

MetaCore利用事例

A biomarker-assisted systems biology approach to explore host-microbiome-drug metabolomics

MetaCoreユーザー会
2019年6月5日

クラリベイト・アナリティクス
ライフサイエンス・ジャパン
ソリューションコンサルタント
門岡 桂史

WHAT IS THE HUMAN MICROBIOME?

The collection of microbes (**microbiota**) - that inhabits the human body, and their genomes.



100 trillion symbiotic microbes (**10 X**)



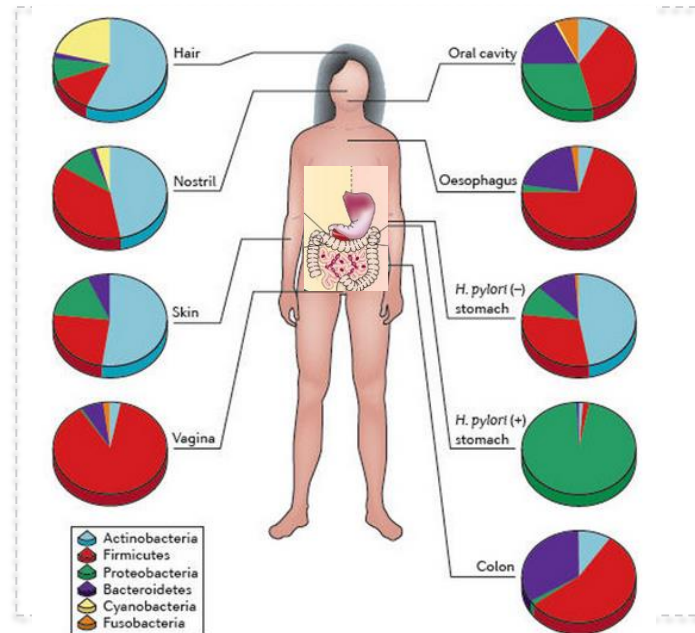
2-20 million unique genes (**100 X**)



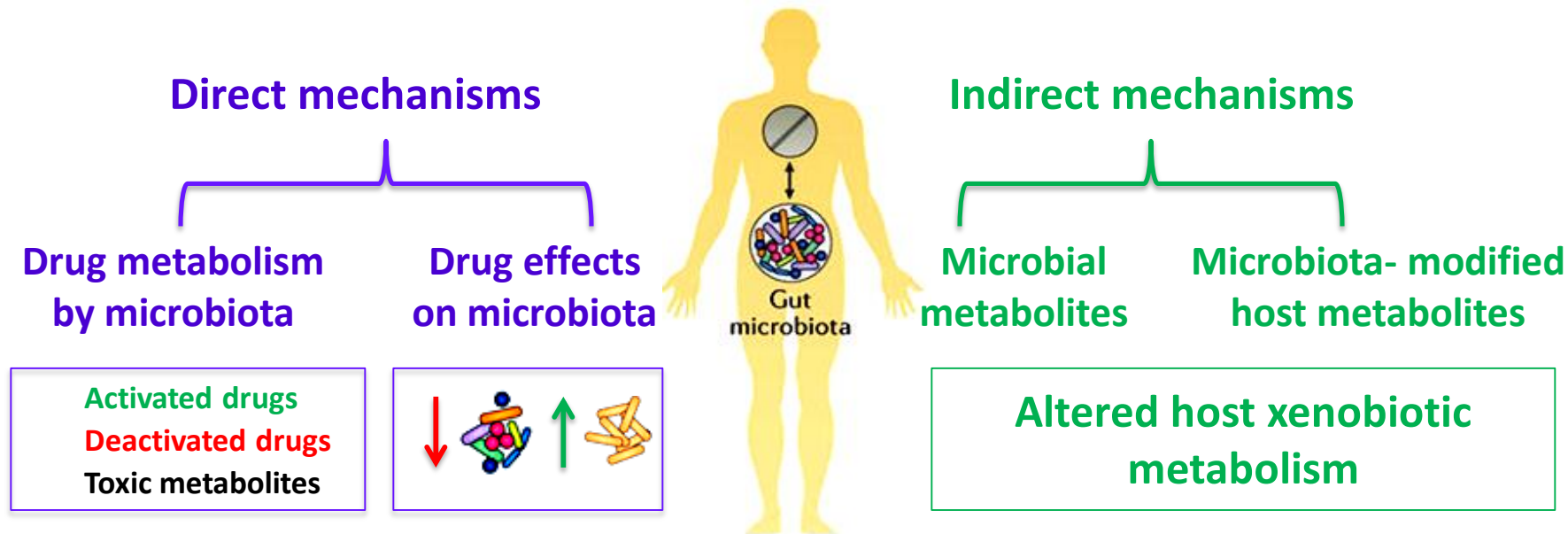
99.9 % Percentage individual humans are identical to one another in terms of host genomes.



80-90 % Percentage individual humans are different from one another in terms of microbiome.



MICROBIOME-HOST-DRUG INTERACTIONS



Based on concepts reported in Nature Reviews Microbiology14, 273–287(2016)

MICROBIOME-HOST-DRUG INTERACTIONS

Microbiome and the immune system

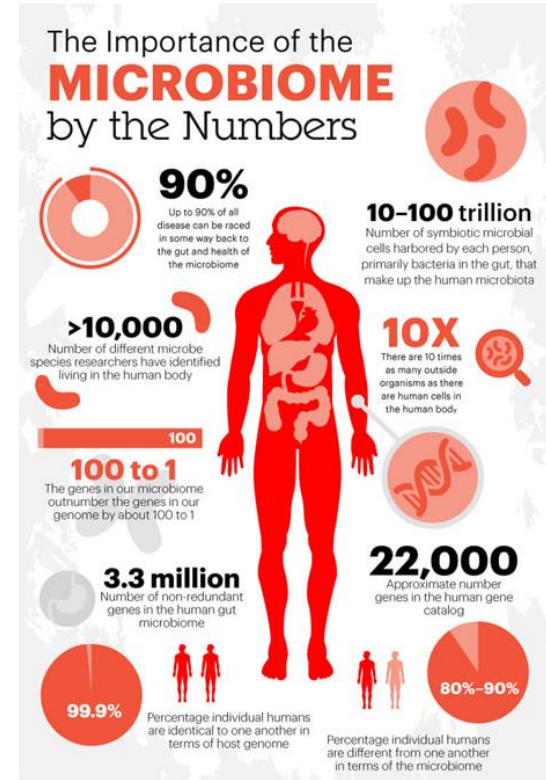
Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients

V. Gopalakrishnan^{1,2,*}, C. N. Spencer^{2,3,*}, L. Nezi^{3,*}, A. Reuben¹, M. C. Andrews¹, T. V. Karpinets³, P. A. Prieto^{1,†}, D. Vicente¹...

* See all authors and affiliations

Science 02 Nov 2017;
eaan4236
DOI: 10.1126/science.aan4236

- Gut microbiome is being extensively studied to understand its impact in gastric and metabolic diseases.
- Further studies have looked into its importance in the immune systems and neurological diseases.
- This recent study from scientists at MD Anderson explored the relationship between anti-PD-1 therapies and microbiome colonies in melanoma patients.
 - Higher diversity and higher abundance of the Ruminococcaceae family bacteria was associated with longer progression-free survival.
 - Low diversity and higher abundance of the Bacteroidales order bacteria was associated with shorter progression-free survival.



ied
s

ate
s

METHODS FOR MINING THE MICROBIOME

TAXONOMIC APPROACHES

METAGENOMICS

- **Sequencing methods**
- The study of the genomes in a microbial community.
- Metagenomic shotgun sequencing studies are used (e.g., 16S rRNA gene amplicon sequencing).
- Suggested that dysbiosis of the gut microbiota may be a key risk factor for many diseases.
- Problems: Poor taxonomic resolution & absence of adequate functional insight.
- Metagenome-wide association studies (MWAS). Study design largely modeled on GWAS to identify taxa and function.

