Metabolomics and its way into hepatology

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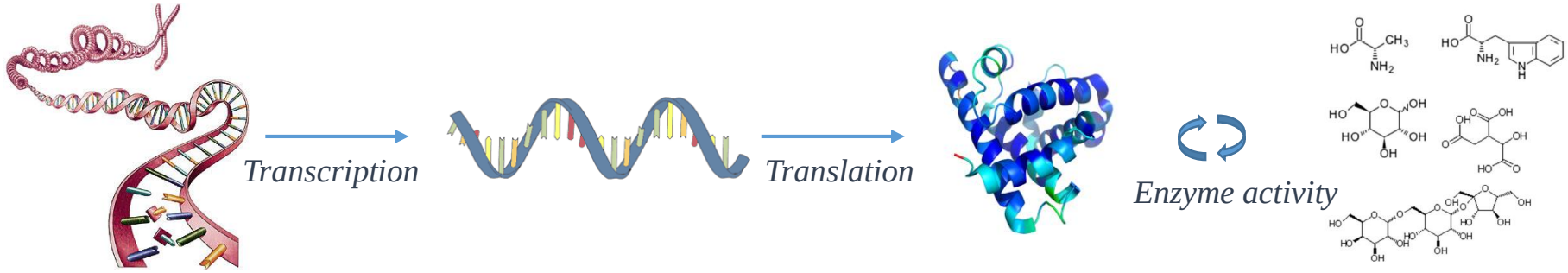
«Omic»-based approaches



Genes	mRNAs	Proteins	Metabolites
Genome	Transcriptome	Proteome	Metabolome
GENOMICS	**TRANSCRIPTOMICS**	**PROTEOMICS**	**METABOLOMICS**

(Genotype) → (Phenotype)

« What might happen »
« How it is regulated »

« What is happening »

Metabolomics deals with the comprehensive analysis of *the set of small molecules or metabolites* present in a given cell, tissue or organism

The metabolomic workflow

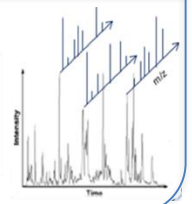
1 Sample preparation

2 Metabolome analysis

GC/MS

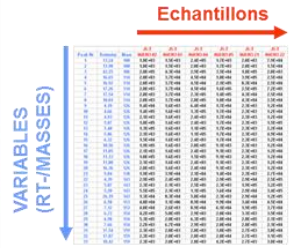
LC/MS

RMN



3 Data processing

Automatic detection of signals

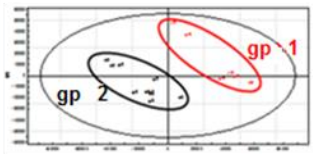


Selection of relevant features

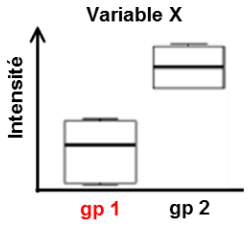
Annotation :
Databases
public / internal

4 Statistical analyses

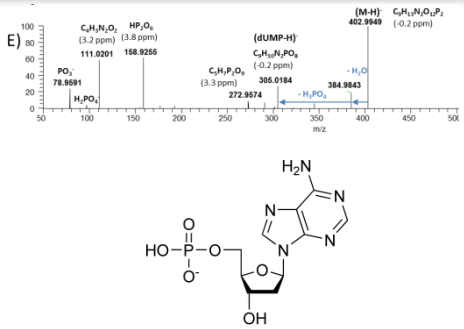
Multivariate



Univariate

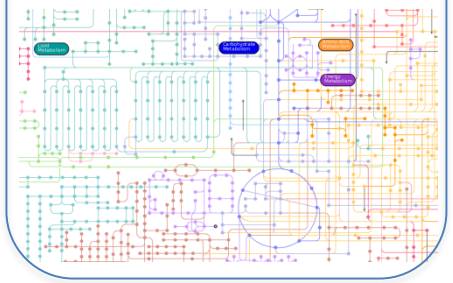


5 Metabolite identification

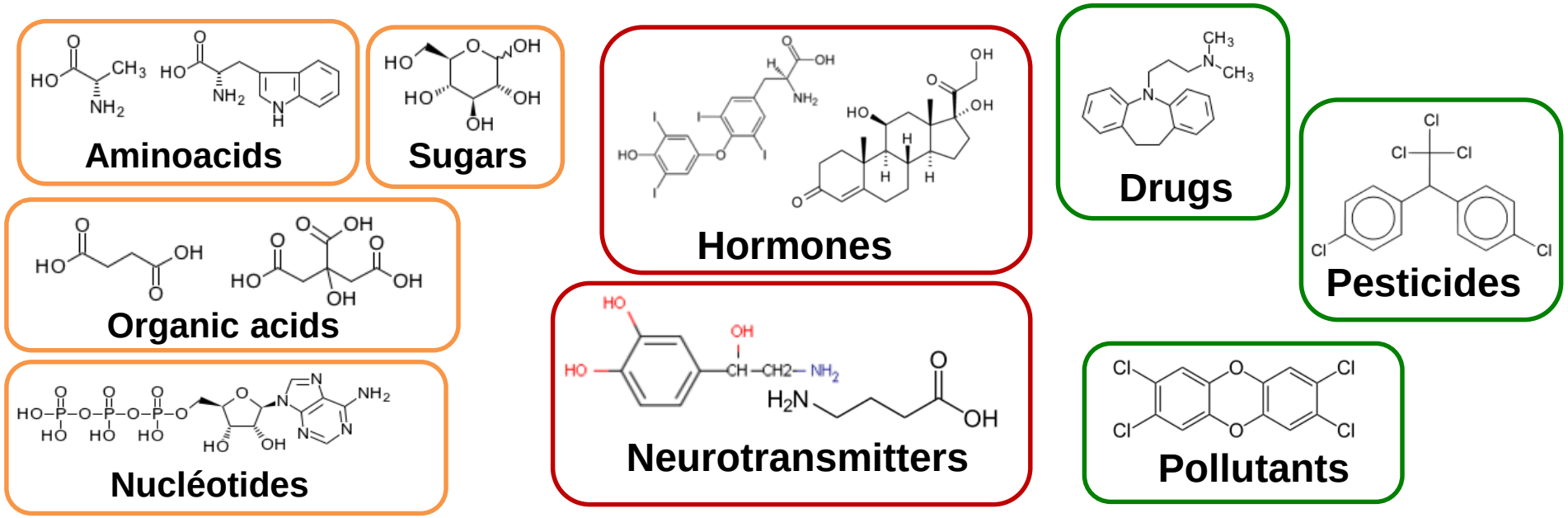


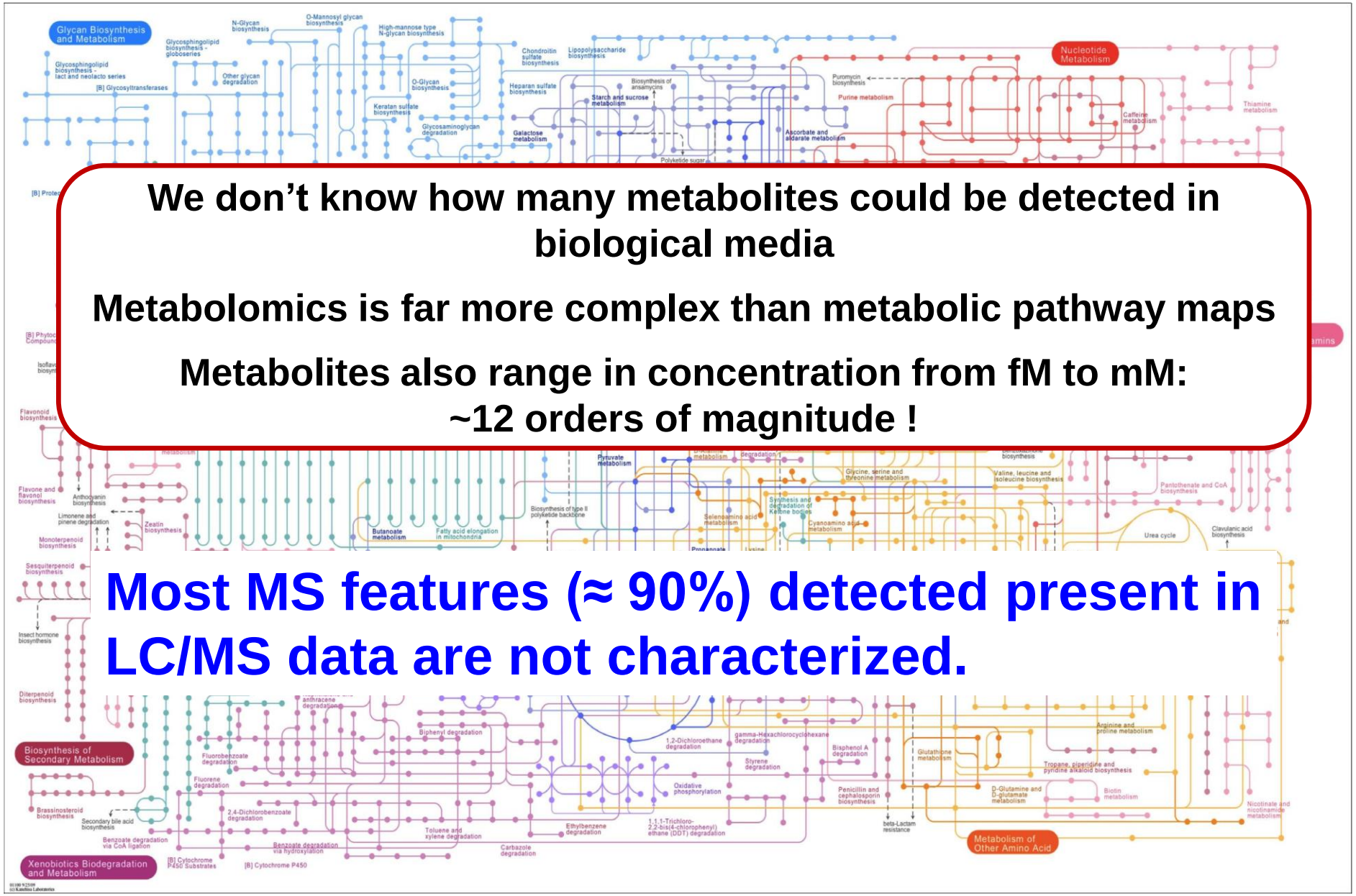
- To confirm annotations
- To characterize unknowns

