

A New Era in Therapies for Rare Diseases

The Alice Approach: Down the Rabbit Hole and Back Up Again

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UPMC | **CHILDREN'S**
HOSPITAL OF PITTSBURGH



Disclosures

Research funding

- NIH
- Ultragenyx
- Biomarin
- Homology
- Shire
- Aeglea
- Alexion
- Reynolds family
- Wilson family
- Burch family
- CHP Foundation
- Moderna
- CoA Therapeutics
- Stealth
- PTC Therapeutics
- Synlogic
- Kriya
- Agios
- LogicBio

Consulting

- BioMarin
- BioLogic
- Synlogic
- JNANA
- Sanofi
- Axcella Health
- Homology
- Agios
- Applied Therapeutics



Why study rare diseases?





Surprising statistics

- 3% of infants are born with a genetic problem
- 0.5% of babies have an inborn error of metabolism
- 40% of infant and childhood mortality
- 50% of childhood hospital admissions
- 25% of adult hospital admissions





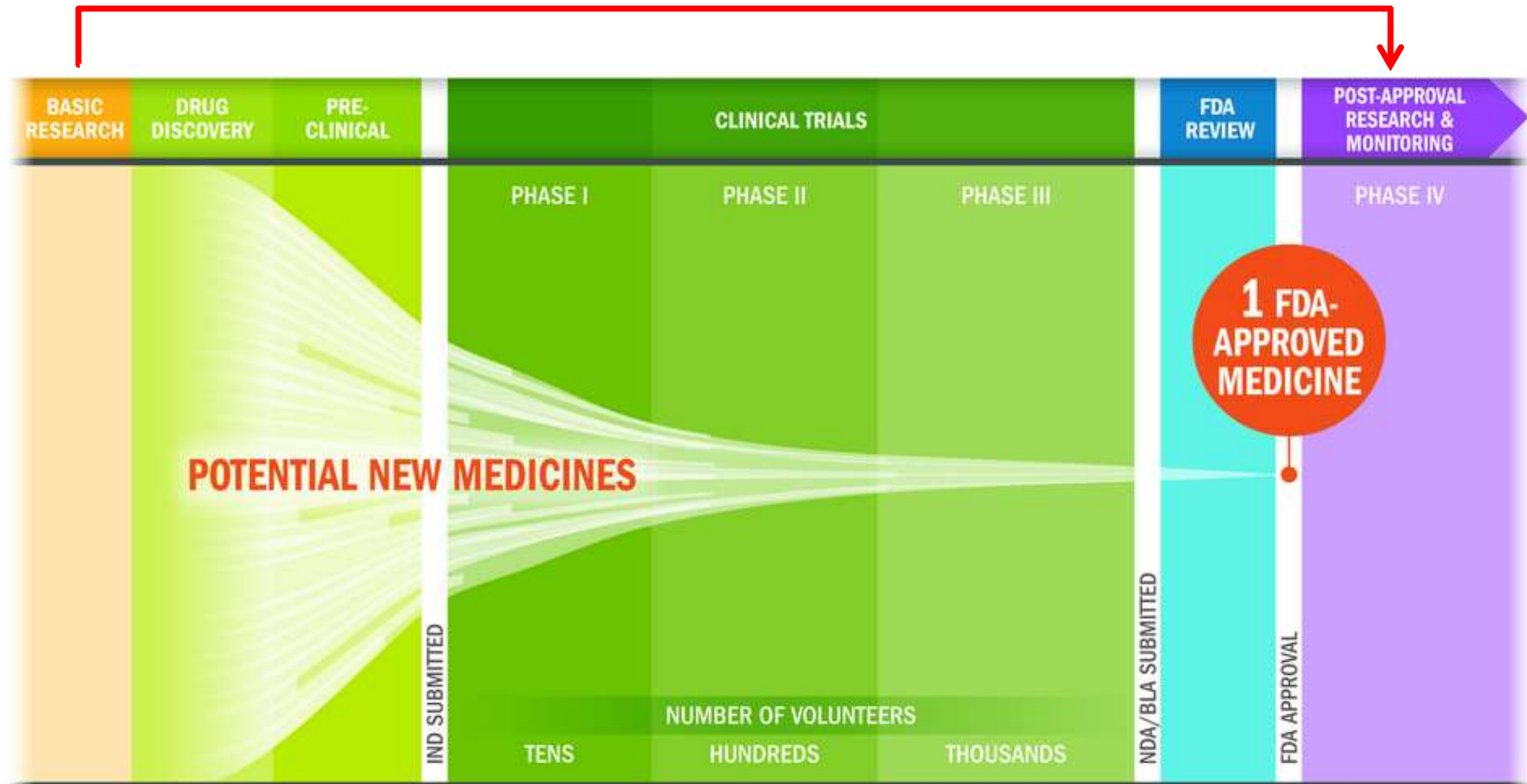
The problem in IEMs

- Metabolites need to proceed to the right place at the right speed at the right time in metabolic pathways
- Blocks have two consequences
 - A deficit of what is supposed to be coming through the pathway
 - Build up of compounds that shouldn't be there behind the block
- Either or both can lead to clinical symptoms



