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

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OBJECTIVE—Metformin, an oral glucose-lowering drug, is taken up in hepatocytes by the organic cation transporter (OCT) 1 and in renal epithelium by OCT2. In these cells, the multidrug and toxin extrusion (MATE) 1 protein, encoded by the *SLC47A1* gene, is responsible for the excretion of metformin into the bile and urine, respectively. We studied the effect of single nucleotide polymorphisms (SNPs) in the *SLC47A1* gene on the A1C-lowering effect of metformin.

RESEARCH DESIGN AND METHODS—We identified all incident metformin users in the Rotterdam Study, a population-based cohort study. Associations between 12 tagging SNPs in the *SLC47A1* gene and change in A1C level were analyzed.

RESULTS—One hundred and sixteen incident metformin users were included in the study sample. The rs2289669 G>A SNP was significantly associated with metformin response. For the other SNPs, no associations were found. For each minor A allele at rs2289669, the A1C reduction was 0.30% (95% CI −0.51 to −0.10; $P = 0.005$) larger. After Bonferroni correction for multiple testing, the P value was 0.045.

CONCLUSIONS—The rs2289669 G>A SNP is associated with a reduction in A1C level, consistent with a reduction in MATE1 transporter activity. These results suggest that the transporter MATE1, encoded by *SLC47A1*, may have an important role in the pharmacokinetics of metformin, although replication is necessary. ***Diabetes*** **58:745–749, 2009**

Metformin is an oral glucose-lowering drug, widely used for the treatment of type 2 diabetes (1). The molecular mechanism of the glucose-lowering effect is not fully understood, although it is known that inhibition of the hepatic gluconeogenesis has an important role (2). Metformin is mainly eliminated by tubular secretion, and hepatic metabolism has a minor role.

Several drug transporters are involved in the distribution and excretion of metformin (3). The role of two organic cation transporters (OCTs), OCT1 and OCT2, is assumed. OCT1 and OCT2 are members of the solute carrier (SLC) 22 family and encoded by the *SLC22A1* and *SLC22A2* genes, respectively, with gene location 6q25.3. OCT1 is expressed in the basolateral membrane of hepatocytes, and the uptake of metformin in the hepatocytes by OCT1 is an essential step for the glucose-lowering effect (4–6). In OCT1 gene knockout mice, the metformin liver concentrations were lower and the glucose-lowering effect impaired (4,7). Genetic variations in the *SLC22A1* gene (R61C, G401S, M420del, and G465R) are associated with differences in metformin plasma levels and glucose concentrations after an oral glucose tolerance test in healthy volunteers (4,7). OCT2 is expressed in the basolateral membrane of the renal epithelium, and transportation of metformin over this membrane may be the first step to tubular secretion (8,9). Genetic variations in *SLC22A2* (T199I, T201M, and A270S) are associated with decreased renal excretion and increased plasma concentrations of metformin (10,11).

Recently, a multidrug and toxin extrusion (MATE) transporter protein family was identified, assigned as the SLC 47 family (12,13). The *SLC47A1* gene, with gene location 17p11.2, encodes the MATE1 transporter. Metformin is one of the substrates of this transporter (14). MATE1 is located in the bile canalicular membrane in the hepatocyte and in the brush border of the renal epithelium and is responsible for the final step of metformin excretion through the bile and urine (12). Another transporter in this family is MATE2-K, encoded by *SLC47A2*. MATE2-K is located in the brush border of the renal epithelium and may also be involved in metformin excretion (14).

The colocalization of OCT1 and MATE1 in the hepatocyte and OCT2 and MATE1 in the renal epithelium suggests that MATE1 may have an important influence on the pharmacokinetics of metformin. The intrahepatic uptake of metformin by OCT1 is an essential step in the glucose-lowering effect, while the excretion out of the hepatocyte into the bile by MATE1 probably averts this. The uptake in the renal epithelium by OCT2 and subsequent excretion by MATE1 are two consecutive steps in the tubular secretion of metformin.

Little is known about the effect of genetic variation in the *SLC47A1* gene on the glucose-lowering effect of metformin. In this prospective, population-based cohort study,

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we assessed the association between tagging single nucleotide polymorphisms (SNPs) in the *SLC47A1* gene and metformin response in Caucasian incident metformin users.

