Identification of Genetic Variation Influencing Metformin Response in a Multi-Ancestry Genome-Wide Association Study in the Diabetes Prevention Program (DPP)

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Identification of genetic variation influencing metformin response in a multi-ancestry genome-wide association study in the Diabetes Prevention Program (DPP)

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ABSTRACT (200 words)

Genome-wide significant loci for metformin response in type 2 diabetes reported elsewhere have not replicated in the Diabetes Prevention Program (DPP). To assess pharmacogenetic interactions in pre-diabetes, we conducted a genome-wide association study (GWAS) in the DPP. Cox proportional hazards models tested associations with diabetes incidence in metformin (MET, n=876) and placebo (PBO, n=887) arms. Multiple linear regression assessed association with one-year change in metformin-related quantitative traits, adjusted for baseline trait, age, sex, and 10 ancestry principal components. We tested for gene-by-treatment interaction. No significant associations emerged for diabetes incidence. We identified four genome-wide significant variants after correcting for correlated traits ($p<9\times10^{-9}$). In MET, rs144322333 near *ENOSF1* (minor allele frequency $[MAF]_{AFR}=0.07$, $MAF_{EUR}=0.002$) was associated with an increase in % glycated hemoglobin (per minor allele β=0.39 [95% CI 0.28, 0.50], $p=2.8\times10^{-12}$). Rs145591055 near *OMSR* (MAF=0.10 in American Indians), was associated with weight loss (kg) (per G allele β=-7.55 [95% CI -9.88, -5.22], $p=3.2\times10^{-10}$) in MET. Neither variant was significant in PBO; gene-by-treatment interaction was significant for both variants ($p_{(G\times T)}<1.0\times10^{-4}$). Replication in individuals with diabetes did not yield significant findings. A GWAS for metformin response in pre-diabetes revealed novel ethnic-specific associations that require further investigation but may have implications for tailored therapy.

INTRODUCTION

Metformin is the most commonly used agent for initial treatment of type 2 diabetes (T2D) with over 100 million users worldwide. Over the years, it has remained the foundation of clinical practice guidelines for the management of diabetes globally because it is cheap, safe, effective, well-tolerated, orally administered, and does not promote weight gain (1-5). However, despite its widespread use, metformin does not work well for everyone; in the ADOPT study, which examined the glycemic durability of three oral glucose-lowering agents, each as monotherapy for T2D, 21% of individuals on metformin failed to meet glycemic goals within the first five years of treatment (6). Moreover, treatment failure rates are as high as 50% in children and adolescents as shown by the TODAY study (7).

The reasons for metformin treatment failure remain unclear and could be related to genetic, pharmacologic, pathophysiologic, or environmental factors. Using the genome-wide complex trait analysis method, the heritability of metformin response has been estimated to be between 20-34%, a value comparable to other complex phenotypes (8). Indeed, genome wide association studies (GWAS) have identified common genetic variants associated with metformin response (9-11). In the first GWAS for metformin response, a variant around the gene encoding the ataxia-telangiectasia mutated kinase (*ATM*) was found to be associated with metformin response in GoDARTS and UKPDS participants (9). Subsequently, in a meta-analysis of over 10,000 participants with a harmonized glycemic measure of metformin response, a genome-wide significant association was observed in an intron of the *SLC2A2* gene encoding the GLUT2 glucose transporter (10). However, neither of these findings was reproduced in the Diabetes Prevention Program (DPP) (10, 12). While the difference in findings could be related to statistical power, one key distinction between the DPP and other studies is that DPP participants

have pre-diabetes and not established T2D. Therefore, it is possible that the influence of genetics on drug response could vary in the pre-diabetes state compared to the more advanced T2D state.

Thus, we performed a GWAS in the DPP to uncover novel variation associated with metformin response in this distinctive multi-ethnic cohort that was selected to be at high risk of T2D by virtue of elevated 2-hour glucose, fasting glucose, and body mass index (BMI). Our objective was to identify genetic variants associated with metformin response, as measured by both diabetes incidence and change in quantitative glycemic and metabolic traits. Through this approach, we were able to examine the genetics of metformin response as it relates to both pre-diabetes and T2D development as well as glycemic response.

RESEARCH DESIGN AND METHODS

Description of participants and DPP study design

The DPP study design and baseline characteristics of the participants have been described previously (13, 14). Briefly, the DPP was a multicenter randomized control trial designed to test the effects of intensive lifestyle modification and pharmacologic intervention on preventing progression to T2D in high-risk individuals. Enrolled participants had a fasting plasma glucose ranging from 95-125 mg/dL (5.3-6.9 mmol/L) and a 2-hour plasma glucose level between 140-199 mg/dL (7.8-11.0 mmol/L) on a 75-g oral glucose tolerance test (OGTT). A total of 3,819 participants were randomized to intensive lifestyle modification (goal weight loss ≥7% and ≥150 min/week of physical activity), standard lifestyle recommendations plus metformin (850 mg twice daily), standard lifestyle recommendations plus troglitazone (400 mg daily), or standard lifestyle recommendations plus placebo. The primary endpoint was diabetes incidence, diagnosed by a fasting glucose of ≥126 mg/dL (7.0 mmol/L) or a 2-hour glucose of ≥200 mg/dL

(11.1 mmol/L) after OGTT and confirmed subsequently on a second test within 6 weeks. The primary study demonstrated that over a mean follow-up of 2.8 years, there was a 58% (95% CI 48-66%) reduction of diabetes incidence in the intensive lifestyle intervention group and a 31% (95% CI 17-43%) reduction in the metformin group compared with placebo (15).

Institutional review board approval was obtained by each participating clinical center. All participants included in this analysis provided written informed consent for the main investigation and for subsequent genetic studies.

Genome-wide genotyping and quality control

DNA was extracted from peripheral blood leukocytes. Genotyping was performed on 3,227 samples using the HumanCore Exome genome-wide array (Illumina, San Diego, CA). We excluded single nucleotide polymorphisms (SNPs) with a call rate <95% or if they failed Hardy-Weinberg Equilibrium (HWE; $p<1.0\times10^{-8}$) within each ethnic group. Samples with discrepant sex, call rate <95%, inbreeding coefficient <-1, and identity-by-state as measured by pi-hat close to 1 were discarded. As 9,730 SNPs and 3,222 samples were additionally genotyped on the Metabochip (Illumina, San Diego, CA), we performed a concordance check, excluding SNPs and samples with a concordance rate <95%. After all quality checks were performed, 3,168 samples remained (Supplemental Figure S1).

Imputation

We performed a two-stage imputation procedure, which consisted of pre-phasing the genotypes into whole chromosome haplotypes followed by imputation itself. The pre-phasing was performed using SHAPEIT2 (16) and IMPUTE2 was utilized for genotype imputation (17). GWIMp-COMPSs can incorporate the contribution of several reference panels (18), and in this work, we used 1000 Genomes Phase3 haplotypes (October 2014) (19). Supplemental Table S1

summarizes the distribution of the 3,168 samples by self-reported race/ethnicity and DPP treatment arm that underwent imputation.

Statistical analyses

Because the study was conceived as a pharmacogenetic study on the influence of genetics on metformin response, we focused our primary analyses on the standard lifestyle recommendations plus metformin (MET) group and the placebo (PBO) group. Continuous variables are presented as mean ± SD and categorical variables as frequency (%). Baseline characteristics were compared with analysis of variance (ANOVA) tests for quantitative variables and chi-square tests for qualitative variables.

Diabetes incidence

A Cox proportional hazards model tested the association between genetic variants and diabetes incidence under an additive genetic model in the metformin and placebo arms. For any genome-wide significant findings, we planned to check and ensure that the proportionality assumptions were met. We evaluated the impact of genetic variation in the metformin arm only, and in a second model, we included a formal interaction test between the metformin and placebo arms. Models were adjusted for age at randomization, sex, and 10 ancestry principal components (PCs) for population structure; secondary models were also adjusted for waist circumference or BMI. We addressed common variants (minor allele frequency [MAF]>1%), both those directly genotyped and imputed. For imputed markers, we excluded variants that had an imputation quality score <0.7, as well as variants that did not pass a filter test ($2 \times MAF \times [1\text{-}MAF] \times n.events > 75$, where n.events is the number of events), as described in the GWASTools package (20). We evaluated for genome-wide significance, defined as $p<5\times10^{-8}$.

Change in quantitative traits

We utilized a multiple linear regression model to test allelic associations, assuming additive effects, with the change in quantitative traits relevant to metformin action at one year; traits included fasting glucose, 2-hour glucose after 75-g OGTT, fasting insulin, insulin sensitivity index (ISI), hemoglobin A1c (HbA1c), and weight. One-year change in each quantitative outcome was defined as one-year minus baseline value. Non-normally distributed traits were natural log transformed. To minimize the influence of outliers, winsorization was performed (at percentiles of 0.5 and 99.5 for normally distributed traits; percentiles of 1 and 99 for natural log transformed traits) (21). Analyses were adjusted for age, sex, first 10 ancestry PCs, and the baseline value of the trait. Similar to the diabetes incidence analyses, we evaluated the impact of genetic variation in the metformin arm only for each of the six outcomes, and in a second model, we tested for a gene-by-treatment interaction for the metformin and placebo arms. We filtered our results to a study-wide MAF>1% and imputation quality ≥0.7. To account for multiple testing and the possible correlation between quantitative traits, we calculated that there were 5.47 effective outcomes, using a previously described method (22), and we set an experiment-wide significance threshold of $p<9\times10^{-9}$ ($5\times10^{-8}/5.47$). For top findings that emerged, we performed an exploratory analysis that evaluated these findings in the intensive lifestyle treatment arm, in order to understand whether the variants act through a shared pathway between metformin and lifestyle intervention.

