Integrating Multi-omics Summary Data Using Mendelian Randomization

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Outline

1. Mendelian randomization (MR)
2. MR methods for multi-omics summary statistics
3. Methods in *p*-value combinations
4. Simulations
5. Analyzing an Alzheimer's disease dataset

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2. MR methods for multi-omics summary statistics
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5. Analyzing an Alzheimer's disease dataset

Finding a causal relationship

Instrumental variable analysis:

Natural experiment

“encouragement”

Example:

We have subjects with lung disease. Some subjects are randomly selected and encouraged to exercise.

We record if the exercise is performed and compute the effect of exercise on outcome.

Rosenbaum, Paul R. “Identification of Causal Effects Using Instrumental Variables: Comment.” *Journal of the American Statistical Association* **91**, no. 434 (1996): 465–68. https://doi.org/10.2307/2291633.

Finding a causal relationship

Mendelian randomization publications using UKBioBank:

ukbiobank.ac.uk/enable-your-research/publications?terms=causal&query=mendelian+randomization#articles

International Journal of Cancer, July 29th 2022
The causal association between smoking, alcohol consumption and risk of bladder cancer: A univariable and multivariable Mendelian randomization study
J Xiong et al

ESC Heart Failure, June 28th 2022
Causal associations between sleep traits and four cardiac diseases: a Mendelian randomization study
Y Yang et al

Frontiers in Nutrition, June 22nd 2022
Causal Relationship of Genetically Predicted Serum Micronutrients Levels With Sarcopenia: A Mendelian Randomization Study
T Sha et al

The Journal of Clinical Endocrinology and Metabolism, June 15th 2022
The Causal Effect of Systolic Blood Pressure Lowering on Vascular Outcomes in Diabetes: A Mendelian Randomization Study
T Hou et al

BMC Psychiatry , June 15th 2022
The causal relationship between sleep traits and the risk of schizophrenia: a two-sample bidirectional Mendelian randomization study
Z Wang et al

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Search Terms

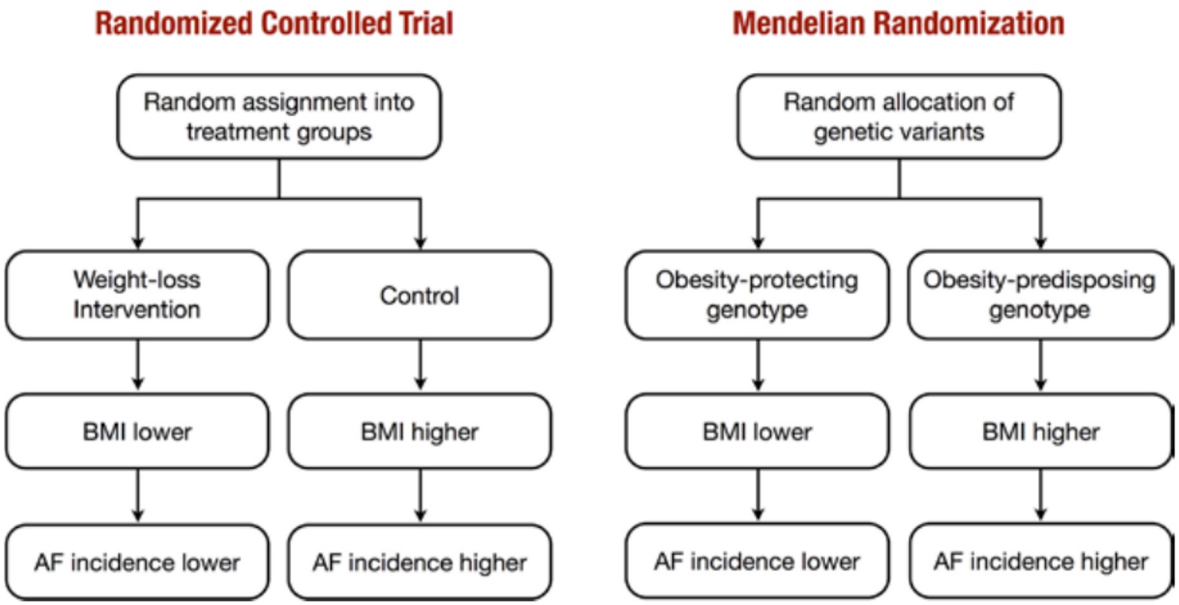
CAUSAL x | MENDELIAN RANDOMIZATION x

Year

2022 (24) | 2021 (29) | 2020 (14) | 2019 (7) | 2018 (3) | 2017 (1)

Finding a causal relationship

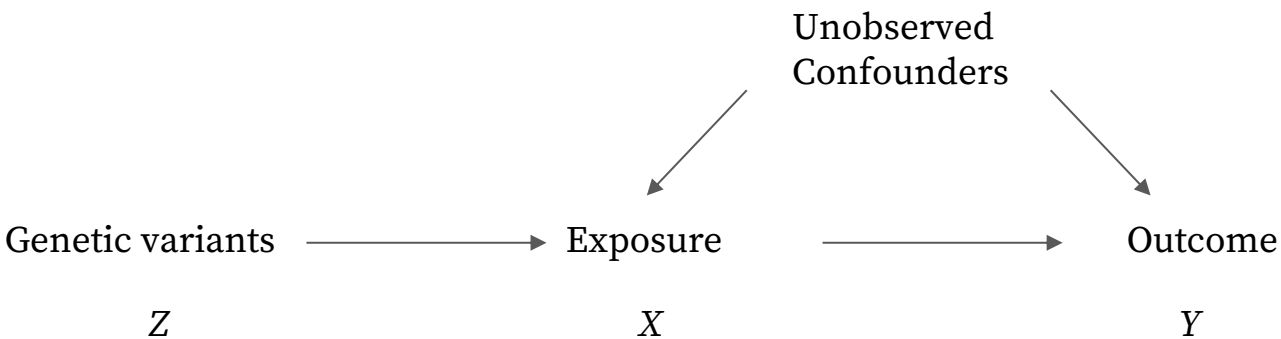
How to find if a higher BMI will cause a higher AF incidence?



AF, atrial fibrillation; BMI, body mass index.

Neeland, I. J. & Kozlitina, J. Mendelian Randomization. *Circulation* **135**, 755–758 (2017).

Finding a causal relationship

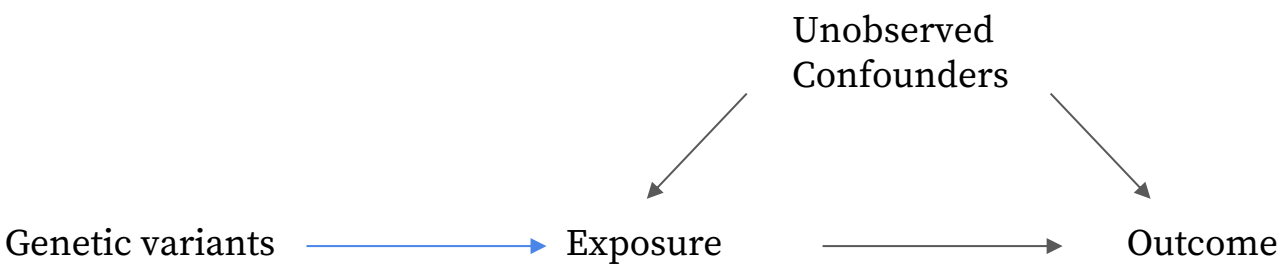


Goal:
Finding casual effect of the exposure on the outcome.

We only have observational data, and a regression of outcome on exposure will be biased since unobserved confounders are unadjusted for.

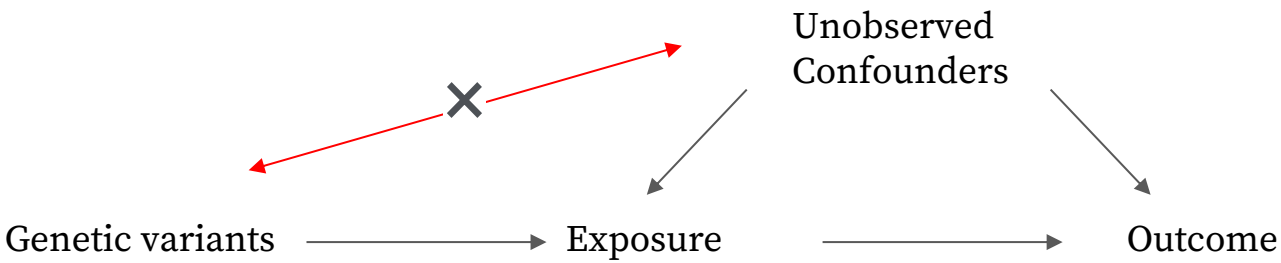
In Mendelian randomization, we will use genetic variants as proxies for the exposure.

Mendelian randomization



Assumptions:
(i) The genetic variant is associated with the exposure.

