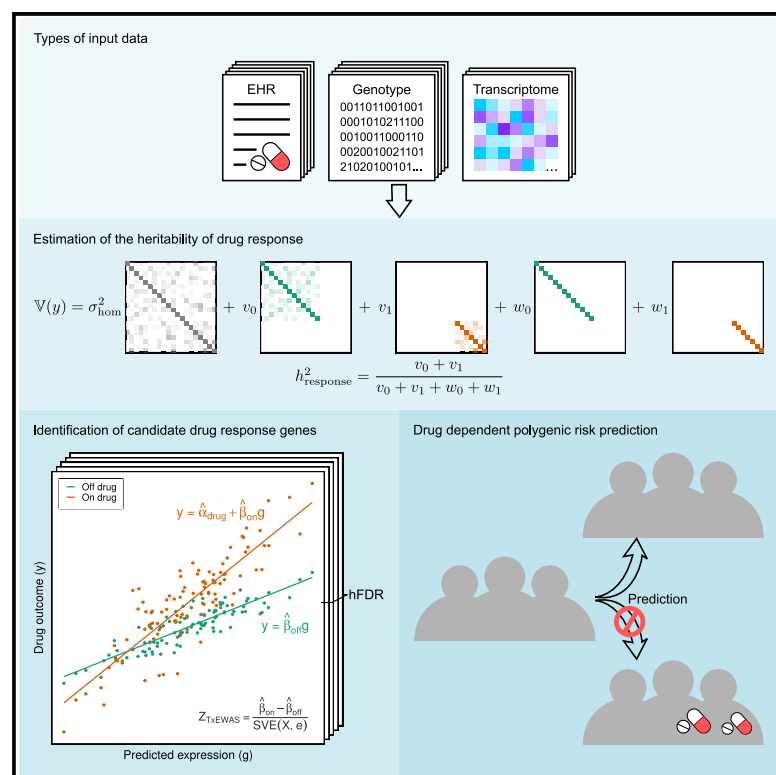


Characterizing the genetic architecture of drug response using gene-context interaction methods

Graphical abstract



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In brief

Sadowski et al. propose a framework to study the genetics of response to commonly prescribed drugs in large biobanks. They quantify the heritability of response to statins, metformin, warfarin, and methotrexate, and identify associated genes. Their analysis also shows the importance of accounting for drug use in genetic risk prediction.

Highlights

- Large biobank data provides insights into the genetic architecture of drug response
- Genome-wide genetic variation broadly modifies drug response
- Hundreds of genes associated with drug response are identified
- Drug use information should be accounted for in genetic risk prediction



Article

Characterizing the genetic architecture of drug response using gene-context interaction methods

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SUMMARY

Identifying factors that affect treatment response is a central objective of clinical research, yet the role of common genetic variation remains largely unknown. Here, we develop a framework to study the genetic architecture of response to commonly prescribed drugs in large biobanks. We quantify treatment response heritability for statins, metformin, warfarin, and methotrexate in the UK Biobank. We find that genetic variation modifies the primary effect of statins on LDL cholesterol (9% heritable) as well as their side effects on hemoglobin A1c and blood glucose (10% and 11% heritable, respectively). We identify dozens of genes that modify drug response, which we replicate in a retrospective pharmacogenomic study. Finally, we find that polygenic score (PGS) accuracy varies up to 2-fold depending on treatment status, showing that standard PGSs are likely to underperform in clinical contexts.

INTRODUCTION

Initiation of drug treatment poses a risk for adverse reactions and long-term side effects, sometimes without guaranteed effectiveness for an individual patient.^{1–6} Genetic testing holds promise for safer and more effective treatment by predicting each individual's specific drug response.^{7–10} To date, several large-effect pharmacogenomic genes have been identified^{11–17}; these genes are commonly tested in the clinic to guide administration and dosing of certain medications,^{18–21} which reduces the incidence of certain severe adverse drug reactions.^{15,17,22,23} More recently, additional pharmacogenomic genes have been identified by genome-wide association studies (GWASs) in randomized controlled trials (RCTs), pharmacogenomic studies nested within large epidemiological cohort studies, or meta-analyses of both.^{24,25} For example, the Clinical Pharmacogenetics Implementation Consortium (CPIC)²⁶—which curates gene-drug pairs and publishes corresponding clinical practice guidelines—

currently lists 119 unique pharmacogenomic genes for 293 drugs with variable levels of evidence or actionability. Additionally, pharmacogenomic studies have reported significant heritability of drug response phenotypes,^{27,28} that most variants and genes discovered in pharmacogenomic GWASs differ from candidate genes,²⁹ and that large-effect variants likely contribute little to the heritability of pharmacogenomic phenotypes.³⁰ However, access to genetic data from RCTs is limited, nested pharmacogenomic studies are rare, and both have much smaller sample sizes than those available in biobanks, so they are generally underpowered to investigate the genetic architecture of drug response.

Even more recently, genome-wide genetic data have been considered for clinical biomarkers of disease risk in the form of polygenic scores (PGSs).^{31–35} PGSs predict disease risk by aggregating many risk alleles identified by GWASs.³⁶ For some diseases, PGSs have comparable performance to current clinical risk-prediction algorithms, at least in European ancestry



Table 1. Drug exposures and responses examined in this work

| Drug exposure | Number of users (non-users) | Description | Primary effects | Side effects |
|---------------|-----------------------------|--|---|---|
| Statins | 56,169 (286,088) | low-density lipoprotein (LDL) cholesterol-lowering therapy prescribed to prevent atherosclerotic cardiovascular disease (ASCVD) events | LDL cholesterol, cardiovascular disease (CVD) ^{49,50} | glucose, hemoglobin A1c, type 2 diabetes (T2D) ^{51–54} |
| Metformin | 8,606 (333,651) | antidiabetic therapy | glucose, A1c ^{55–58} | body mass index (BMI), ^{59,60} LDL cholesterol, CVD ^{61,62} |
| Warfarin | 3,753 (338,504) | anticoagulant therapy | reticulocyte count, hematocrit, plateletcrit, venous thromboembolism (VTE) ^{63–65} | none examined |
| Methotrexate | 1,865 (340,392) | antirheumatic therapy | C-reactive protein (CRP) ^{66,67} | none examined |

individuals.^{37–39} However, very few genome-wide predictions for treatment response have been developed,^{40,41} even though their potential benefits have been discussed extensively.^{42–45}

Here, we build a framework to study genome-wide genetic effects on the primary and side effects of common drugs. Our approach leverages recent and novel methods for gene-environment interaction (GxE). Crucially, our methods apply to passively obtained electronic health records (EHRs), enabling analyses of sample sizes far exceeding randomized controlled trials. We apply our approach to some of the most common drugs in the UK Biobank: statins, metformin, warfarin, and methotrexate. Our methods quantify genome-wide heritability of drug response, identify specific genes modifying drug response, and characterize the implications for clinical use of PGSs. We replicate many of the gene-drug interactions in a longitudinal pharmacogenomic study of statins' effects on LDL cholesterol.^{46,47} Overall, our framework characterizes the genetic architecture of individual-level response to modifiable risk factors in passively obtained EHRs.

RESULTS

Study overview

We apply our framework to 342,257 unrelated white British individuals in the UK Biobank⁴⁸ (STAR Methods). We focus on four commonly prescribed drugs in this dataset: statins, metformin, warfarin, and methotrexate. For each drug, we study phenotypes related to its primary effect as well as phenotypes related to its possible side effects (Table 1).

We first define and develop a novel approach to estimate the aggregate impact of common genetic variation on treatment response (h^2_{response}). h^2_{response} is the SNP heritability of the phenotype change after treatment (Figure 1A, STAR Methods). We estimate this parameter by post-processing results from GxEMM,⁶⁸ which was developed to estimate GxE-based heritability. GxEMM explicitly models treatment-dependent heteroscedasticity, which is essential for unbiased estimates of treatment-dependent heritability. Because this approach aggregates genetic effects across the genome, it is powerful but does not identify specific genes.

Next, we develop a new method called TxEWAS to identify specific genes that modify drug response. TxEWAS is a GxE extension of the transcriptome-wide association study

(TWAS^{69,70}) framework. It genetically imputes gene expression levels using reference transcriptomics data, as in a TWAS, and then tests whether the imputed expression interacts with an environmental ("E") variable (Figure 1B, STAR Methods). A major challenge in TxEWAS is accounting for treatment-dependent heteroscedasticity, which we accomplish using the sandwich variance estimator (SVE)⁷¹ (Figures 1C, S1A, and S1B, STAR Methods). Compared to SNP-level tests, TxEWAS improves interpretability by suggesting possible causal genes and can improve power by aggregating multiple SNP effects and therefore reducing the number of statistical tests.^{69,70}

Like all existing gene-environment interaction models, GxEMM and TxEWAS are susceptible to endogeneity bias because individuals' treatments depend on their baseline phenotypes. We use theory, simulations, and additional data to characterize and account for this bias (Figures S2 and S3, supplemental note). Importantly, we test the gene-statin interaction effects on LDL cholesterol in a retrospective longitudinal pharmacogenomic study, which validates these specific results as well as our cross-sectional approach.

Finally, we study the impact of treatment status, which varies significantly between individuals, on the performance of polygenic scores. We evaluate changes in PGS prediction accuracy by varying the proportion of treated individuals in the training and/or validation data.

Primary effects and side effects of commonly prescribed drugs are heritable

We estimated h^2_{response} for statins' association with LDL cholesterol at 9% (Table 2). This is consistent with a prior estimate of 12% (SE = 9%) derived by comparing first-degree relatives.⁴⁶ Statin-dependent genetic effects on A1c and blood glucose were estimated at 10% and 11%, respectively (Table 2). For comparison, the statin-independent heritabilities for these traits (h^2_{hom}) are 21%, 29%, and 11% for LDL cholesterol, A1c, and blood glucose, respectively (Table 2).

We next found that the h^2_{response} for metformin's associations with LDL cholesterol and BMI are 2% and 17%, respectively (Table 2), and the metformin-independent heritabilities are 11% and 28%, respectively. However, we did not find significant heritability of response to warfarin or methotrexate, which was expected as we have lower power for less common drugs (Table S1).

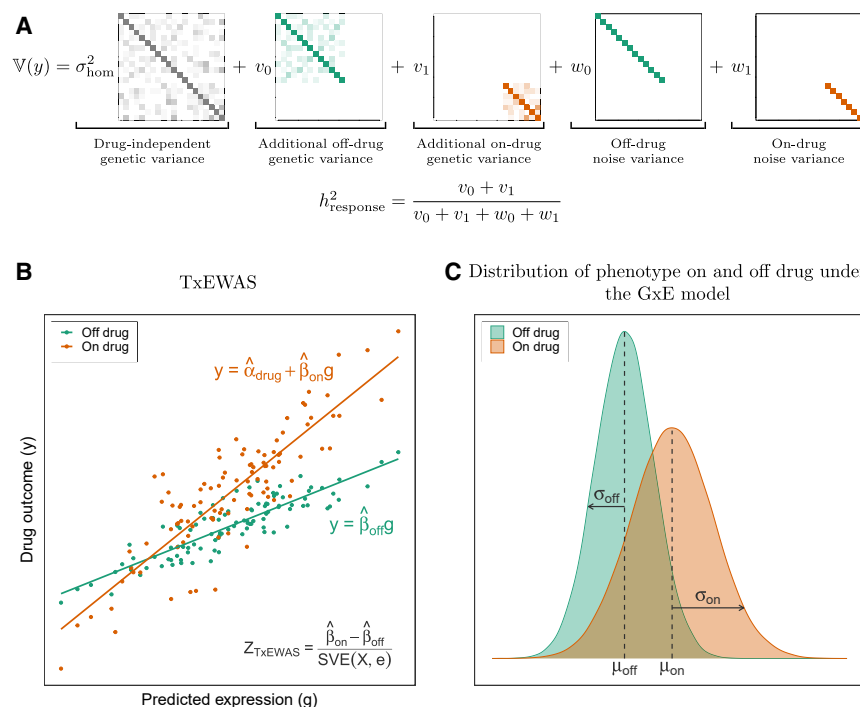


Figure 1. Schematic of the GxE framework to analyze treatment response in cross-sectional data

(A) We use GxEMM to estimate the heritability of treatment response (h_{response}^2) based on the genetic (v) and nongenetic (w) variances specific to treatment status (STAR Methods).

(B) We identify genes that modify treatment response using TxEWAS, a new method to estimate gene-level GxE interaction. TxEWAS genetically imputes gene expression and tests if this gene's effect interacts with some "E."

(C) Statistical interactions with treatment status induce treatment-dependent heteroscedasticity that must be modeled in GxEMM and TxEWAS.

131 interaction effects have opposite signs to the main effects (Figures 3A and S5). That is, statins uniformly buffer these genetic effects on LDL cholesterol. This is consistent with our observation that LDL cholesterol has higher heritability in statin non-users than users (Table S2).

At the time of conducting this study, the NHGRI-EBI GWAS Catalog⁷⁴ reports 10 unique SNP associations with LDL cholesterol change in response to statin therapy,

We next separately evaluated heritabilities in statin users vs. non-users. We observe that the heritability of LDL cholesterol is much higher in individuals who do not take statins (41% vs. 27%, Tables S2 and S3). This suggests that statins mitigate the genetic effects on LDL cholesterol present in untreated individuals (Table S4). We found a qualitatively different pattern for the side effect of statins on A1c and blood glucose, where statin users had comparable or higher heritability (Table S2). This suggests that statins activate or amplify genetic effects on blood sugar compared to untreated individuals. For metformin users, we found significantly higher heritability for BMI (51% vs. 31%) and lower heritability for LDL cholesterol (11% vs. 23%).

Identifying gene-drug interactions for primary and side effects

We next sought to identify specific genes that modify drug response. We found 156 genes that modify statins' association with LDL cholesterol (hierarchical FDR^{72,73}: hFDR < 10%, Figures 2A and S4A, Table S5). These genes include *APOE*, which has been previously implicated in response to statin therapy²⁵ and is listed by CPIC as one of 13 gene-statin pharmacogenes. None of the other 12 CPIC genes overlap our TxEWAS genes, but four of them are within 200 kb of our TxEWAS genes (*CYP2C9*, *HMGCR*, *CETP*, *LDLR*). Overall, this demonstrates some alignment of our results with existing evidence. Additionally, our discovery of many genes that are not in CPIC further documents the complexity of drug response phenotypes.^{29,30}

Of the 156 genes significantly interacting with statins, 131 also have significant additive effects (hFDR < 10%). Interestingly, all

and 2,766 such associations with LDL cholesterol levels (STAR Methods). These SNP associations were mapped—either by the studies or the GWAS Catalog—to 17 and 1,468 genes, respectively. Of the 156 gene-statin interactions identified in the LDL cholesterol TxEWAS, four overlap the 17 genes reported by GWAS for LDL cholesterol change (*SMARCA4*, *APOE*, *PCSK9*, *APOC1*). Those four, as well as 51 additional TxEWAS genes, overlap the 1,468 GWAS genes for LDL cholesterol levels (Figure S6A). Compared to a TWAS for LDL cholesterol (STAR Methods), 135 of the 156 TxEWAS genes are shared, and 21 are new (Figure S6B). These 135 shared genes include the 55 shared with GWAS (Figure S6C).