Replication

For each of the quantitative trait outcomes, we compiled a list of the top 500 genotyped and imputed variants with a MAF>1% and imputation score ≥0.7 for imputed markers, for a total of ~6000 variants (500 markers × 6 traits × 2 models). We explored their relevance in the Metformin Genetics (MetGen) Consortium, which has data from >10,000 participants from over

12 observational studies and clinical trials, in which a pharmacogenetic meta-analysis of metformin response was performed (10). We did additional filtering of variants, excluding those with a minor allele count <10 and with imputation score <0.7 from the Pharmacogenomics of Metformin (PMET) study, which represents the largest cohort comprising MetGen participants. We then calculated the number of effective variants after accounting for correlation between genetic variants (22) and determined the replication significance threshold. In addition, we attempted to replicate our top variant in the Million Veteran Program (MVP) and the Diabetes Multi-omic Investigation of Drug Response (DIAMOND). Full details of replication cohorts and the statistical models utilized in the replication analyses are described in the Supplemental Material and in Supplemental Table S2.

Data and Resource Availability

Genetic data generated and analyzed in the current study are available in dbGap (dbGaP Study Accession: phs000681.v2.p1, https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000681.v2.p1). Phenotype data for the DPP is available through the NIDDK Data Repository. No applicable resources were generated or analyzed during the current study.

RESULTS

Baseline demographics

Table 1 describes the characteristics of the subset of DPP participants in the metformin and placebo arms in whom genome-wide genotyping was available. The mean baseline age was 50.7±10.4 years, 67% were women, and approximately 44% self-reported as non-white. Baseline measurements were consistent with a population at risk of developing T2D. There were no significant differences in baseline measurements by treatment arm. The event rate for diabetes

incidence is reported for each self-reported race/ethnicity group for both the metformin and placebo arms in Supplemental Table S3.

Association of genetic variation with diabetes incidence

In the metformin arm only (n=876, Supplemental Table S4), we performed association analysis of genetic variants with diabetes incidence during the main DPP study, which had a mean follow-up of 3.2 years. While we did not observe any genome-wide significant findings (Supplemental Figure S2A), we identified a genetic locus near *HIF1AN* that reached suggestive significance. The C effect allele of rs2489017 had a frequency of 0.59 in the entire DPP cohort and was notably more common in non-white populations (White: 0.49, African American: 0.83, Asian/Pacific Islander: 0.62, Hispanic/Latino: 0.62, American Indian: 0.78). Per copy of the variant, participants had a decreased risk of progressing to diabetes (HR 0.54, 95% CI 0.43-0.67, $p=9.2\times10^{-8}$). In the placebo group, the variant did not influence diabetes incidence ($p=0.72$), and we noted the presence of an interaction with treatment arm ($p(G\times T)=5.9\times10^{-5}$).

We subsequently combined the metformin and placebo arms (n=1,763, Supplemental Table S4) and utilized a Cox proportional hazards model to assess for a gene-by-treatment arm interaction for the outcome of diabetes incidence. We did not identify any genome-wide significant findings or variants that met a suggestive significance threshold of $p<1\times10^{-6}$ (Supplemental Figure S2B).

Association of genetic variation with one-year change in quantitative traits

With respect to the quantitative trait outcomes, we performed association analysis of genetic variants with one-year change in any of six quantitative traits (fasting glucose, 2-hour glucose after OGTT, fasting insulin, ISI, HbA1c, and weight) across two separate models, one examining the metformin arm only and another testing for a gene-by-treatment arm interaction.

Supplemental Table S3 presents the sample size for each model and quantitative trait outcome tested. Table 2 summarizes the 14 independent signals that met genome-wide significance ($p<5\times10^{-8}$) for at least one quantitative trait, four of which met experiment-wide significance ($p<9\times10^{-9}$): two for change in HbA1c (Supplemental Figure S3A-B) and two for change in weight (Supplemental Figure S4A-B). These results remained the same after additionally adjusting the analyses for BMI (data not shown). The effect sizes and *p*-values for the top findings, stratified by self-reported race/ethnicity, are also presented in Supplemental Table S5. Because we report the effect allele frequencies stratified by self-reported ethnicity/race, we compared these groupings with the ancestry groupings determined by principal components analysis (PCA) and illustrated that the self-identified groups tended to cluster tightly on the PCA plot (Supplemental Figure S5).

The top hit, rs144322333, which associated with change in HbA1c in the metformin treatment group, is a deletion polymorphism near *ENOSF1* (Figure 1) and was mainly found in African American participants with a MAF of 6%, whereas it is present in under 0.2% in White individuals (Table 2). Carriers have a 0.39% increase in one-year change in HbA1c per copy of the deletion, consistent with a worse response to metformin ($p=2.9\times10^{-12}$, Figure 2A). The proportion of variance in one-year change in HbA1c explained by the variant is 5.2%, compared with 10.8% for baseline HbA1c, the strongest predictor of this quantitative outcome in the model.

Since rs144322333 is more common in African American individuals, we performed a subgroup analysis and found that the effect was detectable in this group ($p=1.3\times10^{-6}$, Figure 2B). In fact, African individuals without any copies of the deletion experienced a *decrease* in HbA1c of 0.03% after a year of metformin, whereas those who carried one or two copies of the deletion

experienced *increases* of 0.2% and 0.6%. respectively. Finally, since the association was discovered in the metformin arm only, we also evaluated its impact in the placebo arm, in which there was no significant effect (p=0.32). The interaction with treatment arm was notably significant (p(G×T)=1.4×10^{-6}, Figure 2C), reflecting the differential impact of this genetic variant.

Another novel experiment-wide significant variant was rs145591055 in chromosome 5 near *OMSR* (Figure 3), a gene that encodes the Oncostatin M receptor (Table 2). Carriers of the G effect allele had a 7.6 kg greater decline in weight at one year following initiation of metformin treatment in adjusted analyses (p=3.2×10^{-10}, Figure 4A). The variant has an effect allele frequency of 10% in American Indians, calculated from the entire DPP study (Table 2). While a subgroup analysis in American Indians was limited by sample size (n=21, Figure 4B), we noted that non-carriers experienced a decrease in weight of 0.5 kg at one year, compared with a decrease of 8.6 kg in heterozygous carriers. We also evaluated the influence of this variant in the placebo arm and found that there was no significant effect on weight (per G allele beta=-0.69, p=0.50). There was again a significant gene-by-treatment interaction (p(G×T)=1.5×10^{-5}, Figure 4C).

In the gene × treatment models, we found that rs6838493, in chromosome 4 near *LINC01093* (Supplemental Figure S6), had a differential influence on change in HbA1c (p(G×T)=1.6×10^{-9}, Supplemental Figure S7), in which heterozygous carriers experienced a *rise* in HbA1c at one year in the placebo arm (beta=0.5, p=1.3×10^{-8}) in comparison to a *decline* in HbA1c in the metformin arm (beta=-0.1, p=0.03). The impact of rs148219263, located in chromosome 18 near *CDH20* (Supplemental Figure S8), on one-year change in weight was also different by treatment arm (p(G×T)=4.8×10^{-9}, Supplemental Figure S9). Here, in comparison to

their TT counterparts, TC carriers experienced weight loss in the placebo arm (beta=-7.0, p=2.4×10^{-8}) but did not achieve weight loss after receiving metformin (beta=3.9, p=4.0×10^{-3}).

In an exploratory analysis, we evaluated our top findings from Table 2 in the intensive lifestyle arm. Of the nine variants that emerged from the analysis completed in the metformin arm only, one variant (rs13401282) appeared to have a similar effect on ISI in the lifestyle arm as in the metformin arm (p=0.004, Supplementary Table S6); the other eight variants were non-significant in the lifestyle arm. For the five variants identified through the gene × treatment model that combined the metformin and placebo arms, we tested their relevance in a similar interaction model that combined the placebo and lifestyle arms. We found that four of the five variants demonstrated similar findings between the two gene × treatment models, as illustrated by the magnitude and direction of the beta estimates, as well as the p-values (Supplementary Table S6).

