

# mGWAS-Explorer 2.0 Tutorial

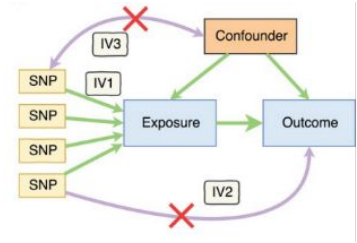
– Crohn's disease case study



# Goal for this tutorial

- Identify the **causal** associations between **metabolites** and **Crohn's disease**

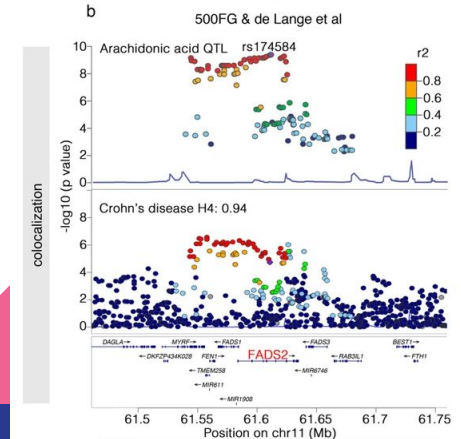
# MR Concept



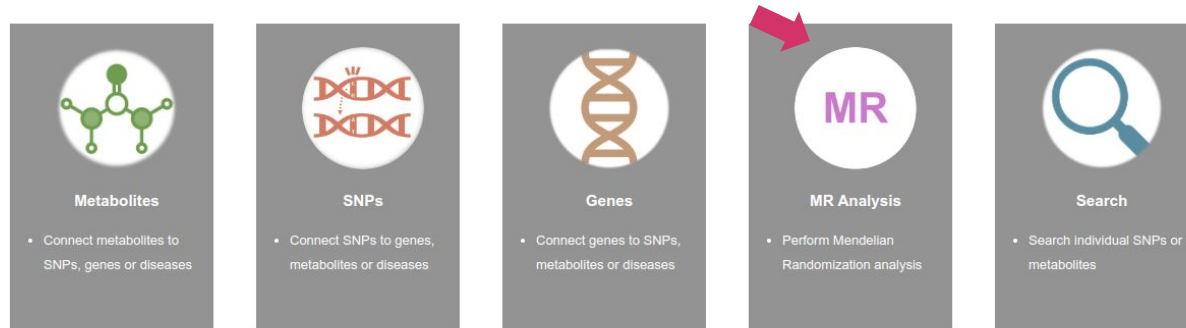
- **Mendelian randomization (MR)** is a research method that uses genetic variants as natural experiments to examine probable **causal** relationships between exposure risk factor and disease outcome.
- Compared to conventional observational research, MR is less susceptible to confounding and reverse causality.
- Assumptions:
  - Relevance assumption (IV1): the instruments must be associated with the exposure risk factor
  - Exclusion restriction assumption (IV2): the only way the IVs influence the outcome is through the relevant exposure risk factor
  - Independence assumption (IV3): the IVs must not associate with confounders

# Background

- Colocalization analysis between **arachidonic acid** mQTL and **Crohn's disease** GWAS's profile strongly supported the hypothesis that arachidonic acid share common genetic variants with Crohn's disease (Chu et al., 2021)
- Therefore, we applied the **Mendelian randomization** method to test the **causal effect** of arachidonic acid on Crohn's disease using mGWAS-Explorer 2.0



# Starting up



- Go to the mGWAS-Explorer homepage ([www.mgwas.ca](http://www.mgwas.ca))
- Click on the “MR” button to enter the data upload page for MR module

# Exposure data (metabolite)

1. Copy and paste “Arachidonic acid” in the search box
2. Select from the drop-down list
3. Click “Search” button

🏠 > Upload

Please use the tabs below to prepare your input. Make sure to click **Submit** button before switching to the next tab.