6 Data visualization

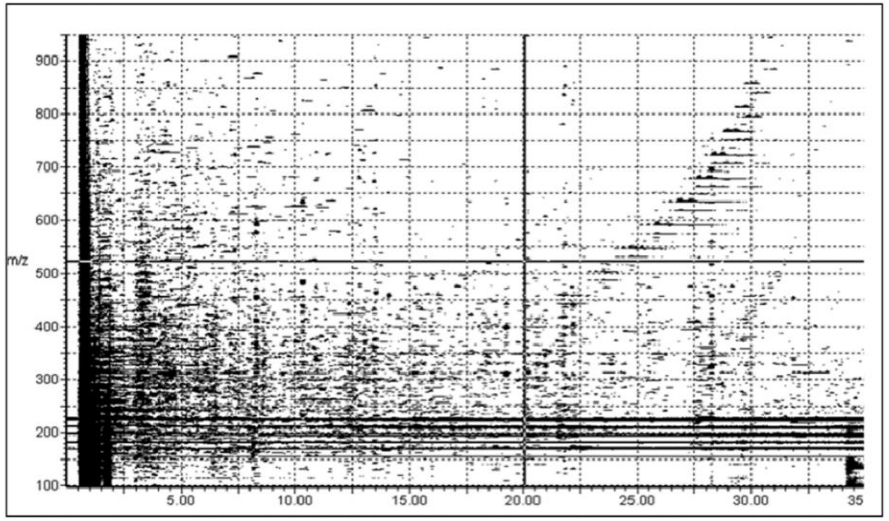


Metabolites??? : Any small organic molecule detectable in the with a molecular weight generally less than 1000 Da (or slightly larger,…)




We don’t know how many metabolites could be detected in biological media
Metabolomics is far more complex than metabolic pathway maps
Metabolites also range in concentration from fM to mM:
~12 orders of magnitude !
Most MS features (≈ 90%) detected present in LC/MS data are not characterized.
Glycan Biosynthesis and Metabolism
Nucleotide Metabolism
Biosynthesis of Secondary Metabolism
Xenobiotics Biodegradation and Metabolism
Metabolism of Other Amino Acid

Annotation of peak lists is required to help for metabolite identification



Samples

Variables (Rt-mass)

Peak Nr	Ret(min)	Mass	J5-T 060303-02	J5-T 060303-03	J5-T 060303-04	J5-T 060303-05	J5-T 060303-21	J5-T 060303-22	
1	13.24	100	1.8E+03	1.5E+03	2.4E+05	1.7E+03	2.0E+03	7.9E+04	?
2	13.98	100	1.8E+03	1.5E+03	2.0E+03	1.7E+03	2.0E+03	1.5E+04	?
3	42.25	106	2.0E+05	4.3E+04	2.9E+05	3.5E+04	1.8E+05	2.0E+03	?
4	16.65	114	2.0E+03	3.7E+04	4.5E+04	1.0E+04	3.9E+05	2.5E+04	?
5	16.92	114	2.0E+03	3.7E+04	4.5E+04	2.1E+05	8.3E+04	2.5E+04	?
6	17.26	114	2.0E+03	3.7E+04	4.5E+04	1.6E+05	2.5E+05	7.2E+05	?
7	17.54	114	2.0E+03	3.7E+04	2.3E+05	1.4E+05	4.3E+04	2.5E+04	?
8	18.01	114	2.0E+03	3.7E+04	2.8E+05	1.0E+04	4.3E+04	2.5E+04	?
9	4.19	126	9.4E+04	1.6E+03	6.4E+04	1.7E+04	2.3E+03	1.2E+04	?
10	4.66	126	1.4E+05	1.6E+03	1.3E+05	1.7E+04	2.3E+03	1.2E+04	?
11	4.93	126	2.1E+03	1.6E+03	2.4E+03	1.7E+04	2.3E+03	1.2E+04	?
12	5.07	126	1.8E+05	1.6E+03	2.4E+03	1.7E+04	2.3E+03	1.5E+05	?
13	5.40	126	1.3E+05	1.6E+03	1.1E+05	1.7E+04	2.3E+03	2.2E+04	?
14	5.86	126	2.1E+03	1.6E+03	1.1E+05	1.7E+04	2.3E+03	4.9E+04	?
15	6.32	126	1.5E+04	1.6E+03	2.4E+03	1.7E+04	2.3E+03	1.2E+04	?
16	10.56	126	1.9E+05	1.6E+03	2.0E+05	9.1E+03	2.3E+03	1.2E+04	?
17	11.05	126	2.1E+03	1.6E+03	2.4E+03	9.1E+03	2.3E+03	1.2E+04	?
18	11.33	126	1.0E+05	1.6E+03	1.5E+05	9.1E+03	2.3E+03	1.2E+04	?
19	11.80	126	2.1E+03	1.6E+03	2.4E+03	9.1E+03	2.3E+03	1.2E+04	?
20	16.36	126	2.0E+03	1.6E+03	2.4E+04	9.1E+03	2.3E+03	1.2E+04	?
21	9.04	138	1.9E+03	3.9E+04	2.1E+04	1.4E+04	2.3E+03	2.7E+04	?
22	4.39	143	2.9E+05	2.4E+05	2.8E+03	2.9E+05	2.9E+04	2.5E+05	?
23	5.07	143	2.3E+03	2.1E+03	2.5E+05	2.3E+03	1.9E+05	3.2E+05	?
24	5.20	143	5.5E+05	2.1E+03	1.1E+05	3.6E+04	2.9E+04	3.4E+05	
25	26.39	143	1.3E+04	8.3E+04	5.8E+04	2.3E+03	2.9E+04	1.2E+05	
26	6.58	153	4.0E+04	1.1E+06	8.9E+04	9.9E+04	3.4E+04	4.5E+04	
27	7.12	153	4.0E+04	2.6E+03	8.9E+04	6.1E+04	1.9E+05	3.7E+04	
28	6.72	154	4.2E+05	5.0E+05	2.9E+03	2.0E+04	3.1E+03	3.9E+04	
29	6.98	154	5.3E+05	2.0E+03	6.3E+05	2.0E+04	2.8E+05	2.1E+05	
30	7.66	154	2.2E+03	6.7E+04	2.9E+03	2.0E+04	2.8E+05	2.1E+04	
31	17.54	159	2.3E+03	2.0E+03	2.8E+03	3.0E+05	2.7E+03	3.8E+04	
32	17.87	159	2.3E+03	2.0E+03	2.8E+03	2.8E+04	2.7E+03	1.1E+05	
33	18.42	159	2.3E+03	2.0E+03	2.8E+03	4.2E+05	2.7E+03	3.8E+04	

10,000+ variables…

…Few hundreds of metabolites ??

1. Matching of experimental m/z to theoretical masses from chemical and biochemical databases: KEGG (www.genome.jp/kegg**), Metlin (**www.metlin.scripps.edu**), HMDB (**www.hmdb.ca**)**

2. Matching of experimental m/z, CID spectra and RT to those of spectral databases

Metabolomics (2007) 3:211–221
DOI 10.1007/s11306-007-0082-2

ORIGINAL ARTICLE

Proposed minimum reporting standards for chemical analysis

Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI)

Lloyd W. Sumner · Alexander Amberg · Dave Barrett · Michael H. Beale · Richard Beger · Clare A. Daykin · Teresa W.-M. Fan · Oliver Fiehn · Royston Goodacre · Julian L. Griffin · Thomas Hankemeier · Nigel Hardy · James Harnly · Richard Higashi · Joachim Kopka · Andrew N. Lane · John C. Lindon · Philip Marriott · Andrew W. Nicholls · Michael D. Reily · John J. Thaden · Mark R. Viant

1. Identified compounds (see below).
2. Putatively annotated compounds (e.g. without chemical reference standards, based upon physicochemical properties and/or spectral similarity with public/commercial spectral libraries).
3. Putatively characterized compound classes (e.g. based upon characteristic physicochemical properties of a chemical class of compounds, or by spectral similarity to known compounds of a chemical class).
4. Unknown compounds—although unidentified or unclassified these metabolites can still be differentiated and quantified based upon spectral data.

Environmental Science & Technology
Viewpoint
pubs.acs.org/est

Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence

Emma L. Schymanski,*,† Junho Jeon,† Rebekka Gulde,†,‡ Kathrin Fenner,†,‡ Matthias Ruff,† Heinz P. Singer,† and Juliane Hollender*,†,‡

Metabolomics (2014) 10:350–353
DOI 10.1007/s11306-014-0656-8

OPINION

Metabolite identification: are you sure? And how do your peers gauge your confidence?

