Genetic Variation in Sodium-glucose Cotransporter 2 and Heart Failure

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Inhibition of sodium-glucose cotransporter 2 (SGLT2) represents an emerging pharmaceutical approach for the treatment of heart failure. The mechanisms by which SGLT2 inhibitors reduce the risk of heart failure are not well understood. The objective of this study was to investigate the association between single nucleotide polymorphisms (SNPs) in the *SLC5A2* gene, encoding SGLT2, and heart failure, and to assess potential mediators of this association. Regression and mediation analyses were conducted with individual participant data of the UK Biobank (n = 416,737) and validated in the cardiovascular high-risk cohort of the LUdwigshafen RIsk and Cardiovascular Health study (LURIC; n = 3316). Two intronic SNPs associated with *SLC5A2* expression were included in a genetic score, which was associated with lower risk of heart failure in UK Biobank (odds ratio 0.97, 95% confidence interval, 0.95–0.99, P = 0.016). This association was also present in participants without type 2 diabetes or coronary artery disease (CAD). The associations of the genetic score with HbA1c, high-density lipoprotein cholesterol, uric acid, systolic blood pressure, waist circumference, and body composition mediated 35% of the effect of the score on heart failure risk. No associations of the genetic SGLT2 score with atherosclerotic cardiovascular disease outcomes or markers of volume status were observed, which was confirmed in the LURIC study. Variations in the gene encoding SGLT2 were associated with the risk of prevalent or incident heart failure. This association was mediated by several mechanisms and did not depend on the presence of type 2 diabetes or previous CAD events.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The mechanisms by which sodium-glucose cotransporter 2 (SGLT2) inhibitors reduce the risk of heart failure are not fully understood. Associations of genetic variants of the SGLT2-encoding gene with heart failure have not been described.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Associations between genetic variants of the SGLT2-encoding gene and heart failure and mediators of this association were determined. Furthermore, the effect of the variants on heart failure risk was investigated in subgroups with and without type 2 diabetes and coronary artery disease (CAD).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ For the first time, an association between genetic variance in SGLT2 and heart failure is described. This association was present in study participants without CAD or type 2 diabetes, and appears to be mediated by distinct metabolic changes, which contributes to the understanding of the mechanism of action of SGLT2 inhibitors.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The results of this study may stimulate further research regarding the mechanism of action of SGLT2 inhibitors in heart failure and beyond diuretic effects.

INTRODUCTION

The pharmacologic effects of inhibiting the sodium-glucose cotransporter 2 (SGLT2) remain incompletely understood. SGLT2 inhibitors have initially been introduced for the treatment of type 2 diabetes.[1] The first cardiovascular outcome trial of SGLT2 inhibitors, EMPA-REG OUTCOME,[2] showed an unexpected reduction in heart failure hospitalizations under treatment with empagliflozin. Studies with canagliflozin[3] and dapagliflozin[4] yielded similar results. In meta-analyses of these trials, the effects of SGLT2 inhibitors on atherosclerotic

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cardiovascular disease (ASCVD) appear moderate; the effect on heart failure is, however, robust and consistent among all subgroups.[5] In the DAPA-HF[6] and the EMPEROR-Reduced[7] trials of patients with heart failure with reduced ejection fraction, significant reductions of worsening heart failure or cardiovascular death were shown under treatment with dapagliflozin and empagliflozin, respectively, which were independent of the presence of type 2 diabetes.[8]

Uncertainty remains regarding the effects of SGLT2 inhibitors on ASCVD outcomes.[9] Furthermore, although the DAPA-HF and the EMPEROR-Reduced trials imply efficacy of SGLT2 inhibitors in heart failure in the absence of type 2 diabetes, this conclusion is based on subgroup analyses.[6,7] Additionally, the proposed mechanisms by which SGLT2 inhibitors reduce the risk of heart failure—including diuretic, metabolic, and effects on the vasculature and the left ventricle[10,11]—are not completely understood.

By using single nucleotide polymorphisms (SNPs) in the *SLC5A2* gene, encoding SGLT2, as proxies for pharmacologic SGLT2 inhibition, the effects of random allocation to these genetic variants were investigated in the UK Biobank.[12] Metabolic in-depth characterization and validation in a cardiovascular high-risk cohort were performed in the LUdwigshafen RIsk and Cardiovascular Health study (LURIC).[13] The objectives of this study were to (1) establish an association between genetic variants at *SLC5A2* and the risk of heart failure, (2) to investigate whether this association is affected by the presence of type 2 diabetes and coronary artery disease (CAD), and (3) to assess potential mechanisms that mediate the reduction of heart failure risk by treatment with SGLT2 inhibitors.

METHODS

Study populations, outcomes, and selection of genetic variants

The UK Biobank is a prospective study, which recruited over 500,000 individuals aged 40–69 years in the United Kingdom between 2006 and 2010 from the general population.[12] In this analysis, only participants who self-reported being of White ancestry were included. Participants with missing values for one of the principal components of ancestry, SNPs in *SLC5A2*, HbA1c, or body mass index (BMI) were excluded.

The LURIC study is a prospective study, which recruited 3,316 White participants in south-west Germany hospitalized for coronary angiography between 1997 and 2000 with extensive metabolic and phenotypic characterization and median follow-up of 9.9 years.[13] Patients with acute medical conditions other than acute coronary syndrome, chronic noncardiac diseases, or malignancies within the last 5 years were excluded. In contrast to the UK Biobank, the LURIC study represents a cohort with high cardiovascular risk and an extremely deep phenotyping, including several markers of glucose metabolism and NT-proBNP, which are not available in the UK Biobank. The majority (77.9%) of participants in LURIC had CAD.

