Variation in the plasma membrane monoamine transporter (PMAT) (encoded by SLC29A4) and organic cation transporter 1 (OCT1) (encoded by SLC22A1) and gastrointestinal intolerance to metformin in type 2 diabetes: an IMI DIRECT study

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OBJECTIVE

Gastrointestinal adverse effects occur in 20–30% of patients with metformin-treated type 2 diabetes, leading to premature discontinuation in 5–10% of the cases. Gastrointestinal intolerance may reflect localized high concentrations of metformin in the gut. We hypothesized that reduced transport of metformin via the plasma membrane monoamine transporter (PMAT) and organic cation transporter 1 (OCT1) could increase the risk of severe gastrointestinal adverse effects.

RESEARCH DESIGN AND METHODS

The study included 286 severe metformin-intolerant and 1,128 metformin-tolerant individuals from the IMI DIRECT (Innovative Medicines Initiative: DIabetes REsearCh on patient straTification) consortium. We assessed the association of patient characteristics, concomitant medication, and the burden of mutations in the *SLC29A4* and *SLC22A1* genes on odds of intolerance.

RESULTS

Women ($P < 0.001$) and older people ($P < 0.001$) were more likely to develop metformin intolerance. Concomitant use of transporter-inhibiting drugs increased the odds of intolerance (odds ratio [OR] 1.72, $P < 0.001$). In an adjusted logistic regression model, the G allele at rs3889348 (*SLC29A4*) was associated with gastrointestinal intolerance (OR 1.34, $P = 0.005$). rs3889348 is the top *cis*-expression quantitative trait locus for *SLC29A4* in gut tissue where carriers of the G allele had reduced expression. Homozygous carriers of the G allele treated with transporter-inhibiting drugs had more than three times higher odds of intolerance compared with carriers of no G allele and not treated with inhibiting drugs (OR 3.23, $P < 0.001$). Use of a genetic risk score derived from rs3889348 and *SLC22A1* variants found that the odds of intolerance were more than twice as high in individuals who carry three or more risk alleles compared with those carrying none (OR 2.15, $P = 0.01$).

CONCLUSIONS

These results suggest that intestinal metformin transporters and concomitant medications play an important role in the gastrointestinal adverse effects of metformin.

Metformin therapy can cause gastrointestinal (GI) discomfort that negatively affects quality of life and adherence to prescribed medications. GI adverse effects usually manifest as nausea, vomiting, diarrhea, flatulence, indigestion, bloating, abdominal discomfort, and stomach ache and occur in 20–30% of metformin-treated subjects with type 2 diabetes, leading to premature discontinuation in 5–10% of the cases (1,2). This inhibits adherence to therapy and may lead to a change of treatment, depriving intolerant patients of effective diabetes therapy. Despite its clinical importance, the underlying pathophysiology of metformin intolerance is not yet clear. However, multiple possible hypotheses have been proposed, including high intestinal metformin concentration (3,4), its effect on the gut microbiota (5), altered transportation of serotonin or direct serotonergic effects (6), and reduced ileal absorption of bile acid salts (7).

Metformin is not metabolized and is excreted unchanged in the urine. At physiologic pH, it is hydrophilic due to the presence of a quaternary ammonium group that results in a net positive charge. Therefore, metformin does not efficiently diffuse across the biological membranes and requires carrier-mediated transport. Multiple solute carrier transporters expressed in membranes of the enterocytes, hepatocytes, and the kidney are reported to be involved in the absorption, distribution, and elimination of metformin. Metformin requires the entire length of the small intestine to be absorbed (8): ~20% of the administered dose is absorbed in the duodenum and 60% in the jejunum and ileum. The remainder reaches the colon and remains unabsorbed. Plasma membrane monoamine transporter (PMAT) and organic cation transporter 1 (OCT1) are reported to play the major role in the intestinal absorption of metformin (9). PMAT is expressed in the apical (luminal) membrane of the enterocytes, but intestinal localization of OCT1 is ambiguous (9–11). An association between reduced-function alleles in *SLC22A1* and concomitant use of OCT1-inhibiting drugs with metformin intolerance has been reported (12,13). An interaction between OCT1 and serotonin transporter (SERT) also plays an important role in the pathophysiology of metformin intolerance (13).

