Identification of Neurotransmitter-Associated and Other Metabotypes: Further Stratification of the Children's Autism Metabolome Project (CAMP) ASD Subjects

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Overview

GOALS:

1) Create metabolism-based diagnostic tests based on reproducible metabotypes

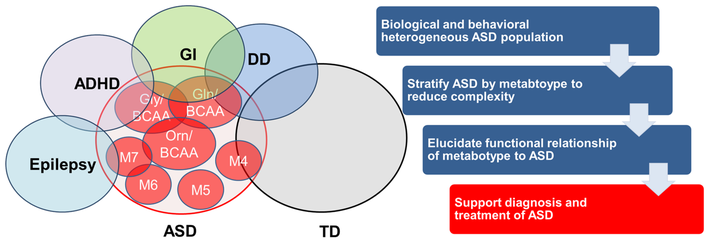
2) Examine the overlap of biochemical processes associated with metabotypes

METHODS: Quantitative LC-MS/MS measurement of plasma metabolites followed by statistical analysis for metabotypes associated with ASD using CAMP subjects

RESULTS: Reproducible metabotypes associated amine, energy, and purine metabolic processes were identified in 41% of CAMP ASD subjects

Introduction

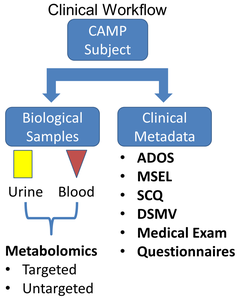
- The Children's Autism Metabolome Project (CAMP, ClinicalTrials.gov: NCT02548442) recruited 1100 children for metaboloimc analyses to identify altered metabolism associated with ASD
- Utilized a metabotyping approach previously published (Biol. Psychiatry 2019; 85:345-354) that identified ASD subjects associated with branched-chain amino acid (BCAA) dysregulation
- Metabotype-based stratification of ASD provides more biochemically homogenous subpopulations that offer the potential for tailored pharmacological, behavioral, and dietary interventions



Children's Autism Metabolome Project CAMP

- 8 clinical sites, research reliable ADOS-2, MSEL, and DSM-V
- Large cohort of fasted subjects allows detection distinct ASD subpopulations using plasma
- Over 600 metadata items per subject from both subject and family medical histories
- Genetic samples available for analysis

Clinical Workflow



CAMP Subjects With Available Plasma Samples

Metric	Training	Test	All
ASD Children	263	263	526
TD Children	107	106	213
N	370	369	739
Prevalence (%)	71.1	71.3	71.2
ASD % Male	77.6	79.5	78.5
TD % Male	60.7	58.5	59.6
ASD Age (Months)	35.7 +/- 7.6	34.5 +/- 7.9	35.1 +/- 7.8
TD Age (Months)	33 +/- 8.6	32 +/- 8.9	32.5 +/- 8.8
Age (range)	18 to 48	18 to 48	18 to 48
DQ ASD	62.2 +/- 17.6	63.5 +/- 17.7	62.9 +/- 17.7
DQ TD	100.3 +/- 15.1	103.4 +/- 17.3	101.8 +/- 16.3

Targeted Metabolomic Analysis Identified Potential Metabotypes

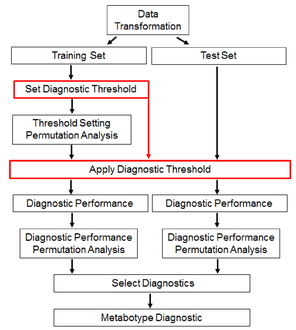
- Targeted non-quantitative analysis of 82 metabolites to identify biochemical areas associated with metabotypes
- Training set of CAMP ASD and TYP subjects evaluated

Metabotypes Identified in Targeted Metabolomic Analysis

Metabolite	SEN	SPEC	PPV	Biochemical Area
α-Ketoglutarate	0.126	1.000	1.000	Energy Homeostasis
Hypoxanthine	0.126	0.988	0.970	Purine Degradation
Pyruvate	0.193	0.965	0.942	Energy Homeostasis
Lactate	0.118	0.976	0.938	Energy Homeostasis
GLY	0.098	0.976	0.926	Amine / Neuro
Taurine	0.142	0.965	0.923	Purine Degradation
ASP	0.094	0.976	0.923	Amine / Neuro
ASN	0.098	0.988	0.962	Amine / Neuro
Succinate	0.094	0.988	0.960	Energy Homeostasis

PPV is reported for the CAMP population (precision)

