OTTERS: A powerful TWAS framework leveraging summary-level reference data

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Abstract

Transcriptome-wide association studies (TWAS) identify genes that influence complex traits through genetic regulation of gene expression. Existing TWAS tools like FUSION require individual-level eQTL reference data from a source like GTEx (n=~100s) to impute gene expression in a test GWAS. Therefore, these TWAS tools are not applicable to enormous summary-level reference eQTL datasets like those generated by the eQTLGen consortium (n=~32K). Development of TWAS methods that can harness summary-level reference data like eQTLGen is valuable not only to enable TWAS in more general settings but also to permit more powerful analyses, since we expect expression prediction to improve with increasing reference sample size. To fill this important gap, we develop a TWAS framework called OTTERS (Omnibus Transcriptome Test using Expression Reference Summary data) that adapts multiple polygenic risk score (PRS) methods to estimate eQTL weights from summary-level eQTL reference data. For each PRS method, OTTERS estimates eQTL weights to impute gene expression per gene and then tests for association between imputed expression and outcome for a GWAS dataset. OTTERS then combines the PRS-based association tests together to create an optimal TWAS p-value. By simulation, we show that OTTERS is powerful across a wide range of models. We also applied OTTERS to conduct a TWAS of cardiovascular disease using GWAS summary data from UK Biobank and summary-level eQTLGen reference data. OTTERS identified 11 additional TWAS risk genes that were missed by FUSION using GTEx reference data. These findings suggest that OTTERS provides a practical and powerful tool for TWAS analysis.

Introduction

A transcriptome-wide association study (TWAS) is a valuable analysis strategy for identifying genes that influence complex traits and diseases through genetic regulation of gene expression[1–5]. Researchers have successfully deployed TWAS analyses to identify risk genes for complex human diseases, including Alzheimer's disease[6–8], breast cancer[9–11], ovarian cancer[12,13],

and cardiovascular disease[14,15]. A typical TWAS consists of two separate stages. In Stage I, TWAS acquires individual-level genetic and expression data from relevant tissues available in a reference dataset like the Genotype-Tissue Expression (GTEx) project[16,17] or the North American Brain Expression Consortium (NABEC)[18], and then trains multivariate regression models on the data treating gene expression as outcome and SNP genotype data (typically cis-SNPs nearby the test gene) as predictors to determine genetically regulated expression (GReX). After the GReX regression models estimate effect sizes of SNP predictors that are in the broad sense of expression quantitative trait loci (eQTLs) in Stage I, Stage II of TWAS proceeds by using these trained eQTL effect sizes to impute GReX within an independent GWAS of a complex human disease or trait. One can then test for association between the imputed GReX and phenotype, which is equivalent to a gene-based association test taking these eQTL effect sizes as corresponding test SNP weights[19–21].

For Stage I of TWAS, a variety of training tools exist for fitting GReX regression models to reference expression and genetic data, including PrediXcan[19], FUSION[20], and TIGAR[22]. While these methods all employ different techniques for model fitting, they all require individual-level reference expression and genetic data to estimate eQTL effect sizes for TWAS. Therefore, these methods cannot be applied to emerging reference summary-level eQTL results such as those generated by the eQTLGen[23] and CommonMind[24] consortia, which provide eQTL effect sizes and p-values relating individual SNPs to gene expression. The development of TWAS methods that can utilize such summary-level reference data is valuable to permit applicability of the technique to broader analysis settings. Moreover, as the power of TWAS generally increases with increasing reference sample size[25], we note that the development of TWAS methods that can utilize summary-level reference data in Stage I can bolster the practical performance of the technique since the sample sizes within summary-level reference datasets are often considerably larger than individual-level reference datasets. For example, the sample size of the summary-based

eQTLGen reference sample is 31,684 for blood whereas the sample size of the individual-level GTEx V6 reference is only 338 for whole blood. Consequently, TWAS analysis leveraging the summary-based eQTLGen dataset as reference likely can provide novel insights into genetic regulation of complex human traits.

In this work, we propose a TWAS framework that can use summary-level reference data to train GReX regression models required for Stage I of analysis. Our method is motivated by a variety of published polygenic risk score (PRS) methods[26–29] that can predict phenotype in a test dataset using summary-level SNP effect-size estimates and p-values based on single SNP tests from an independent reference GWAS. We can adapt these PRS methods for TWAS since eQTL effect sizes are essentially SNP effect sizes resulting from a reference GWAS of gene expression. Thus, our predicted GReX in Stage I of TWAS is analogous to the PRS constructed based on training GWAS summary statistics of single SNP-trait association. Here, we adapt four summary-data based PRS methods — P-value Thresholding with linkage disequilibrium (LD) clumping (*P*+*T*)[26], frequentist LASSO[30] regression based method *lassosum*[27], Bayesian Dirichlet Process Regression model[31] based method *SDPR*[29], and Bayesian multivariate regression model based method *PRS-CS*[28] for TWAS analysis. We apply each of these PRS methods to first train eQTL effect sizes based on a multivariate regression model from summary-level reference expression data, and subsequently use these eQTL effect sizes (i.e., eQTL weights) to impute GReX and then test GReX-trait association in an independent test GWAS for Stage II analysis.

As we will show, the performance of each of these PRS methods for TWAS depends on the underlying genetic architecture for gene expression, with some methods working better for sparse architecture than dense architecture (or vice versa). Since the genetic architecture of expression for a specific gene is unknown a priori, we maximize the performance of TWAS over different possible architectures by proposing a novel TWAS framework called OTTERS (Omnibus Transcriptome Test using Expression Reference Summary data). OTTERS first constructs

individual TWAS tests and p-values using eQTL weights trained by each of the PRS techniques outlined above, and then calculates an optimal test using the aggregated Cauchy association test[32] (ACAT-O) with all individual TWAS p-values (Figure 1). OTTERS is applicable to both summary-level and individual-level test GWAS data within Stage II TWAS analysis.

In subsequent sections, we first describe how to use the PRS methods on summary-level expression data in Stage I TWAS and then describe how we can use the resulting eQTL weights to perform Stage II TWAS using OTTERS. We then evaluate the performance of PRS methods and OTTERS using simulated expression and real genetic data based on patterns in real datasets. We also apply OTTERS to blood eQTL summary-level data (n=31,684) from the eQTLGen consortium[23] and GWAS summary data of cardiovascular disease from the UK Biobank (UKBB)[33]. By comparing OTTERS results to those of FUSION[20] using individual-level GTEx V6 reference data (n=338 for whole blood tissue), we demonstrate that TWAS using large summary-level reference datasets can successfully reveal potential risk genes missed by TWAS based on smaller individual-level reference datasets. Finally, we conclude with a discussion.