Details on genotyping, laboratory procedures, disease outcomes, and their definition are provided in the **Appendix** and **Table S1**.

All genetic variants within the *SLC5A2* gene with a minor allele frequency > 0.01 in the UK Biobank were considered as potential instruments to randomly allocate participants to groups with different magnitudes of SGLT2 expression. SNPs were selected if they were associated with *SLC5A2* expression with at least nominal statistical significance ($P < 0.05$) in any tissue type according to data of the genotype-tissue expression project[14] and were not in linkage disequilibrium ($r^2 < 0.2$). SNPs were selected stepwise, beginning with the SNP with the strongest association with *SLC5A2* expression (**Figure S1**). Assuming an additive model, a genetic SGLT2 score was calculated for each study participant in the UK Biobank and the LURIC study by adding the number of inherited alleles of either selected SNP that were associated with lower expression of SGLT2 (i.e., for 2 selected SNPs, the score ranges between 0 and 4).

Mediation and sensitivity analyses

Mediation analyses were conducted in analogy to a mediation analysis of the EMPA-REG OUTCOME trial.[15] To be considered a mediator of a treatment effect, a variable must be affected by the treatment over time (using inheritance of the selected genetic variants as a proxy), and the change in the variable must affect the outcome (heart failure).[16] Furthermore, in an analysis with the potential mediator as covariate, the reduction in risk due to the treatment must be lower compared with the unadjusted analysis, if the effect is partly mediated by the covariate. The proportion of mediation can be calculated by the difference of the natural logarithm of the unadjusted odds ratio (OR) of the treatment effect minus the natural logarithm of the OR of the treatment effect with the potential mediator as covariate, divided by the natural logarithm of the unadjusted OR of the treatment effect and multiplied by 100.[15]

Continuous traits that were associated with the genetic SGLT2 score at $P < 0.10$ in the UK Biobank were included in the mediation analysis, which therefore should be interpreted as exploratory. In multivariable models, in a stepwise procedure, potential mediators of biologically nonredundant categories were added, beginning with the parameters that explained the largest proportion of mediation, to explore the overall proportion of reduction in risk of heart failure explained by these parameters. All analyses were adjusted for age, gender, BMI, and the first five principal components of ancestry.

As sensitivity analyses, we constructed genetic scores with the selection of genetic variants based on the variants' association with log-transformed HbA1c, representing one major effect of SGLT2 inhibitors (i.e., lowering of blood glucose). The association between every genetic variant and log-transformed HbA1c was measured using linear regression, adjusting for age, gender, BMI, the first five principal components of ancestry, and treatment with insulin. The allele associated with lower HbA1c was defined as exposure allele. In a stepwise procedure, variants with the strongest associations with HbA1c were included in the genetic score if they were not in linkage disequilibrium ($r^2 < 0.2$) with any previously included variant (**Figure S1**). Two scores were calculated for every study participant: for the first score, the number of alleles associated with lower HbA1c were added (unweighted score); and for the second score, the alleles associated with lower HbA1c were multiplied with the conditional association of the variant with HbA1c and then added (weighted score). The weighted score was multiplied by 500 in order to produce effect estimates of comparable magnitude as the unweighted score.

Statistical methods

Associations between the genetic variants and continuous traits were investigated with linear regression, and associations with disease outcomes with logistic regression. All regression analyses were adjusted for age, gender, and BMI in LURIC and additionally for the first five principal components of ancestry in the UK Biobank. Regression analyses of body weight were adjusted for height instead of BMI. HbA1c, NT-proBNP, CRP, lipoprotein(a), and triglycerides were log-transformed before any analyses. In LURIC, 0.1 mg/dL was added to lipoprotein(a) before transformation, as zero values occurred.

Subgroup analyses were conducted by stratifying the population by the presence of type 2 diabetes or CAD. Subgroups were compared by (1) a z test and (2) by introducing an interaction term in the regression analyses (outcome multiplied by the genetic SGLT2 score).

Hardy-Weinberg equilibrium was assessed with the χ^2 test.

The confidence intervals (CIs) for the proportion of mediation were calculated with the percentile method using a bootstrap resampling procedure based on 1,000 bootstrap samples equally sized as the original dataset.

Two-sided P values < 0.05 were considered statistically significant. In analyses with multiple tests of association, the Bonferroni-corrected thresholds for statistical significance are provided. All analyses were performed with Stata IC (version 15; StataCorp, College Station, TX, USA), or R (version 3.6.2, package *meta*; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

An overview of the data sources and analyses is provided in **Figure 1**.

Of the 459,322 participants of White ancestry in the UK Biobank, 416,737 had no missing values and represented the study cohort. The mean age was 65.2 years, 45.5% were men, and 1.8% (n = 7,463) had a diagnosis of heart failure.

In the LURIC study, data of 3,316 participants were analyzed. The mean age was 62.7 years, 69.7% were men, 32.8% (n = 1086) had heart failure, and the median NT-proBNP was 293 pg/mL.

The baseline characteristics are shown in **Table 1** and **Table S2**.

Association of SNPs in *SLC5A2* with *SLC5A2* expression, heart failure, and NT-proBNP

In the UK Biobank, 98 SNPs within the *SLC5A2* gene were identified, of which 7 had a minor allele frequency of > 0.01. In the UK Biobank and the LURIC study, these 7 SNPs were in Hardy–Weinberg equilibrium (P > 0.001 for all, P > 0.05 for all except rs3116150 in LURIC (P = 0.017)).

