Reduced-Function *SLC22A1* Polymorphisms Encoding Organic Cation Transporter 1 and Glycemic Response to Metformin: A GoDARTS Study

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OBJECTIVE—Metformin is actively transported into the liver by the organic cation transporter (OCT)1 (encoded by *SLC22A1*). In 12 normoglycemic individuals, reduced-function variants in *SLC22A1* were shown to decrease the ability of metformin to reduce glucose excursion in response to oral glucose. We assessed the effect of two common loss-of-function polymorphisms in *SLC22A1* on metformin response in a large cohort of patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS—The Diabetes Audit and Research in Tayside Scotland (DARTS) database includes prescribing and biochemistry information and clinical phenotypes of all patients with diabetes within Tayside, Scotland, from 1992 onwards. R61C and 420del variants of *SLC22A1* were genotyped in 3,450 patients with type 2 diabetes who were incident users of metformin. We assessed metformin response by modeling the maximum A1C reduction in 18 months after starting metformin and investigated whether a treatment target of A1C <7% was achieved. Sustained metformin effect on A1C between 6 and 42 months was also assessed, as was the time to metformin monotherapy failure. Covariates were *SLC22A1* genotype, BMI, average drug dose, adherence, and creatinine clearance.

RESULTS—A total of 1,531 patients were identified with a definable metformin response. R61C and 420del variants did not affect the initial A1C reduction ($P = 0.47$ and $P = 0.92$, respectively), the chance of achieving a treatment target ($P = 0.83$ and $P = 0.36$), the average A1C on monotherapy up to 42 months ($P = 0.44$ and $P = 0.75$), or the hazard of monotherapy failure ($P = 0.85$ and $P = 0.56$).

CONCLUSIONS—The *SLC22A1* loss-of-function variants, R61C and 420del, do not attenuate the A1C reduction achieved by metformin in patients with type 2 diabetes. ***Diabetes*** **58:1434–1439, 2009**

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Metformin is recommended as first-line oral treatment in the joint American Diabetes Association (ADA)/European Association for the Study of Diabetes (EASD) guidelines on the treatment of type 2 diabetes (1) and has been shown to be effective in decreasing both micro- and macrovascular disease (2). The glycemic response to metformin is variable, with some people having a marked response and others gaining no benefit. Furthermore, between 5 and 10% of patients are unable to tolerate metformin as a result of gastrointestinal side effects (3). This variation in metformin response may reflect phenotypic differences and variation in drug action or drug distribution. We and others have shown little or no clinical effect of basic phenotypic parameters such as age, sex, or BMI on metformin response (4–6), suggesting that genetic variation in pharmacokinetics or pharmacodynamics of metformin at the molecular level may be important.

Pharmacokinetic studies suggest that metformin, an organic cation, is actively absorbed from the gut and is excreted unchanged in the urine. Recent studies have shown that metformin is actively transported into the liver by organic cation transporter (OCT)1 (encoded by *SLC22A1*) (7,8) and into the renal tubules by OCT2 (encoded by *SLC22A2*) (9). In a previous study, Shu et al. (10) show, in mice lacking *Slc22a1*, that this transporter is necessary for metformin transport into the liver and for metformin to elicit its therapeutic effect. In addition, they describe reduced-function *SLC22A1* polymorphisms in humans that impair the glucose-lowering effect of metformin during an oral glucose tolerance test. We hypothesized that two common reduced-function polymorphisms in *SLC22A1* (420del and R61C) (10) would decrease glycemic response to metformin in patients with type 2 diabetes. In the current study, we assessed a series of drug-response models to metformin including short- and mid-term A1C reduction, reaching the treatment target of A1C <7%, and time to monotherapy failure in a large population-based study of 1,531 subjects residing in Tayside, Scotland.

