Metabolon

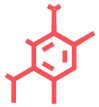
Metabolomics Applications to Human Health

Leiden University Retreat
May 2023

Patrick Arnott, PhD
Greg Michelotti, PhD
Alessandro Busetti, PhD

Metabolon

is the global leader in metabolomics: the next omics revolution



20 years of leadership in metabolomics

Leading expertise in biochemistry and its role in health, wellness, nutrition and disease

Unrivalled capabilities

Unique industrial scale technology platform, knowledgebase and AI/bioinformatics capabilities generate high barriers to entry

Growing services business

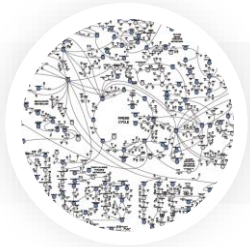
Providing metabolomics solutions that support the research continuum from discovery through clinical trials, to in-market life cycle management

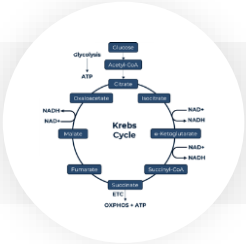
Transitioning into clinical applications

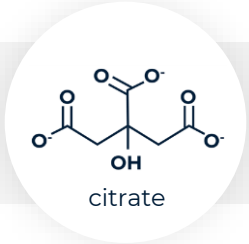
Building a multi-omic disease database for biomarker discovery and clinical test development in rare and common diseases

Metabolon has transformed biochemistry into an omics technology and is delivering this on an industrial scale

The ability to perform massively parallel sampling in biology has been **transformational to researchers' ability to perform large-scale and unbiased biological analysis**



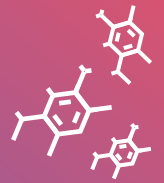




citrate

Any transition from a healthy to disease state requires changes in biochemistry

Metabolites are therefore excellent **biomarkers** for diagnosis and pathogenesis of large numbers of diseases



Metabolism is a collection of biochemical reactions that enable life

These reactions are organized into 100's of highly conserved pathways

Metabolites are the chemical intermediaries and endpoints of these reactions

Metabolomics is the large scale study of these metabolites

Metabolomics is Closer to the Phenotype and Can Help the Understanding of Other Omics

WHAT COULD BE

FUNCTIONAL EXPRESSION OF WHAT IS

Genomics

Ilumina, 10x Genomics, PacBio

Genomics is complicated with limited contribution to the majority of diseases.

Proteomics

Seer, SomaLogic, Olink

Proteomics faces similar complexity—single proteins exist in multiple different forms that dictate activity.

Metabolomics

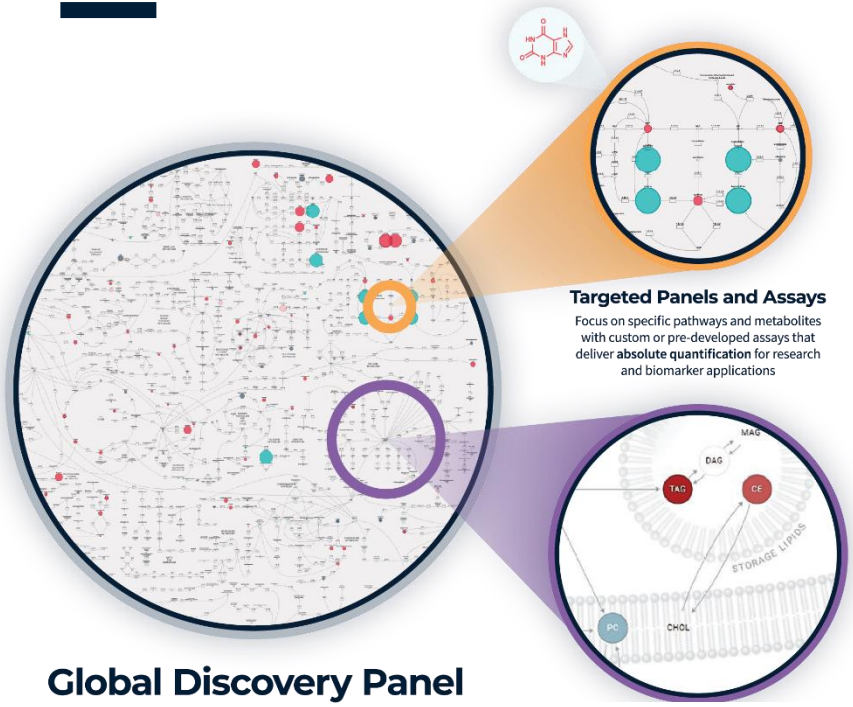
Metabolon

Metabolomics is the definitive representation of the phenotype—associations from diseases to metabolites produce direct links to biological mechanisms.

METABOLOMICS CAN HELP THE FUNCTIONAL UNDERSTANDING OF THE GENOME AND PROTEOME

Metabolomics Solutions

DELIVERING HIGH-QUALITY DATA AND INSIGHTS



Global Discovery Panel

Cast a wide research net with our global metabolomics technology. Identify **5,400+ metabolites*** across **70 major biochemical pathways**.

