Single-cell Mendelian randomisation on human brain disease and behaviour

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Motivation:

- Genetic evidence for drug-target discovery and validation
- Single-cell sequencing to identify cell-types of action

Methods: Single-cell expression quantitative trait loci (eQTL) mapping and Mendelian randomization (MR)

- Single-cell eQTL mapping
- Single-cell MR

Results: Single-cell expression affecting human brain disease and behaviour

Conclusion and Outlook

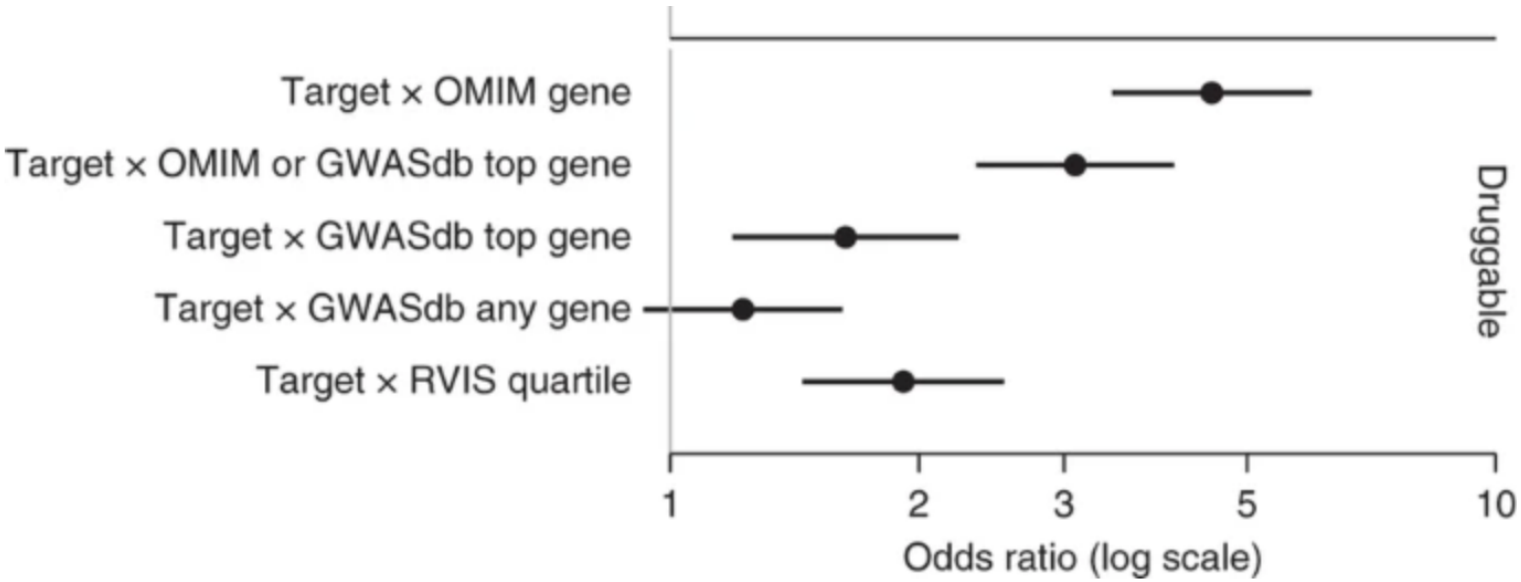
Genetic evidence for drug-target discovery and validation

- Most drugs fail during clinical development
- Most common reason for this is lack of efficacy in Phase II/III trials due to inadequate target validation in early-stage drug discovery
- Of 216 new drugs entering German market between 2011-2017, 75% showed no quantifiable benefit in efficacy over existing drugs for the same indication
- For neurology/psychiatry indications - 94% provided no added efficacy benefit over existing therapies

[Wieseler et al., 2019]

Genetic evidence for drug-target discovery and validation

- Genetic evidence is increasingly used for drug-target discovery, prioritization and validation



[Nelson et al., 2015]

Single-cell sequencing to identify cell-types of action

- Single-cell RNA sequencing (scRNA-seq) allows to measure the transcriptome at single-cell resolution and has given insights into gene-regulation of different cell-types
- In contrast standard bulk-tissue measurements average expression of a transcript over all cell-types
- Linking genotype with scRNA-seq data allows novel insights into genetic regulation of cell-type specific effects

[Cuomo et al., 2023]

How to link cell-type specific gene-expression with disease and behavioral outcomes?

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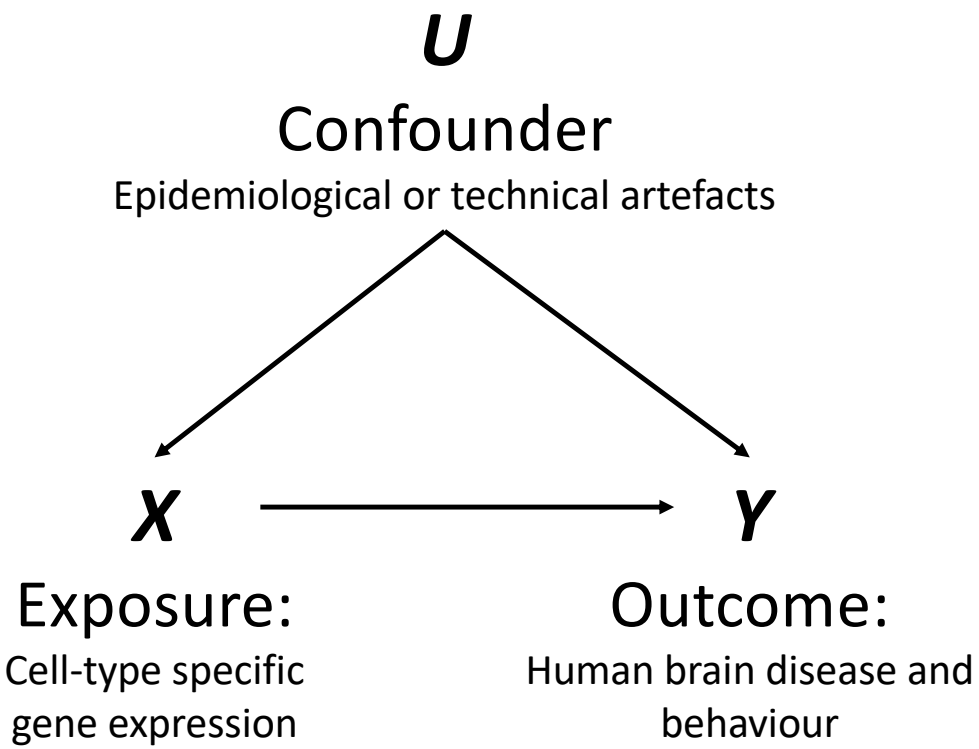
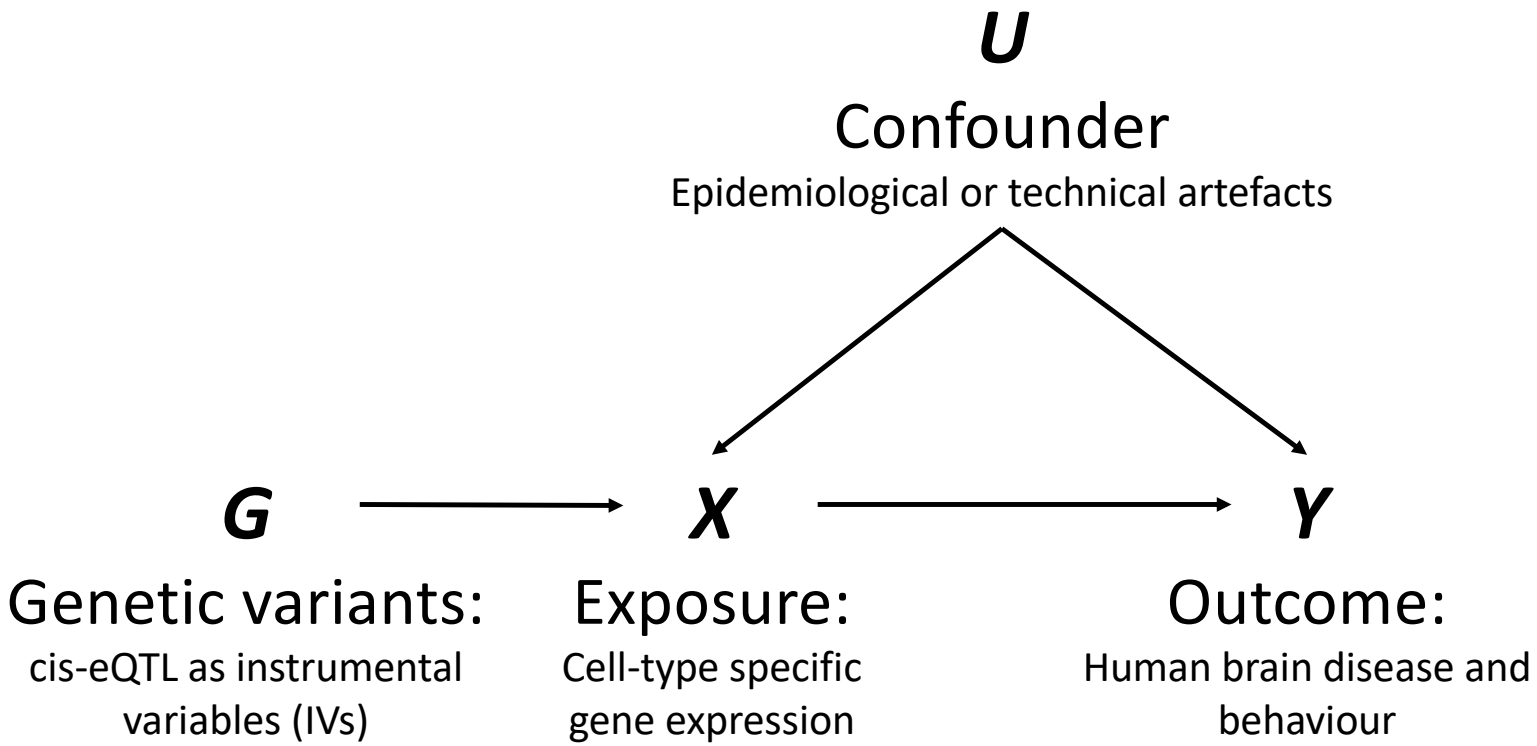