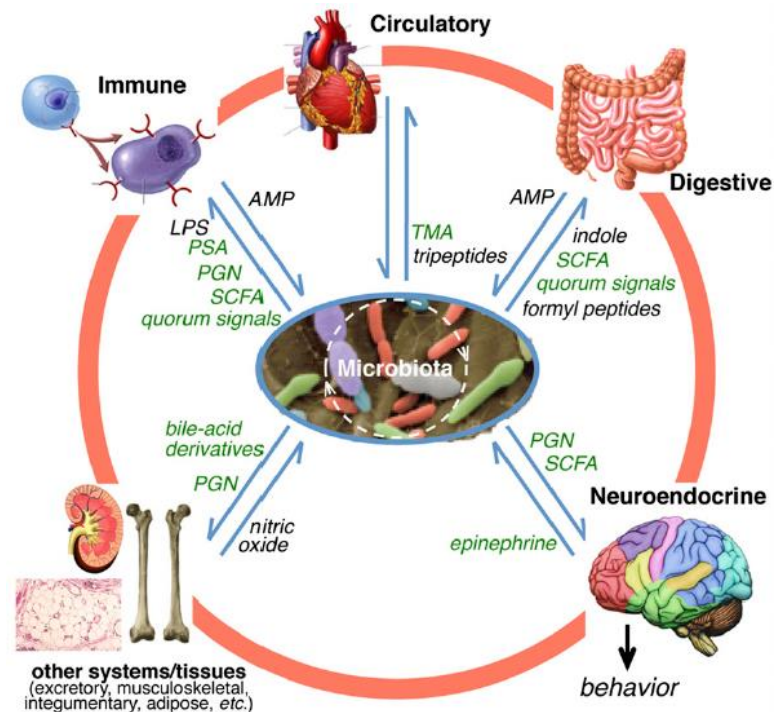
METATRANSCRIPTOMICS

- **Sequencing methods**
- The study of what genes are expressed by the entire microbial community.
- Sheds light on the *active* functional profile of a microbial community.
- Limitations because of the reference genomes needed for alignment approaches, and limitations in the ability of software programs to assemble contigs and supercontigs correctly from short reads data for de novo assembly approaches.

METHODS FOR MINING THE MICROBIOME

METABOLOMICS

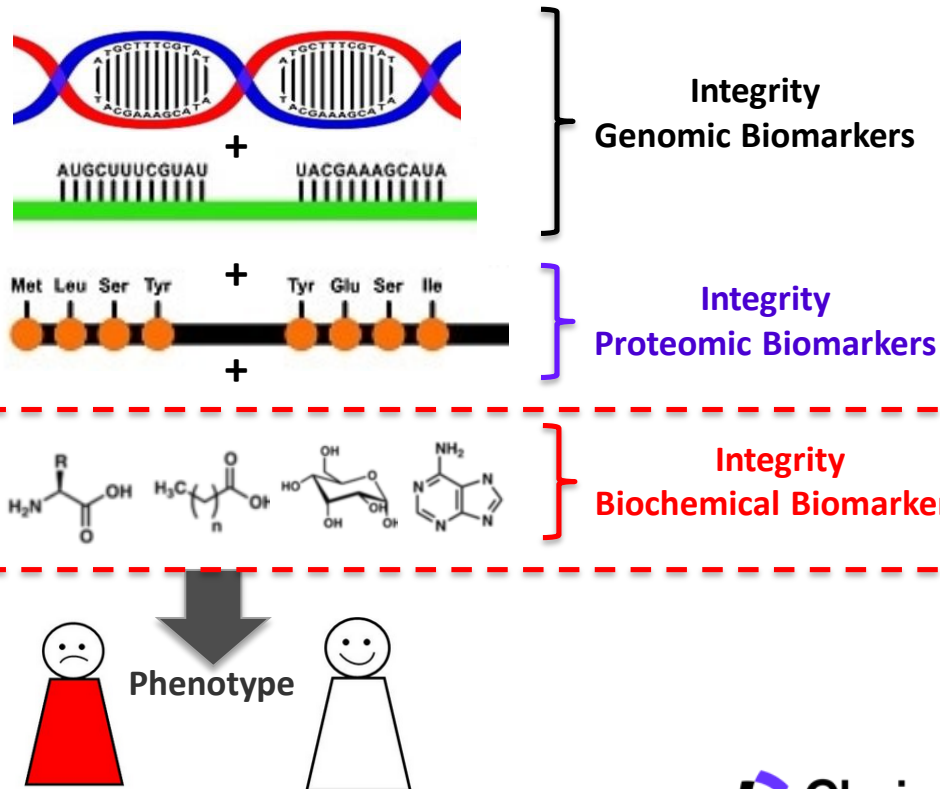
- **Chromatography, Spectrometry, Magnetic Resonance**
- Metabolomics is the comprehensive analysis by which all metabolites of a sample (small molecules released by the organism into the immediate environment) are identified and quantified.
- The metabolome is considered the most direct indicator of the health of an environment or of the alterations in homeostases (i.e., dysbiosis).
- Variation in the production of signature metabolites are related to changes in activity of metabolic routes, and therefore, metabolomics represents an applicable approach to pathway analysis.



*Signals in green promote body's homeostasis

based therapies.

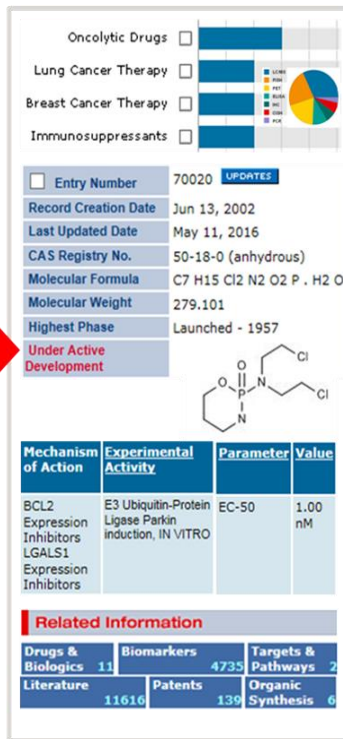
The diagram illustrates the human microbiome ecosystem. A central yellow human silhouette is shown with a circular inset representing the gut microbiota. This inset contains a complex network of colored nodes and arrows, with labels for Genes (pink), RNAs (green), Proteins (cyan), and Metabolites (blue). Arrows radiate from this central hub to various components: Drugs (black arrow pointing up), Genes (pink arrow pointing up-left), Microbes (purple arrow pointing up-right), Microbial Metabolites (blue arrow pointing right), Proteins (cyan arrow pointing down-right), RNAs (green arrow pointing down-left), and Host Metabolites (red arrow pointing left). Two horizontal dashed red lines separate the host from the microbiota and the microbiota from the host's internal environment. An inset in the top right corner provides a detailed view of the microbiota's internal structure, showing a dense cluster of nodes with arrows connecting them to the same set of labels: Drugs, Metabolites, Proteins, Genes, and RNAs.



INTEGRITY: DRUG R&D PIPELINE DATABASE, CREATED BY SCIENTISTS FOR SCIENTISTS

Drug pipeline and competitive intelligence database

Number of records in each knowledge area



572,661	drugs & biologics
6,966	targets
48,008	genomic records
42,560	biomarkers
35,375	synthetic routes
2,620,311	pharmacology records
130,833	experimental models
1,007,355	PK/metabolism records
379,976	clinical studies references
161	disease briefings
31,550	companies & research institutions
2,604,039	references
437,410	patents

*As of 2019/06/03

WHY INTEGRITY BIOMARKERS?

