The Effects of Genetic Polymorphisms in the Organic Cation Transporters OCT1, OCT2, and OCT3 on the Renal Clearance of Metformin

MV Tzvetkov[1], SV Vormfelde[1], D Balen[2], I Meineke[1], T Schmidt[1], D Sehrt[1], I Sabolić[2], H Koepsell[3] and J Brockmöller[1]

Organic cation transporters (OCTs) can mediate metformin transmembrane transport. We explored metformin pharmacokinetics in relation to genetic variations in *OCT1*, *OCT2*, *OCT3*, *OCTN1*, and *MATE1* in 103 healthy male Caucasians. Renal clearance varied 3.8-fold and was significantly dependent on creatinine clearance ($r^2 = 0.42$, $P < 0.0001$), age ($r^2 = 0.09$, $P = 0.002$), and OCT1 polymorphisms. Carriers of zero, one, and two low-activity *OCT1* alleles (Arg61Cys, Gly401Ser, 420del, or Gly465Arg) had mean renal clearances of 30.6, 33.1, and 37.1 l/h, respectively ($P = 0.04$, after adjustment for creatinine clearance and age). Immunohistochemical staining of human kidneys demonstrated OCT1 expression on the apical side of proximal and distal tubules. Increased renal clearance, in parallel with the known decreased hepatic uptake, may contribute to reduced metformin efficacy in low-activity genotypes. Renal OCT1 expression may be important not only in relation to metformin but with respect to other drugs as well.

Interindividual variations in the pharmacokinetics of drugs may be caused by genetic variations in biotransformation and transmembrane transport.[1–5] Metformin is a very important oral antidiabetic drug used in the treatment of type 2 diabetes, and therefore its genetically determined variations in transport through organic cation transporters (OCTs) may have significant medical impact. Metformin is a hydrophilic organic cation (pK_a 12.4) that is not significantly metabolized in humans and may thus be a good probe drug for organic cation transport. More than 98% of the absorbed dose is eliminated by the kidneys.[6,7] The mean estimates of renal clearance varied between 443 and 615 ml/min,[8] indicating extensive tubular secretion. Patients with renal failure may be at risk of metformin-induced lactic acidosis,[9] and inherited or acquired variations in secretory or reabsorbing drug transporters in the kidneys may also contribute to that risk. On the basis of an analysis of intraindividual vs. interindividual variability, it has been suggested that as much as 90% of the interindividual variation in renal metformin clearance may have a genetic basis.[10]

The OCTs belonging to the family of solute carriers SLC22A can transport metformin both *in vitro* and *in vivo*.[11–14] Three isoforms—OCT1, OCT2, and OCT3—with partially overlapping substrate spectra, are known in humans.[2] The genes encoding the three isoforms (*OCT1*, *-2*, and *-3*, also termed *SLC22A1*, *-2*, and *-3*) are clustered together on the long arm of chromosome 6. Population genetics analyses identified numerous single-nucleotide polymorphisms (SNPs) in the three genes.[15–20] Functional amino acid substitutions in the *OCT1* gene may have an effect on hepatic uptake transport of metformin *in vitro*;[14] these substitutions were found to be associated with reduced efficacy and had an effect on the pharmacokinetics of drugs in humans.[14,21–23] In this study, the interindividual variations in renal and total clearance of orally administered metformin were analyzed with respect to inherited genetic variants found in *OCT1*, *OCT2*, *OCT3*, *OCTN1*, and *MATE1*. We analyzed all known nonsynonymous coding variants with frequencies >0.5% among Caucasians and tagged SNPs for all known polymorphisms with a frequency >5% in *OCT1*, *OCT2*, and *OCT3* genes and we analyzed two previously reported variants in *OCTN1* and *MATE1* genes.[24–26] Known or putative covariates such as body mass index, age, creatinine clearance, and plasma testosterone were included as covariates in the genotype–phenotype correlation analysis.

[1]Department of Clinical Pharmacology, University Medical Center, University of Göttingen, Göttingen, Germany; [2]Institute for Medical Research and Occupational Health, Zagreb, Croatia; [3]Institute of Anatomy and Cell Biology, Julius Maximilians University Würzburg, Würzburg, Germany. Correspondence: J Brockmöller (jbrockm@gwdg.de)

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RESULTS

Interindividual variation in metformin pharmacokinetics

The pharmacokinetics of a single 500-mg oral dose of metformin was studied in 103 unselected and unrelated healthy, male Caucasian volunteers with a median age of 25.5 years (range 18–49) and a median body weight of 80.5 kg (range 58–100). All volunteers who had taken metformin were successfully analyzed and are included in this presentation. The mean apparent total metformin clearance was 68.3 l/h, varying from 36.2 l/h to 191.5 l/h (**Table 1**). The mean renal clearance was 32 l/h, varying 3.8-fold with a range from 14.9 l/h to 55.9 l/h (**Table 1** and **Figure 1a**). The mean apparent extrarenal clearance was 36.2 l/h and varied 38-fold with a range from 3.97 l/h to 152 l/h among the participants (**Table 1** and **Figure 1b**). However, on the basis of previous data obtained using radiolabeled metformin[7,8] this extrarenal clearance predominantly reflects lack of bioavailability, whereas systemic clearance proceeds almost entirely through the kidneys. Substantial interindividual variations were also observed in the time to maximum blood concentration, the volume of distribution, and the total amount of metformin recovered in urine (**Table 1**).

Renal metformin clearance was correlated with creatinine clearance ($r^2 = 0.42$, $P < 10^{12}$, **Figure 1a**) and age ($r^2 = 0.09$, $P = 0.002$), but was independent of body mass index and testosterone concentrations in blood ($r^2 < 0.01$, $P > 0.3$).

