Tanja Dujic,[1] Kaixin Zhou,[2] Louise A. Donnelly,[2] Roger Tavendale,[2]
Colin N.A. Palmer,[2] and Ewan R. Pearson[2]

Association of Organic Cation Transporter 1 With Intolerance to Metformin in Type 2 Diabetes: A GoDARTS Study

Diabetes 2015;64:1786–1793 | DOI: 10.2337/db14-1388

PHARMACOLOGY AND THERAPEUTICS

Metformin is the most widely prescribed medication for the treatment of type 2 diabetes (T2D). However, gastrointestinal (GI) side effects develop in ~25% of patients treated with metformin, leading to the discontinuation of therapy in ~5% of cases. We hypothesized that reduced transport of metformin via organic cation transporter 1 (OCT1) could increase metformin concentration in the intestine, leading to increased risk of severe GI side effects and drug discontinuation. We compared the phenotype, carriage of reduced-function OCT1 variants, and concomitant prescribing of drugs known to inhibit OCT1 transport in 251 intolerant and 1,915 fully metformin-tolerant T2D patients. We showed that women and older people were more likely to be intolerant to metformin. Concomitant use of medications, known to inhibit OCT1 activity, was associated with intolerance (odds ratio [OR] 1.63 [95% CI 1.22–2.17], $P = 0.001$) as was carriage of two reduced-function OCT1 alleles compared with carriage of one or no deficient allele (OR 2.41 [95% CI 1.48–3.93], $P < 0.001$). Intolerance was over four times more likely to develop (OR 4.13 [95% CI 2.09–8.16], $P < 0.001$) in individuals with two reduced-function OCT1 alleles who were treated with OCT1 inhibitors. Our results suggest that reduced OCT1 transport is an important determinant of metformin intolerance.

Metformin is recommended as first-line therapy for patients with type 2 diabetes (T2D) (1) and currently is used by >120 million patients worldwide. It ameliorates hyperglycemia by inhibiting hepatic gluconeogenesis, and increasing peripheral glucose uptake (2). It may also increase gut glucose utilization (3). At a molecular level, it has been suggested that metformin interferes with glucagon signaling (4), and, more recently, that it inhibits mitochondrial glycerol-3-phosphate dehydrogenase, leading to the reduction of hepatic gluconeogenesis (5). Activation of AMP-activated protein kinase may mediate metformin effects on lipid metabolism and insulin sensitivity (6). Metformin is recommended as first-line therapy for T2D because of its efficacy, safety (i.e., lack of weight gain and low risk of hypoglycemia), relatively low cost, and potential cardiovascular benefit (7).

Metformin treatment is, however, frequently associated with gastrointestinal (GI) side effects (20–30% of patients) (2), and this can negatively affect quality of life and treatment adherence in T2D patients (8). Severe GI symptoms develop in ~5% of patients, who discontinue the treatment with metformin, which could deprive them of the beneficial effects of the drug. Common metformin GI symptoms include nausea, diarrhea, vomiting, bloating, and abdominal pain (9). The pathophysiology of metformin-induced GI intolerance is unclear, although different hypotheses have been proposed, including stimulation of intestinal serotonin secretion, changes in incretin and glucose metabolism, and bile salt malabsorption (9). It is hypothesized that GI intolerance is related to a high concentration of metformin in the intestine after oral administration of the drug (10,11).

Metformin is an organic cation, and carrier proteins mediate its oral absorption, hepatic uptake, and renal elimination. Several solute carrier transporters, expressed in the membranes of the enterocytes, could be involved in the absorption of metformin from

[1]Department of Biochemistry & Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
[2]Division of Cardiovascular & Diabetes Medicine, Medical Research Institute, University of Dundee, Dundee, Scotland, U.K.
Corresponding author: Ewan R. Pearson, e.z.pearson@dundee.ac.uk.

Received 9 September 2014 and accepted 24 November 2014.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db14-1388/-/DC1.

the intestinal lumen, including organic cation transporter (OCT) 1, plasma membrane monoamine transporter (PMAT), carnitine/cation transporter 1, and OCT3 (12–15). While there are no established common loss-of-function variants of other metformin gut transporters, the human OCT1 gene (*SLC22A1*) is highly polymorphic, and four OCT1 variants, R61C (rs12208357), M420del (rs72552763), G401S (rs34130495), and G465R (rs34059508), showed reduced metformin transport in vitro (16). In addition to genetic variation, a number of commonly prescribed drugs have been shown to inhibit transport via OCT1 in vitro (e.g., tricyclic antidepressants [TCAs], proton pump inhibitors [PPIs], α-adrenoreceptor antagonists, and calcium-channel blockers [verapamil and diltiazem]) (17).

