ONLINE SUPPLEMENT

Genome-wide association study identifies pharmacogenomic variants associated with metformin glycemic response in African American patients with type-2 diabetes

Baojun Wu, PhD[1]*; Sook Wah Yee, PhD[2]*; Shujie Xiao, MS*; Fei Xu, MA[3]; Sneha B. Sridhar, MPH[3]; Mao Yang, MS[1]; Samantha Hochstadt, MS[1]; Whitney Cabral, MS[1]; David E. Lanfear, MD, MS[1]; Monique M. Hedderson, PhD[3]; Kathleen M. Giacomini, PhD[2]; L. Keoki Williams, MD, MBA, MPH[1]

*Denotes equal contribution as the first author

[1]Center for Individualized and Genomic Medicine Research (CIGMA), Department of Internal Medicine, Henry Ford Health System, Detroit, MI

[2]Department of Bioengineering and Therapeutic Sciences and Institute for Human Genetics, School of Pharmacy, University of California San Francisco, San Francisco, CA

[3]Division of Research, Kaiser Permanente Northern California, Oakland, CA

Short running title: Genome-wide pharmacogenomic study of metformin

Corresponding Authors:

Baojun Wu, PhD

Center for Individualized and Genomic Medicine Research (CIGMA)

Henry Ford Health System

1 Ford Place, 2F

Detroit, Michigan 48202

(313) 874-6435 (office)

bwu3@hfhs.org

L. Keoki Williams, MD, MPH

Center for Individualized and Genomic Medicine Research (CIGMA)

Henry Ford Health System

1 Ford Place, 2F

Detroit, Michigan 48202

(313) 874-6693 (office)

kwillia5@hfhs.org

SUPPLMENTARY METHODS AND DISCUSSION

Two separate analyses were performed in this paper. In the first analysis, we did a time-updated assessment of metformin exposure on change in HbA1c. The assumption was that the observed change in HbA1c among individuals taking a daily dose ≥425 mg per day was due to metformin. In this regard, our approach was analogous to pharmacogenomic papers in which the analysis was restricted to the active treatment arm of a clinical trial (exposure-only analysis) and the observed change in the main outcome was attributed to treatment. Our approach was also similar to the first published genome-wide association study (GWAS) for metformin by the GoDARTS and UKPDS Diabetes Pharmacogenetics Study Group and The Wellcome Trust Case Control Consortium (1). In this study, the authors adjusted for medication adherence, whereas we restricted our analysis to those who were exposed (i.e., ≥425 mg per day). The earlier GWAS used treatment initiation but it also allowed for 18 months of follow-up in which to assess the change in HbA1c (i.e., the putative time window before metformin treatment response deteriorates). Because our analysis used the first qualifying exposure window when patients were using metformin exclusively, this exposure window may not have included the time of metformin initiation (Supplementary Figure 1). Nevertheless, the majority of our observations were at the time of treatment initiation (Supplementary Figure S8). Moreover, our empirically observed time of deterioration in metformin treatment response was shorter than presumed in the earlier studies (i.e., we observed 403 days instead of 18 months) (Supplementary Figure S9). However, our main conclusion about rs143276236, our lead variant, was unchanged by restricting our assessment to the first 403 days (Supplementary Table S6). We also showed (Supplementary Figure S2) that our exposure metric had a dose-response relationship with change in HbA1c (i.e., via our first qualifying observation window approach), although the dose-

response relationship that we observed may have been muted as a result of not exclusively focusing on treatment initiation and the immediate ensuing time period (e.g., the first 146 days following treatment initiation, as shown in Supplementary Figure S8 and Supplementary Table S5).

The GoDARTS/UKPDS/WTCCC GWAS also did not directly model the effect of treatment; rather, it adjusted for whether the patients were on metformin alone or on metformin added to a sulfonylurea (1). In our study, we restricted our observation to time periods in which patients were on metformin alone. Therefore, our first analysis had many similarities to the GoDARTS/UKPDS/WTCCC pharmacogenomic GWAS of metformin. However, because we wanted to assess presumed metformin response at times when patients were only using metformin (i.e., without the overlay of concomitant diabetes treatments which could obscure the effect of metformin), we accepted the limitation of assessing the change in HbA1c at times which didn't always include metformin treatment initiation (unlike the GoDARTS/UKPDS/WTCCC pharmacogenomic GWAS of metformin) – See Supplementary Figures S1 and S8. Nevertheless, despite showing that the vast majority of observations were performed within the window of metformin's apparent response (Supplementary Figures S8 and S9), we concede that it is possible that the change in HbA1c modeled in our first analysis was not attributable to metformin. Similarly, the change in HbA1c modeled in the GoDARTS/UKPDS/WTCCC GWAS might also not be fully attributable to metformin for the above reasons (i.e., deterioration in response over time and concomitant diabetes treatments).

For the above reasons, we embarked on a wholly distinct second analytic approach in which we assessed for gene (genotype) x drug interactions. In this second approach, we did directly model the effect of metformin on HbA1c change in the regression model. Because

metformin exposure was directly entered into the model, it was incumbent to assess for variants which modified this relationship. In other words, because metformin was a main effect variable in the model, we had to also include a genotype x metformin interaction term to evaluate for effect modification by variants. Given that we were primarily assessing the joint effect of genotype and the genotype x metformin interaction on change in HbA1c, we used an inverse-variance weighted approach. Similar approaches have been described elsewhere (2). The inclusion of an interaction term also precluded us from adopting the repeated measure approach used in one of our earlier studies (3), as the time-updated approach would have produced an uninterpretable interaction term. Nevertheless, based on the data already presented showing the predictive validity of our exposure measure (Supplementary Figure S2), we felt it acceptable to use the first qualifying observation period for the interaction analysis (again accepting the trade-off between studying the effect of metformin monotherapy albeit at times after treatment initiation vs. using exclusively the time of metformin treatment initiation when other diabetes medications could have been used concomitantly). In these models, we directly modeled the main effect of metformin, genotype, and metformin x genotype interaction (Supplementary Table S4). Metformin exposure was assessed dichotomously in the model (i.e., <425 mg/day vs. ≥425 mg/day). The main effect estimates for metformin in both cohorts clearly demonstrated that the estimated effect of metformin in every model analyzed was consistently associated with a reduction in HbA1c levels (Supplementary Table S4) using the exposure-interval approach previously discussed and presented (Supplementary Figures S1 and S2). Were our metformin exposure term just modeling noise, we would not have expected to see the observed consistent and a statistically significant relationship between metformin exposure and HbA1c reduction. Metformin exposure was also inversely associated with the change in HbA1c in the base model

(i.e., the model that did not include genotype or the interaction term - not shown). This again underscored that our second analytic approach was directly modeling metformin's effect on glycemia. Most importantly, the interaction analysis again identified the same top association, rs143276236 ARFGEF3, but this time more clearly as an effect modifier for metformin treatment response. Only rs143276236 in ARFGEF3 showed a consistent relationship in the KPNC cohort (P=0.06). The parameter estimates for rs14376236 must be considered in the context of metformin exposure in which case the parameter estimates for genotype and the interaction term would be combined. In both DIAMOND and KPNC, the rs14376236 risk allele was associated with increased HbA1c in the setting of "high" metformin exposure.

REFERENCES

1. GoDarts, Group UDPS, Wellcome Trust Case Control C, Zhou K, Bellenguez C, Spencer CC, Bennett AJ, Coleman RL, Tavendale R, Hawley SA, Donnelly LA, Schofield C, Groves CJ, Burch L, Carr F, Strange A, Freeman C, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Dronov S, Duncanson A, Edkins S, Gray E, Hunt S, Jankowski J, Langford C, Markus HS, Mathew CG, Plomin R, Rautanen A, Sawcer SJ, Samani NJ, Trembath R, Viswanathan AC, Wood NW, investigators M, Harries LW, Hattersley AT, Doney AS, Colhoun H, Morris AD, Sutherland C, Hardie DG, Peltonen L, McCarthy MI, Holman RR, Palmer CN, Donnelly P, Pearson ER. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. Nat Genet 2011;43:117-120

2. Aschard H, Hancock DB, London SJ, Kraft P. Genome-wide meta-analysis of joint tests for genetic and gene-environment interaction effects. Hum Hered 2010;70:292-300

3. Williams LK, Padhukasahasram B, Ahmedani BK, Peterson EL, Wells KE, Gonzalez Burchard E, Lanfear DE. Differing effects of metformin on glycemic control by race-ethnicity. J Clin Endocrinol Metab 2014;99:3160-3168

Table S1. Average number of days in the observation period between initial and follow-up HbA1c measurements among study participants stratified by cohort and degree of metformin use.

	African American DIAMOND participants	African American KPNC cohort participants	European American DIAMOND participants
Individuals whose estimated metformin exposures was ≥425 mg/day the during observation period – mean ± SD	238.0 ± 103.8	193.6 ± 56.1	209.9 ± 81.7
Individuals whose estimated metformin exposures was <425 mg/day the during observation period – mean ± SD	283.7 ± 125.2	190.7 ± 54.7	274.3 ± 115.9

Table S2. Meta-analysis of African American and European American populations*

Variant	Chr	Position (Hg38)	Alleles	Nearest Gene	Functional consequence	African American DIAMOND participants		European American DIAMOND participants		Direction of effect	Meta-analysis (P-value)
						MAF	P-value	MAF	P-value		
rs12297682	12	40920062	G/A	CNTN1	Intron Variant	0.052	$4.09x10^{-08}$	0.119	0.9378	+/+	0.0707
rs79162841	12	40926234	G/A	CNTN1	Intron Variant	0.052	$4.09x10^{-08}$	0.114	0.9681	+/+	0.0714

DIAMOND denotes Diabetes Multi-omic Investigation of Drug Response, and MAF, minor allele frequency.

