Variation in the glucose transporter gene SLC2A2 is associated with glycemic response to metformin

Kaixin Zhou1,46, Sook Wah Yee2,46, Eric L Seiser3, Nienke van Leeuwen4, Roger Tavendale1, Amanda J Bennett5, Christopher J Groves5, Ruth L Coleman6, Amber A van der Heijden7, Joline W Beulens8,9, Catherine E de Keyser10, Linda Zaharenko11,12, Daniel M Rotroff13,14, Mattijs Out15,16, Kathleen A Jablonski17, Ling Chen18, Martin Javorsky19, Jozef Židzik19, Albert M Levin20, L Keoki Williams21,22, Tanja Dujic1,23, Sabina Semiz23,24, Michiaki Kubo25, Huan-Chieh Chien2, Shiro Maeda26,27, John S Witte28–31, Longyong Wu28, Ivan Tkáč19, Adriaan Kooy15,16, Ron H N van Schaik32, Coen D A Stehouwer33, Lisa Logie1, MetGen Investigators34, DPP Investigators34, ACCORD Investigators34, Calum Sutherland1, Janis Klovins11,12, Valdis Pirags12,35,36, Albert Hofman10, Bruno H Stricker10,37, Alison A Motsinger-Reif13, Michael J Wagner38, Federico Innocenti3, Leen M ’t Hart4,8,39, Rury R Holman6, Mark I McCarthy5, L Keoki Williams21,22, Tanja Dujic1,23, Sabina Semiz23,24, Michiaki Kubo25, Huan-Chieh Chien2, Shiro Maeda26,27, John S Witte28–31, Longyong Wu28, Ivan Tkáč19, Adriaan Kooy15,16, Ron H N van Schaik32, Coen D A Stehouwer33, Lisa Logie1, MetGen Investigators34, DPP Investigators34, ACCORD Investigators34, Calum Sutherland1, Janis Klovins11,12, Valdis Pirags12,35,36, Albert Hofman10, Bruno H Stricker10,37, Alison A Motsinger-Reif13, Michael J Wagner38, Federico Innocenti3, Leen M ’t Hart4,8,39, Rury R Holman6, Mark I McCarthy5,40,41, Monique M Hedderson42, Colin N A Palmer1, Jose C Florez18,43–45, Kathleen M Giacomini2,29,47 & Ewan R Pearson1,47

Metformin is the first-line antidiabetic drug with over 100 million users worldwide, yet its mechanism of action remains unclear. Here the MetGen Genetics (MetGen) Consortium reports a three-stage genome-wide association study (GWAS), consisting of 13,123 participants of different ancestries. The C allele of rs8192675 in the intron of SLC2A2, which encodes the facilitated glucose transporter GLUT2, was associated with a 0.17% ($P = 6.6 \times 10^{-14}$) greater metformin-induced reduction in hemoglobin A1c (HbA1c) in 10,377 participants of European ancestry. rs8192675 was the top cis expression quantitative trait locus (cis-eQTL) for SLC2A2 in 1,226 human liver samples, suggesting a key role for hepatic GLUT2 in regulation of metformin action. Among obese individuals, C-allele homozygotes at rs8192675 had a 0.33% (3.6 mmol/mol) greater absolute HbA1c reduction than T-allele homozygotes. This was about half the effect seen with the addition of a DPP-4 inhibitor, and equated to a dose difference of 550 mg of metformin, suggesting rs8192675 as a potential biomarker for stratified medicine.

Metformin was commercialized before the modern era of target-based drug discovery. It typically reduces HbA1c by 1–1.5% (11–16 mmol/mol) and has an excellent safety record, but considerable variation exists in how well patients respond to metformin. We have recently established that genetic factors influence glycemic response to metformin, with many common variants across the genome together explaining a substantial proportion of the variation, ranging from 21% to 34%, depending on how glycemic response had been measured. Hypothesis-driven studies of pharmacokinetic variants have shown no consistent results. The only GWAS published to date showed an association with rs11212617 near the ATM locus, which has been replicated.

Here we extended the previous GWAS by an additional 345 samples to a screening set of 1,373 participants. As in our previous report, rs11212617 remained the top signal with no other genome-wide significant hits (Supplementary Fig. 1). We undertook a systematic three-stage replication, with the workflow shown in Supplementary Figure 2. Only rs8192675 in the intron of SLC2A2 was replicated through the first two stages with a combined $P = 1 \times 10^{-7}$ derived from a linear regression meta-analysis of 3,456 participants (Supplementary Data and Supplementary Table 1).

