Spectra Processing, Compound Annotation, Functional Insight and Causal Analysis using MetaboAnalyst 6.0

Jianguo (Jeff) Xia, Associate Professor

Canada Research Chair in Bioinformatics & Big Data Analytics

jeff.xia@mcgill.ca | www.xialab.ca

McGill University, Canada

XiaLab.ca

Empowering researchers through trainings, tools, and AI

McGill UNIVERSITY

Schedule

Part I: 2:15 p.m. – 4:15 p.m

- **2:15 – 3:00:** Background
  - ✓ General introduction
  - ✓ LC-MS & MS/MS spectral processing
  - ✓ From peaks to functions
- **3:00 – 3:20**: Live demo
- **3:20 – 4:15:** Hands on practice

Part II: 4:30 p.m. – 6:30 p.m.

- **4:30 – 5:10**: Background
  - ✓ Data processing
  - ✓ Statistical analysis
  - ✓ Causal analysis
- **5:10 – 5:40**: Live demo
- **5:40 – 6:15**: Hands on practice
- **6:15 – 6:30**: Summary & discussion

Github Repository

- https://github.com/xia-lab/Metabolomics_2024

- Slides (in PDF format);
- Example data;
- Reference literatures;
- Contact information.

Causal Analysis

- mGWAS
- Two-sample Mendelian Randomization (2SMR)

MetaboAnalyst 6.0 Modules

Input Data Type	Available Modules (click on a module to proceed, or scroll down to explore a total of 18 modules including utilities)				
LC-MS Spectra (mzML, mzXML or mzData)			Spectra Processing [LC-MS w/wo MS2]		
MS Peaks (peak list or intensity table)		Peak Annotation [MS2-DDA/DIA]	Functional Analysis [LC-MS]	Functional Meta-analysis [LC-MS]	
Generic Format (.csv or .txt table files)	Statistical Analysis [one factor]	Statistical Analysis [metadata table]	Biomarker Analysis	Statistical Meta-analysis	Dose Response Analysis
Annotated Features (metabolite list or table)		Enrichment Analysis	Pathway Analysis	Network Analysis	
Link to Genomics & Phenotypes (metabolite list)			Causal Analysis [Mendelian randomization]		

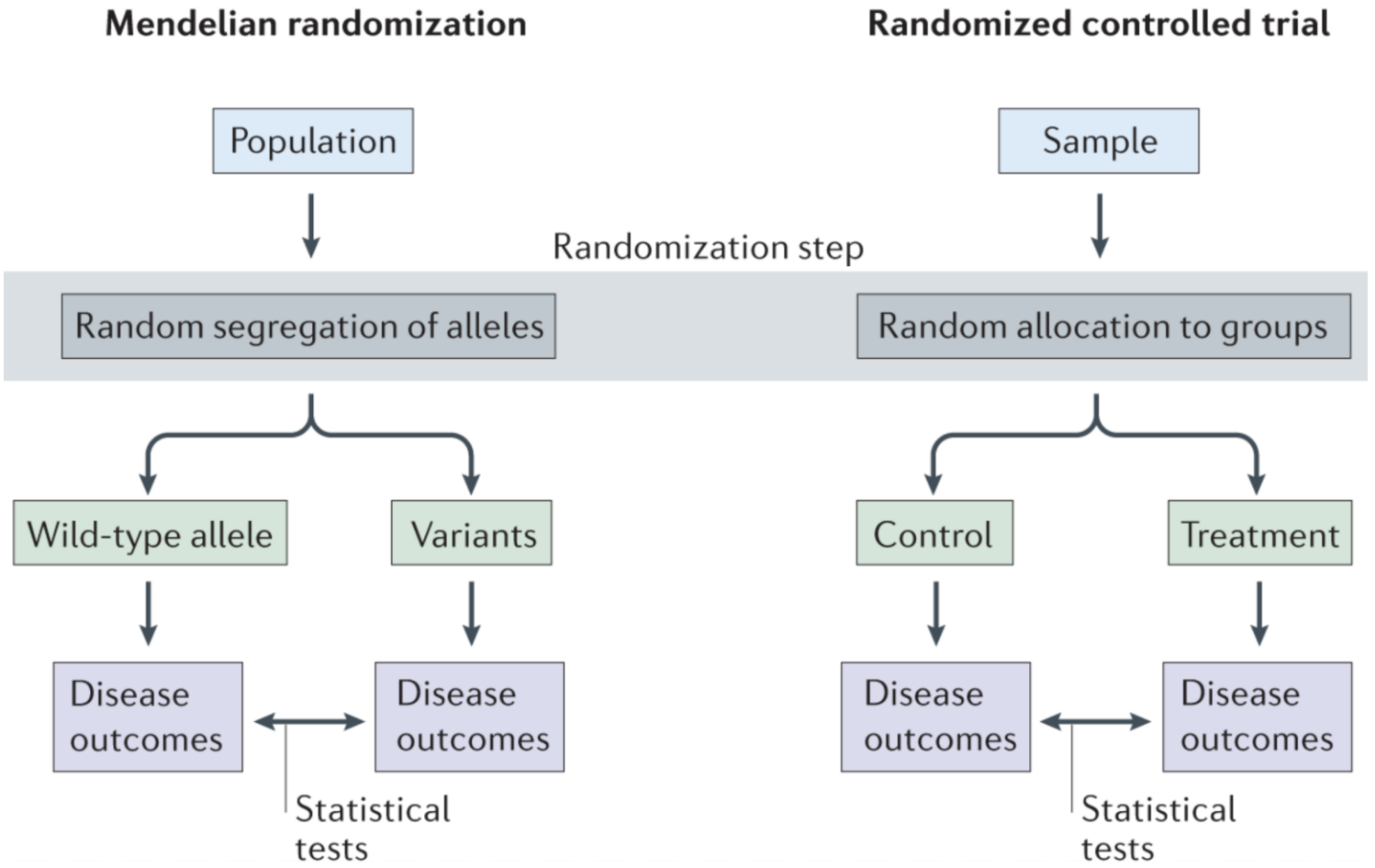
How can we detect causal effects?

- **Key Concept:**
  - If a biomarker / metabolite is causal for a disease / phenotype, the genetic variants which influence the levels of the biomarker should result in a higher risk of the disease
  - Leverage known genetic variants (i.e. SNPs) to eliminates confounders to help detect causal links

➢Mendelian Randomization (MR) analysis

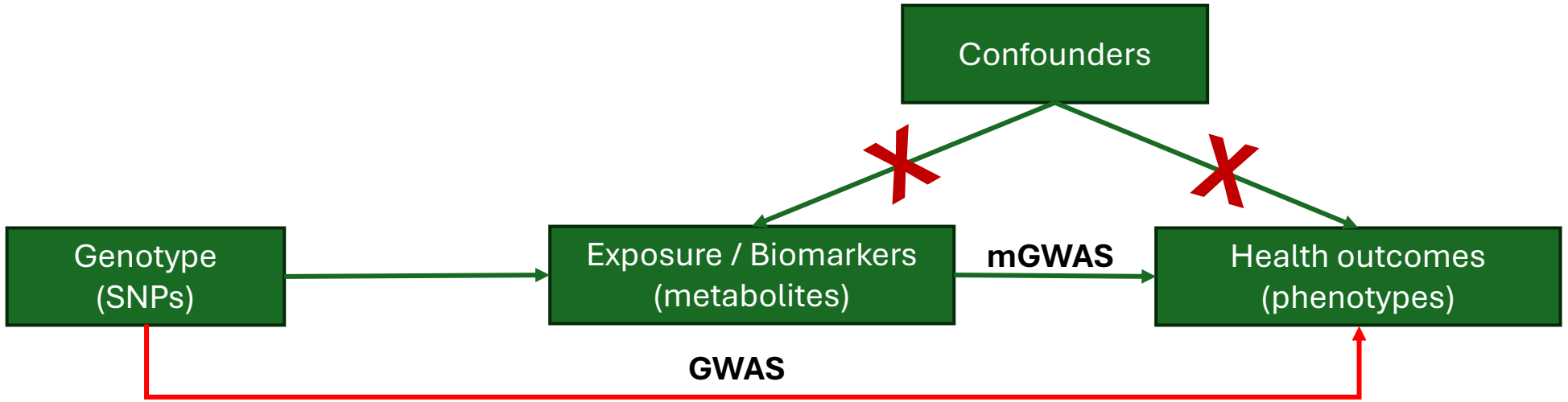
- These genetic variants are called **instrument variables**