RESEARCH DESIGN AND METHODS

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study of 7,983 Caucasians aged ≥55 years in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus Medical Center. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, endocrine, and ophthalmologic diseases. The rationale, ethical approval, and design of this study have been described before (15,16). Seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from 1 January 1991 until 1 January 2008 was available and included the product name of the drug, the anatomical therapeutical chemical code, the amount dispensed, the prescribed dosage regimen, and the date of dispensing (17).

For this study, we used the A1C assessments from the Stichting Trombosedienst en Artsenlaboratorium Rijnmond–Medisch Diagnostisch Centrum (STAR-MDC), which performs all outpatient laboratory assessments for general practitioners in the Rijnmond area of Rotterdam. Hereby, we obtained all outpatient A1C assessments from all participants between 1 April 1997, the time at which a new computer system was introduced at STAR-MDC, and 1 January 2008. The A1C levels were measured by high-performance liquid chromatography on a BiaRad Variant and from October 2004 onwards on a Menarini HA8160, according to professional standards and quality. The STAR-MDC is a CCKL-certified laboratory, and the quality is continuously monitored by internal and external quality-assurance programs.

All participants in the Rotterdam Study, who were incident metformin users in the period between 1 April 1997 and 1 January 2008, were included in this analysis. Incident metformin use was defined as a first-dispensed prescription for metformin in the database, which included all prescriptions from 1 January 1991 onwards. The study sample consisted of all incident metformin users who had a measurement of A1C both in the period of 90 days before the first prescription of metformin and in the period between 30 and 120 days following the first prescription of metformin. Patients who discontinued metformin therapy before the first measurement after 30 days were excluded. We also excluded patients who were coprescribed acarbose, rosiglitazone, pioglitazone, or insulin at the time of one of the two A1C measurements because defined daily doses for these drugs are not similar and these patients most likely differ in their severity of disease. Patients using sulfonylurea were not excluded.

Outcomes. The aim of antihyperglycemic therapy is to reduce plasma glucose levels. The A1C level is the percentage of hemoglobin in the blood that is glycosylated and represents the average glucose level in the preceding period of time. Since the A1C level is a more stable measurement of glycemic control than plasma glucose levels, A1C levels are used more frequently for long-term therapeutic purposes. We analyzed the association between genetic variation in the *SLC47A1* gene and difference in A1C level between the last A1C measurement before start of metformin therapy and the first A1C measurement after 30 days of metformin therapy. The target level for diabetic patients is an A1C level <7% (18).

Cofactors. Characteristics considered as potential determinants affecting the change in A1C level were age, sex, the A1C level at the last measurement before start of metformin, the daily prescribed dose of metformin at the time of the first measurement after start, the change in daily prescribed dose of sulfonylurea, the time from diabetes diagnosis to start of metformin therapy, and the estimated glomerular filtration rate. To make the prescribed doses of different sulfonylurea comparable with each other, we divided the prescribed daily dose by the defined daily dose (17). The defined daily dose is a standardized dosing measure representing the recommended daily dose for the main indication in an adult. For the diabetes diagnosis, the World Health Organization definition was used (19). If patients were diagnosed with diabetes before entrance in the Rotterdam Study, the date of entrance was used. The estimated glomerular filtration rate was estimated from the serum creatinine level at baseline with the Cockcroft-Gault formula.

Genotyping. Participants were genotyped using the Illumina 550k SNP array according to the manufacturer's instruction. Quality controls and results of the genotyping were previously described (20). The tagging SNPs on the array were selected using an algorithm with which, in a Caucasian population, 90% of all phase I and II Hapmap SNPs are covered by at least one SNP on the array (21–23). This coverage arises because genetic variation is transmitted in blocks, in which haplotype alleles exist. Within these haplotypes, variant alleles are associated with each other. This more frequent occurrence of combinations of variant alleles than would be expected from a random formation is called linkage disequilibrium. For this study, we selected the tagging SNPs in the *SLC47A1* gene, including the tagging SNPs within 10 kilobasepairs (kbp) of the gene that were on the array.