Mendelian randomization

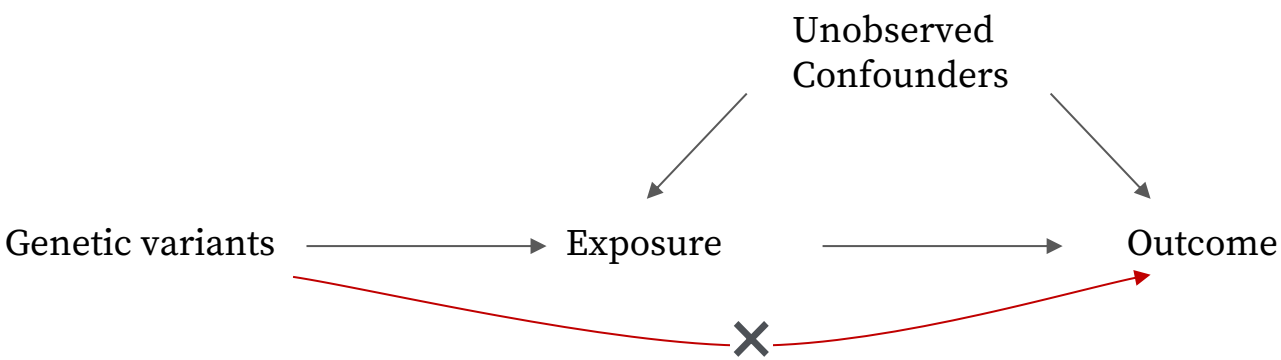


Assumptions:

(i) The genetic variant is associated with the exposure.

(ii) The genetic variant cannot be associated with any confounder that lies within the exposure-outcome relationship.

Mendelian randomization



Assumptions:

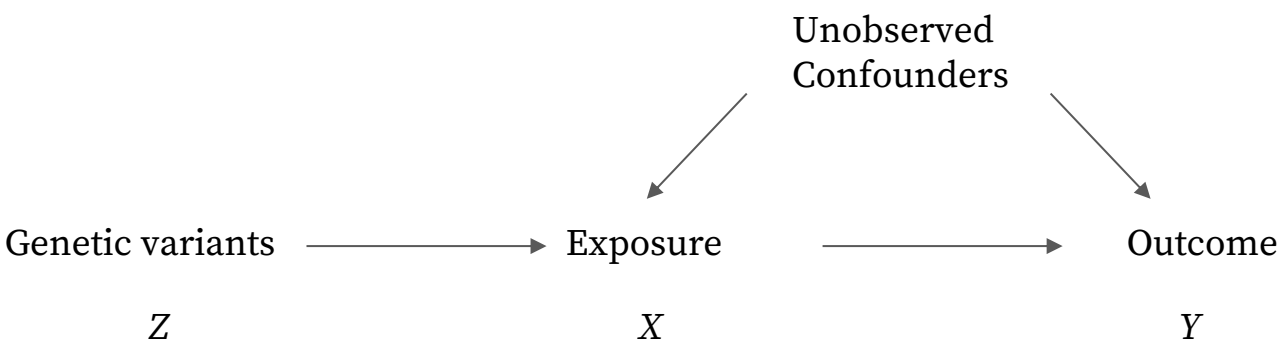
(i) The genetic variant is associated with the exposure.

(ii) The genetic variant cannot be associated with any confounder that lies within the exposure-outcome relationship.

(iii) The genetic variant cannot be associated with the outcome through any pathway other than through the exposure in question.

(Usually, a fourth assumption of linearity or monotonicity is needed for identifiability)

Individual level data vs. summary statistics



Individual-level data:

Individual-level genetic variants Z_i, exposure X_i, and outcome Y_i are available.

Summary statistics:

Summary statistics of regressing exposure on genetic variants $X \sim Z$ and regressing outcome on genetic variants $Y \sim Z$ are available.

General guidelines

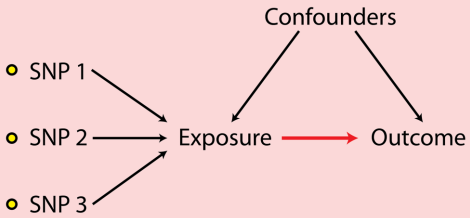
STROBE-MR Guidelines (BMJ 2021)

Guidelines for performing Mendelian randomization investigations (Wellcome Open Research 2020)

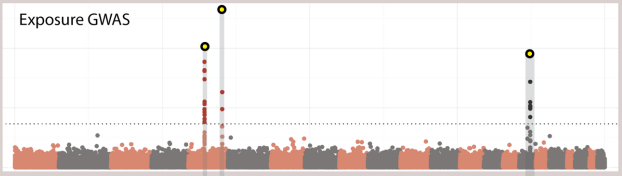
Mendelian randomization (Nature Reviews Methods Primers 2022)

Using the TwoSampleMR package

Objective: Infer the causal effect of the exposure on the outcome



1.



Description

Define instruments: Obtain SNPs that are GWAS significant for the exposure. Ensure that they are independent.

Instruments can be defined from a variety of different sources.

R commands

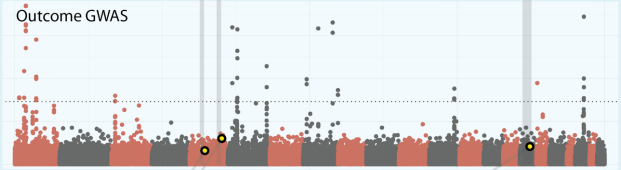
```
extract_instruments()

read_exposure_data()

library(MRInstruments);
  data(gwas_catalog);
  data(aries_mqtl);
  data(gtex_eqtl);
  data(proteomic_qtls);
  data(metab_qtls);

clump_data()
```

2.



Get effects on outcome: Extract the instrument SNPs from the outcome GWAS. If they are not available, use LD proxies instead.

MR Base contains a large database of entire GWAS summary statistics.

```
extract_outcome_data()

read_outcome_data()
```


Gibran Hemani, https://mrcieu.github.io/TwoSampleMR/articles/introduction.html

Using the TwoSampleMR package

3.

	Exposure GWAS				Outcome GWAS			
SNP	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*
rs123456	0.132	A	G	0.28	0.022	A	G	0.26
rs234567	-0.485	G	T	0.41	**0.056**	**T**	**G**	**0.61**
rs345678	0.203	G	C	0.11	**-0.046**	**G**	**C**	**0.88**

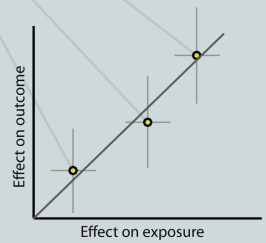
Harmonize effects

	Exposure GWAS				Outcome GWAS			
SNP	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*
rs123456	0.132	A	G	0.28	0.022	A	G	0.26
rs234567	-0.485	G	T	0.41	**-0.056**	**G**	**T**	**0.39**
rs345678	0.203	G	C	0.11	**0.046**	**G**	**C**	**0.12**

Harmonize effects: Ensure that the effect of the SNP on the exposure and the effect of the SNP on the outcome correspond to the same allele.

```
harmonise_data()
```

4.



Perform analysis: Using the harmonized data, perform Mendelian randomization analyses and related sensitivity analyses.

The slope of the regression line corresponds to the causal effect of the exposure on the outcome

```
mr()
mr_singlesnp()
mr_leaveoneout()
mr_heterogeneity()
mr_steiger()
mr_pleiotropy_test()
```


Gibran Hemani, https://mrcieu.github.io/TwoSampleMR/articles/introduction.html

Association between gene expression and phenotype through genotypes

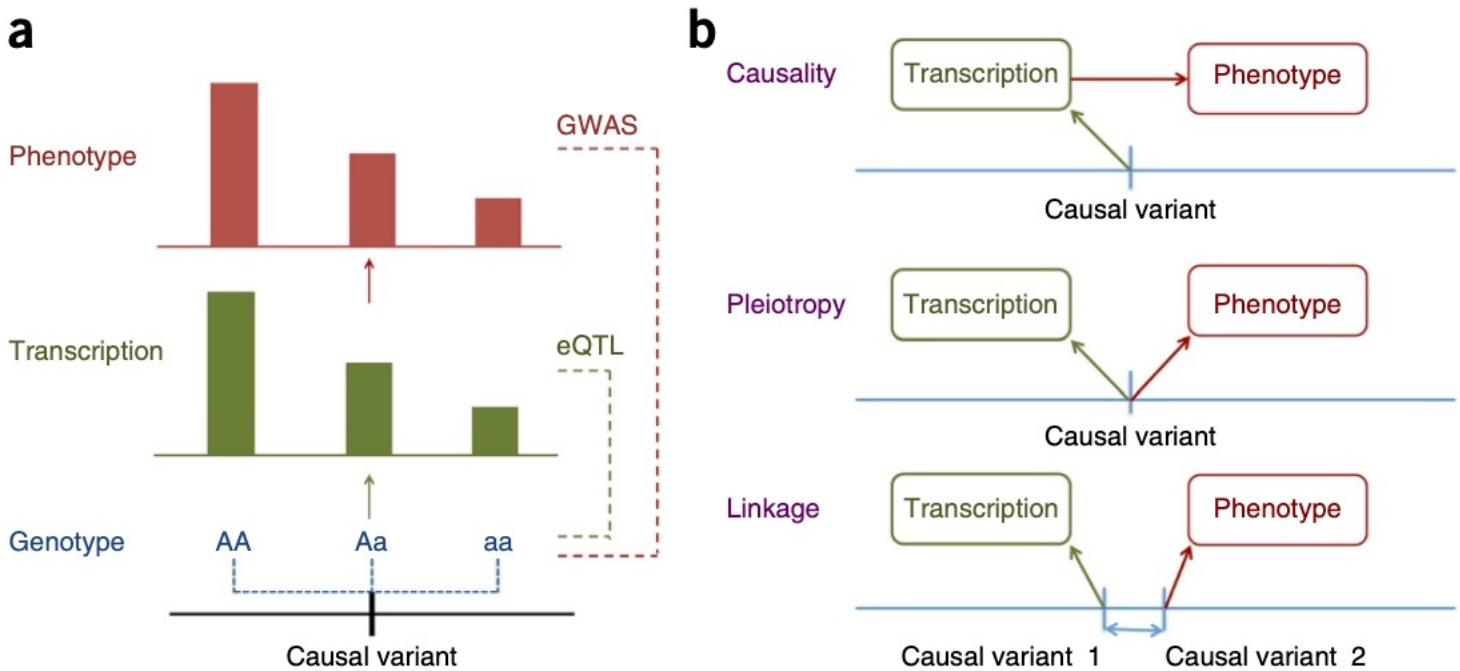


Figure 1 of Zhu, Zhihong, et al. "Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets." Nature genetics 48.5 (2016): 481-487.

