A variant of the glucose transporter gene *SLC2A2* modifies the glycaemic response to metformin therapy in recently diagnosed type 2 diabetes

Wolfgang Rathmann^{1,2} · Klaus Strassburger^{1,2} · Brenda Bongaerts^{1,2} · Oliver Kuss^{1,2,3} · Karsten Müssig^{2,4,5} · Volker Burkart^{2,4} · Julia Szendroedi^{2,4,5} · Jörg Kotzka^{2,6} · Birgit Knebel^{2,6} · Hadi Al-Hasani^{2,6} · Michael Roden^{2,4,5} · for the GDS Group

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Abstract

Aims/hypothesis The aim of this study was to investigate the modifying effect of the glucose transporter (GLUT2) gene *SLC2A2* (rs8192675) variant on the glycaemic response to metformin in individuals recently diagnosed with type 2 diabetes.

Methods Individuals with type 2 diabetes ($n = 508$) from the prospective German Diabetes Study (age [mean $\pm$ SD] 53 ± 10 years; 65% male; BMI 32 ± 6 kg/m², metformin use 57%) underwent detailed metabolic characterisation (hyperinsulinaemic–euglycaemic clamp, IVGTT) during the first year after diagnosis. Participants provided self-reported data from the time of diagnosis. The change in fasting glucose was assessed in relation to *SLC2A2* genotype and glucose-lowering treatment using two-way ANCOVA with gene $\times$ treatment interactions adjusted for age, sex, BMI and diabetes duration.

Results The C variant allele of rs8192675 was associated with a higher prevalence of diabetes symptoms at diabetes diagnosis. In the metformin monotherapy group only, patients with a C allele showed a larger adjusted blood glucose reduction during the first year after diabetes diagnosis than patients with the TT genotype (6.3 mmol/l vs 3.9 mmol/l; genotype difference 2.4 mmol/l, $p = 0.02$; p value for genotype interaction [metformin monotherapy vs non-pharmacological therapy] <0.01). The greater decline in fasting glucose (CC/CT vs TT) in metformin monotherapy persisted after further adjusting for glucose values at diagnosis (genotype difference 1.0 mmol/l, $p = 0.01$; genotype $\times$ treatment interaction $p = 0.06$).

Conclusions/interpretation The variant rs8192675 in the *SLC2A2* gene (C allele) is associated with an improved glucose response to metformin monotherapy during the first year after diagnosis in type 2 diabetes.

Trial registration [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01055093) NCT01055093

Keywords Gene interaction · Metformin · Personalised medicine · Type 2 diabetes

Abbreviations

GDS German Diabetes Study
iAUC C-peptide secretion incremental area under the curve (IVGTT)

M value Insulin sensitivity (from the hyperinsulinaemic–euglycaemic clamp)
NPT Non-pharmacological therapy
SNP Single-nucleotide polymorphism

✉ Wolfgang Rathmann
rathmann@ddz.uni-duesseldorf.de

¹ Institute of Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Auf'm Hennekamp 65, D-40225 Düsseldorf, Germany

² German Center for Diabetes Research (DZD), München-Neuherberg, Germany

³ Institute of Medical Statistics, Heinrich Heine University, Medical Faculty, Düsseldorf, Germany

⁴ Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany

⁵ Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany

⁶ Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany

Research in context

What is already known about this subject?

- The Metformin Genetics (MetGen) Consortium has shown that the rs8192675 single nucleotide polymorphism (SNP) in *SLC2A2*, encoding the glucose transporter GLUT2, has both a pre- and post-treatment effect on HbA_{1c} in individuals receiving metformin therapy. The mechanisms of the improved response of HbA_{1c} to metformin under influence of the SNP are unclear

What is the key question?

- Does the *SLC2A2* (rs8192675) variant have a modifying effect on the glycaemic response to metformin in individuals with recently diagnosed type 2 diabetes?

What are the new findings?

- At diabetes diagnosis, C allele carriers more frequently presented with diabetes symptoms compared with people who were homozygous for the common T allele
- In the year following diabetes diagnosis, C allele carriers (CC or CT genotypes) on metformin monotherapy showed an adjusted greater reduction (ANCOVA adjusted for age, sex, BMI and diabetes duration) in fasting glucose levels compared with individuals homozygous for the T allele
- These genotypic differences were not observed among participants on metformin combination therapy and participants not receiving medical treatment. Furthermore, there were no evident interactions between genotype and glucose-lowering treatment regarding insulin sensitivity

How might this impact on clinical practice in the foreseeable future?