Of the 7 SNPs, 3 were strongly associated with the expression of *SLC5A2* (rs11646054 and rs3116150 in the tibial artery, rs9934336 in the lungs; **Table 2**; **Figure S1**). Although *SLC5A2* is mainly expressed in the kidneys, none of the SNPs was associated with *SLC5A2* expression in kidney tissue.

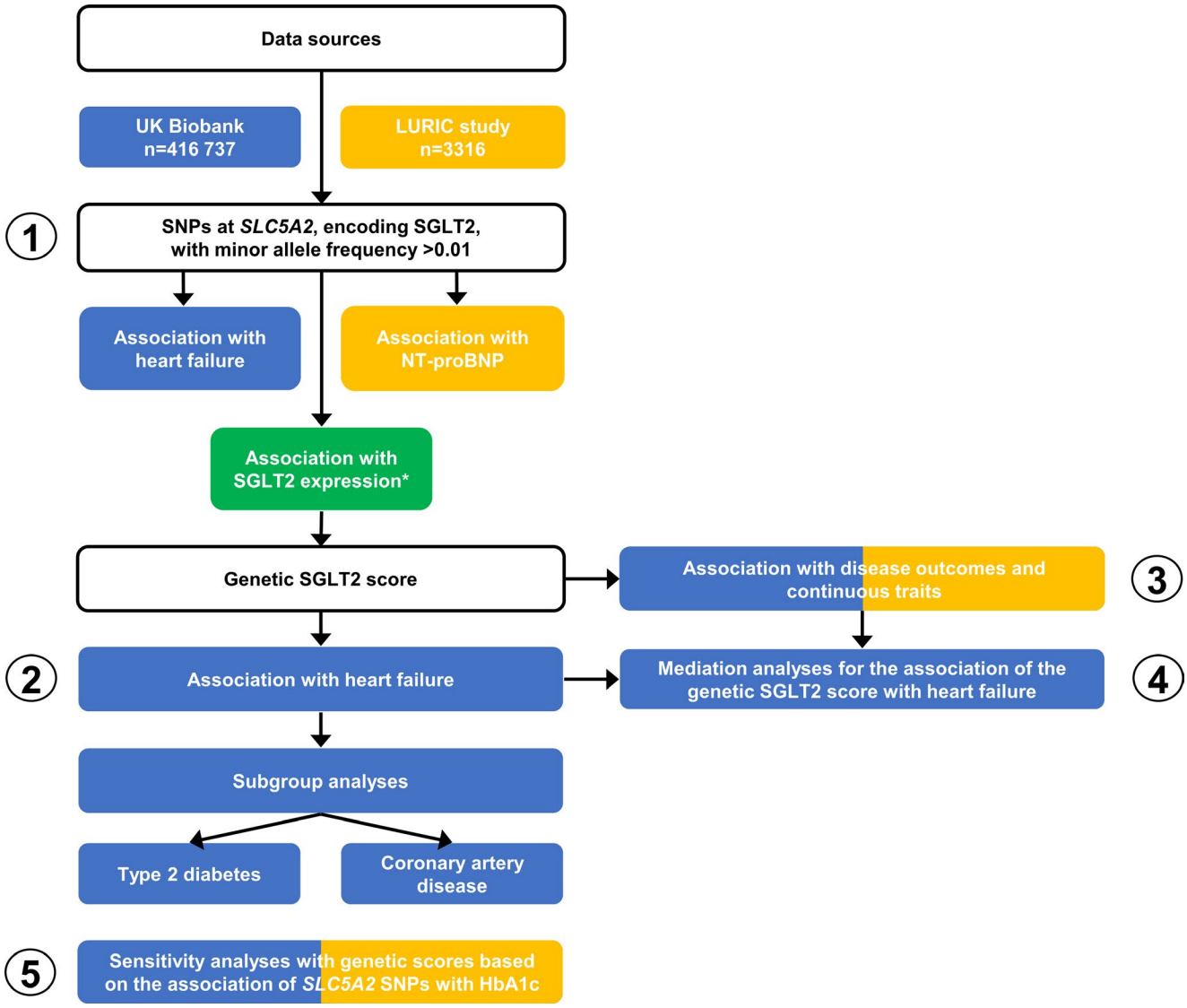

Figure 1 Data sources and analyses. * Data from the GTEx project.[14] GTEx, genotype-tissue expression; LURIC, LUdwigshafen RIsk and Cardiovascular Health study; SGLT2, sodium-glucose cotransporter 2; SNP, single nucleotide polymorphism.

Table 1 Baseline characteristics

	UK Biobank	LURIC
General		
N	416,737	3,316
Male	45.5 (189,822)	69.7 (2,310)
Age, years	65.2 ± 8.0	62.7 ± 10.6
Clinical characteristics		
Systolic blood pressure, mmHg	137.8 ± 18.6	141.1 ± 23.6
Diastolic blood pressure, mmHg	82.0 ± 10.1	81.0 ± 11.5
Body mass index, kg/m^2	27.4 ± 4.7	27.5 ± 4.1
Blood pressure-lowering medication	20.4 (85,097)	86.8 (2,879)
Lipid-lowering medication	17.0 (70,918)	48.6 (1,610)
Glucose-lowering medication	1.0 (4,181)[a]	12.4 (412)
Medical history		
Type 2 diabetes	6.3 (26,227)	39.9 (1,324)
Myocardial infarction	4.0 (16,747)	41.3 (1,370)
Coronary artery disease[b]	5.0 (20,674)	77.9 (2,583)
Heart failure	1.8 (7,463)	32.8 (1,086)
HFpEF	–	15.3 (506)
HFrEF	–	17.5 (580)

Plus-minus values are means ± SD, other values are % (*n*).
LURIC, LUdwigshafen RIsk and Cardiovascular Health study. HFrEF/HFpEF, Heart failure with reduced/preserved ejection fraction.
[a] Includes only treatment with insulin.
[b] Defined as composite of myocardial infarction, coronary revascularization, and coronary death in the UK Biobank, and the presence of a 20% stenosis in at least one of 15 coronary segments in the LURIC study.

