

University of Dundee

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

Dawed, Adem; Zhou, Kaixin; van Leeuwen, Nienke ; Mahajan, Anubha; Robertson, Neil R.; Koivula, Robert W.

Published in:
Diabetes Care

DOI:
[10.2337/dc18-2182](https://doi.org/10.2337/dc18-2182)

Publication date:
2019

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Dawed, A., Zhou, K., van Leeuwen, N., Mahajan, A., Robertson, N. R., Koivula, R. W., Elders, P. J. M., Rauh, S. P., Jones, A. G., Holl, R. W. ., Stingl, J. C., Franks, P. W., McCarthy, M. I., 't Hart, L. M., Pearson, E. R., & IMI DIRECT Consortium (2019). 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. *Diabetes Care*, 42(6), 1027-1033.
<https://doi.org/10.2337/dc18-2182>

General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

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

Running title: Gut metformin transporters and GI intolerance

Adem Y Dawed (PhD)¹, Kaixin Zhou (PhD)¹, Nienke van Leeuwen (PhD)², Anubha Mahajan (PhD)³, Neil Robertson (PhD)^{3,4}, Robert Koivula (PhD)^{4,5}, Petra JM Elders (PhD)⁶, Simone P Rauh (PhD)⁷, Angus G Jones (MD, PhD)⁸, Reinhard W Holl (MD, PhD)⁹, Julia C Stingl (MD, PhD)¹⁰, Paul W Franks (PhD)^{5,11,12}, Mark I McCarthy (MD, PhD)^{3,4,13}, Leen 't Hart (PhD)^{2,7,14}, Ewan R Pearson (MD, PhD)¹; for the IMI DIRECT Consortium.

¹Division of Population Health & Genomics, School of Medicine, University of Dundee, Dundee, UK

²Cell and Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands

³Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK.

⁴Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

⁵Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Skåne University Hospital Malmö, Lund University, Malmö, Sweden.

⁶Department of General Practice and Elderly Care Medicine, Amsterdam Public Health Research Institute, Amsterdam UMC, Amsterdam, the Netherlands

⁷Department of Epidemiology and Biostatistics, Amsterdam Public Health Research Institute, Amsterdam UMC, Amsterdam, the Netherlands.

⁸Institute of Clinical and Biological Sciences, University of Exeter Medical School, Exeter, UK.

⁹Institute of Epidemiology and Medical Biometry (ZIBMT), University of Ulm, Ulm, Germany, German Center for Diabetes Research (DZD).

¹⁰Federal institute for drugs and medical devices, research division, Bonn, Germany.

¹¹Department of Public Health & Clinical Medicine, Umeå University, Umeå, Sweden. paul.franks@med.lu.se.

¹²Department of Nutrition, Harvard School of Public Health, Boston, MA, USA. paul.franks@med.lu.se.

¹³Oxford NIHR Biomedical Research Centre, Oxford University Hospitals Trust, Oxford, UK.

¹⁴Department of Biomedical Data Sciences, section Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Corresponding Author:

Ewan R Pearson

Division of Population Health & Genomics

Level 5, Mailbox 12

Ninewells Hospital and Medical School

Telephone (office): +(44) 01382 383387

Email: E.Z.Pearson@dundee.ac.uk

Word counts

Main body: 3115

Number of references: 50

Number of tables: 1

Number of figures: 3

This is an author-created, uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association (ADA), publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at: <http://care.diabetesjournals.org>.

Abstract

OBJECTIVES 20-30% of patients with metformin treated type 2 diabetes experience gastrointestinal side effects leading to premature discontinuation in 5-10% of the cases. Gastrointestinal intolerance may reflect localised 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 side effects.

RESEARCH DESIGN AND METHODS The study included 286 severe metformin intolerant and 1128 tolerant individuals from the IMI DIRECT 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 (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-eQTL 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 over three times higher odds of intolerance compared to carriers of no G allele and not treated with inhibiting drugs (OR 3.23, $p < 0.001$). Using a genetic risk score derived from rs3889348 and *SLC22A1* variants, the odds of intolerance was more than twice 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 gastrointestinal side effects of metformin.

Metformin therapy can cause gastrointestinal discomfort that negatively affects quality of life and adherence to prescribed medications. Gastrointestinal side effects usually manifest as nausea, vomiting, diarrhoea, flatulence, indigestion, bloating, abdominal discomfort and stomach ache. 20-30% of metformin treated subjects with type 2 diabetes experience gastrointestinal side effects 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): around 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. PMAT and OCT1 are reported to play the major role in the intestinal absorption of metformin (9). While PMAT is expressed in the apical (luminal) membrane of the enterocytes, 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) has also been shown to play an important role in the pathophysiology of metformin intolerance (13).

Whilst PMAT shares extensive substrate and inhibitor overlap with OCTs (14), there are no studies investigating its role in metformin intolerance. Therefore, we 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 side effects. To address this, we used prescribing, biochemistry and clinical data from 286 metformin intolerant and 1128 tolerant individuals from the IMI DIRECT (DIabetes REsearch on patient stratification) consortium (15). Although OCT3 is expressed in the intestine, there are no common functional variants described, and therefore we do not include OCT3 in this analysis.