42,560 biomarkers
~ 2.5 million biomarker uses

Discovery

Pre-clinical

Clinical

Approval

Biomarker's "ROLE"	
• Disease/Toxicity Profiling	
• Risk Factor	• Predicting Treatment Efficacy / Tox.
• Screening	• Monitoring Treatment Efficacy / Tox.
• Staging	• Prediction of Drug Resistance
• Prognosis	• Prediction of Drug Resistance
• Diagnosis, Differential Diagnosis	
• Monitoring Disease Progression	

Biomarker's "VALIDITY"	
1)	Emerging
2)	Experimental
3)	Early studies in humans
4)	Late studies in human or observational studies
5)	Recommended/approved
Biomarker's "TYPE"	
1)	Genomic
2)	Proteomic
3)	Biochemical
4)	Other

METACORE: YOUR SYSTEMS BIOLOGY GPS

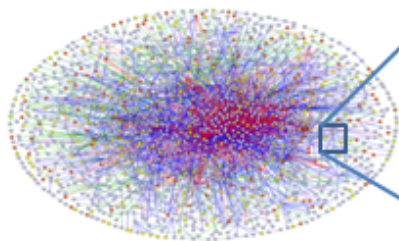
TRUSTED, HIGH QUALITY, MANUALLY-CURATED CONTENT (ERACTIONS, NETWORKS, AND PATHWAY MAPS)

Molecular Interaction

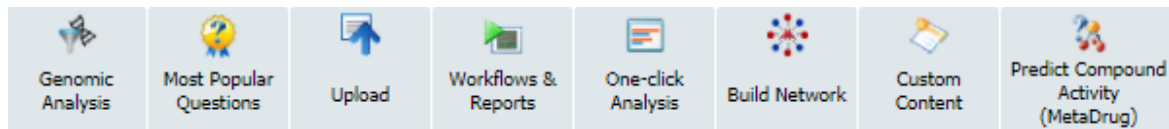


Extracted from scientific papers and patents

Global Interaction Network



>2.3 million interactions

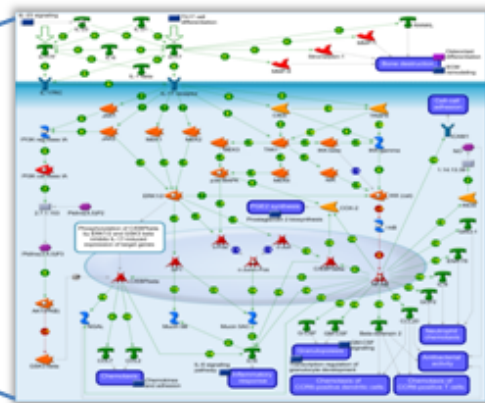


Process Networks



Global view of a process
(typically 100s of network objects)

Canonical Pathway Maps



Focused view on a subset of interactions or processes
(typically 50-60 network objects)

> 1,550 Pathway maps
> 220,000 Gene-disease associations

CASE STUDY

“Exploring Host-Microbiome-Metformin
Metabolomics in Type 2 Diabetes”

MICROBIOME-HOST METFORMIN INTERACTIONS



Nat Med. 2017 Jul;23(7):850-858. doi: 10.1038/nm.4345. Epub 2017 May 22.

Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug.

Wu H¹, Esteve E^{2,3,4}, Tremaroli V¹, Khan MT¹, Caesar R¹, Mannerås-Holm L¹, Ståhlman M¹, Olsson LM¹, Serino M⁵, Planas-Fèlix M⁶, Xifra G^{2,3,4}, Mercader JM⁶, Torrents D^{6,7}, Burcelin R^{8,9}, Ricart W^{2,3,4}, Perkins R¹, Fernández-Real JM^{2,3,4}, Bäckhed F^{1,10,11}.

⊕ Author information

Abstract

Metformin is widely used in the treatment of type 2 diabetes (T2D), but its mechanism of action is poorly defined. Recent evidence implicates the gut microbiota as a site of metformin action. In a double-blind study, we randomized individuals with treatment-naïve T2D to placebo or metformin for 4 months and showed that metformin had strong effects on the gut microbiome. These results were verified in a subset of the placebo group that switched to metformin 6 months after the start of the trial. Transfer of fecal samples (obtained before and 4 months after treatment) from metformin-treated donors to germ-free mice showed that glucose tolerance was improved in mice that received metformin-altered microbiota. By directly investigating metformin-microbiota interactions in a gut simulator, we showed that metformin affected pathways with common biological functions in species from two different phyla, and many of the metformin-regulated genes in these species encoded metalloproteins or metal transporters. Our findings provide support for the notion that altered gut microbiota mediates some of metformin's antidiabetic effects.

MICROBIOME-HOST METFORMIN INTERACTIONS



Type 2 Diabetes:

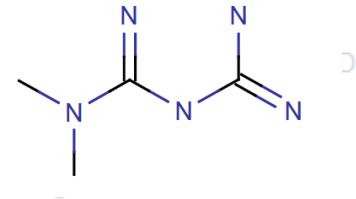
Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug.

- Butyrate producing bacterial Taxa ↓
- Butyrate is the major energy source for microbiome and colonocytes.^{6, Xifra G^{2,3,4}, Mercader JM⁶, Torrents D^{6,7}, Burcelin R^{8,9}, Ricart W^{2,3,4}, Perkins R¹, Fernández-Real JM^{2,3,4}, Bäckhed F^{1,10,11}}
- Colonocytes depend on butyrate for energy production.