RESEARCH DESIGN AND METHODS

Patients were selected from an ongoing study of the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS). (Additional information is available in an online appendix [http://diabetes.diabetesjournals.org/cgi/content/full/db08-0896/DC1].) All incident users of metformin ($n = 3,450$) were included to identify patients informative for the current study following the ascertainment process outlined in Fig. 1. Two study groups were defined, each of which required complete data with respect to sex, age, metformin dose, adherence, and A1C response. The subjects in group 1 received no

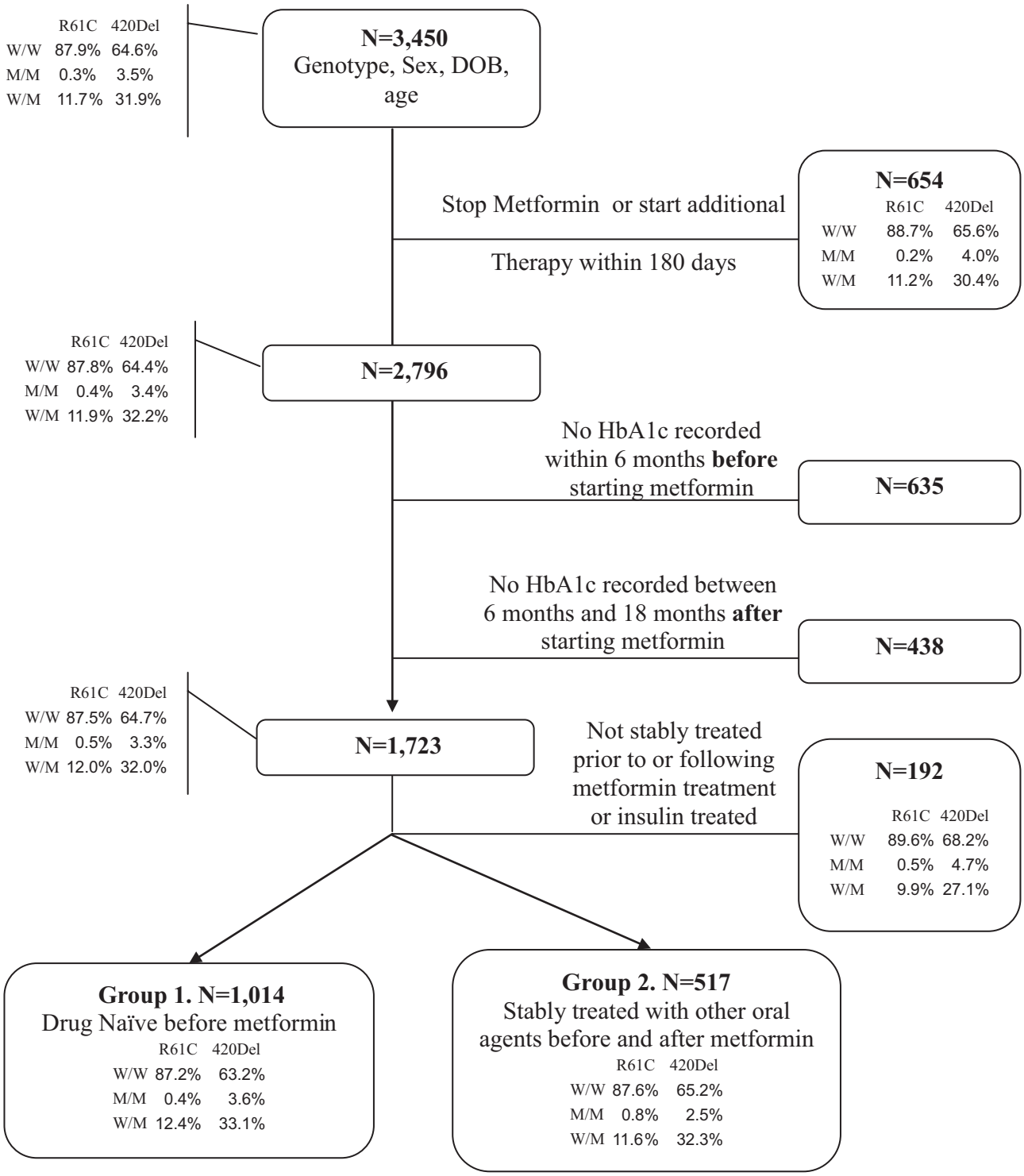

FIG. 1. Flowchart of patients' ascertainment. In the mini genotype frequency tables, W/W denotes the wild-type homozygote, M/M denotes the rare homozygote of CC and Del/Del for R61C and 420del, respectively, and W/M denotes heterozygote. DOB, date of birth.

diabetes-related drug treatment for at least 6 months before their index metformin prescription and were thus considered treatment naive. The subjects in group 2 were stably treated with oral hypoglycemic agents for at least 6 months before starting metformin and until at least the A1C on metformin therapy was measured. Both groups required at least 6 months of metformin use. The study was approved by the Tayside Medical Ethics Committee, and informed consent was obtained from all subjects.