The MetGen Consortium performed the final replication of rs8192675 as a meta-analysis. Measures of glycemic response to metformin were aligned across the cohorts as the absolute HbA1c reduction (expressed as reduction in percentage of HbA1c). Within each cohort, we tested associations with rs8192675 using two multiple linear models with or without the adjustment of baseline HbA1c, in addition to other available clinical covariates (Supplementary Table 2). In the meta-analysis of 10,557 participants of European ancestry (Fig. 1), each copy of the C allele was associated with a greater HbA1c reduction ($P = 2 \times 10^{-8}$, and Cochran's heterogeneity statistic's $P = 0.35$) when adjusting for baseline HbA1c; without adjustment the allelic effect of the C allele was 0.17% ($P = 6.6 \times 10^{-14}$, $P = 0.52$). There was no effect of rs8192675 on the efficacy of metformin in delaying progression to diabetes, or on metformin efficacy in a small insulin-treated cohort (Supplementary Table 3).

We tested the pharmacogenetic effect of rs8192675 in 2,566 participants of non-European ancestries (Supplementary Table 4). The meta-analysis showed that the C allele was associated with a 0.08% greater HbA1c reduction ($P = 0.006$, $P_{het} = 0.63$) when adjusting for
Given the association of rs8192675 with HbA1c before treatment and the fact that rs8192675 is not associated with sulfonylurea response (是如何rs8192675, rs5400 was not significant (是如何r = 2.3 × 10−9); when conditioning on the association with metformin response (是如何beta = 0.13%; s.e.m. = 0.04%, P = 0.001) in the non-obese participants as compared to that of 0.24% (s.e.m. = 0.04%, P = 5.0 × 10−11) in the obese participants.

We performed a locus-wise meta-analysis to narrow down the candidate causal gene and variant list. Variant rs8192675 and its proxies showed the strongest association with HbA1c reduction (Fig. 3}). The linkage disequilibrium (LD) block covered three genes, of which SLC2A2 encodes the facilitated glucose transporter GLUT2, and EIF5A2 and RPL22L1 have little known functionality. Previous GWAS showed that the nonsynonymous rs5400 in SLC2A2 is the main variant associated with glycemic traits such as fasting glucose and HbA1c18,19. Because rs8192675 and rs5400 are in partial LD (D′ = 1; r² = 0.35), here rs5400 was also associated with metformin response (beta = 0.13%; s.e.m. = 0.04%, P = 5.2 × 10−4). However, when conditioning on rs5400, rs8192675 remained strongly associated with metformin response (beta = 0.21%, s.e.m. = 0.04%, P = 2.3 × 10−9); when conditioning on rs8192675, rs5400 was not significant (P = 0.29}). These results suggest that the pharmacogenetic impact of rs8192675 is unlikely to be via the amino acid change of GLUT2 at rs5400.

Given that liver is the most established site of metformin action, we examined whether rs8192675 is an eQTL in 1,226 liver samples of baseline HbA1c, and without the baseline adjustment the allelic effect of the C allele was 0.15% (P = 0.005, Phet = 0.95). In the meta-analysis of 13,123 participants of any ancestry (data not shown), we observed no genetic heterogeneity (Phet > 0.29) between different ethnic groups despite the frequency of the C allele ranging from 24% in Latino populations to around 70% in African-American populations.

We examined whether rs8192675 had an impact on baseline HbA1c, because the effect sizes of its association with glycemic response to metformin differed depending on whether there was adjustment for the baseline HbA1c. In the 10,557 participants of European ancestry, the C allele was associated with a 0.13% (P = 2.6 × 10−8) higher baseline HbA1c but a 0.04% (P = 0.007) lower on-treatment HbA1c, which together contributed to the observed 0.17% (P = 6.6 × 10−14) pharmacogenetic impact on HbA1c reduction in the model without baseline adjustment (Supplementary Fig. 3}).