Darren J. Creek · Warwick B. Dunn · Oliver Fiehn · Julian L. Griffin · Robert D. Hall · Zhentian Lei · Robert Mistrik · Steffen Neumann · Emma L. Schymanski · Lloyd W. Sumner · Robert Trengove · Jean-Luc Wolfender

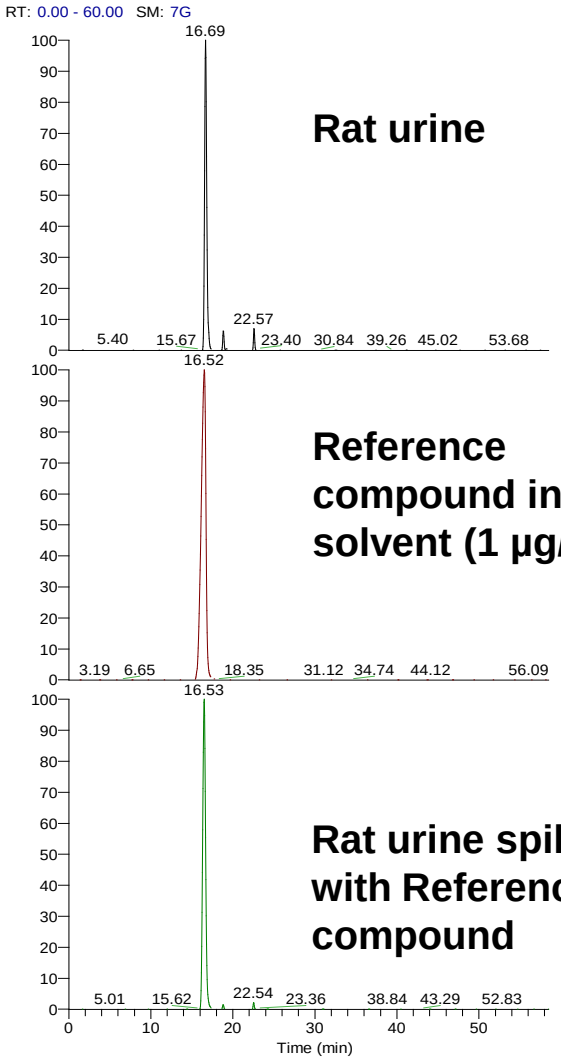
Identification of pantothenic acid in rat urines:

based on reference compound, RT, accurate mass and MS^2

LEVEL 1

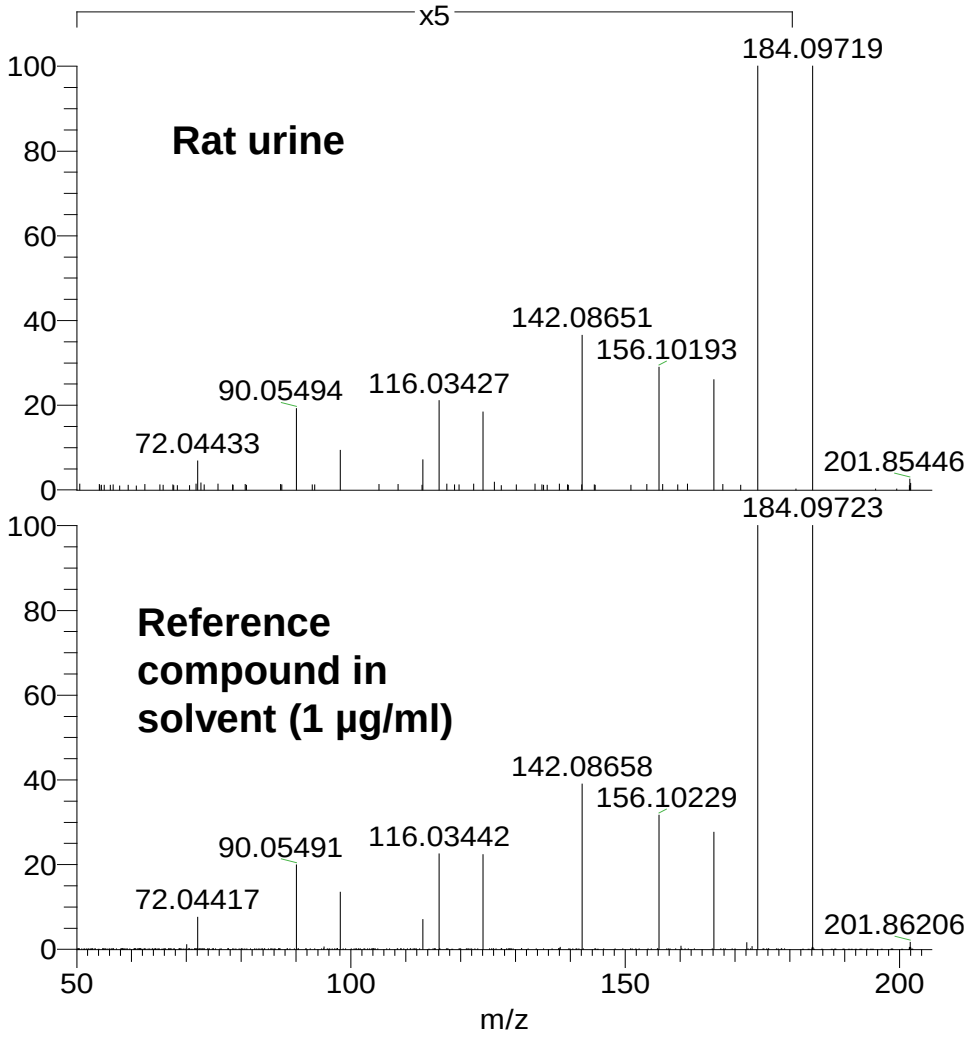
(2 stereosisomers on HMDB)

EIC at *m/z* 220 (ESI+, RP/LC-HRMS)



MS^2 spectra

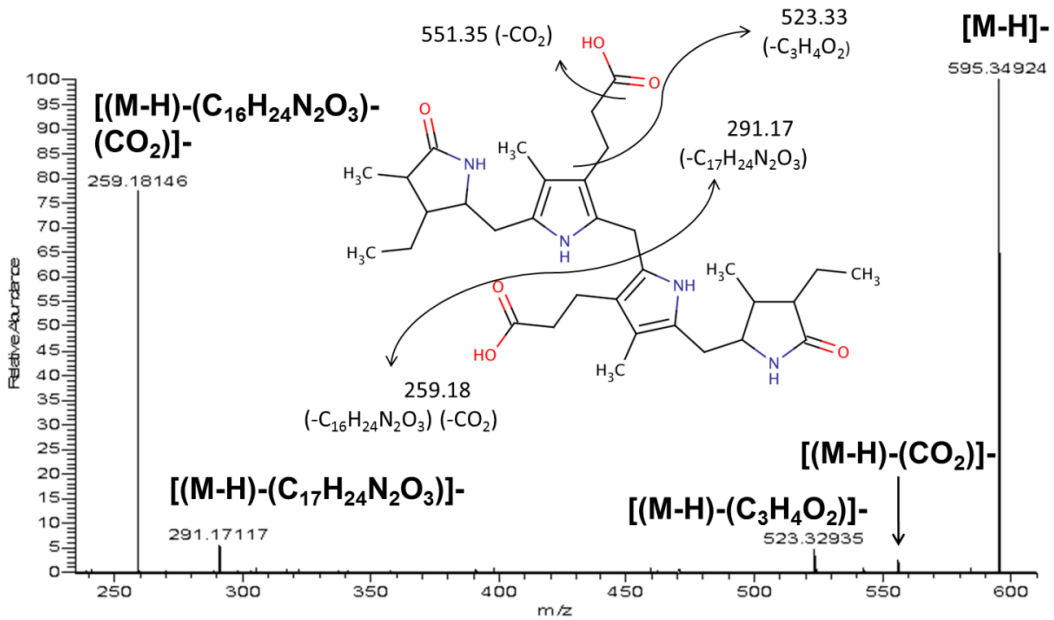
(LTQ-Orbitrap Discovery, at a mass resolution of 7500, FWHM)



(Werner E et al., J. Chromatogr. B, 2008)

Identification of L-Urobilinogen (or L-Stercobilinogen) in human urines (m/z 595.3463)

XCMS output			CAMERA output			Inter-sample correlation	Public database annotation
Variable number	m/z	Retention time (min)	isotopes	adduct	pcgroup		
1806	303.1443	9.33	**	**	531	NA	**
4663	593.3312	9.34	[681][M]+	**	512	NA	L-Urobilin
4668	594.3368	9.34	[681][M+1]+	**	512	NA	**
4679	595.3463	9.40	**[650][M]+**	**[M-H]-**	**394**	**1.00**	C-Curarine / L-Urobilinogen
4682	596.3514	9.40	**[650][M+1]+**	**	**394**	**0.98**	**
4878	631.3256	9.40	**	**[M+Cl]-**	**394**	**0.96**	**
3797	481.2789	9.46	**	**	552	NA	GPCho(10:0/4:0) / GPCho(12:0/2:0)
2763	381.1910	9.53	**	**	627	NA	**
3834	485.1792	9.61	**	**	544	NA	Rutaevin / Nafenopinglucuronide
1255	253.1440	9.67	**	**	556	NA	**