MR analysis: nature's randomized controlled trials

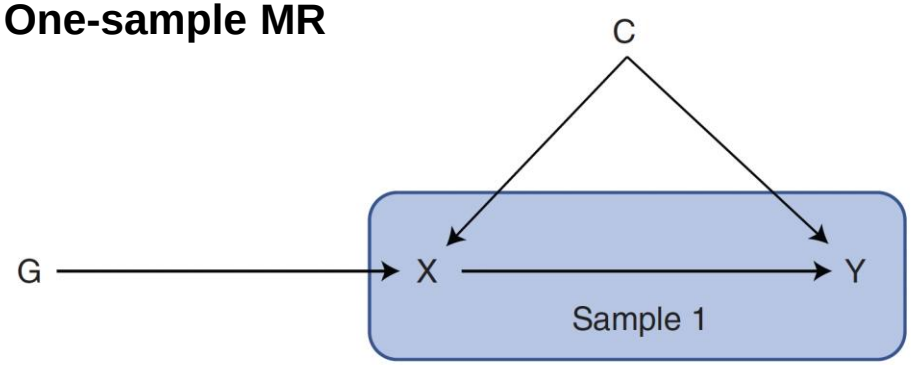


Nat Rev Methods Primers **2**, 6 (2022)

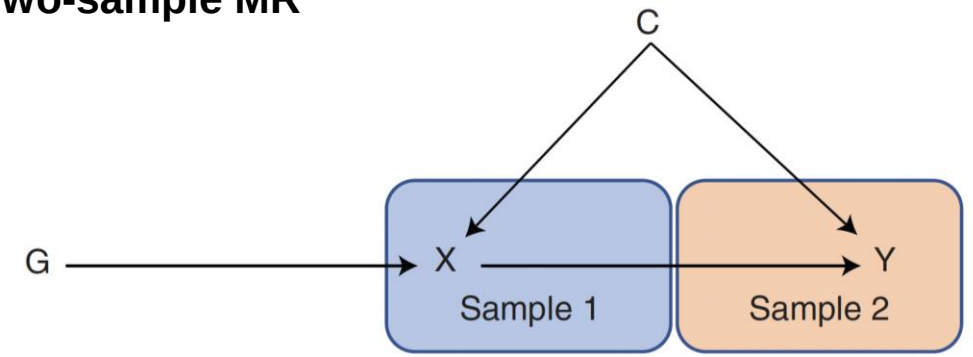
Mendelian randomization concept



One-sample MR



Two-sample MR



Metabolite Genome-Wide Association Study (mGWAS)

Linking the genomics with metabolomics to identify genetic variants affecting metabolite levels

Sample Type	Study #	* Metabolite #	** Metabolite Ratio #	SNP #	SNP–Metabolite Associations #
Blood	57	3992	1265	67,570	30,3090
Urine	5	271	1123	6877	9647
Saliva	1	14	0	1364	1454
Cerebrospinal fluid (CSF)	1	15	0	1178	1182
Mitochondria	1	0	390	194	404
Sum (unique)	65	4147	2388	73,737	313,720

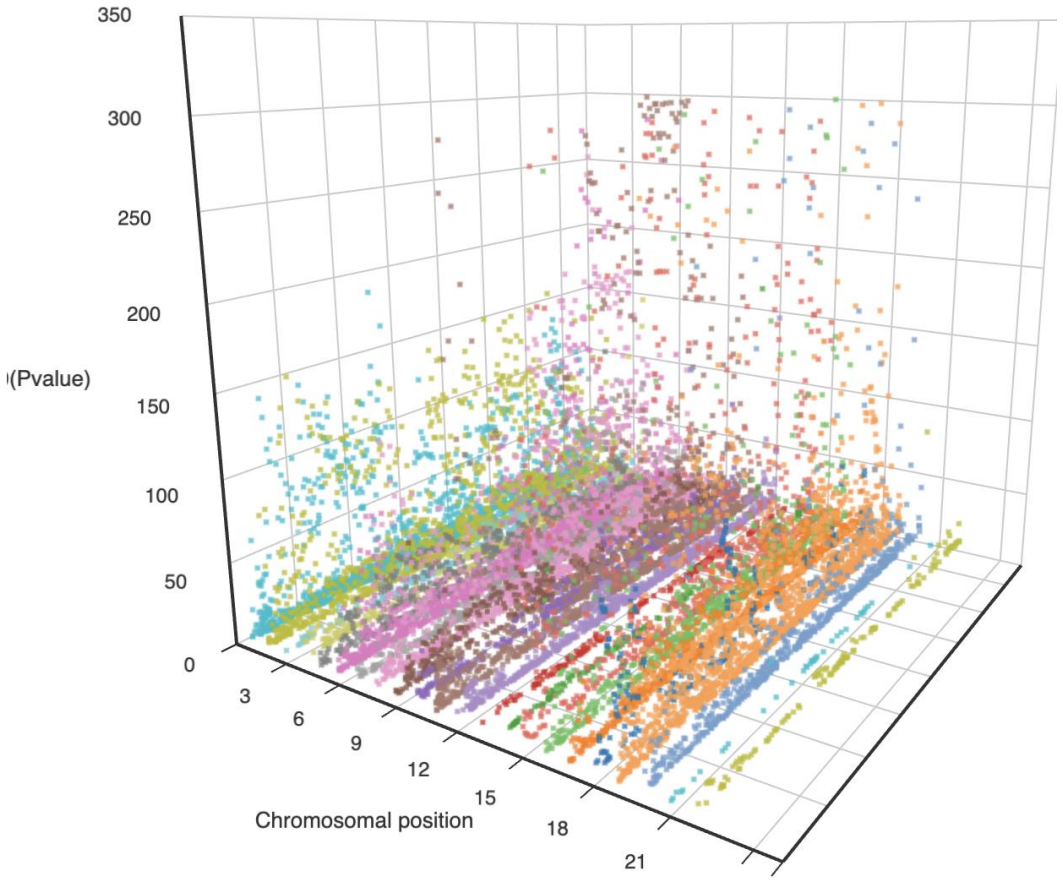
Chang et al. Metabolites **2022**, *12*(6), 526

