TIGAR-V2: Efficient TWAS Tool with Nonparametric Bayesian eQTL Weights of 49 Tissue Types from GTEx V8

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Abstract

Standard Transcriptome-Wide Association Study (TWAS) methods first train gene expression prediction models using reference transcriptomic data, and then test the association between the predicted genetically regulated gene expression and phenotype of interest. Most existing TWAS tools require cumbersome preparation of genotype input files and extra coding to enable parallel computation. To improve the efficiency of TWAS tools, we develop TIGAR-V2, which directly reads VCF files, enables parallel computation, and reduces up to 90% computation cost compared to the original version. TIGAR-V2 can train gene expression imputation models using either nonparametric Bayesian Dirichlet Process Regression (DPR) or Elastic-Net (as used by PrediXcan), perform TWAS using either individual-level or summary-level GWAS data, and implements both burden and

variance-component test statistics for inference. We trained gene expression prediction models by DPR for 49 tissues using GTEx V8 by TIGAR-V2 and illustrated the usefulness of these nonparametric Bayesian DPR eQTL weights through TWAS of breast and ovarian cancer utilizing public GWAS summary statistics. We identified 88 and 37 risk genes respectively for breast and ovarian cancer, most of which are either known or near previously identified GWAS (∼95%) or TWAS (∼40%) risk genes of the corresponding phenotype and three novel independent TWAS risk genes with known functions in carcinogenesis. These findings suggest that TWAS can provide biological insight into the transcriptional regulation of complex diseases. TIGAR-V2 tool, trained Bayesian cis-eQTL weights, and LD information from GTEX V8 are publicly available, providing a useful resource for mapping risk genes of complex diseases.

Keywords:

Transcriptome-wide association study; TWAS; GWAS; cis-eQTL; Nonparametric Bayesian DPR; Elastic-Net; TIGAR

1 Introduction

A transcriptome-wide association study (TWAS) [1–5] is a popular technique widely used for integrating reference transcriptomic data with GWAS data to conduct gene-based association studies. TWAS has been shown to improve the power of identifying GWAS risk loci as well as illustrate the underlying biological mechanism of GWAS loci, for example in studies of schizophrenia [6], age-related macular degeneration [7], and broad types of complex traits [8]. Especially, the risk genes identified by TWAS have genetic effects potentially mediated through gene expression.

The standard two-stage TWAS methods [1–3] first fit gene expression prediction models using the reference transcriptomic and genetic data profiled for the same samples, and then

test the association between the predicted genetically regulated gene expression (GReX) and phenotype of interest for the test GWAS cohort. The TWAS framework enables the advantages of using publicly available reference transcriptomic data such as the Genotype-Tissue Expression (GTEx) project [9, 10] and summary-level GWAS data [11, 12].

However, most of the existing tools [1, 2, 5] require cumbersome preparation of genotype data files and fail to take advantage of parallel computing to improve computational efficiency. These result in difficulties for users who need to train gene expression prediction models by using their own reference transcriptomic and genetic data. Here, we develop a new version of the Transcriptome-Integrated Genetic Association Resource (referred to as TIGAR-V2) that takes genotype data of the Variant Call Format (VCF) as input, conducts cross-validation [13] to evaluate trained gene expression prediction models, and enables parallel computation to enable use on high performance computing clusters.

Additionally, TIGAR-V2 can train gene expression imputation models using either nonparametric Bayesian Dirichlet Process Regression (DPR) [14] or Elastic-Net penalized regression (as used by PrediXcan [1]). TIGAR-V2 can perform TWAS using either individual-level or summary-level GWAS data. Besides the burden type TWAS test [1], the software further implements an additional variance-component test [15] for TWAS that retains power under model misspecification.

To make the TIGAR-V2 a convenient resource for the public, we trained nonparametric Bayesian DPR gene expression prediction models for 49 tissues from the GTEx V8 reference panel [10]. These estimated tissue-specific SNP effect sizes on the quantitative gene expression traits (eQT) are considered as Bayesian eQTL weights per gene and are provided along with this TIGAR-V2 tool, which can be conveniently used for follow-up gene-based association studies using both individual-level and summary-level GWAS data. In our example application studies, we used eQTL weights obtained from transcriptomic data of breast mammary tissue and ovary tissue from the GTEX V8 reference panel along

with publicly available GWAS summary statistics [11, 12] to conduct TWAS for studying breast cancer and ovarian cancer.

In the following sections, we first outline the TIGAR-V2 framework in Section 2.1. We then describe the application of TIGAR-V2 to train gene expression prediction models with the GTEx V8 reference data and TWAS of breast cancer and ovarian cancer in Section 2.2. Application results are described in Section 3. Finally, we conclude with a discussion in Section 4.

2 Material and Methods

2.1 TIGAR-V2 framework

2.1.1 Gene expression prediction model

The standard two-stage TWAS [1–3] first fits gene expression prediction models by taking genotype data ($\mathbf{G}$) of cis-SNPs (within $\pm$1MB of the target gene g) as predictors, assuming the following additive genetic model for expression quantitative traits ($\mathbf{E}_g$) with respect to the target gene g,

$$\mathbf{E}_g = \mathbf{G}\mathbf{w} + \boldsymbol{\epsilon};\ \boldsymbol{\epsilon} \sim N(0, \sigma_\epsilon^2 \mathbf{I}). \tag{1}$$

The cis-eQTL effect size vector $\mathbf{w}$ can be estimated by different regression methods from the reference training data. For example, PrediXcan estimates $\mathbf{w}$ by a general linear regression model with Elastic-Net penalty [1]; FUSION estimates $\mathbf{w}$ by the Bayesian Sparse Linear Mixed Model (BSLMM) [2]; and TIGAR estimates $\mathbf{w}$ by a nonparametric Bayesian DPR model (Text S1) [3, 14].

TIGAR-V2 implements both nonparametric Bayesian DPR methods [14] and general linear regression with Elastic-Net penalty as used by PrediXcan [1] to estimate $\mathbf{w}$, which are eQTL effect sizes in a broad sense not considering whether the SNP has a genome-wide significant eQTL p-value. Additionally, TIGAR-V2 runs 5-fold cross validation [13] with

the reference data by default to provide an average prediction R^2 per gene across 5 folds of validation data (referred to as 5-fold CV R^2). The 5-fold CV R^2 can be used to evaluate if the trained gene expression prediction model is "valid" for follow-up TWAS (e.g., using the threshold of 5-fold CV $R^2 > 0.005$). Here, we use a more liberal threshold than the 0.01 by previous studies to allow more genes to be tested in follow-up TWAS.