Methods

Traditional Two-Stage TWAS Analysis

Stage I of TWAS estimates a GReX imputation model using individual-level expression and genotype data available from a reference dataset. Consider the following GReX imputation model from n individuals and m SNPs (multivariate regression model assuming linear additive genetic effects) within the reference dataset:

$$\boldsymbol{E}_g = \boldsymbol{X}\boldsymbol{w} + \boldsymbol{\epsilon},\ \ \boldsymbol{\epsilon} \sim N(0, \sigma_\epsilon^2 \boldsymbol{I}). \qquad \text{(Equation 1)}$$

Here, $\boldsymbol{E}_g$ is a vector representing gene expression levels of gene g, $\boldsymbol{X}$ is an $n \times m$ matrix of genotype data of SNP predictors proximal or within gene g, $\boldsymbol{w}$ is a vector of genetic effect sizes (referred to as a broad sense of eQTL effect sizes), and $\boldsymbol{\epsilon}$ is the error term. Here, we consider

only cis-SNPs within 1 MB of the flanking 5' and 3' ends as genotype predictors that are coded within $\boldsymbol{X}$[19,20,22]. Once we configure the model in Equation 1, we can employ methods like PrediXcan, FUSION, and TIGAR to fit the model and obtain estimates of eQTL effect sizes ($\widehat{\boldsymbol{w}}$).

Stage II of TWAS uses the eQTL effect sizes ($\widehat{\boldsymbol{w}}$) from Stage I to impute gene expression (GReX) in an independent GWAS and then test for association between GReX and phenotype. Given individual-level GWAS data with genotype data $\boldsymbol{X}_{new}$ and eQTL effect sizes ($\widehat{\boldsymbol{w}}$) from Stage I, the GReX for $\boldsymbol{X}_{new}$ can be imputed by $\widehat{\boldsymbol{GReX}} = \boldsymbol{X}_{new}\,\widehat{\boldsymbol{w}}$. The follow-up TWAS would test the association between $\widehat{\boldsymbol{GReX}}$ and phenotype $\boldsymbol{Y}$ based on a generalized linear regression model, which is equivalent to a gene-based association test taking $\widehat{\boldsymbol{w}}$ as test SNP weights. When individual-level GWAS data are not available, one can apply FUSION and S-PrediXcan test statistics to summary-level GWAS data as follows:

$$Z_{g,FUSION} = \frac{\sum_{j=1}^{J}(\widehat{w}_j Z_j)}{\sqrt{\widehat{\boldsymbol{w}}'\boldsymbol{V}\widehat{\boldsymbol{w}}}}, \quad Z_{g,S-PrediXcan} = \frac{\sum_{j=1}^{J}(\widehat{w}_j \widehat{\sigma}_j Z_j)}{\sqrt{\widehat{\boldsymbol{w}}'\boldsymbol{V}\widehat{\boldsymbol{w}}}} \qquad \text{(Equation 2)}$$

where Z_j is the single variant *Z-score* test statistic in GWAS for the jth SNP, $j = 1, \ldots, J$, for all test SNPs that have both eQTL weights with respect to the test gene g and GWAS Z-scores; $\hat{\sigma}_j$ is the genotype standard deviation of the jth SNP; and $\boldsymbol{V}$ denotes the genotype correlation matrix in FUSION Z-score statistic and genotype covariance matrix in S-PrediXcan Z-score statistic of the test SNPs. In particular, $\hat{\sigma}_j$ and $\boldsymbol{V}$ can be approximated from a reference panel with genotype data of samples of the same ancestry such as those available from the 1000 Genomes Project[34]. If $\widehat{\boldsymbol{w}}$ are standardized effect sizes estimated assuming standardized genotype $\boldsymbol{X}$ and gene expression $\boldsymbol{E}_g$ in Equation 1, FUSION and S-PrediXcan Z-score statistics are equivalent[13]. Otherwise, the S-PrediXcan Z-score should be applied to avoid false positive inflation.

TWAS Stage I Analysis using Summary-Level Reference Data

We now consider a variation of TWAS Stage I to estimate cis-eQTL effect sizes $\widehat{\boldsymbol{w}}$ based on a multivariate regression model (Equation 1) from summary-level reference data. We assume that the summary-level reference data provide information on the association between a single genetic variant *j* ($j = 1, \ldots, m$) and expression of gene *g*. This information generally consists of effect size estimates ($\widetilde{w}_j, j = 1, \ldots, m$) and p-values derived from the following single variant regression models:

$$\boldsymbol{E}_g = \boldsymbol{X}_j w_j + \boldsymbol{\epsilon},\ \ \boldsymbol{\epsilon} \sim N(0, \sigma_\epsilon^2 \boldsymbol{I}),\ j\ =\ 1, \ldots, m. \qquad \text{(Equation 3)}$$

Here, $\boldsymbol{X}_j$ is an $n \times 1$ vector of genotype data for genetic variant j. Since eQTL summary data are analogous to GWAS summary data where gene expression represents the phenotype, we can estimate the eQTL effect sizes $\widehat{\boldsymbol{w}}$ using marginal least squared effect estimates ($\widetilde{w}_j, j = 1, \ldots, m$) and p-values from the QTL summary data as well as reference linkage disequilibrium (LD) information of the same ancestry[26–29]. We describe methods for doing this below.

P+T: The *P+T* method selects eQTL weights by LD-clumping and P-value Thresholding[26]. Given threshold P_T for p-values and threshold R_T for LD R^2, we first exclude SNPs with marginal p-values from eQTL summary data greater than P_T or strongly correlated (LD R^2 greater than R_T) with another SNP having a more significant marginal p-value (or Z-score statistic value). For the remaining selected test SNPs, we use marginal standardized eQTL effect sizes from eQTL summary data as eQTL weights for TWAS in Stage II. We considered $R_T = 0.99$ and $P_T = (0.001, 0.05)$ in this paper and implemented the *P+T* method using PLINK 1.9[35] (see Web Resources). We denote the *P+T* method with P_T equal to 0.001 and 0.05 as *P+T (0.001)* and *P+T (0.05)*, respectively.

Frequentist lassosum: With standardized $\boldsymbol{E_g}$ and **X**, we can show that the marginal least squared eQTL effect size estimates from the single variant regression model (Equation 3) is $\widetilde{\boldsymbol{w}} = \boldsymbol{X}^T\boldsymbol{E}_g/n$ and that the LD correlation matrix is $\boldsymbol{R} = \boldsymbol{X}^T\boldsymbol{X}/n$. That is,

$$\boldsymbol{X}^T\boldsymbol{E}_g = n\widetilde{\boldsymbol{w}} \text{ and } \boldsymbol{X}^T\boldsymbol{X} = n\boldsymbol{R}. \qquad \text{(Equation 4)}$$

By approximating $n\boldsymbol{R}$ by $n\boldsymbol{R_s}$ ($\boldsymbol{R}_s = (1-s)\boldsymbol{R_r} + s\boldsymbol{I}$ with a tuning parameter $0 < s < 1$, a reference LD correlation matrix $\boldsymbol{R_r}$ from an external panel such as one from the 1000 Genomes Project[34], and an identity matrix $\boldsymbol{I}$) in the LASSO[30] penalized loss function, the frequentist *lassosum* method[27] can tune the LASSO penalty parameter and s using a pseudovalidation approach and then solve for eQTL effect size estimates $\widehat{\boldsymbol{w}}$ by minimizing the approximated LASSO loss function requiring no individual-level data (see details in Supplemental Methods).