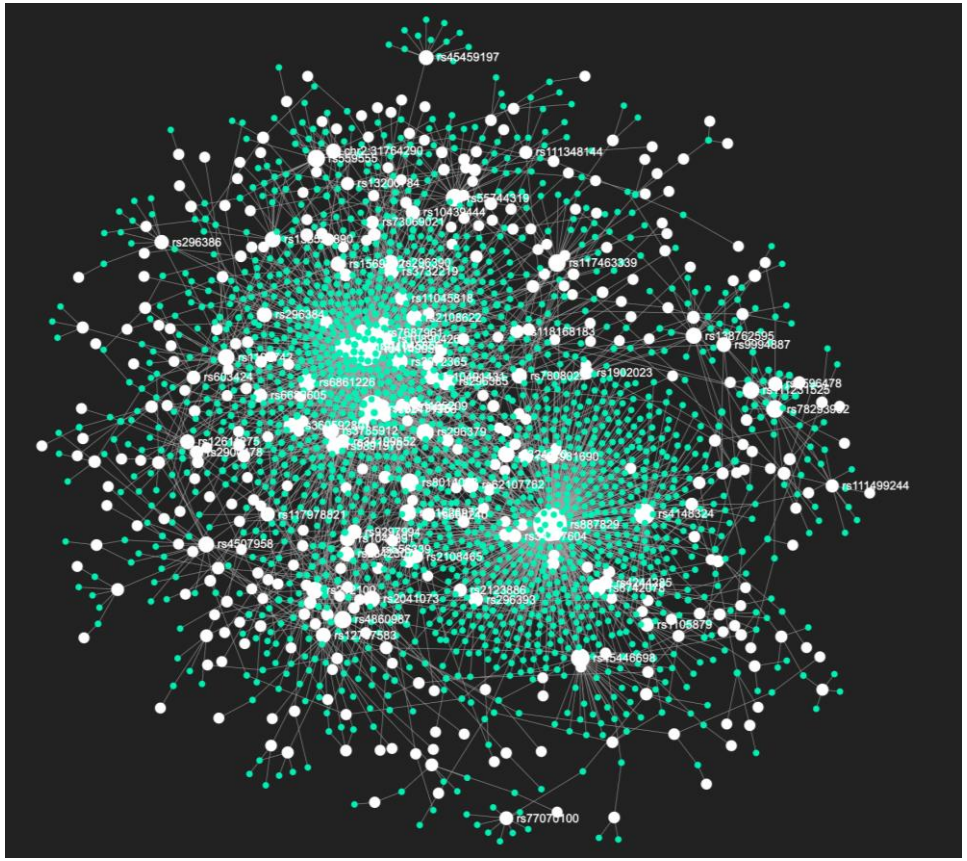
Browse mGWAS studies

ID	Biofluid	Study	Publication	Sample Size	Population	Genotyping Platform	Metabolomics Platform	Cutoff Threshold	Browse
65b	Blood	Viñuela_medRxiv_2021_targeted	Genetic analysis of blood molecular phenotypes reveals regulatory networks affecting complex traits: a DIRECT study	3029	European	Illumina HumanCore array (HCE24 v1.0)	BIOCRATES (AbsoluteIDQ™ p150 kit)	5e-08	View
65a	Blood	Viñuela_medRxiv_2021_untargeted	Genetic analysis of blood molecular phenotypes reveals regulatory networks affecting complex traits: a DIRECT study	3029	European	Illumina HumanCore array (HCE24 v1.0)	Metabolon (LC-MS/MS)	5e-08	View
64	Blood	Qin_medRxiv_2020	Genome-wide association and Mendelian randomization analysis prioritizes bioactive metabolites with putative causal effects on common diseases	8738	European	Illumina genome-wide SNP arrays (HumanCoreExome BeadChip, Human610-Quad BeadChip and HumanOmniExpress)	Thermo Q Exactive Orbitrap	4.5e-12	View
63	Blood	Borges_UKBB_2020	Metabolic biomarkers in the UK Biobank measured by Nightingale Health 2020	500000	European	Affymetrix genome-wide genotyping array	Nightingale NMR	5e-08	View
62	Blood	Montasser_bioRxiv_2021	Leveraging a founder population to identify novel rare-population genetic determinants of lipidome	650	Old Order Amish founder population	Affymetrix 500K array	Agilent (6550 Q-TOF LC/MS)	5e-08	View
61	Mitochondria	Aboulmaouahib_HMG_2021	First mitochondrial genome wide association study with metabolomics	2718	European	Illumina MiSeq	BIOCRATES (AbsoluteIDQ™ p150 kit)	1e-05	View
60	Blood	Harshfield_BM_2021	Genome-wide analysis of blood lipid metabolites in over 5000 South Asians reveals biological insights at cardiometabolic disease loci	13814+5662	European+South Asian	Illumina 660-Quad, Illumina HumanOmniExpress, Affymetrix	Thermo Q Exactive Orbitrap	8.9e-10	View

Chang et al. Metabolites **2022**, *12*(6), 526

Associations between SNPs and compounds/peaks



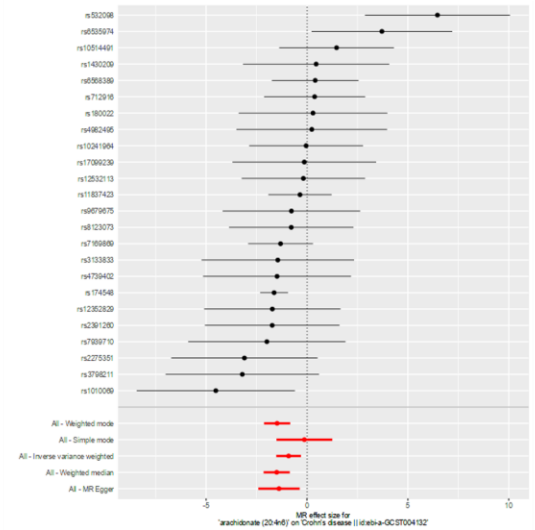


Explore causally links and evidences

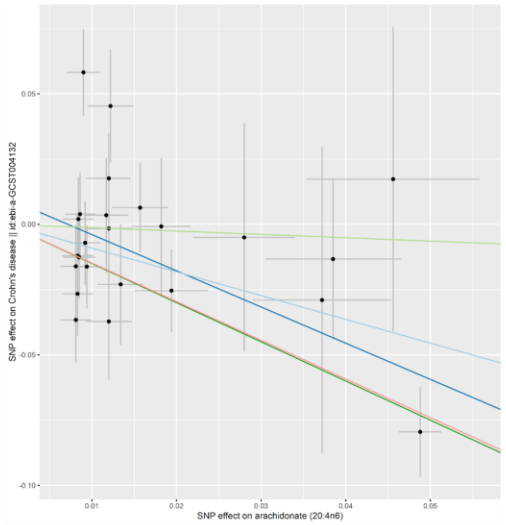
(a)

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	24	-0.91	0.313	0.0036	42.1	23	0.00893	-	-	-
MR Egger	24	-1.39	0.523	0.0146	39.7	22	0.0116	0.00993	0.00877	0.27
Simple mode	24	-0.128	0.711	0.858	-	-	-	-	-	-
Weighted median	24	-1.5	0.323	3.46e-06	-	-	-	-	-	-
Weighted mode	24	-1.48	0.315	9.73e-05	-	-	-	-	-	-

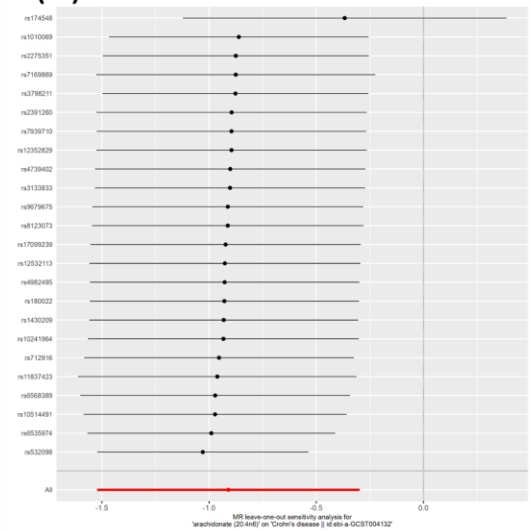
(b)



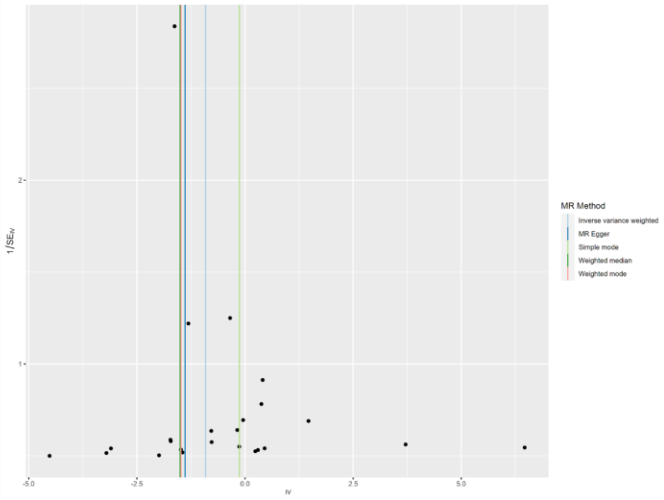
(c)



(d)



(e)

