Multi-omics在队列研究的多样化场景

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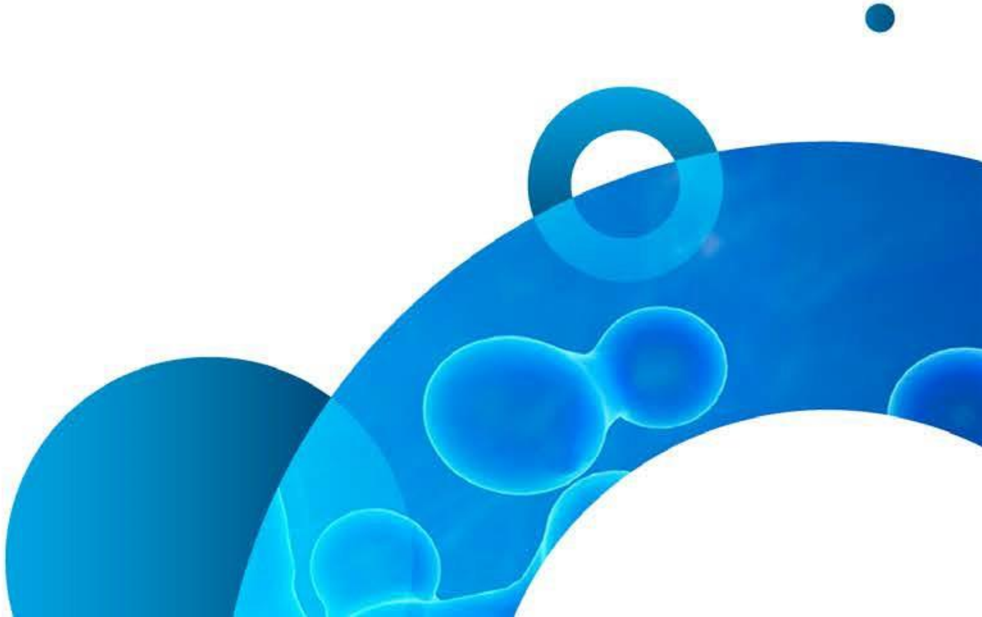


Multi-omics队列研究目标

生物世界

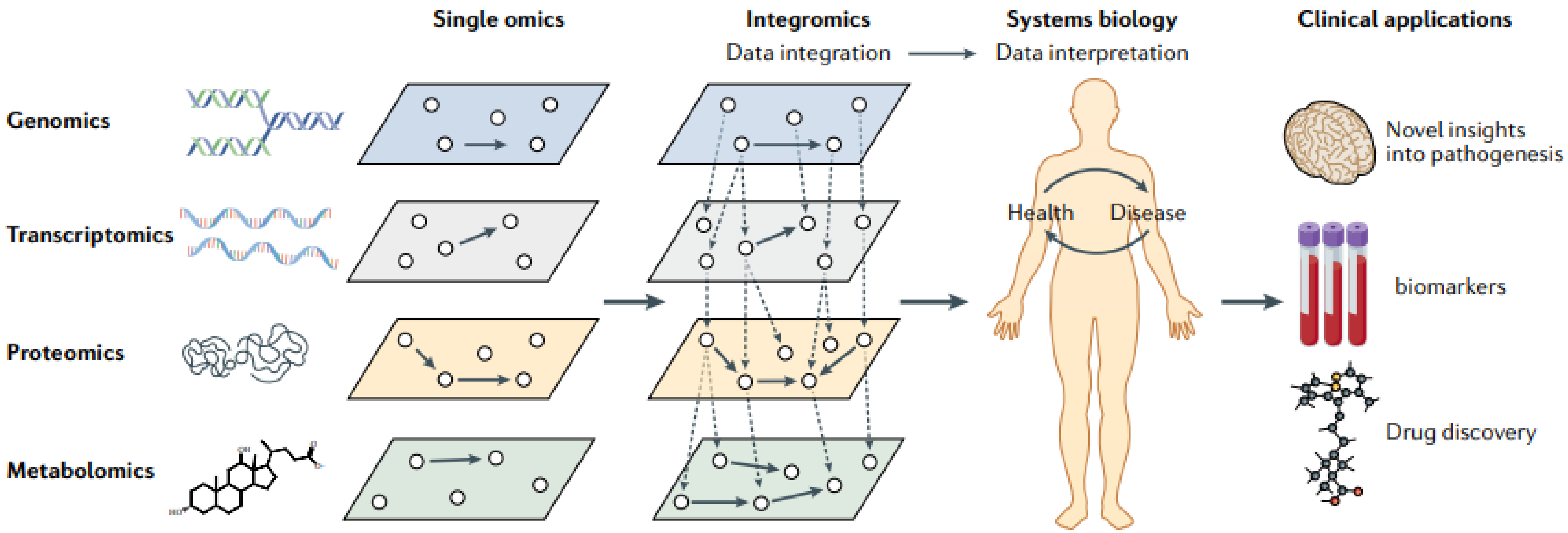
- ✓ 基因组、**转录组**、代谢组…
- ✓ **单细胞组**
- ✓ 微生物组

因果**调**控功能机制

真**实**世界

- ✓ **环境暴露组**
- ✓ **结局**表型组
- ✓ 影像组…

Multi–omics 队列研究场景—Disease based


Single omics
Integromics
Data integration
Systems biology
Data interpretation
Clinical applications
Genomics
Transcriptomics
Proteomics
Metabolomics
Health
Disease
Novel insights into pathogenesis
biomarkers
Drug discovery

