Influence of common polymorphisms in the *SLC5A2* gene on metabolic traits in subjects at increased risk of diabetes and on response to empagliflozin treatment in patients with diabetes

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Objective **Inhibition of the renal sodium–glucose cotransporter 2 (SGLT2) is a novel concept in the therapy of diabetes mellitus. In this study, we first assessed whether common single nucleotide polymorphisms (SNPs) in the SGLT2-encoding gene *SLC5A2* affect diabetes-related metabolic traits in subjects at risk for type 2 diabetes and, second, whether these have pharmacogenetic relevance by interfering with the response to empagliflozin treatment in patients with type 2 diabetes.**

Patients and methods **Samples from a metabolically well-phenotyped cross-sectional study population (total $N = 2600$) at increased risk for type 2 diabetes and pooled pharmacogenetic samples from patients from four phase III trials of empagliflozin (in total: 603 receiving empagliflozin, 305 receiving placebo) were genotyped for five common SNPs (minor allele frequencies $\geq 5\%$) present in the *SLC5A2* gene locus.**

Results **In the cross-sectional study, none of the *SLC5A2* SNPs significantly influenced metabolic traits such as body fat, insulin sensitivity/resistance, insulin release, HbA_{1c}, plasma glucose, or systolic blood pressure when multiple testing was taken into account (all $P \geq 0.0083$). Further, no relevant effect on response to treatment with empagliflozin on HbA_{1c}, fasting glucose, weight, or systolic blood pressure was observed for the SNPs tested in the pharmacogenetic study.**

Conclusion **Common genetic variants in the *SLC5A2* gene neither affects diabetes-related metabolic traits nor have a clinically relevant impact on response to treatment with the SGLT2 inhibitor empagliflozin.** ***Pharmacogenetics and Genomics*** **27:135–142**

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Introduction

The sodium–glucose cotransporter *2* (SGLT2) is responsible for most glucose reabsorption in the kidney and represents the target of a novel class of antidiabetes drugs: the SGLT2 inhibitors [1]. SGLT2 inhibition results in loss of glucose through the urine and in clinically relevant reductions in plasma glucose and HbA_{1c} [2].

SGLT2 is encoded by the *SLC5A2* gene located on human chromosome 16p11.2. Several rare mutations in this gene that cause functional defects in SGLT2 (impaired transport capacity) and result in familial renal glucosuria (FRG) have been described [3]. Studies have identified missense mutations, premature stop codons, and frameshift mutations that are phenotypically associated with FRG [4–6]. None of these mutations were consistently found across studies/families, indicating that they represent private mutations. FRG is therefore an inhomogeneous disease that can have an autosomal recessive or dominant mode of inheritance, and glucosuria that is mild (10–30 g/1.73 m^2/day) or considerable (>150 g/1.73 m^2/day) [3]. Mutations that cause FRG have potentially beneficial effects on the prevalence of

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Table 1 Association of SGLT2 single nucleotide polymorphisms with pathomechanistic risk factors for type 2 diabetes

	SNP rs9924771			*P*-value	SNP rs9934336			*P*-value	SNP rs3813008			*P*-value	SNP rs31161150			*P*-value
Genotype	GG	GA	AA	–	GG	GA	AA	–	GG	GA	AA	–	GG	GA	AA	–
n	1113	901	215	–	1251	843	135	–	1643	543	43	–	1204	881	144	–
Sex (male) (%)	34.6	35.4	35.8	–	35.3	35.3	31.1	–	34.8	36.1	32.6	–	35.4	33.8	39.6	–
Age (years)	40.0 ± 13.1	39.5 ± 12.4	40.3 ± 13.4	–	39.8 ± 12.8	39.9 ± 12.9	40.0 ± 13.2	–	40.1 ± 12.8	39.0 ± 12.9	40.4 ± 14.9	–	39.5 ± 12.7	40.4 ± 13.0	38.8 ± 13.1	–
BMI (kg/m^2)	30.8 ± 9.4	31.4 ± 10.4	31.4 ± 9.7	0.2	31.2 ± 9.9	31.1 ± 10.0	30.2 ± 8.2	0.5	30.9 ± 9.6	31.8 ± 10.5	31.6 ± 10.4	0.2	31.3 ± 10.0	31.0 ± 9.8	30.0 ± 8.3	0.2
Waist circumference (cm)	97.4 ± 19.6	98.6 ± 21.0	98.5 ± 19.2	0.2	97.9 ± 19.9	98.4 ± 20.7	96.8 ± 18.5	0.9	97.7 ± 19.7	98.8 ± 21.3	99.5 ± 20.7	0.2	98.6 ± 20.5	97.4 ± 19.7	96.5 ± 19.0	0.08
Body fat content (%)	33.4 ± 12.8	34.1 ± 12.9	33.7 ± 13.4	0.2	33.8 ± 13.2	33.5 ± 12.7	34.4 ± 11.6	0.9	33.6 ± 12.8	33.8 ± 13.1	35.9 ± 14.4	0.2	33.7 ± 12.8	34.0 ± 13.2	32.6 ± 11.9	0.7
HOMA-IR (10^{-6} molU/l^2)	3.04 ± 2.66	3.31 ± 3.09	3.16 ± 2.74	0.7	3.17 ± 2.82	3.19 ± 3.00	2.83 ± 2.11	0.9	3.10 ± 2.74	3.33 ± 3.22	3.03 ± 2.17	0.6	3.24 ± 2.95	3.07 ± 2.78	3.03 ± 2.43	0.8
ISI (10^{19} l^2/mol^2)	13.9 ± 9.7	13.5 ± 9.8	13.3 ± 9.1	0.6	13.6 ± 9.7	13.8 ± 9.7	13.8 ± 9.1	0.7	13.7 ± 9.7	13.5 ± 9.9	12.8 ± 7.5	0.6	13.5 ± 9.4	13.9 ± 10.1	13.3 ± 9.2	0.7
$AUC_{0-30\,min}$ ins/$AUC_{0-30\,min}$ glc (10^{-9})	50.1 ± 37.2	51.7 ± 36.4	49.1 ± 31.7	0.7	50.5 ± 35.4	51.2 ± 38.6	48.0 ± 30.6	0.7	50.0 ± 36.3	52.3 ± 37.2	51.8 ± 29.6	0.8	51.7 ± 37.1	49.4 ± 36.3	48.9 ± 31.0	0.3
$AUC_{0-120\,min}$ Cpep/$AUC_{0-120\,min}$ glc (10^{-9})	311 ± 108	314 ± 96	324 ± 93	0.06	316 ± 101	310 ± 104	315 ± 91	0.4	311 ± 102	320 ± 102	321 ± 92	0.1	316 ± 98	310 ± 105	312 ± 110	0.2