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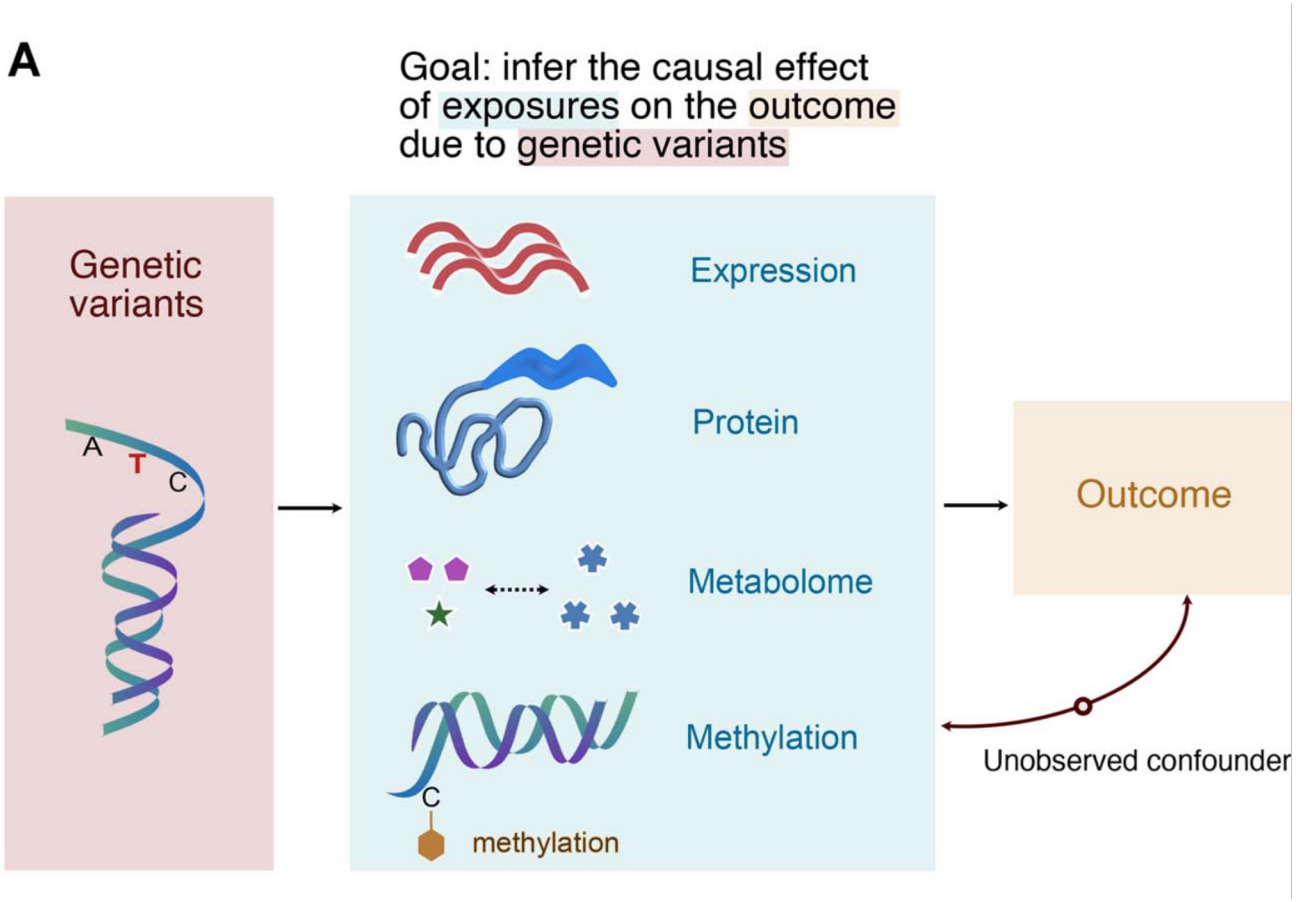
Alzheimer's disease

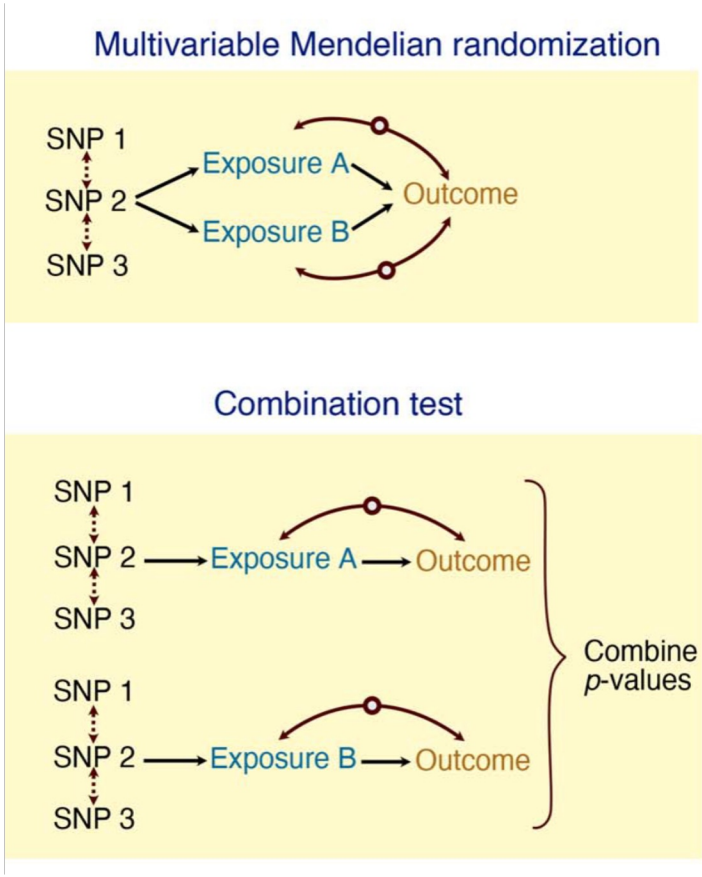
- In 2019, the estimated total worldwide cost of dementia was US$ 1.3 trillion (WHO report)
- Agora is compiling a "Nominated Target List" as investigators nominate genes that are potentially good targets from human genomic, proteomic and/or metabolomic data

Multi-omics Mendelian randomization



Multi-omics Mendelian randomization

In the project, we aim to evaluate which is the better strategy:



Inverse variance-weighted average method

When the genetic variants are uncorrelated, the IVW estimate of the causal effect size of the outcome on the exposure k is

$$\hat{\theta}_{\mathrm{IVW},k} = \frac{\sum_{j=1}^{p} \widehat{\beta}_{Xkj} \widehat{\beta}_{Yj} \widehat{\sigma}_{Yj}^{-2}}{\sum_{j=1}^{p} \widehat{\beta}_{Yj}^{2} \widehat{\sigma}_{Yj}^{-2}},$$

where the standard error of the estimate is

$$\mathrm{se}(\hat{\theta}_{\mathrm{IVW},k}) = \sqrt{\frac{1}{\sum_{j=1}^{p} \widehat{\beta}_{Yj}^{2} \widehat{\sigma}_{Yj}^{-2}}}.$$

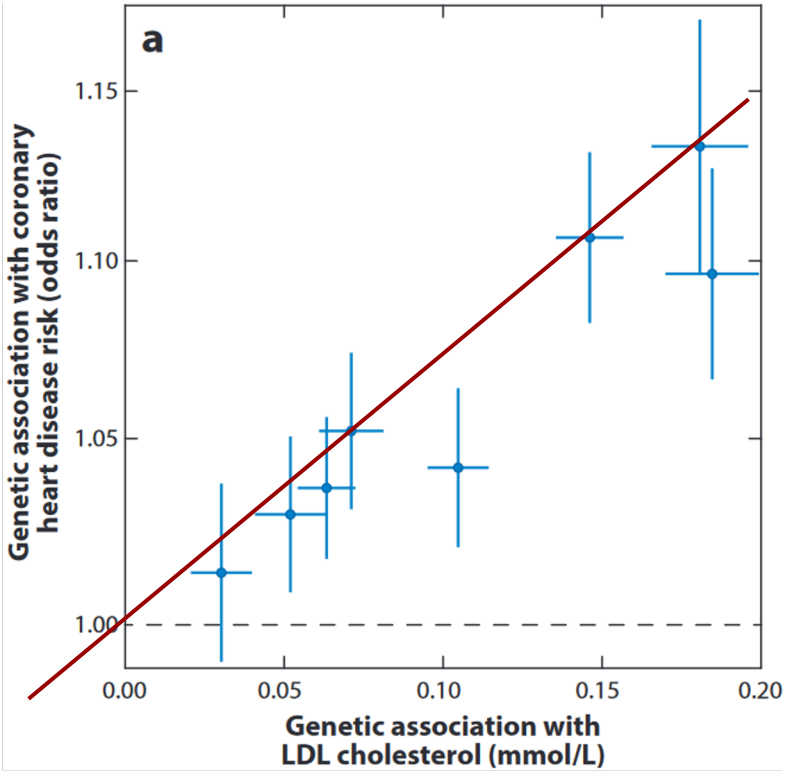


Figure from: Burgess, S., Foley, C. N. & Zuber, V. Inferring Causal Relationships Between Risk Factors and Outcomes from Genome-Wide Association Study Data. *Annual Review of Genomics and Human Genetics* **19**, 303–327 (2018).