- The SNP rs8192675 in the *SLC2A2* gene was associated with an improved glucose response to metformin in patients with recently diagnosed type 2 diabetes

Introduction

The glucose transporter GLUT2 is expressed in the liver, intestine, kidney, pancreatic beta cells and in the central nervous system [1]. GLUT2 variants are associated with increased hyperglycaemia and type 2 diabetes development [2, 3]. The Metformin Genetics (MetGen) Consortium has shown that the single-nucleotide polymorphism (SNP) rs8192675 in the intron of the *SLC2A2* gene encoding GLUT2 has both pre- and post-treatment effects on HbA_{1c} in type 2 diabetes patients on metformin treatment [4]. The C variant allele was associated with higher pre-treatment HbA_{1c} levels [4]. The effect of the variant allele was subsequently influenced by type of glucose-lowering treatment, with lower on-treatment HbA_{1c} values on metformin, but not on the sulfonylurea class of drugs [4].

It is unclear whether the improved response of HbA_{1c} to metformin in the presence of the C variant can be explained by a lower hepatic glucose production or by improved glucose disposal to the liver or peripheral tissues [5].

The aim of the present study was to investigate the interaction between the C allele of the *SLC2A2* gene and metformin therapy in the reduction of hyperglycaemia in patients with recently diagnosed type 2 diabetes.

Methods

Study population and measures The German Diabetes Study (GDS) is a cohort study investigating the natural history of recent-onset diabetes in individuals aged 18 to 69 years [6] and has been approved by the ethics committee of Heinrich Heine University, Düsseldorf, Germany. All participants provided written informed consent. Within the first year after diagnosis, patients undergo detailed metabolic phenotyping, including the Botnia clamp, a 1 h IVGTT followed by a hyperinsulinaemic–euglycaemic clamp [6].

A structured interview was carried out to assess symptoms at the time of diabetes diagnosis and the patient's current glucose-lowering medication. Glucose-stimulated total incremental AUC (iAUC) for C-peptide during the 1 h IVGTT was calculated using the trapezoidal rule, subtracting basal C-peptide from the AUC. Glucose disappearance rate (%/min) represents the net elimination rate after glucose injection and is calculated as the slope of the interval from 6 to 40 min. Whole-body insulin sensitivity (*M* value) is given as space-corrected mean glucose infusion rate during steady state of the clamp divided by body weight in (kg) [6].

Blood glucose concentration was measured by the hexokinase method using an EPOS 5060 Analyser (Eppendorf,

Germany). C-peptide concentrations were analysed using a Siemens Immulite Analyser (Eschborn, Germany). HbA_{1c} was determined by the DCCCT method using HPLC (Bio-Rad Laboratories, Munich, Germany). *SLC2A2* genotyping was conducted using real-time polymerase chain reaction-based allelic discrimination with predeveloped gene-specific assays (ThermoFisher, Darmstadt, Germany).

Statistical analyses Data are presented as mean (SD), median (interquartile range) or percentages. Variables with skewed distributions were log_e-transformed. Logistic and linear multiple regression adjusted for potential confounders (age, sex, BMI and diabetes duration) were used to compare treatment and genotype groups, respectively. Age- and sex-adjusted least squares means were estimated to calculate adjusted genotype effects within each treatment group.

The modifying effect of the *SLC2A2* genotype on the association between glucose-lowering treatment and reduction of fasting blood glucose levels, including interaction terms for *SLC2A2* genotype and glucose-lowering therapy, was analysed in a two-way ANCOVA adjusting for age, sex, BMI, diabetes duration and self-reported fasting glucose at diagnosis. A *p* value <5% was considered to be statistically significant. Analyses were performed with SAS (version 9.4; SAS Institute, Cary, NC, USA).

Results

Clinical and genetic characteristics A total of 508 individuals with type 2 diabetes (age [mean ± SD]: 53 ± 10 years; 65% males; BMI: 32 ± 6 kg/m²) were genotyped (rs8192675 C

Table 1 Clinical characteristics of newly diagnosed type 2 diabetes patients in the GDS according to *SLC2A2* genotype (TT vs TC/CC)

