Multi-omic strategies for transcriptome-wide prediction and association studies

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Abstract

Traditional predictive models for transcriptome-wide association studies (TWAS) consider only single nucleotide polymorphisms (SNPs) local to genes of interest and perform parameter shrinkage entirely with a regularization process. These approaches ignore the effect of distal-SNPs or the functionality of the SNP-gene interaction. Here, we outline multi-omic strategies for transcriptome imputation from germline genetics for testing gene-trait associations by prioritizing distal-SNPs to the gene of interest. In one extension, we identify mediating biomarkers (CpG sites, microRNAs, and transcription factors) highly associated with gene expression and train predictive models for these mediators using their local SNPs. Imputed values for mediators are then incorporated into the eventual model as fixed effects with local SNPs to the gene included as regularized effects. In the second extension, we assess distal eQTLs for their mediation effect through mediators local to these distal-SNPs. Highly mediated distal-eQTLs are then included in the eventual transcriptomic prediction model. We show the utility of these extensions in with simulation analysis and real data applications with TCGA breast cancer data and in ROS/MAP brain tissue data, showing considerable gains in percent variance explained of gene expression and TWAS power to detect gene-trait associations. This integrative approach to transcriptome-wide imputation and association studies aids in understanding the complex interactions underlying genetic regulation within a tissue and identifying important risk genes for various traits and disorders.

Keywords: TWAS, GWAS, eQTL, mediation analysis

1. Introduction

Genomic methods that borrow information from multiple data sources, or omics, offer advantages in interpretability and statistical efficiency and opportunities to understand the flow of information in disease regulation [1, 2]. Transcriptome-wide association studies (TWAS) aggregate genetic information into functionally-relevant testing units that map to genes and their expression in a relevant tissue. This gene-based approach combines the effects of many regulatory variants into a single testing unit that increases study power and more interpretable trait-associated genomic loci [3, 4]. However, traditional TWAS methods like

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PrediXcan and FUSION focus on local genetic regulation of transcription. These methods ignore significant portions of heritable expression that can be attributed to distal genetic variants which may indicate complex mechanisms that contribute to gene regulation.

Recent work in transcriptional regulation has estimated that distal genetic variants can account for at least 70% of the variation in gene expression [5, 6]. This estimation follows directly from Boyle et al's omnigenic model, which proposes that regulatory networks are sufficiently interconnected that a majority of genetic variants in the genome, local or distal, have indirect effects on transcription [6, 7]. Many groups have leveraged this model to identify distal expression quantitative trait loci by testing the effect of a distal-SNP on a gene of interest mediated through a set of genes local to the SNP to conclude that many distal-eQTLs are often eQTLs for many local genes [8, 9, 10, 11, 12]. Furthemore, it has been shown previously that distal-eQTLs in regulatory hotspots are generally cell-type specific [8, 11, 13]. Deep learning methods have employed similar logic to link GWAS-identified variants to nearby regulatory mechanisms for functional hypothesis generation [14]. Furthermore, Zhang et al's recent EpiXcan method shows that incorporating epigenetic information into transcriptomic prediction models generally improves predictive performance and power in detecting gene-trait associations in TWAS [15].

Here, we outline two extensions to TWAS that draw from ideas of eQTL mediation and borrowing information from other omics. Using simulations and data from the The Cancer Genome Atlas (TCGA) [16] and the Religious Orders Study and the Rush Memory and Aging Project (ROS/MAP) [17], we show improvements in both transcriptomic prediction and power to detect gene-trait associations. These **M**ulti-**O**mic **S**trategies for **T**ranscriptome-**W**ide **A**ssociation **S**tudies are curated in the R package MOSTWAS (`www.github.com/bhattacharya-a-bt/MOSTWAS`).

2. Material and Methods

We first outline the two methods proposed in this work, (1) mediator-enriched transcriptome-wide prediction (MeTWAS) and (2) distal-eQTL prioritization via mediation analysis (DePMA). MeTWAS and DePMA are combined in the MOSTWAS R package, available freely at `https://bhattacharya-a-bt.github.io/MOSTWAS`.

2.1. Mediator-enriched TWAS (MeTWAS)

2.1.1. Transcriptomic prediction using MeTWAS

Here, we present mediator-enriched TWAS, or MeTWAS one of the two tools presented in the MOSTWAS R package. Across n samples, consider the vector Y_G of expression a gene G of interest, the matrix $\mathbf{X}_G$ of local SNPs dosages in a 500 kilobase window around gene G, and m_G mediating biomarkers that we estimate to be significantly associated with the expression of gene G via a relevant one-way test of association. These mediating biomarkers can be DNA methylation sites, microRNAs, or transcription factors. Accordingly, let the matrix $\mathbf{X}_{M_j}$ be the local-genotype dosages in a 500 kilobase window around mediator j, $1 \leq j \leq m_G$.

Furthermore, let M_j be the intensity of mediator j (i.e. methylation M-value if j is a CpG site or expression if j is a miRNA or a gene). Prior to any modelling, we scale Y_G and all $M_j,\ 1 \leq j \leq m_G$ to zero mean and unit variance. We also residualize $M_j,\ 1 \leq j \leq m_G$ and Y_G with the covariate matrix $\mathbf{X}_C$ to account for population stratification using principal components of the global genotype matrix and relevant clinical covariates to obtain $\tilde{M}_j,\ 1 \leq j \leq m_G$ and $\tilde{Y}_G$.

Transcriptome prediction in MeTWAS draws from two-step regression, as summarized in **Figure 1**. First, in the training set for a given training-test split, for $1 \leq j \leq m_G$, we model the residualized intensity $\tilde{M}_j$ of training-set specific mediator j with the following additive model:

$$\tilde{M}_j = \mathbf{X}_{M_j,train} w_j + \varepsilon_m, \tag{1}$$

where w_j is the effect-sizes of the SNPs in $\mathbf{X}_{M_j,train}$ on $\tilde{M}_j$ in the training set. As in traditional transcriptomic imputation models [3, 4], we find $\hat{w}_m$ using one of the two following methods with the largest predicted adjusted R^2: (1) elastic net regression with mixing parameter $\alpha = 0.5$ and λ tuned over 5-fold cross validation using glmnet [18], or (2) linear mixed modelling assuming random effects for $\mathbf{X}_{M_j}$ using rrBLUP [19].

For all j, using these optimized predictive models for M_j as denoted by $\hat{w}_{M_j}$, we estimate the genetically regulated intensity (GRIn) of the mediator m_j, denoted GRIn_{m_j}, in the test set. Denote $\hat{\mathbf{M}}$ as the $n \times m$ matrix of estimated GRIn, such that the jth column of $\hat{\mathbf{M}}_j$ is GRIn_{m_j} across all n samples.

Next, we consider the following additive model for the residualized expression of gene G:

$$\tilde{Y}_G = \hat{\mathbf{M}}\beta_M + \mathbf{X}_G w_G + \varepsilon_{Y_G},$$

where β_M is the fixed effect-sizes of GRIn_{m_j} on $\tilde{Y}_G$, $\hat{\mathbf{M}}$ is the matrix of estimated GRIn for all m_j mediators, $\mathbf{X}_G$ are the local-SNPs to gene G, and w_G are the "random" or regularized effect sizes of the local-SNPs. We estimate β_M by traditional ordinary least squares, where $\hat{\beta}_M = \left(\hat{\mathbf{M}}'\hat{\mathbf{M}}\right)^{-1}\hat{\mathbf{M}}'E_g$. Next, using one of the methods outlined above when estimating $\hat{w}_{M_j}$, we can generate estimated effect sizes $\hat{w}_G$ of the local-SNPs on $\tilde{Y}_G$, residualized with $\hat{\mathbf{M}}$.