Data are unadjusted raw data (counts, %, means ± SD).
Associations were analyzed by multiple linear regression analysis (standard least-squares method) in the additive inheritance model. Body fat parameters were tested with sex and age as covariates; insulin sensitivity/resistance indices with sex, age, and BMI as covariates; and insulin release indices with sex, age, BMI, and ISI as covariates.
AUC, area under the curve; Cpep, C-peptide; glc, glucose; HOMA-IR, homeostasis model assessment of insulin resistance; ins, insulin; ISI, insulin sensitivity index; SNP, single nucleotide polymorphism.

overweight and diabetes because of loss of calories in the urine [7]. Thus, it is likely that these mutations are even rarer in patients with diabetes compared with the general population, questioning the importance and feasibility of testing the impact of the *SLC5A2* gene on the efficacy of pharmacologic SGLT2 inhibition.

At a higher frequency, several common single nucleotide polymorphisms (SNPs) have been identified in the *SLC5A2* gene, most of them in noncoding regions (introns), with no established functional impact on transcript expression or protein activity of relevance to clinical phenotypes or type 2 diabetes [8]. In a study based on two populations, SNP rs9934336 was the only common variant that showed nominal associations with glucose concentrations during three-point oral glucose tolerance tests (OGTTs) [8].

To investigate the impact of the *SLC5A2* genetic variants on risk factors for diabetes, such as overweight, insulin resistance, and β-cell failure, we assessed the associations of common SNPs with clinical parameters in an extensively phenotyped cross-sectional study population at increased risk for type 2 diabetes. In addition, we investigated the pharmacogenetic relevance of these SNPs with respect to treatment response in a pool of patients from four phase III clinical trials testing two doses of the SGLT2 inhibitor empagliflozin in patients with type 2 diabetes.

Patients and methods

Study populations

Two study populations were subjected to genotyping and data analysis. A flow chart including sample and SNP selection for the cross-sectional study (A) and the pharmacogenetic study (B) is given in Supplementary Fig. 1 (Supplemental digital content 1, *http://links.lww.com/FPC/B171*).

Cross-sectional study

Data analysis was carried out in 2229 individuals without diabetes recruited from the ongoing Tübingen Family (TÜF) study for type 2 diabetes. TÜF includes more than 2600 nonrelated individuals at increased risk for type 2 diabetes, that is, with a family history of type 2 diabetes, BMI of at least 27 kg/m^2, impaired fasting glycemia, and/or previous gestational diabetes. All TÜF participants underwent an assessment of medical history, smoking status, and alcohol consumption habits, physical examination, routine blood tests, and five-point OGTTs [9]. Recruitment of the current study population was based on complete OGTT and genotype data sets. Patient characteristics stratified by genotype are presented in Tables 1 and 2. The study adhered to the Declaration of Helsinki and all participants provided their informed written consent before participation in the study. The protocol was approved by the Ethics Committee of the University of Tübingen (Tübingen, Germany).

Table 2 Association of SGLT2 single nucleotide polymorphisms with clinically relevant endpoints HbA_{1c}, glucose concentrations, blood pressure, and estimated glomerular filtration rate by genotype in the cross-sectional study