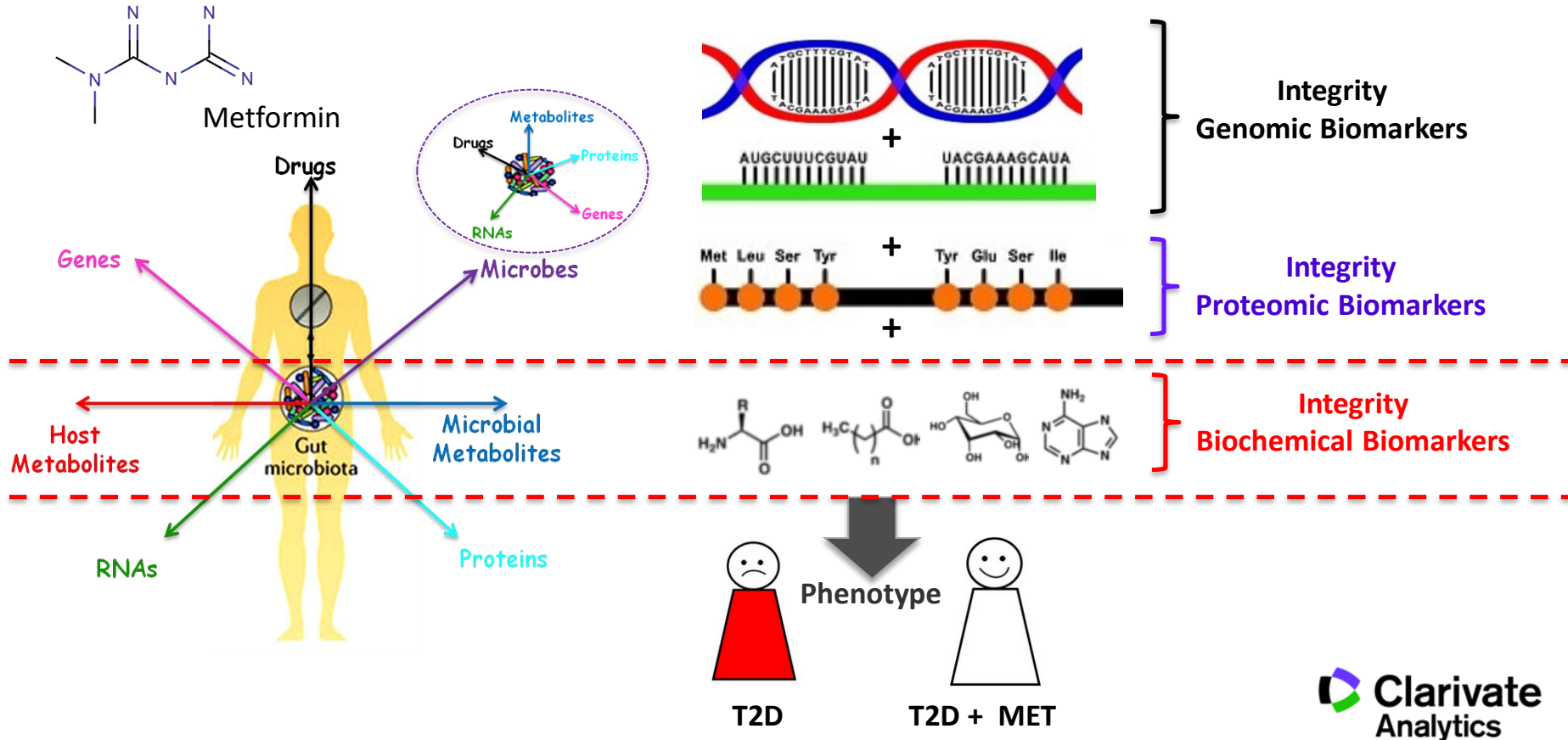
Abstract

Metformin is widely used in the treatment of type 2 diabetes (T2D), but its mechanism of action is poorly understood. It has been hypothesized that metformin's effect on glucose metabolism is mediated by its action on the gut microbiota as a site of metformin action. In a double-blind study, we randomized individuals to placebo or metformin treatment. The composition of the gut microbiota was determined by 16S rRNA sequencing. In a subset of the placebo group that switched to metformin 6 months after the start of the trial, we observed a significant increase in the abundance of gamma-proteobacteria and a decrease in the abundance of firmicutes. Firmicutes Gram-positive

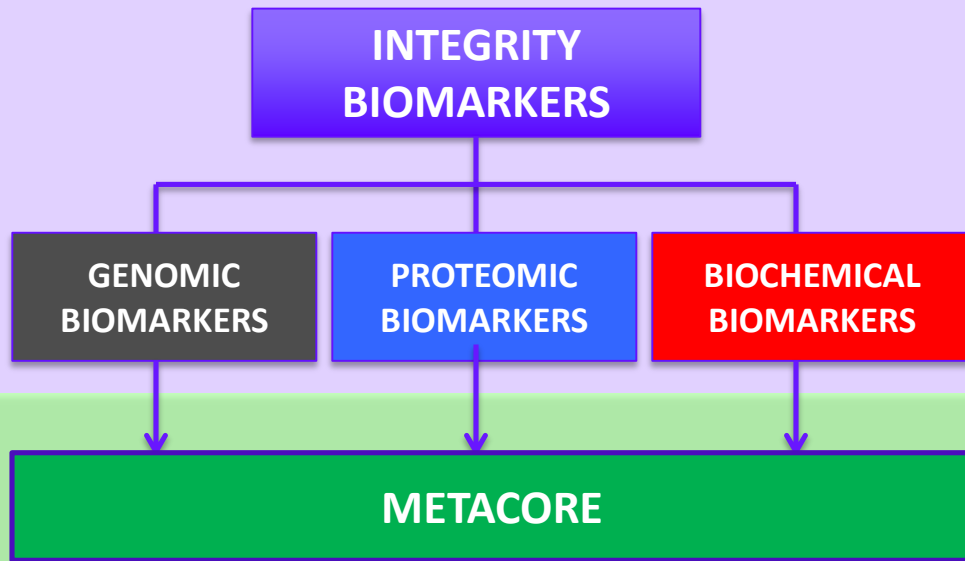
- Gamma-proteobacteria (Gram-negative ↑, LPS ↑)
- Firmicutes Gram-positive ↓
- Many of the metformin-regulated genes in these species encoded **metalloproteins or metal transporters**.



A BIOMARKER-ASSISTED SYSTEMS BIOLOGY APPROACH TO METABOLOMICS



WORKFLOW



Derive

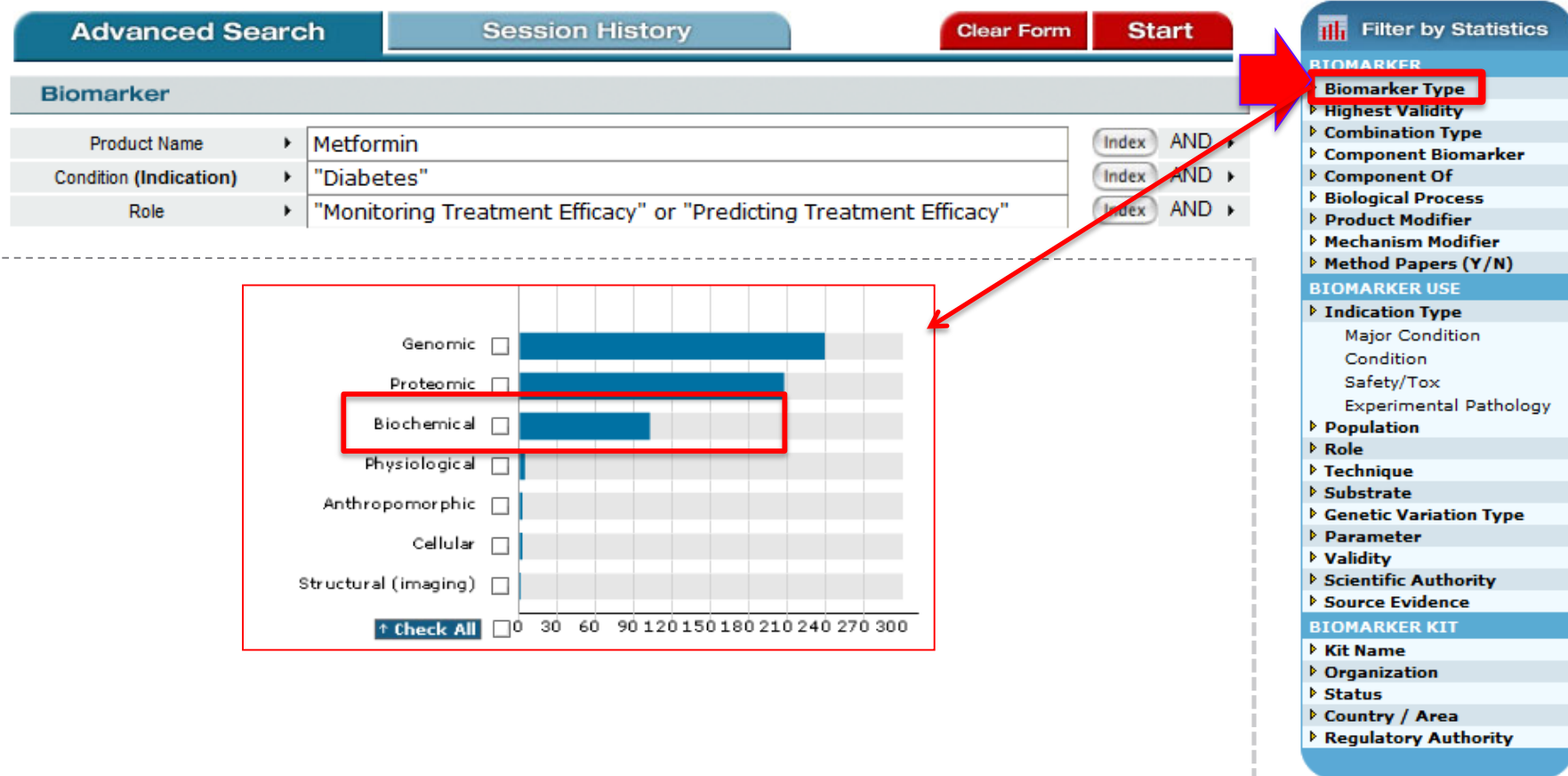
“genomic”
“proteomic”
“metabolomic”
signatures representative
of biology/pharmacology

Analyze

the underlying biology
and metabolic processes
on a “systems” level.

