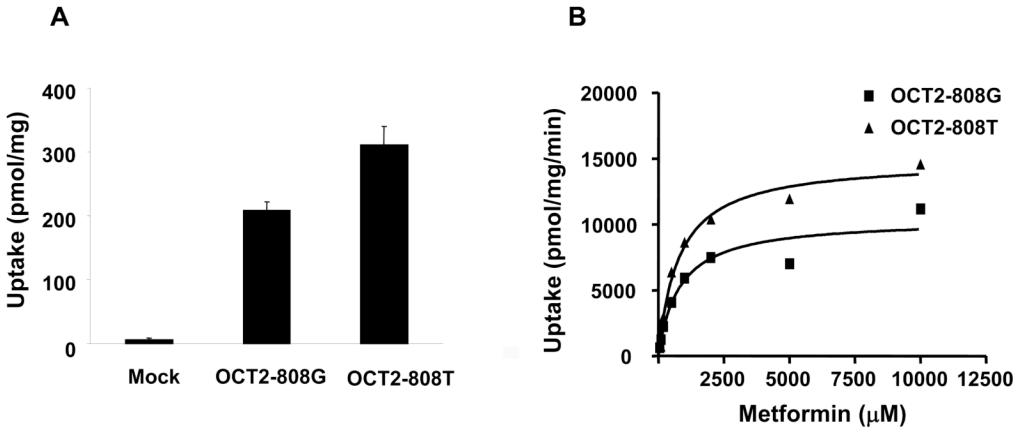

Figure 1. Transport of metformin by reference OCT2 (OCT2-808G) and the variant (OCT2-808T)

(A) HEK-293 cells stably transfected with empty vector, OCT2-808G or OCT2-808T were incubated with 9.25 μM [^{14}C] metformin for 30 seconds and uptake of metformin in these cells was determined. (B) These cells were incubated with 9.25 μM [^{14}C] metformin as well as different concentrations of unlabeled metformin for 30 seconds. Final uptake values were obtained by subtracting the uptake in MOCK cells from that in the cells expressing OCT2 reference or the variant at each corresponding substrate concentration. Studies were performed in triplicate in each individual experiment. Data represent three separate experiments.

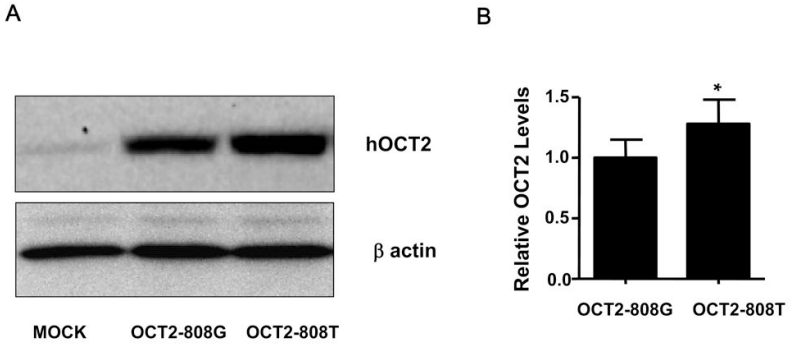

Figure 2. Expression level of OCT2 protein in cells stably transfect with OCT2-808G and OCT2-808T

A. Cells stably transfected with empty vector, OCT2-808G and OCT2-808T were harvested and analyzed by Western blot for levels of OCT2. Anti-β actin was used as the loading control. B. OCT2 levels were quantified by scanning densitometry and expressed as mean ± SD (n=3), with OCT2 reference value set to 1. * indicates that OCT2 level in OCT2-808T was statistically significant from that of OCT2-808G ($p < 0.05$, Student's t-test).

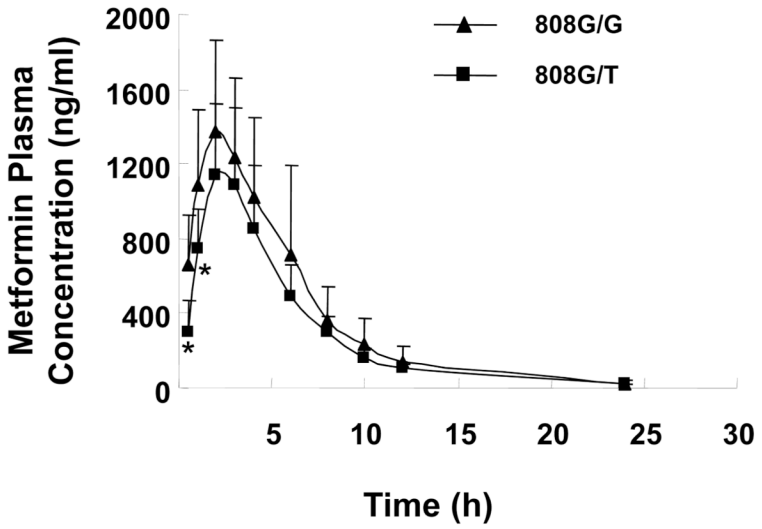

Figure 3. Mean metformin plasma concentration-time curves in individuals with OCT2 genetic variants

A single oral dose of 850 mg metformin HCl tablet was administered to subjects homozygous for the reference allele (808G/G, n=14, triangles) or those heterozygous for the variant allele (808G/T, n=9, squares). Blood was drawn at the indicated time points. Significant differences in metformin plasma concentrations between two genotypes are indicated by asterisks. Data represent mean ± SD.