Effects of genetic polymorphisms on metformin pharmacokinetics

Creatinine clearance and age accounted for 51% of the observed variation in renal clearance. In order to explain the rest of the variability, we analyzed genetic polymorphisms in the OCTs 1, 2, and 3. We analyzed 30 SNPs and one copy number variation in the *OCT1*-to-*3* locus that were selected either on the basis of previously reported functional effects or because they were SNPs tagging the common genetic variants in the locus (see **Supplementary Materials and Methods** and **Table S2** online). The selected SNPs in the *OCT1*-to-*3* gene locus were sufficient to reflect all the genetic variations with allele or haplotype frequencies of >5% among Caucasians. The average call rate was 99.94%, and all the SNPs were in Hardy–Weinberg equilibrium ($P > 0.12$ in χ^2-test). A copy number variation that was previously suggested to lead to the deletion/multiplication of all three *OCT* genes[27,28] was absent in our population, with the three genes being present in two copies per genome in all the subjects.

There were no significant associations between the renal clearance of metformin and the variants analyzed in the *OCT2*, *OCT3*, *OCTN1*, and *MATE1* genes (**Table 2** and **Supplementary Figure S2** online). In contrast, two SNPs in the *OCT1* gene—a promoter-linked SNP (rs1867351) and the Gly465Arg (rs45476695) substitution—were significantly associated with the renal clearance of metformin ($P = 0.03$ and 0.003, respectively). The *OCT1* expression in lymphoblastoid cell lines was strongly associated with the presence of the promoter-linked SNP rs1867351—demonstrated by the fact that *OCT1* expression significantly decreases with the number of C-alleles of rs1867351 ($P < 0.001$; **Supplementary Figure S3** online). However, after adjustment for age and creatinine clearance, the significance of the association between the promoter-linked SNP and metformin renal clearance was no longer evident (**Table 2**).

Next, we analyzed the combined effect of the four amino acid variants known to abolish OCT1 activity partially (Met420del)

Table 1 Metformin pharmacokinetics following a single 500-mg oral dose in 103 healthy male volunteers

Parameter	Mean	95% Confidence intervals of the mean	Range
C_{max} (mg/l)	1.10	1.05–1.16	0.49–1.93
t_{max} (h)	2.19	1.99–2.40	0.62–5.75
$t_{1/2}$ (h)	4.20	4.08–4.32	3.15–7.29
MRT (h)	5.83	5.71–5.95	4.09–7.65
AUC (h·mg/l)	8.09	7.62–8.57	2.61–13.8
Cl/F (l/h)[a] of orally administered metformin	68.3	63.5–73.0	36.2–191.5
CL_{ren} (l/h)	32.0	30.7–33.4	14.9–55.9
Cl_{extren}/F (l/h) of orally administered metformin	36.2	31.8–40.7	3.97–152
V/F (l)	427	382–471	164–2,015
A_e (mg)	253	238–268	104–449

A_e, amount excreted in urine; AUC, area under the concentration–time curve; C_{max}, maximum plasma concentration; Cl/F, total clearance; CL_{ren}, renal clearance; Cl_{extren}, extrarenal clearance/F; MRT, mean residence time; t_{max}, time to maximum plasma concentration; $t_{1/2}$, terminal half-life; V/F, volume of distribution/F.

[a]F stands for bioavailability.

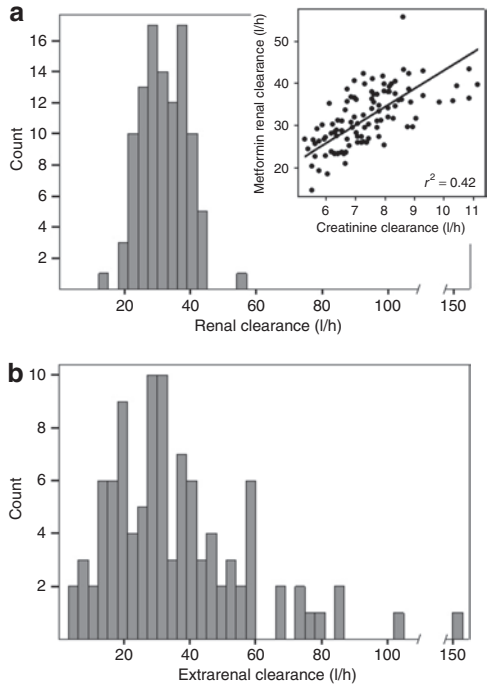

Figure 1 Interindividual variation in renal and extrarenal metformin clearances. The frequency distributions of the individual estimates of (**a**) the renal and (**b**) the extrarenal oral clearance are shown. The insert in the upper right corner illustrates the correlation between renal metformin and creatinine clearances.

Table 2 Renal metformin clearance in relation to *OCT1, OCT2, OCT3, OCTN1*, and *MATE1* polymorphisms