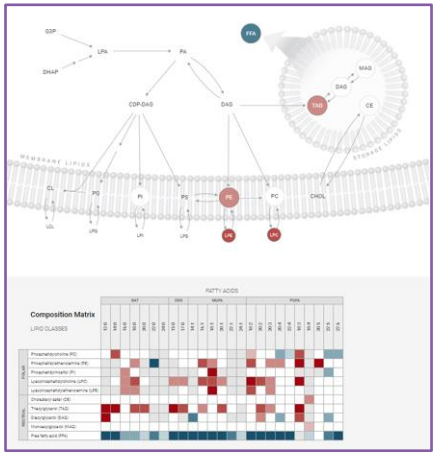
Targeted Panels and Assays

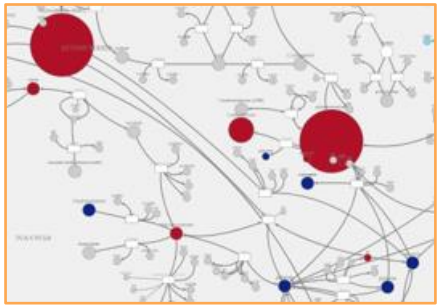
Focus on specific pathways and metabolites with custom or pre-developed assays that deliver **absolute quantification** for research and biomarker applications

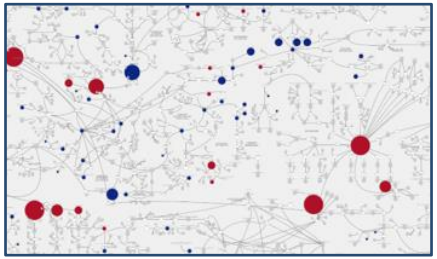
Complex Lipids Targeted Panel

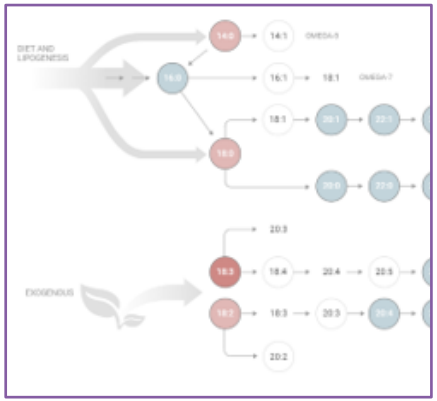
Our insightful lipidomics approach comprehensively quantitates up to **1,100 lipid species/sample**

FIT FOR PURPOSE TOOLS



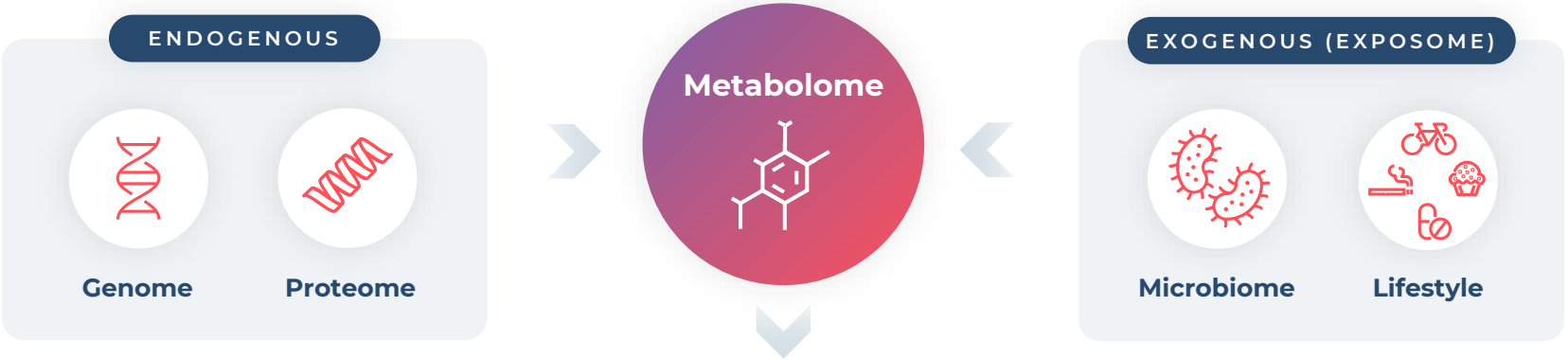






Metabolomics is the Nexus of Gene-environment Interactions with Broad Utility in Understanding Health and Disease

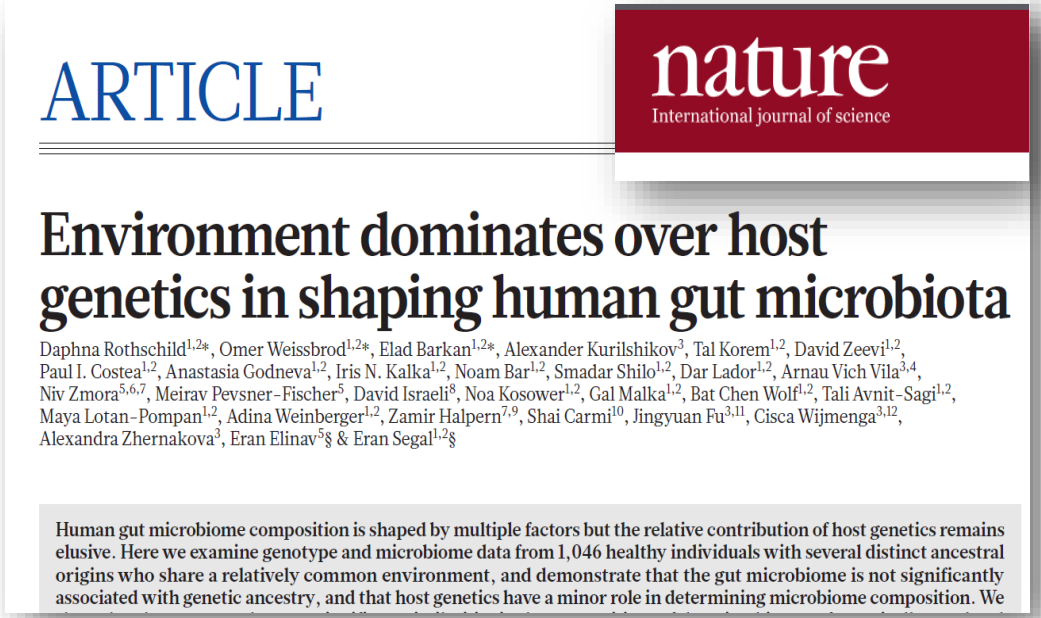
The risks for getting most diseases arise from your metabolism, your environment, and your lifestyle.