We hypothesized that reduced transport of metformin by OCT1 could increase metformin concentration in the intestine, resulting in increased risk of GI intolerance and drug discontinuation. Therefore, we assessed the role of five reduced-function variants in OCT1 (R61C, C88R [rs55918055], G401S, M420del, and G465R), and concomitant use of OCT1-inhibiting drugs in metformin intolerance, in a large cohort of metformin-treated T2D patients from Tayside, Scotland, U.K.

RESEARCH DESIGN AND METHODS

Study Population

In this observational cohort study, we identified patients with T2D who were receiving treatment with metformin, using data from the Genetics of Diabetes Audit and Research Tayside Study (GoDARTS) database. The GoDARTS resource includes nearly 10,000 patients with T2D. Since October 1997, DNA was collected from the patients for genetic studies. Retrospective and prospective longitudinal data are collected on each individual with T2D from the diagnosis of diabetes, including prescribing, biochemistry, and clinical data, which can be obtained in an anonymized form. The GoDARTS study was approved by the Tayside Medical Ethics Committee. Informed consent was obtained from all participants. The use of the GoDARTS bioresource for the study of metformin pharmacogenetics was approved by the Tayside Tissue Bank.

The study included all GoDARTS patients with T2D, who were incident users of metformin in the period from 1 January 1994 to 1 June 2011.

Definition of Intolerance

We established a proxy phenotype of metformin intolerance based upon prescribing patterns. Patients who stopped receiving metformin (immediate-release [IR] formulation) within the first 6 months of treatment and switched to another oral hypoglycemic agent, including metformin slow-release formulations, within 6 months of the last metformin IR prescription, were identified as being intolerant. We excluded patients who switched to treatment with insulin within 6 months of the last metformin prescription, as well as patients who ever received a daily dose of metformin IR or slow-release formulations, of ≥2,000 mg.

Patients who were prescribed a dose of ≥2,000 mg metformin (IR formulation) for >6 months were defined as being tolerant. Patients in both groups with serum creatinine levels >120 μmol/L were excluded from the analysis.

From a total of 6,265 patients who were incident users of metformin with T2D, based on our definition, we classified 251 patients as intolerant and 1,915 patients as tolerant (2,166 patients totally).

Anthropometric and Biochemical Variables

Values closest to metformin index date were obtained for weight, BMI, and serum creatinine levels (within 1 year on either side of the start of metformin treatment), and for HbA_{1c} level (within 6 months prior to the start of metformin treatment). Creatinine clearance was estimated using the Cockcroft-Gault formula (18).

Metformin Dose

A daily dose of metformin was defined as the last prescribed dose for intolerant patients, and as an average dose in the first 6 months of metformin treatment for tolerant patients.

OCT1-Inhibiting Medications

We identified all patients who were prescribed, concomitantly with metformin, medications shown to inhibit OCT1 activity in vitro. This included prescriptions for TCAs (19,20), citalopram (17,19), PPIs (21), verapamil (19,20), diltiazem (19), doxazosin (19,20), spironolactone (19,20), clopidogrel (22), rosiglitazone (23), quinine (17,19), tramadol (19,24), and codeine (25). There were only few or no prescriptions for other OCT1-inhibiting drugs (including prazosin, disopyramide, quinidine, repaglinide, propafenone, ketoconazole, morphine, tropisetron, ondansetron, antipsychotic agents, and tyrosine kinase inhibitors) (17,19,20,23,25–27).

Genotyping

M420del and R61C variants were genotyped previously in the whole of GoDARTS using TaqMan genotyping assays (Applied Biosystems) (28). Genotypes of the other three variants were imputed from existing genome-wide data on 7,319 GoDARTS patients using the 1,000-genome reference panel and IMPUTE2 software (29,30). The imputation quality information values were 0.932, 0.918, and 0.876 for C88R, G410S, and G465, respectively. The imputed genotype data were further supplemented by exome chip data on 4,760 individuals (695 patients with a definable phenotype in this study). High concordance rates between imputed genotypes called at a threshold of 0.9 and exome chip data were observed, as only 0.2%, 0.4%, and 0.5% of variant carriers of C88R, G410S, and G465, respectively, were misclassified by imputed data.

A total of 1,940 patients (90% of 2,166 patients in the study) had available genotype data for the five OCT1 variants. All variants were in line with Hardy-Weinberg equilibrium ($P > 0.05$). The OCT1 diplotypes were estimated using PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) (31).

Statistical Analysis

The differences between quantitative variables were estimated using a *t* test (variables with normal distribution), or a Mann-Whitney *U* test (variables with nonnormal distribution). Comparisons between categorical variables were performed with a χ^2 test or Fisher exact test (in the case where expected frequencies were ≤5). R61C, C88R, G401S, M420del, and G465R variants were analyzed together, according to the number of haplotypes carrying reduced-function alleles: 0, 1, or 2 (OCT1 combined genotype). The combined genotype frequencies between the two groups were compared using the χ^2 test (recessive and dominant model) and the Cochran-Armitage trend test (additive model). Logistic regression analysis was used to assess the effects of the OCT1 combined genotype, and coprescribed medications known to inhibit OCT1 activity in vitro, on the outcome phenotype of metformin intolerance, as defined above. Based on previous studies (32,33), showing a significant effect of the OCT1 combined genotype in individuals with two dysfunctional alleles, a recessive model was assumed. Age, sex, and weight were included as covariates. In a sensitivity analysis, patients were matched for age and sex. Conditional logistic regression was used for the analysis of matched groups. The statistical analyses were performed with SAS software version 9.3 (SAS Institute Inc., Cary, NC). Statistical significance was defined as $P < 0.05$.