Generalized least squares method

The generalized least squares method (GLS) builds upon IVW and accounts for remaining LD within SNPs after LD clumping

$$\widehat{\theta}_{\mathrm{GLS},k} = (\widehat{\boldsymbol{\beta}}_{Xk}^{T}\Omega^{-1}\widehat{\boldsymbol{\beta}}_{Xk})^{-1}(\widehat{\boldsymbol{\beta}}_{Xk}^{T}\Omega^{-1}\widehat{\boldsymbol{\beta}}_{Y}),$$

$$\mathbf{var}\left(\widehat{\theta}_{\mathrm{GLS},k}\right) = (\widehat{\boldsymbol{\beta}}_{Xk}^{T}\Omega^{-1}\widehat{\boldsymbol{\beta}}_{Xk})^{-1},$$

where the covariance matrix Ω is constructed from the unsquared LD matrix R

$$\Omega = (\widehat{\sigma}_{Yi}\widehat{\sigma}_{Yj}r_{ij}) = \begin{bmatrix} \widehat{\sigma}_{Y1} & & \\ & \ddots & \\ & & \widehat{\sigma}_{Yp} \end{bmatrix} R \begin{bmatrix} \widehat{\sigma}_{Y1} & & \\ & \ddots & \\ & & \widehat{\sigma}_{Yp} \end{bmatrix},$$

and r_{ij}'s are the entries in the unsquared LD matrix R.

Summary data-based MR

Applicable when we have only one SNP as instrumental variable

Under the assumption that the GWAS z-value z_Y and QTL z-value z_{Xk} are estimated using independent samples, we have an approximate χ^2 test statistic with one degree of freedom

$$T_{\mathrm{SMR},k} = \frac{z_Y^2 z_{Xk}^2}{z_Y^2 + z_{Xk}^2}.$$

Zhu, Z. et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nature Genetics 48, 481–487 (2016).

Generalized summary data-based MR

$$\widehat{\boldsymbol{\theta}}_k = (\widehat{\theta}_{k1}, \widehat{\theta}_{k2}, \ldots, \widehat{\theta}_{km})^{\mathrm{T}} = \left(\frac{\widehat{\beta}_{\mathrm{Y}1}}{\widehat{\beta}_{\mathrm{X}k1}}, \frac{\widehat{\beta}_{\mathrm{Y}2}}{\widehat{\beta}_{\mathrm{X}k2}}, \ldots, \frac{\widehat{\beta}_{\mathrm{Y}p}}{\widehat{\beta}_{\mathrm{X}kp}} \right)^{\mathrm{T}},$$

$$\mathrm{var}\left(\widehat{\theta}_{kj}\right) = \frac{\widehat{\beta}_{\mathrm{Y}j}^2}{\widehat{\beta}_{\mathrm{X}kj}^2} \left[\frac{\mathrm{var}(\widehat{\beta}_{\mathrm{X}kj})}{\widehat{\beta}_{\mathrm{X}kj}^2} + \frac{\mathrm{var}(\widehat{\beta}_{\mathrm{Y}j})}{\widehat{\beta}_{\mathrm{Y}j}^2} - \frac{\mathrm{var}(\widehat{\beta}_{\mathrm{X}kj})^2}{\widehat{\beta}_{\mathrm{X}kj}^4} \right],$$

$$\mathrm{cov}\left(\widehat{\theta}_{ki}, \widehat{\theta}_{kj}\right) = \frac{r_{ij}\sqrt{\mathrm{var}(\widehat{\beta}_{\mathrm{Y}i})\mathrm{var}(\widehat{\beta}_{\mathrm{Y}j})}}{\widehat{\beta}_{\mathrm{X}ki}\widehat{\beta}_{\mathrm{X}kj}} + \frac{\widehat{\beta}_{\mathrm{Y}i}\widehat{\beta}_{\mathrm{Y}j}}{\widehat{\beta}_{\mathrm{X}ki}\widehat{\beta}_{\mathrm{X}kj}}$$

$$\left[\frac{r_{ij}\sqrt{\mathrm{var}(\widehat{\beta}_{\mathrm{X}ki})\mathrm{var}(\widehat{\beta}_{\mathrm{X}kj})}}{\widehat{\beta}_{\mathrm{X}ki}\widehat{\beta}_{\mathrm{X}kj}} - \frac{\mathrm{var}(\widehat{\beta}_{\mathrm{X}ki})\mathrm{var}(\widehat{\beta}_{\mathrm{X}kj})}{\widehat{\beta}_{\mathrm{X}ki}^2\widehat{\beta}_{\mathrm{X}kj}^2} \right],$$

$$\mathrm{V}_k = \left(\mathrm{cov}\left(\widehat{\theta}_{ki}, \widehat{\theta}_{kj}\right)\right)_{p\times p}.$$

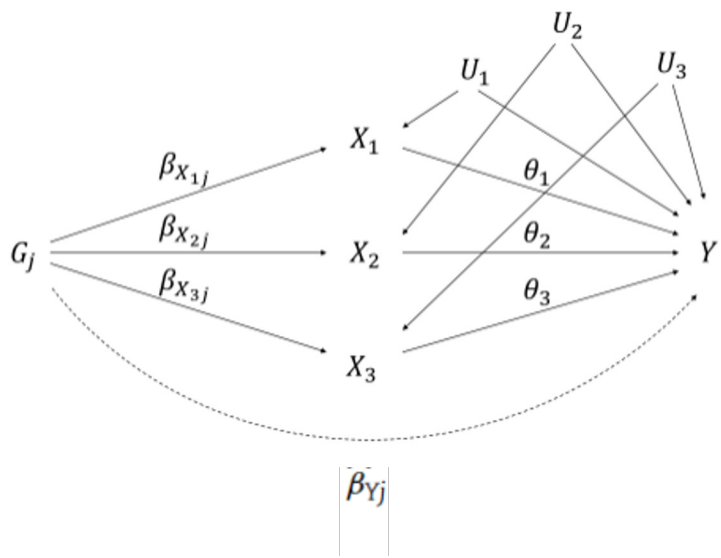
$$\widehat{\theta}_{\mathrm{GSMR},k} = (\mathbf{1}^{\mathrm{T}}\mathrm{V}_k^{-1}\mathbf{1})^{-1}\mathbf{1}^{\mathrm{T}}\mathrm{V}_k^{-1}\widehat{\boldsymbol{\theta}}_{\mathbf{k}},$$

$$\mathrm{var}\left(\widehat{\theta}_{\mathrm{GSMR},k}\right) = (\mathbf{1}^{\mathrm{T}}\mathrm{V}_k^{-1}\mathbf{1})^{-1},$$

$$T_{\mathrm{GSMR},k} = \widehat{\theta}_{\mathrm{GSMR},k}^2 / \mathrm{var}\left(\widehat{\theta}_{\mathrm{GSMR},k}\right).$$

Multivariable MR

$$\widehat{\beta}_{Yj} = \sum_{k=1}^{m} \theta_k \widehat{\beta}_{Xkj} + \epsilon, \quad \textbf{weights} = \widehat{\sigma}_{Yj}^{-2},$$



Rees, J. M. B., Wood, A. M. & Burgess, S. Extending the MR-Egger method for multivariable Mendelian randomization to correct for both measured and unmeasured pleiotropy. *Statistics in Medicine* **36**, 4705–4718 (2017).

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Methods in p-value combinations

Cauchy combination test:

Cauchy distribution

Harmonic mean p-value:

Stable distribution (1,1)

$$T_{\text{Cauchy}} = \sum_{k=1}^{m} w_k \tan\{(0.5 - p_k)\pi\}$$

$$T_{\text{HMP}} = \frac{\sum_{k=1}^{m} w_k}{\sum_{k=1}^{m} w_k / p_k}$$

The p-values do not need to be independent in Cauchy combination test or HMP.

