Untargeted Metabolomic Profiling in Inborn Errors of Metabolism

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Disclosure

A fixed portion of my salary is paid by Baylor Genetics Laboratories but compensation is not tied to laboratory revenue
Outline

• Common practice and limitations of current routine testing for IEMs
• Global **Metabolomic Assisted Pathway Screen** (Global MAPS®) - Methods
• Validation for common IEMs
• Confirmation of DNA variant pathogenecity
• Discovery of Novel Biomarkers
CURRENT RECOMMENDATIONS FOR INTELLECTUAL DISABILITY EVALUATION
AAN Recommendations for Intellectual Disability (2011)

• Screening for inborn errors of metabolism (IEMs) in children with GDD/ID has a yield of between 0.2% and 4.6%, depending on the presence of clinical indicators and the range of testing performed (Class III).

• Testing for congenital disorders of glycosylation has a yield of up to 1.4%, and testing for creatine disorders has a yield of up to 2.8% (Class III).
1st Tier: Non-Targeted screening to identify 54 (60%) treatable IEMs

**Blood:**
- ammonia, lactate
- plasma amino acids
- total homocysteine
- acylcarnitine profile
- copper, ceruloplasmin

**Urine:**
- organic acids
- purines & pyrimidines
- creatine metabolites
- oligosaccharides
- glycosaminoglycans

2nd Tier: Targeted testing to identify 35 (40%) treatable IEMs requiring ‘specific testing’

- according to patient’s symptomatology patient (Table 4) & clinician’s expertise
- utilization of textbooks & digital resources (WebApp: www.treatable-ID.org)
- consider the following biochemical / molecular analyses:
  - whole blood manganese
  - plasma cholesterol
  - plasma 7-dehydro-cholesterol:cholesterol ratio
  - plasma pipecolic acid & urine AASA
  - plasma very long chain fatty acids
  - plasma vitamin B12 & folate
  - serum & CSF lactate:pyruvate ratio
  - enzyme activities (leucocytes): arylsulphatase A, biotinidase, glucocerebrosidase, fatty aldehydes dehydrogenase
  - urine deoxypyridinoline
  - CSF amino acids
  - CSF neurotransmitters
  - CSF: plasma glucose ratio
  - CoQ measurement fibroblasts
  - molecular: CA5A, NPC1, NPC2, SC4MOL, SLC18A2, SLC19A3, SLC30A10, SLC52A2, SLC52A3, PDHA1, DLAT, PDHX, SPR, TH

van Karnebeek CDM et al., Mol Genet & Metab 111:428-38, 2014
Current Challenges

• For undifferentiated phenotypes, such as intellectual disability, seizures, recurrent vomiting, failure to thrive etc. many different tests are needed

• Various fluid types (blood, urine, cerebrospinal fluid) may be needed for diagnosis

• Cost for multiple tests may be prohibitive and many are rare, so no good way to tier testing
Methods/Tests

- HPLC – amino acids
- GC/MS – organic acids
- MS/MS
  - Acylcarnitines
  - Newborn screening
  - Individual specialized tests
    - Purines & Pyrimidines
    - Creatine & guanidinoacetate
    - Pyridoxine responsive seizure panels
    - Bile acids
    - CSF Neurotransmitters
    - Etc!
Rationale for Metabolomic Approach

Biochemistry Advantages
- Any sample type
- Condensed & information rich
- Actionable
- Bridge between genome & phenotype

DNA
RNA
Proteins
Biochemicals
Mechanistic Insight into Phenotype

glucose
cholesterol
threonine
METHODS
Metabolon, a global leader in metabolomics

Pioneering the emerging field of global biochemical pathway analysis for biomarker discovery and the development of innovative diagnostic tests

- Founded in 2000
- Over 150 employees worldwide with expertise in biochemistry, mass spectrometry and software development
- 54,000 sq. ft. facility in Research Triangle Park, NC and Sacramento
- CLIA-certified lab onsite
Biochemical Extraction