Multi–omics roles

基因组学（Genomics）

——What is possible happen

- ✓ 胚系细胞SNP、CNV、InDel等遗传变异
- ✓ 体细胞mutation、InDel等环境突变变异

转录组学（Transcriptomics）

——What appears to be happening

- ✓ 转录本类型：mRNA、miRNA、lncRNA、cirRNA、piRNA等
- ✓ 转录定位：表达、融合、剪切、ceRNA等

代谢组学（Metabolomics）

——What has happened

- ✓ 脂质组、氨基酸类、胆汁酸类、神经递质类等等
- ✓ 宿主代谢物、微生物代谢物

表观基因组学（Epigenomics）

——What appears to be happening

- ✓ 基因组：ATAC、HiC
- ✓ DNA甲基化：5mC、5hmC、6mA等
- ✓ RNA甲基化：m6A等

蛋白质组学（Proteinomics）

——What is happening

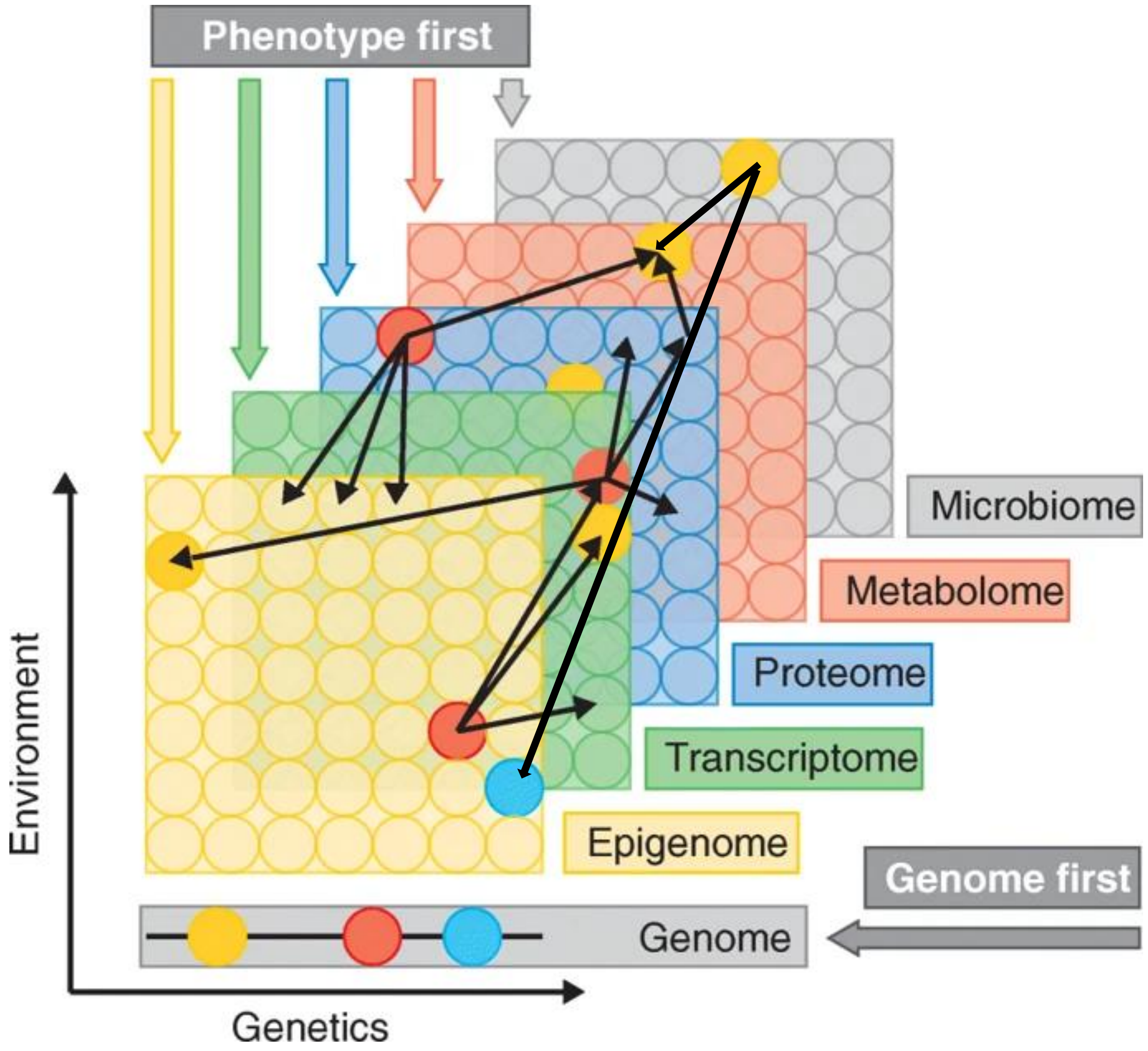
- ✓ 功能蛋白质：酶、转录因子等
- ✓ 修饰蛋白质：糖基化、乙酰化等

微生物组学（Microbiomics）

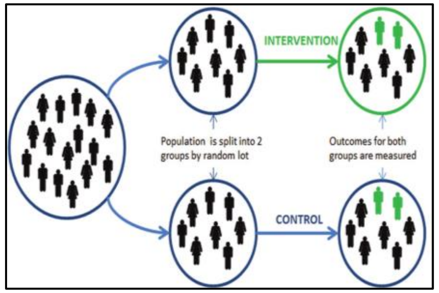
——The second omics：interact

- ✓ 宏基因组、宏转录组、宏蛋白质组、宏代谢组

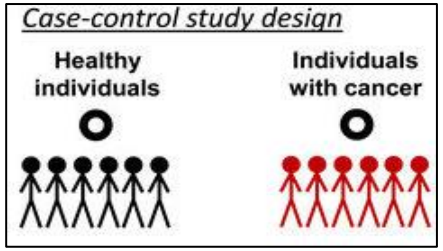
Multi-omics approaches to disease research


Phenotype first
Microbiome
Metabolome
Proteome
Transcriptome
Epigenome
Genome first
Genome
Environment
Genetics

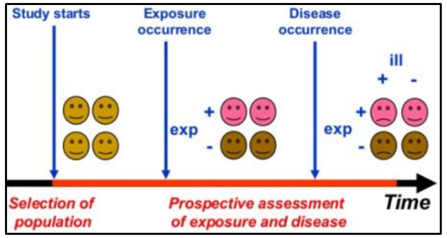
Multi–omics cohort


INTERVENTION
Population is split into 2 groups by random lot
Outcomes for both groups are measured
CONTROL

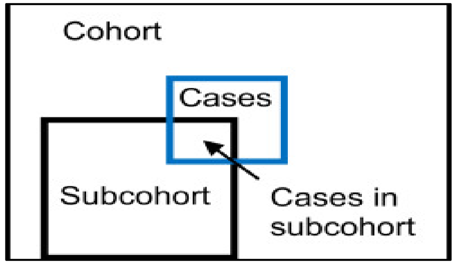
Randomized controlled trial(RCT)
随机对照研究


Case-control study design
Healthy individuals
Individuals with cancer

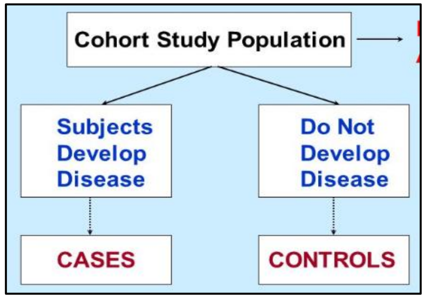
Case-control study
病例对照研究


Study starts
Exposure occurrence
Disease occurrence
ill
+
-
exp
+
-
exp
+
-
Selection of population
Prospective assessment of exposure and disease
Time

Prospective cohort study
前瞻性队列研究

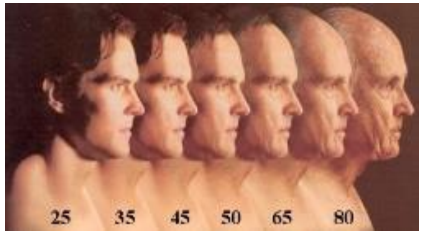

Cohort
Cases
Subcohort
Cases in subcohort

Case-cohort study
病例-队列研究
病例参比式研究


Cohort Study Population
Subjects Develop Disease
Do Not Develop Disease
CASES
CONTROLS

Nested case-control study
巢式病例对照研究
队列内病例对照研究

Cross-sectional study
横断面研究


25
35
45
50
65
80

Longitudinal study
纵贯研究，纵向研究

Multi–omics trajectory to disease research

多组学调控网络构建及因果效应分析

生物学功能通路挖掘及关键调控分子筛选

细胞体外及动物体内分子调控功能机制验证

The Genome first approach

研究目的

研究手段

研究定位的转变

单一GWAS研究的局限性

- ✓侧重相关性结论的挖掘
- ✓遗传模型对于复杂表型的片面性
- ✓高质量队列样本的长周期
- ✓非功能区域易感SNP位点的分子调控功能机制挑战

GWAS多组学研究的广阔性

- ✓ 构建不同纬度的QTL调控network以及易感SNP causal结论的挖掘
- ✓ 不同多组学TWAS、MeWAS、PWAS等融合分析及MR随机化因果推断分析
- ✓ 筛选与表型有关的重要生物学通路及其中关键调控功能分子

