Genome-wide meta-analysis identifies genetic variants associated with glycemic response to sulfonylureas

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Supplementary notes

Cohort descriptions:

GoDARTS: GoDARTS is a longitudinal cohort study established to study the genetics of T2D. Over 18,000 participants were enrolled between December 1998 and August 2012, of whom half of them are diagnosed with T2D and the remaining age and sex matched non-diabetic controls identified from general practice records in Tayside, Scotland[1]. Comprehensive electronic medical records history dating back to 1990 including anthropometric, clinical, prescription and biochemistry is available for each participant through a unique anonymised community health index number provided by the Health Informatics Centre (HIC) in partnership with the University of Dundee and the National Health Service (NHS). Previous analysis from the GoDARTS has delivered crucial PGx findings in diabetes and related traits[2-10]. The GODARTS study was approved by the Tayside Committee for Medical Research Ethics and written, informed consent was obtained from each participant. In this study, participants diagnosed with type 2 diabetes with sulfonylurea prescription as monotherapy or add on to metformin were included. Subjects with a history of insulin use, diagnosed before 35 years of age and baseline HbA1c <7% or >14 % were removed.

PMET1: Pharmacogenomics of Metformin (PMET) cohort 1, was established to study the metformin response of patients with T2D. The participants from the PMET1 cohort 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). The criteria for selecting the participants from GERA cohort have been described previously[11-13]. A detailed description of the cohort and study design can also be found in dbGAP (dbGaP Study Accession: phs000674.v1.p1). Genotyping of the European ancestry was performed using the methods described previously[14-16]. The cohort was imputed using IMPUTE2 and 1000 Genome Phase I. This cohort was used as replication cohort for two metformin pharmacogenomics studies[10,17]. In this genomewide meta-analysis of sulfonylurea response, prescriptions of sulfonylurea added to initial metformin therapy, along with HbA1c levels, dose and adherence were available for genetic association studies.

PMET2: Pharmacogenomics of Metformin (PMET) cohort 2, was established to study the metformin response of patients with T2D. The participants from the PMET2 cohort were selected from the Kaiser Permanente Research Program on Genes, Environment, and Health (RPEGH), using the criteria previously established for PMET1. It was established using the research funding from the US National Institute of Health (NIH) grant (R01-GM117163). In this genomewide meta-analysis of sulfonylurea response, prescriptions of sulfonylurea added to initial metformin therapy, along with HbA1c levels, dose and adherence were available for genetic association studies. Genotypes for PMET2 were imputed to HRC.r1-1 EUR reference genome (GRCh37 build) using the Michigan Imputation Server.

DCS: The DCS is a population based observational study from the West-Friesland region in the Netherlands established with the aim to improve diabetes care through empowerment and education. Details of the DCS is described elsewhere[18]. Patients visit their local DCS centre annually and longitudinal prescribing, biochemistry and health data are available for more than 6,000 T2D subjects of Caucasian ancestry. Each participant has consented to participate in the study and Ethical approval was obtained from the Medical Ethics Committee of the VU Vrije Universiteit Medical Center. For this study, participants diagnosed with type 2 diabetes with sulfonylurea prescription as monotherapy or add on to metformin, HbA1c levels, dose and GWAS data available were included.

Geisinger: Geisinger MyCode Community Health Initiative (MyCode®) is a system-wide research biorepository at Geisinger with more than 265,000 participants enrolled to date. Participants are consented to use their de-identified genetic and EHR data for research purposes.[19,20] The type 2 diabetes cohort in this study were identified using eMERGE algorithm.[21] Data extracted from EHR database included sulfonylureas, HbA1c tests, demographics, vitals. Genotyping was performed on the Illumina Infinium OmniExpress Exome array and GSA-24v1-0 array. Genotypes for both array were imputed to HRC.r1-1 EUR reference genome (GRCh37 build) separately using the Michigan Imputation Server. Only samples with European ancestry were included in the imputation. Centralized quality controls steps were performed[22] to only include variants with an info score > 0.7, call rate > 99% and minor allele frequency > 1% and samples with missingness <5%. This study was exempted by the Geisinger Institutional Review Board for using deidentified data and was approved by MyCode Governing Board for performing genetic study.