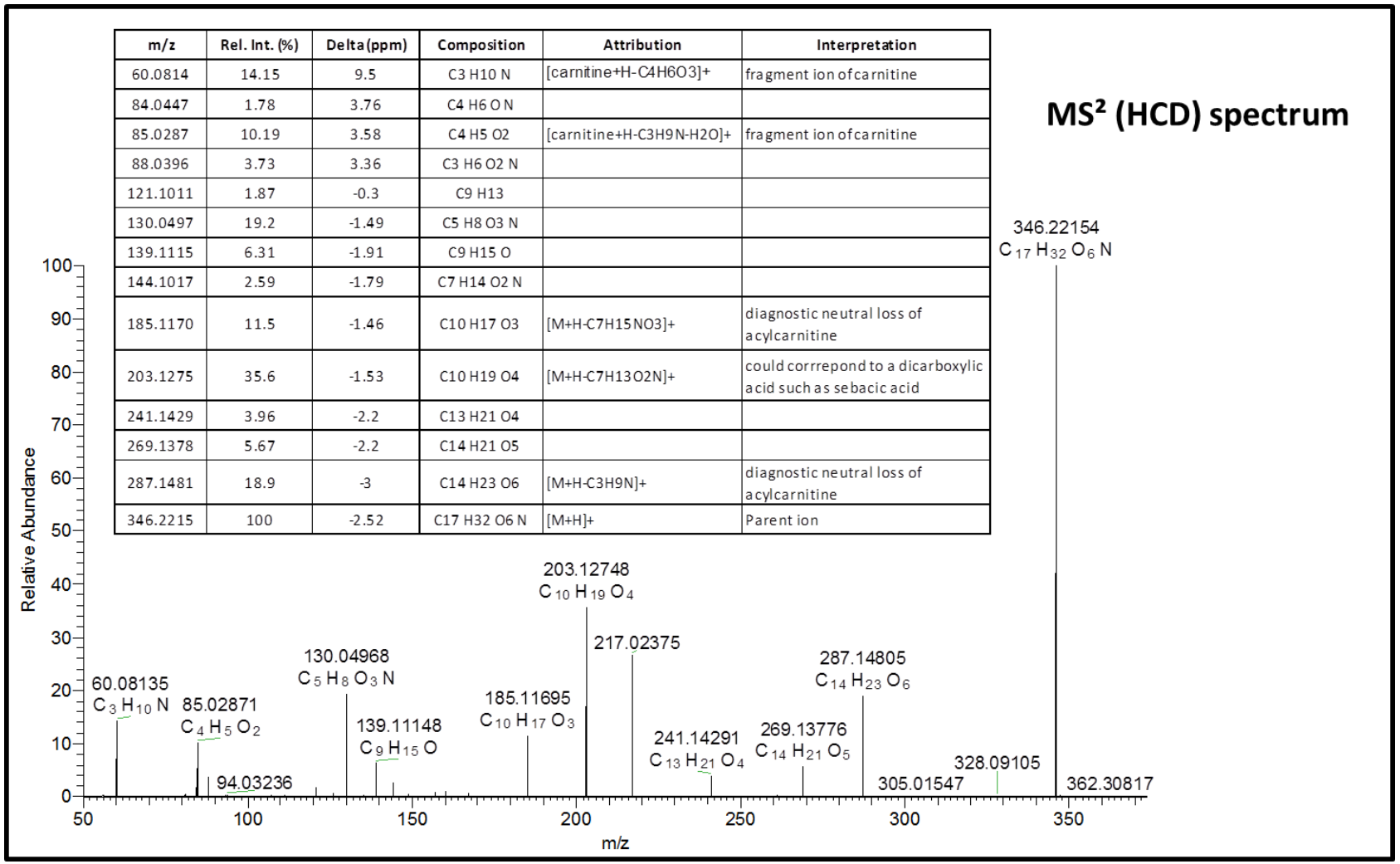
LEVEL 2 by diagnostic evidence

- No reference compound available in our library
- No MS² spectra in MassBank and mzCloud
- No isomers in HMDB and KEGG

(Roux A. et al., Anal. Chem., 2012)

Characterization of a dicarboxylic acylcarnitine in human urines
(m/z 346.2215, ESI pos)

m/z	Rel. Int. (%)	Delta (ppm)	Composition	Attribution	Interpretation
60.0814	14.15	9.5	C3 H10 N	[carnitine+H-C4H6O3]+	fragment ion of carnitine
84.0447	1.78	3.76	C4 H6 O N		
85.0287	10.19	3.58	C4 H5 O2	[carnitine+H-C3H9N-H2O]+	fragment ion of carnitine
88.0396	3.73	3.36	C3 H6 O2 N		
121.1011	1.87	-0.3	C9 H13		
130.0497	19.2	-1.49	C5 H8 O3 N		
139.1115	6.31	-1.91	C9 H15 O		
144.1017	2.59	-1.79	C7 H14 O2 N		
185.1170	11.5	-1.46	C10 H17 O3	[M+H-C7H15NO3]+	diagnostic neutral loss of acylcarnitine
203.1275	35.6	-1.53	C10 H19 O4	[M+H-C7H13O2N]+	could corrrepond to a dicarboxylic acid such as sebacic acid
241.1429	3.96	-2.2	C13 H21 O4		
269.1378	5.67	-2.2	C14 H21 O5		
287.1481	18.9	-3	C14 H23 O6	[M+H-C3H9N]+	diagnostic neutral loss of acylcarnitine
346.2215	100	-2.52	C17 H32 O6 N	[M+H]+	Parent ion



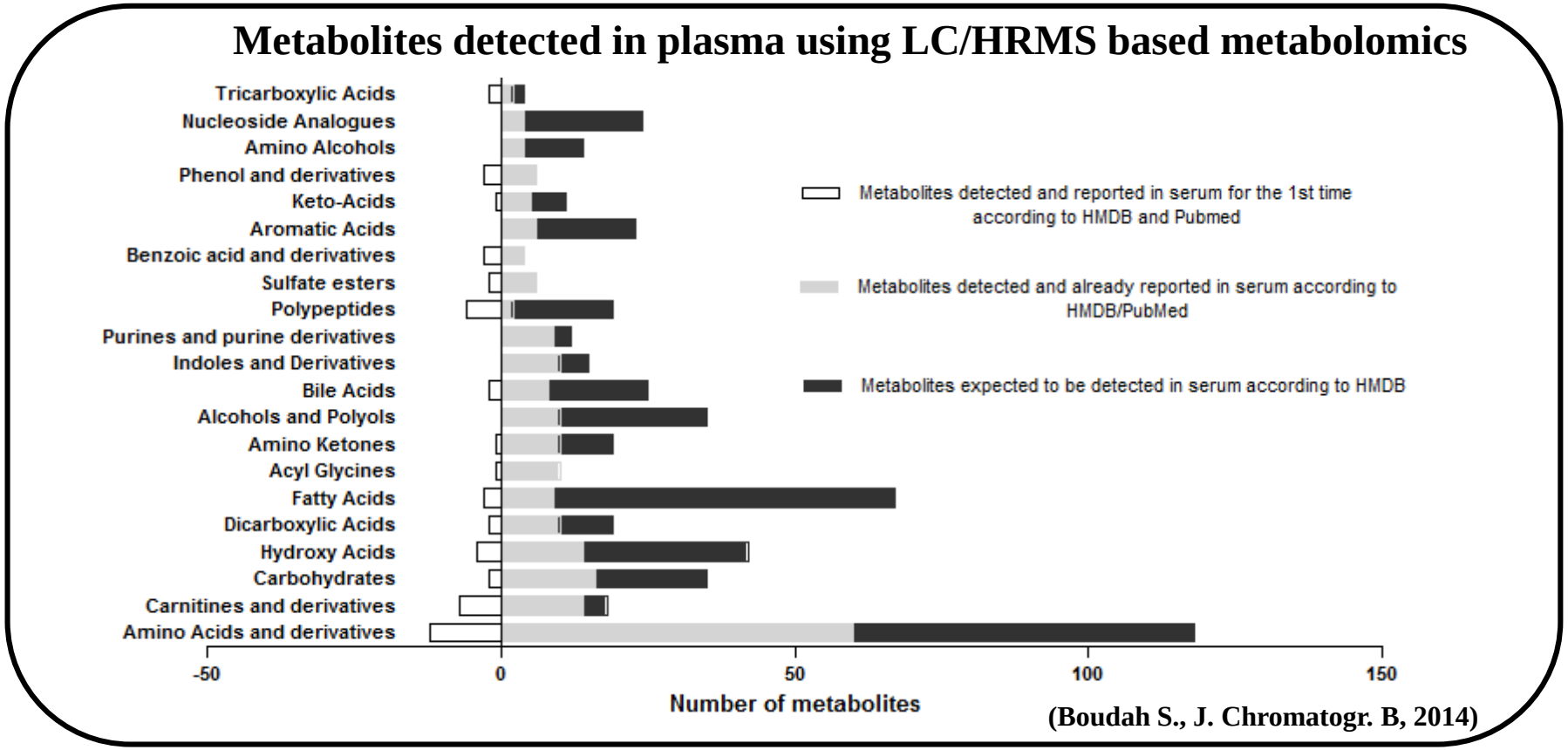
LEVEL 3

Could be sebacylcarnitine. However, the dicarboxylic acid moiety (C10H18O4) is not characterized

Non-targeted metabolomics:

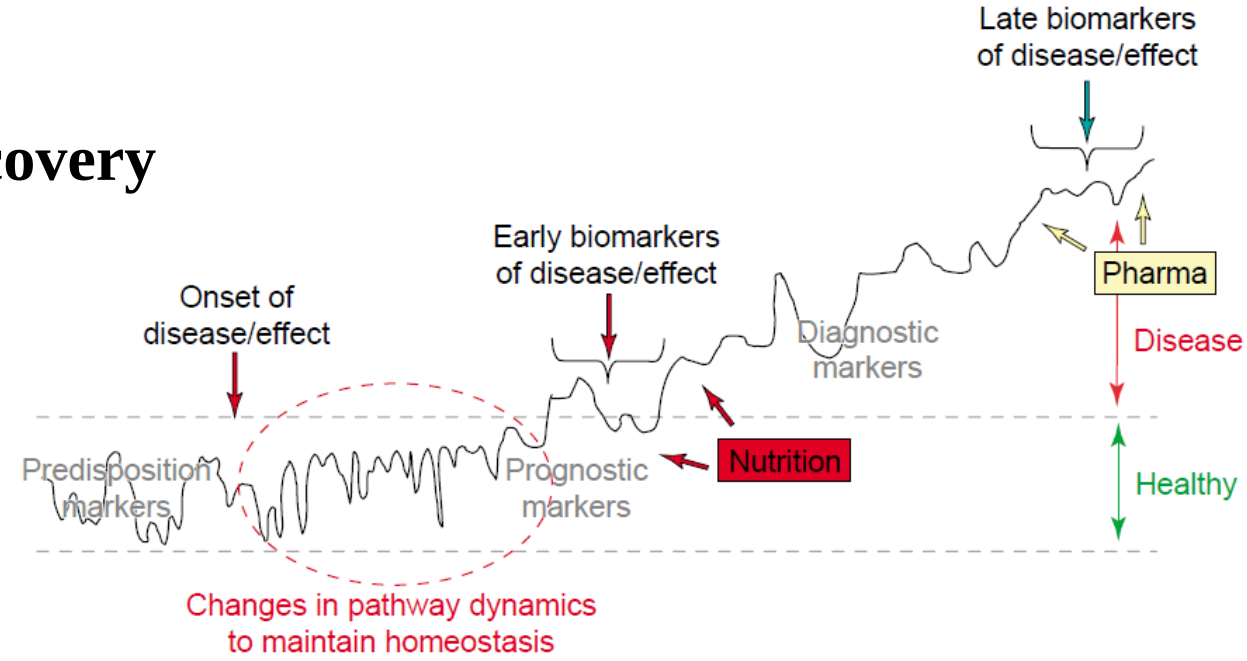
Metabolic phenotype (host-environment interactions)
Impact of the microbiota
Chemical exposome

≈ 250-350 metabolites annotated/identified in humans biofluids from several thousands of features



Why measuring metabolites?