The Genome first approach—样本遗传基线数据

Epigenomics
meGWAS-meQTL

Phenotype
GWAS-asscoiation

Transcriptomics
TGWAS-eQTL、sQTL、roQTL
mRNA/miRNA
/LncRNA/CirRNA/Splice

Single cell RNA-seq
Sc-eQTL

Protein&Glycomics
pGWAS-pQTL
glycGWAS-glycQTL

Metabolome&Microbiomics
mGWAS-mQTL、miGWAS-miQTL

The Genome first approach

科研主旨

关联分析研究

✓QTL分析Cis_与trans分析遗传调控网络network构建

✓精准筛选重要驱动功能基因

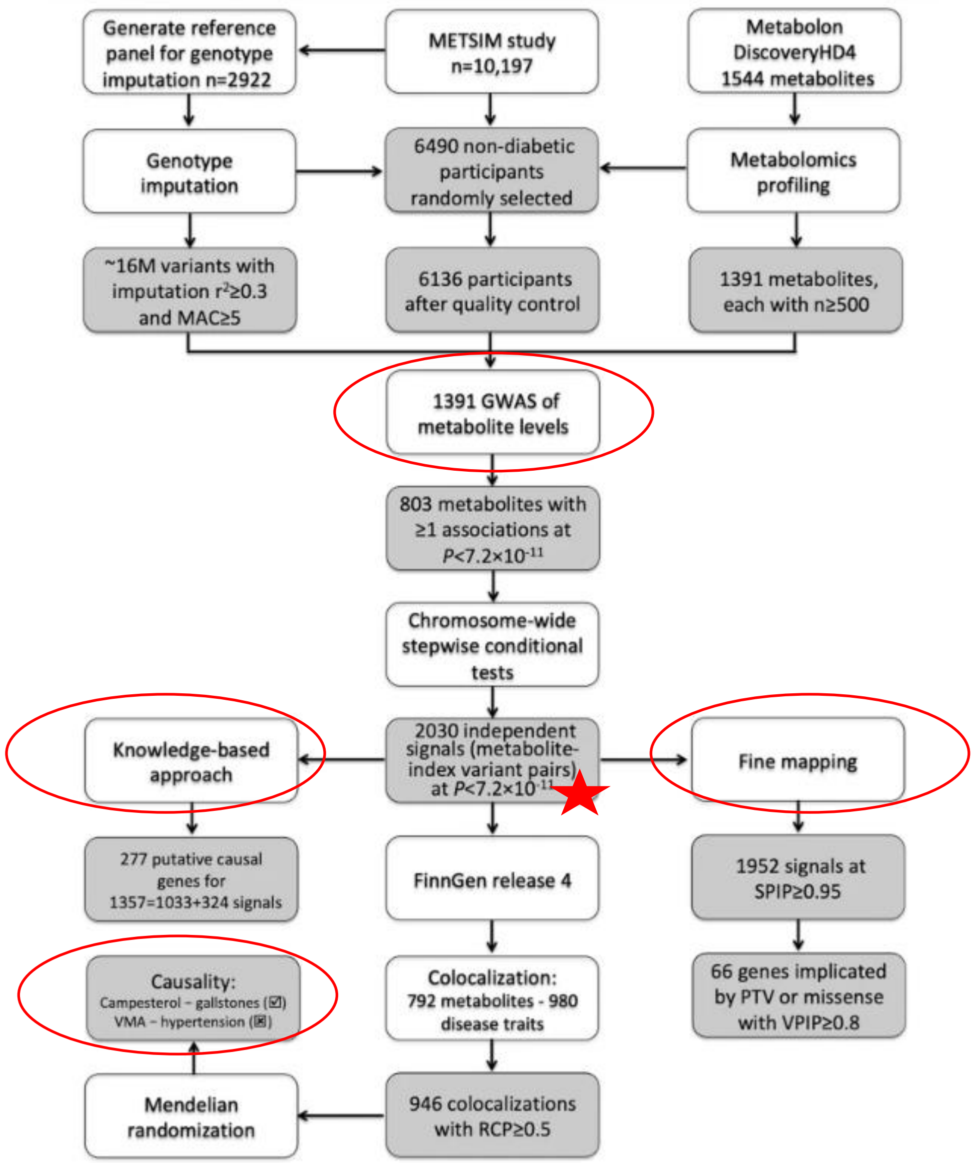
✓以PRS为基础的多组学标记物联合模型优化构建

因果效应分析研究

✓MR分析、COLOC分析、Mediation分析、Condition分析等

✓探讨不同组学与表型组、暴露因素与表型组、不同表型组间因果关系

The Genome first approach—mQTL图谱构建及分子调控机制挖掘



Generate reference panel for genotype imputation n=2922

METSIM study n=10,197

Metabolon DiscoveryHD4 1544 metabolites

Genotype imputation

6490 non-diabetic participants randomly selected

Metabolomics profiling

~16M variants with imputation $r^2 \geq 0.3$ and MAC≥5

6136 participants after quality control

1391 metabolites, each with n≥500

1391 GWAS of metabolite levels

803 metabolites with ≥1 associations at $P<7.2\times10^{-11}$

Chromosome-wide stepwise conditional tests

Knowledge-based approach

2030 independent signals (metabolite-index variant pairs) at $P<7.2\times10^{-11}$

Fine mapping

277 putative causal genes for 1357=1033+324 signals

FinnGen release 4

1952 signals at SPIP≥0.95

Causality:
Campesterol – gallstones (☑)
VMA – hypertension (☒)

Colocalization: 792 metabolites - 980 disease traits

66 genes implicated by PTV or missense with VPIP≥0.8

Mendelian randomization

946 colocalizations with RCP≥0.5

Genome-wide association studies of metabolites in Finnish men identify disease-relevant loci.
Nature communications 2022