METRO: Metformin Response (METRO) cohort was established through funding by GSK (GlaxoSmithKline) in collaboration with University of California San Francisco (UCSF), and Kaiser Permanente Northern California (KPNC) under a collaborative research agreement. The goal of the cohort is to perform a genome-wide association of approximately 1000 extreme responders to metformin. The patients from Kaiser Permanente

Northern California (KPNC), who met the study criteria (see https://www.gsk-studyregister.com/en/trial-details/?id=207926) were invited to participate. All participants provided blood samples for GWAS genotyping. Drug response information was extracted from the electronic health record. Prescriptions of sulfonylurea added to initial metformin therapy, along with HbA1c levels, dose and adherence were available for the study.

HARMONY: The HARMONY trials are randomized, double-blind, placebo controlled, multicentre, phase III studies that evaluated the efficacy and safety of a s.c. injection of Albiglutide as compared with placebo or other hypoglycaemic agents in patients with type 2 diabetes. These studies were conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the Declaration of Helsinki, and all patients provided written informed consent before they participated in the study. Subjects randomised to the glimepiride arm of the HARMONY 3 trial (ClinicalTrials.gov registration number NCT00838903) were included for this study[23].

SUGAR-MGH: The Study to Understand the Genetics of the Acute Response to Metformin and Glipizide in Humans (SUGAR-MGH) is a human perturbation study that enrolled 1,000 subjects who were naïve to type 2 diabetes medications. The study design of SUGAR-MGH has been previously described.[24] Briefly, participants received a single dose of 5 mg glipizide, followed by a two-day course of 500 mg metformin twice daily and a 75-g oral glucose tolerance test one week later. For the glipizide challenge, glucose and insulin levels were subsequently measured at 30, 60, 90, 120, 180, and 240 minutes to construct phenotypes of acute glipizide response. In SUGAR-MGH, 890 subjects underwent genome-wide genotyping using the Illumina Multi-Ethnic Genotyping Array, and high-quality imputation was performed using the TOPMed reference panel. Informed consent was obtained from study participants, and the study protocol was approved by the Partners Human Research Committee (Partners HealthCare, Boston, MA). For the association of rs1234032 and rs10770791 with glipizide response, the covariates were baseline glucose, age, sex, and the first 10 ancestry principal components.

Supplementary Tables:

Supplementary Table 1: List of abbreviations

Abbreviation	Explanation
µg	Microgram
µM	Micrometre
BMI	Body mass index
CEU population	Northern and Western European Ancestry in Utah
Cis-eQTL	Cis-expression quantitative trait locus
DCS	The Hoorn Diabetes Care System
DIRECT	DIabetes REsearCh on patient straTification
DPP4	Dipeptidyl-peptidase 4
EV	Empty vector
GoDARTS	Diabetes Audit and Research in Tayside Scotland
GRCh37	Genome Reference Consortium 37
GTEx	Genotype-Tissue Expression
GWAMA	Genome-Wide Association Meta-Analysis
GWAS	Genome-wide association study
GXYLT1	Glucoside Xylosyltransferase 1
h^2	Heritability estimate
HbA1c	Glycated haemoglobin
HEK293 cells	Human embryonic kidney 293 cells
IC50	Half-maximal inhibitory concentration
LD	Linkage disequilibrium
LDAK	Learning Disabilities Association of Kansas
MAF	Minor allele frequency
MetGen Plus	Metformin Genetics Plus
MHC	Major histocompatibility complex
mL	Millilitre
mmol/mol	Millimoles per mole
mRNA	Messenger ribonucleic acid
NAS	Notch1 antisense transgenic
NOTCH1	Notch homolog 1, translocation-associated
OATP1B1	Solute carrier organic anion transporter family member 1B1
phet	Heterogeneity P value
PMET	Pharmacogenomics of Metformin
REML	Restricted maximum likelihood
SLCO1B1	Solute Carrier Organic Anion Transporter Family Member 1B1
SLCO1B3	Solute Carrier Organic Anion Transporter Family Member 1B3
SNP	Single-nucleotide polymorphism
SUGAR-MGH	Study to understand the genetics of the acute response to metformin and glipizide in humans
UK	United Kingdom

Supplementary Table 2: Baseline characteristics of participants included in each cohort