		Alleles	*n*			Renal clearance mean (SEM) (l/h)				
Gene	Genetic marker	A>B[a]	A/A	A/B	B/B	A/A	A/B	B/B	P^b	Adjusted P^c
OCT1	rs1867351	T>C	64	38	1	31.0 (0.7)	33.7 (1.3)	40.6	0.03	0.11
	R61C	Arg>Cys	85	16	1	31.6 (0.7)	34.5 (1.9)	38.7	0.16	0.07
	rs461473	C>T	84	18	1	31.7 (0.7)	33.5 (1.7)	36.3	0.26	
	L160P	Leu>Phe	59	35	9	32.0 (0.8)	31.9 (1.3)	33.0 (1.9)	0.99	
	G401S	Gly>Ser	99	4		32.1 (0.7)	29.9 (2.7)		0.50	
	M408V	Val>Met	36	45	21	32.6 (1.1)	32.2 (1.1)	31.4 (1.3)	0.51	
	M420del	Met>del	70	30	3	31.6 (0.8)	32.7 (1.1)	36.6 (4.9)	0.23	
	rs4646281	del>ins	35	46	22	33.0 (1.0)	31.9 (1.1)	30.8 (1.3)	0.26	
	G465R	Gly>Arg	97	6		31.6 (0.7)	39.4 (1.8)		0.003	0.36
	rs2297374	G>A	37	48	18	30.7 (1.0)	33.2 (1.1)	31.8 (1.5)	0.35	
	Number of inactive alleles[d]	**Active > inactive**	**51**	**48**	**4**	**30.6 (0.9)**	**33.1 (1.0)**	**37.1 (3.5)**	**0.04**	**0.04**
OCT2	rs10755577	G>A	86	17		32.1 (0.8)	32.0 (1.4)		0.96	
	rs2928035	A>G	88	15		32.3 (0.7)	30.5 (1.8)		0.39	
	Val502	T>C	72	29	2	32.4 (0.8)	31.6 (1.2)	26.9 (3.4)	0.46	
	rs17588242	T>C	49	46	8	32.2 (1.0)	32.0 (1.0)	31.4 (1.7)	0.82	
	rs315996	G>A	70	29	4	31.5 (0.8)	33.0 (1.1)	34.8 (7.7)	0.41	
	A270S	Ala>Ser	84	19		32.3 (0.7)	31.0 (1.6)		0.56	
	Thr130	C>A	32	51	20	31.8 (1.2)	32.1 (1.0)	32.3 (1.5)	0.71	
	rs3127573	T>C	87	16		31.8 (0.7)	33.3 (1.6)		0.42	
	rs316024	C>T	43	46	14	31.4 (1.0)	32.0 (0.9)	34.1 (2.2)	0.48	
	rs316025	C>T	68	30	5	32.2 (0.8)	32.1 (1.3)	30.4 (3.0)	0.87	
	rs316026	G>A	33	50	20	31.0 (1.2)	32.4 (0.9)	32.9 (1.7)	0.45	
	rs662301	G>A	88	13	2	32.1 (0.7)	32.0 (1.3)	28.0 (13.1)	1.00	
	rs533452	C>T	38	53	12	32.9 (1.1)	30.7 (0.8)	35.1 (2.9)	0.51	
	rs17589858	C>G	58	41	4	32.8 (0.9)	30.8 (1.0)	33.9 (2.5)	0.23	
OCT3	rs3120137	G>A	84	19		32.2 (0.8)	31.5 (1.3)		0.65	
	rs3123634	C>T	31	61	11	31.9 (1.4)	32.5 (0.8)	29.8 (2.3)	0.91	
	rs12194182	A>G	61	37	5	32.7 (0.9)	31.4 (1.1)	29.1 (2.7)	0.22	
	rs2292334	C>T	47	49	7	33.3 (1.1)	31.3 (0.9)	28.9 (2.0)	0.06	0.21
	rs2504927	G>A	31	47	25	32.1 (1.4)	31.3 (0.9)	33.3 (1.3)	0.33	
	rs2457576	C>G	45	46	12	32.8 (1.1)	31.3 (1.0)	32.2 (1.9)	0.65	
OCTN1	L503F	Leu>Phe	27	55	21	33.0 (6.5)	31.4 (7.1)	32.5 (6.4)	0.65	
MATE1	rs2289669	G>A	32	54	17	32.3 (6.3)	31.7 (7.2)	32.8 (6.4)	0.95	

Genetic variants showing significant association with renal metformin clearance are in bold.

[a]The major (i.e., more frequent) alleles were designated A and the minor alleles B. A/A, A/B, and B/B represent individuals homozygous for the major allele, heterozygous for the minor allele, and homozygous for the minor allele, respectively.[b]The univariate significances according to the two-sided Jonckheere–Terpstra test are given.[c]The adjusted significances are according to multiple linear regression analysis, including the respective genetic variant analyzed as well as age and creatinine clearance as independent variables. [d]Inactive alleles were defined by the presence of one or more of the following amino acid substitutions: Arg61Cys, Gly401Ser, Met420del, or Gly465Arg. A/A denotes carriers of no inactive allele and B/B denotes carriers of two inactive alleles.

or fully (Arg61Cys, Ser401Gly, and Gly465Arg). The calculated haplotypes of these SNPs were designated as *OCT1* alleles 2 to 5 (*2 to *5, **Figure 2a**). The renal clearance of metformin increased significantly with the number of inactive *OCT1* alleles defined in this manner ($P = 0.038$, **Table 2** and **Figure 2b**). The increase remained significant after adjusting for creatinine clearance and age ($P = 0.032$, **Table 2**). Also, the net clearance by tubular secretion of metformin (calculated as the difference between renal metformin and creatinine clearances for each individual) increased significantly with the number of inactive alleles (the mean values were 23.6, 25.6, and 29.7 for carriers of none, one, and two inactive alleles, respectively; $P = 0.03$). The plasma metformin concentrations in the homozygous carriers of inactive *OCT1* alleles were significantly lower at 10 h after administration (**Figure 2c**), but the values of the area under the concentration–time curve did not significantly differ between the *OCT1* genotypes.