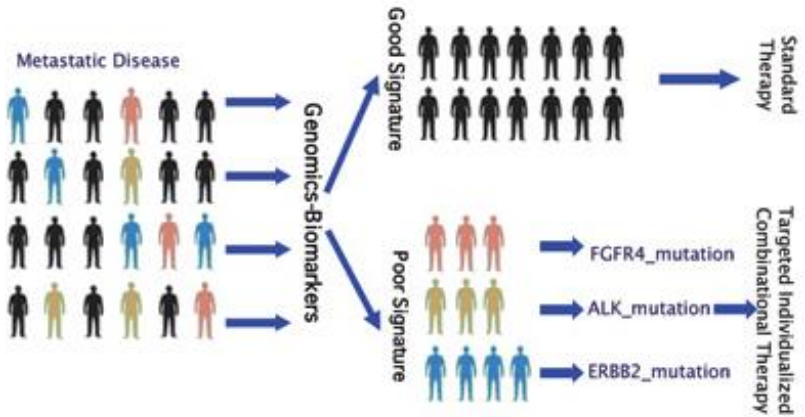
Biomarker discovery



van der Greef et al, Curr Opin Chem Biol 2004

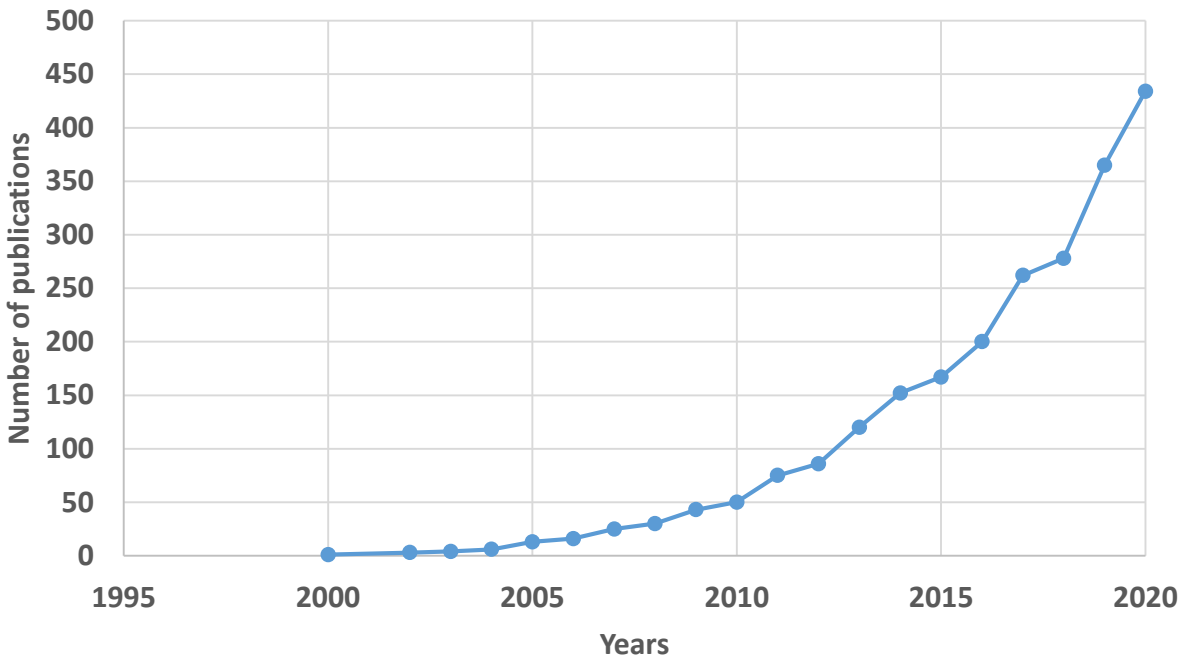
Systems biology

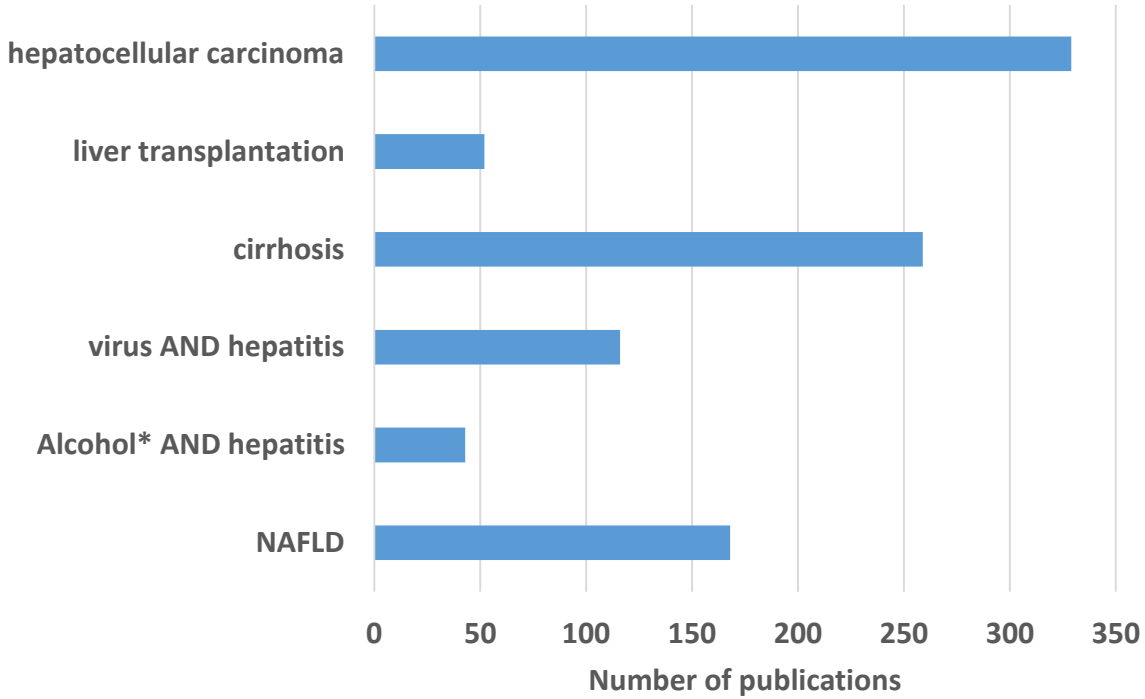
Personalized medecine



Metabolomics in the field of hepatology

> 2000 publications (Pubmed database)





Search query: (metabolom*[Title/Abstract] OR metabonom*[Title/Abstract] OR metabotyp*[Title/Abstract]) AND ((liver disease)[Title/Abstract] OR hepatitis[Title/Abstract] OR cirrhosis[Title/Abstract] OR (liver transplantation)[Title/Abstract] OR (hepatocellular carcinoma)[Title/Abstract])

Recent developments:

multi-omics integrated studies ++
impact of the gut microbiota ++

ARTICLES
https://doi.org/10.1038/s41591-018-0061-3
nature medicine

Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women

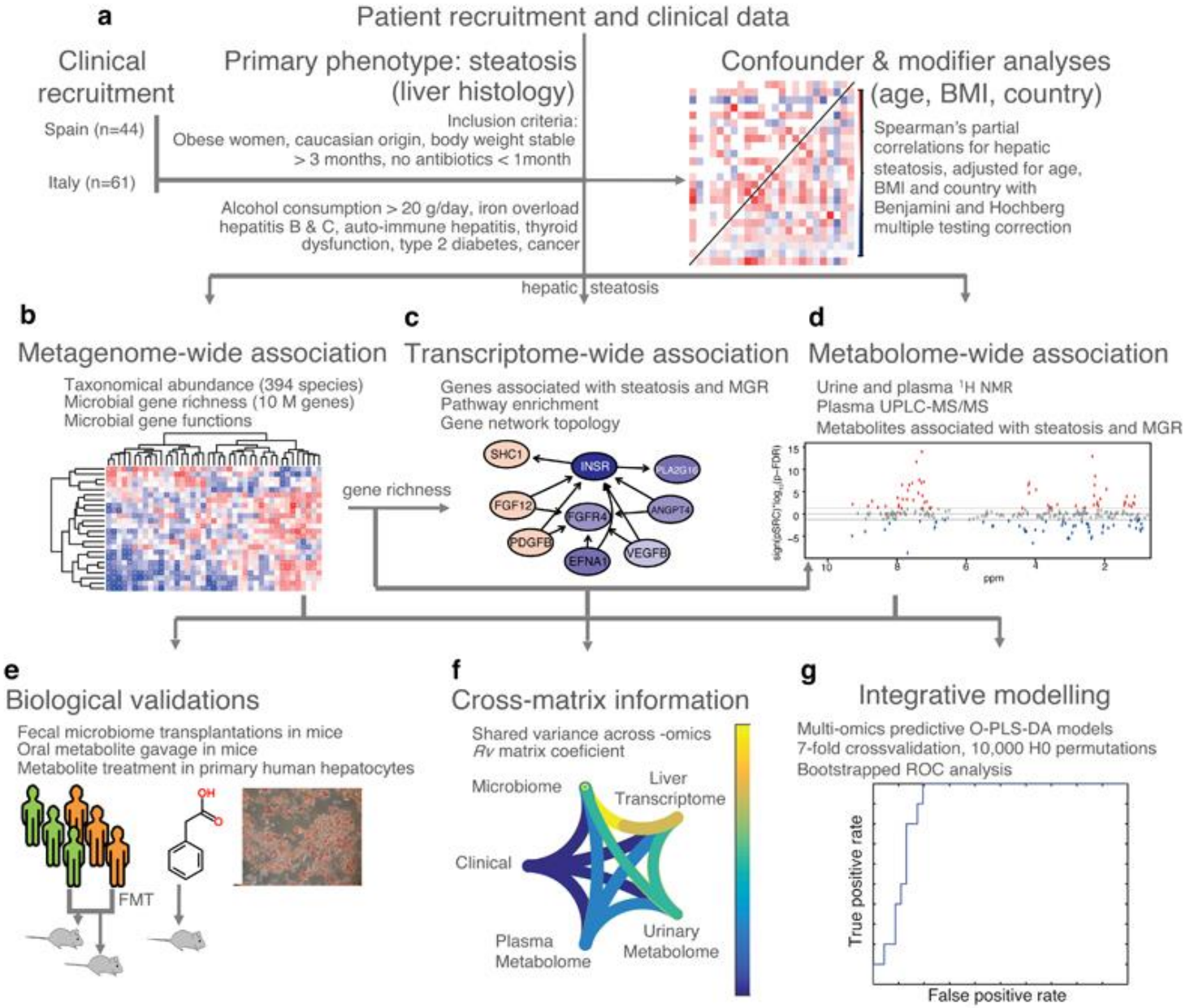
Lesley Hoyles[1,10], José-Manuel Fernández-Real[2,10*], Massimo Federici[3,10*], Matteo Serino[4,5], James Abbott[1], Julie Charpentier[4,5], Christophe Heymes[4,5], Jèssica Latorre Luque[2], Elodie Anthony[6], Richard H. Barton[1], Julien Chilloux[1], Antonis Myridakis[1], Laura Martinez-Gili[1], José Maria Moreno-Navarrete[2], Fadila Benhamed[6], Vincent Azalbert[4,5], Vincent Blasco-Baque[4,5], Josep Puig[2], Gemma Xifra[2], Wifredo Ricart[2], Christopher Tomlinson[1], Mark Woodbridge[1], Marina Cardellini[3], Francesca Davato[3], Iris Cardolini[3], Ottavia Porzio[7,8], Paolo Gentileschi[7], Frédéric Lopez[4,5], Fabienne Foufelle[9], Sarah A. Butcher[1], Elaine Holmes[1], Jeremy K. Nicholson[1], Catherine Postic[6], Rémy Burcelin[4,5*] and Marc-Emmanuel Dumas[1*]