Characteristics	GoDARTS 1	GoDARTS 2	DCS	PMET 1	PMET 2	METRO	GEISINGER	HARMONY
n	1187	1118	600	835	417	434	675	219
Age at diagnosis (years)	58.79 ± 9.65	59.63 ± 10.98	59.18 ± 10.26	58.73±9.39	58.56±9.57	54.36±9.30	57.31 ± 12.87	55.3 ± 9.87
Duration of diabetes* (years)	4.63 [1.24-6.96]	4.05 [1.32-7.00]	2.76 [0.34-5.69]	NA	NA	NA	NA	4.90 [2.8-8.4]
Baseline HbA1c (%)	9.03 ± 1.48	8.92 ± 1.46	7.49± 1.42	8.25±1.56	8.41±1.63	8.28±1.14	8.13±1.45	8.12 ± 0.87
Weight (kg)	84.09 ± 16.36	86.05 ± 17.16	88.27± 16.60	99.98±22.25 (N=431)	98.19±19.34 (N=213)	105.95±20.91 (N=302)	101.89 ± 23.10 (N=674)	93.07 ± 21.18
BMI (kg/m^2)	30.36 ± 5.33	30.52 ± 5.53	29.98± 5.31	33.87±7.13 (N=431)	33.59±6.57 (N=213)	34.96±7.11	36.00 ± 7.31 (N = 674)	32.43 ± 5.44
Sex (women/% women)	47%	43%	44%	45%	46%	40%	47%	42.5%
Treatment HbA1c (%)	7.65 ± 1.40	7.63 ± 1.40	6.78± 0.93	7.03±1.04	6.99±1.08	7.45±1.18	6.85±0.93	7.32 ± 1.08
HbA1c reduction (%)	1.38 ± 1.71	1.29 ± 1.67	0.72± 1.41	1.22±1.67	1.42±1.84	0.83±1.35	1.28±1.36	0.80 ± 0.95
Adherence* (%)	0.98 [0.77-1.0]	0.96 [0.77-1.0]	NA	0.85 [0.78-1.0]	1.0[0.83-1.0]	NA	1.0 [0.83,1.0]	
Drug group (% monotherapy)	41.3%	45.1%	36%	0%	0%	0%	24.1%	0%
Average dose (% of max. BNF)*	0.25 [0.19-0.36]	0.25 [0.17-0.34]	0.5 [0.25-0.5]	Glipizide = 10.36 mg [5-10.2] (N=624); Glyburide = 6.62 mg [2.66-10.0] (N=157)	Glipizide = 10.36 mg (N=363); Glyburide = 7.74 mg (N=64)	Glipizide = 5.98 mg [5-5] (N=400); Glyburide = 3.89 mg [2.5-5] (N=18)	Glipizide = 8.14 mg [6.08 - 10.0]; Glyburide = 6.33 mg [3.58 – 8.14]; Glimepiride = 2.12 mg [1.70 – 3.43]	

*median [IQR]

Supplementary Table 3: Genotyping arrays and models used in each cohort

Cohort	Genotyping platform	Clinical covariates	PubMed citation
GoDARTS 1	Affymetrix Genome-Wide Human SNP array 6.0 (Affymetrix, Santa Clara, CA, USA)	Baseline HbA1c, age at diagnosis, baseline BMI, sex, daily dose, adherence, drug group, 3PCs	29025058
GoDARTS 2	Illumina HumanOminExpress	Baseline HbA1c, age at diagnosis, baseline BMI, sex, daily dose, adherence, drug group, 3PCs	29025058
DCS	Illumina HumanCoreExpress BeadChip	Baseline HbA1c, age at diagnosis, baseline BMI, sex, daily dose, adherence, 3PCs	25287012, 21447662
PMET 1	Affymetrix Axiom genotyping technology	Baseline HbA1c, daily dose, adherence, days to treatment HbA1c, 4PCs	21565264, 27500523
PMET 2	Illumuna OmniExpressExome	Baseline HbA1c, daily dose, adherence, days to treatment HbA1c, 4PCs	None
METRO	Illumina HumanOminExpress	Baseline HbA1c, daily dose, days to treatment HbA1c, 4PCs	None
GEISINGER	Illumina Infinium OmniExpress Exome array (N=542) and GSA-24v1-0 (N=133)	Baseline HbA1c, age at diagnosis, sex, chip, drug group, baseline BMI, days to treatment HbA1c, 5PCs.	None
HARMONY	Illumina	Baseline HbA1c, age at diagnosis, baseline BMI, sex, daily dose, adherence, drug group, 3PCs	24898304

Supplementary Table 4: Linear regression model for HbA1c reduction to SU treatment in the GoDARTS and PMET data.

