METABOLIC PROFILING AND SIGNATURES IN ALS

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Metabolic Profiling: Pathways in Discovery
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Metabolomics is the newest Omics science.

- DNA
- RNA
- Protein
- Biochemicals (Metabolites)

Genomics
- Transcriptomics
- Proteomics
- Metabolomics
THE METABOLOME
KEY CONCEPTS

• DISEASE RESULTS IN SPECIFIC METABOLIC SIGNATURES

• GENETIC AND ENVIRONMENTAL INSULTS INDUCE SPECIFIC AND LONG LASTING CHANGES IN METABOLIC PROFILES

• COMPLEMENTS STUDIES OF GENOME AND PROTEOME

• SUBCATEGORIES OF A DISEASE CAN BE DISCRIMINATED BASED ON METABOLIC FINGERPRINTS

• RESPONSE TO THERAPY AND PROGRESSION OF A DISEASE CORRELATE WITH CHANGES IN THE METABOLOME

• METABOLIC SIGNATURES COULD YIELD DRUG LEADS, TARGETS, MARKERS
The Power of Metabolomics

- Leverages a long history of cellular biochemistry
- Looks at “higher order” information to link genes and proteins to metabolic pathways
- Benefits from the evolutionary conservation of metabolism across species
- Amenable to high-throughput platforms
- Provides most direct linkage to physiological function
Metabolon Process Overview

1. Acquire Samples
2. Sample Repository (& Chemistry)
3. Metabolon Reader
4. Statistical Analysis
5. Data Visualizer
6. User Toolbox

Data Filters:
- EC
- UV
- MS+
- MS-
- MS ei
- ICP

Data Repository

Reference Database

Therapeutic Area Expert Panel

Biology Chemistry Platform Informatics
Breadth of Coverage

Non-Polar
- fats
- waxes
- terpenes/terpenoids, steroids, carotenoids
- fatty acids
- alkaloids

Polar
- phenolics
- alcohols
- nucleosides
- organic acids
- amino acids
- metals
- simple sugars

uv/vis & EC

GC/MS

HPLC/MS

ICP-MS
Statistical treatment

- Assumptions
  - Biological variability is significant
  - Metabolic pool sizes vary over many orders of magnitude

- Resolution
  - For each compound its mean and standard deviation is routinely calculated in the largest possible control group
  - Experimental samples are understood in terms of every compound’s deviation from its norm.
Normalized Response

Normal Tissue

-5 0 +5

Abnormal Tissue

-5 0 +5

Compound 1
Compound 2
Compound 3
Compound 4
Compound 5
Compound 6
Compound 7
Compound 8
Compound 9
Compound 10
Compound 11
Compound 12
Compound 13
Compound 14
Compound 15
Compound 16
Compound 17
Compound 18
Compound 19
Compound 20
Compound 21
Compound 22
Compound 23
Compound 24
From Biochemical Profiles to Metabolic pathways
Experiment 1 - Two plasma sets were profiled for training purposes
- Were able to separate controls from ALS patients

Experiment 2 - A third plasma set was profiled blinded (mixed ALS and controls)
- Half samples un-blinded
- Signatures were established
- A set of predictors for ALS patients on or off Riluzole was determined
- Predictions were made for rest of the samples

Experiment 3 - Cell lines expressing SOD1 WT or mutants were profiled
One of the Variant Peaks identified

Channel 6
Near
69.5 Minutes

A10 in front
(A10 - A9)

Controls in back
(C11 - Series31)

Put cursor over rtion to see identity.

Select Chart,
3D view to rotate
Statistical Analysis

- Class Prediction or supervised learning
- Principal Components Analysis (PCA)
- Discriminant Analysis (PLS-DA)
- Scatter Analysis
Class prediction or supervised learning can classify a subset of small molecules that strongly associate with a disease or its absence (or with disease progression or response to therapy).

The input of this analysis is a set of training profiles (disease and controls).

The output consists of a list of compounds that highly associate with disease or health state.

We assess whether the association is strong and statistically significant.

Compounds with significant association can be used collectively as surrogate markers for disease classification, response to therapy or disease progression.

These compounds will help us understand better the biochemistry of the disease. They will highlight therapeutic targets and leads.
Controls ALS

Association strength

Strong association with ALS

Strong association with controls

Experiment 1 – Class Prediction

Standard deviations above mean

Standard deviations below mean
Principal Components Analysis (PCA)
Discriminant Analysis (PLS-DA)

- 3 SD
- 2 SD
- 1 SD

Deviation from mean

Patient

Controls
ALS
Experiment 2 - A Blinded Analysis

- 59 blood serum samples were collected at Massachusetts General Hospital (MGH)
- The plasma were analyzed blinded
- A training set of 33 samples (16 controls, 12 ALS on drug and 6 ALS not on drug) was un-blinded
- ALS disease profiles were developed
- The remainder were categorized by diagnostic chemical patterns
Profile of ALS patients
(non-Riluzole taking sub-population)

14 peaks found in majority of patients
Experiment 2 -
Profile of ALS patients
(Riluzole taking sub-population)

24 peaks found in patients taking Riluzole
Discriminant Analysis (PLS-DA)
We built class predictors by un-blinding 35 of the samples in experiment 2.

From the set of 24 samples left blinded we were able to assign 21 correctly.
Experiment 3
Profiling of Cell lines expressing SOD mutants

- Profiled cells over-expressing WT or mutant SODs
- These are stably transfected N2A cells
  NV, WT, G37R, G41D, G85R
- Naive (NV) cells represent mock transfected cells (N2A stably transfected with the G418 resistant plasmid).
- WT cells express the human wild type SOD1
- The other three lines express three different SOD1 mutants (G37R, G41D and G85R)
SOD1 Mutant Cell lines

- WT-SOD1
- m1-SOD1
- m2-SOD1
- m3-SOD1
- Naïve

Graph showing the distribution of different cell lines in a high-dimensional space.
It is possible to characterize the physiological status of a biological sample by making a near simultaneous measurement on several hundred biochemical intermediates. This requires making multiple measurements on a single sample, each measuring different physical parameters, followed by an automated data extraction process. These techniques all rely on information management techniques to integrate experimental sample information and physiological knowledge. This ability to cast biochemical status into the physiological realm, metabolomics, is the newest “Omics” science.
Collaborators

**Clinical**

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**PROFILING GROUP**

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