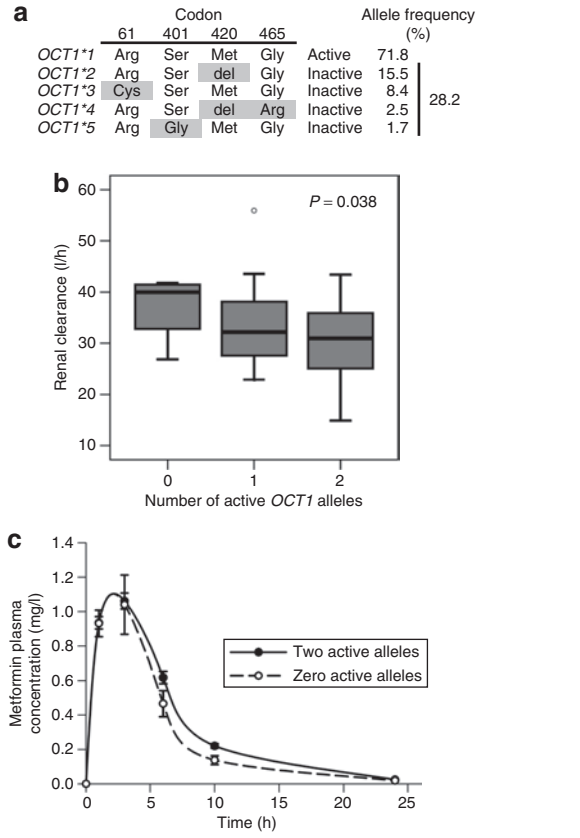

Figure 2 Metformin pharmacokinetics in relation to the number of active *OCT1* alleles. (**a**) Haplotype combination of the four common functional amino acid substitutions in the *OCT1* gene illustrating the presence of the major active and four inactive *OCT1* alleles with a common frequency of 28.2% in Caucasians. Haplotype calculation was performed using PHASE software, version 2.1 (refs. 45, 46). Minor alleles are shown in gray. (**b**) Renal metformin clearance in dependence from the number of active *OCT1* alleles. Significance was calculated using the two-sided Jonckheere–Terpstra trend test. (**c**) Plasma concentration–time curves of metformin in volunteers carrying two or zero active OCT1 alleles. Depicted are concentration means ± SE.*$P = 0.038$ Student's *t*-test.

No association could be observed between any of the studied polymorphisms in the *OCT* genes and additional pharmacokinetics parameters, namely, apparent extrarenal clearance of the orally administered drug, apparent total clearance of metformin, and volume of distribution ($P > 0.22$).

Detection of OCT1 in human kidney

One explanation of the association of *OCT1* polymorphisms with renal clearance is metformin reabsorption by OCT1 on the luminal membrane in the kidney tubules. Therefore, we analyzed the *OCT1* mRNA expression in kidney in relation to liver and small intestine, and we carried out immunohistochemical analysis of human kidneys for OCT1. In line with previous data,[29,30] the highest *OCT1* mRNA expression was observed in the liver (81.3 transcripts per single transcript of TATA-box binding protein). However, we observed the second-highest *OCT1* expression in the total kidney homogenates (Figure 3a). The expression in the kidney was more than fivefold higher than in the small intestine, which is generally accepted as a site of OCT1 expression.[2]

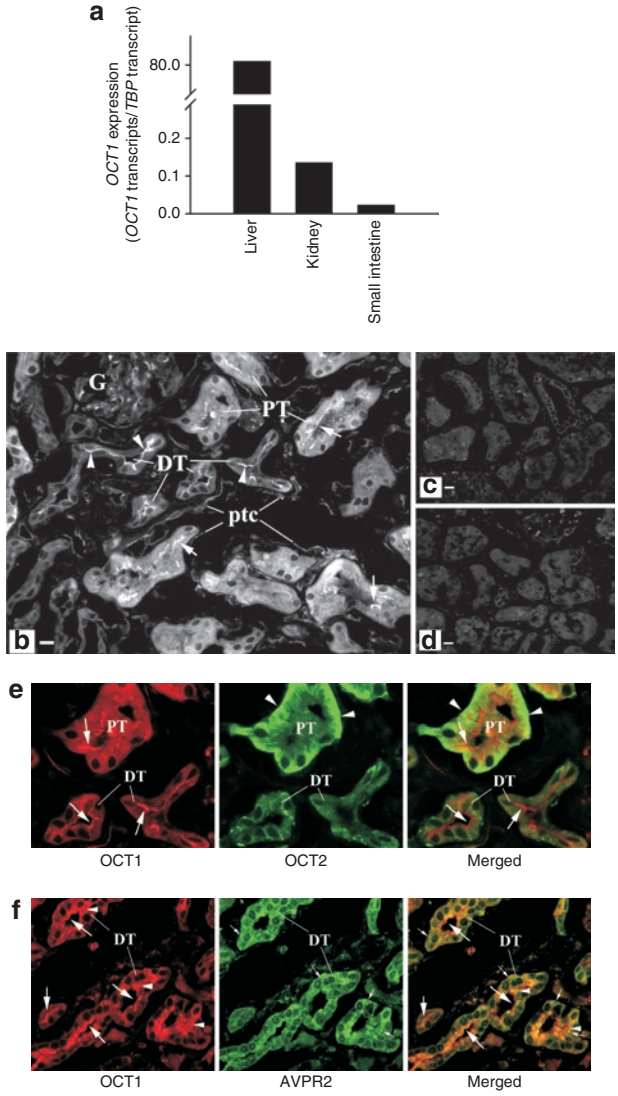

Figure 3 mRNA expression and immunohistochemical detection of OCT1 in human kidney. **(a)** OCT1 mRNA expression measured in total organ homogenates of human liver, kidney, and small intestine. The expression is given as number of mRNA molecules per single mRNA molecule of the TATA-box binding protein (TBP) determined in the same sample as an internal control. (**b**) Immunostaining of OCT1 in the human kidney cortex. OCT1 was stained in the apical membrane of proximal convoluted tubules (PT, arrows) and distal tubules (DT, arrowheads). OCT1 was also weakly stained in nonidentified cells in glomeruli (G) and peritubular capillaries in the cortex (ptc), but was not detected in the proximal tubule segments in the outer stripe and other segments of the nephron (not shown). In the control experiments, the observed OCT1 staining was blocked by the immunizing peptide (**c**), and in the absence of primary antibody no staining was observed (**d**). (**e**) Double immunostaining for OCT1 (red fluorescence) and OCT2 (green fluorescence). In the human kidney cortex, the OCT2 was strongly stained in the basolateral membrane (arrowheads) of proximal tubules (PT) only. The same proximal tubules were stained with the OCT1 antibody, but OCT1 was localized in the apical membrane. (**f**) Double immunostaining for OCT1 (red fluorescence) and arginine vasopressin receptor 2 (AVPR2) as a marker for distal tubules (green fluorescence). OCT1 is stained in the apical membrane (large arrows) and intracellular organelles (arrowheads) of distal tubules (DT). The same distal tubules were stained with the AVPR2 antibody, but they were largely granular and intracellular (small arrows). White bars = 10 µm.

