

SUPPLEMENTAL MATERIAL

Supplemental Table S1. Distribution of 3,168 samples with genomic data by self-reported race/ethnicity and DPP treatment arm that underwent imputation.

Self-reported race/ethnicity	Treatment arm				Total
	MET	ILS	PBO	TROG	
AfrAm	178	173	186	94	631
AsnPI	31	49	38	20	138
Hisp	143	161	147	89	540
AI	21	28	27	3	79
White	507	479	490	304	1780
Total	880	890	888	510	3168

AfrAm=African American; AsnPI=Asian/Pacific Islander; Hisp=Hispanic/Latino; AI=American Indian; MET=metformin; ILS=intensive lifestyle modification; PBO=placebo; TROG=troglitazone

Descriptions of replication cohorts:

The Metformin Genetics (MetGen) Consortium: MetGen consists of 20 research institutions from Europe and the United States with available data for studies of metformin pharmacogenetics from population observational studies and clinical trials. This resource has an excess of 10,000 multi-ethnic individuals with established type 2 diabetes (T2D) across over 12 cohorts in whom a pharmacogenetic meta-analysis of metformin has been performed using a harmonized measure of glycemic response. Additional details regarding the methodology of the meta-GWAS can be found in Zhou *et al.* (1).

The Million Veteran Program (MVP): The MVP is a mega-biobank in the Department of Veteran Affairs (VA) healthcare system that combines survey data, clinical electronic health record information, and biospecimens to enable genomic research. Recently, 318 novel genetic risk loci for T2D were identified in a multi-ethnic analysis that incorporated over 100,000 cases of T2D from MVP (2). For this replication analysis, we included 2733 individuals who self-identified as non-Hispanic African Americans with a baseline HbA1c $\geq 6.5\%$ and received metformin monotherapy for up to 15 months.

Diabetes Multi-omic Investigation of Drug Response (DIAMOND): DIAMOND is an NIH-funded study that aimed to enroll 5500 individuals with T2D from the metropolitan Detroit and the surrounding areas of southeast Michigan for the purposes of understanding the genetic causes of diabetes, diabetes-related traits, and medication response. Individuals with more than two diabetes diagnoses and at least two historical HbA1c tests drawn over 4 months apart were identified from the electronic medical record in the Henry Ford Health System. For this replication analysis, we assessed a subset of 471 individuals who self-reported as African American and had an average daily exposure of ≥ 500 mg metformin in the 120 days prior to the follow-up HbA1c measurement. The follow-up period was between 4-18 months.

Supplemental Table S2. Genotyping platforms and models utilized in each replication analysis.

Cohort	Genotyping and Imputation	Outcome	Covariates
MetGen	See Supplementary Table 2 of Zhou <i>et al.</i> (1) for details of the genotyping platforms and imputation methods used for the meta-GWAS	Change in HbA1c (baseline minus follow-up within 18 months)	Baseline HbA1c, adherence, metformin dose, treatment group (metformin monotherapy vs. add-on to sulfonylurea), 10 ancestry PCs Some covariates were included to varying degrees – see Supplementary Table 2 of Zhou <i>et al.</i> (1) for details of the study-specific models
MVP	Custom Affymetrix Axiom array (MVP 1.0) Imputation using 1000 Genomes Project Phase 3 reference panel	Change in HbA1c (baseline minus follow-up within 15 months)	Baseline HbA1c, age, BMI, metformin dose, number of HbA1c measurements, 10 ancestry PCs
DIAMOND	Axiom Precision Medicine Diversity Array Imputation using the TOPMed reference panel (version R2 on GRC38)	Change in HbA1c (follow-up minus baseline within 18 months)	Baseline HbA1c, age, sex, 10 ancestry PCs

PC=principal components

Supplemental Table S3. Event rate for diabetes incidence by DPP treatment arm and self-reported race/ethnicity information.

	MET		PBO	
Self-reported race/ethnicity	N	# Events (%)	N	# Events (%)
AfrAm	178	36 (20.2)	186	61 (32.8)
AsnPI	31	8 (25.8)	38	12 (32.4)
Hisp	143	34 (23.8)	147	39 (26.5)
AI	21	4 (20.0)	27	8 (29.6)
White	507	101 (25.1)	490	133 (27.1)
Total	880	183 (20.9)	888	253 (28.5)

AfrAm=African American; AsnPI=Asian/Pacific Islander; Hisp=Hispanic/Latino; AI=American Indian; MET=metformin; PBO=placebo.

Supplemental Table S4. Sample sizes for each individual model and outcome tested.

Outcome	MET* (n=880)	PBO* (n=888)	G×T* (n=1768)
Diabetes incidence	876	887	1763
HbA1c	818	803	1621
Weight	829	844	1673
Fasting glucose	821	812	1633
2-hr glucose	817	810	1627
Insulin sensitivity index	808	818	1626
Fasting insulin	802	792	1594

MET=metformin; PBO=placebo; G×T=gene by treatment. *The decrease in sample size from the counts in the column headings is due to data missingness within in each model (i.e., follow-up measurement for the quantitative trait was unavailable, so the value for one-year change could not be calculated).

