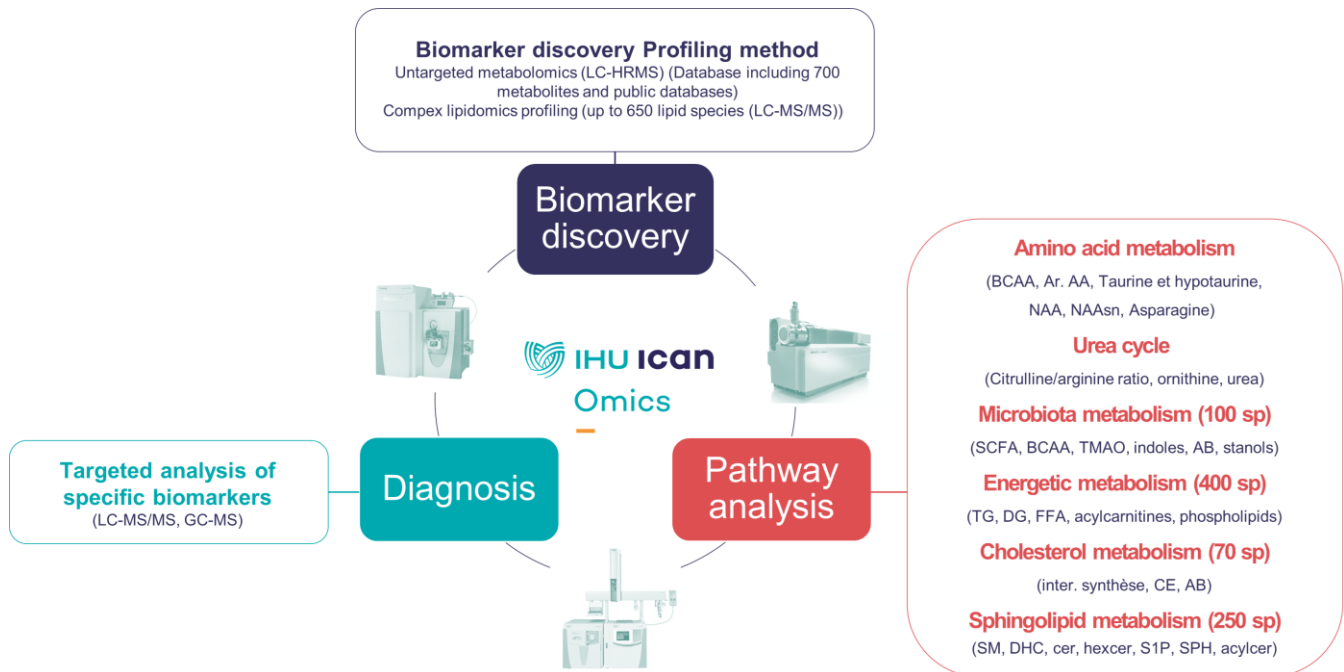


Several approaches were proposed by ICAN Omics metabolomics:

- **Untargeted approach for biomarker discovery**
- **Profiling methods for pathways analysis**
- **Targeted approach for candidate marker validation**



Untargeted approach

Aim: Propose a snapshot of the whole metabolome

List of features annotated based on a local and a public database

Local database includes over 650 metabolites + Public database includes over 10.000 chemical entities from KEGG and HmDB databases

Strategy of annotation and identification steps were based on standards proposed by L.W. Sumner et al. 2007 and 2014.

Sample requirements = 100-150 µl Biofluids (plasma/serum, urine), 100mg Faeces, 1.000.000 cells, 50mg of tissue (Liver, brain, heart...)

Profiling approach

Aim: Multiplex analyses of the metabolites usually linked to microbiota and cardiometabolic diseases

Screening of 100-140 metabolites derived from aminoacids metabolism, tryptophane-kynurenine pathways, methylamine metabolism, phenolic compounds, B-vitamins metabolism, short-chain acylcarnitines, organic acids and indoles