2.1.2 Gene-based association study

With the estimates of cis-eQTL effect sizes $\widehat{\mathbf{w}}$ and individual-level GWAS data of test samples, TIGAR-V2 predicts GReX values by taking estimates of cis-eQTL effect sizes (outputs from the step of training gene expression prediction model) and genotype data (VCF files, $\mathbf{G}_{\text{test}}$) of test samples as inputs, using formula $\widehat{\mathbf{GReX}} = \mathbf{G}_{\text{test}}\widehat{\mathbf{w}}$. TIGAR-V2 implements the burden [16, 17] type TWAS test by testing the association between $\widehat{\mathbf{GReX}}$ and the phenotype of interest (PED format) based on the general linear regression model, with the phenotype as response variable and predicted $\widehat{\mathbf{GReX}}$ as a test covariate. TIGAR-V2 implements the variance-component TWAS test by [15] using the sequence kernel association test (SKAT) framework [18] with variant weights provided by eQTL effect size estimates $\widehat{\mathbf{w}}$. Variance-component TWAS test is recommended if the assumption of the linear relationship between the SNP effect sizes on phenotype and eQTL weights is violated (see Text S2.2). Note that here the eQTL weights $\widehat{\mathbf{w}}$ are specific to the test gene and specific to the tissue type of the reference transcriptomic data.

With summary-level GWAS data (i.e., Z-score statistic values from single variant GWAS tests) of test samples, TIGAR-V2 tests the gene-based association by using both burden [16, 17] and variance-component [15] test statistics, where cis-eQTL effect size estimates $\widehat{\mathbf{w}}$ are taken as variant weights.

In particular, for burden test, we found that the FUSION Z-score statistic [2] as given by Equation (2) will lead to inflated false positive findings if $\widehat{\mathbf{w}}$ are estimated using non-standardized reference data (i.e., centered gene expression and genotype data as described in

Text S1 for Bayesian DPR model); the S-PrediXcan test statistic [19] as given by Equation (3) should be used in this situation instead. We also show that both FUSION and S-PrediXcan test statistics are equivalent if $\widehat{\mathbf{w}}$ are estimated using standardized reference data (Text S2). The S-PrediXcan test statistic is the default test statistic implemented by TIGAR-V2.

$$\widetilde{Z}_{g,\text{FUSION}} = \frac{\sum_{l=1}^{m}(\widehat{w}_l Z_l)}{\sqrt{\widehat{\mathbf{w}}'\mathbf{V}\widehat{\mathbf{w}}}}, \quad \mathbf{V} = \text{Corr}(\mathbf{G}_0) \tag{2}$$

$$\widetilde{Z}_{g,\text{S-PrediXcan}} = \frac{\sum_{l=1}^{m}(\widehat{w}_l \widehat{\sigma}_l Z_l)}{\sqrt{\widehat{\mathbf{w}}'\mathbf{V}\widehat{\mathbf{w}}}}, \quad \widehat{\sigma_l^2} = \text{Var}(\mathbf{G_{0,l}}), \quad \mathbf{V} = \text{Cov}(\mathbf{G}_0). \tag{3}$$

Here, Z_l denotes the Z-score statistic values from single variant GWAS tests (i.e., summary-level GWAS data). The required linkage disequilibrium (LD) covariance matrix (or correlation matrix for FUSION test statistic) among test cis-SNPs ($\mathbf{V}$), and the variance of test cis-SNPs ($\widehat{\sigma_l^2} = Var(\mathbf{G_{0,l}})$) can be obtained from reference genotype data ($\mathbf{G}_0$) such as 1000 genome [20] and GTEx V8 [10].

2.1.3 Tool framework

The tool framework of TIGAR-V2 is shown in Figure 1, where all TWAS steps in TIGAR-V2 are enabled using Python and Bash scripts. Python libraries "pandas" [21, 22], "numpy" [21, 23], "scipy" [24], "sklearn" [25, 26], and "statsmodels" [27] are used to develop TIGAR-V2. Genotype data in VCF saved as one file per chromosome are input genotype files for TIGAR-V2. TABIX tool [28] is used to extract genotype data per target gene efficiently from VCF genotype files. Parallel computation is enabled by using the "multiprocessing" Python library, allowing users to train gene expression prediction models and test gene-based association of multiple genes in parallel.

This new version uses fewer Python library dependencies for easier set up, speeds up computation by using functions from the "numpy" Python library, reduces required memory usage by loading genotype data from VCF files in small block increments with pre-specified genotype data format (either genotype or dosage in the VCF file), and adds the function

to conduct the recently published variance-component gene-based association test [15]. For example, for training gene expression prediction models by Bayesian DPR method with 129 samples, $\sim$ 1800 SNPs per gene, four genes, and a single core, the computation time is reduced by up to 90% and memory usage by up to 50%, compared to the initial TIGAR tool. Training gene expression prediction models using the GTEx V8 reference data requires less than 8GB of memory per gene.

2.2 Reference resource from GTEx V8

2.2.1 Train Bayesian DPR eQTL weights from GTEx V8

The Genotype-Tissue Expression (GTEx) project V8 (dbGaP phs000424.v8.p2) contains comprehensive profiling of whole genome sequencing (WGS) genotype data and RNA-sequencing (RNA-seq) transcriptomic data (15,253 normal samples) across 54 tissue types of 838 donors [9, 10, 29, 30]. GTEx V8 provides ideal reference data for training tissue-specific gene expression prediction models for a diverse of tissue types on human bodies. Both PrediXcan and FUSION tools use GTEx V8 data as the reference data, and provide estimated cis-eQTL weights per gene with respect to 49 tissue types that have >70 samples with profiled WGS genotype and RNA-seq transcriptomic data (Figure 2A) as a public resource for TWAS.

Here, we also train tissue-specific gene expression prediction models for these 49 tissue types using the nonparametric Bayesian DPR method previously implemented in TIGAR. WGS genotype data of cis-SNPs within $\pm$1MB around gene transcription start sites (TSS) of the target gene were used as predictors. In particular, variants with missing rate $< 20\%$, minor allele frequency > 0.01, and Hardy-Weinberg equilibrium p-value $> 10^{-5}$ were considered for fitting the gene expression prediction models. Gene expression data of Transcripts Per Million (TPM) per sample per tissue were downloaded from the GTEx portal. Genes with > 0.1 TPM in ≥ 10 samples were considered. Raw gene expression data (TPM) were then adjusted for age, body mass index (BMI), top five genotype principal

components, and top probabilistic estimation of expression residuals (PEER) factors [31]. The raw gene expression data of breast mammary tissue were further adjusted for *ESR1* expression following previous TWAS analysis of breast cancer [32].