Research Design and Methods

Study population

286 metformin intolerant (cases) and 1128 metformin tolerant (controls) subjects were identified from prescribing data in the IMI DIRECT consortium from participating centres 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 centres.

All metformin intolerant (cases) and metformin tolerant (controls) had: type 2 diabetes diagnosed clinically, a creatinine clearance ≥ 60 mL/min at metformin exposure, and were white Europeans aged between 18-90 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 1000 mg daily metformin for up to 6 weeks, who also reported gastrointestinal side effects on the metformin treatment as the reason for switching or where gastrointestinal side 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 above 500 mg daily despite an HbA1c > 7% (53 mmol/mol) and who either reported gastrointestinal side effects on more than 500 mg, or where gastrointestinal side effects were clearly documented in the clinical record as a reason for transfer.

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

Definition of metformin tolerance

Metformin tolerant individuals were defined as those treated with ≥ 2000 mg of metformin per day for more than a year (excluding modified release formulations of metformin) and report no side effects.

Clinical covariates

Weight, height and creatinine were defined as the closest measured values within 180 days prior to 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 report gastrointestinal symptoms of metformin intolerance for cases, and for controls it is the date when patients start 2000 mg of metformin. Daily dose was the last dose during ITE for cases, and it was determined as the mean dose of prescriptions encashed during the first six months of metformin therapy for controls.

Concomitant medications

Gut metformin transporters have strong substrate and inhibitor overlap (16). Therefore, we 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 half-maximal inhibitory concentration (IC₅₀) values. Accordingly the use of any of the following medications with metformin were 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), dysopyramide (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 (UOXF) using the Illumina Human Core Exome chip v1.0 (HCE24 v1.0). 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 in order to highlight poorly performing genetic markers and samples prior to imputation.

Samples were excluded for any of the following reasons: call rate less than 95%, heterozygosity greater than 4 standard deviations (SD) from the mean, high correlation to another sample ($\hat{\rho} \geq 0.2$) or identified as ethnic outlier from constructed axes of genetic variation from principal components analysis implemented in the Genome-wide Complex Trait Analysis (GCTA) software (v1.24.7) (27) using the 1000 Genome as a reference. Further filtration was performed to remove: non-autosomal markers, duplicate markers (sharing the same positions), markers with minor allele frequency (MAF) <1%, Hardy–Weinberg equilibrium (HWE) p-value < 0.0001 and call rate < 98%. Imputation to the 1000 Genomes Phase 3 CEU reference panel was performed with ShapeIt (v2.r790) (28) and Impute2 (v2.3.2) (29).

Single nucleotide polymorphism selection

As there are no functionally characterised common nonsynonymous 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%), G401S (3.1%)) were also extracted from the genome-wide data. There was no deviation from HWE for any polymorphism ($p > 0.05$).

Statistical methods

Categorical data are presented as frequency (percentage) and continuous variables as mean \pm SD if normally distributed or as median and inter quartile range (IQR) otherwise. Students 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 cases

and controls was done using X^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 the covariates included using SNPTEST (version 2.5.2) (31). Association of the intronic rs3889348 G>A in *SLC29A4* was explored assuming additive genetic model. *SLC22A1* variants (M420del, R61C, G401S) were grouped together by summing up the number of risk alleles. 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 less than 0.025 was considered statistically significant.

Expression quantitative trait locus (eQTL) analyses

We investigated whether rs3889348 is a cis-QTL in the gut using eQTL datasets comprising a total of 246 colon transverse and 122 terminal ileum samples from the Genotype-Tissue expression (GTEx) data release V6 (32). Tissue procurement, gene expression analysis, genotyping and eQTL analysis have been described previously (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 to 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 1128 tolerant subjects who had complete data on history of concomitant medications. Forty percent of metformin intolerant subjects were taking one or more cation transporter inhibitory drugs compared to 24% in tolerant subjects ($p < 0.0001$) (Table 1). In a logistic regression model adjusted for age, gender, weight and concomitant use of these drugs increased the odds of being intolerant by 70% (OR = 1.72 [1.26-2.32], $p < 0.001$) (Supplementary Table 1). When the individual drug or drug groups were explored, concomitant use of metformin with either PPIs, TCAs or codeine increased the odds of metformin intolerance significantly (Figure 1). The number of subjects who have been coprescribed metformin with transporter inhibiting drugs are shown 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 [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 (Supplemental Table 3). In addition, no significant interaction between rs3889348, the use of metformin transporter inhibiting drugs and any of the other clinical variables (age, gender) 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 not treated with transporter inhibiting drugs as the reference group, carriers of one and two G alleles treated with transporter inhibiting drugs had more than two (2.44 [1.30-4.78]) and three (3.23 [1.71-

6.39]) fold higher odds of intolerance, respectively, 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 carried out an analysis on the association between two reduced function (R61C, G401S) and one loss of function (M420del) *SLC22A1* SNPs and metformin intolerance 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 [0.84-2.12], $p = 0.21$).