- UHPLC-MS/MS (+ESI) Early/Polar
- UHPLC-MS/MS (+ESI) Late/Lipid
- UHPLC-MS/MS (-ESI)
- UHPLC (HILIC)-MS/MS

Library Search
- RT, Mass, MS/MS
Data Reduction
- Compound ID
QA/QC

Biochemical Extraction

Pathway Visualization: Metabolync™ plugin to Cytoscape developed to overlay analyte findings onto metabolic pathways

Statistical Analysis

Interpretation

Biomarkers
Mechanistic Understanding
Drug MoA
Cellular Characteristics
Biochemical Extraction

UHPLC-MS/MS (+ESI) Early/Polar
UHPLC-MS/MS (+ESI) Late/Lipid
UHPLC-MS/MS (-ESI)
UHPLC (HILIC)-MS/MS

Library Search
RT, Mass, MS/MS
Data Reduction
Compound ID
QA/QC

Statistical Analysis

Enrich 50-1500 Da small molecule analytes

Biomarkers
Mechanistic Understanding
Drug MoA
Cellular Characteristics

Interpretation
Biochemical Extraction

- Biochemical Extraction
- UHPLC-MS/MS (+ESI) Early/Polar
- UHPLC-MS/MS (+ESI) Late/Lipid
- UHPLC-MS/MS (-ESI)
- UHPLC (HILIC)-MS/MS

Library Search
- RT, Mass, MS/MS
- Data Reduction
- Compound ID
- QA/QC

• Q exactive MS/MS - Orbitrap based MS/MS (Thermo)
• Accurate to 1 ppm vs 100 ppm for quadrupole analyzers
• Cost ~2X quadrupole

Biomarkers
- Mechanistic Understanding
- Drug MoA
- Cellular Characteristics

Interpretation
Biochemical Extraction

- UHPLC-MS/MS (+ESI) Early/Polar
- UHPLC-MS/MS (+ESI) Late/Lipid
- UHPLC-MS/MS (-ESI)
- UHPLC (HILIC)-MS/MS

Library Search
RT, Mass, MS/MS
Data Reduction
Compound ID
QA/QC

•Library of ~2500 human metabolites

Statistical Analysis

Interpretation

Biomarkers
Mechanistic Understanding
Drug MoA
Cellular Characteristics
Biochemical Extraction

Pathway Visualization: Metabolync™ plugin to Cytoscape developed to overlay analyte findings onto metabolic pathways

Biomarkers
Mechanistic Understanding
Drug MoA
Cellular Characteristics

Interpretation
Metabolon’s Approach to Metabolomics - a Comprehensive Survey of the Metabolome
Extensive Metabolite Library

High resolution biochemistry surveys central metabolism & peripheral pathways that drive attributes of phenotype.
Normal metabolomics result

- High
- Low
- -1.5 - +1.5
- Molecule in library but not detected
- Molecule not in library

Circle size relative to Z-score
Global MAPS

Global Metabolomic Assisted Pathway Screen

• Metabolic pathway screen for perturbations in levels of analytes and relative abundance
  – Screens for >2500 small molecules (50-1500 Da)
  – Z-scores provided (not absolute values)
• Small molecule metabolomic analysis
  – Plasma
    • ~750-900 analyte identifications per plasma sample
  – CSF
    • ~300 analyte identifications per CSF sample
  – Urine
    • ~1200 analyte identifications per urine sample
Limitations of test

1. Inappropriate for identification of
   • Analytes >1500 Da or <50 Da
     • Proteins/large peptides
     • Complex oligosaccharides
     • Large lipids
     • Elements (K, Na, etc.)