Bayesian SDPR: Bayesian DPR method[31] as implemented in TIGAR[22] estimates $\widehat{\boldsymbol{w}}$ for the underlying multivariate regression model in Equation 1 by assuming a normal prior $N(0, \sigma_w^2)$ for w_j and a Dirichlet process prior[36] $DP(H, \alpha)$ for σ_w^2 with base distribution H and concentration parameter α. *SDPR*[29] assumes the same DPR model but can be applied to estimate the eQTL effect sizes $\widehat{\boldsymbol{w}}$ using only eQTL summary data (see details in Supplemental Methods).

Bayesian PRS-CS: The *PRS-CS* method[28] assumes the following normal prior for w_j and non-informative scale-invariant Jeffreys prior on the residual variance $\sigma_\epsilon{}^2$ in Equation 1

$$w_j \sim N\left(0, \frac{\sigma_\epsilon^2}{n}\psi_j\right), \;\; p(\sigma_\epsilon^2) \propto \sigma_\epsilon{}^2; \;\; \psi_j \sim Gamma(a, \delta_j), \delta_j \sim Gamma(b, \phi),$$

where local shrinkage parameter ψ_j has an independent gamma-gamma prior and ϕ is a global-shrinkage parameter controlling the overall sparsity of $\boldsymbol{w}$. *PRS-CS* sets hyper parameters a = 1 and b = 1/2 to ensure the prior density of w_j to have a sharp peak around zero to shrink small effect sizes of potentially false eQTL towards zero, as well as heavy, Cauchy-like tails which asserts little influence on eQTLs with larger effects. Posterior estimates $\widehat{\boldsymbol{w}}$ will be obtained from

eQTL summary data (i.e., marginal effect size estimates $\tilde{\boldsymbol{w}}$ and p-values) and reference LD correlation matrix $\boldsymbol{R}$ by Gibbs Sampler (see details in Supplemental Methods). We set ϕ as the square of the proportion of causal variants in the simulation and as 10^{-4} per gene in the real data application.

OTTERS Framework

As shown in Figure 1, OTTERS first trains GReX imputation models per gene *g* using *P+T, lassosum, SDPR,* and *PRS-CS* methods that each infers cis-eQTLs weights using cis-eQTL summary data and an external LD reference panel of similar ancestry (Stage I). Once we derive cis-eQTLs weights for each training method, we can impute the respective GReX using that method and perform the respective gene-based association analysis in the test GWAS dataset using the formulas given in Equation 2 (Stage II). We thus derive a set of TWAS p-values for gene *g*; one p-value for each training model that we applied. We then use these TWAS p-values to create an omnibus test using the ACAT-O[32] approach that employs a Cauchy distribution for inference (see details in Supplemental Methods). We refer to the p-value derived from ACAT-O test as the OTTERS p-value.

Simulation Study

We used real genotype data from gene *ABCA7* (2,156 SNPs with minor allele frequency > 5%) within 1894 whole genome sequencing (WGS) samples from the Religious Orders Study and Rush Memory and Aging Project (ROS/MAP) cohort[37,38] and Mount Sinai Brain Bank (MSBB) study[39]. We randomly split samples into 568 training (30%) and 1326 testing samples (70%). From the genotype data, we simulated various proportions of casual cis-eQTL, $p_{causal} = (0.001, 0.01, 0.1, 0.2)$, as well as the proportions of gene expression variance explained by such causal eQTL, $h_e^2 = (0.05, 0.1, 0.2, 0.5)$. We generated gene expression data using the multivariate regression model in Equation 1, where $\boldsymbol{X}_g$ represents the standardized genotype

matrix of the randomly selected causal eQTL, $\boldsymbol{w} \sim N(0, h_e^2)$, and $\boldsymbol{\epsilon}_g \sim N(0, (1-h_e^2)\boldsymbol{I})$. We then generated phenotypes from gene expression using the model $\boldsymbol{Y} = \beta \boldsymbol{E}_g + \boldsymbol{\epsilon}$, $\beta \sim N(0,1)$, $\boldsymbol{\epsilon} \sim N(0, (1-h_p^2)\boldsymbol{I})$, where h_p^2 denotes the proportion of phenotypic variance explained by simulated gene expression. We considered the following combinations $(h_e^2, h_p^2) = (0.05, 0.8), (0.1, 0.4), (0.2, 0.15), (0.5, 0.07)$ for generating expression and phenotype data. After simulating the individual-level gene expression and phenotype data, we then generated eQTL summary data and reference LD panels from training samples, and summary-level GWAS data for test samples. We generated 1000 replicates for each simulation scenario.

In Stage I of our TWAS analysis, we applied *P+T (0.001)*, *P+T (0.05)*, *lassosum*, *SDPR*, and *PRS-CS* methods to estimate eQTL weights using eQTL summary data and the reference LD panel. In Stage II of the TWAS, we imputed GReX using estimated eQTL weights in our test GWAS sample and conducted gene-based association using both individual-level and summary-level GWAS datasets. In addition to constructing gene-based association tests based on eQTL weights from each training method, we further constructed the corresponding OTTERS p-values as well. We evaluated the performance of the training methods on eQTL reference data in terms of test R^2 that was the squared Pearson correlation coefficient between imputed GReX and simulated gene expression. We evaluated TWAS power as the proportion of 1,000 repeated simulations with TWAS p-value $< 2.5 \times 10^{-6}$ (genome-wide significance threshold adjusting for testing 20K independent genes). Additionally, to evaluate the type I error, we conducted null simulations 10^6 times where phenotypes were randomly simulated from $N(0,1)$. We randomly picked one simulated replicate from the scenario with $h_e^2 = 0.2$ and $p_{causal} = 0.01$ and then permuted the eQTL weights for TWAS in these null simulations.

GReX Imputation Accuracy of Training Methods in GTEx V8 Blood Samples

To evaluate the imputation accuracy of *P+T (0.001)*, *P+T (0.05)*, *lassosum*, *SDPR*, and *PRS-CS* methods in real data, we downloaded GTEx V8 (dbGaP phs000424.v8.p2) which contains comprehensive profiling of WGS genotype data and RNA-sequencing (RNA-seq) transcriptomic data across 54 tissue types of 838 donors. To impute gene expression in GTEx, we applied our training models to summary-level eQTL reference data from the eQTLGen consortium[23] based on meta-analysis across 37 individual cohorts (n=31,684), consisted with 25,482 blood (80.4%) and 6,202 peripheral blood mononuclear cell (19.6%) samples. We considered SNPs with minor allele frequency (MAF) > 0.01, Hardy–Weinberg P value >0.0001, call rate >0.95, genotype imputation r^2 > 0.5 and observed in at least 2 cohorts[23]. We only considered cis-eQTL (within $\pm$1MB around gene transcription start sites (TSS)) with test sample size > 3000. Marginal standardized eQTL effect sizes were approximated by $\widetilde{w}_j \approx Z_j/\sqrt{median(n_{g,j})}$, where $median(n_{g,j})$ denotes the median sample size of all cis-eQTLs for the target gene g. The median cis-eQTL sample size per gene was also taken as the sample size value required by *lassosum, SDPR,* and *PRS-CS* methods. Since eQTLGen summary data were obtained by meta-analysis of 37 cohorts, the sample size per test SNP could vary across all cis-eQTLs of the test gene. The median cis-eQTL sample size ensures a robust performance for applying those eQTL summary data based methods.