RESULTS

Metformin Intolerance Phenotype

The characteristics of metformin-intolerant and metformin-tolerant patients are shown in Table 1. Intolerant patients were on average 10 years older ($P < 0.001$), were more commonly women ($P < 0.001$); had lower weight and BMI ($P < 0.001$), lower creatinine clearance levels ($P < 0.001$), and lower HbA_{1c} levels ($P = 0.003$); and were treated with a lower metformin dose ($P < 0.001$).

To assess whether we had identified a group of patients who developed GI side effects after starting treatment with metformin, we explored the difference in use of GI drugs that could be prescribed for GI side effects (compound alginates, antispasmodics, antidiarrheals, and antiemetics) between the two groups before and after the commencement of metformin therapy (Supplementary Table 1). Consistent with a prescribing pattern suggesting GI intolerance, more patients were treated with antidiarrheal medications after initiation of metformin therapy in the intolerant group than in the tolerant group. Although not statistically significant, there was also a similar trend for higher use of antispasmodic agents and other drugs altering gut motility, as well as antiemetics, after the initiation of metformin therapy in the intolerant group compared with the tolerant group (Supplementary Table 1).

OCT1-Interacting Drugs and Metformin Intolerance

Almost half of the intolerant patients (47.8%) were taking OCT1-inhibiting drugs compared with 32.7% of tolerant patients ($P < 0.001$) (Table 1). In a logistic regression analysis, adjusted for age, sex, and weight, the concomitant use of OCT1-inhibiting medications was significantly associated with intolerance (odds ratio [OR] 1.63 [95% CI 1.22–2.17], $P = 0.001$) (data for other covariates are not shown).

Next, we explored differences in concomitant treatment with individual OCT1-inhibiting drug/drug class between intolerant and tolerant patients. The numbers of patients receiving different OCT1-inhibiting medications are shown in Supplementary Table 2. ORs for the

Table 1—Baseline characteristics of metformin-intolerant and metformin-tolerant patients

	Intolerant group (*n* = 251)	Tolerant group (*n* = 1,915)	*P*
Age (years)	67.8 ± 10.5	58.0 ± 10.8	<0.001
Age at diagnosis (years)	62.8 ± 10.5	54.8 ± 10.5	<0.001
Sex			<0.001
Females	141 (56.2)	767 (40.1)	
Males (*n*)	110	1,148	
Weight (kg)	82.8 ± 17.1	92.2 ± 18.5	<0.001
BMI (kg/m^2)	30.6 ± 5.8	32.6 ± 6.2	<0.001
HbA_{1c}			0.003
%	8.3 (7.7–9.5)	8.7 (7.8–9.9)	
mmol/mol	67 (61–80)	72 (62–85)	
Creatinine (μmol/L)	87.2 ± 13.9	87.0 ± 14.5	0.808
Creatinine clearance (mL/min)	75.4 (58.2–92.8)	97.4 (77.6–121.6)	<0.001
Antidiabetic drug-naive	136 (54.2)	1,173 (61.3)	0.031
Use of OCT1-inhibiting drugs	120 (47.8)	627 (32.7)	<0.001
Metformin daily dose (mg)	1,000 (1,000–1,000)	1,000 (1,000–1,500)	<0.001

Data are presented as the mean ± SD, *n* (%), or median (interquartile range), unless otherwise indicated.

association of the individual drugs/drug classes with intolerance are shown in a forest plot in Fig. 1. The concomitant use of citalopram, PPIs, verapamil, doxazosin, and codeine was significantly associated with metformin intolerance. Interestingly, patients treated with verapamil had seven times higher odds of developing intolerance (OR 7.44 [95% CI 2.09–26.5], $P = 0.002$). A high proportion of intolerant patients were using PPIs (28.3%). As PPIs are used to treat indigestion and reflux, and more intolerant patients were receiving PPIs before the initiation of metformin therapy than tolerant patients (56 [22.3%], intolerant patients vs. 232 [12.1%] tolerant patients, $P < 0.001$), it is possible that the result for PPIs is confounded by prior GI symptoms. Therefore, we studied the use of histamine H_2-receptor antagonists (H2RAs) between intolerant and tolerant patients, as these are used for the same indication as PPIs, yet do not inhibit OCT1 (19). There were no significant differences in the percentage of patients treated with H2RAs (19 [7.6%] intolerant patients vs. 117 [6.1%] tolerant patients, $P = 0.370$) in the intolerant group and the tolerant group, suggesting that the result seen for PPIs does reflect OCT1 inhibition.