Statistical analysis. Deviations from Hardy-Weinberg equilibrium and differences in genotypes between patients who continued and discontinued metformin therapy were analyzed using χ^2 tests. We used one-way ANOVA to test for differences in average time between the last A1C measurement and start of metformin therapy and in the average time between metformin start and the first A1C measurement after start. Linear regression was used to analyze differences in A1C change between genotypes. For each polymorphism, we calculated the association between the number of variant alleles and the difference in A1C change. We adjusted for multiple testing with the Bonferroni correction, multiplying the *P* value by the number of independent tests. Two or more SNPs that were in strong linkage disequilibrium ($r^2 > 0.80$) were counted as one independent test. For the associations that were statistically significant after Bonferroni correction, we calculated separately the difference between patients with one variant allele and those with the wild-type genotype and the difference between patients with two variant alleles and those with the wild-type genotype. The analyses were performed with SPSS software (version 11.0.1; SPSS, Chicago, IL).

TABLE 1
Baseline characteristics of the study population (n = 116)

Sex	
Male	47 (41)
Female	69 (59)
Age (years)	76.8 ± 6.7
A1C level (%)*	8.3 ± 1.2
BMI (kg/m^2)†‡	28.3 ± 3.7
Creatinine level (μmol/l)†§	82.5 ± 14.4
Sulfonylurea use*	
Glibenclamide	17 (14.7)
Tolbutamide	31 (26.7)
Gliclazide	7 (6.0)
Glimepiride	17 (14.7)

Data are means ± SD or n (%). *At the time of the last A1C measurement before start of metformin therapy. †At the time of entrance in the Rotterdam Study. ‡n = 114. §n = 88.

RESULTS

One hundred and eighty-one participants of the Rotterdam Study were incident metformin users between 1 April 1997 and 1 January 2008 and had an A1C measurement both in the period of 90 days before start and in the period between 30 and 120 days after the start of metformin therapy. Seven patients were excluded because they were prescribed insulin at the time of one of the A1C measurements, and six patients were excluded because they were prescribed acarbose (n = 1), rosiglitazone (n = 3), or pioglitazone (n = 2). Blood samples for genotyping were not available for 34 patients, and 18 patients discontinued metformin therapy before the first A1C measurement in the period between 30 and 120 days after start. Eventually, we included 116 incident metformin users in the analysis, for whom the change in A1C levels was available (Table 1). The average initial starting dose was (means ± SD) 648 ± 310 mg metformin. At the time of the first A1C measurement after start, the participants were prescribed, on average, 741 ± 358 mg metformin.

The average time from the last A1C measurement before start and start of metformin therapy was 12 ± 16 days and the average time from start of metformin therapy to the first measurement after start was 66 ± 25 days. These times did not differ significantly between genotypes. The average A1C level before start of metformin therapy was

TABLE 2
Genotyped polymorphisms in the *SLC47A1* gene*

SNP		AA	Aa	aa	Minor allele frequency	Hardy-Weinberg equilibrium (*P*)
rs894680	G>A	43	58	15	0.38	0.51
rs2018675	C>T	43	57	16	0.38	0.67
rs2440154	G>A	50	52	14	0.34	0.93
rs2440155	T>C	77	35	4	0.19	0.99
rs16960201	—	116	0	0	0	—
rs2453568	C>T	58	45	13	0.31	0.35
rs2244280	G>A	73	36	7	0.22	0.38
rs2289669	G>A	36	58	21	0.43	0.78
rs1961669	A>G	79	32	4	0.17	0.73
rs2453594	T>C	73	36	7	0.22	0.38
rs2453589	A>G	41	56	19	0.38	0.91
rs2165894	A>G	68	39	9	0.25	0.32

*Genotyping failed in some participants. Therefore, not all numbers add up to 116. A, variant allele with the major allele frequency; a, with minor allele frequency.

8.3 ± 1.2% and decreased to 7.7 ± 1.1% after start of metformin therapy.

We identified nine tagging SNPs in the *SLC47A1* gene and three tagging SNPs (rs2453594, rs2453589, and rs2165894) in the 10-kbp-downstream region (Table 2). There were no tagging SNPs in the 10-kbp-upstream region. For the SNP rs16960201, no genetic variation was found in the study population. The SNPs rs2441054 and rs2453568 ($r^2 = 0.84$, $D' = 0.97$) and the SNPs rs2441055 and 1961669 ($r^2 = 0.85$, $D' = 0.96$) were in linkage disequilibrium. For the other SNPs, no linkage disequilibrium was found ($r^2 < 0.8$). The genotype distributions of the eleven tagging SNPs were in Hardy-Weinberg equilibrium. In the Caucasian sample of Hapmap, 11 tagging SNPs cover 25 of 32 (78%) Hapmap SNPs ($r^2 > 0.80$) in the selected gene region (22).