Methods in p-value combinations

Cauchy combination test:

Cauchy distribution

Harmonic mean p-value:

Stable distribution (1,1)

Minimum p-value:

Uniform distribution

Fisher combination test:

(Fisher_chisq) chi-squared distribution

(Fisher_gamma) gamma distribution to approximate the null distribution can *account for the correlation between p-values*

$$T_{\text{Cauchy}} = \sum_{k=1}^{m} w_k \tan\{(0.5 - p_k)\pi\}$$

$$T_{\text{HMP}} = \frac{\sum_{k=1}^{m} w_k}{\sum_{k=1}^{m} w_k / p_k}$$

$$T_{\text{MinP}} = \min_{k \in \{1 \ldots m\}} p_k$$

$$T_{\text{Fisher}} = -2 \sum_{k=1}^{m} \ln(p_k)$$

Methods in p-value combinations

Cauchy combination test:

Cauchy distribution

$$T_{\text{Cauchy}} = \sum_{k=1}^{m} w_k \tan\{(0.5 - p_k)\pi\}$$

Harmonic mean p-value:

Stable distribution (1,1)

$$T_{\text{HMP}} = \frac{\sum_{k=1}^{m} w_k}{\sum_{k=1}^{m} w_k / p_k}$$

Minimum p-value:

Uniform distribution

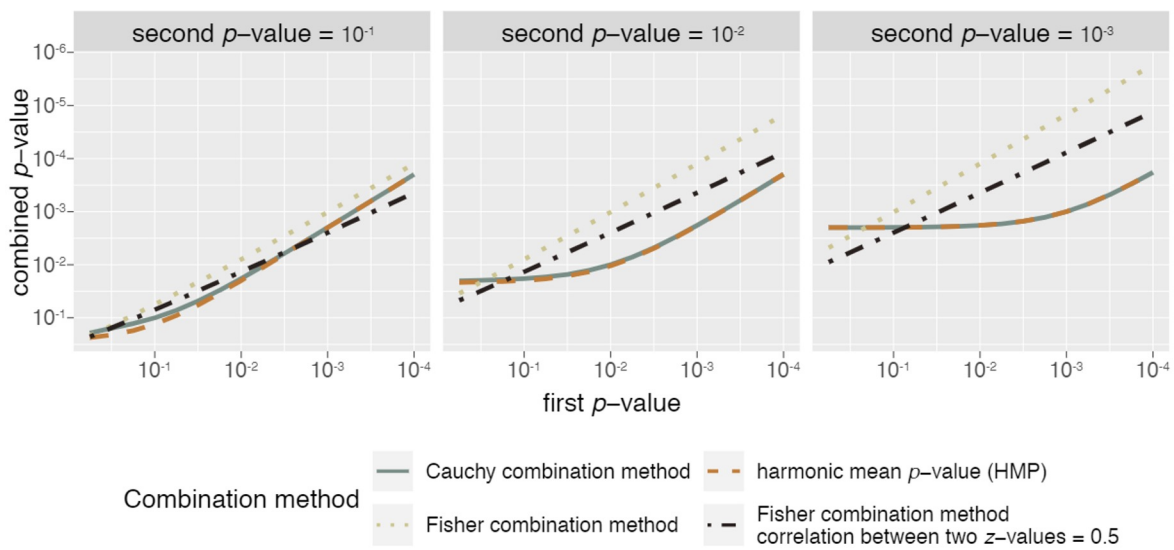
$$T_{\text{MinP}} = \min_{k \in \{1 \ldots m\}} p_k$$

Fisher combination test:

chi-squared distribution

gamma distribution

$$T_{\text{Fisher}} = -2 \sum_{k=1}^{m} \ln(p_k)$$



Fisher combination test: Fisher_gamma

- Plugging in $\boldsymbol{\gamma}_{Xk}$ and $\mathbf{z}_Y$ to the GLS estimator, we have

$$\widehat{\theta}_{\mathrm{GLS},k} = (\widehat{\boldsymbol{\gamma}}_{Xk}^T \mathrm{R}^{-1} \widehat{\boldsymbol{\gamma}}_{Xk})^{-1} (\widehat{\boldsymbol{\gamma}}_{Xk}^T \mathrm{R}^{-1} \mathbf{z}_Y),$$

$$\mathrm{var}\left(\widehat{\theta}_{\mathrm{GLS},k}\right) = (\widehat{\boldsymbol{\gamma}}_{Xk}^T \mathrm{R}^{-1} \widehat{\boldsymbol{\gamma}}_{Xk})^{-1},$$

$$z_{\mathrm{GLS},k} = \frac{\widehat{\theta}_{\mathrm{GLS},k}}{\sqrt{\mathrm{var}\left(\widehat{\theta}_{\mathrm{GLS},k}\right)}},$$

and

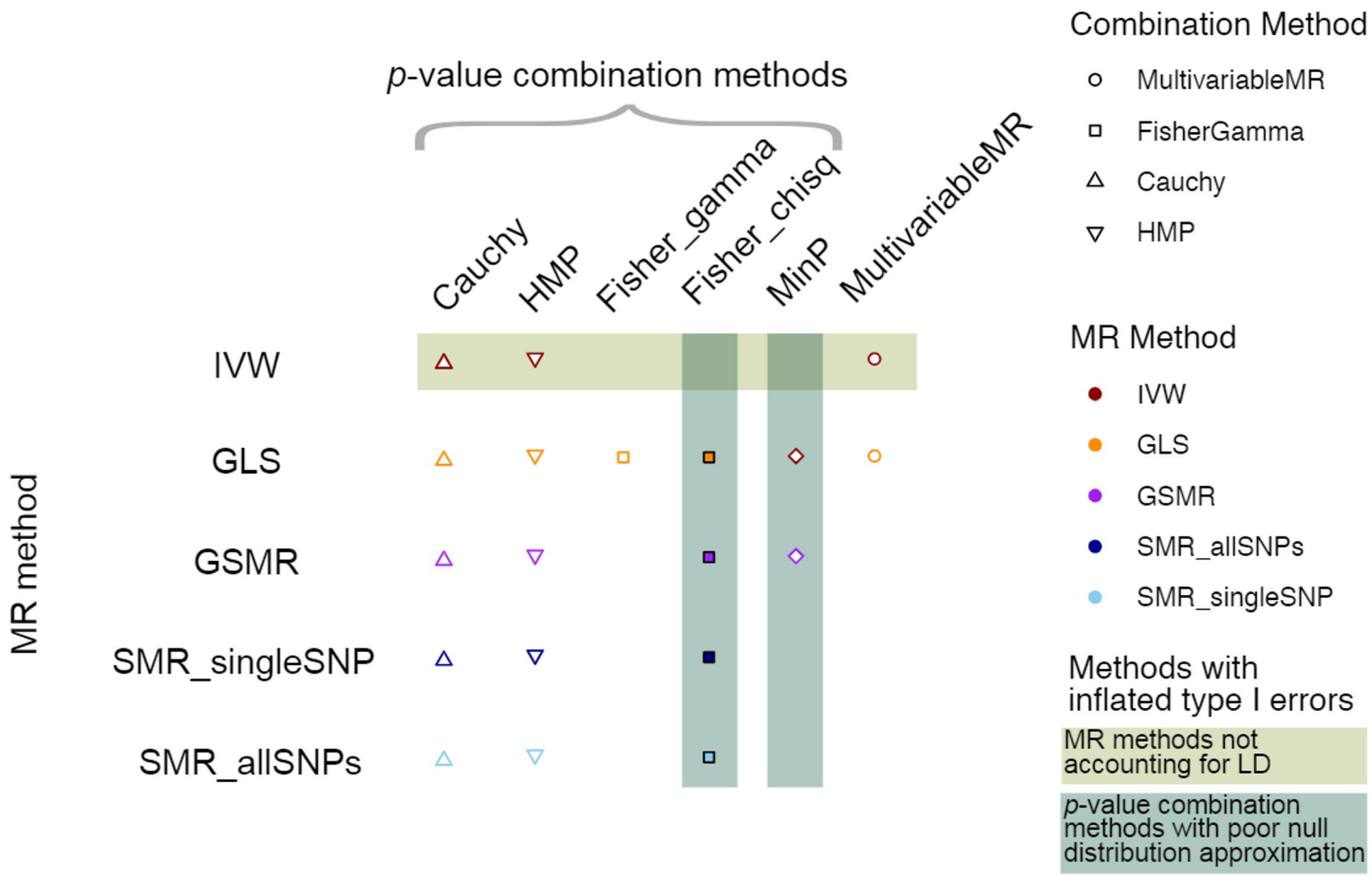
$$\begin{aligned}
&\mathrm{cov}\left(z_{\mathrm{GLS},k_1}, z_{\mathrm{GLS},k_2}\right) \\
=&\mathrm{cov}\left(\frac{\widehat{\boldsymbol{\beta}}_{Xk_1}^T \mathrm{R}^{-1} \mathbf{z}_Y}{\sqrt{\widehat{\boldsymbol{\beta}}_{Xk_1}^T \mathrm{R}^{-1} \widehat{\boldsymbol{\beta}}_{Xk_1}}}, \frac{\widehat{\boldsymbol{\beta}}_{Xk_2}^T \mathrm{R}^{-1} \mathbf{z}_Y}{\sqrt{\widehat{\boldsymbol{\beta}}_{Xk_2}^T \mathrm{R}^{-1} \widehat{\boldsymbol{\beta}}_{Xk_2}}}\right) \\
=&\left(\frac{\widehat{\boldsymbol{\beta}}_{Xk_1}^T \mathrm{R}^{-1}}{\sqrt{\widehat{\boldsymbol{\beta}}_{Xk_1}^T \mathrm{R}^{-1} \widehat{\boldsymbol{\beta}}_{Xk_1}}}\right) \mathrm{R} \left(\frac{\widehat{\boldsymbol{\beta}}_{Xk_2}^T \mathrm{R}^{-1}}{\sqrt{\widehat{\boldsymbol{\beta}}_{Xk_2}^T \mathrm{R}^{-1} \widehat{\boldsymbol{\beta}}_{Xk_2}}}\right)^T.
\end{aligned}$$