2. Analytes requiring special extraction/chromatographic separation
   • Homocysteine (requires reductant treatment)

3. Screening tool to identify metabolic perturbations

4. Values are not quantitative

5. Not for acute assessments

If you are interested in a specific compound we can provide information on detection rates, accuracy, and analyte stability.
Sample collection/validation

• Retrospectively collected from stored lab samples
  • Na-Heparin treated plasma, stored -20 C for up to 3 months
  • 83% from Texas Children’s Hospital

• “Normal Controls”
  • Patient that came to our lab for testing but for whom no abnormal analytes were detected
  • Roughly age and sex matched to known patient samples
Overview of Plasma Samples

• 200 total
  • 128 from patients with diagnosis of IEM
  • 72 “Normal Controls”

• 27 different IEMs

• Majority of patients on treatment
Overview of samples

- **200 total**
  - 128 from patients with diagnosis of IEM
  - 72 “Normal Controls”
- **27 different IEMs**
- **Majority of patients on treatment**
Current analyte detections possible in our lab

<table>
<thead>
<tr>
<th>Category</th>
<th>#Analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>28</td>
</tr>
<tr>
<td>Acylcarnitines</td>
<td>28</td>
</tr>
<tr>
<td>Bile acids</td>
<td>13</td>
</tr>
<tr>
<td>Small panel</td>
<td>14</td>
</tr>
</tbody>
</table>
Average metabolomic detection in plasma

- Human metabolites: 463
- Other: 403
- #Analytes
  - Amino acids: 26 (BCM only: 2, Both: 24)
  - Acylcarnitines: 20 (BCM only: 8, Both: 12)
  - Bile acids: 11 (BCM only: 2, Both: 9)
  - Small panel: 10 (BCM only: 4, Both: 6)
## Average metabolomic detection in plasma

The diagram illustrates the distribution of metabolites detected in plasma, categorized by type and detection method. Here is the breakdown:

### Human metabolites
- **Other**: 403
- **Human metabolites**: 463
- **Other metabolites**: 67

### Metabolomic Detection

<table>
<thead>
<tr>
<th>Category</th>
<th>BCM only</th>
<th>Both</th>
<th>Metabolomics only</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>2</td>
<td>26</td>
<td>70</td>
<td>102</td>
</tr>
<tr>
<td>Acylcarnitines</td>
<td>8</td>
<td>20</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>Bile acids</td>
<td>2</td>
<td>11</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Small panel</td>
<td>4</td>
<td>10</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>2</td>
<td>11</td>
<td>66</td>
<td>89</td>
</tr>
<tr>
<td>Lysolipids</td>
<td>2</td>
<td>10</td>
<td>58</td>
<td>70</td>
</tr>
<tr>
<td>Nucleotides</td>
<td>2</td>
<td>31</td>
<td>31</td>
<td>64</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>2</td>
<td>26</td>
<td>26</td>
<td>54</td>
</tr>
<tr>
<td>Steroids/sterols</td>
<td>2</td>
<td>22</td>
<td>22</td>
<td>46</td>
</tr>
<tr>
<td>Vitamins/cofactors</td>
<td>2</td>
<td>16</td>
<td>16</td>
<td>44</td>
</tr>
<tr>
<td>TCA cycle</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>16</td>
</tr>
</tbody>
</table>

This data highlights the prominence of fatty acids and lysolipids in metabolomic detection.
Clinical validation experiments

• Intra-assay precision
  • median=10.47% (IQR= 5.55-22.04%)

• Linear detection
  • 34 acylcarnitines and amino acids
  • median r=0.9, IQR r= 0.84-0.95

• Stability
  • Plasma separation time course studies- 30 mins to >1 day
Expected IEM-related analyte elevations were detected

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Disease-related findings</th>
<th>Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria</td>
<td>phenylalanine</td>
<td></td>
</tr>
</tbody>
</table>
Expected IEM-related analyte elevations were detected

