SUMMIT: An integrative approach for better transcriptomic data imputation improves causal gene identification

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MD Anderson Cancer Center
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Research goal

My long-term research goal is to develop new methods, theories, and software to:

- identify likely causal risk factors and biomarkers for a complex disease (prostate cancer, Alzheimer's disease, etc.)
- enhance risk prediction to advance precision medicine

Research Interests: causal inference (Mendelian randomization), machine learning, statistical genetics (polygenic risk score, integrative analysis, TWAS, PWAS)

Data we work on: UK Biobank (genotype, risk factors, & disease status), GTEx (splicing, gene expression, & genotype), ROS/MAP (protein & genotype), GWAS summary data, functional annotations, DNA methylation

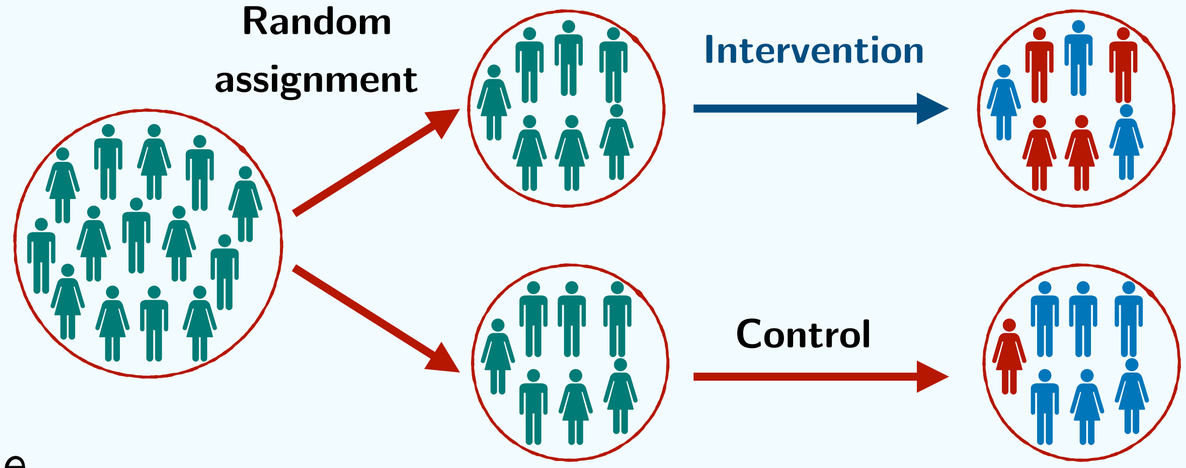
Outline

- **Background**
- New method: SUMMIT
- Results
- Extension

Causal inference in observational data

Does X (risk factor) cause Y (complex disease)?

- **Example:** Does smoking cause lung cancer?
- Randomized clinical trial
  - Gold standard
  - Randomization balances participant characteristics between the groups
- **Challenges:** randomized clinical trial would be both not feasible and unethical



Causal inference in observational data

Example: identify causal biomarkers for a complex disease

Why:

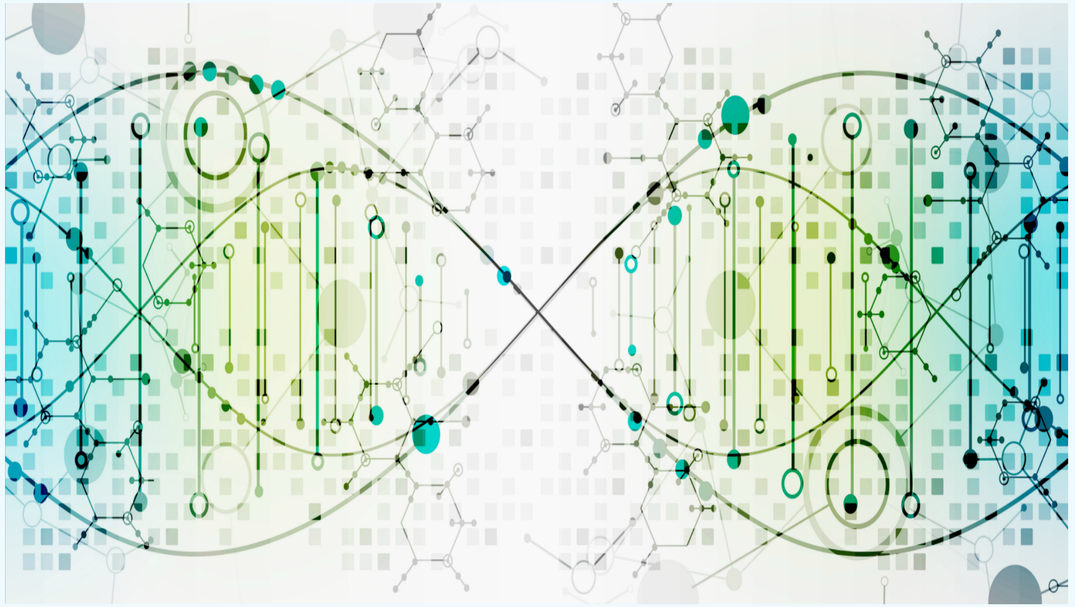
- understand the etiology
- drug development

Challenges:

- the number of biomarkers is large
- biomarkers are correlated

Goal:

identify likely causal biomarkers by using observational data



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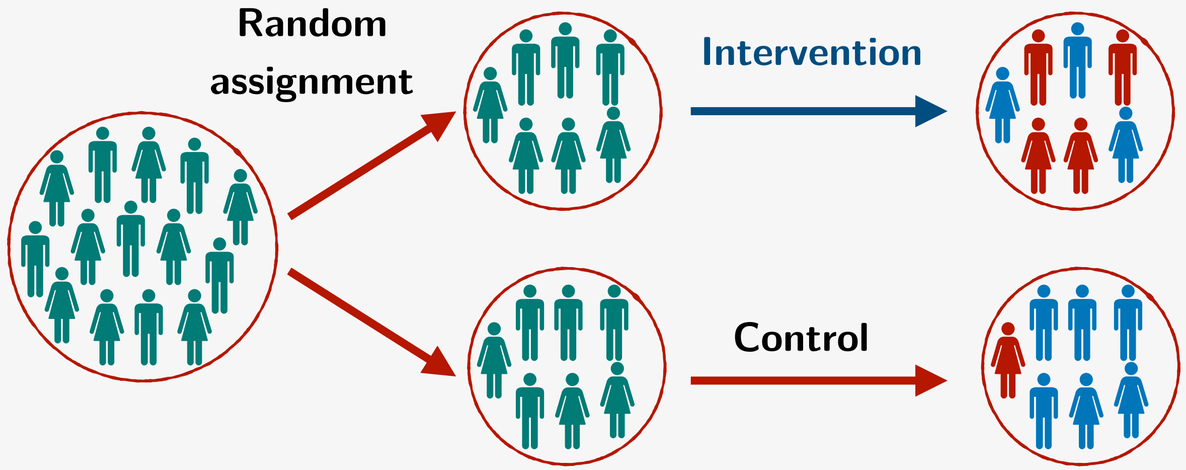
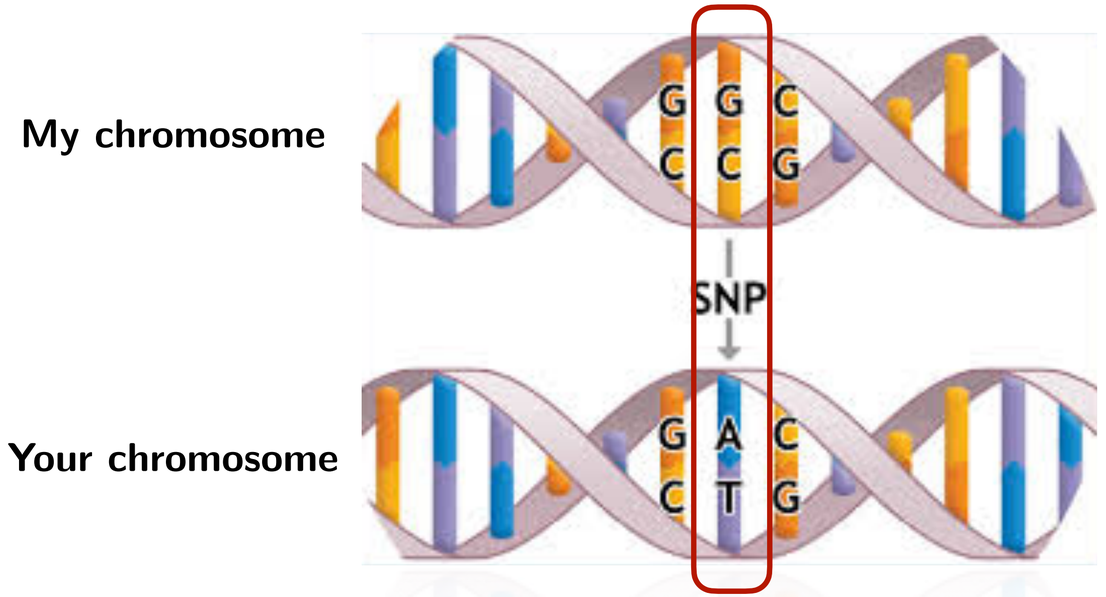
Mendelian randomization

Randomized clinical trial

- Gold standard
- Randomization balances participant characteristics between the groups



- Genome: genetic information encoded in 23 chromosome pairs
- SNP
  - variation in a single base pair
  - inherited randomly and fixed at conception