Then we can use a Gamma distribution approximation in Yang *et al.* 2016 when the Fisher combination statistic is the sum of dependent chi-squared statistics:

$$T_{\mathrm{Fisher}} = -2 \sum_{k=1}^{m} \ln(p_k)$$

Mendelian randomization (MR) methods and combination methods

The methods we investigate in the simulation are marked by dots:



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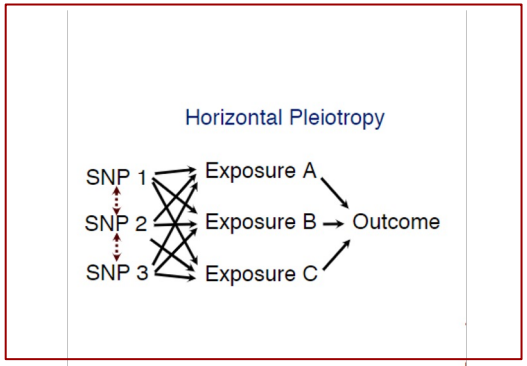
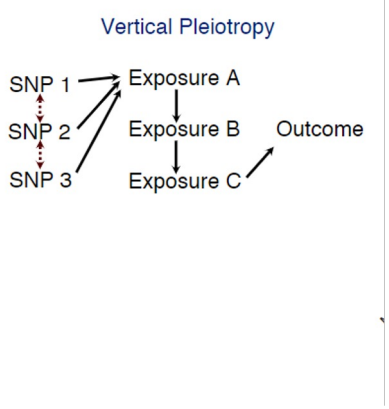
Simulation settings: **Horizontal** vs. Vertical Pleiotropy





$$Z_j \sim \text{Binom}(2, 0.3) \text{ for } j = 1, \ldots, p,$$

$$r_{ij}^2 = 0, 0.01, \text{or } 0.2 \text{ for } i \neq j,$$

$$X_1 = \frac{\alpha}{p} \sum_{j=1}^{p} Z_j + U + \epsilon_{X_1},$$

$$X_2 = 2X_1 - U + \epsilon_{X_2},$$

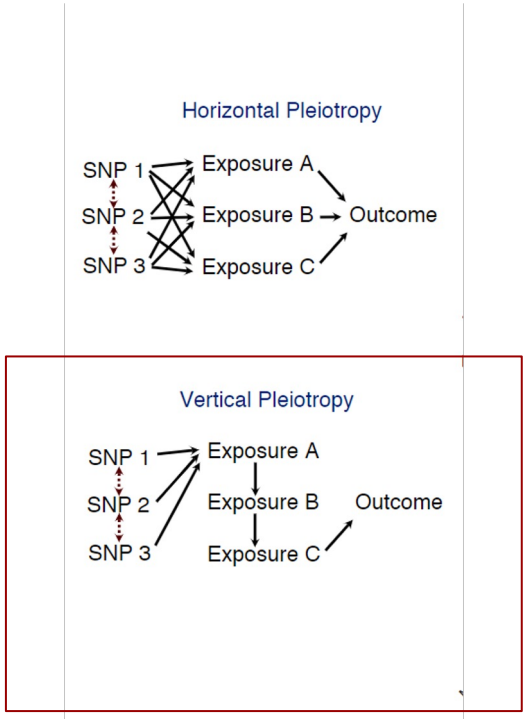
$$X_3 = -0.5X_2 + U + \epsilon_{X_3},$$

$$Y_B \sim \text{Binom}(1, \tau), \text{ where } \text{logit}(\tau) = -2 + \beta_X X_3 - U,$$

$$Y_C = \beta_X X_3 - U + \epsilon_Y,$$

$$U \sim \mathcal{N}(0, 1), \ \epsilon_{X_k} \sim \mathcal{N}(0, 1), \ \epsilon_Y \sim \mathcal{N}(0, 1) \text{ independently}.$$

Simulation settings: Horizontal vs. **Vertical** Pleiotropy



$$Z_j \sim \text{Binom}(2,\ 0.3) \text{ for } j = 1, \ldots, p,$$

$$r_{ij}^2 = 0, 0.01, \text{or } 0.2 \text{ for } i \neq j,$$

$$X_1 = \frac{\alpha}{p} \sum_{j=1}^{p} Z_j + U + \epsilon_{X_1},$$

$$X_2 = -\frac{\alpha}{p} \sum_{j=1}^{\lfloor p/2 \rfloor} Z_j - U + \epsilon_{X_2},$$

$$X_3 = \frac{\alpha}{p} \sum_{j=\lfloor p/2 \rfloor + 1}^{p} Z_j - U + \epsilon_{X_3},$$

$$Y_B \sim \text{Binom}(1, \tau), \text{ where } \text{logit}(\tau) = -2 + \beta_X \sum_{k=1}^{3} X_k + U,$$

$$Y_C = \beta_X \sum_{k=1}^{3} X_k + u_i + \epsilon_Y,$$

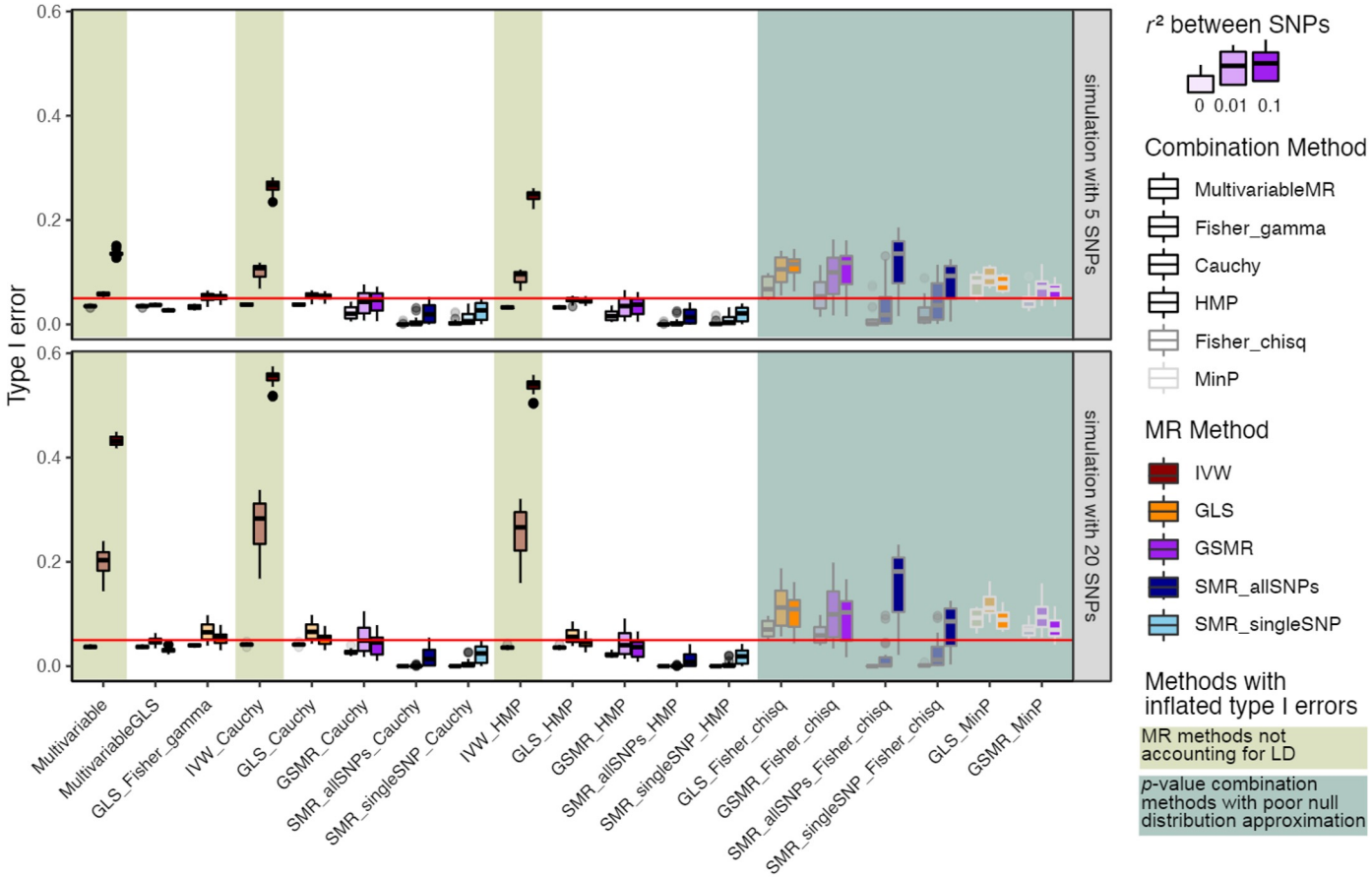
$$U \sim \mathcal{N}(0,1),\ \epsilon_{X_k} \sim \mathcal{N}(0,1),\ \epsilon_Y \sim \mathcal{N}(0,1) \text{ independently}.$$

Simulation settings

- (i) Pleiotropy: horizontal or vertical;
- (ii) Number of SNPs used as IVs: 5 or 20;
- (iii) Overlap between samples involved in the GWAS studies and the QTL studies: 0 (two-sample), 0.5 (half of the samples overlap) or 1 (one-sample);
- (iv) Use same samples or independent samples to calculate QTLs belonging to multiple omics biomarkers under a two-sample setting;
- (v) Outcome: continuous (Y_C) or binary (Y_B). When the outcome is binary, we also need to decide whether we will only use control samples to estimate the QTLs;
- (vi) LD between different SNPs r_2: 0, 0.01 or 0.2;
- (vii) Strength of IV α, which is proportional to the association between IVs and exposures: 0.5, 1 or 2;
- (viii) Effect size of the pleiotropic association between exposure and outcome β_X: 0 or 0.1.