Exposures Outcomes

Enter metabolite  
e.g., arachidonic acid

Arachidonic acid

Arachidonic acid

>> Search

Advanced Filter

Reset

Metabolite ↑↓	SNP ID ↑↓	Chr ↑↓	Position ↑↓	A1 ↑↓	A2 ↑↓	Beta ↑↓	SE ↑↓	P-value ↑↓	PMID ↑↓	Action
arachidonate (20:4n6)	rs174548	11	61571348	C	G	0.0488	0.0025	1.430e-84	24816252	Delete
arachidonate (20:4n6)	rs174556	11	61580635	T	C	-0.0488	0.0025	2.980e-84	24816252	Delete
arachidonate (20:4n6)	rs174547	11	61570783	T	C	0.048	0.0025	8.760e-84	24816252	Delete
arachidonate (20:4n6)	rs174555	11	61579760	T	C	0.0481	0.0025	6.580e-82	24816252	Delete
arachidonate (20:4n6)	rs174550	11	61571478	T	C	0.0475	0.0025	7.430e-82	24816252	Delete
arachidonate (20:4n6)	rs174546	11	61569830	T	C	-0.0476	0.0025	7.910e-82	24816252	Delete
arachidonate (20:4n6)	rs174549	11	61571382	A	G	-0.048	0.0025	8.180e-82	24816252	Delete
arachidonate (20:4n6)	rs174545	11	61569306	C	G	0.0476	0.0025	8.310e-82	24816252	Delete
arachidonate (20:4n6)	rs174574	11	61600342	A	C	-0.0475	0.0025	1.230e-81	24816252	Delete
arachidonate (20:4n6)	rs102275	11	61557803	T	C	0.0475	0.0025	2.040e-81	24816252	Delete
arachidonate (20:4n6)	rs174535	11	61551356	T	C	0.0475	0.0025	2.050e-81	24816252	Delete
arachidonate (20:4n6)	rs174536	11	61551927	A	C	0.0475	0.0025	2.050e-81	24816252	Delete
arachidonate (20:4n6)	rs174537	11	61552680	T	G	-0.0475	0.0025	2.050e-81	24816252	Delete
arachidonate (20:4n6)	rs1535	11	61567072	A	G	0.0469	0.0025	1.690e-79	24816252	Delete

Data uploaded:  exposure  outcome

Proceed

- Note: use advanced filter to select a mGWAS study
  - Here we chose Shin et al. *Nature genetics* (2014), PMID: 24816252

Please use the tabs below to prepare your input. Make sure to click **Submit** button before switching to the next tab.

Exposures Outcomes

Enter metabolite  
e.g., arachidonic acid

Arachidonic acid

>> Search

Advanced Filter

Reset

Metabolite ↑↓	SNP ID ↑↓	Chr ↑↓	Position ↑↓	P-value ↑↓	PMID ↑↓	Action
arachidonate (20:4n6)	rs174548	11	61571348	1.430e-84	24816252	Delete
arachidonate (20:4n6)	rs174556	11	61580635	2.980e-84	24816252	Delete
arachidonate (20:4n6)	rs174547	11	61570783	8.760e-84	24816252	Delete
arachidonate (20:4n6)	rs174555	11	61579760	6.580e-82	24816252	Delete
arachidonate (20:4n6)	rs174550	11	61571478	7.430e-82	24816252	Delete
arachidonate (20:4n6)	rs174546	11	61569830	7.910e-82	24816252	Delete
arachidonate (20:4n6)	rs174549	11	61571382	8.180e-82	24816252	Delete
arachidonate (20:4n6)	rs174545	11	61569306	8.310e-82	24816252	Delete
arachidonate (20:4n6)	rs174574	11	61600342	1.230e-81	24816252	Delete
arachidonate (20:4n6)	rs102275	11	61557803	2.040e-81	24816252	Delete
arachidonate (20:4n6)	rs174535	11	61551356	2.050e-81	24816252	Delete
arachidonate (20:4n6)	rs174536	11	61551927	2.050e-81	24816252	Delete
arachidonate (20:4n6)	rs174537	11	61552680	2.050e-81	24816252	Delete
arachidonate (20:4n6)	rs14536	11	61507072	1.600e-79	24816252	Delete