*The dependent variable was the rank-based inverse normal transformation of the change in HbA1c. P-values are the association results for a given genotype adjusted for patient age, sex, eGFR, and baseline glycated hemoglobin levels. Association and meta-analysis results for African American participants utilized regions homozygous for African ancestry. The analysis was also restricted to individuals with an average daily level of metformin exposure ≥425 mg during the time of observation. A $P<5.0x10^{-8}$ was the threshold for genome-wide significance.

Table S3. Characteristics of discovery and replication groups used in the secondary analysis and whose average daily metformin exposure was <425 mg/day

	Discovery Group	Replication Groups	
	African American DIAMOND participants (n=203)	**African American KPNC cohort participants (n=55)**	**European American DIAMOND participants (n=94)**
Age (years) – mean ± SD*	53.2 ± 11.5	53.6 ± 12.6	58.9 ± 11.4
Male sex – no. (%)	66 (32.5)	16 (29.0)	45 (47.9)
BMI – mean ± SD†	36.4 ± 7.6 (n=143)	34.8 ± 7.1 (n=45)	34.7 ± 7.3 (n=67)
Proportion of African ancestry – mean ± SD‡	81.1 ± 11.9	80.6 ± 12.5	1.0 ± 2.4
Baseline HbA1c level – mean ± SD§	7.4 ± 1.7	6.8 ± 0.9	7.1 ± 1.3
Change in HbA1c on metformin treatment – mean ± SD‖	-0.22 ± 1.44	-0.07 ± 1.10	0.04 ± 1.20
Metformin use per day (mg/day) – mean ± SD**	224.2 ± 120.0	333.9 ± 78.7	209.6 ± 132.3
eGFR – mean ± SD‡‡	97.2 ± 21.3 (n=203)	94.8 ± 21.1 (n=55)	89.2 ± 18.4 (n=94)

DIAMOND denotes Diabetes Multi-omic Investigation of Drug Response; KPNC, Kaiser Permanente Northern California; SD, standard deviation; and BMI, body mass index.

*Age at the time of metformin response was assessed.

†BMI was available for 152 (71%), 46 (82%), and 72 (72%) of the African American DIAMOND participants, the African American KPNC participants, and the European American DIAMOND participants, respectively.

‡Using the approach described in the methods, proportion of African ancestry refers to the proportion of each individual's genome determined to be of African continental origin. This was then averaged over all participants to determine the average proportion across the subset of participants.

§The result of the first HbA1c level in the interval used to assess metformin response.

‖Absolute change in the HbA1c percent in the observation period.

**Average estimated metformin daily use in milligrams per day during the 120-day period preceding the 2nd HbA1c measurement used in the observation interval. This is among the study sample with an average metformin use <425 mg/day.

‡‡ Based on creatinine levels drawn within 6 months of the observation period.

Table S4. Genome-wide significant genotype x drug associations for change in HbA1c levels among African American DIAMOND and KPNC participants on metformin monotherapy*

Variant	Chr	Position	Alleles	Nearest Gene	Functional consequence	Cohort	MAF	Metformin parameter estimate†	Metformin P-value	Genotype Parameter estimate†	Genotype P-value	Genotype x Drug Parameter estimate†	Genotype x Drug Interaction P-value	Joint Test P-value‡
rs150979496	18	8501049	T/C	*THEMIS3P*	None Known	DIAMOND	0.013	-0.10	0.26	-0.18	0.65	2.07	$4.99x10^{-5}$	$6.71x10^{-9}$
						KPNC	NA	NA	NA	NA	NA	NA	NA	NA
rs143276236	6	138176343	A/C	*ARFGEF3*	Intron Variant	DIAMOND	0.028	-0.21	0.02	-0.07	0.82	1.27	$6.03x10^{-4}$	$1.08x10^{-8}$
						KPNC	0.032	-0.17	0.24	0.27	0.46	0.22	0.59	0.06
rs137899285	17	42070230	G/A	*ZNF385C*	Intron Variant	DIAMOND	0.019	-0.18	0.03	2.11	$1.35x10^{-7}$	-1.43	$2.07x10^{-3}$	$1.15x10^{-8}$
						KPNC	0.016	-0.35	0.01	0.21	0.78	0.06	0.95	0.62
rs144431612	16	58966992	A/G	*AC092378.1*	None Known	DIAMOND	0.012	-0.19	0.03	0.10	0.79	1.69	$2.40x10^{-4}$	$1.15x10^{-8}$
						KPNC	0.02	-0.32	0.02	0.16	0.77	0.34	0.59	0.19
rs138642807	9	109970865	T/G	*PALM2-AKAP2*	Intron Variant	DIAMOND	0.013	-0.07	0.43	0.43	0.30	1.42	$6.57x10^{-3}$	$1.72x10^{-8}$
						KPNC	0.019	-0.25	0.09	0.39	0.48	-0.18	0.76	0.59
rs146090661	9	109975903	G/A	*PALM2-AKAP2*	Intron Variant	DIAMOND	0.013	-0.07	0.43	0.43	0.30	1.42	$6.57x10^{-3}$	$1.72x10^{-8}$
						KPNC	0.019	-0.25	0.09	0.39	0.48	-0.18	0.76	0.59
rs55831341	15	90223306	T/C	*SEMA4B*	Intron Variant	DIAMOND	0.013	-0.10	0.21	0.96	0.02	0.68	0.171	$1.77x10^{-8}$
						KPNC	0.018	-0.10	0.46	-0.24	0.58	0.25	0.63	0.86
rs59699971	12	40649666	G/A	*MUC19*	None Known	DIAMOND	0.032	-0.20	0.03	0.08	0.80	1.10	$2.33x10^{-3}$	$2.81x10^{-8}$
						KPNC	0.039	-0.25	0.07	-0.23	0.62	-0.1	0.84	0.21
rs115567566	12	40671268	G/A	*MUC19*	None Known	DIAMOND	0.032	-0.20	0.03	0.07	0.82	1.10	$2.25x10^{-3}$	$3.24x10^{-8}$
						KPNC	0.037	-0.18	0.19	-0.17	0.72	-0.22	0.66	0.13
rs141012141	6	138258422	T/C	*ARFGEF3*	Intron Variant	DIAMOND	0.022	-0.21	0.02	-0.53	0.15	1.81	$3.16x10^{-5}$	$3.48x10^{-8}$
						KPNC	0.033	-0.19	0.17	-0.02	0.96	0.2	0.68	0.66
rs80340144	6	138258595	T/C	*ARFGEF3*	Intron Variant	DIAMOND	0.022	-0.21	0.02	-0.53	0.15	1.81	$3.16x10^{-5}$	$3.48x10^{-8}$
						KPNC	0.033	-0.19	0.17	-0.02	0.96	0.2	0.68	0.66

DIAMOND denotes Diabetes Multi-omic Investigation of Drug Response; KPNC, Kaiser Permanente Northern California; MAF, minor allele frequency; and NA, not available.

*The dependent variable was the rank-based inverse normal transformation of the change in HbA1c. P-values are the joint association results for both genotype and genotype x metformin interaction (i.e., the full model vs. the reduced model without genotype and interaction term). The interaction term included metformin exposure dichotomized as <425 mg/day and ≥425 mg per day. All results were adjusted for patient age, sex, metformin exposure, renal function (eGFR), and baseline glycated hemoglobin levels. Association and meta-analysis results for African American participants were restricted to regions homozygous for African ancestry. The genome-wide significance threshold for the joint test was $P<5.0x10^{-8}$.

†Parameter estimates for genotype represent the approximate effect size of the genotype (coded 0, 1, and 2 for the number of copies of the effect allele). A negative parameter estimate suggests the variable is associated with a reduction in HbA1c over the observation period, and a positive parameter estimate suggests that the variable increases HbA1c. The interaction parameter estimate represents the change in the aforementioned relationship between low (<425 mg/day) and high (≥425 mg per day) metformin exposure. The effect of genotype is therefore considered within the context of the degree of metformin exposure (i.e., a combination of the main effect and interaction term for high metformin exposure).

Table S5. Genome-wide significant associations with change in HbA1c levels among individuals on metformin therapy stratified by time of observation with respect to treatment initiation.*