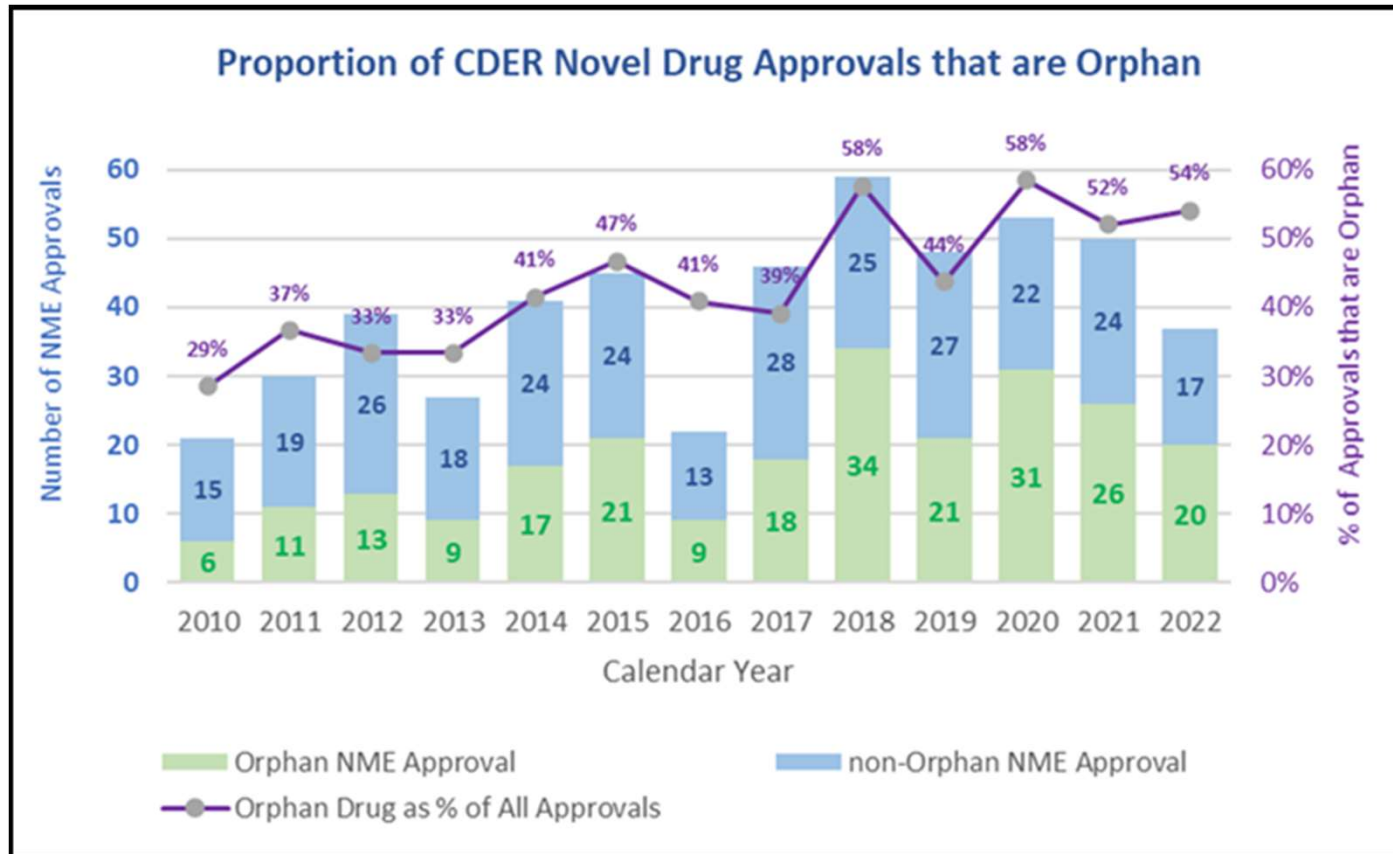


Drug development pipeline





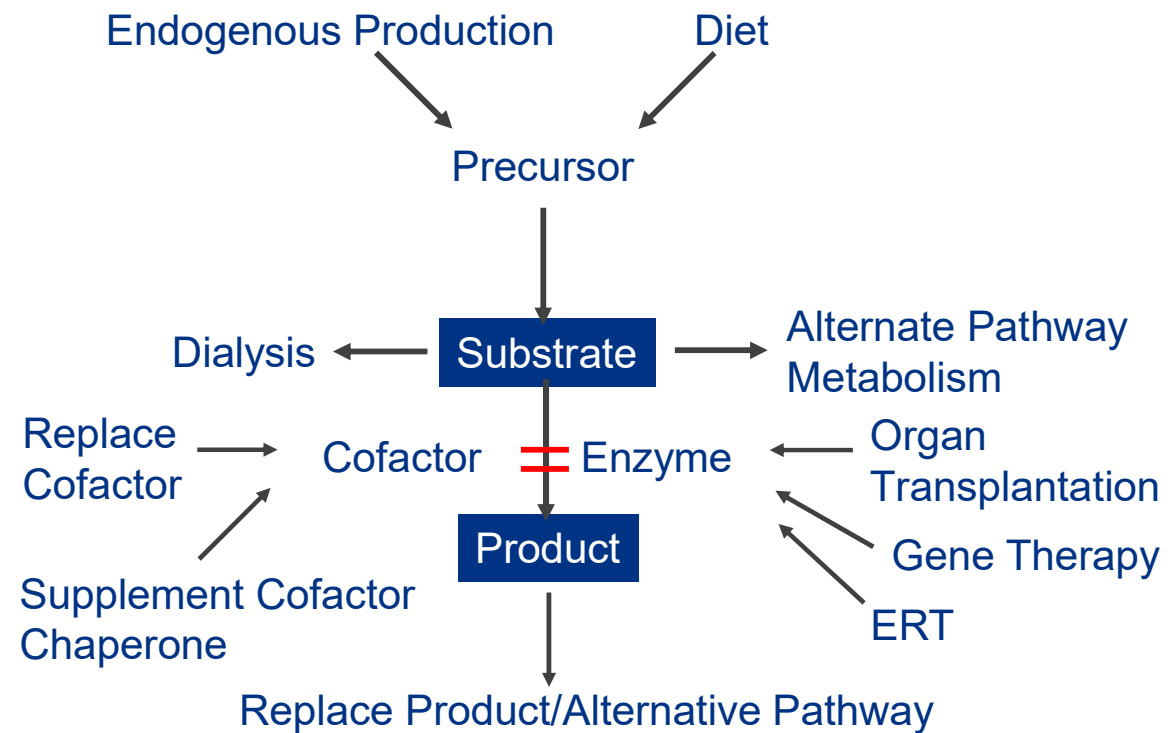
Center for Drug Evaluation and Research





- Replace/substitute/stabilize enzyme
- Replace product
- Reduce substrate
- Remove toxin
- Supplement cofactor
- Activate alternative pathways for metabolism
- Provide alternative substrates

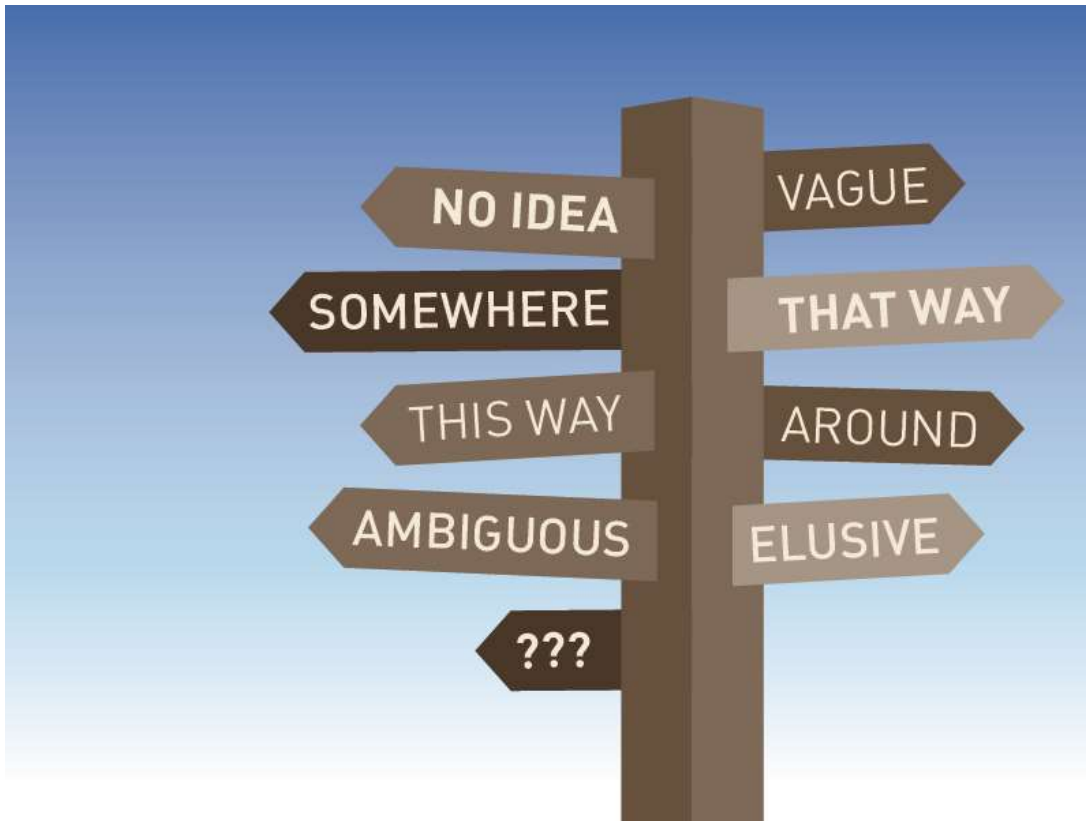
Options for therapy







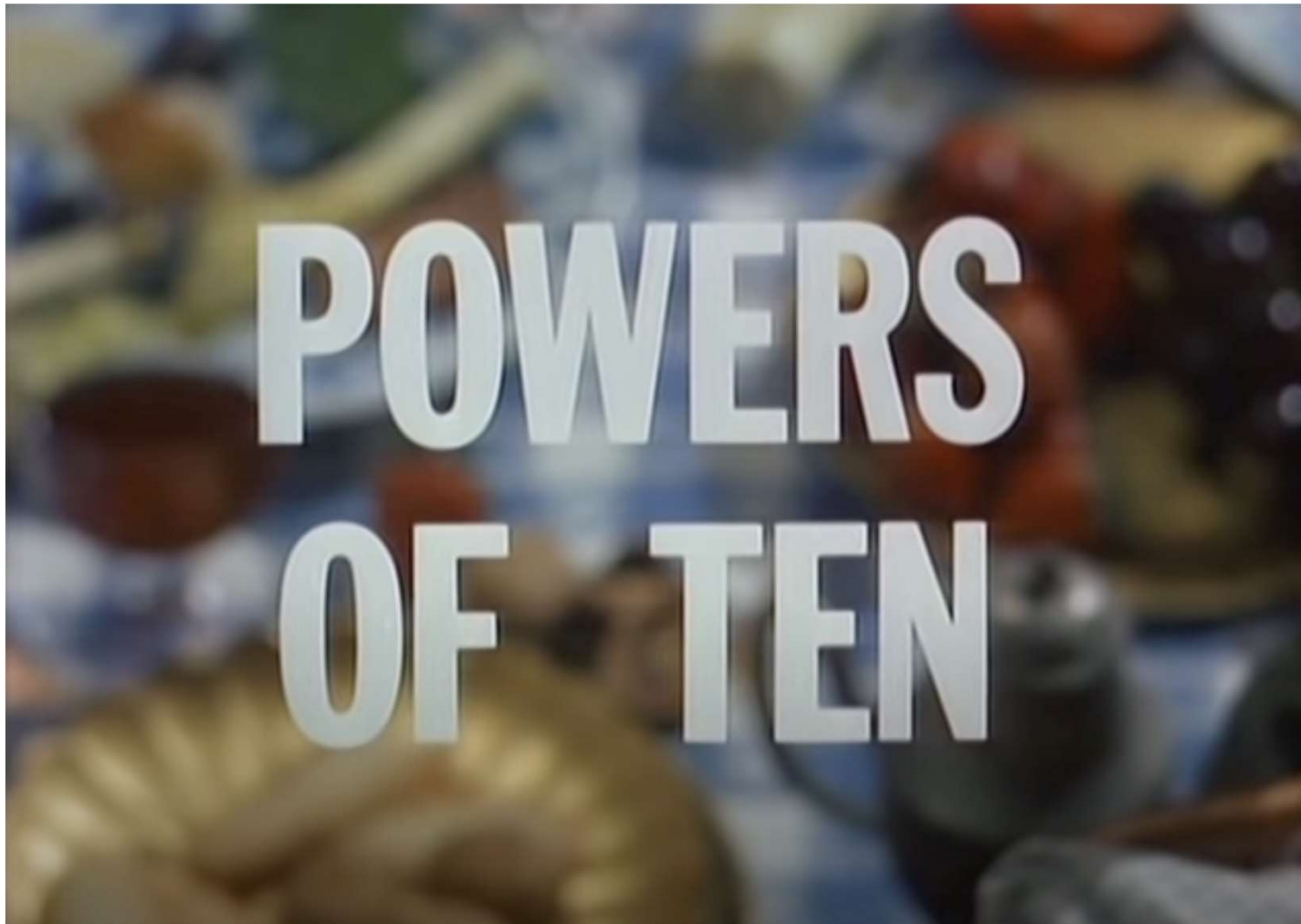
The right way?