We searched our lead findings in the Genotype-Tissue Expression (GTex) project (https://gtexportal.org) (23) and eQTLconsortium (https://eqtlgen.org) (24) to evaluate for expression quantitative trait loci (eQTL) associations. We observed that rs17083791, which was associated with a greater decline in weight at one year following metformin, is a cis-eQTL associated with higher expression of *KIAA0825*, the nearest gene, in fibroblasts (normalized effect size [NES] 0.411, p=2.7×10^{-12}), subcutaneous adipose tissue (NES 0.285, p=7.9×10^{-7}), skeletal muscle (NES 0.241, p=5.0×10^{-5}), and visceral adipose tissue (NES 0.229, p=1.6×10^{-4}). In blood samples in eQTLconsortium, we noted that the variant was also a cis-eQTL for *MCTP1* (p=7.3×10^{-189}). We also evaluated the top variants in the pancreatic islet genotype tissue-expression resource (TIGER, http://tiger.bsc.es), which aggregates >500 human islet genomic datasets (25), but there were no notable findings. Finally, we searched top variants against

phenotypes associated with T2D using the Type 2 Diabetes Knowledge Portal (https://t2d.hugeamp.org). We found that the G allele of rs145591055, which was associated with greater one-year weight change on metformin, was marginally associated with a higher BMI-adjusted waist-hip ratio (beta=0.079, p=0.001).

Independent replication

We sought to replicate our pharmacogenetic findings in additional cohorts in which metformin response has been defined. As there are few cohorts of patients with pre-diabetes, our first approach was to explore the relevance of the variants in MetGen, in which the outcome was glycemic response, as measured by baseline minus minimum on-treatment HbA1c within 18 months after metformin initiation (10). Out of ~6000 variants, only 3050 had available genotype information in MetGen, resulting in 2610 effective variants; we thus set a significance threshold of $p<1.9\times10^{-5}$ (0.05/2610). After filtering, results were available in MetGen for only 6 of the 14 genome-wide significant variants from our DPP GWAS, none of which replicated (Table 3).

Because the study cohorts in MetGen were predominately European ancestry, we sought to replicate our top HbA1c finding in African American cohorts. We first examined 2733 participants with established T2D from MVP who self-reported as non-Hispanic African Americans and had received metformin monotherapy for up to 15 months. The top variant, rs144322333, which had a MAF of 7% and an imputation score of 0.89 in MVP, was not significantly associated with change in HbA1c (beta=0.05, p=0.15).

We also sought replication in DIAMOND, an observational population-based cohort with electronic medical record-linked clinical data on individuals with T2D on metformin monotherapy. We identified a subset of 471 individuals who self-reported as African American and were calculated to have a reliable exposure of at least 500 mg metformin daily as

monotherapy for treatment of T2D in the 120 days preceding the follow-up HbA1c measurement. In DIAMOND, rs144322333 had a MAF of 8% and had an imputation score of 0.98. Per copy of the deletion, carriers had a 0.13% reduction in HbA1c lowering over one year in DIAMOND, indicating a worse metformin response, which was similar in direction to that seen in the DPP, but smaller in magnitude and also not reaching statistical significance (p=0.19).

DISCUSSION

To date, GWAS have uncovered several genetic loci influencing the glycemic response to metformin, but these were largely performed in European populations with established T2D (9-11). When these variants were assessed in the DPP, the reported associations were not confirmed for the outcomes of diabetes incidence and other relevant physiologic parameters such as insulin sensitivity, fasting glucose, HbA1c, or oral disposition index (10, 12). One possible explanation for this inconsistency is that metformin response is defined differently in a disease cohort, in which the measure of response is the achievement of a HbA1c ≤7%, compared to a pre-diabetic cohort, in which diabetes incidence or quantitative traits are the studied outcomes. Furthermore, genetic variation may influence drug response differently in individuals with pre-diabetes, who likely have better pancreatic β-cell function.

Thus, we undertook a GWAS in the DPP, examining the outcomes of diabetes prevention as well as one-year change in six quantitative traits known to be affected by metformin therapy. The design of the DPP is unique for studying metformin pharmacogenetics, as it includes both a placebo and metformin arm, permitting the use of an interaction test to assess genetic variants that may have a differential response based on drug exposure. In assessing diabetes incidence, we did not identify any genome-wide significant variants. We acknowledge that the relatively

modest sample size of the DPP with a limited range of impaired glucose tolerance may have restricted our ability to detect an interaction effect. However, we did observe an association that met a suggestive threshold that could be investigated in the future or meta-analyzed with other cohorts.

Our quantitative trait analyses yielded several genome-wide significant associations, after correcting for multiple testing. *ENOSF1*, the closest gene to the top variant rs144322333, encodes the enolase superfamily member 1, a mitochondrial enzyme involved in the catabolism of L-fucose, a sugar found on cellular glycoproteins. Interestingly, *ENOSF1* is next to a kinase, YES1, which has been shown to regulate the activity of organic cation transporters OCT1 (*SLC22A1*) and OCT2 (*SLC22A2*) that play a critical role in metformin disposition and elimination in the kidney and liver, respectively (26, 27). Moreover, LINC01093 near rs186681623, has been shown to be related to several liver phenotypes, including fibrosis (28) and hepatocellular carcinoma (29), and may play a role in modulating metformin's action on the liver.

Many of our genome-wide significant findings were ancestry-specific (rs144322333 near *ENOSF1*, rs145591055 near *OSMR,* rs6838493 near *LINC01093*). We further illustrate this through subgroup analyses and demonstrate the persistent effect of top variants of interest after stratifying by self-reported ethnicity/race. Based on these stratified analyses, it appeared that the ancestry-specificity of our findings was mainly driven by allele frequency, since similar trends were observed across subgroups. We acknowledge that this may have been a consequence of conducting our analyses in a multi-ancestry cohort, which could have biased our results toward variants with concordant effects across subgroups. One limitation of this approach is that variants with opposite effects in different subgroups could be potentially missed. We also estimated that

our top genetic finding (rs144322333) explained 5.2% of the variance in one-year change in HbA1c, which was half of that of baseline HbA1c, supporting the notion that the contribution of genetics is modest compared to a traditional predictor of drug response.

In the metformin arm of the DPP, the average weight loss was 2.1 kg and was relatively stable throughout the study follow-up period (15). In comparison, the association of rs145591055 (near *OSMR*) variation with one-year change in weight was 7.6 kg, over three times greater in magnitude. Individuals who carry this variant, which has an effect allele frequency of 10% in American Indians, appear to derive a significant weight-loss benefit from metformin, which is especially relevant given the high incidence and prevalence of T2D in this population (30, 31). Moreover, the G allele was reported in the T2D Knowledge Portal to be marginally associated with a higher BMI-adjusted waist-hip ratio, also suggesting that this is a group of individuals that would benefit from metformin. However, the underlying mechanism responsible for this weight effect is not clear and requires further investigation. We also evaluated our top findings in the GTex project, the eQTLconsortium, and the TIGER portal. Interestingly, we observed that the variant rs17083791 was a *cis*-eQTL for the nearest gene *KIAA0825* in several relevant tissues, suggesting that changes in *KIAA0825* gene expression may modulate metformin-related weight loss. The same variant was also a strong *cis*-eQTL for *MCTP1*, which codes for a protein involved in calcium ion binding, though the significance of this finding is unknown. While these genomic resources can be useful for functional follow-up of variants, the majority of participants are of European ancestry and thus, their utility is limited for variants that are common only in specific populations.

In an exploratory analysis, we evaluated the top findings from the quantitative trait analysis using the intensive lifestyle arm of the DPP. There were five variants for which the

association with the quantitative trait was similar in the lifestyle arm as in the metformin arm. Prior studies have suggested that the intensive lifestyle intervention of the DPP worked similarly to metformin in improving insulin sensitivity (32). Therefore, it is possible that the mechanism of action of these variants may be shared between metformin and lifestyle. On the other hand, the remaining nine variants that met genome-wide significance were non-significant when evaluated in the lifestyle arm, illustrating they may be specific to metformin response.

Finally, we took a comprehensive approach to replicate findings that emerged from our GWAS, though this was a major challenge due to the lack of a suitable replication cohort of individuals with pre-diabetes who received longitudinal metformin exposure. We evaluated approximately the top 500 variants for each quantitative trait analyses in MetGen, the largest meta-analysis examining glycemic response to metformin in T2D (10). Our efforts did not yield replication, but this could be explained by the composition of MetGen as previously discussed. We subsequently identified two African American cohorts (MVP and DIAMOND) for replication of our top variant rs144322333. Though the study populations again largely reflected individuals with T2D, we constructed our models to reflect a similar length of metformin exposure as in the DPP and included similar covariates as in our quantitative trait analyses. Despite imputation on different reference panels in these cohorts, we reassuringly observed similar MAFs for rs14432233; however, neither replication produced a nominally significant result. We acknowledge the possibility that given the low study-wide MAF of our top findings and the small sample size, our findings could represent false positive results.

Our work underscores the importance of conducting multi-ancestry pharmacogenetic studies to improve upon treatment algorithms across diverse populations in the future. A prior study based on pharmacy fill data reported that African American adults appear to have a better

glycemic response to metformin compared with European Americans (33), and the TODAY study showed that African American youth with T2D on metformin had poorer glycemic response to metformin (7). Though these studies were based on self-reported race/ethnicity information, we demonstrated in the DPP that there is clustering of the self-reported ethnicity/race groups along the genetic ancestry PCs. Thus, the identification of variants in our study that are more prevalent in African American populations suggests that there may be a heritable component to these differences in response to metformin.