Variant	Chr	Position†	Alleles	Overlapped Gene	Functional consequence	DIAMOND cohort (Days 0-146)‡		DIAMOND cohort (Days 147-403)‡		DIAMOND cohort (All time periods)‡		KPNC cohort (All time periods)‡	
						Parameter estimate§	P-value	Parameter estimate§	P-value	Parameter estimate§	P-value	Parameter estimate§	P-value
rs114486706	1	241016709	G/T	RGS7	intronic	1.89	2.28x10-9	-0.63	2.84x10-1	0.83	2.36x10-3	-0.08	7.64x10-1
rs79788364	1	241037134	A/G	RGS7	intronic	2.26	4.64x10-9	0.92	3.56x10-1	1.6	2.90x10-5		
rs116779606	1	241042129	A/G	RGS7	intronic	2.26	4.64x10-9			1.77	3.40x10-5		
rs149823580	1	241053607	T/C	RGS7	intronic	2.26	4.64x10-9	0.92	3.56x10-1	1.6	2.90x10-5		
rs188225980	2	34730109	A/G	LINC01320	intronic	1.6	4.99x10-8	0.12	7.91x10-1	0.83	5.04x10-4		
rs367561476	2	34736504	T/C	LINC01320	intronic	1.53	2.81x10-8	0.29	3.89x10-1	0.77	2.33x10-4	0.43	2.46x10-1
rs116625636	2	34740124	G/T	None	None	1.6	4.99x10-8	0.29	3.89x10-1	0.77	3.10x10-4	0.47	2.48x10-1
rs115087207	2	34744862	T/C	None	None	1.53	2.81x10-8	0.29	3.89x10-1	0.77	2.33x10-4	0.43	2.46x10-1
rs561603314	2	34751198	G/A	None	None	1.6	4.73x10-9	0.78	8.16x10-2	1.23	3.26x10-7	-0.3	4.16x10-1
rs150661296	3	126396514	A/G	CFAP100	intronic	1.82	5.73x10-9	0.24	6.57x10-1	1.17	1.86x10-5	-0.28	2.87x10-1
rs372612573	4	60756991	T/G	LINC02496	intronic	2.23	3.27x10-8	-0.06	9.26x10-1	1.14	4.54x10-4	-0.22	5.89x10-1
rs373559260	4	60767039	A/G	LINC02496	intronic	2.23	3.27x10-8	-0.06	9.26x10-1	1.14	4.54x10-4	-0.22	5.89x10-1
rs192608778	4	60770172	A/C	LINC02496	intronic	2.23	3.27x10-8	-0.06	9.26x10-1	1.14	4.54x10-4	-0.22	5.89x10-1
rs371351518	4	60772243	T/C	LINC02496	intronic	2.23	3.27x10-8	-0.06	9.26x10-1	1.14	4.54x10-4	-0.22	5.89x10-1
rs78024857	4	60805717	T/C	None	None	2.23	3.27x10-8	-0.06	9.26x10-1	1.14	4.54x10-4	-0.22	5.89x10-1
rs187409470	4	60810423	T/C	None	None	2.23	3.27x10-8	-0.06	9.26x10-1	1.14	4.54x10-4	-0.22	5.89x10-1
rs74321975	4	60811218	A/G	None	None	2.23	3.27x10-8	-0.06	9.26x10-1	1.14	4.54x10-4	-0.22	5.89x10-1
rs11725978	4	66203996	T/C	None	None	1.32	1.71x10-8	0.46	1.04x10-1	0.77	6.73x10-6	0.06	7.68x10-1
rs79606041	4	172570614	A/C	GALNTL6	intronic	2.2	2.30x10-8	-0.11	8.00x10-1	0.89	1.33x10-3	-0.08	7.71x10-1
rs78727414	4	172573853	C/A	GALNTL6	intronic	2.2	2.30x10-8	-0.11	8.00x10-1	0.89	1.33x10-3	-0.08	7.71x10-1
rs76404446	4	172577406	C/A	GALNTL6	intronic	2.2	2.30x10-8	-0.11	8.00x10-1	0.89	1.33x10-3	-0.08	7.71x10-1
rs146112909	4	172616838	G/A	GALNTL6	intronic	2.2	2.30x10-8	-0.88	1.10x10-1	0.75	1.68x10-2	-0.03	9.07x10-1
rs189272622	4	172617852	A/G	GALNTL6	intronic	2.2	2.30x10-8	-0.88	1.10x10-1	0.75	1.68x10-2	-0.03	9.07x10-1
rs115987514	4	172618492	C/T	GALNTL6	intronic	2.2	2.30x10-8	-0.88	1.10x10-1	0.75	1.68x10-2	-0.03	9.07x10-1
rs186716246	4	172619017	T/C	GALNTL6	intronic	2.2	2.30x10-8	-0.88	1.10x10-1	0.75	1.68x10-2	-0.03	9.07x10-1
rs114587664	4	172623453	G/A	GALNTL6	intronic	2.2	2.30x10-8	-0.88	1.10x10-1	0.75	1.68x10-2	-0.03	9.07x10-1
rs76516598	4	172623879	T/C	GALNTL6	intronic	2.2	2.30x10-8	-0.88	1.10x10-1	0.75	1.68x10-2	-0.03	9.07x10-1
rs76726085	4	172626847	C/T	GALNTL6	intronic	2.2	2.30x10-8	-0.88	1.10x10-1	0.75	1.68x10-2	-0.03	9.07x10-1
rs143276236	6	138176343	A/C	ARFGEF3	intronic	1.15	9.59x10-6	1.7	4.29x10-4	1.2	6.39x10-9	0.6	7.22x10-3
rs116251012	6	138191883	T/C	ARFGEF3	intronic	1.08	1.39x10-5	1.3	3.51x10-3	1.08	4.80x10-8	0.49	9.21x10-3
rs144864037	7	40414401	G/A	SUGCT	intronic	1.77	2.62x10-9	0.01	9.93x10-1	1.12	2.57x10-5	-0.15	5.70x10-1
rs75085341	7	43533893	C/T	HECW1	intronic	1.02	3.76x10-8	-0.34	3.75x10-1	0.58	3.11x10-4	-0.11	4.86x10-1
rs112717443	7	43547824	A/C	HECW1	intronic	1.02	3.76x10-8	-0.34	3.75x10-1	0.57	4.86x10-4	-0.11	4.86x10-1
rs138172419	7	43563707	G/A	HECW1	3utr	1.2	5.09x10-9	-0.52	2.04x10-1	0.64	4.58x10-4	0.06	7.50x10-1
rs111465018	7	43564547	G/A	HECW1	3utr	1.2	5.09x10-9	-0.52	2.04x10-1	0.64	4.58x10-4	0.06	7.50x10-1
rs113045159	7	43570078	C/T	None	None	1.2	5.09x10-9	-0.52	2.04x10-1	0.64	4.58x10-4	0.06	7.50x10-1
rs112805322	7	43570751	A/G	None	None	1.2	5.09x10-9	-0.52	2.04x10-1	0.64	4.58x10-4	0.06	7.50x10-1
rs148953186	7	43571886	T/G	None	None	1.2	5.09x10-9	-0.52	2.04x10-1	0.64	4.58x10-4	0.06	7.50x10-1
rs534994513	7	43572068	G/A	None	None	1.2	5.09x10-9	-0.26	5.64x10-1	0.73	9.34x10-5	0.06	7.50x10-1
rs115327228	7	90995459	A/G	CDK14	intronic	1.61	3.19x10-8	-0.76	3.96x10-1	1.08	1.68x10-4		
rs114142114	7	91018332	G/A	CDK14	intronic	1.61	3.19x10-8	-0.76	3.96x10-1	1.08	1.68x10-4		
rs186413984	8	118097005	A/G	EXT1	intronic	2.31	8.88x10-9	0.27	7.83x10-1	1.77	2.34x10-6	-0.27	3.52x10-1
rs34639288	9	75530297	A/C	None	None	2.07	2.49x10-8	0.55	5.54x10-1	1.49	4.62x10-5	-0.33	3.85x10-1
rs17643494	9	75530666	C/T	None	None	2.07	2.49x10-8	0.55	5.54x10-1	1.49	4.62x10-5	-0.33	3.85x10-1