Immunohistochemistry showed OCT1 expression in the apical and subapical domains of both proximal and distal tubules in human kidney (**Figure 3b–d**). The detection of OCT1 was performed using a previously well-characterized antibody.[31] Specificity was further confirmed under our staining conditions (**Supplementary Figure S4** online). Next, we stained human kidney slices with OCT1 antibody alone, with OCT1 and OCT2 antibodies, or with OCT1 antibody and an antibody to detect arginine vasopressin receptor 2. The staining intensity of OCT1 in the distal tubule was heterogeneous, that is, strong in some of the tubules, as shown in **Figure 3**, but weak or absent in other tubule profiles (not shown). Different staining patterns were observed with the OCT1 and OCT2 antibodies in the proximal tubules (**Figure 3e**). In confirmation of previously reported data,[32] OCT2 was localized predominantly on the basolateral membranes, whereas the OCT1 was localized predominantly on the apical membranes. OCT1 was also colocalized with arginine vasopressin receptor 2, a marker for distal tubules[33] (**Figure 3f**). The location of OCT1 in apical membranes of proximal and distal tubules indicates that OCT1 may be involved in the reabsorption of metformin.

DISCUSSION

In a healthy population, a wide variation in metformin renal clearance was found (14.9–55.9 l/h). This finding of variable pharmacokinetics was analyzed with respect to genetic polymorphisms in the *OCT* genes *1*, *2*, and *3*. Polymorphisms in *OCT1*, but not in *OCT2* or *OCT3*, were associated with the renal clearance of metformin (**Table 2**). Low-function *OCT1* amino acid substitutions Arg61Cys, Ser401Gly, Met420del, and Gly465Arg, and the *OCT1* promoter-linked variant rs1867351, were associated with an increase in the renal clearance of metformin by ~20% and ~30%, respectively. These amino acid substitutions, which are known to reduce maximum transport velocity for metformin or to impair the membrane localization of OCT1,[14] were associated with increased renal clearance of metformin in a gene-dose dependent manner, i.e., clearance increased by every additional low-activity allele (**Table 2** and **Figure 2**). Our data therefore suggest that a reduction in *OCT1* expression or activity may increase renal excretion of metformin.

OCT1 is known to transport metformin *in vitro*,[14,34] and amino acid substitutions in this gene were shown to affect the pharmacokinetics of metformin.[21] Confirming the data of Motohashi *et al.*,[32] we found that OCT1 was expressed at a low level in a total human kidney mRNA preparation (**Figure 3a**). Using immunohistochemical staining with an antibody well characterized for its specificity toward OCT1, we detected human OCT1 at the apical cell side of proximal and distal tubules, suggesting localization and a functional role for OCT1 in the luminal membrane (**Figure 3**). The location of OCT1 in the luminal membrane in distal tubules suggests that OCT1 may contribute to metformin reabsorption in the tubule. This provides the most straightforward explanation for the finding from our study data that low-activity OCT1 variants are associated with increased renal elimination of metformin.

A possible alternative explanation for this observation is the existence of a cross-regulation between the hepatic and renal transport of metformin as has been discussed by Jonker *et al.*,[35] who attempted to explain the observed increase of renally eliminated tetraethylammonium in *Oct1* knockout mice after intravenous injection of this compound. It is possible that decreased expression or lowered activity of OCT1 in humans causes upregulation in the renal excretion of metformin by upregulating the expression of transporters that are involved in metformin excretion. A hypothetical mechanism behind such a phenomenon is the so-called metabolic crosstalk. It implies that, in humans, the accumulation of substances expressing OCT1 variants may cause upregulation of metformin-translocating transporters such as OCT2. However, the mechanisms mediating this effect remain unclear. Therefore, OCT1-mediated reabsorption is the better-supported concept.

Furthermore, a haplotype in the *OCT1* promoter that is associated with decreased *OCT1* expression in lymphoblast cell lines in a gene-dependent and dose-dependent manner (**Supplementary Figure S3a** online), was also associated with an increase in renal clearance of metformin (**Supplementary Figure S3b** online and **Table 2**). This interesting finding, however, requires validation. First, the association was not present after adjusting for age and creatinine clearance (**Table 2**). Second, the effect of this haplotype on *OCT1* expression may be tissue dependent, as is known to be the case for other transporters,[36,37] and the effect observed in lymphoblastoid cell lines might not be representative of that seen in the kidney or liver.

We observed only a weak quantitative effect of the *OCT1* polymorphisms on renal clearance (**Table 2**, **Figures 2** and **Supplementary Figure S3b** online) as compared with the rather strong effects of the same polymorphisms on *OCT1* expression (**Supplementary Figure S3a** online) and activity.[14,16,20] The explanation may be that only a relatively small fraction of metformin—ultrafiltrated or secreted in the proximal tubule—is reabsorbed by *OCT1*. Variable posttranslational regulation of OCTs[38] or variable expression or activity of other polyspecific OCTs may additionally contribute to the observed interindividual variations in renal metformin excretion. Other polyspecific OCTs, e.g., OCTN1, OCTN2, MATE1, and MATE2-K, may be involved in the renal excretion of metformin in humans. The limited number of polymorphisms in the *OCTN1* and *MATE1* genes analyzed here showed no association with the renal or extrarenal clearance of orally administered metformin. Our data do not exclude that the recently reported association of the *MATE1* rs2289669 SNP with the efficacy of metformin[24] may be explained by an influx transport of metformin into hepatocytes or muscle cells mediated by MATE1. MATE1 is strongly expressed in the liver and skeletal muscles.[39] As far as we know, the rs2289669 SNP is not *per se* functional. Our population may have had minor differences with respect to ethnic background as compared with the population studied by Becker *et al.*,[24] and the rs2289669 SNP may be differentially linked with the causal functional variant.

On the other hand, it can be assumed that the effects of OCT1 genetic polymorphisms on metformin pharmacokinetics and pharmacodynamics are additive. Genetic variations leading to a reduction in OCT1 activity are known to decrease the

efficacy of metformin by reducing the metformin uptake in hepatocytes.[14] Here, the same nonfunctional or low-activity OCT1 variants resulted in higher elimination of metformin by the kidney and thereby to lower systemic exposure to metformin. This may further reduce metformin efficacy in OCT1-deficient patients. However, this is an assumption that requires further systematic analyses.