The Alice approach



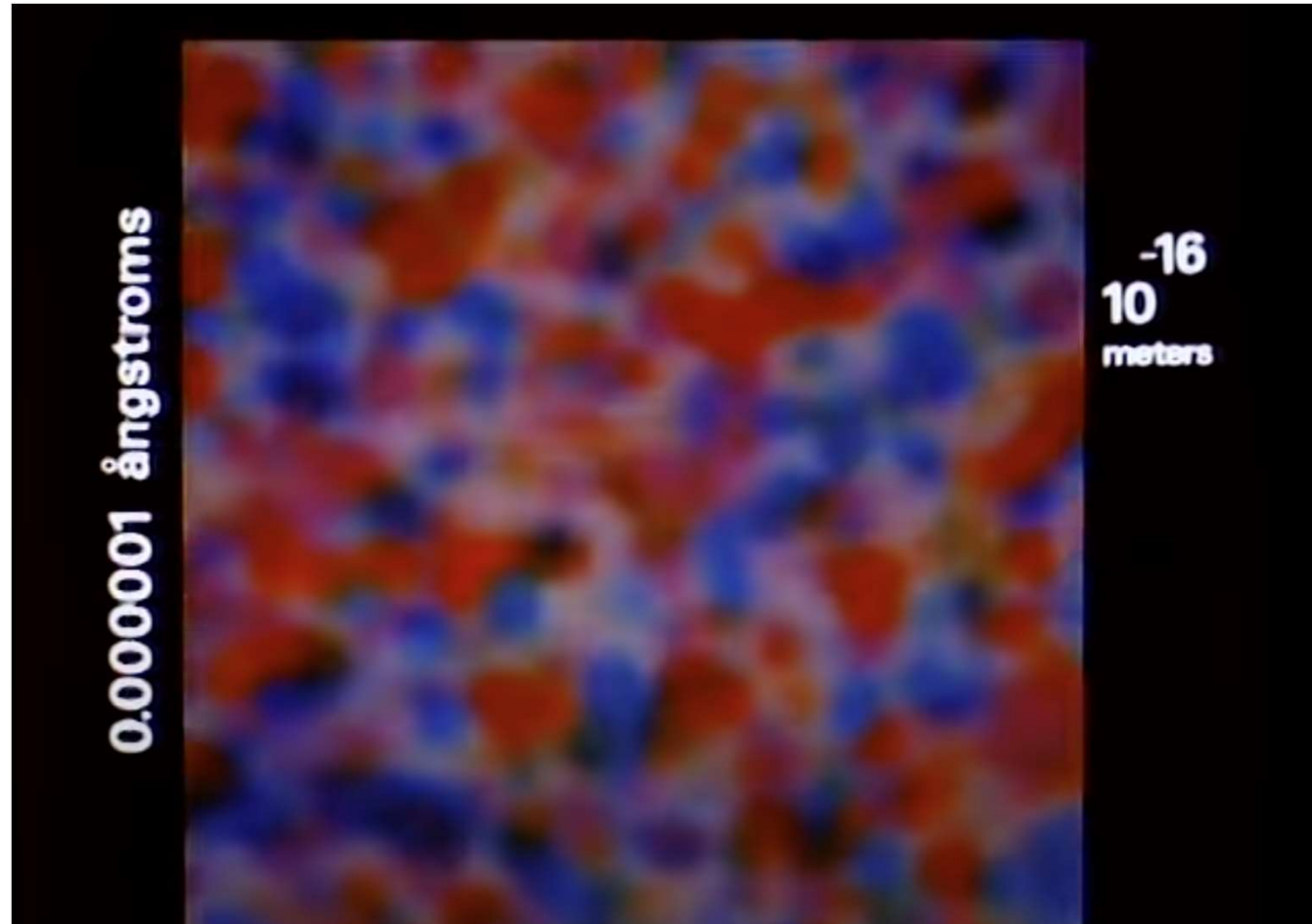


<https://www.google.com/search?client=safari&rls=en&q=powers+of+10+video&ie=UTF-8&oe=UTF-8#fpstate=ive&vld=cid:f8c8d350,vid:0fKBhvDjuy0>





Down the rabbit hole





Back up again





'Omics



Phenomics

The study of observable traits.

Examples:

- Observable symptoms
- Morphological differences.
- Behavioural traits.
- Developmental patterns.

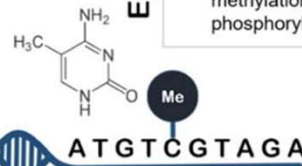


Genomics

The study of sequence level DNA modifications.

Examples:

- Single nucleotide sequence variation (base changes / substitutions / insertions / deletions)
- Structural variation (copy number variants)
- Chromosomal variation (segment aneusomy / whole genome or whole chromosome alterations)

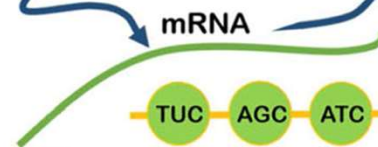


Epigenomics

The study of non-sequence level DNA modifications.

Examples:

- DNA methylation
- RNA modifications (e.g. methylation and phosphorylation)
- Histone modifications (e.g. methylation, acetylation, phosphorylation)



Transcriptomics

The study of RNA transcript abundance and expression.

Examples:

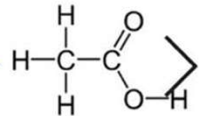
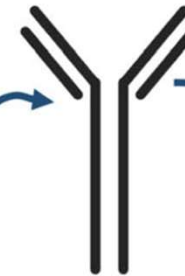
- Coding RNA transcripts (messenger RNA)
- Non coding RNA transcripts (e.g. circular RNA, micro RNA, long non coding RNA, transfer RNA, ribosomal RNA, small nucleolar RNA, small nuclear RNA, small interfering RNA.)

Proteomics

The study of protein abundance and expression.

Examples:

- Antibodies (e.g. Immunoglobulins)
- Enzymes (e.g. Polymerases)
- Messenger (e.g. growth hormones)
- Structural component (e.g. actin)
- Transport/storage (e.g. ferritin)



Metabolomics

The study of low molecular weight compounds in a cell.

Examples:

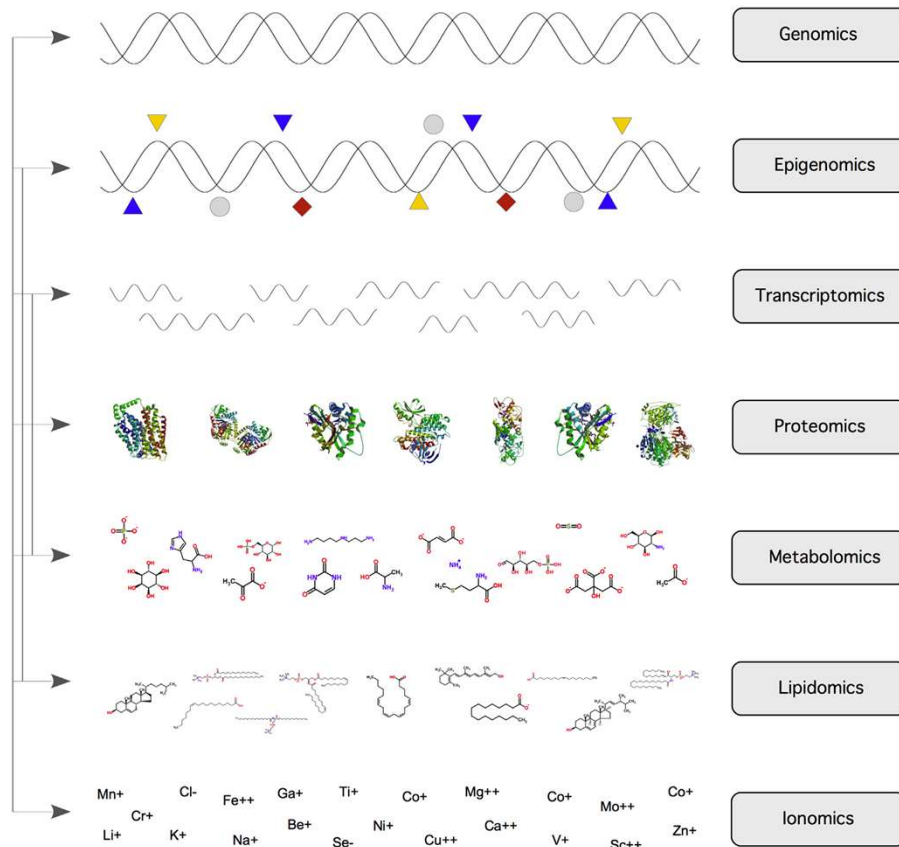
- Thousands of metabolites have been documented in biological samples. The example above in this figure is acetic acid.

Kerr K, et al. Orphanet J Rare Dis. 15: 107 (2020)

The comprehensive study of the roles, relationships, and actions of various types of molecules in cells of an organism



