Development of a plasma metabotype-based biomarker test battery to screen for children at risk of ASD

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Overview

Goals:
- Identify alterations in metabolism helpful to stratify CAMP ASD subjects into subpopulations of similar metabolic phenotypes (i.e metabotypes)
- Develop a quantitative screening approach based on measurement of plasma metabolites to identify children at risk of ASD and inform on more precise intervention
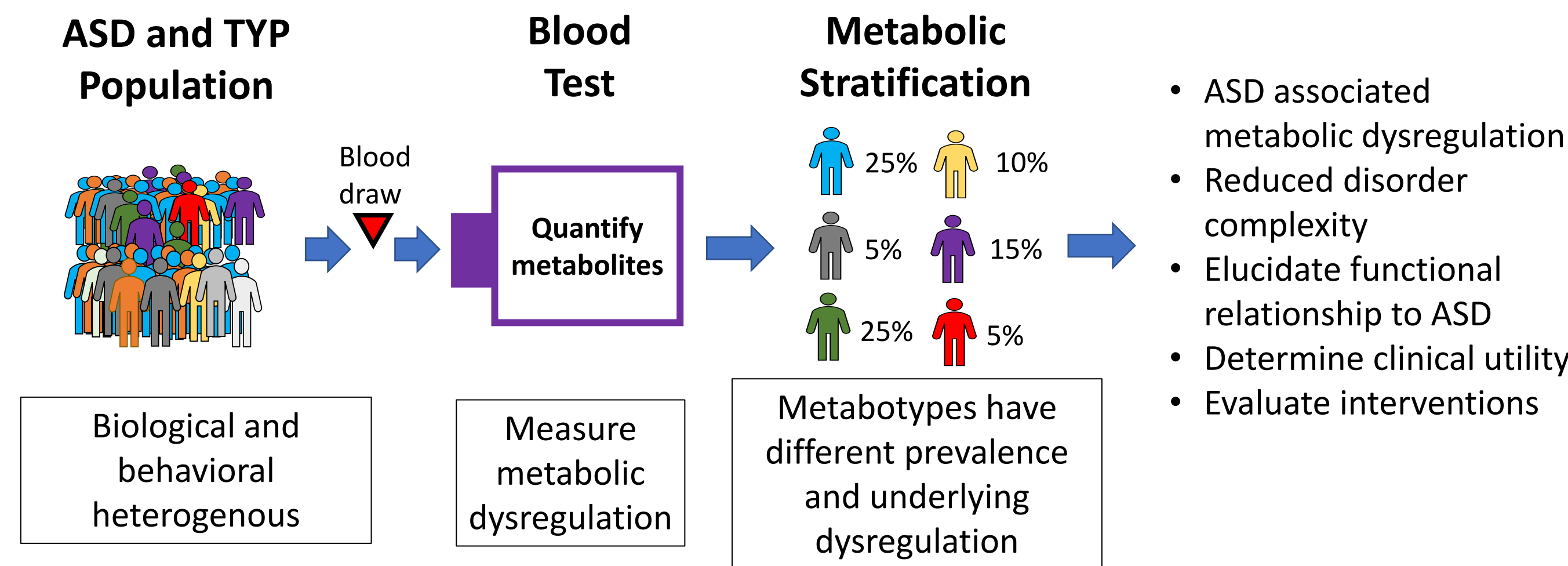
Methods:
- CLIA/CAP certified quantitative LC-MS/MS measurement of 42 plasma metabolites
- Computation to develop test of metabolic dysregulation associated with risk of ASD

Results:
- Metabolites and ratios of metabolites identified dysregulation associated with 8 clusters of metabotypes. Clusters represent metabotypes that identify similar dysregulation.
- A test battery based on a subset of these ratios identified ASD CAMP subjects with 63% sensitivity and 90% specificity.

Concept:

- ASD associated metabolic dysregulation
- Reduced disorder complexity
- Elucidate functional relationship to ASD
- Determine clinical utility
- Evaluate interventions

Children's Autism Metabolome Project (CAMP)

- ClinicalTrials.gov Identifier: NCT02548442
- 8 clinical sites, assessments: ADOS-2, MSEL, and DSM-V, family medical history
- Overnight fasted blood draw in heparin tubes, plasma separated and store at -80 C
- Funded by NIH and the Nancy Lurie Marks Family Foundation