	HbA_{1c} (%)	HbA_{1c} (mmol/mol)	Fasting glucose (mmol/l)	120-min glucose (mmol/l)	$AUC_{0-120\,min}$ glucose (mmol/l)	Systolic blood pressure (mmHg)	eGFR (ml/min/1.73 m²)
SNP rs9924771							
GG	5.40±0.46	36±5.0	5.16±0.53	6.34±1.56	14.7±3.1	126±18	93.4±21.8
GA	5.42±0.47	36±5.1	5.17±0.57	6.34±1.65	14.8±3.2	125±18	95.6±22.7
AA	5.42±0.42	36±4.6	5.12±0.50	6.35±1.51	14.8±2.8	126±17	94.6±22.1
P-value	0.4		0.4	0.6	1.0	0.5	0.2
SNP rs9934336							
GG	5.42±0.44	36±4.8	5.17±0.54	6.36±1.60	14.8±3.1	126±18	94.5±22.7
GA	5.41±0.48	36±5.2	5.16±0.55	6.33±1.62	14.8±3.1	125±18	94.8±22.0
AA	5.36±0.50	35±5.5	5.11±0.52	6.24±1.41	14.5±3.1	125±17	91.4±18.8
P-value	0.2		0.3	0.6	0.6	0.2	0.9
SNP rs3813008							
GG	5.40±0.46	36±5.0	5.16±0.53	6.36±1.59	14.8±3.0	126±18	93.8±22.1
GA	5.44±0.46	36±5.0	5.19±0.57	6.30±1.61	14.7±3.2	125±18	96.4±22.8
AA	5.42±0.38	36±4.2	5.04±0.49	6.24±1.53	14.2±2.9	127±18	94.1±20.2
P-value	0.1		0.9	0.3	0.2	0.3	0.1
SNP rs3116150							
GG	5.41±0.47	36±5.1	5.15±0.56	6.33±1.61	14.7±3.1	125±17	95.0±22.1
GA	5.41±0.44	36±4.8	5.17±0.52	6.33±1.55	14.8±3.0	126±18	93.8±22.2
AA	5.42±0.46	36±5.0	5.23±0.58	6.49±1.70	15.2±3.2	128±20	93.6±23.7
P-value	0.7		0.0226	0.2	0.0272	0.0083	0.3

Data are unadjusted raw data (mean±SD). Associations were analyzed by multiple linear regression analysis (standard least-squares method) in the additive inheritance model. All parameters were tested with sex, age, and BMI as covariates. Nominal associations ($P<0.05$) are marked in bold.
AUC, area under the curve; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease); HbA_{1c}, hemoglobin A1c; SNP, single nucleotide polymorphism.

Pharmacogenetic study

Data were pooled from patients with type 2 diabetes from four placebo-controlled phase III clinical trials that evaluated the safety and efficacy of empagliflozin 10 and 25 mg as monotherapy or as add-on therapy to (i) pioglitazone alone or in combination with metformin or (ii) metformin or metformin plus sulfonylurea. Details of the studies from which data were extracted for retrospective genetic analyses are provided in Supplementary Table 1 (Supplemental digital content 2, *http://links.lww.com/FPC/B172*). The monotherapy study included a sitagliptin active comparator group. On the basis of the different modes of action, there is no scientific rationale to explore the effect of *SLC5A2* on response to sitagliptin and because of the comparably low number of patients in this treatment group ($n=71$), the respective (underpowered) data are not presented. Study design and primary and secondary endpoints have been reported [10–13]. Of 2705 patients randomized in the four trials, 979 patients provided informed consent for pharmacogenetic analyses. The baseline characteristics of the patients included in the pharmacogenetic analyses were similar to those of the overall pool (data not shown) and are presented stratified by genotype in Table 3.

Genotype analysis

On the basis of publicly available data from the 1000 Genomes Project (*http://browser.1000genomes.org/index.html*), we selected six common SNPs [minor allele frequencies (MAFs) ≥ 0.05] to cover the SLC5A2 locus (gene region plus 2 kb of the 5′-flanking region): rs9924771 G/A, rs11646054 G/C, rs3116149 G/A, and rs9934336 G/A in intron 1, and rs3813008 G/A and rs3116150 G/A in intron 5. Three of these, that is, rs9934336, rs3813008, and rs3116150, were reported by Enigk *et al.* [8]. In both studies, DNA was extracted from whole-blood samples and DNA sequences encompassing the SNPs were amplified by PCRs.

Genotyping in the cross-sectional study

SNPs rs3116149, rs9934336, rs3813008, and rs3116150 were genotyped by mass spectrometry using the massARRAY platform from Sequenom and the manufacturer's iPLEX software (Sequenom, Hamburg, Germany). Two SNPs, rs9924771 and rs11646054, resisted multiplex assay design for massARRAY. For rs9924771, but not for rs11646054, a TaqMan assay for allelic discrimination could be designed (Applied Biosystems, Foster City, California, USA). Of the five SNPs ultimately genotyped, rs3116149 was shown to be monomorphic (all patients homozygous for the major G-allele) and, therefore, was excluded from the analyses.

Genotyping in the pharmacogenetic study

SNPs rs3116149, rs9934336, rs3813008, rs11646054, rs3116650, and rs9924771 were genotyped by allelic discrimination using TaqMan PCR assays (Applied Biosystems). The TaqMan assay for rs9924771 resulted in clusterplots of limited quality (possibly because of multiple repetitive sequences near the SNP position). Therefore, rs9924771 was excluded from the analyses in the pharmacogenetic study.

Thus, five SNPs were analyzed in at least one of the two studies and these SNPs cover ~83% of the common genetic variation within the gene locus.