Supplemental Table S5. Effect size and *p*-values, stratified by self-reported race/ethnicity, for the 14 independent genome-wide significant findings for one-year change in quantitative traits in the DPP.

rsid	Chr	Position*	Nearest gene	EA	NEA	Trait	EAF [†]	Self-reported race/ethnicity	N	Beta	SE	<i>p</i>
Metformin only model												
rs144322333	18	705550	<i>ENOSF1</i>	C	CTGTT	HbA1c, %	0.013	All	818	0.39	0.06	2.9E-12
							0.065	AfrAm	166	0.32	0.06	1.3E-06
							0.009	Hisp	138	1.05	0.22	3.8E-06
rs145591055	5	38849463	<i>OSMR</i>	G	A	Weight, kg	0.014	All	829	-7.55	1.19	3.2E-10
							0.018	AsnPI	28	6.18	18.75	7.5E-01
							0.064	Hisp	139	-5.56	1.60	6.8E-04
							0.103	AI	21	-3.20	2.79	2.9E-01
							0.007	White	473	-10.71	3.02	4.4E-04
rs13401282	2	207810690	<i>CPO</i>	A	T	ISI, ln	0.029	All	808	0.44	0.08	1.7E-08
							0.119	AfrAm	167	0.42	0.08	2.2E-07
							0.015	Hisp	134	-0.25	0.36	4.9E-01
rs186681623	13	81546608	<i>LINC00377</i>	C	T	Weight, kg	0.068	All	829	-2.66	0.47	2.0E-08
							0.121	AfrAm	169	-1.98	0.61	1.5E-03
							0.074	AsnPI	28	-7.65	3.11	2.9E-02
							0.122	Hisp	139	-3.19	1.13	5.4E-03
							0.161	AI	21	-5.24	2.68	9.8E-02
rs17083791	5	93863813	<i>KIAA0825</i>	G	A	Weight, kg	0.036	White	473	-3.83	0.87	1.4E-05
							0.149	All	829	-1.85	0.33	3.3E-08
							0.052	AfrAm	169	-1.65	0.95	8.3E-02
							0.194	AsnPI	28	0.42	1.53	7.9E-01
							0.210	Hisp	139	-2.08	0.83	1.3E-02
rs9931871	16	19928315	<i>GPRC5B</i>	G	A	Fasting glucose, mmol/L	0.367	AI	21	-3.19	2.39	2.3E-01
							0.137	White	473	-1.71	0.44	1.2E-04
							0.011	All	821	0.68	0.12	3.5E-08
							0.049	AfrAm	168	0.62	0.13	5.9E-06
							0.012	All	818	0.35	0.06	3.9E-08
rs549305231	6	19497504	<i>LOC101928519</i>	A	G	HbA1c, %	0.046	AfrAm	166	0.40	0.07	4.9E-08
							0.005	Hisp	138	-1.22	1.24	3.3E-01
							0.020	All	802	-0.47	0.08	4.1E-08
rs73944532	2	104691634	<i>LOC100287010</i>	A	G	Fasting insulin, ln	0.075	AfrAm	165	-0.50	0.09	1.4E-07
							0.007	Hisp	134	-0.13	0.30	6.6E-01