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Mendelian randomization

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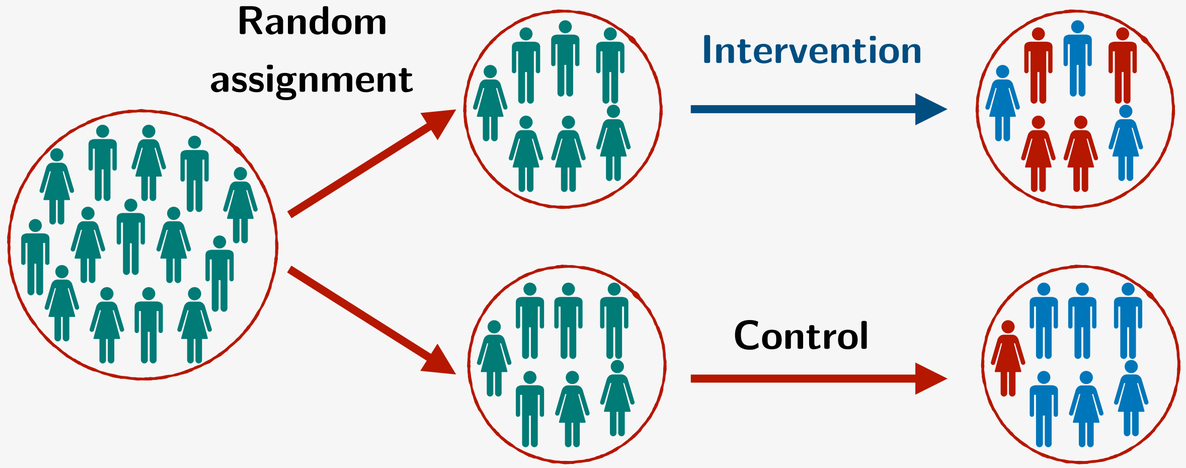
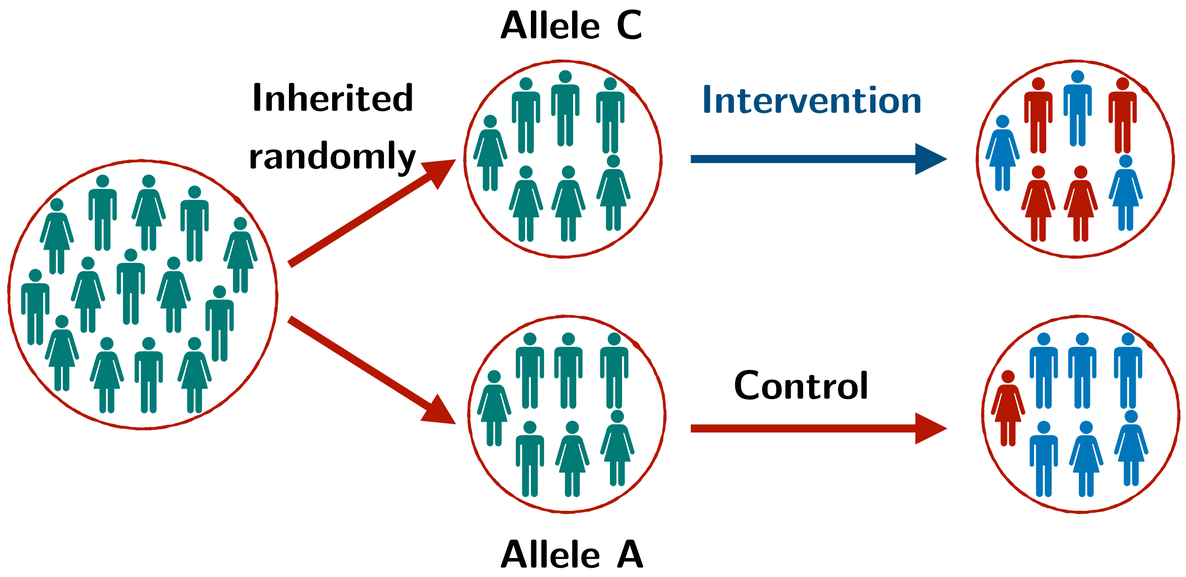
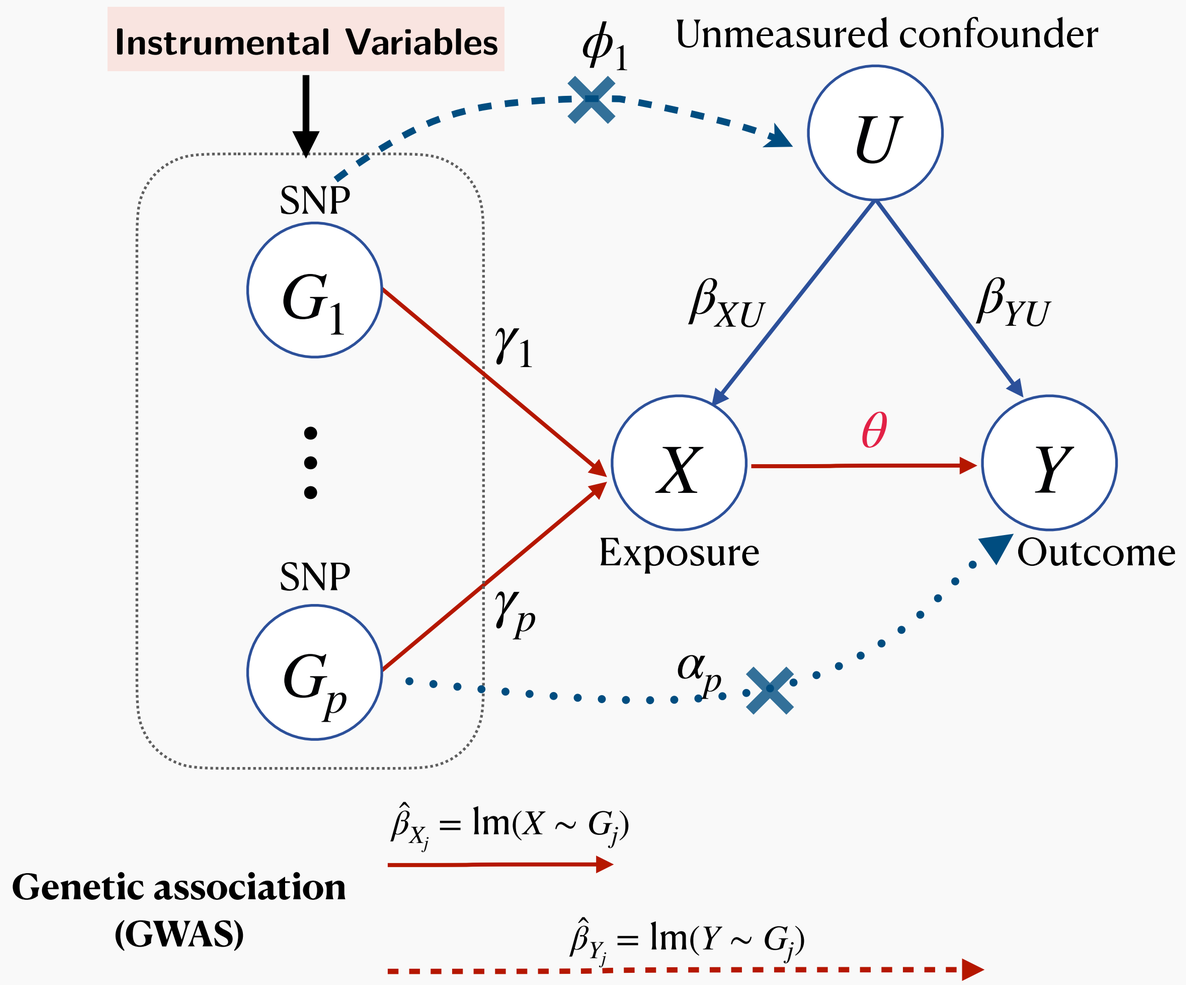


Hypothetical example

- Allele A: not smoking
- Allele C: smoking
- Not associated with unmeasured confounding factors (e.g., drinking)
- No direct effect on the outcome (e.g., lung cancer)



Mendelian randomization



Structure equation model:

$$\beta_{X_j} = \gamma_j + \phi_j \cdot \beta_{XU}$$

$$\beta_{Y_j} = \beta_{Y_j,\mathrm{M}} + \beta_{Y_j,\mathrm{D}} = \theta \cdot \beta_{X_j} + (\alpha_j + \phi_j \cdot \beta_{YU})$$

SNP j is a valid instrumental variable (IV) if

- **Relevance:** $\gamma_j \neq 0$
- **Independence:** $\phi_j = 0$
- **Exclusion restriction:** $\alpha_j = 0$

For a valid IV SNP j:

$$\beta_{X_j} = \gamma_j$$

$$\beta_{Y_j} = \theta \cdot \beta_{X_j}$$

Two-sample summary-data MR

Two-sample MR setup:

	Original data	Summary data
Exposure GWAS	$\left\{(X_i^*, G_{ij}^*)\right\}_{i=1}^{n_X}$	$\left\{(\hat{\beta}_{X_j}, \sigma_{X_j})\right\}_{j=1}^{p}$
Outcome GWAS	$\left\{(Y_i, G_{ij})\right\}_{i=1}^{n_Y}$	$\left\{(\hat{\beta}_{Y_j}, \sigma_{Y_j})\right\}_{j=1}^{p}$

Strengths of two-sample MR:

- Increase the power
- Expand the scope of MR studies

Inverse variance weighted (IVW) estimator:

- Assume all IVs are valid
- Assume no measurement error: $\hat{\beta}_{X_j} = \beta_{X_j}$
- $\hat{\beta}_{Y_j} = \theta \cdot \hat{\beta}_{X_j} + \epsilon_j$
- The IVW estimator:

$$\hat{\theta}_{\texttt{IVW}} = \frac{\sum_{j=1}^{p} \hat{\beta}_{X_j}\hat{\beta}_{Y_j}/\sigma_{Y_j}^2}{\sum_{j=1}^{p} \hat{\beta}_{X_j}^2/\sigma_{Y_j}^2}$$

Identify likely causal gene expression

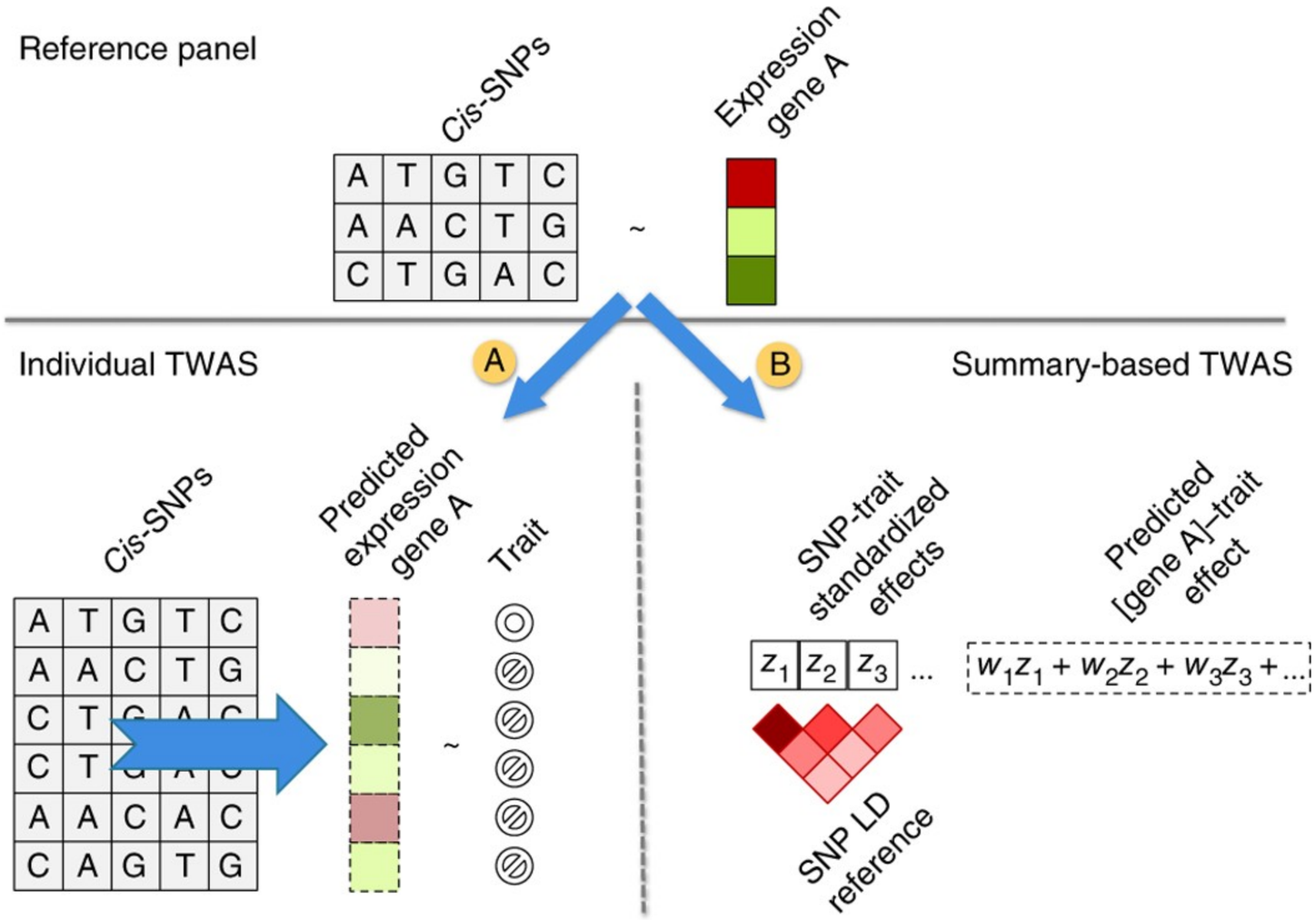
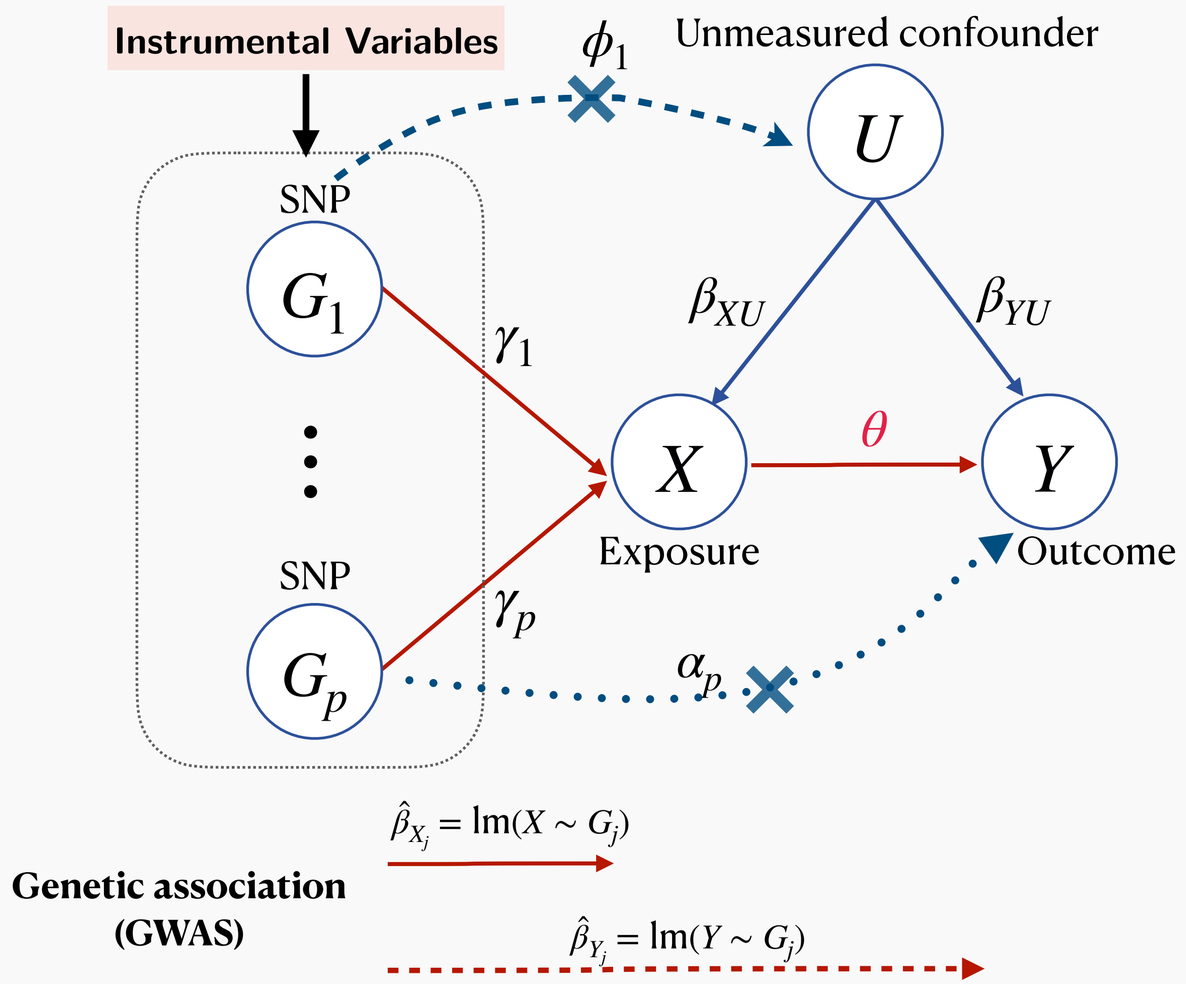