Using eQTLGen, we trained 5 GReX imputation models: *P+T (0.001), P+T (0.05), lassosum, SDPR,* and *PRS-CS* using a reference LD panel from GTEx V8 WGS samples[40]. LD blocks were determined externally by ldetect[41] for *lassosum* and *PRS-CS*, while internally for *SDPR* which ensure that SNPs in one LD block do not have nonignorable correlation (R^2 > 0.1) with SNPs in other blocks. For each training model, we then imputed GReX in 315 test samples of whole blood tissue that are in GTEx V8 but were not part of GTEx V6 (as GTEx V6 samples were used in the eQTLGen meta-analysis to generate the reference summary data). For these

315 samples, we compared imputed GReX to observed expression levels. We considered trained imputation models with test $R^2 \geq 0.01$ as "valid" models. For such models, we compared the test R^2 across the five training models.

TWAS of Cardiovascular Disease using Summary-Level eQTLGen Data and UKBB GWAS Data

We applied our OTTERS framework to the summary-level GWAS data of Cardiovascular Disease from UKBB (n=459,324, case fraction = 0.319)[33], which were generated by BOLT-LMM based on the Bayesian linear mixed model per SNP[42] with assessment center, sex, age, and squared age as covariates. Although BOLT-LMM was derived based on a quantitative trait model, it can be applied to analyze case-control traits and has well-controlled false positive rate when the trait is sufficiently balanced with case fraction ≥ 10% and samples are of the same ancestry[33]. The tested dichotomous cardiovascular disease_phenotype includes a list of sub-phenotypes: hypertension, heart/cardiac problem, peripheral vascular disease, venous thromboembolic disease, stroke, transient ischaemic attack (tia), subdural haemorrhage/haematoma, cerebral aneurysm, high cholesterol, and other venous/lymphatic disease.

We used the eQTL summary data from the eQTLGen consortium[23] and applied the same quality-control procedures to this reference dataset as outlined in the previous section. We trained 5 GReX imputation models: *P+T (0.001), P+T (0.05), lassosum, SDPR, and PRS-CS* with eQTLGen reference data using the LD panel from 838 GTEx V8 WGS samples[40]. Using eQTL weights trained under each of the training models, we applied Z-score statistics as used by FUSION and S-PrediXcan (Equation 2) to obtain TWAS statistics for every eligible gene across the genome. We then performed genomic control[43] for TWAS test statistics generated under each specific training model and then combined the adjusted p-values across training models using ACAT-O to obtain our OTTERS test statistics and p-values. Genes with OTTERS p-values < 2.998×10^{-6} (Bonferroni corrected significance level) were identified as significant TWAS genes for cardiovascular risk. We compared our OTTERS results with the TWAS results shown on

TWAS hub obtained by FUSION using the same UKBB GWAS summary data of cardiovascular disease but using a smaller individual-level reference expression dataset from GTEx V6 (n=338).

Results

Simulation Results

Figures 2A and 2B provide the test R^2 and TWAS power, respectively, of our OTTERS framework across a range of simulation models that varied the proportion of true causal cis-eQTL (p_{causal}), gene expression heritability (h_e^2), and proportion of phenotype variance explained by gene expression (h_p^2). As shown in these figures, we observed that the training method with optimal performance depended on the underlying genetic architecture of gene expression as well as the effect that expression had on phenotype. In situations where true cis-eQTLs were sparse ($p_{causal} = 0.001$) and the gene expression heritability was relatively small, the *P+T (0.001)* method performed the best with the highest test R^2 and TWAS power among all individual methods. However, when gene expression heritability increased in a sparse eQTL model, the training methods *lassosum, SDPR, PRS-CS* outperformed the *P+T* methods with higher test R^2 and TWAS power. Generally, we observed that *lassosum* performed better when the true eQTL signal was sparse with $p_{causal} = (0.001, 0.01)$ and *SDPR* performed better when true eQTL was less sparse with $p_{causal} = (0.1, 0.2)$. Meanwhile, although *PRS-CS* was not the top performer in all simulation settings, this particularly training method performed robustly well across all considered scenarios. LD-clumping as described in the Methods section of the *P+T* method with R_T=0.99 was performed for all individual methods in both simulation and real studies. Based on our simulation and application results, this clumping step included in all training methods did not influence the GReX imputation accuracy and TWAS power (Figures S1-S2).

Compared to TWAS power of individual methods, the omnibus OTTERS achieved the highest or the second highest TWAS power in almost all scenarios (Figure 2B). Overall, these results demonstrated that OTTERS aggregates the strength of these complementary individual

training methods that each assumes different underlying models, and thereby produces a powerful test that is robust across a range of genetic architecture and mechanisms. We note that, while we present TWAS power in Figure 2B using summary-level GWAS data, we observed similar TWAS power and trends using individual-level GWAS data (Figure S3). We also present type I error rates of OTTERS as well as individual methods at different significant levels in Table S1. Overall, OTTERS had well-controlled type I errors across all significance levels considered. The corresponding quantile-quantile (QQ) plots of OTTERS p-values (Figure S4) further demonstrate the method is well calibrated.

GReX Imputation Accuracy in GTEx V8 Blood Samples

Comparing imputed gene expression to observed gene expression in GTEx V8 blood samples for each training method, we observed that *PRS-CS* obtained the most "valid" models (test R^2≥0.01) for genes. Among 16,699 tested genes, *PRS-CS* obtained 10,337 valid genes compared to 9,816 genes by *P+T (0.001)* (5.0% less valid genes than PRS-CS), 9,662 genes by *P+T (0.05)* (6.5% less valid genes), 8,718 genes by *lassosum* (15.7% less valid genes), and 9,670 genes by *SDPR* (6.5% less valid genes) (Table 1). Among the "valid" gene models obtained by each method, the ones trained by *PRS-CS* have the highest median GReX test R^2. The *P+T (0.001)* method obtained the second most "valid" gene models with the second largest median GReX test R^2, as compared to *P+T (0.05)*, *lassosum*, and *SDPR* (Table 1).

By comparing test R^2 per "valid" gene model by *PRS-CS* versus the other methods (Figure 3A), we observed that *PRS-CS* had the best overall performance for imputing GReX as it provided the most "valid" gene models with higher GReX imputation accuracy compared to other methods. We also note that the GReX imputation performance by *PRS-CS* is not sensitive to the choice of the global shrinkage parameter ϕ employed by this particular training model (Figure S5). In regards to comparing the test R^2 among the other four training methods, we observed that the

two *P+T* methods obtained similar test R^2 per "valid" gene model. Meanwhile, the test R^2 per valid gene varied widely among the *P+T* methods, *lassosum*, and *SDPR* (Figure S6), suggesting that none of these four were optimal and their performance likely depended on underlying genetic architecture (which was consistent with our simulation results).