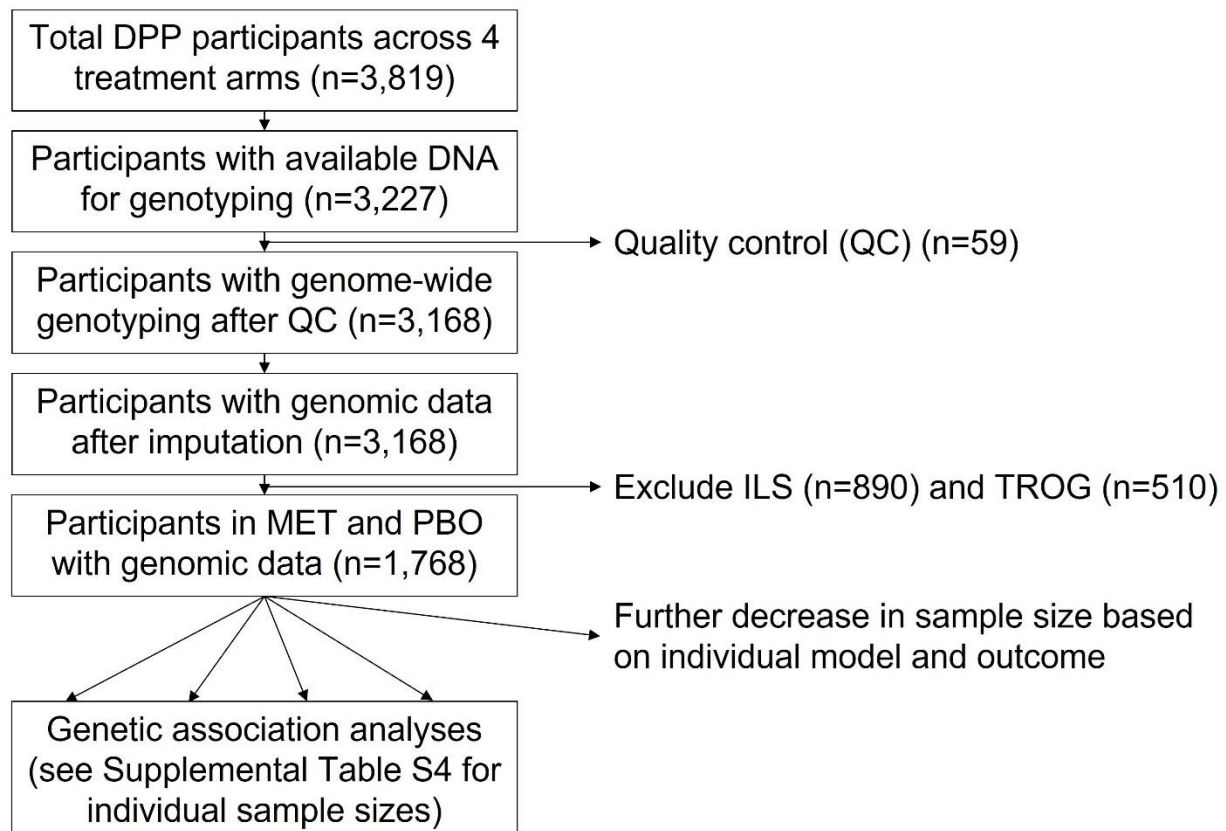
rsid	Chr	Position*	Nearest gene	EA	NEA	Trait	EAF†	Self-reported race/ethnicity	N	Beta	SE	p
Gene × Treatment model‡												
rs6838493	4	185879789	<i>LINC01093</i>	A	T	HbA1c, %	0.011	All	1621	-0.66	0.11	1.6E-09
							0.041	AfrAm	330	-0.80	0.14	6.0E-08
rs148219263	18	58808522	<i>CDH20</i>	C	T	Weight, kg	0.012	All	1673	-6.92	1.22	1.9E-08
							0.007	AfrAm	342	-4.09	6.50	5.3E-01
							0.011	Hisp	275	-0.37	2.98	9.0E-01
							0.017	White	947	-8.81	1.50	5.6E-09
rs75147163	14	57626769	<i>EXOC5</i>	A	G	Fasting glucose, mmol/L	0.022	All	1633	-0.73	0.13	1.4E-08
							0.053	AfrAm	334	-1.05	0.25	4.2E-05
							0.022	Hisp	267	-0.36	0.39	3.6E-01
							0.015	White	925	-0.53	0.18	3.0E-03
rs78075715	4	6613716	<i>MAN2B2</i>	C	T	HbA1c, %	0.013	All	1621	-0.51	0.09	1.4E-08
							0.063	AfrAm	330	-0.53	0.11	1.4E-06
rs12314996	12	24567467	<i>SOX5</i>	A	G	HbA1c, %	0.016	All	1621	-0.47	0.08	3.9E-08
							0.062	AfrAm	330	-0.51	0.11	4.5E-06
							0.007	Hisp	266	-0.24	0.60	6.9E-01
rs143203347	7	107384199	<i>CBLL1</i>	C	G	Fasting glucose, mmol/L	0.015	All	1633	-0.92	0.17	4.1E-08
							0.062	AfrAm	334	-0.92	0.21	1.6E-05
							0.011	Hisp	267	-0.42	0.62	5.0E-01

EA=Effect allele; NEA=Non-effect allele; EAF=effect allele frequency. AfrAm=African American; AsnPI=Asian/Pacific Islander; Hisp=Hispanic/Latino; AI=American Indian; ISI=insulin sensitivity index. *GRCh37 assembly. †This is the effect allele frequency taken from Table 2. For each variant, we only included stratified analyses when the effect allele frequency was greater than 0.005 in the self-reported race/ethnicity group. ‡Beta estimates and standard errors (SE) are reported for the interaction term rather than the main effect of metformin.

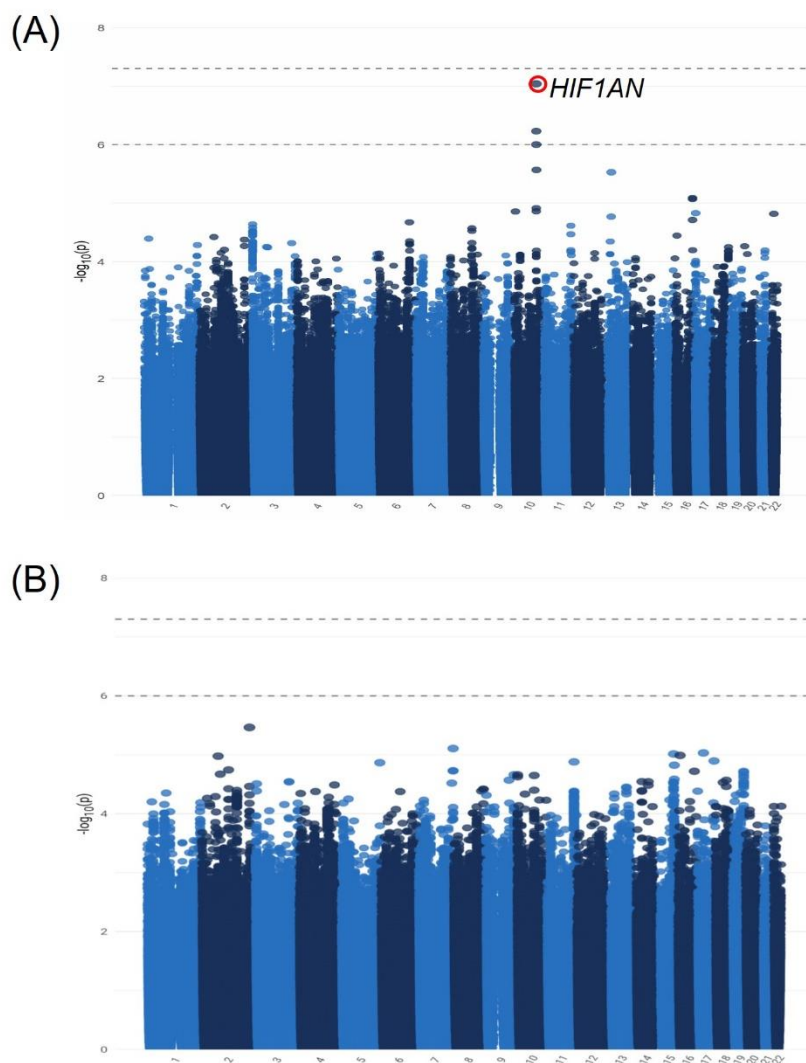
Supplemental Table S6. Evaluation of the 14 independent genome-wide significant findings from Table 2 in the lifestyle treatment arm of the DPP.