A GRS was then generated from *SLC29A4* and *SLC22A1* variants by summing up the number of risk alleles for each individual. Compared to those with no risk allele, metformin treated subjects with type 2 diabetes having two risk alleles had nearly a two-fold (1.93[1.10-3.65]) increased odds of GI intolerance. Those who carry 3 or more risk alleles had more than twice (2.15[1.20-4.12]) the odds of intolerance (Figure 2).

Sensitivity analysis

There was a big difference in sample size between metformin intolerant and tolerant subjects. In addition, there were significant difference in age and gender between cases and controls. Therefore, we carried out a sensitivity analysis by comparing the intolerant group (n=237) with 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 Table 5 and 6).

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 utilizing the publicly available

data set from the GTEx portal (Version 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 ($\beta = -0.45$, $p = 1.4 \times 10^{-08}$) (Supplemental Figure 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 organic cation transporter 1, and metformin related GI side effects has been previously reported (12, 13, 36). However, the data on intestinal localization of OCT1 is 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 it 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 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 show that those who carry two or more variants at either *SLC29A4* or *SLC22A1*, were two-fold 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 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 Figure 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 is also shown to inhibit diamine oxidase (DAO), 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 has also been observed in serotonin reuptake transporter (SERT) knock-out mice (39, 40). In addition, a recent study from the GoDARTS 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 more than 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 glands of the stomach. Binding of histamine to the H1, H2 and H4 receptors that are highly expressed in the gut, stimulate gastric acid secretion, increase 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 GLP-1 levels in subjects with type 2 diabetes and without

type 2 diabetes treated with metformin (47) have also been reported and this might increase GI side effects (48) (Figure 3).

In this study we observed increased risk of intolerance with older age, female sex, lower weight and lower creatinine levels. Concomitant use of metformin with the PPIs and TCAs also increase the risk of intolerance. These findings are largely consistent with the results of previous studies, providing further evidence for clinical practice (12, 35). The FDA Adverse Events Reporting System (AERS) suggested that women experience more adverse effects than men (49). Several factors can contribute to these differences. Gender based variability in intestinal expression of drug transporters may result in variability in gut drug concentrations. Women have also 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 risk of metformin associated gastrointestinal intolerance, and that combined with the previously reported *SLC22A1* variants, this genotype profile can increase odds of metformin intolerance over 2-folds. 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 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 be 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 a well-powered independent studies.

Acknowledgements

We are very grateful to all participants who took part in these studies.

Author contributions

A.D, analysed data, wrote manuscript, performed research. K.Z analysed data, designed research, revised manuscript. N.van L, collected data and revised manuscript. A.M, analysed data and revised manuscript. N.R, analysed data and revised manuscript. R.K, collected data and revised manuscript. P.E, collected data and revised manuscript. S.R, collected data and revised manuscript. A.J, designed research, collected data and revised manuscript. R.H, designed research, collected data and revised manuscript. J.S, designed research, collected data and revised manuscript. P.F, designed research, performed research, revised manuscript. M.M, designed research, analysed data and revised manuscript. L.'t H, designed research, collected data, analysed data, and revised manuscript. E.R.P designed research, performed research, analysed data, wrote manuscript and revised manuscript

A.D and E.P are guarantors of this work

Funding

The work leading to this publication has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. ERP holds a Wellcome Trust New Investigator Award (102820/Z/13/Z)

Conflict of interests

The authors claim no conflicts of interest

References

1. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial. *Am J Med.* 1997 Dec;103(6):491-7.
2. Hirst JA, Farmer AJ, Ali R, Roberts NW, Stevens RJ. Quantifying the effect of metformin treatment and dose on glycaemic control. *Diabetes Care.* 2012 Feb;35(2):446-54.
3. Bailey CJ, Wilcock C, Scarpello JH. Metformin and the intestine. *Diabetologia.* 2008 Aug;51(8):1552-3.
4. Wilcock C, Bailey CJ. Accumulation of metformin by tissues of the normal and diabetic mouse. *Xenobiotica.* 1994 Jan;24(1):49-57.
5. Napolitano A, Miller S, Nicholls AW, Baker D, Van Horn S, Thomas E, et al. Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus. *PLoS One.* 2014;9(7):e100778.
6. Yee SW, Lin L, Merski M, Keiser MJ, Gupta A, Zhang Y, et al. Prediction and validation of enzyme and transporter off-targets for metformin. *J Pharmacokinet Pharmacodyn.* 2015 Oct;42(5):463-75.
7. Yi F, Sun J, Lim GE, Fantus IG, Brubaker PL, Jin T. Cross talk between the insulin and Wnt signaling pathways: evidence from intestinal endocrine L cells. *Endocrinology.* 2008 May;149(5):2341-51.
8. Vidon N, Chaussade S, Noel M, Franchisseur C, Huchet B, Bernier JJ. Metformin in the digestive tract. *Diabetes Res Clin Pract.* 1988 Feb 19;4(3):223-9.
9. Han TK, Proctor WR, Costales CL, Cai H, Everett RS, Thakker DR. Four cation-selective transporters contribute to apical uptake and accumulation of metformin in Caco-2 cell monolayers. *J Pharmacol Exp Ther.* 2015 Mar;352(3):519-28.
10. Han TK, Everett RS, Proctor WR, Ng CM, Costales CL, Brouwer KL, et al. Organic cation transporter 1 (OCT1/mOct1) is localized in the apical membrane of Caco-2 cell monolayers and enterocytes. *Mol Pharmacol.* 2013 Aug;84(2):182-9.
11. Muller J, Lips KS, Metzner L, Neubert RH, Koepsell H, Brandsch M. Drug specificity and intestinal membrane localization of human organic cation transporters (OCT). *Biochem Pharmacol.* 2005 Dec 05;70(12):1851-60.
12. Dujic T, Zhou K, Donnelly LA, Tavendale R, Palmer CN, Pearson ER. Association of Organic Cation Transporter 1 With Intolerance to Metformin in Type 2 Diabetes: A GoDARTS Study. *Diabetes.* 2015 May;64(5):1786-93.
13. Dujic T, Zhou K, Tavendale R, Palmer CN, Pearson ER. Effect of Serotonin Transporter 5-HTTLPR Polymorphism on Gastrointestinal Intolerance to Metformin: A GoDARTS Study. *Diabetes Care.* 2016 Nov;39(11):1896-901.
14. Duan H, Hu T, Foti RS, Pan Y, Swaan PW, Wang J. Potent and Selective Inhibition of Plasma Membrane Monoamine Transporter by HIV Protease Inhibitors. *Drug Metab Dispos.* 2015 Nov;43(11):1773-80.
15. Koivula RW, Heggie A, Barnett A, Cederberg H, Hansen TH, Koopman AD, et al. Discovery of biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes: rationale and design of the epidemiological studies within the IMI DIRECT Consortium. *Diabetologia.* 2014 Jun;57(6):1132-42.
16. Engel K, Wang J. Interaction of organic cations with a newly identified plasma membrane monoamine transporter. *Mol Pharmacol.* 2005 Nov;68(5):1397-407.