The Epigenomics first approach—功能调控中介数据

暴露因素调控通路机制

Exposomics

EWAS

Genomics

遗传调控通路机制

Transcriptomics

与mRNA/miRNA/LncRNA/cirRNA转录表达调控机制

Proteinomics

与蛋白翻译、酶活性调控机制

Metabolomics

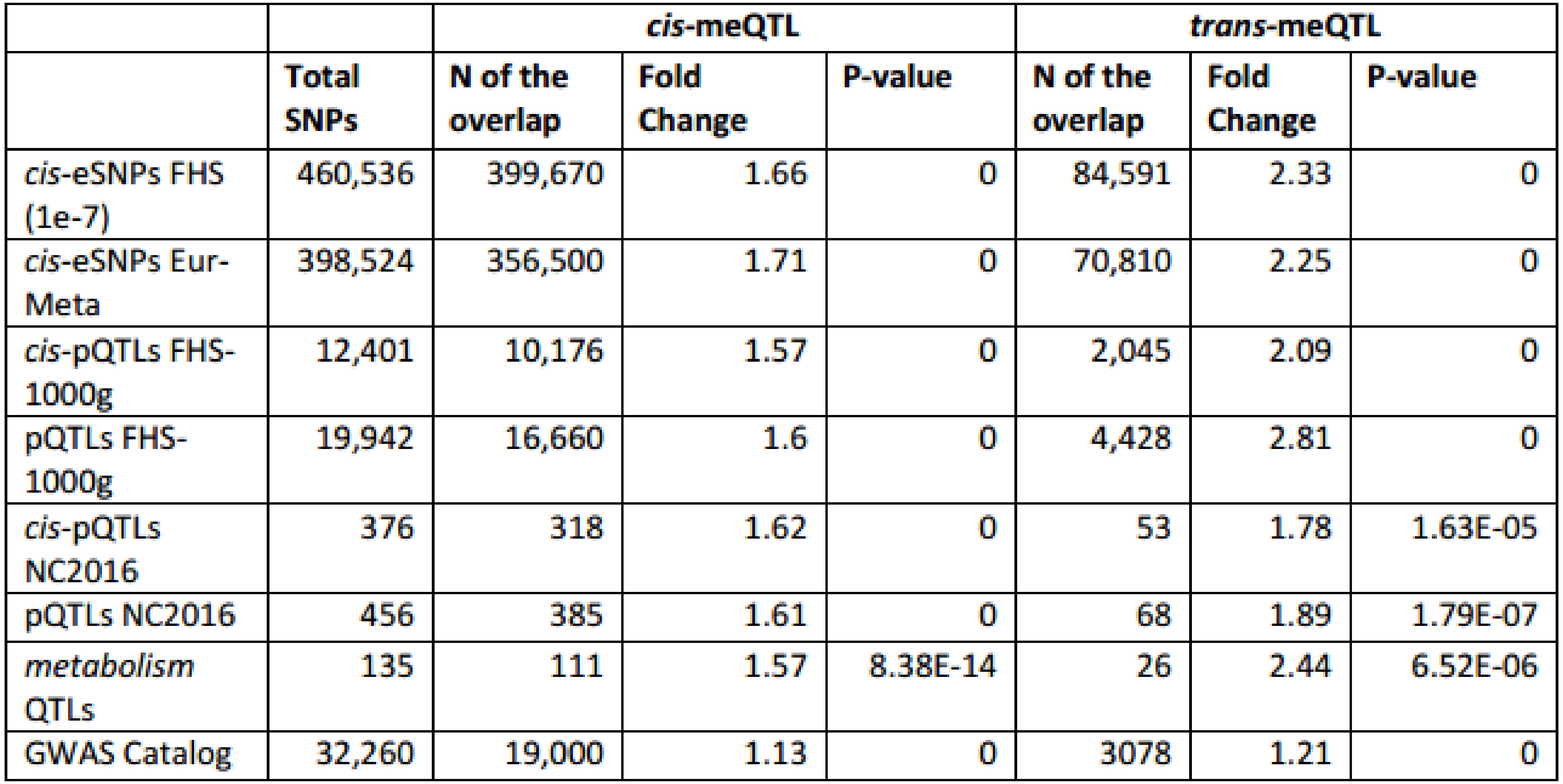
与代谢产物的交互调控机制

Microbiomics

宿主内环境暴露变量与宿主表观的调控机制

The Epigenomics first approach

87% of cis-eQTL SNPs, 82% of cis-pQTL SNPs, and 59% of GWAS Catalog SNPs are also cis-meQTL variants



		cis-meQTL			*trans*-meQTL		
	Total SNPs	**N of the overlap**	**Fold Change**	**P-value**	**N of the overlap**	**Fold Change**	**P-value**
cis-eSNPs FHS (1e-7)	460,536	399,670	1.66	0	84,591	2.33	0
cis-eSNPs Eur-Meta	398,524	356,500	1.71	0	70,810	2.25	0
cis-pQTLs FHS-1000g	12,401	10,176	1.57	0	2,045	2.09	0
pQTLs FHS-1000g	19,942	16,660	1.6	0	4,428	2.81	0
cis-pQTLs NC2016	376	318	1.62	0	53	1.78	1.63E-05
pQTLs NC2016	456	385	1.61	0	68	1.89	1.79E-07
metabolism QTLs	135	111	1.57	8.38E-14	26	2.44	6.52E-06
GWAS Catalog	32,260	19,000	1.13	0	3078	1.21	0

The Epigenomics first approach—eQTM分析及分子调控功能机制验证

Discovery Phase (Human Blood)

DNA methylation Microarray
1st Cohort : 40 MDD vs. 40 CTL
|Delta Beta| > 0.2; $p < 3.6 \times 10^{-8}$

RNA Expression Microarray
2nd Cohort : 20 MDD vs. 20 CTL
|Log$_2$ fold change| > 1; $p < 0.01$

Gene: *BICD2*

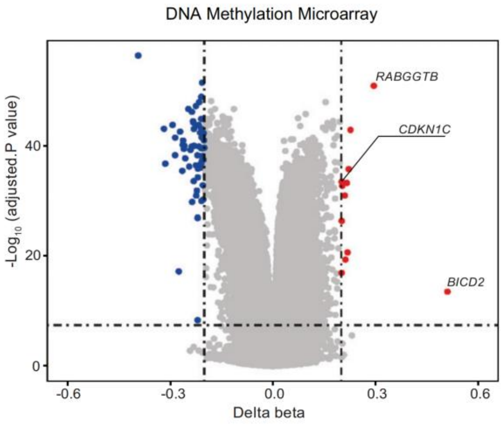
Replication Phase (Human Blood)

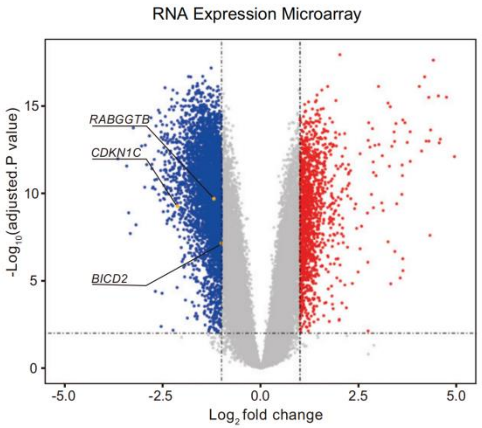
Sequenom MassARRAY
3rd Cohort: 528 MDD vs. 818 CTL