OCT1 Genotypes and Metformin Intolerance

We explored the linkage disequilibrium among the five OCT1 variants by haplotype analysis using directly genotyped exome chip data. As shown in Supplementary Table 3, C88R and G465R substitutions only occurred with M420del variant, while R61C and G401S substitutions occurred only on the wild-type background of other polymorphisms, which is in line with previous studies in Caucasians (16,27,33). Further diplotype data in Supplementary Table 4 showed that the number of reduced-function haplotypes in each patient could be simply characterized by the total number of variant alleles in R61C, M420del, and G401S (OCT1 combined genotype).

The numbers of patients in the intolerant and tolerant groups according to the number of deficient OCT1 alleles are shown in Supplementary Table 5. There was a significant difference in the combined genotype frequency between the two groups in the recessive model ($P < 0.001$).

The combined genotype was added to the logistic regression model, adjusted for age, sex, weight, and the overall use of OCT1-inhibiting drugs (Table 2). In addition to the concomitant treatment with OCT1-inhibiting medications, the presence of two reduced-function alleles was independently associated with intolerance to metformin (OR 2.41 [95% CI 1.48–3.93], $P < 0.001$). When patients were grouped according to the combination of OCT1 genotype and the use of OCT1-inhibiting drugs, carriers of two low-activity alleles who were also treated with OCT1-interacting drugs had fourfold higher odds of intolerance compared with patients with one or no deficient alleles who were not being treated with OCT1 inhibitors (OR 4.13 [95% CI 2.09–8.16], $P < 0.001$) (Table 3).

Sensitivity Analysis

Since there were large differences in age and sex between case patients and control patients, we carried out a sensitivity analysis by comparing the intolerant group ($n = 231$) with age- and sex-matched subgroups of tolerant patients ($n = 709$). In this sensitivity analysis, we confirmed the main findings obtained with the larger tolerant group (Supplementary Fig. 1 and Supplementary Tables 6 and 7).

To guard against potential bias originating from the imputed data, we performed another sensitivity analysis in a subset of 660 patients using only directly genotyped data by TaqMan and exome chip. The carriage of two dysfunctional OCT1 alleles showed significant association

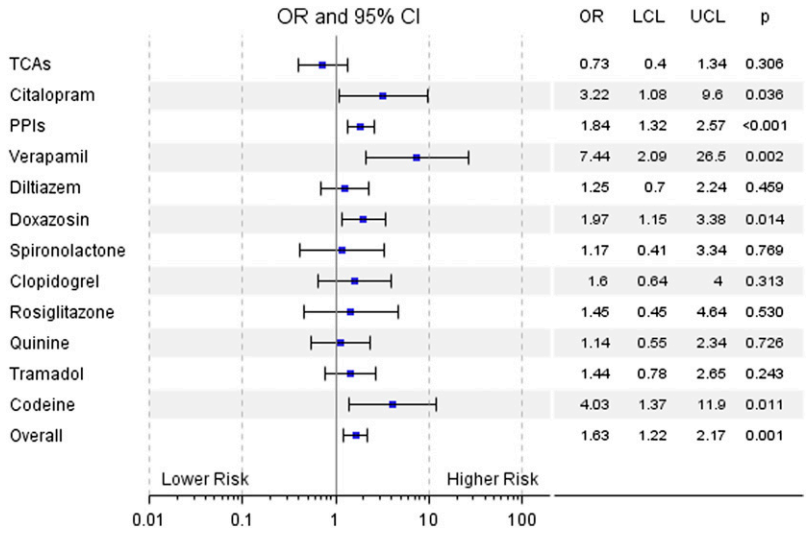

Figure 1—Association of the individual drugs and drug classes with metformin intolerance. The analysis was adjusted for age, sex, weight, and cotreatment with other OCT1 inhibitors. LCL, lower confidence limit; UCL, higher confidence limit.

Table 2—Logistic regression model of metformin intolerance

	OR (95% CI)	*P*
Age	1.10 (1.08–1.12)	<0.001
Sex (females vs. males)	1.85 (1.33–2.57)	<0.001
Weight	0.99 (0.98–1.00)	0.064
Use of OCT1-inhibiting drugs	1.64 (1.20–2.25)	0.002
Two reduced-function OCT1 alleles	2.41 (1.48–3.93)	<0.001

Logistic regression analysis included 205 intolerant and 1,650 tolerant patients.

with intolerance even in this much smaller cohort (OR 2.99 [95% CI 1.40–6.38], $P = 0.005$; Supplementary Table 8).