17. Ahlin G, Chen L, Lazorova L, Chen Y, Ianculescu AG, Davis RL, et al. Genotype-dependent effects of inhibitors of the organic cation transporter, OCT1: predictions of metformin interactions. *Pharmacogenomics J.* 2011 Dec;11(6):400-11.
18. Ahlin G, Karlsson J, Pedersen JM, Gustavsson L, Larsson R, Matsson P, et al. Structural requirements for drug inhibition of the liver specific human organic cation transport protein 1. *J Med Chem.* 2008 Oct 09;51(19):5932-42.
19. Nies AT, Hofmann U, Resch C, Schaeffeler E, Rius M, Schwab M. Proton pump inhibitors inhibit metformin uptake by organic cation transporters (OCTs). *PLoS One.* 2011;6(7):e22163.
20. Li L, Song F, Tu M, Wang K, Zhao L, Wu X, et al. In vitro interaction of clopidogrel and its hydrolysate with OCT1, OCT2 and OAT1. *Int J Pharm.* 2014 Apr 25;465(1-2):5-10.
21. Bachmakov I, Glaeser H, Fromm MF, Konig J. Interaction of oral antidiabetic drugs with hepatic uptake transporters: focus on organic anion transporting polypeptides and organic cation transporter 1. *Diabetes.* 2008 Jun;57(6):1463-9.
22. Tzvetkov MV, Saadatmand AR, Lotsch J, Tegeder I, Stingl JC, Brockmoller J. Genetically polymorphic OCT1: another piece in the puzzle of the variable pharmacokinetics and pharmacodynamics of the opioidergic drug tramadol. *Clin Pharmacol Ther.* 2011 Jul;90(1):143-50.
23. Tzvetkov MV, dos Santos Pereira JN, Meineke I, Saadatmand AR, Stingl JC, Brockmoller J. Morphine is a substrate of the organic cation transporter OCT1 and polymorphisms in OCT1 gene affect morphine pharmacokinetics after codeine administration. *Biochem Pharmacol.* 2013 Sep 01;86(5):666-78.
24. Nies AT, Koepsell H, Damme K, Schwab M. Organic cation transporters (OCTs, MATEs), in vitro and in vivo evidence for the importance in drug therapy. *Drug Transporters: Springer;* 2011. p. 105-67.
25. Tzvetkov MV, Saadatmand AR, Bokelmann K, Meineke I, Kaiser R, Brockmoller J. Effects of OCT1 polymorphisms on the cellular uptake, plasma concentrations and efficacy of the 5-HT(3) antagonists tropisetron and ondansetron. *Pharmacogenomics J.* 2012 Feb;12(1):22-9.
26. Minematsu T, Giacomini KM. Interactions of tyrosine kinase inhibitors with organic cation transporters and multidrug and toxic compound extrusion proteins. *Mol Cancer Ther.* 2011 Mar;10(3):531-9.
27. Yang J, Manolio TA, Pasquale LR, Boerwinkle E, Caporaso N, Cunningham JM, et al. Genome partitioning of genetic variation for complex traits using common SNPs. *Nat Genet.* 2011 Jun;43(6):519-25.
28. Delaneau O, Zagury JF, Marchini J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat Methods.* 2013 Jan;10(1):5-6.
29. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* 2009 Jun;5(6):e1000529.
30. Christensen MM, Brasch-Andersen C, Green H, Nielsen F, Damkier P, Beck-Nielsen H, et al. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. *Pharmacogenet Genomics.* 2011 Dec;21(12):837-50.
31. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet.* 2007 Jul;39(7):906-13.
32. The Genotype-Tissue Expression (GTEx) project. *Nat Genet.* 2013 Jun;45(6):580-5.
33. Gamazon ER, Wheeler HE, Shah KP, Mozaffari SV, Aquino-Michaels K, Carroll RJ, et al. A gene-based association method for mapping traits using reference transcriptome data. *Nat Genet.* 2015 Sep;47(9):1091-8.