Health: It's Not All About Your Genes

Metabolomics highlights normal and abnormal chemical reactions that are the root cause of health and disease

Metabolic impact—not just genetics—has a foundational role in determining health



ARTICLE

nature
International journal of science

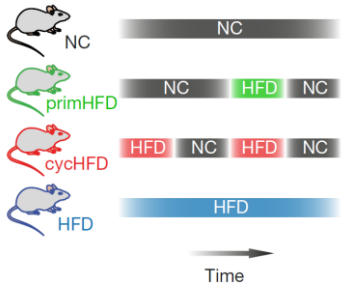
Environment dominates over host genetics in shaping human gut microbiota

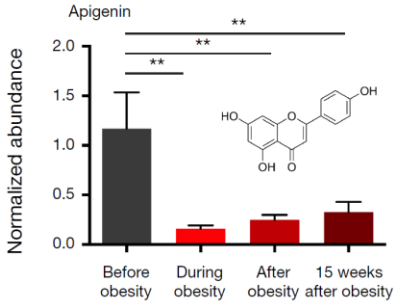
Daphna Rothschild[1,2*], Omer Weissbrod[1,2*], Elad Barkan[1,2*], Alexander Kurilshikov[3], Tal Korem[1,2], David Zeevi[1,2], Paul I. Costea[1,2], Anastasia Godneva[1,2], Iris N. Kalka[1,2], Noam Bar[1,2], Smadar Shilo[1,2], Dar Lador[1,2], Arnau Vich Vila[3,4], Niv Zmora[5,6,7], Meirav Pevsner-Fischer[5], David Israeli[8], Noa Kosower[1,2], Gal Malka[1,2], Bat Chen Wolf[1,2], Tali Avnit-Sagi[1,2], Maya Lotan-Pompan[1,2], Adina Weinberger[1,2], Zamir Halpern[7,9], Shai Carmi[10], Jingyuan Fu[3,11], Cisca Wijmenga[3,12], Alexandra Zhernakova[3], Eran Elinav[5]§ & Eran Segal[1,2]§

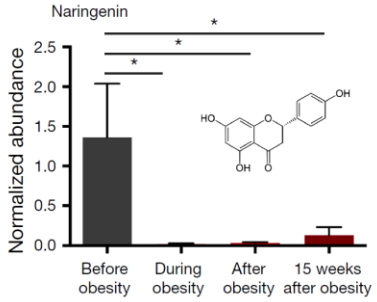
Human gut microbiome composition is shaped by multiple factors but the relative contribution of host genetics remains elusive. Here we examine genotype and microbiome data from 1,046 healthy individuals with several distinct ancestral origins who share a relatively common environment, and demonstrate that the gut microbiome is not significantly associated with genetic ancestry, and that host genetics have a minor role in determining microbiome composition. We

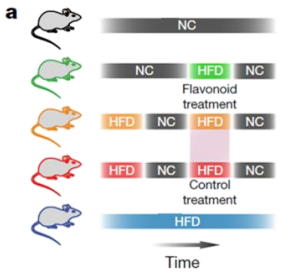
Rothschild et al, Nature, 2018 Mar 8;555(7695):210-215. DOI:10.1038/nature25973

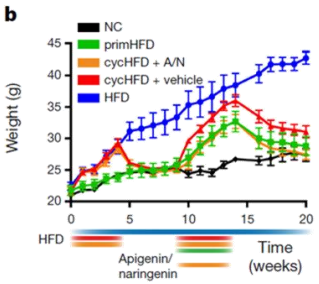
Untargeted and unbiassed profiling elucidates novel mechanisms











CHALLENGE

Dieting individuals fail to maintain weight loss.

- Dieting individuals fall into a cycle of repeated weight loss and weight gain.
- Investigators hypothesize that the preceding weight gain/weight loss cycle predisposed the mice to accelerated weight gain after receiving a HFD, and that the microbiome may play a role in this effect. To test, turned to microbiome and metabolite profiling.

METABOLOMIC INSIGHT

Flavonoid metabolism is indicative of weight gain.

- Obesity drives a dramatic shift in microbial profile, where some of those microbes were either lost or gained. Once the animal loses weight, only 45% of those changes were reversed back to the pre-obesity state, while the rest of the microbial changes persist weeks after obesity.
- Among those that persist are the flavonoids (apigenin and naringenin) levels reduced during obesity and persist for 15 weeks after obesity (equivalent to 26 years of human life).