Table 3 Demographic and baseline characteristics in the pharmacogenetic study by single nucleotide polymorphisms

	n	Sex (male) (%)	Age (years)	BMI (kg/m^2)	Body weight (kg)	Baseline HbA_{1c} (%)	Baseline HbA_{1c} (mmol/mol)	Fasting glucose (mmol/l)	eGFR (ml/min 1.73 m^2)	Systolic blood pressure (mmHg)
SNP rs9934336										
GG	572	53.8	56.6 ± 10.3	31.31 ± 5.63	87.44 ± 20.11	8.01 ± 0.84	64 ± 9.2	8.8 ± 2.0	84.60 ± 19.95	131.0 ± 14.6
AG	362	54.7	57.4 ± 10.3	31.54 ± 5.61	89.39 ± 19.91	7.97 ± 0.87	64 ± 9.5	8.8 ± 1.9	81.62 ± 21.93	132.8 ± 15.4
AA	45	55.6	58.6 ± 11.9	30.44 ± 5.34	84.51 ± 16.59	8.07 ± 0.78	65 ± 8.5	9.7 ± 2.6	79.89 ± 18.69	136.3 ± 19.6
SNP 3813008										
GG	727	54.1	57.0 ± 10.2	31.49 ± 5.43	88.26 ± 19.51	8.01 ± 0.86	64 ± 9.4	8.9 ± 2.0	83.89 ± 20.02	132.2 ± 15.4
AG	233	52.8	57.2 ± 10.9	30.87 ± 6.13	86.90 ± 20.91	7.98 ± 0.83	64 ± 9.1	8.7 ± 1.9	81.31 ± 22.68	130.9 ± 14.5
AA	18	77.8	54.8 ± 9.8	31.92 ± 5.90	92.07 ± 22.66	7.82 ± 0.76	62 ± 8.3	8.1 ± 2.3	85.04 ± 20.49	130.4 ± 14.5
SNP 3116150										
GG	625	51.2	56.0 ± 10.6	30.92 ± 5.58	85.96 ± 19.94	8.03 ± 0.87	64 ± 9.5	8.7 ± 2.0	84.15 ± 21.75	130.6 ± 15.4
AG	320	58.3	58.3 ± 9.9	32.15 ± 5.79	91.85 ± 20.04	7.97 ± 0.82	64 ± 9.0	8.9 ± 1.9	81.91 ± 18.63	133.6 ± 14.6
AA	305	66.7	60.7 ± 8.4	31.89 ± 4.31	90.58 ± 15.04	7.75 ± 0.7	61 ± 7.9	9.0 ± 1.7	80.84 ± 18.62	136.9 ± 13.9
SNP 3116149										
GG	846	54.1	57.2 ± 10.3	31.47 ± 5.49	88.36 ± 19.59	7.99 ± 0.85	64 ± 9.3	8.8 ± 2.0	83.18 ± 19.96	132.2 ± 15.4
AG	128	55.5	55.9 ± 10.7	30.58 ± 6.29	85.86 ± 21.38	8.01 ± 0.86	64 ± 9.4	8.7 ± 2.0	83.95 ± 25.07	129.4 ± 13.9
AA	4	50.0	55.3 ± 6.2	32.00 ± 7.60	90.18 ± 37.19	7.50 ± 0.44	58 ± 4.8	6.8 ± 1.6	80.90 ± 25.16	140.3 ± 7.7
SNP rs11646054										
GG	353	52.4	56.2 ± 10.8	30.79 ± 5.62	86.11 ± 19.6	8.01 ± 0.87	64 ± 9.5	8.8 ± 2.1	83.23 ± 23.04	130.7 ± 15.0
CG	435	53.1	57.6 ± 10.2	31.61 ± 5.64	88.99 ± 20.34	7.98 ± 0.85	64 ± 9.3	8.8 ± 1.9	81.96 ± 17.95	132.2 ± 15.6
CC	191	60.2	57.1 ± 10.0	31.81 ± 5.46	89.36 ± 19.16	7.99 ± 0.82	64 ± 9.0	8.8 ± 1.9	86.39 ± 21.69	133.3 ± 14.3

Data are unadjusted raw data (counts, %, mean ± SD).
eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A1c; SNP, single nucleotide polymorphism.

Clinical measurements and calculations

In the cross-sectional study, five-point OGTTs were performed and HbA_{1c}, plasma glucose, serum insulin, serum C-peptide, and bioimpedance-derived body fat content were measured as described previously [16]. The estimates of insulin sensitivity/resistance [insulin sensitivity index (ISI) [17]; and homeostasis model assessment of insulin resistance [18]] and insulin secretion [area under the curve $(AUC)_{0–30\,min}$ insulin/$AUC_{0–30\,min}$ glucose and $AUC_{0–120\,min}$ C-peptide/$AUC_{0–120\,min}$ glucose] were calculated as reported earlier [19]. The estimated glomerular filtration rate (eGFR) was calculated according to the prediction equation of the Modification of Diet in Renal Disease study [20]. The laboratory measurements performed in the pharmacogenetic study have been described [10–13].

Statistical analyses

If not indicated otherwise, data are presented as mean ± SD. Hardy–Weinberg equilibrium was tested using χ^2-tests with 1 *d.f.*

Data analysis in the cross-sectional study

SNP effects were primarily tested in the additive inheritance model. Secondarily, genotypic and dominant inheritance models were applied. Continuous variables with a skewed distribution were $\log_e$-transformed before statistical analysis. Multiple linear regression analyses were carried out using the standard least-squares method. In the regression models, the trait of interest (glucose concentration, body fat, insulin sensitivity/resistance, insulin release, or blood pressure) was chosen as the outcome variable, the SNP genotype as an independent variable, and sex, age, BMI, and ISI as confounding variables where appropriate. The selection of confounding variables was based on knowledge from large epidemiological studies and on results of univariate and multivariate analyses in the TÜF study. The statistical software JMP 10.0 (SAS Institute, Cary, North Carolina, USA) was used for all analyses.

