PRECISION MEDICINE

Effect of Serotonin Transporter 5-HTTLPR Polymorphism on Gastrointestinal Intolerance to Metformin: A GoDARTS Study

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

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OBJECTIVE

The mechanism causing gastrointestinal intolerance to metformin treatment is unknown. We have previously shown that reduced-function alleles of organic cation transporter 1 (OCT1) are associated with increased intolerance to metformin. Considering recent findings that serotonin reuptake transporter (SERT) might also be involved in metformin intestinal absorption, and the role of serotonin in gastrointestinal physiology, in this study we investigated the association between a common polymorphism in the SERT gene and metformin gastrointestinal intolerance.

RESEARCH DESIGN AND METHODS

We explored the effect of composite SERT 5-HTTLPR/rs25531 genotypes, L*L* (L_AL_A), L*S* (L_AL_G, L_AS), and S*S* (SS, SL_G, L_GL_G), in 1,356 fully tolerant and 164 extreme metformin-intolerant patients by using a logistic regression model, adjusted for age, sex, weight, OCT1 genotype, and concomitant use of medications known to inhibit OCT1 activity.

RESULTS

The number of low-expressing SERT S* alleles increased the odds of metformin intolerance (odds ratio [OR] 1.31 [95% CI 1.02–1.67], $P = 0.031$). Moreover, a multiplicative interaction between the OCT1 and SERT genotypes was observed ($P = 0.003$). In the analyses stratified by SERT genotype, the presence of two deficient OCT1 alleles was associated with more than a ninefold higher odds of metformin intolerance in patients carrying the L*L* genotype (OR 9.25 [95% CI 3.18–27.0], $P < 10^{-4}$); however, it showed a much smaller effect in L*S* carriers and no effect in S*S* carriers.

CONCLUSIONS

Our results indicate that the interaction between OCT1 and SERT genes might play an important role in metformin intolerance. Further studies are needed to replicate these findings and to substantiate the hypothesis that metformin gastrointestinal side effects could be related to the reduced intestinal serotonin uptake.

Metformin is a first-line antihyperglycemic agent, and the most widely used type 2 diabetes drug. It has major clinical advantages over other therapies because of its proven safety record, it does not induce hypoglycemia or weight gain, and has possible cardiovascular benefits (1). The most common adverse effect of metformin

[1]*Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[2]*Division of Molecular & Clinical Medicine, School of Medicine, University of Dundee, Dundee, Scotland, U.K.*

Corresponding author: Ewan R. Pearson, e.z.pearson@dundee.ac.uk.

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treatment is gastrointestinal (GI) upset, which occurs in ~30% of patients, limiting compliance. In 5% of patients treated with metformin, GI symptoms are intolerable and warrant the discontinuation of the drug (2). The mechanism of metformin GI side effects is not clear. Various pathophysiological hypotheses have been proposed, including metformin-induced release of serotonin in the intestinal mucosa (3), reduced absorption of bile salts (4), an increase in glucagon-like peptide-1 concentrations (5), and more recently, changes in the gut microbiome (6).

Metformin side effects might be related to a high concentration of metformin in the gut after oral administration (7). We have recently shown that reduced-function alleles of organic cation transporter (OCT) 1, as well as concomitant treatment with medications known to inhibit OCT1 activity, are risk factors for metformin intolerance in a large cohort of patients with type 2 diabetes treated with metformin (8). OCT1 is one of the several cation-selective transporters expressed in the enterocytes, which could be involved in metformin absorption (9–11). Other potentially involved transporters are OCT3 and plasma membrane monoamine transporter (PMAT). Interestingly, a recent study (11) showed that OCT1, PMAT, serotonin reuptake transporter (SERT; 5-HTT), and choline high-affinity transporter, and not OCT3, contribute to the apical uptake of metformin into Caco-2 cell monolayers and, thus, potentially to intestinal metformin absorption. The choline high-affinity transporter is not expressed in the human intestine (11), and there are no established common loss-of-function variants of PMAT. On the other hand, the expression of SERT is modulated by genetic variants, most notably the serotonin transporter-linked polymorphic region (5-HTTLPR) variant, a well-established 43-bp insertion/deletion polymorphism in the promoter region. Moreover, a recent study (12) showed that metformin can inhibit serotonin uptake by OCT1, OCT3, and SERT at concentrations that may be achieved in the human intestine after oral administration. These findings contribute to the hypothesis of serotonin-mediated GI adverse effects after metformin treatment, as metformin inhibition of serotonin uptake could result in increased GI side effects (12).