TWAS of Cardiovascular Disease

Using OTTERS, we performed TWAS of cardiovascular disease in the summary-level UKBB GWAS for 16,678 genes by applying the Z-score statistics as in Equation 2 (as used by FUSION and S-PrediXcan) using eQTL weights trained from the reference eQTLGen consortium data and reference LD from GTEx V8. We obtained TWAS p-values for each gene per individual training method (*P+T (0.001), P+T (0.05), lassosum, SDPR, and PRS-CS*). We then combined these results using the ACAT-O test to obtain the OTTERS p-value. In total, we identified 37 significant TWAS genes using either OTTERS or any of the individual training methods. To identify independently significant TWAS genes, we calculated the R^2 between the GReX predicted by *PRS-CS* for of each pair of genes. For a pair of genes with the predicted GReX $R^2 > 0.5$, we only kept the gene with the smaller TWAS p-value in our final TWAS results.

OTTERS obtained 22 independently significant TWAS genes (Table 2, Figure 3B), compared to 17 by *P+T (0.001)*, 13 by *P+T (0.05)*, 10 by *lassosum*, 15 by *SDPR*, and 18 by *PRS-CS*. Among the 22 TWAS risk genes identified by OTTERS, gene *RP11-378A13.1* (OTTERS p-value = 1.58e-06) was not within 1 MB of any known GWAS risk loci with genomic-control corrected p-value < 5×10^{-8} in the UKBB summary-level GWAS data. This novel risk gene *RP11-378A13.1* was also identified to be a significant TWAS risk gene in blood tissue for systolic blood pressure, high cholesterol, and cardiovascular disease by FUSION. We compared the OTTERS results using summary-level reference data from eQTLGen to FUSION results shown in TWAS hub (see Web Resources) based on smaller individual-level reference data from GTEx V6. FUSION identified 18 out of these 22 genes for cardiovascular disease

considering all available tissue types in GTEx reference data. However, only 7 of them (*CLCN6b, ZDHHC18, PSRC1, RP11-378A13.1, CAMK1D, MTHFSD, OPRL1*) were detected by FUSION using the GTEx reference data of whole blood tissue. The remaining 11 (*NPPA, ABCF3, CPEB2, NT5C2, TNNT3, C11orf49, SIPA1, MAPKAPK5-AS1, FES, USP36, RASIP1*) were identified by FUSION using GTEx reference data of other tissue types, such as artery, thyroid, adipose visceral, and nerve tibial, which might be more directly related to cardiovascular disease[44–46]. For example, the most significant gene *FES* (OTTERS p-value = 9.05e-30) was identified by FUSION using GTEx reference data of artery tibial, thyroid, and adipose visceral omentum tissues, and was also identified as a TWAS risk gene for high blood pressure that are strongly related to cardiovascular disease[47]. Genes *ABCF3*, *NT5C2* and *TNNT3* were identified by FUSION using artery aorta, which is in charge of bringing oxygenated blood from the left ventricle to the rest of the body[48].

Our OTTERS method also identified 4 novel risk genes (*HCG4B, PRELID1P1, RP3-473L9.4, EDN3*) that were not detected by FUSION, where *EDN3* (Endothelin 3, a member of the endothelin family) was shown to be active in the cardiovascular system and play an important role in the maintenance of blood pressure or generation of hypertension[49].

By comparing OTTERS results with the ones obtained by individual methods (Table 2; Figure 3B; Figure S7), we found that all individual methods contributed to the OTTERS results. For example, *RP11-378A13.1* was only detected by *PRS-CS* while *ABCF3*, *CPEB2*, *CAMK1D*, and *MTHFSD* were only identified by *lassosum* and *SDPR* and the novel risk gene *EDN3* was only identified by the *P+T* methods. These results were consistent with our simulation study results that showed that different individual methods were optimal depending on underlying genetic architecture. We do note that there were a handful of genes identified by an individual method that were not significant using OTTERS (Table S2). Nonetheless, the omnibus test

borrows strength across all individual methods, thus generally achieves higher TWAS power and identifies the group of most robust TWAS risk genes.

By examining the quantile-quantile (QQ) plots of TWAS p-values, we observed a moderate inflation for all methods (Figure S8). Such inflation in TWAS results is not uncommon[50–52], which could be due to similar inflation in the GWAS summary data and not distinguishing the pleiotropy and mediation effects for considered gene expression and phenotype of interest[53] (Figure S9). We observed a notable inflation in the GWAS p-values of cardiovascular disease from UKBB (Figure S9) as we estimated the LD score regression[54] intercept to be 1.1 from the GWAS summary data.

Computational Time

The computational time per gene of the different PRS method depends on the number of initial variants considered within and around the target gene. Thus, we calculated the computational time and memory usage for 4 groups of genes that assumed the number of initial variants were <2000, from 2000 to 3000, from 3000 to 4000, and >4000, respectively. Among 16,699 tested genes with both eQTL summary statistics and GTEx LD reference data, the median number of initial variants per gene is 3152, and the proportion of genes in each group is 10.3%, 33.4%, 34.5%, and 21.8%, respectively. For each group, we randomly selected 10 genes on Chromosome 4 to evaluate the average computational time and memory usage per gene. We benchmarked the computational time and memory usage of each method on one Intel(R) Xeon(R) processor (2.10 GHz). The evaluation was based on 1000 MCMC iterations for SDPR and PRS-CS (default) without parallel computation (Table S3). We show that *P+T* and *lassosum* are computationally more efficient than *SDPR* and *PRS-CS*, whose speed are impeded by the need of MCMC iterations. Between the two Bayesian methods, SDPR implemented in C++ uses significantly less time and memory than PRS-CS implemented in Python.

Discussion

To the best of our knowledge, our OTTERS framework represents the first omnibus TWAS tool that can leverage summary-level expression and genotype results from a reference sample, thereby robustly expanding the use of TWAS into more settings. To this end, we adapted and evaluated five different PRS methods assuming different underlying models, including the relatively simple method *P+T*[26] with two different p-value thresholds 0.001 and 0.05, the frequentist method *lassosum*[27], as well as the Bayesian methods *PRS-CS*[28] and *SDPR*[29]. In simulation studies, we demonstrated that the performance of each of these five PRS methods depended substantially on the underlying genetic architecture for gene expression, with *P+T* methods generally performing better for sparse architecture whereas the Bayesian methods performing better for denser architecture. Consequently, since genetic architecture of gene expression is unknown apriori, we feel this justifies the use of the omnibus TWAS test implemented in OTTERS for practical use as this test had near-optimal performance across all simulation scenarios considered. In our real data application using UKBB GWAS summary-level data, we compared OTTERS TWAS results using reference eQTL summary data from eQTLGen consortium to FUSION TWAS results using a substantially smaller individual-level reference dataset from GTEx. OTTERS identified 11 significant TWAS risk genes that were missed by FUSION using individual-level GTEx reference data of blood tissue, suggesting that the use of larger reference datasets like eQTLGen in TWAS can identify novel findings. Interestingly, the genes missed by FUSION were instead detected using individual-level GTEx reference data of other tissue types that are more directly related to cardiovascular disease.

Among all individual methods, *P+T* is the most computationally efficient method. The Bayesian methods *SDPR* and *PRS-CS* require more computation time than the frequentist method *lassosum* as the former set of methods require a large number of MCMC iterations for model fit. By comparing the performance of these five methods in terms of the imputation accuracy

in simulations and real applications, we conclude that although *PRS-CS* showed the best overall performance, none of these methods were optimal across different genetic architectures. We found that all methods provided distinct and considerable contributions to the final OTTERS TWAS results. These results demonstrate the benefits of OTTERS in practice, since OTTERS can combine the strength of these individual methods to achieve the optimal performance.