A pathway enrichment analysis with ConsensusPathDB⁷⁵ of the 156 interacting genes found enrichments mostly in cholesterol and lipoprotein metabolism pathways, as well as regulatory and transcription pathways (Table S6). For example, the two most significant Wikipathways were "Metabolic pathway of LDL, HDL, and TG, including diseases" and "Statin inhibition of cholesterol production," and the top KEGG and Reactome pathways also included cholesterol-related biology.

We next tested which genes modify the side effects of statins on A1c and blood glucose, and we identified 53 and 6, respectively (Figures 2B, 2C, S4B, and S4C). 28 of the 53 genes with statin-dependent effects on A1c lie in the highly complex MHC region, and it is likely that many of these are not causal.⁷⁶ One example gene outside the MHC is *GIPR*, which is known to regulate insulin levels in the presence of elevated glucose in mice⁷⁷ and is associated with increased risk of hyperinsulinemia after an antipsychotic treatment.⁷⁸ All six genes that modify statins' association with glucose overlap the statin-dependent A1c loci (Figure 2C).

Table 2. Statistically significant drug-independent heritability and heritability of drug response estimates

| Drug/response | h^2_{hom} | h^2_{response} | h^2_{response} p value |
|------------------|--------------------|-------------------------|-----------------------------------|
| Statins | | | |
| LDL cholesterol | 0.21 | 0.089 | 1.13×10^{-30} |
| A1c | 0.29 | 0.102 | 1.79×10^{-6} |
| glucose | 0.11 | 0.111 | 2.26×10^{-4} |
| Metformin | | | |
| LDL cholesterol | 0.11 | 0.023 | 0.016 |
| BMI | 0.28 | 0.170 | 2.51×10^{-4} |

Corresponding estimates for all tested drug exposures and responses can be found in [Table S1](#).

Of the 53 genes with statin-dependent effects on A1c, 36 have significant additive effects. However, unlike the genes modifying statins' association with LDL cholesterol, only four of them have an interaction effect with opposite sign to the main effect ([Figures 3B and S7](#)). Similarly, only three of the six genes with statin-dependent effects on glucose exhibit significant additive effects, and all of these effects have the same sign as the corresponding interaction effects ([Figure 3C](#)). Broadly, this suggests that some genetic effects on A1c and glucose are amplified by statins, while others are dampened.

We next analyzed metformin, warfarin, and methotrexate for gene-drug interactions. Although these have many fewer users ([Table 1](#)), we identified three gene-warfarin interactions effects on reticulocyte count (*HIF3A*, implicated in the response to hypoxia⁷⁹; *ITGA1*, shown to be upregulated at high oxygen levels in the environment⁸⁰; and *AL049542.1*) and one gene-methotrexate interaction effect on C-reactive protein levels (*C6orf164*).

The results are highly concordant if we exclude individuals who take combinations of the aforementioned drugs ([Figure S8; Table S5, supplemental note](#)).

Replicating gene-drug interactions in a pharmacogenomic study

The UK Biobank EHR data are passively obtained from an observational cohort and may suffer from confounding due to endogeneity in treatment status—sick individuals are more likely to be on treatments, and this may be driven in part by genetics. Therefore, we validated our approach by replicating the gene-level interactions for statins' primary effect in a pharmacogenomic study. The replication study analyzed statin-induced LDL cholesterol change in 34,874 statin users from the Kaiser Permanente GERA cohort (Genetic Epidemiology Research on Adult Health and Aging)^{46,47} ([STAR Methods](#)). Of the 156 significant genes that we identified from cross-sectional data, 155 could be studied in the replication cohort ([STAR Methods](#)). We found that 36/155 genes replicated ($\text{hFDR} < 10\%$, [Table S5](#)) and that the remaining genes were significantly enriched for low p values < 0.1 (binomial test p value = 0.002).

Two of the 36 replicated interacting genes (*APOE* and *APOC1*) were previously reported in GWASs of LDL cholesterol change in response to statin therapy. These two and 16 more were reported by GWASs for LDL cholesterol levels. We then studied the remaining 18/36 genes using pathway analysis, which re-

vealed enrichments in plasma lipoprotein processes, statin inhibition of cholesterol production, and cholesterol metabolism ([Table S7](#)). All of the 36 replicated interacting genes were identified by the standard TWAS for LDL cholesterol levels.

Gene-drug interactions impact polygenic prediction accuracy

Gene-drug interactions may reduce the performance of ordinary PGSs, which are additive genetic predictors and must compromise between optimizing for users and non-users. We assessed the impact of this bias on PGS performance by varying the proportion of individuals on a drug in the training and/or testing cohorts, keeping the training sample size fixed. We focused on statins and metformin because they had treatment response heritability ([Table S1, STAR Methods](#)).

First, we evaluated PGSs for A1c as a function of statin use ([Table 3](#)). We find that statin users are better predicted by the PGS trained on statin users, and vice versa for statin non-users. Numerically, prediction accuracy of the PGS for A1c in statin users increases by 31% when it is trained in treated vs. untreated individuals. While this is intuitive, it need not hold in general. For example, if two groups share identical genetic effects but have different levels of non-genetic noise, the PGS should always be trained in the less-noisy group. Indeed, we observe this pattern for LDL cholesterol, which has higher heritability in statin non-users than users, and we find that training a PGS in non-users is optimal for predicting in users ([Table 3](#)). We performed extensive simulations to confirm these results ([Table S8, STAR Methods](#)). Finally, we evaluated an “agnostic” PGS built from a mix of users and non-users without accounting for statins, and we found that this PGS performed worst of all ([Table 3](#)). This illustrates an unappreciated limitation of standard approaches to building PGSs in biobanks.

We found qualitatively similar results for PGSs dependent on metformin, though they had smaller sample size and were weaker: BMI for users were better predicted using the PGS built from users, while LDL cholesterol was always better predicted using non-users ([Table 3](#)).

Overall, our results demonstrate that the accuracy of polygenic prediction is significantly affected by the distribution of drug use status in both GWAS and prediction cohorts, and that the optimal approach varies across drugs and phenotypes. In particular, when genetic effects on an outcome are simply buffered by a drug, the optimal PGS for both users and non-users will be trained solely in drug non-users. This is because the genetic effects are perfectly correlated between groups, yet larger in the non-user group. Conversely, if the drug does not simply buffer baseline genetic effects, then the genetic correlation between users and non-users will decrease, and it becomes more important to match drug use status between GWAS and prediction cohorts. We hypothesize that the former scenario will be more frequent when studying the primary effect of a drug, and the latter will be more frequent for side effects; this hypothesis is consistent with our observation for statins' associations with LDL cholesterol and A1c. In general, the optimal PGS will depend on the net effect of these parameters and the available sample sizes for users and non-users ([Table S9](#)).

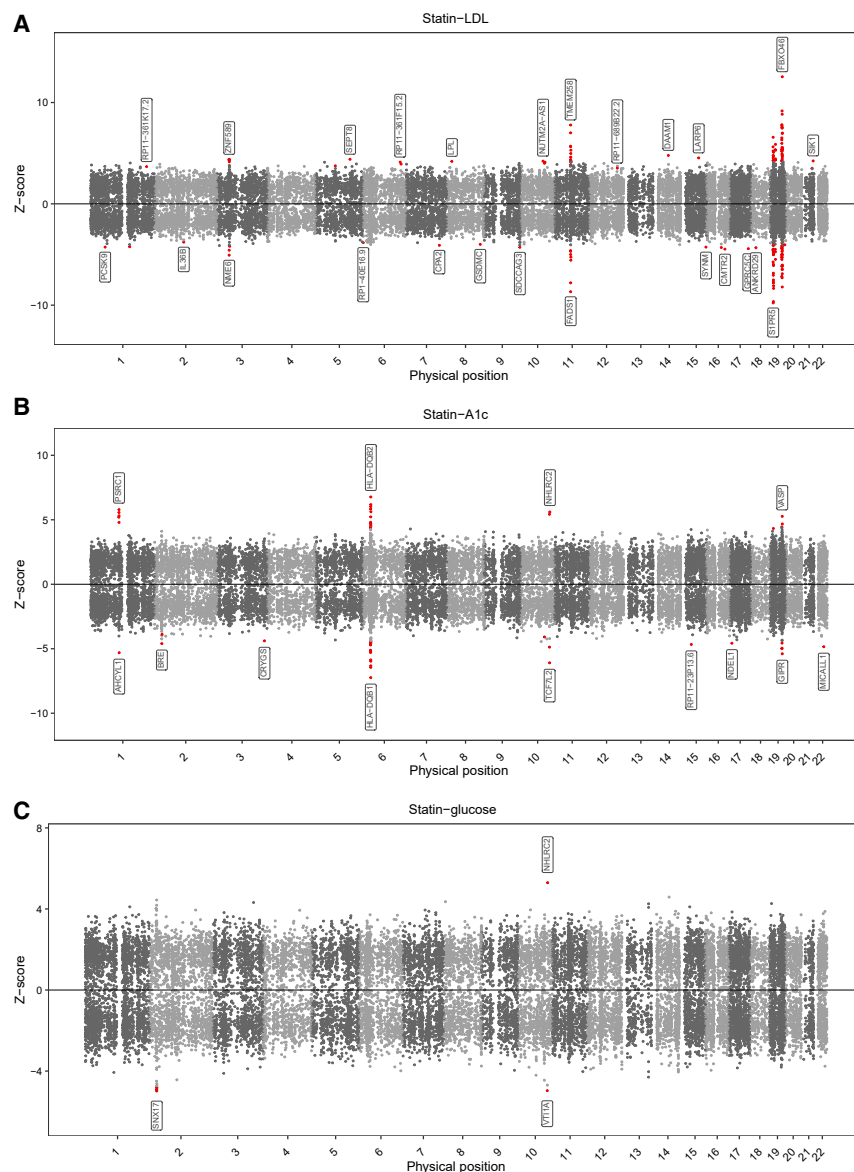


Figure 2. Manhattan plots of gene-statin interactions for low-density lipoprotein cholesterol and for hemoglobin A1c and blood glucose

(A) Manhattan plot of gene-statin interaction effects for LDL cholesterol (primary effect). Each point represents a single gene, with physical position plotted on the x axis and standardized effect size plotted on the y axis. The most extreme effect across tissues is shown for each gene. Significant associations are highlighted in red, and the strongest associations on each chromosome are labeled.

(B and C) Same as (A), but for A1c and blood glucose (side effects).

Our results suggest that genome-wide genetic variation broadly modifies drug response. This is an important extension of pharmacogenomic studies, which usually focus on large-effect genes directly involved in drug metabolism. This extension is consistent with the overall arc of human genetic studies, where first large-effect genes are identified and then, as sample sizes grow and methods mature, genome-wide signals are identified.^{81,82}

We observe a large, but not complete, overlap between statin-interacting genes identified with TxEWAS for LDL cholesterol and TWAS and GWAS associations with baseline LDL cholesterol (STAR Methods). This result calls for caution, since it could be explained by endogeneity (Figure S2, supplemental note) or model misspecification, which are one of the main concerns in all GxE studies. Nonetheless, reassuringly, many of these genes replicated in a longitudinal study, and some have additional forms of experimental evidence.^{83–85}

DISCUSSION

We quantified the genome-wide contribution of genetic variation to drug response (h^2_{response}) for some of the most commonly prescribed drugs worldwide. We identified specific genes driving this variation, and we validated the gene-statin interaction effects on LDL cholesterol in a longitudinal pharmacogenomic study. We found that such genetic effects on drug response have downstream implications for PGSSs, which are moving toward clinical use. In particular, we showed that current PGSSs will often underperform in the clinic because they are biased toward untreated individuals. While our paper focuses on drug treatments, we note that our novel framework can characterize the genetic basis of any covariate's effect, including sex/gender, age, or modifiable risk factors.

An interesting finding is *PCSK9*—an LDL cholesterol-lowering drug target, which we observed to have a stronger effect in statin users than non-users (Figure 3A). This interaction effect did not replicate in our longitudinal study, which could be due to insufficient power in this study, or due to endogeneity in our discovery analysis. There is a possibility of endogeneity bias because *PCSK9* has a strong effect on baseline LDL cholesterol (supplemental note). On the other hand, known facts about *PCSK9* make it an interesting candidate for a statin pharmacogene: (1) loss-of-function and gain-of-function variants in *PCSK9* are known to reduce and elevate LDL cholesterol levels, respectively,^{86,87} and (2) statin therapy has been shown to increase serum *PCSK9* levels, which can buffer statin LDL cholesterol-lowering effects.^{84,87} Furthermore, a genetic study⁸⁸ identified loss-of-function variants associated with improved LDL cholesterol response to statins. However, the strongest association reported in this study was

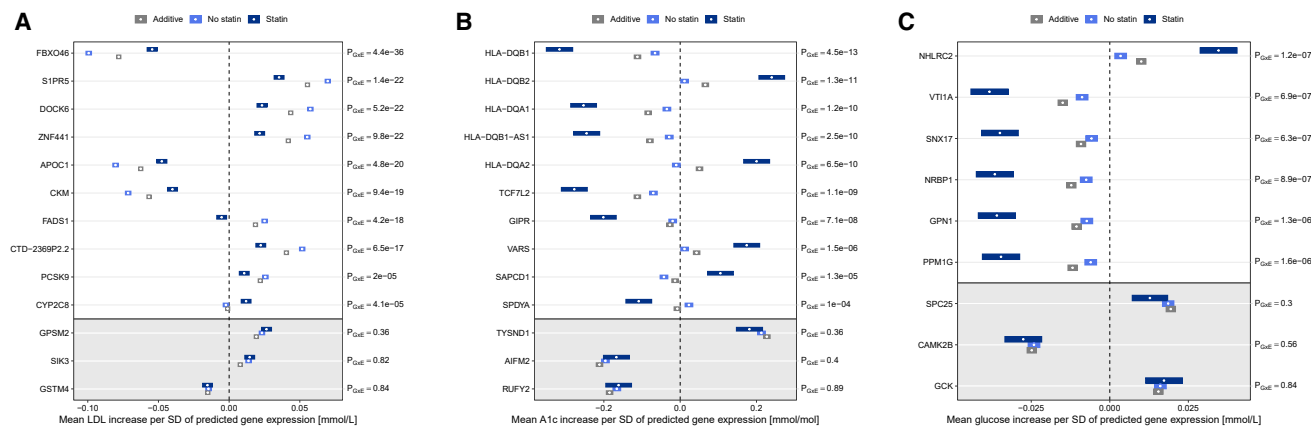


Figure 3. Gene-statin interaction effect sizes for low-density lipoprotein cholesterol, hemoglobin A1c, and blood glucose

(A) Estimated effect sizes of selected genes on LDL cholesterol in statin users and non-users and from a standard additive model. Color boxes depict standard errors around effect size estimates. Reported p values $P_{G \times E}$ are for the gene-statin interaction effects. The top 10 genes have significant interaction effects; for comparison, the bottom three genes are only additively significant.