Shotgun sequencing of fecal metagenome
Host phenome (hepatic transcriptome, plasma and urine metabolomics)

Molecular networks linking the gut microbiome and the host phenome to hepatic steatosis.

Steatosis: low microbial gene richness, hepatic inflammation, dysregulation of aromatic and branched chain AA metabolism.

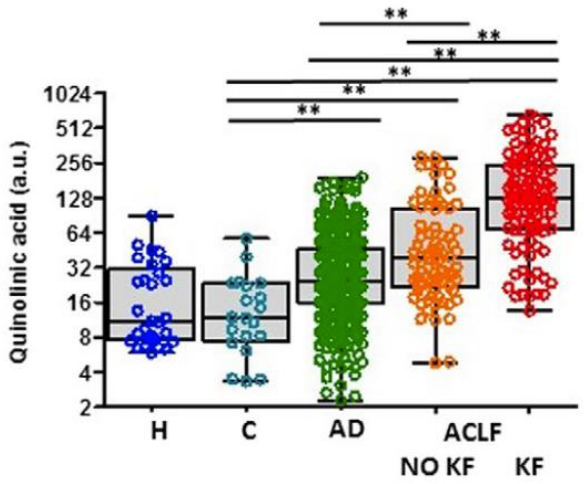
Microbiota transplants and chronic treatment with phenylacetic acid trigger steatosis and dysregulation of BCAA metabolism

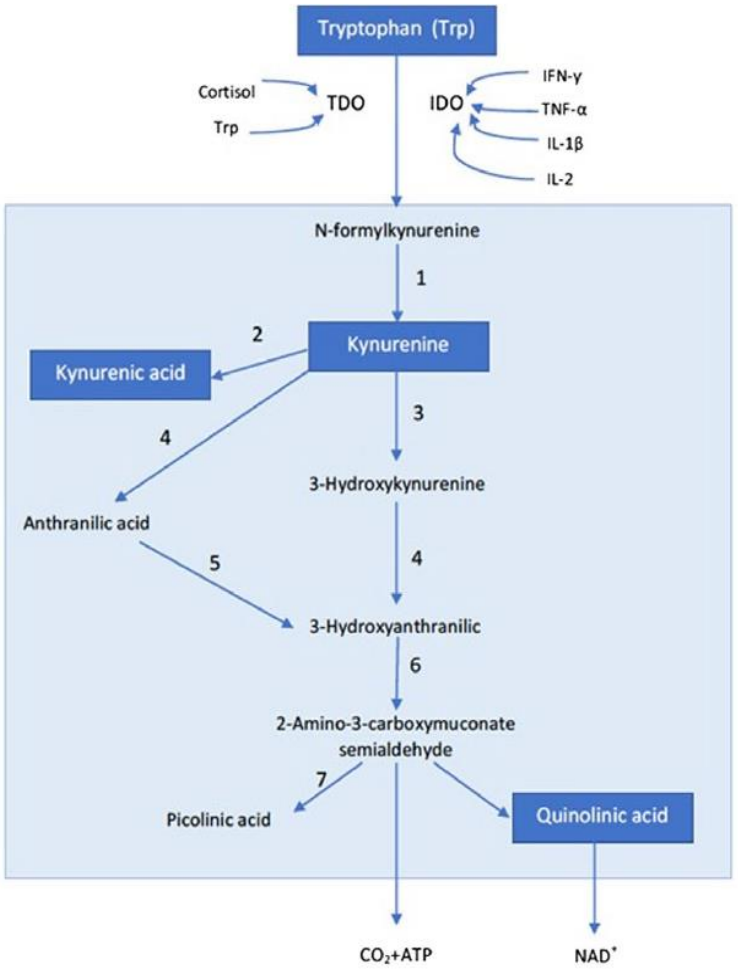


Orchestration of Tryptophan-Kynurenine Pathway, Acute Decompensation, and Acute-on-Chronic Liver Failure in Cirrhosis

Joan Clària,[1,2*] Richard Moreau,[1,3*] François Fenaille,[4] Alex Amorós,[1] Christophe Junot,[4] Henning Gronbaek,[5] Minneke J. Coenraad,[6] Alain Pruvost,[7] Aurélie Ghettas,[7] Emeline Chu-Van,[4] Cristina López-Vicario,[2] Karl Oettl,[8] Paolo Caraceni,[9] Carlo Alessandria,[10] Jonel Trebicka,[1,11,12] Marco Pavesi,[1] Carme Deulofeu,[1] Agustin Albillos,[13] Thierry Gustot,[14] Tania M. Welzel,[12] Javier Fernández,[1,2] Rudolf E. Stauber,[8] Faouzi Saliba,[15] Noémie Butin,[4] Benoit Colsch,[4] Christophe Moreno,[14] François Durand,[3] Frederik Nevens,[16] Rafael Bañares,[17] Daniel Benten,[18] Pere Ginès,[2] Alexander Gerbes,[19] Rajiv Jalan,[20] Paolo Angeli,[1,21] Mauro Bernardi,[9] and Vicente Arroyo[1]; for the CANONIC Study Investigators of the EASL Clif Consortium, Grifols Chair and the European Foundation for the Study of Chronic Liver Failure (EF Clif)

HEPATOLOGY, VOL. 69, NO. 4, 2019





Kynurenine pathway is activated in patient with acute decompensation and acute-on-chronic liver failure

Confirmation by a quantitative LC-MS/MS assay for kynurenic acid, quinolinic acid, kynurenine and tryptophan on 234 samples (validation cohort)

JOURNAL
OF HEPATOLOGY

Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF

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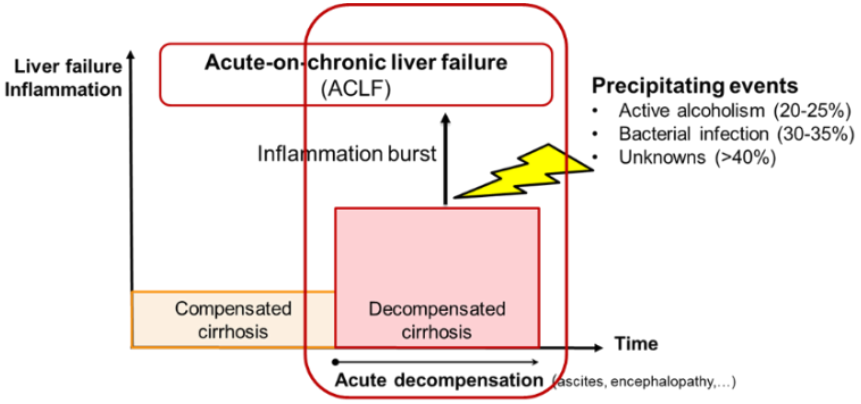