To enable the use of OTTERS by the public, we provide an integrated tool (see Data and Code Availability) to: (1) Train GReX imputation models (i.e., learn eQTL weights in Stage I) using eQTL summary data by *P+T*, *lassosum*, *SDPR*, and *PRS-CS*; (2) Conduct TWAS (i.e., testing gene-trait association in Stage II) using both individual-level and summary-level GWAS data with the learned eQTL weights; (3) Apply ACAT-O to aggregate the TWAS p-values from individual training methods. Since the existing tools for *P+T*, *lassosum*, *SDPR*, and *PRS-CS* were originally developed for PRS calculations, we adapted and optimized them for training GReX imputation models for genome-wide genes in our OTTERS tool. For example, we integrate TABIX[55] and PLINK[35] tools in OTTERS to extract input data per target gene more efficiently. We also enable parallel computation in OTTERS for training GReX imputation models and testing gene-trait association of multiple genes.

The OTTERS framework does have its limitations. First, training GReX imputation models by all individual methods on average cost ~20 minutes for all 5 training models per gene, which might be computationally challenging for studying eQTL summary data of multiple tissue types and for ~20K genome-wide genes. Users might consider prioritize *P+T(0.001)*, *lassosum,* and *PRS-CS* training methods as these three provide complementary results in our studies. Second, the currently available eQTL summary statistics are generally derived from individuals of European descent. Our OTTERS trained GReX imputations model based on these eQTL summary statistics, and the resulting imputed GReX, could consequently have attenuated cross-population predictive performance[56]. This will further limit the transferability of our TWAS results

across populations. Third, our OTTERS cannot provide the direction of the identified gene-phenotype associations, which should be referred to the sign of the TWAS Z-score statistic per training method. Last, our current OTTERS tool only considers cis-eQTL effects because of computational demands of considering millions of trans-eQTL and the low availability of highly powered trans-eQTL reference datasets. Extension of OTTERS to enable cross-population TWAS and incorporation of trans-eQTL effects is part of our ongoing research but out of the scope of this work.

In conclusion, our novel OTTERS framework using large-scale eQTL summary data has the potential to identify more significant TWAS risk genes than standard TWAS tools that use smaller individual-level reference transcriptomic data. This tool provides the opportunity to leverage not only available public eQTL summary data of various tissues for conducting TWAS of complex traits and diseases, but also the emerging summary-level data of splicing QTLs, methylation QTLs, metabolomics QTLs, and protein QTLs. For example, OTTERS could be applied to perform proteome-wide association studies using summary-level reference data of genetic-protein relationships such as those reported by the SCALLOP consortium[57], and epigenome-wide association studies using summary-level reference data of methylation-phenotype relationships reported by Genetics of DNA Methylation Consortium (GoDMC) (see Web Resources). The wide application feasibility of integrating summary-level QTL data and GWAS data make our OTTERS tool valuable in current multi-omics studies of complex traits and diseases.

Figure Titles and Legends

Figure 1. OTTERS framework.

OTTERS estimates cis-eQTL weights from eQTL summary data and reference LD panel using four imputation models (Stage I) and performing ACAT-O test to combine gene-based association test p-values from individual methods with individual/summary level test GWAS data (Stage II).

Figure 2. Test R^2 (A) and TWAS power (B) comparison in simulation studies by 5 individual methods: *P+T(0.001), P+T(0.05), lassosum, SDPR, PRS-CS*, and *OTTERS*.

Various proportions of true casual cis-eQTL $p_{causal} = (0.001, 0.01, 0.1, 0.2)$ and gene expression heritability $h_e^2 = (0.05, 0.1, 0.2, 0.5)$ were considered in the simulation studies. The proportion of phenotype variance explained by gene expression was chosen with respect to h_e^2 values, that is, $(h_e^2, h_p^2) = (0.05, 0.8), (0.1, 0.4), (0.2, 0.15), (0.5, 0.07)$. TWAS was conducted using simulated summary-level GWAS data.

Figure 3. Test R^2(A) by *PRS-CS* versus *P+T(0.001)*, *P+T(0.05), lassosum*, and *SDPR* with 315 GTEx V8 test samples and Manhattan plot (B) of TWAS results by OTTERS using GWAS summary-level statistics of cardiovascular disease.

Test R^2(A) by *PRS-CS* versus *P+T(0.001), P+T(0.05)*, *SDPR,* and lassosum with 315 GTEx V8 test samples, with different colors denoting whether the imputation R^2 > 0.01 only by PRS-CS (red), only by the y axis method (green), or both methods (blue). Genes with $R^2 \leq 0.01$ by both methods were excluded from the plot. Manhattan plot (B) of TWAS results by OTTERS using GWAS summary-level statistics of cardiovascular disease and imputation models fitted based on eQTLGen summary statistics. Independently significant TWAS risk genes are labelled.

Tables

Table 1. Test R^2 in 315 whole blood tissue samples from GTEx V8.

	P+T(0.001)	P+T(0.05)	Lassosum	SDPR	PRS-CS
# Genes with $R^2 > 0.01$	9816	9662	8718	9670	10337
Median R^2 [a]	0.044	0.0430	0.0416	0.0418	0.0517

a: median R^2 among genes with test $R^2 > 0.01$ per method.

Table 2. Independent TWAS risk genes of cardiovascular disease identified by OTTERS, with the corresponding TWAS p-values by individual methods. Reference eQTL summary data from eQTLGen consortium and GWAS summary data from UKBB were used. Significant p-values are in bold.