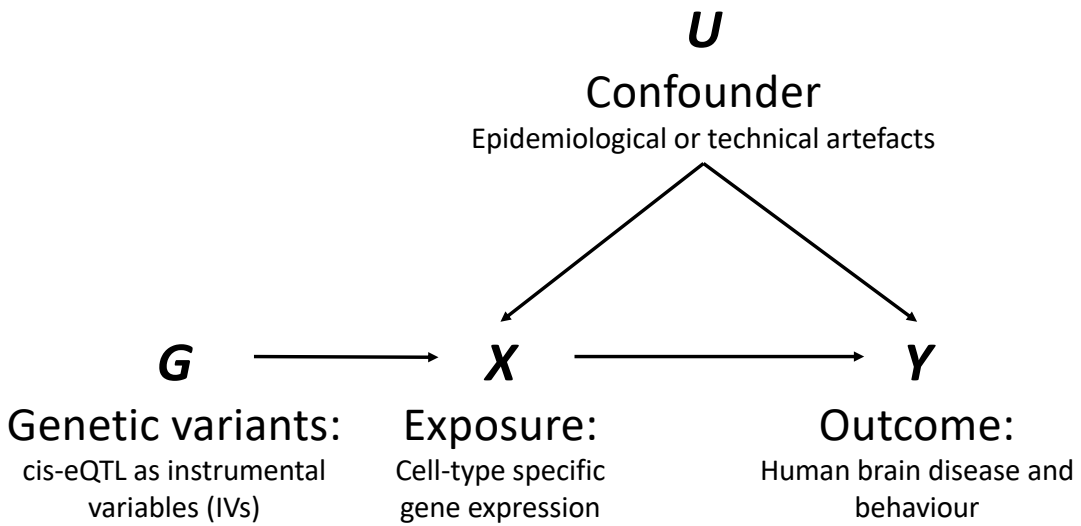
Two-sample summary-level Mendelian randomization (MR)



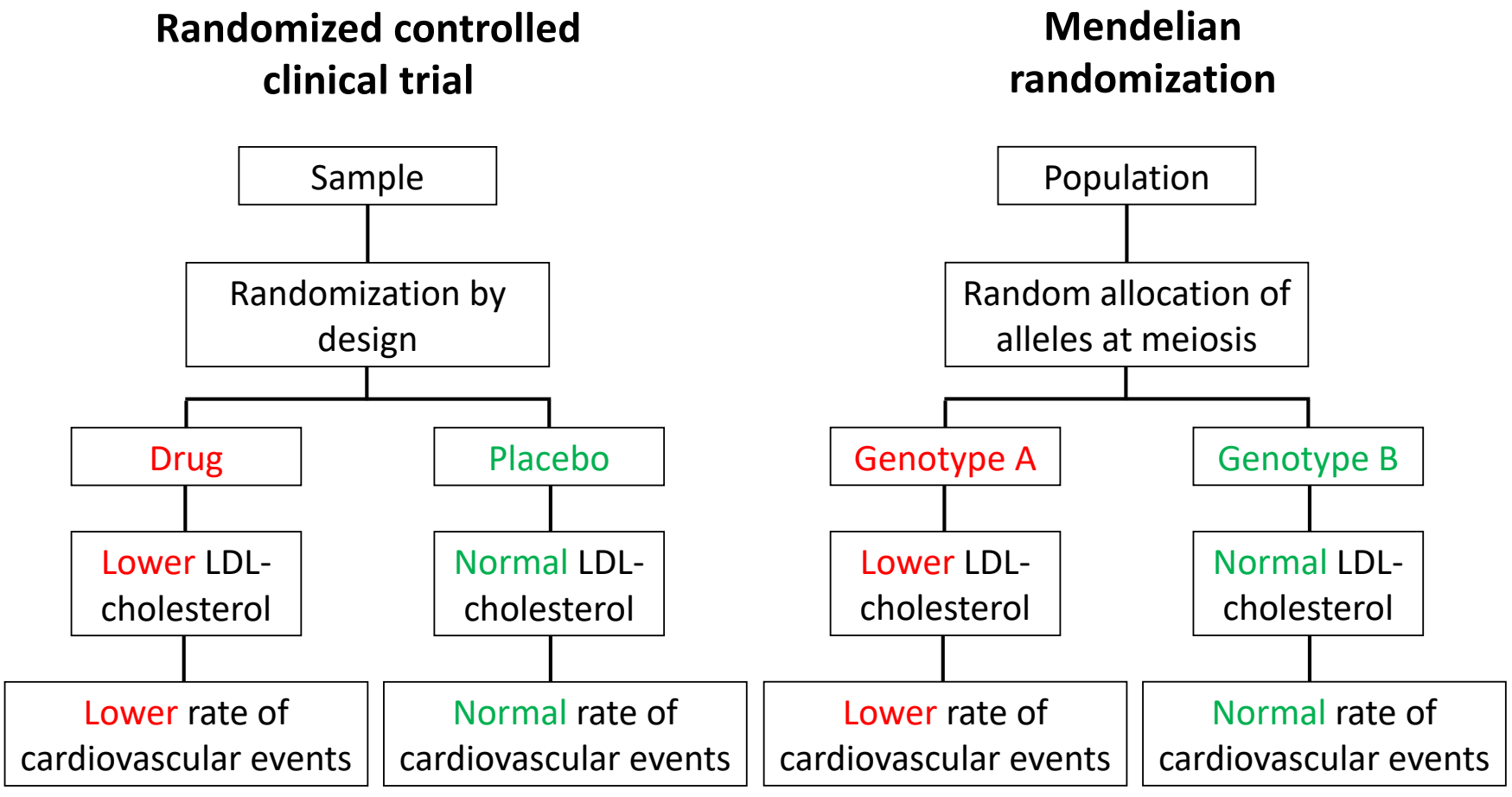
The small print: Instrumental variable (IV) assumptions

- If all genetic variants used as IVs are valid, MR can estimate the true causal effect unbiased from any confounders U.
- A genetic variant is a **valid IV** if the following criteria hold:

IV1 **Relevance**: The variant is associated with the exposures
IV2 **Exchangeability**: The variant is independent of the confounders U of the exposure-outcome associations
IV3 **Exclusion restriction**: The variant is independent of the outcome Y conditional on the exposure X and confounder U



Analogy: Randomized controlled clinical trial and MR



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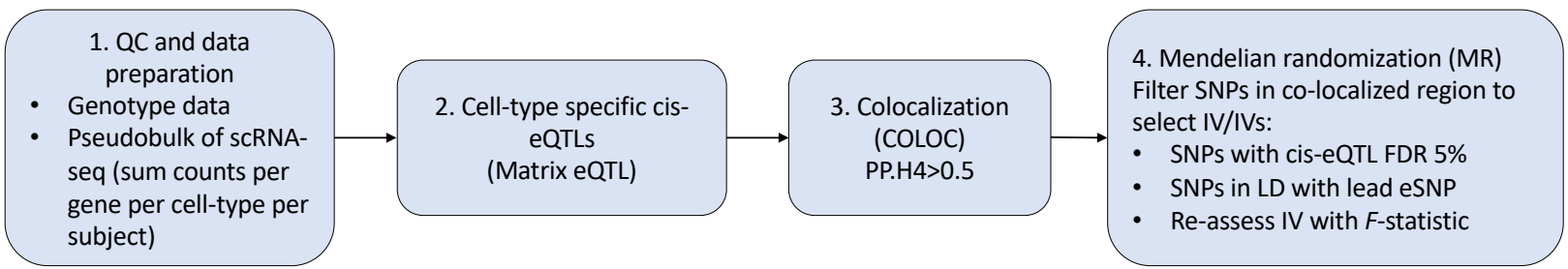
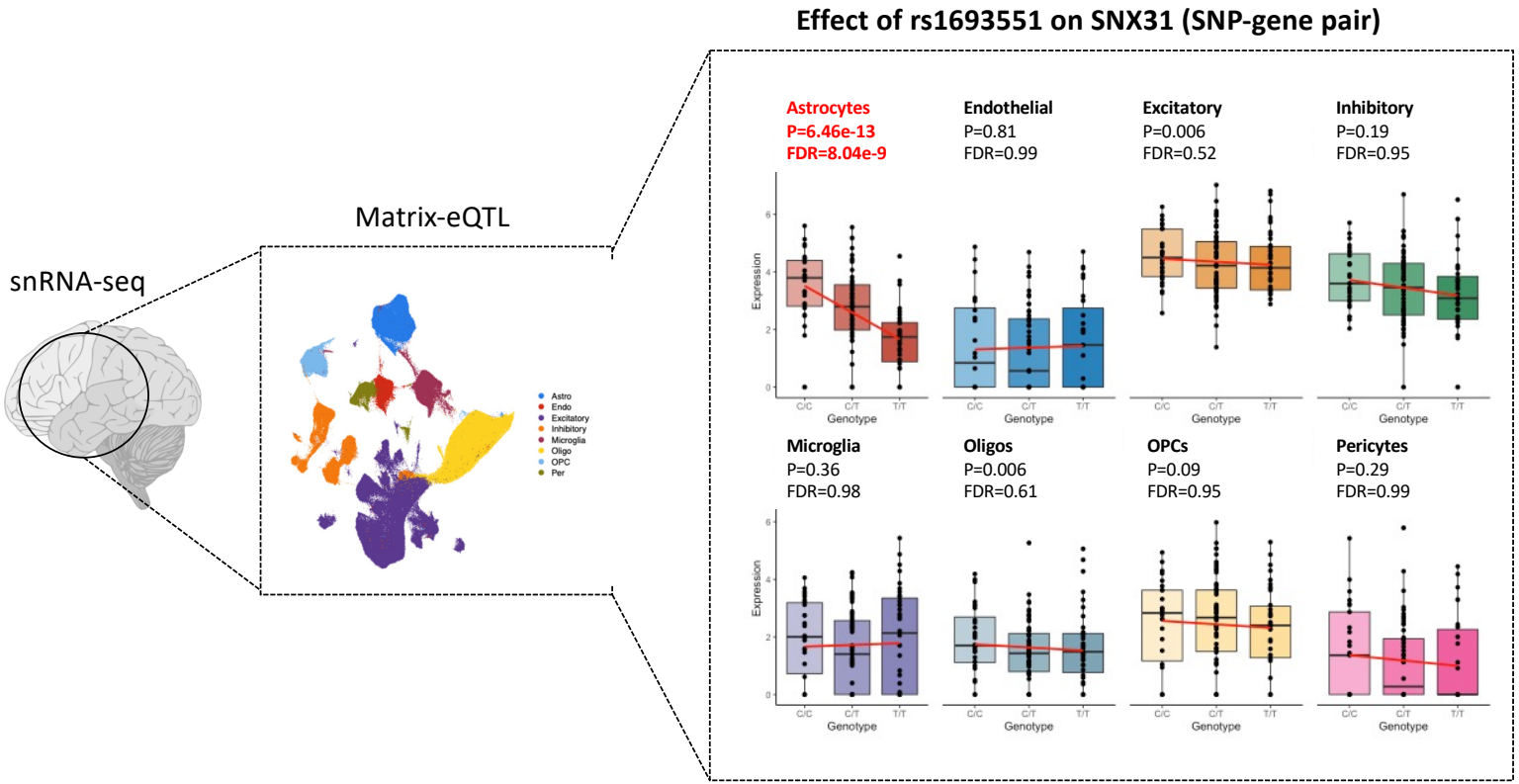
Analysis plan