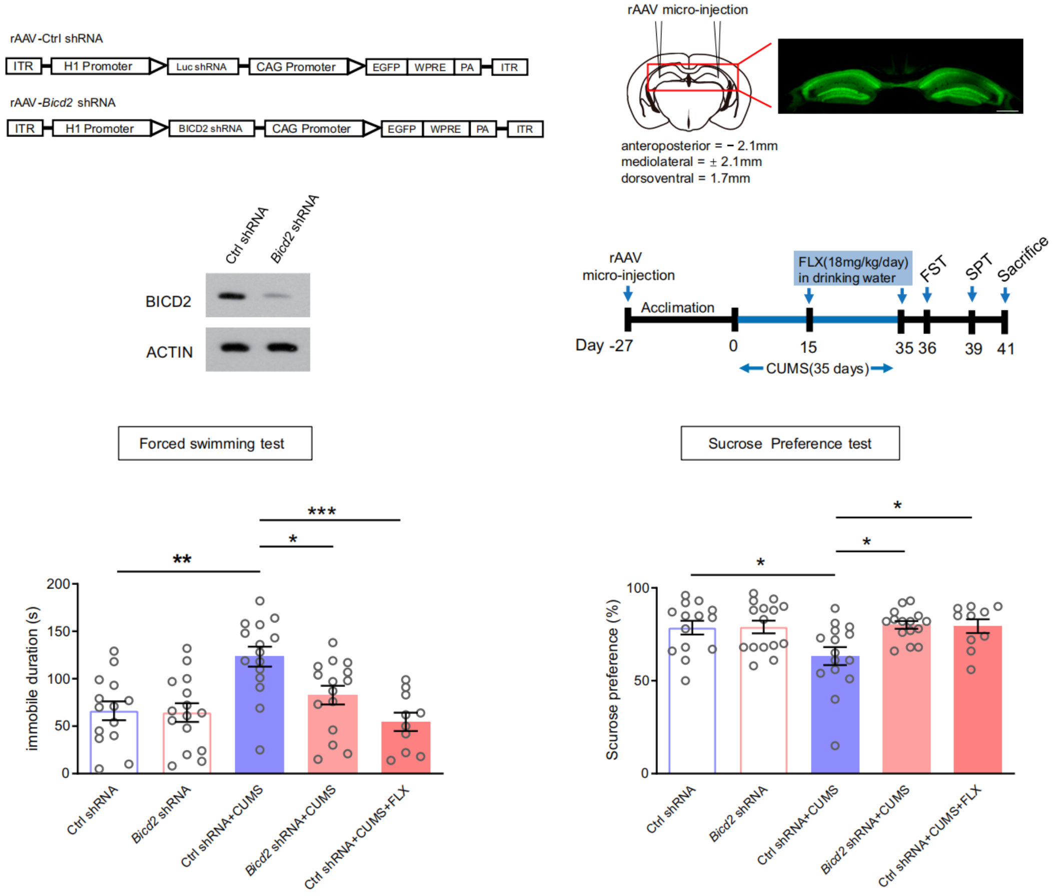
MSE-qPCR/qPCR: Combined analysis of DNA methylation & mRNA expression
4th Cohort: 30 MDD vs. 30 CTL

Functional study Phase (Mouse Blood & Brain)

- Validation of human results in CUMS model ★
- Mapping of brain regions with altered *Bicd2* DNA methylation and mRNA expression ★
- Functional studies in vivo & in vitro ★

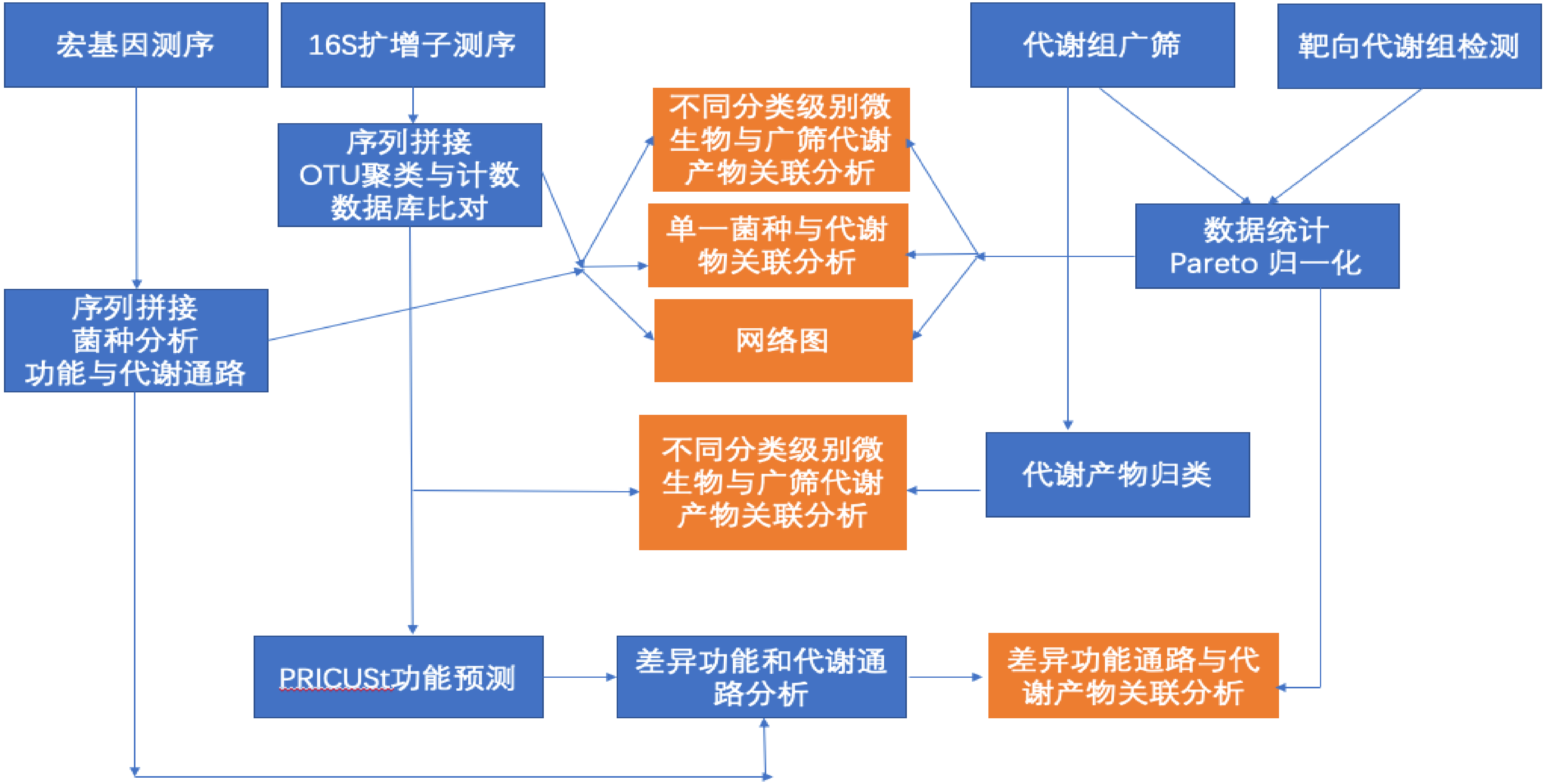






Elevated BICD2 DNA methylation in blood of major depressive disorder patients and reduction of depressive-like behaviors in hippocampal Bicd2-knockdown mice *PNAS 2022 July*