rsid	Chr	Position*	Nearest gene	EA	NEA	Trait	EAF†	Metformin only model			Lifestyle only model		
								Beta	SE	p	Beta	SE	p
rs144322333	18	705550	<i>ENOSF1</i>	C	CTGTT	HbA1c, %	0.013	0.39	0.06	2.9E-12	0.04	0.06	5.3E-01
rs145591055	5	38849463	<i>OSMR</i>	G	A	Weight, kg	0.014	-7.55	1.19	3.2E-10	1.08	1.31	4.1E-01
rs13401282	2	207810690	<i>CPO</i>	A	T	ISI, ln	0.029	0.44	0.08	1.7E-08	0.26	0.09	4.1E-03
rs186681623	13	81546608	<i>LINC00377</i>	C	T	Weight, kg	0.068	-2.66	0.47	2.0E-08	0.79	0.61	2.0E-01
rs17083791	5	93863813	<i>KIAA0825</i>	G	A	Weight, kg	0.149	-1.85	0.33	3.3E-08	0.03	0.47	9.5E-01
rs9931871	16	19928315	<i>GPRC5B</i>	G	A	Fasting glucose, mmol/L	0.011	0.68	0.12	3.5E-08	-0.07	0.12	5.4E-01
rs549305231	6	19497504	<i>LOC101928519</i>	A	G	HbA1c, %	0.012	0.35	0.06	3.9E-08	-0.12	0.11	2.8E-01
rs73944532	2	104691634	<i>LOC100287010</i>	A	G	Fasting insulin, ln	0.020	-0.47	0.08	4.1E-08	-0.15	0.10	1.3E-01
rsid	Chr	Position*	Nearest gene	EA	NEA	Trait	EAF†	Gene × Treatment model‡ (metformin and placebo)			Gene × Treatment model‡ (lifestyle and placebo)		
								Beta	SE	p	Beta	SE	p
rs6838493	4	185879789	<i>LINC01093</i>	A	T	HbA1c, %	0.011	-0.66	0.11	1.6E-09	-0.54	0.12	5.9E-06
rs148219263	18	58808522	<i>CDH20</i>	C	T	Weight, kg	0.012	-6.92	1.22	1.9E-08	6.31	1.96	1.3E-03
rs75147163	14	57626769	<i>EXOC5</i>	A	G	Fasting glucose, mmol/L	0.022	-0.73	0.13	1.4E-08	-0.75	0.12	1.1E-09
rs78075715	4	6613716	<i>MAN2B2</i>	C	T	HbA1c, %	0.013	-0.51	0.09	1.4E-08	-0.40	0.09	7.9E-06
rs12314996	12	24567467	<i>SOX5</i>	A	G	HbA1c, %	0.016	-0.47	0.08	3.9E-08	-0.35	0.10	2.8E-04
rs143203347	7	107384199	<i>CBLL1</i>	C	G	Fasting glucose, mmol/L	0.015	-0.92	0.17	4.1E-08	-0.78	0.19	5.0E-05