2.1.2. Transcriptomic imputation with MeTWAS

In an external GWAS panel, if individual SNPs are available, we construct the mediator-enriched genetically regulated expression (MeGReX) of gene G directly using $\hat{w}_G$ and $(\hat{w}_j, \hat{\beta}_j),\ 1 \leq j \leq m_G$:

$$MeGReX_G = \sum_{j=two1}^{m_G} \mathbf{X}_{M_j,GWAS}\hat{w}_{M_j}\hat{\beta}_{M,j} + \mathbf{X}_{G,GWAS}\hat{w}_G,$$

where $\mathbf{X}_{M_j,GWAS}$ and $\mathbf{X}_{G,GWAS}$ are the SNPs in the GWAS panel local to mediator j and gene G, respectively. $MeGREX_G$ can be used in downstream tests of association.

2.2. Distal-eQTL prioritization via mediation analysis (DePMA)

2.2.1. Transcriptomic prediction using DePMA

Expression prediction in DePMA hinges on up-weighting distal-eQTLs to the gene of interest via mediation analysis, adopting methods from previous studies [9, 12, 10]. We first split data for gene expression, SNP dosages, and any potential mediators into k training-testing splits. Based on the minor allele frequencies of SNPs and sample size, we recommend a low number of splits (i.e. $k \leq 5$).

In the training set, we identify mediation test triplets that consist of (1) a gene of interest G with expression Y_G (scaled to zero mean and unit variance), (2) a distal eSNP s in association with G at a user-defined P-value threshold (default of $P = 10^{-6}$) with dosages X_s, and (3) a set of m biomarkers local to s that are associated with s at a user-defined P-value threshold (default of FDR-adjusted $P = 0.05$) with intensities in the m columns of $\mathbf{M}$. The columns of $\mathbf{M}$ are scaled to zero mean and unit variance. Consider the following mediation model for $1 \leq j \leq m$:

$$\begin{aligned} Y_G &= X_s\beta_s + \mathbf{M}\beta_{\mathbf{M}} + \mathbf{X}_C\beta_C + \varepsilon_{Y_G} \\ M_j &= X_s\alpha_{M_j} + \mathbf{X}_C\alpha_{C,j} + \varepsilon_{M_j}. \end{aligned} \tag{2}$$

Here, we have $\beta_{\mathbf{M}}$ as the effects of the M mediators local to s on Y_G adjusting for the effects from s and the covariates and $\alpha_{\mathbf{M}} = (\alpha_{M_1}, \ldots, \alpha_{M_m})'$ as the effects of s on mediators M_j, for $1 \leq j \leq m$. We assume that $\varepsilon_{Y_G} \sim N(0, \sigma^2)$ and $\varepsilon_{\mathbf{M}} \sim \mathbf{N}_m(0, \mathbf{\Sigma}_M)$, where $\mathbf{\Sigma}_M$ may have non-zero off-diagonal elements that represent non-zero covariance between mediator intensities. Further, we assume that ε_{Y_G} and $\varepsilon_{\mathbf{M}}$ are independent. We define the total mediation effect (TME) [20] of SNP s as

$$TME = \alpha_{\mathbf{M}}^T\beta_{\mathbf{M}}.$$

We are interested in SNPs with large TME, which we prioritize with the test of $H_0 : \ TME = 0$. We assess this hypothesis with a permutation test, as more direct methods of computing standard errors for the estimated TME are often biased [21, 12], obtaining a permutation P-value. We also provide an option to estimate an asymptotic approximation to the standard error of TME and conduct a Wald-type test for $TME = 0$. This asymptotic option is significantly faster at the cost of inflated false positives (see **Supplemental Materials** and **Supplemental Figure 1**). Corresponding to the t testing triplets identified, we obtain a vectors of length t of TMEs and P-values for each distal eSNP to G. We estimate Storey et al's q-value for each test to adjust for multiple testing. For the predictive model, we select distal SNPs with $TME \neq 0$ at a given q-value threshold ($q < 0.10$ as a default) and include them with all local SNPs in a design matrix. We then find estimated SNP weights using either elastic net or weighted least squared regression.

2.2.2. Transcriptomic imputation with DePMA

In an external GWAS panel, if individual SNPs are available, we construct the genetically regulated expression (GReX) of gene G directly using $\hat{w}_G$ and $\hat{w}_t$:

$$GReX_G = \mathbf{X}_{t,GWAS}\hat{w}_t + \mathbf{X}_{G,GWAS}\hat{w}_G,$$

where $\mathbf{X}_{t,GWAS}$ is the matrix of dosages of the t distal-SNPs and $\mathbf{X}_{G,GWAS}$ is the matrix of dosages of the local SNPs to gene G in the external GWAS panel. $GREX_G$ can be used in downstream tests of association. If individual SNPs are not available, the weighted burden test can be employed using summary statistics with permutation follow-up test [4].

2.3. Tests of associations

If individual SNPs are not available, then the weighted burden Z-test proposed by Gusev et al can be employed [4] using summary statistics. Briefly, we compute

$$\tilde{Z} = \frac{\mathbf{W}Z}{(\mathbf{W}\Sigma_{s,s}\mathbf{W}^T)^{1/2}}. \tag{3}$$

Here, Z is the vector of Z-scores of SNP-trait associations for SNPs used in estimating $\hat{w}_{M_j}$ and $\hat{w}_G$. The matrix W is defined as $\Sigma_{e,s}\Sigma_{s,s}^{-1}$, the product of the covariance matrix between all SNPs and the expression of gene G and the covariance matrix among all SNPs. These covariance matrices are estimated from the reference panel used to estimate $\hat{w}_{M_j}$ and $\hat{w}_G$. and $\hat{w}_G$. The test statistic $\tilde{Z}$ can be compared to the standard Normal distribution for inference. We implement a permutation test conditioning on the GWAS effect sizes to assess whether the same distribution of $\hat{w}_G$ effect sizes could yield a significant association by chance [4]. We permute $\hat{w}_G$ 1,000 times without replacement and recompute the weighted burden test statistic to generate a permutation null distribution for $\tilde{Z}$. This permutation test is only conducted for overall associations at a user-defined significance level.

Lastly, we also implement a test to assess the information added from distal-eSNPs in the weighted burden test beyond what we find from local SNPs. This added-last test can be thought of as a group added-last test in regression analysis, applied here to GWAS summary statistics. Let Z_l and Z_d be the vector of Z-scores from GWAS summary statistics from local and distal-SNPs identified by a MOSTWAS model. The local and distal-SNP effects from the MOSTWAS model are represented in $\mathbf{w}_l$ and $\mathbf{w}_d$. Formally, we test whether the weighted Z-score $\tilde{Z}_d \equiv \mathbf{w}_d^T Z_d$ from distal-SNPs is significantly larger than 0 given the observed weighted Z-score from local SNPs $\tilde{Z}_l \equiv \mathbf{w}_l^T Z_l$, drawing from the assumption that $(\tilde{Z}_l, \tilde{Z}_d)$ follow a bivariate Normal distribution. Namely, we conduct a two-sided Wald-type test for the null hypothesis:

$$H_0 : \mathbf{w}_d^T\mathbf{Z}_d|\mathbf{w}_l^T\mathbf{Z}_l = \tilde{Z}_{l,\text{obs}} = 0.$$

Under the null hypothesis, we can derive that the distribution of $\tilde{Z}_d|\tilde{Z}_l = \tilde{Z}_{l,\text{obs}}$ is normally distributed with mean and variance determined from the observed local $\tilde{Z}_l$-score, the SNP-effect size vectors $\mathbf{w}_l$ and $\mathbf{w}_d$, and components of the linkage disequilibrium as estimated from the reference panel [22]. Full details and derivation for this added-last test are given in **Supplemental Methods**.