Considering that different expression or activity of SERT might contribute to high interindividual variability in GI intolerance to metformin, in this study we investigated the role of a common SERT triallelic 5-HTTLPR polymorphism in intolerance to metformin, and explored the potential interaction between SERT (*SLC6A4*) and OCT1 (*SLC22A1*) genes.

RESEARCH DESIGN AND METHODS

Study Population and Definition of Intolerance

The study population was previously described in detail (8). Briefly, the study included patients with type 2 diabetes from the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS), who were prescribed metformin for the first time in the period from 1 January 1994 to 1 June 2011. A surrogate phenotype of metformin intolerance was defined based on the discontinuation of metformin therapy within the first 6 months of treatment (immediate release [IR] form) and a switch to another oral hypoglycemic agent, including metformin slow-release forms, within 6 months of the last metformin IR prescription. Intolerant patients were compared with patients who were defined as tolerant based on treatment with ≥2,000 mg of the metformin IR form for >6 months.

Clinical cofactors, including anthropometric and biochemical parameters, metformin daily dose, and the use of OCT1-inhibiting medications were defined previously (8).

Genotyping

Genotyping of five OCT1 reduced-function variants (R61C, C88R, G401S, M420del, and G465R) and the classification of individuals based on the number of haplotypes carrying reduced-function alleles were described in our previous study (8).

The 5-HTTLPR polymorphism in a SERT gene (*SLC6A4*) is characterized by long (L) and short (S) alleles. The S allele has been associated with lower SERT expression and function (13). A single nucleotide polymorphism (SNP), rs25531 A > G, located within this region, further modulates SERT expression, with L_A carriers having higher SERT expression, and L_G carriers having lower SERT expression, similar to that in S allele carriers (14). In this study, we predicted the 5-HTTLPR polymorphism based on published machine learning method of vertex discriminant analysis validated for Northern European populations (15). This method uses eight variants in partial linkage disequilibrium with 5-HTTLPR to predict three genotypes, LL, SL, and SS (15). Seven of eight SNPs, and rs25531, were imputed from existing genome-wide data on 7,319 GoDARTS participants using the 1,000 genome reference panel and the software IMPUTE2. The imputation quality information values were between 0.88 and 1.00. All SNPs were in line with the Hardy-Weinberg equilibrium ($P > 0.05$). Considering that the L_G allele has the same expression as the S allele, the triallelic 5-HTTLPR genotypes were coded as L*L* (L_AL_A), L*S*(L_AL_G, L_AS), and S*S* (SS, SL_G, L_GL_G).

Statistical Analysis

Differences in quantitative variables between two groups were compared using a *t* test or Mann-Whitney *U* test, depending on the distribution normality, and categorical variables were compared using a χ^2 test. For testing the significance of the additive genetic model, groups of quantitative variables were compared using ANOVA for trend or Jonckheere's trend test, depending on the distribution normality, and categorical variables were compared using the Cochran-Armitage trend test. The logistic regression model was used to analyze the association of genotypes with metformin intolerance, with age, sex, weight, and the concomitant use of OCT1-inhibiting medications as covariates (8). On the basis of the findings of our previous study, the effect of two deficient OCT1 alleles was assessed (recessive model) (8), and for the triallelic 5-HTTLPR polymorphism, an additive genetic model was used. The multiplicative interaction was assessed by adding an interaction term to the regression model. Statistical analyses were conducted using SAS version 9.3 software (SAS Institute Inc., Cary, NC), and the statistical significance level was set at $P < 0.05$.

RESULTS

A total of 1,356 tolerant patients and 164 intolerant patients with available OCT1 and SERT genotype data were included in the study (Table 1). Patients differed in baseline characteristics, in