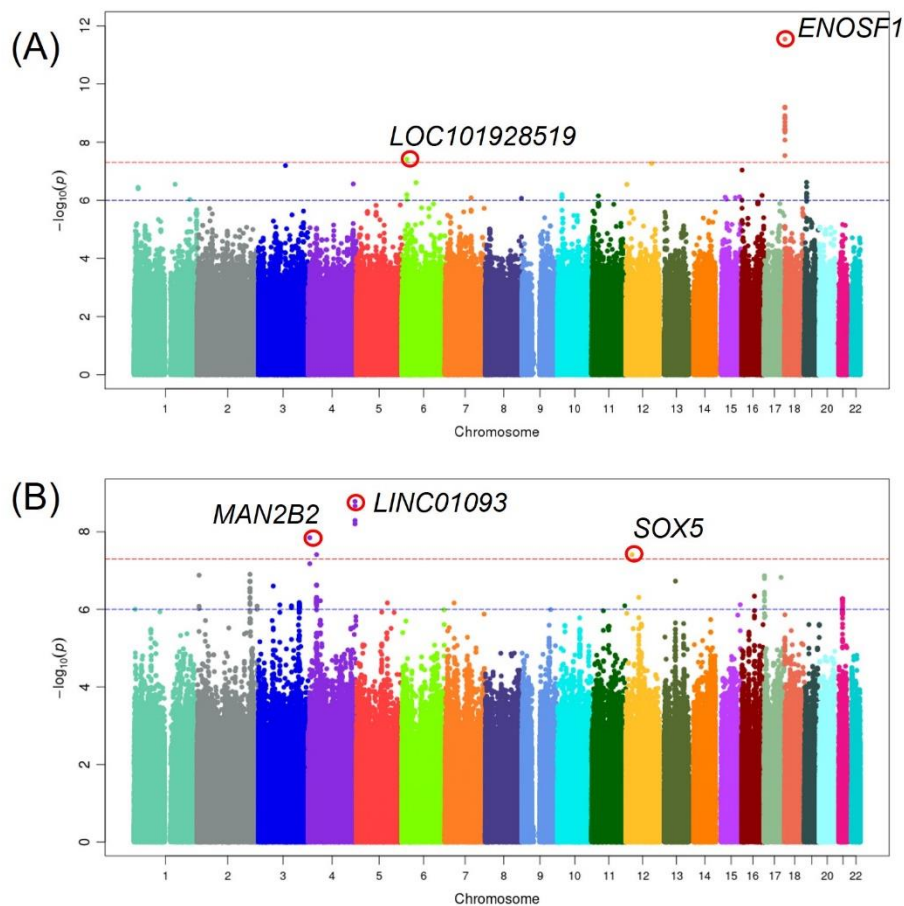
EA=Effect allele; NEA=Non-effect allele; EAF=effect allele frequency. ISI=insulin sensitivity index. *GRCh37 assembly. †This is the effect allele frequency taken from “All” participants in Table 2. ‡Beta estimates and standard errors (SE) are reported for the interaction term rather than the main effect of metformin or lifestyle.



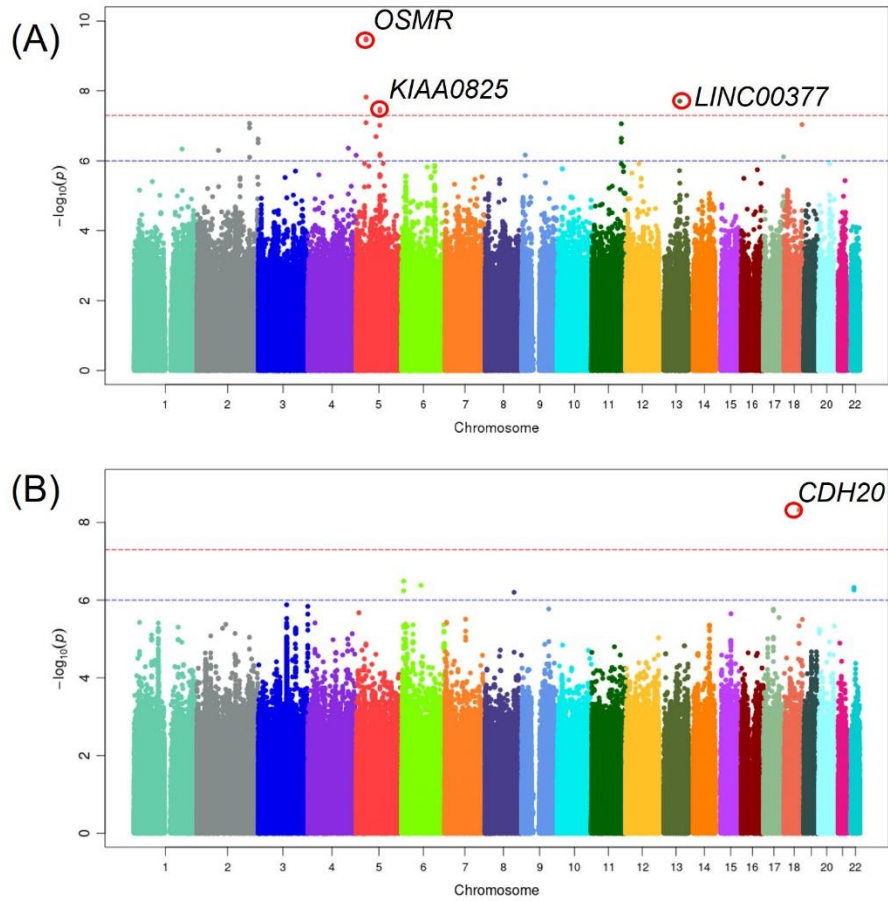
Supplemental Figure S1. Flowchart illustrating the sample size reduction in the DPP leading up to genetic association analyses. MET=metformin; ILS=intensive lifestyle modification; PBO=placebo; TROG=troglitazone.