2.4. Simulation framework

We first conducted simulations to assess the predictive capability and power to detect gene-trait associations under various phenotype (h^2_p), local heritability of expression ($h^2_{e,l}$), distal heritability of expression ($h^2_{e,d}$), and proportion of causal local ($p_{c,l}$) and distal ($p_{c,e}$) SNPs for MeTWAS and DePMA. We considered two scenarios for each combination of $(h^2_p, h^2_{e,l}, h^2_{e,d}, p_{c,l}, p_{c,e})$: (1) the leveraged association between the distal-SNP and gene of interest exists in both the reference and imputation panel, and (2) the leveraged association between distal-SNP and gene of interest exists in the reference panel but is null in the imputation panel.

Using TCGA data, we extracted 2,592 SNPs local to the gene *ESR1* on Chromosome 6 and 1,431 SNPs local to the gene *FOXA1*. We generated (1) a reference panel with sample size 400 with simulated SNPs, expressions, and one mediators and (2) a GWAS panel of 1,500 samples with simulated SNPs and phenotypes using the following data generating process, modified from Mancuso et al's framework [23]:

We estimated the linkage disequilibrium LD matrix of the SNPs X_G with n samples and p SNPs, as follows with regularization to ensure LD is positive semi-definite:

$$LD = \frac{1}{n} X_G^T X_G + \frac{1}{10} I_p.$$

We computed the Cholesky decomposition of LD for faster sampling [23]. We simulated SNPs for a 400-sample reference panel $X_{g,ref}$ and 1,500-sample GWAS panel $X_{g,GWAS}$.

We then simulated effect sizes for $p_{c,l}$ of the 2,592 local SNPs $w_{g,l}$ from a standard Normal distribution. We generated locally heritable expression

$$E_{g,l} = X^T_{G,ref} w_{g,l} + \varepsilon_l,$$

with $\varepsilon_l \sim N(0, 1 - h^2_{e,l})$ and $w_{g,l}$ scaled to ensure the given $h^2_{e,l}$. Similarly, we simulated effect sizes for $p_{c,d}$ of the 1,431 distal-SNPs $w_{g,d}$ and generated the distally heritable intensity of the mediator $M_{g,d}$. We constructd the distally heritable expression $E_{g,d}$ by scaling $M_{g,d}$ by $\beta \sim N(0,1)$ and adding random noise that scales distal heritability to $h^2_{e,d}$. We lastly formed the total expression $E_g = E_{g,l} + Eg, d$.

Next, we simulated the phenotype in the GWAS panel such that the variance explained in the phenotype reflects only that due to genetics. We drew a causal effect size from gene expression $\alpha \sim N(0,1)$. We computed the "unobserved" gene expression in the GWAS panel as

$$E_{g,GWAS} = X^T_{g,GWAS,local} w_{g,l} + X^T_{g,GWAS,distal} w_{g,d} \beta.$$

Here, we also considered a "null" case as well, where the distal eQTLs are not detected in the GWAS panel (i.e. $w_{g,d} = 0$ for all distal-SNPs). GWAS summary statistics were computed in this step for downstream weighted burden testing. We then fitted predictive models using MeTWAS, DePMA, and local-only models (i.e. FUSION [4]), computed the adjusted predictive R^2 in the reference panel, and tested the gene-trait association in the GWAS panel using a weighted burden test. The association study power was defined as the proportion of gene-trait association tests with $P < 2.5 \times 10^{-6}$, the Bonferroni-corrected significance threshold for testing 20,000 independent genes. With these simulated datasets, we also assessed the power of the distal added-last test by computing the proportion of significant distal associations conditional on the local association at FDR-adjusted $P < 0.05$.

2.5. Data acquisition

2.5.1. Multi-omic data from TCGA-BRCA

We retrieved genotype, RNA expression, miRNA expression, and DNA methylation data for breast cancer indications in The Cancer Genome Atlas. Birdseed genotype files of 914 subject were downloaded from the Genome Data Commons (GDC) legacy (GRCh37/hg19) archive. Genotype files were merged into a single binary PLINK file format (BED/FAM/BIM) and imputed using the October 2014 (v.3) release of the 1000 Genomes Project dataset as a reference panel in the standard two-stage imputation approach, using SHAPEIT v2.87 for phasing and IMPUTE v2.3.2 for imputation [24, 25, 26]. We excluded variants (1) with a minor allele frequency of less that 1% based on genotype dosage, (2) that deviated significantly from Hardy-Weinberg equilibrium ($P < 10^{-8}$) using appropriate functions in PLINK v1.90b3 [27, 28], and (3) located on sex chromosomes. Final TCGA genotype data was coded as dosages, with reference and alternative allele coding as in dbSNP.

TCGA level-3 normalized RNA-seq expression data, miRNA-seq expression data, and DNA methylation data collected on Illumina Infinium HumanMethylation450 BeadChip were downloaded from the Broad Institute's GDAC Firehose (2016/1/28 analysis archive). We intersected to the subset of samples assayed for genotype (4,564,962 variants), RNA-seq (15,568 genes), miRNA-seq (1,046 miRNAs), and DNA methylation (485,578 CpG sites), resulting in a total of 563 samples. We only consider the autosome in our analyses. We adjusted gene and miRNA expression and DNA methylation by relevant covariates (5 principal components of the genotype matrix, tumor stage at diagnosis, and age).

2.5.2. Multi-omic data from ROS/MAP

We retrieved imputed genotype, RNA expression, miRNA expression, and DNA methylation data from The Religious Orders Study and Memory and Aging Project (ROS/MAP) Study for samples derived from human pre-frontal cortex [29, 30, 31].We excluded variants (1) with a minor allele frequency of less that 1% based on genotype dosage, (2) that deviated significantly from Hardy-Weinberg equilibrium ($P < 10^{-8}$) using appropriate functions in PLINK v1.90b3 [27, 28], and (3) located on sex chromosomes. Final ROS/MAP genotype data was coded as dosages, with reference and alternative allele coding as in dbSNP. We intersectted

to the subset of samples assayed for genotype (4,141,537 variants), RNA-seq (15,857 genes), miRNA-seq (247 miRNAs), and DNA methylation (391,626 CpG sites), resulting in a total of 370 samples. We only consider the autosome in our analyses. We adjusted gene and miRNA expression and DNA methylation by relevant covariates (20 principal components of the genotype age at death, and sex).

2.5.3. Summary statistics for downstream association studies

For association testing, we downloaded iCOGs GWAS summary statistics for breast cancer-specific survival for women of European ancestry [32]. Funding for BCAC and iCOGS came from: Cancer Research UK [grant numbers C1287/A16563, C1287/A10118, C1287/A10710, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565], the European Union's Horizon 2020 Research and Innovation Programme (grant numbers 634935 and 633784 for BRIDGES and B-CAST respectively), the European Community's Seventh Framework Programme under grant agreement n° 223175 [HEALTHF2-2009-223175] (COGS), the National Institutes of Health [CA128978] and Post-Cancer GWAS initiative [1U19 CA148537, 1U19 CA148065-01 (DRIVE) and 1U19 CA148112 - the GAME-ON initiative], the Department of Defence [W81XWH-10-1-0341], and the Canadian Institutes of Health Research CIHR) for the CIHR Team in Familial Risks of Breast Cancer [grant PSR-SIIRI-701]. All studies and funders as listed in Michailidou et al [33, 34] and in Guo et al [32] are acknowledged for their contributions.

For association testing, we downloaded GWAS summary statistics for risk of late-onset Alzheimer's disease from the International Genomics of Alzheimer's Project (IGAP) [35]. We also downloaded GWAS and genome-wide association by proxy (GWAX) summary statistics for risk of major depressive disorder (MDD) from the Psychiatric Genomics Consortium [36] and the UK Biobank [37], respectively.