Given the association of rs8192675 with HbA1c before treatment with metformin, we assessed whether this variant was marking a general ability to respond to any antihyperglycemic treatment. Therefore we studied the pharmacogenetic impact of rs8192675 in 2,654 participants treated with sulfonylureas (Supplementary Table 5}), another commonly used class of antidiabetic drug13,14. As in metformin users, the C allele was also associated with a higher baseline HbA1c in these users of sulfonylureas (beta = 0.15%, P = 3.1 × 10−4). However, in contrast to the case for users of metformin, the C allele remained associated with a higher on-treatment HbA1c (beta = 0.09%, P = 0.006) in the users of sulfonylureas, which resulted in no net pharmacogenetic impact (beta = 0.04%, P = 0.44) on sulfonylurea-induced HbA1c reduction. These data suggest that rs8192675 is marking a genetic defect in glucose metabolism in type 2 diabetes that is ameliorated by metformin treatment but not by sulfonylurea treatment. The fact that rs8192675 is not associated with sulfonylurea response strongly supports a specific role for this variant on glycemic response to metformin, rather than simply reflecting the higher pretreatment (baseline) HbA1c seen in carriers of this C allele. In addition, the association with metformin-induced HbA1c reduction remained significant (P = 2 × 10−8, Fig. 1) after adjustment for baseline HbA1c, corroborating a specific effect on response beyond its effect on baseline glycemia.

Metformin is particularly recommended for the treatment of diabetes in obese individuals owing to its beneficial effect on body weight15–17. Therefore, we explored whether the pharmacogenetic impact of rs8192675 varied by body mass index (BMI) in the MetGen cohorts (n = 7,581 participants). BMI was associated with HbA1c reduction (beta = −0.01%; P = 1.7 × 10−4) but not rs8192675 genotype (P = 0.52). Adjusting for BMI did not attenuate the observed pharmacogenetic effect of rs8192675 (Supplementary Table 6}). When we stratified participants into nonobese (BMI < 30 kg/m²) and obese (BMI ≥ 30 kg/m²) groups, there was a significant (P = 0.02) gene by BMI group interaction (Fig. 2). The pharmacogenetic effect size of the C allele was 0.13% (s.e.m. = 0.04%, P = 0.001) in the non-obese participants as compared to that of 0.24% (s.e.m. = 0.04%, P = 5.0 × 10−11) in the obese participants.

Figure 1 Pharmacogenetic impact of rs8192675 on metformin response in participants of European ancestry. The forest plots show meta-analyses of association test results for metformin-induced change in HbA1c in a total of 10,557 participants from 10 MetGen cohorts. Results from linear regression models with (left) and without (right) adjustment for baseline HbA1c are presented. The x axis represents the impact on metformin-induced HbA1c reduction of each copy of the C allele. HbA1c was measured in percentage.
European ancestry. In Figure 2 we show rs8192675 as the top cis-eQTL for SLC2A2, with the C allele associated with decreased (P = 4.2 × 10−12) expression. In the 48 tissues examined by the Genotype–Tissue Expression (GTEx) Project, SLC2A2 was sufficiently expressed in seven tissues (Supplementary Table 7). rs8192675 showed a significant (P = 5.7 × 10−4) impact on SLC2A2 expression in the 271 samples of transformed fibroblasts, but no other significant associations. Beyond GTEx, we sought additional eQTL evidence for other tissues that have been implicated in metformin action or glucose homeostasis. We found directionally consistent and supportive evidence of rs8192675 or its proxies being associated with glucose homeostasis in and the differing impact of common GLUT2 variants in various physiological states (Fig. 4). Patients with Fanconi–Bickel syndrome exhibit low fasting glucose but high postprandial glucose. In parallel, the C allele of rs8192675 that is associated with reduced SLC2A2 expression is associated with faster fasting glucose and HbA1c among individuals of normal glycaemia. We found that, in patients with type 2 diabetes, the expression-decreasing C allele of rs8192675 was associated with higher HbA1c before treatment with either metformin or sulfonylureas. This deleterious genetic effect of rs8192675 on HbA1c was reversed with metformin treatment (C allele associated with lower on-treatment HbA1c and therefore better response to metformin), but not by sulfonylurea treatment.