Rs9934336 exhibited the strongest association with *SLC5A2* expression ($P = 2.8*10^{-27}$). As rs9934336 and rs11646054 were in linkage disequilibrium ($r^2 = 0.272$), rs11646054 was not selected for further analyses. The genetic SGLT2 score was calculated using rs9934336 and rs3116150 ($r^2 = 0.106$; both intronic variants).

Four of the 7 SNPs with minor allele frequency > 0.01 were associated with the diagnosis of heart failure at $P \leq 0.05$ in the UK Biobank. Rs11646054 and rs9934336 were significantly associated with lower NT-proBNP in LURIC ($P < 0.001$ and $P = 0.016$, respectively) in participants without CAD (**Table 2**; for all LURIC participants: **Table S3**).

Heart failure in subgroups by type 2 diabetes and CAD

Per inherited allele of the genetic SGLT2 score, the risk of heart failure was significantly lower in the UK Biobank with an OR of 0.97 (95% CI, 0.95–0.99, $P = 0.016$).

The association of the SGLT2 score with heart failure was stronger in participants without CAD (0.95 (95% CI, 0.92–0.98), $P = 0.002$), but was neutral in participants with CAD (1.00 (95% CI, 0.97–1.04), $P = 0.832$). The *z* test indicated statistically significant differences in the reduction of heart failure comparing participants with vs. without CAD ($P = 0.032$), as did the interaction term analysis ($P = 0.021$).

The association of the SGLT2 score with heart failure reached statistical significance in participants without type 2 diabetes (0.97 (95% CI, 0.94–0.99), $P = 0.017$), but did not in those with type 2 diabetes (0.99 (95% CI, 0.95–1.04), $P = 0.816$). The *z* test and the interaction term analysis did not indicate statistically significant differences between participants with vs. without type 2 diabetes ($P = 0.330$ and $P = 0.316$, respectively; **Figure 2**).

Association of the genetic SGLT2 score with disease outcomes and continuous traits

In the UK Biobank, there were no significant associations of the genetic SGLT2 score with any class of ASCVD (myocardial infarction, stroke, and peripheral artery disease), chronic kidney disease, or with the risk of cardiovascular or all-cause death (**Figure 3**). This observation was confirmed in the LURIC study (**Figure S2**). In the UK Biobank, there was a nominally significant association of the SGLT2 score with reduced risk of type 2 diabetes (OR 0.98, 95% CI, 0.97–1.00, $P = 0.018$), and an increased risk for deep vein thrombosis (1.03, 95% CI, 1.01–1.05, $P = 0.003$; **Figure 3**).

The genetic SGLT2 score was associated with lower HbA1c in the UK Biobank ($P < 0.001$) and the LURIC study ($P = 0.038$), respectively. Several markers of glucose metabolism were nominally significantly reduced or tended to be decreased in the LURIC study (fasting glucose ($P = 0.095$), glucose after oral glucose tolerance test (after 1 hour and 2 hours: $P = 0.074$ and $P = 0.023$), insulin ($P = 0.083$), proinsulin ($P = 0.017$), and homeostasis model assessment index ($P = 0.016$)). In the UK Biobank, the SGLT2 score was associated with higher high-density lipoprotein (HDL) cholesterol ($P = 0.013$), a trend toward lower triglycerides ($P = 0.086$), lower uric acid ($P = 0.017$), lower waist circumference ($P = 0.003$), higher systolic blood pressure, and lower body fat percentage and body impedance ($P < 0.001$ for all). In neither study, markers of renal function or volume status (hemoglobin, hematocrit, total protein, and albumin) were associated with the SGLT2 score (**Table S4**).

Mediation analyses

Of the associations of the SGLT2 score with continuous traits in the UK Biobank, those with $P < 0.10$ were included in the mediation analyses (**Table S5**). All included parameters were observationally associated with heart failure at $P < 0.001$, with each association being directionally opposite to the association of the SGLT2 score with the respective parameter. HbA1c mediated 11.9% (95% CI, 3.7–47.1%) of the effect of the SGLT2 score on heart failure, HDL cholesterol 10.3% (1.2–30.8%), uric acid 10.0% (–2.5 to 15.4%), and triglycerides 8.7% (6.2–11.2%). By adding the parameters with the largest proportion of mediation of each biologically nonredundant category in a multivariable analysis, the effect on heart failure mediated by the changes in HbA1c, HDL cholesterol, uric acid, systolic blood pressure, waist circumference, and body impedance was 35.0% (7.9–155.8%). The results are graphically displayed in **Figure 4**.