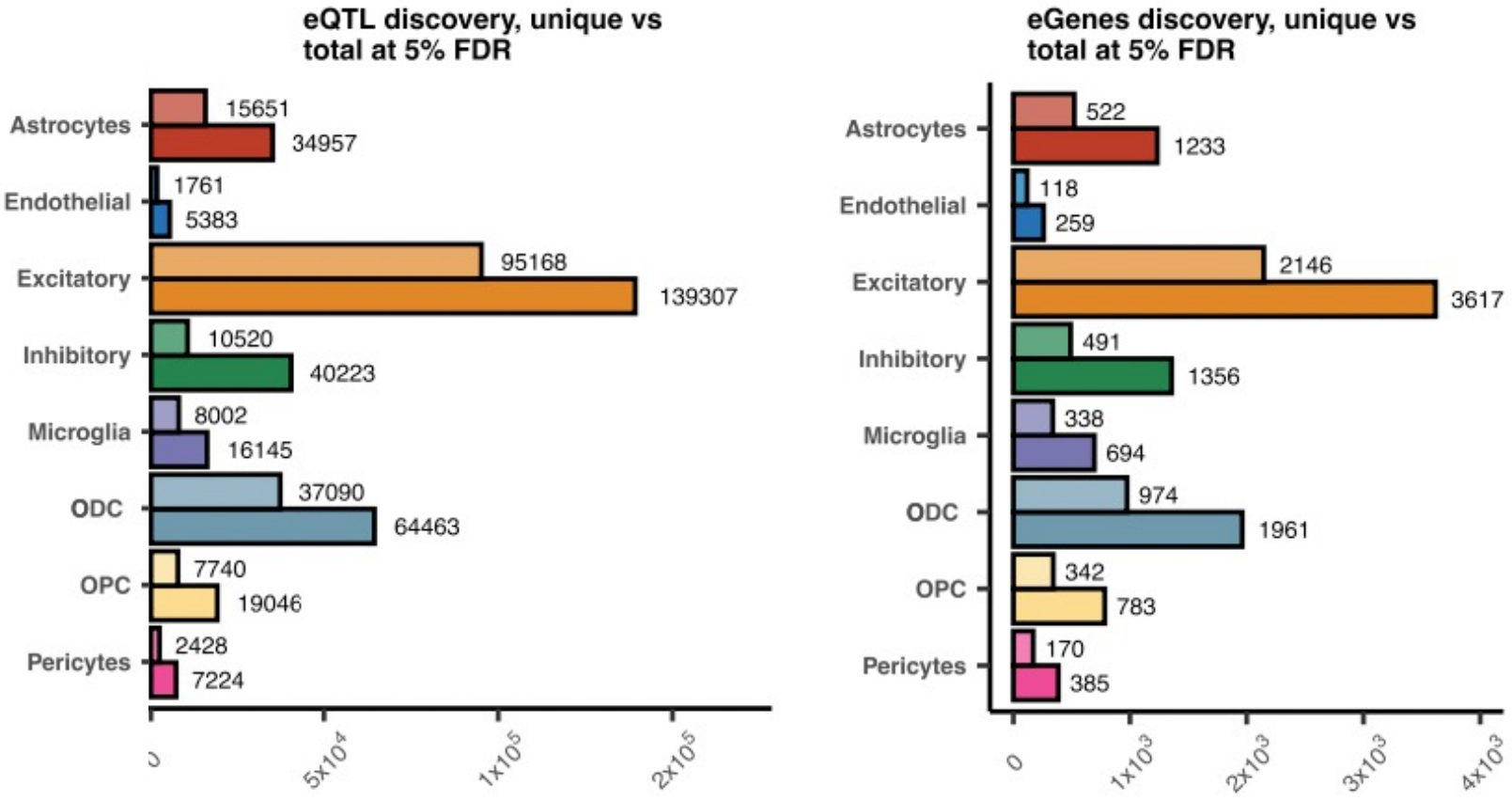
Pre-processing

- Post QC n=128 with 4,500 cells/subject
- 587k nuclei across the sample set
- Integrated, clustered and cell-types annotated using canonical markers (8 major cell-types)

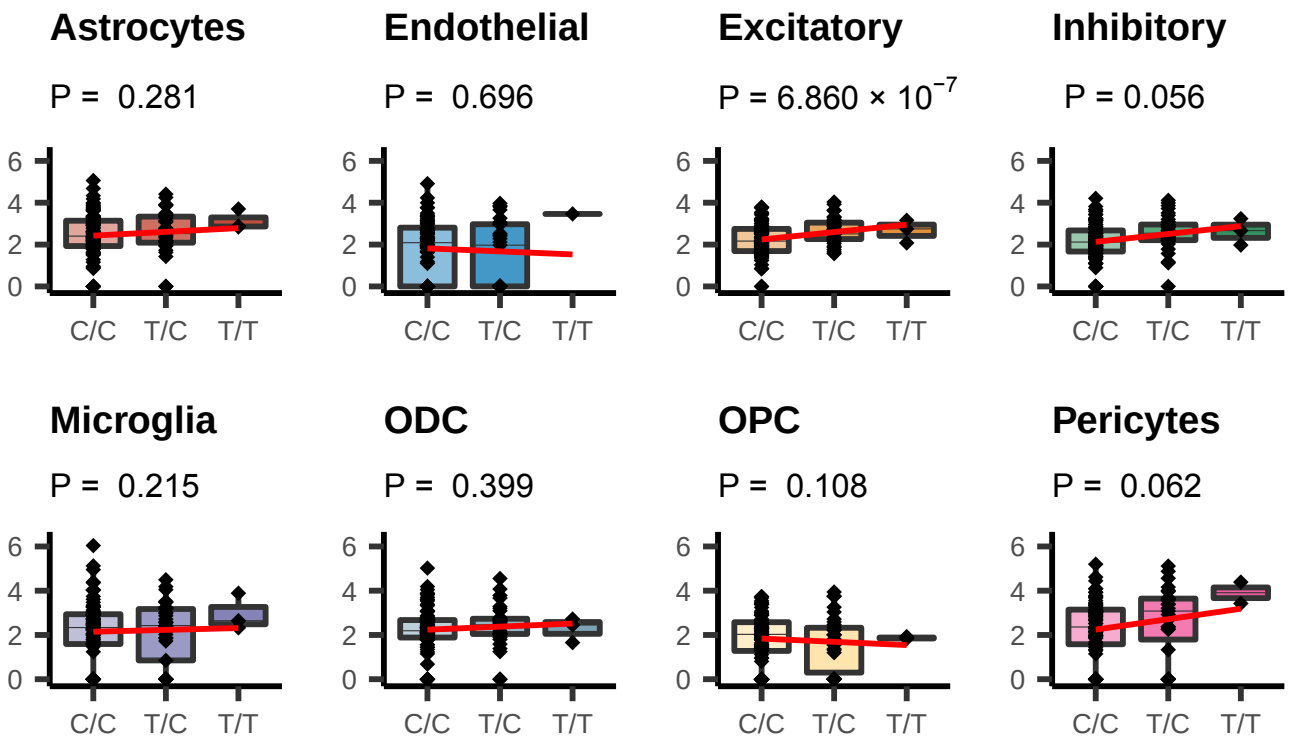
Single cell-type cis-expression quantitative trait loci (eQTL) mapping



