Biomarker analysis using LC-MS/MS

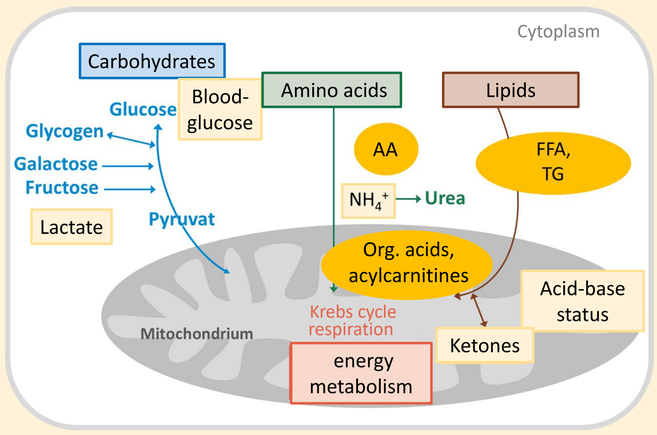
Sabrina Sailer, Tereza Vlasáková, Benjamin Natha, Markus A. Keller

Institute of Human Genetics, Biochemical Genetics, contact: markus.keller@i-med.ac.at

Inborn Errors of Metabolism: The Diversity of Metabolites

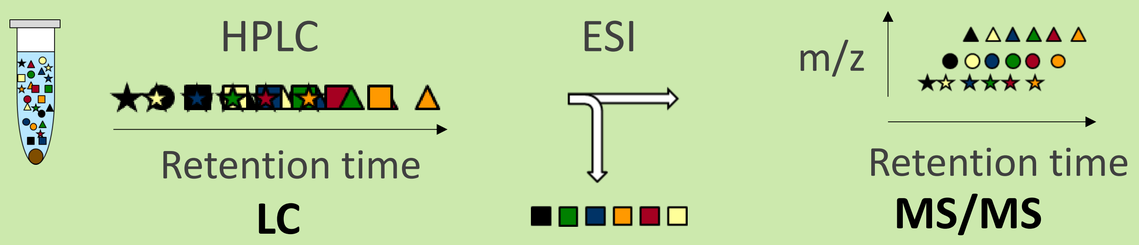
- Human genome: 2,9 x 10^9 base pairs [1]
- Proteins: 20.000-25.000
- endogenous metabolites: 222.860 [2]
- Disease-associated metabolites: 22.600
- Currently, more than 1,500 inherited metabolic disorders have been described [3].

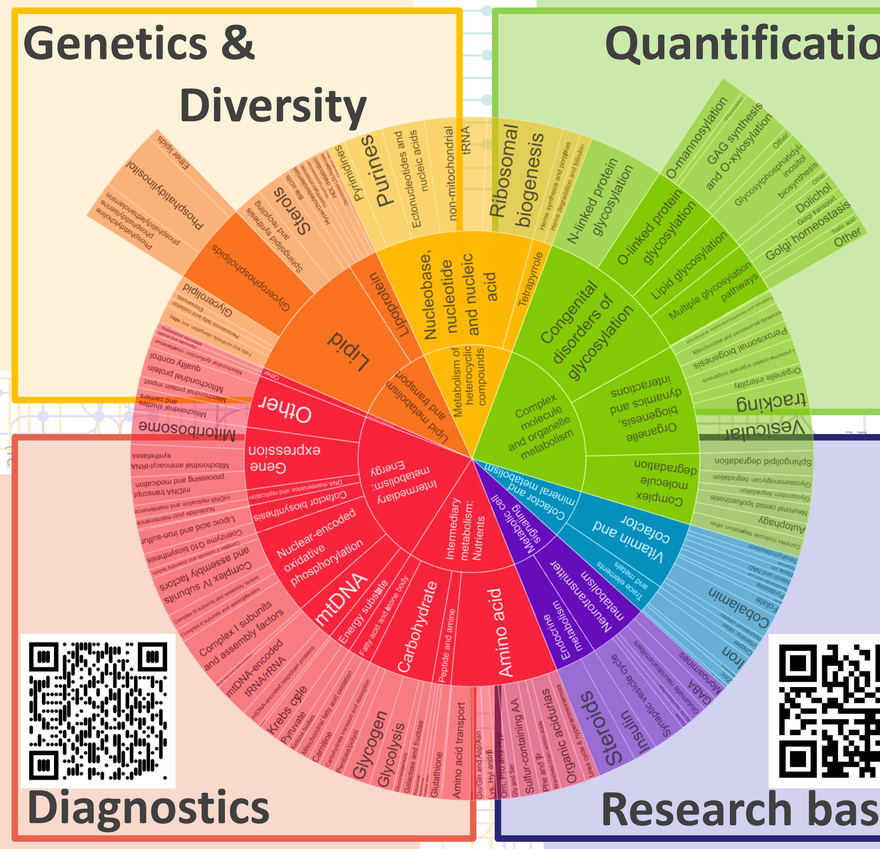
- Pathomechanisms: (i) Accumulation of toxic substrates or non-degraded substrates, (ii) Deficiency of the reaction product, (iii) Excess of the reaction product, (iv) Disturbance of energy supply



Mass spectrometric analysis by electrospray ionization (ESI)