**Data Filter Dialog** ×

Target Column: PMID

Value Criterion: (Character) Containing

24816252

Action:  Remove  Keep

Submit

Data uploaded:  exposure  outcome

Proceed

# Outcome data (disease)

1. Copy and paste “Crohn’s disease” in the search box
2. Select from the drop-down list
3. Click “Search” button
4. After both exposure and outcome data are uploaded, click “Proceed” button

Home > Upload

Please use the tabs below to prepare your input. Make sure to click **Submit** button before switching to the next tab.

Exposures **Outcomes**

Enter disease  
e.g., Crohn's disease

Crohn's disease | ebi-a-GCST003044  
Crohn's disease | ebi-a-GCST004132  
Crohn's disease | ieu-a-10  
Crohn's disease | ieu-a-11  
Crohn's disease | ieu-a-12  
Crohn's disease | ieu-a-13  
Crohn's disease | ieu-a-14  
Crohn's disease | ieu-a-30

ID ↑↓	Trait ↑↓	Number of Cases ↑↓	Number of Controls ↑↓	Sample Size ↑↓	Number of Variants ↑↓	Year ↑↓	PMID ↑↓
<a href="#">ebi-a-GCST004132</a>	Crohn's disease	28072	40266	9457998	2017	<a href="#">28067908</a>	

of 1) << 1 >> 15

Here we chose de Lange et al.  
*Nature genetics* (2017), PMID:  
28067908

Data uploaded:  exposure  outcome

[Proceed](#)



# Run MR

Here we chose five commonly used MR methods: MR Egger, weighted median, Inverse variance weighted, simple mode, and weighted mode

[Home](#) > [Upload](#) > [Parameters](#) > [Results](#)

Process your data below

① LD Clumping	<input type="radio"/> Do not check for LD between SNPs <input checked="" type="radio"/> Use clumping to prune SNPs for LD
② LD Proxies	<input checked="" type="checkbox"/> Use proxies Minimum LD $R^2$ value: <input type="text" value="0.8"/> <input checked="" type="checkbox"/> Allow palindromic SNPs MAF threshold for aligning palindromes: <input type="text" value="0.3"/>
③ Allele Harmonization	<input type="radio"/> Assume all alleles are presented on the forward strand <input checked="" type="radio"/> Try to infer the forward strand alleles using allele frequency information <input type="radio"/> Correct the strand for non-palindromic SNPs, but drop all palindromic SNPs
④ Methods Selection	Methods <input type="text" value="Selections"/>

- Maximum likelihood
- MR Egger
- MR Egger (bootstrap)
- Simple median
- Weighted median

Previous

Proceed



# MR results

Home > Upload > Parameters > Results

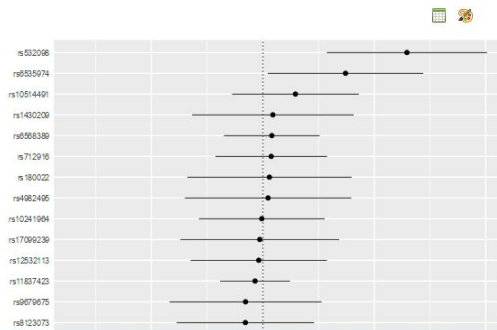
Users can toggle between tabs to view different plots.