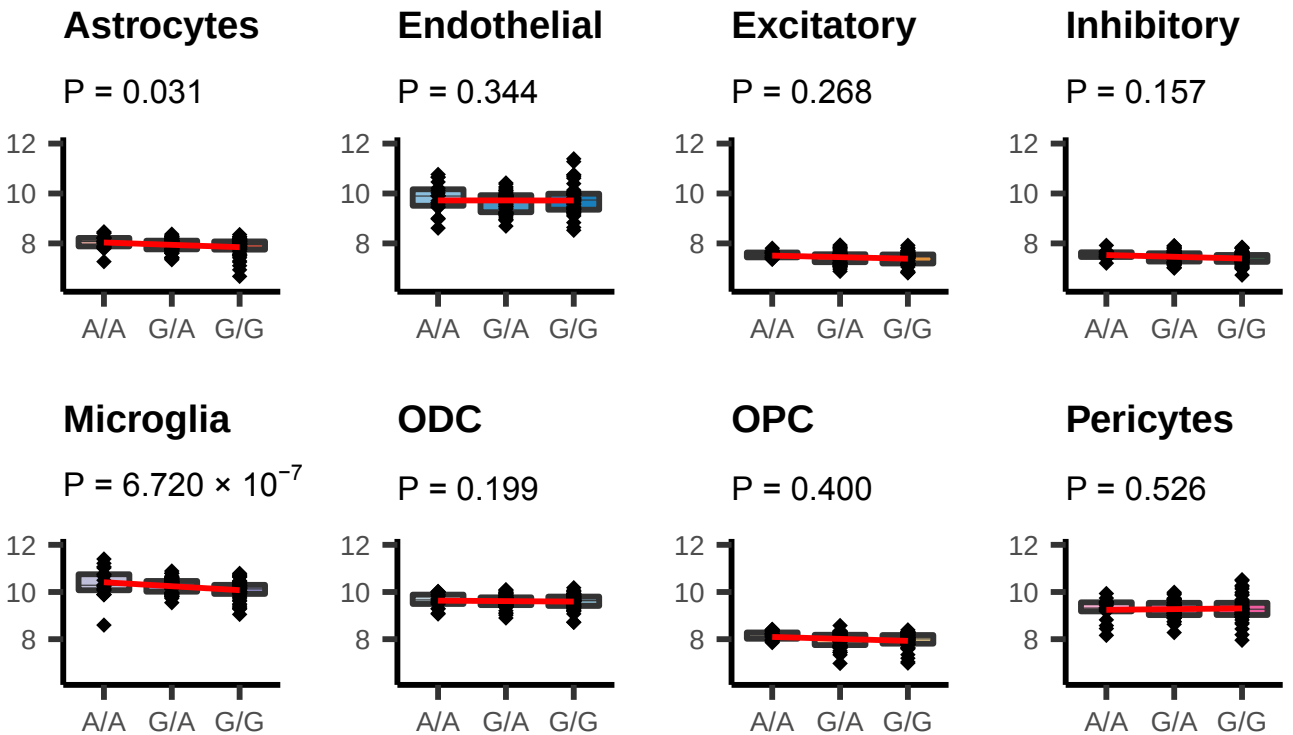
Single cell-type cis-eQTL mapping



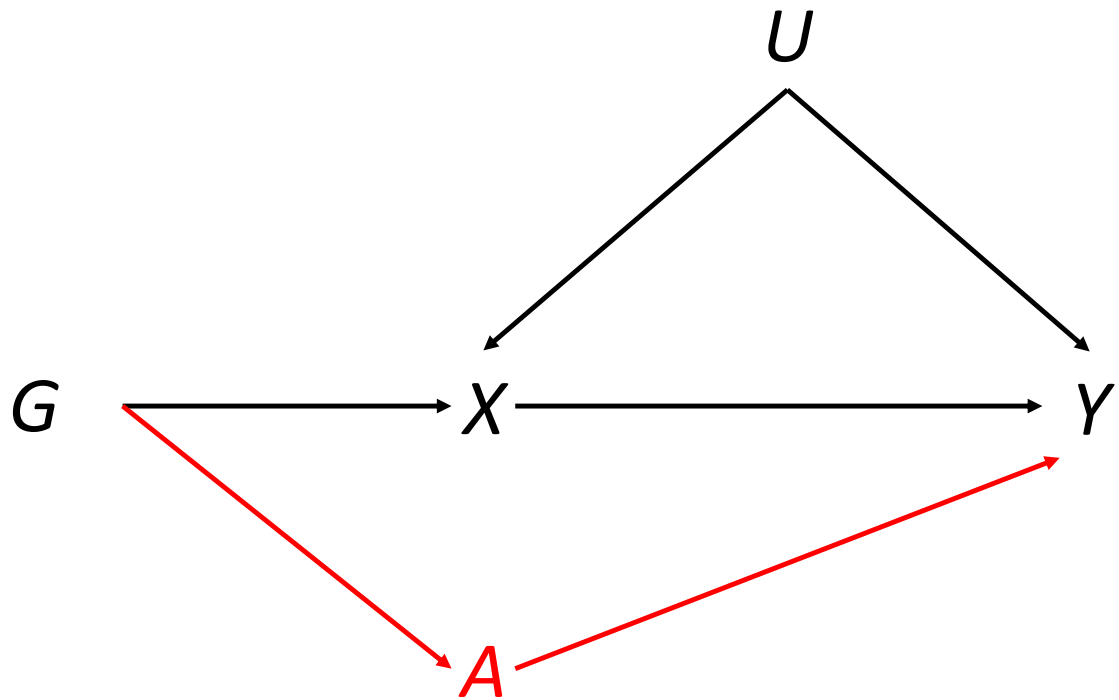
Genetic regulation of cell-type specific effects rs1716183 / OGFOD2



Genetic regulation of cell-type specific effects rs10792832 / PICALM

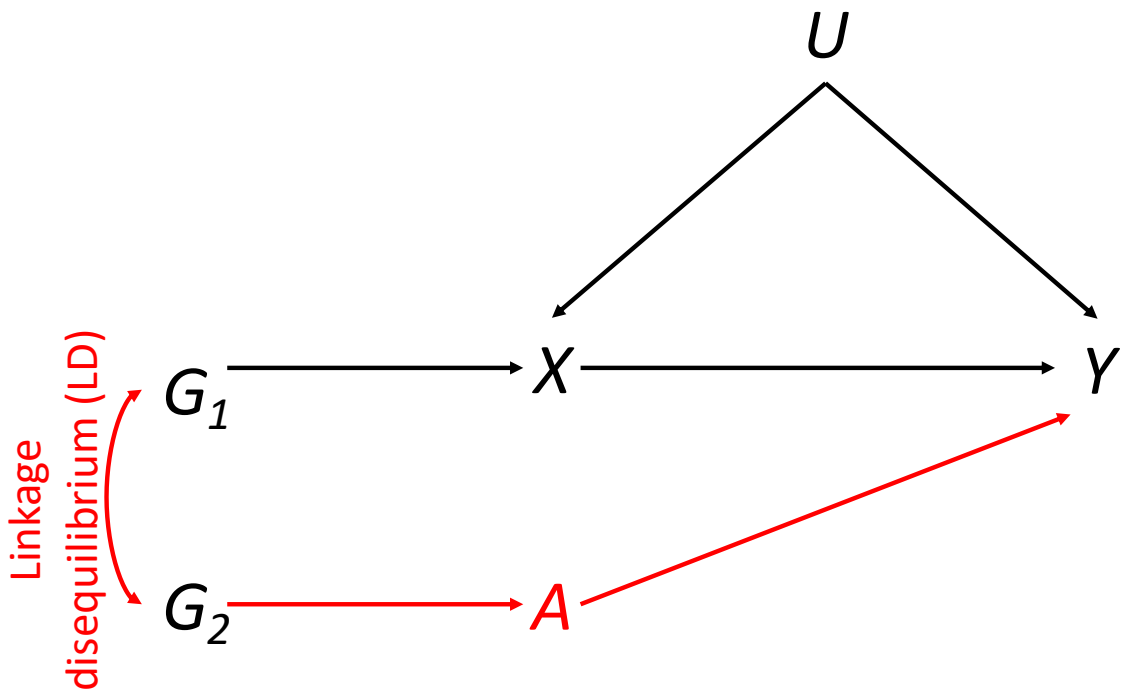


MR and genetic confounding



There is no pleiotropic pathway A that directly connects G with Y.

Drug-target/cis-MR and genetic confounding

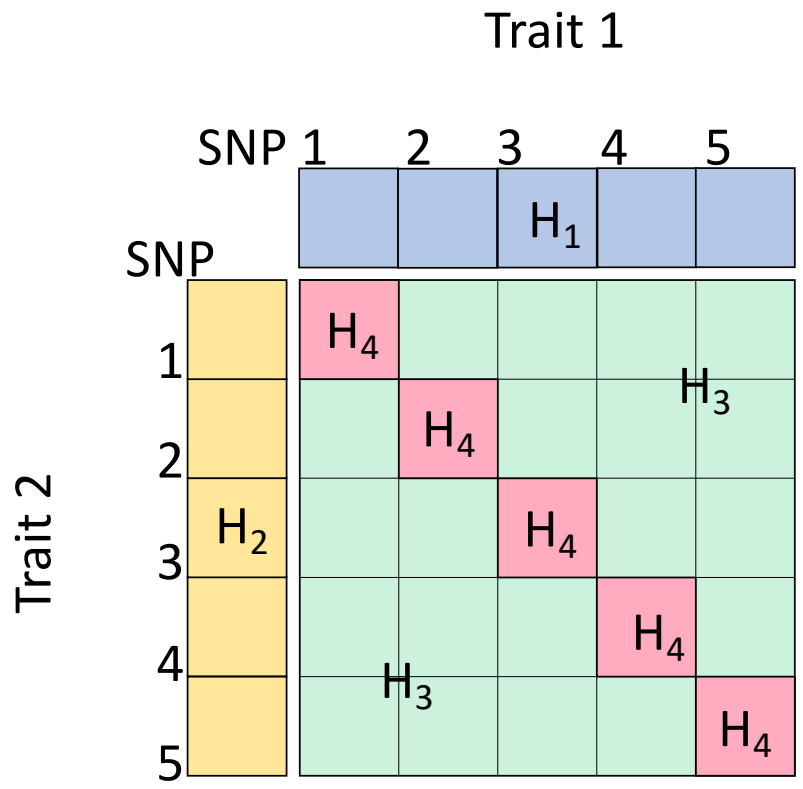


- Colocalisation is a necessary filtering step to ensure cis-MR results are not confounded by LD [Zuber et al., 2022]

Colocalisation

Bayesian approach to test if two traits share the same genetic architecture in a region of interest [Giambartolomei et al., 2014]