rs11145263	9	77072363	T/C	RFC5P1	intronic	1.36	3.54x10-8	-0.91	5.02x10-2	0.57	9.09x10-3	-0.05	8.64x10-1
rs12342624	9	77074196	C/T	None	None	1.36	3.54x10-8	-0.91	5.02x10-2	0.57	9.09x10-3	-0.03	9.32x10-1
rs184479153	9	85312566	A/G	None	None	2.03	1.91x10-8	-0.11	9.00x10-1	1.45	7.04x10-6		
rs12003146	9	85341110	A/G	None	None	2.03	1.91x10-8	-0.11	9.00x10-1	1.45	7.04x10-6	-0.3	2.74x10-1
rs138642807	9	109970865	T/G	PALM2-AKAP2	intronic	2.97	1.71x10-9	1.37	8.08x10-3	1.88	1.25x10-8	0.2	4.43x10-1
rs146090661	9	109975903	G/A	PALM2-AKAP2	intronic	2.97	1.71x10-9	1.37	8.08x10-3	1.88	1.25x10-8	0.2	4.43x10-1
rs141546505	9	112741961	A/G	None	None	-0.21	6.18x10-1	3.09	1.25x10-8	1.18	7.1x10-5		
rs11595482	10	5284044	T/C	AKR1C7P	intronic	0.86	2.16x10-9	0.32	1.49x10-1	0.66	6.10x10-8	0.17	1.33x10-1
rs187380871	10	53441814	A/G	None	None	1.67	2.77x10-8	-1.02	2.77x10-1	1.06	3.89x10-4	-0.37	1.79x10-1
rs567455990	10	53453958	A/G	None	None	1.67	2.77x10-8	-1.02	2.77x10-1	1.06	3.89x10-4	-0.37	1.79x10-1
rs147104885	10	53459665	A/G	None	None	1.67	2.77x10-8	-1.02	2.77x10-1	1.06	3.89x10-4	-0.37	1.79x10-1
rs116376432	10	53460271	A/G	None	None	1.67	2.77x10-8	-1.02	2.77x10-1	1.06	3.89x10-4	-0.37	1.79x10-1
rs79510070	10	53474901	A/G	None	None	1.67	2.77x10-8	-1.02	2.77x10-1	1.06	3.89x10-4	-0.37	1.79x10-1
rs149911554	10	106606746	A/G	SORCS1	intronic	1.77	8.99x10-5	1.52	2.76x10-2	1.54	4.99x10-8	0.44	7.75x10-2
rs114019854	10	106606829	T/C	SORCS1	intronic	1.77	8.99x10-5	1.52	2.76x10-2	1.54	4.99x10-8	0.44	7.75x10-2
rs72901015	11	19037610	T/C	None	None	2.16	2.23x10-8	-0.32	6.34x10-1	1.14	2.39x10-4	1.37	8.30x10-2
rs72901024	11	19039367	G/T	None	None	2.16	2.23x10-8	-0.32	6.34x10-1	1.14	2.39x10-4	1.37	8.30x10-2
rs61282406	11	19040598	A/G	None	None	2.16	2.23x10-8	-0.32	6.34x10-1	1.14	2.39x10-4	1.37	8.30x10-2
rs117546020	11	19043099	T/C	None	None	2.16	2.23x10-8	-0.32	6.34x10-1	1.14	2.39x10-4	1.37	8.30x10-2
rs59224730	11	19045282	T/C	None	None	2.16	2.23x10-8	-0.32	6.34x10-1	1.14	2.39x10-4	1.37	8.30x10-2
rs72887932	11	19048218	T/C	None	None	2.16	2.23x10-8	-0.32	6.34x10-1	1.14	2.39x10-4	1.37	8.30x10-2
rs114749533	11	126076784	T/C	None	None	1.47	2.18x10-8	-0.25	5.81x10-1	0.89	3.98x10-5	0.15	4.58x10-1
rs59699971	12	40649666	G/A	None	None	1.37	1.32x10-7	1.18	8.96x10-4	1.16	9.57x10-9	-0.32	9.77x10-2
rs115567566	12	40671268	G/A	None	None	1.36	1.40x10-7	1.18	1.06x10-3	1.16	1.13x10-8	-0.39	4.82x10-2
rs12297682	12	40920062	G/A	CNTN1	intronic	0.61	6.15x10-4	1.73	1.01x10-6	0.85	4.09x10-8	-0.24	1.51x10-1
rs79162841	12	40926234	G/A	CNTN1	intronic	0.61	6.15x10-4	1.73	1.01x10-6	0.85	4.09x10-8	-0.24	1.51x10-1
rs16967563	15	38844896	T/G	C15orf53	intronic	2.02	9.01x10-10	0.24	7.27x10-1	1.26	6.48x10-5	-0.39	2.87x10-1
rs12442064	15	38848055	T/G	C15orf53	intronic	2.02	9.01x10-10	0.24	7.27x10-1	1.26	6.48x10-5	-0.39	2.87x10-1
rs143877366	15	38851677	G/A	AC022929.2	non-coding	2.02	9.01x10-10	0.24	7.27x10-1	1.26	6.48x10-5	-0.39	2.87x10-1
rs2624265	15	38856448	C/T	C15orf53	intronic	1.66	3.97x10-8	0.24	7.27x10-1	1.05	3.89x10-4	-0.15	6.32x10-1
rs115028136	15	74292658	T/G	CCDC33	intronic	2.37	1.08x10-8	-0.24	5.40x10-1	0.61	2.20x10-2	0.03	9.16x10-1
rs114235489	16	14680836	C/T	PLA2G10	intronic	2.09	1.68x10-8			1.83	6.64x10-7	0.3	2.25x10-1
rs142017898	16	83500006	C/T	CDH13	intronic	1.5	3.57x10-5	1.51	4.53x10-4	1.52	1.15x10-8	-0.09	6.88x10-1
rs148248781	17	950446	T/C	NXN	intronic	2.16	3.76x10-10	0.38	3.39x10-1	1.19	2.55x10-6	0.39	2.14x10-1
rs114633775	17	13550273	T/C	HS3ST3A1	intronic	1.66	1.43x10-8	-0.47	3.25x10-1	0.85	4.68x10-4	0.3	2.21x10-1
rs148594865	17	32992680	G/A	SPACA3	intronic	1.68	4.43x10-8	0.09	8.61x10-1	1.05	1.29x10-4	0.25	2.61x10-1
rs149676225	17	33015085	A/G	ASIC2	intronic	1.91	1.35x10-8	1.47	2.73x10-2	1.68	6.07x10-8	0.12	6.63x10-1
rs116218041	18	5548091	G/A	EPB41L3	intronic	1.51	3.08x10-8	-0.23	6.86x10-1	0.87	6.90x10-4	-0.21	2.79x10-1
rs150979496	18	8501049	T/C	THEMIS3P	intronic	2.55	2.75x10-10	1.4	4.35x10-2	1.9	5.04x10-9		
rs11873080	18	31830105	T/C	TRAPPC8	3utr	1.75	1.39x10-8	-0.37	2.55x10-1	0.6	3.84x10-3	-0.21	2.82x10-1
rs137955599	18	31872462	C/T	TRAPPC8	intronic	1.75	1.39x10-8	-0.46	1.84x10-1	0.6	4.65x10-3	-0.21	2.82x10-1
rs11872942	18	31923411	G/A	TRAPPC8	intronic	1.75	1.39x10-8	-0.38	2.69x10-1	0.65	2.33x10-3	-0.21	2.82x10-1
rs114258339	18	31983689	G/A	AC009831.4	non-coding	1.75	1.30x10-8	-0.38	2.73x10-1	0.61	5.70x10-3	-0.14	4.80x10-1
rs146377398	18	59571394	T/G	CCBE1	intronic	1.21	1.47x10-8	0.43	2.84x10-1	0.96	5.48x10-7	-0.09	6.01x10-1
rs28501251	18	59578637	A/G	CCBE1	intronic	1.21	1.47x10-8	0.43	2.84x10-1	0.96	5.48x10-7	-0.06	7.51x10-1
rs112849128	19	2013254	T/C	BTBD2	intronic	2.15	1.43x10-8	-0.99	2.92x10-1	0.99	3.81x10-3	-0.42	2.03x10-1
rs146349680	19	36902201	A/G	ZNF829	intronic	1.99	3.58x10-8	0.13	7.63x10-1	0.94	4.50x10-4		
rs112014215	19	38879908	T/C	SIRT2	intronic	2.28	2.16x10-8	0.24	6.01x10-1	1.08	4.02x10-5	0.14	6.33x10-1

DIAMOND denotes Diabetes Multi-omic Investigation of Drug Response; KPNC, Kaiser Permanente Northern California.
*The dependent variable was the rank-based inverse normal transformation of the change in HbA1c. P-values are the association results for a given genotype adjusted for patient age, sex, renal function (eGFR), and baseline glycated hemoglobin levels. Association results for African American participants were restricted to regions homozygous for African ancestry and to individuals whose level of daily metformin exposure was ≥425 mg at the time of observation.
†Position based on human genome reference build GRCh38.
‡Days represent the interval in which metformin was assessed with respect to treatment initiation
§Parameter estimates represent the approximate effect size of the genotype (coded 0, 1, and 2 for the number of copies of effect allele). A negative parameter estimate suggests that the effect allele is associated with a reduction in HbA1c over the observation period, and a positive parameter estimate is associated with an increase in HbA1c.

Table S6. Genome-wide significant associations with change in HbA1c levels among individuals on metformin therapy stratified by time of observation with respect to treatment initiation – dichotomized at day 403 post initiation.*

Variant	Chr	Position†	Alleles	Overlapped Gene	Functional consequence	DIAMOND cohort (Days 0-403)‡		DIAMOND cohort (Days >403)‡		DIAMOND cohort (All time periods)‡		KPNC cohort (All time periods)‡	
						Parameter estimate§	P-value	Parameter estimate§	P-value	Parameter estimate§	P-value	Parameter estimate§	P-value
rs4072190	3	17137600	A/G	None	None	1.42	4.62x10-8			1.4	7.59x10-8	-1.16	2.66x10-2
rs188113809	5	103431031	G/T	PDZPH1P	intronic	1.29	3.64x10-8			1.26	1.03x10-7	0.18	4.55x10-1
rs10060711	5	141461919	T/G	PCDHGA1	intronic	1.12	4.98x10-8			1.06	4.42x10-7	0.09	7.25x10-1
rs145484670	5	141469912	T/C	PCDHGA1	intronic	1.16	4.12x10-8			1.11	3.42x10-7	0.18	5.23x10-1
rs143276236	6	138176343	A/C	ARFGEF3	intronic	1.32	9.72x10-9	1.16	1.18x10-1	1.2	6.39x10-9	0.6	7.22x10-3
rs116251012	6	138191883	T/C	ARFGEF3	intronic	1.15	1.09x10-7	1.16	1.18x10-1	1.08	4.80x10-8	0.49	9.21x10-3
rs138642807	9	109970865	T/G	PALM2-AKAP2	intronic	2.08	3.89x10-9	1.34	2.91x10-1	1.88	1.25x10-8	0.2	4.43x10-1
rs146090661	9	109975903	G/A	PALM2-AKAP2	intronic	2.08	3.89x10-9	1.34	2.91x10-1	1.88	1.25x10-8	0.2	4.43x10-1
rs11595482	10	5284044	T/C	AKR1C7P	intronic	0.69	1.63x10-8	-0.59	5.98x10-1	0.66	6.10x10-8	0.17	1.33x10-1
rs149911554	10	106606746	A/G	SORCS1	intronic	1.77	1.82x10-6	1.2	7.45x10-2	1.54	4.99x10-8	0.44	7.75x10-2
rs114019854	10	106606829	T/C	SORCS1	intronic	1.77	1.82x10-6	1.2	7.45x10-2	1.54	4.99x10-8	0.44	7.75x10-2
rs59699971	12	40649666	G/A	None	None	1.28	8.97x10-10	-1.33	1.75x10-1	1.16	9.57x10-9	-0.32	9.77x10-2
rs115567566	12	40671268	G/A	None	None	1.27	1.08x10-9	-1.33	1.75x10-1	1.16	1.13x10-8	-0.39	4.82x10-2
rs12317274	12	40677115	A/G	None	None	1.16	9.90x10-9	-1.33	1.75x10-1	1.06	7.91x10-8	-0.39	4.82x10-2
rs78193290	12	40709548	T/C	CNTN1	intronic	1.25	2.17x10-9	-0.66	1.75x10-1	0.94	4.80x10-7	-0.37	5.27x10-2
rs115655845	12	40746819	A/G	CNTN1	intronic	1.55	8.28x10-9	-1.33	1.75x10-1	1.36	1.27x10-7		
rs74439828	12	40775855	G/A	CNTN1	intronic	1.42	7.37x10-9	-1.33	1.75x10-1	1.27	8.05x10-8	-0.09	6.59x10-1
rs12308484	12	40822784	A/G	CNTN1	intronic	1.1	4.72x10-8	-1.33	1.75x10-1	1.02	2.40x10-7	-0.16	4.40x10-1
rs12297682	12	40920062	G/A	CNTN1	intronic	0.89	3.48x10-8	-0.23	7.59x10-1	0.85	4.09x10-8	-0.24	1.51x10-1
rs79162841	12	40926234	G/A	CNTN1	intronic	0.89	3.48x10-8	-0.23	7.59x10-1	0.85	4.09x10-8	-0.24	1.51x10-1
rs142017898	16	83500006	C/T	CDH13	intronic	1.57	2.20x10-9			1.52	1.15x10-8	-0.09	6.88x10-1
rs149676225	17	33015085	A/G	ASIC2	intronic	1.71	2.07x10-8			1.68	6.07x10-8	0.12	6.63x10-1
rs150979496	18	8501049	T/C	THEMIS3P	intronic	2.11	1.93x10-9	0.55	7.01x10-1	1.9	5.04x10-9		

DIAMOND denotes Diabetes Multi-omic Investigation of Drug Response; KPNC, Kaiser Permanente Northern California.