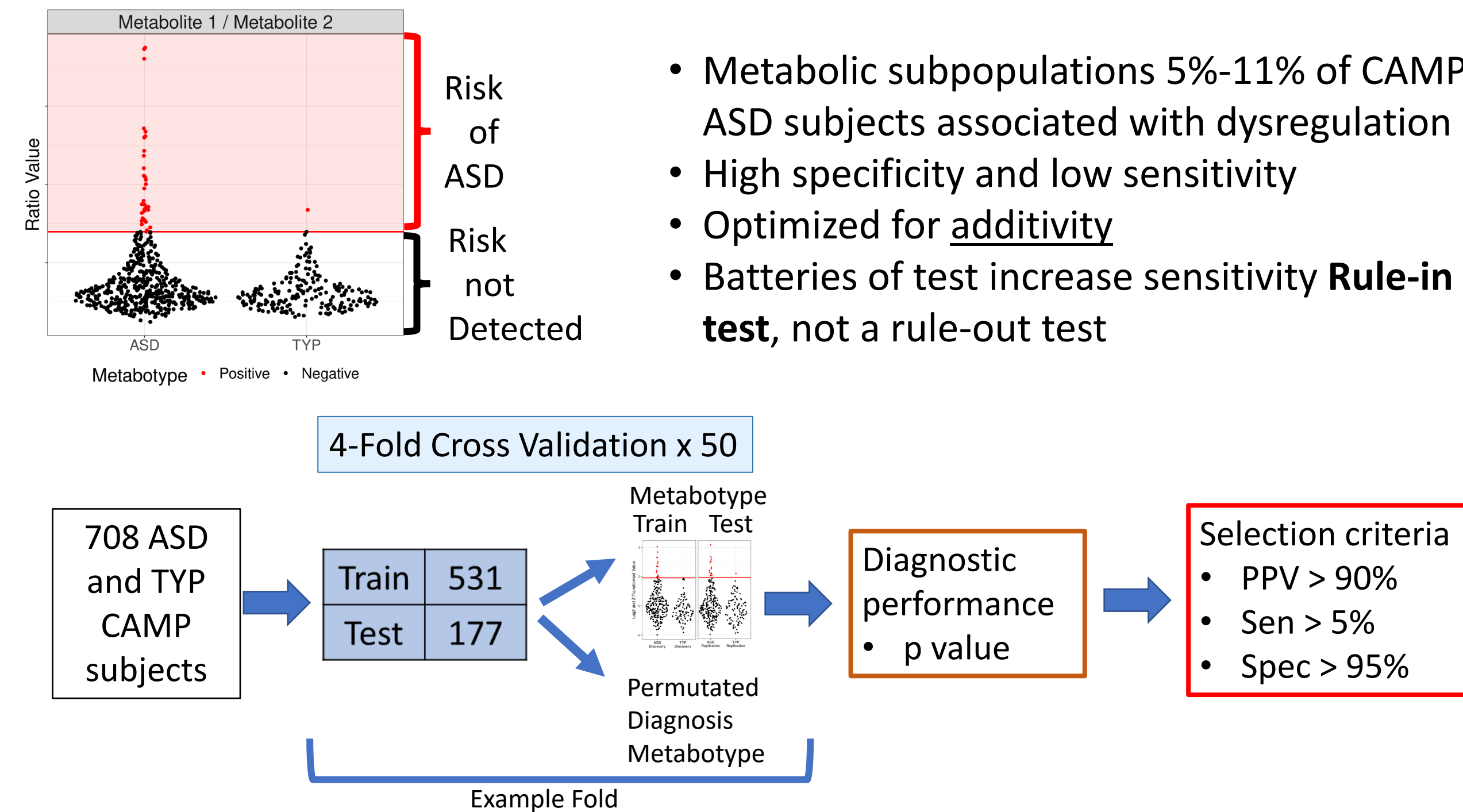### CAMP Study Population Demographics

Metric	Value
No. ASD Children	499
No. TYP Children	209
ASD vs TYP Prevalence, %	70.5
ASD male[a], %	79
TYP male[a], %	59.3
ASD Age[b], mean (SD), months	35.1 (7.8)
TYP Age[b], mean (SD), months	32.6 (8.7)
Age range, months	18 to 48