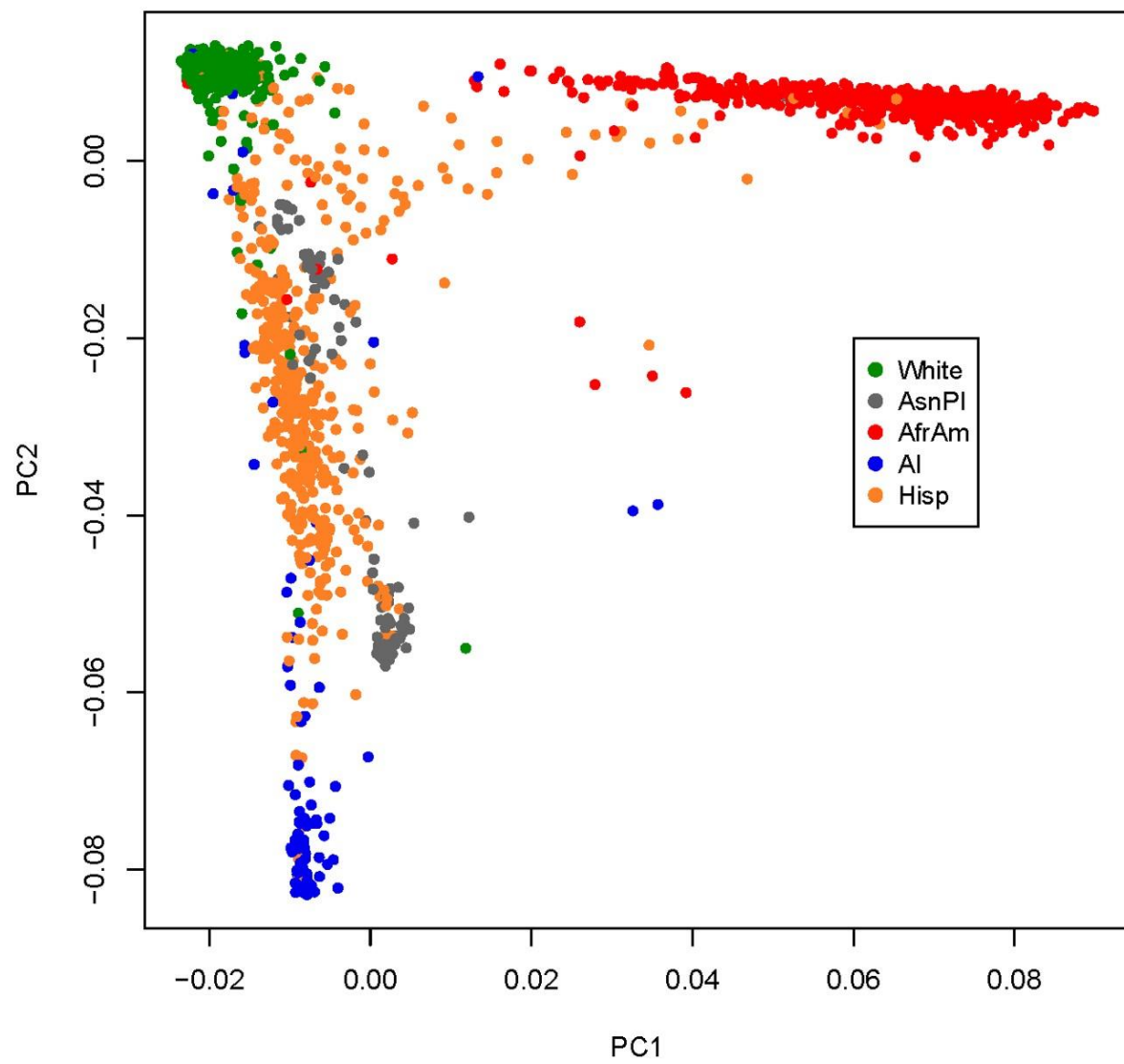
Supplemental Figure S2. Manhattan plot of genome-wide results from single marker association with diabetes incidence (A) using an additive genetic model in 876 individuals in the metformin arm only and (B) testing a gene \times treatment interaction in the placebo and metformin arms in 1,763 individuals. For each panel, the two dotted lines indicate the suggestive significance ($p < 1 \times 10^{-6}$) and genome-wide significance ($p < 5 \times 10^{-8}$). Top loci are circled in red with the closest gene labeled.