Although PMAT shares extensive substrate and inhibitor overlap with OCTs (14), no studies have investigated its role in metformin intolerance. We therefore hypothesized that reduced transport of metformin by major transporters of metformin, PMAT and/or OCT1, could increase intestinal metformin concentration and subsequently increase the risk of GI adverse effects. To address this, we used prescribing, biochemistry, and clinical data from 286 metformin-intolerant and 1,128 metformin-tolerant individuals from the IMI DIRECT (Innovative Medicines Initiative: DIabetes REsearCh on patient straTification) consortium (15). Although OCT3 is expressed in the intestine, no common functional variants are described, and we therefore did not include OCT3 in this analysis.

RESEARCH DESIGN AND METHODS

Study Population

We identified 286 metformin-intolerant (case) and 1,128 metformin-tolerant (control) subjects from prescribing data in the IMI DIRECT consortium from participating centers across northern Europe (15). Each participant consented to participate in the study, and ethical approval was obtained from the medical ethics committees of the respective centers.

All metformin-intolerant (case) and metformin-tolerant (control) subjects had a clinical diagnosis of type 2 diabetes, a creatinine clearance ≥60 mL/min at metformin exposure, and were white Europeans aged between 18 and 90 years at recruitment.

Definition of Metformin Intolerance

The metformin intolerance phenotype was defined in two ways: firstly, individuals who switched to an alternative agent within 6 months of stopping metformin (including modified-release metformin) after having had up to 1,000 mg daily metformin for up to 6 weeks, who also reported GI adverse effects on the metformin treatment as the reason for switching or where GI adverse effects were clearly documented in the clinical record as a reason for transfer. In an alternative definition, intolerant individuals were defined as those who could not increase their metformin immediate-release dose >500 mg daily despite an HbA_{1c} >7% (53 mmol/mol) and who reported GI adverse effects on >500 mg or where GI adverse effects were clearly documented in the clinical record as a reason for transfer.

Where the patient was asked to recall adverse effects, the intolerance event was limited to be within the last 5 years; if adverse effects were documented from clinical records, then there was no time limit. Participants who did not recall being on metformin or having adverse effects were excluded (unless clearly documented in clinical records).

Definition of Metformin Tolerance

Metformin-tolerant individuals were defined as those treated with ≥2,000 mg of metformin daily for more than a year (excluding modified-release formulations of metformin) and reported no adverse effects.

Clinical Covariates

Weight, height, and creatinine were defined as the closest measured values within 180 days before the index intolerance event (ITE), and BMI was calculated as weight in kg/height in m^2. The ITE was defined as the date when patients reported GI symptoms of metformin intolerance for case subjects, and for control subjects it was the date when patients started 2,000 mg of metformin. Daily dose was the last dose during ITE for case subjects and was determined as the mean dose of prescriptions encashed during the first 6 months of metformin therapy for control subjects.

Concomitant Medications

Gut metformin transporters have strong substrate and inhibitor overlap (16). We therefore identified medications prescribed together with metformin previously reported to inhibit the PMAT and/or OCTs, proteins that mediate transmembrane trafficking of their target molecules and are required for metformin absorption in the gut. These drugs are selected based on their reported IC_{50} values. Accordingly, the use of any of the following medications with metformin was investigated: tricyclic antidepressants (TCAs) (17,18), proton pump inhibitors (PPIs) (19), citalopram (18), verapamil (17,18), diltiazem (18), doxazosin (17,18), spironolactone (17,18), clopidogrel (20), rosiglitazone (21), quinine (18), tramadol (18,22), codeine

(23), disopyramide (24), quinidine (21), repaglinide (21), propafenone (17), ketoconazole (17), morphine (22,23), tropisetron (25), ondasetrone (25), antipsychotic agents (17), and tyrosine kinase inhibitors (26).

Genotyping

DNA samples from participants were genotyped at the University of Oxford using the Illumina HumanCoreExome-24 v1.0 BeadChip. Genotype calling was performed using the GenCall algorithm in the GenomeStudio software supplied by Illumina. Data were subjected to a series of standard quality control analyses to highlight poorly performing genetic markers and samples before imputation.