CAMP sites
8 Premier Children's Hospitals
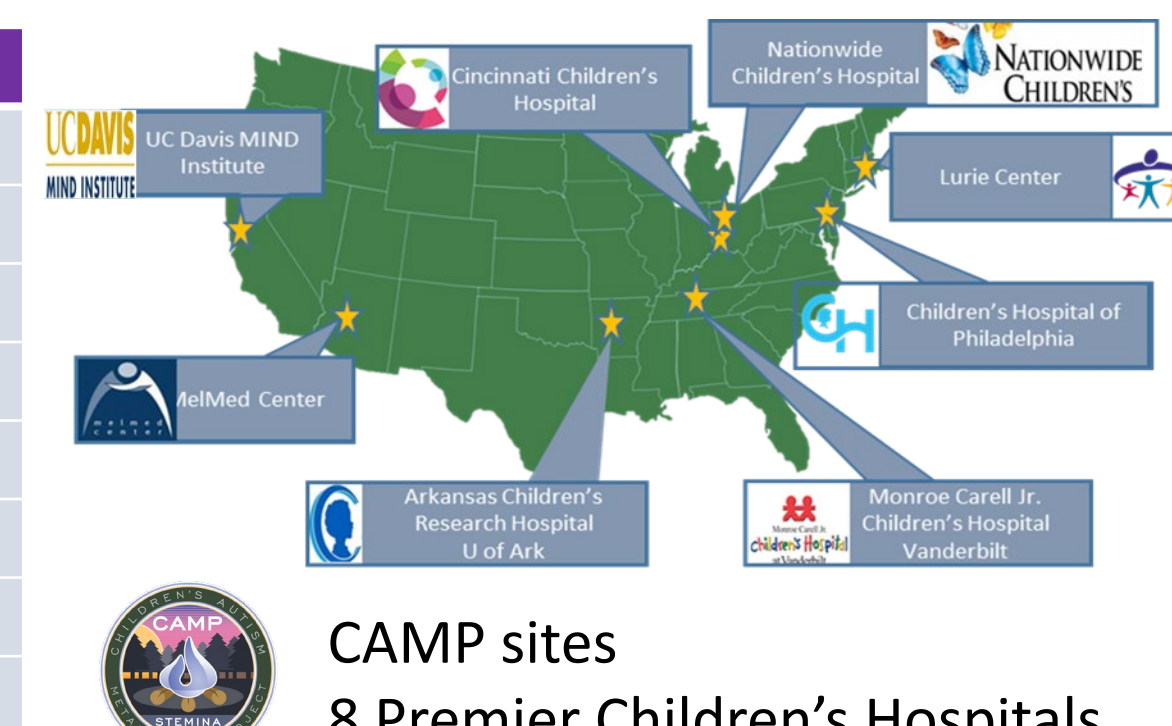
[a,b,c]Indicates a comparison with statistically significant difference between ASD and TYP populations (p-value < 0.05). Abbreviations: TYP, typically developing; ASD, autism spectrum disorder

https://neuropointdx.com/studies/

Approach to Metabotype Test Development

- Metabolic subpopulations 5%-11% of CAMP ASD subjects associated with dysregulation
- High specificity and low sensitivity
- Optimized for additivity
- Batteries of test increase sensitivity **Rule-in test**, not a rule-out test

4-Fold Cross Validation x 50

708 ASD and TYP CAMP subjects

Train	531
Test	177

Diagnostic performance
- p value

Selection criteria
- PPV > 90%
- Sen > 5%
- Spec > 95%

Metabotyping Metabolic Dysregulation Associated with ASD

- 42 Metabolites quantified from heparin plasma samples
  - Amines and organic acids

- Generated 1764 unique ratios of metabolites

- A subset of 125 metabotype tests met minimum diagnostic criteria
  - 79% of CAMP ASD participants