VALUE REVEALED

Novel flavonoid pathway elucidated in brown adipose tissue.

- High fat diet there is a dramatic shift in bacterial composition to favor flavonoid degradation.
- Flavonoids are involved in energy expenditure, reduced flavonoids = reduced energy expenditure.
- Mechanism: expression of the major thermogenic factor in brown adipose tissue, uncoupling protein 1 (Ucp1) is directly linked to flavonoids in a concentration-dependent manner.

Thaiss C., et al. Nature (2016) 540.7634. doi:10.1038/nature20796

Metabolomics—Connecting Cancer Genetics to Phenotype

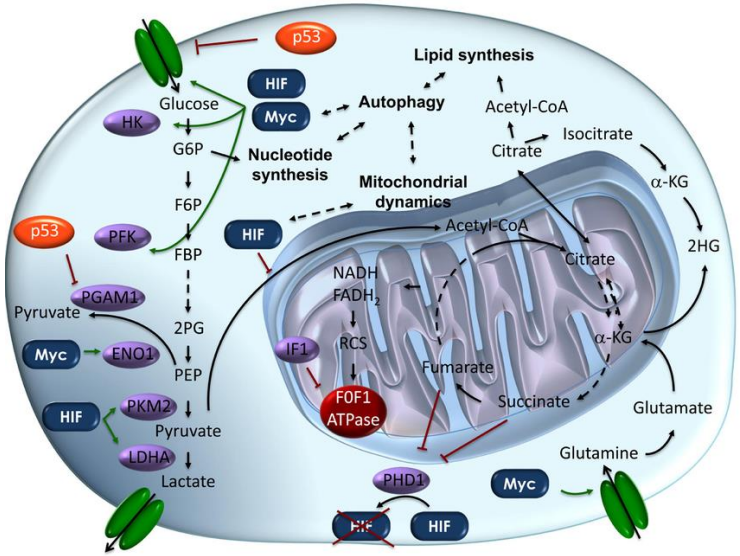
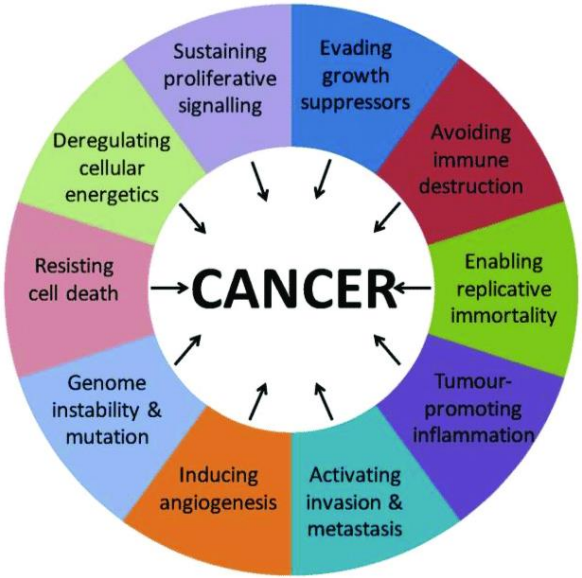


Figure from: Corrado M, Scorrano L, Campello S. Changing perspective on oncometabolites: from metabolic signature of cancer to tumorigenic and immunosuppressive agents. Oncotarget. 2016;7(29)
This open-access article is distributed under the terms of the Creative Commons Attribution License (CC BY 2.5).

- Each hallmark of cancer reveals interaction and engagement with diverse metabolic processes—from angiogenesis to immune evasion, metabolic pathways are of fundamental importance.
- Genetic drivers of cancer-profiling as diagnostic tools have been in the mainstream.
- Metabolomics
  - Reveals phenotypic characteristics underlying gene perturbation
  - Fills gaps not enabled by genetic profiling
  - Allows translatability of findings across model systems due to phylogenetic conservation of metabolic networks

Metabolomics—Connecting Cancer Genetics to Phenotype

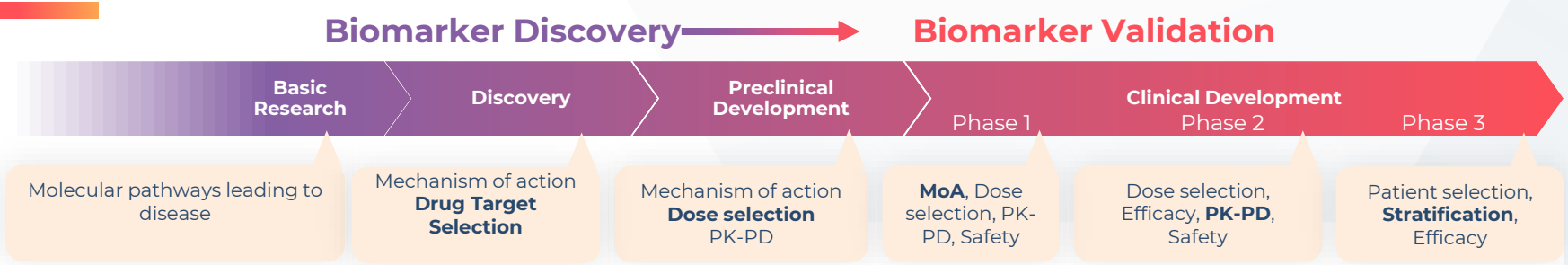


- Each hallmark of cancer reveals interaction and engagement with diverse metabolic processes—from angiogenesis to immune evasion, metabolic pathways are of fundamental importance.
- Genetic drivers of cancer-profiling as diagnostic tools have been in the mainstream.
- Metabolomics
  - Reveals phenotypic characteristics underlying gene perturbation
  - Fills gaps not enabled by genetic profiling
  - Allows translatability of findings across model systems due to phylogenetic conservation of metabolic networks