Five-fold cross validation was conducted by default to obtain 5-fold CV R^2 per gene per tissue. Only "significant" gene expression prediction models with 5-fold CV $R^2 > 0.005$ were retained in the output files. The estimated Bayesian cis-eQTL weights from these "significant" gene expression prediction models can be used to conduct TWAS using both individual-level and summary-level GWAS data, and are shared with the public along with our TIGAR-V2 tool.

Further, we compared gene expression prediction models trained from GTEx V8 [1, 19, 33] by nonparametric Bayesian DPR method to the ones (i.e., PredictDB models) trained from the same GTEx V8 reference data by Elastic-Net method using the PrediXcan tool.

2.2.2 Application TWAS of breast and ovarian cancer

We used TIGAR-V2 to conduct TWAS of breast and ovarian cancer by using the Bayesian cis-eQTL weights estimated from GTEx V8 [10] of breast mammary tissue and ovary tissue and summary-level GWAS data [11, 12]. The GWAS summary data of breast and ovarian cancer were respectively obtained from the Breast Cancer Association Consortium (BCAC) with 122,977 cases and 105,974 controls of European ancestry [11] and the Ovarian Cancer Association Consortium (OCAC) with 22,406 cases and 40,941 controls of European ancestry [12]. We also compared with TWAS results using eQTL weights given by Elastic-Net method (i.e., PrediXcan), which were also generated by our TIGAR-V2 tool.

3 Results

3.1 Bayesian DPR eQTL weights from GTEx V8

From the GTEx V8 reference data as described in Section 2.2, a total of 1,104,305 significant gene expression prediction models with 5-fold CV $R^2 > 0.005$ were successfully trained by TIGAR (using the nonparametric Bayesian DPR method), for genes on the autosomal chromosomes of 49 tissue types. The average and median number of gene expression prediction models obtained per tissue type was ∼22.5K. The corresponding Bayesian DPR eQTL weights (which are effect sizes of cis-SNPs in the fitted gene expression prediction models by nonparametric Bayesian DPR method) are publicly available along with our TIGAR-V2 tool.

3.1.1 Model over-fitting due to small training sample sizes

We present the median 5-fold CV R^2 and the median training R^2 per tissue type by TIGAR in Figure 2B and 2C, respectively. The nonparametric Bayesian DPR method over-fitted gene expression prediction models with training data, resulting in inflated training R^2 (Figure 2C). Here, median 5-fold CV R^2 values demonstrate gene expression prediction performances with independent test data (Figure 2B).

Surprisingly, we observed that larger median 5-fold CV R^2 values were obtained for tissue types with smaller sample size (Figures 2(B, C)). For example, the top median 5-fold CV R^2 values (~ 0.04) were obtained for kidney cortex tissue (cyan bar), various brain tissues (yellow bars), and uterus tissue (hot pink bar), which all have sample sizes ~ 100. Whereas, muscle skeletal, skin, and whole blood tissues that have relatively larger sample sizes $400 \sim 600$ have median 5-fold CV $R^2 \approx 0.02$. This trend is further demonstrated in the density plots of 5-Fold CV R^2 and training R^2 by TIGAR for all tissues (Figure S1).

We suspect this controversial trend is mainly due to model over-fitting with small training sample sizes. To further investigate this, we take the gene expression prediction models

fitted with breast ($N = 337$) and ovarian ($N = 140$) tissue types as examples. First, we down-sampled breast tissue samples to 140 to match with the sample size of ovarian tissue. Second, we trained both PrediXcan Elastic-Net and TIGAR nonparametric Bayesian DPR models on the down-sampled breast tissue data. Third, we made density plot of the 5-fold CV R^2 and training R^2 for genes that have 5-fold CV R^2 greater than various threshold, $(0.005, 0.01, 0.05, 0.1, 0.2)$ (Figures S2 and S3).

We found that the same over-fitting issue existed for both PrediXcan Elastic-Net and TIGAR DPR methods. That is, the down-sampled breast tissue with the same sample size 140 as the ovarian tissue showed similar density distributions with respect to training R^2, which had larger median training R^2 than the breast tissue with sample size 337. As for the 5-fold CV R^2, genes with 5-fold CV $R^2 > 0.2$ had similar distributions between down-sampled and original breast tissues, whereas other groups of genes had similar distributions between down-sampled breast tissue and ovarian tissue that are of the same training sample size. We think this is mainly driven by genes with relatively small expression heritability. Since TIGAR DPR method has higher power to fit gene expression precision models for genes with relatively small expression heritability, the TIGAR training results are affected more by this over-fitting issue.

3.1.2 Comparison with PrediXcan eQTL weights

Additionally, we compared the gene expression prediction model training results by TIGAR with the ones by PrediXcan (i.e., PredictDB models generated by the PrediXcan tool) using the same GTEx V8 reference data [1, 19, 33]. From boxplots of median values (Figure S4) and density plots (Figure S5) of 5-Fold CV R^2 and training R^2 by PrediXcan, we observed the similar trend that relatively larger median 5-Fold CV R^2 and median training R^2 values were obtained with relatively smaller training sample sizes. These findings are consistent with our TIGAR training results (Figure 2) as well as down-sample investigation in Section 3.1.1.

As shown in Figure S6, more consistent findings were obtained by PrediXcan and TIGAR for genes that were of relatively larger sample sizes (yellowish colours) and relatively higher expression heritability. We found TIGAR had consistently better performance with fitting valid gene expression prediction models for genes of relatively smaller expression heritability, especially when the training sample size is relatively large (yellowish colours). In particular, the higher median 5-fold CV R^2 shown in Figure S4 by PrediXcan is based on the group of valid genes with 5-fold CV $R^2 > 0.005$ that is $< 50\%$ of the valid genes by TIGAR (Figure 3A). These findings are consistent with previous studies [3].

3.1.3 Computation cost by TIGAR-V2

The training computation costs in CPU hours per chromosome per tissue type with GTEx V8 reference data by TIGAR-V2 are shown in Figure 3B. The computation cost per chromosome ranged from 5 CPU hours to over 474, with a median of 50.6 and mean of 69.1, which is mainly due to various numbers of genes per chromosome and various sample sizes per tissue type. That is, with sample size ~ 300, the average computation time for training a nonparametric Bayesian DPR gene expression prediction model per gene with 5-fold cross-validation is only ~ 4 minutes by TIGAR-V2. The computation complexity is linear with respect to training sample size.