IGAP is a large two-stage study based on GWAS on individuals of European ancestry. In stage 1, IGAP used genotyped and imputed data on 7,055,881 single nucleotide polymorphisms (SNPs) to meta-analyse four previously-published GWAS datasets consisting of 17,008 Alzheimer's disease cases and 37,154 controls (The European Alzheimer's disease Initiative – EADI the Alzheimer Disease Genetics Consortium – ADGC The Cohorts for Heart and Aging Research in Genomic Epidemiology consortium – CHARGE The Genetic and Environmental Risk in AD consortium – GERAD). In stage 2, 11,632 SNPs were genotyped and tested for association in an independent set of 8,572 Alzheimer's disease cases and 11,312 controls. Finally, a meta-analysis was performed combining results from stages 1 and 2.

2.6. Model training and association testing in TCGA-BRCA and ROS/MAP

Using both TCGA-BRCA and ROS/MAP multiomic data, we first identifed associations between SNPs and mediators (transcription factor genes, miRNAs, and CpG methylation sites), mediators and gene expression, and SNPs and gene expression using MatrixEQTL [38]. These QTL analyses were adjusted for 10 principal components of the genotype matrix to account for population stratification, along with other relevant covariates (tumor stage and age for TCGA-BRCA, age and sex and smoking status for ROS/MAP). For MeTWAS modeling, we considered the top 5 mediators associated with the gene of interest. For DePMA

models, we considered all distal-SNPs associated with gene expression at raw $P < 10^{-6}$ and any local mediators at FDR-adjusted $P < 0.05$. Local windows for all models were set to 0.5 Mb. For association testing, we consider only genes with significant non-zero estimated total heritability by GCTA-LDMS [39] and cross-validation adjusted $R^2 > 0$ across 5 folds. The MeTWAS or DePMA model with larger cross-validation R^2 was considered as the final MOSTWAS model for a given gene. All other modeling options in MeTWAS and DePMA were set to the defaults provided by the MOSTWAS package.

Using TCGA-BRCA models, we conducted TWAS burden testing [22, 4] in iCOGs GWAS summary statistics for breast cancer-specific survival in a cohort of women of European ancestry. We subjected TWAS-identified loci at Benjamini-Hochberg [40] FDR-adjusted $P < 0.05$ to permutation testing, and any loci that persisted past permutation testing to distal variation added-last testing.

Using ROS/MAP models, we first conducted TWAS burden testing in GWAS summary statistics for late-onset Alzheimer's disease risk from IGAP, prioritized 14 known risk loci identified from literature [35, 41, 42, 43]. We subjected TWAS-identified loci at Benjamini-Hochberg [40] FDR-adjusted $P < 0.05$ to permutation testing, and any loci that persisted past permutation testing to distal variation added-last testing. We similarly conducted TWAS for risk of major depressive disorder (MDD) using GWAS summary statistics from PGC (excluding data from 23andMe and the UK Biobank) with the necessary follow-up tests. For any TWAS-identified loci that persisted permutation in PGC, we further conducted TWAS in GWAX summary statistics for MDD risk in the UK Biobank [37] for replication.

3. Results

3.1. Overview of MOSTWAS

MOSTWAS incorporates two methods to include distal-eQTLs in transcriptomic prediction: Mediator-enriched TWAS (MeTWAS) and distal-eQTL prioritization via mediation analysis (DePMA). As large proportions of total heritable gene expression are explained by distal-eQTLs local to regulatory hotspots [6, 9, 11, 12], we used data-driven approaches to either identify mediating regulatory biomarkers (MeTWAS) or distal-eQTLs mediated by local biomarkers (DePMA) to increase predictive power and power to detect gene-trait associations. These methods are summarized graphically in **Figure 1**.

[Figure 1 about here.]

- MeTWAS first identifies m mediators (e.g. CpG sites, miRNAs, gene coding for transcription factors, etc) such that the intensity (methylation or expression levels) of these mediators are associated to the mRNA expression. A model for the genetically regulated intensities (GRIn) is estimated using the local SNPs to these mediators, and the GRIn of these mediators are imputed into the training set. The final gene expression model is estimated by incorporating the GRIn of the mediators as fixed effects and the local SNPs to the gene as regularized effects (see **Methods** for more details).

- DePMA first identifies testing triplets of the gene of interest, a distal eSNP (SNP in an eQTL) to the gene, and any associated mediators local to the eQTL. We estimate the total mediation effect (TME) of the eQTL on the gene through the set of mediators and test the two-sided hypothesis of $H_0 : TME = 0$ with one of two options (**Supplemental Figure 1**). Any distal-eSNP with a significant TME is included with the SNPs local to the gene of interest to form the final design matrix. A model including all local SNPs and all significant distal-eSNPs is fit to the expression of the gene using either elastic net or linear mixed modeling (see **Methods** for more details).

If individual genotype data is available in an external GWAS panel, using either a MeTWAS or DePMA model, we impute expression as a linear combination of the genotypes. If only summary statistics are available in the GWAS panel, the Imp-G weighted burden testing framework[22], as implemented in Gusev et al[4], can be applied. We further implement a permutation test to assess whether the overall association is significant conditional on the GWAS effect sizes[4] and an added-last test that assesses the added information from distal-SNPs given the weighted Z-score from local SNPs (see **Methods** and **Supplemental Materials**).

3.2. Simulation analysis

We first conducted simulations to assess the predictive capability and power to detect gene-trait associations under various settings for phenotype heritability (h^2_p), local heritability of expression ($h^2_{e,l}$), distal heritability of expression ($h^2_{e,d}$), and proportion of causal local ($p_{c,l}$) and distal ($p_{c,e}$) SNPs for MeTWAS and DePMA. We considered two scenarios for each combination of ($h^2_p, h^2_{e,l}, h^2_{e,d}, p_{c,l}, p_{c,e}$): (1) the leveraged association between the distal-SNP and gene of interest exists in both the reference and imputation panel, and (2) the leveraged association between distal-SNP and gene of interest exists in the reference panel but does not exist in the imputation panel. Using genetic data from TCGA-BRCA, we used the 2,592 SNPs local to the gene *ESR1* on Chromosome 6 to generate local eQTLs and the 1,431 SNPs local to the gene *FOXA1* to generate distal eQTLs for a 400-sample eQTL reference panel and 1,500-sample GWAS imputation panel (see **Methods**). Though the choice of these loci were arbitrary for simulations, there is evidence that *ESR1* and *FOXA1* are highly co-expressed in breast tumors and local-eQTLs of *FOXA1* have been shown to be distal-eQTLs of *ESR1*[44]. We believe these loci served as a strong reference for these simulations.

In these simulation studies, we found that MOSTWAS methods performed well in prediction across different causal proportions and local and distal mRNA expression heritabilities. Furthermore, across all simulation settings, we observed that MOSTWAS showed greater or equal power to detect gene-trait associations as local-only models. We saw that as the proportion of total expression heritability that is attributed to distal genetic variation, the positive difference in predictive performance between the best MOSTWAS model and the local-only model increased (**Supplemental Figure 3**). Similarly, we found that, under the setting that distal variation contributes to trait heritability, the best MOSTWAS model has greater power to detect gene-trait associations than the local-only model, with the advantage in power over local-only models increasing with increased distal expression heritability (**Figure 2A**). Under the null case that distal

variation influences expression in the reference panel but does not affect the trait in the GWAS panel, we find that local-only and MOSTWAS models perform similarly. At low causal proportion ($p_c = 0.01$) and low trait heritability ($h^2_p = 0.2$), local-only models have a modest advantage in TWAS power over MOSTWAS models (shown in **Supplemental Data**). This difference is mitigated at larger causal proportions and trait heritabilities (**Figure 2B**). We also find that the power of the distal added-last increase significantly as both the sample sizes of the eQTL reference panel and the GWAS imputation panel increases. At a sample size of 10,000 in the GWAS panel with summary statistics (a suitably large GWAS), we obtain greater than 65% power to detect significant distal associations given the local association at eQTL sample sizes of greater than 200 (**Supplemental Figure 2**). Overall, these results demonstrated the advantages of MOSTWAS methods for modeling the complex genetic architecture of transcriptomes, especially when distal variation has a discernibly large effect on the heritability of both the gene and trait of interest. Full simulation results are provided in **Supplemental Data** at `www.github.com/bhattacharya-a-bt/mostwas_paper`. The MOSTWAS package also contains functions for this simulation framework.