Figure reproduced and adapted from: El-Tanani M, Dakir E-H, Raynor B, Morgan R. Mechanisms of Nuclear Export in Cancer and Resistance to Chemotherapy. Cancers. 2016; 8(3):35. https://doi.org/10.3390/cancers8030035 This open-access article is distributed under the terms of the Creative Commons Attribution License (CC BY 4.0).

Metabolon: A Strategic Partner

FROM BASIC RESEARCH THROUGH CLINICAL



Metabolon Portfolio

Global Discovery Panel
5,400+ metabolites
(relative quantification)

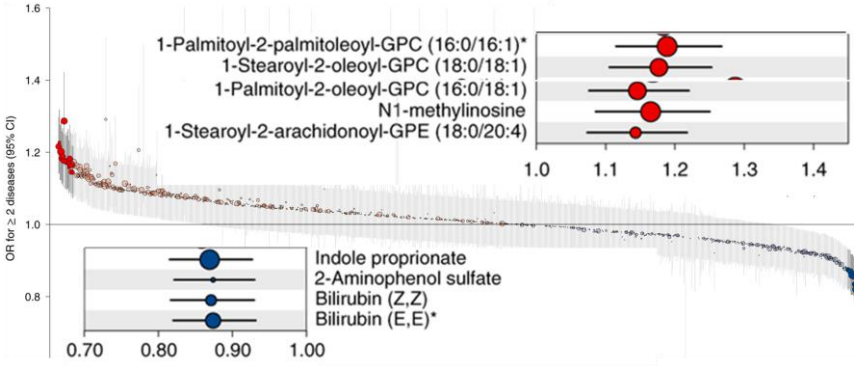
Targeted Panels and Assays
1 to 20 metabolites per assay
(absolute quantification)

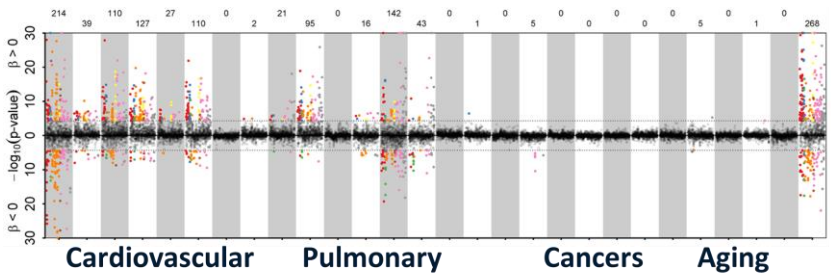
How Investigators Incorporate Our Data Into Their Workflow

- Discovery: highly powered population studies to identify signatures of disease
- Functionally map disease causing genes
- Biomarker assessment of target engagement and pharmacodynamics
- Biomarkers of response for clinical trials
- Novel therapeutic or diagnostic targets
- Understand functional outputs of the microbiome that influence health

Discovery: Highly Powered Population Studies To Identify Signatures Of Disease

Actionable antecedents of multimorbidity identified





Mirrored Manhattan-like plot showing the p-values from Cox proportional hazard models

CHALLENGE

New clinical strategies are needed for complex disease with multimorbidity profiles.

- There are current gaps in knowledge on common modifiable factors that underpin simultaneous multiple chronic conditions for more effective prevention and management of multimorbidity.
- Metabolomics was used to identify pathways at baseline shared across multiple incident conditions to identify biochemical perturbations that predispose individuals to multimorbidity.

METABOLOMIC INSIGHT

Two-thirds of associated metabolites are shared among diseases.

- A total of 420 (65.6%) metabolites were associated with at least 2 different diseases or all-cause mortality ($P < 0.001$; while only 220 (34.6%) metabolites were associated with one disease only.
- A high connectivity among cardiometabolic and respiratory diseases including coronary heart disease, heart failure, type 2 diabetes, cerebral stroke, peripheral artery disease, renal and liver diseases, chronic obstructive pulmonary disease, and lung cancer across different biochemical classes of metabolites was observed.

VALUE REVEALED

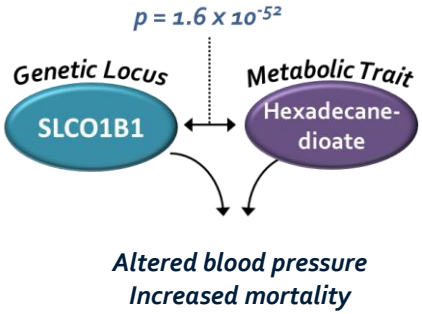
Novel biomarkers and pathways of multimorbidity were identified, accelerating therapeutics.