- Clustered metabotypes

- Optimized a battery of 23 tests to identify ASD subjects at least 90% specificity
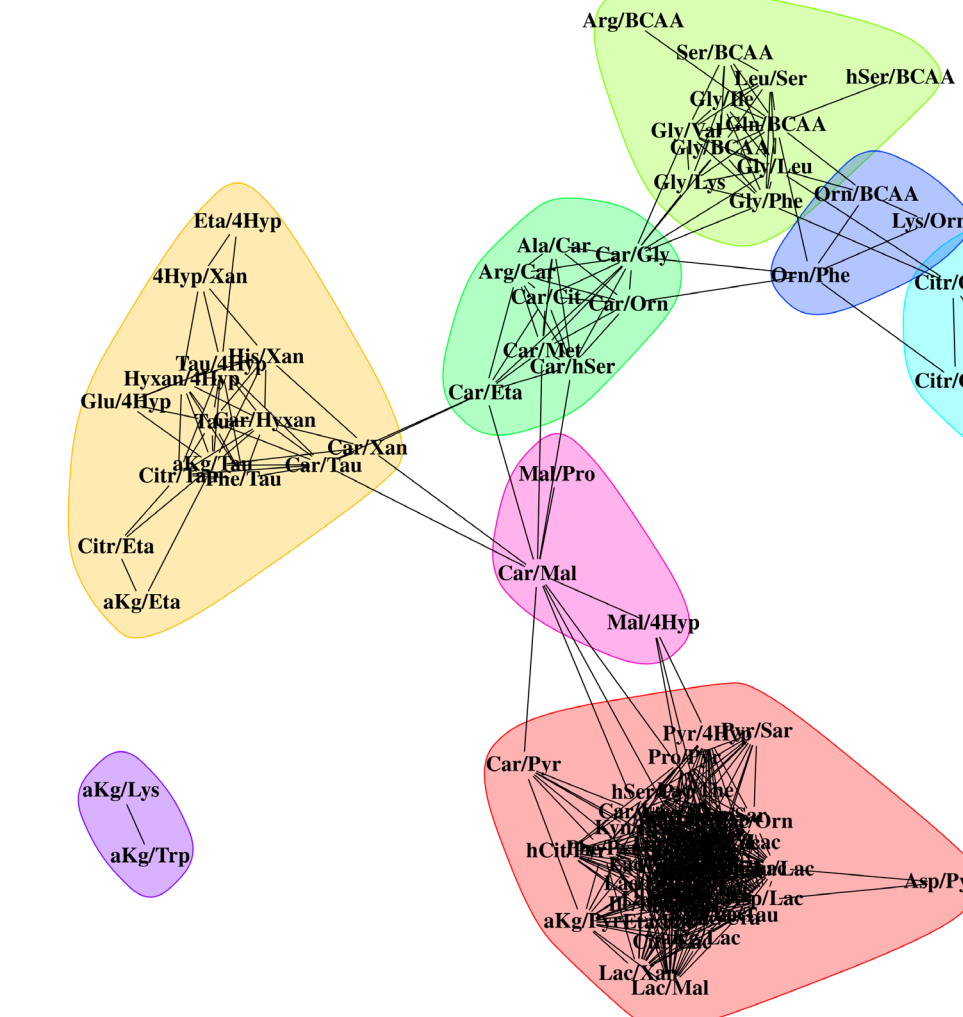
Test Battery Metabotypes

Metabotype	Sensitivity	Specificity
GABA BCAA panel	7.8%	98.0%
Glutamine BCAA panel	9.8%	98.5%
Glycine BCAA panel	8.0%	99.0%
Ornithine BCAA panel	8.4%	98.0%
Alanine/a-Ketoglutarate	6.0%	100.0%
Ethanolamine/4-Hydroxyproline	5.2%	100.0%
Ethanolamine/a-Ketoglutarate	7.8%	98.5%
Ethanolamine/Carnitine	5.4%	100.0%
Ethanolamine/Kynurenine	7.2%	98.5%
Glycine/Citrate	10.1%	99.5%
Lactate/Glutamic acid	9.1%	100.0%
Lactate/Homoserine	5.6%	100.0%
Malate/Proline	5.8%	99.5%
Ornithine/Carnitine	6.6%	100.0%
Ornithine/Lysine	7.8%	99.0%
Phenylalanine/Citrate	9.5%	98.6%
Pyruvate/Aspartic acid	5.8%	99.5%
Pyruvate/Homocitrulline	8.2%	99.0%
Pyruvate/Sarcosine	6.8%	99.0%
Taurine/a-Ketoglutarate	9.7%	98.1%
Taurine/Phenylalanine	6.4%	99.0%
Tryptophan/a-Ketoglutarate	9.1%	98.5%
Xanthine/4-Hydroxyproline	5.4%	99.5%