Strengths of our study include the diverse composition of the DPP, which includes nearly 45% non-white individuals by self-report and represents a cohort of individuals at a higher risk of developing T2D in the US. Participants were well-characterized under standardized clinical trial protocols, and the genetic data was obtained using the highest standards for genotyping and imputation at the time. As described, limitations of our study are the small sample size, which is a frequent problem in pharmacogenomic GWAS (34) and the lack of replication of our findings in patients with diabetes, though this may reflect differences in genetic modulation of metformin response between the pre-diabetes and diabetes state. However, we were exhaustive in our attempts to identify independent cohorts, including the largest African American cohorts with diabetes known to us. Future directions should include additional follow-up and meta-analyses in additional independent ancestry-specific cohorts as they arise, to advance work in this area.

In conclusion, we have identified several genome-wide significant associations with metformin response, as measured by change in quantitative traits, in a well-defined multi-ancestry clinical trial cohort. We believe that we have generated a valuable resource that can be utilized for future genetic investigation and to gain insight into the genetic underpinnings of interindividual differences in metformin response in a population at risk for developing T2D.

Finally, we illustrate the peremptory need to generate suitable pharmacogenomic and transcriptomic resources in diverse populations.

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Author Contributions. JHL, JAP, KAJ, SS, TIP, and JCF contributed to the conception and design the study. Genotyping, quality control, and imputation of the genetic data were performed by LC, MH, and JMM. GWAS analyses were performed by JAP, KAJ, and TIP. JHL, JAP, KAJ, SS, JNT, JMM, QP, PWF, RLH, SEK, WCK, TIP, and JCF contributed to data interpretation. AYD, SWY, KMG, ERP, AG, AMH, SX, and LKW contributed to the replication analyses

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TABLES

Table 1. Baseline characteristics of 1,768 participants in the DPP in the metformin (MET) and placebo (PBO) arms with genome-wide genotyping.

Baseline Characteristic[*]	**Total**	**PBO**	**MET**	***p*-value**[**]
n	1768	888	880	
Age in years	50.7±10.4	50.6±10.4	50.9±10.3	0.538
Men	578 (32.7)	281 (31.6)	297 (33.8)	0.345
Women	1190 (67.3)	607 (68.4)	583 (66.3)	
Self-reported Race/Ethnicity				
White	997 (56.4)	490 (55.2)	507 (57.6)	0.746
Black	364 (20.6)	186 (20.9)	178 (20.2)	
Hispanic/Latino	290 (16.4)	147 (16.6)	143 (16.3)	
Asian/Pacific Islander	69 (3.9)	38 (4.3)	31 (3.5)	
American Indian	48 (2.7)	27 (3.0)	21 (2.4)	
Weight (kg)	94.7±19.8	94.9±20.0	94.5±19.6	0.692
HbA1c (%)[†]	5.917±0.501	5.920±0.495	5.915±0.507	0.832
Fasting insulin (pmol/L)[#†]	167 (111, 236)	167 (111, 229)	167 (111, 236)	0.595[***]
Fasting glucose (mmol/L)	5.940±0.462	5.956±0.466	5.923±0.458	0.138
ISI[#†]	0.161 (0.112, 0.235)	0.161 (0.113, 0.236)	0.161 (0.110, 0.234)	0.762[***]

[*] Data are Means±SD for continuous variables and n (%) for categorical variables unless noted otherwise
[**] *p*-values from F test for continuous variables and Chi-square for categorical variables
[***] *p*-value from Kruskal-Wallis test
[#] Median (25th, 75th percentile)
†n=1766 for Total; n=887 for PBO; n=879 for MET

Table 2. 14 independent genome-wide significant findings ($p<5\times10^{-8}$) for one-year change in quantitative traits in the DPP.

rsid	Chr	Position*	Nearest gene	EA	NEA	Trait	N	Beta (95% CI)	*p*	Effect allele frequency					
										All†	AfrAm‡	AsnPI‡	Hisp‡	AI‡	White‡
Metformin only model															
rs144322333	18	705550	*ENOSF1*	C	CTGTT	HbA1c, %	818	0.39 (0.28, 0.50)	2.9×10^{-12}	0.013	0.065	0.001	0.009	0.0001	0.002
rs145591055	5	38849463	*OSMR*	G	A	Weight, kg	829	-7.55 (-9.87, -5.22)	3.2×10^{-10}	0.014	0.001	0.018	0.064	0.103	0.007
rs13401282§	2	207810690	*CPO*	A	T	ISI, ln	808	0.44 (0.29, 0.59)	1.7×10^{-8}	0.029	0.119	0.003	0.015	0.006	0.002
rs186681623	13	81546608	*LINC00377*	C	T	Weight, kg	829	-2.66 (-3.58, -1.74)	2.0×10^{-8}	0.068	0.121	0.074	0.122	0.161	0.036
rs17083791	5	93863813	*KIAA0825*	G	A	Weight, kg	829	-1.85 (-2.50, -1.20)	3.3×10^{-8}	0.149	0.052	0.194	0.210	0.367	0.137
rs9931871	16	19928315	*GPRC5B*	G	A	Fasting glucose, mmol/L	821	0.68 (0.44, 0.93)	3.5×10^{-8}	0.011	0.049	<0.0001	0.003	<0.0001	<0.0001
rs549305231	6	19497504	*LOC101928519*	A	G	HbA1c, %	818	0.35 (0.23, 0.48)	3.9×10^{-8}	0.012	0.046	<0.0001	0.005	<0.0001	0.0003
rs73944532	2	104691634	*LOC100287010*	A	G	Fasting insulin, ln	802	-0.47 (-0.63, -0.30)	4.1×10^{-8}	0.020	0.075	0.005	0.007	0.001	0.002
Gene × Treatment model‖															
rs6838493	4	185879789	*LINC01093*	A	T	HbA1c, %	1621	-0.66 (-0.87, -0.44)	1.6×10^{-9}	0.011	0.041	0.004	0.005	0.004	0.002
rs148219263	18	58808522	*CDH20*	C	T	Weight, kg	1673	10.73 (7.15, 14.30)	4.8×10^{-9}	0.012	0.007	0.011	0.011	0.0009	0.017
rs75147163	14	57626769	*EXOC5*	A	G	Fasting glucose, mmol/L	1633	-0.73 (-0.98, -0.48)	1.4×10^{-8}	0.022	0.053	0.018	0.022	0.0002	0.015
rs78075715	4	6613716	*MAN2B2*	C	T	HbA1c, %	1621	-0.51 (-0.68, -0.33)	1.4×10^{-8}	0.013	0.063	<0.0001	0.002	<0.0001	0.0004
rs12314996	12	24567467	*SOX5*	A	G	HbA1c, %	1621	-0.47 (-0.63, -0.30)	3.9×10^{-8}	0.016	0.062	<0.0001	0.007	0.001	0.002

rs143203347	7	107384199	*CBLL1*	C	G	Fasting glucose, mmol/L	1633	-0.92 (-1.24, -0.59)	4.1×10^{-8}	0.015	0.062	0.0002	0.011	<0.0001	<0.0001

EA=Effect allele; NEA=Non-effect allele; 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 for the number of participants in the model (N) that was calculated based on imputation. ‡Effect allele frequency breakdown by self-reported race/ethnicity is for all 3,168 participants with genome-wide genotyping, calculated based on imputation. §rs13401282 is also associated with fasting insulin (beta [95% CI] = -0.39 [-0.53, -0.25], $p=4.65\times10^{-8}$). G×T models contain an exposure term for the treatment arm and a G×T interaction term. Results are filtered to $p(G\times T)<5\times10^{-8}$ and $p(SNP)<1\times10^{-4}$. ‖Beta estimates and confidence intervals (CI) are reported for the interaction term rather than the main effect of metformin.

Table 3. Attempt at replication of genome-wide significant findings from the DPP in the Metformin Genetics Consortium.

DPP Model	Trait	rsid	DPP Beta*	MetGen (n)	MetGen Beta*	MetGen *p*-value
MET	Weight, kg	rs186681623	-2.66 (greater weight loss)	10519	-0.05 (decreased HbA1c reduction)	0.10
MET	Weight, kg	rs17083791	-1.85 (greater weight loss)	12578	0.004 (greater HbA1c reduction)	0.79
MET	Fasting insulin, ln	rs73944532	-0.47 (greater decrease in fasting insulin)	7048	0.03 (greater HbA1c reduction)	0.84
G×T	Weight, kg	rs148219263	10.73 (reduced weight loss)	10519	-0.04 (decreased HbA1c reduction)	0.39
G×T	Fasting glucose, mmol/L	rs75147163	-0.73 (greater decrease in fasting glucose)	10288	-0.02 (decreased HbA1c reduction)	0.70
G×T	HbA1c, %	rs12314996	-0.47 (greater decrease in HbA1c)	7048	0.19 (greater HbA1c reduction)	0.24

*In the DPP, the beta estimates represent one-year change in the quantitative trait as calculated by follow-up value minus baseline value. In MetGen, the outcome was change in HbA1c (%), defined as baseline value minus follow-up value within 18 months (will have opposite signs of beta estimates for change in HbA1c compared to that in the DPP). For each analysis and cohort, the impact of the variant on the metformin response outcome is indicated in parentheses.