To correct for multiple testing, we applied Bonferroni correction. In the cross-sectional study, a *P*-value less than 0.0025 resulting from correction for 20 null hypotheses tested in parallel given by five traits (glucose concentration, body fat, insulin sensitivity/resistance, insulin secretion, and blood pressure) and four SNPs (rs9934336, rs3813008, rs3116150, and rs9924771) was considered significant. Associations with *P*-values above these Bonferroni-corrected α-levels and less than 0.05 are indicated as nominal.

Data analysis in the pharmacogenetic study

SNP effects were primarily tested in the genotypic inheritance model. Secondarily, additive and dominant inheritance models were applied. Data from randomized patients in the four trials who were treated with at least one dose of study medication, had a baseline HbA_{1c} measurement, had genetic polymorphism data available, and fulfilled the genetic mismatch quality control criteria were pooled. In cases where endpoint data were not available, the last observed value was carried forward. The homogeneity of the treatment effect on the change from baseline after 24 weeks in the genotype subgroups was investigated using analysis of covariance for the endpoints HbA_{1c} (primary endpoint), fasting glucose, weight, and systolic blood pressure. Assuming different modes of inheritance, all models included the baseline of

the endpoint in question, baseline HbA_{1c}, baseline eGFR, region, background medication, treatment, genetic variant, and the interaction of genetic variant and treatment. SAS, version 9.2 (SAS Institute Inc.) was used for all analyses in the pharmacogenetic study. A *P*-value less than 0.0025 resulting from correction for 20 null hypotheses tested in parallel given by four traits and five SNPs (rs3116149, rs9934336, rs3813008, rs11646054, rs3116650) was considered significant. Associations with *P*-values above these Bonferroni-corrected α-levels and less than 0.05 are indicated as nominal.

Results

Cross-sectional study

We have analyzed the impact of common *SLC5A2* SNPs (rs9934336, rs9924771, rs3813008, and rs3116150) on glucose concentrations, insulin sensitivity/resistance, insulin release, body fat, and blood pressure in 2229 subjects at increased risk for type 2 diabetes. The study population was middle-aged (mean ± SD age: 39.8 ± 12.9 years), obese (BMI: 31.2 ± 9.9 kg/m^2), and about 1/3 were men. All four SNPs were in Hardy–Weinberg equilibrium ($P \geq 0.1$). After appropriate adjustments and correction for multiple testing, none of the SNPs significantly influenced BMI, waist circumference, bioimpedance-derived body fat content, homeostasis model assessment of insulin resistance, ISI, or insulin secretion as estimated by $AUC_{0–30\,min}$ insulin/$AUC_{0–30\,min}$ glucose and $AUC_{0–120\,min}$ C-peptide/$AUC_{0–120\,min}$ glucose ($P_{additive} \geq 0.06$ all; Table 1). Furthermore, none of the SNPs were associated significantly with HbA_{1c}, fasting glucose, glucose concentration during OGTT, systolic blood pressure, or eGFR ($P_{additive} \geq 0.0083$ all; Table 2). Notably, minor A-allele carriers of SNP rs3116150 showed elevated fasting glucose concentrations ($P_{additive} = 0.0226$), elevated AUC of glucose during the OGTT ($P_{additive} = 0.0272$), and elevated systolic blood pressure ($P_{additive} = 0.0083$) without reaching the Bonferroni-corrected level of significance ($P < 0.0025$) (Table 2). The nominal effects of rs3116150 on fasting glucose and the AUC of glucose were still evident in the genotypic model ($P = 0.0392$ and $P = 0.0199$, respectively), but were lost in the dominant model ($P \geq 0.09$). This SNP's nominal effect on systolic blood pressure was observed in the genotypic and dominant models ($P_{genotypic} = 0.0248$, $P_{dominant} = 0.0286$).

In addition, the total study population was divided into normal glucose-tolerant individuals ($n = 1558$) and prediabetic individuals ($n = 671$), and the SNPs for association with HbA1c, fasting, and 2-h glucose concentrations in normal glucose-tolerant ($n = 1558$) and prediabetic ($n = 671$) subjects were tested separately. Data are shown in Supplementary Table 2 (Supplemental digital content 3, *http://links.lww.com/FPC/B173*).

Pharmacogenetic study

The clinical responses in the genotyped subgroups were compared with those in the overall population of patients from the four phase III trials. The number of patients and genotype frequencies of SGLT2 polymorphisms according to race in the analysis population are shown in Supplementary Table 3 (Supplemental digital content 4, *http://links.lww.com/FPC/B174*). Treatment responses in the pharmacogenetic subgroup were almost identical to those of the corresponding treatment groups in the overall population, suggesting that the pharmacogenetic subgroups were representative for the entire study population (data not shown). All five SNPs analyzed were in Hardy–Weinberg equilibrium. The baseline characteristics of the participants in the pharmacogenetic study stratified by genotype are shown in Table 3. For SNP rs3116149 and rs3813008, the genotype frequencies of homozygous minor allele carriers was low, as expected from the MAF (~5–7%) and the *a priori* exclusion of SNPs with MAFs below 0.05 according to the 1000 Genomes Project. For rs3116149, only four homozygous minor A-allele carriers and for rs3813008, 18 homozygous carriers were found. Because of the low minor allele frequency of some SNPs, unexpected numerical differences were observed for some endpoints, which were not significant in all relevant genetic models.