The highly variable extrarenal clearance of orally administered metformin was not dependent on the *OCT1* polymorphisms, although it is well known that human *OCT1* is predominantly expressed in the liver,[2] as confirmed by our gene expression analyses (**Figure 3**). However, this is not a discrepancy, given that previous pharmacokinetics studies with oral and intravenous administration have shown that 99.9% of intravenously administered metformin is excreted in the urine (as determined by radioactive tracers) as compared with only 51.6% of the orally administrated drug.[7] Therefore, for elimination of systemically available metformin, hepatic clearance of the unchanged parent drug seems to play only a marginal role—accounting for <1% of the total elimination of the drug. In addition, no metabolites of metformin have been identified in humans.[8] These data suggest that the variability in the apparent extrarenal clearance of orally administered metformin observed in our study (**Table 1** and **Figure 1b**) may almost exclusively reflect interindividual differences in the bioavailability of metformin—probably because of variable membrane transport through the gut epithelium.

In our study, we observed high variability in the bioavailability of metformin (reflected by extrarenal clearance, **Figure 1b**). Some of the OCTs analyzed here are expressed in gut epithelium[2] and may mediate drug uptake into the blood, but polymorphisms in these genes were not associated with variations in apparent extrarenal clearance. However, the design of our study was optimized to analyze variations in renal clearance. Therefore, we cannot exclude the possibility that genetic polymorphisms in the OCTs can cause minor variations in metformin bioavailability.

In contrast to previous studies on metformin pharmacokinetics (reviewed by Scheen[8]), we studied a significantly greater number of subjects, which was motivated by our interest in pharmacokinetic variations in a large population. As is usual in pharmacokinetics studies involving a large population, we have drawn fewer blood samples per individual. Despite this relatively sparse sampling, the pharmacokinetics parameters analyzed in our study corresponded very well with those determined in previous studies. For instance, Tucker *et al.*[40] measured a value for clearance of orally administered metformin as 79.1 l/h and a volume of distribution/F of 442 l, both of which were very close to our estimates of 68.3 l/h and 427 l, respectively. Other parameters, such as time to maximum plasma concentration, maximum plasma concentration, and, in particular, renal clearance, corresponded equally well with existing data.[7,21,40,41] This confirms that our sample is representative and that the sparse sampling schedule did not result in systematic errors.

In line with previous data,[40] we observed a correlation between renal metformin clearance and creatinine clearance. The variation in creatinine clearance accounted for 42% of the variation observed in metformin renal clearance, and the genetic polymorphisms in *OCT1* accounted for 9.7%. Calculations based on repeated measurements in five subjects[7] suggest that the genetic component accounts for >90% of the interindividual variations in the renal clearance of metformin.[10] Interindividual variations in creatinine clearance, however, also have a significant genetic component.[42] Consequently, metformin clearance may be determined both indirectly by genetic factors affecting glomerular filtration (reflecting creatinine clearance) and by genetic factors specifically affecting tubular secretion and/or reabsorption of organic cations, such as polymorphisms in the *OCT1* gene.

The finding in this study of an increase in renal metformin excretion in individuals with amino acid variants of human OCT1 with low or missing transport is in apparent contradiction to data described by Shu *et al.*[21] However, for several reasons, the studies are not directly comparable. First, Shu *et al.* used a higher metformin dose and a formulation with a significantly lower bioavailability (excreted fraction between 18% and 28% as compared with ~50% in our study and in other previously published studies on metformin pharmacokinetics[8]). Second, our study was performed with male subjects only, whereas Shu *et al.* included both male and female volunteers. Third, in our study a fivefold higher number of subjects was investigated, including four homozygous carriers of the low-activity alleles.

In our study population of Caucasian men, we were not able to confirm the effects of genetic polymorphisms in OCT2 on the renal clearance of metformin. Metformin is readily transported by OCT2 (refs. 11,18), OCT2 is strongly expressed in the kidneys and is located at the basolateral membranes of renal proximal tubules (ref. 29 and **Figure 3e**), and metformin is predominantly eliminated by renal excretion in humans.[7] We therefore considered OCT2 to be the most critical transporter for metformin tubular secretion and metformin to be an appropriate probe drug with which to study the functional consequences of genetic variants in human OCT2 *in vivo*. We included 14 SNPs to completely tag the frequent polymorphisms (minor allele frequency ≥5%) in the coding regions, the regulatory regions, and the introns of the *OCT2* gene. Three common variants in the coding region of *OCT2* in Caucasians[18]—an alanine$_{270}$-to-serine substitution and synonymous SNPs in valine$_{502}$ and threonine$_{130}$ codons—were also analyzed. Nevertheless, we have not been able to see a statistically significant association of genetic variants in OCT2 with the renal elimination of metformin. Similarly, previous studies did not find any relationship between common coding variants in *OCT2* and metformin uptake *in vitro*[18] or glucose-lowering effects of metformin.[43] However, recent studies by Song *et al.* and Wang *et al.* showed significantly lower renal clearances of metformin in homozygous OCT2 serine$_{270}$ carriers as compared with homozygous alanine$_{270}$ carriers.[22,23] In our unselected Caucasian study sample, there were no homozygous serine$_{270}$ carriers. Consistent with the data from the studies of Song *et al.* and Wang *et al.* in the heterozygous serine$_{270}$ carriers, we observed a trend toward reduced renal clearance.[22,23] However, the reduction was less than that found in the two previous studies. With a frequency of the minor allele of 0.9% (**Supplementary Table S1** online), the homozygous serine$_{270}$ genotype is expected to be present in 0.8% of Caucasians; this

may explain only a limited part of the variability in the renal and total clearance values of orally administered metformin in that population. In addition, there may be an interethnic difference in the effect of this variation that is apparently functional by itself,[22] but that may be amenable to modulation by linkage with other variants and/or by other nongenetic factors. However, this does not detract from the fact that the alanine$_{270}$-to-serine substitution is probably the most important polymorphism to focus on in future large pharmacogenomic studies on metformin.