FIGURE LEGEND

Figure 1. Regional association plot of rs144322333 for one-year change in HbA1c. The red line indicates the experiment-wide significance threshold of $p<9\times10^{-9}$ and the blue line indicates a suggestive significance threshold of $p<1\times10^{-6}$.

Figure 2. (A) Box plot illustrating the mean change in HbA1c (one-year minus baseline) by rs144322333 genotype in the metformin only arm, n=818. (B) Stratified analyses by self-reported race/ethnicity. *P*-value was calculated for the subgroup of African American individuals, n=166. (C) Comparison of the influence of rs144322333 genotype on the mean change in HbA1c in all subjects 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.

Figure 3. Regional association plot of rs145591055 for one-year change in weight. The red line indicates the experiment-wide significance threshold of $p<9\times10^{-9}$ and the blue line indicates a suggestive significance threshold of $p<1\times10^{-6}$.

Figure 4. (A) Box plot illustrating the mean change in weight (one-year minus baseline) by rs145591055 genotype in the metformin only arm, n=829. (B) Box plot stratified by self-reported race/ethnicity. (C) Comparison of the influence of rs144322333 genotype on the mean change in weight in all subjects 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.

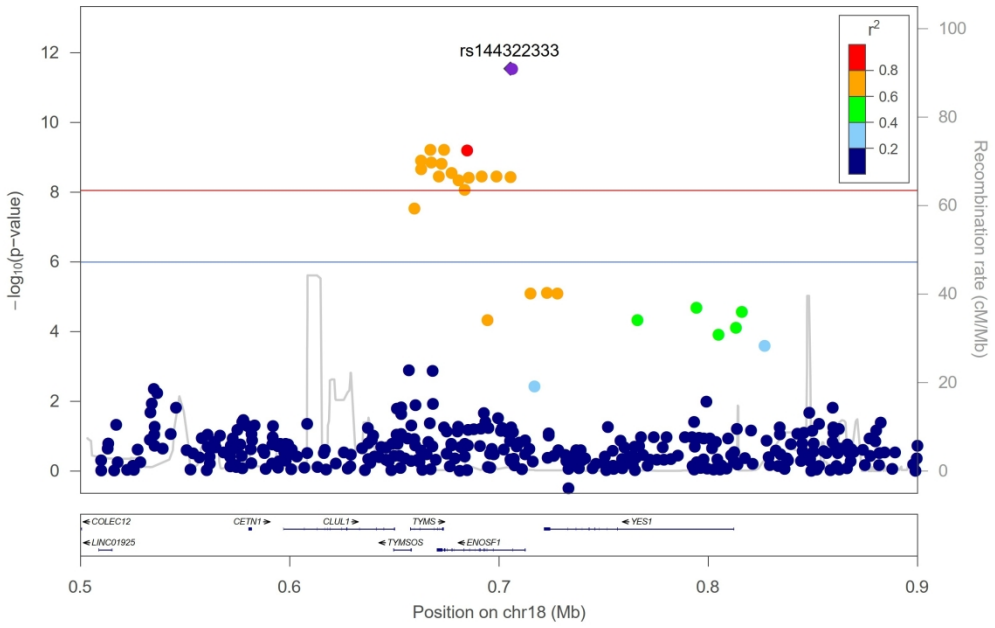

Figure 1. Regional association plot of rs144322333 for one-year change in HbA1c. The red line indicates the experiment-wide significance threshold of p<9×10-9 and the blue line indicates a suggestive significance threshold of p<1×10-6.

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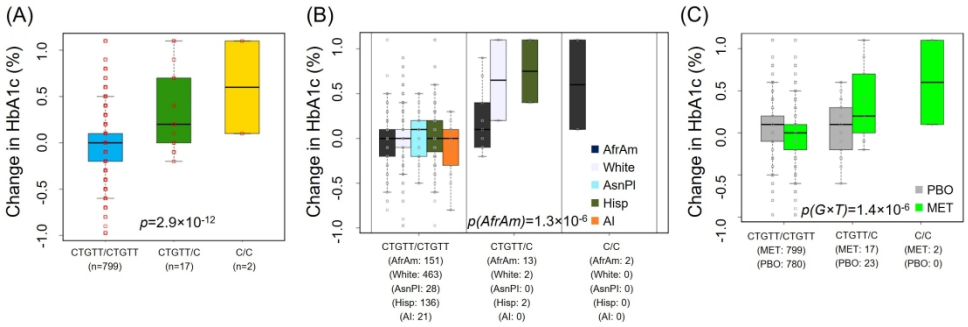

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238x79mm (330 x 330 DPI)

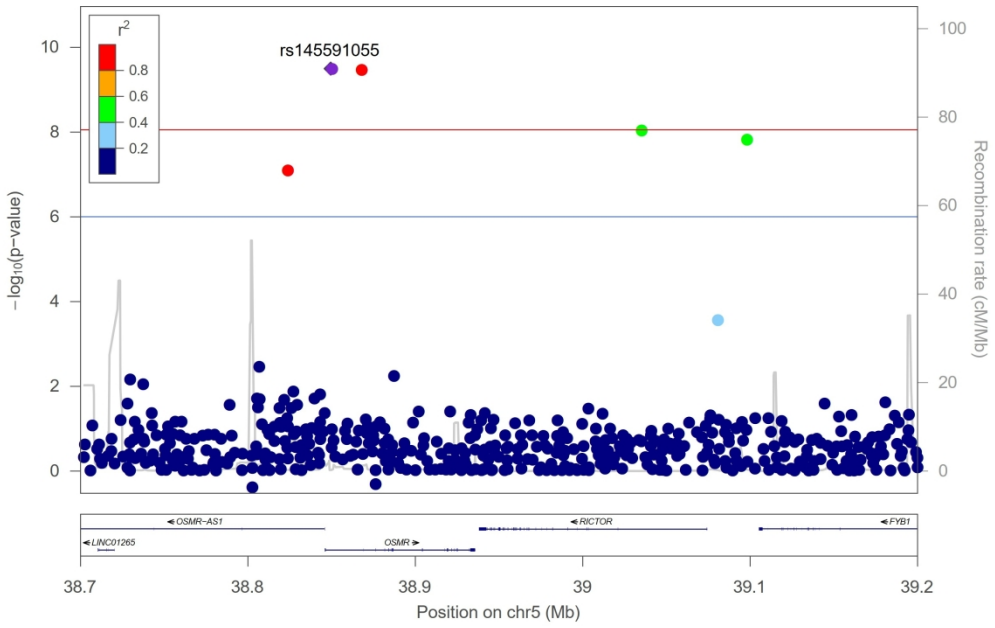

Figure 3. Regional association plot of rs145591055 for one-year change in weight. The red line indicates the experiment-wide significance threshold of p<9×10-9 and the blue line indicates a suggestive significance threshold of p<1×10-6.

242x152mm (330 x 330 DPI)

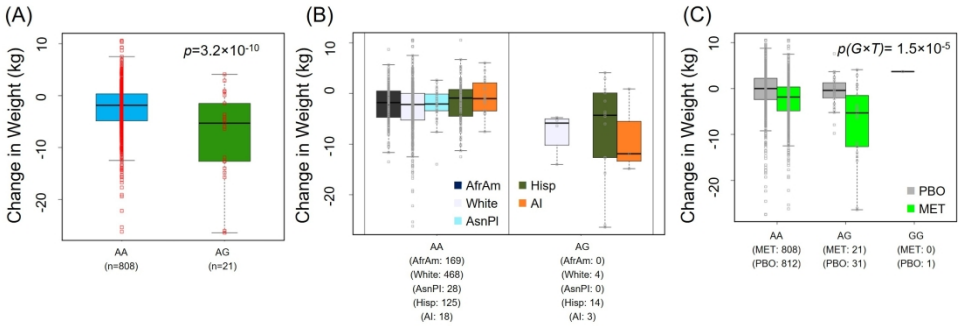

Figure 4. (A) Box plot illustrating the mean change in weight (one-year minus baseline) by rs145591055 genotype in the metformin only arm, n=829. (B) Box plot stratified by self-reported race/ethnicity. (C) Comparison of the influence of rs144322333 genotype on the mean change in weight in all subjects 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.

237x79mm (330 x 330 DPI)

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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.