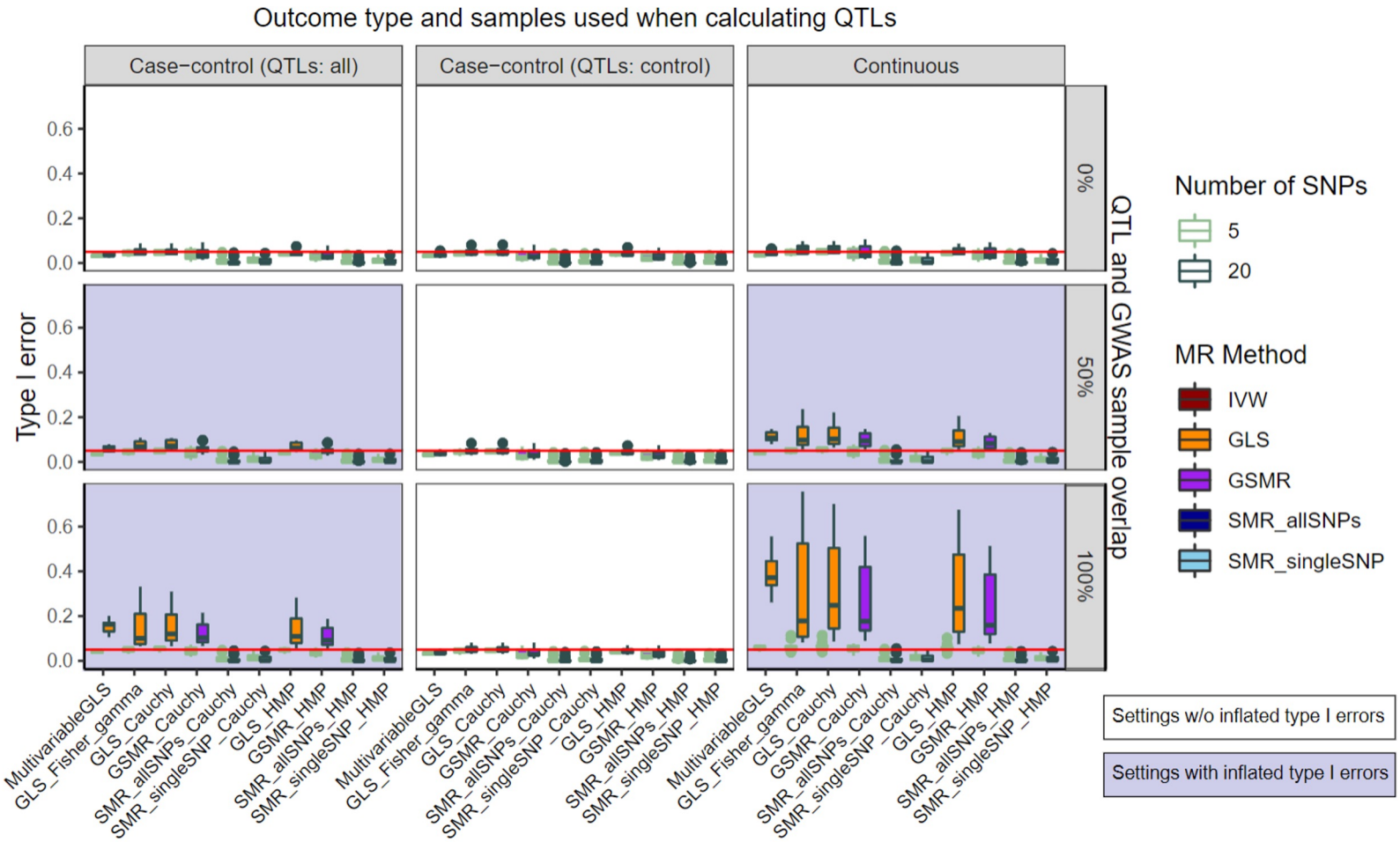
A comparison of the type I error across methods

The unshaded methods can control type I error for a large number of simulation settings

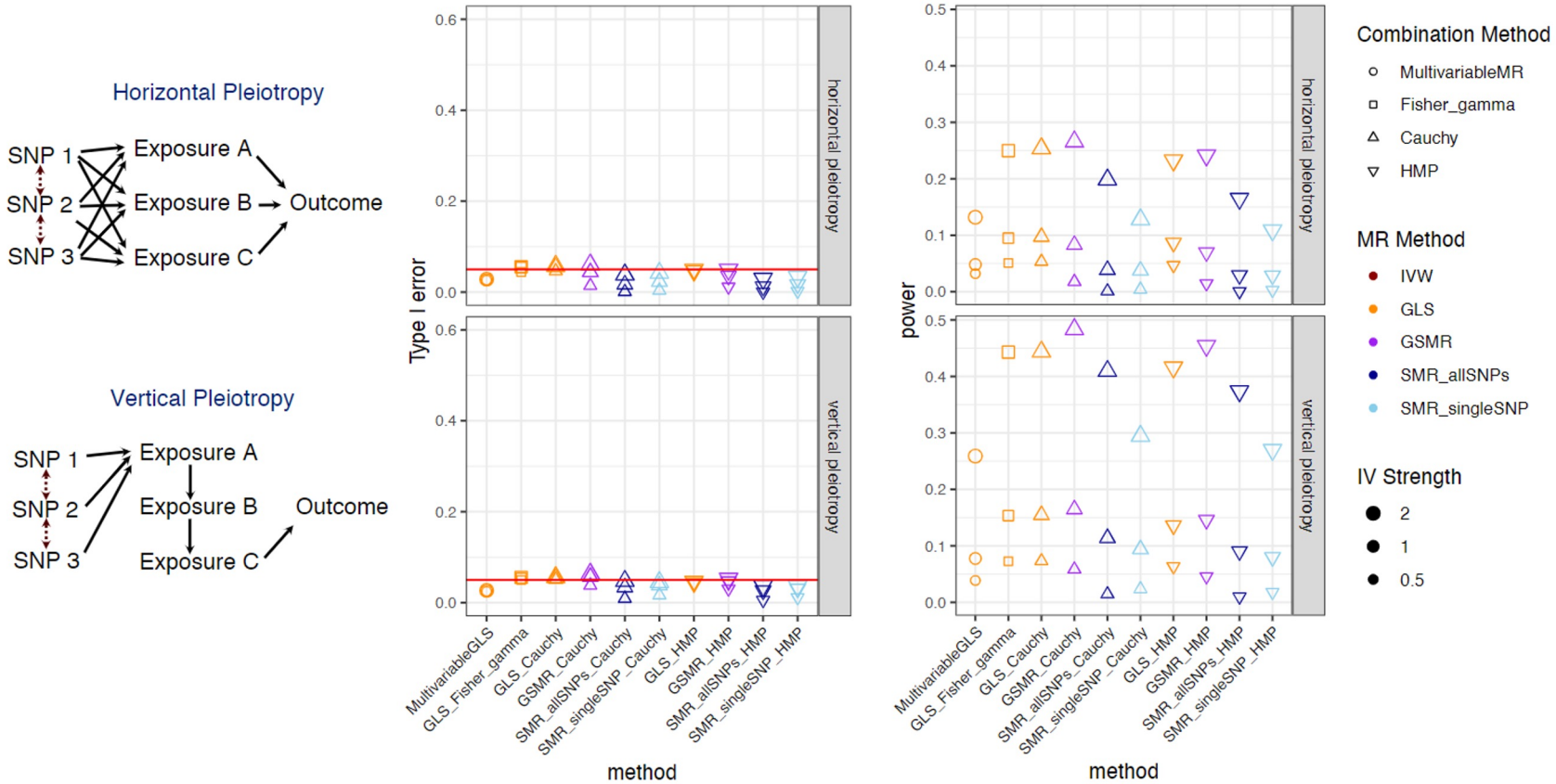


Type I error when the QTL and the GWAS datasets are simulated with various degrees of overlap