The SNP rs2289669 G>A, with a minor allele frequency of 0.43, was significantly associated with a decrease in A1C level after start of metformin therapy (Table 3). For each minor A allele, the decrease in A1C level was 0.30% (95% CI −0.51 to −0.10; $P = 0.005$) more (Table 4). For the other tagging SNPs, no significant associations were found. After Bonferroni correction for multiple testing, this association remained significant ($P = 0.045$).

The rs2289669 genotype distributions did not differ significantly between patients who continued metformin therapy and those who discontinued at the time of the A1C measurement after start ($\chi^2 = 1.61$, $P = 0.45$). There was a trend that the decrease in dose of coprescribed sulfonylurea was larger in patients with the AA genotype than in patients with the GG genotype (Table 5), although this association was not significant ($P = 0.08$).

DISCUSSION

This population-based cohort study in diabetic patients is the first one in which the role of MATE1 in the glucose-lowering effect of metformin was assessed. We identified that the SNP rs2289669 was associated with the A1C-lowering effect of metformin. The decrease in A1C level was 0.3% larger per copy of the A allele. These results suggest that polymorphisms in MATE1 may have a role in the pharmacokinetics of metformin and, accordingly, with the glucose-lowering effect. As metformin is recommended as first-line treatment for type 2 diabetes, these results may be valuable for daily clinical practice (18).

TABLE 3
Difference in change of A1C after start of metformin therapy per genotype

SNP	Adjusted difference in A1C change (%)*	*P*	*P* after Bonferroni correction†
rs894680	−0.15	0.19	1.00
rs2018675	0.029	0.80	1.00
rs2440154	0.11	0.35	1.00
rs2440155	0.23	0.10	0.90
rs16960201	—	—	—
rs2453568	0.09	0.42	1.00
rs2244280	0.23	0.062	0.56
rs2289669	−0.30	0.005	0.045
rs1961669	0.16	0.27	1.00
rs2453594	0.26	0.036	0.32
rs2453589	0.12	0.28	1.00
rs2165894	0.28	0.019	0.17

*Additive model (number of variant allele and dose effect), adjusted for age, sex, A1C level before start, prescribed dose of metformin, change in prescribed doses of sulfonylurea, time from diagnosis of diabetes to start of metformin therapy, and estimated glomerular filtration rate. †We corrected for nine independent tests because one tagging SNP had no genetic variation and 2 × 2 tagging SNPs were in linkage disequilibrium.

The average prescribed daily dose of metformin at the time of the first A1C measurement after start was 741 mg. The guidelines recommend an initial daily dose of 1,500–2,000 mg, and this dose may be increased after 10–15 days to at most 3,000 mg a day. The reason for the low doses of metformin used in this study may be that the average age of the study population is 77 years, and physicians are prudent to prescribe high doses of metformin in this elderly population because of potential adverse effects. The average decrease in A1C level (0.6%) is less than what would be expected when recommended doses are prescribed, and this may explain why the decrease in A1C level in patients with the GG genotype was near zero and did not differ significantly from zero.

A reduced efflux of metformin in the renal brush border due to an impaired MATE1 transporter will lead to an increase in metformin plasma levels and possibly to a larger decrease in glucose levels. Similarly, a reduced efflux from the hepatocyte will lead to higher metformin levels in the hepatocyte and a stronger inhibition of the gluconeogenesis, resulting in lower glucose levels. The

TABLE 4
Difference in change of A1C after start of metformin therapy for polymorphism rs2289669

rs2289669	*n**	Unadjusted average change in A1C (%)	Adjusted difference in A1C change (%)†	95% CI	*P*
GG	36	−0.28	ref.		
GA	58	−0.59	−0.32	−0.65 to 0.01	0.055
AA	21	−0.87	−0.66	−1.19 to −0.14	0.015
Additive model‡			−0.30	−0.51 to −0.10	0.005

*In one participant, genotyping for rs2289669 failed.†Adjusted for age, sex, A1C level before start, prescribed dose of metformin, change in prescribed doses of sulfonylurea, time from diagnosis of diabetes to start of metformin therapy, and estimated glomrular filtration rate. ‡Number of variant alleles.