Models of drug response

Initial A1C reduction. Linear regression was used to model two quantitative A1C reduction measurements derived from different treatment A1C definitions: *1*) the minimum A1C recorded from baseline to 18 months after the index prescription while the patient was on stable treatment (no additional therapy initiated or metformin stopped) and *2*) the average A1C reflecting the area under the curve of A1C within the same period. The corresponding A1C reduction was defined as the difference between the baseline and treatment A1C. The baseline A1C was taken as the nearest A1C within the 180 days before the index metformin prescription.

Treatment target A1C <7%. Logistic regression was used to model the ability of patients to reach a treatment target of A1C <7% within 18 months after the start of metformin given their baseline A1C >7%.

Sustained A1C response. In prospective trials of metformin monotherapy (e.g., A Diabetes Outcome Progression Trial [ADOPT]) (11) following the

TABLE 1
Baseline characteristics of the two study groups

	Group 1			Group 2			
	N	Mean	SD	*N*	Mean	SD	*P*
Male sex (%)	1,014	52.7	—	517	62.9	—	<0.0001
Age diagnosed (years)	1,014	57.1	10.7	517	58.3	9.66	0.02
Age treated with metformin (years)	1,014	59.1	11.2	517	63.1	10.5	<0.0001
BMI (kg/m^2)	990	32.5	5.7	509	29.5	4.9	<0.0001
Baseline A1C (%)	1,014	8.6	1.6	517	9.2	1.4	<0.0001
Average dose (g)	1,014	1.24	0.4	517	1.28	0.4	0.08
Adherence (%)	1,014	75.9	18.0	517	77.9	16.7	0.06
Creatinine clearance (ml/min)	983	97.7	34.0	493	83.9	30.0	<0.0001

initial response for 6 months, there was a gradual deterioration in glycemic control as diabetes progressed. The rate of deterioration of A1C will also reflect the patients' ability to consistently respond to metformin treatment. In the current study, we identified patients on metformin monotherapy for more than 3.5 years and performed unbalanced repeated-measurement analysis on their A1C, measured between 6 and 42 months.

Time to monotherapy failure or reaching a dose of 2 g/day. In our observational study, the primary care physicians will increase the prescribed metformin dose over time to achieve the treatment target or maintain the A1C level. Therefore, the time to reach a metformin monotherapy dose of 2 g/day will provide an additional measure of drug response. Similarly, the time to start additional therapy (monotherapy failure) will also reflect metformin efficacy. In the current study, we used Cox regression to model time to metformin monotherapy failure and time to a composite event of either reaching a 2 g/day dose of metformin or monotherapy failure. For both analyses, individuals were censored at study end or after 5 years of metformin monotherapy.

Covariates. In the models of initial drug response, apart from the genetic factors, the following covariates were included: baseline A1C, average prescribed dose, average adherence, creatinine clearance, BMI, and the study group (defined in Fig. 1), if applicable. In the repeated-measures analysis, the dose used was the dose in the 3 months preceding each A1C measure; other covariates were as per the initial drug response. For the Cox regression models, baseline A1C, average adherence, creatinine clearance, and genotype were used as covariates. For the definition of these covariates, please see the online appendix.

Genotyping. We genotyped R61C (rs12208357) and 420del variants of *SLC22A1* by Taqman-based allelic discrimination (see the online appendix for primer and probe sequences and quality-control data). The minor allele frequencies were 6.7% for R61C and 19.8% for 420del. The R61C mutant only occurred on the wild-type allele at codon 420. Both variants were in Hardy-Weinberg equilibrium ($P > 0.05$).

Statistical analysis. Allele frequencies of the two variants were compared between each subgroup and the original sample in Fig. 1, with a 2 d.f. χ^2 test. Both linear regression analysis of A1C reduction and logistic regression analysis of achieving treatment target A1C were carried out in PLINK under an additive model with all the covariates included (http://pngu.mgh.harvard.edu/purcell/plink/) (12). For the unbalanced repeated-measurement analysis of A1C between 6 and 42 months, the SAS (version 9.1) Proc Mixed module was used with covariates of baseline A1C, time of the measurement, the average dose in the 3 months before each measurement, an additive genetic effect, and a gene-by-time interaction term. The Cox proportional hazards regression analysis of time to monotherapy failure was conducted with the survival package in the statistical software R 2.8.0 (http://www.r-project.org), again including all of the covariates with the additive genetic model.