3.2 TWAS of breast and ovarian cancer

From the gene expression prediction model training results in the above Section 3.1 by TIGAR, we respectively obtained 22,781 and 22,823 valid gene expression prediction models with 5-fold CV $R^2 > 0.005$ by using the nonparametric Bayesian DPR method for breast ($N = 337$) and ovarian ($N = 140$) tissue types (Figure S7). Using GWAS summary statistics of breast cancer and ovarian cancer [11, 12] and Bayesian *cis*-eQTL weights estimated with respect to the corresponding tissue type, TIGAR using our Bayesian eQTL weights respectively detected 88 and 37 significant TWAS genes (p-values $< 2.5 \times 10^{-6}$) for

breast and ovarian cancer (Figure 4). Of these significant genes, 17 were identified as risk genes of both breast and ovarian cancer (Table S1). TWAS computation costs in CPU hours per chromosome per tissue type by TIGAR-V2 for both Bayesian DPR and Elastic-Net weights are shown in Figure S8.

3.2.1 Independently significant TWAS risk genes by TIGAR

Out of these 88 significant TWAS genes for breast cancer by TIGAR, 20 genes are known GWAS risk genes of breast cancer [11, 34–41], 64 are located within 1MB region of a previously identified GWAS loci of breast cancer [11, 34–41] (Table S2), and 35 genes are identified by previous TWAS [32, 42–46]. Similarly, out of these 37 significant TWAS genes for ovarian cancer by TIGAR, 35 genes are located on chromosome 17 including two known GWAS risk genes (*NSF* and *PLEKHM1*) [12, 47, 48], 33 genes are located within 1MB of these two known GWAS risk genes (Table S3), and 13 genes (including *NSF* [49]) are identified by previous TWAS [32, 49, 50]. The known GWAS risk genes are curated from GWAS Catalogue [51] containing at least one significant SNP within or ±1MB around the gene region.

Since TWAS is conducted using genotype data within ±1MB region of the test gene (i.e., test region), genes with overlapped test regions often have highly correlated GReX values (see locus-zoom plots around the top significant TWAS genes in Figure S9). Thus, these nearby significant TWAS genes are often not representing independent associations. In Tables 1 and 2, we listed the most significant genes among genes that have shared test regions, which represent the independently significant TWAS risk genes. For breast cancer, 31 out of all 34 independent TWAS risk genes (Table 1) were either identified by previous GWAS/TWAS or within ±1MB region of previously identified risk genes of breast cancer. For example, TIGAR identified *L3MBTL3* (previously identified by GWAS [11] and TWAS [43–46]) and an additional 6 significant genes within the 1MB region of *L3MBTL3*. Of the independent TWAS genes of breast cancer, 17 (54%) have been identified by

previous TWAS using PrediXcan and FUSION [32, 42–46].

Similarly, as shown in Table 2, TIGAR identified 4 independent significant TWAS genes for ovarian cancer. In particular, TWAS risk gene *RP11-798G7.8* on chromosome 17 was identified by previous TWAS [32] and lies within 1MB of known GWAS risk gene *PLEKHM1* [12, 47]. Interestingly, all independent TWAS risk genes of ovarian cancer by TIGAR (*PRC1-AS1*, *UBE2MP1*, *RP11-798G7.8*, and *FRG1EP*) are also TWAS risk genes of breast cancer [11, 32, 52], which demonstrates likely pleiotropy effect for these TWAS risk genes.

3.2.2 Novel findings by TIGAR

TIGAR identified 3 novel independent TWAS risk genes (*KLHL25*, *UBE2MP1*, and *FRG1EP*) for breast cancer. Gene *KLHL25* has known biological functions involved in carcinogenesis, while genes *UBE2MP1* and *FRG1EP* are near such a gene [53–57]. Interestingly, genes *UBE2MP1* and *FRG1EP* were also identified for ovarian cancer by TIGAR (Table S11), and all three genes are involved with biological functions in carcinogenesis, either directly or indirectly. The protein encoded by *KLHL25* was reported acting as an adaptor protein for a suspected lung cancer tumour-suppressing protein *CUL3* to form an enzyme complex that targets ACLY, a protein often over-expressed in cancers, for degradation [55]. Pseudogene *UBE2MP1* was found to have a significant expression-methylation-correlation difference between normal and cancerous breast tissue [53]. *UBE2MP1* was also found to be amplified in gastric cancers with amplified copy number variations in the 16p11.2 region, a mutation found to be associated with shorter overall survival [57], and was predicted to be a driver of lung adenocarcinoma [56]. The test region of *FRG1EP* overlaps with the test region of pseudogene *ANKRD20A21P*, another TWAS risk gene identified by TIGAR, which has been implicated as a potentially important lncRNA regulator of endometrial carcinogenesis [54].

3.2.3 Compare with TWAS results by PrediXcan

Additionally, we compared with the TWAS results of breast and ovarian cancer obtained by using *cis*-eQTL weights estimated by Elastic-Net method as used by PrediXcan (Tables S8 and S9), which were generated using the PrediXcan function enabled in our TIGAR-V2 tool. We respectively obtained 11,095 and 12,337 valid gene expression prediction models for breast and ovary tissue types by using the PrediXcan function, about half of the number of valid gene expression prediction models by TIGAR (Figure S7). This is consistent with our above comparison of trained valid gene expression models by TIGAR and PrediXcan (Figure 3A, Section 3.1.2).

As a result, PrediXcan detected 56 significant (32 independent) TWAS genes for breast cancer and 4 significant (2 independent) TWAS genes for ovarian cancer (Figure S10, Tables S4 and S5). Respective 30 out of 32 and 2 out of 2 of the independent TWAS risk genes by PrediXcan for breast and ovarian cancer were either identified by previous corresponding GWAS or within 1MB region of a known GWAS risk gene (Tables S6 and S7). Potentially novel TWAS risk genes by PrediXcan and TIGAR that were not identified by previous GWAS are presented in Table S11.

Even though there were respective 18 (56.25%) and 2 (100%) independent TWAS genes of breast and ovarian cancer by PrediXcan also identified by TIGAR (see Figure S11 and Tables S8, S9, S10), only TIGAR identified the novel TWAS genes *UBE2MP1* and *FRG1EP* shared by both breast and ovarian cancer and the known GWAS risk genes *FGF10* [11, 58] and *TOX3* [36, 38] of breast cancer. Other exclusive independent TWAS genes identified by TIGAR include lncRNA *RP11-758M4.4* which was shown to be a potential biomarker of breast cancer [59], *RPS23* which was found to be over-expressed in advanced colorectal adenocarcinomas [60], and *ZNF404* whose dysregulation was linked to breast cancer pathogenesis by eQTL analyses [61, 62].