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</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria</td>
<td>phenylalanine</td>
<td></td>
</tr>
<tr>
<td>3-MCC def. Hydroxyisovaleroylcarnitine (C5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-methylcrotonylglycine</td>
<td></td>
</tr>
<tr>
<td>Arginemia</td>
<td>homoarginine</td>
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</tr>
<tr>
<td></td>
<td>arginine</td>
<td></td>
</tr>
<tr>
<td>Glutaric aciduria type 1</td>
<td>glutaroylcarnitine (C5)</td>
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</tr>
<tr>
<td></td>
<td>glutarate</td>
<td></td>
</tr>
<tr>
<td>HMG coA lyase def.</td>
<td>3-methylglutaroylcarnitine (C6)</td>
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</tr>
<tr>
<td></td>
<td>beta-hydroxyisovalerate</td>
<td></td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>methionine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-methylthioadenosine</td>
<td></td>
</tr>
<tr>
<td>MCAD def.</td>
<td>hexanoylglycine (C6)</td>
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</tr>
<tr>
<td></td>
<td>octanoylcarnitine (C8)</td>
<td></td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>propionylcarnitine (C3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methylcitrate</td>
<td></td>
</tr>
<tr>
<td>Thymidine phosph. def.</td>
<td>2'-deoxyuridine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5,6-dihydrothymine thymidine</td>
<td></td>
</tr>
</tbody>
</table>
PKU

PHENYLALANINE
TYROSINE
METABOLISM

THYROID HORMONE SYNTHESIS
Methylmalonic acidemia

- Isoleucine
- Leucine
- Valine
- Propionyl CoA
- 2-methylcitrate
- Methylmalonyl CoA
- Methylmalonyl Mutase Deficiency
- Succinyl CoA

Analyte Class:
- Nucleotide
- Lipid
- Energy
- Cofactor
- Carbohydrate
- Amino acid
- Branch chain amino acid
Methylmalonic acidemia

Isoleucine, Leucine, Valine → Propionyl CoA → 2-methylcitrate → Methylmalonyl CoA → Succinyl CoA

2-methylmalonyl carnitine

propionylcarnitine

2-methylcitrate

Z-score

Analyte Class
Plasma metabolomic analysis successfully screened for 20 different IEMs

- **Urea cycle disorders**
  - Arginase def
  - Argininosuccinate lyase def
  - Citrullinemia
  - Ornithine transcarbamylase def

- **Amino acid disorders**
  - Homocystinuria (CBS)
  - Maple syrup urine disease
  - Phenylketonuria

- **Fatty acid oxidation disorders**
  - MCAD
  - VLCAD

- **Organic acidemias**
  - 3-methylcrotonyl-CoA carboxylase def
  - Cobalamin disorders
  - Glutaric acidemia type I
  - HMG-CoA lyase def
  - Isovaleric acidemia
  - Methy malonic acidemia
  - Propionic acidemia

- **Other**
  - Guanidinoacetate methyltransferase def
  - Holocarboxylase synthetase def
  - Thymidine phosphorylase def
  - TMLHE def
Untargeted metabolomic analysis for the clinical screening of inborn errors of metabolism

Marcus J. Miller¹ · Adam D. Kennedy² · Andrea D. Eckhart² · Lindsay C. Burrage¹ · Jacob E. Wulff² · Luke A.D. Miller² · Michael V. Milburn² · John A. Ryals² · Arthur L. Beaudet¹ · Qin Sun¹ · V. Reid Sutton¹ · Sarah H. Elsea¹
FUNCTIONAL VALIDATION OF DNA VARIANTS OF UNCERTAIN SIGNIFICANCE

Neurologic phenotypes
Case 1 – Citrate Transporter Deficiency

EXOME Findings
- **trans mutations in SLC13A5**
  - c.997C>T (p.R333X) and c.680C>T (p.T227M)
- Disorder: AR citrate transporter def.
Case 2 - Aromatic Amino Acid Decarboxylase Deficiency

- 4 year old infant with developmental delay and hypotonia; initial presentation 11 months
- Tests performed previously—VLCFA, LSD panel, urine MPS, CMA, PAA, UOA, ACP, NH3, lactate, CK, CSF glucose/protein, muscle biopsy, ETC analysis, mitochondrial genome/depletion, MRI brain
- WES – 2 VUS (trans), c.286G>A (p.G96R) and c.260C>T (p.P87L) in the DDC gene
Case 2 - Pathway and Results