- Large putatively protective role for gut microbial diversity from biochemical profiling.
- Impaired glucose homeostasis, low-grade inflammation, lipoprotein metabolism, liver and kidney function were identified as common actionable antecedents of multimorbidity.
- https://omicscience.org/apps/mwasdisease/

Pietzner M, et al. Nature Medicine. 2021: 471–479. DOI:10.1038/s41591-021-01266-0

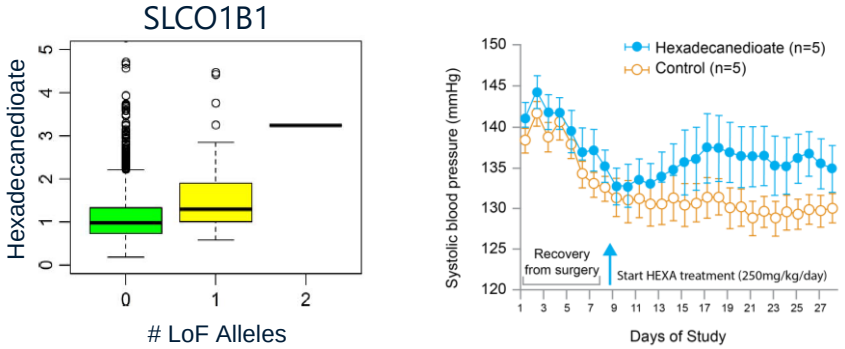
Functionally Map Disease Causing Genes

New therapeutic insights to treat hypertension

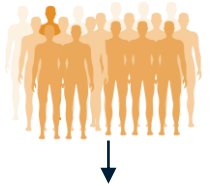


Altered blood pressure
Increased mortality

Metabolomic + cohorts and translational studies



Sequencing + metabolomics



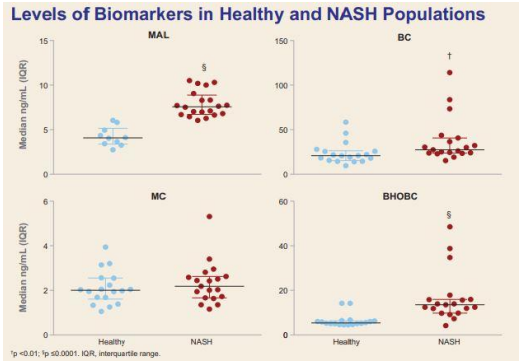
Gene variant (SLCO1B1) + metabolite (HEXA) for high blood pressure

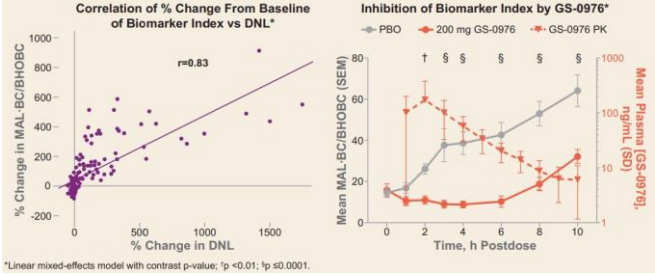
CHALLENGE	METABOLOMIC INSIGHT	VALUE REVEALED
New targets desperately needed An estimated 1.13 billion people worldwide have hypertension. Fewer than 1 in 5 people with hypertension are properly managed with current therapies. Leads to premature death and new targets are needed for effective therapies.	**Metabolomics identifies a metabolite causal for high blood pressure (BP) and functionally maps associated gene of unknown function** *Can metabolomics help identify important genes faster?* *Test case: high BP:* As of 2020, over 1.5 billion people are reported to have high BP; 2/3 have not achieved target therapeutic control with current therapeutics Hexadecanedioate (HEXA) was highly associated with BP in three cohorts and shown to be causal.	**New paradigm for finding targets and unlocking human health and disease:** Identification of a novel target; avenues into gene function and the understanding of disease etiology by integrating omic technologies into a deeply phenotyped populations

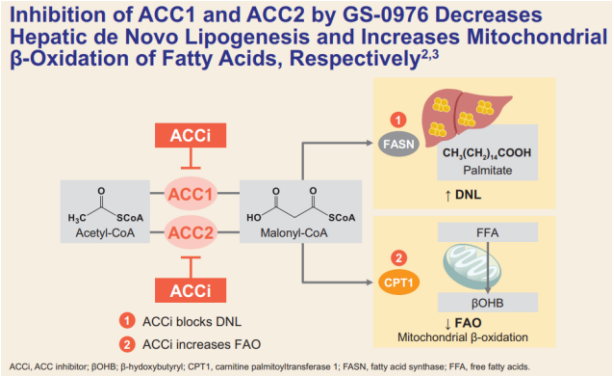
S.Y. Shin et al., Nat Genet (2014) 46:543-550 DOI:10.1038/ng.2982
E Link et al., N. Engl J Med (2008) 359:789-799 DOI:10.1056/NEJMoa0801936;
C. Menni et al., Hypertension (2015) 66:433-429 DOI:10.1161/HYPERTENSIONAHA.115.05544
B. Yu et al., Sci Adv (2016) 2:e1600800. DOI:10.1126/sciadv.1600800
Menni et al. (2017) PLoS ONE 12(4): e0175479. DOI:10.1371/journal.pone.0175479

Biomarker assessment of target engagement and pharmacodynamics

Acetyl-CoA carboxylase inhibitor clinical evaluation







CHALLENGE

- Simplified approach to assessing new non-alcoholic fatty liver disease (NASH) therapeutics
- Typical approach to hepatic de novo lipogenesis (DNL) and FAO – use specialized stable isotope tracing studies – limit wider application
- To evaluate a panel of plasma metabolites as potential pharmacodynamic (PD) markers of GS-0976