Clinical characteristics	TT	TC/CC	<i>p</i> value CC/TC vs TT
<i>n</i>	292	216	—
Sex (% male)	66	65	0.840
At time of diabetes diagnosis (self-reported)			
Age (years)	53 ± 10	53 ± 11	0.860
BMI (kg/m ²) (<i>n</i> = 482)	33 ± 6	34 ± 7	0.206
Fasting blood glucose (FBG) (mmol/l) (<i>n</i> = 255)	10.9 ± 5.1	11.9 ± 5.8	0.221
Symptoms (at diabetes diagnosis):			
Polyuria (%)	25	35	0.022*
Nocturia (%)	21	28	0.097
Increased thirst (%)	29	38	0.033*
Blurred vision (%)	15	21	0.084
Weight loss (%)	11	17	0.053
At time of baseline study visit (structured interview and physical examination)			
Days since diabetes diagnosis	182 ± 98	175 ± 96	0.479
BMI (kg/m ²)	31 ± 6	32 ± 6	0.220
FBG, baseline investigation (mmol/l) (<i>n</i> = 508)	7.1 ± 1.6	7.0 ± 1.6	0.282
FBG, baseline investigation (mmol/l) (subgroup with self-report at diagnosis: <i>n</i> = 255)	7.3 ± 1.8	7.0 ± 1.6	0.107
Improvement in FBG (FBG diagnosis – FBG baseline) (mmol/l) (<i>n</i> = 225)	3.6 ± 5.1	4.9 ± 5.7	0.079
HbA _{1c} , baseline (%) (mmol/mol)	6.4 ± 0.9 (46)	6.4 ± 0.9 (46)	0.584
<i>M</i> value (mg [kg body weight] ^{−1} min ^{−1})	6.0 (4.3, 7.6)	6.1 (4.3, 7.8)	0.331
C-peptide (iAUC) ^a ; (nmol/l × min)	35 (23, 50)	35 (22, 56)	0.670
Glucose disappearance rate ^b (%/min)	0.79 ± 0.27	0.82 ± 0.28	0.261
Metformin user (%)	55	59	0.520
Metformin dosage (mg/day) (users only)	(1515 ± 706)	(1462 ± 582)	0.320

Data are percentages, mean ± SD or median (25th; 75th percentile)

Blood glucose values, BMI and symptoms at diabetes diagnosis are based on self-reports. The *p* values were adjusted for age, sex, BMI and diabetes duration

Conversion factor for glucose (mg/dl to mmol/l): 0.0555

^a The iAUC is for 1 h IVGTT

^b IVGTT

**p* < 0.05

allele carriers: allele frequency 24%; exact Hardy–Weinberg p value 0.71). Overall, 227 (45%) were treated with metformin monotherapy, 61 (12%) received metformin in combination with other glucose-lowering drugs (mostly dipeptidyl peptidase-4 inhibitors) and 220 (43%) did not receive pharmacological therapy (NPT). The clinical characteristics of participants at time of diagnosis and at baseline (study visit) stratified by genotype are shown in Table 1. Self-reported data on BMI and fasting glucose at diagnosis was not available for all patients. However, the characteristics of patients with and without self-reported data were similar (data not shown).

Participants with the TC and CC genotypes more frequently had diabetes-related symptoms at time of diagnosis ($p < 0.05$) (Table 1). No significant differences were found for age, sex, BMI, fasting glucose at diagnosis, baseline fasting glucose, HbA_{1c} or metabolic measures (M value, C-peptide iAUC, glucose disappearance rate) between the genotype groups.

Association between genotype and metabolic variables

Figure 1 shows the age- and sex-adjusted change in fasting glucose between diabetes diagnosis and baseline stratified by genotype and treatment groups. Patients on combination therapy had the largest change in glucose values followed by those on metformin monotherapy and non-pharmacological therapy. In metformin monotherapy only, patients with a C allele showed a larger blood glucose reduction than patients with the TT genotype (6.2 mmol/l vs 3.9 mmol/l; genotype difference: 2.3 mmol/l; $p = 0.02$; p value for genotype interaction (metformin monotherapy vs NPT) = 0.01) (Fig. 1a).

Glucose disappearance rate during the IVGTT was highest in the NPT group, followed by the metformin monotherapy and combination therapy groups (Fig. 1b).

When analysed according to genotype, only the metformin monotherapy group showed a statistically significant difference (0.82%/min vs 0.75%/min; genotype difference: +0.07%/min; $p = 0.04$; p value for genotype interaction [metformin monotherapy vs NPT] = 0.11). C-peptide secretion was also highest in the NPT group, followed by the metformin monotherapy and combination therapy groups. C-peptide secretion was significantly higher in patients with TC and CC genotypes than in those with the TT genotype in the metformin monotherapy group (36 vs 29 iAUC [nmol/l $\times$ min]; relative change +25%, $p = 0.04$; p value for genotype interaction [metformin monotherapy vs NPT] = 0.08), but not in the other groups (Fig. 1c).

After adjusting for age, sex, BMI and diabetes duration, two-way ANCOVA confirmed that the C allele of the *SLC2A2* gene was associated with a reduction in fasting blood glucose in the metformin monotherapy group (genotype difference: 2.4 mmol/l, $p = 0.02$; genotype $\times$ treatment interaction $p < 0.01$). After additionally adjusting for self-reported fasting glucose at diagnosis, the effect diminished, but was still statistically significant (genotype difference: 1.0 mmol/l; $p = 0.01$; genotype $\times$ treatment interaction $p = 0.06$).