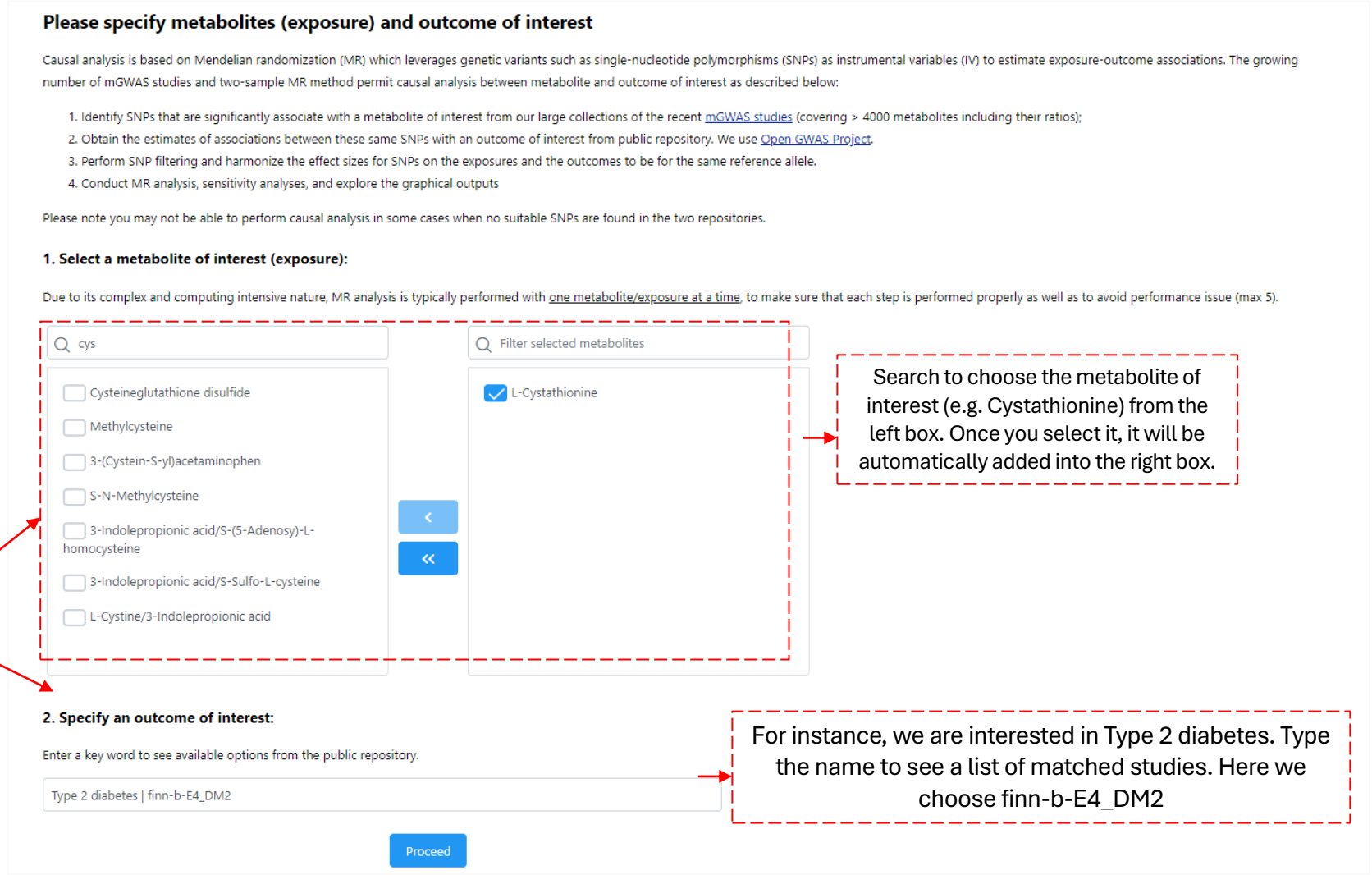


Potential causal associations between ~4000 metabolites and >200 diseases

Diseases	Exposure / Metabolites	SNP	Effect size	SE	Pval
Atherosclerotic heart disease	serine	5	-0.0279495	0.00203363	5.5606E-43
	sm c24:0	5	0.02832925	0.00217185	6.8912E-39
	sm (oh) c22:2	5	0.02907336	0.0022289	6.8912E-39
Inflammatory bowel disease	x-11381	3	-0.8251061	0.04109667	1.1679E-89
	nervonoylcarnitine (c24:1)*	3	-0.9093196	0.04529115	1.1679E-89
	margaroylcarnitine (c17)*	2	-1.0991236	0.06922023	8.9079E-57
	linoleoylcarnitine (c18:2)*	2	-0.8909735	0.05611142	8.9079E-57
	x-24241	1	-1.2770696	0.09285142	4.8253E-43
	histidine betaine (hercynine)*	1	1.24068493	0.09046718	8.3483E-43
	pc aa c32:2	5	0.46386518	0.03590836	3.5615E-38
	pc ae c34:2	5	0.4087556	0.03164226	3.5615E-38
	1-oleoyl-2-eicosapentaenoyl-gpc (18:1/20:5)	4	-0.2323055	0.0195418	1.3726E-32
	arachidoylcarnitine (c20)*	1	-0.9189255	0.07831706	8.5934E-32
	nisinate (24:6n3)	4	-0.3856061	0.03335131	6.4236E-31
Asthma	x-11381	3	0.04781557	0.0027428	4.6252E-68
	pc ae c36:3	8	-0.021023	0.00120984	1.2411E-67
	1-oleoyl-2-eicosapentaenoyl-gpc (18:1/20:5)	4	0.02003149	0.00134398	3.0763E-50
	linoleoylcarnitine (c18:2)*	2	0.05415016	0.00374454	2.1338E-47
	docosatrienoate (22:3n6)*	3	0.0276776	0.00206776	7.3688E-41
	1-eicosapentaenoyl-gpc (20:5)	3	0.02112966	0.00162433	1.0981E-38
	1-palmitoyl-2-eicosapentaenoyl-gpc (16:0/20:5)	3	0.02159433	0.00166005	1.0981E-38
	1-dihomo-linolenoyl-gpe (20:3n3 or 6)	3	-0.016672	0.00128165	1.0981E-38

Live Demo

Specify metabolite and phenotype of interest



Users should first select an exposure (i.e., metabolites) and an outcome (i.e., diseases) of interest.

Search to choose the metabolite of interest (e.g. Cystathionine) from the left box. Once you select it, it will be automatically added into the right box.

For instance, we are interested in Type 2 diabetes. Type the name to see a list of matched studies. Here we choose finn-b-E4_DM2

SNP Filtering & Harmonization

- Multiple SNPs could be identified as potential instrumental variables (IV) from the mGWAS and GWAS studies.

- To perform proper 2SMR, the IVs should be
  - Independent (i.e. not correlated with each other)
  - Showing strong effect (i.e. significant p-values)
  - No horizontal pleiotropy (i.e. affect the outcome only through the metabolite).

- Users need to carefully examine SNPs and apply different filtering and harmonization methods for each criterion

SNP Filtering and harmonization

To properly conduct two-sample MR analysis, the instrumental variables (IV) should be **independent** (i.e. not correlated with each other), showing **strong effect** (i.e. significant p-values), and **no horizontal pleiotropy** (i.e. affect the outcome only through the metabolite). The step provides following procedures to facilitate proper MR analysis:

- Acquisition of independent IVs by performing linkage disequilibrium (LD) clumping.
- In cases where the SNP query is absent in the outcome GWAS, a proxy SNP in LD with the input SNP, utilizing the 1000 Genomes Project (phase 3).
- Harmonizing exposure and outcome data to make sure that the effects of the SNPs on exposure and outcome are associated with the same allele. You should also review the table below to perform further harmonization based on other metadata (such as population, study info, etc)
- To control horizontal pleiotropy, you should manually exclude SNPs that are associated with multiple metabolites.