GoDARTS (2,314)			
Variable	**beta**	**se**	**p**
Statin use	0.22	0.09	0.02
rs10770791C	0.24	0.06	1.66×10^{-05}
rs1234032C	-0.25	0.08	0.003
Baseline HbA1c	0.75	0.02	$<2 \times 10^{-16}$
Sex	0.32	0.06	1.31×10^{-08}
Age at diagnosis	0.02	0.003	1.13×10^{-07}
BMI	-0.01	0.005	0.07
Dose	-0.13	0.02	1.08×10^{-16}
Statin use*rs10770791C	-0.24	0.08	0.001
PMET (n = 1,252)			
Statin use	-0.05	0.11	0.64
rs10770791C	0.21	0.07	0.005
rs1234032C	-0.37	0.09	<0.0001
Baseline HbA1c	0.86	0.02	<0.0001
Statin use*rs10770791C	-0.12	0.09	0.19

Sex: male Vs Female, dose: average dose (% of max. BNF)

Supplementary Table 5: Inhibition potencies of atorvastatin and simvastatin acid for SLCO1B1- and SLCO1B3-mediated glyburide uptake. The experiments were repeated once to obtain IC_{50} mean +/- SD (the inhibition concentration at 50% glyburide uptake).

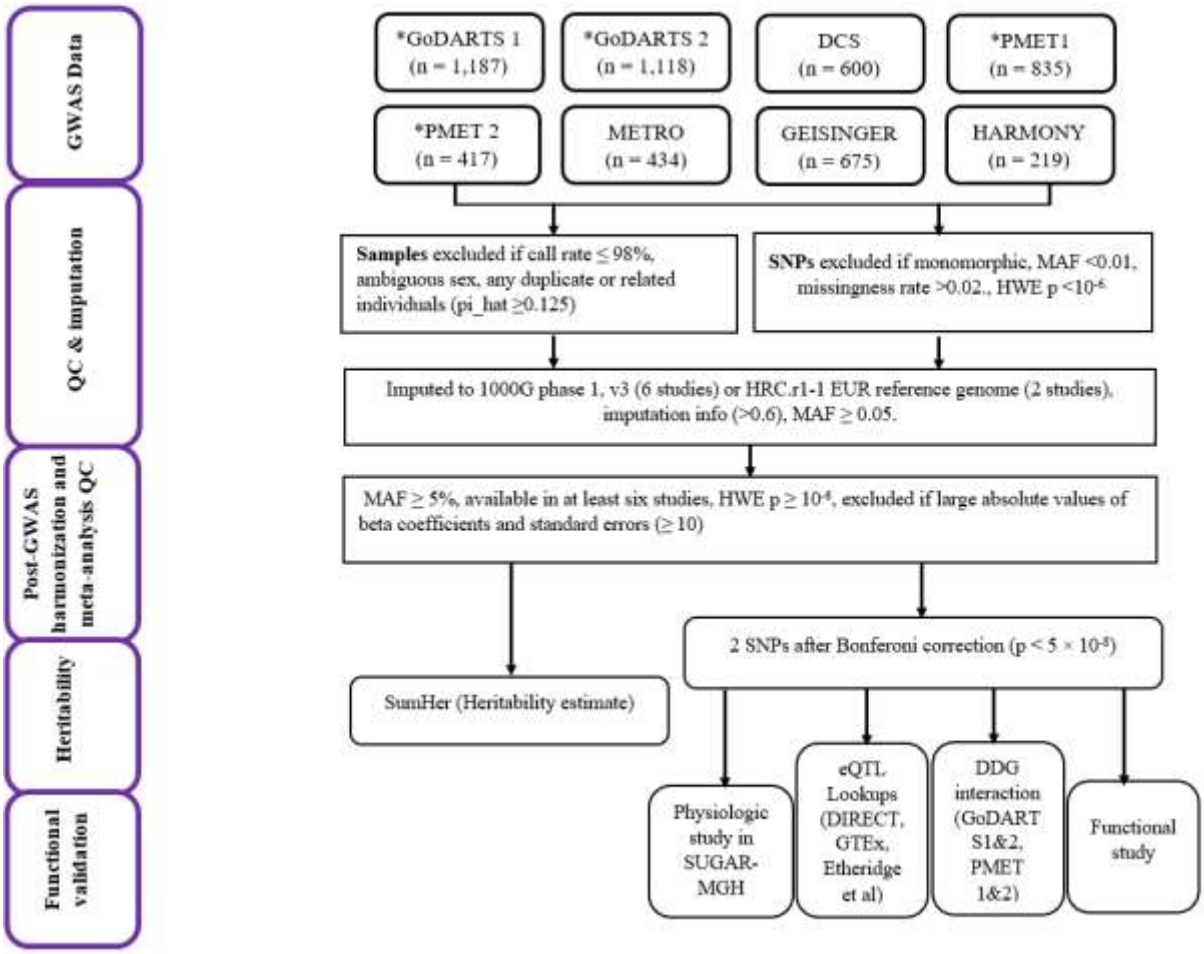
Substrate	**Inhibitor**	**OATP1B1 (IC_{50}, µM)**	**OATP1B3 (IC_{50}, µM)**
Glyburide	Atorvastatin (acid)	0.2 ± 0.1	1.7 ± 1.3
Glyburide	Simvastatin (acid)	2.9 ± 0.2	2.3 ± 1.9