(B and C) Same as (A), but for A1c and blood glucose. See also Figures S5 and S7.

estimated with just three carriers, and the effect of a loss-of-function variant is easier to predict than the combined effect of small and moderate effect variants, which we studied. A more recent genetic study⁸⁹ reported variants with statin-dependent effects on PCSK9 levels, and stronger causal effects of PCSK9 on LDL cholesterol in statin non-users compared to statin users. This is in line with our result. However, this study evaluated statin interactions on small preselected sets of variants and suffered similar limitations as ours in regards to observational data.

Importantly, our results identify an unappreciated limitation of the clinical use of PGSs, which are systematically less predictive in treated individuals than healthy controls. This complements other known limitations of PGSs, including limited transferability across socio-economic status, age, sex,^{90,91} and ancestry,^{92,93} which could also be driven in part by gene-environment interactions.⁹⁴ On the other hand, our results pave a path to developing context-specific PGSs that incorporate context-specific genetic effects, which could directly predict an individual's response to common drugs by the difference between their on-drug PGS and their off-drug PGS.

Limitations of the study

Our study has several limitations. First, it has focused on the UK Biobank, which is a cross-sectional cohort with non-random allocation of drugs. This raises the possibility of endogeneity biases causing false positives or false negatives, where our results reflect causes of drug prescription rather than its consequences. Nonetheless, we have validated many of our results in a longitudinal pharmacogenomic study, which took steps to reduce biases from dosing and baseline LDL cholesterol levels. Robust replication for one drug-outcome pair does not imply that our results will replicate for every such pair. Nonetheless, it is an important reassurance that our exploratory approach has value to partly recover longitudinal analyses. More importantly, for many traits, the results are inconsistent with simple endogeneity-driven biases because we observed both positive and negative interaction effects (supplemental note). A related limitation is that our cross-

sectional approach to estimate heritability provides only a lower bound to h^2_{response} (supplemental note). In the future, methods to formally account for such endogeneity would give more precise estimates of treatment response heritability. Second, large-scale projects, like the UK Biobank, measure many shallow phenotypes but often do not measure the most clinically relevant phenotypes for a specific study. For this study, for example, we were unable to extract relevant phenotypes to investigate statin-induced myopathy, and we were only able to extract proxies for the primary clinical measure of warfarin's effect (international normalized ratio⁹⁵). If the number of drug users in a large biobank is relatively small, as in our warfarin study, bespoke small-scale studies can have more power to model genetic effects on drug response.⁹⁶ Third, biobanks often lack detailed information about drug dose for many or all participants. Our study in the UK Biobank did not account for drug dose, which is an important limitation, even though we were able to validate an important part of our discoveries in a pharmacogenomic study that did correct for it. Fourth, patients are often on multiple drugs simultaneously. Results of our secondary analysis, which excluded individuals on combinations of drugs considered in this study, showed high concordance with results of the primary analysis. However, more targeted studies can improve by including information about important drugs taken concurrently. Fifth, statistical genetic interactions need not reflect biochemical interactions between a gene and drug. This contrasts with large-effect pharmacogenomic genes, which typically encode enzymes that directly metabolize the drug. Sixth, the TxEWAS method is liable to detect genes or tissues that are merely correlated with causal genes of tissues. In the future, established TWAS methods to fine-map causal genes⁷⁶ or tissues^{97–99} could be adapted to the TxEWAS setting. Seventh, due to biases in available data, we analyzed only individuals of European ancestry. For example, GxEMM requires tens of thousands of samples for robustness, and TxEWAS requires reference genomic data which currently is heavily biased to European ancestries.¹⁰⁰ More ancestrally diverse data are needed to obtain more generally applicable results. Nonetheless, we expect that our qualitative conclusions

Table 3. Prediction accuracy of polygenic scores trained in drug users, non-users, and a 50:50 mixture of both agnostic to treatment status

| Training | Prediction accuracy (incremental R^2 [%] [SE]) | | | | | | | |
|----------|--|--------------|-------------|-------------|-----------------|-------------|---------------|-----------------|
| | Statins | | | | Metformin | | | |
| | LDL cholesterol | | A1c | | LDL cholesterol | | BMI | |
| | On drug | Off drug | On drug | Off drug | On drug | Off drug | On drug | Off drug |
| On drug | 7.18 (0.30) | 12.60 (0.36) | 3.36 (0.23) | 2.32 (0.18) | 1.32 (0.37) | 3.24 (0.54) | 0.919 (0.286) | 0.0040 (0.0592) |
| Off drug | 7.98 (0.32) | 14.87 (0.39) | 2.56 (0.20) | 5.79 (0.28) | 2.75 (0.51) | 5.43 (0.67) | 0.041 (0.080) | 0.0077 (0.0636) |
| Agnostic | 5.86 (0.29) | 10.49 (0.36) | 2.60 (0.20) | 3.75 (0.23) | 2.25 (0.44) | 5.00 (0.66) | 0.255 (0.164) | 0.0931 (0.1078) |

See also Table S9.

about the genetic architecture of treatment response will apply to all individuals. Eighth, since estimated heritability depends on the choice of covariates, and since our analyses always adjust for the main effect of the specific drug being tested, our estimates of h_{hom}^2 for an outcome depend on the drug being tested. Similar issues apply to ordinary additive heritability estimates¹⁰¹ (supplemental note, Table S10). Nonetheless, this does not affect our conclusions about h_{response}^2 .

Despite these limitations, we provided evidence for substantial polygenic contributions to drug response and showed how large-scale cross-sectional studies like the UK Biobank can be used to estimate genetic effects on drug response. Although validation with randomized controlled trials is needed before drawing definitive conclusions about causal genetic effects on treatment response, our results demonstrate that cross-sectional data can generate compelling hypotheses on genetic modifiers and statistical predictions for treatment response. It is important, as advances in pharmacogenomics cannot be made with sole input from randomized controlled trials,²⁴ which have strict inclusion and exclusion criteria, cannot always be performed due to ethical issues and are limited to small sample sizes due to high cost. Based on these results, we envision that novel PGS approaches incorporating treatment information will provide actionable clinical guidelines for optimizing primary effects and minimizing harmful side effects of drugs.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Michal Sadowski (michalsadowski@ucla.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table.
- All original code has been deposited at https://github.com/michalsad/txewas_scripts and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.S., J.F.A., A.W.D., and N.Z.; methodology, M.S., J.M., A.W.D., and N.Z.; software, M.S., M.T., and A.W.D.; validation, T.H. and A.O.-O.; resources, M.T., T.H., A.O.-O., R.B., A.P., and N.C.; writing—original draft, M.S.; writing—review & editing, M.S., M.T., A.O.-O., R.B., S.S., A.W.D., and N.Z.; supervision, J.F.A., S.S., A.W.D., and N.Z.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|--|---|--|
| Deposited data | | |
| UK Biobank Resource | UK Biobank | https://www.ukbiobank.ac.uk/ |
| Response to statins (LDL cholesterol percent change) GWAS summary statistics | Oni-Orisan et al. ^{46,47} | GWAS Catalog: GCST009821 |
| GTEEx (context-by-context) eQTL weights | Thompson et al. ⁹⁸ | http://gusevlab.org/projects/fusion/#multi-context-content-expression |
| LDL cholesterol change in response to statin therapy GWAS associations | Oni-Orisan et al., ⁴⁷ Barber et al., ¹⁰² Chasman et al., ¹⁰³ Deshmukh et al., ¹⁰⁴ Postmus et al. ¹⁰⁵ | GWAS Catalog: GCST010338, GCST009821, GCST000635, GCST001408, GCST001425, GCST002675 |
| LDL cholesterol levels GWAS associations | Sollis et al. ⁷⁴ | GWAS Catalog: GCST90002412, GCST008238, GCST008593, GCST90010364, GCST90179477, GCST90025954, GCST90134520, GCST007204, GCST90092883, GCST90255425, GCST90102272, GCST90255492, GCST011683, GCST007141, GCST004541, GCST007131, GCST010245, GCST011417, GCST010204, GCST009757, GCST008990, GCST000234, GCST000866, GCST007442, GCST007689, GCST90319533, GCST003361, GCST006004, GCST008988, GCST90239612, GCST90278638, GCST90278111, GCST90292747, GCST90293039, GCST90140947, GCST90244006, GCST90104175, GCST90134487, GCST90134501, GCST90134504, GCST000131, GCST000151, GCST000134, GCST000283, GCST003303, GCST003216, GCST001645, GCST008935, GCST90019512, GCST90319696, GCST90101741, GCST90101745, GCST009043, GCST000132, GCST006612, GCST90079033, GCST90018961, GCST90018741, GCST90296607, GCST008037, GCST000807, GCST000975, GCST000287, GCST000282, GCST004920, GCST90090990, GCST011347, GCST007848, GCST002898, GCST003662, GCST004233, GCST004236, GCST90244653, GCST008077, GCST000759, GCST002222, GCST002220, GCST009147, GCST009150, GCST008676, GCST90255506, GCST90255507, GCST90255505, GCST90239655, GCST90239659, GCST90239660, GCST90239658, GCST90239656, GCST90239657 |
| Software and algorithms | | |
| TxEWAS | this paper | https://doi.org/10.5281/zenodo.12192083 |
| GxEMM | Dahl et al. ⁶⁸ | https://github.com/andywdahl/gxemm |
| NetworkX | Hagberg et al. ¹⁰⁶ | https://github.com/networkx/networkx |
| bigstatsr | Prive et al. ^{107,108} | https://privefl.github.io/bigstatsr |
| GCTA | Yang et al. ¹⁰⁹ | https://yanglab.westlake.edu.cn/software/gcta |
| sandwich | Zeileis et al. ^{110,111} | https://sandwich.r-forge.r-project.org |
| TreeQTL | Peterson et al. ^{72,73} | http://bioinformatics.org/treeqtl |
| FUSION | Gusev et al. ⁷⁰ | http://gusevlab.org/projects/fusion |
| ConsensusPathDB | Kamburov et al. ⁷⁵ | http://cpdb.molgen.mpg.de |

METHOD DETAILS

UK Biobank data

Analyses presented in this work were performed in the UK Biobank population of 342,257 unrelated white British individuals.

Samples

The 342,257 unrelated white British individuals were identified by performing the following steps. From among 488,363 UK Biobank participants, we retained putative “White British” individuals using field 22006–0.0 ($n = 409,692$). We then filtered out 199 individuals with excess genotype missingness (>0.05), 312 individuals with a mismatch between self-reported and genetic sex, 999 individuals with excess heterozygosity (≥ 5 standard deviations above the mean), and 90 individuals who requested their data be redacted. We then removed 629 individuals related to ten or more individuals (KING coefficient $\geq 2^{-9/2}$) as a preprocessing step to the application of the maximal_independent_set algorithm implemented in the NetworkX Python package.¹⁰⁶

In contrast to Bycroft et al.,¹¹² who estimated kinships using approximately 92,000 common SNPs with small loadings onto the first few principal components (PCs) in the full sample (including multiple ancestries; see S3.7 of Bycroft et al.), we estimated kinships using 561,780 common SNPs in a sample of European ancestry individuals. The close relatives the UK Biobank identified in field 22021–0.0 are a subset of our more conservative approach: we identified all 81,218 related individuals in this subsample identified by the UK Biobank plus an additional 3,261 not identified by Bycroft et al.

Genotypes

For heritability and PGS analyses, we used 579,566 UK Biobank variants with minor allele frequency (MAF) larger than 0.01, Hardy-Weinberg equilibrium (HWE) test p value below 10^{-10} , and imputation INFO score of 1. For the TxEWAS analysis, UK Biobank SNPs that matched eQTLs trained in the GTEx consortium¹¹³ were used.

Phenotypes

Individuals who take statins were identified by UK Biobank field 20003–0.0–47 using the following codes: 1140861958, 1140861970, 1141146138, 1140888594, 1140888648, 1140910632, 1140910654, 1141146234, 1141192410, 1141192414, 1141188146, 1140881748 and 1140864592. There were 56,169 such subjects within the UK Biobank population of 342,257 unrelated white British individuals. Individuals who take metformin ($n = 8,606$) were identified by codes 1140884600 and 1141189090 in the same UK Biobank field. Warfarin users ($n = 3,753$) were identified by codes 1140888266 and 1140910832; and methotrexate users ($n = 1,865$) by codes 1140869848 and 1140910036.

LDL cholesterol, glucose, A1c, BMI, hematocrit, plateletcrit, reticulocyte count and C-reactive protein levels were retrieved from UK Biobank fields 30780–0.0, 30740–0.0, 30750–0.0, 21001–0.0, 30030–0.0, 30090–0.0, 30240–0.0 and 30710–0.0, respectively.

C-reactive protein levels were inverse normally transformed before fitting the models. For other traits, we discarded measurements greater than five standard deviations from the mean, with the assumption that such extreme levels were results of non-modeled circumstances.

CVD was defined as in Thompson et al.¹¹⁴ The T2D disease status and the VTE status were extracted from the UK Biobank EHRs. The former was defined using ICD10 code E11. The latter, using ICD10 codes I26, I80.1, I80.2, I81 and I82.0, and OPCS procedure code L90.2.¹¹⁵ For testing associations with drug use, we only retained diagnoses recorded after the date of the initial assessment with the UK Biobank initiative (when the information about medication use was collected), which resulted in 29,393 (278,675), 18,193 (297,399), 7,356 (332,403) cases (controls) for CVD, T2D and VTE, respectively.

Covariates

The main analyses reported in this work were performed using the following covariates: age, sex, birth date, Townsend deprivation index, and the first 16 genetic PCs.⁹² We additionally accounted for the measuring device type when an outcome required it, which was the case for hematocrit, plateletcrit, and reticulocyte count. All non-binary covariates were standardized (transformed to mean-zero, variance 1) before calculating interaction variables.

Heritability of treatment response

GxEMM quantifies the heritability contributed by genome-wide additive effects and genome-wide GxE effects. The general GxEMM model for phenotype y of an individual i in environment k (i.e., $Z_i = k$) is:

$$y_i|Z_i = k \sim \sum_q X_{iq} \alpha_q + \sigma_{\text{hom}} \sum_s G_{is} \beta_s + \sqrt{v_k} \sum_s G_{is} \gamma_{sk} + \sqrt{w_k + \sigma_\epsilon^2} \epsilon_i$$

In this model, X are covariates (indexed by q) with fixed effects α , and G is a matrix of SNPs (indexed by s), with additive effects β . We assume that β and the noise, ϵ , are i.i.d. standard normal, and the additive heritability is determined by the genetic and noise variances, σ_{hom}^2 and σ_ϵ^2 . GxEMM additionally captures SNP-environment interaction effects, γ , which are also assumed i.i.d. standard normal. Further, GxEMM allows environment-specific genetic (v_k) and noise (w_k) variances. If the phenotype is scaled to variance 1, $\sigma_{\text{hom}}^2 = h_{\text{hom}}^2$.

Here, we use treatment status as the “environment” in order to quantify the heritability due to treatment-specific effects. We approximate the heritability of treatment response, $\Delta y_i = y_i(Z_i = 1) - y_i(Z_i = 0)$, by:

$$h_{\text{response}}^2 := \frac{V_0 + V_1}{V_0 + V_1 + W_0 + W_1} \leq \frac{V_0 + V_1}{V_0 + V_1 + W_0 + W_1 - 2W_{01}} = h^2(\Delta y)$$

where w_{01} captures the covariance in effect sizes for unmodeled risk factors between treated/untreated states (Supplemental Note).