1. LD Clumping	◉ Do not check for LD between SNPs ○ Use clumping to prune SNPs for LD
2. LD Proxies	◉ Do not use proxies ○ Use proxies and allow palindrome SNPs (advanced settings)
3. Allele Harmonization	○ Assume all alleles are presented on the forward strand ◉ Try to infer the forward strand alleles using allele frequency information ○ Correct the strand for non-palindromic SNPs, but drop all palindromic SNPs

Submit Proceed

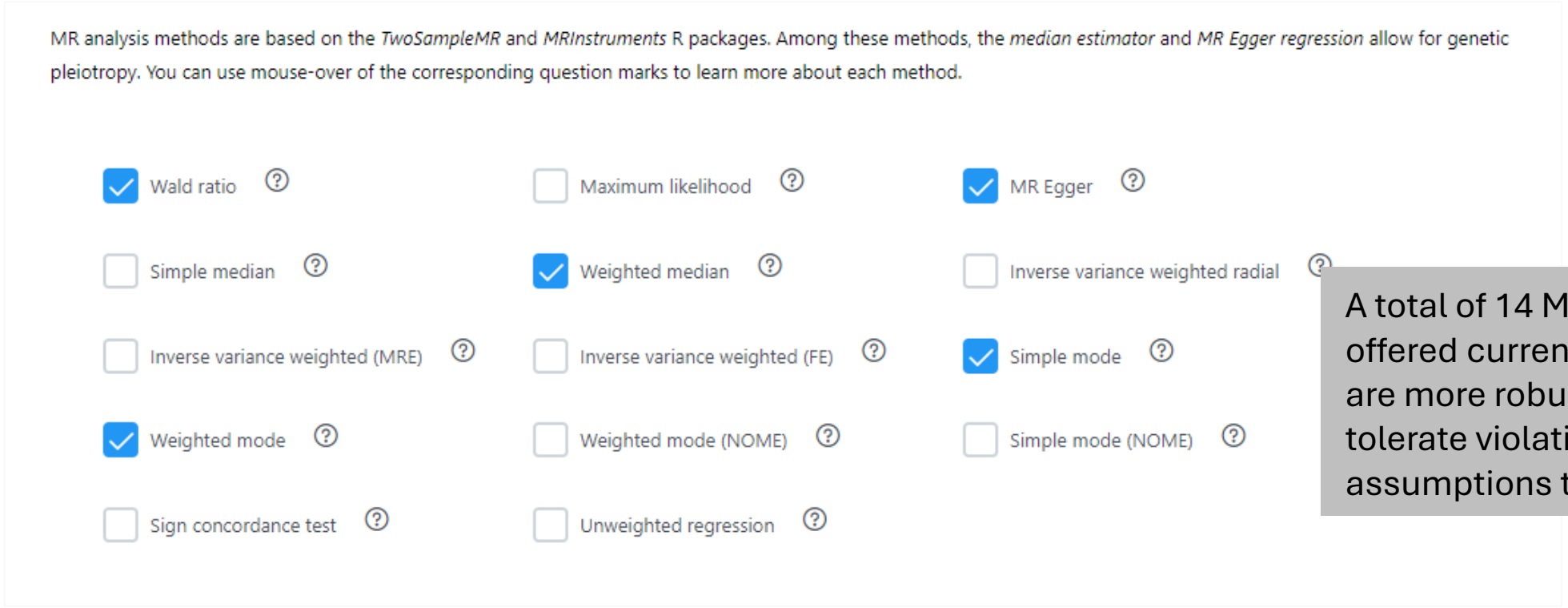
Harmonization steps require intensive computing and also access via remote server.

It could take a long time or time out. Please be patient

SNP ID	Associated Metabolites	Nearest Gene	P-value	Biofluid	Population	Study	
L-Cystathionine (7)							
rs117782586	L-Cystathionine	JRKL	4.637e-08	Blood	European	28263315	☑
rs146276253	L-Cystathionine	ANKRD13C	3.436e-08	Blood	European	28263315	☑
rs150320192	L-Cystathionine	SRSF11	3.566e-08	Blood	European	28263315	☑

Details from the mGWAS studies

Estimating the causal link via MR analysis



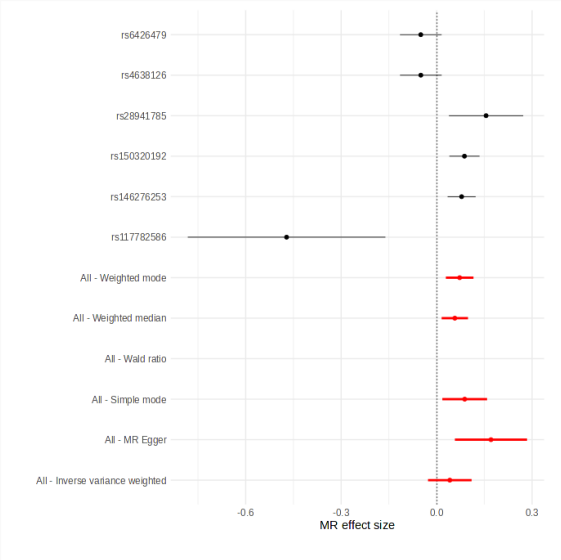
A total of 14 MR methods are offered currently. Some of them are more robust and can better tolerate violations of the assumptions to certain degree

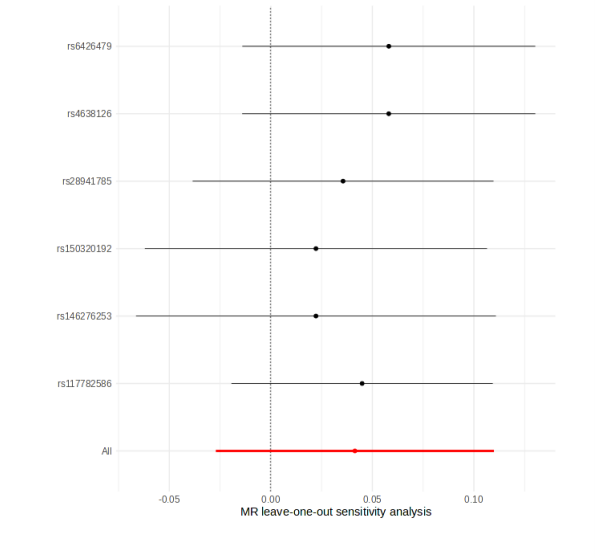
- Mouse over the question marks for each method to see their main features.
- You can also find more detailed introduction on the forum:

https://omicsforum.ca/t/what-are-the-differences-between-the-mr-analysis-methods/1045

Graphical outputs from MR analysis

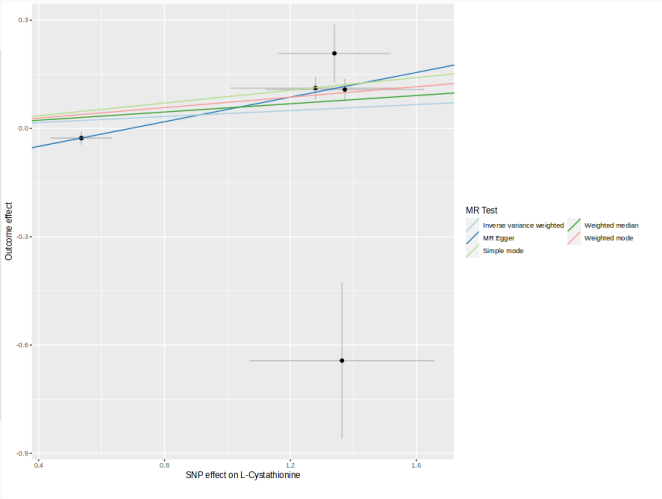
Forest plot compares the causal effect calculated using the methods that include all the SNPs to using each SNP separately.

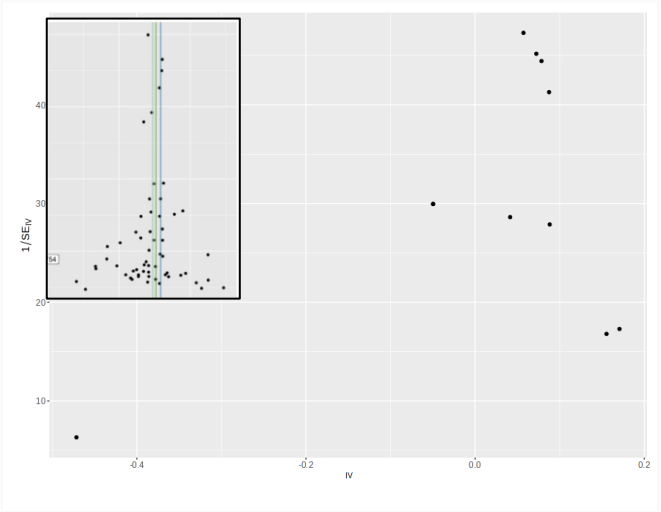




Leave one out sensitivity analysis: assesses whether a single SNP is having a disproportionately larger impact on an association. Each dot represents the MR analysis excluding that specific SNP using IVW method.