Figure: Workflow of TWAS[1]

1 Gusev, Alexander, et al. "Integrative approaches for large-scale transcriptome-wide association studies." *Nature Genetics* 48.3 (2016): 245-252.

Mendelian randomization



Structure equation model:

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Motivation

- The size of the expression reference panels primarily determines the number of analyzable genes, and hence the power of TWASs
- The average number of expression models increased from **4,570 (v6p)** to **7,213 (v8)** for one popular TWAS method PrediXcan when the average sample size increased from 160 (v6p) to 332 (v8)
- The existing methods are based on individual-level expression reference panel with limited sample size;
- eQTLGen consortium has conducted the largest meta-analysis involving 31,684 blood samples from 37 cohorts

Q: How can we build expression prediction models using summary-level expression reference panel with large sample size?

Outline

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- **New method: SUMMIT**
- Results
- Extension

SUMMIT: Overview

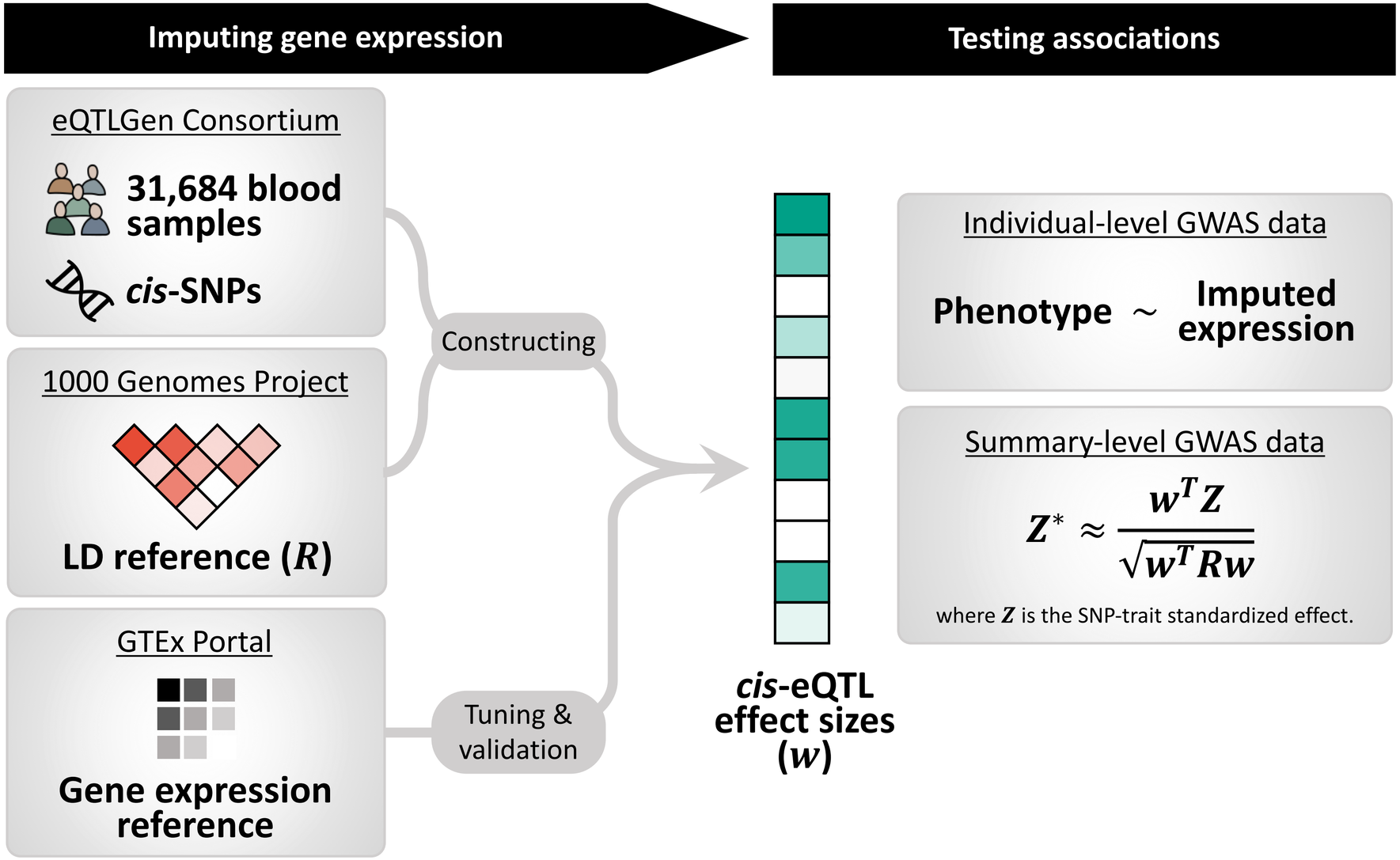

Figure: Workflow of SUMMIT

SUMMIT

Notation and model setup

$$\mathbf{Y} = \sum_{j=1}^{p} w_j \mathbf{X}_j + \epsilon$$

- $\mathbf{Y}$ is the gene expression levels; $\mathbf{X} = (\mathrm{X}_1^{'}, \cdots, \mathrm{X}_p^{'})'$ is the $N \times p$ standardized genotype matrix of p cis-SNPs around the gene; $\mathbf{w} = (w_1, \cdots, w_p)'$ is the cis-eQTL effect size, which can be estimated by

$$f(\mathbf{w}) = \frac{(\mathbf{Y} - \mathbf{X}\mathbf{w})'(\mathbf{Y} - \mathbf{X}\mathbf{w})}{N} + J_\lambda(\mathbf{w}) = \frac{\mathbf{Y}'\mathbf{Y}}{N} + \mathbf{w}'\left(\frac{\mathbf{X}'\mathbf{X}}{N}\right)\mathbf{w} - 2\mathbf{w}'\frac{\mathbf{X}'\mathbf{Y}}{N} + J_\lambda(\mathbf{w})$$

SUMMIT

Notation and model setup

$$f(\mathbf{w}) = \frac{\mathbf{Y}'\mathbf{Y}}{N} + \mathbf{w}'\mathbf{R}\mathbf{w} - 2\mathbf{w}'\mathbf{r} + J_\lambda(\mathbf{w}),$$

Not depend on w

- $J_\lambda(\cdot)$ is a penalty term; such as LASSO, elastic net, MCP, SCAD, and MNet
- $\mathbf{r} = \mathbf{X}'\mathbf{Y}/N = (r_1, \cdots, r_p)'$ is p-dimensional vector of standardized marginal effect size for cis-SNPs (i.e., correlation between cis-SNPs and gene expression levels)
- $\mathbf{R} = \mathbf{X}'\mathbf{X}/N$ is the linkage disequilibrium (covariance) matrix of the cis-SNPs.
- The objective function is

$$\tilde{f}(\mathbf{w}) = \mathbf{w}'\tilde{\mathbf{R}}\mathbf{w} - 2\mathbf{w}'\tilde{\mathbf{r}} + \theta\mathbf{w}'\mathbf{w} + J_\lambda(\mathrm{w})$$

Ensure a unique solution upon optimization

SUMMIT

Estimating the standardized marginal effect size $\tilde{r}$:

$$\tilde{r}_j = Z_j / \sqrt{N_j - 1 + Z_j^2},$$

- where Z_j and N_j are the z-score and sample size for cis-SNP j, respectively.
- Z_j and N_j are provided by eQTL summary-level data (such as eQTLGen; **publicly available**)

Estimating the LD matrix $\tilde{R}$:

We can estimate LD matrix $\tilde{R}$ from a reference panel (such as 1000 Genomes Project data; **publicly available**)

High dimensionality problem:

- Instead of using sample correlation matrix, we use the shrinkage estimator of the LD matrix
- Stabilize results by shrinking the off-diagonal entries toward zero (the magnitude depends on the genetic distance)

SUMMIT

When individual-level GWAS data (genotype data $\mathsf{X}_{\mathsf{new}}$, phenotype $\mathsf{P}_{\mathsf{new}}$, and covariance matrix $\mathsf{C}_{\mathsf{new}}$) are available

- one can apply a generalized linear regression model to test $H_0 : \beta = 0$

$$f(E[\mathsf{P}_{\mathsf{new}} \,|\, \mathsf{X}_{\mathsf{new}}, \mathsf{C}_{\mathsf{new}}]) = \alpha \mathsf{C}_{\mathsf{new}} + \beta \mathsf{X}_{\mathsf{new}} \hat{\mathsf{w}},$$

- where $\mathsf{X}_{\mathsf{new}} \hat{\mathsf{w}}$ is the predicted genetically regulated expression for the trait of interest.