CHR	ID	OTTERS	P+T(0.001)	P+T(0.05)	lassosum	SDPR	PRS-CS
1	CLCN6[a]	**1.68E-17**	**7.31E-09**	**1.90E-08**	**1.72E-14**	**4.21E-18**	**1.31E-11**
1	NPPA[b]	**3.51E-08**	**2.81E-09**	**9.25E-09**	3.26E-02	9.20E-01	**1.71E-07**
1	ZDHHC18[a]	**2.80E-06**	**1.62E-06**	**1.82E-06**	**7.44E-05**	1.48E-05	**1.25E-06**
1	PSRC1[a]	**1.26E-07**	**6.21E-07**	**2.30E-06**	1.05E-02	1.21E-01	**3.20E-08**
2	RP11-378A13.1[a]	**1.58E-06**	2.02E-02	2.40E-02	1.43E-01	4.63E-01	**3.96E-07**
3	ABCF3[b]	**6.27E-08**	3.14E-04	5.47E-04	**1.58E-08**	**1.58E-06**	4.52E-05
4	CPEB2[b]	**6.64E-07**	4.22E-03	3.31E-03	**2.10E-06**	**1.81E-07**	5.82E-05
6	HCG4B[c]	**8.30E-07**	8.40E-01	**7.61E-01**	1.33E-05	**2.11E-07**	1.47E-02
6	PRELID1P1[c]	**5.37E-10**	4.37E-02	**1.46E-10**	3.65E-05	**1.66E-09**	**8.76E-08**
10	CAMK1D[a]	**2.52E-07**	2.62E-02	3.59E-02	**1.59E-06**	**6.58E-08**	2.03E-05
10	NT5C2[b]	**5.59E-07**	**2.33E-06**	3.76E-06	5.64E-06	**1.74E-06**	**1.63E-07**
11	TNNT3[b]	**1.19E-10**	**6.15E-07**	**2.04E-06**	**2.47E-08**	**2.98E-11**	**1.58E-07**
11	C11orf49[b]	**1.27E-06**	**1.88E-07**	**3.18E-07**	2.30E-02	2.25E-03	2.88E-04
11	SIPA1[b]	**9.44E-07**	**1.14E-06**	**1.16E-06**	3.46E-06	**1.90E-06**	**3.90E-07**
12	RP3-473L9.4[c]	**2.46E-07**	**6.29E-07**	**7.46E-07**	**2.44E-07**	**1.37E-07**	**2.85E-07**
12	MAPKAPK5-AS1[b]	**7.67E-09**	**2.72E-06**	5.67E-06	**6.87E-08**	**1.97E-09**	2.87E-05
15	FES[b]	**9.05E-30**	**7.99E-10**	**6.59E-09**	**4.60E-24**	**2.26E-30**	**3.92E-20**
16	MTHFSD[a]	**1.37E-07**	1.59E-02	1.36E-02	**1.12E-06**	**3.54E-08**	7.51E-04
17	USP36[b]	**1.02E-10**	2.49E-04	3.56E-04	1.34E-05	4.70E-06	**2.55E-11**
19	RASIP1[b]	**7.11E-07**	**2.11E-06**	**2.48E-06**	5.92E-02	**2.15E-06**	**2.10E-07**
20	EDN3[c]	**2.38E-07**	**6.60E-08**	**5.95E-08**	1.64E-04	8.44E-03	3.31E-02
20	OPRL1[a]	**1.59E-06**	**1.80E-06**	4.23E-06	4.83E-05	7.34E-05	**4.45E-07**

a: Risk gene of UKBB cardiovascular disease in TWAS-hub identified using GTEx whole blood tissue.

b: Risk genes of UKBB cardiovascular disease in TWAS-hub identified using other GTEx tissue types.

c: Novel risk gene

Description of Supplemental Data

Supplemental data include 9 figures, 3 tables, and supplemental methods.

Declaration of Interests

The authors declare no competing interests.

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Web Resources

ACAT: https://github.com/yaowuliu/ACAT

eQTLGen: https://www.eqtlgen.org

FUSION: http://gusevlab.org/projects/fusion

GoDMC: http://www.godmc.org.uk

lassosum: https://github.com/tshmak/lassosum

OTTERS: https://github.com/daiqile96/OTTERS

PLINK: https://www.cog-genomics.org/plink

PRS-CS: https://github.com/getian107/PRScs

SDPR: https://github.com/eldronzhou/SDPR

TWAS hub: http://twas-hub.org

Data and Code Availability

eQTLGen consortium data are available from their portal website (https://www.eqtlgen.org). UK Biobank summary-level GWAS data are available through the Alkes Group (https://alkesgroup.broadinstitute.org/UKBB). Individual-level GTEx reference data are available through dbGap (Accession phs000424.v8.p2). ROS/MAP/MSBB WGS data used in our simulation studies are available through Synapse with data access application (https://www.synapse.org/#!Synapse:syn10901595). All source code and scripts used in this study are available through OTTERS Github page (https://github.com/daiqile96/OTTERS).