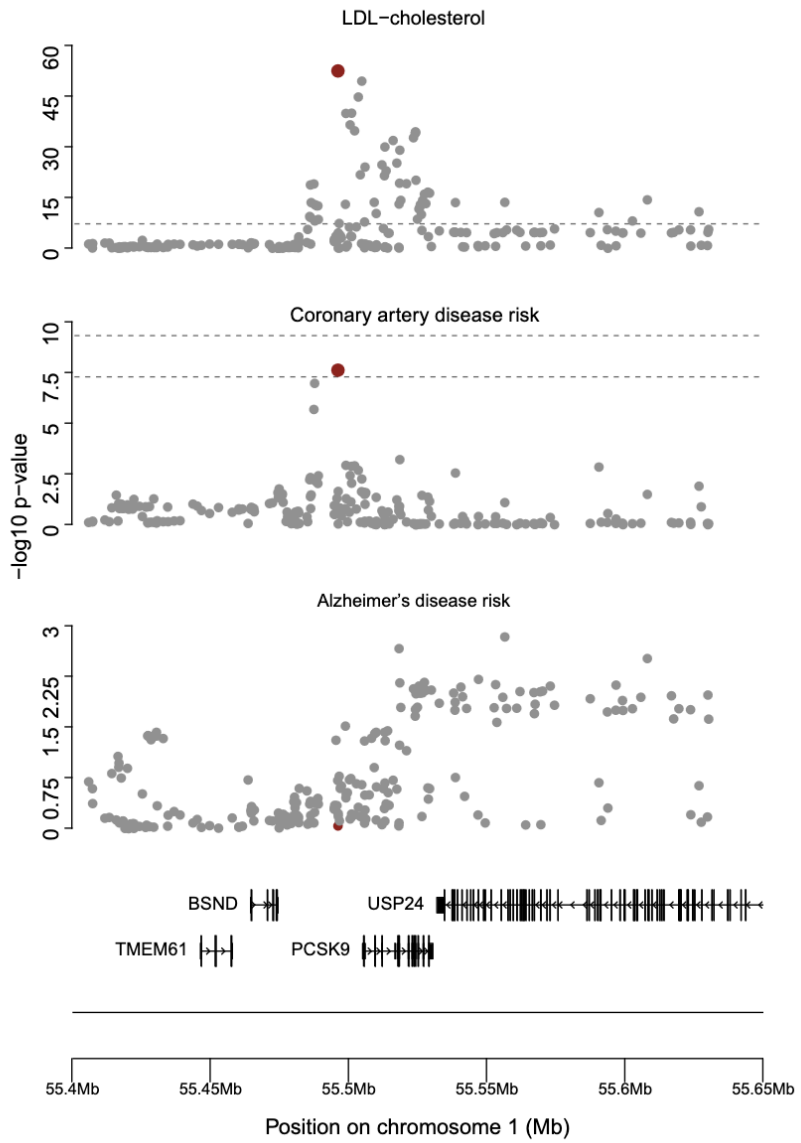
- H_1: Association with trait 1
- H_2: Association with trait 2
- H_3: Association with trait 1 and 2, but different SNP
- H_4: Association with trait 1 and 2, one shared SNP



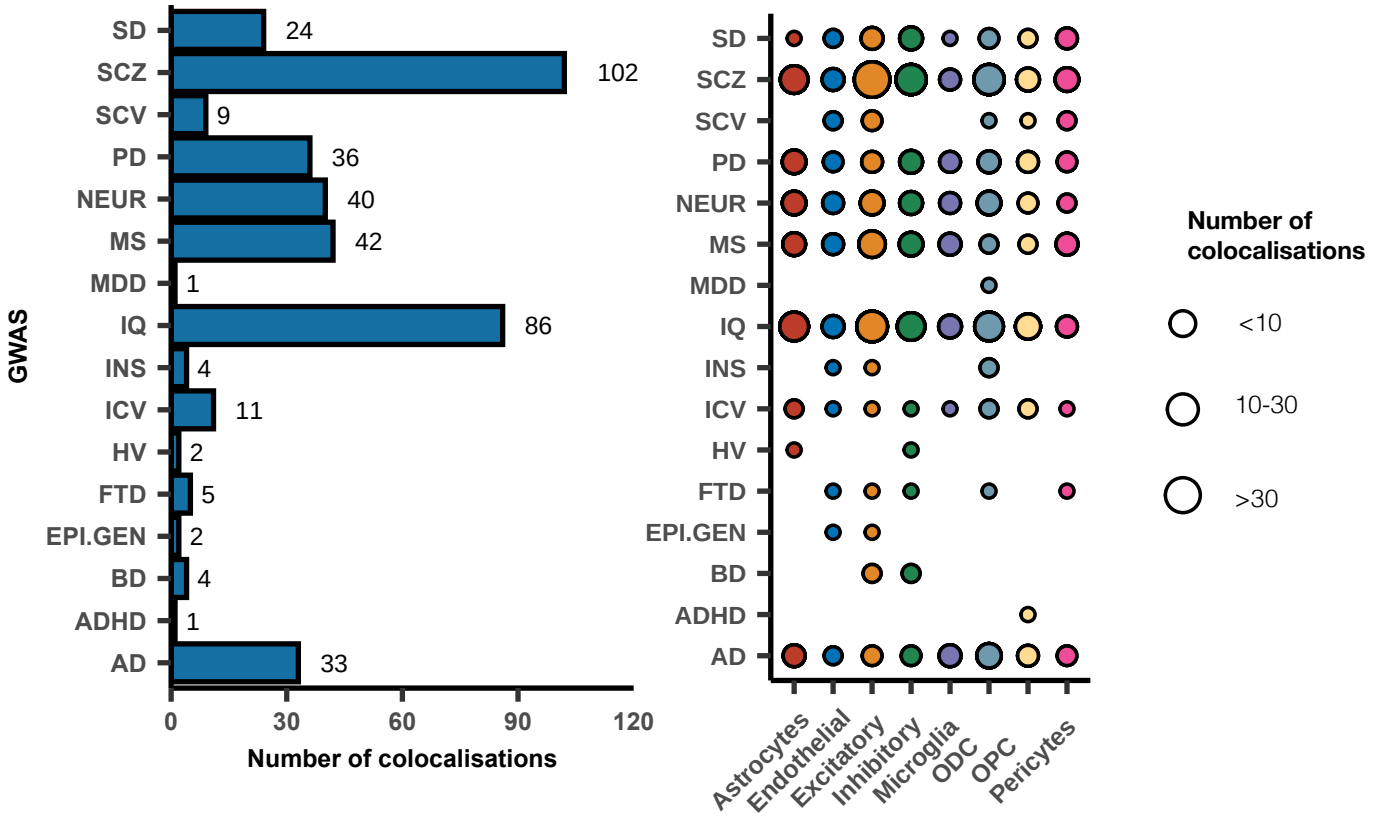
Colocalization to support MR

PCSK9 inhibitors

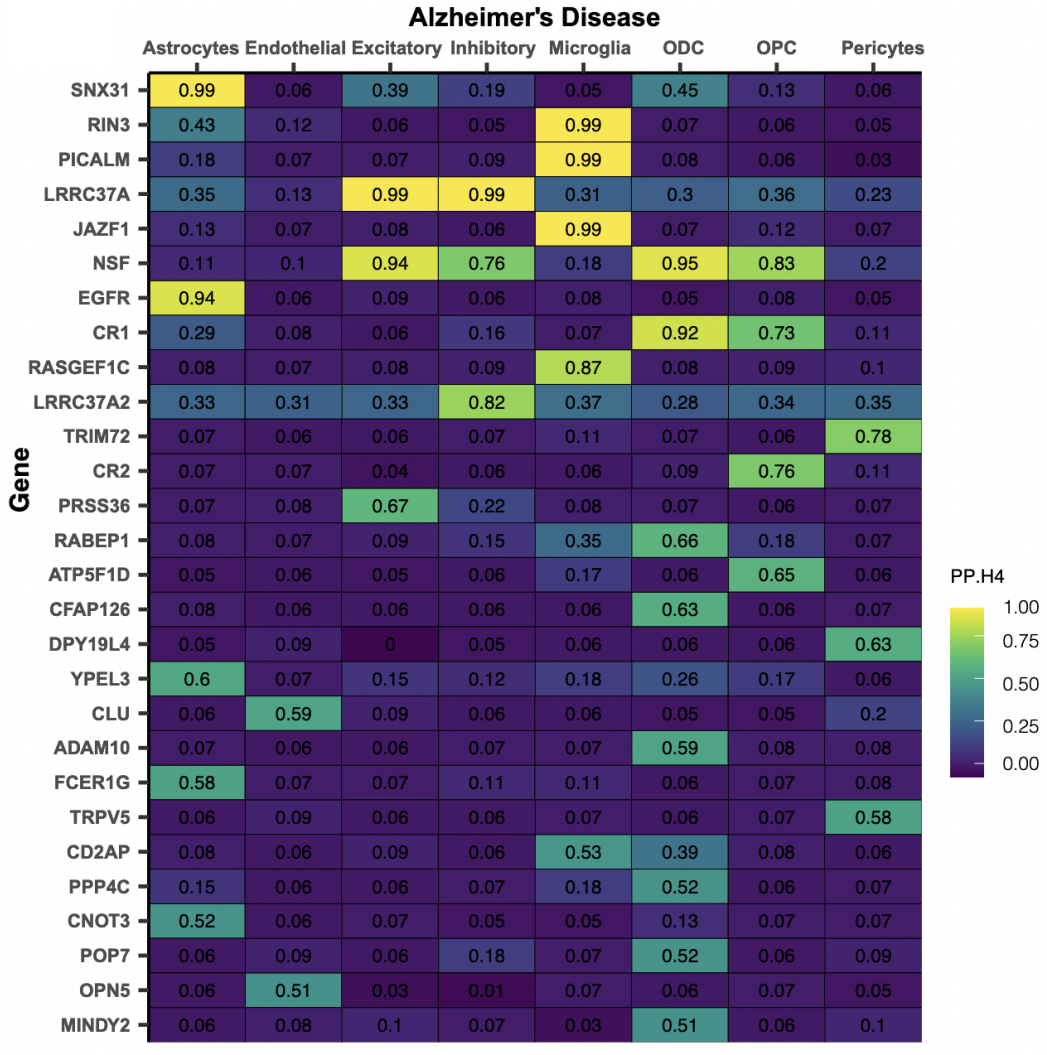
- *PCSK9*-gene region: PCSK9 is an enzyme that binds to and degrades the receptor for low-density lipoprotein particles (LDL)
- Exposure: LDL-cholesterol
- Primary outcome: Coronary artery disease
- Secondary outcome: Alzheimer's disease [Zuber et al., 2022]