	Treatment arm				
Self-reported race/ethnicity	**MET**	**ILS**	**PBO**	**TROG**	**Total**
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 ≥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 *et al.* (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 *et al.* (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	*p*
Metformin only model												
rs144322333	18	705550	*ENOSF1*	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	*OSMR*	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	*CPO*	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	*LINC00377*	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
							0.036	White	473	-3.83	0.87	1.4E-05
rs17083791	5	93863813	*KIAA0825*	G	A	Weight, kg	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
							0.367	AI	21	-3.19	2.39	2.3E-01
							0.137	White	473	-1.71	0.44	1.2E-04
rs9931871	16	19928315	*GPRC5B*	G	A	Fasting glucose, mmol/L	0.011	All	821	0.68	0.12	3.5E-08
							0.049	AfrAm	168	0.62	0.13	5.9E-06
rs549305231	6	19497504	*LOC101928519*	A	G	HbA1c, %	0.012	All	818	0.35	0.06	3.9E-08
							0.046	AfrAm	166	0.40	0.07	4.9E-08
							0.005	Hisp	138	-1.22	1.24	3.3E-01
rs73944532	2	104691634	*LOC100287010*	A	G	Fasting insulin, ln	0.020	All	802	-0.47	0.08	4.1E-08
							0.075	AfrAm	165	-0.50	0.09	1.4E-07
							0.007	Hisp	134	-0.13	0.30	6.6E-01

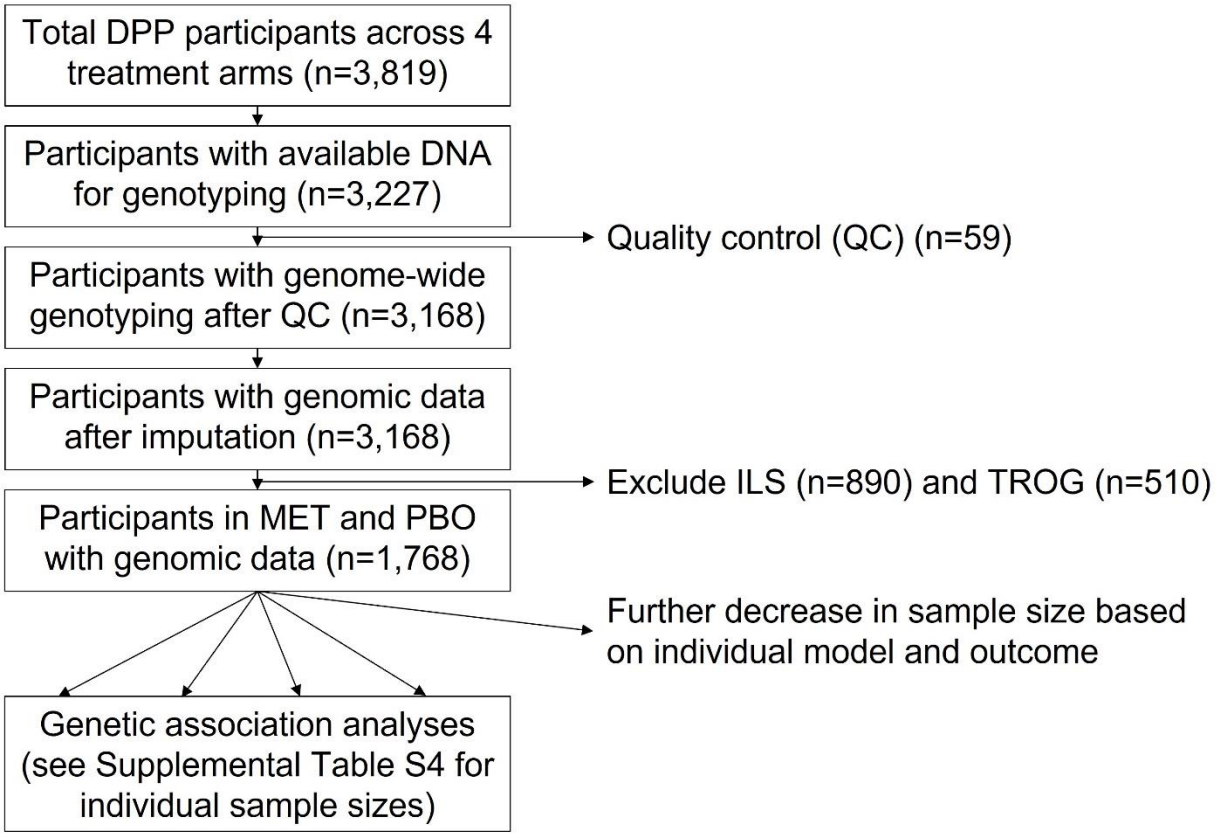
rsid	Chr	Position*	Nearest gene	EA	NEA	Trait	EAF†	Self-reported race/ethnicity	N	Beta	SE	*p*
Gene × Treatment model‖												
rs6838493	4	185879789	*LINC01093*	A	T	HbA1c, %	0.011	All	1621	-0.66	0.11	1.6E-09
							0.041	AfrAm	330	-0.80	0.14	6.0E-08
rs148219263	18	58808522	*CDH20*	C	T	Weight, kg	0.012	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	*EXOC5*	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	*MAN2B2*	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	*SOX5*	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	*CBLL1*	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.

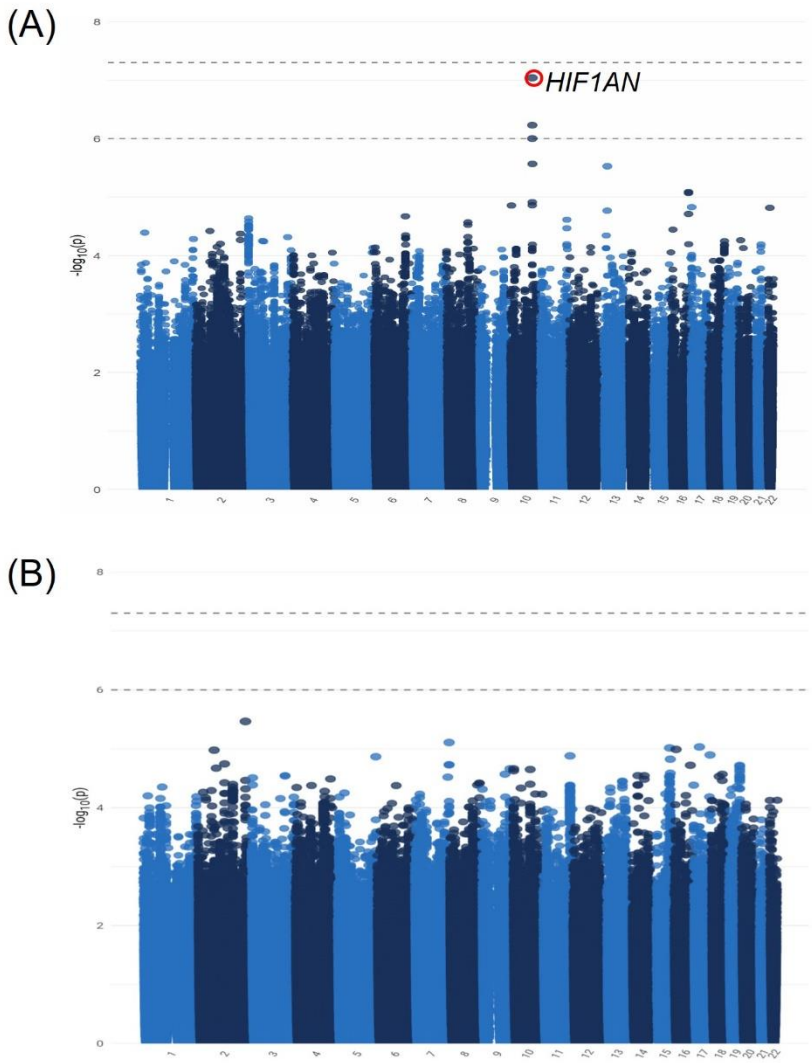
Supplemental Table S6. Evaluation of the 14 independent genome-wide significant findings from Table 2 in the lifestyle treatment arm of the DPP.

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

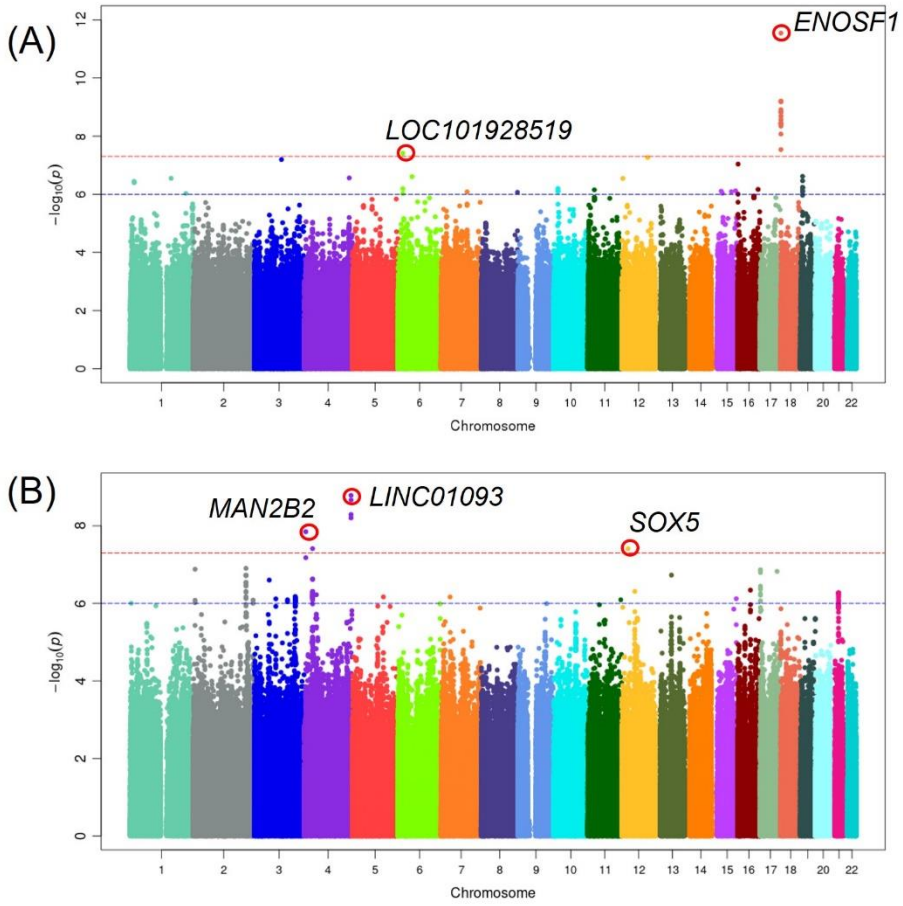
EA=Effect allele; NEA=Non-effect allele; EAF=effect allele frequency. ISI=insulin sensitivity index. *GRCh37 assembly. †This is the effect allele frequency taken from "All" participants in Table 2. ‖Beta estimates and standard errors (SE) are reported for the interaction term rather than the main effect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