Scatter plot shows the relationships between SNP effects on exposure vs on the outcome. The slopes indicating the causal association





Funnel plot: Funnel shape will become more obvious with many SNPs (i.e. green box inset). Its asymmetry and wider spread may suggest horizontal pleiotropy.

Mendelian randomization results

L-Cystathionine

Methods	SNP Count	Causal Effect Estimates			Heterogeneity Tests			Horizontal Pleiotropy		
		Beta	SE	P value	Q	Q_df	Q_pval	Egger Intercept	SE	P value
Inverse variance weighted	6	0.041396	0.034939	0.23609	35.367	5	1.2709e-06	-	-	-
MR Egger	6	0.17071	0.057839	0.041906	14.006	4	0.0072767	-0.1179	0.047732	0.068948
Simple mode	6	0.088203	0.032068	0.040286	-	-	-	-	-	-
Weighted median	6	0.057145	0.021665	0.0083484	-	-	-	-	-	-
Weighted mode	6	0.072379	0.0219	0.021358	-	-	-	-	-	-

- The MR results are organized per metabolite (exposure).
- For metabolite, it shows the SNPs instrumental variables, along with their corresponding causal effect estimates, standard errors and p-values.
- Key values such as the MR-Egger regression intercept and its corresponding p-value are presented.
- Not all methods selected from the previous page would yield results depending on the data used.