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

	Intolerant group (n = 164)	Tolerant group (n = 1,356)	P*
Age (years)	68.8 ± 9.7	58.4 ± 10.6	<0.001
Age at diagnosis (years)	63.5 ± 9.9	55.2 ± 10.3	<0.001
Females/males (% female)	94/70 (57.3)	545/811 (40.2)	<0.001
Weight (kg)	81.7 ± 15.5	92.1 ± 18.3	<0.001
BMI (kg/m^2)	30.4 ± 5.4	32.6 ± 6.1	<0.001
HbA_{1c}			<0.001
%	8.1 (7.7–9.2)	8.8 (7.8–9.9)	
mmol/mol	65 (61–77)	73 (62–85)	
Creatinine (μmol/L)	87.4 ± 14.4	87.2 ± 14.4	0.831
Creatinine clearance (mL/min)	74.4 (57.4–91.4)	97.7 (77.0–120.7)	<0.001
Antidiabetes drug naive	86 (52.4)	831 (61.3)	0.029
Use of OCT1-inhibiting drugs†	83 (50.6)	450 (33.2)	<0.001
Metformin daily dose (mg)	1,000 (1,000–1,000)	1,000 (1,000–1,500)	<0.001

Data are reported as the mean ± SD, median (interquartile range), or n (%), unless otherwise indicated. *P values refer to the significance of t test, Mann-Whitney U test, or a χ^2 test for data presented as the mean ± SD, median (interquartile range), or n (%), respectively. †Number of individuals concomitantly treated with OCT1-inhibiting drugs, including proton pump inhibitors, tricyclic antidepressants, citalopram, verapamil, diltiazem, doxazosin, spironolactone, clopidogrel, rosiglitazone, quinine, tramadol, and codeine.

line with our previous study (8). The OCT1 or SERT genotypes were not associated with study participants' baseline characteristics, with the exception of a lower percentage of the antidiabetes drug-naive patients in the group with two deficient OCT1 alleles compared with one or no deficient OCT1 allele carriers (Supplementary Table 1, P = 0.003).

The numbers of individuals in each genotype group are shown in Supplementary Table 2. In addition to the association of the two deficient OCT1 alleles with intolerance (recessive model, P = 0.001), which is in line with our previous report (8), there was a significant difference in the SERT genotype frequencies between the intolerant and tolerant groups (additive model, P = 0.019).

In the logistic regression analysis model adjusted for the clinical covariates age, sex, and weight, the number of S* alleles was associated with higher odds of metformin intolerance (odds ratio [OR] 1.28 [95% CI 1.01–1.63], P = 0.040). This effect was greater after adding the OCT1 genotype and OCT1-inhibiting medications to the model (Table 2; OR 1.31 [95% CI 1.02–1.67], P = 0.031). Furthermore, we tested the interaction between the OCT1 and SERT genotypes. A negative multiplicative interaction was observed between the two genes (P = 0.003), which is visually presented in Fig. 1. This shows the joint effects of OCT1 and SERT genotypes compared with the reference genotype group (the combination of one or no deficient OCT1 alleles and the L*L* genotype). In the analysis stratified by SERT genotypes, the presence of two deficient OCT1 alleles was associated with a more than ninefold higher odds of metformin intolerance (OR 9.25 [95% CI 3.18–27.0], $P < 10^{-4}$) in individuals with L*L* genotype, whereas there was no significant association in L*S* carriers (OR 2.11 [95% CI 0.99–4.50], P = 0.054) and the S*S* genotype group (OR 0.45 [95% CI 0.09–2.20], P = 0.325) (Table 3). On the other hand, when patients were stratified according to the OCT1 genotypes, the number of S* alleles increased intolerance in carriers of one or no deficient OCT1 allele (OR 1.48 [95% CI 1.15–1.92], P = 0.003), but showed opposite effect in two deficient OCT1 allele carriers (OR 0.33 [95% CI 0.13–0.82], P = 0.017) (Table 3).

Table 2—Results of logistic regression model for metformin intolerance

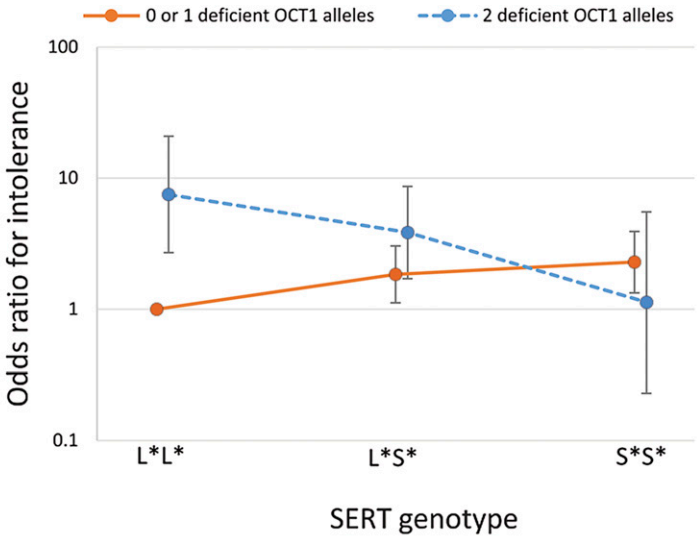
	OR (95% CI)	P
Age	1.11 (1.08–1.13)	<0.001
Sex (females vs. males)	1.82 (1.26–2.65)	0.002
Weight	0.99 (0.97–1.00)	0.031
Use of OCT1-inhibiting drugs	1.75 (1.22–2.49)	0.002
Two reduced-function OCT1 alleles	2.27 (1.31–3.92)	0.003
Number of SERT S* alleles	1.31 (1.02–1.67)	0.031