*The dependent variable was the rank-based inverse normal transformation of the change in HbA1c. P-values are the association results for a given genotype adjusted for patient age, sex, renal function (eGFR), and baseline glycated hemoglobin levels. Association results for African American participants were restricted to regions homozygous for African ancestry and to individuals whose level of daily metformin exposure was ≥425 mg at the time of observation.

†Position based on human genome reference build GRCh38.

‡Days represent the interval in which metformin was assessed with respect to treatment initiation

§Parameter estimates represent the approximate effect size of the genotype (coded 0, 1, and 2 for the number of copies of effect allele). A negative parameter estimate suggests that the effect allele is associated with a reduction in HbA1c over the observation period, and a positive parameter estimate is associated with an increase in HbA1c.

Table S7. Genome-wide significant associations for change in HbA1c levels among individuals on metformin monotherapy with the time from metformin initiation as an additional covariate*

Variant	Chr	Position†	Alleles	Overlapped Gene	Functional consequence	DIAMOND cohort (discovery set)				KPNC cohort (replication set)				Direction of effect§	Meta-analysis P-value
						No.	MAF	Parameter estimate‡	P-value	No.	MAF	Parameter estimate‡	P-value		
rs189016655	2	8008449	T/C	LINC00298	intronic	268	0.011	1.88	$2.99x10^{-8}$	229	0.017	-0.4	0.130	+/-	0.028
rs188113809	5	103431031	G/T	PDZPH1P	intronic	289	0.022	1.25	$2.25x10^{-8}$	214	0.023	0.15	0.508	+/+	$8.56x10^{-6}$
rs189243819	5	103487923	A/G	PDZPH1P	intronic	289	0.021	1.28	$4.14x10^{-8}$	214	0.023	0.15	0.508	+/+	$1.63x10^{-5}$
rs180771604	5	103487924	G/A	PDZPH1P	intronic	289	0.021	1.28	$4.14x10^{-8}$	214	0.023	0.15	0.508	+/+	$1.63x10^{-5}$
rs143276236	6	138176343	A/C	ARFGEF3	intronic	292	0.027	1.13	$9.09x10^{-9}$	227	0.024	0.46	$3.47x10^{-2}$	+/+	$1.36x10^{-8}$
rs149871228	7	65442084	A/G	None	None	272	0.015	1.57	$3.40x10^{-8}$	224	0.016	0.24	0.379	+/+	$9.82x10^{-6}$
rs138642807	9	109970865	T/G	PALM2-AKAP2	intronic	285	0.012	1.77	$3.09x10^{-8}$	221	0.018	0.22	0.395	+/+	$3.73x10^{-5}$
rs146090661	9	109975903	G/A	PALM2-AKAP2	intronic	285	0.012	1.77	$3.09x10^{-8}$	221	0.018	0.22	0.395	+/+	$3.73x10^{-5}$
rs11595482	10	5284044	T/C	AKR1C7P	intronic	281	0.089	0.65	$3.26x10^{-8}$	224	0.109	0.14	0.177	+/+	$2.58x10^{-6}$
rs59699971	12	40649666	G/A	None	None	288	0.031	1.15	$4.61x10^{-9}$	219	0.041	-0.34	$6.97x10^{-2}$	+/-	0.006
rs115567566	12	40671268	G/A	None	None	287	0.031	1.14	$5.77x10^{-9}$	218	0.039	-0.4	$3.63x10^{-2}$	+/-	0.011
rs12317274	12	40677115	A/G	None	None	287	0.033	1.05	$3.31x10^{-8}$	218	0.039	-0.4	$3.63x10^{-2}$	+/-	0.016
rs12297682	12	40920062	G/A	CNTN1	intronic	287	0.052	0.85	$1.52x10^{-8}$	219	0.055	-0.21	0.189	+/-	0.001
rs79162841	12	40926234	G/A	CNTN1	intronic	287	0.052	0.85	$1.52x10^{-8}$	219	0.055	-0.21	0.189	+/-	0.001
rs142017898	16	83500006	C/T	CDH13	intronic	287	0.017	1.45	$1.20x10^{-8}$	219	0.025	-0.01	0.955	+/-	$1.46x10^{-4}$
rs149676225	17	33015085	A/G	ASIC2	intronic	305	0.011	1.75	$5.70x10^{-9}$	204	0.02	0.13	0.627	+/+	$2.87x10^{-5}$
rs150979496	18	8501049	T/C	THEMIS3P	intronic	300	0.012	1.82	$9.98x10^{-9}$					+/?	

DIAMOND denotes Diabetes Multi-omic Investigation of Drug Response; KPNC, Kaiser Permanente Northern California; MAF, minor allele frequency.

*The dependent variable was the rank-based inverse normal transformation of the change in HbA1c. P-values are the association results for a given genotype adjusted for patient age, sex, renal function (eGFR), baseline glycated hemoglobin levels, and time from metformin initiation. Association results for African American participants were restricted to regions homozygous for African ancestry and to individuals whose level of daily metformin exposure was ≥425 mg at the time of observation.

†Position based on human genome reference build GRCh38.

‡Parameter estimates represent the approximate effect size of the genotype (coded 0, 1, and 2 for the number of copies of effect allele). A negative parameter estimate suggests that the effect allele is associated with a reduction in HbA1c over the observation period, and a positive parameter estimate is associated with an increase in HbA1c.

§Direction of effect recapitulates the parameter estimate direction of both cohorts, such that when the symbols are in the same direction (+ or -) indicates that both cohorts had effect estimates in the same direction. A "?" indicates that an effect estimate could not be estimated in a given cohort.

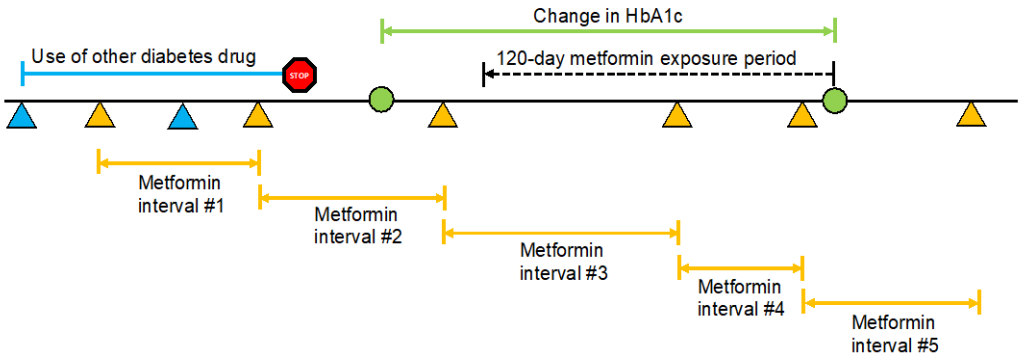

Figure S1. Schematic demonstrating how metformin exposure was related to the change in glycosylated hemoglobin (HbA1c) levels. Yellow triangles represent the time of metformin fills, and blue triangles represent the time of other diabetes medication fills. The red octagon represents the discontinuation of the other diabetes medication and the blue line represents the time interval during which the individual was another overlapping diabetes treatment. For the purposes of relating metformin exposure to the change in HbA1c, this study used the individual's first qualifying time period on metformin monotherapy. The green circles indicate the first time period in which HbA1c levels were measured more than 120 days apart while on metformin monotherapy; the change in HbA1c was the difference in these measurements for this time (green line). To estimate metformin exposure, we used the number of days between metformin fills (yellow lines), and the amount of metformin dispensed at each fill (yellow triangles). A 120-day period of metformin exposure (dashed black line) was assessed by anchoring on the date of the 2nd HbA1c measurement and averaging daily metformin use for the preceding 120 days.