[Figure 2 about here.]

3.3. Real data application in breast cancer tumors

We applied MOSTWAS in the context of breast tumor multi-omics and disease outcomes, motivated by recent GWAS and TWAS into breast cancer-specific survival [45, 33, 34, 32, 46]. Breast tumor eQTL studies have also revealed several signficant distal-eQTLs in trait-associated loci, many of which are in regulatory or epigenetic hotspots [46, 47], making breast tumors a natural setting for MOSTWAS application. Using TCGA-BRCA data on germline SNPs, tumor mRNA expression, DNA methylation, and miRNA expression, we trained MeTWAS, DePMA, and traditional local-only predictive models for the mRNA expression of all genes with germline heritability $h^2 > 0$ at $P < 0.05$. Estimates of heritability for genes were considerably larger when we considered distal variation using MOSTWAS methods (mean heritabilities in **Supplemental Table 1**). We also found that MeTWAS and DePMA perform better in cross-validation R^2 in cross-validation, with larger numbers of models at $R^2 \geq 0.01$ using MOSTWAS methods than local-only models (**Figures 3A-C**). Mean predictive R^2 for local-only models was 0.011 (25% to 75% inter-quartile interval (0.0,0.013)), for MeTWAS models was 0.028 (0.013, 0.032), and for DePMA models was 0.051 (0.019, 0.068).

In addition to cross-validation, we used 351 paired samples in TCGA-BRCA with genotype and mRNA expression data that were not used in model training to test the portability of MOSTWAS models in independent external cohorts. As shown in **Figure 3A**, DePMA models obtain the highest predictive adjusted R^2 in the external cohort (mean 0.016, 25% to 75% inter-quartile interval (0.003, 0.018)), with local-only models (0.013, (0.00,0.013)) outperforming MeTWAS models (0.011, (0.002, 0.012)), considering only genes that attained cross-validation adjusted $R^2 \geq 0.01$ using a given method. Overall, among genes with cross-validation adjusted $R^2 \geq 0.01$, 37 out of 280 genes achieved external predictive $R^2 \geq 0.01$ using local-only models, 89 out of 709 using MeTWAS, and 787 out of 1,185 using DePMA (**Figure 2A-C**).

Lastly, we conducted association studies for breast cancer-specific survival using local-only and the MOSTWAS model with largest R^2 trained in TCGA-BRCA and summary-level GWAS data from iCOGs. Here, we constructed the weighted burden test, as described above and in Pasaniuc et al and Gusev et al [22, 4]. We prioritized genes with Benjamini-Hochberg adjusted $P < 0.05$ for permutation testing. Of the 122 genes that had cross-validation $R^2 \geq 0.01$ in TCGA-BRCA using both local-only and MOSTWAS models, we found 2 survival associations at Benjamini-Hochberg FDR-adjusted 0.05 using both local-only and MOSTWAS models, with the strength of association marginally larger with the MOSTWAS model in each case (**Supplemental Figure 5**). QQ-plots for TWAS Z-statistics are provided in **Supplemental Figure 6B** for both local-only and MOSTWAS models. Overall, using all heritable genes with cross-validation R^2 with the best MOSTWAS model in TCGA-BRCA, we identified 21 survival-associated loci at Benjamini-Hochberg[40] FDR adjusted $P < 0.05$. Of these 21 loci, 11 persisted when subjected to permutation testing at a significance threshold of FDR-adjusted $P < 0.05$ (**Figure 3C** and **Supplemental Table 2**). Our results in TCGA-BRCA showed improved transcriptomic prediction using MOSTWAS over local-only modeling and the strength of MOSTWAS to detect gene-trait associations that are influenced by distal variation.

3.3.1. Functional hypothesis generation with MOSTWAS

An advantage of MOSTWAS is its ability to aid in functional hypothesis generation for mechanistic follow-up studies. The added-last test allows users to identify genes where trait association from distal variation is significant given the strength of the local association. For 8 of the TWAS-associated 11 loci, at FDR-adjusted $P < 0.05$ we found significant distal variation added-last associations (see **Supplemental Methods** and **Supplemental Table 2**), suggesting that distal variation may contribute to the gene-trait association. All 8 of these loci showed distal association with the gene of interest mediated through a set of four transcription factors (*NAA50*, *ATP6V1A*, *ROCK2*, *USF3*), all highly interconnected with the critical MAPK pathway[48, 49, 50, 51, 52, 53]. These regulatory sites serve as an example of how distal genomic regions can be prioritized for functional follow-up studies to elucidate the mechanisms underlying the SNP-gene-trait associations.

3.4. Real data application in brain tissue

We also applied MOSTWAS to transcriptomic data on samples of prefrontal cortex, a tissue that has been used previously in studying neuropsychiatric traits and disorders with TWAS [54, 55]. There has been ample evidence in brain tissue, especially the prefrontal cortex, that non-coding variants (up to 80%) regulate distal genes, providing a prime example to assess MOSTWAS [54, 56]. Using ROS/MAP data on germline SNPs, tumor mRNA expression, DNA methylation, and miRNA expression, we trained MeTWAS, DePMA, and traditional local-only predictive models for the mRNA expression of all genes with germline heritability $h^2 > 0$ at $P < 0.05$. Consistent with results in TCGA-BRCA, estimates of heritability for genes were considerably larger when we considered distal variation using MOSTWAS methods (**Supplemental Table 1**). We also find that MeTWAS and DePMA perform better in cross-validation R^2 than local-only models

(**Figures 3D-F**). Mean predictive R^2 for local-only models was 0.029 (25% to 75% inter-quartile interval (0.0,0.015)), for MeTWAS models was 0.079 (0.019, 0.082), and for DePMA models was 0.045 (0.013, 0.037).

In addition to cross-validation, we used 87 samples in ROS/MAP with genotype and mRNA expression data that were not used in model training to test the portability of MOSTWAS models in independent external cohorts. As shown in **Figure 3A**, DePMA models obtain the highest predictive adjusted R^2 in the external cohort (0.042 (25% quantile 0.009, 75% quantile 0.057)), with MeTWAS models (0.040 (0.010, 0.054)) outperforming local-only models (0.031 (0.007, 0.039)), considering only genes that attained cross-validation adjusted $R^2 \geq 0.01$ using a given method. Overall, among genes with cross-validation adjusted $R^2 \geq 0.01$, 187 out of 267 genes achieved external predictive $R^2 \geq 0.01$ using local-only models, 683 out of 911 using MeTWAS, and 2,135 out of 2,934 using DePMA (**Figure 2D-F**).

We next conducted association tests for known Alzheimer's disease risk loci using local-only and the best MOSTWAS model (comparing MeTWAS and DePMA cross-validation R^2) trained in ROS/MAP and summary-level GWAS data from IGAP. From literature, we identified 14 known common and rare loci of late-onset Alzheimer's disease [35, 41, 42, 43], 11 of which had MOSTWAS models with cross-validation $R^2 \geq 0.01$. Five of these 11 loci (*APOE*, *CLU*, *PLCG2*, *SORL1*, *ZCWPW1*) showed significant association at Benjamini-Hochberg FDR-adjusted $P \leq 0.05$ (**Supplemental Table 3**). We also compared these all 11 associations to those identified by local-only models and by latent Dirichlet process regression (DPR) as implemented in TIGAR [57], with raw P-values of association shown in **Figure 4B**. MOSTWAS showed stronger associations at 8 of these loci than both local-only and DPR models. We followed up on the 5 significantly associated loci using the permutation and added-last tests (see **Methods** and **Supplemental Materials**). The added-last test assesses whether the association from distal loci, given the strength of the association in the local locus, is significant. Three of these loci (*APOE*, *SORL1*, *ZCWPW1*) persisted permutation testing at FDR-adjusted $P < 0.05$ and showed significant associations with distal variants, given the association with local variants, at FDR-adjusted $P < 0.05$ (**Supplemental Table 3**).