3.2.4 Cis-eQTL weights by PrediXcan and TIGAR

To investigate the reason of why PrediXcan and TIGAR lead to different TWAS findings, we took three TWAS risk genes shared by both breast and ovarian cancer as examples. In particular, *FRG1EP* was only identified by TIGAR for both breast and ovarian cancer, while *LRRC37A4P* and *PRC1-AS1* were identified by both PrediXcan and TIGAR for both breast and ovarian cancer. Pseudogene *LRRC37A4P* on chromosome 17 lies within 1MB downstream from the known risk gene *PLEKHM1* of breast cancer and ovarian cancer [12, 47]. Gene *PRC1-AS1* on chromosome 15 is a long non-coding RNA (lncRNA) gene previously identified as being associated with breast carcinoma [11, 52]. Regulation of *PRC1-AS1* is known to differ with respect to different types of breast cancers [63] and increased expression of *PRC1-AS1* lncRNA is associated with hepatocellular carcinoma [64].

We plotted the cis-eQTL weights estimated by Elastic-Net (PrediXcan) and Bayesian DPR method (TIGAR) from GTEX V8 for these three example TWAS risk genes. We observed that Bayesian estimates generally had non-zero values for all SNPs within the test region while Elastic-Net estimates had non-zero values for < 100 SNPs within the test region that had effect sizes (i.e., weights) of relatively larger magnitudes. These results match with the assumptions by the nonparametric Bayesian DPR (TIGAR) and Elastic-Net methods (PrediXcan). We can see that PrediXcan would miss the risk gene if only test SNPs with nonsignificant GWAS p-values are of non-zero weights such as *FRG1EP* (Figure S12), while otherwise both PrediXcan and TIGAR would have similar power to identify the risk genes such as *LRRC37A4P* and *PRC1-AS1* (Figures S13 and S14).

Overall, these TWAS results not only validated our TIGAR-V2 tool with findings consistent with previous GWAS and TWAS of breast cancer and ovarian cancer, but also identified novel risk genes that were shown to be possibly involved in the biological mechanisms of oncogenesis.

4 Discussion

In this work, we develop a new version of the TIGAR tool [3] with improved computation efficiency, referred to as TIGAR-V2. Compared to the initial TIGAR tool, this new version reduces up to 90% computation time and up to 50% memory usage. TIGAR-V2 can efficiently train gene expression prediction models by using both nonparametric Bayesian DPR and Elastic-Net (as used by PrediXcan) methods, as well as construct gene-based association tests using either individual-level or summary-level GWAS data. Gene-based associated tests implemented in TIGAR-V2 include both burden statistics (based on FUSION [2] and S-PrediXcan [19] Z-score test statistics) and variance-component statistics.

We trained gene expression prediction models of 49 tissue types with the GTEx V8 reference data by using the nonparametric Bayesian DPR method. We demonstrated the usefulness of these trained models by performing TWAS of breast and ovarian cancer by integrating the estimated cis-eQTL weights of relevant tissue types with the relevant GWAS summary statistics. Compared to the cis-eQTL weights estimated by PrediXcan with the GTEx V8 data and TWAS results by PrediXcan, our Bayesian cis-eQTL weights lead to not only a larger number of significant TWAS risk genes but also interesting novel TWAS risk genes with potential pleiotropy effects for breast and ovarian cancer. With a larger number of "valid" gene expression prediction model trained by the nonparametric Bayesian DPR method, TIGAR is expected to identify a larger number of TWAS risk genes than PrediXcan.

TIGAR-V2 tool still has its limitations such as considering only cis-eQTL data and assuming a two-stage model for TWAS. There are many other alternative TWAS tools available to address these two limits. For example, BGW-TWAS [4] uses both cis- and trans- genotype data to train gene expression prediction model of the target gene, CoMM [65] and PMR-Egger [5, 66] assume a joint model with reference and test data that can achieve higher power when both data sets are homogeneous.

In conclusion, TIGAR-V2 tool along with Bayesian cis-eQTL weights and reference LD covariance data (European ancestry) estimated from the GTEx V8 reference data are freely shared with the public on GitHub and Synapse. Given the convenience of directly loading VCF genotype data saved per chromosome, flexibility of using different training models and TWAS test statistics, and efficient computation enabled by Python source code, we believe our improved TIGAR-V2 tool will provide a useful resource for mapping risk genes of complex diseases by TWAS.

Supplemental Data

Supplemental data include 2 text sections describing the nonparametric Bayesian DPR model and derivations of the FUSION and S-PrediXcan Z-score test statistics, 11 tables, and 13 figures.

Acknowledgements

RP and JY are supported by National Institutes of Health (NIH/NIGMS) grant award R35GM138313. MPE was supported by NIH/NIGMS grant award R01GM117946 and NIH/NIA grant award RF1AG071170.

Declaration of Interests

The authors declare no competing interests.

Web Resources

- TIGAR-V2: `https://github.com/yanglab-emory/TIGAR`

- GTEx V8 resources generated by TIGAR are available on Synapse: https://www.synapse.org/TIGAR_V2_Resource_GTExV8
- GTEx portal: https://gtexportal.org/home/
- eQTL weights trained by PrediXcan with GTEx V8: https://predictdb.org

Data and Code Availability

- TIGAR-V2 tool generated in this study is available at GITHUB https://github.com/yanglab-emory/TIGAR.
- All Bayesian eQTL weights of 49 tissue types from GTEx V8 and reference LD files from GTEx V8 WGS samples of European ancestry are available at Synapse https://www.synapse.org/TIGAR_V2_Resource_GTExV8.
- GTEx V8 data are available from dbGaP with accession phs000424.v8.p2.
- GWAS data for studying breast cancer is available from Breast Cancer Association Consortium (BCAC), http://bcac.ccge.medschl.cam.ac.uk/bcacdata/oncoarray/oncoarray-and-combined-summary-result/gwas-summary-results-breast-cancer-risk-2017/.
- GWAS data for studying ovarian cancer is available from Ovarian Cancer Association Consortium (OCAC), ftp://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/PhelanCM_28346442_GCST004415/.

Tables

Table 1: Independent TWAS risk genes of breast cancer identified by TIGAR, with three novel risk genes of bold text.