34. Gamazon ER, Segre AV, van de Bunt M, Wen X, Xi HS, Hormozdiari F, et al. Using an atlas of gene regulation across 44 human tissues to inform complex disease- and trait-associated variation. *Nat Genet.* 2018 Jul;50(7):956-67.
35. Dujic T, Causevic A, Bego T, Malenica M, Velija-Asimi Z, Pearson ER, et al. Organic cation transporter 1 variants and gastrointestinal side effects of metformin in patients with Type 2 diabetes. *Diabet Med.* 2016 Apr;33(4):511-4.
36. Tarasova L, Kalnina I, Geldnere K, Bumbure A, Ritenberga R, Nikitina-Zake L, et al. Association of genetic variation in the organic cation transporters OCT1, OCT2 and multidrug and toxin extrusion 1 transporter protein genes with the gastrointestinal side effects and lower BMI in metformin-treated type 2 diabetes patients. *Pharmacogenet Genomics.* 2012 Sep;22(9):659-66.
37. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther.* 2002 Aug;302(2):510-5.
38. Zhou M, Xia L, Wang J. Metformin transport by a newly cloned proton-stimulated organic cation transporter (plasma membrane monoamine transporter) expressed in human intestine. *Drug Metab Dispos.* 2007 Oct;35(10):1956-62.
39. Gershon MD. Review article: serotonin receptors and transporters -- roles in normal and abnormal gastrointestinal motility. *Aliment Pharmacol Ther.* 2004 Nov;20 Suppl 7:3-14.
40. Mawe GM, Hoffman JM. Serotonin signalling in the gut--functions, dysfunctions and therapeutic targets. *Nat Rev Gastroenterol Hepatol.* 2013 Aug;10(8):473-86.
41. Scarpello JH, Hodgson E, Howlett HC. Effect of metformin on bile salt circulation and intestinal motility in type 2 diabetes mellitus. *Diabet Med.* 1998 Aug;15(8):651-6.
42. Kelly OB, Mroz MS, Ward JB, Colliva C, Scharl M, Pellicciari R, et al. Ursodeoxycholic acid attenuates colonic epithelial secretory function. *J Physiol.* 2013 May 01;591(9):2307-18.
43. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. *Appl Environ Microbiol.* 2014 Oct;80(19):5935-43.
44. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. *Diabetologia.* 2016 Mar;59(3):426-35.
45. Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, et al. An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut.* 2014 May;63(5):727-35.
46. Kinaan M, Ding H, Triggle CR. Metformin: An Old Drug for the Treatment of Diabetes but a New Drug for the Protection of the Endothelium. *Med Princ Pract.* 2015 Jul; 24(5): 401–15
47. Preiss D, Dawed A, Welsh P, Heggie A, Jones AG, Dekker J, et al. Sustained influence of metformin therapy on circulating glucagon-like peptide-1 levels in individuals with and without type 2 diabetes. *Diabetes Obes Metab.* 2017 Mar;19(3):356-63.
48. Bettge K, Kahle M, Abd El Aziz MS, Meier JJ, Nauck MA. Occurrence of nausea, vomiting and diarrhoea reported as adverse events in clinical trials studying glucagon-like peptide-1 receptor agonists: A systematic analysis of published clinical trials. *Diabetes Obes Metab.* 2017 Mar;19(3):336-47.
49. Tran C, Knowles SR, Liu BA, Shear NH. Gender differences in adverse drug reactions. *J Clin Pharmacol.* 1998 Nov; 38(11):1003-9.
50. Soldin OP, Mattison DR. Sex Differences in Pharmacokinetics and Pharmacodynamics. *Clin Pharmacokinet.* 2009; 48(3): 143–57.

Tables

Table 1. Baseline characteristics of metformin tolerant and intolerant subjects.

| Variable | Metformin Tolerant (n = 1,128) | Metformin Intolerant (n = 286) | <i>p</i> |
|--|-----------------------------------|-----------------------------------|----------|
| 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 |
| Males/female (male %) | 696/432 (61.7%) | 117/169 (40.9%) | <0.0001 |
| 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 (mg)* at diagnosis | 1500 (1000-2000) | 1000 (500-1000) | <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 |

ITE: index intolerance event *Median (IQR). Dose was calculated as the last dose during ITE for cases, and it was determined as the mean dose of prescriptions encashed during the first six months of metformin therapy for controls.

Figure legends

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

Figure 2. Association of a genetic risk score derived from *SLC29A4* (PMAT) and *SLC22A1* (OCT1) with metformin intolerance. OR: odds ratio; GRS: genetic risk score. Bars indicate standard errors around the mean.