Diagram showing the pathway from 3-Methoxytyrosine to VMA, involving COMT, SAH, SAM, AADC, L-Dopa, Dopamine, HVA, 5HTP, Serotonin, and 5-HIAA. The patient's results are highlighted in red, showing abnormal Z scores for VMA compared to controls.
Dopa-containing medications

Aromatic amino acid decarboxylase deficiency

- High
- Low
- -1.5 - +1.5
- Molecule in library but not detected
- Molecule not in library

Circles size related to Z-score

- vanillylmandelate (VMA)
- 3-methoxy-4-hydroxyphenylglycolaldehyde
- acetyl CoA
- acetone
- fumarate
- 3-methoxy-4-hydroxyphenylglycol
- acetooacetate
- 3-methoxy-4-hydroxyphenylglycolaldehyde
- metanephrine
- homovanillate (HVA)
- normetanephrine
- 4-fumarly-acetate
- 4-maleyl-acetate
- normetanephrine
- 4-dihydroxyphenyllactate
- homogentisate
- Microbiome
- 4-hydroxyphenylpyruvate
- 3-(4-hydroxyphenyllactate
- methoxy-4-hydroxyphenylglycolaldehyde
- homogentisate
- Microbiome
- SULF
- dopamine
- 3-O-sulfate
- 3-methoxytyrosine
- -methoxytyramine
- dopamine
- 3-O-sulfate
- 3-methoxytyrosine
- 3-methoxytyramine sulfate
- dopamine
- 3-O-sulfate
- 3-methoxytyrosine
- 3-methoxytyramine
- dopamine
- 3-O-sulfate
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- dopamine
- 3-O-sulfate
- 3-methoxytyrosine
- 3-methoxytyramine
- dopamine
- 3-O-sulfate
- 3-methoxytyrosine
- 3-methoxytyra
Case 3

• 19 month old male
  – Global developmental delay
  – Hypotonia
  – Abnormal movements
  – Abnormal MRI (delayed myelination)
  – Oculomotor apraxia
  – Facial hemangioma
  – Constipation

• Prior normal workup
  – Microarray
  – Metabolic workup
    • Plasma amino acids
    • Lactate
    • Ammonia
    • Urine organic acids
    • CSF amino acids
    • CSF neurotransmitter profile

Whole exome sequencing and metabolomics ordered
Case 3 WES Results

- Single heterozygous pathogenic variant
  - *UROC1*, novel c.1448_1449delCT (p.S483fs)
    - Urocanase deficiency [MIM #276880]
- Two heterozygous VUS
  - *ABAT*, novel: c.454C>T (p.P152S) and c.1393G>C (p.G465R)
    - GABA transaminase deficiency [MIM #613163]

Single heterozygous VUS

- *ACAD9*, ACAD9 deficiency
- *ATM*, Ataxia-telangiectasia
- *UPB1*, Beta-ureidopropionase deficiency
- *DARS*, Hypomyelination with brainstem and spinal cord involvement and leg spasticity
- *CSPP1*, Joubert syndrome 21
- *HERC2*, Mental retardation, autosomal recessive 38
- *TH*, Segawa syndrome, recessive
- *SPG11*, Spastic paraplegia 11, autosomal recessive
- *AP4B1*, Spastic paraplegia 47, autosomal recessive
Case 3 WES Results

• Single heterozygous pathogenic variant
  —\textit{UROC1}, novel c.1448\_1449delCT (p.S483fs)
    • Confirmed by Sanger sequencing: Coverage = 100%
      » Father heterozygous
      » Mother negative
    • Urocanase deficiency [MIM #276880 ]

• Two heterozygous VUS
  — \textit{ABAT}, novel: c.454C>T (p.P152S) and c.1393G>C (p.G465R)
    • GABA transaminase deficiency [MIM #613163 ]
Case 3 - Metabolomic Results

- No significant alterations of molecules in histidine pathway.
- Second pathogenic variant likely not present.
- Diagnosis likely not urocanase deficiency.
Case 3 WES Results

