

Overview

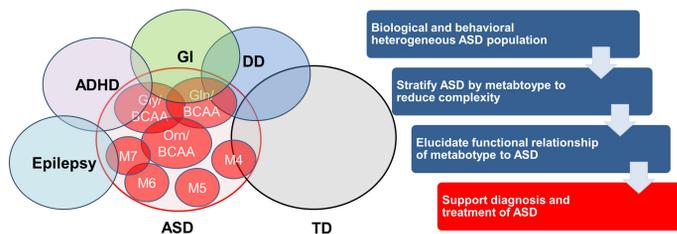
PURPOSE: Develop metabolite-based metatype diagnostic tests to support ASD diagnosis.

METHODS: Quantitative measure of 31 amines. Diagnostics developed in a training set of 348 subjects and evaluated in an independent test set of 362 subjects.

RESULTS: Amino Acid Dysregulation Metatypes (AADM) identified altered metabolism in 15.4% of ASD subjects that is detectable with a specificity of 97% and a PPV of 93%.

Introduction

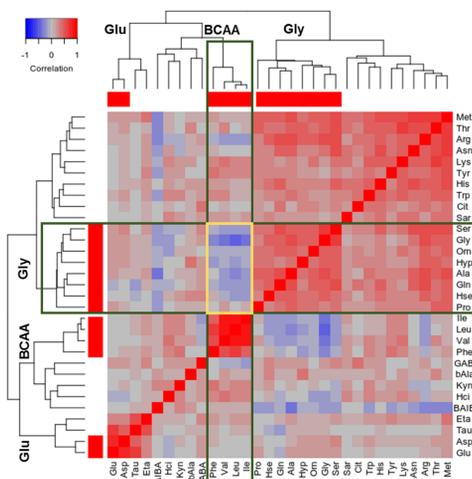
Metabolism based analysis can identify metabolites associated with an individual's inherited biochemistry as well as capture the interactions of the gut microbiome, dietary, and environmental factors. We designed the Children's Autism Metabolome Project (CAMP), which enrolled 1100 subjects ages 18 to 48 months, to discover clinically significant metabolic phenotypes associated with ASD. We conducted quantitative LC-MS/MS to identify metabolic subtypes able to stratify ASD into groups based on shared metabolic phenotypes. A metatype based test can support earlier diagnosis, development and selection of more precise therapeutic interventions, as well as better understanding of the efficacy of current interventions within metatypes.



Metatypes

- Subpopulations based on shared patterns of metabolism
- Allow development of metabolic based tests to identify distinct ASD populations

Discovery of Amino Acid Dysregulation Metatype



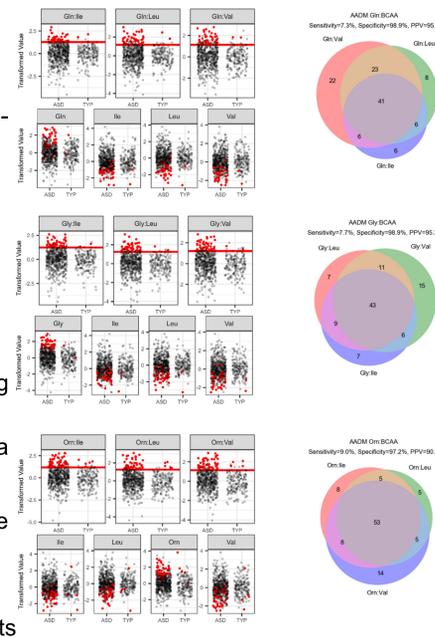
Metabolite Correlations within ASD Reveal Distinct Clusters of Amine Metabolites

- Hierarchical clustering of pairwise Pearson correlation identify metabolites with co-regulated metabolism
- Three clusters of metabolites with positive correlations were identified related to branched chain amino acids (BCAAs), glycine and glutamine
- Intersection of the glycine and BCAA clusters [green boxes] yielded a block of negative correlations [yellow box]