Additional 'omics

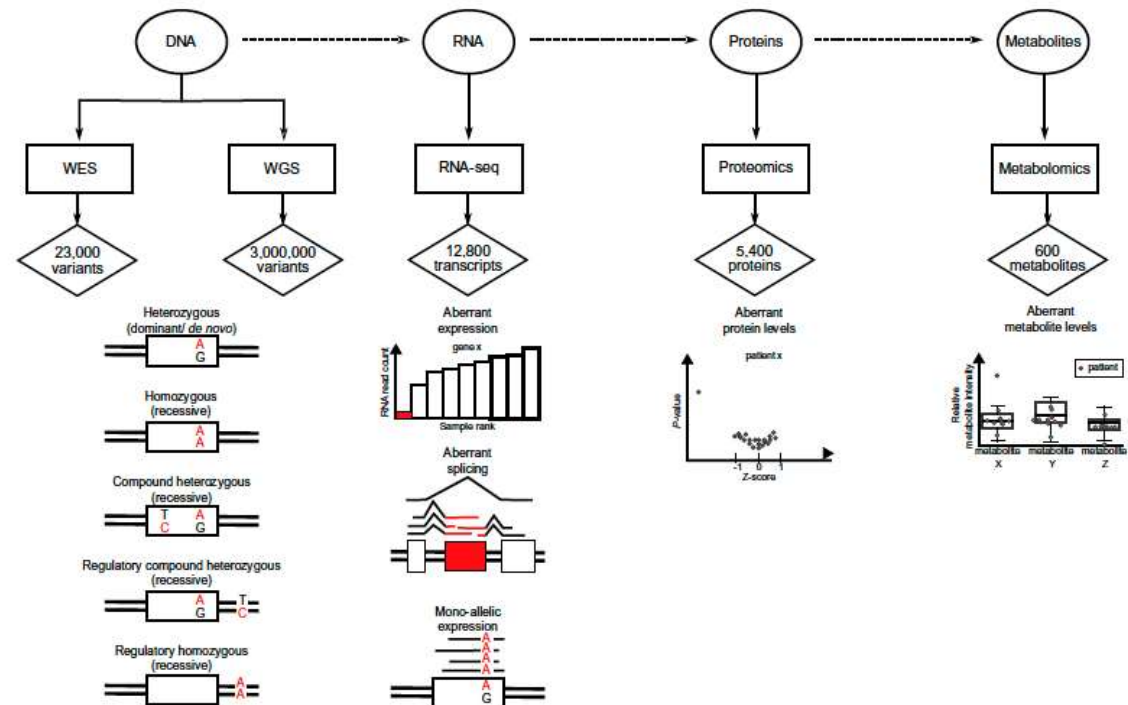


- Technology driven
- Nucleic acid based 'omics are most robust
- Proteomics and metabolomics remain more limited in spectrum
- Statistical methods of integration are in infancy
- Multivectoral



A numbers game

- Most information, but most variation at DNA level
- RNA sequence is limited to the transcriptome, but can identify splicing variants
- Protein identification is more limited but often the closest biomolecule to a disease
- Metabolomics most limited in scope but reveals patterns that are useful beyond a single diagnostic metabolite



Kremer, et al. 2018. *J Inherit Metab Dis*, 41: 525-32.



Goals of multi-omics approach

- Improve diagnosis
- Understand pathophysiology
- Study disease variability
- Identify biomarkers
- Monitor therapies





Combined D,L-2 hydroxyglutaric aciduria

An integrated example

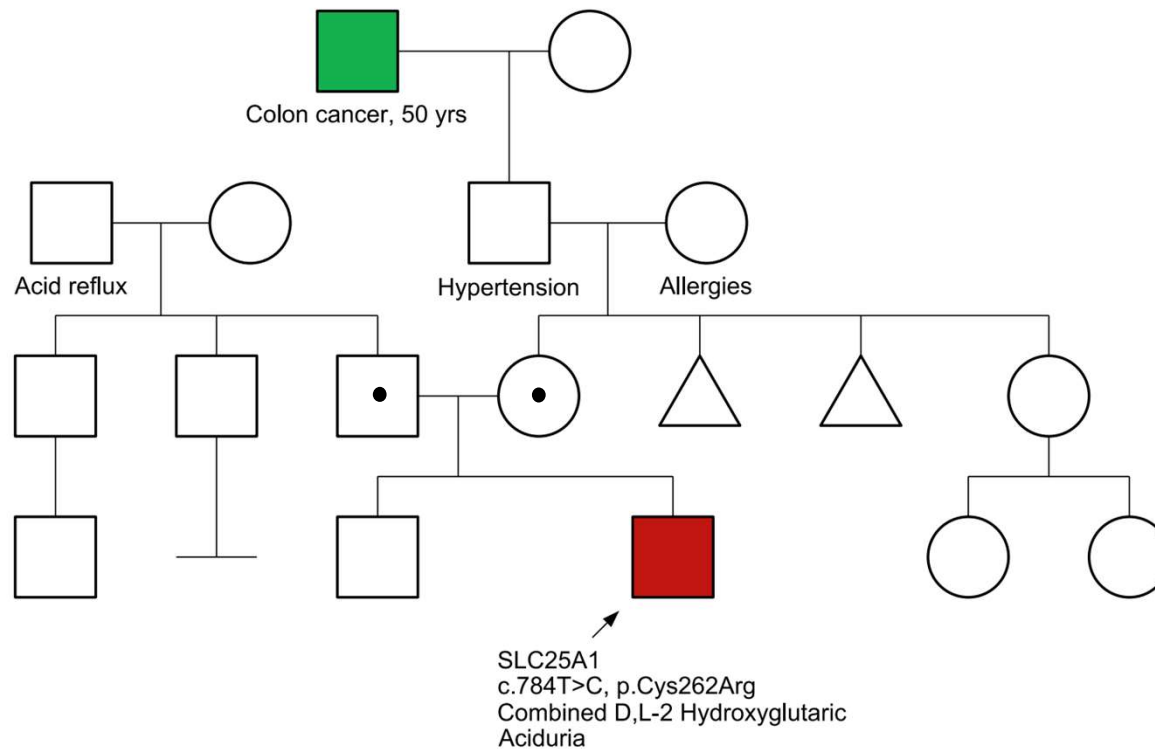
- Chief complaint
 - Hypotonia, apnea, feeding issues
 - Progressive developmental delay, seizures, profound swallowing and aspiration
- Labs
 - Plasma amino acid: Unremarkable
 - Very long chain fatty acid: **Reduced C22, C24 and C26**
 - Urine organics screen: **TCA metabolites and 2-hydroxyglutaric**

Metabolites	UOA_1	UOA_2
2-hydroxyglutaric + Artifact	3.24	
2-hydroxyglutaric		1.38
Succinic		1.35
Internal standard	1	1



Whole exome sequencing

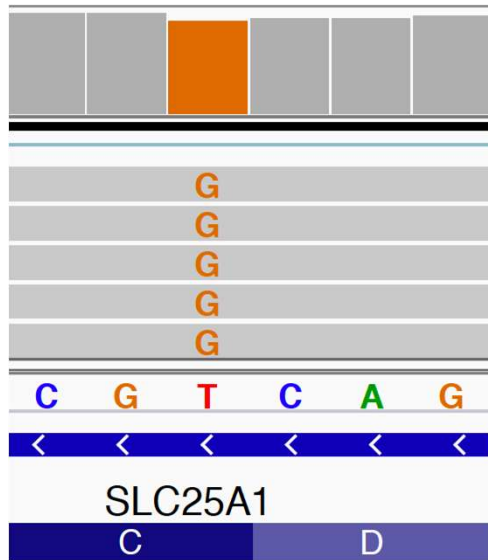
Homozygous likely pathogenic variant in *SLC25A1*



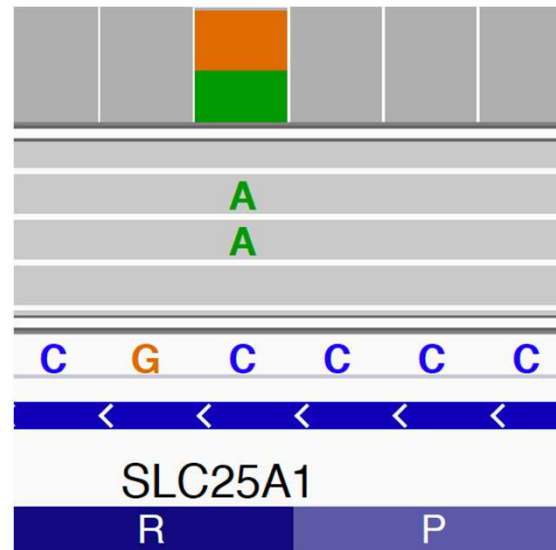


RNA-seq shows abnormal splicing

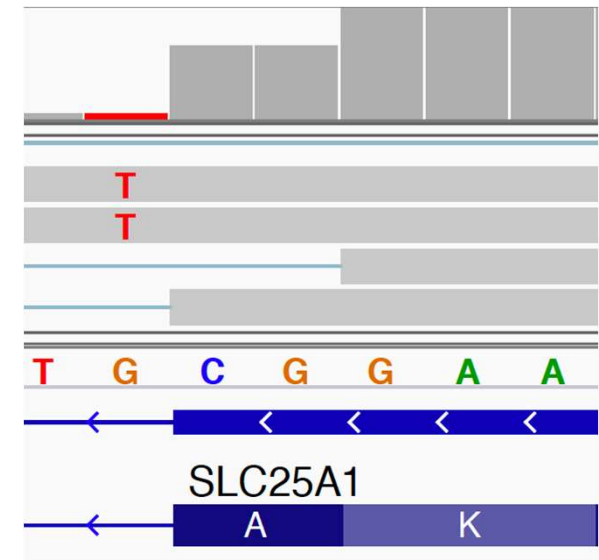
FB893
c.784T>C



FB897
c.844C>T (p.Arg282Cys)