Logistic regression analysis included 164 intolerant and 1,356 tolerant patients.

CONCLUSIONS

In the first study of genetic and phenotypic determinants of metformin intolerance, we showed that variants of the highly polymorphic OCT1 gene are associated with severe intolerance leading to the discontinuation of metformin therapy (8). We hypothesized this based on the possible role of OCT1 in metformin intestinal absorption. Our later prospective study demonstrated the relationship between OCT1-deficient alleles and common GI side effects of metformin, thus replicating earlier findings and extending them also to the milder intolerance phenotype (16). The mechanism for this association, however, was unclear. On the basis of the recent findings that metformin may alter serotonin uptake by gut transporters (12), and considering the role of serotonin in GI physiology, here we focused on the effect of a common and well-established SERT 5-HTTLPR functional polymorphism on metformin intolerance.

We found that the low-expressing S* allele of the SERT gene is associated with increased intolerance to metformin, although this effect was smaller than that seen to be associated with two OCT1-deficient alleles. The 5-HTTLPR polymorphism has been extensively studied previously, and there is a possible association of 5-HTTLPR alleles with irritable bowel syndrome (17), psychiatric traits (18), and antidepressant drug response (19). Although the results of the pharmacogenetic studies have been inconsistent, evidence from reviews and meta-analyses suggest that the L allele is a predictor of better response to selective serotonin reuptake inhibitors (SSRIs) in Caucasian populations (19). On the other hand, in the meta-analysis of nine studies, the S allele was significantly



Numbers of intolerant/tolerant individuals

	SERT genotype		
OCT1 genotype	L*L*	L*S*	S*S*
0 or 1 deficient OCT1 allele	26/362	68/605	47/298
2 deficient OCT1 alleles	8/20	13/51	2/20

Figure 1—Joint effects of OCT1 and SERT genotypes on metformin intolerance. The combination one or no deficient OCT1 alleles/L*L* is used as a reference group. The numbers in each genotype group are presented for the intolerant and tolerant individuals as "intolerant/tolerant."

associated with more total adverse effects after SSRI treatment, and showed a trend of association with GI side effects induced by SSRIs (20), which is in line with our results.

In humans, serotonin is predominantly synthesized in the enterochromaffin cells of the gut mucosa. Here it mediates many GI functions, including motility, secretion, and vasodilation by activating afferent neurons in the lamina propria (21). Serotonin has been involved in the pathophysiology of a number of GI disorders, and drugs targeting serotonin receptors have been used in the treatment of GI symptoms (21). Previously, it has been shown that metformin can induce serotonin release from the intestinal mucosa, in a dose-dependent manner, without effect on 5-HT_3 receptors (3). In this study, the effect of metformin on serotonin reuptake was not explored (3). However, recent in vitro findings showed that metformin can inhibit serotonin uptake by SERT and other cation transporters (12). Thus, metformin could increase serotonin extracellular concentrations, resulting in prolonged serotonergic signaling in the intestine and increased GI side effects (12). Although in this study, metformin inhibited serotonin uptake by OCT1 more strongly than that by SERT (12), another in vitro study showed conversely that metformin is not a significant inhibitor of OCT1-mediated serotonin transport (22). Beside this, SERT has much higher expression than OCT1 in the human intestine (11,23), implying that although metformin is a weak SERT inhibitor, it could inhibit SERT at the high concentrations achieved in the gut after oral administration (24). In addition, it has been proposed that the inhibition of intestinal SERT may contribute to GI adverse effects commonly observed with SSRI treatment (25), and possibly also to the side effects of other drugs that may act as SERT inhibitors (26).