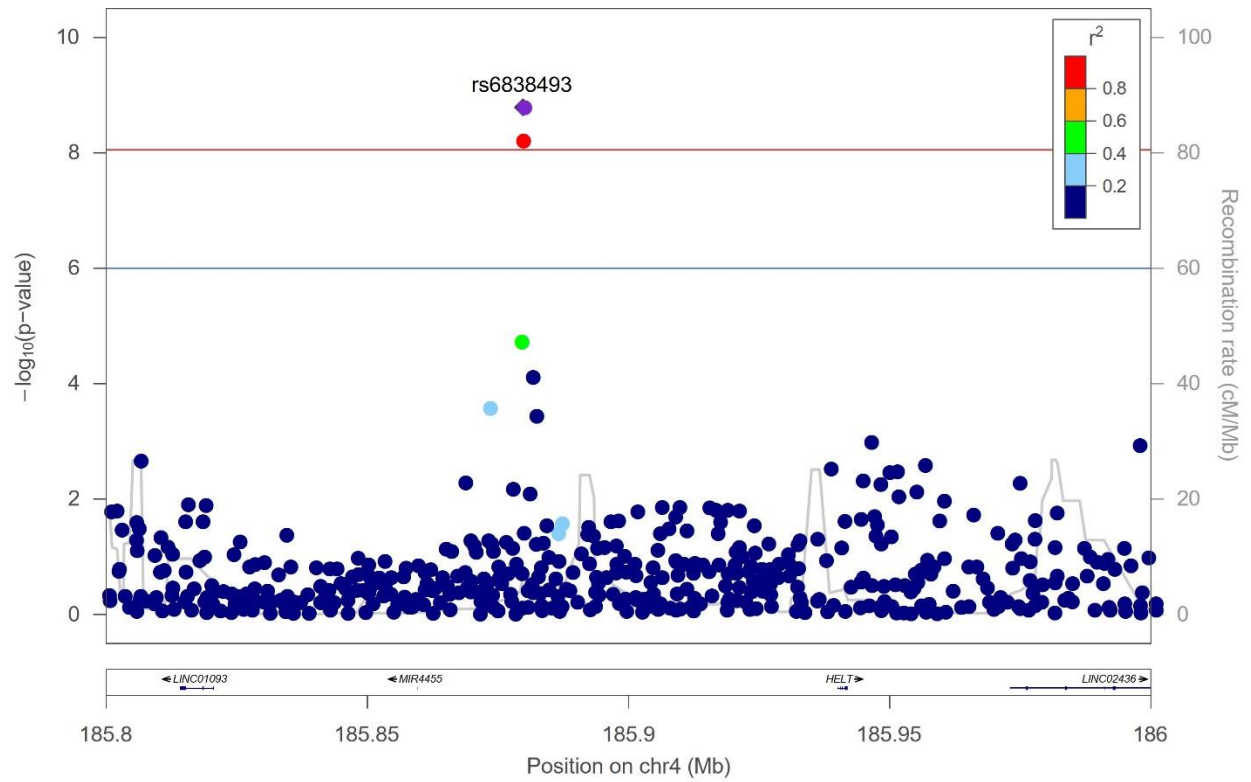
Supplemental Figure S3. Manhattan plots of genome-wide results from single marker association with one-year change in HbA1c (A) using an additive genetic model in 818 individuals in the metformin arm only and (B) testing a gene \times treatment interaction in the placebo and metformin arms in 1,621 individuals. The blue line indicates suggestive significance ($p < 1 \times 10^{-6}$) and the red line indicates genome-wide significance ($p < 5 \times 10^{-8}$). Top loci are circled in red with the closest gene labeled.



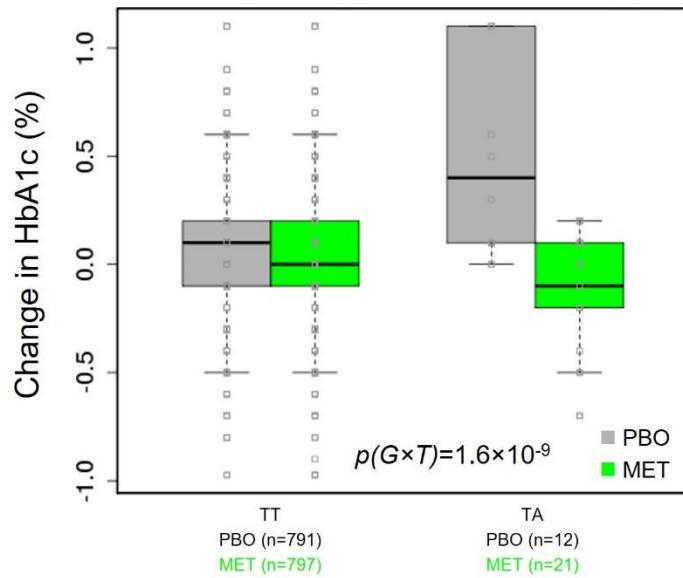
Supplemental Figure S4. Manhattan plots of genome-wide results from single marker association with one-year change in weight (A) using an additive genetic model in 829 individuals in the metformin arm only and (B) testing a gene \times drug interaction in the placebo and metformin arms in 1,673 individuals. The blue line indicates suggestive significance ($p < 1 \times 10^{-6}$) and the red line indicates genome-wide significance ($p < 5 \times 10^{-8}$). Top loci are circled in red with the closest gene labeled.