For warfarin, methotrexate, and metformin, we studied a sample of 30,000 individuals that included all users of that drug and an accordingly-sized random draw of non-users. To assess stability of our results, we repeated the analysis five times by randomly re-sampling non-users, and reported results from the sample with median additive heritability (h_{hom}^2 , Figure S9). Because statins are much more common, we instead randomly split all 342,257 individuals into 11 non-overlapping subsets and meta-analyzed the results. This is a common approach employed in biobank-scale datasets to reduce computational complexity.¹¹⁶

Genes responsible for variable drug response

TxEWAS extends the transcriptome-wide association study (TWAS^{69,70}) framework to test gene-environment interactions. The TxEWAS framework involves two major steps: First, gene expression levels of each gene are genetically imputed using a reference dataset. Second, the interaction effect, γ , between imputed gene expression and the drug is tested in the regression model:

$$y_i = \beta_0 + \sum_j \beta_{1j} X_{ij} + \sum_j \beta_{2j} Z_i X_{ij} + \sum_j \beta_{3j} g_i X_{ij} + \beta_4 g_i + \beta_5 Z_i + \gamma g_i Z_i + \epsilon_i,$$

where $\epsilon_i \sim \mathcal{N}(0, \sigma^2)$; Z_i and g_i are the drug use indicator and imputed expression of some gene for individual i , respectively, and X is a matrix of covariates.

For binary phenotypes, the interaction effect is tested in the logistic regression model with the same covariates (Supplemental Note). In both models, the variance of the effect size estimates is estimated with the sandwich variance estimator to control for heteroskedasticity and/or misspecification of the functional form of the environmental factor^{117,118} (Figure S1, Supplemental Note).

In this study, we imputed gene expression into the UK Biobank using 48 tissues from the GTEx consortium, and we used hierarchical FDR^{72,73} (hFDR < 10%) to account for multiple hypothesis testing across genes and tissues. Hierarchical FDR has been shown to properly control the false discovery rate across contexts when there are multiple hypothesis tests being run for a given group, in this case, a gene.^{72,73,119} It also boosts power in cases where a gene has a significant association in multiple contexts. Finally, since TxEWAS is liable to detect genes that are merely correlated with the causal gene due to the genetic LD structure in the proximity of the causal gene, we define TxEWAS association loci by adding consecutive genes until there is no gene within 500 kb from the last added gene.

Gene expression prediction models

In TxEWAS, gene expression (or rather its genetic component) is imputed as a linear combination of genetic variants (SNPs). The coefficients used for the imputation are referred to as “weights” or “eQTL weights” and are calculated on a per-gene basis by fitting a linear model of gene expression onto the gene’s *cis*-genotypes in an external reference dataset. We fit each model using the elastic net, as it has been found to be the most robust across a wide range of genetic architectures.¹²⁰ We used package bigstatsr¹⁰⁷ to fit each model using 10-fold cross-validation, and after determining whether the expression of a gene was significantly predicted using *cis*-genotypes at a nominal p value of 0.05, we retrained the model using the entire set of individuals to generate a final set of weights. The weights has been deposited at <http://gusevlab.org/projects/fusion/#multi-context-content-expression> under the “GTEx (context-by-context)” download link.

Replication in a pharmacogenomic study

We initially discovered gene-drug associations in cross-sectional data using TxEWAS. To validate these discoveries, we performed an ordinary TWAS on the change in LDL cholesterol after statin initiation in an external pharmacogenomic study (we term this analysis PGx TWAS, Table S11). More concretely, we used data from a longitudinal study of 28,616 individuals with European ancestries from the Kaiser Permanente GERA cohort (Genetic Epidemiology Research on Adult Health and Aging).^{46,47} In this study, the phenotype was rigorously characterized utilizing electronic health records, and the analysis was adjusted for carefully selected covariates. For every TxEWAS interacting gene identified in the UK Biobank cohort, we calculated the PGx TWAS statistic in all available GTEx tissues, and employed an hFDR correction to call statistically significant genes at FDR < 10%.

Implications for polygenic scores

PGSs are weighted sums of risk alleles optimized to predict some training dataset. This makes PGSs depend on characteristics of the training data, such as ancestry,⁹³ age,⁹⁰ or sex.⁹¹ We assessed the accuracy of PGSs as a function of drug use, including PGSs trained in users and tested in non-users and vice-versa. In the main analysis, we varied the proportion of individuals on a drug in the training cohort, keeping the sample size fixed. We evaluated additional scenarios in the Supplemental Note. We fit PGSs using a fast implementation of penalized linear regression with the lasso penalty^{92,108} and we measured prediction accuracy by the incremental R^2 over baseline covariates. Standard errors around the estimates were calculated using bootstrap.

Simulating polygenic scores

We performed realistic simulations to examine two scenarios observed in real data. Scenario 1 mimicked statin-LDL, where “on” genetic effects are buffered to be half the size of “off” effects. As expected, we found that training a PGS in “off” individuals is optimal regardless of the test set (Table S8). Scenario 2 mimicked statin-A1c, where “on” and “off” effects are highly correlated but vary randomly in magnitude. As expected, we found that training a PGS in training samples matching the test samples is optimal in this scenario. These simulations show how prediction accuracy of a PGS depends on the genetic correlation and heritability between the train and test dataset and explain the discordant results for statins’ effects on A1c and LDL cholesterol (Table S9). In both cases, we simulated the unexposed population to have 40% heritability. In the exposed population, we either (Scenario 1) divided all genetic effect sizes by two, reflecting systemic buffering of the unexposed effects, or (Scenario 2) randomly deflated (with probability 0.4) or inflated (with probability 0.6) each individual genetic effect by a random fraction between 0.2 and 1. We then performed PGS analyses as in the real data by varying the distribution of drug use in the train and test populations.

GWAS data

GWAS associations with LDL cholesterol change in response to statin therapy, and LDL cholesterol levels were collected from the NHGRI-EBI GWAS Catalog⁷⁴ on 04/17/2024.

Associations with LDL cholesterol change in response to statin therapy were identified using the following names of traits: “Response to statin therapy”, “Response to statins (LDL cholesterol change)”, “Response to statins (LDL cholesterol percent change)”.^{102–105}

Associations with LDL cholesterol levels were identified using the following names of traits: “LDL cholesterol levels”, “Low-density lipoprotein cholesterol levels”, “Direct low-density lipoprotein cholesterol levels”, “LDL cholesterol”, “Direct low-density lipoprotein levels (UKB data field 30780)”.

Only associations that reached the genome-wide significance level of p value $< 5 \times 10^{-8}$ were considered. For gene-level comparisons, lists of associated genes were created for each of the above phenotypes by taking the union of the “reported genes” and “mapped genes” columns of a given summary table.

TWAS for LDL cholesterol levels

TWAS for LDL cholesterol levels was performed in the UK Biobank analogously to the TxEWAS for statins’ association with LDL cholesterol (see above), only no interaction terms were included in the model. TWAS tests were performed in 48 tissues from the GTEx consortium, and significant genes were called at $hFDR < 10\%$.

QUANTIFICATION AND STATISTICAL ANALYSIS

General statistical analysis

Unless otherwise specified, all analyses were conducted using custom scripts implemented in the R programming language.

Heritability estimation with GxEMM

GxEMM heritability estimates and their standard errors were calculated using the GxEMM package.⁶⁸ Specifically, we fit the GxEMM’s Free model using REML. p values for h^2_{response} were obtained by testing for non-additive heritability, i.e., by testing whether the off-drug (v_0) and on-drug (v_1) genetic variances are both zero. Because GxEMM does not scale to the whole UK Biobank, we perform meta-analysis across 11 non-overlapping subsets of data (method details). More specifically, we use inverse variance weighting to meta-analyze heritabilities and Fisher’s method to meta-analyze p values.

Heritability estimation with GCTA

Estimates of the additive heritability and their standard errors reported in Table S3 were calculated using the GCTA software.¹⁰⁹ Sample sizes of metformin users who were not statin users were: 1,584 for LDL cholesterol, 1,337 for A1c, and 1,243 for blood glucose. Sample sizes of the rest of the drug user groups were matched to those available for users of statins and metformin: 6,572 for LDL cholesterol, 5,800 for A1c, and 5,157 for blood glucose.

Estimates of the additive heritability and their standard errors reported in Table S10 were calculated using the GCTA software. Similarly to GxEMM, GCTA only scales up to tens of thousands samples. Thus, for the analyses that did not include drug use as covariates or included statin use (which has high frequency of $\sim 16\%$ in our dataset), we randomly split all 342,257 individuals into 11 non-overlapping subsets and meta-analyzed the results, as described above for GxEMM. For the analyses which included metformin use as a covariate, we estimated the additive heritability in a sample of 30,000 individuals that included all users of the drug and an accordingly-sized random draw of non-users. As in the corresponding GxEMM analysis, we repeated the analysis five times by randomly resampling non-users, and reported results from the sample with median estimate.

GxE tests with TxEWAS

The TxEWAS model of a continuous response is fitted using R’s `lm()` function, and the model of a binary response using the `glm()` function with the parameter family = “binomial” and logit link function. For both models, robust standard errors are calculated using

White's estimator implemented in the `vcovHC()` function from R package `sandwich`.^{110,111} p values are obtained from a two-sided Wald test. Hierarchical FDR correction from package `TreeQTL`^{72,73} is applied to p values across genes tested for multiple tissues, and significant genes are called at 10% FDR.

Replication analysis

TWAS on summary statistics from our replication pharmacogenomic study ([method details](#)) was performed using FUSION⁷⁰ and the LD reference data for individuals of European ancestry provided with the software. The LD reference was calculated based on genotypes from the 1000 Genomes Project, which provided fewer SNPs than the UK Biobank. As a result, FUSION could not build expression models for some of the genes that were identified with TxEWAS using the individual-level UK Biobank data. This is why, in the main analysis, we report replication rate relative to 155 genes, and not all 156 genes identified in the statin-LDL TxEWAS.

Pathway enrichment analysis

All pathway enrichment analyses presented in this work were performed with ConsensusPathDB.⁷⁵

Supplemental information

**Characterizing the genetic architecture of drug
response using gene-context interaction methods**

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Supplemental References

1 Supplemental Figures

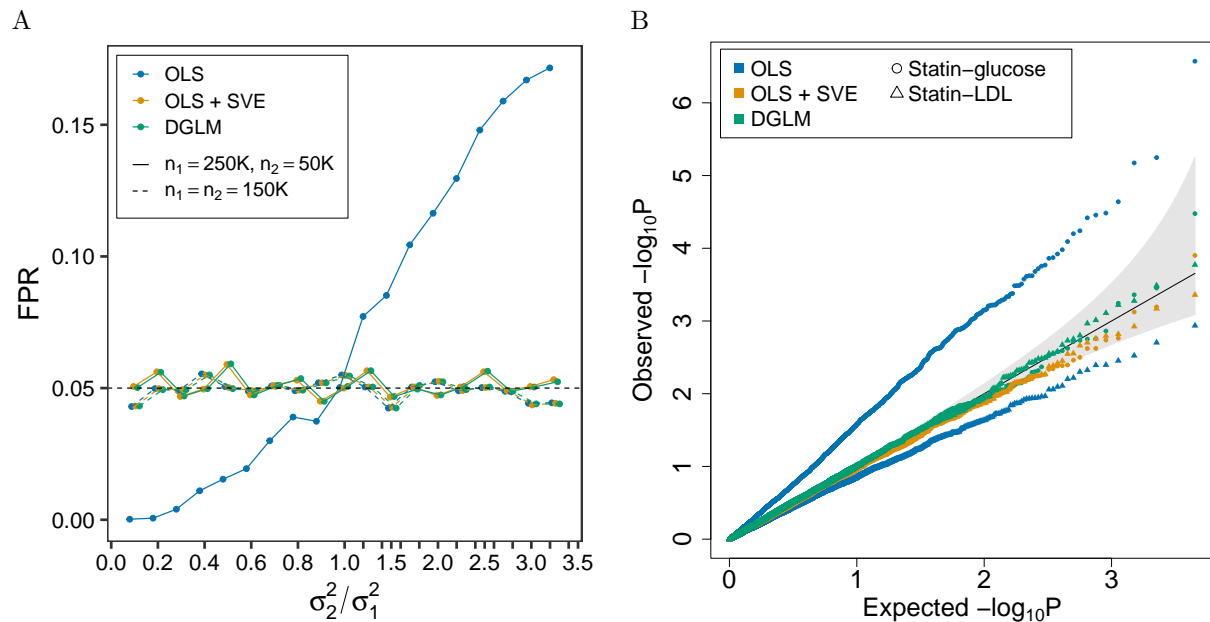


Figure S1. Evaluation of the type I error rate for the GxE effect estimated with the OLS model, the OLS model using robust standard errors (OLS + SVE) and the DGLM; related to Figure 1 and STAR Methods. (A) False positive rate (FPR) of GxE as a function of the ratio between phenotype variances in two environments: unexposed (of size n_1 and phenotype variance σ_1^2), and exposed (of size n_2 and phenotype variance σ_2^2). The nominal FPR of 5% is marked by the black dashed line. (B) Quantile-quantile plot comparing the null expected p values (x-axis) to the observed GxE p values after permuting real data from the UK Biobank (y-axis). The permutation permutes imputed expression of 4,516 genes and then tests their interaction with statins on blood glucose (circles) or LDL cholesterol (triangles).

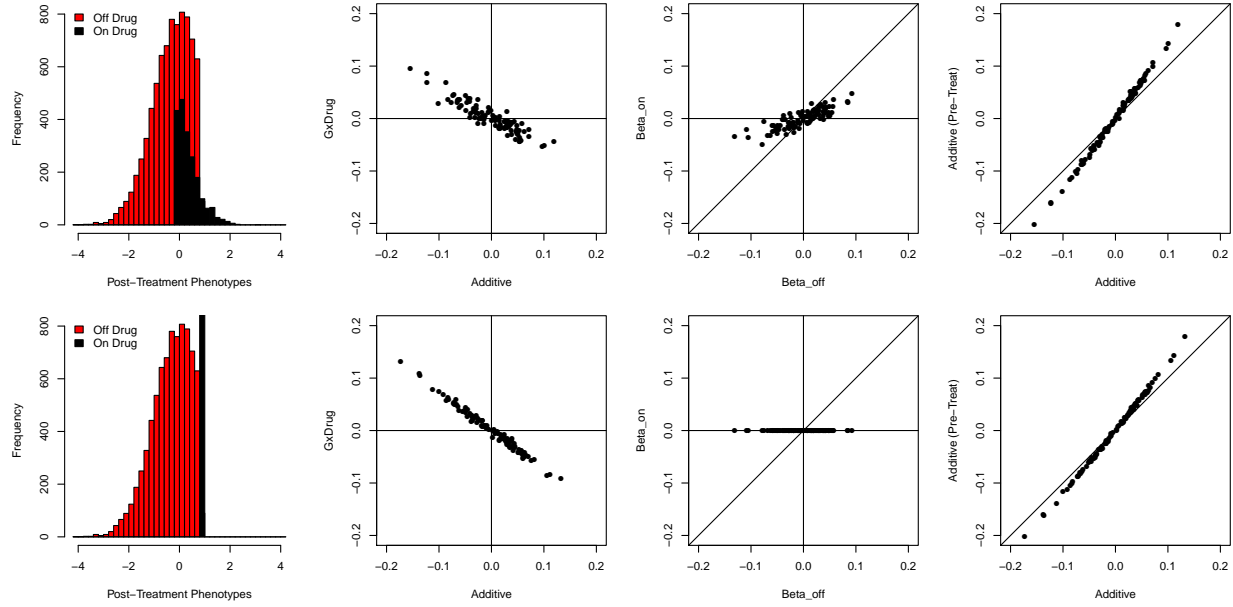


Figure S2. Endogeneity bias simulations; related to Figure 3. Top row: Simulation assumes that treatments have an equal additive effect on all individuals. Bottom row: Simulation assumes that treatments return all individuals to the treatment threshold, regardless their initial phenotypes. First column: Cross-sectional phenotype distribution, stratified by treatment status. Second column: Comparison of estimated additive vs interaction effect sizes. Third column: Comparison of estimated effect sizes in treated vs untreated individuals. Fourth column: comparison of additive effect estimated on pre-treatment phenotypes vs cross-sectionally observed phenotypes containing a mix of treated and untreated individuals.