To test our definition of intolerance, we carried out additional analyses, excluding intolerant patients who had more than two metformin prescriptions, and excluding patients who transitioned to metformin slow-release formulations. The exclusion of these two small subgroups did not alter the results (data not shown).

DISCUSSION

A number of studies have investigated the effects of genetic variants on metformin efficacy (34), including the first genome-wide association study of metformin response performed by our group (35). However, so far, only one small study (36) has examined the pharmacogenetics of metformin GI side effects in T2D patients, and no studies have investigated the effect of coprescribing on the risk of metformin intolerance. To our knowledge, this is the first study that addressed the genetics of metformin intolerance, and the role of OCT1, in a large cohort of T2D patients. We found that concomitant use of drugs that inhibit OCT1 increases metformin intolerance, with some drugs such as verapamil increasing the odds of intolerance sevenfold. In addition, we showed a significant effect of OCT1 genotype on intolerance: patients carrying two OCT1 reduced-function alleles had more than twice the odds of intolerance compared with patients carrying one or no deficient alleles. Together, these results point to a key role of metformin transport by OCT1 in mediating GI intolerance with metformin treatment.

Although our results provide strong evidence that the inhibition of OCT1 transport increases the risk of GI intolerance, the precise mechanism for this remains uncertain. Results from earlier studies (14,37,38) suggested basolateral localization of OCT1 in enterocytes; however, a recent study (15) demonstrated apical localization of OCT1 in Caco-2 cell monolayers and in mouse and human enterocytes. If OCT1 has a role in the efflux of metformin (either via the basolateral or apical route), then inhibition could increase metformin concentration in the enterocytes. Alternatively, if OCT1 has a role in the transport of metformin from the lumen into the enterocytes, then OCT1 inhibition could increase luminal metformin concentration. Although proposed hypotheses of metformin GI intolerance are inconclusive, and it is unclear whether adverse effects could be attributed to the drug presence in the mucosa or in the lumen, increased metformin concentrations in the gut may affect intestinal serotonin concentration (39), bile salt absorption (40), or potentially may alter the microbiome (41).

An alternative mechanism for the increased metformin intolerance with reduced OCT1 transport is that OCT1 inhibition is altering systemic concentrations of metformin, and it is the higher circulating metformin concentrations that result in metformin intolerance. However, the role of OCT1 in metformin pharmacokinetics has been extensively studied, and the data are unclear. There were no differences in the pharmacokinetic properties of metformin between $Oct1^{-/-}$ and $Oct1^{+/+}$ mice after oral application (42). On the other hand, results of the studies on OCT1 reduced-function variants and metformin pharmacokinetics in humans have been contradictory (32,33,42). In a study by Christensen et al. (33), the number of reduced-function alleles was associated with lower trough metformin levels. It has been suggested that a decrease in metformin levels could be a combined result of reduced intestinal absorption, an increased renal clearance (32), and decreased distribution (33). In contrast, in a study (42) in 20 healthy volunteers, patients carrying one or more OCT1 reduced-function alleles had slightly higher metformin plasma concentrations than patients with wild-type OCT1. However, in this study, only two patients had two inactive alleles, and it has been suggested that major changes may be seen only in subjects carrying two OCT1 low-activity alleles (recessive model) (33,43). This is in line with our results, as a significant effect of OCT1 genotype on metformin intolerance was observed only in the recessive model. Additional studies are needed to clarify contradictory

Table 3—Joint effects of OCT1 genotype and OCT1 interacting drugs on intolerance

	OR (95% CI)	*P*
Carriers of one or no reduced-function allele not treated with OCT1-inhibiting drugs*	1.00	
Carriers of one or no reduced-function allele treated with OCT1-inhibiting drugs†	1.62 (1.16–2.26)	0.005
Carriers of two reduced-function alleles not treated with OCT1-inhibiting drugs‡	2.27 (1.13–4.58)	0.022
Carriers of two reduced-function alleles treated with OCT1-inhibiting drugs§	4.13 (2.09–8.16)	<0.001

Analysis was adjusted for age, sex, and weight. *Ninety-three intolerant and 1,030 tolerant patients. †Eighty-four intolerant and 516 tolerant patients. ‡Twelve intolerant and 70 tolerant patients. §Sixteen intolerant and 34 tolerant patients.

findings on the effect of OCT1 variants on metformin pharmacokinetics, as well as response (16,28,33).

We show that the use of concomitant drugs that inhibit OCT1 transport in vitro increases the risk of metformin intolerance. Many drugs used clinically have been identified as OCT1 inhibitors in vitro (17). We included 10 drugs and 2 drug classes in our analysis, based on their frequency of use in the study cohort and their reported half-maximal inhibitory concentration (IC_{50}) values. Strong inhibitory effects on OCT1-mediated metformin transport in vitro were demonstrated for verapamil (20), rosiglitazone (23), PPIs (21), and clopidogrel (22). For other included drugs and drug classes in our study, in vitro inhibition measurements with metformin as the OCT1 substrate were not performed. However, based on the IC_{50} values for 4-[4-(dimethylamino)styryl]-*N*-methylpyridinium (ASP) transport, spironolactone, most of the TCAs, diltiazem, doxazosin, and citalopram were classified as strong OCT1 inhibitors (19,20), while quinine and tramadol were weaker inhibitors (17,19,24). A recent study (25) showed that codeine could also significantly inhibit OCT1-mediated uptake. Metformin transport, however, may be more sensitive to inhibition compared with the model substrates because of its lower apparent affinity (20).