Samples were excluded for any of the following reasons: call rate <95%, heterozygosity >4 SD from the mean, high correlation to another sample (pi-hat ≥0.2), or identification as an ethnic outlier from constructed axes of genetic variation from principal components analysis implemented in Genome-wide Complex Trait Analysis (GCTA) software (v1.24.7) (27) using the 1000 Genomes as a reference. Further filtration was performed to remove nonautosomal markers, duplicate markers (sharing the same positions), markers with minor allele frequency (MAF) <1%, Hardy-Weinberg equilibrium P value <0.0001, and call rate <98%. Imputation to the 1000 Genomes Phase 3 CEU (Northern Europeans from Utah) reference panel was performed with ShapeIt (v2.r790) (28) and Impute2 (v2.3.2) (29).

Single Nucleotide Polymorphism Selection

As there are no functionally characterized common nonsynonymous single nucleotide polymorphisms (SNPs) in the *SLC29A4* gene, the tagging intronic SNPs, rs3889348 and rs2685753 (r^2 = 0.57, D' = 1), had been previously shown to be associated with trough steady-state metformin concentration (30). Therefore, the rs3889348 G>A genotype was extracted from existing genome-wide data. The frequency of the minor allele (A) of rs3889348 was 38%. Data for previously reported missense *SLC22A1* variants M420del (18.6%), R61C (7.1%), and G401S (3.1%) were also extracted from the genome-wide data. There was no deviation from Hardy-Weinberg equilibrium for any polymorphism ($P > 0.05$).

Statistical Methods

Categorical data are presented as frequency (percentage) and continuous variables as mean ± SD if normally distributed or as median and interquartile range (IQR) otherwise. The Student t test and the Mann-Whitney U test were used to compare differences in quantitative variables distributed normally or not, respectively. Comparison of categorical variables between case subjects and control subjects was done using χ^2 test. Logistic regression was used to estimate the association of independent variables with metformin intolerance. Multivariate logistic regression analyses of metformin intolerance were performed with all of the covariates included using SNPTEST (v2.5.2) (31). Association of the intronic rs3889348 G>A in *SLC29A4* was explored assuming an additive genetic model. *SLC22A1* variants M420del, R61C, and G401S were grouped together by summing the number of risk alleles. A combined unweighted genetic risk score (GRS) was generated as 0, 1, or 2 according to the number of reduced-function alleles in each individual. The combined genotype was then added to the multivariate analyses assuming an additive model. A two-tailed P value of <0.025 was considered statistically significant.

Expression Quantitative Trait Locus Analyses

We investigated whether rs3889348 is a *cis*-quantitative trait locus (QTL) in the gut using expression QTL (eQTL) data sets comprising 246 colon transverse and 122 terminal ilium samples from the Genotype-Tissue Expression (GTEx) data release v6 (32). Tissue procurement, gene expression analysis, genotyping, and eQTL analysis have been previously described (32–34).

RESULTS

Phenotypic Differences Between Tolerant and Intolerant Subjects

The characteristics of tolerant and intolerant subjects are presented in Table 1. Women ($P < 0.001$) and older people at diagnosis or at ITE ($P < 0.001$) were more likely to be metformin intolerant. Compared with tolerant subjects, metformin-intolerant individuals had lower weight ($P < 0.001$), lower creatinine clearance ($P = 0.036$), and were treated with a lower metformin dose ($P < 0.001$).

Concomitant Medications and Intolerance

This analysis was performed on 237 metformin-intolerant and 1,128 metformin-tolerant subjects who had complete data on history of concomitant medications. The analysis showed 40% of metformin-intolerant subjects were taking one or more cation transporter inhibitory drugs compared with 24% of tolerant subjects ($P < 0.0001$) (Table 1). A logistic regression model adjusted for age, sex, and weight showed concomitant use of these drugs increased the odds of being intolerant by 70% (odds ratio [OR] 1.72 [95% CI, 1.26–2.32], $P < 0.001$) (Supplementary Table 1). When the individual drug or drug groups were explored, concomitant use of metformin with PPIs, TCAs, or codeine increased the odds of metformin intolerance significantly (Fig. 1). The number of subjects who were coprescribed metformin with transporter-inhibiting drugs is reported in Supplementary Table 2.