• Single heterozygous pathogenic variant
  – UROC1, novel c.1448_1449delCT (p.S483fs)
    • Confirmed by Sanger sequencing: Coverage = 100%
      » Father heterozygous
      » Mother negative
  • Urocanase deficiency [MIM #276880 ]

• Two heterozygous VUS
  – ABAT, novel: c.454C>T (p.P152S) and c.1393G>C (p.G465R)
    • c.454C>T (p.P152S), novel, inherited from mother
    • c.1393G>C (p.G465R), novel, inherited from father
  • Both variants predicted to be deleterious using sift and polyphen
  • GABA transaminase deficiency [MIM:#613163]
GABA transaminase (ABAT) deficiency (Plasma!)

2-pyrrolidinone - a new biomarker for ABAT deficiency
Exome + Metabolomics

• 180 Cases with both clinical exome and clinical metabolomic testing
• Assessed diagnostic rate of platforms
• Assessed when metabolomics contributed to variant re-classification [Alaimo, ASHG 2017]
Contribution of Metabolomics to Genomics

Contribution of Metabolomics:
- Provided Information: 37.8%
- Did not aid in the case: 62.2%

Diagnosis:
- Molecularly Solved: 49.5%
- Did not aid in the case: 50.5%
Contribution of Metabolomics to Genomics

Contribution of Metabolomics

- Provided Information: 62.2%
- Did not aid in the case: 37.8%

Areas Improved

- Rules Out Variants from Diagnosis: 75%
- Variant Classification Change: 14.7%
- Confirms Molecular Diagnosis: 19.1%
Variants Improved for Diagnosis

- **Rules Out Variant from Diagnosis**
- **Variant Classification Change**
- **Confirms Molecular Diagnosis**

**Variant Classification Changes**

- **B** = benign
- **LB** = likely benign
- **VUS** = variant of uncertain significance
- **LP** = likely pathogenic
- **P** = pathogenic

Areas Improved:

- **19.1%**
- **14.7%**
- **75%**

**Contributions of Metabolomics to Variant Interpretation**
Peroxisomal Biogenesis disorders (PBD)

FURTHER VALIDATION AND NEW DISCOVERIES
PBD are a clinical spectrum of disease

**Disease Phenotype**
- Classic Zellweger
- Neonatal Adrenoleukodystrophy
- Infantile Refsum

**Biochemical Phenotype**
- **↑↑ VLCFA’s**
  - Absent or deficient peroxisomes
- **↑ or normal VLCFA’s**
  - Reduced # peroxisomes

**PEX alleles**
- **Severe hypomorphic or null**
- **Mild hypomorphic**

**Disease Severity**

Pictures from Inborn Metabolic Diseases 4th edition and SIMD NAMA
Mild PBD

- 7 year old
- Phenotype mimicking Usher syndrome with hearing loss and pigmentary retinopathy, normal cognition, diagnosed by research sequencing study for Usher
- *PEX1* G843D homozygote
Zellweger-spectrum disorders

Metabolomics

~650 named molecules identified in each plasma sample, N=19
Results: Untargeted metabolomic analysis

- Pipecolic Acid
- C24 (VLCFA)
- 7-HOCA (bile acid)
- Hexadecanedioate
- Octadecanedioate
- Eicosanodioate
- Docosadioate
- 1-(1-enyl-palmitoyl)-GPC*
- 1-(1-enyl-oleoyl)-GPC*
- 1-(1-enyl-stearoyl)-GPC*
- 1-O-hexadecyl-GPC*

*Plasmalogens
Results: Sphingomyelins as new biomarkers for PBD-ZSD

- Palmitoyl Sphingomyelin
- Palmitoleoyl Sphingomyelin
- Oleoyl Sphingomyelin
- Nervonoyl Sphingomyelin
- Myristoyl Sphingomyelin
- Euricoyl Sphingomyelin
- Eicosenoyl Sphingomyelin
- Behenoyl Sphingomyelin*
- Sphingomyelin

Z Score
NEW DISCOVERIES!
Oral Carnitine is converted to Trimethylamine N-oxide (TMAO)

Carnitine

Gut Microbiota

TMAO

Oral Carnitine is converted to Trimethylamine N-oxide (TMAO)

Diet & baseline production of TMAO

Dietary influence of carnitine-challenge on TMAO production

Plasma TMAO elevations in Global MAPS validation cohort

TMAO Identified?