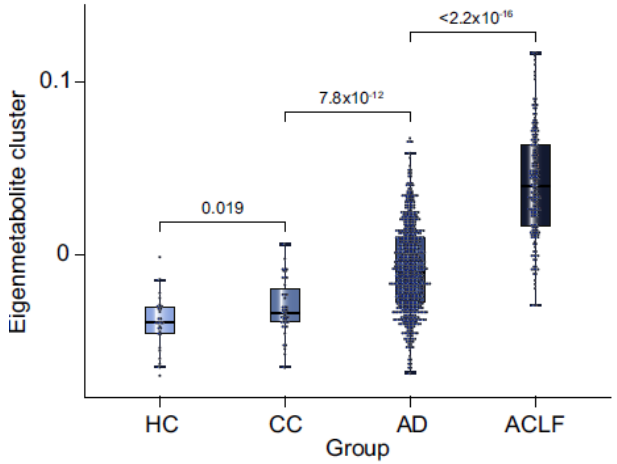

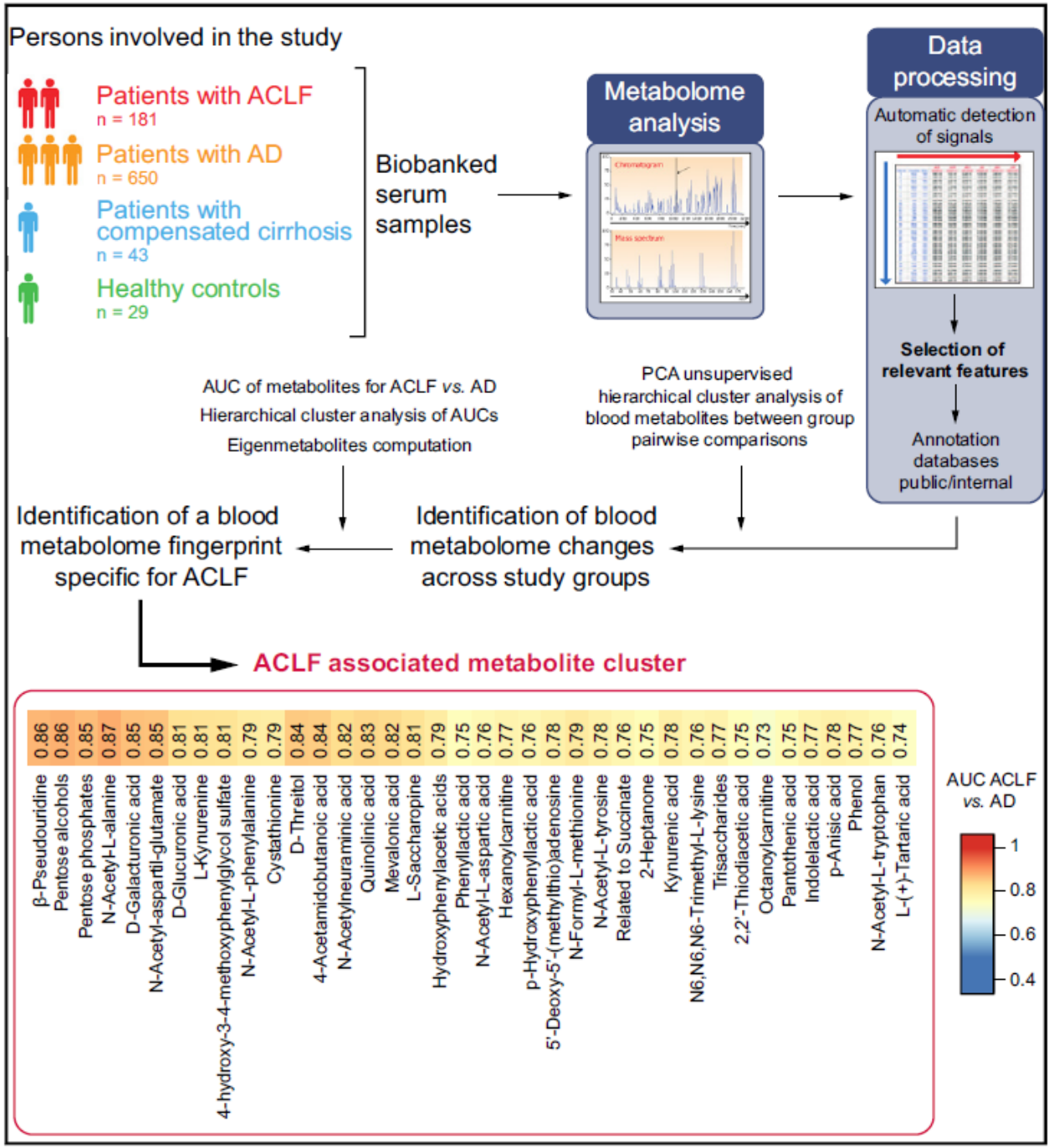

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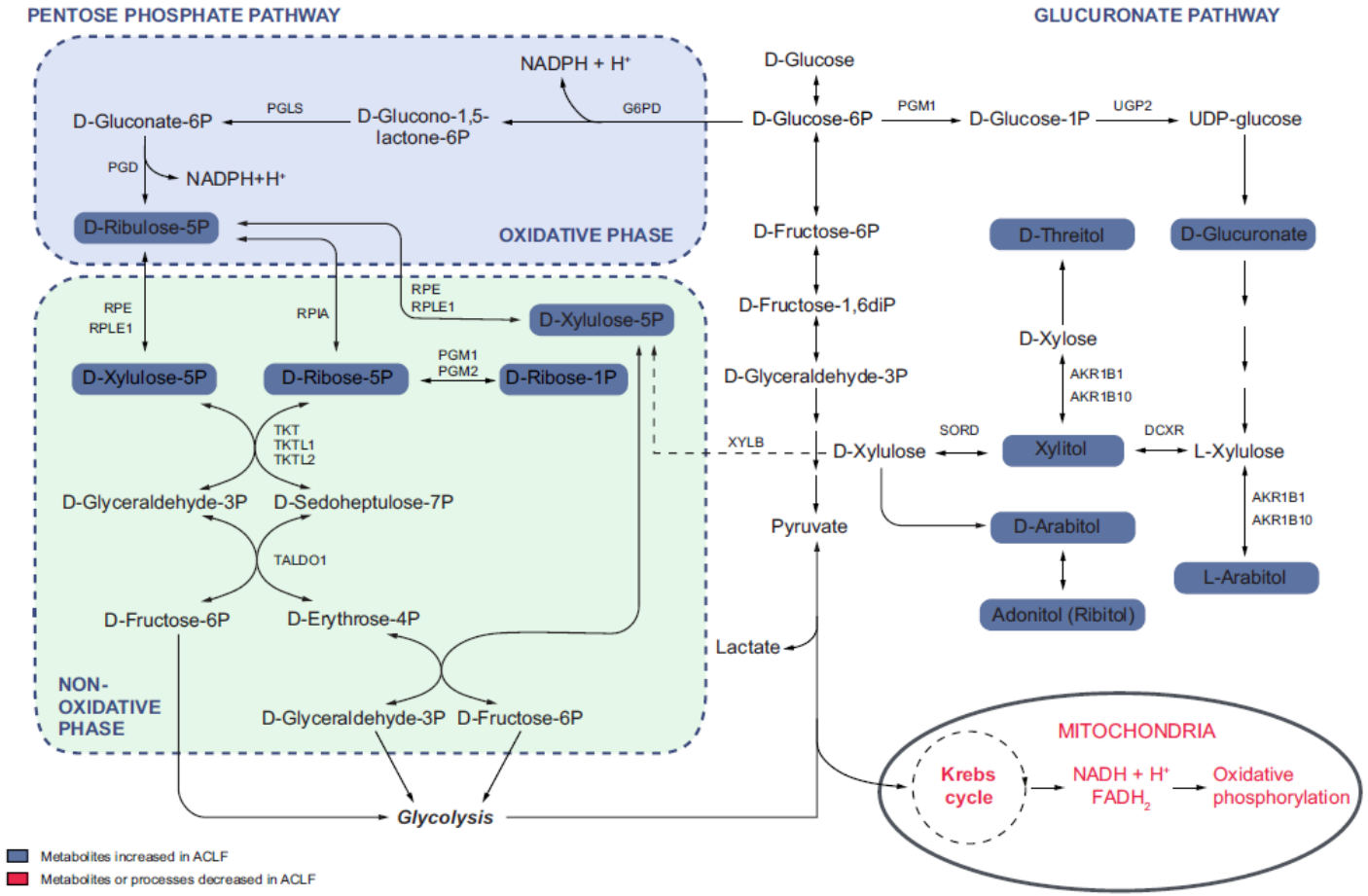
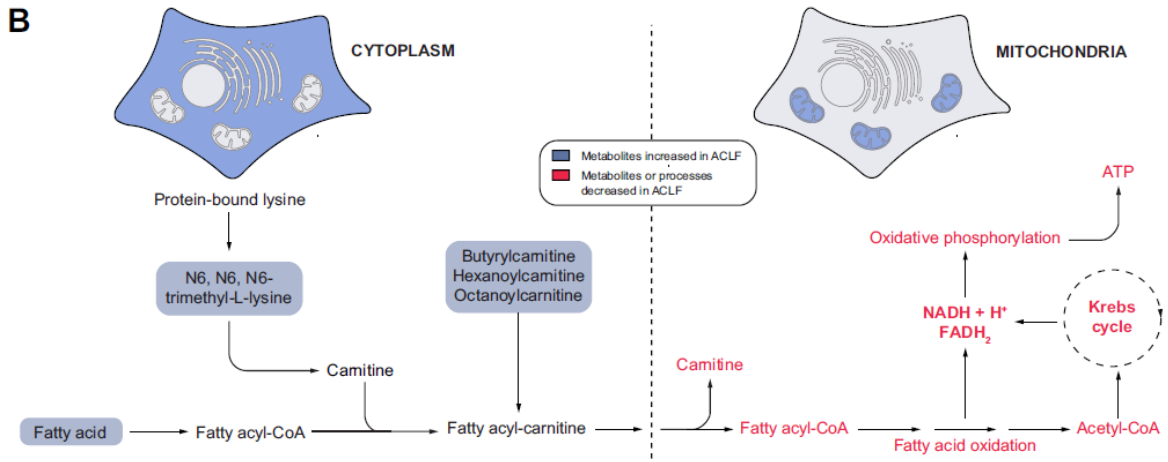
The ACLF associated metabolite cluster correlates with systemic inflammation.