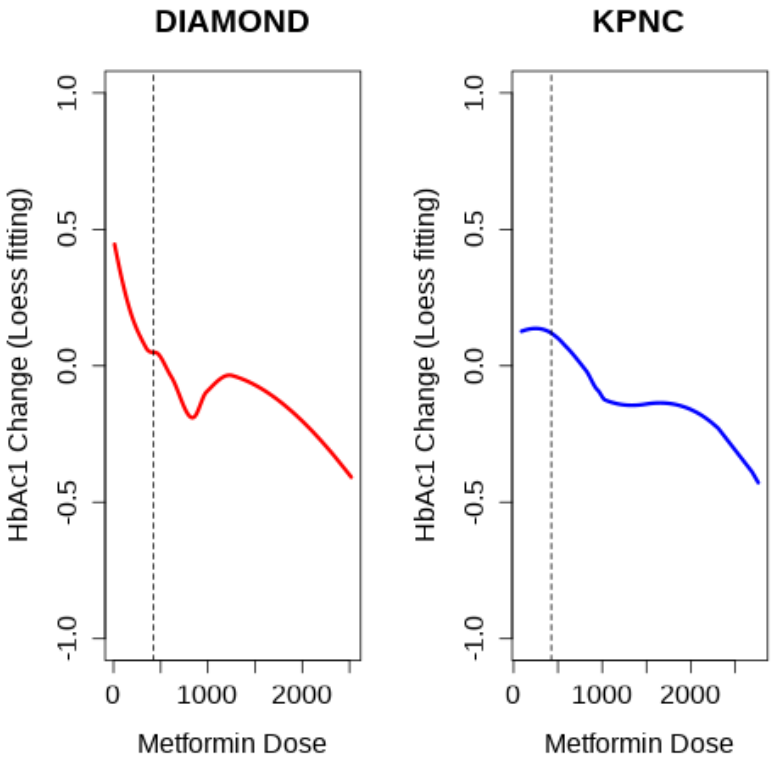

Figure S2. Relationship between estimated daily metformin use and change in HbA1c adjusted for the baseline HbA1c level using localized regression. The vertical line represents the 425mg/day threshold used in the study to delineate low vs. high metformin use. DIAMOND denotes the analysis in the Diabetes Multi-omic Investigation of Medication Response cohort (left), and KPNC denotes the analysis in the Kaiser Permanente Northern California cohort (right).

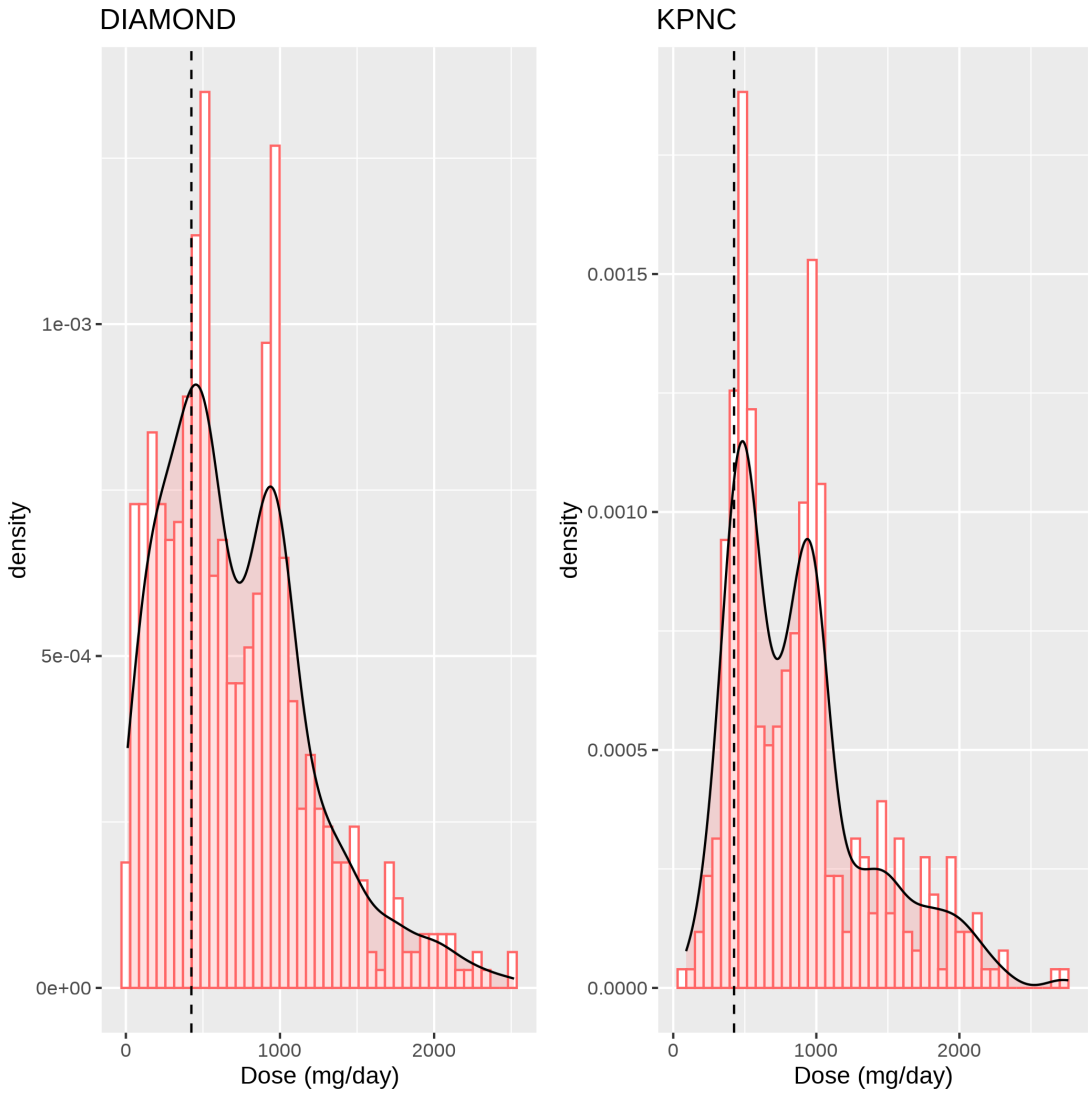

Figure S3. Distribution of estimated daily amount of metformin taken among participants in the DIAMOND cohort (left) and the KPNC cohort (right). The vertical line represents the 425mg/day threshold used in the study to delineate low vs. high metformin use. DIAMOND denotes the Diabetes Multi-omic Investigation of Medication Response cohort, and KPNC denotes the Kaiser Permanente Northern California cohort.

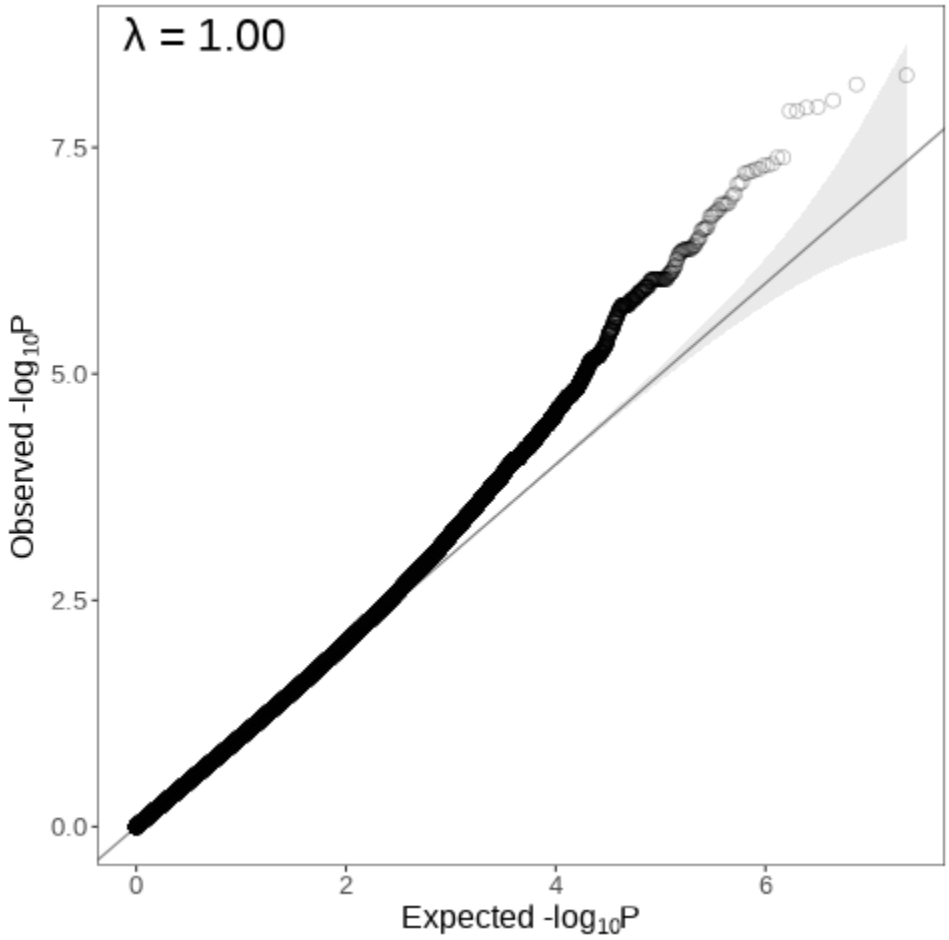

Figure S4. Q-Q plot of the genome-wide association discovery analysis for change in HbA1c levels. The discovery set comprised African American participants on metformin monotherapy from the Diabetes Multi-omic Investigation of Drug Response (DIAMOND) cohort. Plotted P-values represent the observed and expected levels statistical significance from the association analysis. Lambda represents genomic inflation and was calculated as the ratio of the observed median chi-squared distribution to the expected chi-squared distribution. A lambda values >1 represent inflation, and conversely, values close to one represent less inflation.