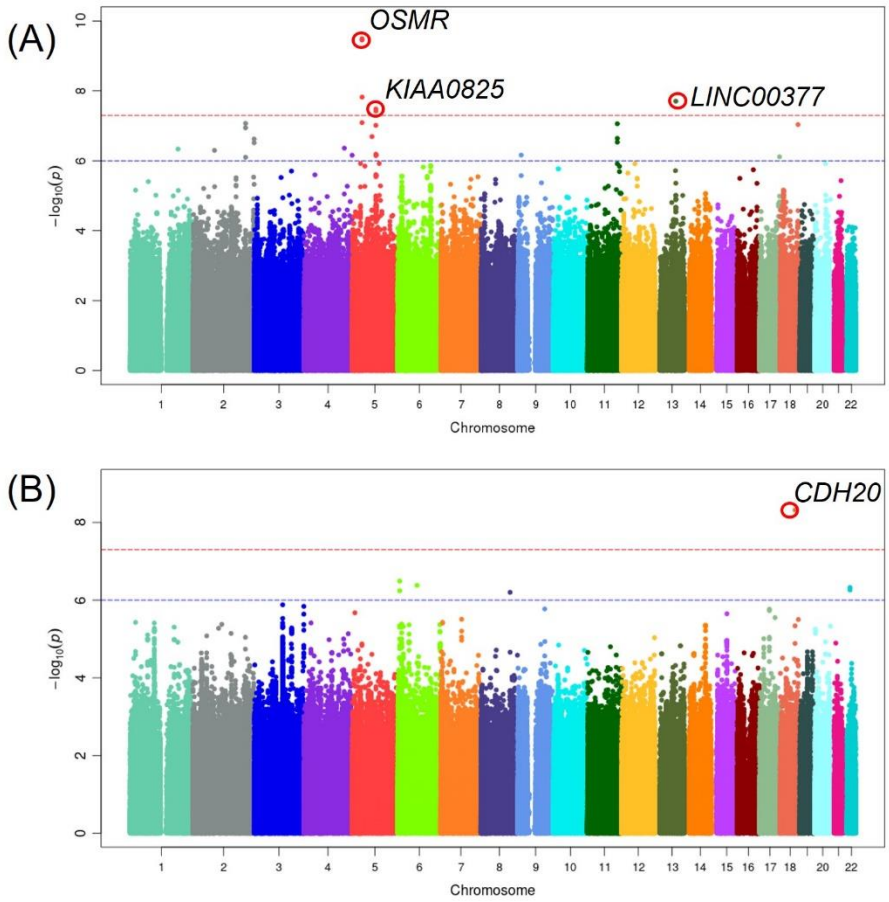
Supplemental Figure S1. Flowchart illustrating the sample size reduction in the DPP leading up to genetic association analyses. MET=metformin; ILS=intensive lifestyle modification; PBO=placebo; TROG=troglitazone.



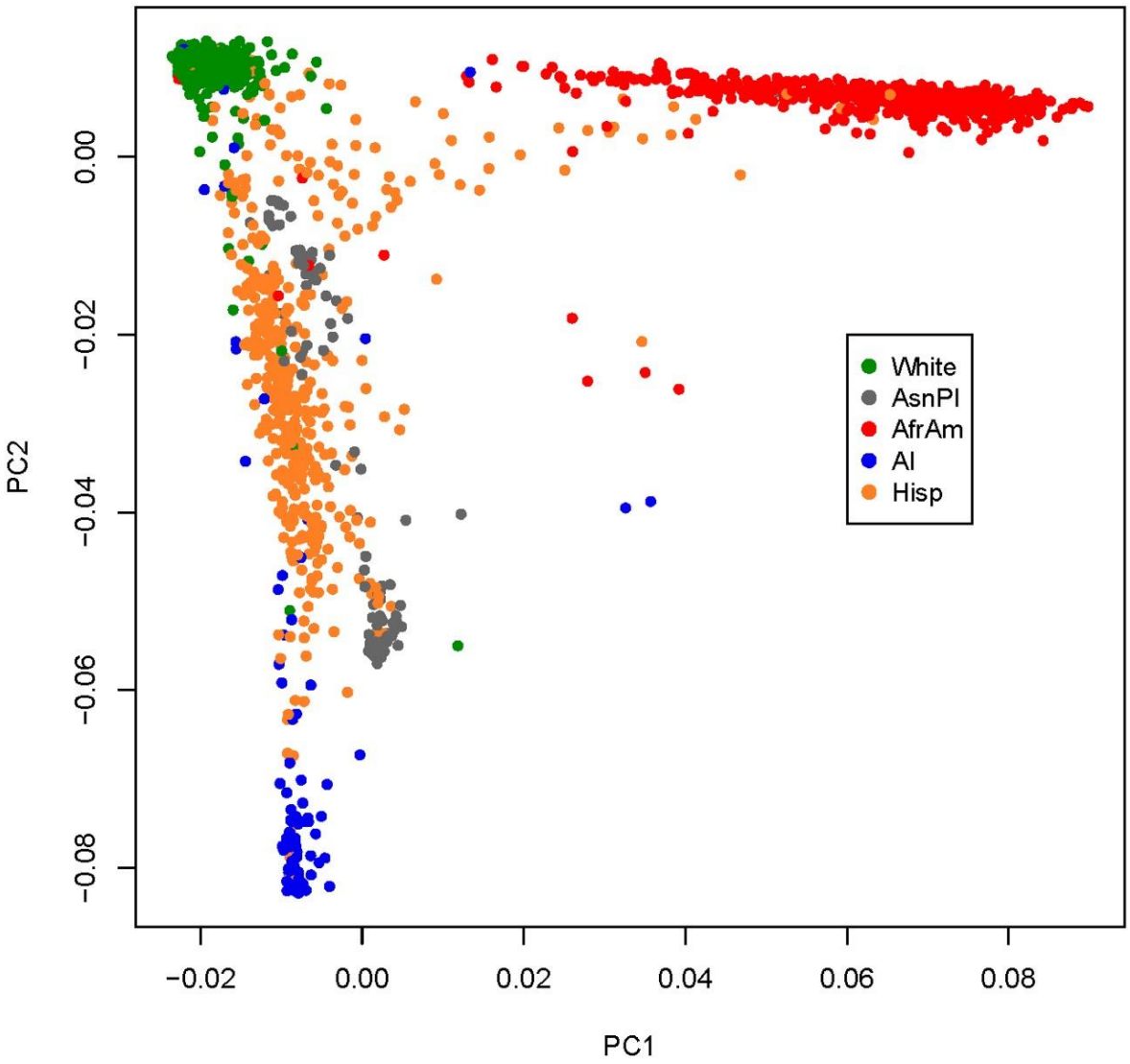
Supplemental Figure S2. Manhattan plot of genome-wide results from single marker association with diabetes incidence (A) using an additive genetic model in 876 individuals in the metformin arm only and (B) testing a gene × treatment interaction in the placebo and metformin arms in 1,763 individuals. For each panel, the two dotted lines indicate the suggestive significance ($p<1\times10^{-6}$) and genome-wide significance ($p<5\times10^{-8}$). Top loci are circled in red with the closest gene labeled.