RESULTS

As shown in the sample ascertainment flowchart of Fig. 1, from the initial 3,450 patients we were able to identify 1,531 informative patients for the current study, of whom 1,014 were treatment naive (group 1). Patients were excluded for the following reasons: 654 patients did not complete 6 months of metformin treatment, 1,083 patients did not have sufficient A1C measurements, and 192 patients could not be classified into either study group. The genotype frequencies in any excluded subgroup never differed significantly from the two study groups or the initial cohort, suggesting that the two variants have no influence on metformin intolerance–caused withdrawal or ascertainment bias.

Baseline characteristics of the patients in study groups 1 and 2 are provided in Table 1. In keeping with the fact that the patients in group 2 were primarily treated with sulfonylureas, they formed a slimmer cohort with a longer duration of diabetes. There was no significant difference in these baseline parameters by R61C or 420del genotype.

In the linear regression model (Table 2) of the minimum treatment A1C achieved, the A1C reduction was independently associated with the baseline A1C, creatinine clearance, and metformin adherence. The subjects in group 2 responded less well than those in group 1, in keeping with their longer duration of diabetes. BMI was not included in the final model because it was not associated with the response and its inclusion had no effect on the other measures. In this model, neither the 420del nor the R61C genotype of *SLC22A1* was associated with metformin response ($P = 0.47$ and $P = 0.92$, respectively). Similar results were seen even when both study groups were analyzed separately (data not shown). The alternative linear regression model of the average A1C reduction still shows no association between the genotypes and drug response (supplementary Table A1).

Table 3 shows results of the logistic regression analysis, which again suggest that neither the 420del nor the R61C variant has a significant effect on metformin response. However, when the treatment-naive group was analyzed alone, the R61C genotype, but not the 420del, was associated with metformin response (odds ratio 1.56; $P = 0.036$), which suggests that carriers of the loss-of-function C-allele have a greater likelihood of treatment success.

To further investigate whether the two variants have any cumulative effect, we combined the genotypes of the two

TABLE 2
Linear regression model of maximum A1C reduction

	420del		R61C	
	Coefficient	*P*	Coefficient	*P*
Genotype	0.034	0.470	0.007	0.919
Baseline A1C	0.743	<0.0001	0.743	<0.0001
Creatinine clearance	−0.175	0.025	−0.175	0.025
Average dose	−0.004	0.595	−0.004	0.582
Adherence	0.081	<0.0001	0.081	<0.0001
Group	−0.314	<0.0001	−0.315	<0.0001

The coefficients are for the average dose per 100 mg, for creatinine clearance per 100 ml/min, and for adherence per 10% change. The genotypes were coded as the dosage of mutant alleles.

TABLE 3
Logistic regression model of treatment to A1C target

	420del		R61C	
	OR	*P*	OR	*P*
Genotype	0.978	0.8307	1.163	0.3554
Baseline A1C	0.748	<0.0001	0.747	<0.0001
Creatinine clearance	0.524	0.0003	0.523	0.0002
Average dose	0.979	0.1726	0.979	0.1690
Adherence	1.134	0.0006	1.134	0.0006
Group	0.448	<0.0001	0.448	<0.0001

The coefficients are for the average dose per 100 mg, for creatinine clearance per 100 ml/min, and for adherence per 10% change. The genotypes were coded as the dosage of mutant alleles. OR, odds ratio.

variants to make a compound genotype in an additive manner (0, no mutant allele at either site; 1, one mutant allele at either site; and 2, a sum of two or more mutant alleles at the two sites). When the compound genotypes were tested in the above models, no significant results were observed (data not shown).

In the unbalanced repeated-measurement analysis of the A1C assessed between 6 and 42 months, a clear A1C increase with time is seen ($P < 0.0001$), as shown in supplementary Table A2. Neither the R61C nor the 420del variant had a significant genotypic effect on the mean A1C level ($P = 0.75$ and $P = 0.44$, respectively). The genotype-by-time interaction term in the model also reveals that the two variants had no significant effect on the rate of A1C deterioration ($P = 0.38$ and $P = 0.82$, respectively).

Figure 2 is the Kaplan-Meier plot of the proportions of patients with metformin monotherapy failure by genotype. The Cox regression model shown in supplementary Table A3 confirms that neither the 420del nor the R61C variant has a genotypic effect on time to monotherapy failure (hazard ratio 0.98 [95% CI 0.82–1.18], $P = 0.85$, and 1.08 [0.83–1.43], $P = 0.56$, respectively). The two variants also did not have any genotypic effect on the time to the composite event of monotherapy failure or dose threshold (supplementary Table A3).