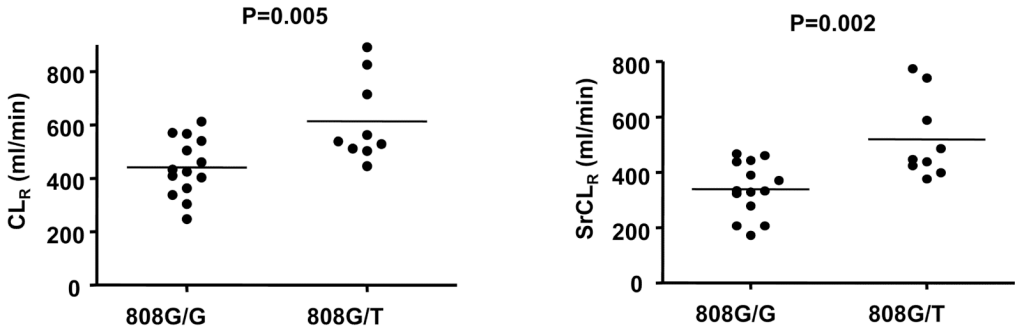

Figure 4. Effect of OCT2 genotype on renal clearance (CL_R) or net tubular secretion ($SrCL_R$) of metformin

The metformin CL_R or $SrCL_R$ of each individual is shown graphically. Note that there were significant differences in CL_R (p= 0.005) and $SrCL_R$ (p= 0.002) between genotype groups.

Table 1

Summary of metformin pharmacokinetic parameters in individuals homozygous for the OCT2 reference allele (808G/G) and heterozygous for the variant allele (808G/T).

	808G/G (n=14)	808G/T (n=9)	P value
T_{max} (hr)	2.0 (2.0-6.0)	2.0 (1.0-3.0)	0.425
C_{max} (ng/mL)	1456 ± 511	1170 ± 340	0.171
AUC_{last} (ng.hr/mL)	8670 ± 3436	6485 ± 2215	0.106
AUC_{inf} (ng.hr/mL)	8821 ± 3489	6604 ± 2195	0.105
V/F (L)	445 ± 206	485 ± 232	0.667
CL/F (mL/min)	1464 ± 603	1912 ± 894	0.164
$t_{1/2}$ (hr)	3.54 ± 0.75	2.95 ± 0.60	0.059
CL_R (mL/min)	441 ± 108	614 ± 158	0.005
CL_{CR} (mL/min)	102 ± 23	94 ± 26	0.468
$SrCL_R$ (mL/min)	340 ± 97	519 ± 148	0.002

Note: Data were obtained after oral administration of 850 mg of metformin HCL tablet. Median (range) used for T_{max} and arithmetic mean (SD) used for other parameters.

T_{max} = Time of maximal plasma concentration; C_{max} = maximal plasma concentration; AUC_{last} = area under the plasma concentration versus time curve from 0 to last time point; AUC_{inf} = area under the plasma concentration versus time curve from 0 to infinity; CL/F= apparent plasma clearance; V/F = apparent oral volume of distribution; $t_{1/2}$ = plasma terminal elimination half-life; CL_R = renal clearance; CL_{CR} = creatinine renal clearance; $SrCL_R$.= renal clearance by secretion.

Table 2

Summary of SNPs that are in linkage with rs316019 (808G>T) (r^2>0.8) of the OCT2 gene in two HapMap populations, CEU (CEPH, Utah residents with ancestry from northern and western Europe) and CHB (Han Chinese). The haplotype frequency is determined using the Haploview (version 4.1).

	Position	Intron 1	Intron 2	Intron 2	Intron 2	Exon 4	Intron 4	Intron 4	Intron 9	Haplotype frequency	Haplotype designation
	HapMap/dbSNP	rs3912162	rs9346814	rs316009	rs9364551	rs316019	rs16891232	rs316020	rs315978 (rs1315321)		
HapMap Population	CEU	C	G	G	C	G	T	C	C	0.916	A
		C	G	A	C	T	T	T	T	0.075	B
	CHB	C	G	G	C	G	T	C	C	0.878	A
		T	A	G	T	T	C	C	C	0.100	C
		C	G	A	C	T	T	T	T	0.022	B

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