Sensitivity analyses

As sensitivity analyses, two genetic scores were constructed by selecting SNPs in *SLC5A2* according to their association with

Table 2 Associations of SNPs in *SLC5A2* with *SLC5A2* expression, heart failure, and NT-proBNP

SNP	EA	NEA	EAF	*P* value eQTL for *SLC5A2* (tissue; normalized effect size)[a]	OR heart failure (95% CI)	*P* value heart failure	Coefficient NT-proBNP (95% CI)[b]	*P* value NT-proBNP
rs11646054	G	C	0.59	$9.1*10^{-14}$ (tibial artery; −0.37)	0.965 (0.933–0.997)	0.034	−0.29 (−0.45 to −0.13)	< 0.001
rs144413428	A	G	0.02	–	0.875 (0.759–1.009)	0.066	−0.03 (−0.83 to 0.78)	0.950
rs3116149	A	G	0.05	–	0.960 (0.892–1.033)	0.277	−0.36 (−0.75 to 0.04)	0.082
rs3116150	G	A	0.76	$1.5*10^{-9}$ (tibial artery; −0.35)	0.962 (0.926–0.999)	0.047	−0.16 (−0.40 to 0.09)	0.204
rs3813007	A	T	0.99	–	0.857 (0.736–0.998)	0.047	−0.69 (−1.44 to 0.05)	0.069
rs3813008	A	G	0.15	–	0.956 (0.912–1.001)	0.056	−0.13 (−0.36 to 0.11)	0.287
rs9934336	A	G	0.28	$2.8*10^{-27}$ (lung; −0.52)	0.964 (0.929–1.000)	0.050	−0.22 (−0.39 to 0.04)	0.016

CI, confidence interval; EA, effect allele; EAF, effect allele frequency; eQTL, expression quantitative trait locus; NEA, other allele; OR, odds ratio; SNPs, single nucleotide polymorphisms.
SNPs in *SLC5A2* with minor allele frequency > 0.01 in the UK Biobank are shown. The association with heart failure was investigated in the UK Biobank, the association with NT-proBNP in LURIC study participants without coronary artery disease (n = 733). Data on *SLC5A2* expression were obtained from the genotype-tissue expression project. The effect allele was defined as the allele associated with lower SGLT2 expression or if not available, with lower risk of heart failure.
[a]The lowest *P* value for each SNP as eQTL for *SLC5A2* in any tissue with the respective tissue type is provided. [b]Given as natural logarithm.

HbA1c (**Figure S1**, **Table S6**). Four SNPs were combined in an unweighted and weighted score.

The results of the sensitivity analyses are shown in **Figures S3–S5** and **Tables S7–S9**. Overall, the sensitivity analyses produced directionally similar results as the main analyses, however, the association between the scores and heart failure was weaker, making mediation analyses with the weighted score not reasonable. Of note, the mediation analyses indicated a larger proportion of the effect of the unweighted SGLT2 score on heart failure being mediated by HbA1c. However, this has to be interpreted with caution as the genetic variants were selected due to their association with HbA1c, and the score was not significantly associated with the risk of heart failure.

DISCUSSION

The study shows an association between genetic variants in the gene encoding SGLT2 and the risk of heart failure. This association was present in participants without type 2 diabetes or previous CAD events, and was mediated by different metabolic and physiological traits. The genetic variants were associated with several markers of glucose metabolism and NT-proBNP. No associations with ASCVD events were observed in two independent cohorts with low and high cardiovascular risk. Our findings have several potential implications.

Genetic variation in SGLT2 and heart failure

First, the association of genetic variants at *SLC5A2* with heart failure strengthens the pathophysiologic rationale to use SGLT2 inhibitors as adjunctive pharmacologic principle in the treatment of heart failure. Because inherited traits result in lifelong exposure to the associated changes, all heart failure cases of this study have to be considered incident cases. Therefore, the results of this study support the findings of the cardiovascular outcomes trials of empagliflozin,[2] canagliflozin,[3] and dapagliflozin,[4] in which the majority of participants did not have heart failure at baseline. Furthermore, our study suggests that the effect of SGLT2 inhibitors on heart failure is at least in part mediated by mechanisms involving SGLT2. Ongoing clinical studies will show whether treatment with SGLT2 inhibitors indeed results in long-term benefits beyond the timeframe of a randomized clinical trial, and whether the reduction in heart failure risk is a class effect of SGLT2 inhibitors.[17–20]

The missing associations between the SNPs included in the genetic SGLT2 score and *SLC5A2* expression in the kidneys may be due primarily to limited availability of data with only 85 available samples from the kidney cortex.[14] In contrast, in the tissue types where associations of the SNPs with *SLC5A2* expression were found, the sample size was considerably larger with n = 578 in the lungs and n = 663 in the tibial artery.

Genetic variation in SGLT2 is associated with heart failure independently of type 2 diabetes

Second, our study shows that the association of genetic variants with heart failure is independent of the presence of type 2 diabetes. In subgroup analyses of the DAPA-HF and the EMPEROR-Reduced trials, patients with and without type 2 diabetes had comparable reductions of the primary outcome of worsening heart failure or cardiovascular death.[6–8] The association of genetic