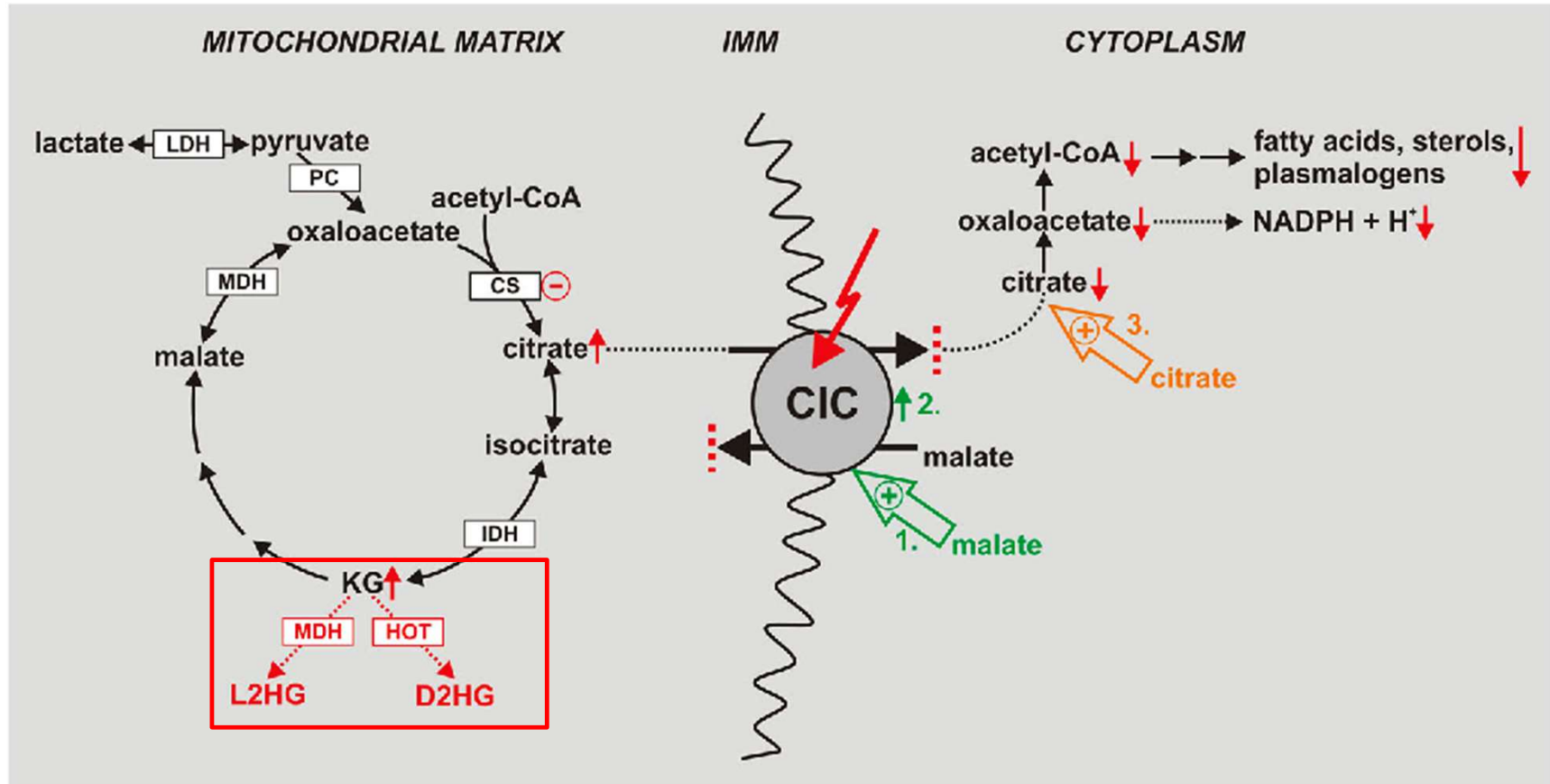


FB897
c.821+1G>A





SLC25A1 encodes mitochondrial citrate carrier (CIC)