Gene	Chr	Start	End	Z-score	P-value
FCGR1B[b]	1	121087345	121096310	-16.77	4.12e-63
KLHDC7A[a]	1	18480982	18486126	-6.04	1.56e-09
MTX1P1[b]	1	155230975	155234325	5.21	1.92e-07
AC010136.2[b]	2	217978707	217992615	-6.52	6.80e-11
CASP8[a]	2	201233443	201287711	-6.51	7.56e-11
EOMES[b]	3	27715949	27722711	6.07	1.28e-09
PSMD6-AS2[b]	3	64004022	64012148	-5.38	7.50e-08
FAM114A1[b]	4	38867677	38945739	-4.82	1.41e-06
FGF10[a]	5	44303544	44389706	6.60	4.13e-11
SLC22A5[b]	5	132369752	132395614	6.53	6.63e-11
ANKRD55[b]	5	56099678	56233359	-5.63	1.85e-08
RPS23[b]	5	82273358	82278577	4.77	1.86e-06
L3MBTL3[a]	6	130013699	130141451	6.37	1.93e-10
RP11-758M4.4[b]	8	74798784	74866939	5.06	4.17e-07
PIDD1[a]	11	799191	809646	-6.64	3.04e-11
CCDC91[a]	12	28133249	28581511	-7.77	7.76e-15
RP11-116D17.4[b]	12	115318657	115320405	-5.36	8.40e-08
CTD-2325P2.4[b]	14	68627166	68628445	-5.09	3.65e-07
RCCD1[a]	15	90955796	90963125	-6.29	3.26e-10
MAN2C1[b]	15	75358201	75368154	-4.85	1.25e-06
KLHL25	15	85759323	85795030	-4.73	2.22e-06
TOX3[a]	16	52438005	52547802	10.98	4.82e-28
UBE2MP1	16	35169692	35170241	-5.31	1.13e-07
LRRC37A4P[b]	17	45506741	45550335	6.08	1.20e-09
CBX8[a]	17	79792132	79801683	5.76	8.46e-09
TOM1L1[b]	17	54899387	54960627	4.77	1.84e-06
SSBP4[a]	19	18418864	18434387	8.53	1.47e-17
ZNF404[b]	19	43872363	43884051	5.41	6.31e-08
FRG1EP	20	29480147	29497179	5.39	6.95e-08
DNAJB7[b]	22	40859549	40861617	-9.22	2.89e-20
TMEM184B[b]	22	38219291	38273034	4.92	8.72e-07

a known GWAS risk genes of breast cancer.

b genes within 1MB of known GWAS risk genes of breast cancer.

Table 2: Independent TWAS risk genes of ovarian cancer identified by TIGAR, with two novel risk genes of bold text.

Gene	Chr	Start	End	Z-score	P-value
PRC1-AS1[a]	15	90972860	90988624	4.95	7.56e-07
UBE2MP1	16	35169692	35170241	5.77	7.88e-09
RP11-798G7.8[a]	17	45531577	45533838	-7.21	5.77e-13
FRG1EP	20	29480147	29497179	-4.99	6.19e-07

a genes within 1MB of known GWAS risk genes of ovarian cancer.

Figure Titles and Legends

Figure 1. TIGAR-V2 framework. Including TWAS steps of training gene expression prediction models from reference data, predicting GReX with individual-level GWAS data, and testing gene-based association with both individual-level and summary-level GWAS data.

Figure 2. Trained gene expression prediction models from GTEx V8 reference data of 49 tissue types, by TIGAR using the nonparametric Bayesian DPR method. A) Number of subjects (i.e., training sample size), B) Median 5-Fold CV R^2, and C) Median Training R^2, per tissue type.

Figure 3. Proportion of valid gene expression prediction models by TIGAR vs. PrediXcan (A) and computation costs by TIGAR-V2 (B). The same colour codes with respect to different tissue types as used in Figure 2 were used here. Computation times were in CPU Hours per chromosome per tissue type for training gene expression prediction models.

Figure 4. Manhattan plots of TWAS results by TIGAR for studying breast and ovarian cancer. (A) TWAS results of breast cancer with with 88 significant risk genes. Significant gene *FCGR1B* of breast cancer (p-value: 4.12×10^{-63}) was removed from (A) to reduce the upper limit of the y-axis. (B) TWAS results of ovarian cancer with 37 significant risk genes.