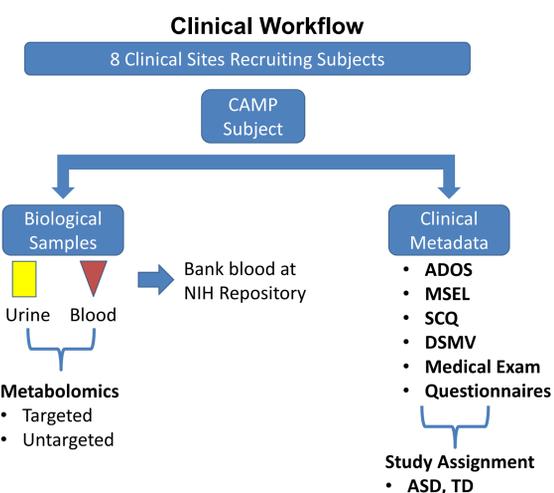
- Analyzed ratios of BCAAs and metabolites within glycine cluster negatively correlated to BCAAs
- Ratios can uncover biological properties not evident with individual metabolites as well as increase the signal

Identification of AA/BCAA imbalance metatypes associated with ASD

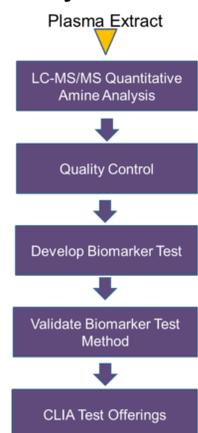
- A novel metabotyping approach was applied to ratios of negatively correlated metabolites in the training set to develop diagnostic thresholds (box plots, red lines) that defined subpopulations (red points) of ASD subjects with a high positive predictive value (PPV)
- The correlation of the BCAAs with each other ($\rho = 0.86 \pm 0.02$) and the overlap of affected-subjects (Venn diagrams) suggested diagnostic panels using ratios based on a single numerator and the BCAAs as denominators could create a diagnostic test for a BCAA related dysregulation metatype
- Test panels were created where each ratio must be positive to consider a subject metatype positive (Venn diagram, Intersection)
- All thresholds were set in the training set of subjects



Children's Autism Metabolome Project (CAMP)



Bioanalytical Workflow

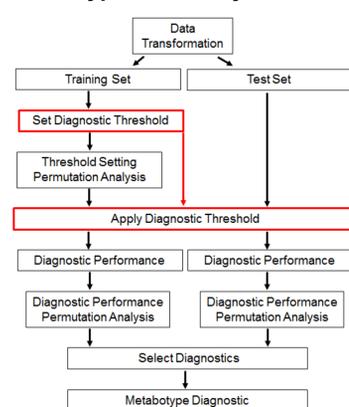


Demographics of 710 CAMP Subjects Analyzed

Metric	Training Set	Test Set	Combined Sets
ASD Children	260	273	533
TYP Children	88	89	177
ASD Prev. (%)	74.7	75.4	75.1
ASD % Male	76.2	78.4	77.3
TYP % Male	64.8	61.8	63.3
ASD Age (Mos)	36 +/- 7.5	34.6 +/- 7.9	35.3 +/- 7.7
TYP Age (Mos)	32.3 +/- 8.5	31.4 +/- 9.1	31.9 +/- 8.8
Age (range)	18 to 48	18 to 48	18 to 48
DQ ASD	62.2 +/- 19.3	63.5 +/- 20.2	62.9 +/- 19.8
DQ TYP	97.6 +/- 14.9	99.5 +/- 18.4	98.6 +/- 16.7

Subject composition of training and tests. Means +/- Standard Deviation.

Metatype Discovery Workflow



AADMs Identifies Common BCAA Metabolic Imbalance

AADM	SEN _{Train}	SEN _{Test}	SPEC _{Train}	SPEC _{Test}	PPV _{Train}	PPV _{Test}
Ala:BCAA	0.085	0.059	0.932	0.966	0.786	0.842
Gln:BCAA	0.073	0.073	0.989	0.989	0.950	0.952
Gly:BCAA	0.073	0.081	0.989	0.989	0.950	0.957
Hse:BCAA	0.112	0.205	0.932	0.921	0.829	0.889
Orn:BCAA	0.081	0.099	0.977	0.966	0.913	0.900
Ser:BCAA	0.096	0.106	0.966	0.944	0.893	0.853
Hyp:BCAA	0.077	0.066	0.966	0.910	0.870	0.692

Table Diagnostic performance metrics of AADM Test Panels.