Genetic Variation in the Gut Metformin Transporters and Metformin Intolerance

In a logistic regression model, carriers of the G allele had 1.39 (95% CI 1.15–1.69, $P < 0.001$) times higher odds of being intolerant to metformin (unadjusted). When rs3889348 was added to a model adjusted for age, sex, weight, and genetic substructure, the presence of the G allele was independently associated with metformin intolerance (OR 1.34 [1.09–1.65], $P = 0.005$) (Supplementary Table 1). No statistically significant difference in any of the baseline phenotypes by genotype was observed (Supplementary Table 3). In addition, no significant interaction between rs3889348, the use of metformin transporter-inhibiting drugs, and any of the other clinical variables (age, sex) was observed.

We then grouped subjects based on the combination of *SLC29A4* genotype and concomitant use of metformin transporter-inhibiting drugs. Taking those with no risk allele and who were not treated with transporter-inhibiting drugs as the reference group, carriers of one and two G alleles who were treated with transporter-inhibiting drugs had more than twofold (2.44 [95% CI 1.30–4.78]) and threefold (3.23 [1.71–6.39]) higher odds of intolerance, respectively,

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

Variable	Metformin tolerant (n = 1,128)	Metformin intolerant (n = 286)	P
Age at diabetes diagnosis (years)	55.88 ± 9.44	58.62 ± 10.65	<0.0001
Age at ITE (years)	60.73 ± 9.84	64.63 ± 9.91	<0.0001
Sex			<0.0001
Males	696 (61.7)	117 (40.9)	
Females	432 (38.3)	169 (59.1)	
Weight (kg)	94.57 ± 18.91	88.84 ± 17.75	<0.0001
BMI (kg/m^2)	32.11 ± 6.01	31.60 ± 5.95	0.19
Creatinine (μmol/dL)	79.89 ± 16.09	78.41 ± 19.33	0.25
Creatinine clearance (mL/min)	85.17 ± 19.36	82.23 ± 29.44	0.04
Dose at diagnosis (mg)*	1,500 (1,000–2,000)	1,000 (500–1,000)	<0.0001
Duration of diabetes (years)	4.0 (1.7–7.0)	4.0 (2.0–9.0)	0.09
Use of metformin transporter-inhibiting drugs	274 (24.29)	95 (40.08)	<0.0001

Continuous data are presented as mean ± SD or median (IQR) and categorical data as n (%). *Dose was calculated as the last dose during ITE for case subjects and was determined as the mean dose of prescriptions encashed during the first 6 months of metformin therapy for control subjects.

after adjusting for age, sex, and weight (Supplementary Table 4).

The association between *SLC22A1* genotypes and metformin intolerance has been previously reported (12,35). We analyzed the association between two reduced-function (R61C and G401S) and one loss-of-function (M420del) *SLC22A1* SNPs and metformin intolerance by using a combined unweighted GRS. In a logistic regression model adjusted for age, sex, weight, genetic substructure, and concomitant use of transporter-inhibiting drugs, the *SLC22A1* GRS was not statistically significantly associated with metformin intolerance (OR 1.35 [95% CI 0.84–2.12], P = 0.21).

A GRS was then generated from *SLC29A4* and *SLC22A1* variants by summing the number of risk alleles for each individual. Compared with those with no risk allele, metformin-treated subjects with type 2 diabetes who had two risk alleles had nearly a twofold (1.93 [95% CI 1.10–3.65]) increased odds of GI intolerance. Those who carried three or more risk alleles had more than twice (2.15 [1.20–4.12]) the odds of intolerance (Fig. 2).

Sensitivity Analysis

There was a big difference in sample size between metformin-intolerant and metformin-tolerant subjects. In addition, there were significant differences in age and sex between case subjects and control subjects. We therefore performed a sensitivity analysis by comparing the intolerant group (n = 237) with an age- and sex-matched subgroup of tolerant subjects (n = 711). The main findings from the larger metformin-tolerant group were confirmed in this sensitivity analysis (Supplementary Tables 5 and 6).