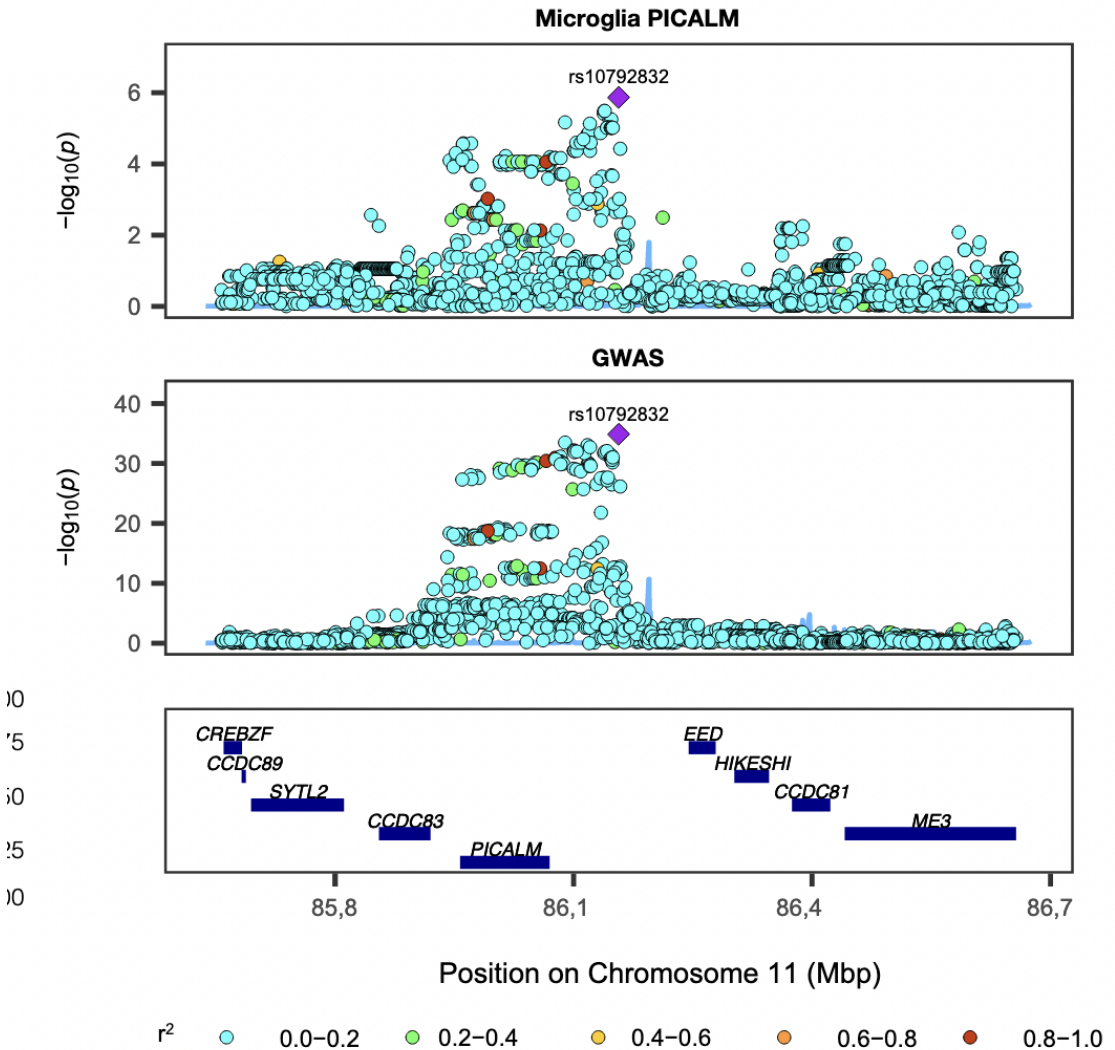
Colocalization for the selection of valid IVs



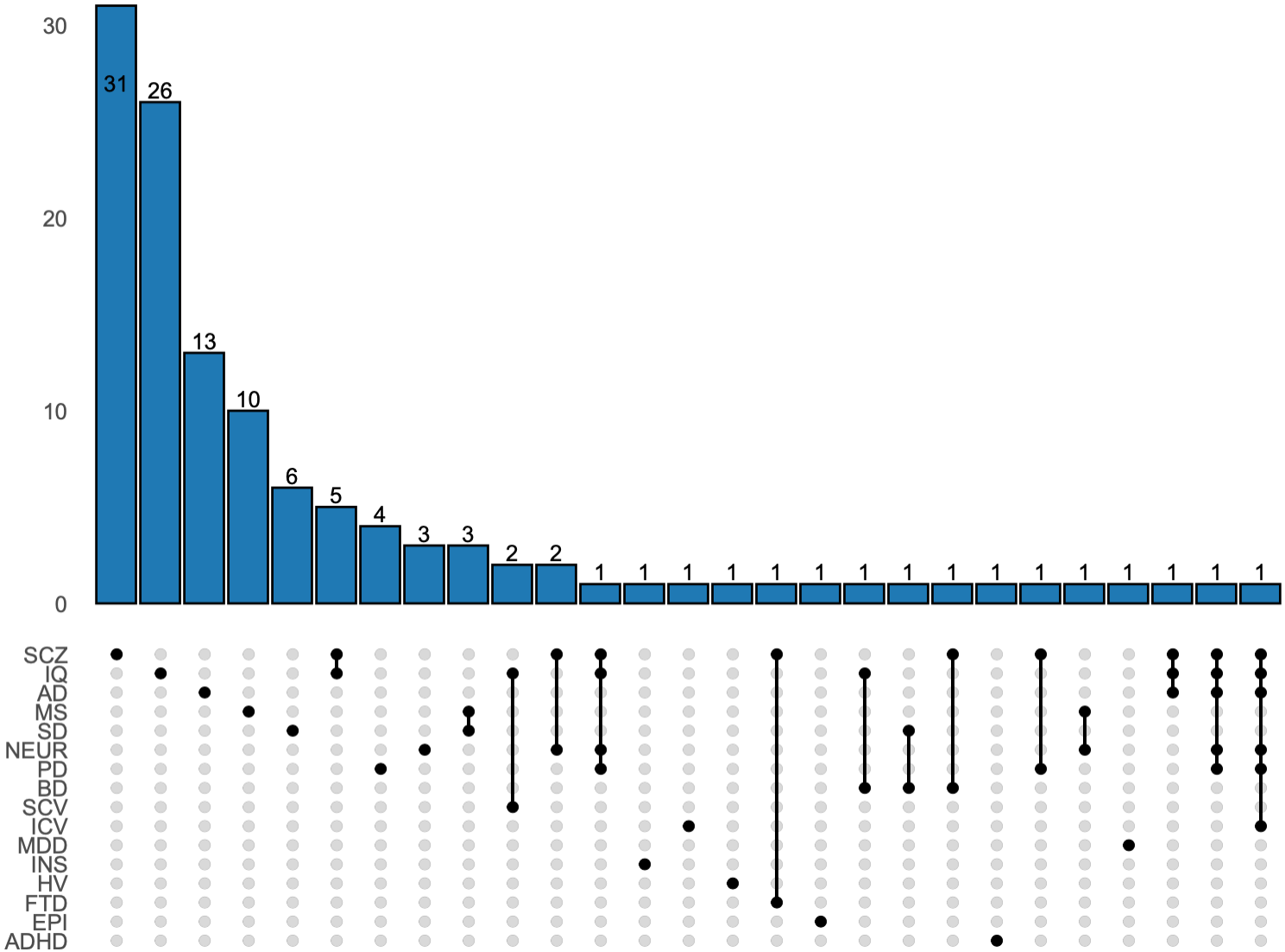
Colocalization example: Alzheimer's Disease (AD)



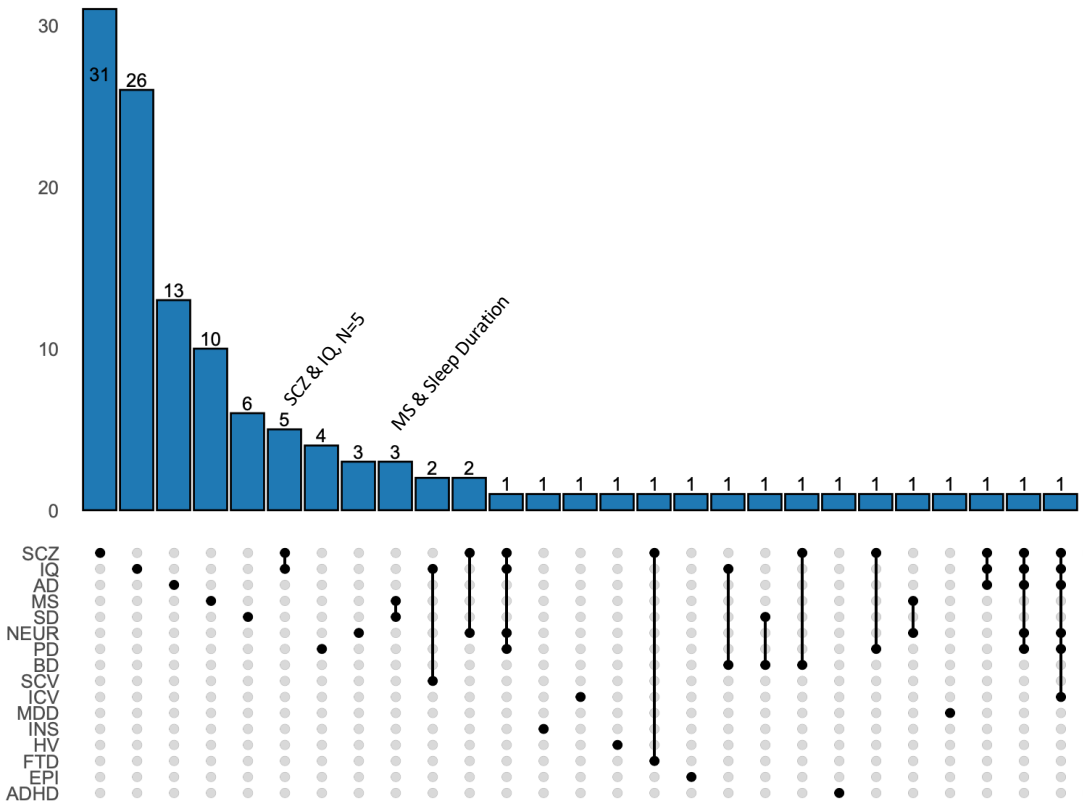
Colocalization example: AD and PICALM in Microglia