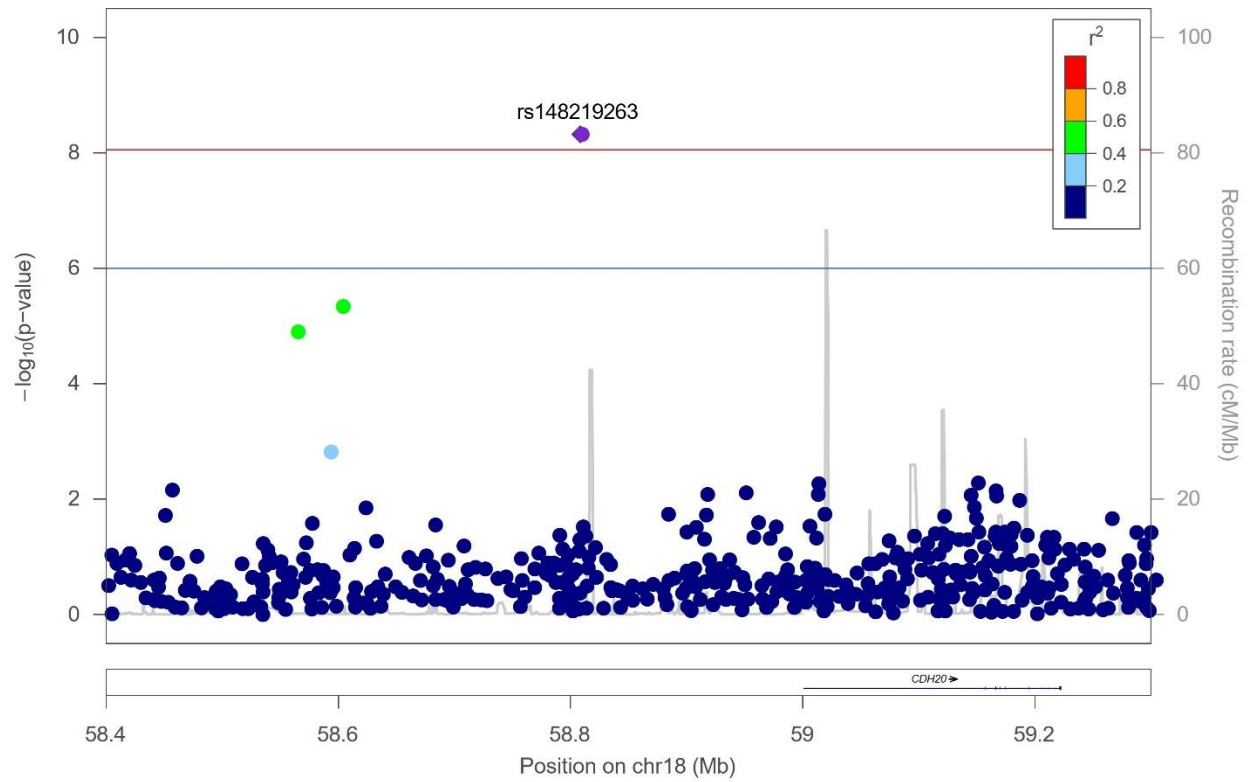
Supplemental Figure S5. Self-reported race/ethnicity (colored circles) displayed on a background plot of the first two genetic ancestry principal components.



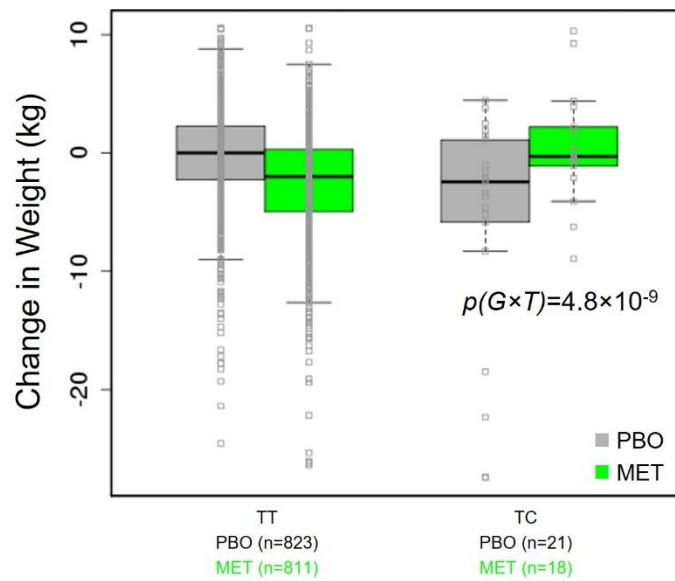
Supplemental Figure S6. Regional association plot of rs6838493 for one-year change in HbA1c. The red line indicates the experiment-wide significance threshold of $p < 9 \times 10^{-9}$ and the blue line indicates a suggestive significance threshold of $p < 1 \times 10^{-6}$.



Supplemental Figure S7. Comparison of the influence of rs6838493 genotype on the mean change in HbA1c (one-year minus baseline) in the metformin (MET, n=818) and placebo (PBO, n=803) arms. The interaction p -value is reported. For the purposes of generating the box plots, fractional alleles were converted to “hard calls” by rounding to the nearest integer.



Supplemental Figure S8. Regional association plot of rs148219263 for one-year change in weight. The red line indicates the experiment-wide significance threshold of $p < 9 \times 10^{-9}$ and the blue line indicates a suggestive significance threshold of $p < 1 \times 10^{-6}$.



Supplemental Figure S9. Comparison of the influence of rs148219263 genotype on the mean change in weight (one-year minus baseline) in the metformin (MET, n=829) and placebo (PBO, n=844) arms. The interaction p -value is reported. For the purposes of generating the box plots, fractional alleles were converted to “hard calls” by rounding to the nearest integer.

References

1. Zhou K, Yee SW, Seiser EL, et al. Variation in the glucose transporter gene SLC2A2 is associated with glycemic response to metformin. *Nat Genet* 2016;48:1055-1059
2. Vujkovic M, Keaton JM, Lynch JA, et al. Discovery of 318 new risk loci for type 2 diabetes and related vascular outcomes among 1.4 million participants in a multi-ancestry meta-analysis. *Nat Genet* 2020;52:680-691