We see striking differences between drug classes, with large effects particularly seen for verapamil, PPIs, doxazosin, codeine, and citalopram. We report the largest effect of coprescribing on metformin intolerance with verapamil use, with an OR of 7.44 for intolerance. This is in keeping with the in vitro findings showing a very potent inhibitory effect of verapamil on OCT1 (20). While it is possible that the use of PPIs may reflect GI problems prior to starting metformin therapy, we show that the use of H2RAs, which are used for the same indication as PPIs, are not associated with intolerance. The effect of all OCT1-inhibiting drugs remains significant despite the removal of PPI use from the analysis (OR 1.46 [95% CI 1.08–1.99], $P = 0.016$), and there is no reason why patients selected to be treated with drugs such as verapamil, doxazosin, or citalopram should have increased GI symptoms, other than by interaction with metformin use.

It is possible that the drugs reported to inhibit OCT1 also inhibit other cation-selective transporters involved in metformin absorption, or distribution and clearance, thus contributing to metformin intolerance in addition to OCT1 inhibition alone. This may explain the additional impact of the use of these drugs on intolerance risk in patients already carrying two loss-of-function OCT1 variants. Although most of the studied inhibiting drugs have a higher affinity to OCT1 compared with OCT2 and OCT3 (17,44), PPIs are shown to inhibit OCT1, OCT2, and OCT3 with similar IC_{50} values (21). There is also a large overlapping of substrates and inhibitors between PMAT and OCTs (45). Although extensive in vitro data for potential PMAT inhibitors are lacking, verapamil, for instance, exhibited similar inhibitory potencies toward PMAT and OCTs (45).

We observed a higher use of antiemetic drugs (drugs used in nausea and vertigo) in the intolerant group prior to and after the initiation of metformin therapy. It is unclear the reason for this and it may reflect the small numbers in these groups. However, adjustment for prior use of antiemetics, as well as the exclusion of patients who had used these agents, did not significantly affect our results. From 14 intolerant patients with prior use of these drugs, 8 were treated with prochlorperazine, and 4 with domperidone. It is interesting, however, that prochlorperazine also showed weak inhibition of ASP transport by OCT1 (19), and domperidone has recently been identified as an OCT2 inhibitor (46).

Other identified significant risk factors for metformin intolerance in our study were older age and female sex, with a trend toward lower weight. Estimated creatinine clearance was calculated based on an equation with these three variables, and thus was significantly different between the two groups, although creatinine levels were similar. Our results are in agreement with the findings of a small study (36) that investigated the genetics of common metformin GI side effects. The presence of side effects in this prospective study correlated positively with age, and 76% of 53 case patients were women. Interestingly, the authors analyzed seven variants in OCT1, OCT2, and MATE1 genes, reporting that, of these, two common OCT1 polymorphisms in high linkage disequilibrium with each other were associated with an increased prevalence of metformin side effects (M408V and 8-base pair insertion), while R61C, M420del, and G465R variants, or OCT1 haplotypes, showed no association (36). However, M408V showed normal uptake of metformin in vitro (16), and the function of 8-base pair insertion is unknown. In contrast to our study, the phenotype used was that of mild intolerance, and no interacting drugs were studied. The small sample size of the previous study means that the power to identify the effects of the OCT1 reduced-function variants was limited.

The main limitation of our study is the surrogate phenotype of metformin GI intolerance based on the discontinuation of the drug in the first months of treatment. However, patients were switched to another oral hypoglycemic agent, so they did not stop metformin therapy because of sufficient glycemic control; and their HbA_{1c} values were lower than those in tolerant patients, suggesting that patients did not stop metformin therapy because of insufficient glycemic control in the first months of treatment either. However, we cannot rule out other possible reasons for stopping therapy with metformin, mainly other non-GI side effects (e.g., rash or headache) or other reasons unrelated to metformin intolerance. Nevertheless, GI symptoms are the most common side effects of metformin, and 5% of patients are not able to tolerate treatment because of their severity (2). This number is in line with our findings, as we identified 251 incident metformin users from a total of 6,265 as being intolerant (4%). We also show that the intolerant group has increased use of

antidiarrheal drugs and a tendency for higher use of other GI drugs, after metformin initiation, supporting the evidence that they are having GI problems. Using a similar approach, we previously successfully developed a proxy phenotype for statin intolerance (47).

