Profiling the Secretome of iPSC Cardiomyocytes Using High Resolution-Mass Spectrometry for Leigh Syndrome Biomarker Discovery

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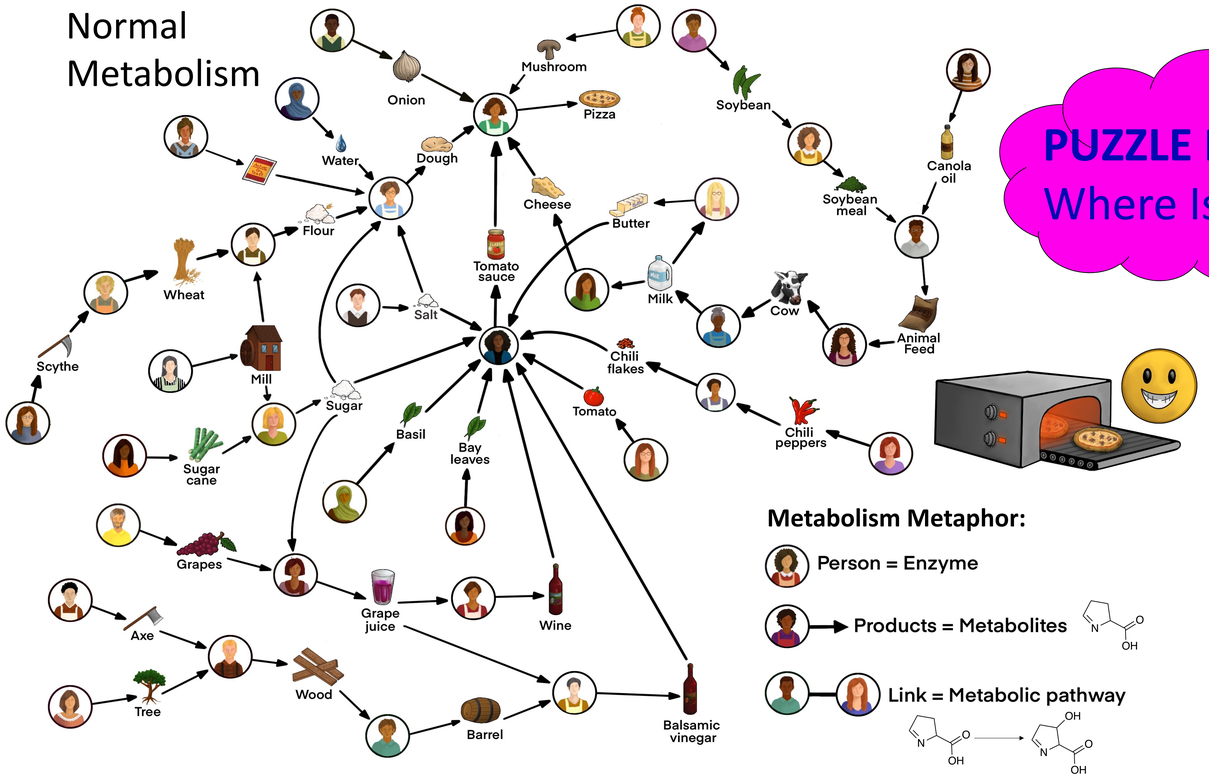
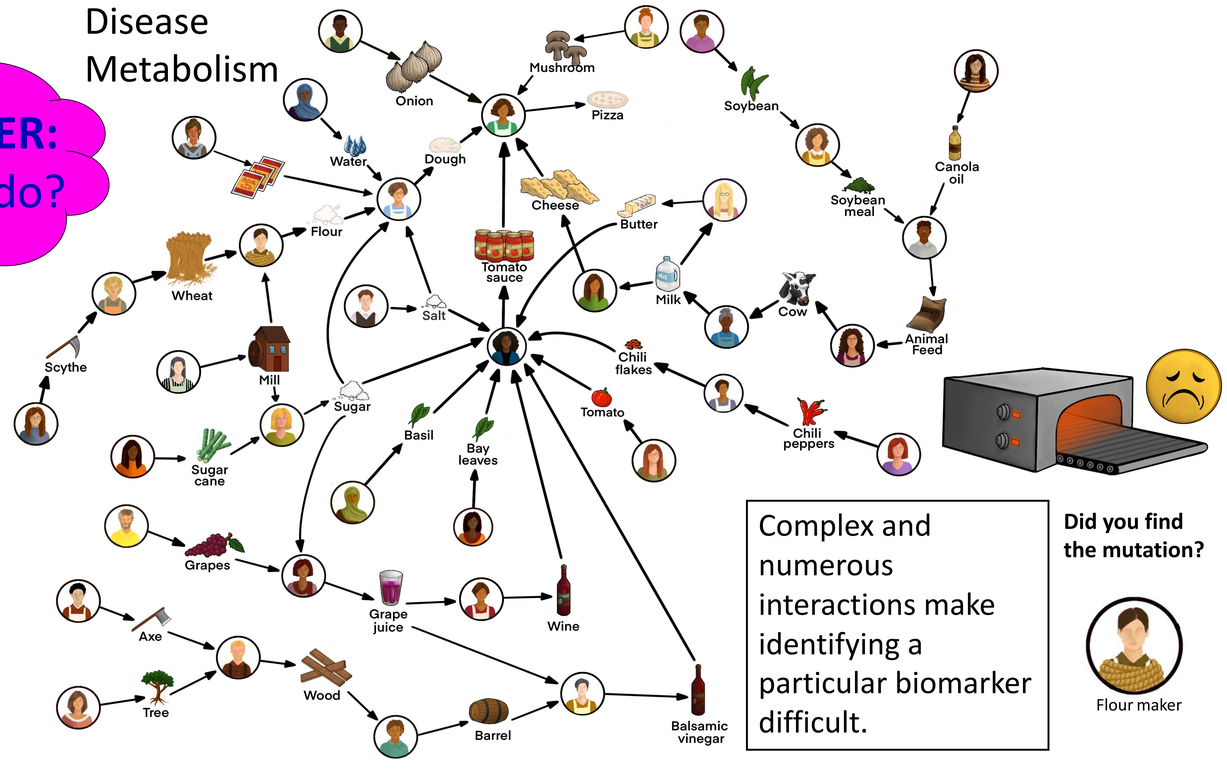
Normal Metabolism