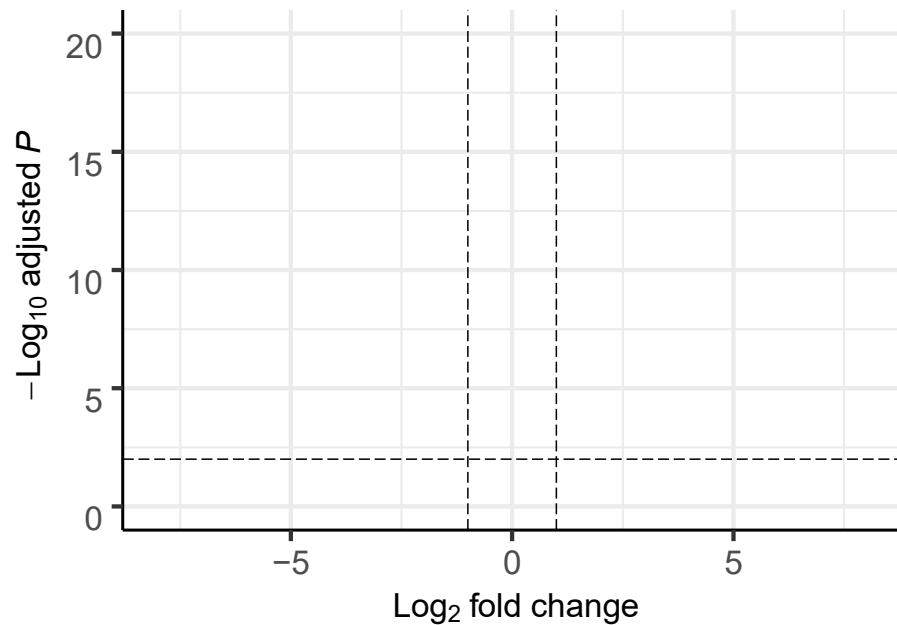


Muhlhausen C. et al. (2014) JIMD



TCA gene expression unchanged

Patients vs. Controls



Downregulated
1453

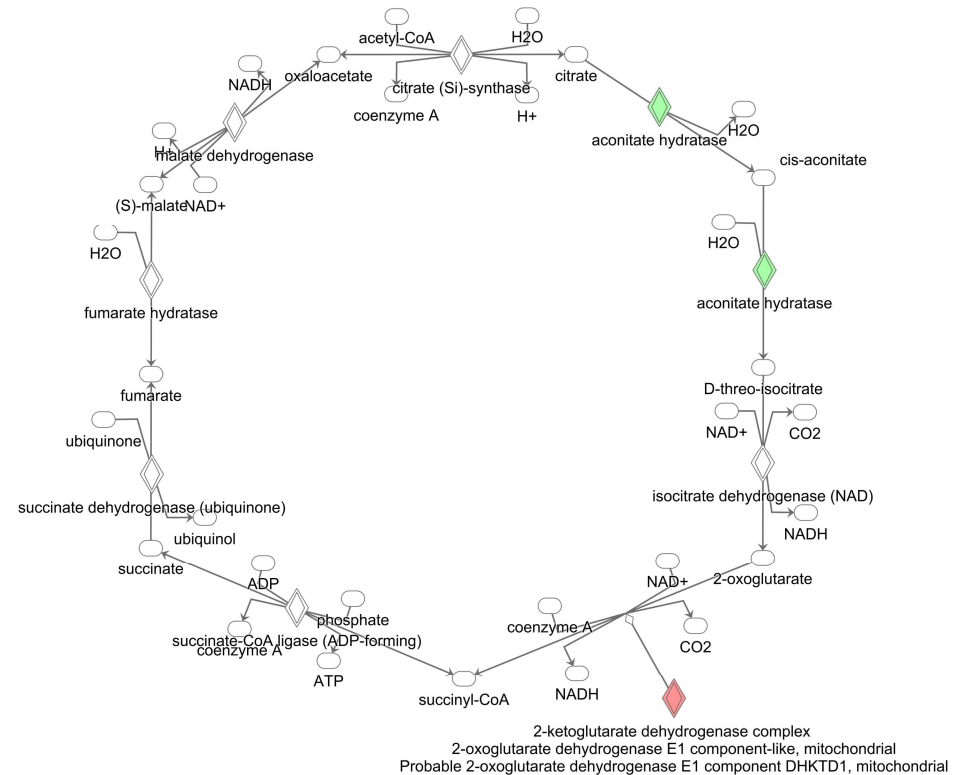
Upregulated
1167

NS

Log₂ FC

p-value

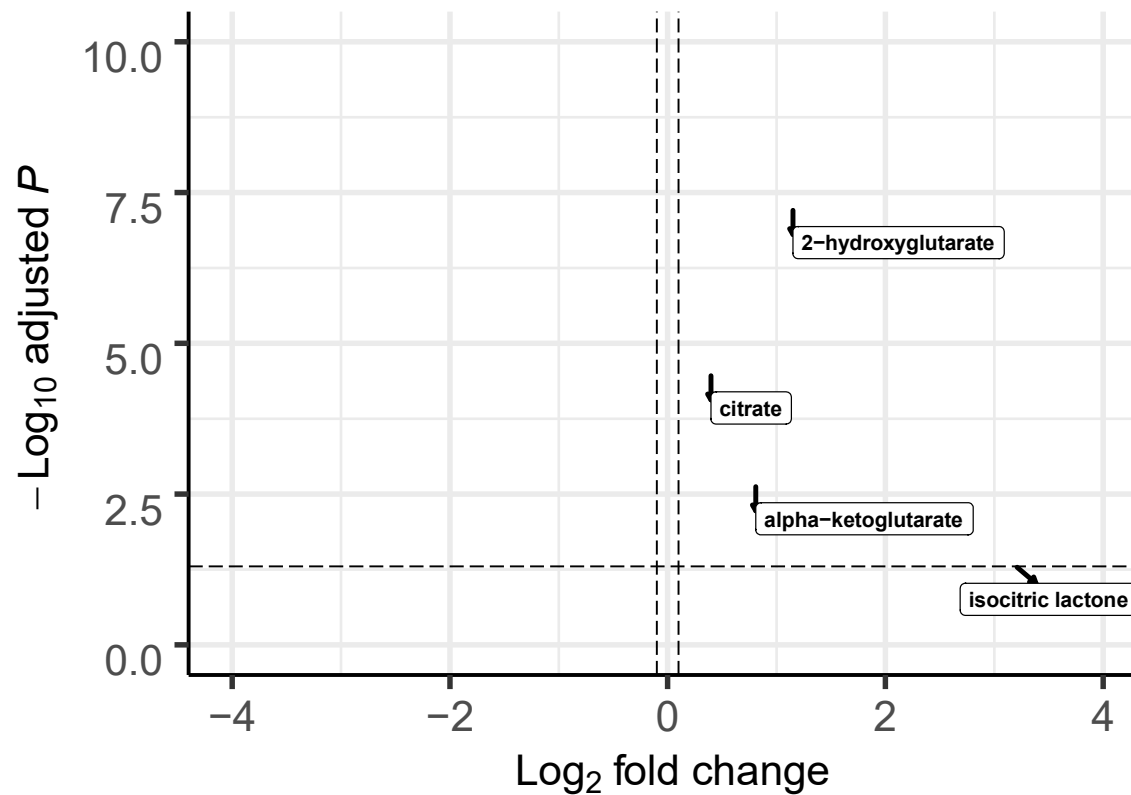
p-value and log₂ FC





Metabolomics validates biochemical phenotype

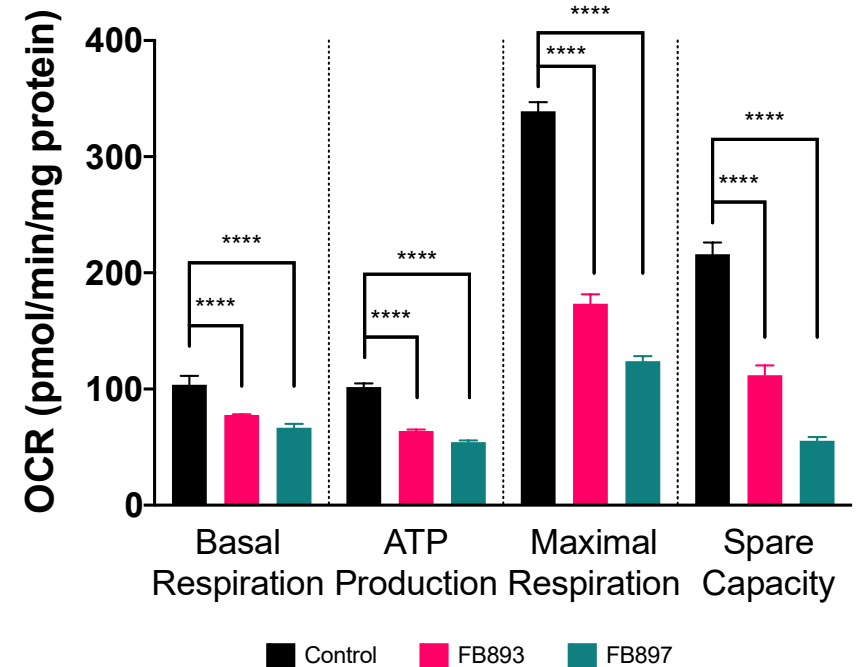
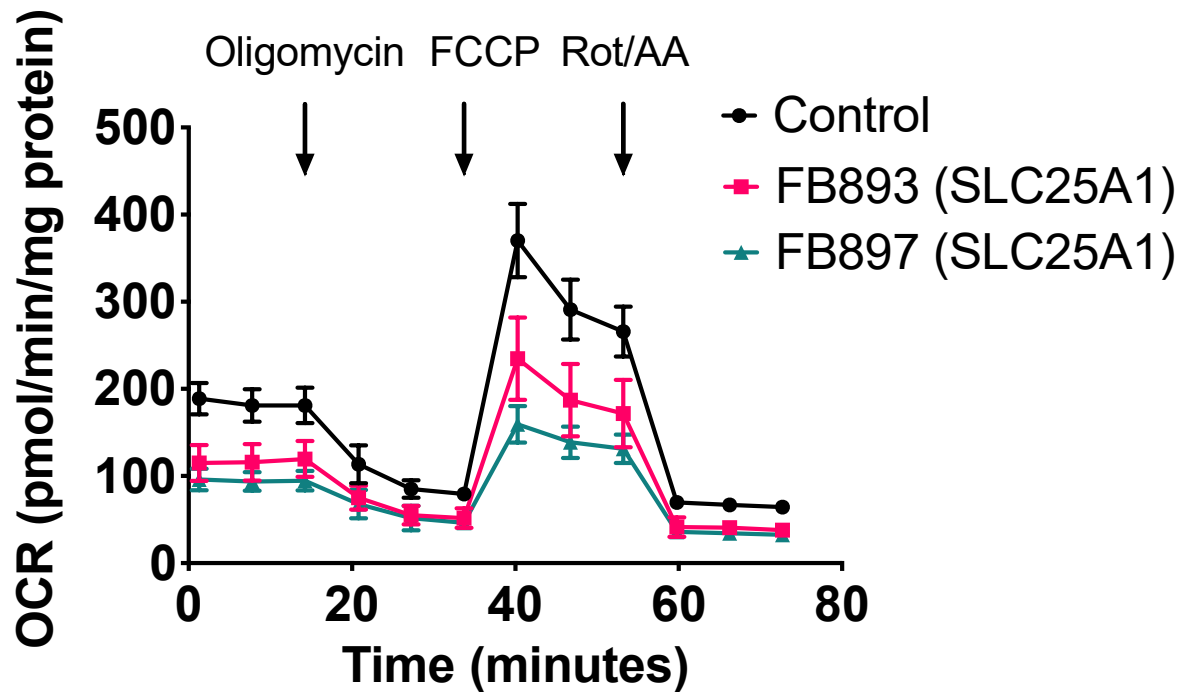
Patients vs. Controls





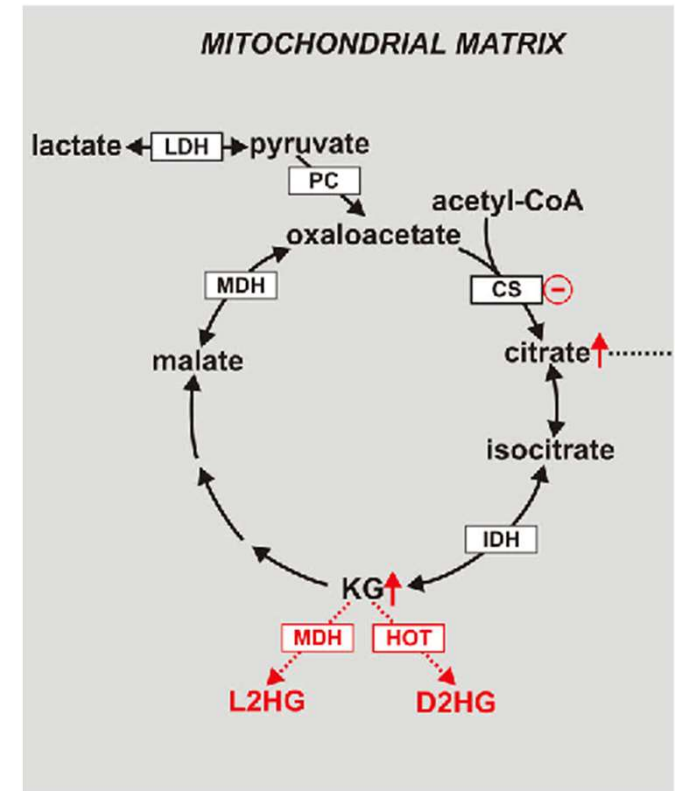
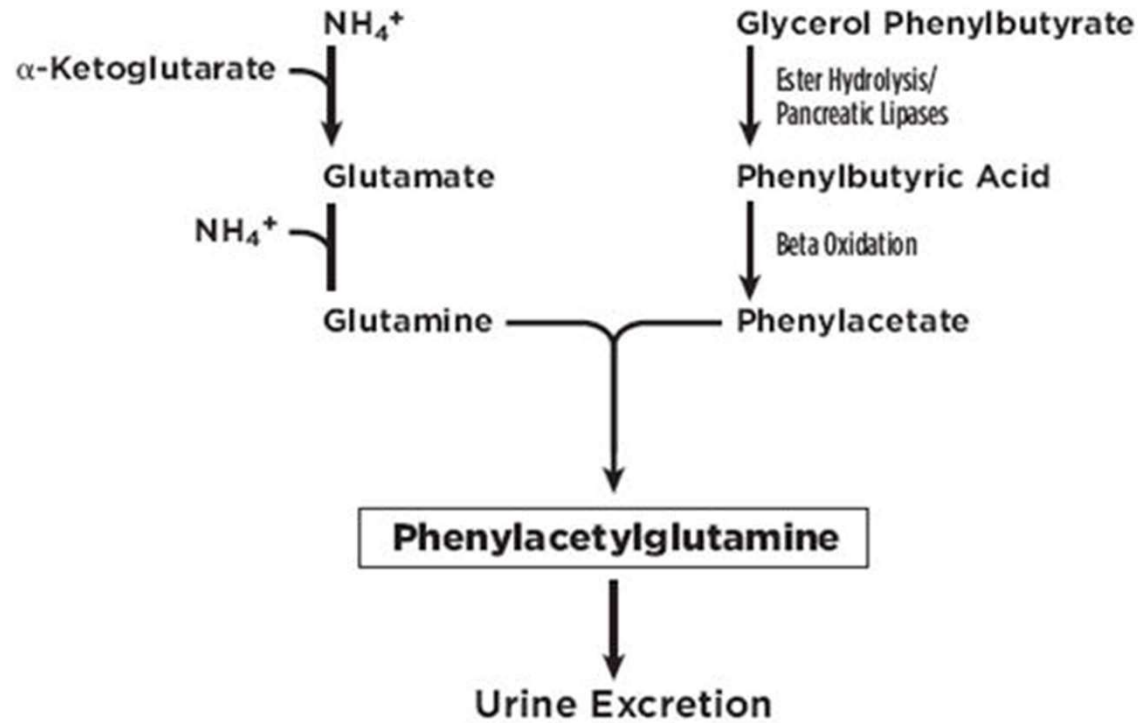
A bioenergetic deficit in patient fibroblasts