## Mendelian Randomization

Methods	MR Results				Heterogeneity Tests			Horizontal Pleiotropy		
	Number of SNPs	Beta	SE	P value	Q	Q_df	Q_pval	Egger Intercept	SE	P value
Inverse variance weighted	20	-0.248	0.421	0.555	33	19	0.024	-	-	-
MR Egger	20	-0.333	0.95	0.73	33	18	0.0167	0.00131	0.0131	0.921
Simple mode	20	0.0753	0.695	0.915	-	-	-	-	-	-
Weighted median	20	-0.257	0.467	0.582	-	-	-	-	-	-
Weighted mode	20	-0.0589	0.557	0.917	-	-	-	-	-	-

OK  
Please see MR results below.

Forest Plot Scatter Plot Funnel Plot Leave-one-out Sensitivity Analysis



### Tips

The forest plot compares the causal effect calculated using the methods that include all the SNPs to using...

Previous

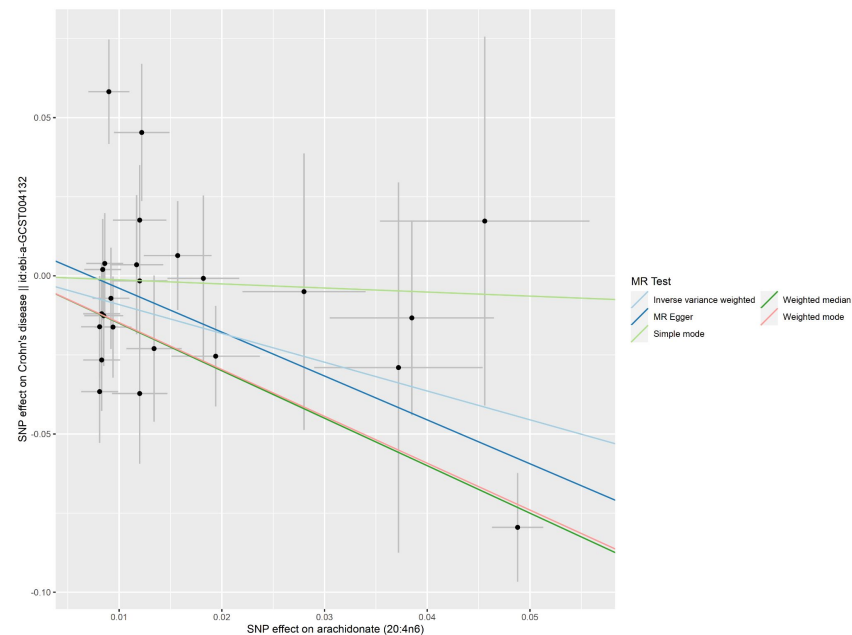
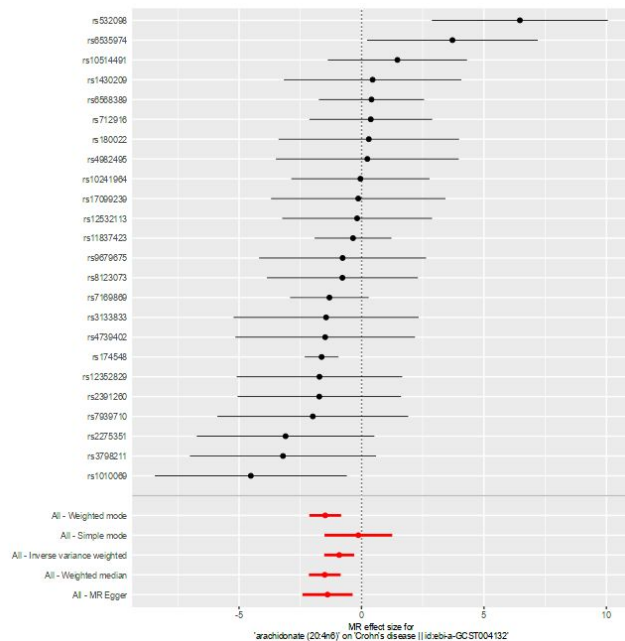
Download