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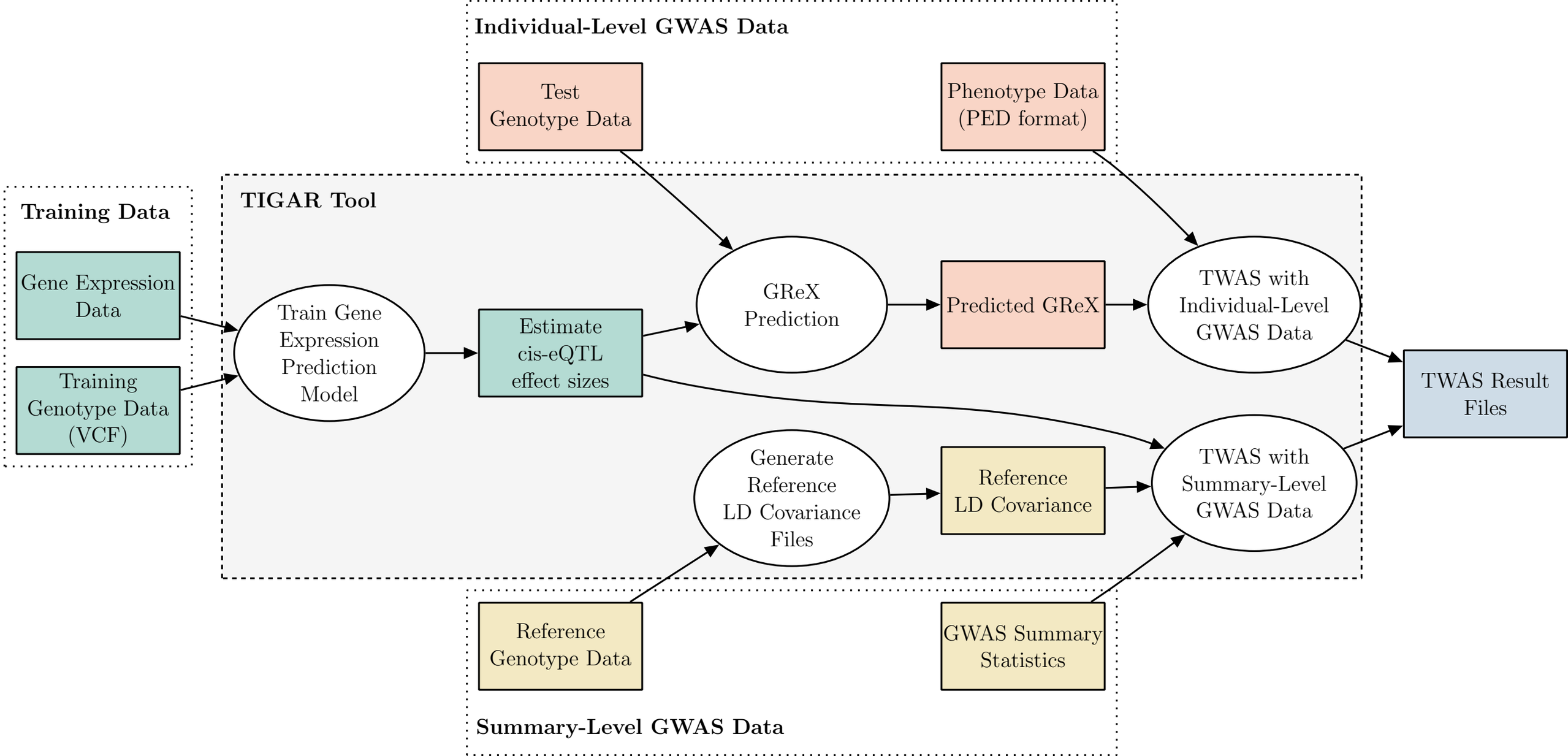
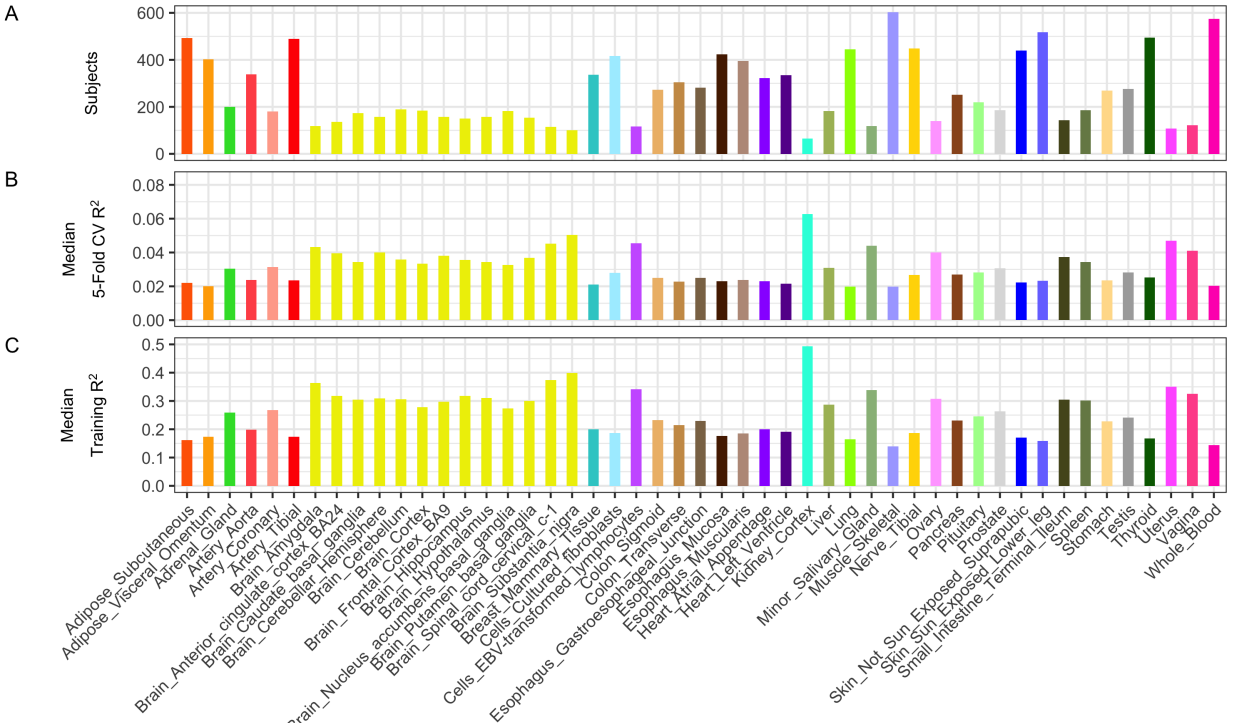
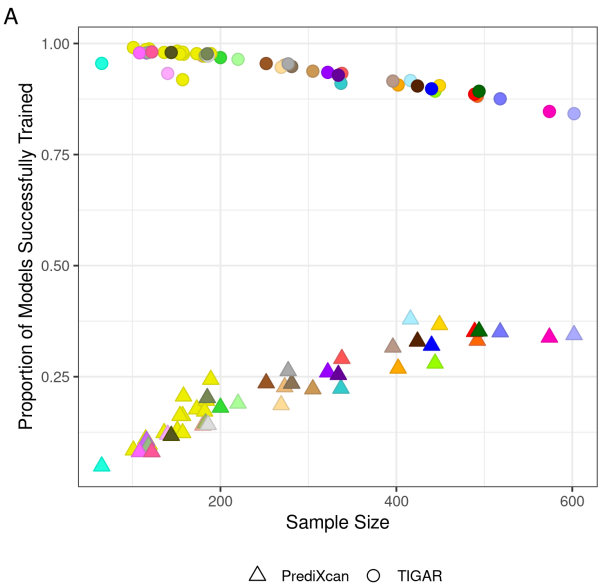
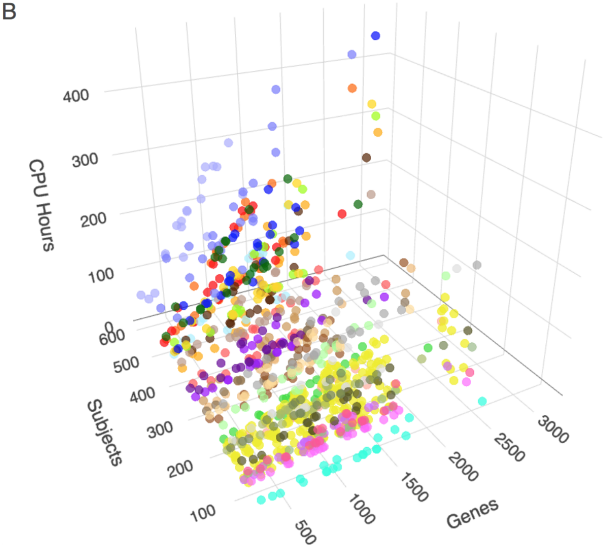
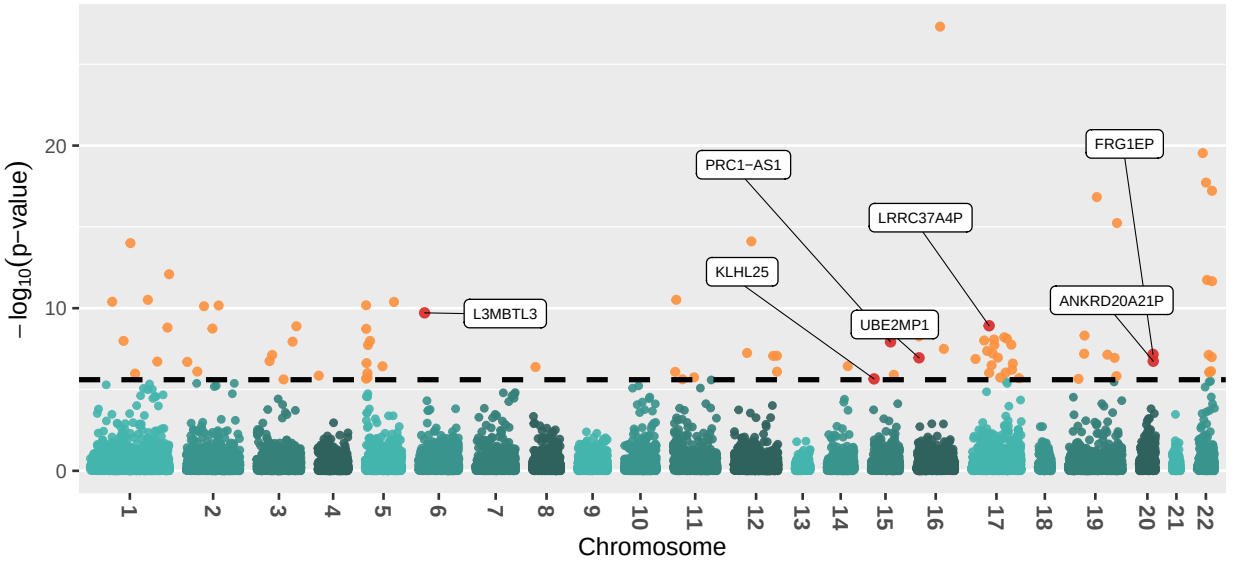