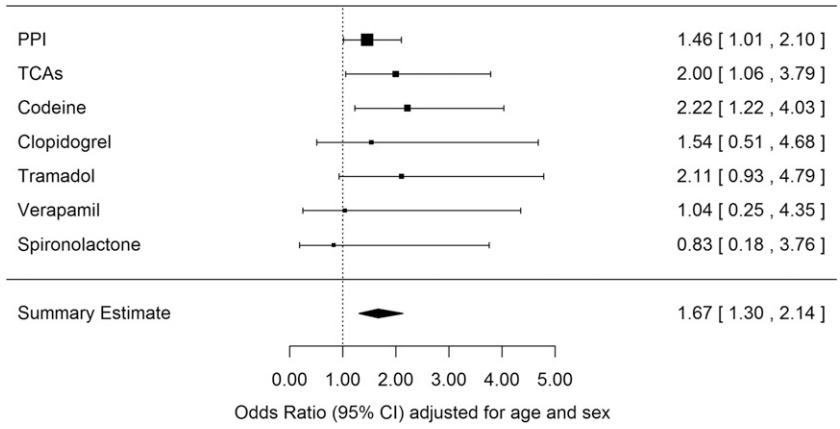

Figure 1—Association of individual intestinal metformin transporter-inhibiting drugs with intolerance.

rs3889348 Is Associated With Altered PMAT Expression in the Gut

Given PMAT is one of the major metformin transporters in the gut, we explored the possibility that the intronic SNP rs3889348 is a *cis*-eQTL in the intestine by using the publicly available data set from the GTEx portal (v6p) (32). The G allele of rs3889348 (associated with higher risk of intolerance) was significantly associated with lower expression of *SLC29A4* in the terminal ileum of the small intestine ($\beta = -0.42$, $P = 2.1 \times 10^{-04}$) and the transverse colon ($\beta = -0.45$, $P = 1.4 \times 10^{-08}$) (Supplementary Fig. 1). rs3889348 is the top *cis*-eQTL for *SLC29A4* in the transverse colon.

CONCLUSIONS

Intestinal absorption of metformin is modulated by the function of cation transporters expressed in the gut. An association between reduced-function alleles in the *SLC22A1*, encoding OCT1, and metformin-related GI adverse effects has been previously reported (12,13,36). However, the data on intestinal localization of OCT1 are ambiguous, with mixed reports suggesting in the apical (10) and basolateral (11,37) sides. In addition to OCT1, PMAT also contributes to the intestinal absorption of metformin. PMAT is abundantly expressed in the human intestine and is concentrated on the tips of the mucosal epithelial layer (38). Carriers of the G allele at this locus (rs3889348) had significantly reduced expression of *SLC29A4* in the gut (32). This could lead to higher luminal concentration of metformin. In this current

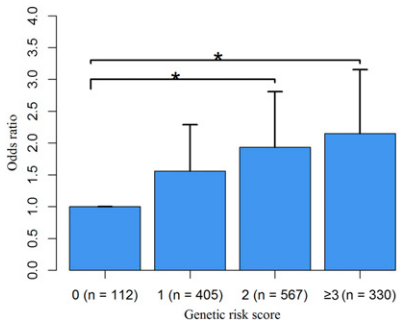

Figure 2—Association of a GRS derived from *SLC29A4* (PMAT) and *SLC22A1* (OCT1) with metformin intolerance. Bars indicate SE around the mean. $*P < 0.05$.

study, we demonstrated a significant association of the G allele of an intronic SNP, rs3889348, in *SLC29A4* encoding PMAT, with higher odds of GI intolerance after metformin therapy. Each copy of the G allele was associated with 1.34 times higher odds of metformin intolerance. We also showed that those who carried two or more variants at *SLC29A4* or *SLC22A1* were twofold more likely to have GI intolerance. Given that PMAT is apically located, this finding suggests that intolerance is driven by increased luminal concentration of metformin rather than by increased enterocyte concentration and direct toxicity to the enterocytes.