The Microbiomics first approach



宏基因测序

16S扩增子测序

代谢组广筛

靶向代谢组检测

序列拼接
OTU聚类与计数
数据库比对

不同分类级别微生物与广筛代谢产物关联分析

单一菌种与代谢物关联分析

网络图

数据统计
Pareto 归一化

序列拼接
菌种分析
功能与代谢通路

不同分类级别微生物与广筛代谢产物关联分析

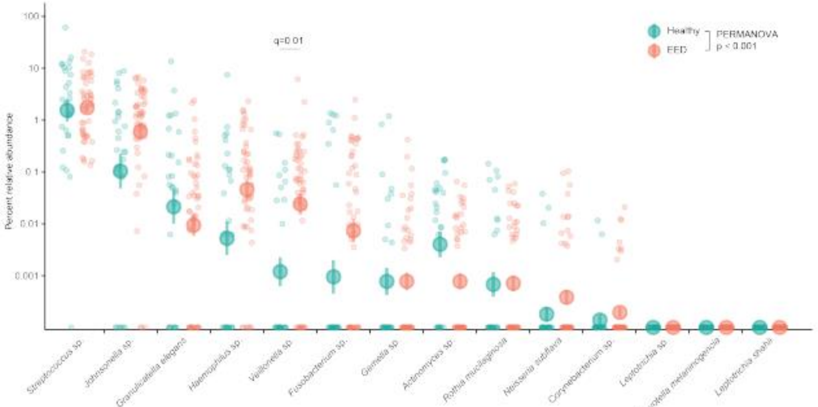
代谢产物归类

PRICUSt功能预测

差异功能和代谢通路分析

差异功能通路与代谢产物关联分析

The Microbiomics first approach —宏基因组&蛋白质组联合分析及分子调控机制验证



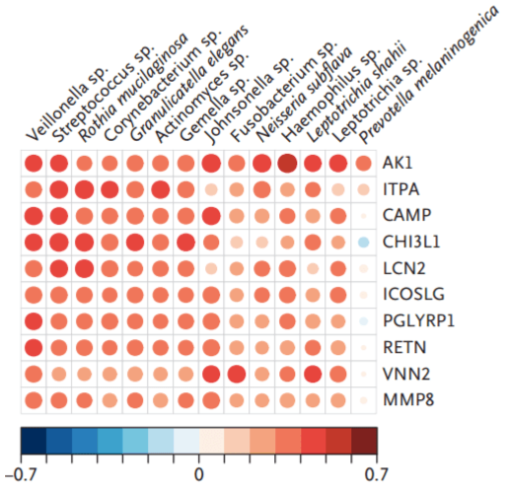
Relative abundances of core duodenal taxa in the fecal microbiota of children living in Mirpur who have healthy growth phenotypes (n=27) and those with EED (n=48)

分析 36 名环境肠功能障碍（EED）患儿的十二指肠微生物群，鉴定出 14 个不属于典型肠道病原体的 EED 核心细菌类群

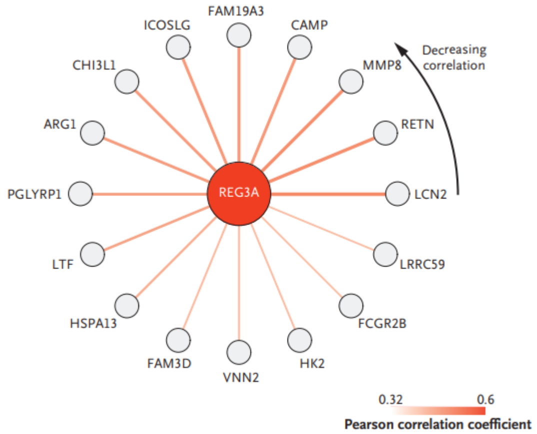
特征菌群的绝对水平，与患儿生长负相关，与参与免疫炎症应答的十二指肠蛋白（如 LCN2）正相关，且在患儿粪便中的含量不同于健康儿童 ★

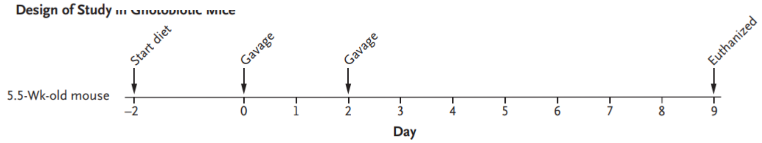
和 EED 核心菌相关的十二指肠蛋白，与血浆 REG3A 等显著相关

定植小鼠 EED 十二指肠菌群的部分分离菌（含大部分 EED 核心菌）可能通过促进十二指肠炎症应答，参与 EED 的发生发展，从而导致发育不良 ★



The top 10 positive correlations between members of the 14 core taxa and duodenal proteins


REG3A
FAM19A3
CAMP
MMP8
RETN
LCN2
LRRC59
FCGR2B
HK2
VNN2
FAM3D
HSPA13
LTF
PGLYRP1
ARG1
CHI3L1
ICOSLG
Decreasing correlation
0.32
0.6
Pearson correlation coefficient


Design of Study
5.5-Wk-old mouse
Start diet
Gavage
Gavage
Euthanized
−2
0
1
2
3
4
5
6
7
8
9
Day

A shows the design of the experiment in which gnotobiotic mice

Duodenal microbiota in stunted undernourished children with enteropathy
NEJM 2020