When only summary-level GWAS data are available

- one can apply a burden-type test:

$$\tilde{Z} = \mathsf{Z} \hat{\mathsf{w}} / \sqrt{\hat{\mathsf{w}}' \mathsf{V} \hat{\mathsf{w}}},$$

- where Z is the vector of z-scores for all cis-SNPs and V is the LD matrix of analyzed SNPs

SUMMIT

Cauchy combination test to integrate information from K models

$$T = \sum_{j=1}^{K} \tilde{R}_j^2 \tan\{(0.5 - p_j)\pi\},$$

- where p_j the p-value for model j and $\tilde{R}_j^2$ is calculated by $R_j^2 / \sum_{j=1}^{k} R_j^2$.
- T approximately follows a standard Cauchy distribution, and the p-value can be calculated as $0.5 - \arctan(T)/\pi$.
- The Cauchy combination test has been widely used, key benefit:
  - p-value approximation is accurate for highly significant results (which are of interest),
  - no need to estimate the correlation structure among the combined p-values.

Outline

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- New method: SUMMIT
- **Results**
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Methods to be compared

SUMMIT: SUMMIT with the cis-eQTL summary-level data from eQTLGene (31,684 blood samples)

Lassosum: a popular polygenic risk score method Lassosum with the eQTLGene

Single tissue method:

PrediXcan: Elastic Net with GTEx v8 samples (individual-level data; 670 blood samples)

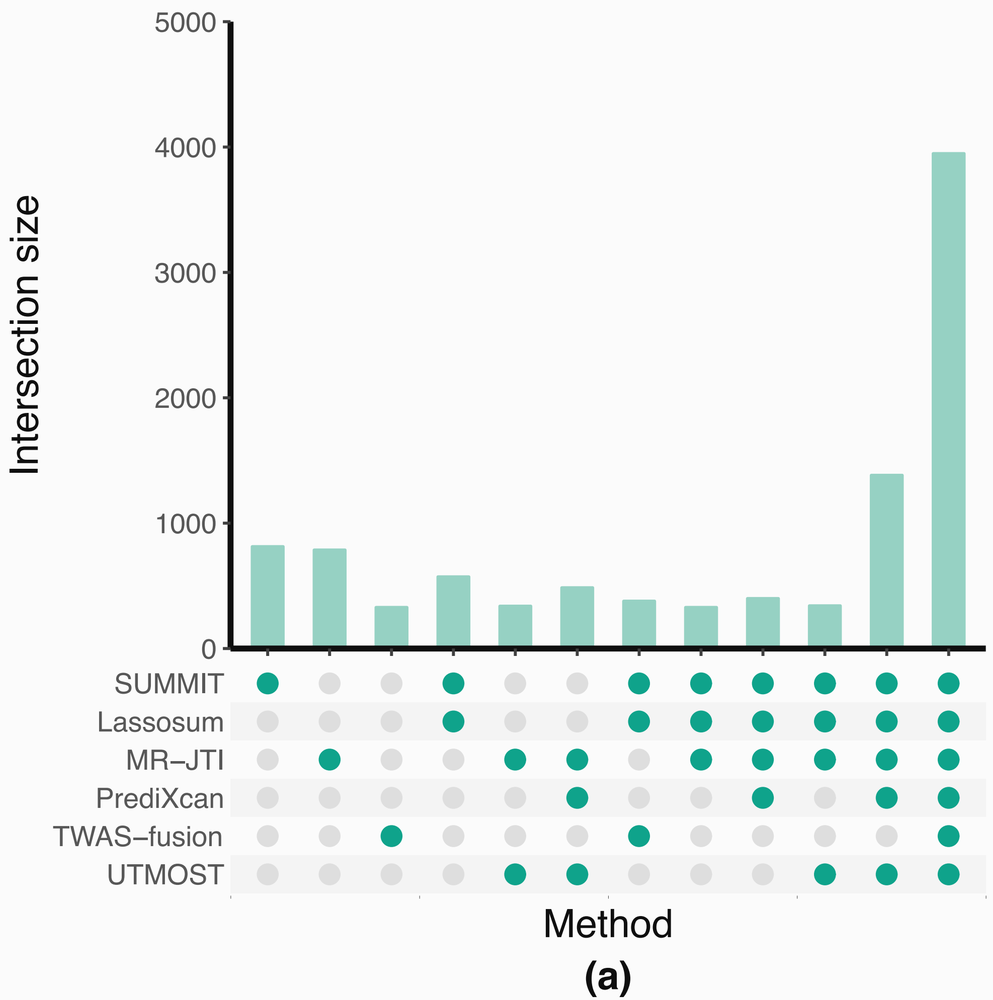
TWAS-fusion: several methods, including BLUP, BSLMM, Elastic Net, LASSO, and TOP1 with GTEx v8 samples

Cross-tissue method:

MR-JTI: GTEx v8 samples (all available tissues)

UTMOST: GTEx v8 samples (all available tissues)

SUMMIT improves the expression imputation accuracy

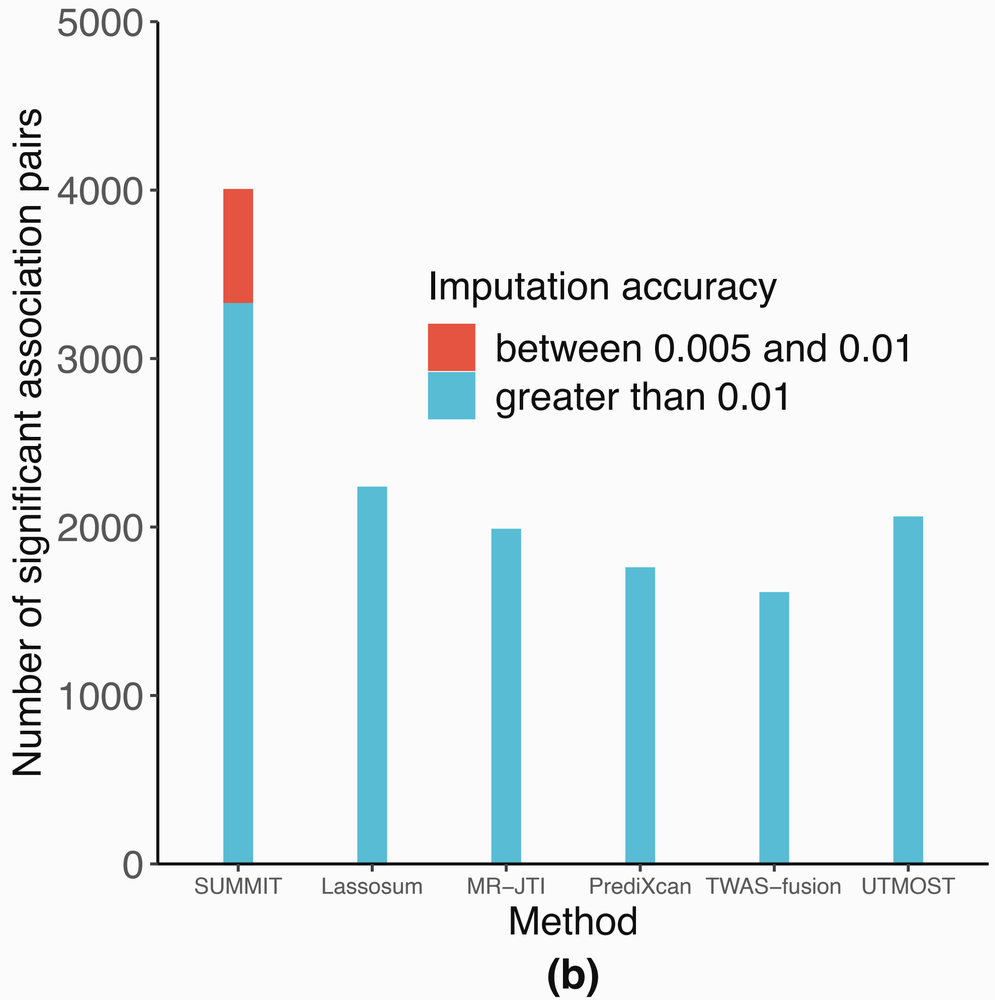


Number of genes with $R^2 \geq 0.01$:

- SUMMIT: 9,749
- Bechmark methods: Lassosum: 8,249; MR-JTI: 9,576; TWAS-Fusion: 5,411; PrediXcan: 7,512; UTMOST: 7,236

SUMMIT achieved higher prediction accuracy in different quantiles compared with all benchmark methods (by Kolmogorov-Smirnov test)

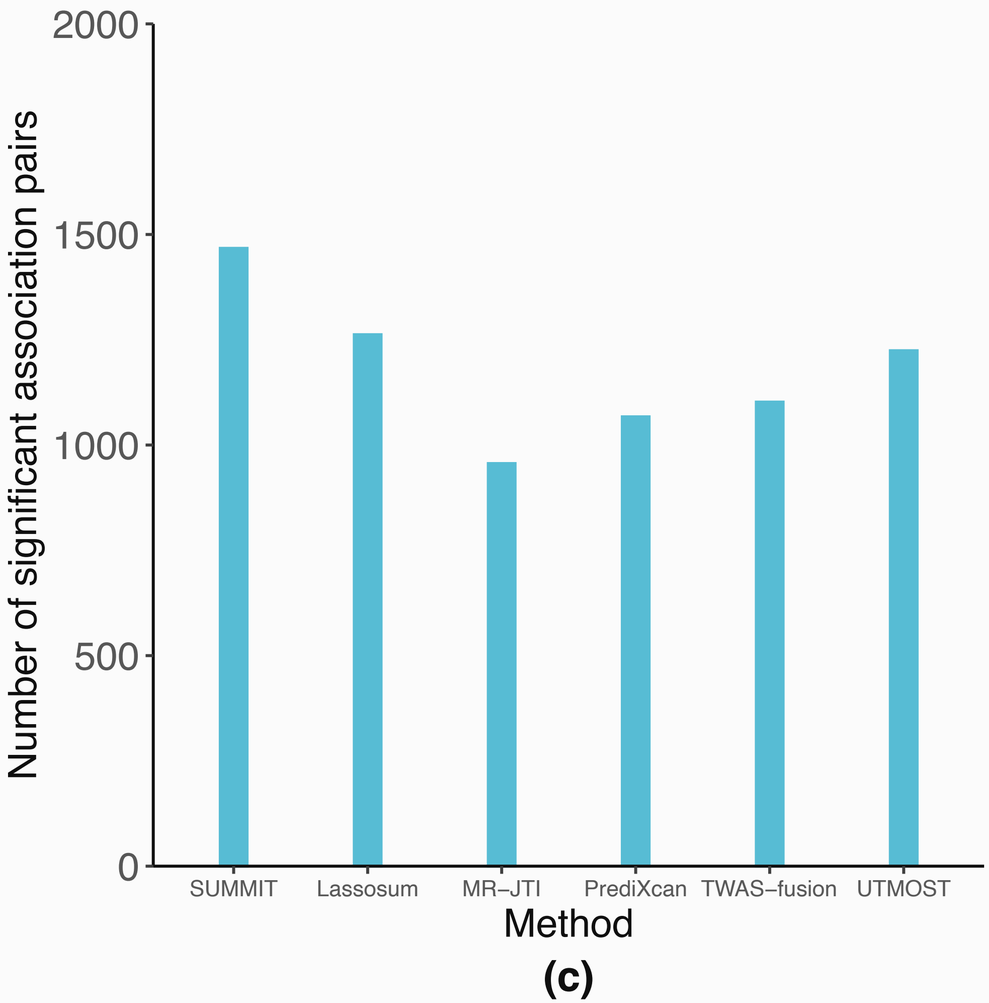
SUMMIT identifies more associations than competing methods