No Diagnosis
- Yes (n=44)
- No (n=26)
- Total (n=70)

Organic Acidemia
- Yes (n=23)
- No (n=1)
- Total (n=24)
Plasma TMAO elevations in Global MAPS validation cohort

TMAO Identified?

- No Diagnosis (n=70)
  - Yes (n=44)
  - No (n=26)

- Organic Acidemia (n=24)
  - Yes (n=23)
  - No (n=1)

$p = 1.1 \times 10^{-8}$
Meat restriction and oral carnitine supplementation

• Clinical notes on 19 of 24 patients
• All on Chronic PO carnitine (range 17-145 mg/kg/day)
• All highly discouraged from consuming meat
Meat restriction and oral carnitine supplementation

• Clinical notes on 19 of 24 patients
• All on Chronic PO carnitine (range 17-145 mg/kg/day)
• All highly discouraged from consuming meat

<table>
<thead>
<tr>
<th>Disorder</th>
<th>3-methylhistidine (average z-score)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>organic acidemia</td>
<td>-0.70</td>
<td>5.02E-04</td>
</tr>
<tr>
<td>urea cycle</td>
<td>-0.73</td>
<td>1.82E-05</td>
</tr>
<tr>
<td>pku</td>
<td>-0.57</td>
<td>7.96E-03</td>
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<tr>
<td>no diagnosis</td>
<td>0.00</td>
<td>NA</td>
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</table>
Plasma Carnitine is decreased in patients with organic acidemias

$p = 0.004$
Plasma Carnitine is decreased in patients with organic acidemias

$p = 0.004$

$R^2 = 0.46$
$p = 0.0009$
TEST REPORTING
Reporting Format

• Analysis of data
• Interpretation provided by laboratory director
• Tables of analytes with Z-score >+2 or <-2
  – Human metabolites
  – Drugs
  – Xenobiotics
  – Dietary
• Analytes categorized by pathway
INTERPRETATION:

We understand this is a 3 year old male with developmental delay, ocular motor apraxia, and seizures. Molecular testing revealed a de novo 12q11.2 duplication (381 Kb). This region contains 8 genes: RNASET, RNAE8E, SOLO, SNF219, HMRNPC, RPGRIP1, SUPT16H, and CHD8. His family history includes two brothers, ages 14 months and 5 years old, who are also affected. The 14 month old has a similar clinical presentation, while the 5 year old suffered from delayed speech development which has since resolved. In addition, his 56 year old maternal grandmother has a history of Crohn’s disease and Type 2 diabetes. Analysis of plasma amino acids, urine organic acids, plasma acylcarnitines, and plasma creatine/guanidinoacetate were negative. Plasma was submitted for analysis of perturbations in metabolic pathways that may be relevant to these clinical symptoms and molecular findings.

All significant analytes are listed in Tables 1-4. N6-succinyladenoine is significantly elevated. Accumulation of this compound is associated with adenylosuccinase deficiency (OMIM 103050), a disorder of de novo purine synthesis. Suggest urine purine analysis for confirmation of this finding. Needs clinical correlation.

Results are dependent upon sample quality, diet, medications, and other physiological conditions. Use of special diets or supplements may mask metabolic abnormalities. Clinical indications, medications, and diet are required for proper interpretation. An expanded report is available upon request.