METABOLOMIC INSIGHT

- Metabolomics to improve diagnostic performance
- Custom-targeted metabolite panel developed for accurate quantification
- Malonate (MAL), butyryl carnitine (BC), and β-OH-butyrylcarnitine (BHOBC) were elevated in NASH patient's vs healthy subjects
- In 10 fasted healthy subjects administered GS-0976 20 mg, there was a median 2.2-fold increase (range 1.3–3.1; $p < 0.01$) in BHOBC 4 h post-dose, which reversed to pre-dose levels at 10 h
- Percent changes in MAL, BC, and BHOBC correlated well with percent change in DNL ($p < 0.001$)

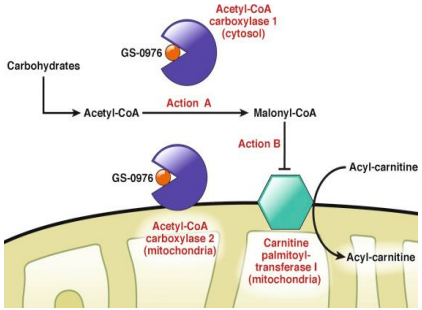
VALUE REVEALED

- New possible biomarker identified
- BHOBC was a PD biomarker of GS-0976
- MAL, BC, BHOBC, and a simple index accurately measured GS-0976 suppression of DNL
- These metabolites show promise in evaluating GS-0976-related activity

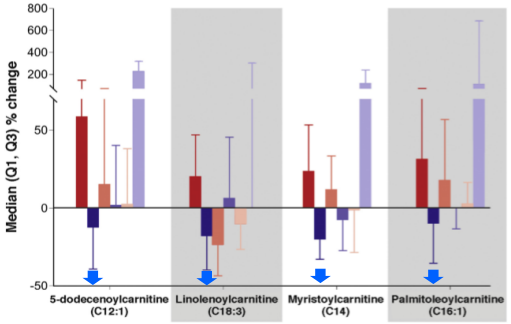
Tarrant, Jacqueline M., et al. "Activity of Acetyl-CoA Carboxylase Inhibitor GS-0976. (2017)"

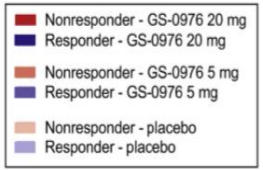
Biomarkers Of Response For Clinical Trials

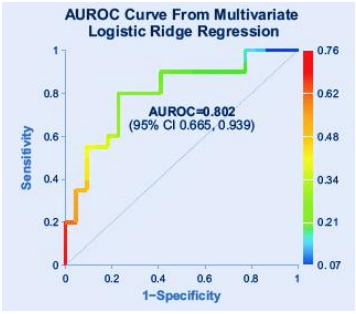
Novel NASH program



Proposed mechanism of action by GS-0976







Panel of 4 ACs had good AUROC for diagnosing MRI-R at Week 12 (change from baseline)

CHALLENGE

- Markers of clinical response needed
- NASH efficacy assessment is limited to imaging or biopsy with no reliable blood biomarker options
- Sought plasma biomarkers of response in Phase 2 trial for NASH with novel molecule, acetyl-CoA carboxylase inhibitor (ACCi)

METABOLOMIC INSIGHT

- Biomarkers of response
- Acylcarnitines (AC) changed with imaging, providing candidate markers for development
- Developed a targeted, assay panel of 22 ACs
- Relative changes from baseline in AC levels between magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF) responders and MRI-PDFF nonresponders were significantly different at week 12 in dosed group

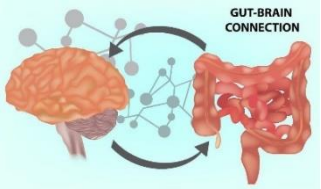
VALUE REVEALED

- New potential targets identified
- A panel of metabolites are promising early biomarkers of imaging response
- Mechanistically linked to ACCi and liver fat content
- Provides confidence to advance program with an expanded toolbox of biomarkers

Loomba, Rohit, et al. Gastroenterology 155.5 (2018): 1463-1473.
Charlton, Michael, et al. Presented at EASL: The International Liver Congress™ 2019, April 10–14, 2019, Vienna, Austria