(b)

- Using Bonferroni correction for all methods
- Based on GWAS summary statistics of 24 traits ($N_{\text{total}} \approx 5{,}600{,}000$ without adjusting for sample overlap across studies
- When focused on genes with $R^2 \geq 0.01$; SUMMIT achieved better results (the differences are significant by the paired Wilcoxon rank test)
- SUMMIT can analyze genes with low heritability, which often have large causal effect sizes on the trait

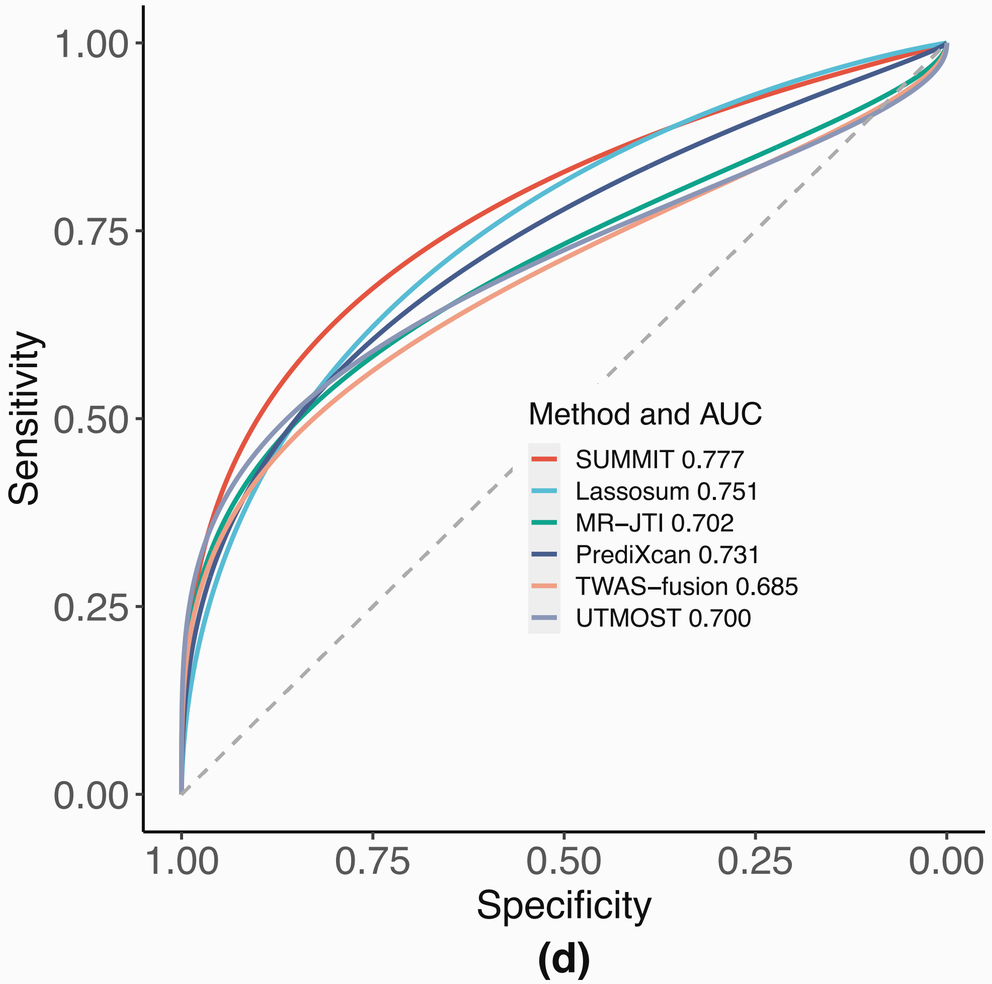
SUMMIT identifies more associations than competing methods



(c)

- When focused on genes that can be analyzed by all the methods; SUMMIT still achieved better results

SUMMIT achieves higher predictive power for identifying “silver standard” genes



(d)

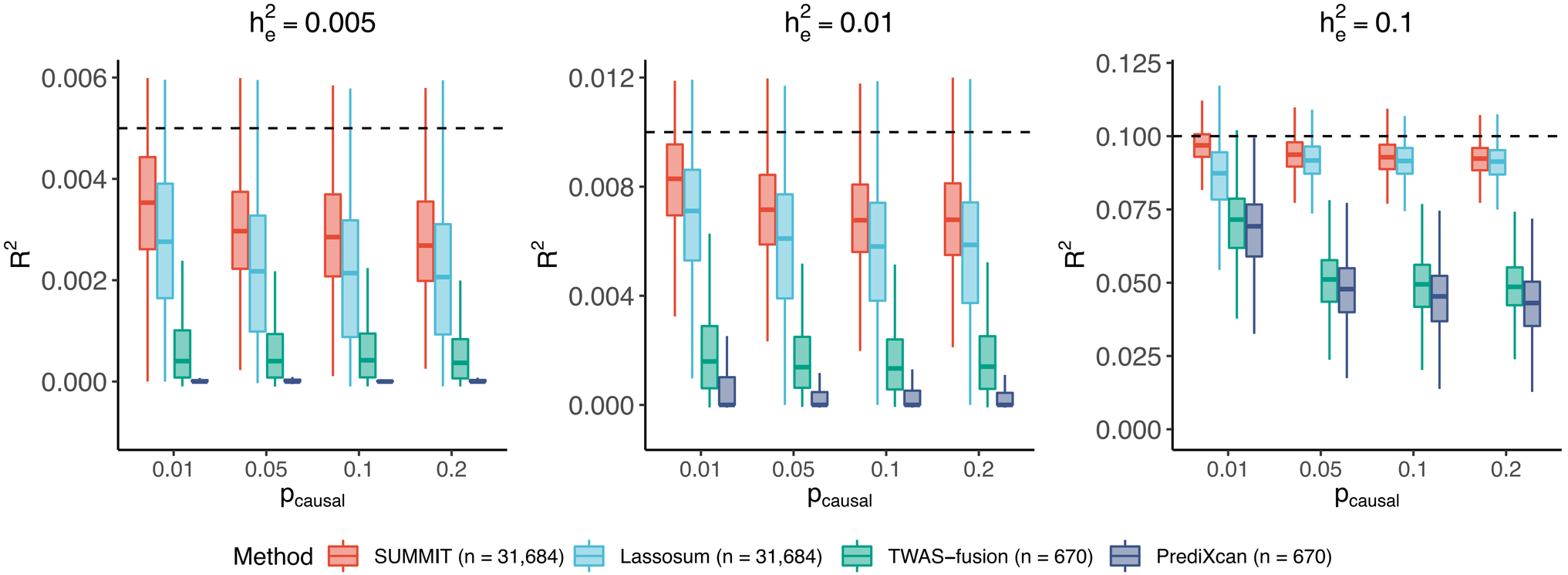
- Following Barbeira et al., we used a set of 1,258 likely causal gene-trait pairs curated by using the Online Mendelian Inheritance in Man (OMIM) database and a set of 29 gene-trait pairs based on rare variant results from exome-wide association studies
- Provide orthogonal information that is independent of the GWAS results
- All methods performed relatively good; SUMMIT achieved the highest AUC

Simulation settings

Using UK Biobank

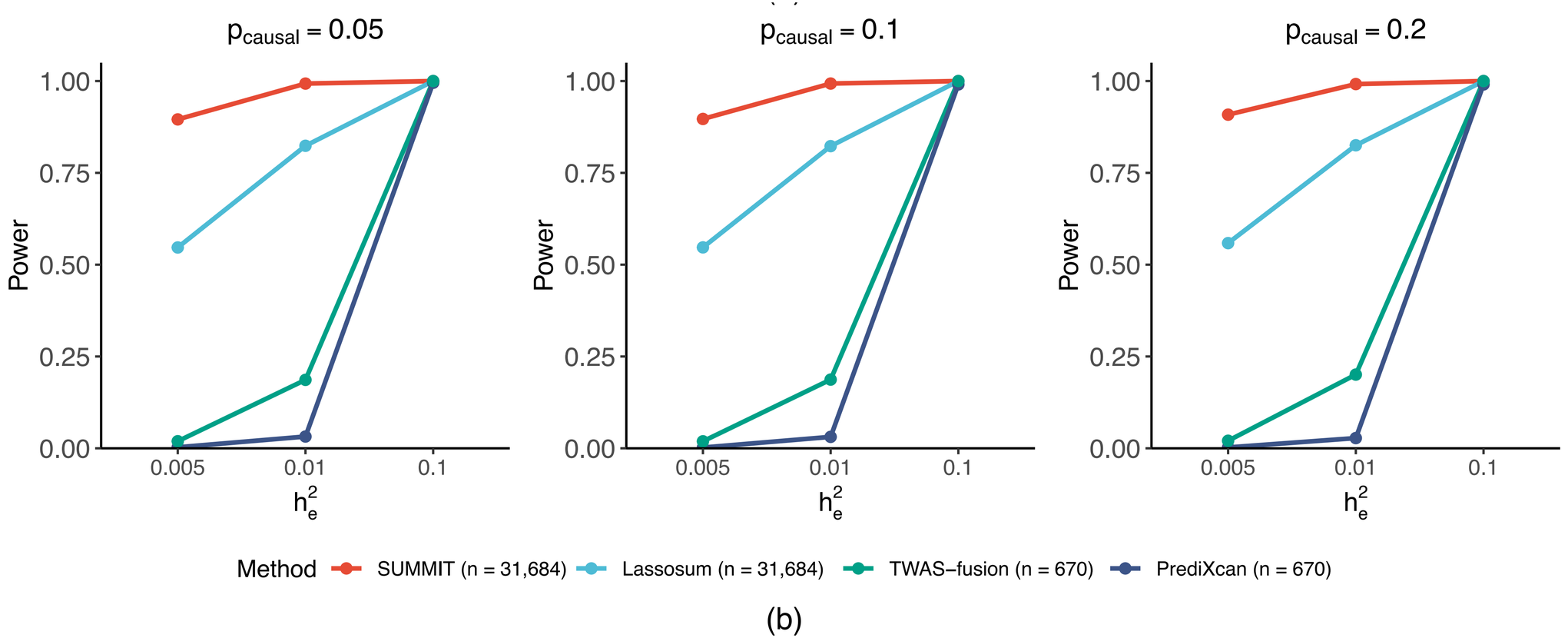
- Randomly selected genotype data from unrelated white British individuals as training data (to match with the sample size of real data analyses)
- 10,000 unrelated white British individuals as test data
- $\mathrm{E}_g = \mathrm{Xw} + \epsilon_e$
- $\mathrm{Y} = \beta \mathrm{E}_g + \epsilon_p$
- $\epsilon_e \sim N(0, 1 - h_e^2)$, and $\epsilon_p \sim N(0, 1 - h_p^2)$
- h_e^2 : expression heritability (i.e., the proportion of gene expression variance explained by SNPs)
- h_p^2: phenotypic heritability (i.e., the proportion of phenotypic variance explained by gene expression levels)