There are a number of putative mechanisms whereby increased luminal metformin may increase GI intolerance to metformin (outlined in Fig. 3). Firstly, a higher concentration of metformin in the gut has been shown to inhibit uptake of histamine and serotonin, leading to increased luminal concentration of these biogenic amines (13). Metformin also inhibits diamine oxidase, an enzyme that degrades histamine, at therapeutic doses (6). Biogenic amines play an important role in the GI pathophysiology. Elevated levels of serotonin and histamine in the GI tract cause GI symptoms such as nausea, vomiting, and diarrhea (6,39). Serotonin is produced mainly in the gut and stored in the enterochromaffin cells of the epithelium. Its release activates gut sensory neurons that will increase intestinal motility, secretion, and sensation (39,40). Increased colon motility and softening of stool consistency have also been observed in serotonin reuptake transporter (SERT) knockout mice (39,40). In addition, a recent study from the GoDARTS (Genetics of Diabetes Audit and Research in Tayside Scotland) cohort showed association of a composite SERT genotype, 5-HTTLPR (5-hydroxy tryptamine [serotonin] transporter-linked polymorphic region)/rs25531, with intolerance to metformin in subjects with type 2 diabetes (13). In this study, carriers of the low-expressing SERT S* alleles had >30% increased odds of metformin intolerance (OR 1.31 [95% CI 1.02–1.67], $P = 0.031$). Histamine is a monogenic amine stored in the enterochromaffin-like cells within the gastric