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 oxidase, an enzyme that metabolize 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 increased luminal metformin level. Increased level of metformin increases the level of biogenic amines, affect the gut microbiota and elevate bile acid levels. These may cause symptoms of gastrointestinal side effects.

Figures

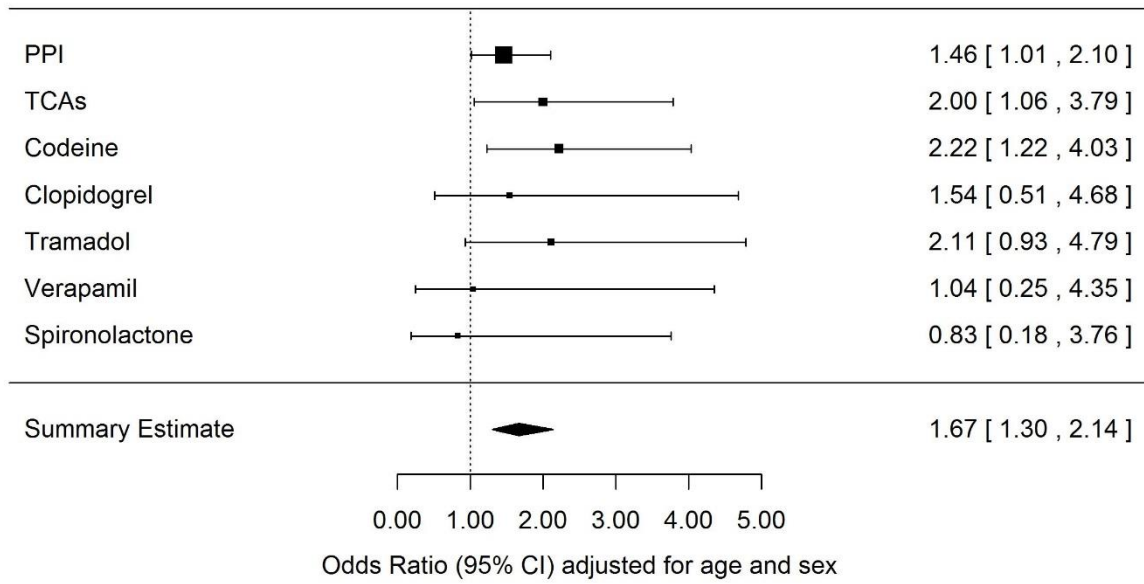


Figure 1

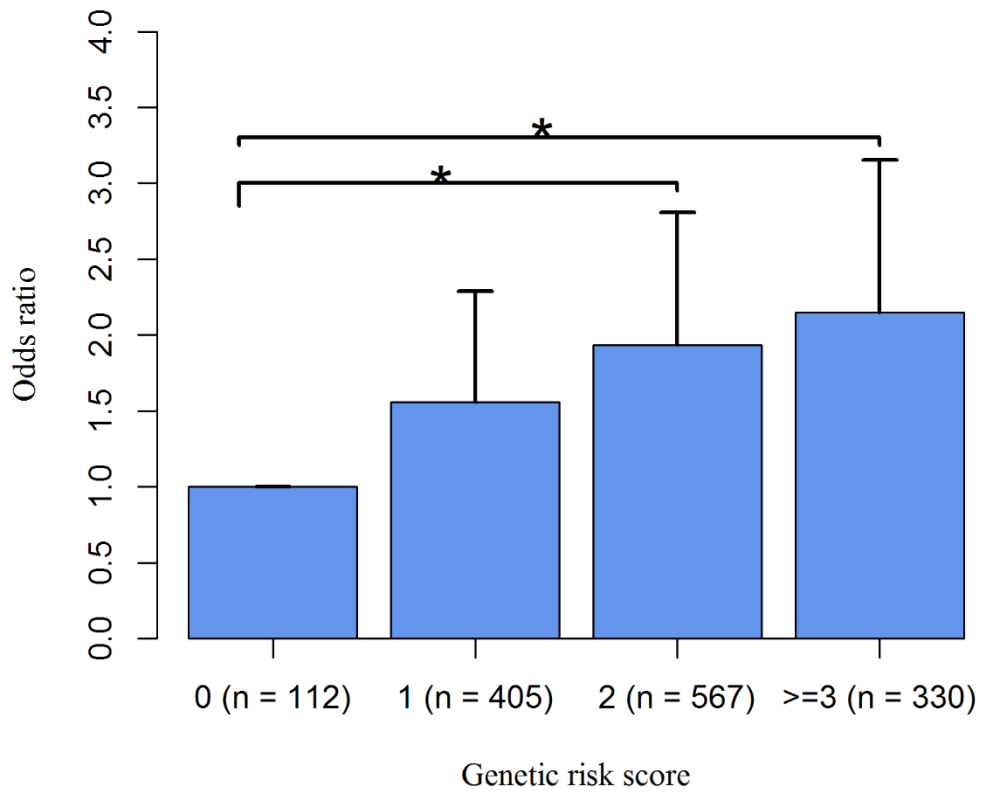


Figure 2

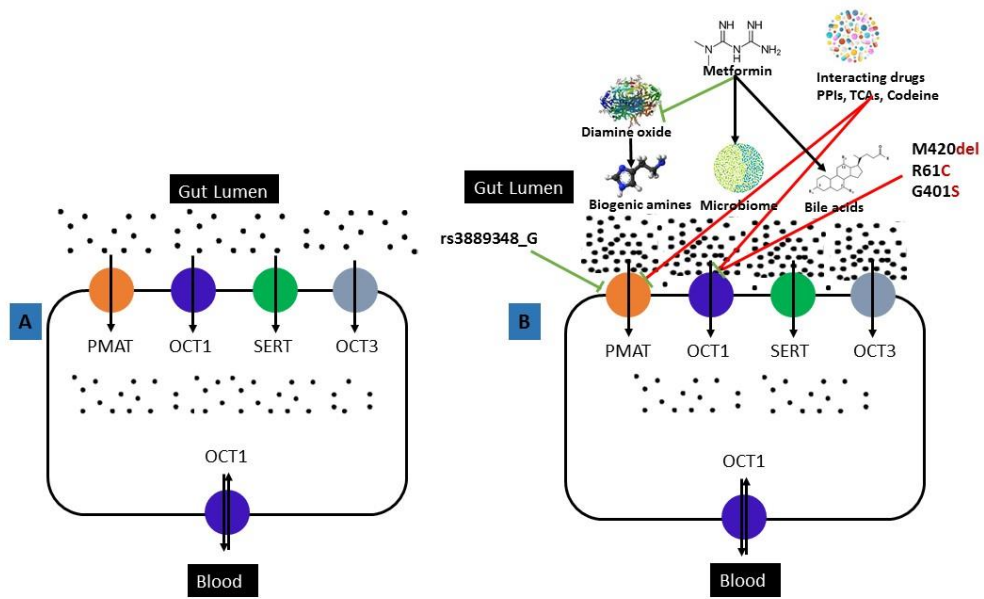


Figure 3

Supplemental Tables

Supplemental Table 1. Logistic regression model of metformin intolerance.

| Variable | OR [CI] | <i>p</i> |
|---|-----------------|------------------------|
| Age at ITE (years) | 1.04[1.03-1.06] | 7.44×10^{-08} |
| Gender | 2.31[1.72-3.10] | 2.52×10^{-08} |
| Weight (kg) | 1.00[0.99-1.00] | 0.20 |
| Use of metformin transporter inhibiting drugs | 1.72[1.26-2.32] | 5.17×10^{-04} |
| rs3889348 | 1.34[1.09-1.65] | 0.005 |

Gender coded as women vs men. ITE: index intolerance event

Supplementary Table 2. Number of patients treated concomitantly with metformin transporter inhibiting drugs in the intolerant and tolerant groups.

| Drug/ Drug Class | Intolerant group (237) | Tolerant group (1128) | <i>p</i> |
|------------------|------------------------|-----------------------|----------|
| PPI | 58 (24.5%) | 156 (13.8%) | <0.001 |
| Codeine | 22 (9.3%) | 35 (3.1%) | <0.001 |
| TCA | 17 (7.2%) | 33 (2.93%) | 0.003 |