No interaction was observed between the genotypes and the change from baseline HbA_{1c} after 24 weeks of treatment for the SNPs considered ($P_{additive} \geq 0.2132$). Application of the genotypic and dominant models did not result in different findings ($P_{genotypic} \geq 0.1698$, $P_{dominant} \geq 0.1607$). In addition, comparison of the treatment effect of empagliflozin on HbA_{1c} over placebo between the genetic variants of the investigated SNPs showed no differences ($P_{additive} \geq 0.0958$). The differences in the responses to empagliflozin (changes from baseline HbA_{1c}, fasting glucose, weight, and systolic blood pressure versus placebo after 24 weeks of treatment) between the SGLT2 genotype groups in the pharmacogenetic study using a genotypic model are shown in Supplementary Table 4 (Supplemental digital content 5, *http://links.lww.com/FPC/B175*).

With respect to the other endpoints, that is, changes from baseline fasting glucose, body weight, and systolic blood pressure after 24 weeks of treatment, no significant interaction effects with the genotypes were observed ($P_{additive} \geq 0.0142$). There were nominal associations of rs3116150 ($P_{additive} = 0.0147$ and $P_{genotypic} = 0.0043$) and rs11646054 ($P_{additive} = 0.0142$ and $P_{genotypic} = 0.0350$) with systolic blood pressure mainly driven by empagliflozin 10 mg. Considering the differences in treatment effects between the genotypes, nominal associations of rs3116149 with fasting glucose in empagliflozin 25 mg ($P_{genotypic} = 0.0310$), and systolic blood pressure ($P_{genotypic} = 0.0337$) driven mainly by the homozygous minor A-allele carriers ($n = 4$) were no longer observed in the additive or dominant inheritance models ($P \geq 0.05$) (Table 4).

Table 4 Empagliflozin treatment response (change versus placebo after 24 weeks of treatment) in SGLT2 genotypes groups assuming the genotypic model and single nucleotide polymorphism association in the pharmacogenetic study