PUZZLE POSTER:
Where Is Waldo?

Disease Metabolism



Metabolism Metaphor:

- Person = Enzyme
- Products = Metabolites
- Link = Metabolic pathway

Complex and numerous interactions make identifying a particular biomarker difficult.

Did you find the mutation?

Flour maker

Challenges in Biomarker Discovery in Metabolism

What is metabolism?

Metabolism is the culmination of all the life sustaining reactions.

What is a biomarker?

A biomarker is a metabolite whose presence suggests an underlying problem with metabolism.

What is a Leigh Syndrome?

Leigh Syndrome (LS) is a mitochondrial disorder, that affects a person's energy metabolism. LS is a rare disease occurring in only 1 in 40,000 people. LS is difficult to diagnose because it can be caused by many mutations and methods of diagnosis (ex. genetic screening) are inefficient and expensive. There is currently no cure and no known biomarker(s).

What are challenges with Biomarker Discovery?

Biomarker discovery requires statistical comparisons between groups with and without the phenotype. Metabolites can fluctuate based on external factors (diet, gender(hormones), circadian rhythm, and others), which can affect the resulting metabolite concentrations. The fluctuations in metabolite concentration can affect the analysis.

What Where the Qualifications for Narrowing the Features of Importance?

The list of qualities that metabolites must fulfil to be considered potential biomarkers included the following:

- Abundance >15,000 and quality score >85
- Frequency of metabolite occurrence in samples
- Retention time between 2-30 minutes
- Exact mass (ex: 180.156 g/mol glucose) is present in the Human Metabolomic Database (HMDB) and matches naturally occurring metabolites

Qualification		Control:	Leigh Syndrome:
Masses with Abundance >5,000 Quality score >70	=	4,533 (All features)	4,843 (All features)
Masses with Abundance >15,000 Quality score >85	=	1,537	1,614
Unique to cells (control or LS)	=	135	155
Feature is present in majority of samples	=	2 (all 4 control)	3 (>3 LS samples)

MS/MS Fragmentation Pattern for Glutathione

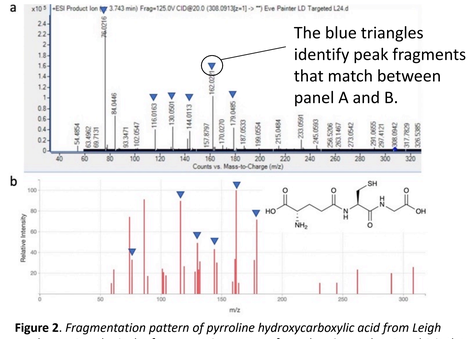
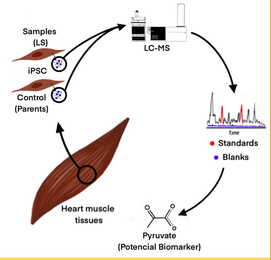


Figure 2. *Fragmentation pattern of pyrroline hydroxycarboxylic acid from Leigh syndrome*. Panel A is the fragmentation pattern from the LS samples. Panel B is the fragmentation pattern of pyrroline hydroxycarboxylic acid from the HMDB.

Biomarker discovery using **iPSCs** in **metabolomics** has many **challenges**, because of current analytical techniques. **Improving** analytical **approaches** and **metabolic databases** will allow for more effective **biomarker discovery**

Are Induced Pluripotent Stem Cells a Useful tool for Metabolomics?

IPSCs, can reduce organismal level variably, however this may not be an effective method due to incomplete metabolic databases, limiting biomarker discovery despite the technique's benefits.



MS/MS Fragmentation Pattern for Pyrroline Hydroxycarboxylic acid

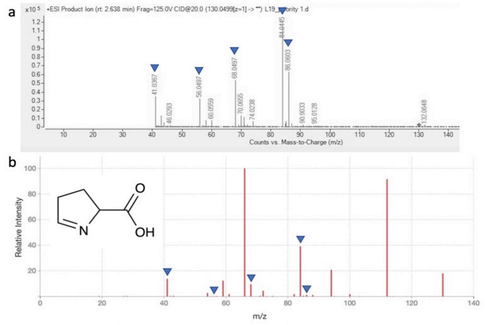


Figure 8. *Fragmentation pattern of glutathionate(1-) from Leigh syndrome*. Panel A is the fragmentation pattern from the LS samples. Panel B is the fragmentation pattern of glutathionate(1-) from PubChem.

What is Went Wrong? Who is Tied Up?

Spot the Affected Person (Mutated Enzyme)

Look for products that appear in a higher concentration in comparison to normal metabolism.

First step in identifying a Biomarker

- A biomarker is a metabolite that may appear in a higher concentration than normal, or is novel, suggesting an underlying problem with metabolism.
- Statistical differences in features (unidentified metabolites) between the control and the diseased phenotype, can be used to determine prospective biomarkers.

Reducing "Organismal Level Variability" using Induced Pluripotent Stem Cells

Biological variation in metabolites are key for determining statistic changes termed "organismal-level variability" which can be reduced by induced pluripotent stem cells (iPSCs). IPSCs can reduce the affect of external factors through in vitro experimentation (test tube cells). IPSCs are stem cells that can be induced to become a particular cell type. For example, cardiomyocytes which are heart muscle cells.