Simulation results



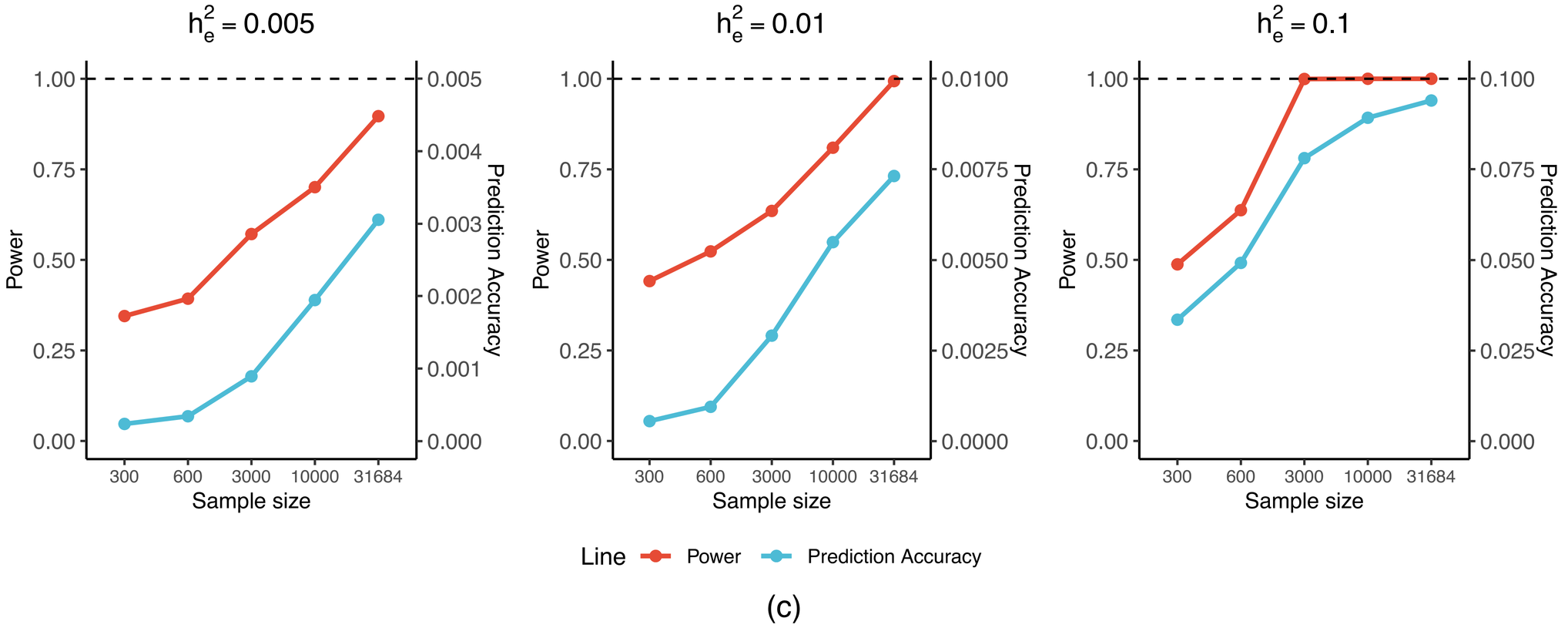
(a)

Simulation results



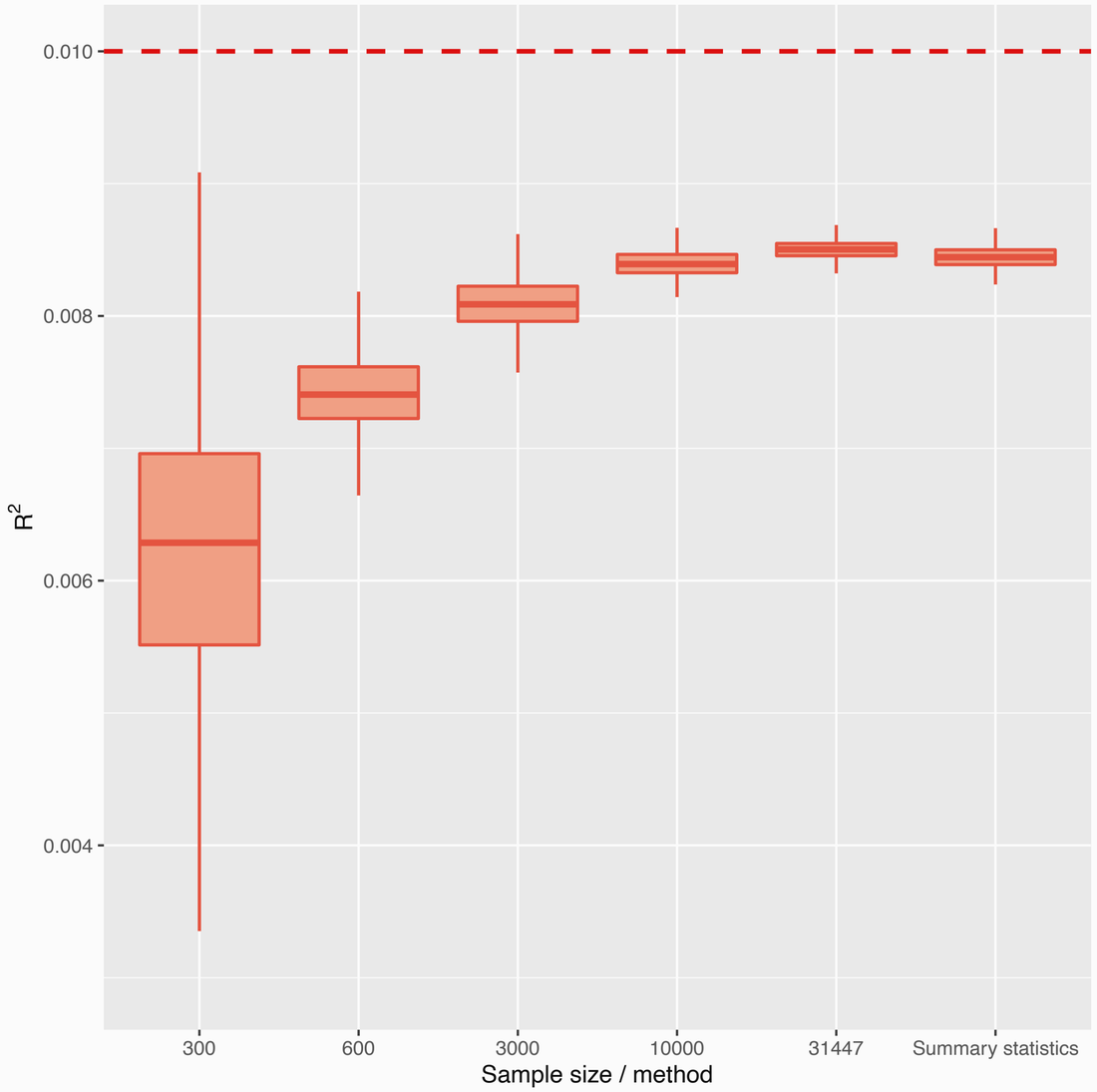
(b)

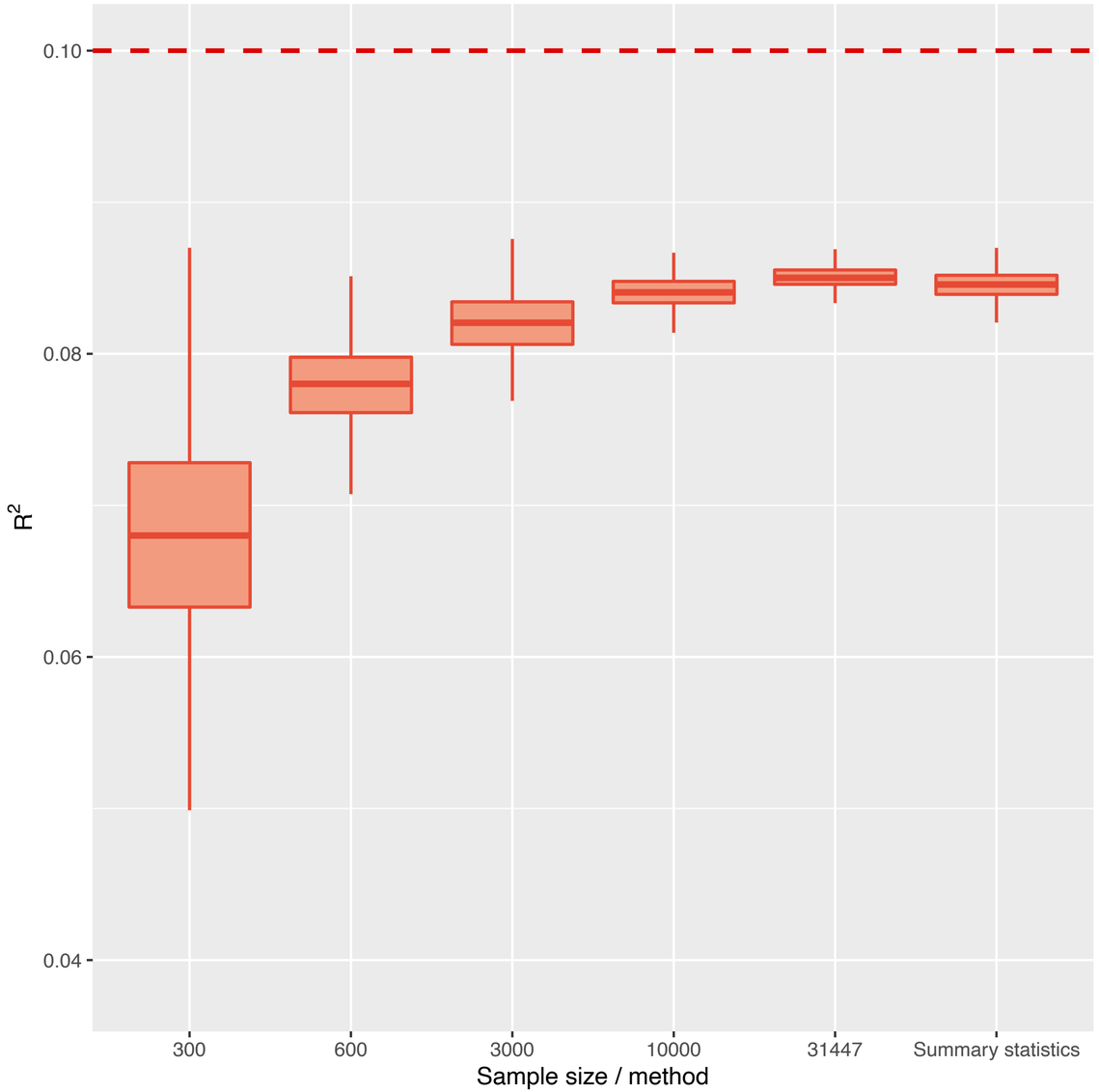
Simulation results



(c)

Simulation results





Online database

chongwulab.shinyapps.io

Mail - Wu,Chong - Outlook | SUMMIT - Online LaTeX Editor Overleaf | Integration of summary data from GWAS and eQTL studies predicts... | SUMMIT | SUMMIT: An integrative approach for better transcriptomic data imp... | screenshot on mac - Google Search