Metabotype Workflow



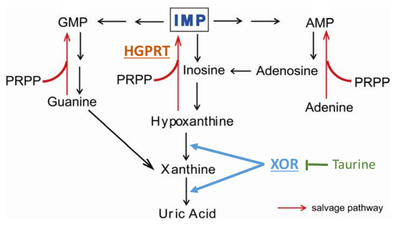
Metabotypes using Quantitative Assays

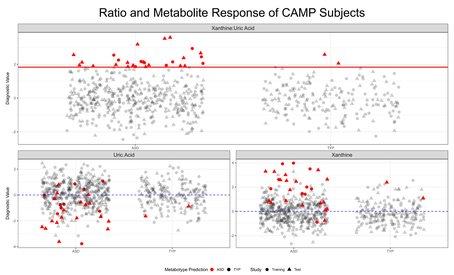
- Quantitative LC-MS/MS assays developed based on metabolomics results
- The CAMP samples were divided between a training set and an independent validation (test) set

Purine Degradation Related Metabotype

- Metabolites measured: xanthine, hypoxanthine, inosine, uric acid, and taurine
- Hemolyzed samples with hemoglobin levels >200 mg/dl were excluded from analysis due to interference
- A single reproducible metabotype was identified in 6.3% of CAMP ASD subjects

Ratio	SEN		SPEC		PPV		Biochemical Area
	Train	Test	Train	Test	Train	Test	
Xanthine / Urate	0.044	0.083	1.000	0.979	1.000	0.900	Purine Degradation





- Xanthine Oxidoreductase (XOR) is required for catabolism of purines
- Elevated xanthine is correlated with taurine (ρ=.64), an amine which reduces XOR activity and is evaluated in MOCOS (molybdenum cofactor required by XOR) deficiency
- The data suggests that biomarkers of defective sulfite metabolism may provide a link to understanding the biology in a subset of children with ASD

References

Smith AM, King JJ, West PR, Ludwig MA, Donley ELR, Burrier RE, Amaral DG. **Amino Acid Dysregulation Metabotypes: Potential Biomarkers for Diagnosis and Individualized Treatment for Subtypes of Autism Spectrum Disorder**. Biol Psychiatry. 2019, 85(4)345:354; PMID: 30446206

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Energy Homeostasis Related Metabotypes

- Metabolites measured: α-ketoglutarate, lactate, pyruvate, succinate, alanine and phenylalanine
- Lactate, pyruvate, and alanine are commonly used to assess mitochondrial bioenergetic function

Ratio	SEN		SPEC		PPV		Biochemical Area
	Train	Test	Train	Test	Train	Test	
α-Ketoglutarate / Lactate	0.074	0.134	0.981	0.981	0.905	0.946	Energy Homeostasis
α-Ketoglutarate / ALA	0.070	0.061	0.981	0.991	0.900	0.941	Energy Homeostasis
Lactate / ALA	0.051	0.073	0.990	0.991	0.929	0.950	Energy Homeostasis
Lactate / PHE	0.058	0.084	0.990	0.981	0.938	0.917	Energy Homeostasis

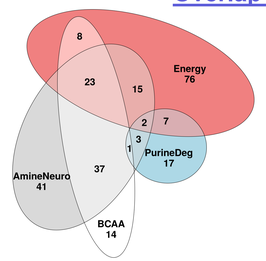
- 22.3% of CAMP ASD identified by an energy related metabotype

Novel Amine Associated Metabotypes

- Metabolites measured: 32 amine containing and amino acid metabolites
- Test for amine related metabotypes not previously published (Smith et al. 2019)
- Five amine metabotypes identified 21.5% of CAMP ASD subjects
- Altered neurotransmission, mitochondrial biology, nitrogen metabolism may be associated with metabotypes.

Ratio	SEN		SPEC		PPV		Biochemical Area
	Train	Test	Train	Test	Train	Test	
ASN / GLY	0.069	0.073	0.990	0.990	0.947	0.950	Amine / Neuro
GLY / PHE	0.062	0.065	1.000	0.990	1.000	0.944	Amine / Neuro
HIS / LEU	0.088	0.084	0.980	0.980	0.920	0.917	Amine / Neuro
KYN / ORN	0.050	0.057	0.990	0.990	0.929	0.938	Amine / Neuro
LYS / ORN	0.065	0.050	0.990	0.990	0.944	0.929	Amine / Neuro

Overlap of Metabotype Positive Subjects



- 41% of CAMP ASD subjects identified in the training and test sets
  - Metabotype positive subjects that share multiple metabotypes may have more complex metabolic phenotypes requiring further study
  - Subjects identified by a single amine, purine, or energy process metabotype may have a specific metabolic dysregulation

Summary

- The large ASD CAMP cohort allows detection of reproducible metabotypes with a prevalence > 5%
- Biological processes associated metabotypes were identified in 41% of CAMP ASD subjects
  - Mitochondrial biology/energy metabolism
  - Diverse amino acid pathways related to amino acid homeostasis
  - Purine catabolism
- Future Studies
  - Develop additional metabolic panels based on ROS and gut microbiome associated metabolism
  - Utilize additional computational approaches to identify metabolic changes predictive of ASD
  - Continue CAMP subject metadata for associations with metabotype positive populations
  - Launch clinical studies of dietary interventions within BCAA metabotype positive ASD subjects