DISCUSSION

In this study, we have defined the response to metformin in 1,531 patients with type 2 diabetes. Contrary to our hypothesis, we show that two established loss-of-function variants in *SLC22A1*, encoding OCT1, did not impair initial glycemic response to metformin, the mid-term A1C control, or the rate of metformin monotherapy failure.

In our models, the A1C reduction is primarily determined by the baseline A1C. In addition, metformin response is increased with enhanced adherence and reduced creatinine clearance, consistent with the renal clearance of metformin. These covariates would act to increase availability of metformin, and this demonstrates the utility of these models in detecting factors such as genotypes that might alter drug availability. The nonsignificant but negative correlation of the dose with response is likely to reflect a higher dose given to those who seem less likely to respond. We show no genotypic effect of the two *SLC22A1* variants tested in any model. Our study was adequately powered to detect a clinically useful difference in metformin response (QUANTO, http://hydra.usc.edu/GxE/). Assuming an additive model for the 420del genotype (minor allele frequency [MAF] 0.20) and 1,500 subjects, the linear regression analysis had a 90% power ($\alpha = 0.05$) to detect a difference in A1C reduction of 0.15% per copy of the variant allele. For the R61C genotype (MAF 0.07), the analysis had an 80% power to detect a difference in A1C reduction of 0.2% per copy of the variant allele.

Our study design is observational, and the results could be prone to bias. In particular, the decision to commence metformin and the rate of dose titration were determined by the patients and their physicians. We had no direct measure of intolerance. However, we have shown that there is no variation in genotype frequency in those who did not achieve 6 months of metformin prescriptions compared with those who were included in the final cohorts; thus, any potential bias due to drug withdrawal is minimal. A further limitation is the fact that we are unable to measure serum metformin concentrations or to look at change in other measures such as insulin sensitivity. However, we believe that the study sample size of 1,531

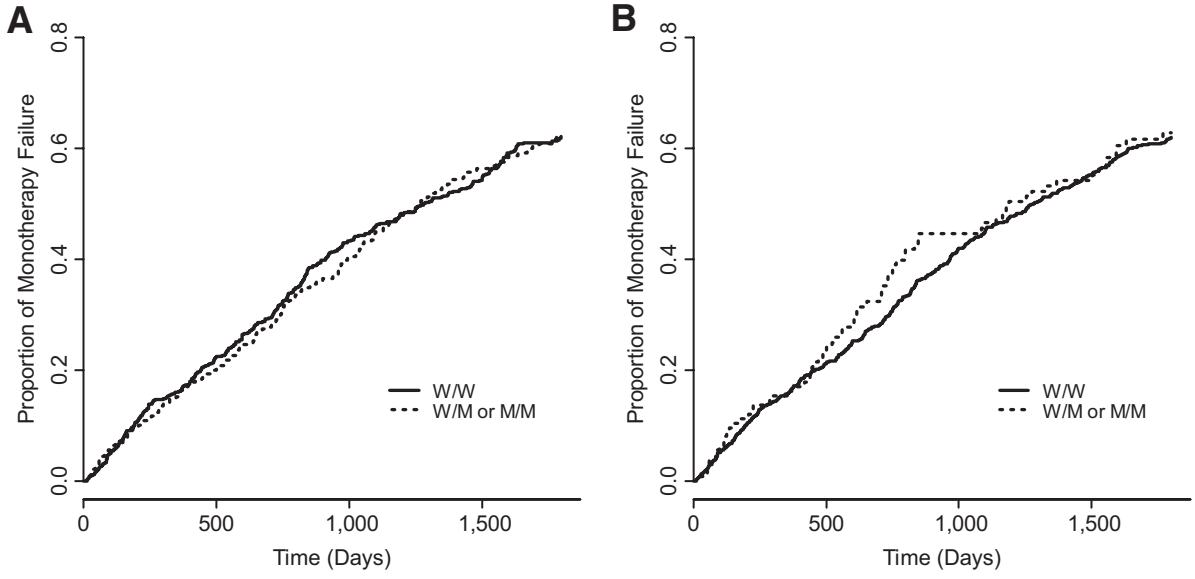

FIG. 2. Kaplan-Meier plots showing the proportions of metformin monotherapy failure by genotypes of *SLC22A1* variants 420del (*A*) and R61C (*B*). The solid lines represent wild-type homozygote genotype (W/W), and the dotted lines represent loss-of-function allele carrier (W/M or M/M).

patients provides adequate power to see a true genotypic effect, and we have used a similar approach successfully to show that *TCF7L2* variants affect sulfonylurea response (13).