Individual-Level GWAS Data
Test Genotype Data
Phenotype Data (PED format)
Training Data
Gene Expression Data
Training Genotype Data (VCF)
TIGAR Tool
Train Gene Expression Prediction Model
Estimate cis-eQTL effect sizes
GReX Prediction
Predicted GReX
TWAS with Individual-Level GWAS Data
TWAS Result Files
Generate Reference LD Covariance Files
Reference LD Covariance
TWAS with Summary-Level GWAS Data
Reference Genotype Data
GWAS Summary Statistics
Summary-Level GWAS Data


A
Subjects
600
400
200
0
B
Median
5-Fold CV R2
0.08
0.06
0.04
0.02
0.00
C
Median
Training R2
0.5
0.4
0.3
0.2
0.1
0.0
Adipose_Subcutaneous
Adipose_Visceral_Omentum
Adrenal_Gland
Artery_Aorta
Artery_Coronary
Artery_Tibial
Brain_Amygdala
Brain_Anterior_cingulate_cortex_BA24
Brain_Caudate_basal_ganglia
Brain_Cerebellar_Hemisphere
Brain_Cerebellum
Brain_Cortex
Brain_Frontal_Cortex_BA9
Brain_Hippocampus
Brain_Hypothalamus
Brain_Nucleus_accumbens_basal_ganglia
Brain_Putamen_basal_ganglia
Brain_Spinal_cord_cervical_c-1
Brain_Substantia_nigra
Breast_Mammary_Tissue
Cells_Cultured_fibroblasts
Cells_EBV-transformed_lymphocytes
Colon_Sigmoid
Colon_Transverse
Esophagus_Gastroesophageal_Junction
Esophagus_Mucosa
Esophagus_Muscularis
Heart_Atrial_Appendage
Heart_Left_Ventricle
Kidney_Cortex
Liver
Lung
Minor_Salivary_Gland
Muscle_Skeletal
Nerve_Tibial
Ovary
Pancreas
Pituitary
Prostate
Skin_Not_Sun_Exposed_Suprapubic
Skin_Sun_Exposed_Lower_leg
Small_Intestine_Terminal_Ileum
Spleen
Stomach
Testis
Thyroid
Uterus
Vagina
Whole_Blood

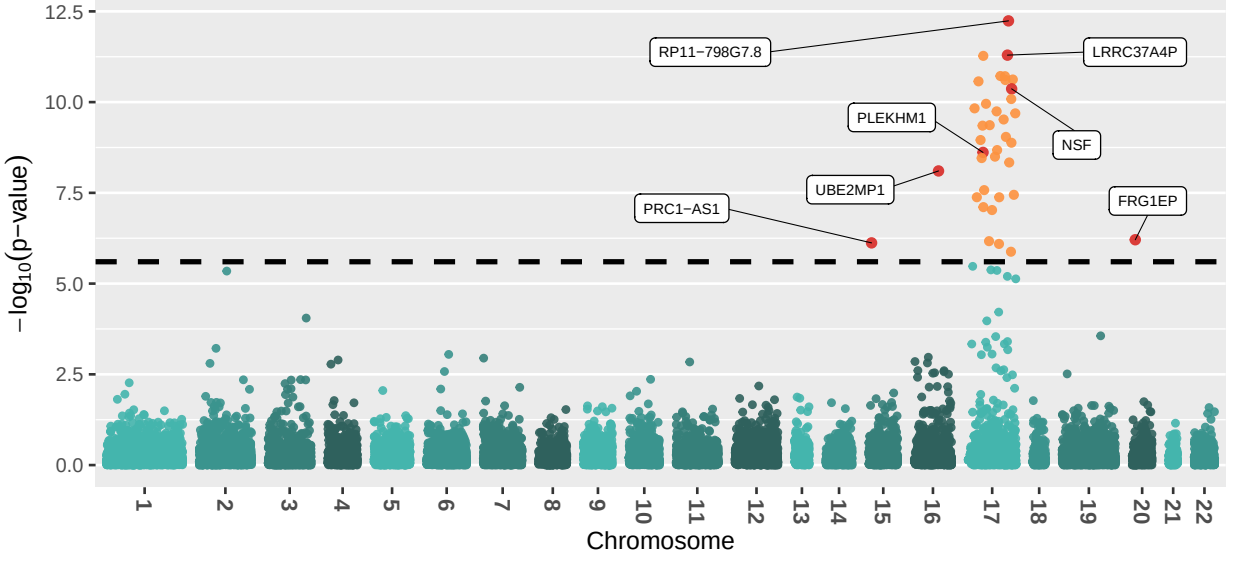

A
Proportion of Models Successfully Trained
1.00
0.75
0.50
0.25
200
400
600
Sample Size
PrediXcan
TIGAR


B
CPU Hours
400
300
200
100
0
Subjects
600
500
400
300
200
100
Genes
500
1000
1500
2000
2500
3000

A


20
10
0
−log10(p−value)
L3MBTL3
PRC1−AS1
KLHL25
UBE2MP1
LRRC37A4P
FRG1EP
ANKRD20A21P
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Chromosome

B


12.5
10.0
7.5
5.0
2.5
0.0
−log10(p−value)
RP11−798G7.8
LRRC37A4P
PLEKHM1
NSF
UBE2MP1
PRC1−AS1
FRG1EP
1
2
3
4
5
6
7
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9
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11
12
13
14
15
16
17
18
19
20
21
22
Chromosome