SUMMIT | Real-data results | Query | About

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Real-data results

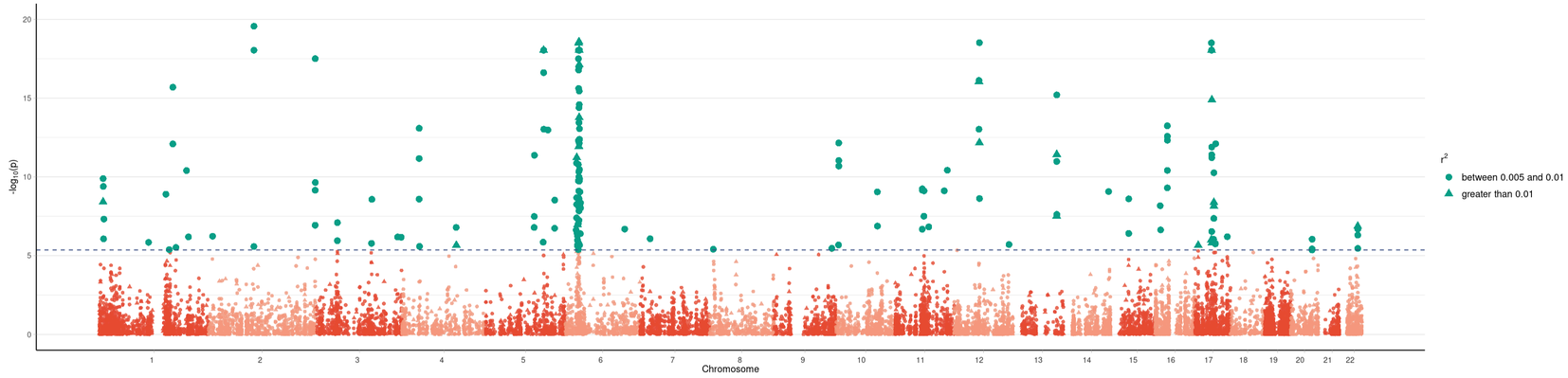
Trait/Disease: Asthma

Method: SUMMIT

Cutoff: Bonferroni-corrected cutoff

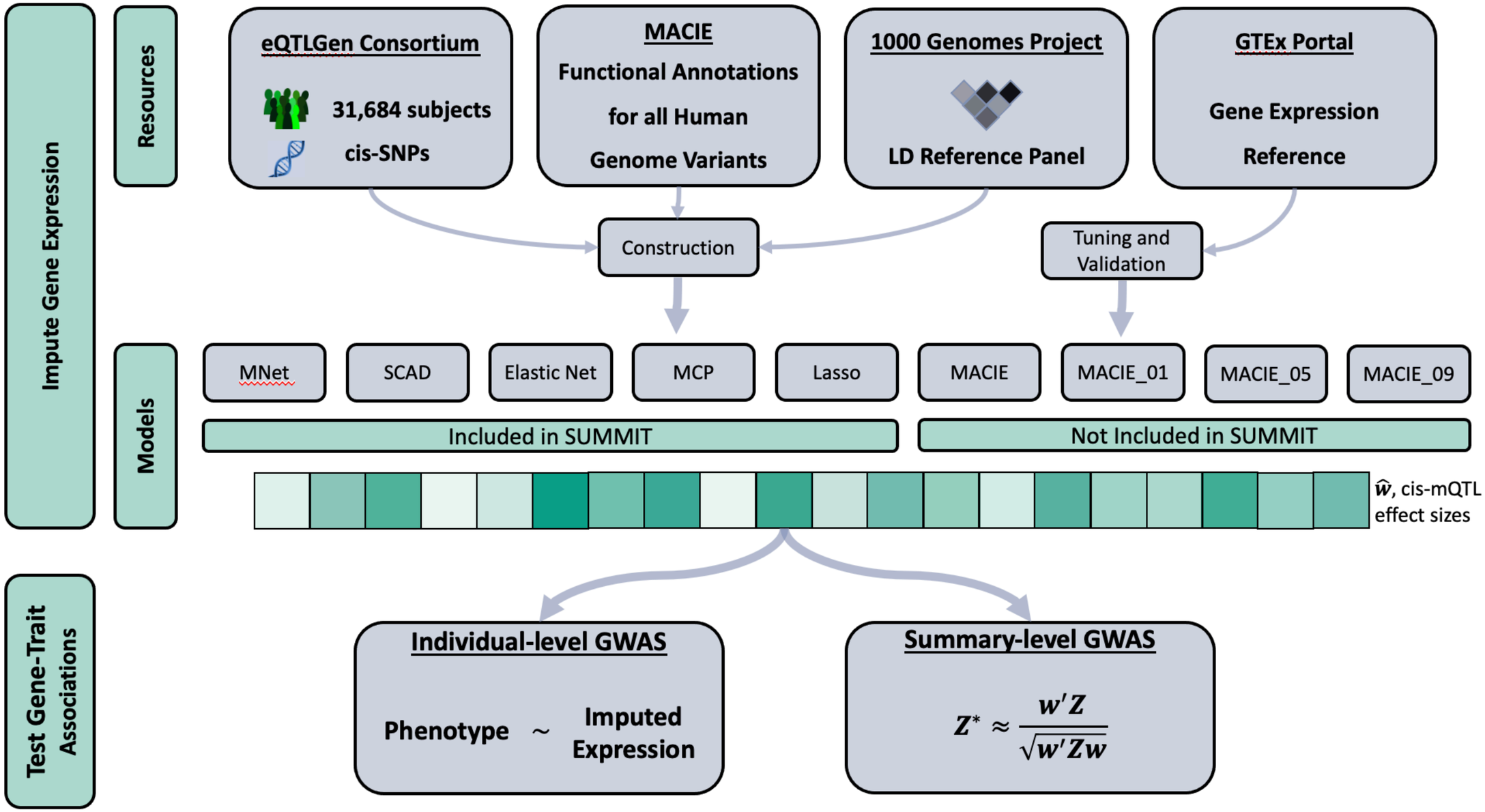
☐ Show gene annotation in Manhattan plot

Download gene list



gene ID	gene name	p-value	chromosome	position	R2
ENSG00000072682	P4HA2	3.297e-22	5	131579269	0.006
ENSG00000073605	GSDMB	1.817e-57	17	38068477	0.395
ENSG00000172057	ORMDL3	1.250e-56	17	38080574	0.298
ENSG00000172346	CSDC2	1.342e-07	22	41965256	0.009
ENSG00000172568	FNDC9	1.816e-07	5	156770668	0.018
ENSG00000172638	EFEMP2	7.663e-10	11	65637487	0.072
ENSG00000172992	DCAKD	4.305e-08	17	43119590	0.253
ENSG00000174123	TLR10	2.571e-09	4	38779235	0.018
ENSG00000174130	TLR6	6.788e-12	4	38841887	0.025
ENSG00000176973	FAM89B	3.123e-08	11	65340744	0.049
ENSG00000179344	HLA-DQB1	1.893e-134	6	32631702	0.708
ENSG00000179428	AC073072.5	8.403e-07	7	22766126	0.015
ENSG00000179639	FCER1A	4.115e-06	1	159268759	0.032
ENSG00000074800	ENO1	4.035e-10	1	8930184	0.014
ENSG00000180902	D2HGDH	6.968e-10	2	242691112	0.053
ENSG00000181004	BBS12	2.188e-06	4	123659977	0.008
ENSG00000182134	TDRKH	1.253e-09	1	151753237	0.075
ENSG00000182742	HOXB4	1.780e-06	17	46655174	0.032
ENSG00000186470	BTN3A2	2.087e-09	6	26371966	0.632

Extension: SUMMIT-FA



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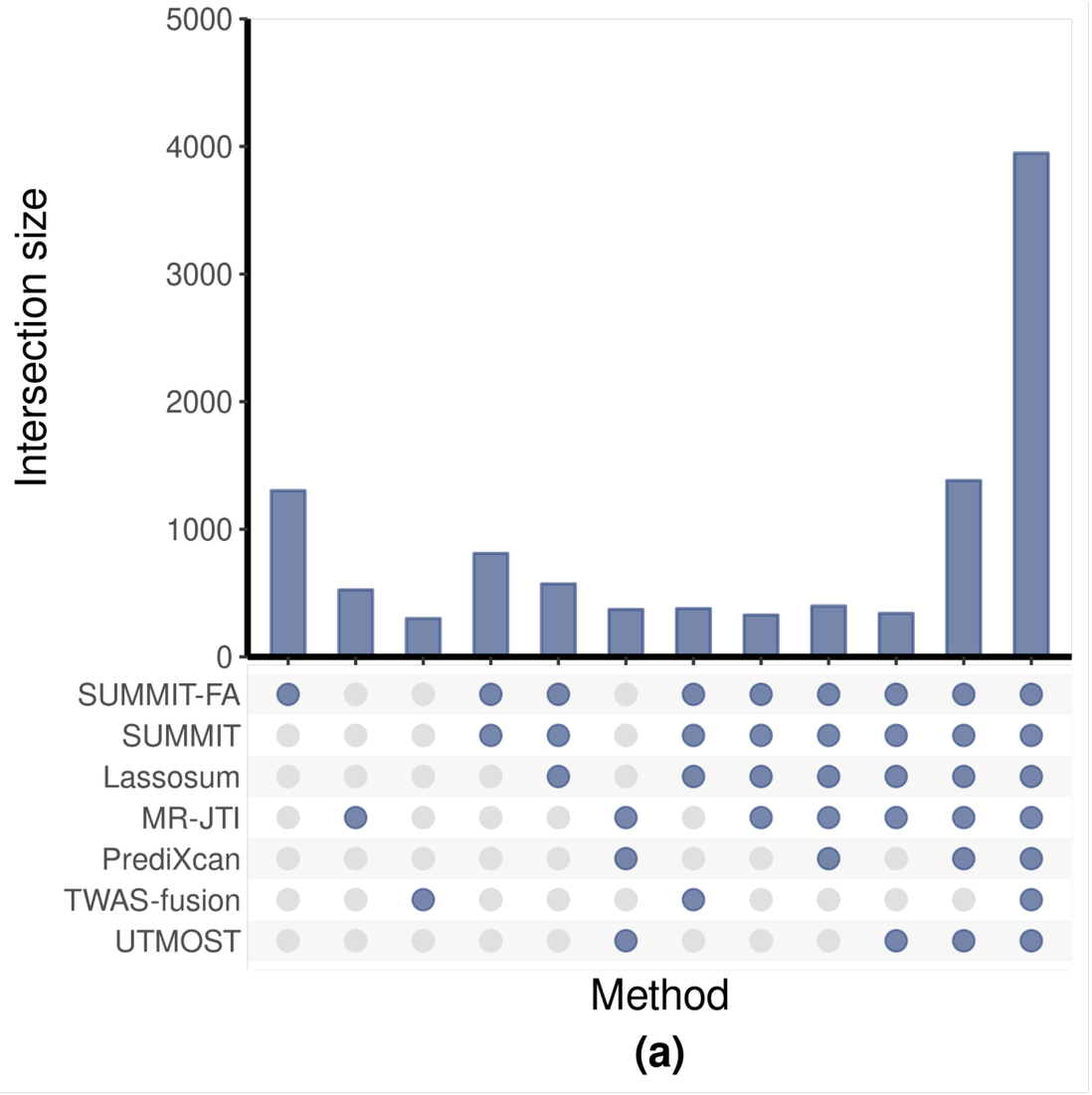