| Tramadol | 10 (4.2%) | 18 (1.6%) | 0.004 |
| Clopidogrel | 5 (2.1%) | 14 (1.24%) | 0.46 |
| Spirolactone | 3 (1.3%) | 9 (0.8%) | 0.75 |
| Verapamil | 3 (1.3%) | 8 (0.7%) | 0.64 |

Data are presented as numbers of patients (percentages). TCAs, tricyclic antidepressants; PPIs, proton pump inhibitors.

Supplemental Table 3. Population characteristics by rs3889348 genotype.

| | rs3889348 genotype | | | <i>p</i> |
|---|--------------------|------------------|------------------|----------|
| | AA (n = 205) | GA (n = 681) | GG (n = 532) | |
| Age at ITE (years) | 61.03 ± 10.50 | 61.34 ± 10.0 | 61.94 ± 9.73 | 0.21 |
| Age at diagnosis (years) | 55.97 ± 10.32 | 56.31 ± 9.67 | 56.78 ± 9.65 | 0.27 |
| Gender (women %) | 36.6% | 42.6% | 45.1% | 0.11* |
| Weight (kg) | 97.21 ± 19.39 | 92.53 ± 18.22 | 93.15 ± 19.43 | 0.06 |
| BMI (kg/m ²) | 32.77 ± 6.02 | 31.89 ± 6.09 | 31.88 ± 5.96 | 0.15 |
| Height (m) | 1.72 ± 0.10 | 1.71 ± 0.10 | 1.71 ± 0.10 | 0.29 |
| Creatinine (µmol/dL) | 80.51 ± 15.57 | 79.09 ± 16.29 | 79.90 ± 17.80 | 0.95 |
| Creatinine clearance (mL/min) | 84.75 ± 18.95 | 84.98 ± 18.55 | 84.04 ± 20.80 | 0.52 |
| HbA1c (%) | 8.21 ± 1.59 | 8.18 ± 1.62 | 8.39 ± 1.75 | 0.11 |
| Diabetes duration (years) [†] | 4 [1.16-7.00] | 4 [2.00-7.19] | 4 [1.78-7.00] | 0.78‡ |
| Dose (mg) [†] | 1000 (1000-1500) | 1000 (1000-1500) | 1000 (1000-1500) | 1.00‡ |
| Drug naïve (%) | 44.1% | 51.7% | 51.4% | 0.14* |
| Use of metformin transporter inhibiting drugs (%) | 22.4% | 27.6% | 28.8% | 0.27* |