We then conducted a transcriptome-wide association study for risk of major depressive disorder (MDD) using summary statistics from the Psychiatric Genomics Consortium (PGC) genome-wide meta-analysis that excludes data from the UK Biobank and 23andMe [36]. QQ-plots for TWAS Z-statistics are provided in **Supplemental Figure 6B** for both local-only and MOSTWAS models. Overall, using all heritable genes with cross-validation R^2 with the best MOSTWAS model in ROS/MAP, we identified 102 MDD risk-associated loci with FDR-adjusted $P < 0.05$ that persisted when subjected to permutation testing at an FDR-adjusted significance threshold of $P < 0.05$ (colored red in **Figure 3D**). We downloaded genome-wide association study by proxy (GWAX) summary statistics from the UK Biobank [37] for replication analysis of loci identified using PGC summary statistics. We found that 7 of these 102 loci (labelled in **Figure 3D**) also show an association in UK Biobank GWAX that is in the same direction as in PGC. Summary statistics for TWAS associations in PGC and UK Biobank are provided in **Supplemental Table 4**. It is important to note the UK Biobank dataset is not a GWAS dataset as it defines a case of MDD as any subject who

has the disorder or a first-degree relative with MDD, leading to lower power to detect associations in this dataset. Nonetheless, we believe that strong prediction of predictive models in independent cohorts and TWAS results across two independent cohorts shows the strength of provides an example of the robustness of models using MOSTWAS.

[Figure 3 about here.]

[Figure 4 about here.]

We observed that MOSTWAS models generally had higher predictive R^2 that local-only models both in training and independent cohorts. We also found that MOSTWAS has recapitulated 5 known Alzheimer's risk loci that were not detected by local-only modeling (both PrediXcan [3] and TIGAR[57]), 3 of which had significant distal associations using our added-last test. We also illustrated that the MOSTWAS detected MDD-risk loci that were replicable across independent GWAS and GWAX cohorts[36, 37].

3.5. Comparison of computational time

To assess the difference in computational burden between local-only, MeTWAS, and DePMA modelling, we randomly selected a set of 50 genes that are heritable across all three models from TCGA-BRCA and computed per-gene time for fitting using a 24-core, 3.0 GHz processor. We found that MeTWAS (mean of 225 seconds per gene) and DePMA (mean 312 seconds per gene) took approximately 6-10 times longer to fit than a traditional local-only model (mean 36 seconds) (**Supplemental Figure 4**). Model-fitting here includes heritability estimation, estimating the SNP-expression weights, and cross-validation. We have implemented parallel options within a given gene and recommend fitting an entire set of genes on an RNA-seq panel via a batch computing approach[58, 59]. Using parallel implementation with 5 cores and batch computing, we analyzed 15,568 genes from TCGA-BRCA in approximately 28 hours.

4. Discussion

Here, through a variety of simulations and real applications in two settings, we have shown that multi-omic methods that prioritize distal variation in TWAS gave added predictive performance and power to detect cell-specific gene-trait associations [8, 11, 13], especially when distal variation contributed to trait heritability. We proposed two methods (MeTWAS and DePMA) for identifying and including distal genetic variants in gene expression prediction models. We have provided implementations of these methods in the MOSTWAS (Multi-omic Strategies for Transcriptome-Wide Association Studies) R package, available freely on Github. MOSTWAS contains functions to train expression models with both MeTWAS and DePMA and outputs models with 5-fold cross-validation $R^2 \geq 0.01$ and significant germline heritability. The package also contains functions and documentation for simulation analyses [23], the weighted burden and follow-up permutation and distal-SNPs added last tests for TWAS [22, 4] using GWAS summary statistics, and file-formatting. We also provide guidelines for parallelization to lessen computational time.

Not only does MOSTWAS improve transcriptomic imputation both in- and out-of-sample, it also provides a test for the identification of heritable mediators that affects eventual transcription of the gene of interest. These identified mediators can give some insight into the underlying mechanisms for SNP-gene-trait associations to improve detection of gene-trait associations and prioritize functional follow-up studies. Using MOSTWAS and iCOGs summary-level GWAS statistics for breast cancer-specific survival[32], we identified 11 survival-associated loci that are enriched for p53 binding and oxidoreductase activity pathways [60, 61]. These loci include two genes (*MAP3K6* and *MAP4K5*) encoding mitogen-activated protein kinases, which are signalling transduction molecules involved in the progression of aggressive breast cancer hormone subtypes [62]. TWAS using MOSTWAS models was able to recapitulate 5 out of 14 known Alzheimer's disease risk loci in IGAP GWAS summary statistics statistics[35], which were not recoverable with local-only models. We showed the utility of the distal-SNPs added last test to prioritize significant distal SNP-gene-trait associations from follow-up. In PGC GWAS summary-level data for major depressive disorder [36], we found 102 risk loci, 7 of which were replicated in independent GWAX summary statistics from the UK Biobank [37]. Three of these seven loci (*SYT1*, *CACNA2D3*, *ADAD2*) encode important proteins involved in synaptic transmission in the brain and RNA editing. Studies have shown that variation at these loci may lead to loss of function at synapses and RNA editing that lead to psychiatric disorders [63, 64, 65, 66, 67]. All survival- or risk-associated loci identified by MOSTWAS were not detected using local-only models.

A considerable limitation of MOSTWAS is the increased computational burden over local-only modelling, especially in DePMA's permutation-based mediation analysis for multiple genome-wide mediators. We believe a Monte-Carlo resampling method will aid in scalability by making some standard distributional assumptions on the effect sizes of SNPs and mediators in the DePMA mediation model [68]. Nevertheless, we believe that MOSTWAS's gain in predictive performance and power to detect gene-trait associations may outweigh this computational time. Another limitation of MOSTWAS is the general lack of rich multi-omic panels, like TCGA-BRCA and ROS/MAP, that provide a large set of mediating biomarkers that may be mechanistically involved in gene regulation. However, we believe that mRNA expression data could be re-used as mediator data to identify distal-eQTLs local to genes that code for transcription factors [9, 5, 12].

In conclusion, MOSTWAS provides a user-friendly and intuitive tool that extends transcriptomic imputation and association studies to include distal genetic variants for improved predictiion for TWAS expression models and increased TWAS power. MOSTWAS enables users to utilize rich reference multi-omic datasets for enhanced gene mapping to better understand the genetic etiology of polygenic traits and diseases with more direct insight into functional follow-up studies.

5. Supplemental Data

6. Declaration of Interests

The authors have no competing interests.