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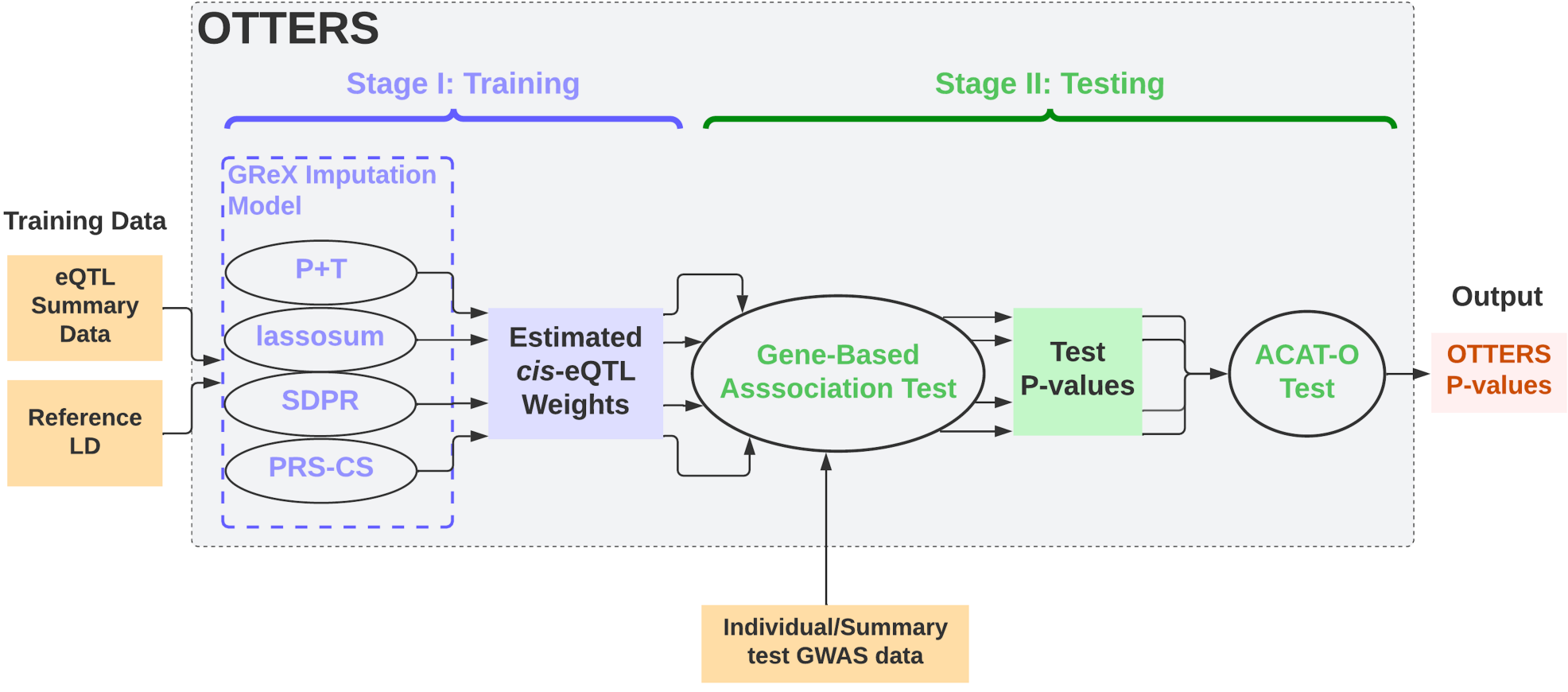
OTTERS
Stage I: Training
Stage II: Testing
GReX Imputation Model
Training Data
eQTL Summary Data
Reference LD
P+T
lassosum
SDPR
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ACAT-O Test
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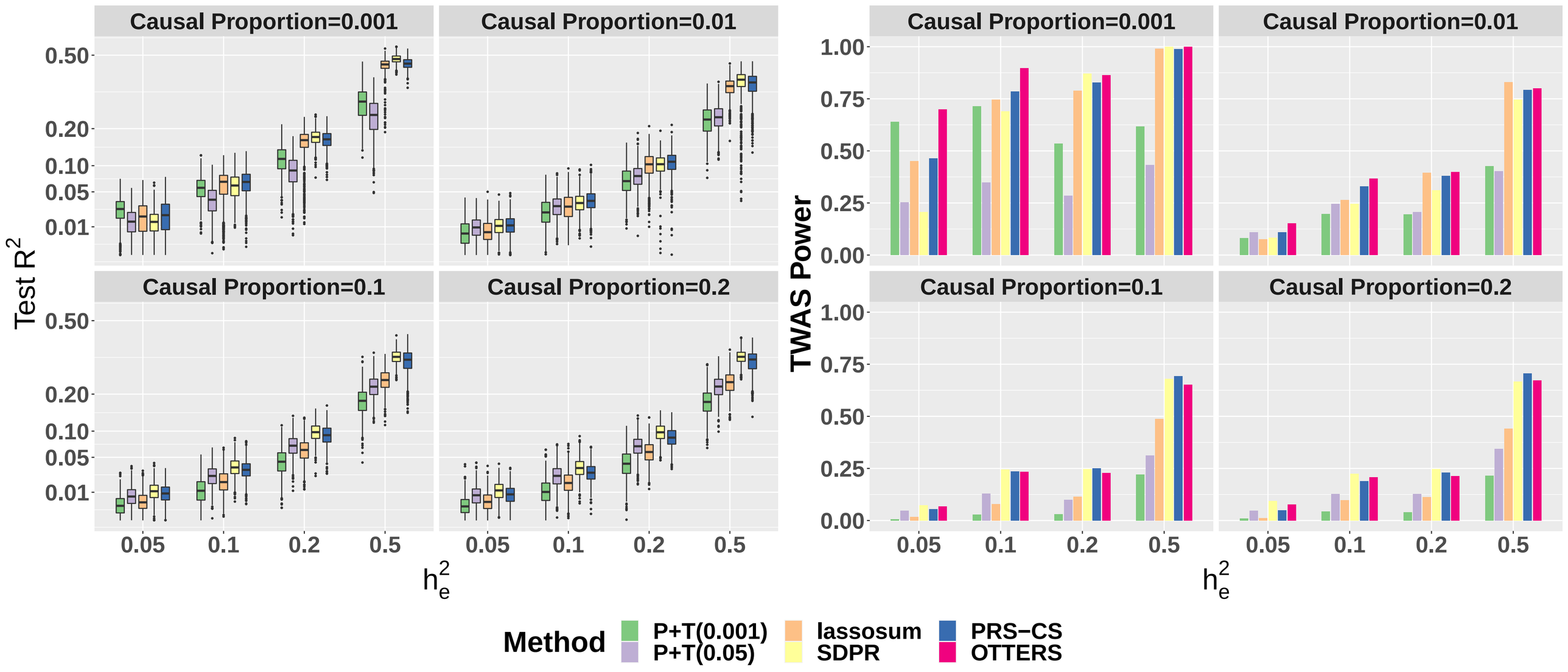
OTTERS P-values
Individual/Summary test GWAS data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Causal Proportion=0.01
Causal Proportion=0.1
Causal Proportion=0.2
Test R^2
0.50
0.20
0.10
0.05
0.01
TWAS Power
1.00
0.75
0.50
0.25
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0.05
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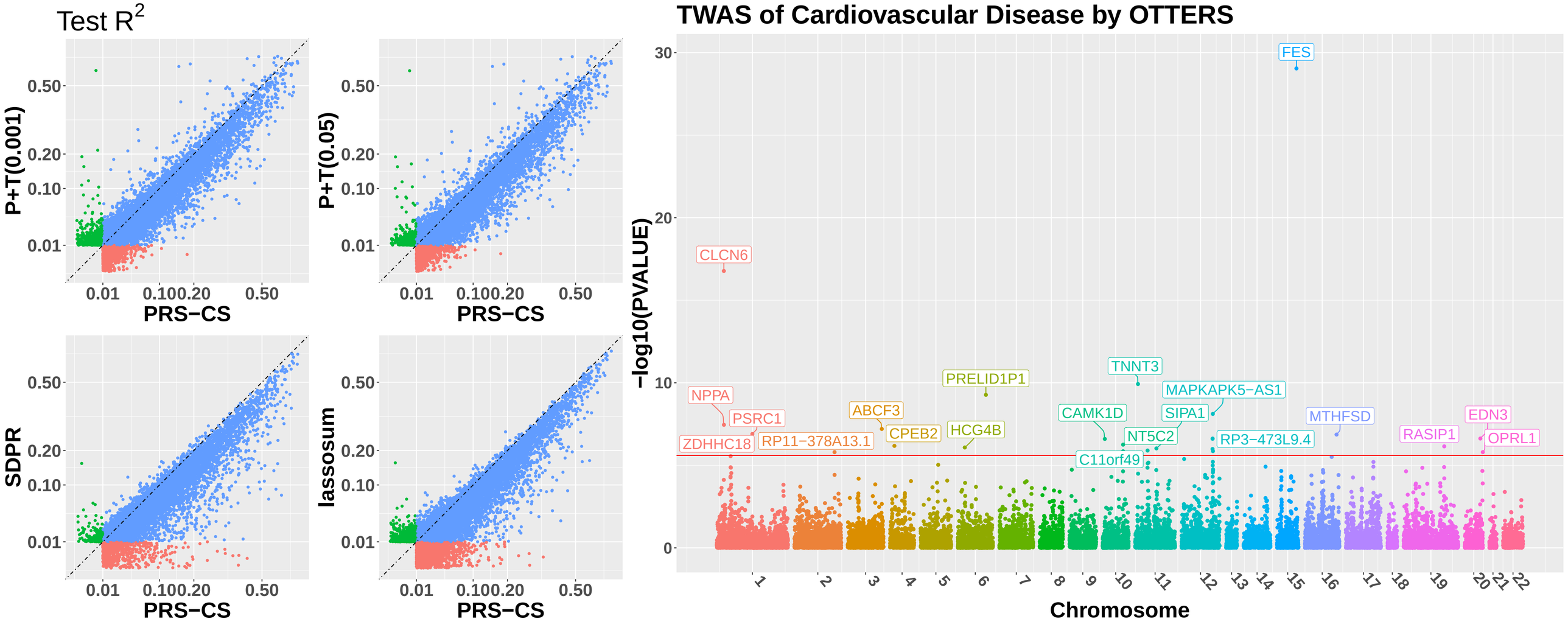
Method
P+T(0.001)
P+T(0.05)
lassosum
SDPR
PRS−CS
OTTERS


Test R2
P+T(0.001)
P+T(0.05)
SDPR
lassosum
PRS−CS
0.01
0.10
0.20
0.50
TWAS of Cardiovascular Disease by OTTERS
−log10(PVALUE)
Chromosome
FES
CLCN6
NPPA
PSRC1
ZDHHC18
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CPEB2
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