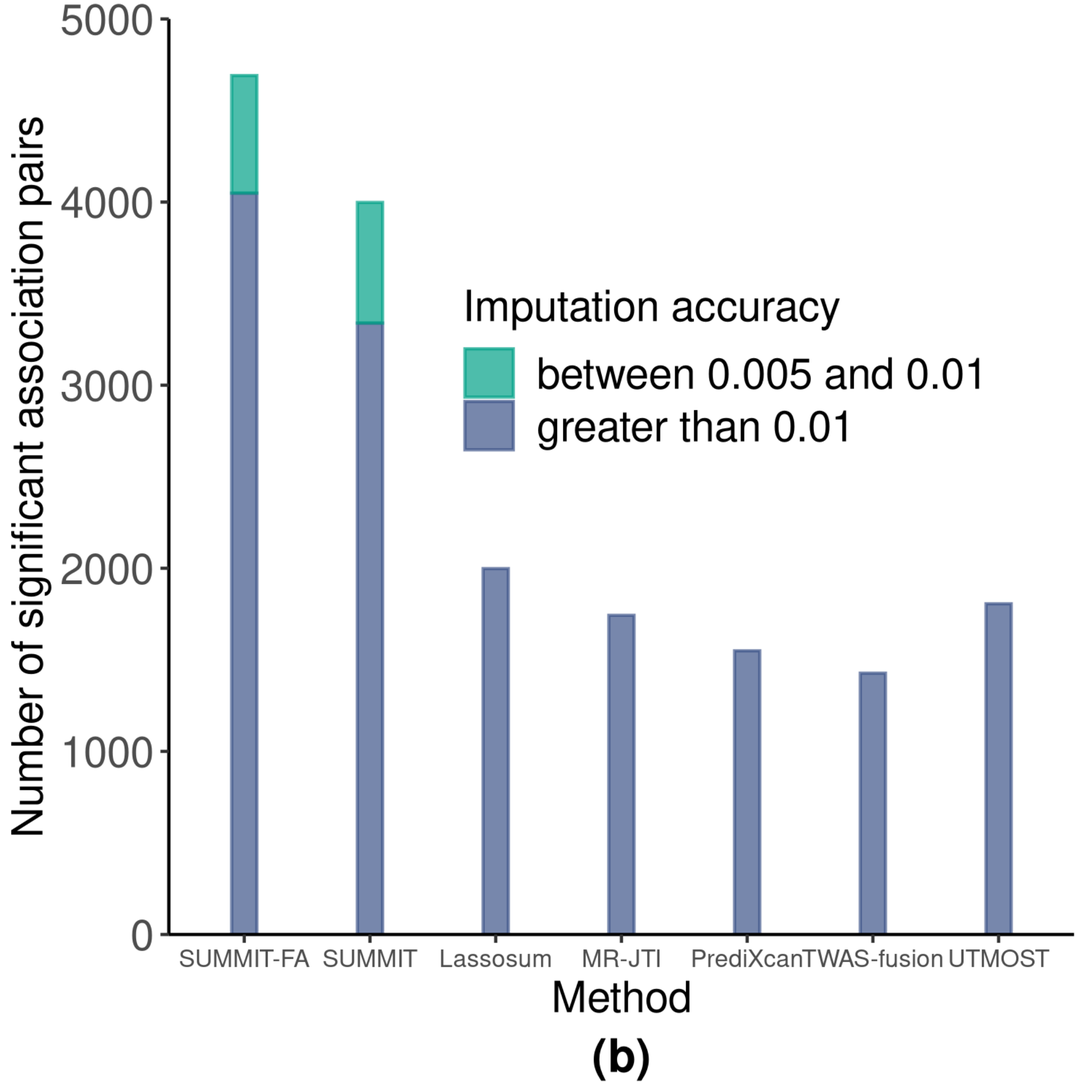

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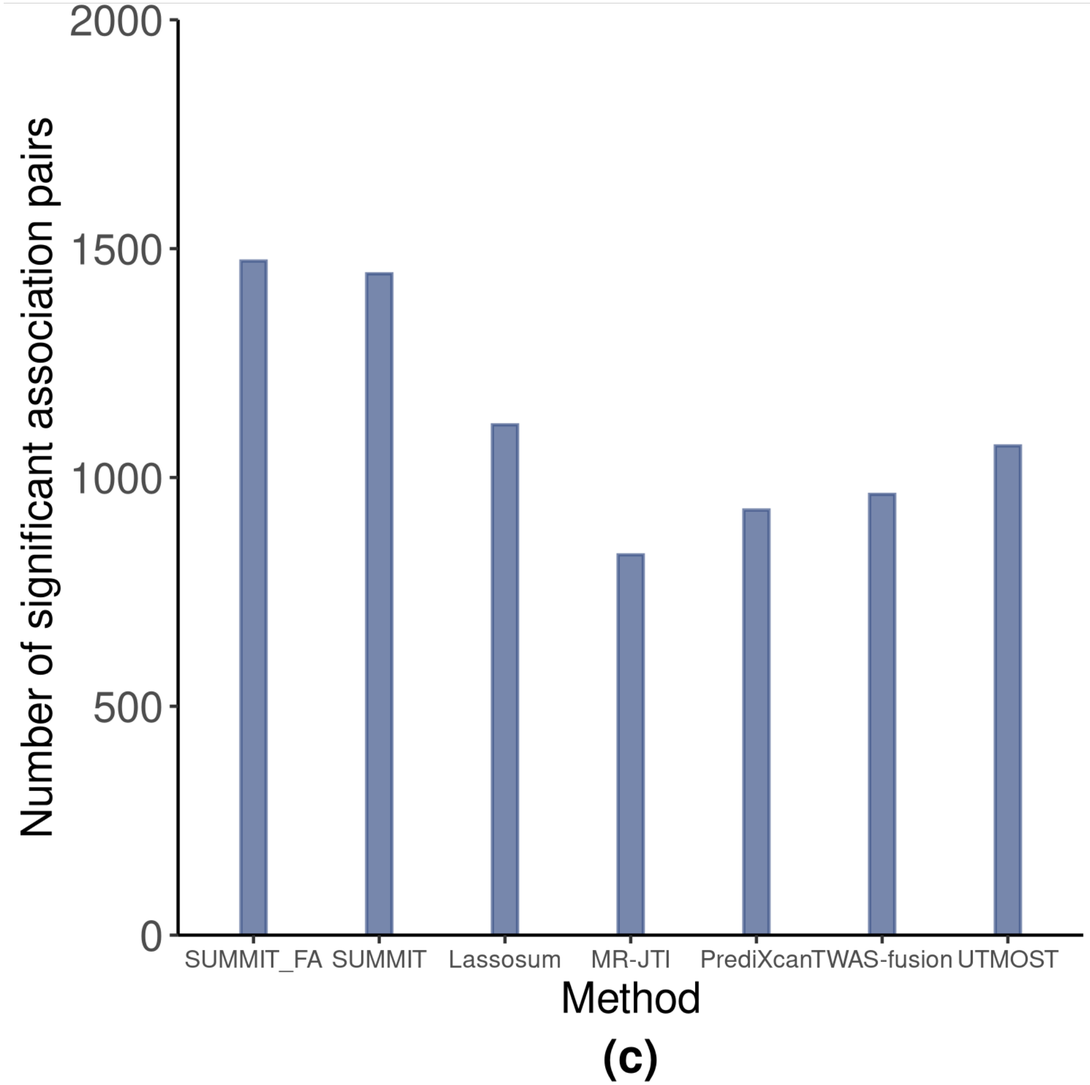

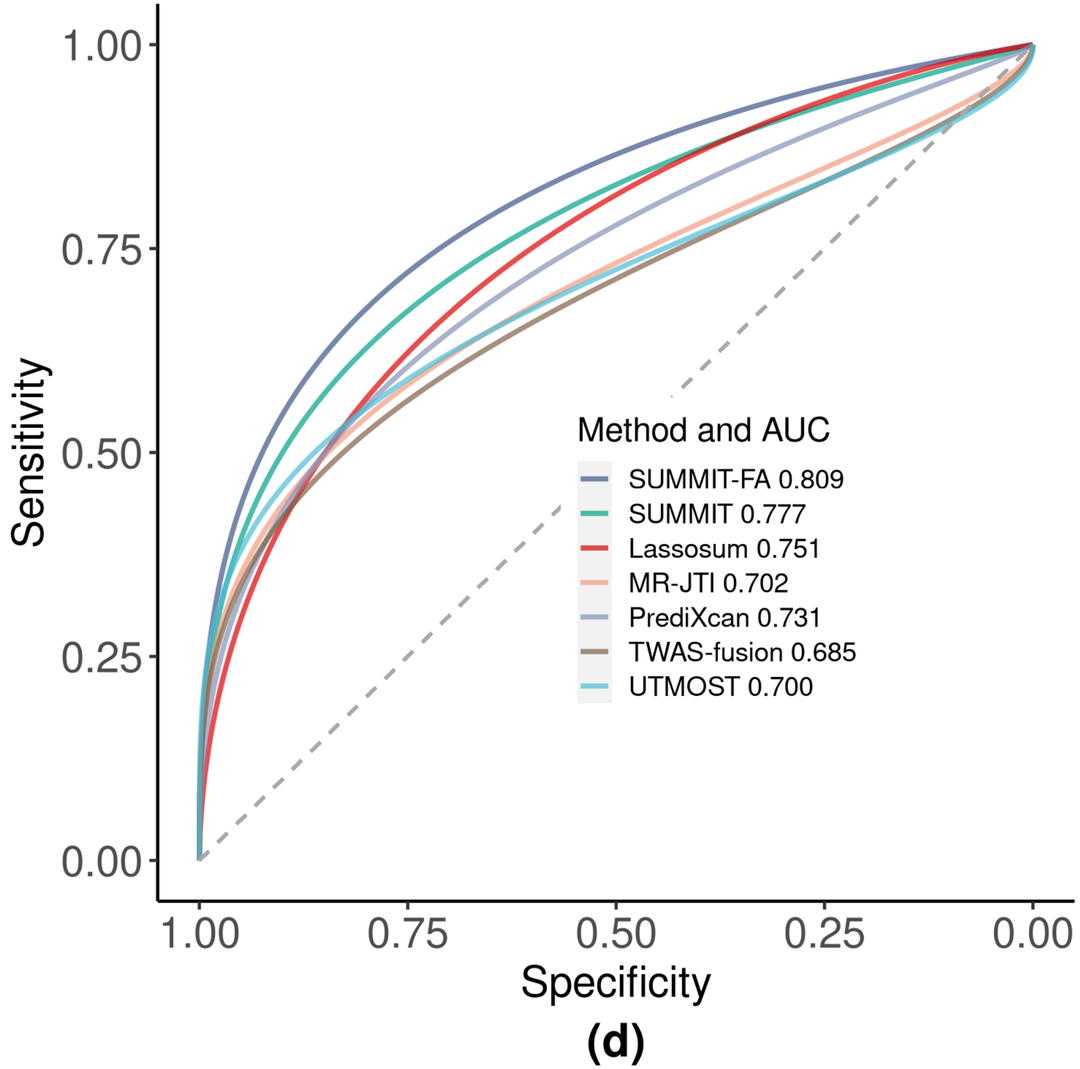

Summary

- By leveraging eQTL data with large sample-size, SUMMIT improves the accuracy of expression prediction in blood, successfully builds expression prediction models for genes with low expression heritability, and outperforms benchmark methods for identifying risk genes
- TWAS methods, including SUMMIT, can be viewed as one type of gene-based Mendelian randomization (MR) and can provide valid causal interpretations only when all genetic variants used in the expression prediction models are valid instrumental variables **(Strong and uncheckable assumption)**

Summary

- Besides complementary analyses (such as fine-mapping and colocalization), robust inference with weak assumptions are needed
- SUMMIT can be extended to other omics data (proteins, DNA methylation, and metabolites)
- Multi-ethnicity: Improve the robustness and performance (transfer learning)
- Multi-ethnicity: Identify ethnicity-specific and pan-ethnicity likely causal biomarkers

Acknowledgements

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Zhang, Zichen, Ye Eun Bae, Jonathan R. Bradley, Lang Wu, and Chong Wu. "SUMMIT: An integrative approach for better transcriptomic data imputation improves causal gene identification." *Nature Communications* **13**, 6336 (2022).

NIH

biobank uk

Thank you!

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