- Identify enriched pathways
- Identify enriched metabolic processes
- Understand the relationships between metabolites, genes/proteins and drugs.

BIOMARKERS TO MONITOR /PREDICT TREATMENT EFFICACY OF METFORMIN



UPLOAD METABOLIC DATA

Genomic Analysis

Most Popular Questions

Upload

Workflows & Reports

One-click Analysis

Upload Experiments with Gene or Protein IDs

Upload Metabolites

Upload Interactions

Upload Structures

Upload Genomic Variants

Data Analysis Wizard

Step 1

Click "browse" to select file(s) to upload:

Next >>

Browse... Metformin_Bioch_BMKs_n.txt

Data format

Warning: do not mix IDs in the same column.

Warning: Currently, Excel 2007 files are not supported. To upload your file, please save it as a text file with tab separated fields or an older Excel version.

Metabolic Parser recognizes the following metabolite identifiers:

- Chemical Name
- Formula
- Molecular Weight
- SMILES
- InChI
- CAS Number
- KeGG ID
- PubChem Compound ID
- Compound ID

Data Analysis Wizard (Metabolic parser)

Step 2

i Only first 10 lines of your file are shown. Use horizontal scrolling if needed.

<< Back

Next >>

Use checkboxes against each row to specify table header lines

Specify the column types in your file:

File data

Experiments name prefix

Metformin_Bioch

Type

Chemical Name

Name

Name



Name



octadecenoyl



carnitine



acylcarnitine



2-Aminoadipic acid



25-Hydroxycholesterol



8,11,14-Eicosatrienoic acid

ENRICHMENT ANALYSIS IN METABOLIC NETWORKS

File
Edit
View
Tools
Help

Activate/Deactivate

Home
My Data
EXPERIMENTS
Microbiome-Metformin

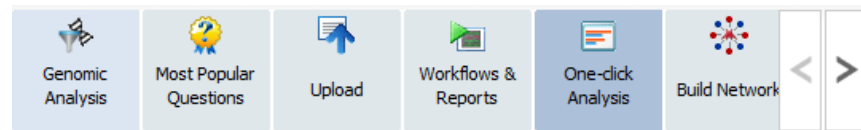
Name	Type	
EXPERIMENTS		
Microbiome-Metformin		
Metformin_Bioch_BMKs_InDiabetes	MX	1
Antibiotic_Bioch_BMKs_n	MX	1
Probiotic_Bioch_BMKs_n	MX	1
Met_Probio_Antibio_Common_Bioch_BMKs	MX	1

Home
Active Data

Name	Type	
[..] Active Data		
Met_Probio_Antibio_Common_Bioch_BMKs	MX	1

To explore linked series of chemical reactions

The reactants, products, and intermediates of an enzymatic reaction are known as metabolites, which are modified by a sequence of chemical reactions catalyzed by enzymes.



Enrichment Ontologies

Scores and ranks entities in functional ontologies most relevant in activated dataset(s).

Ontologies

- [Pathway Maps](#)
- [Map Folders](#)
- [Process Networks](#)
- [Diseases \(by Biomarkers\)](#)
- [Disease Biomarker Networks](#)
- [Drug Target Networks](#)
- [Toxic Pathologies](#)
- [Drug and Xenobiotic Metabolism Enzymes](#)
- [Toxicity Networks](#)
- [Metabolic Networks](#)
- [Metabolic Networks \(Endogenous\)](#)

Public Ontologies

- [GO Processes](#)
- [GO Molecular Functions](#)
- [GO Localizations](#)



TOP ENRICHED METABOLIC NETWORKS

Experiments

Experiment name	Species	Network Objects
<input checked="" type="checkbox"/> Metformin_Bloch_BMKs_InDiabetes		156

Settings

Threshold: 0

P-value: 1

Signals: ☒ up ☐ down ☐ both

[more options](#)

Metabolic Networks

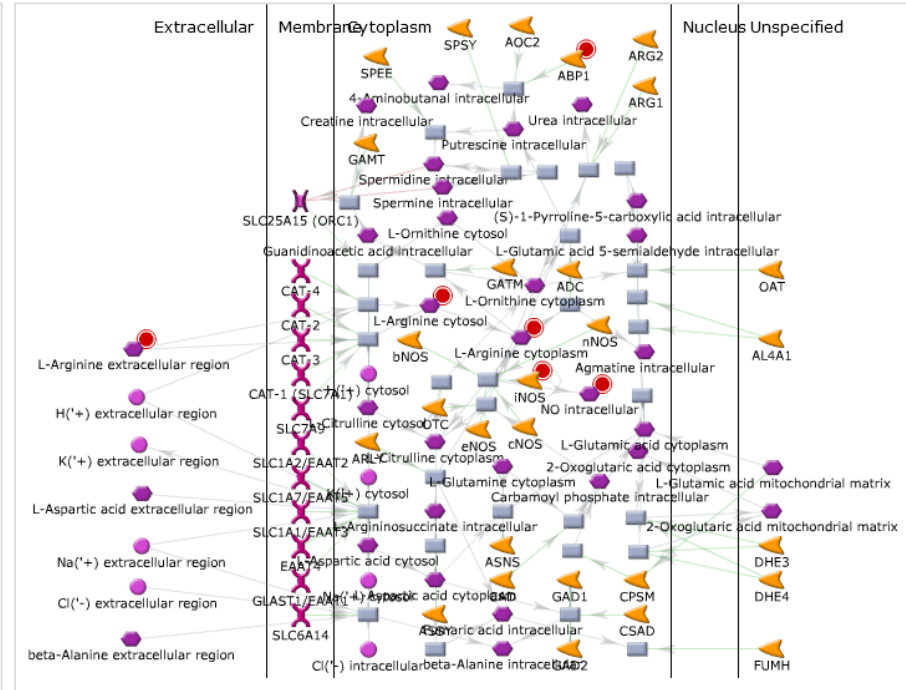
Export

#	Networks
1	L-ornithine pathways and transport
2	L-serine pathways and transport
3	Aminoacid metabolism Arginine metabolism and transport
4	Glucosylceramide pathways and transport
5	1-palmitoyl-sn-glycero-3-phosphocholine pathway
6	1-oleoyl-sn-glycero-3-phosphocholine pathway
7	Aminoacid metabolism Asparagine, Aspartic acid, Arginine metabolism and transport
8	1-icosatrienoyl-sn-glycero-3-phosphocholine pathway
9	1-docosahexaenoyl-glycerol 3-phosphocholine pathway
10	Glutamic acid pathways and transport

Total results: 50

#	FDR	Ratio
e-8	4.581e-6	11/124
e-7	4.629e-5	10/130
e-6	4.629e-5	9/111
e-6	4.629e-5	8/83
e-6	4.629e-5	8/83
e-6	6.707e-5	9/120
e-6	2.183e-4	7/76
e-5	5.225e-4	8/122
e-5	7.783e-4	8/131