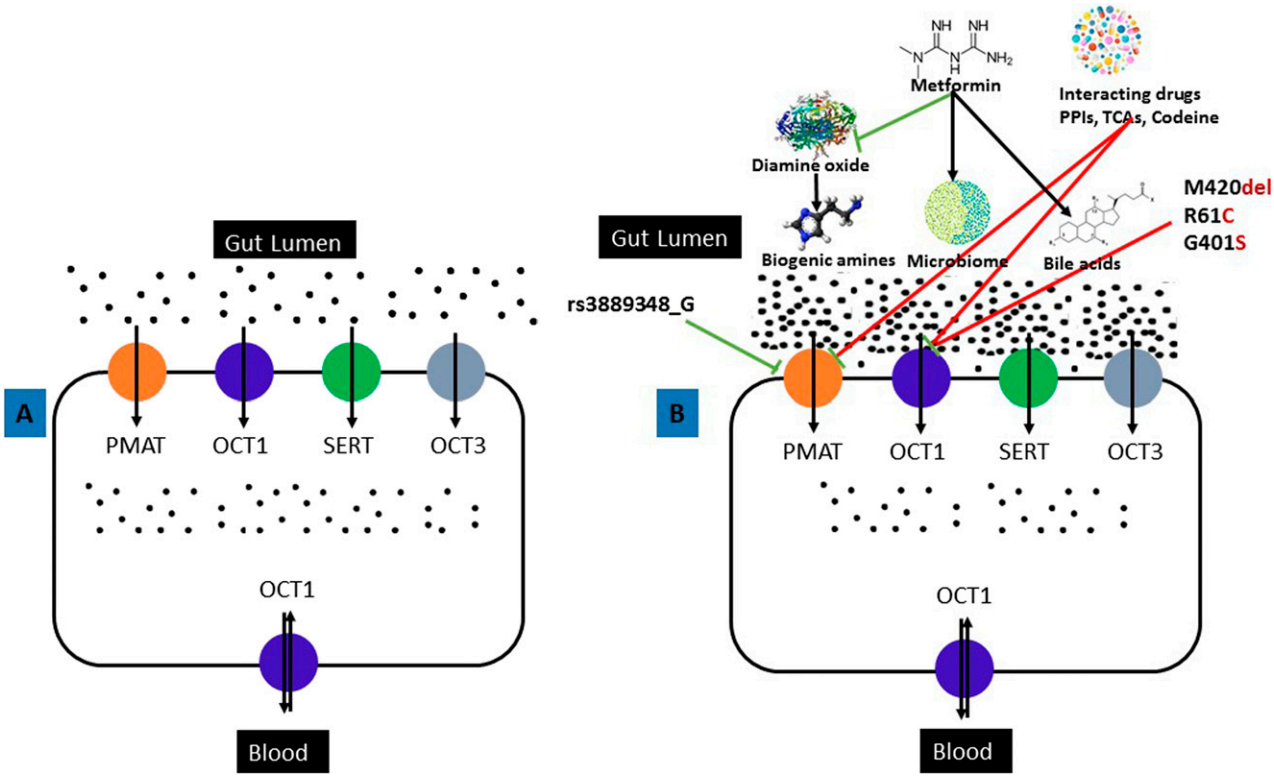

Figure 3—Possible mechanisms for metformin intolerance. *A*: Metformin is absorbed from the gut lumen via cation transporters such as PMAT, OCT1, SERT, and OCT3. *B*: Increased level of metformin in the gut lumen is observed when metformin is taken with cation transporter-inhibiting drugs such as PPIs, TCAs, and codeine. These drugs competitively inhibit metformin uptake by the cation transporters. Metformin is also shown to inhibit diamine oxide, an enzyme that metabolizes biogenic amines. In addition, transport capacity of the cation transporters could be reduced in carriers of reduced function (420del, 61C, 401S in *SLC22A1*) or low-expressing alleles (rs3889348_G in *SLC29A4*) and hence increase luminal metformin level. The increased level of metformin increases the level of biogenic amines, affects the gut microbiota, and elevates bile acid levels. These may cause symptoms of GI adverse effects.

glands of the stomach. Binding of histamine to the H1, H2, and H4 receptors that are highly expressed in the gut stimulates gastric acid secretion and increases intestinal motility and smooth muscle inflammation (6).

In addition to the potential role of local concentrations of serotonin and histamine, increased luminal concentrations of metformin could also cause intolerance by other mechanisms that need to be explored. For example, intolerance could be mediated by a reduction in bile acid reabsorption in the ileum leading to elevated bile acid levels in the colon (41), which is known to cause GI disturbances (42). In addition, metformin affects composition and function of the gut microbiota favoring the growth of some species like *Akkermansia* (5,43–46). Furthermore, increased levels of active and total glucagon-like peptide 1 levels in subjects with type 2 diabetes and without type 2 diabetes treated with metformin (47) were also reported, and this might increase GI adverse effects (48) (Fig. 3).

In this study, we observed an increased risk of intolerance with older age, female sex, lower weight, and lower creatinine levels. Concomitant use of metformin with PPIs and TCAs also increases the risk of intolerance. These findings are largely consistent with the results of previous studies, providing further evidence for clinical practice (12,35). The U.S. Food and Drug Administration Adverse Events Reporting System suggested that women experience more adverse effects than men (49). Several factors can contribute to these differences. Sex-based variability in intestinal expression of drug transporters may result in variability in drug concentrations in the gut. Women also have slower gastric emptying, altered bile composition, and slower intestinal transit time than men (50). These factors could in turn affect the rate and/or extent of absorption of oral medications and hence local drug concentrations in the gut. For a better understanding of the basic mechanisms of sex differences in metformin intolerance, future studies should be designed with a primary focus on this topic.

In summary, we have identified a variant that alters intestinal expression of the cation transporter PMAT (*SLC29A4*) that increases the risk of metformin-associated GI intolerance. Combined with the previously reported *SLC22A1* variants, this genotype profile can increase the odds of metformin intolerance more than twofold. The apical location of PMAT means that reduced expression will result in increased luminal metformin concentration, suggesting that metformin intolerance is caused by this increased luminal concentration rather than by increased enterocyte concentration.

A limitation of this study was the definition for metformin-induced GI intolerance. Even though we examined patient reports and clinical records for GI intolerance as a reason for stopping metformin and switching to other medications, there could have been other reasons for stopping metformin such as comorbidities that might cause GI disturbance. In addition, initial conclusions drawn from this study need validation and replication in well-powered independent studies.

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Author Contributions. A.Y.D. analyzed data, wrote the manuscript, and performed research. K.Z. analyzed data, designed research, and revised the manuscript. N.v.L., R.K., P.J.M.E., and S.P.R. collected data and revised the manuscript. A.M. and N.R. analyzed data and revised the manuscript. A.G.J., R.W.H., and J.C.S. designed research, collected data, and revised the manuscript. P.W.F. designed and performed research and revised the manuscript. M.I.M. designed research, analyzed data, and revised the manuscript. L.M.'tH. designed research, collected and analyzed data, and revised the manuscript. E.R.P. designed and performed research, analyzed data, and wrote and revised the manuscript. A.Y.D. and E.R.P. are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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