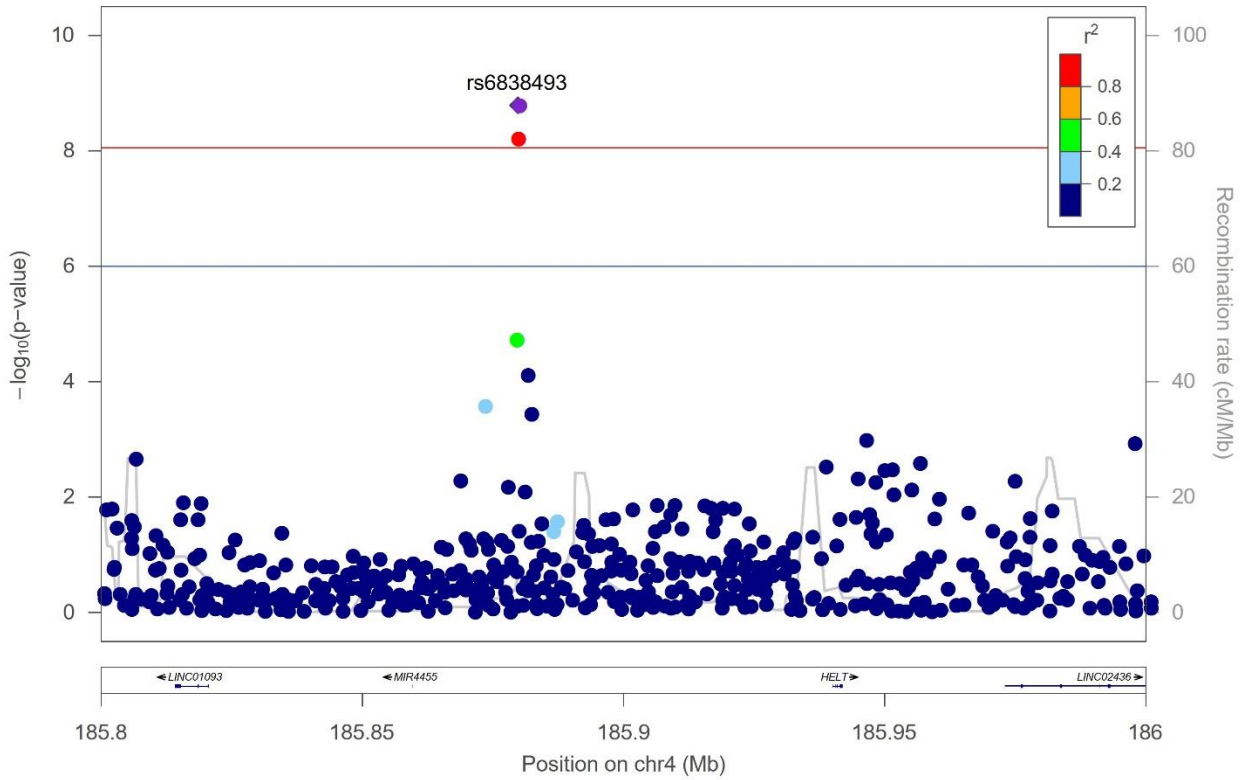
Supplemental Figure S3. Manhattan plots of genome-wide results from single marker association with one-year change in HbA1c (A) using an additive genetic model in 818 individuals in the metformin arm only and (B) testing a gene × treatment interaction in the placebo and metformin arms in 1,621 individuals. The blue line indicates suggestive significance ($p<1\times10^{-6}$) and the red line indicates genome-wide significance ($p<5\times10^{-8}$). Top loci are circled in red with the closest gene labeled.



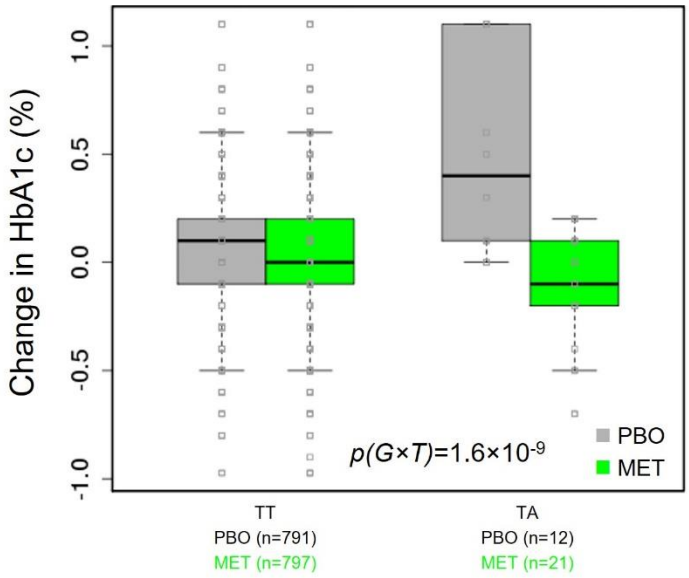
Supplemental Figure S4. Manhattan plots of genome-wide results from single marker association with one-year change in weight (A) using an additive genetic model in 829 individuals in the metformin arm only and (B) testing a gene × drug interaction in the placebo and metformin arms in 1,673 individuals. The blue line indicates suggestive significance ($p<1\times10^{-6}$) and the red line indicates genome-wide significance ($p<5\times10^{-8}$). Top loci are circled in red with the closest gene labeled.



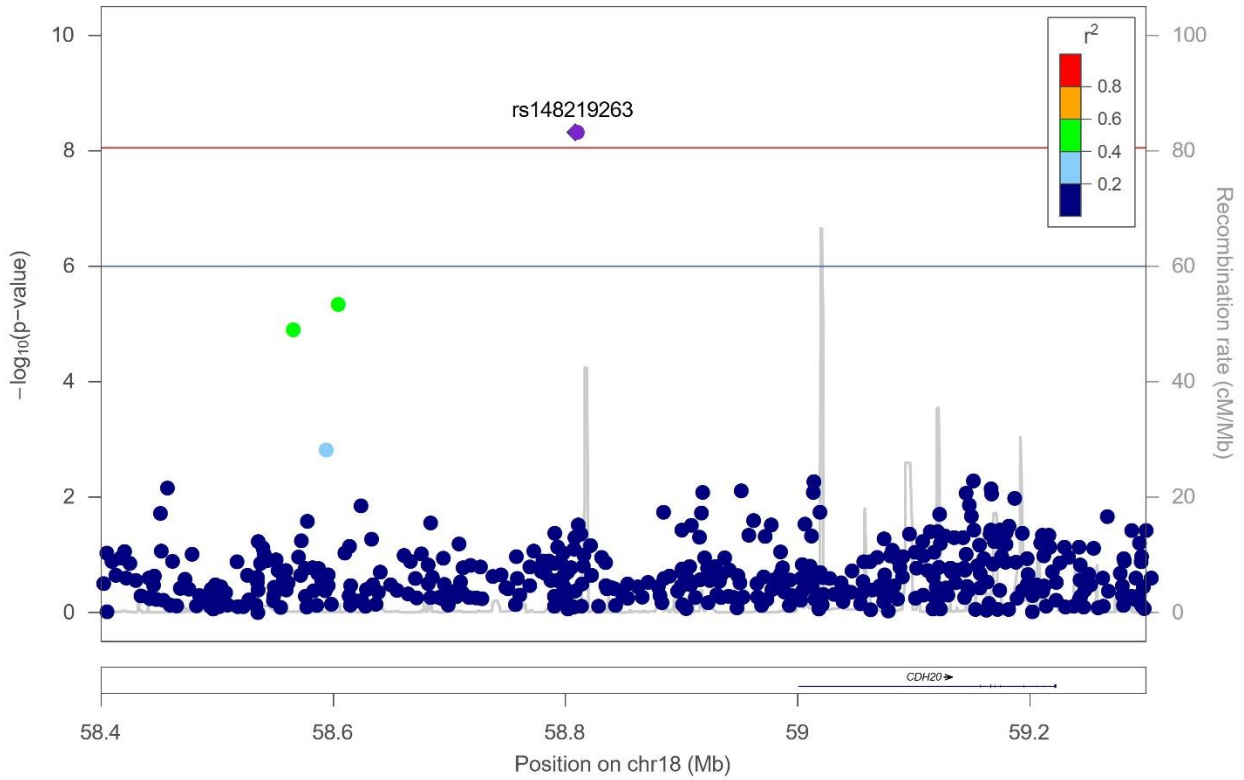
Supplemental Figure S5. Self-reported race/ethnicity (colored circles) displayed on a background plot of the first two genetic ancestry principal components.



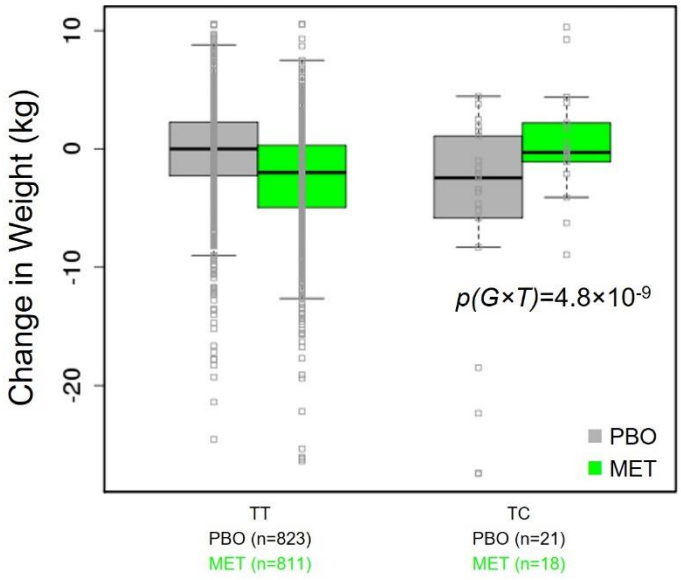
Supplemental Figure S6. Regional association plot of rs6838493 for one-year change in HbA1c. The red line indicates the experiment-wide significance threshold of $p<9\times10^{-9}$ and the blue line indicates a suggestive significance threshold of $p<1\times10^{-6}$.



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 $p<9\times10^{-9}$ and the blue line indicates a suggestive significance threshold of $p<1\times10^{-6}$.



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