Aminoacid metabolisms (41) Alanine Acetyl-Glycine Arginine Asparagine Aspartate Citrulline Creatine Creatinine Methionine-Sulfoxide Glutamic acid Glycine Histidine Homocysteine Isoleucine Kynurenine Leucine Glutamine Lysine Proline Serine Methionine N-acetyl-alanine N-acetyl-glutamate N-acetyl-tyrosine N-Acetyl-L-phenylalanine 5-Oxoprolin N-alpha-acetyl-arginine Ornithine Phenylalanine Threonine-Homoserine Tryptophan Tyrosine Valine N6,N6,N6-Trimethyl-L-lysine N(pi)-Methyl-L-histidine Hexanoylglycine N-Acetyl-L-Leucine 2-Furoylglycine Cinnamoylglycine alpha-N-Phenylacetyl-L-glutamine Total DMA (ADMA-SDMA)	Tryptophan-kynurenine metabolisms (21) 2-aminoadipic acid 3-Hydroxykynurenine 3-Hydroxytryptophane 3-indolepropionate Anthranilic acid Hydroxyanthranilic acid Indole-3-acetate Indole-3-lactate Kynurenic acid Kynurenine Melatonin N-acetyl-tryptophane Niacinamide (B3) Nicotinic acid (B3) N-methylnicotinamide Picolinic acid Quinaldic Quinolinic acid Serotonin Tryptophan Xanthurenic acid	Organic acids (34) 2-hydroxyoctanoate Lactate 2-OH-butyrate Adipate Succinate Aconitate (Cis&trans) Citrate-Isocitrate Fumarate Hippurate Homovanillate Hydroxyhippurate Pipecolate Suberate Urocanate 4-Aminobutanoate 4-Acetamidobutanoate 2-Hydroxy-3-methylbutyric acid 3-Hydroxy-3-methylbutyric acid 2-Hydroxyhexanoic acid Malate 4-Hydroxybenzoate 3-Hydroxybenzoate 4-Acetamidobutanoate 2-Oxoglutarate Mandelate 3-Phenylactic Acid 3-(4-Hydroxyphenyl)lactate 2-Methylhippuric acid Ferulic acid Alpha-Hydroxyhippuric Acid Syringic acid Glutarate Pimelic acid Hydrocinnamic Acid	Indoles (9) 5-methoxyindoleacetate 5-Hydroxyindoleacetate (5-HIAA) IndoxylSulfate 3-Indolebutyric acid 3-Indolepropionic acid Indole-3-carboxylic acid Indole-3-pyruvic acid Indole-3-carbaldehyde Indole-3-acetaldehyde	
	Purine and pyrimidine metabolisms (22) 1-Methyladenosine 3'-deoxyguanosine 7-methylguanine adenine cytidine cytosine Deoxycytidine deoxyinosine deoxyuridine Guanine Guanosine Hypoxanthine inosine Methylcytidine Pseudouridine thymidine thymine Uracil Urate uridine 3-Methyladenine Xanthine	B-vitamins (10) 6-Methylnicotinamide Pyridoxic acid (Vit B6) Ascorbic acid (Vit C.) N-methylnicotinamide p-aminobenzoic acid (Vit B10) Pantothenic acid (B5) Pyridoxal (Vit B6) Pyridoxamine (Vit B6) Riboflavin (Vit B2) Thiamine (Vit B1)	Phenol, nutrient-related and exogene metabolites (8) 4-Nitrophenol Trigoneiline Dihydrocaffeic acid Theobromine Caffeine Acetaminophen Vanillin Metformin	Methylamine and polyamines metabolisms (9) 2-PhenylEthylamine Betaine Butyrobetaine Carnitine Choline TMAO N-Acetyl-putrescine Diethanolamine Acetylcholine

Targeted Approach

Aim: Analyses of published markers linked to microbiota activity and cardiometabolic diseases

3 available methods:

- 27 metabolites derived from aminoacids metabolism
- 19 metabolites from the tryptophane-kynurenine pathways
- 6 metabolites derived from dietary choline metabolism
- 6 SCFA metabolites*

Sample requirements per method = 50µl Biofluids (Plasma/Serum/Urine)*, 50mg of faeces, 500.000 cells

Validation only for faeces, caecal content

Tryptophan-tryptophan pathways (19)

2-aminoadipic acid
3-Hydroxyanthranilic acid
3-Hydroxykynurenine
Anthranilic acid
Kynurenic acid
Kynurenine
Picolinic acid
Quinaldic
Quinolinic acid
Tryptophan
Xanthurenic acid
Serotonin
Melatonin
Nicotinamide
Hydroxy-tryptophan
Nicotinate
Indole-3-acetate
Indole-3-lactate
Indole-propionate

Aminoacids metabolism (27)

Alanine
Arginine
Aspartic acid
Glutamic acid
Glycine
Lysine
Leucine
Methionine
Phenylalanine
Proline
Serine
Threonine
Tyrosine
Valine
Cystine
Isoleucine
Acetylarginine
ADMA
SDMA
Asparagine
Citrulline
Cysteine
Glutamine
Hippurate
Histidine
Homocysteine
Taurine

Dietary choline metabolisms (6)

Trimethylamine N-oxide	TMAO
Choline	Choline
L-carnitine	AC-C0
Betaine	betaine
Gamma-Butyrobetaine	γ-Bb
Acetyl-carnitine	AC-C2

Short chain fatty acids (6)

Acetate
Propionate
Butyrate
Isobutyrate
Valerate
Isovalerate

The pipeline of analyses were standardized as follow :