In the treatment-naive group, our logistic regression found an increase of treatment success in the C-allele carriers of an R61C variant, though only of nominal significance without multiple test correction. It is worth noting that those C-allele carriers also showed a consistent trend to better metformin response in the other A1C outcome models: higher A1C reduction in the linear regression model and lower mean A1C outcome from the repeated-measurement analysis. This effect is in the direction opposite that in the findings of Shu et al. and, although not definitive, would certainly suggest that our inability to show loss of metformin response does not simply reflect lack of power.

In mice, Slc22a1 was shown to be an important transporter of metformin into the liver and, to a lesser extent, into the small intestine (7,8). Subsequently, compared with wild type, mice lacking Slc22a1 have reduced lactic acidosis (14) and loss of glucose reduction (10) when treated with metformin. Shu et al. (10) have also identified a number of rare and relatively common *SLC22A1* polymorphisms in humans and demonstrated that 12 human, glucose tolerant carriers of these variants showed a reduced efficacy of metformin in lowering glucose excursions in an oral glucose tolerance test compared with that in eight control subjects. It is interesting that in a subsequent pharmacokinetic study, the serum metformin concentrations are increased in these same individuals (15), which in mice is explained by decreased hepatic clearance. In contrast to the findings of the study by Shu et al., in a small study of patients with type 2 diabetes defined by response or nonresponse to metformin, variation in *SLC22A1* was not consistently associated with response (16).

The difference between our study and that of Shu et al. could be explained by key differences in the sample ascertainment and response models. We have studied patients with type 2 diabetes and in a real-world setting assessed A1C reduction in association with prolonged oral metformin use; this compares with a more controlled study in a few subjects with normal glucose tolerance who are given just two doses of oral metformin and assessed by response to oral glucose. It is generally accepted that metformin primarily acts to suppress hepatic glucose output (17) and, therefore, that fasting glucose may be a better measure of efficacy than the dynamic response to oral glucose used by Shu et al. There is an ongoing controversy over the pharmacological mechanism of metformin. Metformin has been shown to increase non–insulin-mediated glucose clearance, and this effect is responsible for as much glucose reduction as the effect on hepatic glucose production (17). Furthermore, metformin has also been shown to augment insulin-mediated glucose uptake into the periphery (18) and decrease glucose absorption from the gut (19). Given that carriers of the loss-of-function *SLC22A1* variants had increased serum metformin concentrations (15), this may augment the nonhepatic actions of metformin—an effect that might be more apparent in patients with diabetes compared with control subjects. This is an area where further study is required.

Although in this observational study there is no apparent reduction in the ability of metformin to lower A1C in patients with type 2 diabetes carrying the R61C or 420del loss-of-function polymorphisms in *SLC22A1*, this does not rule out an effect of *SLC22A1* variation on metformin response. A more complete investigation of *SLC22A1* variants would be required to fully assess the effect of the gene on metformin response given that variants with a more severe loss of function have been described (20). However, we have studied the two most common loss-of-function variants described in Europeans, and any additional known or unknown variants would be very rare. The observational pharmacogenetic approach that we have used in this study requires a large sample size, and the study of rarer loss-of-function polymorphisms would lack power in our current dataset. A more carefully controlled, intensively phenotyped prospective study of metformin response ideally selected by *SLC22A1* genotype will have less variation in the response phenotype and, hence, would be better powered to detect subtle effects of *SLC22A1* variation on metformin response.

In conclusion, we have shown no clinically evident reduction in the ability of metformin to lower A1C in patients with type 2 diabetes with two common loss-of-function polymorphisms in *SLC22A1*. A number of other OCTs have recently been implicated in the transport of metformin: *SLC22A2 (OCT2)* (9,21), *MATE* (22), and *PMAT* (23). These are clearly of interest in the regulation of metformin response; in particular, *SLC22A2* is involved in renal excretion of metformin and has been shown to have a greater affinity for metformin than *SLC22A1* (9). In addition to a further study of *SLC22A1*, a detailed pharmacogenetic study of these metformin transporters is also required.

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