NeuroPoint DX

Point. Act. Thrive.

Metabolism Based Diagnosis and Precision Medicine for Autism Spectrum Disorders

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Chief Executive Officer
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Powered by Stemina

Stemina: World Leader in Biomarker Discovery

Founded in 2007 in Madison, Wisconsin, with a strong proprietary IP portfolio, subject-experienced scientists and exclusive focus on metabolomics-based diagnostics for autism and drug discovery.

First in class development: no US or global tests are equivalent to, or similar to, NPDX ASD. Stemina has more than 10 years experience in metabolomics research and development and is the recognized biotechnology leader in metabolomics and *in vitro* diagnostics in the US.

CLIA certified laboratory provides autism diagnostic test NPDX ASD under the brand NeuroPointDX. The test is being offered to patients in an early access program at limited sites.

Stemina's metabolomics R&D has delivered tests for different applications (drug discovery) that are currently sold to global pharmaceutical, chemical, tobacco and cosmetics companies and US government agencies (EPA).

Autism Spectrum Disorders

- Autism spectrum disorders (ASD) are complex neurodevelopment disorders characterized by impaired social skills, communication and cognitive deficits, and repetitive behaviors
- ASD is very diverse with significant differences in social and cognitive deficits, genetics and metabolism across the spectrum
- NPDX ASD is the first blood test that identifies a biological basis for autism based on the largest clinical study of the metabolism of children with ASD, the Children's Autism Metabolome Project (CAMP)

Oct 03, 2018

Variability in Autism Diagnostic Gene Panels Sparks Push for Standardization

ARTICLE: IN-DEPTH—in Neurological & Psychological Disorders

Researchers found that diagnostic autism NGS panels offered by 21 clinical labs have very little overlap and are working to create a standardized gene list.

- Despite extensive studies, *Genetic tests detect only 1-5% of classic ASD**, physicians rely on standardized behavioral screens and questionnaires for diagnosis at *an average age of 4.5 years*
- An extensive study of the metabolism of children with ASD was needed for another perspective and greater understanding of the disorder

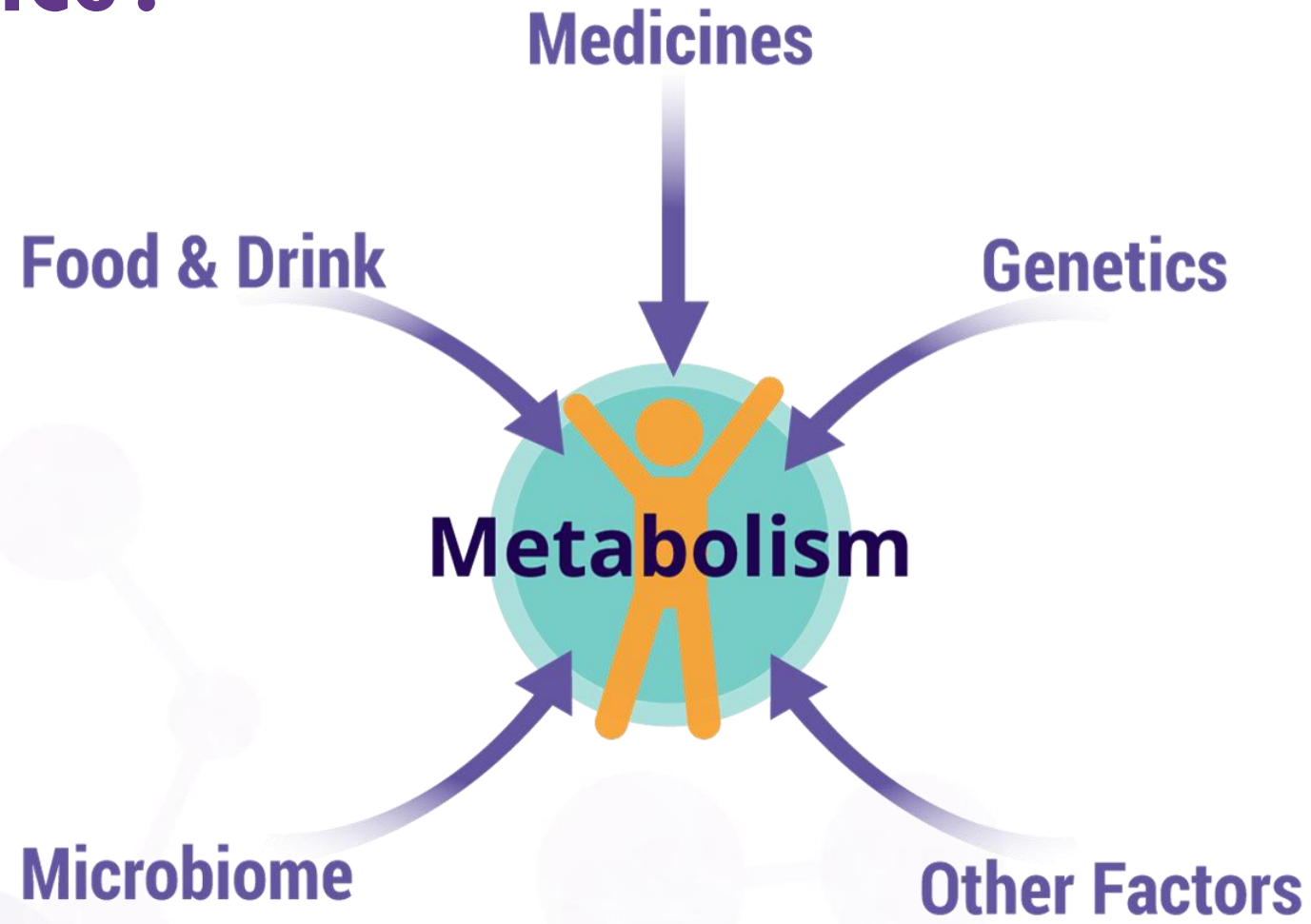
* ASD without Fragile-X, Down, Tuberous Sclerosis or other genetic co-morbidities

Metabolism of ASD and Precision Medicine in Autism

- Our approach is to identify differences in the metabolism of children with autism across the spectrum using metabolomics
  - Earlier diagnosis as young as 18 months
  - Inform more precise treatment based on dysregulation
  - Retest to assess changes

What is Metabolomics?