The Proteomics first approach

蛋白质组&代谢组

调控功能机制的验证

✓显著差异蛋白及代谢物的相关性分析、KEGG通路联合分析、PCA比较分析等

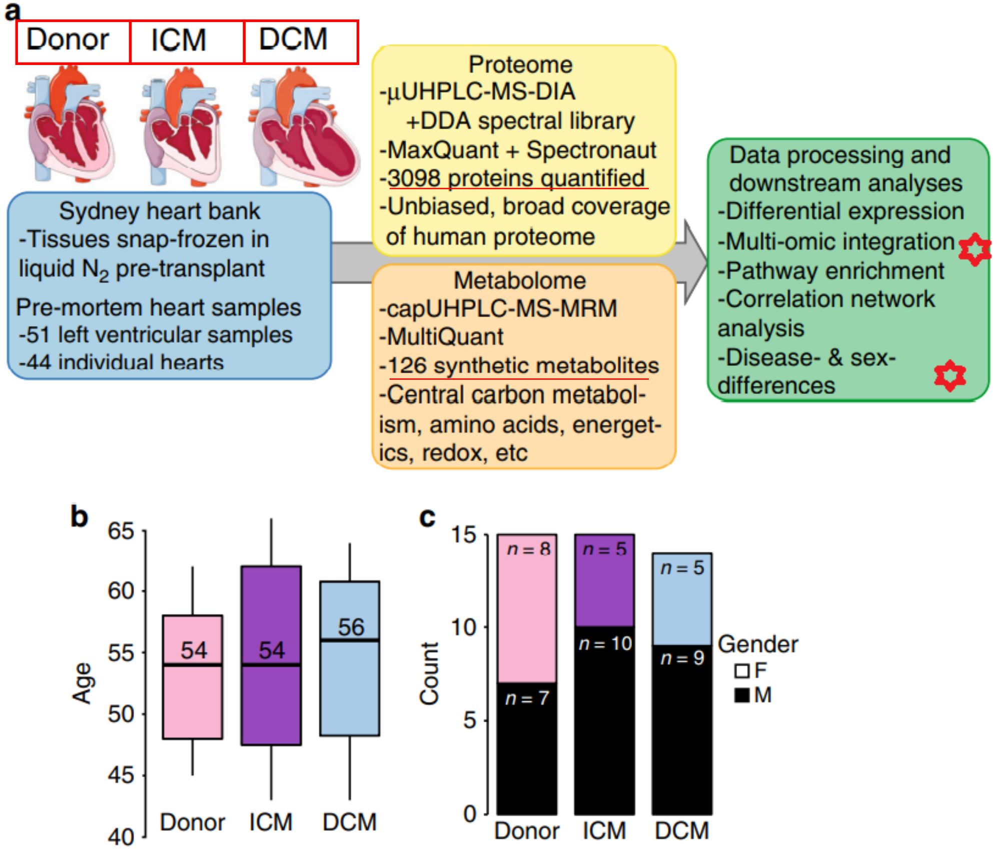
✓系统描绘蛋白至代谢的调控过程，挖掘关键蛋白与代谢物上下游调控通路

多组学联合预测模型构建

✓单一组学标记物、多组学标记物联合，基于机器学习算法进行预测模型构建及AUC效率比较

✓分子表型聚类分析，构建不同的分子病理模型及对应的生物机制探讨

The Proteomics first approach —分子调控功能机制研究


a
Donor
ICM
DCM
Sydney heart bank
-Tissues snap-frozen in liquid N_2 pre-transplant
Pre-mortem heart samples
-51 left ventricular samples
-44 individual hearts
Proteome
-µUHPLC-MS-DIA
+DDA spectral library
-MaxQuant + Spectronaut
-3098 proteins quantified
-Unbiased, broad coverage of human proteome
Metabolome
-capUHPLC-MS-MRM
-MultiQuant
-126 synthetic metabolites
-Central carbon metabolism, amino acids, energetics, redox, etc
Data processing and downstream analyses
-Differential expression
-Multi-omic integration
-Pathway enrichment
-Correlation network analysis
-Disease- & sex-differences
b
Age
65
60
55
50
45
40
54
54
56
Donor
ICM
DCM
c
Count
15
10
5
0
n = 8
n = 7
n = 5
n = 10
n = 5
n = 9
Donor
ICM
DCM
Gender
□F
■M

研究设计

疾病表型：不同病因心肌病（缺血性心肌病、扩张性心肌病）的生物调控机制差异

特色：围绕不同性别对应疾病组织进行了蛋白质组学及代谢组差异分析

单一组学差异分析

蛋白质组差异分析：根据ICM\DCM分别与Donor组间分析，筛选差异蛋白质及相关生物学特征

代谢组差异分析：根据ICM\DCM分别与Donor组间分析，筛选差异代谢物及相关生物学特征

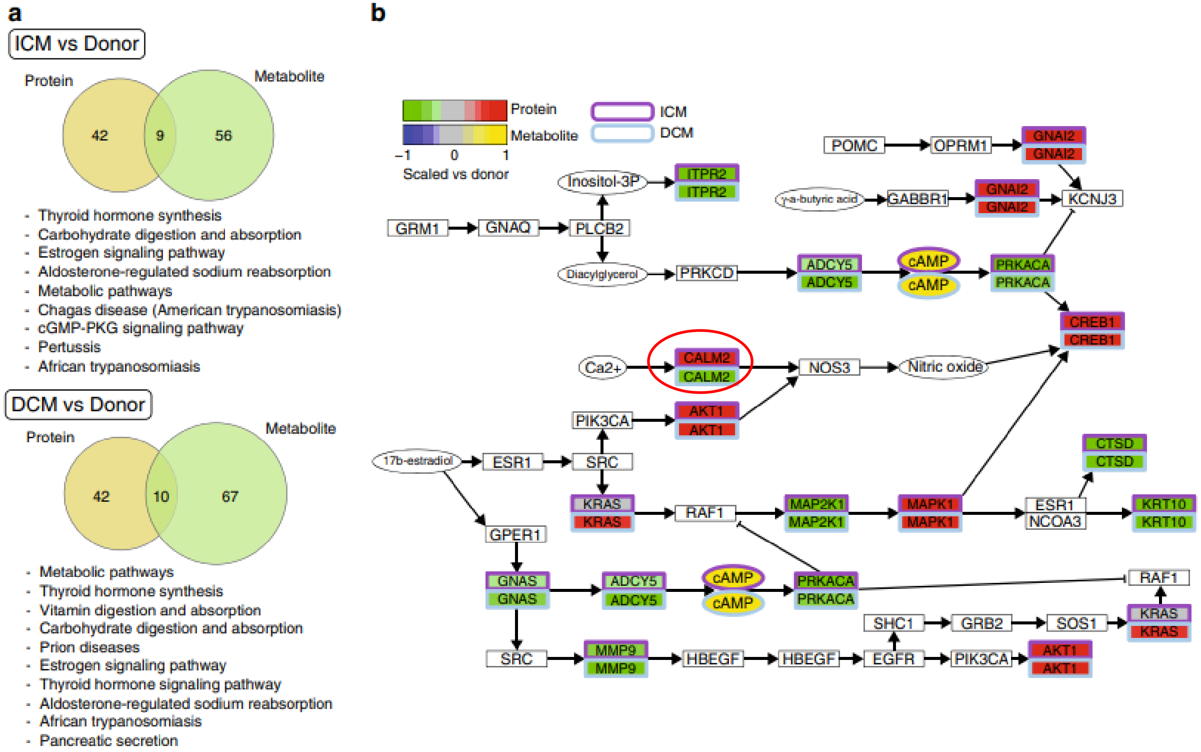
蛋白质组&代谢组联合分析

KEGG通路联合分析：对 ICM 和 DCM 的蛋白质和代谢物水平上进行KEGG通路分析,显示了两者在蛋白质和代谢物水平上调节的共同途径

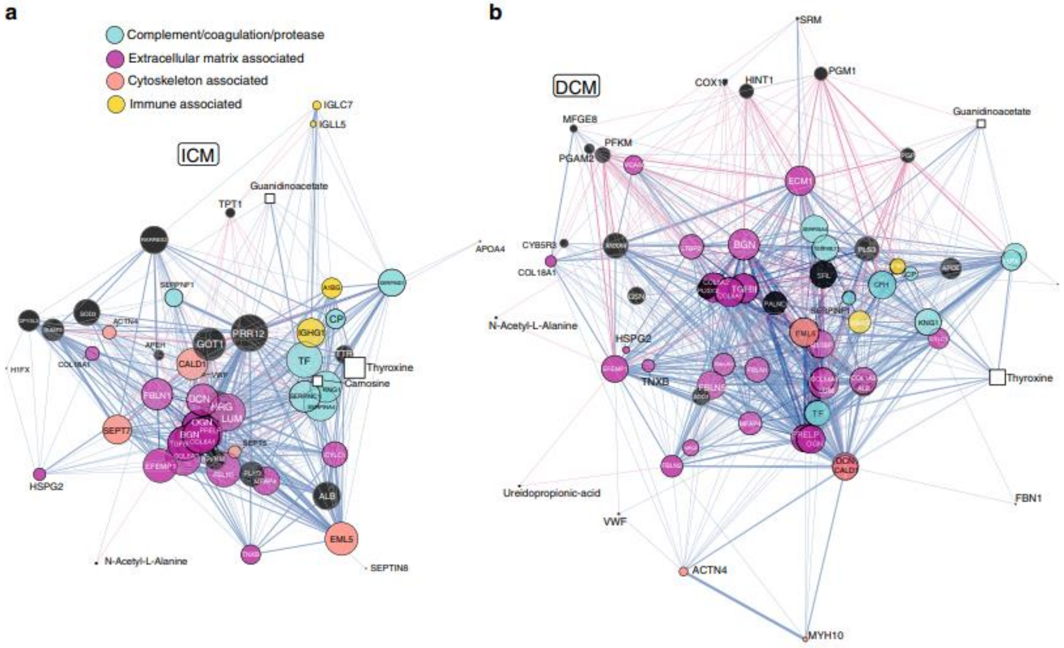
显著差异蛋白质与代谢物相关性分析：在相关性分析基础上构建网络调控图，筛选关键节点蛋白质及代谢物