In humans, GLUT2 is a facilitative glucose transporter that is highly produced in the liver, kidney, small intestine and islets, and to a lesser extent in certain brain regions and other tissues. Defects in the GLUT2–encoding gene could potentially alter glucose homeostasis at any or all of these sites. Metformin’s main site of action is widely believed to be the liver, where it primarily acts to suppress hepatic glucose production and lipidogenesis. In mice with GLUT2 inactivation, glucose and glucose-6-phosphate accumulated in the cytoplasm owing to reduced glucose efflux, resulting in increased expression of nuclear ChREBP, 1-pyruvate kinase and lipogenic genes. Our eQTL data in liver samples (Fig. 3) and corresponding reporter assays (Supplementary Fig. 4) showed that the C allele at rs8192675 was associated with lower expression of SLC2A2. This suggests that the variant may lead to similar effects on hepatic gene expression in humans, which will be potentially modulated by metformin’s well-described effect on hepatic glucose production and lipogenesis. An alternative explanation could be that reduced SLC2A2 expression owing to rs8192675 is associated with reduced glucose-mediated glucose clearance (glucose effectiveness) owing to a decreased ability for glucose to enter the liver. This is seen in mice lacking GLUT2 in the liver, and is an effect that is improved by metformin treatment, although the mechanism for this is not understood.

Metformin is also increasingly believed to exert some of its beneficial effects by acting on the intestines to increase gut glucose uptake and non-oxidative glucose disposal, as well as increasing bile acid reabsorption, GLP-1 secretion and altering the microbiome. In leptin-deficient (ob/ob) mice, metformin has been shown to increase translocation of Glut2 to the apical surface resulting in improved glucose homeostasis. In light of the interaction we observed between rs8192675 and BMI on metformin response, obese humans are reported to have altered GLUT2 localization in the fasting state.
by metformin treatment. The clinically appreciable impact in obese patients suggests that rs192675 has the potential to be a biomarker for stratified medicine.

METHODS
Methods and any associated references are available in the online version of the paper.

Accession codes. Part of the unpublished eQTL data set 4 (Supplementary Table 8), which covers the SLC2A2 locus, has been deposited in the Figshare: http://dx.doi.org/10.6084/m9.figshare.3438362. Phenotype and genotype data used in the first-stage GWAS screening have been deposited at the European Genome-phenome Archive: EGAS000001001875 and EGAD00001000282, respectively.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
Conception and design of the study: E.R.P. and K.M.G.; data analysis: K.Z., S.W.Y., E.L.S., N.v.L., A.A.v.d.H., J.W.B., C.E.d.K., L.Z., D.M.R., M.O., K.A.J., L.C., M.J., A.M.L., L.K.W., T.D. and A.A.-M.-R.; data collection and genotyping: S.W.Y., C.S., R.T., A.J.B., C.J.G., R.L.C., L.L., L.K.W., T.D., S.S., M.K., M.M.H., H.-C.C., E.L.S., M.S., J.S.W., L.W., J.Z., I.T., A.K., R.H.N.V.S., C.D.A.S., J.K., V-P., A.H., R.H.S., M.J.W., L.M.H., I.C.F., R.R.H., M.I.M. and C.N.A.P.; manuscript writing: E.R.P., K.Z., S.W.Y. and K.M.G.; with contributions from all authors on the final version.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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These authors jointly directed this work. Correspondence should be addressed to E.R.P. (e.z.pearson@dundee.ac.uk) or K.M.G. (kathy.giacomini@ucsf.edu).
ONLINE METHODS

Data access. The three liver eQTL data sets published previously are available with Gene Expression Omnibus (GEO) accession numbers: GSE39036, GSE25933 and GSE9588.

Studies and samples. Both GWAS screening and the first-stage replication analyzed participants with type 2 diabetes of European ancestry from the GoDARTS cohort. The current GWAS screening used 1,373 participants, which included data from 345 samples released after our initial GWAS report on 1,028 participants12. The first-stage replication included up to 1,473 samples from the remaining GoDARTS participants depending on the call rate and genotyping assay. The second-stage replication consisted of 1,223 participants of European ancestry from the UKPDS study. The final replication and meta-analysis was conducted within the MetGen Consortium which included an extra 6,488 participants of European ancestry and 2,566 participants of non-European ancestry. Detailed information on the MetGen participants is provided in Supplementary Table 2. Of note, about 50% of the MetGen cohort is from PMT, which represents ethnically diverse US populations. These cohorts were used extensively in our multiethnic analysis for replication purposes. Participants from the largest PPT cohort, PMT2, were selected from the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, a subsample of the Kaiser Permanente Research Program on Genes, Environment, and Health (RPEGH)36. Three MetGen cohorts, GoDARTS, UKPDS and DCS, also provided data on response to sulfonylureas. All human research was approved by the relevant institutional review boards, and all participants provided written informed consent.