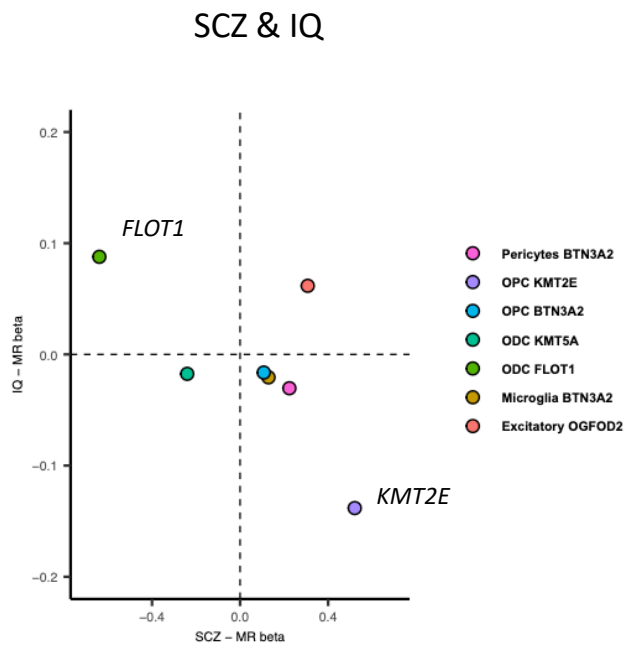


MR results: Overview



MR results for more than one phenotype: SCZ and IQ





MR-Results: Key highlights

Outcome	Gene	Cell-type	MR $p-$value	MR beta	eQTL FDR
AD	**PICALM**	**Microglia**	**3.03E-36**	**Negative**	**6.26E-08**
AD	EGFR	Astrocytes	1.70E-07	Positive	0.001
Parkinson	GPNMB	Astrocytes	3.01E-06	Positive	0.03

- *PICALM* modulates AD risk via clearance of Aβ and tau (Van Acker at al., Molecular Neurodegen 2019)
- Reduction in *PICALM* expression increases tau deposition. (Ando et al., Acta Neuropath 2020)
- Suggests *PICALM* activation as a potential therapeutic strategy in AD

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- Fine mapped in 2022 using on bulk brain tissue eQTLs (Bellenguez at al., Nat Genet, 2022) but no data on directionality or cell type
- *EGFR* inhibition reduces Aβ/tau pathology and reactive astrocytes in several models of AD (Lee et al., Aging Cell 2021)
- Several new BBB penetrant *EGFR* inhibitors (Tavassoly et al., Molecular Pharm 2021)

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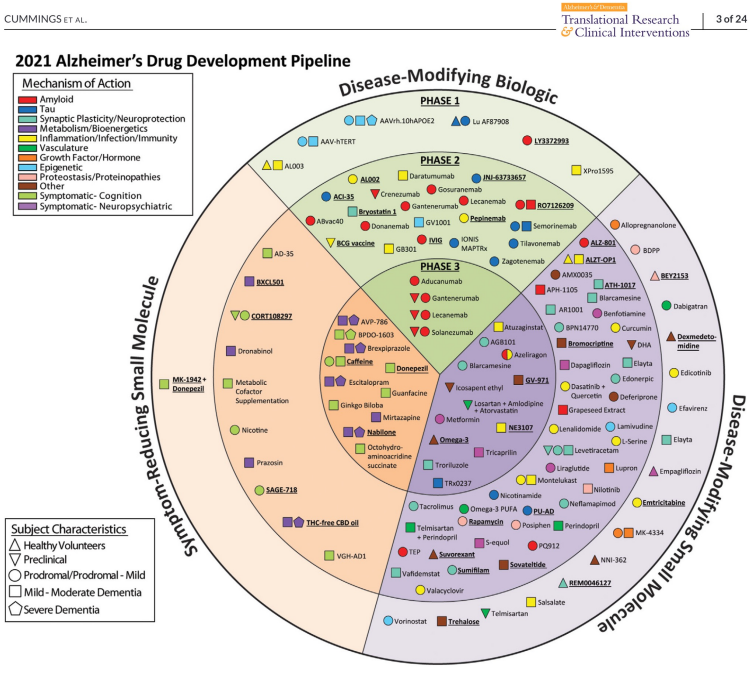
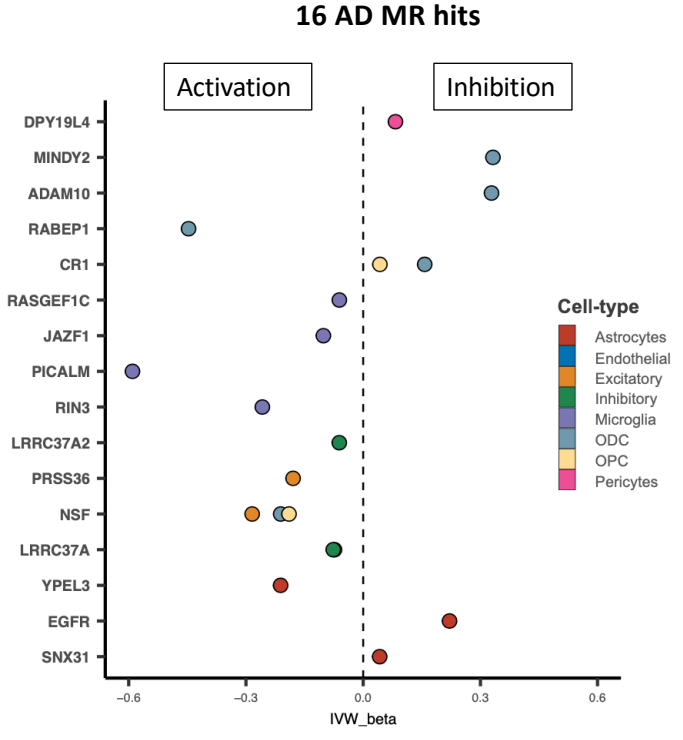
- Loss of *GPNMB* activity results in loss of cellular internalization of fibrillar alpha synuclein and reduced PD pathogenecity (Diaz-Ortiz et al., Science 2022)
- Levels of *GPNMB* in plasma correlate with PD severity (potential biomarker)
- Suggests *GPNMB* inhibition as a potential therapeutic strategy in PD

Focus on Alzheimer's disease: Drug development pipeline





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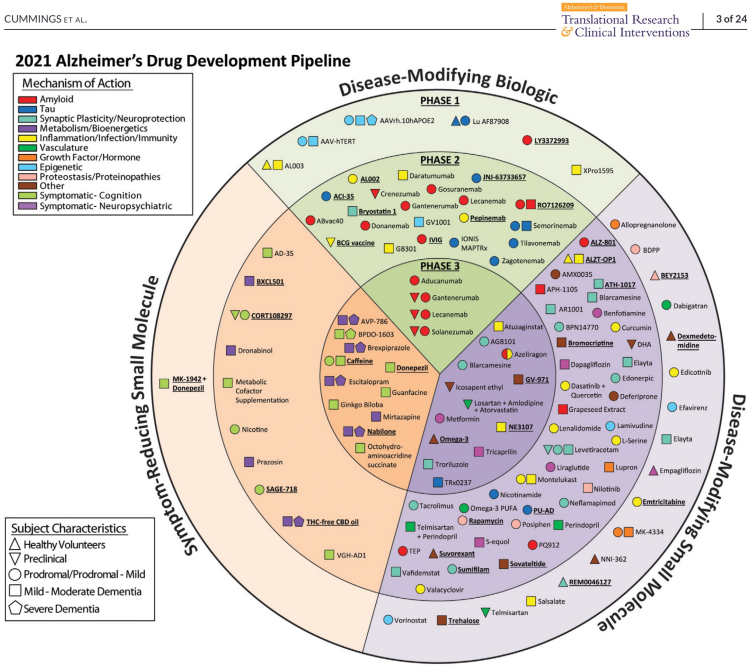