In conclusion, the renal clearance of metformin showed substantial interindividual variation, but far greater was the estimated variation in metformin bioavailability (**Figure 2**). Variation in creatinine clearance accounted for 42% of the observed variation in the renal clearance of metformin, and genetic variants in OCT1 accounted for a further 10%, whereas no effect was observed for the common genetic variants in OCT2 and OCT3. The detected location of OCT1 in the luminal membrane of the distal tubule suggests that OCT1 may mediate reabsorption of metformin and that the low or absent reabsorption in the carriers of inactive OCT1 variants leads to an increase in renal metformin excretion.

METHODS

A detailed description of the study and the analytical methods used is available in the **Supplementary Materials and Methods** online.

Subjects, study design, and determination of metformin pharmacokinetics. An unselected sample of 103 healthy male volunteers was enrolled in the study. All participants were of Caucasian German ethnicity. Each volunteer received a single 500-mg oral dose of metformin (Glucophage; Merck KGaA, Darmstadt, Germany). Blood samples were drawn prior to metformin administration and at 1, 3, 6, 10, and 24 h after. Total urine was collected over 0–1, 1–3, 3–6, 6–10, and 10–24 h after drug administration. Metformin plasma concentrations were quantified by liquid chromatography with tandem mass spectrometry using a PerkinElmer/Sciex HPLC system and API4000 mass spectrometer (Applied Biosystems, Darmstadt, Germany), and pharmacokinetics data analyses were performed by noncompartmental methods using WinNonlin software (Pharsight, Mountain View, CA). The study protocol was approved by the ethics committee of the University of Göttingen, and all volunteers gave their written informed consent to participate in the study.

Selection and genotyping of SNPs. Five functional amino acid substitutions (four in OCT1 and one in OCT2) and 25 haplotype tagging SNPs were selected on the basis of linkage analyses of the HapMap CEU population (see **Supplementary Figure S1**). Polymorphisms in the other OCTs OCTN1 L503F and MATE1 rs2289669 were selected on the basis of previous reports involving the functional effects on and the associations with[25,26] the efficacy of metformin treatment,[24] respectively. Selected polymorphisms were genotyped by single-base primer extension, PCR-restriction fragment length polymorphism, real-time PCR, and sequencing. In addition, we genotyped a copy number variation that had previously been suggested in order to delete/multiply the entire *OCT1*-to-*3* locus.[27,28] We sequenced the *SLC22A2* gene to a greater extent because of the presumed significant role of OCT2 in the renal excretion of metformin.

Immunohistochemical detection. A previously characterized polyclonal antiserum was used for the detection of human OCT1 (ref. 31), and a previously characterized monoclonal antibody was used for the detection of human OCT2 (ref. 44) in healthy human kidney tissue samples obtained from surgery of tumor-affected kidneys in local hospitals in Zagreb. The use of human tissues in these studies was approved by institutional and hospital ethics committees.

Statistical analyses. Prior to the commencement of the study, renal clearance of metformin was defined as the primary parameter of this study. The nonparametric, two-sided Jonckheere–Terpstra test (for comparing three genotype groups) and the Mann–Whitney *U* test (for comparing two groups) were used to test for genotype-dependent differences in renal and extrarenal clearances of orally administered metformin and in gene expression rates. Significant genotype associations were adjusted for demographic and clinical covariates using linear regression analyses. The approach to the combined analysis of the four low-to-no-function *OCT1* genotypes was defined on the basis of previous data.[14] Because these data clearly showed functionality and because we performed only one combined genotype type of analysis of this kind, it was performed without correction for multiple testing. All other genotype–phenotype correlation analyses were considered explorative and were not adjusted for multiple testing.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

1. Ho, R.H. & Kim, R.B. Transporters and drug therapy: implications for drug disposition and disease. *Clin. Pharmacol. Ther.* **78**, 260–277 (2005).
2. Koepsell, H., Lips, K. & Volk, C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm. Res.* **24**, 1227–1251 (2007).
3. Seithel, A., Glaeser, H., Fromm, M.F. & König, J. The functional consequences of genetic variations in transporter genes encoding human organic anion-transporting polypeptide family members. *Expert Opin. Drug. Metab. Toxicol.* **4**, 51–64 (2008).
4. Vormfelde, S.V. *et al*. Torsemide renal clearance and genetic variation in luminal and basolateral organic anion transporters. *Br. J. Clin. Pharmacol.* **62**, 323–335 (2006).
5. Vormfelde, S.V., Toliat, M.R., Schirmer, M., Meineke, I., Nurnberg, P. & Brockmoller, J. The polymorphisms Asn130Asp and Val174Ala in OATP1B1 and the CYP2C9 allele *3 independently affect torsemide pharmacokinetics and pharmacodynamics. *Clin. Pharmacol. Ther.* **83**, 815–817 (2008).
6. Beckmann, R. Absorption, distribution in the organism and elimination of metformin. *Diabetologia* **5**, 318–324 (1969).
7. Pentikäinen, P.J., Neuvonen, P.J. & Penttilä, A. Pharmacokinetics of metformin after intravenous and oral administration to man. *Eur. J. Clin. Pharmacol.* **16**, 195–202 (1979).
8. Scheen, A.J. Clinical pharmacokinetics of metformin. *Clin. Pharmacokinet.* **30**, 359–371 (1996).
9. Wang, D.S., Kusuhara, H., Kato, Y., Jonker, J.W., Schinkel, A.H. & Sugiyama, Y. Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. *Mol. Pharmacol.* **63**, 844–848 (2003).
10. Leabman, M.K. & Giacomini, K.M. Estimating the contribution of genes and environment to variation in renal drug clearance. *Pharmacogenetics* **13**, 581–584 (2003).
11. Dresser, M.J., Xiao, G., Leabman, M.K., Gray, A.T. & Giacomini, K.M. Interactions of n-tetraalkylammonium compounds and biguanides with a human renal organic cation transporter (hOCT2). *Pharm. Res.* **19**, 1244–1247 (2002).