Genotyping and quality control. Genotyping for the GWAS screening and the first-stage CardioMetabochip replication in GoDARTS cohort has been described before by WTCCC2 and DIAGRAM12,37. Standard quality-control procedures were applied to both data sets to filter SNPs with minor allele frequency (MAF) < 1% or call rate < 98% or Hardy–Weinberg equilibrium deviation (P < 10−8). Samples with call rate <98% or extra heterozygosity (more than 3 s.d. away from the mean) or correlated with another sample (identity by descent (IBD) > 0.125) were filtered out. In-house genotyping of the GoDARTS samples in the first-stage replication was performed with Sequenom MassArray for 66 SNPs and TaqMan-based Allelic Discrimination assays for nine SNPs. Details of the SNP selection procedure are described in Supplementary Data. All 75 SNPs had call rate >90% and no deviation from Hardy–Weinberg equilibrium (P > 0.005). The second-stage genotyping of the UKPDS sample was carried out in duplicate runs using standard TaqMan assays. All the SNPs were in Hardy–Weinberg equilibrium (P > 0.05), and only samples with concordant genotypes from both runs were analyzed. The third-stage replication used high-quality genotypes from either TaqMan assay or GWAS imputed data on rs8192675 (Supplementary Table 2).

Assessment of glycemic response to metformin and sulfonylureas. As with our previous GWAS12, two correlated measures of glycemic response to metformin were used in the current GWAS screening and the first-stage replication. A quantitative measure of HbA1c reduction (baseline minus on-treatment HbA1c) and a categorical measure of whether achieving a target of treatment HbA1c ≤ 7% were used for genetic association tests. Therefore only participants with type 2 diabetes and a baseline HbA1c > 7% were included. Baseline HbA1c was measured within 6 months before metformin start, and on-treatment HbA1c was taken as the minimum achieved within 18 months after metformin start.

In the second-stage replication and the meta-analysis in the third-stage replication, we opted to maximize the sample size by synchronizing the measurement of metformin efficacy in a wider spectrum of participants with type 2 diabetes (including those with baseline HbA1c < 7%) across the MetGen. Therefore only the quantitative outcome of HbA1c reduction was used to assess the glycemic response to metformin. To maintain relative clinical homogeneity, only participants with type 2 diabetes on metformin, sulfonylurea or metformin as an add-on therapy to another oral agent were included.

Data from two MetGen cohorts, which used alternative measures of glycemic response, were not included in the current meta-analyses, but the results are shown in Supplementary Table 4. In the DPP cohort of prediabetes participants, Cox proportional hazards regression was used to evaluate the genetic impact on the time to diabetes incidence8. In the HOME cohort, a multiple linear regression was used to test the genetic association with the difference in daily dose of insulin because metformin was used in conjunction with insulin in these participants38.

Assessment of glycemic response to sulfonylureas adopted a similar approach as the quantitative outcome of metformin response in the MetGen. Baseline HbA1c and on-treatment HbA1c were captured in a similar manner as those in defining metformin response. Only participants with type 2 diabetes who were on sulfonylurea monotherapy or using sulfonylurea as an add-on therapy to metformin were included. All participants had a baseline HbA1c > 7%.

Statistical analysis. In the GWAS screening and first-stage replication, each SNP was tested for association with the continuous measure and categorical measure of glycemic response to metformin separately with PLINK software using linear and logistic regression respectively39. Baseline HbA1c, adherence, metformin dose, creatinine clearance and treatment scheme (whether on metformin monotherapy or dual therapy of metformin add-on to sulfonylureas) and the first 10 principal components from EIGENSTRAT were used as covariates40. Statistical evidence of the two associations at each SNP was averaged by taking the geometric mean of the two P values in cases in which the direction of effect was consistent (for example more HbA1c reduction and more likely to achieve the treatment target both indicate better response).