Our study has a potential clinical impact. Our data suggest that concomitant therapy with OCT1-inhibiting drugs, like PPIs and verapamil, is a risk factor for development of intolerance, and this could be avoided by prescribing alternative medications that do not interact with OCT1 to patients experiencing side effects. This is particularly true for the 8% of the population with two inactive OCT1 alleles, who are more than fourfold more likely to develop intolerance with coprescribed OCT1-inhibiting drugs. Avoiding OCT1-inhibiting drugs in these individuals would prevent the reduced efficacy associated with suboptimal metformin dosage, and the potential cessation of metformin treatment and its substitution with second-line therapies. This needs to be established in a clinical trial before such a recommendation can be implemented in practice.

In conclusion, we have identified age, female sex, reduced-function alleles of OCT1, and the concomitant use of OCT1-inhibiting medications as risk factors for metformin intolerance. Future prospectively designed studies are needed to substantiate our findings and to identify other possible predictive biomarkers of metformin intolerance.

Acknowledgments. The authors thank all of the participants who took part in this study; the general practitioners; the Scottish School of Primary Care for their help in recruiting the participants; and the whole team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

Funding. The Wellcome Trust United Kingdom Type 2 Diabetes Case Control Collection (GoDARTS) cohort was funded by The Wellcome Trust, and informatics support was provided by the Chief Scientist Office, Scotland. This work was specifically supported by a European Foundation for the Study of Diabetes Albert Renold Travel Fellowship to T.D. E.R.P. received a Wellcome Trust New Investigator Award (102820/Z/13/Z).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. T.D. analyzed and interpreted the data, and wrote the manuscript. K.Z., L.A.D., R.T., and C.N.A.P. analyzed the data, and critically assessed and reviewed the manuscript. E.R.P. designed the study, interpreted the data and wrote the manuscript. E.R.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Inzucchi SE, Bergenstal RM, Buse JB, et al.; American Diabetes Association (ADA); European Association for the Study of Diabetes (EASD). Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care 2012;35:1364–1379

2. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002;137:25–33

3. Mithieux G, Rajas F, Zitoun C. Glucose utilization is suppressed in the gut of insulin-resistant high fat-fed rats and is restored by metformin. Biochem Pharmacol 2006;72:1757–1762

4. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 2013;494:256–260

5. Madiraju AK, Erion DM, Rahimi Y, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 2014;510:542–546

6. Rena G, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: old or new insights? Diabetologia 2013;56:1898–1906

7. Scarpello JH, Howlett HC. Metformin therapy and clinical uses. Diab Vasc Dis Res 2008;5:157–167

8. Florez H, Luo J, Castillo-Florez S, et al. Impact of metformin-induced gastrointestinal symptoms on quality of life and adherence in patients with type 2 diabetes. Postgrad Med 2010;122:112–120

9. Bouchoucha M, Uzzan B, Cohen R. Metformin and digestive disorders. Diabetes Metab 2011;37:90–96

10. Wilcock C, Bailey CJ. Accumulation of metformin by tissues of the normal and diabetic mouse. Xenobiotica 1994;24:49–57

11. Bailey CJ, Wilcock C, Scarpello JH. Metformin and the intestine. Diabetologia 2008;51:1552–1553

12. Zhou M, Xia L, Wang J. Metformin transport by a newly cloned proton-stimulated organic cation transporter (plasma membrane monoamine transporter) expressed in human intestine. Drug Metab Dispos 2007;35:1956–1962

13. Nakamichi N, Shima H, Asano S, et al. Involvement of carnitine/organic cation transporter OCTN1/SLC22A4 in gastrointestinal absorption of metformin. J Pharm Sci 2013;102:3407–3417

14. Müller J, Lips KS, Metzner L, Neubert RH, Koepsell H, Brandsch M. Drug specificity and intestinal membrane localization of human organic cation transporters (OCT). Biochem Pharmacol 2005;70:1851–1860

15. Han TK, Everett RS, Proctor WR, et al. Organic cation transporter 1 (OCT1/mOct1) is localized in the apical membrane of Caco-2 cell monolayers and enterocytes. Mol Pharmacol 2013;84:182–189

16. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. J Clin Invest 2007;117:1422–1431

17. Nies AT, Koepsell H, Damme K, Schwab M. Organic cation transporters (OCTs, MATEs), in vitro and in vivo evidence for the importance in drug therapy. Handb Exp Pharmacol 2011;105–167

18. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41

19. Ahlin G, Karlsson J, Pedersen JM, et al. Structural requirements for drug inhibition of the liver specific human organic cation transport protein 1. J Med Chem 2008;51:5932–5942

20. Ahlin G, Chen L, Lazorova L, et al. Genotype-dependent effects of inhibitors of the organic cation transporter, OCT1: predictions of metformin interactions. Pharmacogenomics J 2011;11:400–411