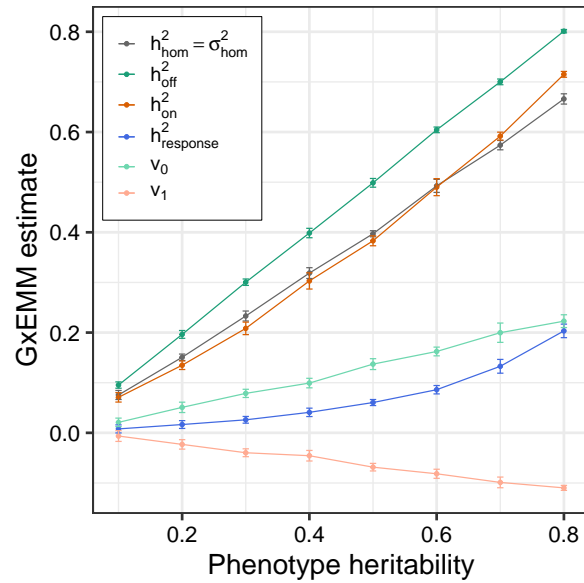


Figure S3. GxEMM estimates in the presence of the buffering effect of a drug; related to Table 2 and Figure 3. Error bars represent standard deviation over 10 simulations.

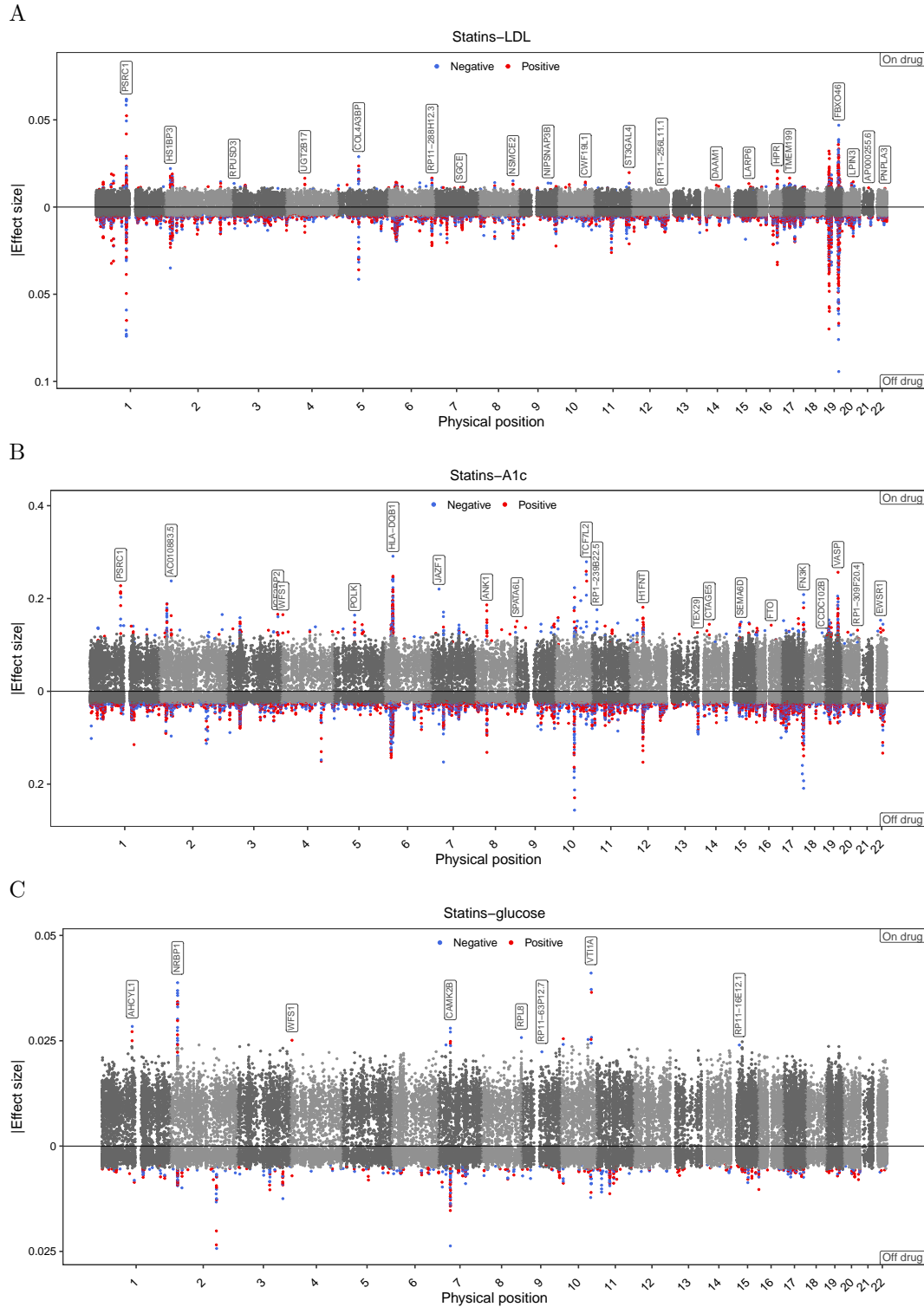


Figure S4. Manhattan plots of TWAS associations for LDL cholesterol, and A1c and blood glucose, obtained in statin users (top) and statin non-users (bottom); related to Figure 2. (A) LDL cholesterol. Each point represents a single gene, with physical position plotted on the x-axis and effect size plotted on the y-axis. The most extreme effect across tissues is shown for each gene. Significant associations are highlighted in blue (negative effects) and red (positive effects), and the strongest on-drug associations on each chromosome are labeled. (B) and (C) follow (A), but for A1c and blood glucose.

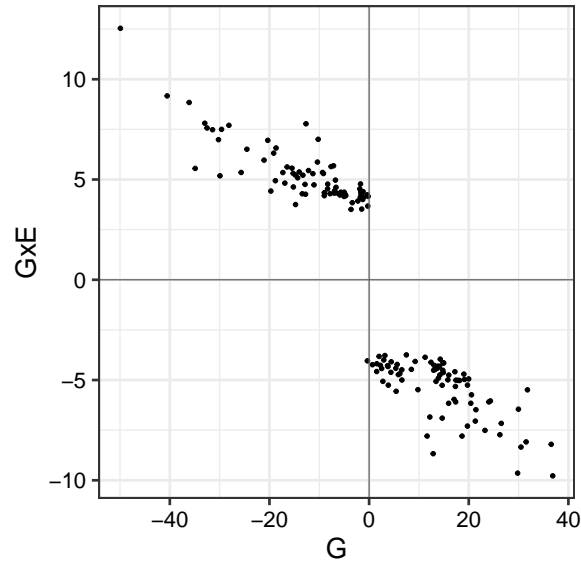


Figure S5. Z-scores for the main (G) and interaction (GxE) effects of genes whose interactions with statins were significantly associated with LDL cholesterol in TxEWAS; related to Figure 3. For each gene, we plot the estimates corresponding to the tissue with the strongest interaction p-value.

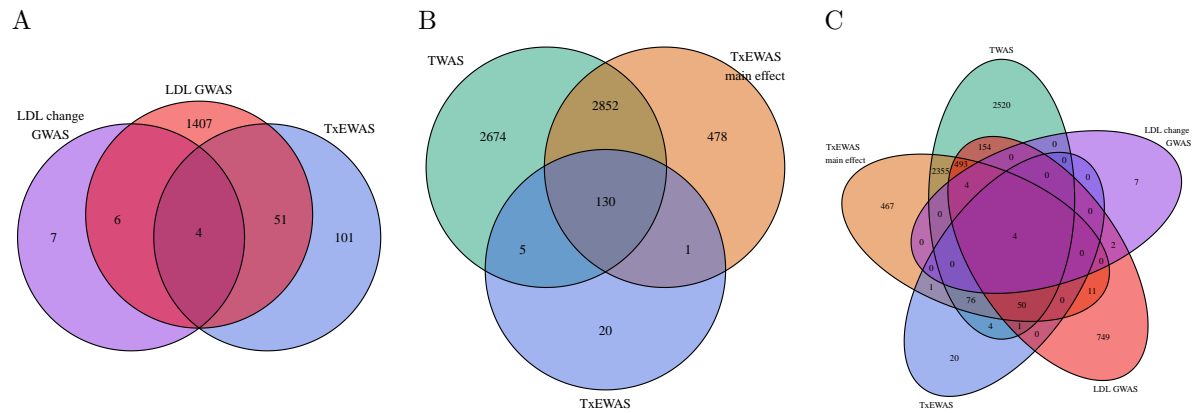


Figure S6. Overlap between genes identified or reported in the four following studies: (1) TxEWAS of statin interactions for LDL cholesterol (TxEWAS), (2) TWAS for LDL cholesterol (TWAS), (3) GWAS for LDL cholesterol change in response to statin therapy (LDL change GWAS), and (4) GWAS for LDL cholesterol (LDL GWAS); related to Figure 2. (A) Overlap between 1, 3 and 4. (B) Overlap between 1, 2, and main genetic effects from 1. (C) Overlap between 1, 2, 3, 4, and main genetic effects from 1.

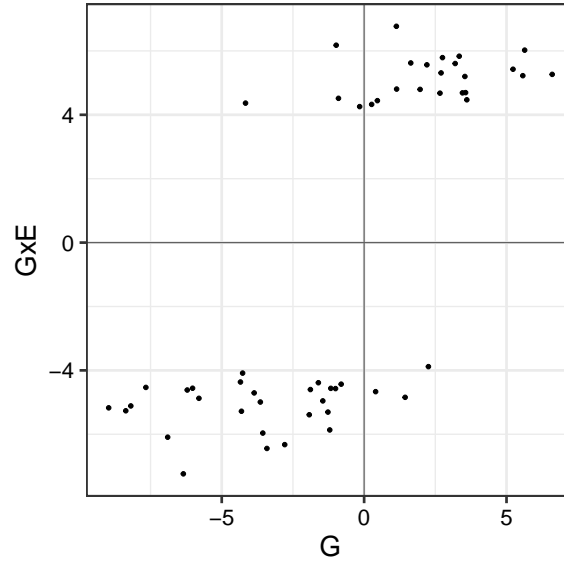


Figure S7. Z-scores for the main (G) and interaction (GxE) effects of genes whose interactions with statins were significantly associated with A1c in TxEWAS; related to Figure 3. For each gene, we plot the estimates corresponding to the tissue with the strongest interaction p-value.

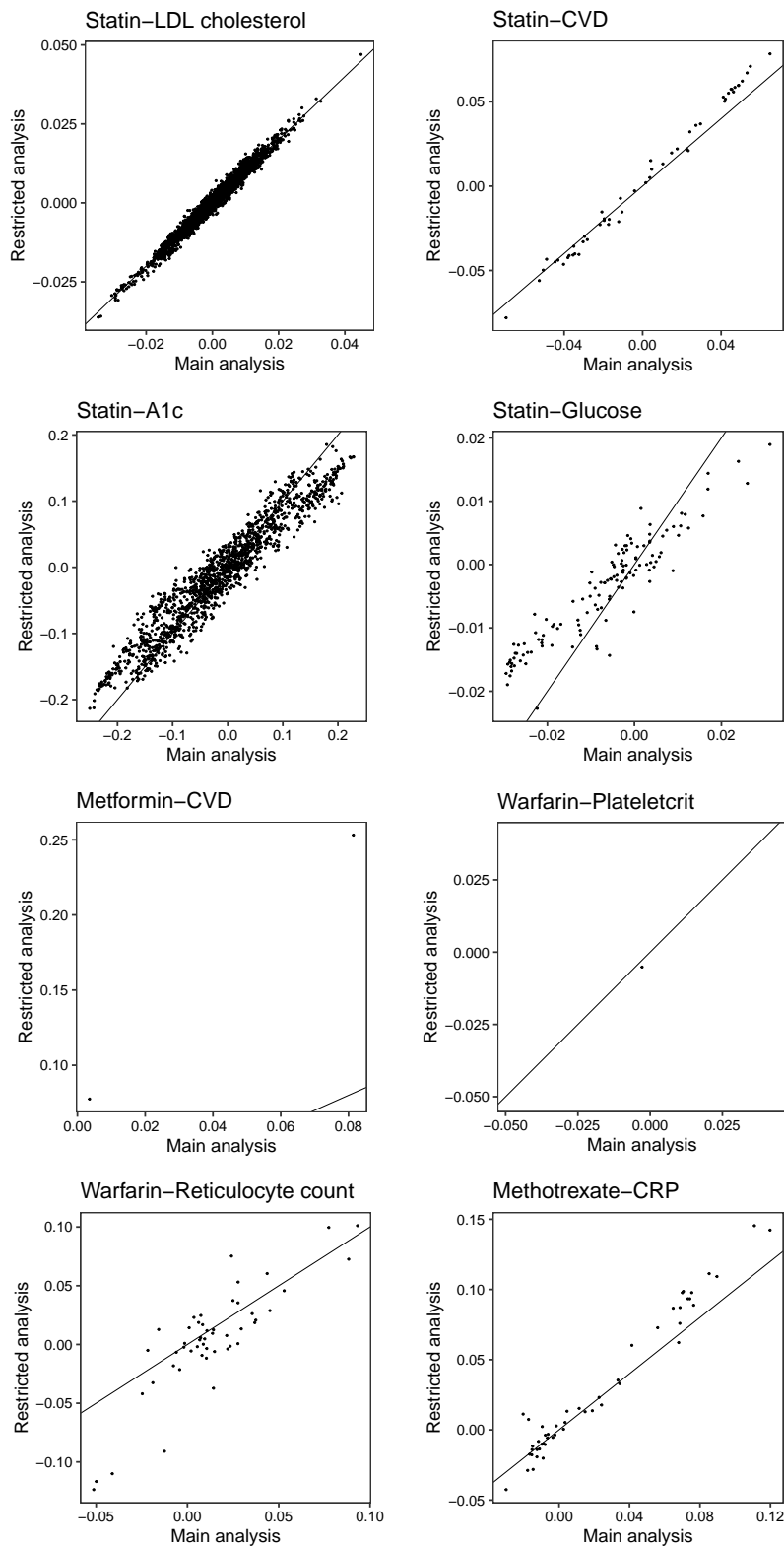


Figure S8. TxEWAS effect sizes of gene-drug interactions significantly associated with a phenotype either in the main analysis (using all users of a certain drug in the population studied) or the restricted analysis (using users of a certain drug who are non-users for the other drugs considered in the study); related to Figure 2. Effect sizes in all tested tissues are shown.