In the second- and third-stage replications, association with HbA1c reduction was tested with multiple linear regression. Within each cohort, two linear models were fitted either with or without adjustment for baseline HbA1c. Baseline HbA1c has been shown to be the strongest predictor of metformin-induced HbA1c reduction in pharmaco-epidemiological studies41. Adjusting for baseline HbA1c could reduce the confounding of measurement error in baseline HbA1c and increase the statistical power for pharmacogenetic studies42. However, if a variant is associated with baseline HbA1c, adjusting for baseline HbA1c would lead to a reduced estimate of its pharmacogenetic effect compared to a model that did not adjust for the baseline HbA1c. Therefore we presented both models in the current study. Other clinical factors such as creatinine clearance (or other measurement of kidney function) and treatment adherence, metformin dose, creatinine clearance and treatment scheme were included as covariates where available (Supplementary Table 2). Association results from individual cohorts were combined by a fixed-effect inverse-variance–weighted meta-analysis as applied in GWAMA43. Cochran’s heterogeneity statistic’s P value was reported as Phet.

For the genetic association tests with response to sulfonylureas, multiple linear regression was used to assess the association between rs8192675 and baseline HbA1c, on-treatment HbA1c, HbA1c reduction and baseline-adjusted HbA1c reduction. Treatment scheme (whether on sulfonylurea monotherapy or using sulfonylurea as add-on treatment to metformin) was included as a covariate when modeling sulfonylurea-induced HbA1c reduction. Association test results from the three cohorts were combined with fixed-effect inverse-variance–weighted meta-analysis in GWAMA.

Locus-wise association was performed with GWAS imputed data of 7,223 participants available in the GoDARTS and PMT2-EU. Software IMPUTE2 was used to impute the post-quality-control GWAS data for the 1-Mb region flanking rs8192675 against the 1000 Genomes reference panel44. Only SNPs with high imputation quality (info > 0.9 and MAF > 0.02) in both cohorts were tested for association with SNPTEST45. Summary statistics from GoDARTS and PMT2-EU were combined with fixed-effect inverse-variance–weighted meta-analysis in GWAMA.

To evaluate the translational potential of rs8192675, we derived an unbiased estimate of its allelic effect by excluding the discovery cohort in the meta-analysis. This effect size was aligned to the clinical impact observed in the PMT2-EU, which was the biggest replication cohort and used the median average daily dose in the MetGen. The average daily dose and dosing impact in PMT2-EU were 962 mg/d and an extra 0.6% HbA1c reduction per gram metformin, respectively. The evaluation of rs8192675 genotype by BMI group interaction was performed with linear regression by adjusting for treatment group, sex and study cohort.
Expression quantitative trait locus analyses. We used four liver eQTL data sets comprising a total of 1,226 livers samples from individuals of European ancestry (Supplementary Table 8). Tissue procurement, gene expression analysis, genotyping and eQTL analyses have been described previously for three of the data sets46–48. The fourth data set was contributed by E. Schadt (E. Schadt, C. Molony, E. Chudin, K. Hao, X. Yang et al., personal communication). Genotypes were imputed to the 1000 Genome reference panel with IMPUTE2. Expression probe sequences were mapped to ENSEMBL genes and only the common genes across all data sets were included for subsequent analyses. Within each data set, the genome-wide eQTL analysis was run with an additive genetic model including data-set-specific covariates to examine cis-associations within a 100-kb flanking window. Results from the four data sets were then combined with a modified meta test statistic which was calculated using the following approach: $t_{\text{meta}} = (\sum w_{i}d_{i})/\sqrt{\sum w_{i}^{2}}$, where $i$ = data sets 1–4 and $n$ = sample size49. $P$ values were generated by assuming the meta test statistics were normally distributed; a Benjamini–Hochberg multiple-testing correction was applied to the $P$ values. For the current study, we extended the cis-association tests to all SNPs within a 1-Mb window of SLC2A2 and report the locus-wise $P$ values of the meta test statistic.

We investigated whether rs8192675 is a cis-eQTL in other tissues in the GTEx data release V6. Because of the sample size limitation, rs8192675 is not a genome-wide significant cis-eQTL for SLC2A2 in any of the tissues examined. However, given the strong evidence of the variant being a cis-eQTL in the large liver samples reported in this study, we considered a directionally consistent association with $P < 0.05$ as supportive evidence. The eQTL data for islet and intestine were acquired through contacting the authors of the original publications50,51. The transcript levels of SLC2A2 were determined using TaqMan probe (ID Hs01096908_m1). The relative expression level of SLC2A2 transcript was calculated by the comparative method ($\Delta\Delta C_{t}$) normalized to the housekeeping gene GAPDH, as described previously51.

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