12. Kimura, N. *et al*. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. *Drug Metab. Pharmacokinet*. **20**, 379–386 (2005).
13. Kimura, N., Okuda, M. & Inui, K. Metformin transport by renal basolateral organic cation transporter hOCT2. *Pharm. Res*. **22**, 255–259 (2005).
14. Shu, Y. *et al*. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J. Clin. Invest*. **117**, 1422–1431 (2007).
15. Kang, H.J. *et al*. Identification and functional characterization of genetic variants of human organic cation transporters in a Korean population. *Drug Metab. Dispos*. **35**, 667–675 (2007).
16. Kerb, R. *et al*. Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. *Pharmacogenetics* **12**, 591–595 (2002).
17. Lazar, A., Gründemann, D., Berkels, R., Taubert, D., Zimmermann, T. & Schömig, E. Genetic variability of the extraneuronal monoamine transporter EMT (SLC22A3). *J. Hum. Genet*. **48**, 226–230 (2003).
18. Leabman, M.K. *et al*. Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *Pharmacogenetics* **12**, 395–405 (2002).
19. Sakata, T. *et al*. Novel single nucleotide polymorphisms of organic cation transporter 1 (SLC22A1) affecting transport functions. *Biochem. Biophys. Res. Commun*. **313**, 789–793 (2004).
20. Shu, Y. *et al*. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc. Natl. Acad. Sci. USA*. **100**, 5902–5907 (2003).
21. Shu, Y. *et al*. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin. Pharmacol. Ther*. **83**, 273–280 (2008).
22. Song, I.S. *et al*. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. *Clin. Pharmacol. Ther*. **84**, 559–562 (2008).
23. Wang, Z.J., Yin, O.Q., Tomlinson, B. & Chow, M.S. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. *Pharmacogenet. Genomics* **18**, 637–645 (2008).
24. Becker, M.L., Visser, L.E., van Schaik, R.H., Hofman, A., Uitterlinden, A.G. & Stricker, B.H. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study. *Diabetes* **58**, 745–749 (2009).
25. Peltekova, V.D. *et al*. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat. Genet*. **36**, 471–475 (2004).
26. Urban, T.J. *et al*. Effects of genetic variation in the novel organic cation transporter, OCTN1, on the renal clearance of gabapentin. *Clin. Pharmacol. Ther*. **83**, 416–421 (2008).
27. Pinto, D., Marshall, C., Feuk, L. & Scherer, S.W. Copy-number variation in control population cohorts. *Hum. Mol. Genet*. **16** Spec No. 2,R168–R173 (2007).
28. Redon, R. *et al*. Global variation in copy number in the human genome. *Nature* **444**, 444–454 (2006).
29. Gorboulev, V. *et al*. Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol*. **16**, 871–881 (1997).
30. Zhang, L., Dresser, M.J., Gray, A.T., Yost, S.C., Terashita, S. & Giacomini, K.M. Cloning and functional expression of a human liver organic cation transporter. *Mol. Pharmacol*. **51**, 913–921 (1997).
31. Nies, A.T., Herrmann, E., Brom, M. & Keppler, D. Vectorial transport of the plant alkaloid berberine by double-transfected cells expressing the human organic cation transporter 1 (OCT1, SLC22A1) and the efflux pump MDR1 P-glycoprotein (ABCB1). *Naunyn Schmiedebergs Arch. Pharmacol*. **376**, 449–461 (2008).
32. Motohashi, H. *et al*. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J. Am. Soc. Nephrol*. **13**, 866–874 (2002).
33. Kim, D., Wang, M., Cai, Q., Brooks, H. & Dressler, G.R. Pax transactivation-domain interacting protein is required for urine concentration and osmotolerance in collecting duct epithelia. *J. Am. Soc. Nephrol*. **18**, 1458–1465 (2007).
34. Wang, D.S., Jonker, J.W., Kato, Y., Kusuhara, H., Schinkel, A.H. & Sugiyama, Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J. Pharmacol. Exp. Ther*. **302**, 510–515 (2002).
35. Jonker, J.W. *et al*. Reduced hepatic uptake and intestinal excretion of organic cations in mice with a targeted disruption of the organic cation transporter 1 (Oct1 [Slc22a1]) gene. *Mol. Cell. Biol*. **21**, 5471–5477 (2001).
36. Kobayashi, D. *et al*. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metab. Dispos*. **33**, 94–101 (2005).
37. Zamber, C.P. *et al*. Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics* **13**, 19–28 (2003).
38. Ciarimboli, G. *et al*. Individual PKC-phosphorylation sites in organic cation transporter 1 determine substrate selectivity and transport regulation. *J. Am. Soc. Nephrol*. **16**, 1562–1570 (2005).
39. Otsuka, M., Matsumoto, T., Morimoto, R., Arioka, S., Omote, H. & Moriyama, Y. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc. Natl. Acad. Sci. USA*. **102**, 17923–17928 (2005).
40. Tucker, G.T., Casey, C., Phillips, P.J., Connor, H., Ward, J.D. & Woods, H.F. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. *Br. J. Clin. Pharmacol*. **12**, 235–246 (1981).
41. Noel, M. Kinetic study of normal and sustained release dosage forms of metformin in normal subjects. *Res. Clin. Forums* **1**, 35–45 (1979).
42. Fox, C.S. *et al*. Genomewide linkage analysis to serum creatinine, GFR, and creatinine clearance in a community-based population: the Framingham Heart Study. *J. Am. Soc. Nephrol*. **15**, 2457–2461 (2004).
43. Shikata, E. *et al*. Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. *J. Hum. Genet*. **52**, 117–122 (2007).
44. Biermann, J. *et al*. Characterization of regulatory mechanisms and states of human organic cation transporter 2. *Am. J. Physiol. Cell Physiol*. **290**, C1521–C1531 (2006).
45. Stephens, M. & Donnelly, P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet*. **73**, 1162–1169 (2003).
46. Stephens, M., Smith, N.J. & Donnelly, P. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet*. **68**, 978–989 (2001).