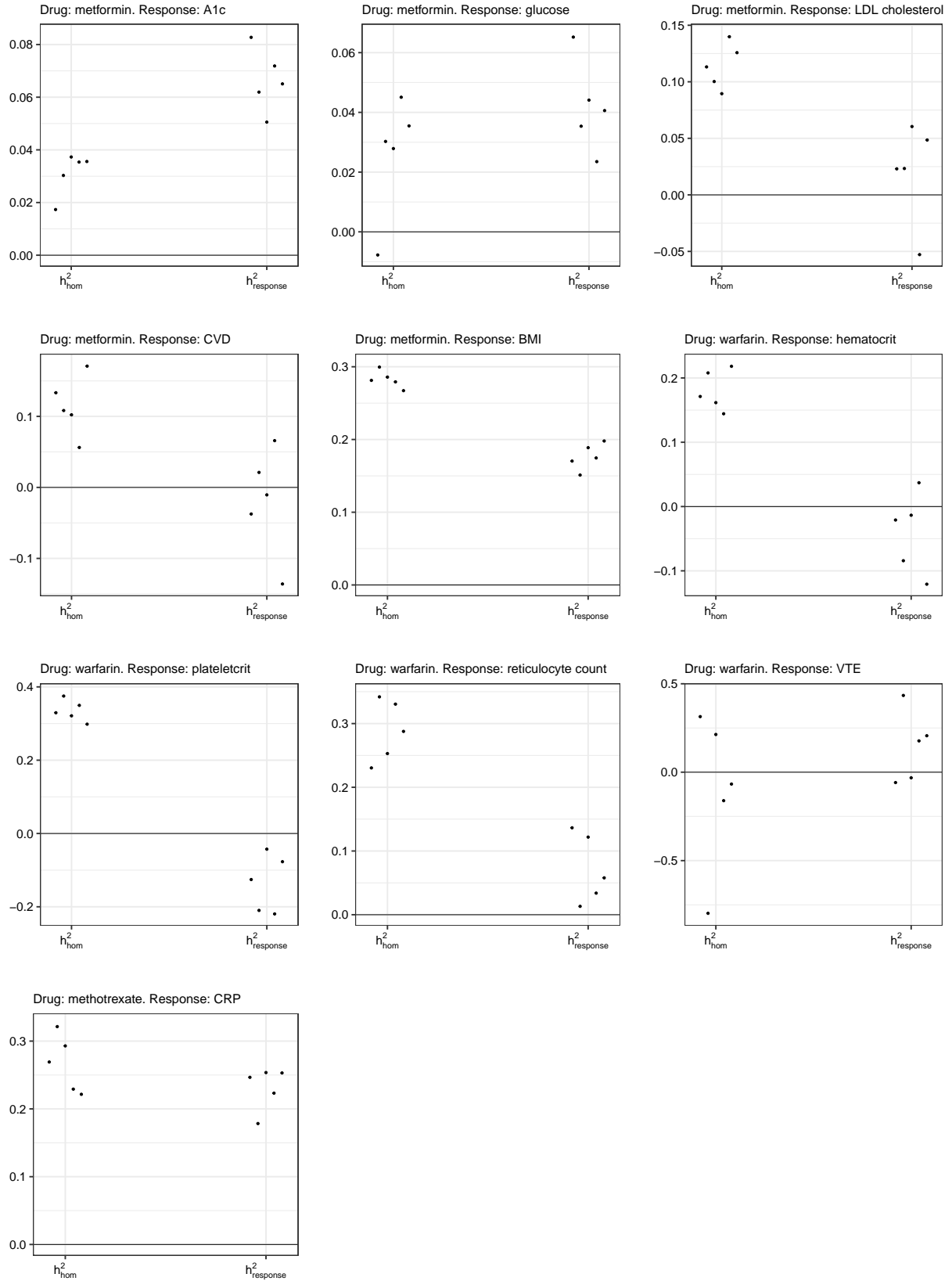


Figure S9. Estimation of drug-independent heritability (h^2_{hom}), and heritability of drug response (h^2_{response}) repeated five times with randomly resampled non-users; related to STAR Methods.

2 Supplemental Tables

Table S1. Drug-independent heritability (h^2_{hom}), and heritability of drug response (h^2_{response}) for a range of drug exposures and responses; related to Table 2.

| Drug exposure | Response | h^2_{hom} | h^2_{response} | h^2_{response} p value |
|---------------|--------------------|--------------------|-------------------------|-----------------------------------|
| Statins | LDL cholesterol | 0.208 | 0.0892 | 1.13×10^{-30} |
| | CVD | 0.086 | 0.0118 | 0.797 |
| | A1c | 0.285 | 0.1018 | 1.79×10^{-6} |
| | Glucose | 0.112 | 0.1114 | 2.26×10^{-4} |
| | T2D | 0.319 | -0.0119 | 0.936 |
| Metformin | A1c | 0.035 | 0.0719 | 0.516 |
| | Glucose | 0.030 | 0.0354 | 0.907 |
| | LDL cholesterol | 0.113 | 0.0230 | 0.016 |
| | CVD | 0.108 | 0.0211 | 0.924 |
| | BMI | 0.281 | 0.1703 | 2.51×10^{-4} |
| Warfarin | Hematocrit | 0.171 | -0.0209 | 0.991 |
| | Plateletcrit | 0.329 | -0.1257 | 0.291 |
| | Reticulocyte count | 0.288 | 0.0579 | 0.627 |
| | VTE | -0.067 | 0.2062 | 0.840 |
| Methotrexate | CRP | 0.269 | 0.2466 | 0.337 |

Table S2. Off-drug (h_{off}^2) and on-drug (h_{on}^2) heritability estimates for a range of drug exposures and responses; related to Table 2. Note that h_{off}^2 and h_{on}^2 are generally higher than h_{hom}^2 , because they additionally capture genetic effects specific to on-drug and off-drug individuals, respectively.

| Drug exposure | Response | h_{off}^2 (SE) | h_{on}^2 (SE) |
|---------------|--------------------|-------------------------|------------------------|
| Statins | LDL cholesterol | 0.4125 (0.0080) | 0.270 (0.034) |
| | CVD | 0.1029 (0.0739) | 0.075 (0.081) |
| | A1c | 0.3170 (0.0075) | 0.286 (0.034) |
| | Glucose | 0.0924 (0.0068) | 0.213 (0.033) |
| | T2D | 0.2820 (0.4547) | 0.119 (0.035) |
| Metformin | A1c | 0.3040 (0.0568) | 0.081 (0.147) |
| | Glucose | 0.0758 (0.0302) | 0.057 (0.106) |
| | LDL cholesterol | 0.2294 (0.0317) | 0.114 (0.075) |
| | CVD | 0.1975 (0.3253) | 0.067 (0.137) |
| | BMI | 0.3073 (0.0300) | 0.513 (0.073) |
| Warfarin | Hematocrit | 0.2647 (0.0296) | 0.153 (0.187) |
| | Plateletcrit | 0.3936 (0.0231) | 0.180 (0.112) |
| | Reticulocyte count | 0.3385 (0.0209) | 0.337 (0.138) |
| | VTE | 0.1386 (0.5809) | 0.185 (0.604) |
| Methotrexate | CRP | 0.3457 (0.0202) | 0.549 (0.248) |

Table S3. Heritability of LDL cholesterol, A1c and glucose in individuals on statin-metformin drug combinations, estimated by the (additive) GCTA model; related to Table 2. A small number of metformin users who do not take statins (1,583) has not allowed for reliable estimation in this group.

| | | LDL cholesterol | | A1c | | Glucose | |
|-----------|-----------|-----------------|---------------|---------------|---------------|---------------|---------------|
| | | Statins | | Statins | | Statins | |
| | | Non-users | Users | Non-users | Users | Non-users | Users |
| Metformin | Non-users | 0.534 (0.074) | 0.335 (0.077) | 0.401 (0.085) | 0.391 (0.082) | 0.121 (0.087) | 0.025 (0.092) |
| | Users | 0.000 (0.281) | 0.244 (0.074) | 0.000 (0.347) | 0.101 (0.077) | 0.860 (0.397) | 0.088 (0.090) |

Table S4. Variance decomposition with GxEMM for a range of drug exposures and responses; related to Table 2.

| Drug exposure | Response | σ_{hom}^2 (SE) | $v_{0(\text{off})}$ (SE) | $v_{1(\text{on})}$ (SE) | $w_{0(\text{off})}$ (SE) | $w_{1(\text{on})}$ (SE) |
|---------------|--------------------|------------------------------|--------------------------|-------------------------|--------------------------|-------------------------|
| Statins | LDL cholesterol | 0.2077 (0.0090) | 0.1414 (0.0110) | -0.0483 (0.0216) | 0.4973 (0.0068) | 0.4528 (0.0214) |
| | CVD | 0.0860 (0.0400) | -0.0049 (0.0501) | 0.0315 (0.1101) | 0.7637 (0.2682) | 1.4621 (0.4331) |
| | A1c | 0.2849 (0.0132) | -0.0840 (0.0138) | 0.2989 (0.0698) | 0.4328 (0.0049) | 1.4629 (0.0698) |
| | Glucose | 0.1116 (0.0131) | -0.0450 (0.0137) | 0.3361 (0.0695) | 0.6600 (0.0052) | 1.6615 (0.0694) |
| | T2D | 0.3187 (0.0481) | -0.0825 (0.0647) | 0.0473 (0.0983) | 0.2676 (0.7135) | 2.7246 (0.3624) |
| Metformin | A1c | 0.0354 (0.0328) | 0.0398 (0.0352) | 0.0394 (0.1348) | 0.1720 (0.0144) | 0.8505 (0.1368) |
| | Glucose | 0.0303 (0.0334) | -0.0054 (0.0344) | 0.0886 (0.2220) | 0.3037 (0.0113) | 1.9657 (0.2222) |
| | LDL cholesterol | 0.1132 (0.0233) | 0.0778 (0.0337) | -0.0513 (0.0436) | 0.6415 (0.0264) | 0.4803 (0.0412) |
| | CVD | 0.1083 (0.0929) | 0.0518 (0.1310) | -0.0072 (0.1826) | 0.6507 (1.1650) | 1.4190 (1.2552) |
| | BMI | 0.2813 (0.0292) | -0.0618 (0.0350) | 0.2706 (0.0828) | 0.4948 (0.0216) | 0.5222 (0.0782) |
| Warfarin | Hematocrit | 0.1712 (0.0427) | -0.0023 (0.0459) | -0.0241 (0.1808) | 0.4694 (0.0190) | 0.8145 (0.1807) |
| | Plateletcrit | 0.3295 (0.0369) | 0.0108 (0.0409) | -0.1570 (0.1009) | 0.5243 (0.0199) | 0.7850 (0.1082) |
| | Reticulocyte count | 0.2877 (0.0372) | 0.0400 (0.0417) | 0.0389 (0.1369) | 0.6404 (0.0203) | 0.6433 (0.1338) |
| | VTE | -0.0671 (0.3474) | 0.1863 (0.4042) | 0.4819 (1.3094) | 0.7404 (1.4170) | 1.8327 (4.2795) |
| Methotrexate | CRP | 0.2692 (0.0536) | 0.0550 (0.0563) | 0.2986 (0.2573) | 0.6135 (0.0189) | 0.4670 (0.2559) |

Table S6. Enrichment of gene-statin interactions for LDL cholesterol in pathway-based gene sets at 1% significance level; related to Figure 2.

| Pathway | Source | Genes | <i>p</i> value |
|---|--------------|--|----------------|
| Herpes simplex virus 1 infection | KEGG | ZNF627, ZNF235, ZNF234, ZNF233, ZNF222, TYK2, ZNF225, ZNF700, NECTIN2, ZNF433, ZNF284, ZNF45, ZNF223, ZNF224, NXF1, ZNF226, ZNF227, ZNF229, ZNF589, ZNF440, ZNF441, ZNF155, ZNF112 | 3.01e-12 |
| Cholesterol metabolism | KEGG | PCSK9, LPL, ANGPTL8, APOE, APOC1 | 5.76e-05 |
| Generic Transcription Pathway | Reactome | ZNF627, ZNF235, ZNF234, ZNF233, ZNF45, SMARCA4, APOE, ZNF700, ZNF433, ZNF222, ZNF223, ZNF224, ZNF225, ZNF226, ZNF227, ZNF589, GATAD2A, ZNF440, ZNF441, ZNF155, PPP1R13L, ZNF112 | 5.78e-05 |
| Plasma lipoprotein assembly, remodeling, and clearance | Reactome | PCSK9, LPL, APOE, ANGPTL8, APOC1 | 1.59e-04 |
| RNA Polymerase II Transcription | Reactome | ZNF627, ZNF235, ZNF234, ZNF233, ZNF45, SMARCA4, APOE, ZNF700, ZNF433, ZNF222, ZNF223, ZNF224, ZNF225, ZNF226, ZNF227, ZNF589, GATAD2A, ZNF440, ZNF441, ZNF155, PPP1R13L, ZNF112 | 2.81e-04 |
| Omega-3 fatty acid metabolism | EHMN | FADS1, FADS3, FADS2 | 2.88e-04 |
| Gene expression (Transcription) | Reactome | ZNF627, ZNF235, ZNF234, ZNF233, ZNF45, SMARCA4, APOE, ZNF700, DNMT1, ZNF433, ZNF222, ZNF223, ZNF224, ZNF225, ZNF226, ZNF227, ZNF589, PPP1R13L, ZNF440, ZNF441, ZNF155, GATAD2A, ZNF112 | 4.64e-04 |
| Nectin/Necl trans heterodimerization | Reactome | NECTIN2, PVR | 4.66e-04 |
| Alpha Linolenic Acid and Linoleic Acid Metabolism | SMPDB | FADS1, FADS2 | 7.72e-04 |
| Metabolic pathway of LDL, HDL and TG, including diseases | Wikipathways | LPL, PCSK9 | 1.15e-03 |
| Plasma lipoprotein remodeling | Reactome | LPL, APOE, ANGPTL8 | 1.21e-03 |
| Linoleate metabolism | EHMN | FADS1, CYP2C8, FADS3, FADS2 | 1.33e-03 |
| Omega-6 fatty acid metabolism | EHMN | FADS1, FADS3, FADS2 | 1.54e-03 |
| Statin inhibition of cholesterol production | Wikipathways | LPL, APOE, APOC1 | 1.54e-03 |
| Linoleic acid (LA) metabolism | Reactome | FADS1, FADS2 | 1.60e-03 |
| Plasma lipoprotein clearance | Reactome | PCSK9, APOE, APOC1 | 2.12e-03 |
| Chylomicron remodeling | Reactome | LPL, APOE | 2.12e-03 |
| oleate biosynthesis | HumanCyc | FADS1, FADS2 | 2.12e-03 |
| eicosapentaenoate biosynthesis | HumanCyc | FADS1, FADS2 | 3.37e-03 |
| Omega-9 FA synthesis | Wikipathways | FADS1, FADS2 | 4.10e-03 |
| alpha-linolenic acid (ALA) metabolism | Reactome | FADS1, FADS2 | 4.89e-03 |
| alpha-linolenic (omega3) and linoleic (omega6) acid metabolism | Reactome | FADS1, FADS2 | 4.89e-03 |
| Assembly of active LPL and LIPC lipase complexes | Reactome | LPL, ANGPTL8 | 4.89e-03 |
| Omega-3-Omega-6 FA synthesis | Wikipathways | FADS1, FADS2 | 5.75e-03 |
| LKB1 signaling events | PID | MARK4, SIK1, CDC37 | 6.12e-03 |
| Plasma lipoprotein assembly | Reactome | APOE, APOC1 | 8.69e-03 |
| Deregulated CDK5 triggers multiple neurodegenerative pathways in Alzheimer's disease models | Reactome | CAST, CDC25A | 9.79e-03 |
| Neurodegenerative Diseases | Reactome | CAST, CDC25A | 9.79e-03 |