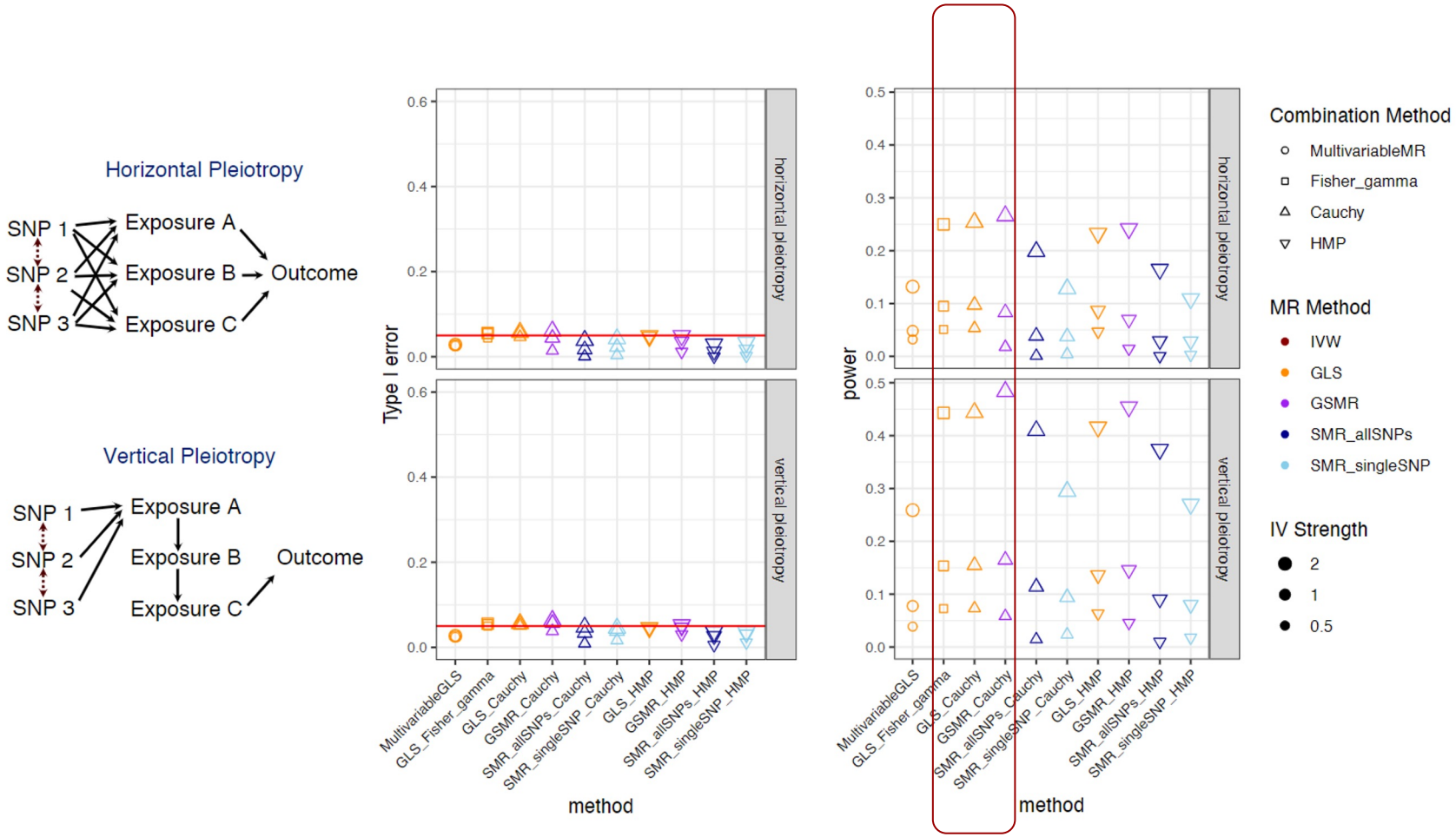
We can control type I error either when there is no overlap between samples *(row of grid)* or when outcome type is binary and only control samples are used to estimate QTLs *(column of grid).*



Type I error and power of selected combinations of MR methods and combination methods



Methods that have the highest powers



Outline

1. Mendelian randomization (MR)
2. MR methods for multi-omics summary statistics
3. Methods in *p*-value combinations
4. Simulations
5. Analyzing an Alzheimer's disease dataset

Prioritization of genes linked to Alzheimer's disease using expression, proteomics, and metabolomics biomarkers

(iv) Select a list of 584 genes with some evidence of association to Alzheimer's disease from Agora genes at https://agora.adknowledgeportal.org/genes.

(v) Within the Agora genes, select 41 genes where all three omics biomarkers (expression, proteomics and metabolomics) are present.

(vi) Select 27 genes that at least one of eQTL, pQTL and metQTL is significant (P-value $< \frac{0.05}{41}$).

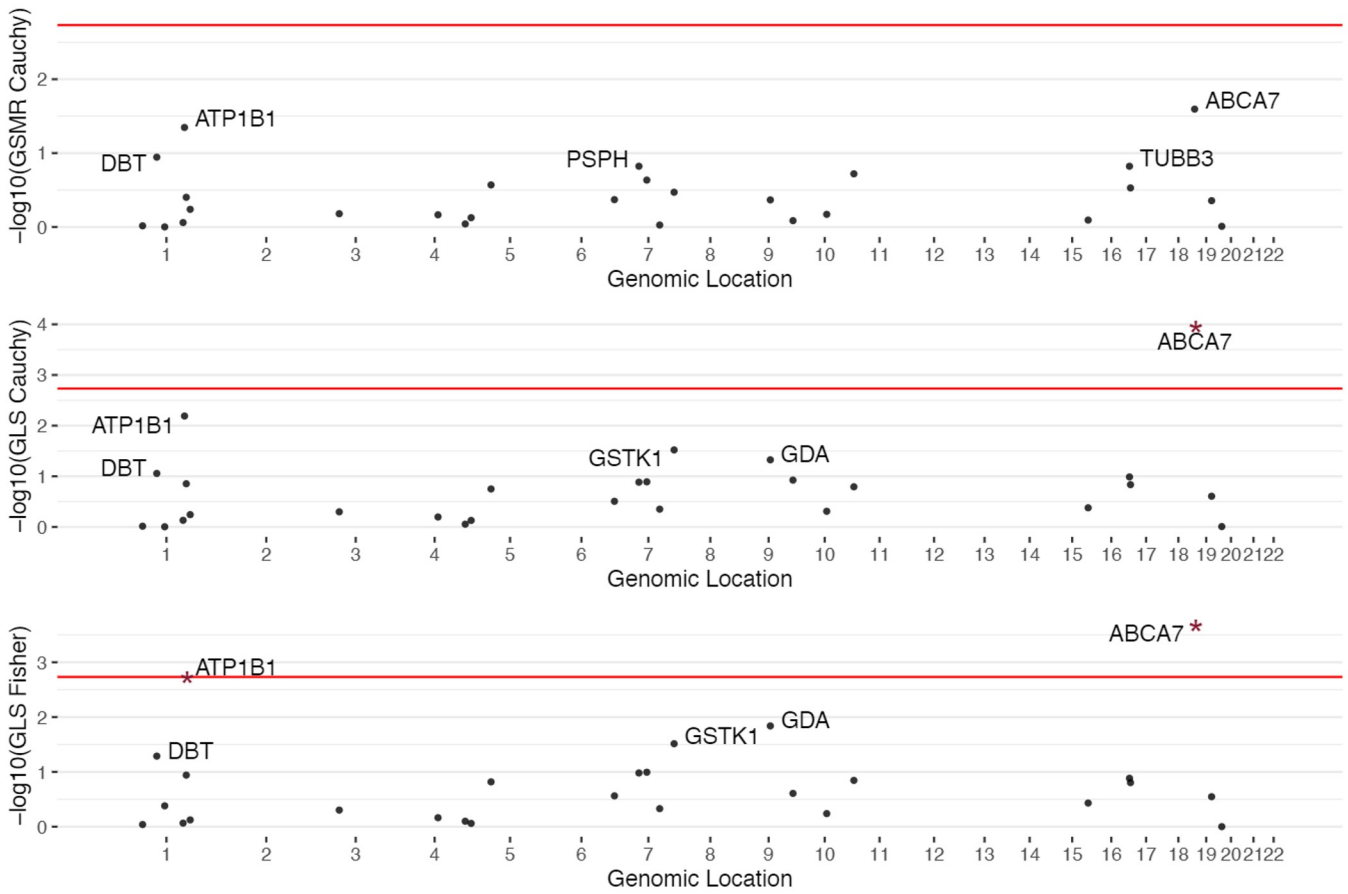
(vii) For each gene, select cis-SNPs (distance between gene and SNP less than 100 kb) and perform LD clumping in each omics biomarker (select SNP with the smallest QTL when possible, $r^2 < 0.2$, distance > 100 kb).

(viii) Perform MR analysis for each omics biomarker using the exposure datasets and the outcome datasets.

(ix) Integrate P-values using combination tests.

Prioritization of genes linked to Alzheimer's disease using expression, proteomics, and metabolomics biomarkers

GLS_Fisher can discover two significant genes *ABCA7* and *ATP1B1*



Discussion

- Prioritization of genes in a genome-wide analysis achieves an increase in power at the expense of deeper interrogations of relationship between biomarkers
- Hard to differentiate causality from pleiotropy and linkage
- Three-sample MR (Zhao *et al.* 2019) could be adopted in the future

Summary

- We propose combination tests that aggregate p-values related to a gene after Mendelian randomization (MR) analyses probing the causal effect (or pleiotropic effect) of omics biomarkers on the outcome.
- Both in simulations and a real example, the combination tests are more powerful in gene prioritization than the multivariable MR framework.
- We recommend using Fisher combination test with gamma distribution approximation. Its power is the highest among the compared methods especially when only weak IVs can be selected from the variants.

Thank you!

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