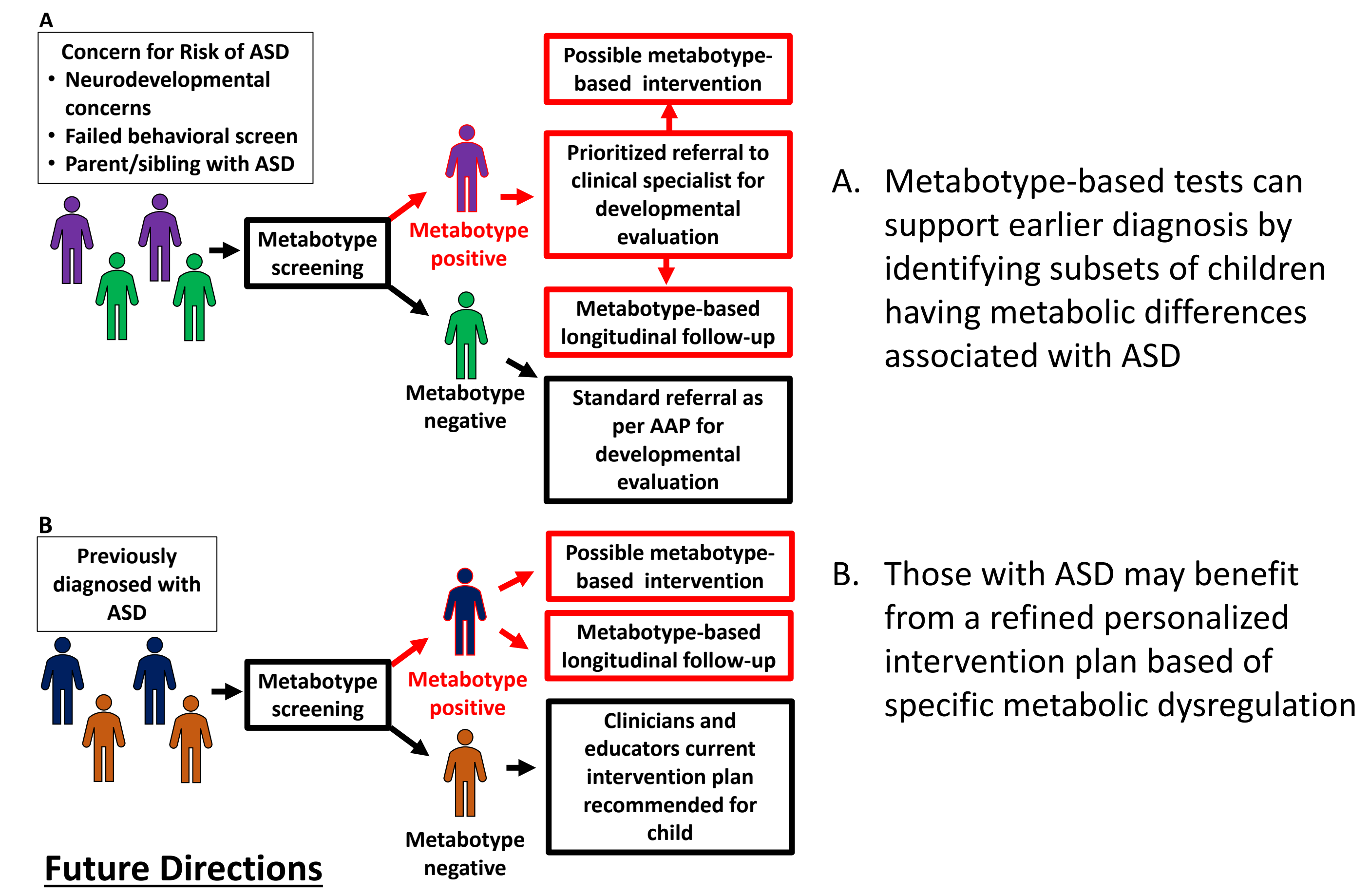## Metabolic Clusters Indicate Dysregulation of Amine and Energy Metabolism

Metabotype community analysis based on metabotype positive CAMP subjects identifies clusters of shared metabolic dysregulation

- 8 clusters highlight potential dysregulation in amino acid and energy metabolism in ASD when compared to TYP

- These clusters may be associated with underlying pathophysiology of ASD

Metabolomics Test Optimized to Detected Each Cluster of Dysregulation

- 23 metabotype tests selected that identify metabolic dysregulation represented each cluster
- **Optimized test battery increased the overall sensitivity to 63% (95%CI, 58%-67%) with a specificity of 90% (95%CI 84-93%).**

A Metabotype Testing Strategy to Support Diagnosis and Intervention

A. Metabotype-based tests can support earlier diagnosis by identifying subsets of children having metabolic differences associated with ASD

B. Those with ASD may benefit from a refined personalized intervention plan based of specific metabolic dysregulation

Future Directions

- Add biochemical domains through continued development of LC-MS/MS methods to identify additional metabotypes and increase sensitivity beyond 63%
- Additional longitudinal and prospective studies to improve our testing strategy and clinical application
- Clinical study of the first paired therapy to address a specific metabolic dysregulation

Supporting Publications

Smith, A. M., et al.. (2019). Amino Acid Dysregulation Metabotypes: Potential Biomarkers for Diagnosis and Individualized Treatment for Subtypes of Autism Spectrum Disorder. Biological Psychiatry, 85(4), 345–354. https://doi.org/10.1016/j.biopsych.2018.08.016

Smith, A. M., et al.. (2020). A Metabolomics Approach to Screening for Autism Risk in the Children's Autism Metabolome Project. Autism Research : Official Journal of the International Society for Autism Research, 13(8), 1270–1285. https://doi.org/10.1002/aur.2330