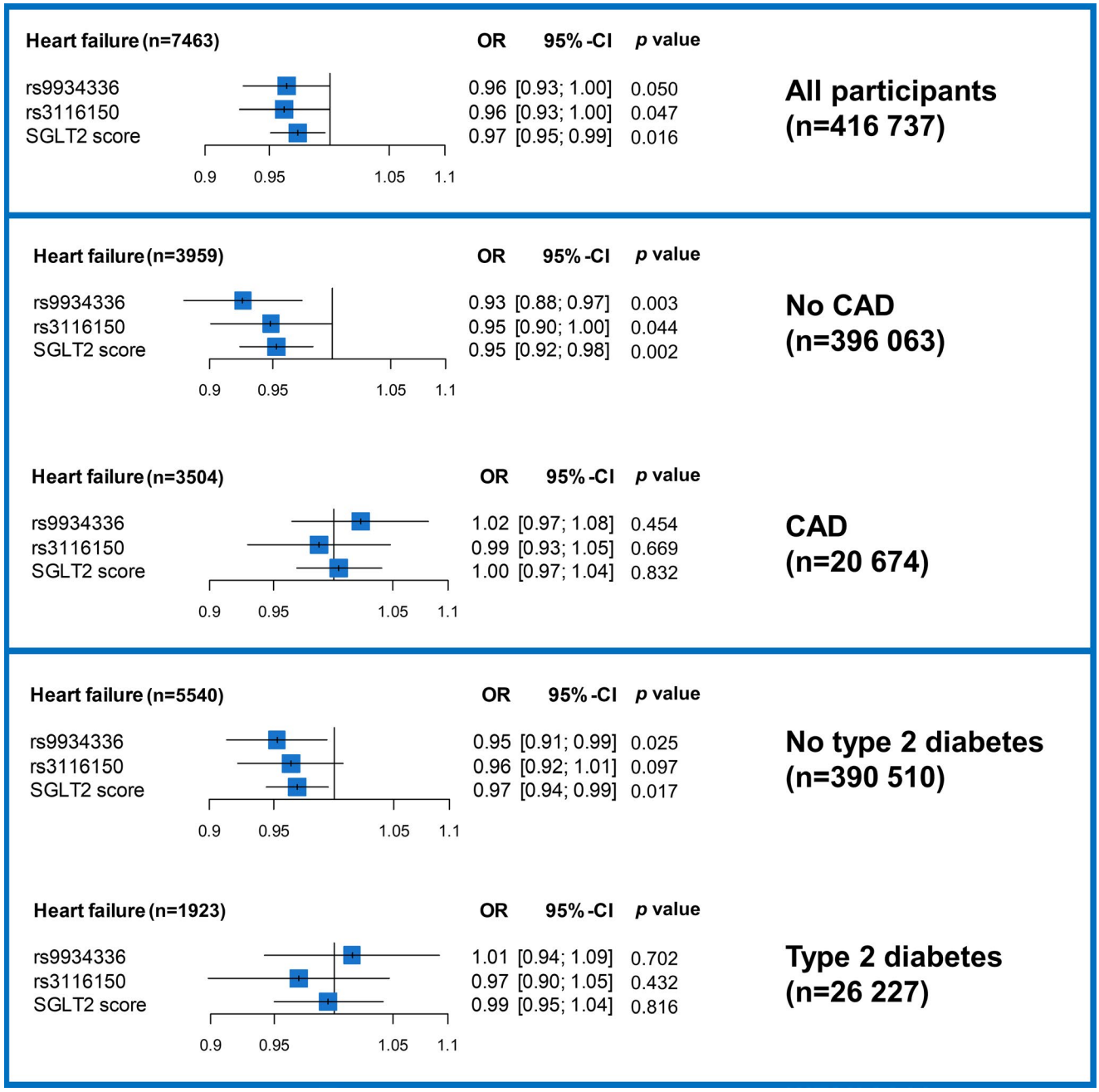

Figure 2 Association of the genetic sodium-glucose cotransporter 2 (SGLT2) score with heart failure. Shown are the associations of the genetic SGLT2 score and the included single nucleotide polymorphisms (SNPs) with heart failure in the UK Biobank for all participants and stratified by coronary artery disease (CAD) and type 2 diabetes.

variation in SGLT2 and heart failure in patients without type 2 diabetes implies that the mechanisms that lead to a lower risk of heart failure also occur in normoglycemic individuals.

Genetic variants in *SLC5A2* are not associated with ASCVD events

Third, several metabolic and physiological pathways mediated the association of the genetic variants in *SLC5A2* and heart failure. The genetic SGLT2 score was associated with lower risk of heart failure in participants without CAD. The score was not associated with ASCVD events in study participants with low or high cardiovascular risk. These findings may indicate that a reduction of macrovascular atherosclerotic events is not primarily responsible for the observed effects of SGLT2 inhibitors on heart failure. The missing association between the genetic variants in *SLC5A2* with heart failure or NT-proBNP in study participants with documented CAD is most likely due to the comparably weak effects of the genetic variants compared with the strong impact of prevalent CAD on the occurrence and progression of heart failure. In contrast, the

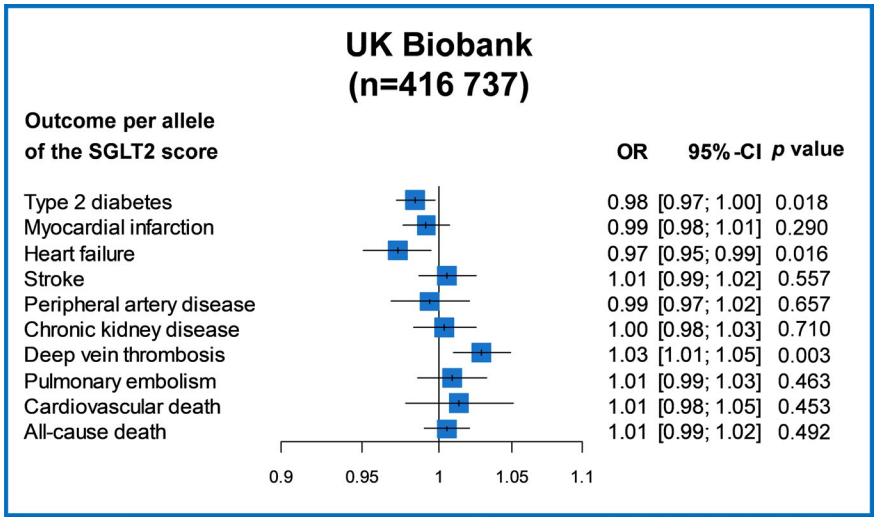

Figure 3 Associations of the genetic sodium-glucose cotransporter 2 (SGLT2) score with outcomes in the UK Biobank. The Bonferroni-corrected threshold for statistical significance is 0.05/10 = 0.005.