	HbA_{1c} (%)	HbA_{1c} (mmol/mol)	Fasting glucose (mmol/l)	Weight (kg)	Systolic blood pressure (mmHg)
SNP rs9934336					
G/G (10 mg Empa–Placebo)	−0.67 ± 0.08	−7.3 ± 0.9	−1.90 ± 0.18	−1.93 ± 0.27	−2.44 ± 1.12
P-value	< 0.0001		< 0.0001	< 0.0001	0.0295
A/G (10 mg Empa–Placebo)	−0.61 ± 0.10	−6.7 ± 1.1	−1.41 ± 0.23	−2.13 ± 0.36	−4.95 ± 1.45
P-value	< 0.0001		< 0.0001	< 0.0001	0.0007
A/A (10 mg Empa–Placebo)	−0.67 ± 0.27	−7.3 ± 3.0	−2.55 ± 0.64	−1.22 ± 0.98	−6.93 ± 3.99
P-value	0.0132		< 0.0001	0.2141	0.0827
G/G (25 mg Empa–Placebo)	−0.73 ± 0.08	−8.0 ± 0.9	−2.00 ± 0.19	−2.44 ± 0.29	−3.56 ± 1.16
P-value	< 0.0001		< 0.0001	< 0.0001	0.0023
A/G (25 mg Empa–Placebo)	−0.69 ± 0.10	−7.5 ± 1.1	−1.88 ± 0.22	−2.84 ± 0.35	−6.65 ± 1.41
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
A/A (25 mg Empa–Placebo)	−0.64 ± 0.28	−7.0 ± 3.1	−2.91 ± 0.67	−3.62 ± 1.03	−5.35 ± 4.19
P-value	0.0254		< 0.0001	0.0005	0.2023
P-value (genotype × treatment interaction)	0.9869		0.2424	0.4460	0.3954
SNP 3813008					
G/G (10 mg Empa–Placebo)	−0.59 ± 0.07	−6.4 ± 0.08	−1.68 ± 0.16	−1.70 ± 0.24	−3.01 ± 1.00
P-value	< 0.0001		< 0.0001	< 0.0001	0.0026
A/G (10 mg Empa–Placebo)	−0.79 ± 0.12	−8.6 ± 1.3	−1.97 ± 0.29	−2.81 ± 0.44	−5.50 ± 1.78
P-value	< 0.0001		< 0.0001	< 0.0001	0.0021
A/A (10 mg Empa–Placebo)	−1.04 ± 0.44	−11.4 ± 4.8	−2.32 ± 1.04	−2.10 ± 1.59	−1.26 ± 6.48
P-value	0.0181		0.0258	0.1881	0.8454
G/G (25 mg Empa–Placebo)	−0.72 ± 0.07	−7.9 ± 0.8	−2.07 ± 0.16	−2.55 ± 0.25	−5.33 ± 1.02
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
A/G (25 mg Empa–Placebo)	−0.63 ± 0.12	−6.9 ± 1.3	−1.78 ± 0.29	−2.90 ± 0.44	−3.74 ± 1.79
P-value	< 0.0001		< 0.0001	< 0.0001	0.0373
A/A (25 mg Empa–Placebo)	−1.33 ± 0.42	−14.5 ± 4.6	−2.08 ± 0.99	−2.60 ± 1.52	−0.20 ± 6.17
P-value	0.0015		0.0355	0.0865	0.9735
P-value (genotype × treatment interaction)	0.1698		0.5030	0.2793	0.3511
SNP 3116150					
G/G (10 mg Empa–Placebo)	−0.60 ± 0.07	−6.6 ± 0.8	−1.73 ± 0.18	−1.68 ± 0.27	−4.59 ± 1.09
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
A/G (10 mg Empa–Placebo)	−0.71 ± 0.11	−7.8 ± 1.2	−1.90 ± 0.25	−2.57 ± 0.38	−4.14 ± 1.54
P-value	< 0.0001		< 0.0001	< 0.0001	0.0071
A/A (10 mg Empa–Placebo)	−0.69 ± 0.25	−7.5 ± 2.7	−1.29 ± 0.58	−1.37 ± 0.89	8.88 ± 3.60
P-value	0.0049		0.0258	0.1224	0.0138
G/G (25 mg Empa–Placebo)	−0.69 ± 0.07	−7.5 ± 0.8	−1.92 ± 0.17	−2.55 ± 0.26	−4.50 ± 1.06
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
A/G (25 mg Empa–Placebo)	−0.78 ± 0.11	8.5 ± 1.2	−2.33 ± 0.27	−3.08 ± 0.41	−6.58 ± 1.68
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
A/A (25 mg Empa–Placebo)	−0.59 ± 0.27	−6.4 ± 3.0	−1.42 ± 0.63	−1.62 ± 0.96	−0.04 ± 3.90
P-value	0.0279		0.0241	0.0919	0.9914
P-value (genotype × treatment interaction)	0.8518		0.6082	0.2958	0.0043
SNP 3116149					
G/G (10 mg Empa–Placebo)	−0.62 ± 0.06	−6.8 ± 0.7	−1.74 ± 0.15	−1.93 ± 0.23	−3.36 ± 0.93
P-value	< 0.0001		< 0.0001	< 0.0001	0.0003
A/G (10 mg Empa–Placebo)	−0.76 ± 0.16	−8.3 ± 1.7	−1.71 ± 0.39	−2.10 ± 0.59	−5.98 ± 2.42
P-value	< 0.0001		< 0.0001	0.0004	0.0137
A/A (10 mg Empa–Placebo)	−0.02 ± 1.03	−0.2 ± 11.3	−5.49 ± 2.43	−4.98 ± 3.72	7.06 ± 15.16
P-value	0.9847		0.0240	0.1811	0.6414
G/G (25 mg Empa–Placebo)	−0.69 ± 0.06	−7.5 ± 0.7	−1.98 ± 0.15	−2.75 ± 0.23	−5.27 ± 0.95
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
A/G (25 mg Empa–Placebo)	−0.79 ± 0.16	−8.6 ± 1.7	−2.04 ± 0.37	−1.90 ± 0.56	−3.37 ± 2.29
P-value	< 0.0001		< 0.0001	0.0007	0.1422
A/A (25 mg Empa–Placebo)	−0.24 ± 0.90	−2.6 ± 9.8	−6.54 ± 2.11	−5.14 ± 3.24	22.84 ± 13.18
P-value	0.7874		0.0020	0.1125	0.0835
P-value (genotype × treatment interaction)	0.8993		0.3051	0.4678	0.1077
SNP rs11646054					
G/G (10 mg Empa–Placebo)	−0.69 ± 0.10	−7.5 ± 1.1	−1.82 ± 0.23	−1.78 ± 0.36	−5.82 ± 1.46
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
C/G (10 mg Empa–Placebo)	−0.62 ± 0.09	−6.8 ± 1.0	−1.78 ± 0.21	−2.20 ± 0.32	−4.14 ± 1.28
P-value	< 0.0001		< 0.0001	< 0.0001	0.0013
C/C (10 mg Empa–Placebo)	−0.65 ± 0.13	−7.1 ± 1.4	−1.59 ± 0.31	−1.74 ± 0.48	1.83 ± 1.94
P-value	< 0.0001		< 0.0001	0.0003	0.3433
G/G (25 mg Empa–Placebo)	−0.72 ± 0.10	−7.9 ± 1.1	−1.99 ± 0.23	−2.79 ± 0.35	−5.77 ± 1.41
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
C/G (25 mg Empa–Placebo)	−0.69 ± 0.09	−7.5 ± 1.0	−2.15 ± 0.21	−2.76 ± 0.33	−5.35 ± 1.33
P-value	< 0.0001		< 0.0001	< 0.0001	< 0.0001
C/C (25 mg Empa–Placebo)	−0.74 ± 0.14	−8.1 ± 1.5	−1.70 ± 0.33	−2.07 ± 0.51	−1.56 ± 2.06
P-value	< 0.0001		< 0.0001	<0.0001	0.4495
P-value (genotype × treatment interaction)	0.9865		0.8340	0.6091	0.0350

ANCOVA results at week 24 for the change from baseline (LOCF, except for systolic blood pressure which was LOCF-H) for combined analysis data assuming a genotypic genetic model. All sitagliptin data were excluded from the analysis. The model includes the baseline of the considered endpoint, baseline HbA_{1c}, baseline eGFR (Modification of Diet in Renal Disease), region, background medication, treatment, genetic variant, and genetic variant × treatment. Estimated treatment response of the genotype groups (mean ± SE) and the corresponding *P*-values as well as *P*-values for genotype × treatment interaction are given for each SNP.