*chi-square test for independence, ‡kruskal-Wallis one-way analysis of variance, †Median (IQR). ITE: index intolerance event

Supplemental Table 4. Joint effect of *SLC29A4* (PMAT) genotype and metformin transporter inhibiting drugs on metformin intolerance.

| | Intolerant/Tolerant | OR [95% CI] | <i>p</i> |
|--|---------------------|------------------|----------|
| Carries no risk allele and not treated with metformin transporter inhibiting drugs | 15/141 | 1 | --- |
| Carries one risk allele and not treated with metformin transporter inhibiting drugs | 67/408 | 1.52 [0.86-2.88] | 0.17 |
| Carries two risk alleles and not treated with metformin transporter inhibiting drugs | 60/305 | 1.76 [1.01-3.35] | 0.04 |
| Carries no risk allele and treated with metformin transporter inhibiting drugs | 8/37 | 1.74 [0.64-4.46] | 0.25 |
| Carries one risk allele and treated with metformin transporter inhibiting drugs | 44/137 | 2.44 [1.30-4.78] | 0.007 |
| Carries two risk alleles and treated with metformin transporter inhibiting drugs | 43/100 | 3.23 [1.71-6.39] | < 0.001 |

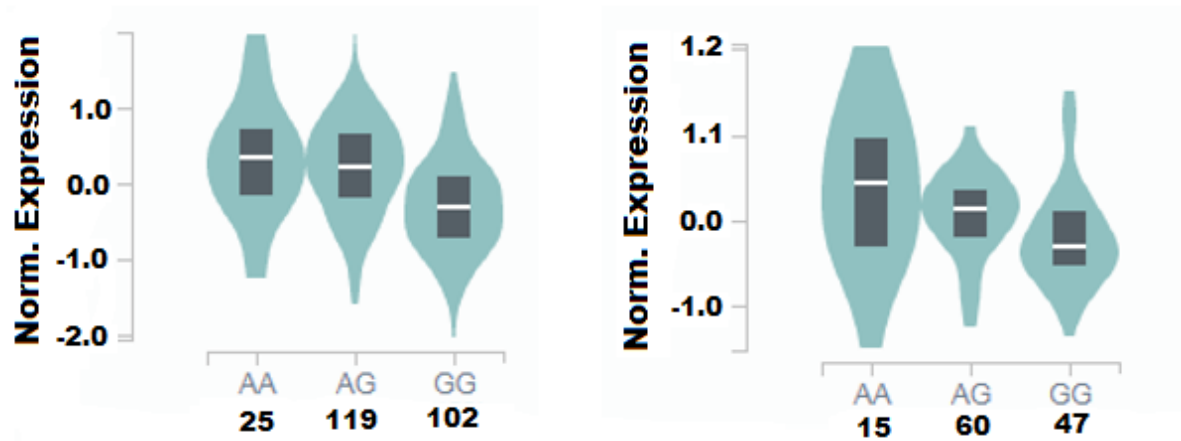
Supplementary Table 5. Joint effects of PMAT genotype and metformin transporter inhibiting drugs on intolerance after matching subjects for age and gender.

| | OR (95% CI) | <i>p</i> |
|---|-----------------|----------|
| Weight | 0.99[0.98-1.00] | 0.08 |
| Use of metformin transporter inhibiting drugs | 1.75[1.28-2.38] | <0.001 |
| rs3889348_G | 1.26[1.02-1.59] | 0.03 |

Supplemental Table 6. Joint effect of *SLC29A4* (PMAT) genotype and metformin transporter inhibiting drugs on metformin intolerance after matching patients for age and gender. 237 cases and 711 controls (1 case ~3 controls).

| | Intolerant/Tolerant | OR [95% CI] | <i>p</i> |
|---|---------------------|-----------------|----------|
| Carries no risk allele and not treated with metformin transporter inhibiting drugs | 15/93 | - | - |
| Carries no risk allele and treated with metformin transporter inhibiting drugs | 8/18 | 2.76[0.99-7.38] | 0.05 |
| Carries one risk allele and not treated with metformin transporter inhibiting drugs | 67/234 | 1.78[0.99-3.37] | 0.06 |
| Carries one risk allele and treated with metformin transporter inhibiting drugs | 60/189 | 1.97[1.09-3.76] | 0.03 |
| Carries two risk allele and not treated with metformin transporter inhibiting drugs | 44/100 | 2.73[1.45-5.37] | 0.003 |
| Carries two risk alleles and treated with metformin transporter inhibiting drugs | 43/77 | 3.46[1.82-6.88] | <0.001 |

Supplemental Figures



Supplemental Figure 1. Boxplot of association between rs3889348 genotype and *SLC29A4* (PMAT) expression in the gut, colon transverse (left side) and terminal ileum of the small intestine (right side).