much more potent inhibition of SGLT2 by pharmacological interventions in the clinical trials was able to mediate benefits on heart failure outcomes in patients with CAD.[5]

A mediation analysis of the EMPA-REG OUTCOME trial suggested that the reduction of cardiovascular death was mainly mediated by markers that reflect changes in plasma volume (i.e., hematocrit and hemoglobin).[15] Furthermore, empagliflozin causes natriuresis with a potentially favorable diuretic profile in the short term.[21] However, the genetic SGLT2 score was not associated with the same markers of plasma volume that explained the largest part of mediation in the mentioned mediation analysis of the EMPA-REG OUTCOME trial.[15] Potential explanations for this

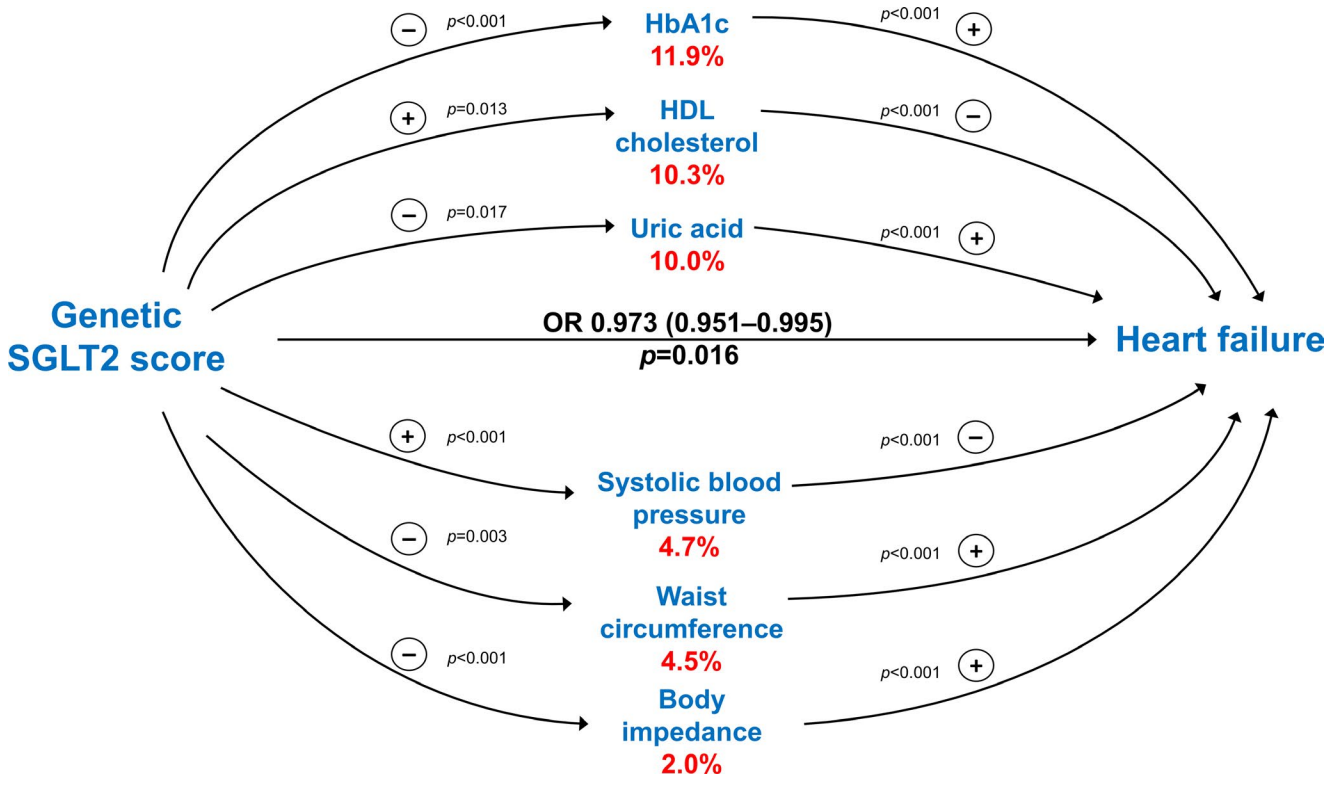

Figure 4 Mediation analyses. The given percentage below each potential mediator indicates the proportion by which heart failure is reduced due to changes of the respective mediator. "−" denotes decrease, "+" denotes increase. *P* values on the left side are obtained from linear regression, and *P* values on the right side from logistic regression of heart failure with the potential mediator as independent variable.