RESULTS:

1. Significantly altered analytes (z-score ≥2 or ≤-2) possibly related to the patient’s phenotype

<table>
<thead>
<tr>
<th>Analyte</th>
<th>z-score</th>
<th>Superpathway</th>
<th>Subpathway</th>
<th>HMDB ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Purine Metabolism, Adenine containing</td>
<td>HMB00912</td>
</tr>
<tr>
<td>N6-succinyladenosine</td>
<td>7.3</td>
<td>Nucleotide</td>
<td>Lysolipid</td>
<td></td>
</tr>
<tr>
<td>2-palmitoyl-GPE (16:0)*</td>
<td>3.1</td>
<td>Lipid</td>
<td>Lysolipid</td>
<td></td>
</tr>
<tr>
<td>phenylacetyl/glycine</td>
<td>2.4</td>
<td>Amino Acid</td>
<td>Phenylalanine and Tyrosine Metabolism</td>
<td>HMB00821</td>
</tr>
<tr>
<td>1-dihomo-linolenyl-GPE (20:3n6) *</td>
<td>2.4</td>
<td>Lipid</td>
<td>Lysolipid</td>
<td></td>
</tr>
<tr>
<td>metatoc</td>
<td>2.3</td>
<td>Nucleotide</td>
<td>Pyrimidine Metabolism, Metabotropic containing</td>
<td>HMB00226</td>
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<tr>
<td>dimethylglycine</td>
<td>2.3</td>
<td>Amino Acid</td>
<td>Glycine, Serine and Threonine Metabolism</td>
<td>HMB00092</td>
</tr>
<tr>
<td>isoavilery/glycine</td>
<td>2.0</td>
<td>Amino Acid</td>
<td>Leucine, Isoeucine and Valine Metabolism</td>
<td>HMB00678</td>
</tr>
<tr>
<td>tyrosine</td>
<td>2.0</td>
<td>Amino Acid</td>
<td>Phenylalanine and Tyrosine Metabolism</td>
<td>HMB00198</td>
</tr>
<tr>
<td>butyrylcarnitine (C4)</td>
<td>-2.2</td>
<td>Lipid</td>
<td>Fatty Acid Metabolism (also BCAA Metabolism)</td>
<td>HMB02013</td>
</tr>
<tr>
<td>3-methoxytyrosine</td>
<td>-4.0</td>
<td>Amino Acid</td>
<td>Phenylalanine and Tyrosine Metabolism</td>
<td>HMB01434</td>
</tr>
</tbody>
</table>
Summary Global MAPS

• Identifies all common IEMs studied to date screened on PAA/UOA/ACP

• Screening tool for undifferentiated phenotypes
  – Developmental Delay/Intellectual disability/Hypotonia
  – Seizures (non-specific)

• Does not replace PAA, UOA, etc. for diagnostic testing or management nor can it detect large molecules (MPS, CDG)

• Validate DNA results and can identify IEMs for which no biochemical testing available (Citrate transporter deficiency)

• Potential to diagnose neurotransmitter disorders on a plasma specimen (AADC & GABA transaminase)

• Discovery of Novel Biomarkers for IEMs (PBDs)

• Understand effects of therapies in IEMs
What does Global MAPS test for?

- Disorders of amino acid metabolism: plasma amino acids $220
- Organic acidemias: urine organic acids and acylglycines $500
- Purine disorders: urine purine panel $260
- Pyrimidine disorders: (urine pyrimidine panel $280
- Neurotransmitter disorders: plasma thymidine; urine pyrimidines; plasma/urine creatine & guanidinoacetate; csf: succinyladenosine, 5HIAA, HVA, 3OMD, lactate, & glucose $1330 (exclusive of LP costs)
- Cholesterol Metabolism & PBDs $850
- Creatine disorders: plasma/urine creatine and guanidinoacetate $280
- Bile acid disorders: plasma/urine bile acids $917
- Urea cycle disorders: plasma amino acids & urine orotic acid $300
- Fatty acid oxidation disorders: acylcarnitine profile, acylglycines, & urine organic acids $770
- Certain mitochondrial disorders:
  - MNGIE: plasma thymidine $200

Total cost: > $5000!