Core functional nodes and sex-specific pathways in human ischaemic and dilated cardiomyopathy
NATURE COMMUNICATIONS | (2020) Article

The Proteomics first approach —分子调控功能机制研究


a
ICM vs Donor
Protein
Metabolite
42
9
56
- Thyroid hormone synthesis
- Carbohydrate digestion and absorption
- Estrogen signaling pathway
- Aldosterone-regulated sodium reabsorption
- Metabolic pathways
- Chagas disease (American trypanosomiasis)
- cGMP-PKG signaling pathway
- Pertussis
- African trypanosomiasis
DCM vs Donor
Protein
Metabolite
42
10
67
- Metabolic pathways
- Thyroid hormone synthesis
- Vitamin digestion and absorption
- Carbohydrate digestion and absorption
- Prion diseases
- Estrogen signaling pathway
- Thyroid hormone signaling pathway
- Aldosterone-regulated sodium reabsorption
- African trypanosomiasis
- Pancreatic secretion
b
Protein
Metabolite
ICM
DCM
−1
0
1
Scaled vs donor
POMC
OPRM1
GNAI2
Inositol-3P
ITPR2
γ-a-butyric acid
GABBR1
KCNJ3
GRM1
GNAQ
PLCB2
Diacylglycerol
PRKCD
ADCY5
cAMP
PRKACA
CREB1
Ca2+
CALM2
NOS3
Nitric oxide
PIK3CA
AKT1
17b-estradiol
ESR1
SRC
CTSD
KRAS
RAF1
MAP2K1
MAPK1
ESR1
NCOA3
KRT10
GPER1
GNAS
SHC1
GRB2
SOS1
MMP9
HBEGF
EGFR

Pathways analysis


a
Complement/coagulation/protease
Extracellular matrix associated
Cytoskeleton associated
Immune associated
ICM
Guanidinoacetate
Thyroxine
Carnosine
HSPG2
N-Acetyl-L-Alanine
SEPTIN8
b
DCM
SRM
PGM1
HINT1
COX1
Guanidinoacetate
MFGE8
PFKM
PGAM2
APOA4
CYB5R3
COL18A1
N-Acetyl-L-Alanine
HSPG2
TNXB
Thyroxine
Ureidopropionic-acid
VWF
FBN1
ACTN4
MYH10

Network analysis

多维度multi-omics研究体系的发展趋势

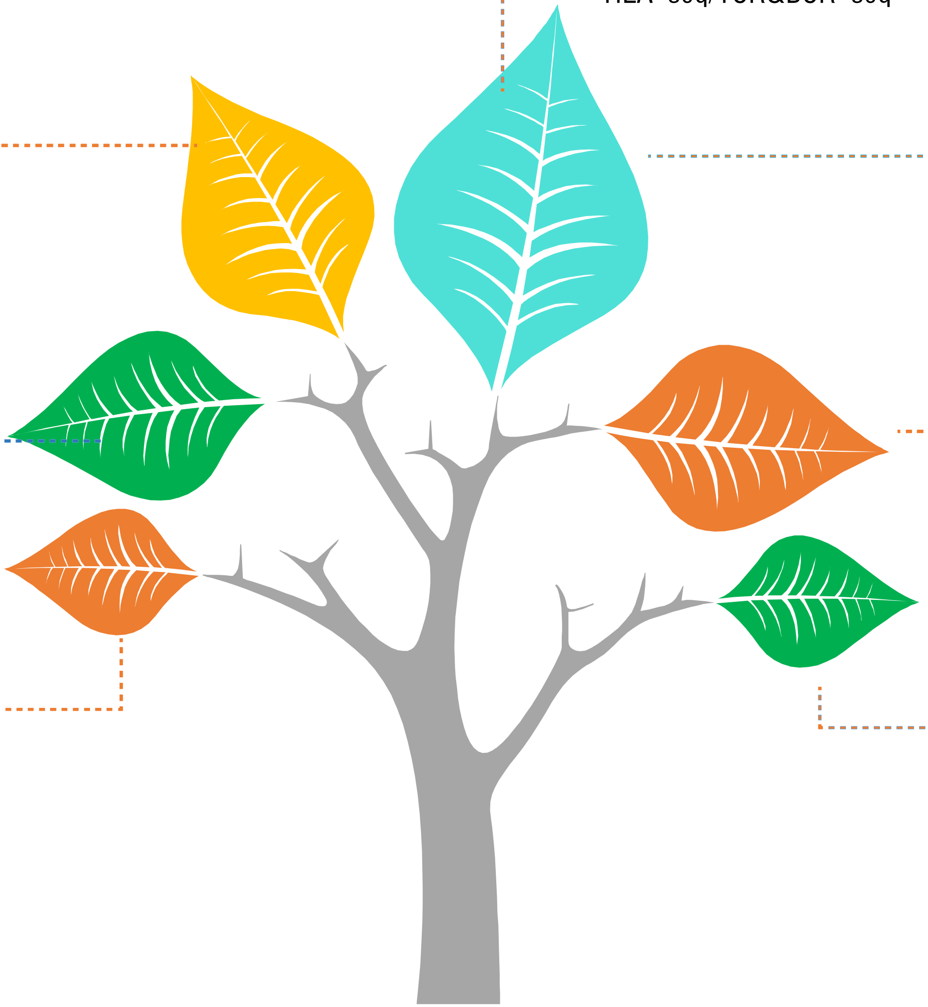
趋势

一维研究体系：
Bulk 多组学
Bulk-multiomics

二维研究体系：
单细胞多组学
Single cell-multiomics

三维研究体系：
空间多组学
Spatial-multiomics

博淼技术服务项目一览图



基因组学服务

- GWAS芯片/WES-seq/Target NGS-seq
- Massarray /Multi-PCR NGS/Taqman /KASP SNP分型
- 16S扩增子测序/宏基因组测序
- HLA-seq/TCR&BCR-seq

表观基因组学服务

- EWAS芯片
- Multi-PCR NGS靶向DNA甲基化定量
- Massarray 靶向DNA甲基化定量

转录组学服务

- 转录组NGS/表达谱芯片
- RT-qPCR靶向转录本定量

单细胞组学服务

- 单细胞转录组测序
- 单细胞免疫组库测序
- 单细胞ATAC测序&转录组测序
- 空间转录组测序

代谢组学服务

- 非靶向代谢组
- 非靶向脂质组
- 靶向代谢组项目系列

蛋白质组学服务

- DIA定量蛋白质组/iTRAQ&TMT定量蛋白质组/Label free定量蛋白质组
- PRM靶向蛋白/Olink蛋白定量/ELISA
- 修饰蛋白质组
- 高密度自身抗体蛋白芯片

多组学联合研究服务

- GWAS&多组学技术服务
- EWAS&多组学技术服务
- 微生物基因组&代谢组技术服务
- 蛋白质组&代谢组技术服务

感谢各位的聆听

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