Table S7. Enrichment of replicated and not reported in LDL cholesterol GWAS gene-statin interactions for LDL cholesterol in pathway-based gene sets at 1% significance level; related to Figure 2.

| Pathway | Source | Genes | <i>p</i> value |
|---|--------------|---|----------------|
| Herpes simplex virus 1 infection - Homo sapiens (human) | KEGG | ZNF224, ZNF226, ZNF227, ZNF229, ZNF235, NECTIN2, ZNF284, ZNF112 | 6.63e-08 |
| Nectin/Necl trans heterodimerization | Reactome | NECTIN2, PVR | 1.24e-05 |
| Plasma lipoprotein assembly | Reactome | APOE, APOC1 | 2.45e-04 |
| Statin inhibition of cholesterol production | Wikipathways | APOE, APOC1 | 6.58e-04 |
| Adherens junctions interactions | Reactome | NECTIN2, PVR | 6.58e-04 |
| Nectin adhesion pathway | PID | NECTIN2, PVR | 7.64e-04 |
| Plasma lipoprotein clearance | Reactome | APOE, APOC1 | 8.20e-04 |
| NR1H3 & NR1H2 regulate gene expression linked to cholesterol transport and efflux | Reactome | APOE, APOC1 | 1.19e-03 |
| NR1H2 and NR1H3-mediated signaling | Reactome | APOE, APOC1 | 1.89e-03 |
| Cholesterol metabolism - Homo sapiens (human) | KEGG | APOE, APOC1 | 2.15e-03 |
| Generic Transcription Pathway | Reactome | APOE, ZNF224, ZNF226, ZNF227, ZNF235, ZNF112 | 2.32e-03 |
| Apoptosis-related network due to altered Notch3 in ovarian cancer | Wikipathways | APOE, BCL3 | 2.53e-03 |
| Cell-cell junction organization | Reactome | NECTIN2, PVR | 2.62e-03 |
| Plasma lipoprotein assembly, remodeling, and clearance | Reactome | APOE, APOC1 | 3.25e-03 |
| RNA Polymerase II Transcription | Reactome | APOE, ZNF224, ZNF226, ZNF227, ZNF235, ZNF112 | 4.03e-03 |
| Cell junction organization | Reactome | NECTIN2, PVR | 5.38e-03 |
| Gene expression (Transcription) | Reactome | APOE, ZNF224, ZNF226, ZNF227, ZNF235, ZNF112 | 6.75e-03 |
| C-type lectin receptor signaling pathway - Homo sapiens (human) | KEGG | RELB, BCL3 | 9.22e-03 |

Table S8. Simulation of polygenic prediction in individuals on and off a treatment; related to Table 3.

| PGS | Prediction accuracy (R^2) | | | |
|--------------|-------------------------------|----------|------------|----------|
| | Scenario 1 | | Scenario 2 | |
| | On drug | Off drug | On drug | Off drug |
| on-drug-PGS | 0.0881 | 0.2654 | 0.3613 | 0.2091 |
| off-drug-PGS | 0.0940 | 0.2838 | 0.2548 | 0.2955 |
| all-PGS | 0.0942 | 0.2844 | 0.3379 | 0.2699 |

Table S9. Prediction accuracy of a PGS as a function of training population (rows) and test population (columns); related to Table 3. In order, training populations/rows are: drug users; non-users, subsampled to match the number of drug users; a similarly subsampled 50:50 mixture of users and non-users without adjustment for treatment as a covariate; the same 50:50 mixture with adjustment; and a 50:50 mixture of users and subsampled non-users pooled together, with adjustment. The last two rows report prediction accuracy of combinations of above PGSs tested jointly (Section 3.4).

| PGS | Prediction accuracy (Incremental R^2 [%](SE)) | | | | | | | |
|---|---|-----------------|----------------|----------------|-----------------|----------------|------------------|---------------------|
| | Statins | | | | Metformin | | | |
| | LDL cholesterol | | A1c | | LDL cholesterol | | BMI | |
| | On drug | Off drug | On drug | Off drug | On drug | Off drug | On drug | Off drug |
| on-drug-PGS | 7.18 (0.30) | 12.60 (0.36) | 3.36 (0.23) | 2.32 (0.18) | 1.32 (0.37) | 3.24 (0.54) | 0.919 (0.286) | 0.0040 (0.0592) |
| off-drug-PGS | 7.98 (0.32) | 14.87 (0.39) | 2.56 (0.20) | 5.79 (0.28) | 2.75 (0.51) | 5.43 (0.67) | 0.041 (0.080) | 0.0077 (0.0636) |
| agnostic-PGS | 5.86 (0.29) | 10.49 (0.36) | 2.60 (0.20) | 3.75 (0.23) | 2.25 (0.44) | 5.00 (0.66) | 0.255 (0.164) | 0.0931 (0.1078) |
| adjusted-PGS | 7.67 (0.32) | 13.59 (0.38) | 2.80 (0.21) | 3.75 (0.22) | 2.14 (0.45) | 5.24 (0.67) | 0.193 (0.141) | -0.0205 (0.0370) |
| adjusted-all-PGS | 8.62 (0.32) | 15.66 (0.42) | 4.70 (0.26) | 5.62 (0.25) | 3.01 (0.53) | 6.63 (0.74) | 0.212 (0.148) | 0.0477 (0.0843) |
| on-drug-PGS + off-drug-PGS | 8.15 (0.34) | 14.93 (0.40) | 4.12 (0.24) | 6.02 (0.28) | 2.74 (0.51) | 5.42 (0.66) | 0.959 (0.299) | 0.0120 (0.0851) |
| adjusted-all-PGS + on-drug-PGS + off-drug-PGS | 8.65 (0.33) | 15.82 (0.40) | 4.72 (0.26) | 6.82 (0.30) | 3.12 (0.55) | 6.70 (0.79) | 0.950 (0.305) | 0.0341 (0.1029) |

Table S10. Heritability of LDL cholesterol, A1c and glucose estimated with the additive GCTA model with adjustment for statin or metformin use, or without adjustment; related to Table 2.

| Drug added to covariates | h^2_{GCTA} (SE) | | |
|-----------------------------|--------------------------|-----------------|-----------------|
| | LDL cholesterol | A1c | Glucose |
| None | 0.2268 (0.0054) | 0.2837 (0.0055) | 0.0938 (0.0055) |
| Statins | 0.3780 (0.0055) | 0.2767 (0.0055) | 0.0892 (0.0055) |
| Metformin | 0.2115 (0.0179) | 0.1521 (0.0176) | 0.0476 (0.0167) |

3 Supplemental Note

3.1 Variance decomposition analysis with GxEMM

3.1.1 The GxEMM model

We quantify heritable effects on drug response using GxEMM, a linear mixed model for genome-wide gene-environment interactions [S1]. GxEMM quantifies the heritability contributed by genome-wide additive effects and genome-wide GxE effects. The general GxEMM model is:

$$\begin{aligned} y &= X\alpha + G\beta + (G * Z)\gamma + (I * Z)\epsilon \\ \beta_l &\stackrel{\text{iid}}{\sim} \mathcal{N}\left(0, \frac{1}{L}\sigma_{\text{hom}}^2\right) \\ \gamma_{(l,i)} &\stackrel{\text{iid}}{\sim} \mathcal{N}\left(0, \frac{1}{L}V\right) \\ \epsilon_i &\stackrel{\text{iid}}{\sim} \mathcal{N}(0, W) \end{aligned}$$

In this model, the known data are:

- y is the quantitative phenotype
- X are covariates with fixed effects α , like age or sex.
- G is a centered and scaled matrix of genome-wide genotypes
- Z is matrix of context features. In our study, $Z_i = (0, 1)$ if individual i is treated, and $Z_i = (1, 0)$ if individual i is untreated
- $*$ is the column-wise Khatri-Rao product, which forms the interaction between two design matrices. For example, each column of $G * Z$ is of the form $G_{\cdot j} \circ Z_{\cdot k}$, where \circ takes the element-wise product between SNP j and context feature k

The random effects and their corresponding variance components are:

- β_l is the effect of SNP l that is shared across contexts. σ_{hom}^2 is the additive genetic variance—i.e., the size of $\sum_l \beta_l$
- $\gamma_{(lk)}$ is the effect of SNP l that is specific to context k . $v_k := V_{kk}$ is the genetic variance specific to context k —i.e., the size of $\sum_l \gamma_{(lk)}$
- $\epsilon_{(ik)}$ is the noise for individual i from context k . $w_k := W_{kk}$ is the noise variance in context k .

Finally, the cross-context covariance terms are:

- $v_{12} := V_{12}$ is the genetic covariance between contexts. Because Z is binary in our setting, this term be ignored WLOG—it can be folded in with σ_{hom}^2 [S1]
- $w_{12} := W_{12}$ is the noise covariance between contexts 1 and 2. In our setting with binary Z , this term cannot be identified—an individual either has noise ϵ_1 or ϵ_2 , but we cannot observe the covariance between these terms in cross-sectional data.
- **Comparing to main text parameters.** In the main text, we use simpler notation to simplify the presentation. Specifically, v_0 and v_1 in the main text correspond to V_{11} and V_{22} here, respectively (and likewise for w_0 , and w_1). W_{12} appears as w_{01} . V_{12} does not appear in the main text.

While neither v_{12} nor w_{12} can be identified in our cross-sectional data, they are different in an important way. Specifically, v_{12} can be assumed 0 WLOG, because it is already captured in σ_{hom}^2 . However, w_{12} cannot be assumed to be zero—we simply have no data to learn about this parameter.

To unpack the model, imagine studying genetic effects on LDL cholesterol across statin users and non-users. A SNP s that equally increases LDL cholesterol in both groups has a homogeneous effect ($\beta_s > 0$) but has no drug-specific effects ($\gamma_{s1} = \gamma_{s2} = 0$), so s contributes to σ_{hom}^2 but not v_1 or v_2 . Conversely, a SNP s' that increases LDL cholesterol only in statin users has $\beta_{s'} = 0$ and $\gamma_{s'2} > \gamma_{s'1} = 0$, so s' contributes to v_2 but not σ_{hom}^2 or v_1 . Finally, $w_2 > w_1$ means that statin users have higher non-genetic LDL cholesterol variance.

In various special cases, GxEMM is similar or identical to other methods that fit genome-wide GxE heritability [S2, S3, S4]. For example, the method from [S3] applies to categorical environments and continuous phenotypes and, thus, would apply to our analyses of quantitative phenotypes (such as LDL cholesterol or A1c) but would not apply to our analyses of binary disorders (such as T2D or CVD). Finally, GxEMM reduces to the ordinary additive heritability model when $V = 0$ and $w_1 = w_2$, i.e., when neither genetic nor nongenetic variance depends on the environment.

3.1.2 Using GxEMM to estimate treatment response heritability

If we had measures of a phenotype before and after a treatment, we could directly calculate the change in phenotype, Δy , and then estimate its heritability using standard heritability estimation methods. This would be ideal, as the change in phenotype captured in Δy cancels out the contribution of all covariates and unmodelled noise that do not depend on treatment status.

In contrast, we are interested in the setting where we only measure each individual's phenotype before or after treatment. This is motivated by large cross-sectional biobank data like UK Biobank, where most individuals are only observed at one time point. Here, we show how to approximate the heritability of Δy in this setting using GxEMM.

How is this possible if we never observe Δy ? Imagine we only see individual i pre-treatment ($E_i = 0$), but that we see their relative j post-treatment ($E_j = 1$). Intuitively, we can use individual j 's post-treatment phenotype to proxy for individual i 's post-treatment phenotype. More specifically, individual j will be a proxy for the genetic part of individual i . Intuitively, individual j cannot inform the nongenetic part of individual i 's phenotype; mathematically, this is equivalent to our above observation that w_{12} is not identified.

To declutter notation, consider a single individual's phenotype y and genotype vector g . Let γ_1 indicate the effects of all S SNPs in untreated individuals ($E = 0$), and let γ_2 indicate the effects of the SNPs in treated individuals ($E = 1$). Informally define Δy as $y(E = 1) - y(E = 0)$, i.e., the phenotype change after an individual is treated. Δy is unobserved because we only observe either the treated or untreated state. Our goal is to estimate the heritability of this unobserved phenotype using GxEMM. Under the GxEMM model defined above, we have:

$$\begin{aligned} \Delta y &= y(E = 1) - y(E = 0) \\ &= (g\beta + g\gamma_2 + \epsilon_2) - (g\beta + g\gamma_1 + \epsilon_1) \\ &= g(\gamma_2 - \gamma_1) + (\epsilon_2 - \epsilon_1) \implies \\ \mathbb{V}(\Delta y) &= \text{tr}(g^T g \mathbb{V}(\gamma_2 - \gamma_1)) + \mathbb{V}(\epsilon_2 - \epsilon_1) \\ &= \text{tr}\left(g^T g \frac{1}{L} I_L (v_{11} + v_{22})\right) + (w_{11} + w_{22} - 2w_{12}) \\ &= (v_{11} + v_{22}) + (w_{11} + w_{22} - 2w_{12}) \end{aligned}$$

GxEMM can estimate all of these parameters—except for w_{12} . Intuitively, it captures the covariance in effect sizes for unmodelled risk factors between treated/untreated states. We can safely assume that w_{12} is nonnegative: Otherwise, the majority of unmodelled nongenetic risk factors would have opposite effects in the treated/untreated contexts. For example, if smoking status was the only unmodelled risk factor for LDL cholesterol, then $w_{12} < 0$ implies smoking becomes protective after statin administration. We emphasize that this is a biological assumption, not a mathematical assumption.

Therefore, we can estimate the heritability of treatment response by:

$$\begin{aligned}
h_{\text{response}}^2 &:= h^2(\Delta y) \\
&= \frac{v_{11} + v_{22}}{v_{11} + v_{22} + w_{11} + w_{22} - 2w_{12}} \\
&\geq \frac{v_{11} + v_{22}}{v_{11} + v_{22} + w_{11} + w_{22}}
\end{aligned}$$

We call this “conservative” in the main text to emphasize that the heritability is underestimated when $w_{12} > 0$, and in this sense the inequality is mathematically conservative. But, again, we will overestimate h^2 in the unlikely case where $w_{12} < 0$.