# MR results

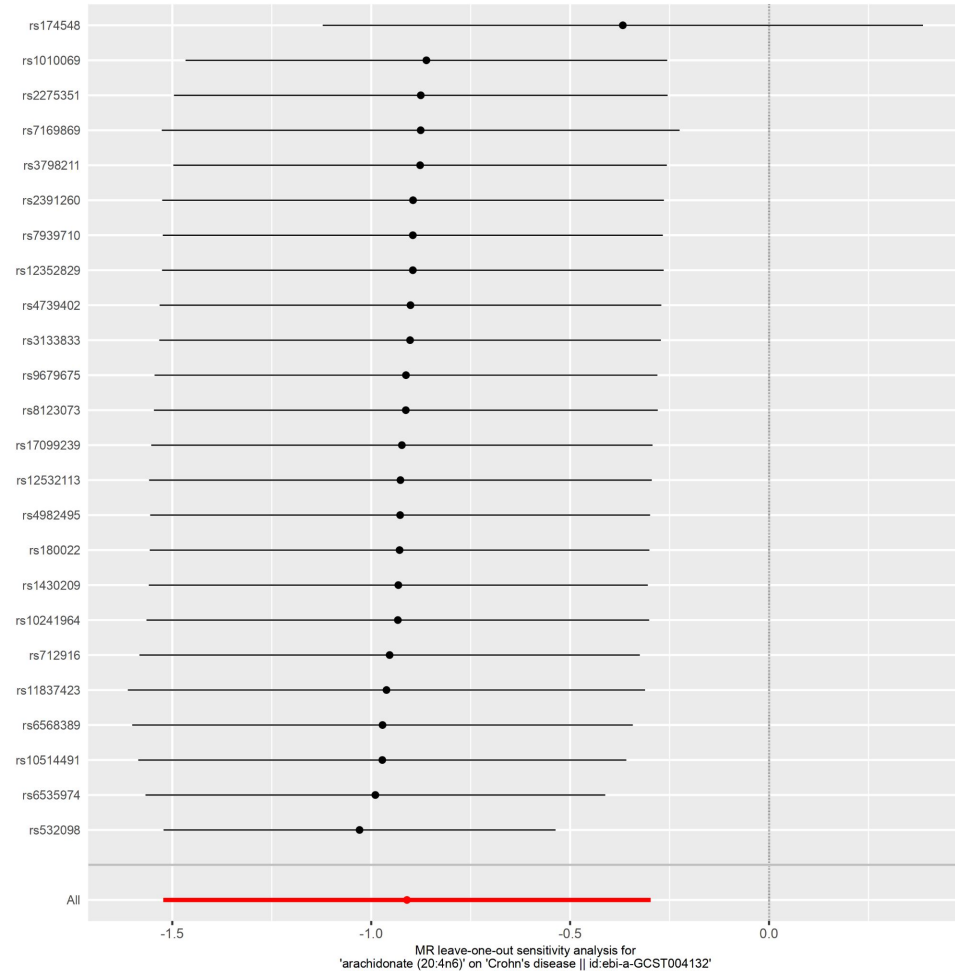
Methods	MR Results			
	Number of SNPs	Beta	SE	P value
Inverse variance weighted	24	-0.91	0.313	0.0036
MR Egger	24	-1.39	0.523	0.0146
Simple mode	24	-0.128	0.646	0.844
Weighted median	24	-1.5	0.335	7.55e-06
Weighted mode	24	-1.48	0.322	0.000126

Consistently shows that the decrease in **arachidonic acid** level had a causal effect on **Crohn's disease**