Arginine metabolism and transport



BUILD NETWORK FOR YOUR BIOMARKERS/EXPERIMENTAL DATA

The screenshot displays the Clarivate Analytics software interface, specifically the 'Build Network' workflow. The interface is divided into several sections:

- Top Navigation Bar:** Includes 'File', 'Edit', 'View', 'Tools', and 'Help' menus. Below the menus are icons for 'Activate/Deactivate' and a list of navigation tabs: 'Home', 'My Data', '[...]', and 'Microbiome-Metformin'.
- Left Panel (Data List):** A table listing data items under the 'Microbiome-Metformin' tab. The table has columns for 'Name', 'Type', and a date. The items are:

Name	Type	Date
T2D_Bioch_BMKs_Diag...	MX	12/04
Metformin_Bioch_BMKs...	MX	12/03
Microbiome-Metformin		12/03
Antibiotic_Bioch_BM...	MX	11/29
Metformin Bioch BM...	MX	11/29
- Right Panel (Build Network):** Contains a list of options for building a network:
 - Build Network for Single Gene/Protein/Compound or a List
 - Build Network for Your Experimental Data** (highlighted with a red box and labeled 'Step 1')
 - Build Network for a Disease
 - Build Network for a Process
 - Merge Networks
- Bottom Panel (Model Pathways):** Includes a section for 'Model Pathways' with a warning: 'Warning. This is a calculation intensive activated experiment(s). We strongly'.
- Network options (Step 2):** A sub-panel on the right showing options for building the network:
 - Choose building algorithm:** A dropdown menu set to 'Analyze network'.
 - Number of nodes in a network:** A text input field set to '50'.
 - ☒ **Use canonical pathways** (processing takes longer for large datasets)
 - [Show additional options](#)
 - Build network** button

ANALYZE RESULTS

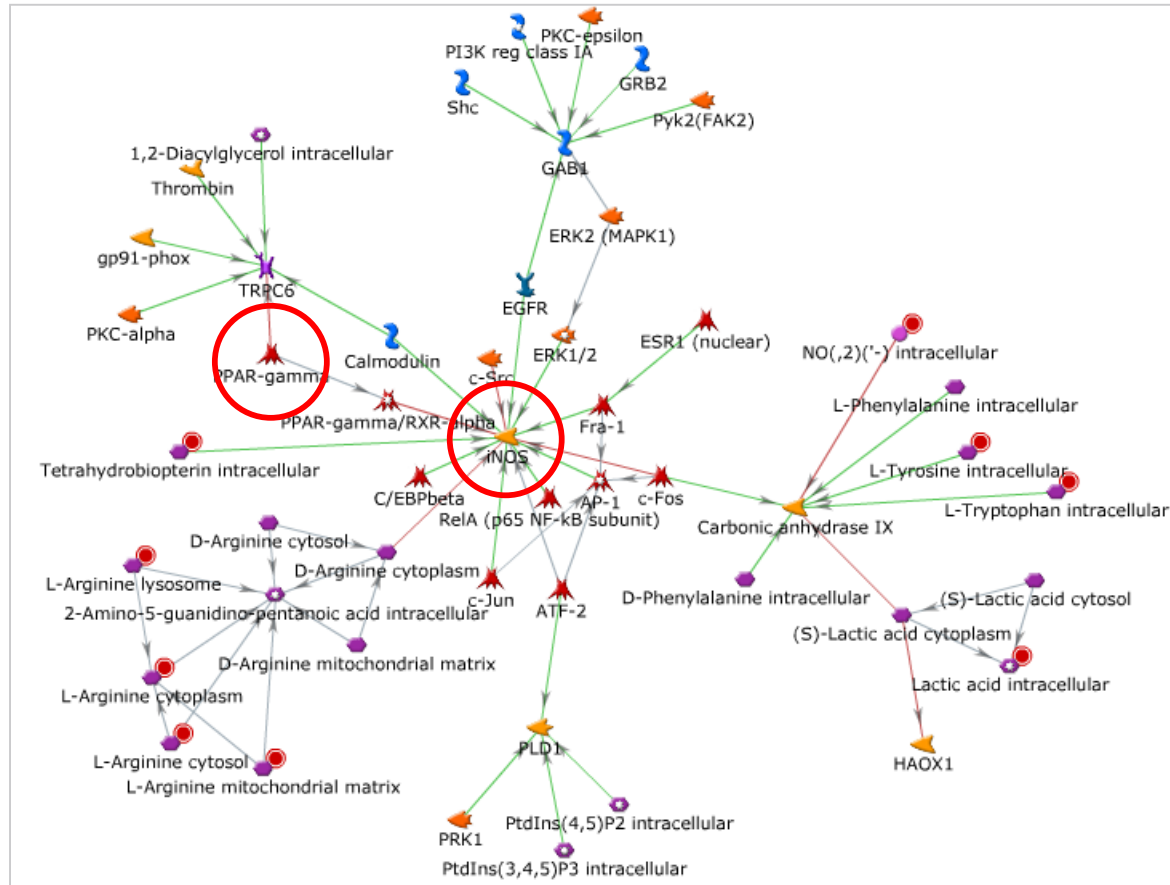


Networks in List with 30 networks List

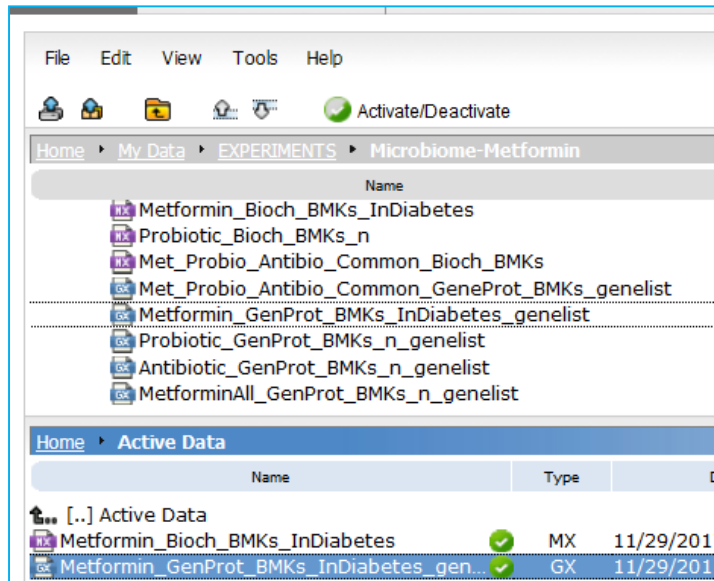


Save list Get report Export Delete Merge P-value for networks Show excluded objects Rebuild										
	#	Name	GO processes	# Nodes		Pathways	p-Value ↑	zScore	gScore	Edit
				Total	Seed					
<input type="checkbox"/>	1	NO(2)(-) intracellular, L-Tryptophan intracellular, L-Tyrosine intracellular, Tetrahydrobiopterin intracellular, iNOS	response to oxygen-containing compound (93.3%; 3.759e-24), response to hormone (80.0%; 3.300e-23), response to endogenous stimulus (83.3%; 1.234e-20), response to organonitrogen compound (76.7%; 6.007e-20), response to organic cyclic compound (76.7%; 1.019e-19)	50	9	0	1.15e-19	46.78	46.78	
<input type="checkbox"/>	2	Arachidonic acid extracellular region, Eicosapentaenoic acid intracellular, D-Glucose intracellular, 25-Hydroxycholesterol intracellular, Ca(2+) cytosol	response to organic cyclic compound (77.1%; 4.631e-23), cellular response to endogenous stimulus (77.1%; 5.358e-23), response to lipid (74.3%; 2.280e-22), response to endogenous stimulus (80.0%; 4.869e-22), cellular response to organic substance (88.6%; 9.240e-22)	50	8	0	4.29e-17	41.12	41.12	
<input type="checkbox"/>	3	Cholesterol cytoplasm, Triacylglycerols intracellular, Cholesterol Golgi apparatus, Cholesterol lysosome, Cholesterol endoplasmic reticulum		50	5	0	3.45e-14	59.54	59.54	
<input type="checkbox"/>	4	Cholesterol mitochondrion, Cholesterol		50	5	0	1.26e-13	53.84	53.84	