Seahorse Bioanalyzer studies



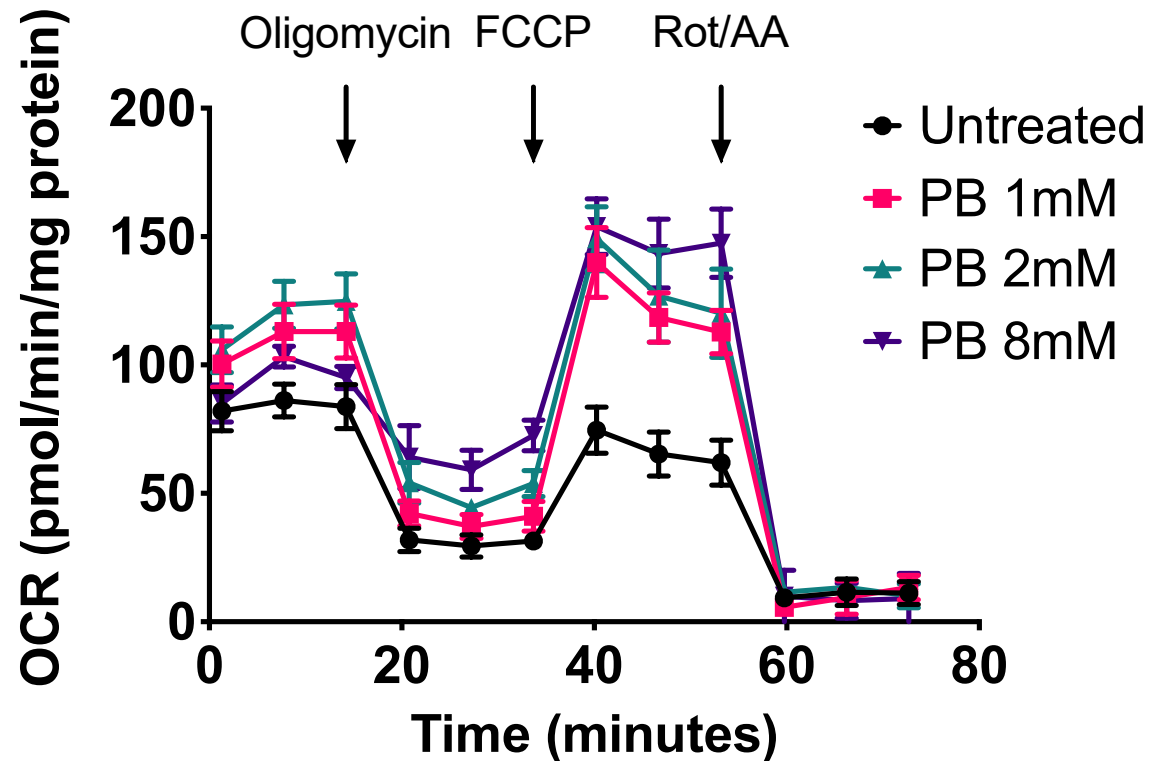


Phenylbutyrate depletes mitochondrial 2KGA



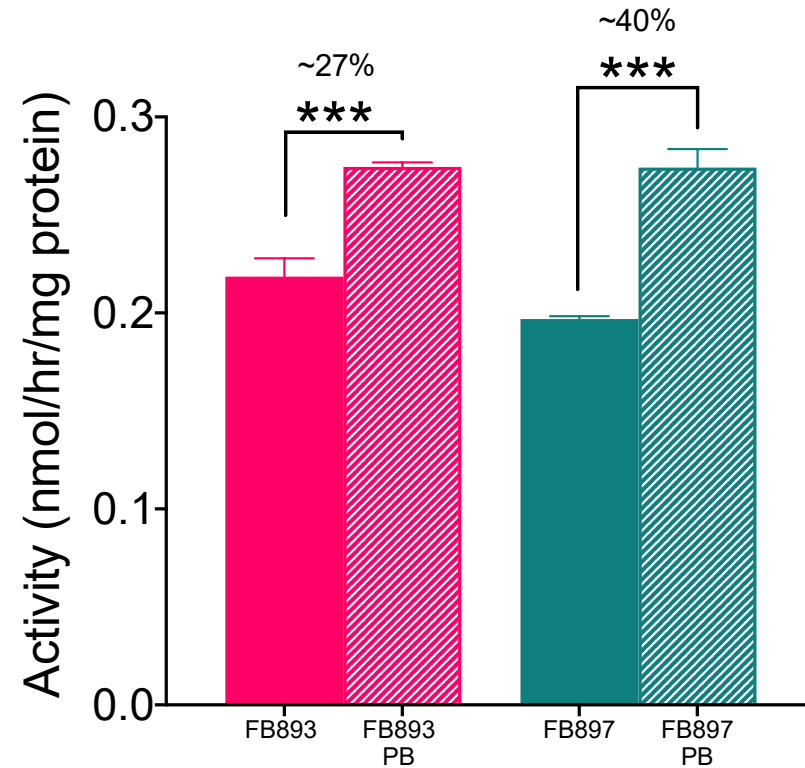
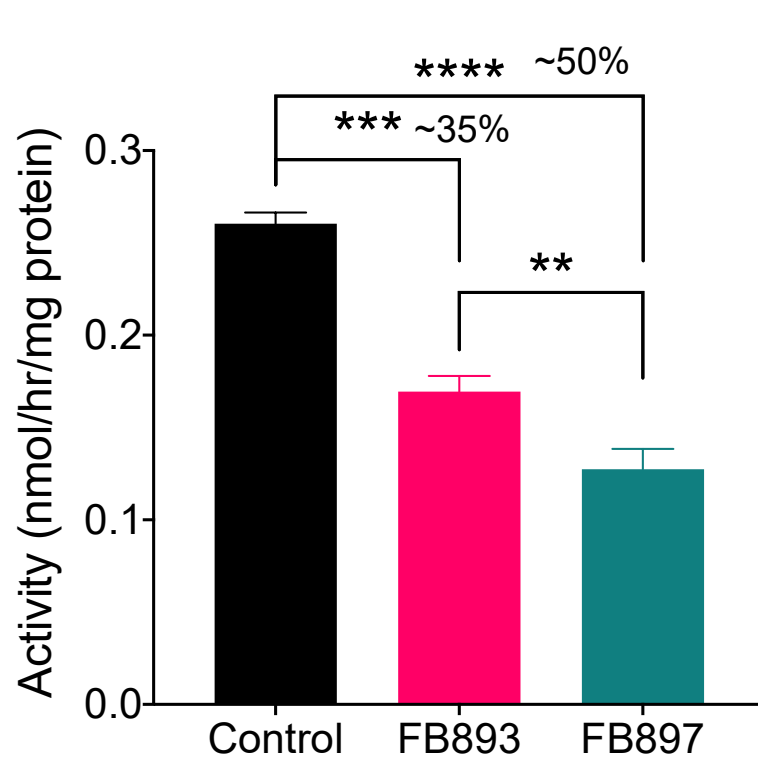


PB improves patient fibroblast bioenergetics





PB improves fibroblast fatty acid oxidation

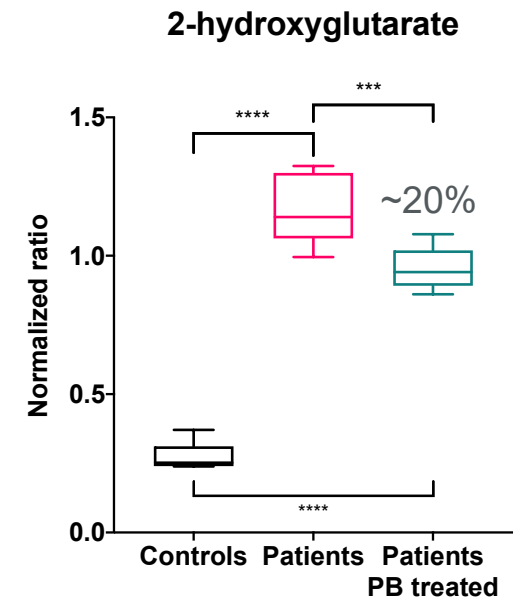
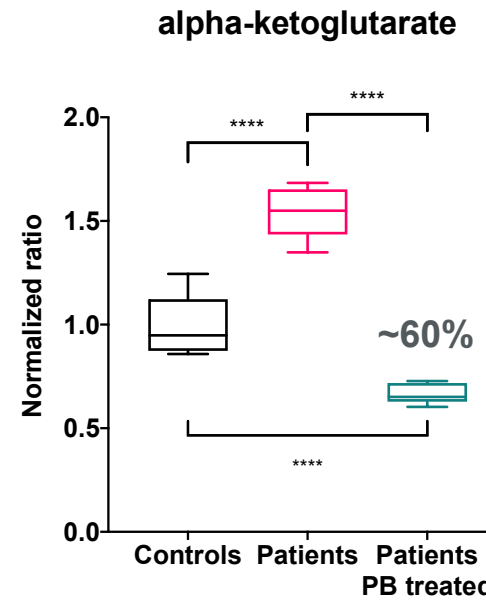
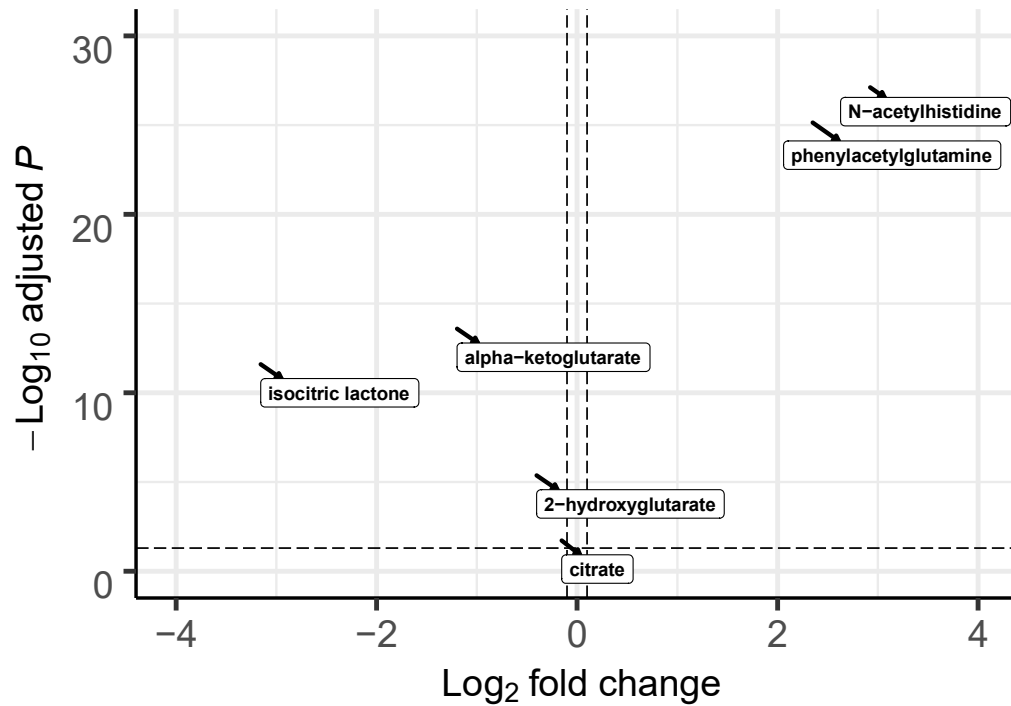