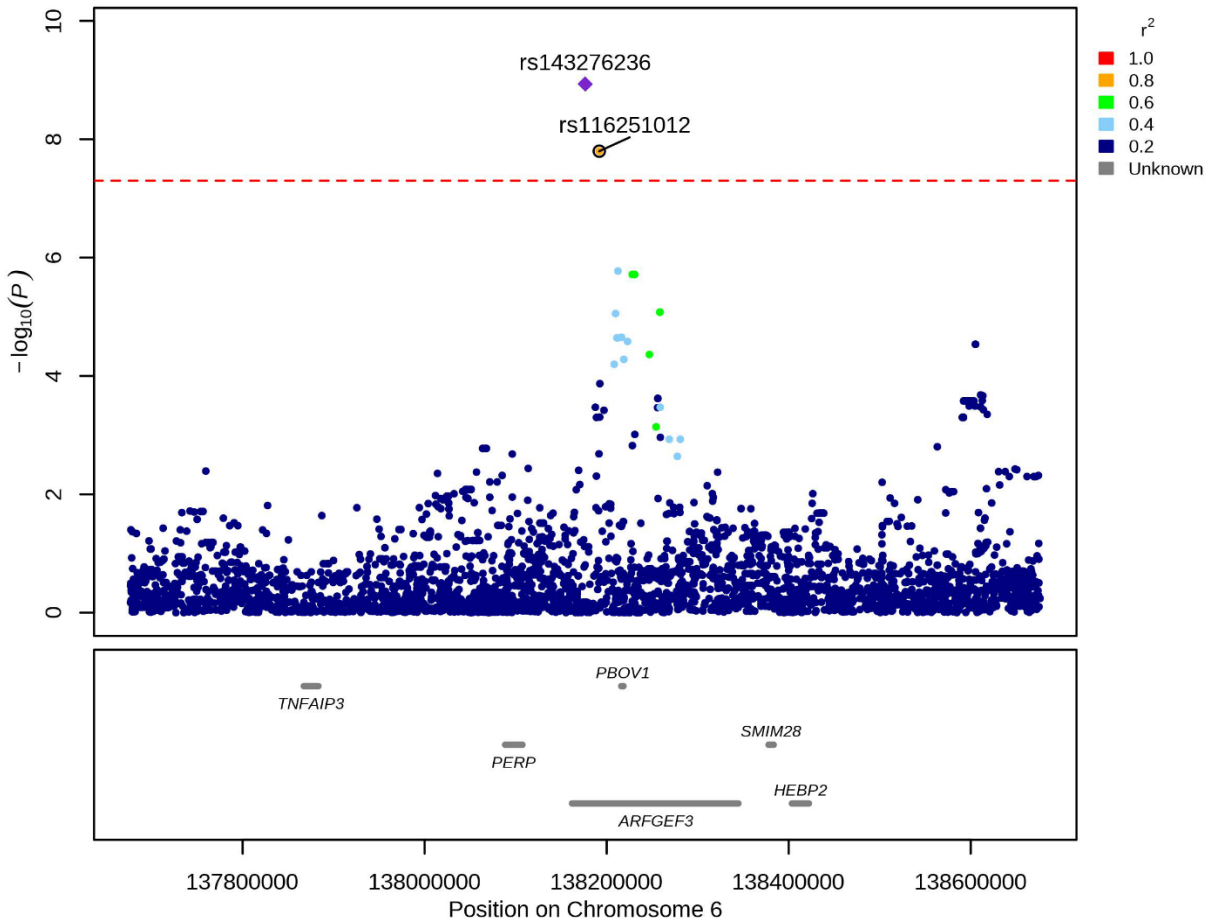

Figure S5. Locus zoom plot of the meta-analyzed association signal on chromosome 6 among African American participants from the Diabetes Multi-omic Investigation of Medication Response (DIAMOND) and Kaiser Permanente Northern California (KPNC) cohorts. DIAMOND and KPNC cohorts. The top replicated variant, rs143276236, is shown (purple diamond). The dashed line indicates the threshold for genome-wide statistical significance (P-value<5 x 10^{-8})

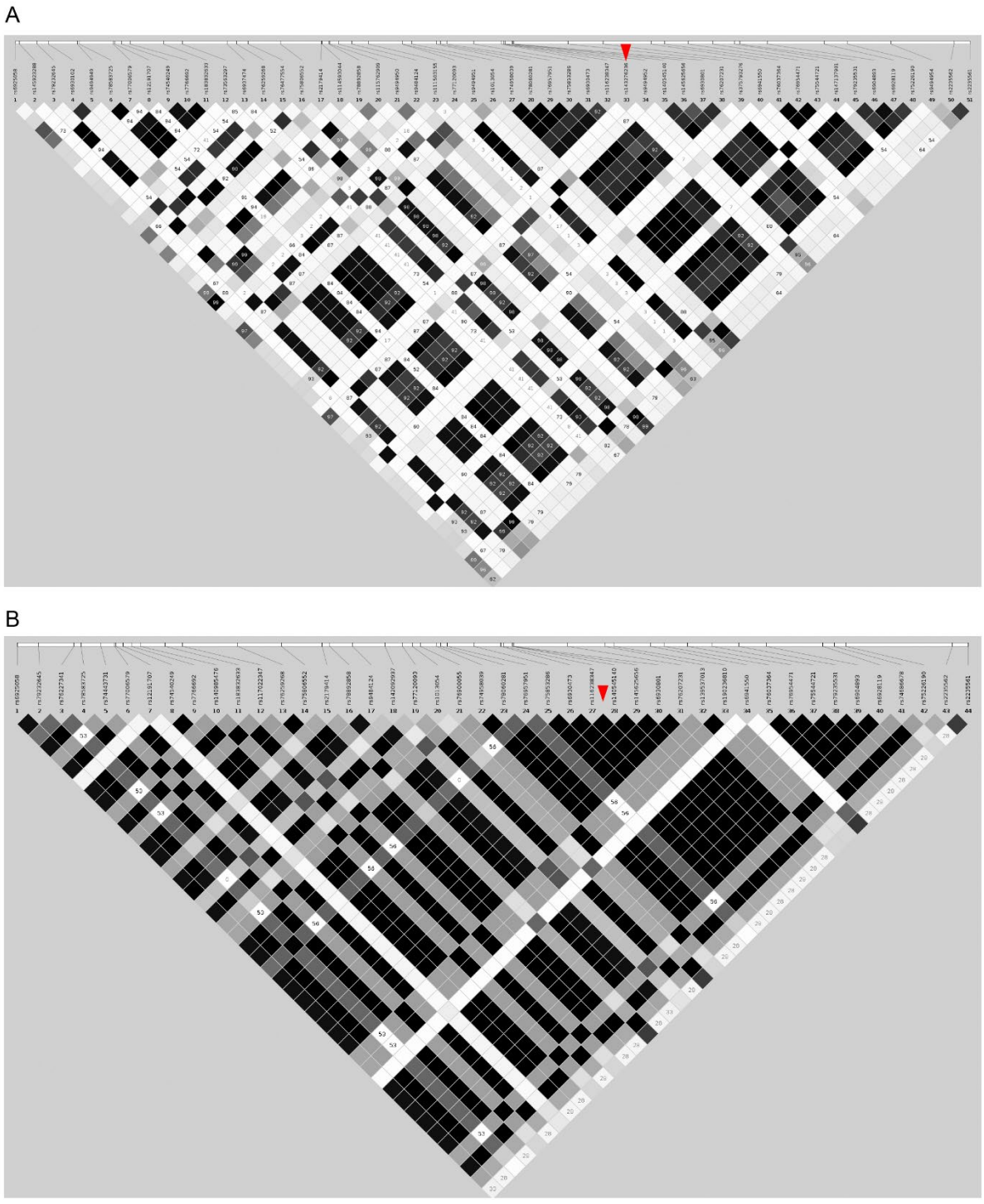

Figure S6. Haplotype plot of the region surrounding variant rs143276236 in the gene *ARFGEF3* (i.e., the top association from the primary analysis) on chromosome 6 among African American DIAMOND participants (**A**) and among European American DIAMOND participants (**B**). The location of variant rs143276236 is denoted by a red triangle. The top plot represents a region homozygous for African ancestry, and the lower plot represents the same region homozygous for European ancestry.

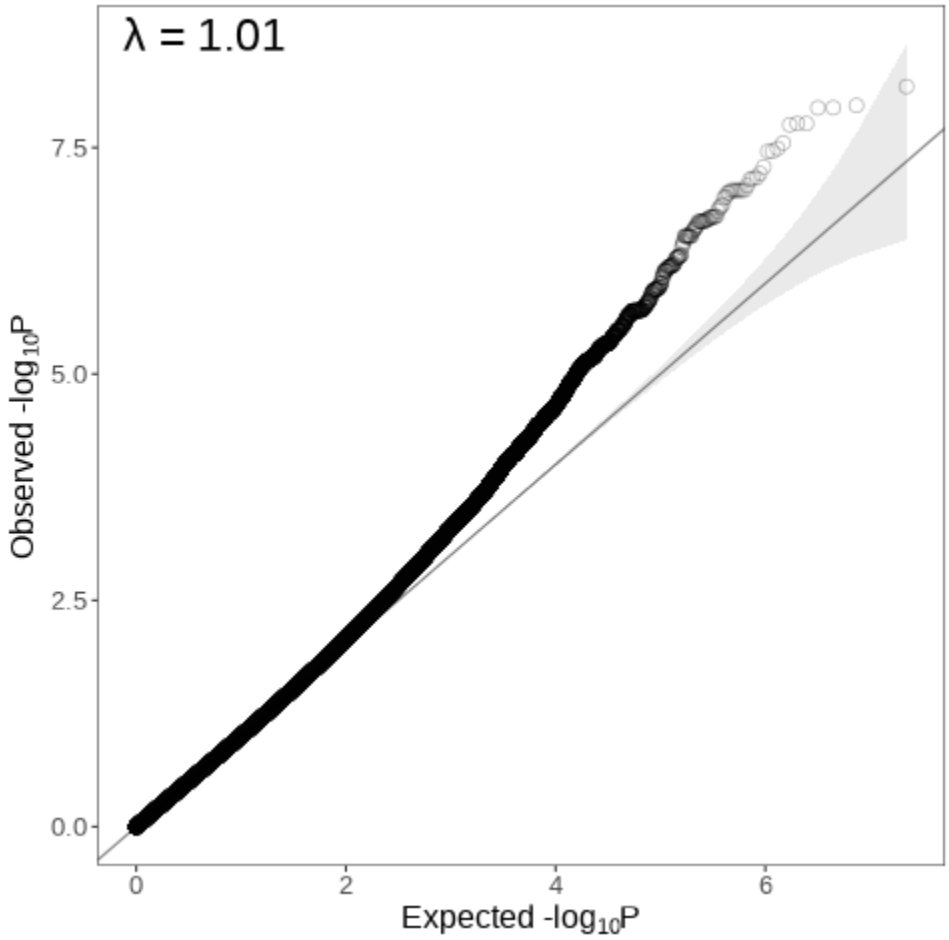

Figure S7. Q-Q plot of the genome-wide association analysis for gene x metformin interactions on the change in HbA1c levels. The analytic set comprised African American participants on metformin monotherapy from the Diabetes Multi-omic Investigation of Drug Response (DIAMOND) cohort. Association P-values represent differences in model fit between a full model with all variables included and the reduced model without the genotype and genotype x metformin interaction terms (in other words, the significance of the joint effect of both variables on model fit). Lambda represents genomic inflation and was calculated as the ratio of the observed median chi-squared distribution to the expected chi-squared distribution. A lambda values >1 represent inflation, and conversely, values close to one represent less inflation.