21. Nies AT, Hofmann U, Resch C, Schaeffeler E, Rius M, Schwab M. Proton pump inhibitors inhibit metformin uptake by organic cation transporters (OCTs). PLoS One 2011;6:e22163

22. Li L, Song F, Tu M, et al. In vitro interaction of clopidogrel and its hydrolysate with OCT1, OCT2 and OAT1. Int J Pharm 2014;465:5–10

23. Bachmakov I, Glaeser H, Fromm MF, König J. Interaction of oral antidiabetic drugs with hepatic uptake transporters: focus on organic anion transporting polypeptides and organic cation transporter 1. Diabetes 2008;57:1463–1469

24. Tzvetkov MV, Saadatmand AR, Lötsch J, Tegeder I, Stingl JC, Brockmöller J. Genetically polymorphic OCT1: another piece in the puzzle of the variable pharmacokinetics and pharmacodynamics of the opioidergic drug tramadol. Clin Pharmacol Ther 2011;90:143–150

25. Tzvetkov MV, dos Santos Pereira JN, Meineke I, Saadatmand AR, Stingl JC, Brockmöller J. Morphine is a substrate of the organic cation transporter OCT1 and polymorphisms in OCT1 gene affect morphine pharmacokinetics after codeine administration. Biochem Pharmacol 2013;86:666–678

26. Minematsu T, Giacomini KM. Interactions of tyrosine kinase inhibitors with organic cation transporters and multidrug and toxic compound extrusion proteins. Mol Cancer Ther 2011;10:531–539
27. Tzvetkov MV, Saadatmand AR, Bokelmann K, Meineke I, Kaiser R, Brockmöller J. Effects of OCT1 polymorphisms on the cellular uptake, plasma concentrations and efficacy of the 5-HT(3) antagonists tropisetron and ondansetron. Pharmacogenomics J 2012;12:22–29
28. Zhou K, Donnelly LA, Kimber CH, et al. Reduced-function SLC22A1 polymorphisms encoding organic cation transporter 1 and glycemic response to metformin: a GoDARTS study. Diabetes 2009;58:1434–1439
29. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 2009;5:e1000529
30. Howie B, Marchini J, Stephens M. Genotype imputation with thousands of genomes. G3 (Bethesda) 2011;1:457–470
31. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575
32. Tzvetkov MV, Vormfelde SV, Balen D, et al. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther 2009;86:299–306
33. Christensen MM, Brasch-Andersen C, Green H, et al. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. Pharmacogenet Genomics 2011;21:837–850
34. Todd JN, Florez JC. An update on the pharmacogenomics of metformin: progress, problems and potential. Pharmacogenomics 2014;15:529–539
35. Zhou K, Bellenguez C, Spencer CC, et al.; GoDARTS and UKPDS Diabetes Pharmacogenetics Study Group; Wellcome Trust Case Control Consortium 2; MAGIC investigators. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. Nat Genet 2011;43:117–120
36. Tarasova L, Kalnina I, Geldnere K, et al. Association of genetic variation in the organic cation transporters OCT1, OCT2 and multidrug and toxin extrusion 1 transporter protein genes with the gastrointestinal side effects and lower BMI in metformin-treated type 2 diabetes patients. Pharmacogenet Genomics 2012;22:659–666
37. Jonker JW, Wagenaar E, Mol CA, 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 2001;21:5471–5477
38. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. J Pharmacol Exp Ther 2002;302:510–515
39. Cubeddu LX, Bönisch H, Göthert M, et al. Effects of metformin on intestinal 5-hydroxytryptamine (5-HT) release and on 5-HT3 receptors. Naunyn Schmiedebergs Arch Pharmacol 2000;361:85–91
40. Carter D, Howlett HC, Wiernsperger NF, Bailey CJ. Differential effects of metformin on bile salt absorption from the jejunum and ileum. Diabetes Obes Metab 2003;5:120–125
41. Napolitano A, Miller S, Nicholls AW, et al. Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus. PLoS One 2014;9:e100778
42. Shu Y, Brown C, Castro RA, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharmacol Ther 2008;83:273–280
43. Zolk O. Current understanding of the pharmacogenomics of metformin. Clin Pharmacol Ther 2009;86:595–598
44. Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. Pharm Res 2007;24:1227–1251
45. Engel K, Wang J. Interaction of organic cations with a newly identified plasma membrane monoamine transporter. Mol Pharmacol 2005;68:1397–1407
46. Wittwer MB, Zur AA, Khuri N, et al. Discovery of potent, selective multidrug and toxin extrusion transporter 1 (MATE1, SLC47A1) inhibitors through prescription drug profiling and computational modeling. J Med Chem 2013;56:781–795
47. Donnelly LA, Doney AS, Tavendale R, et al. Common nonsynonymous substitutions in SLCO1B1 predispose to statin intolerance in routinely treated individuals with type 2 diabetes: a go-DARTS study. Clin Pharmacol Ther 2011;89:210–216