As we observed a significant interaction between OCT1 and SERT genotypes, we performed analyses stratified by each genotype. Interestingly, in the analyses stratified by SERT genotype, two OCT1-deficient alleles had a high effect in patients carrying the L*L* genotype, a much smaller effect in L*S* genotype carriers, and no effect in the S*S* genotype carriers. Furthermore, the low-expressing S* allele was associated with intolerance only in patients with one or no deficient OCT1 allele, and showed opposite direction in the carriers of two deficient OCT1 alleles. It can be hypothesized that low activity of OCT1, possibly the main intestinal transporter of metformin, results in increased metformin concentrations in the gut, which can inhibit SERT and thus cause high extracellular serotonin levels and GI intolerance. On the other hand, although the number of low-expressing SERT S* alleles was associated with intolerance per se, presumably also due to higher serotonin extracellular levels, the S* allele showed a protective effect in the presence of two low-activity OCT1 alleles. This contradictory finding possibly could be explained by desensitization of serotonin receptors, which may occur as a consequence of greatly increased interstitial serotonin concentrations, in the

Table 3—Stratified analyses according to OCT1 and SERT genotypes

	OR (95% CI)	*P*
Effect of two deficient OCT1 alleles—analysis stratified for SERT genotype		
SERT genotype		
L*L* carriers*	9.25 (3.18–27.0)	<0.0001
L*S* carriers†	2.11 (0.99–4.50)	0.054
S*S* carriers‡	0.45 (0.09–2.20)	0.325
Effect of the number of SERT S* alleles—analysis stratified for OCT1 genotype		
OCT1 genotype		
0 or 1 deficient alleles carriers§	1.48 (1.15–1.92)	0.003
2 deficient alleles carriers‖	0.33 (0.13–0.82)	0.017

Analyses were adjusted for age, sex, weight, and use of OCT1-inhibiting medications. *Thirty-four intolerant patients and 382 tolerant patients. †Eighty-one intolerant patients and 656 tolerant patients. ‡Forty-nine intolerant patients and 318 tolerant patients. §One hundred forty-one intolerant patients and 1,265 tolerant patients. ‖Twenty-three intolerant patients and 91 tolerant patients.

case of low SERT expression (27) and high SERT inhibitor concentrations (28). However, the small numbers of patients especially in some of these genotype-stratified analyses preclude drawing strong conclusions about the observed interaction. SERT is expressed at both apical and basolateral membranes of the enterocytes, with predominant apical expression (11). However, there is ambiguity around the localization of the OCT1 in the enterocytes, as it has been suggested to be located basolaterally (9,29), and conversely, in a recent study, apically (10). Thus, it is unclear whether increased mucosal or luminal metformin concentrations could contribute to the GI adverse effects. Nevertheless, the results of our study suggest a plausible hypothesis for GI intolerance of metformin, which should be explored further.

In addition to the small sizes of groups in the stratified analyses, there are several other limitations of our study that need to be acknowledged. First, we used a surrogate phenotype for metformin GI intolerance based on the discontinuation of metformin in the first months of treatment. We ensured that patients were switched to another oral hypoglycemic agent; thus, the cessation of metformin treatment was not due to improvement in glycemic control. However, there could be other reasons for stopping metformin treatment, including other side effects or other reasons not related to drug intolerance. This could result in some imprecision in the definition of phenotype categories, although GI intolerance represents the most common adverse effect of metformin treatment. Furthermore, it would be interesting to explore the effect of concomitant treatment with SSRIs on metformin intolerance, and their interaction with SERT as well as OCT1 genotypes. However, we were not able to do this due to the small number of patients who were treated with SSRIs. In addition, SSRIs could also act as OCT1 inhibitors, and citalopram has been included among the overall OCT1-inhibiting drugs. Finally, considering the relatively small size of our study, the novel findings of our study should be considered preliminary and require independent replication. Beside this, as clearly genetic studies alone cannot infer molecular mechanisms of drug effects, further in vitro and in vivo studies are needed to explore the proposed hypothesis of metformin GI intolerance.

In conclusion, our results indicate that the SERT genotype and the interaction between OCT1 and SERT genes might play an important role in GI intolerance to metformin. Further studies are needed to replicate our preliminary findings as well to substantiate the proposed interaction between metformin and serotonin disposition in the intestine, and to elucidate the exact mechanisms of GI intolerance to metformin.

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