- Separation of compounds based on physicochemical properties using high-performance liquid chromatography (HPLC)
- Mass spectrometric separation of analytes according to their mass-to-charge ratio (m/z)
- Generation of characteristic fragment ions by collision-induced dissociation (CID)





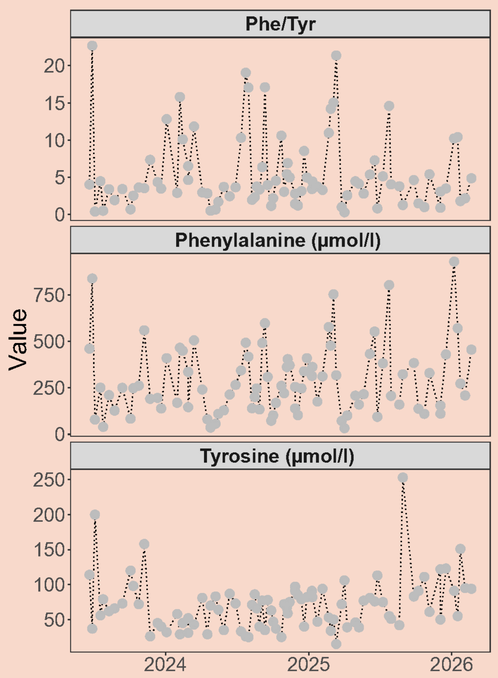
Advantage:
1. Accuracy: Identification and differentiation of analytes based on their mass-to-charge ratio (m/z) and fragmentation patterns.
2. Efficiency: Simultaneous analysis of numerous metabolites within a short time.
3. High degree of automation.

Acylcarnitines ISO 15189

Biomarkers for 28 diseases [4]
- Carnitin-Transporter deficiency: CPT1, CPT2 & CACT
- β-oxidation defects: SCADD, MCADD, VLCADD, LCHADD
- Organic acidurias: PA, MMA, GA
- Ketogenic diet for epilepsy patients i.e. C4OH – ketosis marker

Aminoc acids ISO 15189

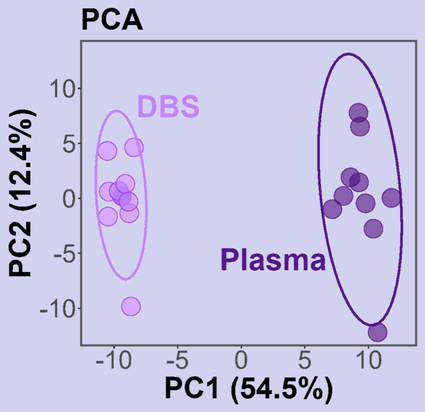
Biomarkers for > 50 diseases [5]
- Phenylalanine & Tyrosine: PKU
- Branched chain amino acids
- Schwefelhaltige Aminosäuren Hypermethioninämie

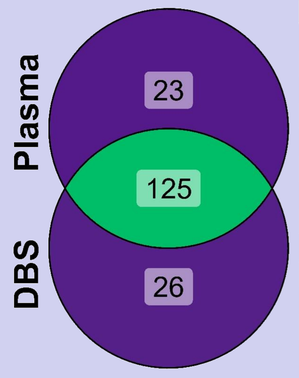


PKU follow up controls over the past years.

Mitochondrial β-oxidation during training. Use of acylcarnitines to fuel the body.

Know your matrix: Lipid composition in DBS and Plasma

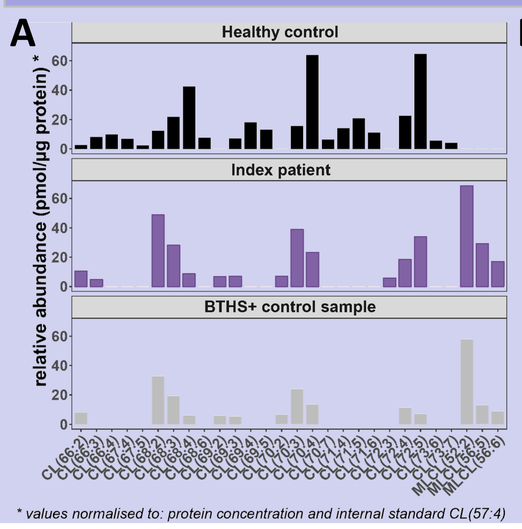




Lipid profiles from DBS and plasma are not fully overlapping, with unique features identified in each matrix.

Lipids and diagnostics

Lipids represent a highly heterogeneous class of metabolites due to the wide variety of possible side-chain configurations (~ 50.000 distinct species) [6].
- Barth Syndrom: mitochondrial lipids – Cardiolipins
- Peroxisomal linked diseases: phospholipid analysis of etherlipids and plasmalogens



Cardiolipn analysis in **A)** patient derived fibroblasts and **B)** DBS cards show distinct patterns in Barth Syndrom.

References

1. Pray et al, 2008, Nature Education; 2. https://hmdb.ca/; 3. Ferreira et al, 2021, J. Inherit. Metab. Dis.; 4. MJ Miller et. al., 2021, Genetics in Medicine; 5. Ling et al., 2023, Signal Transduct. Target. Ther.; 6. https://www.lipidmaps.org/databases/lmsd/overview