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8. Accession Numbers

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List of Figures

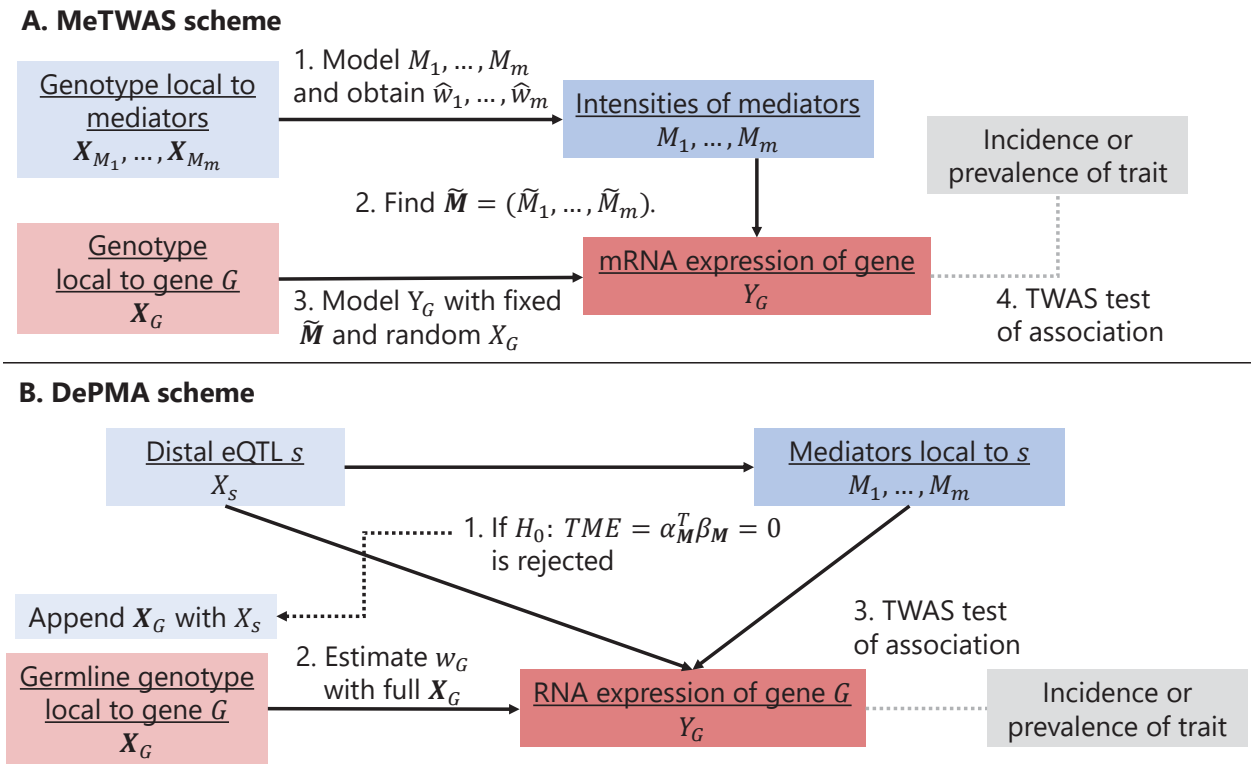

Figure 1: *Modeling schemes implemented in MOSTWAS.* (A) Two-step least squares for mediator-enriched TWAS (MeTWAS). (B) Distal-eQTL prioritization by via mediation analysis (DePMA).

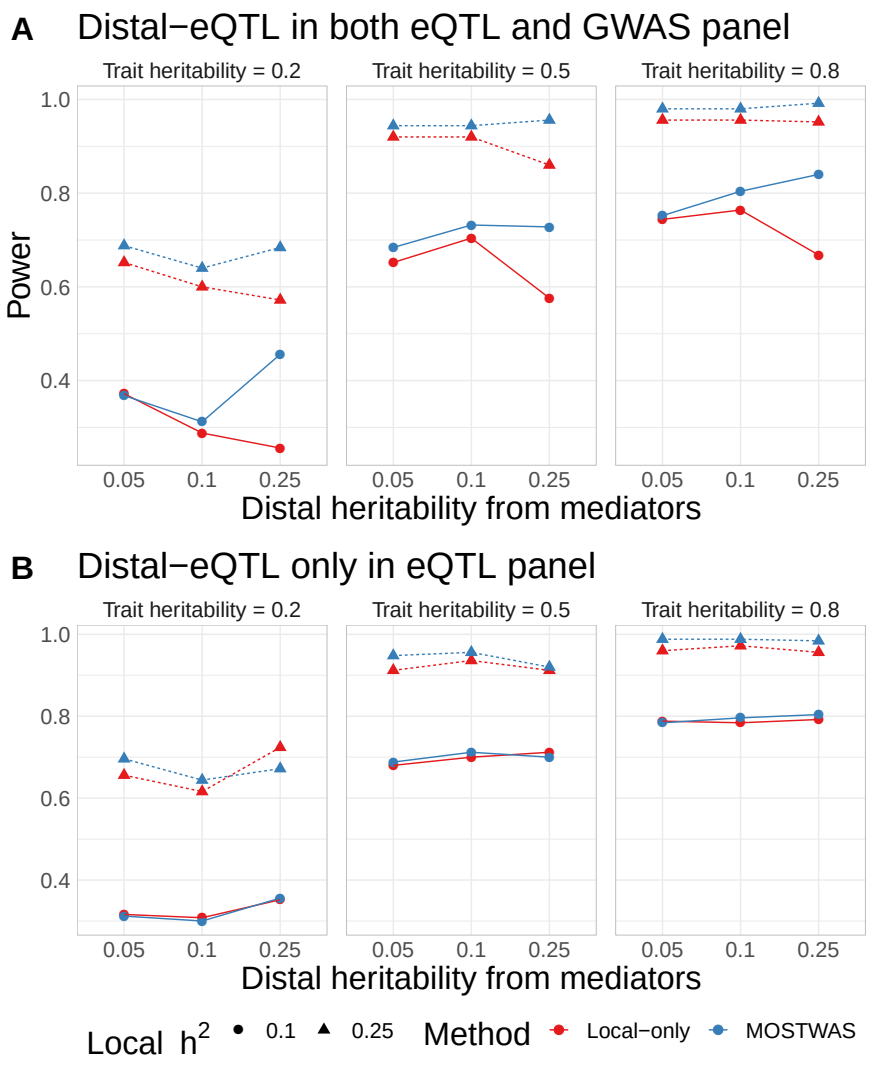

Figure 2: *Comparison of TWAS power via simulations using MOSTWAS and local-only models.* (A) Proportion of gene-trait associations at $P < 2.5 \times 10^{-6}$ using local-only (red) and the most predictive MOSTWAS (blue) models across various local and distal expression heritabilities, trait heritability, and causal proportions. (B) Proportion of significant gene-trait associations across the same simulation parameters with no distal effect on the trait in the simulated external GWAS panel.