In the fully adjusted model, there was no difference between genotypes within the metformin monotherapy group for the glucose disappearance rate (genotype difference +0.07%/min, $p = 0.05$; genotype $\times$ treatment interaction 0.11). In fully adjusted analyses, C allele carriers in the metformin monotherapy group also showed no difference in C-peptide secretion (relative change: +21%, $p = 0.07$; genotype $\times$ treatment interaction $p = 0.14$). Finally, there were no interactions of genotype and glucose-lowering therapy with regard to measures of insulin sensitivity (M value) or baseline HbA_{1c} (data not shown).

Fig. 1 Change in (a) fasting glucose (from diabetes diagnosis until the baseline investigation during first year after diagnosis); (b) glucose disappearance rate (baseline); and (c) C-peptide secretion (iAUC at baseline) by *SLC2A2* genotype and treatment in patients with recently diagnosed

type 2 diabetes in the GDS according to least squares means (95% CI) adjusted for age and sex. The key in (a) applies to all parts. Genotype $\times$ treatment interaction for metformin monotherapy vs NPT: (a) $p = 0.01$, (b) $p = 0.11$, (c) $p = 0.08$

Discussion

This study shows that the SNP rs8192675 in the *SLC2A2* gene (C allele) was associated with an improved glucose response to metformin monotherapy in patients with newly diagnosed type 2 diabetes. These findings are in line with a three-stage genome-wide association study including 13,123 type 2 diabetes patients from the MetGen Consortium, which reported that having a C allele was related to higher baseline HbA_{1c} values independent of the type of glucose-lowering therapy, and to a significantly larger on-treatment HbA_{1c} reduction for metformin users only [4].

SLC2A2 encodes the glucose transporter isoform GLUT2, which is expressed in liver, kidney, intestine, pancreatic islet beta cells and the central nervous system (neurons, astrocytes) [1]. The glucose-lowering effect of metformin is believed to result mainly from decreased hepatic glucose output through inhibition of gluconeogenesis, but also from increased muscle glucose uptake [7]. Furthermore, metformin may also act through alterations in the gut microbiome [7]. Gene expression data from 1226 human liver samples revealed that *SLC2A2* expression is decreased in people with the C allele, which most likely results in less GLUT2 activity in the liver [4]. This genetic alteration may be positively modulated by metformin. A possible explanation for the present finding could be that glucose clearance is reduced in C allele carriers, including a decreased ability of glucose to enter the liver, which is improved by metformin therapy. Furthermore, GLUT2 is also responsible for hepatic glucose release [8]. It is conceivable that people with the C allele variant of the *SLC2A2* gene may be more sensitive to the effects of metformin on the liver.

In early type 2 diabetes, insulin secretion is commonly increased because of the prevailing insulin resistance [9]. Over time, insulin secretion begins to decline and glycaemic control worsens [9].

In the present study, neither C-peptide stimulation nor glucose disappearance rate was increased in C allele carriers with metformin monotherapy. It is noteworthy that it has previously been reported that metformin improves glucose disappearance as assessed by IVGTT in women with polycystic ovary syndrome [10].

Our results should be interpreted in the context of some limitations. First, data on diabetes-related symptoms and fasting glucose values at time of diabetes diagnosis relied on self-reports only. Second, metformin blood concentrations were not measured. Third, while the primary endpoint was the change of blood glucose from diagnosis to baseline, for various other variables there were no data available at time of diabetes diagnosis. Therefore, the corresponding tests and *p* values should be interpreted as hypotheses-generating.

In conclusion, in this study we found a modifying effect of the *SLC2A2* gene on reduction of fasting glucose by metformin monotherapy in patients with recently diagnosed type 2 diabetes.

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Some of the data were presented at the 53rd Annual Conference of the DDG (German Diabetes Society) in 2018.

Data availability The datasets generated during and/or analysed during the current study are not publicly available due to ethical constraints in consideration of participants' privacy and intellectual property protection but are available from the corresponding author on reasonable request.

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Duality of interest WR reports receiving consulting fees for attending educational sessions or advisory boards from AstraZeneca, Boehringer Ingelheim and NovoNordisk and institutional research grants from NovoNordisk. MR reports receiving consulting fees from Boehringer Ingelheim, Eli Lilly, Merck, NovoNordisk, Poxel and Sanofi. No other potential conflicts of interest relevant to this article were reported.

Contribution statement WR designed the study and wrote the manuscript. KS designed the study and analysed the data. BK, JK and HAH carried out the genetic analyses. All authors acquired and interpreted the data, critically reviewed the manuscript, provided technical or material support and approved the final version to be published. WR and KS are the 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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