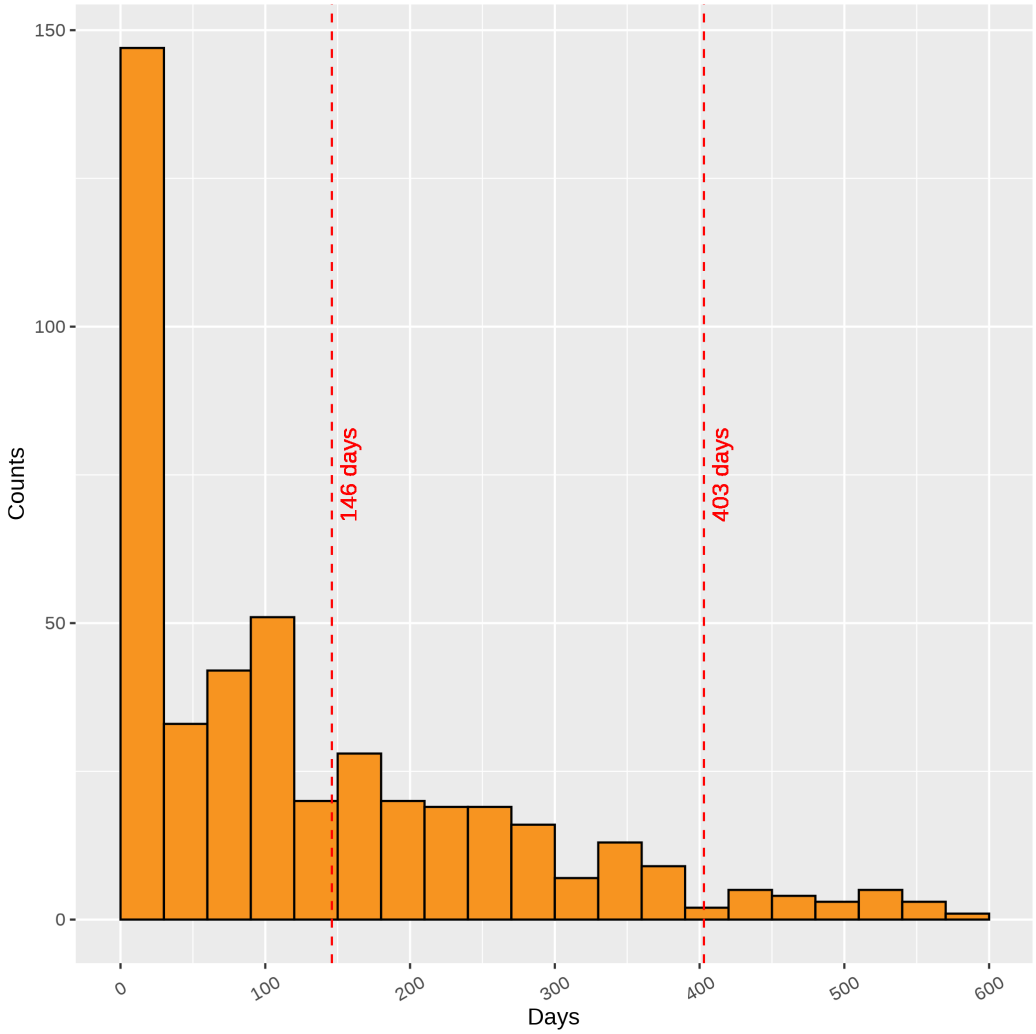

Figure S8. Distribution of assessment periods with respect to metformin initiation from the Diabetes Multi-omic Investigation of Drug Response (DIAMOND) discovery group. Days represent the difference in the time from metformin initiation to the time of effect assessment. The vertical lines time periods in which the effect of metformin is most evident after treatment initiation (also see **Figure S9**).

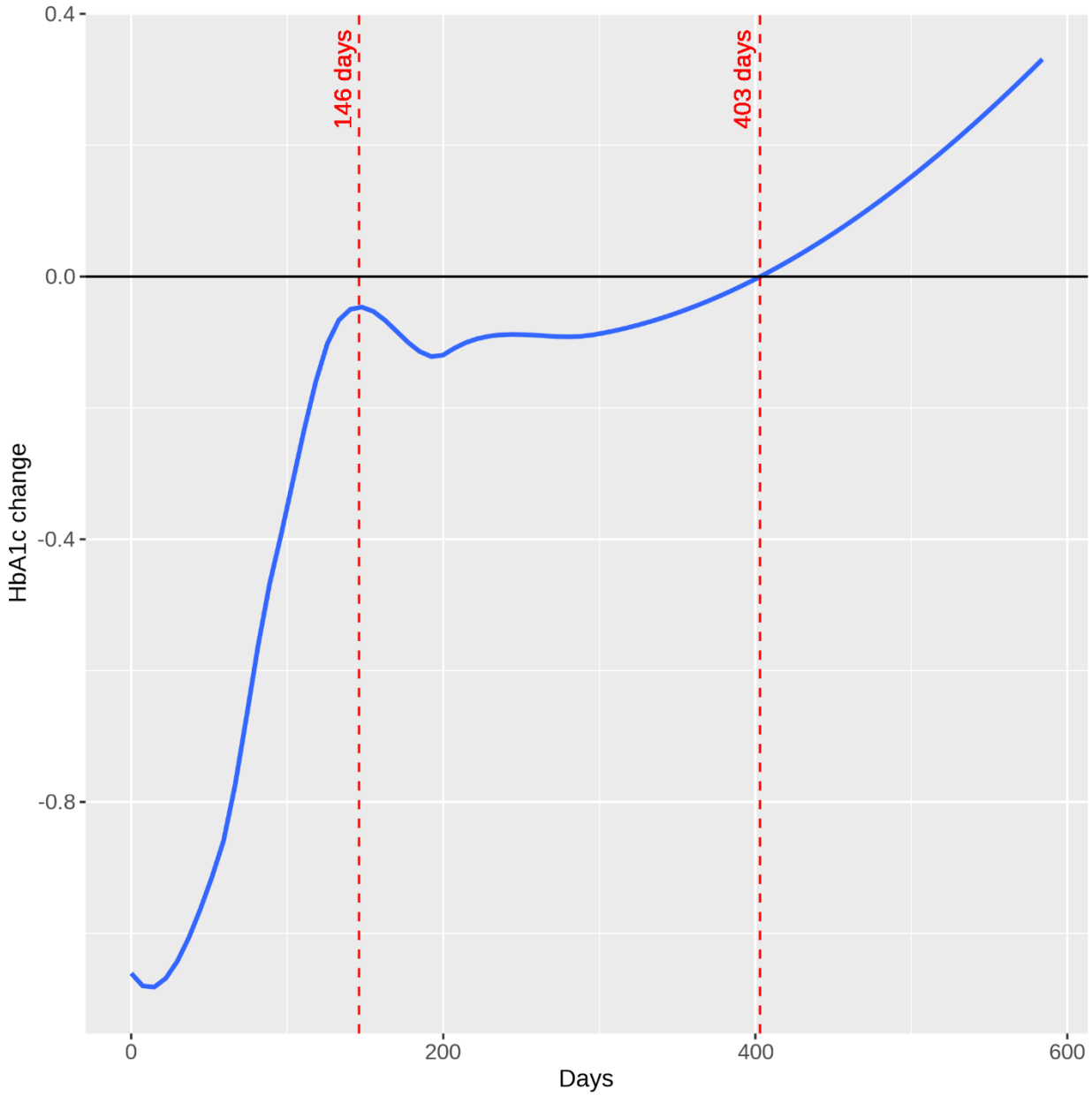

Figure S9. The effect of metformin on HbA1c improvement following the time of treatment initiation (day 0). Local regression was used to estimate the effect of metformin treatment on the change in HbA1c levels (y-axis) with respect to the time in days following metformin initiation (x-axis). In other words, each day represented the estimated effect that metformin would have on blood glycemia (over the ensuing 120 days) if observation began on that day with respect to when metformin treatment was first started. The sample was restricted to individuals whose estimated metformin usage was ≥425 mg/day. The vertical dashed lines indicate times in which the effect of metformin appeared to change. The largest effect of metformin on HbA1c levels appeared within the first 146 days following treatment initiation; however, metformin's effect on reducing HbA1c appeared to persist until 403 days following initiation.