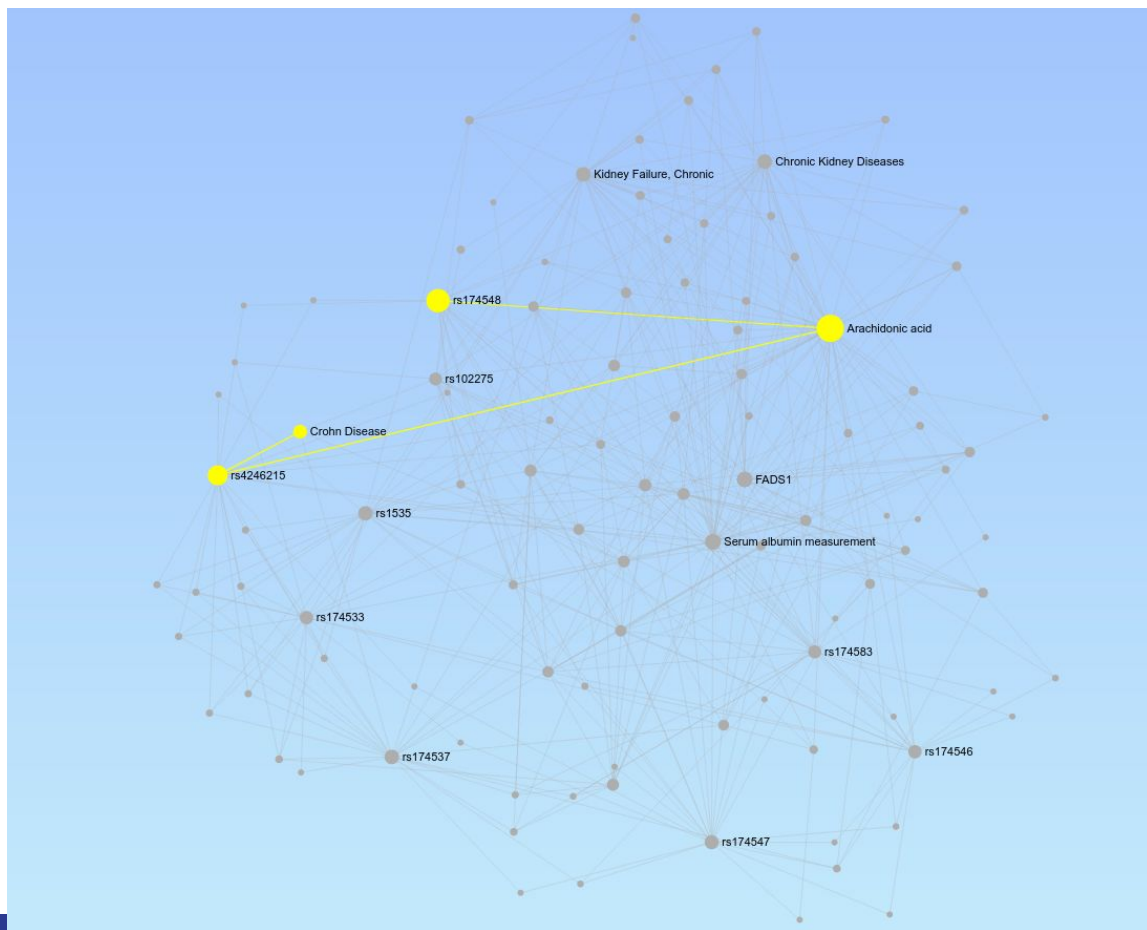
# Leave-one-out plot

- Each black point represents the MR method applied to estimate the causal effect of AA on CD excluding that particular SNP from the analysis
- Exclusion of **rs174548** leads to larger changes in the result