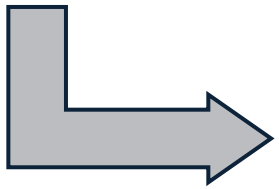
Hands-on time

Summary & Conclusion

The evolution of data analytics

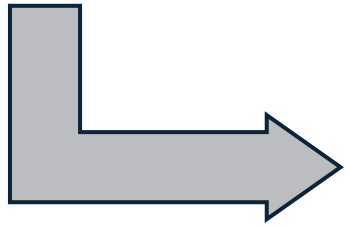
Elite Analytics

- Programming skills
- Statistical knowledge



Visual Analytics

- Cloud/web technology
- Broad access



Conversational Analytics

- AI-augmented
- General researchers

An ecosystem for omics data analysis

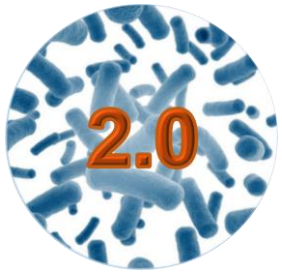
LC-MS spectra

RNA-seq

Microbiome

MetaboAnalyst

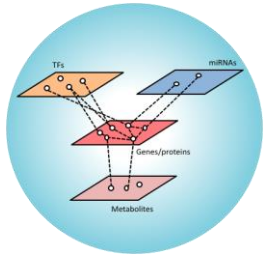
ExpressAnalyst



MicrobiomeAnalyst

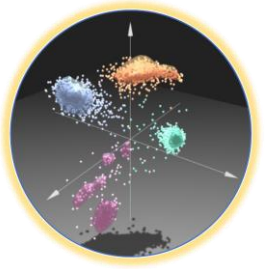
Signature Lists

Normalized Tables



OmicsNet

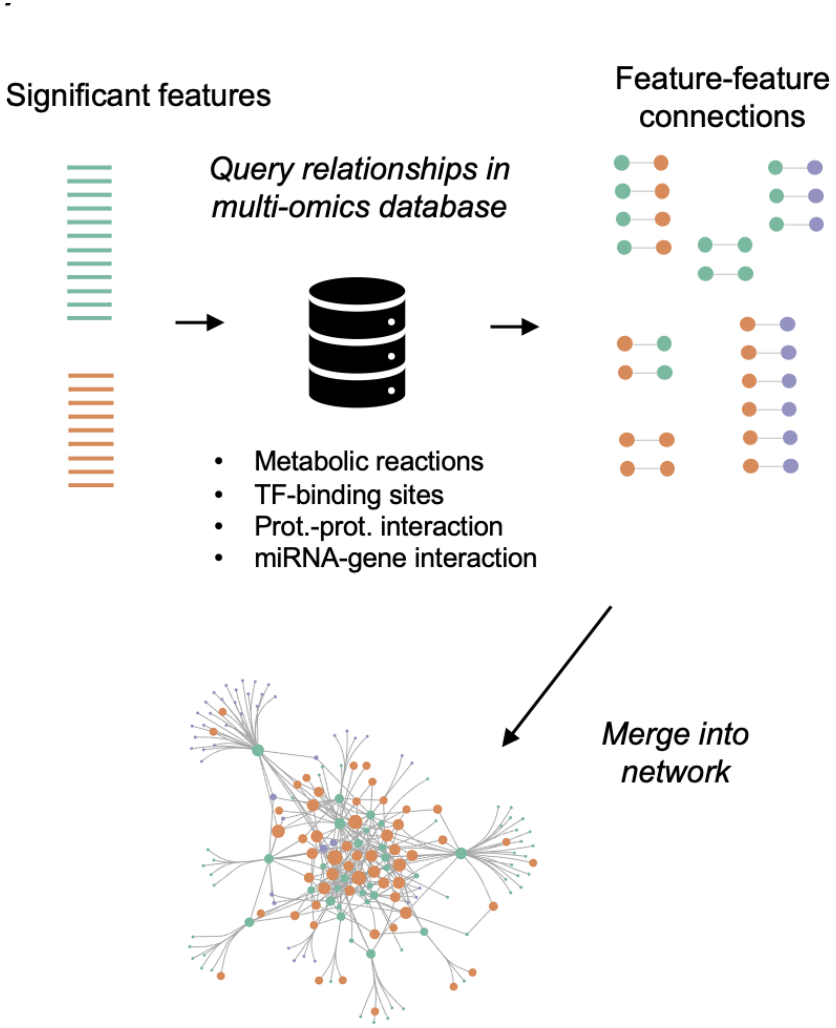
Qualitative Integration



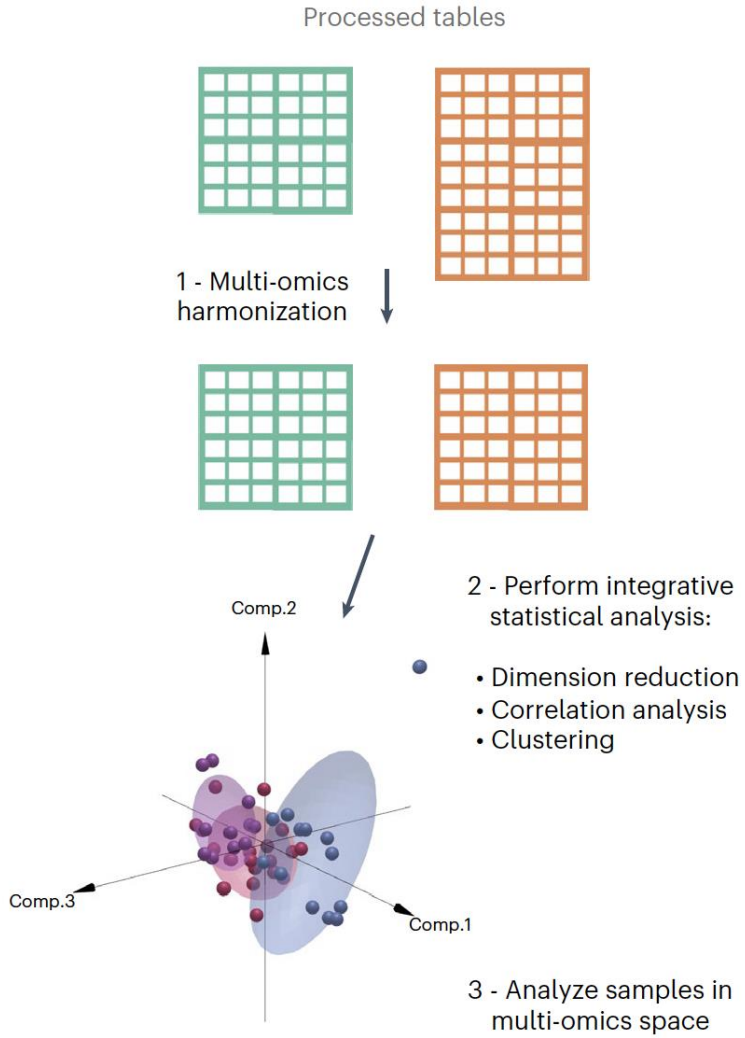
OmicsAnalyst

Quantitative Integration

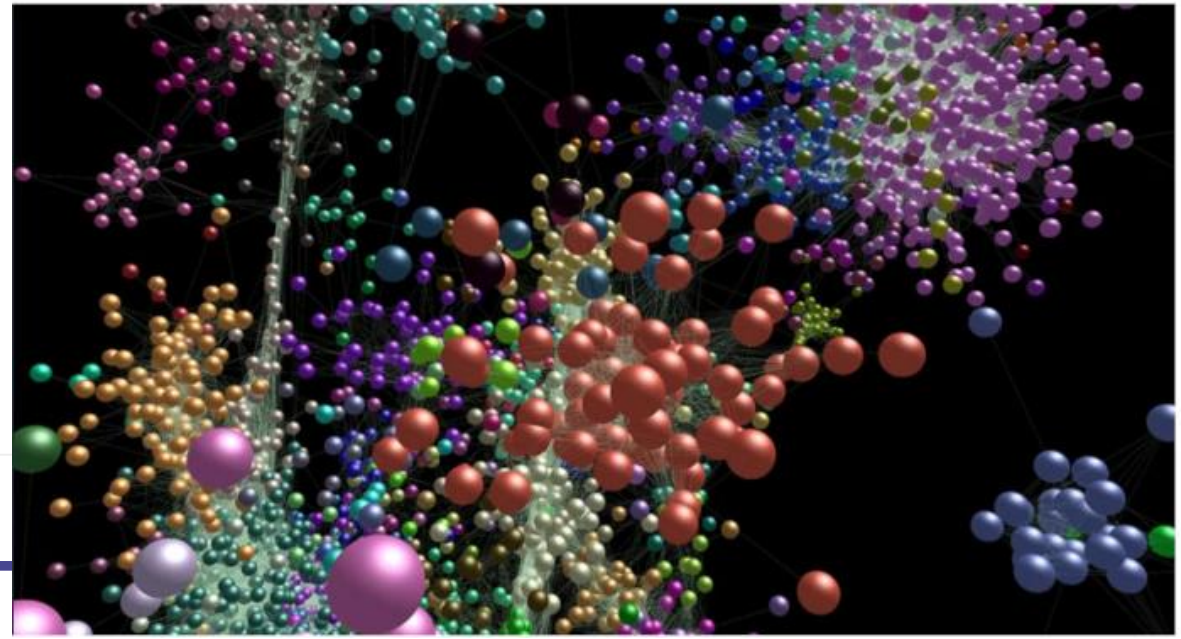
General workflow for multi-omics



Qualitative Integration



Quantitative Integration



nature protocols

Explore content | About the journal | Publish with us

nature > nature protocols > protocols > article

Protocol | Published: 14 February 2024

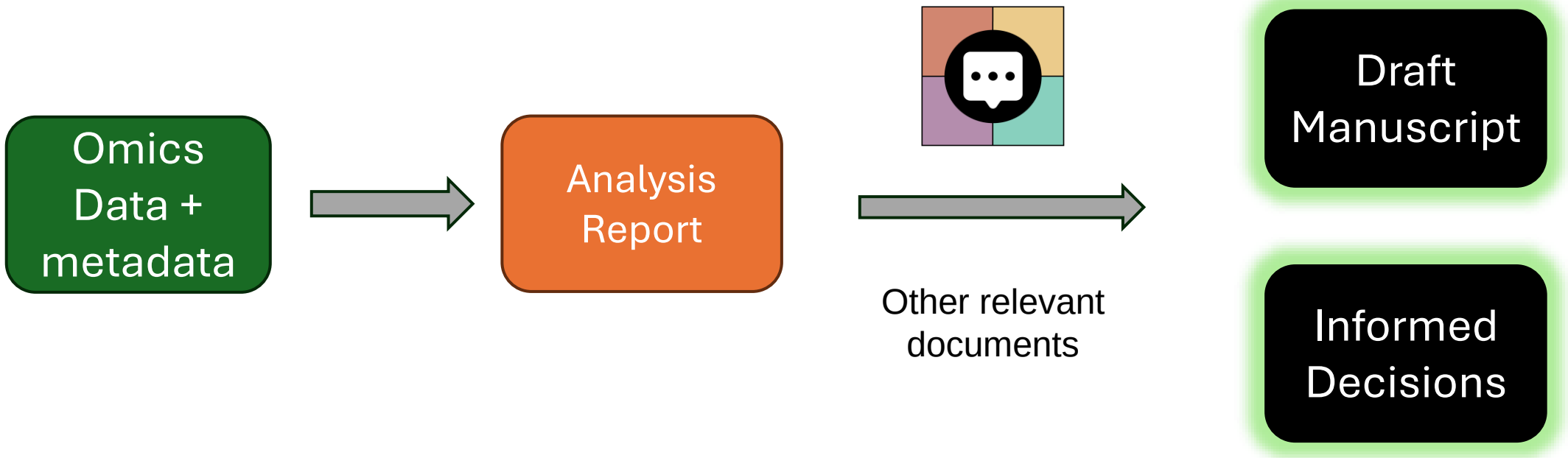
Web-based multi-omics integration using the Analyst software suite

Jessica D. Ewald, Guangyan Zhou, Yao Lu, Jelena Kolic, Cara Ellis, James D. Johnson, Patrick E. Macdonald & Jianguo Xia

Nature Protocols (2024) | Cite this article

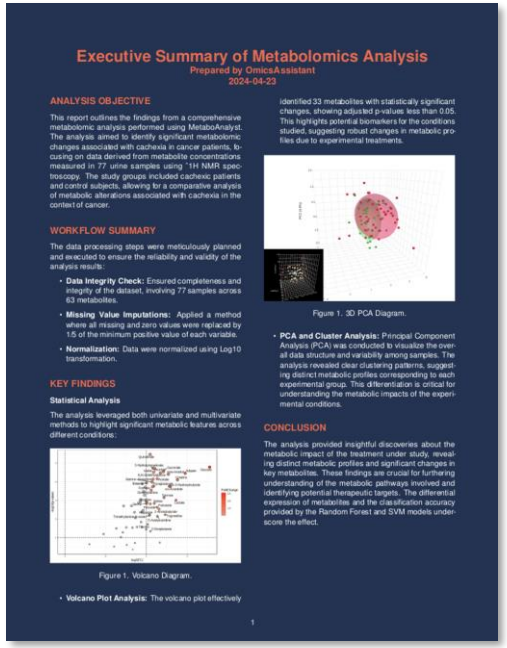
https://www.nature.com/articles/s41596-023-00950-4

Leveraging AI co-pilot for productivity



Towards conversational analytics

Validated workflow



Executive Summary of Metabolomics Analysis

Prepared by OmicsAssistant

2024-04-23

ANALYSIS OBJECTIVE

This report outlines the findings from a comprehensive metabolomic analysis performed using MetaboAnalyst. The analysis aimed to identify significant metabolomic changes associated with cachexia in cancer patients, focusing on data derived from metabolite concentrations measured in 77 urine samples using ^{1}H NMR spectroscopy. The study groups included cachexic patients and control subjects, allowing for a comparative analysis of metabolic alterations associated with cachexia in the context of cancer.

WORKFLOW SUMMARY

The data processing steps were meticulously planned and executed to ensure the reliability and validity of the analysis results:

- **Data Integrity Check:** Ensured completeness and integrity of the dataset, involving 77 samples across 63 metabolites.
- **Missing Value Imputations:** Applied a method where all missing and zero values were replaced by 1/5 of the minimum positive value of each variable.
- **Normalization:** Data were normalized using Log10 transformation.