TABLE 5
Cofactors by the rs2289669 polymorphism

	GG	GA	AA
Sex (male)	18 (50)	22 (38)	7 (33)
Age (years)	75.3 ± 7.0	77.9 ± 6.5	75.6 ± 6.1
A1C level before start (%)	8.3 ± 0.9	8.3 ± 1.4	8.4 ± 1.1
Prescribed metformin dose (mg)	853 ± 476	662 ± 262	757 ± 320
Sulfonylurea use (%)	22 (61)	33 (57)	13 (62)
Change in sulfonylurea dose (DDD)*	−0.01 ± 0.53	−0.17 ± 0.61	−0.27 ± 0.52
Time from diabetes diagnosis (years)	5.5 ± 4.4	5.6 ± 4.8	4.7 ± 3.7
Estimated glomerular filtration rate (ml/min)	74 ± 19	68 ± 17	68 ± 14
BMI (kg/m^2)	28.9 ± 3.9	28.1 ± 3.8	27.6 ± 3.2

Data are means ± SD or *n* (%). *P = 0.08 for trend. DDD, defined daily dose.

rs2289669 G>A polymorphism was associated with an increased glucose-lowering effect, implying that the gene with the A allele encodes a MATE1 efflux transporter less effective in transporting metformin. This SNP is located in an intron, and the SNP does not code for an amino acid change. Most likely, the SNP rs2289669 is in linkage disequilibrium with a SNP causing the reduced MATE1 functioning, although we cannot exclude that it has a direct effect, e.g., by affecting gene expression.

One previous study assessed the effect of a SNP in the *SLC47A1* gene on MATE1 expression (24). The authors identified a SNP in the promoter region (G-32A) that downregulates the basal promoter activity. Whether this SNP affects metformin efflux is unknown. Four glutamate amino acids in MATE1 were found to have an important role in substrate recognition, although genetic variation in the nucleotides encoding these amino acids has not been described (25).

In population-based studies, bias may affect the obtained results. At the time of the first A1C measurement after start, there was a trend toward lower doses of coprescribed sulfonylurea in patients with the AA genotype. This is in line with the results of our study. The glucose-lowering effect of metformin was stronger in patients with the AA genotype, and these patients require less antidiabetes drugs to reach their target levels. In our analyses, we adjusted for these changes in prescribed doses of sulfonylurea. The A1C measurements in this study were done in regular clinical practice. If discontinuation of metformin therapy and measurement of A1C levels were dependent on the genotype, bias might have occurred. However, no differences in genotype frequency were found for rs2289669 between patients who continued metformin until the first A1C measurement and patients who discontinued. Bias may also have occurred if there were differences in frequency of A1C level measurements. However, the time from start of metformin therapy until the first A1C measurement did not differ between genotypes, and neither the prescribing physician nor the patient was aware of the genetic variation in the *SLC47A1* gene. Selection bias is unlikely because we identified all incident metformin users in the Rotterdam Study and we collected information prospectively, without prior knowledge of the study hypothesis. Whether patients give permission to isolate their DNA for scientific research is most likely not associated with genetic variation in the *SLC47A1* gene, making selection bias unlikely either.

The Rotterdam Study is a population-based cohort study on chronic diseases and not primarily designed to assess the effects of metformin therapy. We identified 116 patients who started metformin treatment during follow-up. This limited sample size may result in both false-negative results and chance findings. The SNP rs2289669 was the SNP with the highest minor allele frequency. Post hoc power analyses with $\alpha = 0.00556$ (0.05 divided by nine independent tests) and $\beta = 0.8$ revealed that this sample size could identify changes in A1C levels for the other SNPs ranging from 0.44 to 0.56%, dependent on the minor allele frequency. Therefore, it is possible that we had false-negative results. We avoided chance findings by adjusting for multiple testing with the Bonferroni correction. Replication of these results in a prospective observational study or trial is necessary.

To conclude, we found an association between the SNP rs2289669 in the *SLC47A1* gene, encoding the MATE1 transporter, and the glucose-lowering effect of metformin. In incident metformin users the decrease in A1C level was 0.30% larger per copy of the A allele. These results suggest that MATE1 may have an important role in the pharmacokinetics and pharmacodynamics of metformin. This is the first epidemiological study assessing the role of MATE1 in metformin response, and replication of these results is necessary.

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