ANCOVA, analysis of covariance; eGFR, estimated glomerular filtration rate; Empa, empagliflozin; HbA_{1c}, hemoglobin A1c; LOCF, last observation carried forward; SNP, single nucleotide polymorphism.

Discussion

With respect to individualized medicine and causal therapies for common multifactorial diseases such as type 2 diabetes, hypertension, and cardiovascular disease, pharmacogenetic investigations are considered to be of crucial importance to overcome low or adverse response [21,22]. With respect to SGLT2 inhibition, it was recently discussed that common variants in the *SLC5A2* gene may be a promising target for pharmacogenetic studies [23]. Our study is the first to address the response to treatment with empagliflozin in carriers of common *SLC5A2* SNPs.

In our cross-sectional study, we did not detect any significant association between the tested SNPs and plasma glucose concentrations, insulin sensitivity/resistance, insulin release, body fat, or systolic blood pressure. Only one SNP, rs3116150, showed a nominal association with plasma glucose and blood pressure. In contrast to previous findings [8], where nominal associations were found between rs9934336 and glucose concentrations during OGTT, no such associations were found in this study population at risk for type 2 diabetes.

In the patients participating in the pharmacogenetic study treated with two dosages of the SGLT2 inhibitor empagliflozin, no significant and clinically relevant influences of the common *SLC5A2* SNPs on HbA_{1c}, fasting glucose, body weight, or systolic blood pressure could be detected. Nominal associations with systolic blood pressure were observed with rs3116150, rs11646054, and rs3116149 and with fasting blood glucose with rs3116149. Most of these findings were not consistent for both dosages and were driven by few minor allele carriers. As there were nominal associations with systolic blood pressure in both substudies, additional independent replication of this finding may help to prove whether this is a real association or only a statistical type-1 error. The strongest association observed in the cross-sectional study with rs3116150 points to a glucose-lowering effect of the major allele (suggesting some loss of SGLT2 function in major allele carriers). However, this is unlikely because urinary glucose excretion as a result of SGLT2 dysfunction is a rare event, even in a population at risk for diabetes. Thus, from a mechanistic point of view, the nominal associations observed in this study point in the direction of statistical type-1 errors. Further studies to confirm the association would be needed to clarify this point.

Even though functional studies on the impact of the tested SNPs on protein function and/or gene expression are lacking, our clear-cut negative results in the cross-sectional setting, as well as on the therapeutic response to empagliflozin, question the clinical relevance of common genetic variations in the *SLC5A2* gene. It has been reported that SGLT1 can, at least partly, compensate for SGLT2 defects limiting renal glucose excretion [24,25]. Thus, it is conceivable that upregulation of SGLT1 may have masked possible effects of the common *SLC5A2* SNPs. The realization of such a compensatory mechanism would, however, not increase the clinical relevance of the *SLC5A2* SNPs.

Limitations of the study are the retrospective nature of the analyses and the lack of 24-h urinary glucose excretion measurements, which represent the most sensitive means to assess the pharmacodynamic effects of *SLC5A2* SNPs [26]. An additional point is that our analyses do not cover 100% of the common genetic variation present in the *SLC5A2* locus, and rs11646054, a common SNP that was also not analyzed in the study by Enigk *et al.* [8], may be of pharmacogenetic relevance. Empagliflozin was the only SGLT2 inhibitor tested. Therefore, it is unknown whether these negative observations are limited to empagliflozin or can be considered a class effect. The strength of our analyses is the use of two unrelated studies to investigate the potential effects of genetic variability of *SLC5A2*: a well-phenotyped cross-sectional study of subjects at increased risk for type 2 diabetes and a pool of patients with type 2 diabetes from well-matched phase III studies. A longitudinal follow-up study is needed and would further strengthen our findings of a lack of association with the SNPs investigated.

Conclusion

We provide the first evidence that common genetic variants in the *SLC5A2* gene do not affect diabetes-related metabolic traits in subjects at increased risk of type 2 diabetes or have a clinically relevant impact on the treatment response to the SGLT2 inhibitor empagliflozin in patients with type 2 diabetes.

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H.Z., A.H., and H.S. were involved in planning the studies, analyzed the data from the cross-sectional study, and wrote the manuscript. M.B. and L.B. carried out the statistical analysis of the pharmacogenetic study and reviewed/edited the manuscript. U.C.B., H.J.W., and H.U.H. contributed

toward the pharmacogenetic study design and interpretation of data, and reviewed/edited the manuscript.

Conflicts of interest

A.H. is an employee of Eli Lilly and Company. H.Z., M.B., L.B., U.C.B. and H.J.W. are employees of Boehringer Ingelheim. H-U.H. is an advisory board member for Daiichi-Sankyo, Sanofi, Boehringer Ingelheim, and Roche. H.S. is an advisory board member for Boehringer Ingelheim.

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