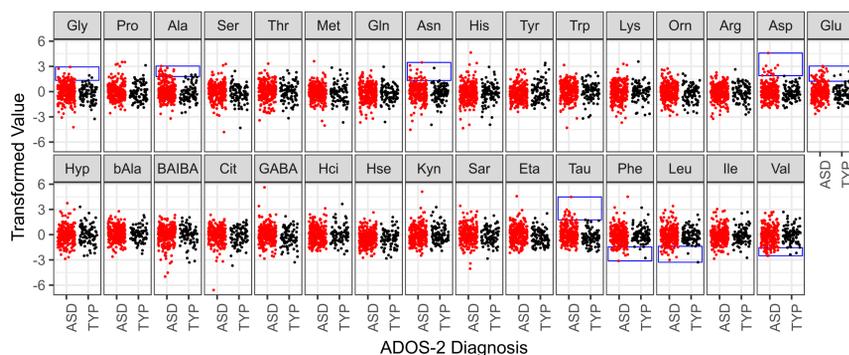
Reproducible Test Panels

- Minimum criteria SEN > 0.05, PPV > 0.90, SPEC > 0.95 in both training and test sets
- Panels based on Gln, Gly, and Orn identify metatypes in the test set

Amino Acid Profiles Suggest Metabolic Subpopulations

Analysis of amine-containing metabolites between ASD and TYP study populations suggest little difference in abundance at population level

- Analysis of covariance was performed on 31 amine containing metabolites in the training set of subjects to test the effect of gender or diagnoses controlling for subject age on metabolite means
- No significant differences were identified in metabolite abundance values for diagnosis, age, sex, gender or their interactions

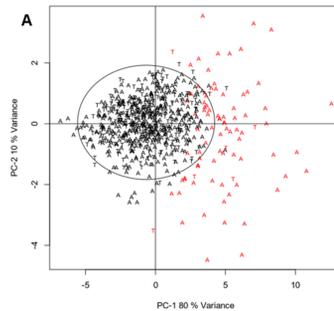


Analysis of boxplots reveal subpopulations

- Revealed metabolites with distinct outlier populations only present in ASD [blue boxes]
- Populations suggestive of metatypes comprised of at least 5% of training set ASD population

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AADMs Define a Diagnostic for BCAA Dysregulation Associated with ASD



• A metabolic phenotype related to imbalances in BCAA metabolism (Union, Venn Diagram) in 15.4% of ASD subjects was identified by the combined test panels

• 81% (71/88) of the AADM-positive subjects (red) were separated from the unaffected subjects (PCA Scores Plot, A=ASD, T=TYP, Ellipse = 95% Confidence Interval from Hoetellings T²)

CLIA Based Laboratory AADM Test

NeuroPoint Dx CLIA based offerings

- Quantitative assay to measure 32 amine containing compounds including amino acids
- AADM diagnostic test for BCAA imbalances: **NPDX ASD AADM Panel 1**
- NPDX ASD AADM Panel 1 identifies 28.2% of CAMP ASD subjects with specificity of 90% and PPV of 90%

Summary

- CAMP is the largest clinical study designed to identify changes in metabolism associated with ASD
- Metatype analysis identifies alterations in metabolism not evident in means based analysis
- Metabolites in ratios with BCAAs uncover imbalances in amino acid metabolism that describe reproducible metabolic subpopulations associated with ASD
- Ratios combined into diagnostic panels create diagnostics with increased specificity
- Metatype diagnostic panels based on Gln, Gly, and Orn in ratios with the BCAAs identify a reproducible metatype of BCAA dysregulation associated with ASD
- The BCAA metatype (AADM) is identifiable in 15.4 % of CAMP ASD subjects with a specificity of 96.6% and PPV of 93.1%
- These metatype test panels are currently available as a CLIA-based test