It also reflects:

- increased proteolysis and lipolysis,
- changes in intracellular metabolism in response to the proliferation of innate immunity cells,
- Reduced mitochondrial oxydation,





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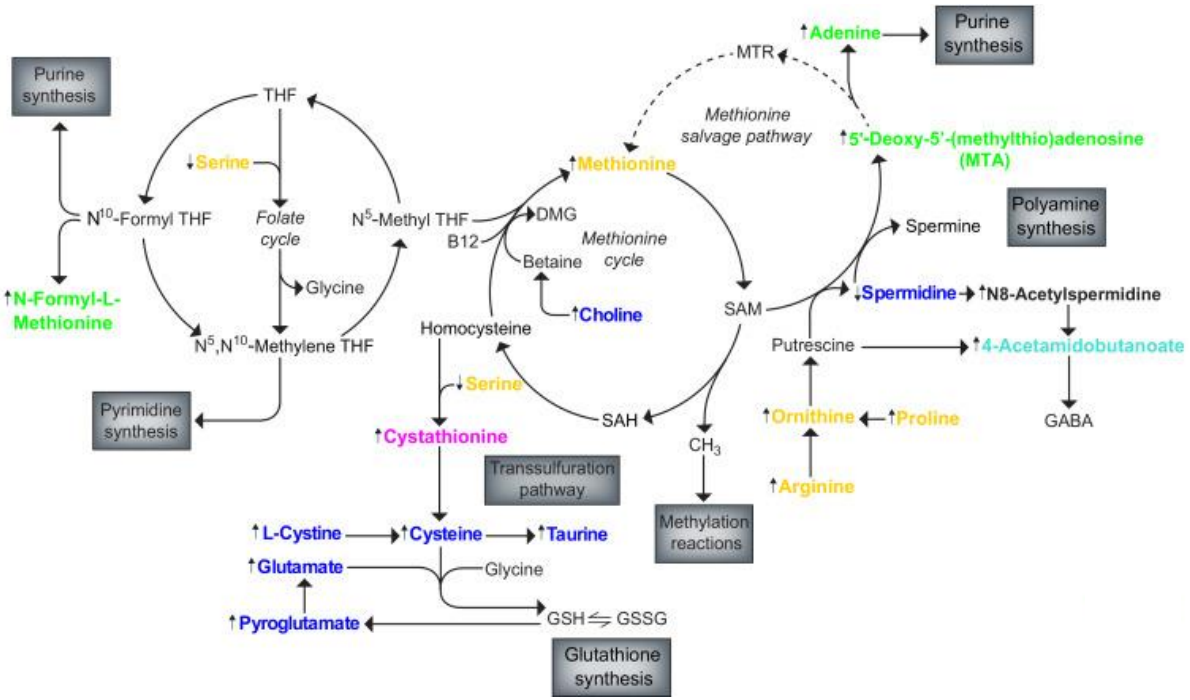
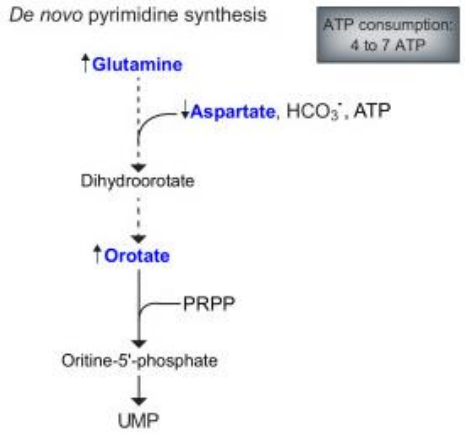
Assessing the role of amino acids in systemic inflammation and organ failure in patients with ACLF

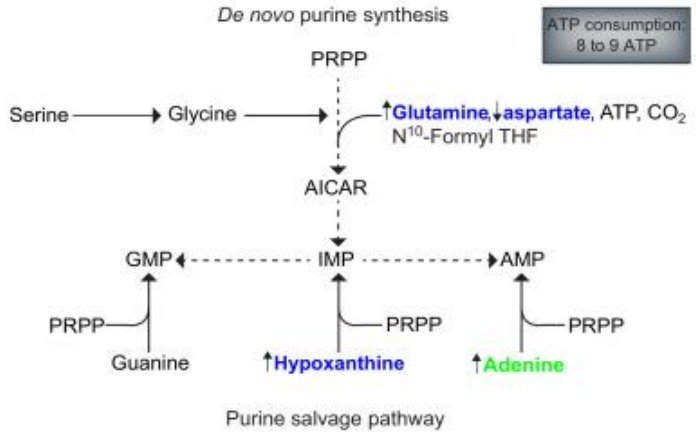
Giacomo Zaccherini[1,2,†], Ferran Aguilar[1,†], Paolo Caraceni[2], Joan Clària[1,3,4], Juan José Lozano[4], François Fenaille[5], Florence Castelli[5], Christophe Junot[5], Anna Curto[1], Chiara Formentin[6], Emmanuel Weiss[1,7], Mauro Bernardi[2], Rajiv Jalan[1,8], Paolo Angeli[1,6], Richard Moreau[1,9,10,*,‡], Vicente Arroyo[1,‡]

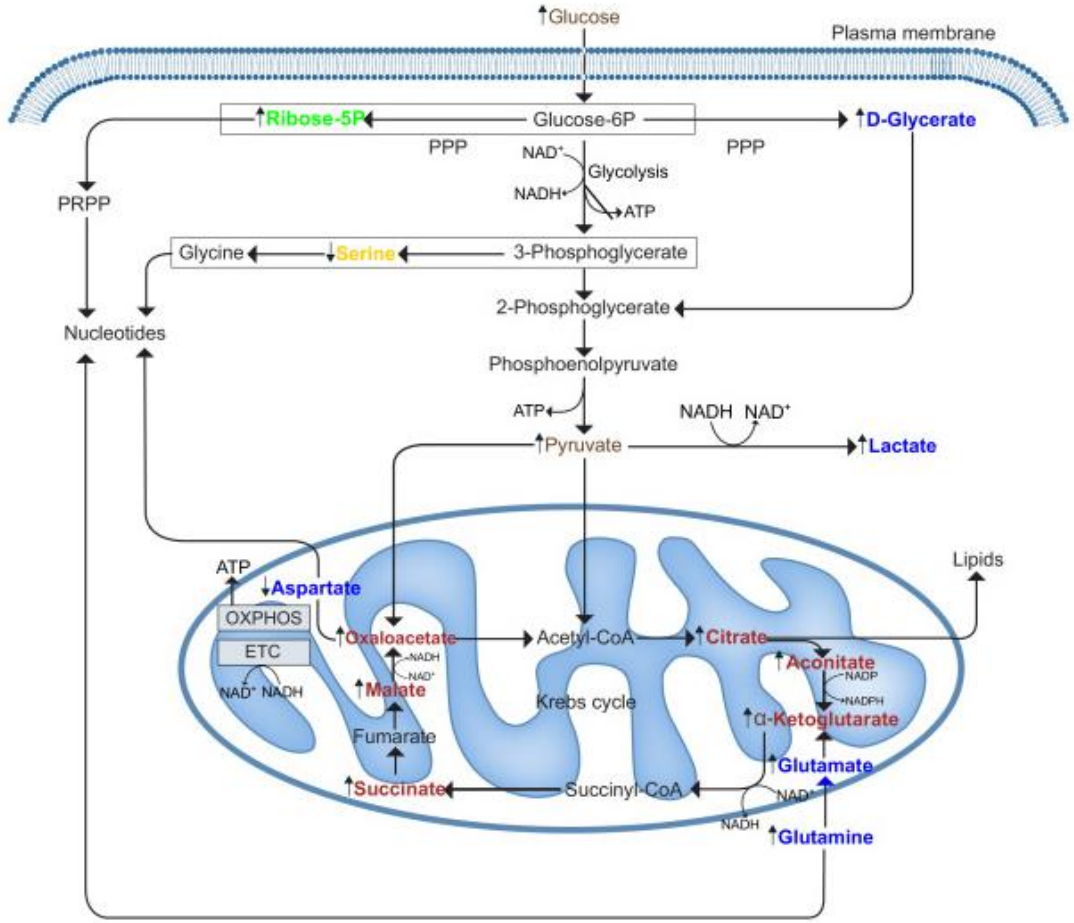
Reanalysis of the blood metabolomic data of the CANONIC study

- Blood AA fuel protein and nucleotide synthesis required for intense systemic inflammation.
- Extensive catabolism of ketogenic AA to produce energy substrates in peripheral organs









Which questions do we now address?

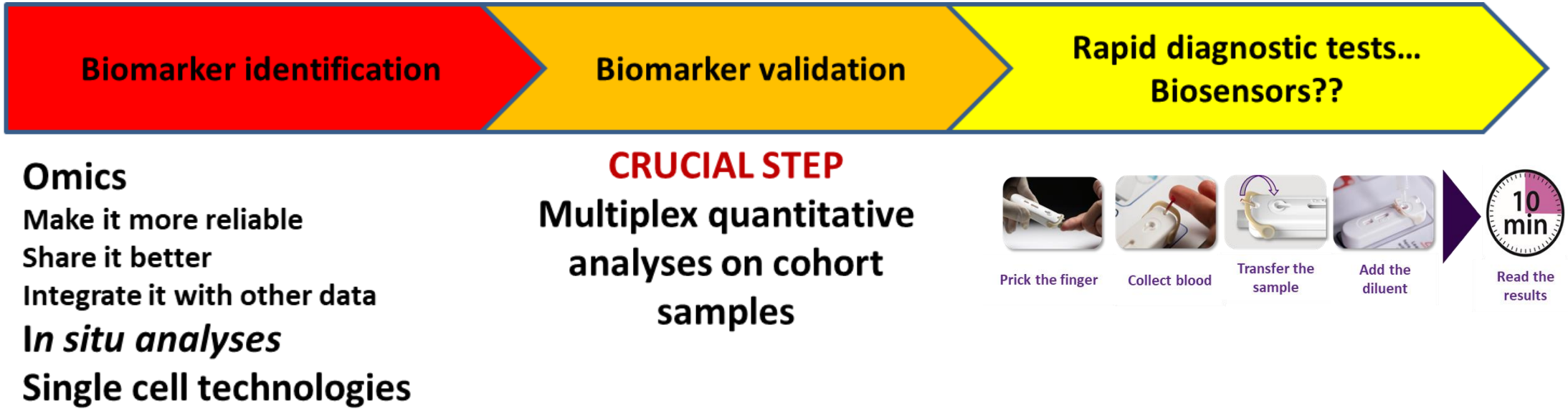
- Improve stratification power regarding the decompensation of cirrhosis
- Provide new insights into pathophysiology of ACLF (biomarkers of precipitants and organ failure)
- Response to treatment
- Pronostic biomarkers that could be translated into clinics

Take home messages

Metabolomics tools are nowadays enough mature to be used in the field of clinical research.

Improvement of data interoperability and reusability of untargeted metabolomic data is a key priority, which has to be addressed in a context of permanent and rapid technological evolution.

How to translate complex metabolomic signatures into clinics and care practice?



Many thanks for your attention,

to my colleagues,

François Fenaille

Florence Castelli

Benoit Colsch

Etienne Thévenot

Sebastian Burz

Sylvain Dechaumet

Emeline Chu-Van

to partners of the Microb-Predict and Decision projects

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