Supplementary Table 6: Stratified analysis by statins

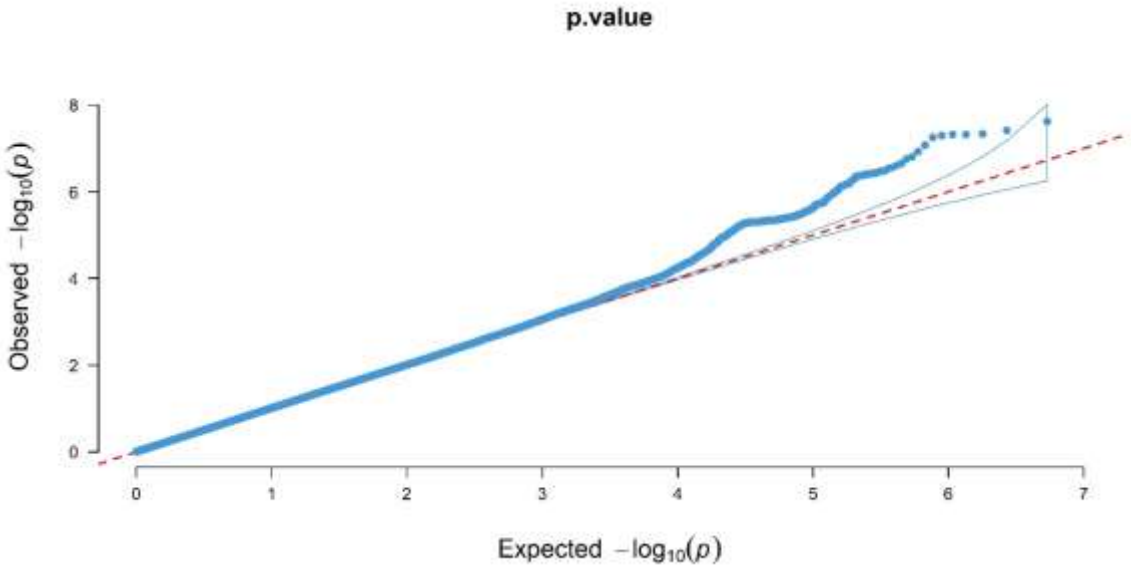
GoDARTS (n = 2,314)							
SU with Statins (1,227)					SU without statins (1,087)		
Variable	**beta**	**se**	**p**		**beta**	**se**	**p**
rs10770791C	0.001	0.05	0.99		0.24	0.06	5.76×10^{-05}
Baseline HbA1c	0.75	0.03	$<2 \times 10^{-16}$		0.76	0.23	$<2 \times 10^{-16}$
Sex	0.24	0.07	0.91×10^{-04}		0.40	0.09	3.16×10^{-06}
Age at diagnosis	0.02	0.004	8.24×10^{-05}		0.02	0.004	4.7×10^{-04}
BMI	-0.005	0.007	0.50		-0.01	0.008	0.08
Dose	-0.15	0.02	5.06×10^{-11}		-0.11	0.02	6.51×10^{-06}
PMET (n = 1,252)							
SU with Statins (869)					**SU without statins (383)**		
Variable	**beta**	**se**	**p**		**beta**	**se**	**p**
rs10770791C	0.09	0.04	0.04		0.21	0.09	0.016
Baseline HbA1c	0.84	0.02	<0.0001		0.88	0.034	<0.0001
Meta-analysis (n = 3,566)							
rs10770791C	0.05	0.03	0.11		0.23	0.05	<0.0001

Sex: male Vs Female, dose: average dose (% of max. BNF)

Supplementary Figures:



Supplementary Figure 1: A flowchart overview of the entire GWAS QC, meta-analysis and validation steps. DDG: drug-drug-gene, *: individual genetic and prescription data is available for follow-up studies.



Supplementary Figure 2: The relationship between observed and expected p values (Q-Q plot) (λ = 1.008).

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