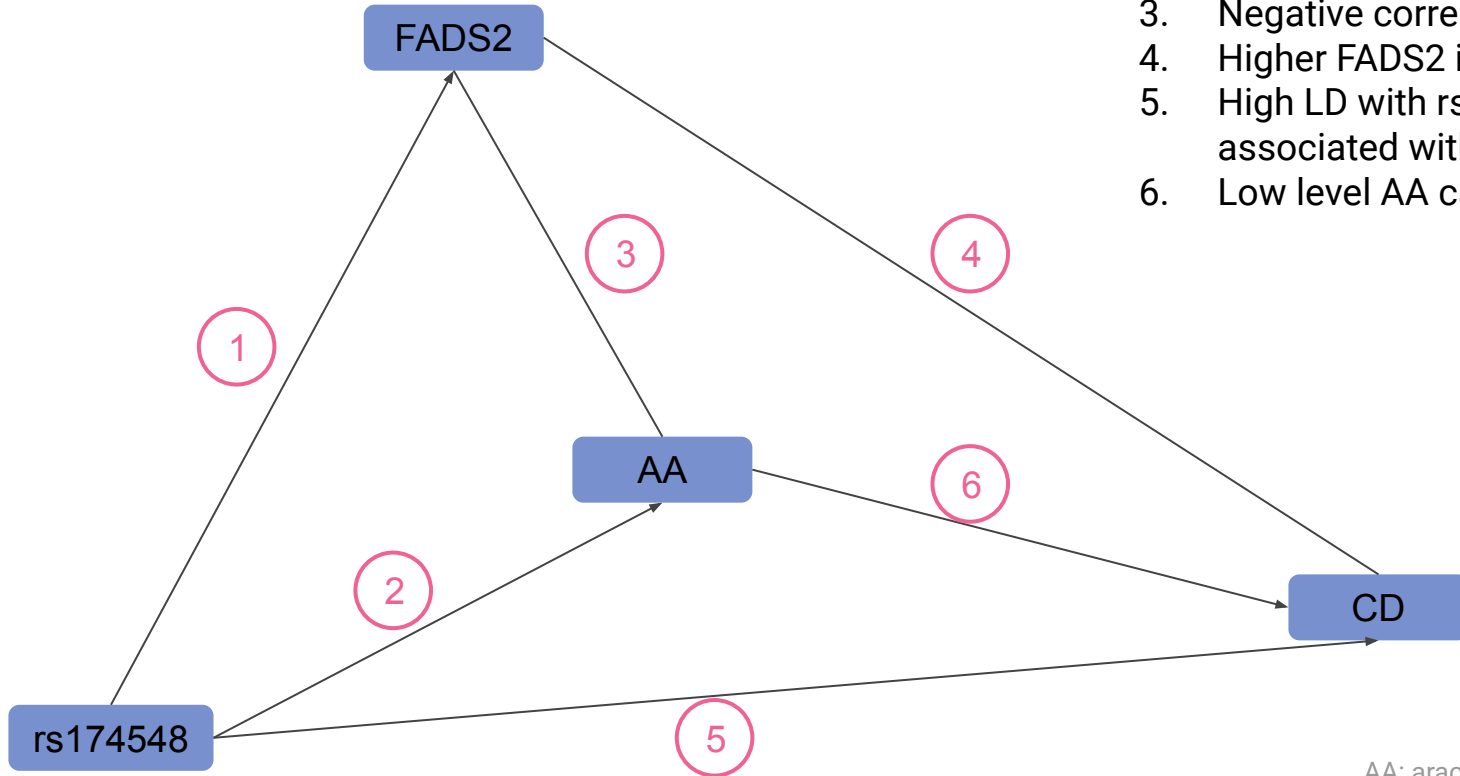


# Network-mapping

- Input:
  - rs174548
    - SNP-metabolite
    - SNP-gene (EUR,  $r^2 > 0.7$ )
    - SNP-disease
  - arachidonic acid
    - metabolite-SNP
    - metabolite-gene
    - metabolite-disease
- Degree filter > 1
- Shortest path view



# Mechanistic link



1. Higher FADS2 level (allele G), eQTL
2. Lower AA level (allele G)
3. Negative correlation
4. Higher FADS2 in CD
5. High LD with rs4246215, associated with CD
6. Low level AA causal to CD

AA: arachidonic acid;  
CD: Crohn's disease

## In summary

mGWAS-Explorer 2.0 is able to provide multiple evidence that arachidonic acid is causally associated with Crohn's disease, suggesting it is a potential therapeutic target.