Olivia D'annibale



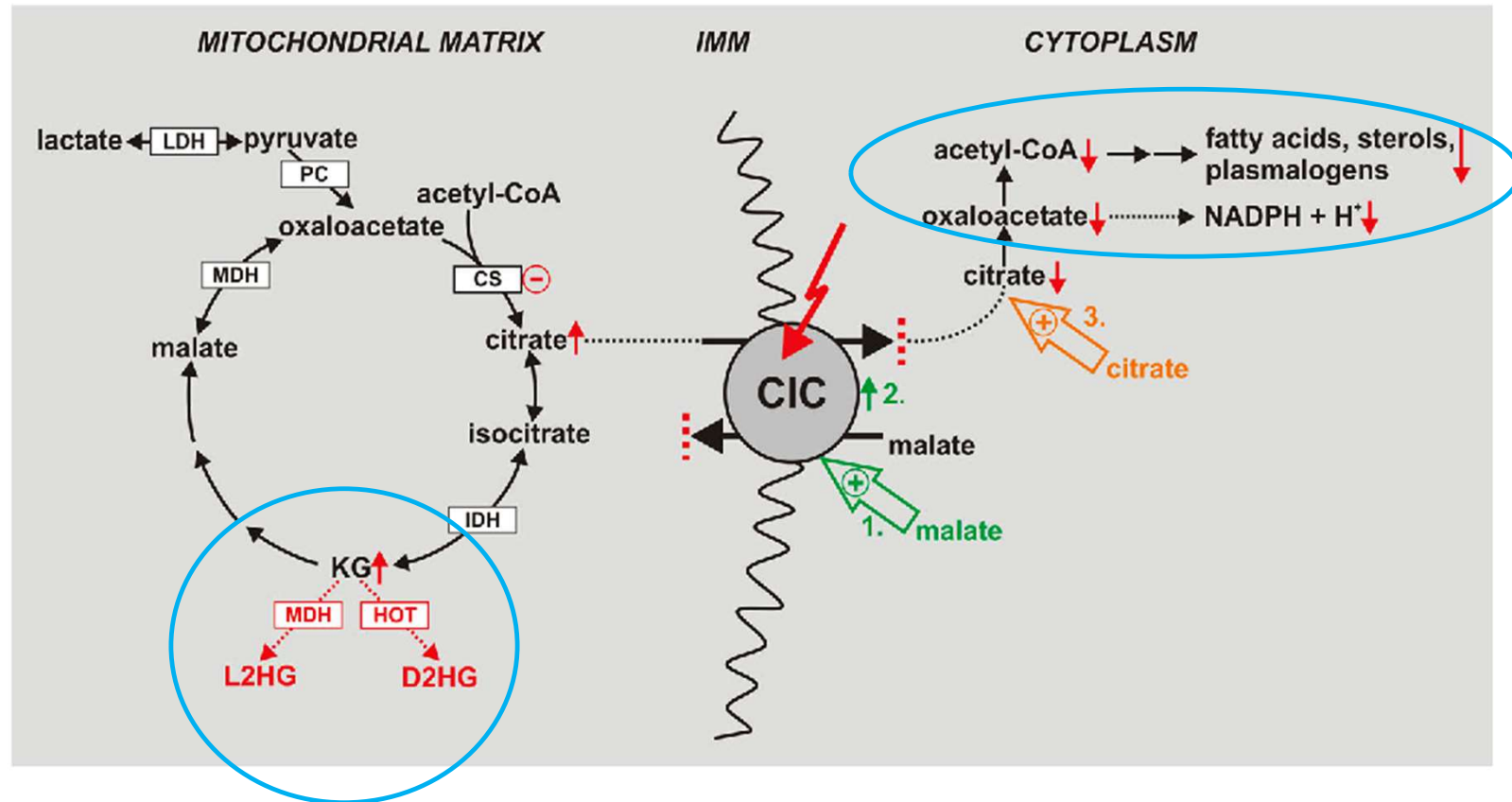
PB reduces 2-KG and 2-HG

Patients-PB treated vs. Patients-Untreated





Potential complex lipid synthesis defect in DL2HG

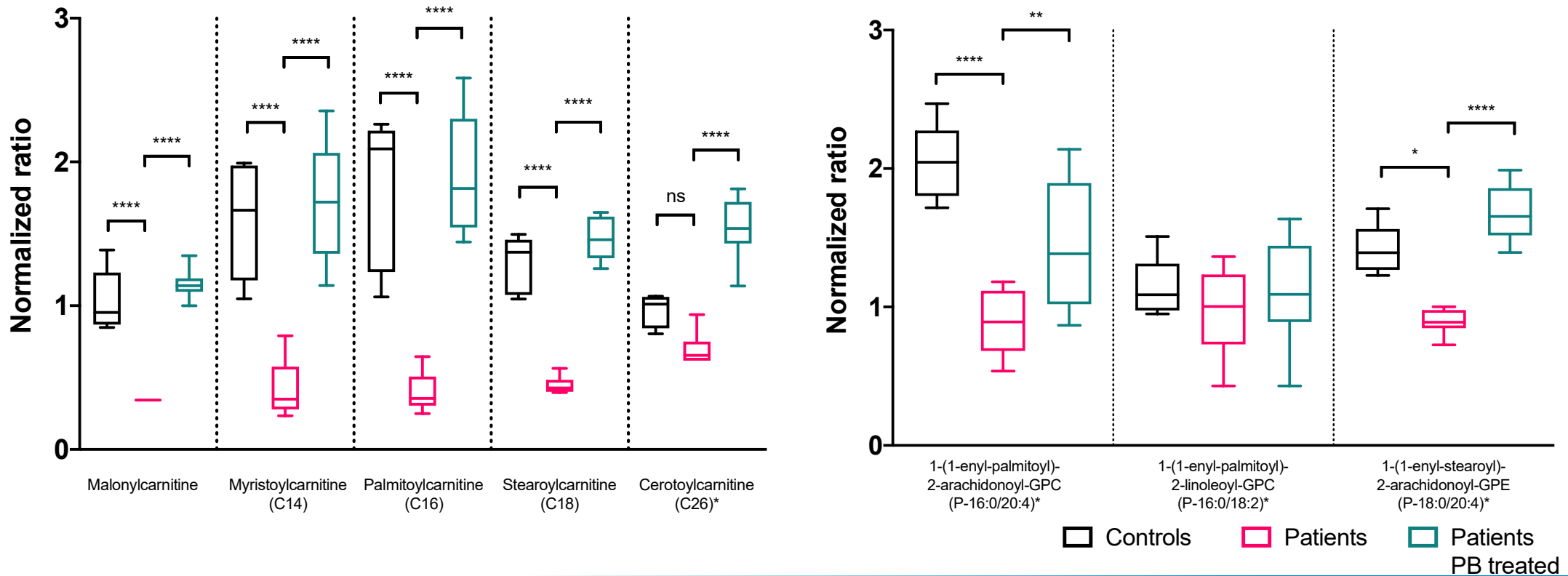


Phenylbutyrate

Muhlhausen C. et al. (2014) JIMD



Dysregulated ACNs and complex lipids





Summary

- Traditional drug development pipelines don't work for rare diseases
- Extensive understanding of pathophysiology of IEMs provides insight into target and options for designing drugs
- Multiomics provides unique insights into IEMs
- New technologies make screening candidate drugs easier
- Still need FDA/EMA recognition of novel trial design for approval of drugs for rare diseases



Thanks to my lab and collaborators



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Hulya Bayir
Valeria Kagan
Eric Goetzman
Yijen Wu
Abbe de Vallejo
Rob Nicholls



Thank you