Finally, we note that these calculations ignore endogeneity in treatment status. This is hiding in our informal definition of Δy , which imagines that we observe an individual in $E = 0$ or $E = 1$ state at random. However, when the treatment is prescribed based on y itself, we are ignoring a subtle form of dependence between E and G (that is more pernicious than mere G-E correlation, which does not generally cause bias in GxEMM [S1]). This is worth theoretically solving in future work; here, we use simulations to evaluate the implications of this potential bias.

3.1.3 Negative estimates of drug-specific genetic variance

Some of the drug-specific genetic variance estimates obtained in this study are negative (Table S4). We demonstrate in simulation that such observations are expected when a drug has a buffering effect.

We simulated 10,000 non-users and 5,000 users of a hypothetical drug. A phenotype of each non-user was simulated as a sum of minor-allele effects at 1000 SNPs plus a noise term drawn from a normal distribution with a zero mean and a variance selected to obtain a particular heritability of the phenotype (h^2). Each of the SNPs was drawn independently from a binomial distribution $B(2, p)$ with p representing the minor allele frequency, which was drawn uniformly from a range between 0.2 and 0.5. SNP effects were drawn from a normal distribution with zero mean and variance $\sigma_g^2 = h^2/1000$. Users’ phenotypes were generated similarly, with the only difference being that the effects of a randomly selected half of the SNPs were shrunk twofold, representing a buffering effect of the drug.

We find that GxEMM produces a negative value of drug-specific genetic variance (v_1) on average across simulation replicates (Figure S3). We note that all heritability parameters (h_{off}^2 , h_{on}^2 , and h_{response}^2) remain non-negative, as expected. This is consistent with our empirical results, where v_1 may be negative but no heritability estimate is significantly less than zero (Tables S1 and S2).

We note that although buffering is a plausible cause for negative v_1 in the context of our work, the above simulation does not prove that this buffering model is the only possible model to explain negative v_1 . More generally, parameters v_0 and v_1 should be interpreted as offsets from h_{hom}^2 , and thus can be negative when one group has higher genetic variance than another. That is, the total genetic variance in the on-drug group is $h_{\text{hom}}^2 + v_1$, and this should be nonnegative; but if h_{hom}^2 is larger than the genetic variance in this group, then v_1 should be negative. v_0 and v_1 are internal model parameters that are a means to the directly interpretable heritability parameters that we focus on in the main text, h_{off}^2 , h_{on}^2 , and h_{response}^2 .

3.1.4 Dependence of heritability estimates on covariates

In our analysis, estimates of h_{hom}^2 for a given outcome vary depending on which drug we test for interaction (Table S1). This is because we always adjust for the main effect of a specific drug being tested. This is important to avoid false positive gene-drug interactions, but can substantially affect the phenotype variance components because drug effects can be large.

This dependence on the choice of covariates is common to all heritability estimation methods and has been discussed before [S5]. To illustrate this point, we show that the ordinary additive estimates of heritability calculated with GCTA [S6] exhibit similar behavior to the corresponding h_{hom}^2 estimates (Table S10).

3.2 TxEWAS for detection of gene-drug interactions

3.2.1 The TxEWAS model

To identify specific genes involved in drug response from cross-sectional data, we use a newly developed statistical framework, TxEWAS, which extends the transcriptome-wide association study (TWAS [S7, S8]) framework. TxEWAS addresses shortcomings of SNP-level GxE studies—it improves power by reducing the number of tests, and interpretability by directly suggesting possible causal genes.

The TxEWAS framework involves two major steps: First, gene expression levels of each gene are genetically imputed using a reference dataset. Second, the interaction effect between imputed gene expression and the drug is tested.

In case where a response y_i is continuous, the interaction effect (β_6) is tested in the linear regression model:

$$y_i \sim \mathcal{N} \left(\beta_0 + \sum_j \beta_{1j} c_{i,j} + \sum_j \beta_{2j} e_i c_{i,j} + \sum_j \beta_{3j} g_i c_{i,j} + \beta_4 g_i + \beta_5 e_i + \beta_6 g_i e_i, \sigma^2 \right),$$

where e_i and g_i are drug use indicator and imputed expression of some gene for individual i , respectively; and $c_{i,j}$ is an element of a matrix of covariates \mathbf{C} . β_4 and β_5 are what we call “main” or “additive” effects of a gene and drug, respectively.

In case where the response is binary, the interaction effect is tested in the logistic regression model:

$$y_i \sim \text{Bernoulli} \left(\text{logit}^{-1} \left(\beta_0 + \sum_j \beta_{1j} c_{i,j} + \sum_j \beta_{2j} e_i c_{i,j} + \sum_j \beta_{3j} g_i c_{i,j} + \beta_4 g_i + \beta_5 e_i + \beta_6 g_i e_i \right) \right).$$

The variance of the effect size estimates is estimated with the robust sandwich variance estimator (SVE). In the linear regression-based test, their role is to control for both heteroscedasticity [S9] and misspecification of the functional form of the environmental factor [S10, S11]. In the logistic regression-based test, they are meant to account for the latter. The bias caused by the violation of the homoscedasticity assumption of the linear regression model is a specifically important concern in GxE studies. We therefore perform extensive simulations and permutation analyses to test that the TxEWAS model is calibrated (Section 3.2.2).

We note that even though in this work we use TxEWAS to detect gene-drug interactions, any environment can be tested with this method.

3.2.2 TxEWAS performance in simulations

In the presence of environment-conditional heteroscedasticity, testing multiple genetic variants for interaction with the environmental factor in a simple linear regression model results in an inflated or deflated false positive rate (FPR), depending on the relation between group size and phenotypic variation [S9]. We performed a simulation to assess the size of this bias in a dataset of the size and characteristics of the UK Biobank (Figure S1A).

In the simulation, we considered a binary environmental variable that divided observations into two groups of sizes n_1 and n_2 , and phenotype variances σ_1^2 and σ_2^2 . We simulated the phenotype as a Gaussian random variable with the corresponding group variances, but with no mean effects. The genotype was drawn from a binomial distribution with the same probability 0.4 of success for both groups. We fitted to this data GxE models that included the genotype, the environmental factor and the product of those two as covariates. For every selected value of the ratio σ_2^2/σ_1^2 , we ran 5,000 such simulations, and calculated the FPR for the GxE effect as the proportion of simulations where the nominal p value for this effect was less than 0.05.

Group sizes used in the simulation were chosen based on the number of statin users in the UK Biobank ($n_1 = 285,822$ and $n_2 = 56,132$). Examples of σ_2^2/σ_1^2 values that we encountered in the UK Biobank when stratifying individuals by statin use were: 0.68 for LDL cholesterol, 1.96 for blood glucose, or 3.00 for A1c.

The simulation shows that if the smaller (larger) group is characterized by the larger (smaller) variance of the response, the FPR for the GxE model fitted with ordinary least-squares (OLS) is inflated (deflated).

Within a realistic range of parameter values, the FPR can reach zero or increase threefold (Figure S1A). On the other hand, if the groups have equal sizes, the model is well calibrated. The bias can be controlled by using robust standard errors estimated with the sandwich variance estimator (SVE). This approach gives virtually the same results as the double generalized linear model (DGLM), which correctly models the phenotypic variance as a function of covariates, and is more computationally efficient than the latter.

Taking advantage of those facts, TxEWAS performs a linear regression-based interaction test and controls for heteroskedasticity with the SVE.

To investigate the type I error rate for TxEWAS in real data, we performed a permutation analysis where we randomly shuffled imputed expression of 4,516 liver genes across subjects. We selected statin for drug exposure, and LDL cholesterol and blood glucose for responses to examine both deflation and inflation biases ($\sigma_2^2/\sigma_1^2 = 0.68$ and $\sigma_2^2/\sigma_1^2 = 1.96$, respectively). The analysis demonstrates that the TxEWAS model is well calibrated, even though p values estimated with the simple linear regression-based interaction test are heavily deflated or inflated, depending on the examined response (Figure S1B).

3.2.3 Combinations of treatments

Patients are often on multiple drugs simultaneously, for example, in the cohort studied in this work, out of the 8,606 individuals who reported taking metformin 6,946 also reported taking statins. This makes accompanying treatments an important potential confounder in our analyses. To test their impact on GxE, we repeated TxEWAS for each drug excluding individuals on the other drugs we consider—e.g., in the TxEWAS for statins, we excluded individuals taking metformin, warfarin, or methotrexate. We observe that the original results remain largely unaffected (Figure S8). For example, the statins-LDL analysis identified 127 interaction genes, from which 113 were among the 156 interaction genes found in the original analysis. Of those 127 genes, 126 could be studied in the replication cohort, and 35 replicated at hFDR < 10%. The remaining genes were enriched for low p values < 0.1 (binomial test=0.002).

3.3 Endogeneity bias

Like all existing gene-environment interaction models, GxEMM and TxEWAS are susceptible to endogeneity bias. In practice, drugs are not administered at random. When the causes of drug prescription are intertwined with causal effects on the focal phenotype, this is called endogeneity. In general, endogeneity can induce complex biases in statistical analyses.

A particularly relevant example to our analysis is when a drug is prescribed to lower a phenotype that exceeds a certain level. Statin therapy for high LDL cholesterol is such an example. Below we simulate two different effects that such a therapy could have in the population (first column in Figure S2): 1) the drug lowers the phenotype of every treated individual by the same amount, and 2) the drug is administered at a dose that achieves the same target level of the phenotype in all treated individuals. Modeling both simulated phenotypes with the GxE model yields GxE effects which are starkly negatively correlated with additive effects (second column in Figure S2), even though the phenotypes were generated in the absence of gene-drug interactions. We further show that the heritability of the change of the second phenotype (we selected one for simplicity) is substantial.

These simulation results are consistent with our empirical results for statins and LDL cholesterol. Indeed, endogeneity bias is surely present in our analyses and affects their results. However, to verify that our approach has ability to discover true gene-drug interactions, we replicated our gene-statin interaction effects on LDL cholesterol in a pharmacogenomic study. This study evades endogeneity bias by examining statin-induced LDL cholesterol change and adjusting for statin dose and other carefully selected covariates.

Furthermore, in other analyses presented in this work, e.g. the analysis of gene-statin interactions on A1c, we identify interaction effects whose signs are the same as the corresponding main genetic effects, and others whose signs are opposite to the corresponding main effects. This result is not consistent with the discussed generative model, where endogeneity bias manifests as interaction effects whose signs are determined by the corresponding main genetic effects—they are either all the same as the main effects or opposite to them. This is a further indication that our approach to analyzing cross-sectional data can generate valid hypotheses on genetic modifiers and statistical predictions for treatment response.

However, we do note that endogeneity induced through other generative processes may not have the same interaction effect property and it is a shortcoming of our approach.

3.3.1 Simulating endogenous “E”

Here, we perform simulations to understand a particular form of endogeneity that is surely present in our analyses: statins are prescribed for individuals with higher levels of LDL cholesterol. We are concerned with the impact of this endogeneity on our gene-drug interaction tests in the absence of any genetic interactions at baseline. Therefore, we simulate LDL cholesterol as a purely additive genetic trait, as is standard in complex trait genetics:

$$\begin{aligned} y &\sim G\beta + \epsilon \\ \beta_l &\stackrel{\text{iid}}{\sim} \mathcal{N}(0, \sigma_g^2/L) \\ \epsilon_l &\stackrel{\text{iid}}{\sim} \mathcal{N}(0, \sigma_e^2) \end{aligned}$$

We use $N = 10,000$ samples, $L = 100$ SNPs, $\sigma_g^2 = .5$, and $\sigma_e^2 = .5$. Note that this is a special case of our GxEMM model, where $V = 0$ and $W = \sigma_e^2 I_2$. That is, the genetic heterogeneity is absent, and the noise is i.i.d. across contexts.

The novel part of our simulation is adding a drug effect in a way that depends on y . Specifically, we assume the drug (E) is administered to individuals above the 80th percentile of the LDL cholesterol distribution. We consider two different models for the drug effect:

- Homogeneous across individuals. In this case, the phenotype y is modified by:

$$y \leftarrow y + E\beta_E$$

where E is a 0-1 indicator of drug use status, and we take $\beta_E = -1$. (This is one standard deviation on the pre-treatment phenotype scale.)

- Projecting individuals to the treatment threshold: all individuals with y_i above the threshold are returned directly to the threshold.

These operations are visualized in the post-treatment phenotype histograms in the left column of Figure S2. These two scenarios are intended to represent two different realistic treatment effects: in the homogeneous case, everyone gets the same effect; in the threshold case, everyone is given a drug dosage/regimen to achieve a target phenotype.

After simulating the data, we then perform a series of regressions to understand the impact on effect size estimates. First, we compare the additive genetic effects (from regressing post-treatment phenotypes on G) to the interaction genetic effects (the interaction term from regression post-treatment phenotypes on $G \times E$). As expected based on our real data analyses in Figure S5, we find that GxE effects are starkly negatively correlated with additive effects (second column of Figure S2). This reflects systematic buffering of genetic effects after treatment, which can also be seen when we compare the effect size estimates from only treated vs only untreated individuals (third column of Figure S2). Finally, we observe that the additive effect estimates are modestly reduced when fitted to post-treatment phenotypes rather than pre-treatment phenotypes (fourth column).

We next fit GxEMM with HE regression to estimate $h^2(\Delta y)$ from the same simulations (with the Homogeneous ‘E’ effect for simplicity). We found that $h^2(\Delta y)$ was 13.8% under this model (on average over 100 simulations, standard error=0.6%). These simulations assume that baseline LDL cholesterol has heritability of 50%. When we instead assume baseline heritability of 20%, we found that $h^2(\Delta y)$ was 4.3% (on average over 100 simulations, standard error=0.4%). Qualitatively, these results are consistent with the observed $h^2(\Delta y)$ for the LDL cholesterol response to statins that we observe in practice (9%) because the LDL cholesterol heritability likely lies in the range of 20-50%.

3.4 The impact of gene-drug interactions on polygenic prediction accuracy

We assessed transferability of PGSs between samples that are of similar genetic ancestry but differ by the drug use status.

We used four samples for training: 1. drug users (50% of all users of a given drug), 2. non-users (randomly subsampled to match the size of sample 1), 3. a 50:50 mixture of users and non-users (randomly subsampled to together match the size of sample 1), 4. merged samples 1 and 2.

We call the PGSs trained in samples 1 and 2, “on-drug-PGS” and “off-drug-PGS”, respectively. We trained two PGSs using sample 3: “agnostic-PGS”, where we used the standard covariates (age, sex, birth date, Townsend deprivation index, and the first 16 genetic PCs), and “adjusted-PGS”, where we added the drug use status to the standard covariates. The drug use status was also added when training PGSs in sample 4, which we call “adjusted-all-PGS”.

We fitted PGSs using a fast implementation of penalized linear regression with the lasso penalty [S12, S13], and we measured prediction accuracy by the incremental R^2 over baseline covariates (Table S9). Standard errors around the estimates were calculated using bootstrap.

We also calculated the incremental R^2 for models including more than one PGS as regressors: 1) on-drug-PGS and off-drug-PGS, and 2) on-drug-PGS, off-drug-PGS and adjusted-all-PGS (Table S9).

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