KEY FINDINGS

Statistical Analysis

The analysis leveraged both univariate and multivariate methods to highlight significant metabolic features across different conditions:

Figure 1. Volcano Diagram.

- **Volcano Plot Analysis:** The volcano plot effectively identified 33 metabolites with statistically significant changes, showing adjusted p-values less than 0.05. This highlights potential biomarkers for the conditions studied, suggesting robust changes in metabolic profiles due to experimental treatments.

Figure 1. 3D PCA Diagram.

- **PCA and Cluster Analysis:** Principal Component Analysis (PCA) was conducted to visualize the overall data structure and variability among samples. The analysis revealed clear clustering patterns, suggesting distinct metabolic profiles corresponding to each experimental group. This differentiation is critical for understanding the metabolic impacts of the experimental conditions.

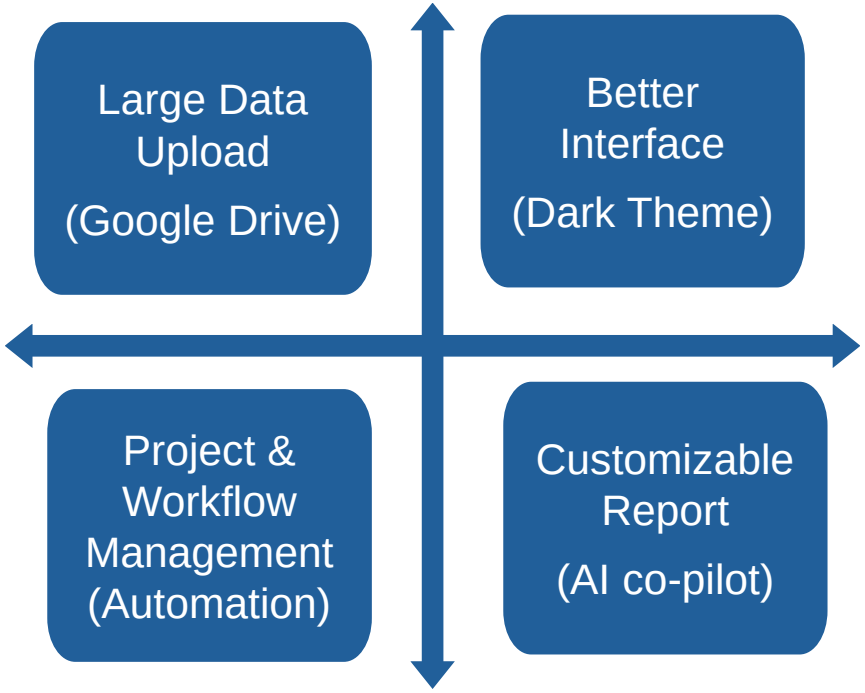
CONCLUSION

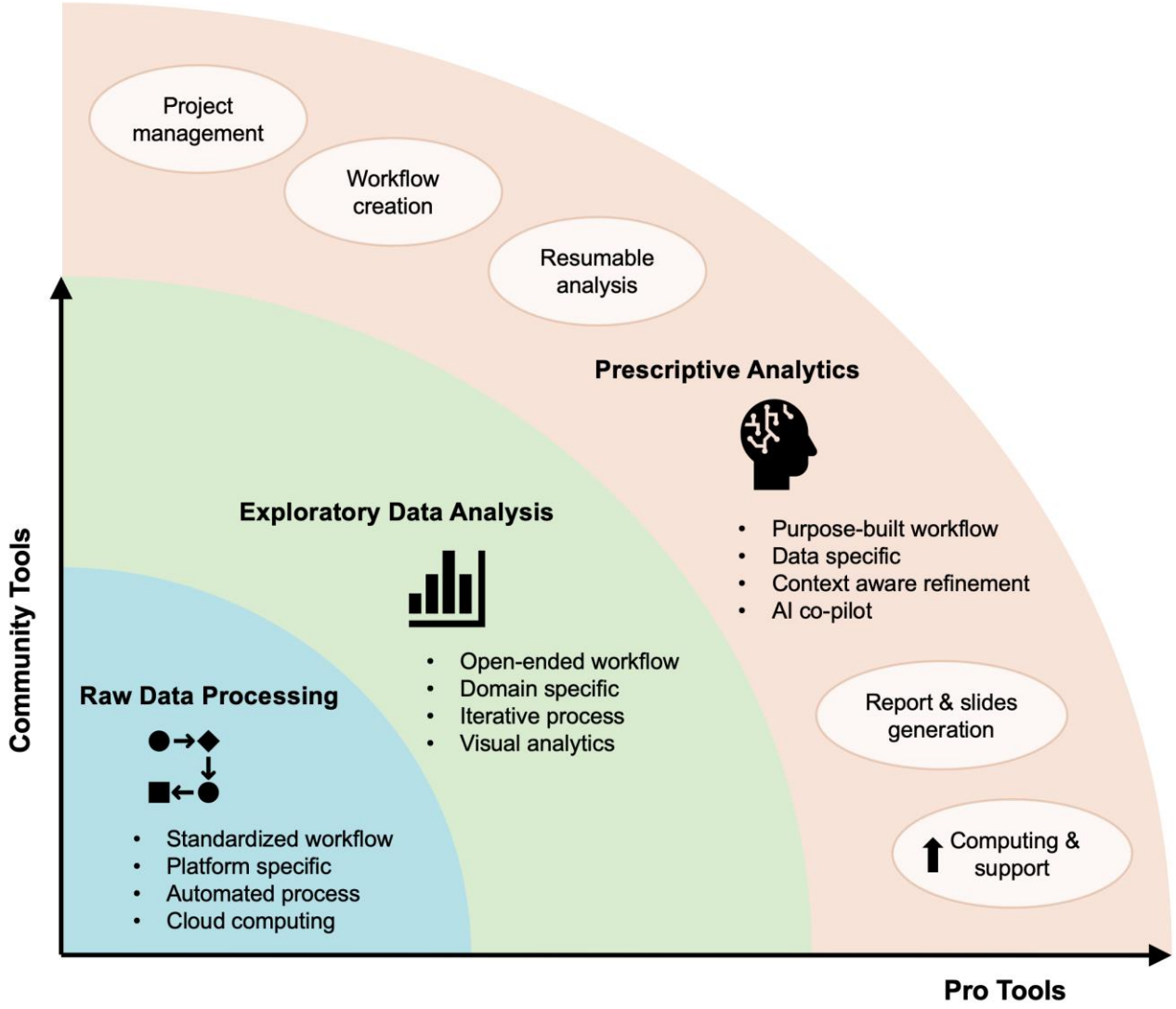
The analysis provided insightful discoveries about the metabolic impact of the treatment under study, revealing distinct metabolic profiles and significant changes in key metabolites. These findings are crucial for furthering understanding of the metabolic pathways involved and identifying potential therapeutic targets. The differential expression of metabolites and the classification accuracy provided by the Random Forest and SVM models underscore the effect.

1

Pathway Name	Total	Hits (all)	Hits (sig.)	Expected	P(Fisher)	P(Gamma)	Details
Vitamin E metabolism	54	38	15	5.0563	0.030024	0.025523	View
Carnitine shuttle	72	25	10	6.7418	0.06554	0.028334	View
Bile acid biosynthesis	82	51	17	7.6781	0.10222	0.029459	View
De novo fatty acid biosynthesis	106	17	6	9.9254	0.22659	0.042666	View

Key Features in “Pro” version





Acknowledgements

If you have any questions, please read/post into OmicsForum (https://omicsforum.ca)

Contact us:

- zhiqiang.pang@xialab.ca
- jeff.xia@xialab.ca

Omics Data Science course:

- Summer Bootcamp
  - Aug. 5 - 9, **9:30 - 16:30**
- Regular Session
  - Saturday morning **9:30 - 12:00**, Sept. - Nov.

TMIC The Metabolomics Innovation Centre
NIH The Common Fund
INNOVATION Canada Foundation for Innovation / Fondation canadienne pour l'innovation
Canada Research Chairs www.chairs-chaires.gc.ca
GenomeCanada
NSERC CRSNG
McGill