ANALYZING GENERATED NETWORKS



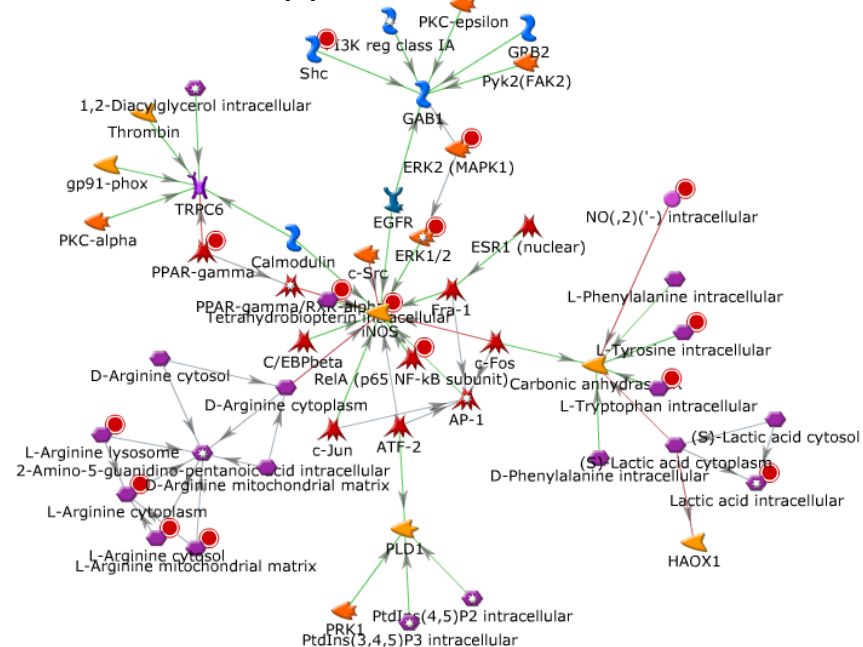
ACTIVATE GENOMICS /PROTEOMICS BIOMARKER DATA



The screenshot shows the Clarivate Analytics software interface. The top menu bar includes File, Edit, View, Tools, and Help. Below the menu is a toolbar with icons for file operations and a green 'Activate/Deactivate' button. The main window displays a hierarchical view of data under 'My Data' > 'EXPERIMENTS' > 'Microbiome-Metformin'. A list of files is shown, including 'Metformin_Bioch_BMKs_InDiabetes', 'Probiotic_Bioch_BMKs_n', 'Met_Probio_Antibio_Common_Bioch_BMKs', 'Met_Probio_Antibio_Common_GeneProt_BMKs_genelist', 'Metformin_GenProt_BMKs_InDiabetes_genelist', 'Probiotic_GenProt_BMKs_n_genelist', 'Antibiotic_GenProt_BMKs_n_genelist', and 'MetforminAll_GenProt_BMKs_n_genelist'. Below this, the 'Active Data' section shows a table with columns for Name, Type, and Date. Two files are listed as active: 'Metformin_Bioch_BMKs_InDiabetes' (MX, 11/29/201) and 'Metformin_GenProt_BMKs_InDiabetes_gen...' (GX, 11/29/201).



Look which genomic and proteomic biomarkers appear on networks



FINDINGS

Wu et al

Type 2 Diabetes:

- Butyrate producing bacterial Taxa ↓
- Butyrate is the major energy source for microbiome and colonocytes.
- Colonocytes depend on butyrate for energy production.

Metformin Rx:

- Gram-negative ↑ , LPS ↑
- Gram-positive ↓

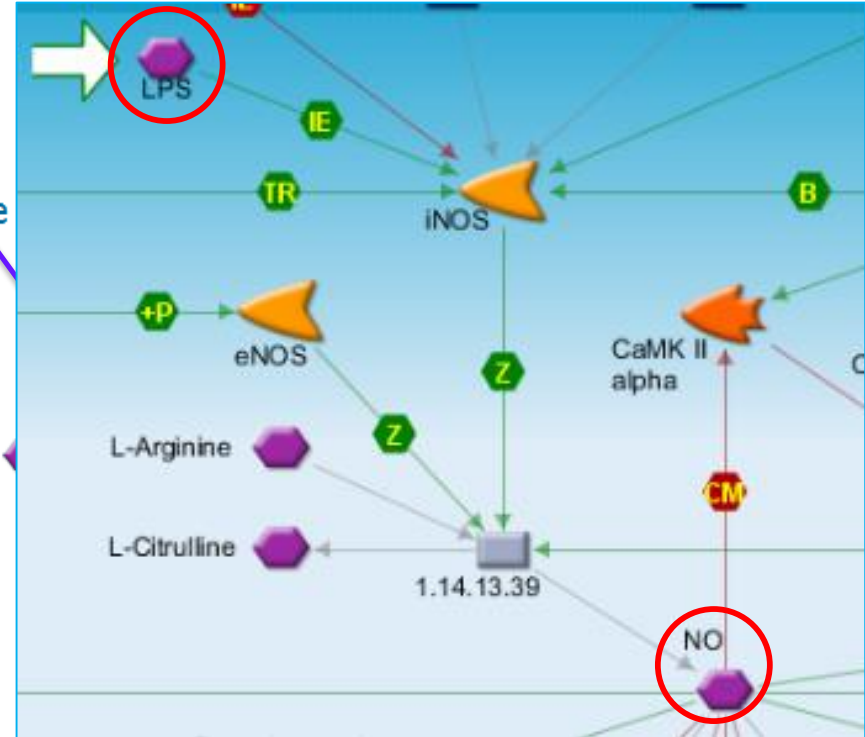
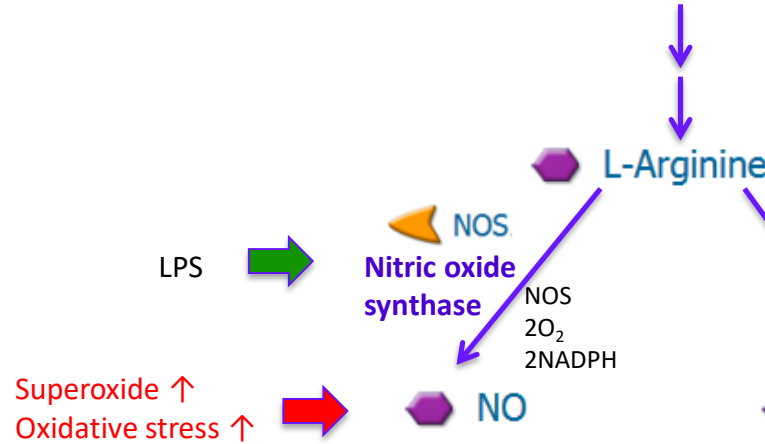
Study Findings

Prioritized metabolic processes of interest:

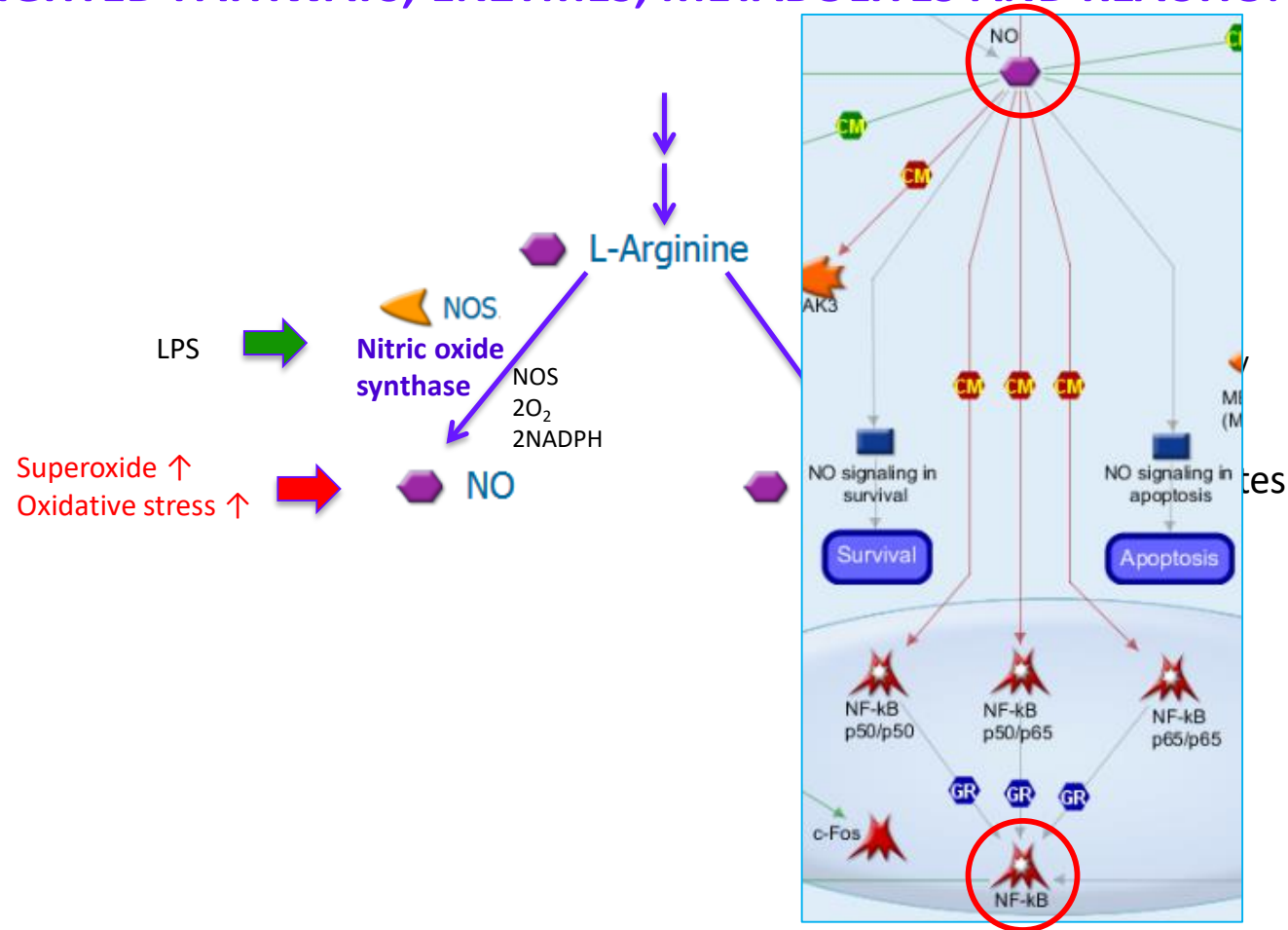
- Arginine and ornithine metabolism and transport.
- NO metabolism
- Fatty acid metabolism
- Amino acid metabolism



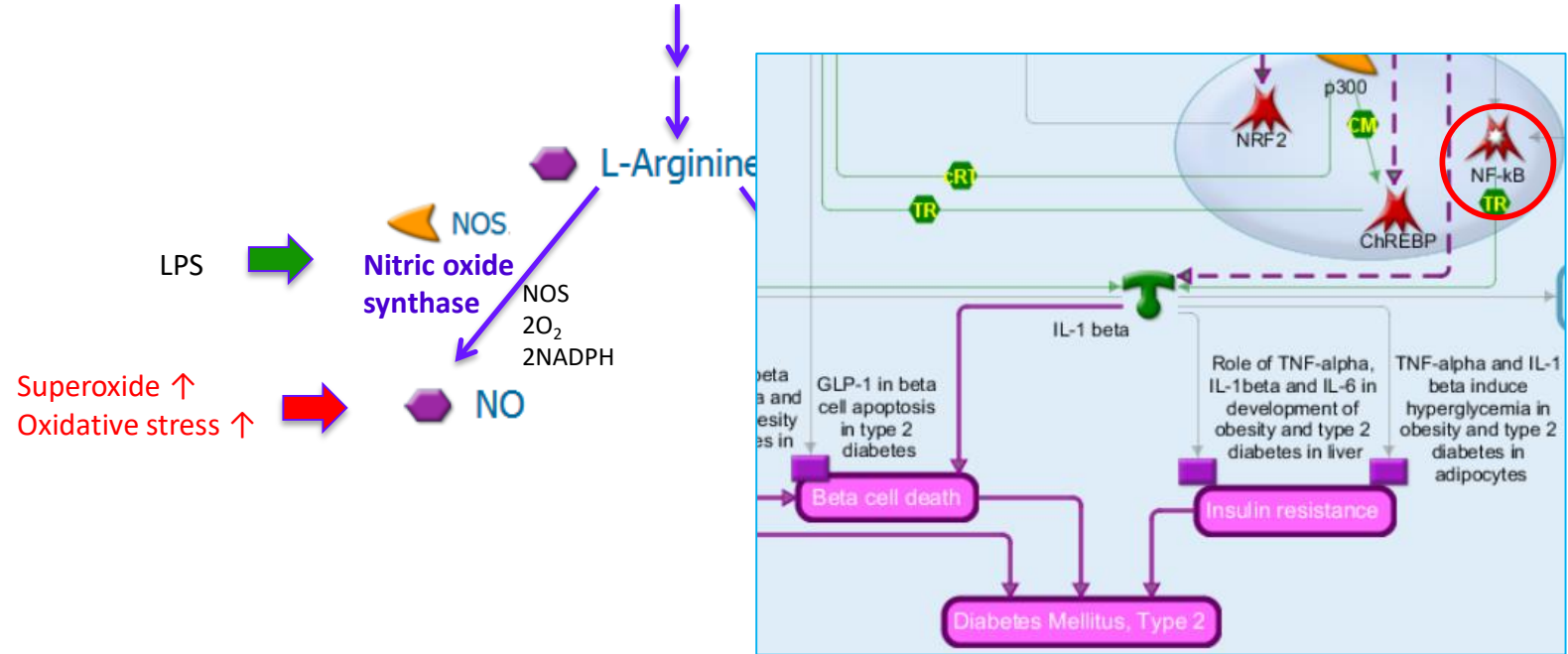
HIGHLIGHTED PATHWAYS, ENZYMES, METABOLITES AND REACTIONS



HIGHLIGHTED PATHWAYS, ENZYMES, METABOLITES AND REACTIONS

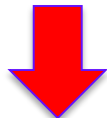


HIGHLIGHTED PATHWAYS, ENZYMES, METABOLITES AND REACTIONS

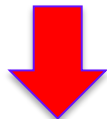


CONCLUSIONS

Network Analysis Using A Biomarkers Approach



- Arginine and ornithine metabolism and transport
- NO metabolism
- Fatty acid metabolism
- Amino acid metabolism



Testable hypothesis: Metformin maybe normalized NO and butyrate production through affect bacterial colonization in the gut.

CONCLUTIONS