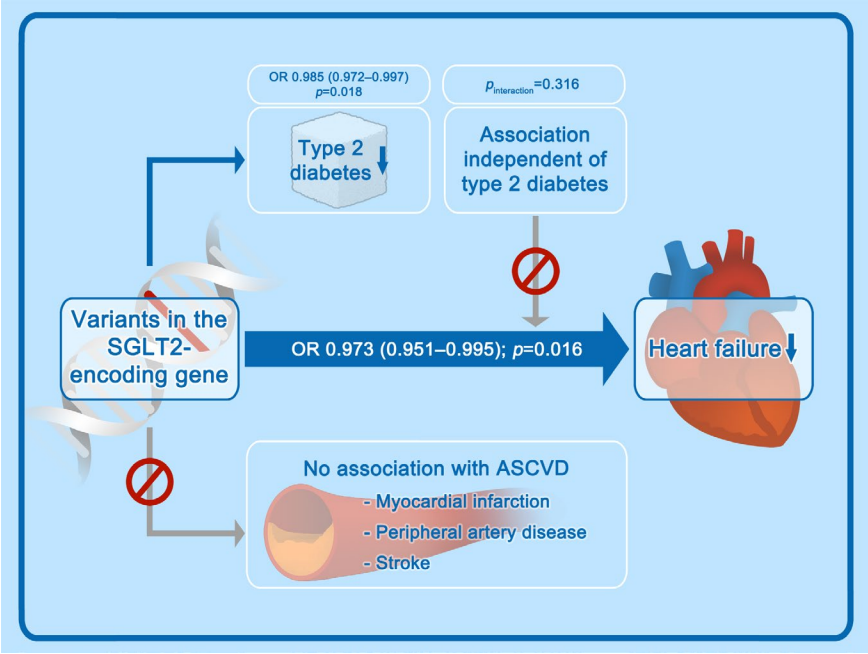

Figure 5 Genetic variation in SGLT2 and heart failure. Genetic variants in *SLC5A2*, encoding sodium-glucose cotransporter 2 (SGLT2), are associated with lower risk of type 2 diabetes and heart failure. The association with lower risk of heart failure is independent of the presence of type 2 diabetes. The genetic variants are not associated with atherosclerotic cardiovascular disease (ASCVD) outcomes. This figure was created by Isabell Katzmann.

discrepancy include developmental compensation and the comparably modest effects of the genetic variants as compared with the relatively strong effects of pharmacologic SGLT2 inhibition.

The three mediators, HbA1c, HDL cholesterol, and uric acid, explained 25.8% of the association between the genetic SGLT2 score and the risk of heart failure. Although type 2 diabetes increases the probability of developing heart failure, a reduction of HbA1c is not consistently associated with a reduction of cardiovascular events or heart failure in clinical trials.[22] The effect on heart failure mediated by HbA1c may therefore reflect mechanisms other than the development and progression of atherosclerosis, such as changes in myocardial fuel utilization, as shown in a porcine nondiabetic model.[22,23] It can only be speculated on the potential underlying mechanisms mediated by HDL cholesterol and uric acid. Interventions that increase HDL cholesterol[24–27] or lower uric acid[28] have failed to show a cardiovascular benefit to date.

Most of the metabolic and physiological changes associated with the genetic SGLT2 score were aligned with the changes induced by treatment with SGLT2 inhibitors[2] (i.e., lower HbA1c, higher HDL cholesterol, lower uric acid, and, indicating lower visceral adiposity, lower waist circumference and body fat percentage). However, whereas systolic blood pressure decreases with SGLT2 inhibitor treatment, genetic variation in SGLT2 led to higher systolic blood pressure. This effect remained directionally consistent when the analysis was restricted to participants without blood pressure-lowering medication. The underlying mechanism remains elusive. The lack of association of the genetic variants with parameters reflecting diuretic effects may be part of the explanation, as the blood pressure reduction with pharmacologic SGLT2 inhibition may be in part due to diuresis.

Last, the association of the genetic SGLT2 score with an increased risk of deep vein thrombosis may warrant surveillance of patients treated with SGLT2 inhibitors in this regard. A meta-analysis of randomized trials with SGLT2 inhibitors found no increased risk of venous thromboembolic events[29]; however, the currently available follow-up period of patients treated with SGLT2 inhibitors is limited.[2–4]

Limitations

First, the associations of the genetic variants with metabolic and physiological measures did align with the changes observed under SGLT2 inhibitor therapy with the exception of systolic blood pressure, which may have limited their use as instrumental variables. Second, the calculation of the genetic SGLT2 score was based on the assumption of additive effects of the genetic variants on *SLC5A2* expression referring to the genotype-tissue expression project data, but neither gene expression nor protein function have been measured in our study. Third, our results only apply to the consequences of SGLT2 inhibition, and not to potential off-target effects of SGLT2 inhibitors. Fourth, the association of the SGLT2 score with heart failure in the UK Biobank could neither be replicated in the LURIC study nor in the independent dataset of the HERMES consortium.[30] Future replication is wanted to support the validity of our findings. Fifth, the calculated contribution of the main mediators on heart failure was 35%, with the majority of mechanisms therefore not being reflected in our mediation analyses. Sixth, our results only apply to populations of European descent. Last, the SNPs included in the genetic SGLT2 score are also associated with the expression of the *TGFB1I1* gene, however, pathways involving the encoded protein transforming growth factor beta 1 induced transcript 1, which

could possibly confound the association between the *SLC5A2* variants and heart failure are not completely understood.[31]

CONCLUSIONS

In conclusion, we found an association between genetic variants in the *SLC5A2* gene, encoding SGLT2, and heart failure, which strengthens the role of SGLT2 inhibitors as adjunctive pharmacologic principle in the prevention and treatment of heart failure independently of type 2 diabetes. This association was mediated by different metabolic and physiological changes, and the genetic variants were not associated with ASCVD events (**Figure 5**).

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

J.L.K. wrote the manuscript. J.L.K. and U.L. designed the research. J.L.K., A.M.M., W.M., M.E.K., A.N., M.B., T.S., and U.L. performed the research. J.L.K., A.M.M., W.M., and M.E.K. analyzed the data.

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