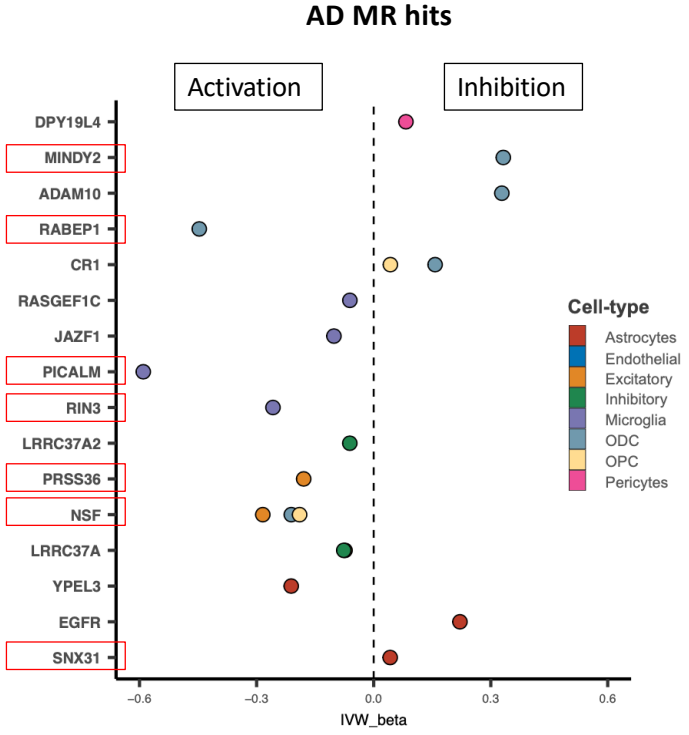

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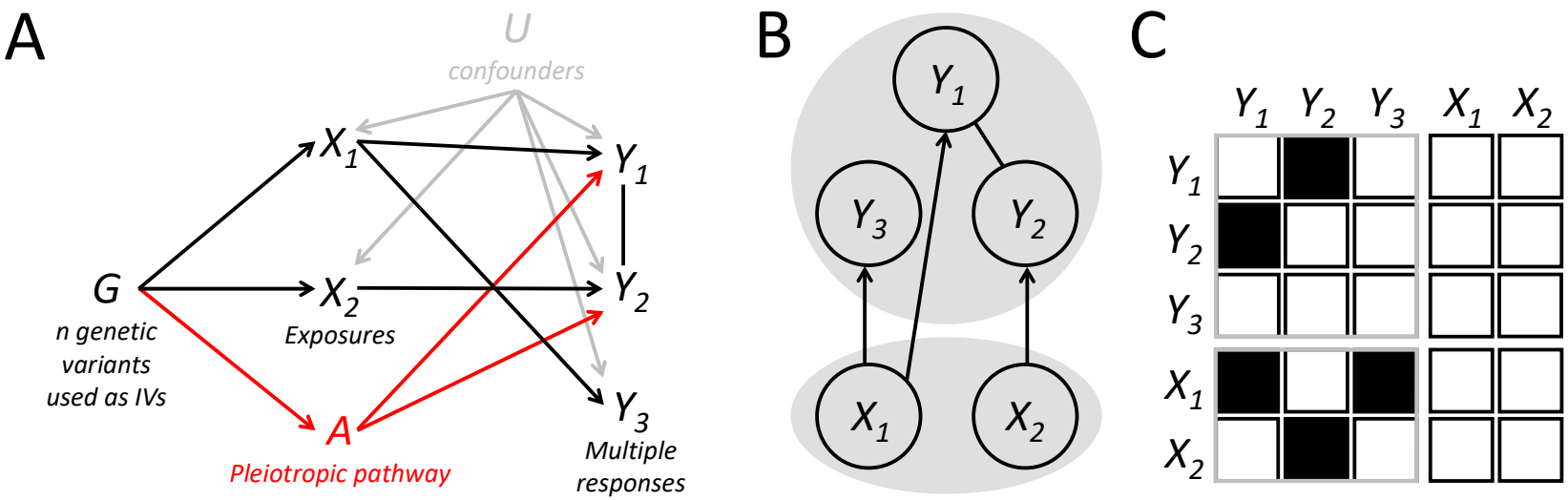
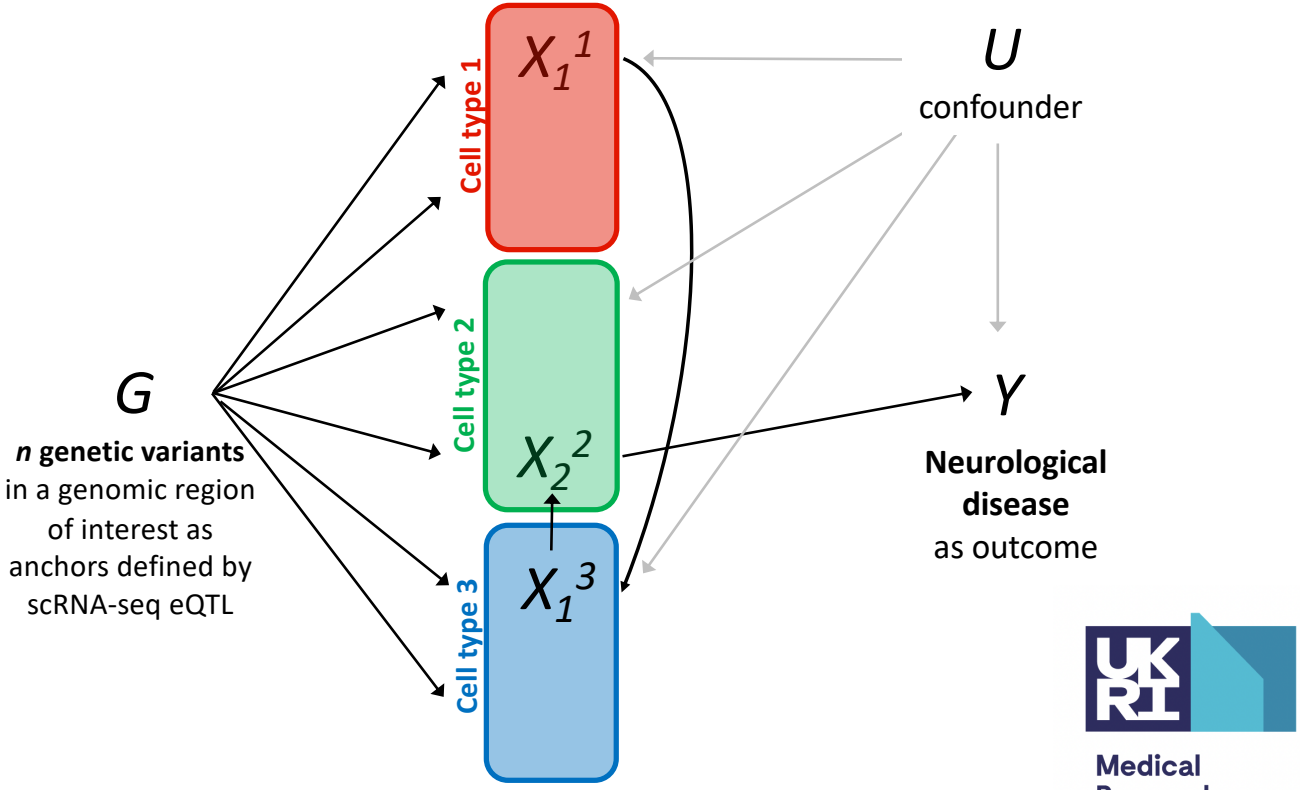
- Principled framework for single-cell Mendelian randomization target discovery in human brain phenotypes
- All samples were non-diseased control human brain samples
- Genetic evidence for 118 genes across 23 human brain diseases and behavioral traits
- 21 genes contribute to > 2 brain phenotypic outcomes (shared therapeutic strategies)
- Approach provides information on the direction of the relationship to inform therapeutic approach
- Causal mechanism and cell-types of action can inform more tailored pre-clinical experiments that may translate better to human disease and biomarker discovery

Outlook: Multi-response MR (MR^2) to identify shared and distinct causes of disease



Outlook: Causal network of gene-expression across different cell-types

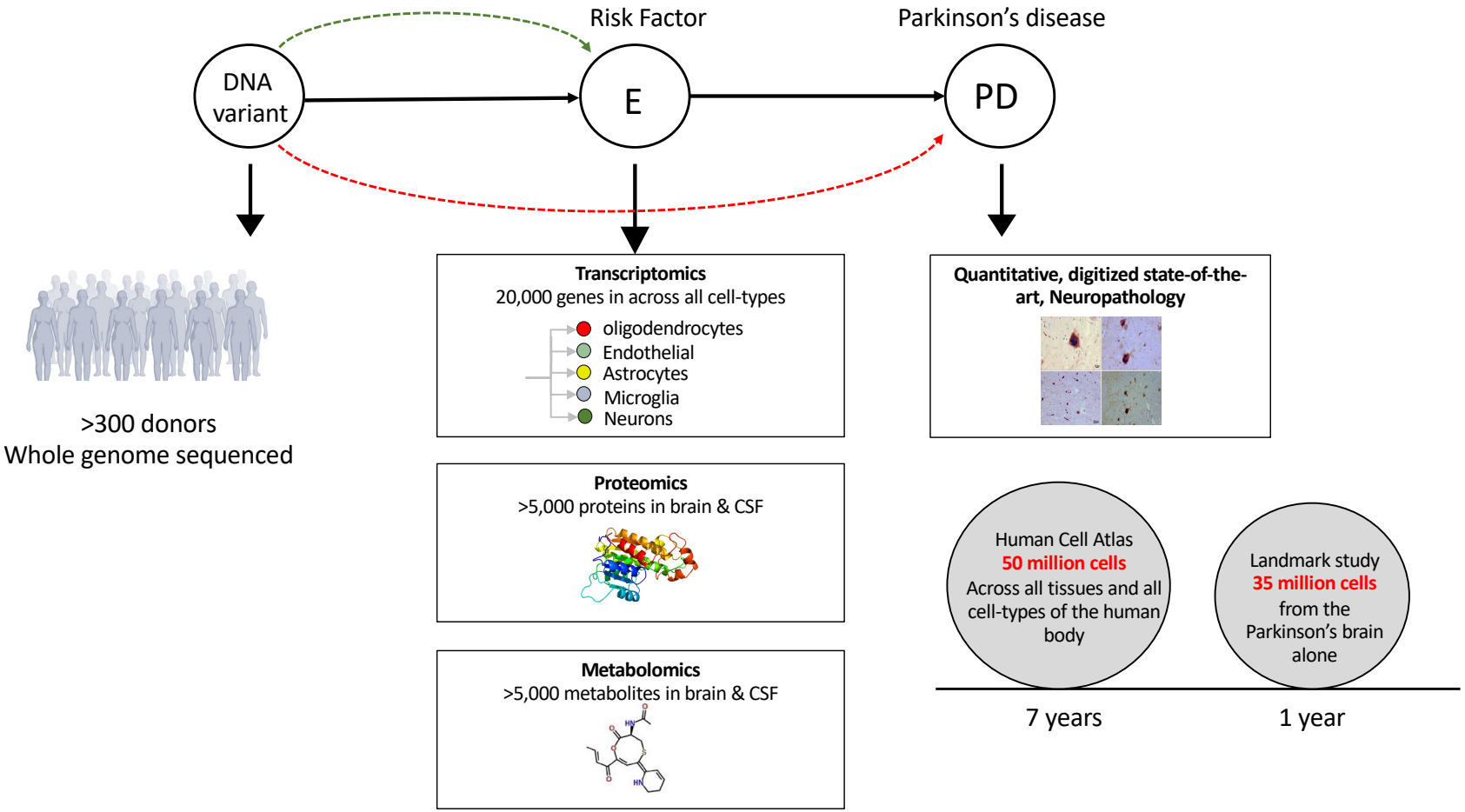


Example network
how transcripts interact in different cell types

Medical Research Council

Better Methods, Better Research

Outlook: LANDMARK



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- Alexi Nott
- Hun Ko Joeng
- Julian Bryios
- Dheeraj Malhotra

- Better Methods, Better Research
- Neurosciences and Mental Health

Thanks to all the patients and their families who so generously donated their brains after death.

New Results

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Single-cell Mendelian randomisation identifies cell-type specific genetic effects on human brain disease and behaviour

Alexander Haglund, Verena Zuber, Yifei Yang, Maya Abouzeid, Rahel Feleke, Jeong Hun Ko, Alexi Nott, Ann C. Babtie, James D. Mills, Louwai Muhammed, Liisi Laaniste, Djordje O. Gveric, Daniel Clode, Susanna Pagni, Ravishankara Bellampalli, Alyma Somani, Karina McDade, Jasper J. Anink, Lucia Mesarosova, Eleonora Aronica, Maria Thom, Sanjay M. Sisodiya, Prashant K. Srivastava, Dheeraj Malhotra, Julien Bryois, Leonardo Bottolo, Michael R. Johnson

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