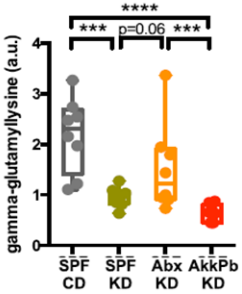
Novel Therapeutic Or Diagnostic Targets

Metabolites deliver actionable insights in microbiome research

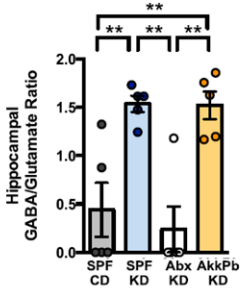


Metabolomics

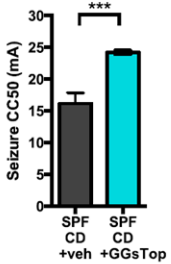
Serum GGTs

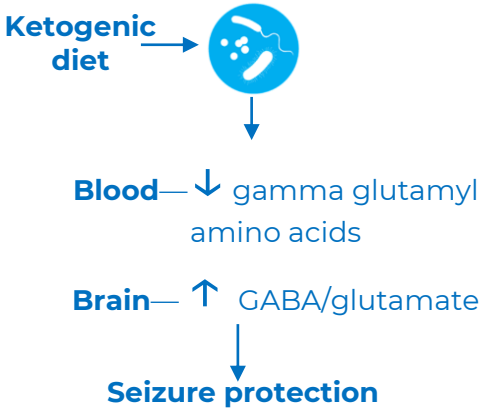


Brain GABA/Glu



Inhibit GGT





CHALLENGE	METABOLOMIC INSIGHT	VALUE REVEALED

Need for treatment strategies

- The ketogenic diet (KD) is an effective treatment for refractory epilepsy; it was a mystery how it worked
- Researchers at UCLA found that the KD works through the gut microbiome
- How host bacteria produce this benefit was unclear

Metabolomics identifies a marker that illuminated efficacy

- Diet reduces ketogenic gamma-glutamyl amino acids (GGTs) in seizure protected mice (serum)
- Serum levels lead to shift in brain GABA/Glu ratio
- Certain strains mimic diet effect through GGT activity as does inhibition of GGT activity with oral inhibitor

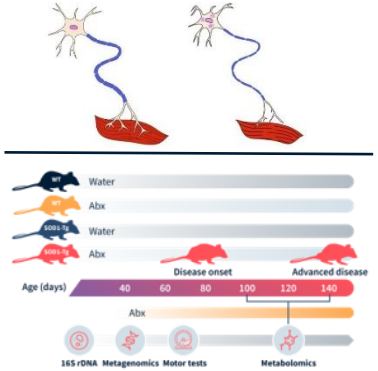
New target and biomarker identified

- A molecular understanding of how the KD works was revealed
- The findings open the potential for more targeted and consistently effective approaches
- This work spawned a new company focused on metabolic targets of neurologic disorders.

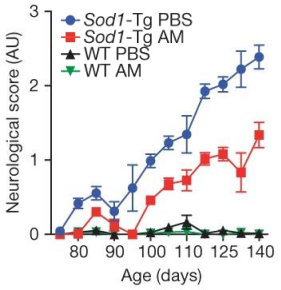
Olson, Christine A., et al. 'The gut microbiota mediates the anti-seizure effects of the ketogenic diet.' Cell 173.7 (2018): 1728-1741.

Understand Functional Outputs Of The Microbiome That Influence Health

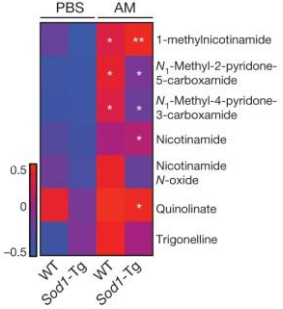
The role of bacterially generated nicotinamide (NAM) to treat amyotrophic lateral sclerosis (ALS)



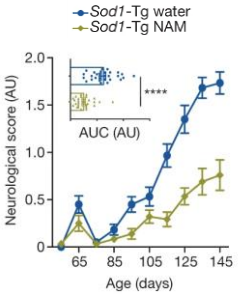
Depleting the microbiome of *Sod1*-Tg ALS mouse model exacerbates motor symptoms



Improved neurological score with *A. muciniphila* (AM)

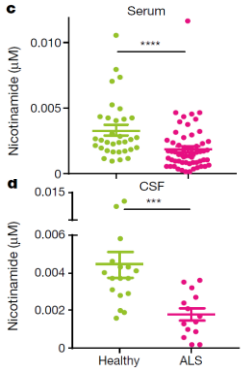


AM treatment ↑ serum & cerebral spinal fluid (CSF) NAM level in ALS mice



Improved neurological score of ALS mice treated with NAM

NAM levels are ↓ in ALS patients



CHALLENGE

- Treatment strategies needed
- ALS is a neurodegenerative disorder that is characterized by the premature death of motor neurons; few treatment options
- Factors, such as gut metabolites that can permeate the blood brain barrier (BBB), have been postulated to modify the course of the disease

METABOLOMIC INSIGHT

Metabolomics identifies NAM biosynthesis in AM treated ALS mice

- Metagenomic shotgun sequencing revealed that the gut microbial composition of *Sod1*-Tg mice diverged from WT even before the appearance of motor dysfunction, particularly *A. muciniphila* (AM).
- AM colonization ameliorated motor degeneration
- AM colonization ↑ nicotinamide (NAM) levels that improved motor performance in ALS mice

VALUE REVEALED

Potential treatment target identified

- Motor dysfunction associated with ALS may be rescued with NAM
- Translational potential as NAM levels are ↓ in ALS patients

Blacher E, et al. Nature. 2019;572(7770):474–80. DOI:10.1038/s41586-019-1443-5

Summary

- We offer unparalleled coverage of all biochemical pathways and continue to actively expand our capacity, library, and insights.
- We have deep expertise across the entire arc of sample analysis to provide support for the broadest range of questions.
- Our technology has been instrumental in functionally mapping over 800 genetic variants relevant across all disease processes.
- Biochemical profiling is uniquely suited to elucidate small molecule messengers from microbial metabolism that influence every organ system and biological process.
- Every disease requires a metabolic change positioning our technology as a cornerstone molecular phenotyping tool.