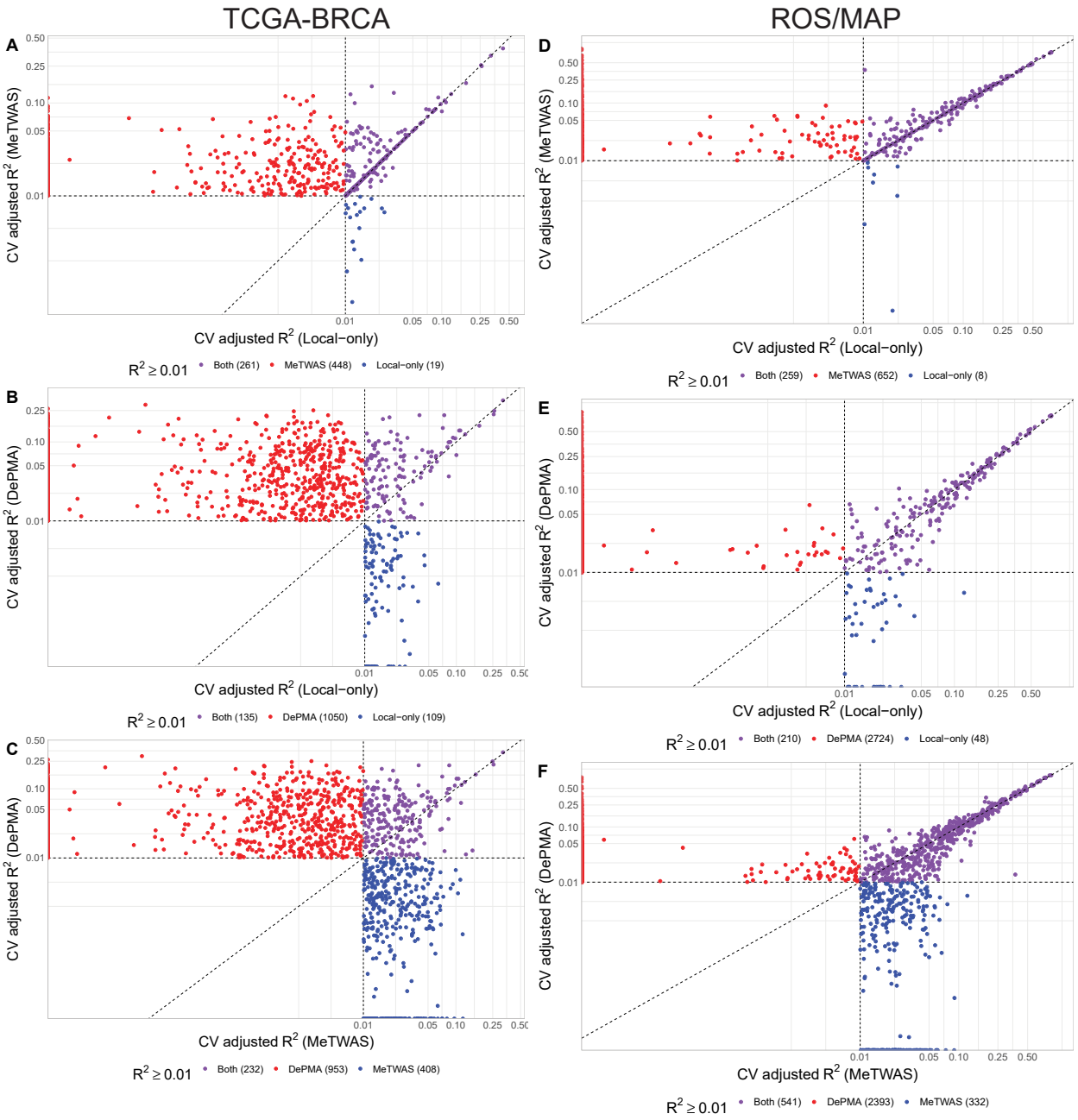

Figure 3: *Comparison of predictive adjusted R^2 in cross-validation using local-only, MeTWAS, and DePMA models.* If a given gene does not have $h^2 > 0$ with $P < 0.05$, we set the predictive adjusted R^2 to 0 here for comparison. We compare local-only and MeTWAS in TCGA-BRCA (A) and ROS/MAP (D), local-only and DePMA in TCGA-BRCA (B) and ROS/MAP (E), and MeTWAS and DePMA in TCGA-BRCA (C) and ROS/MAP (F).

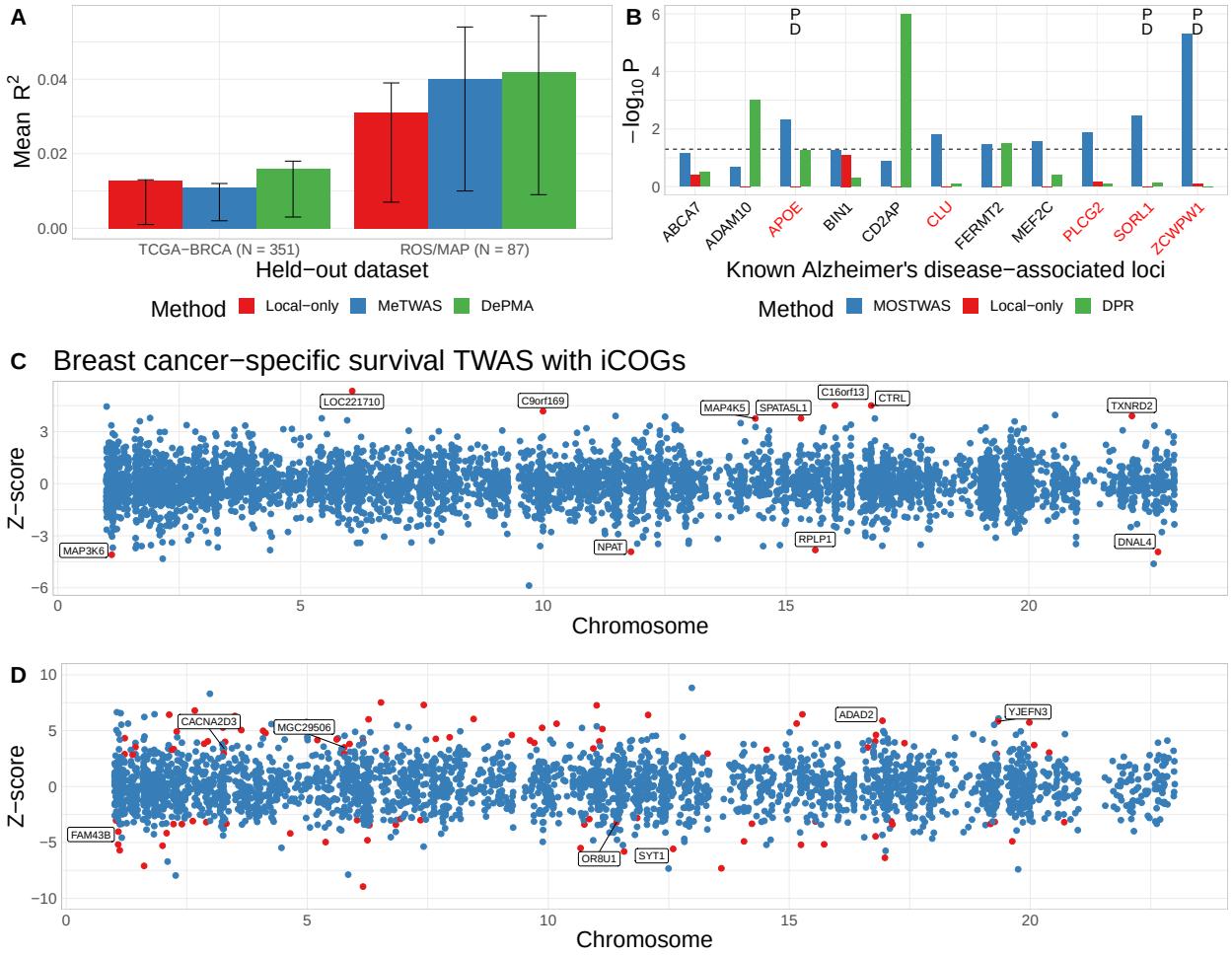

Figure 4: *External validation of MOSTWAS and gene-trait associations using MOSTWAS models.* (A) Predictive adjusted R^2 in held-out cohorts from TCGA-BRCA and ROS/MAP in local-only, MeTWAS, and DePMA models that have in-sample significant heritability and cross-validation $R^2 \geq 0.01$. The interval shows the 25% and 75% quantiles for external cohort predictive R^2. (B) Associations with 11 known Alzheimer's risk lock, as identified in literature, using MOSTWAS, local-only, and TIGAR Dirichlet process regression (DPR). (C) TWAS associations for breast cancer-specific survival using GWAS summary statistics from iCOGs. Loci are colored and labelled if the overall association achieves FDR-adjusted $P < 0.05$. Loci are labelled with 'P' if the permutation test achieves FDR-adjusted $P < 0.05$ and 'D' if the added-last test achieves FDR-adjusted $P < 0.05$. (D) TWAS associations for major depressive disorder risk using GWAS summary statistics from PGC. Loci are colored red if the overall association achieves FDR-adjusted $P < 0.05$ and the permutation test also achieves FDR-adjusted $P < 0.05$. We label the 12 loci that were independently validated with UK Biobank GWAX summary statistics at FDR-adjusted $P < 0.05$ for both the overall association test and permutation test.