Metabolomics measures changes in metabolites, such as sugars and amino acids in blood, urine, and/or tissue to provide diagnosis based on combined factors for more precise treatment



Important Biological Concepts in Autism

As Noted by Dr Rossignol*

- "Several metabolic abnormalities have been reported to contribute to or cause a potentially reversible form of autism"
  - E.g. Cerebral Folate Deficiency
- "The goal is to screen for these abnormalities, identify them, and start treating them"
- "Testing can be done by certain biomarkers (laboratory tests that may identify these abnormalities)"
- "ASD has a clear biological basis with features of known medical disorders (e.g. in my opinion, it is not just a psychiatric disorder)"

*Dr Rossignol, TACA National Conference 2018 presentation (Slide 8)

No Precision in Treatment Choices: Need to Identify Biomarkers to Diagnose and Inform Treatment

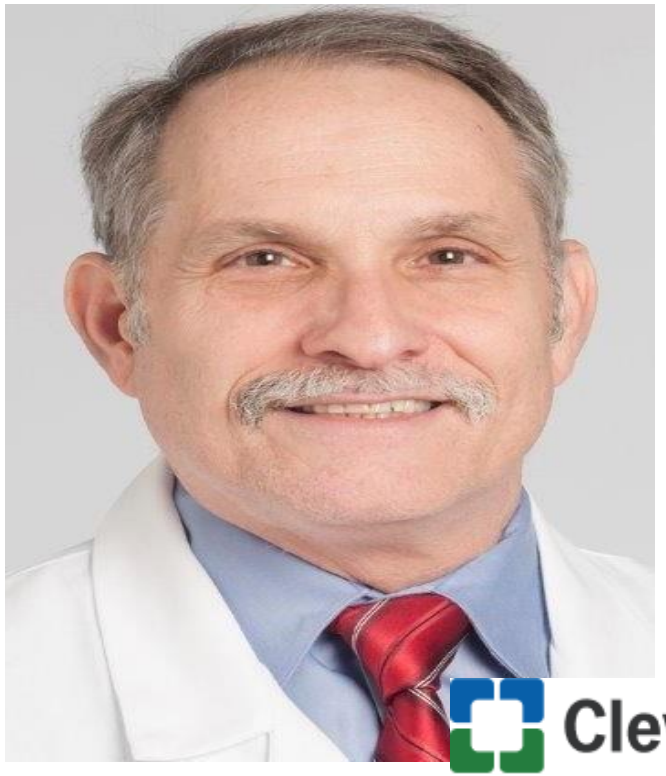
Many interventions are taken by families and physicians in treating ASD

- ABA therapy
- Modified diet
- Vitamins and supplements
- Hyperbaric therapy
- Attention deficit medicine
- Psychotropic drugs

Key Collaborators

MIND INSTITUTE

David G. Amaral, PhD
Research Director, M.I.N.D. Institute
University of California - Davis



Cleveland Clinic

Marvin Natowicz, MD, PhD
Medical geneticist and clinical pathologist, Cleveland Clinic
Professor of Pathology, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Proof of Concept Study with Dr. Amaral: Metabolomics can Identify ASD

OPEN ACCESS Freely available online

PLOS ONE

Metabolomics as a Tool for Discovery of Biomarkers of Autism Spectrum Disorder in the Blood Plasma of Children

Paul R. West[1]*, David G. Amaral[2], Preeti Bais[3], Alan M. Smith[1], Laura A. Egnash[1], Mark E. Ross[1], Jessica A. Palmer[1], Burr R. Fontaine[1], Kevin R. Conard[1], Blythe A. Corbett[4], Gabriela G. Cezar[1¤], Elizabeth L. R. Donley[1], Robert E. Burrier[1]

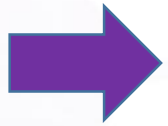
1 Stemina Biomarker Discovery, Madison, Wisconsin, United States of America, **2** The M.I.N.D. Institute and Department of Psychiatry and Behavioral Sciences, University of California Davis, Davis, California, United States of America, **3** The Jackson Laboratory for Genomic medicine, University of Connecticut Health Center, Farmington Connecticut, United States of America, **4** Department of Psychiatry, Psychology and Kennedy Center, Vanderbilt University, Nashville, Tennessee, United States of America

MIND I, MIND II and ACHRI Studies

- In 3 banked blood studies (495 subjects) we obtained multivariate signature for an ASD vs Typ test
  - Balanced accuracies of ~80% using about 80 metabolites
  - Metabolites used in each study had many common features
    - Computational models were different and did not overlap well
    - Differences between studies may account for some of this

	MIND I		MIND II		ACHRI	
	Typical	Autistic	Typical	Autistic	Typical	Autistic
Group Size	30	52	93	180	115	103
Age (y)	5.6	5.4	3.1	3	5.3	6
Sex (male, %)	87	79	69	83	69	83
Developmental Status	114	67	106	62	-	-
Dietary Status	Fasted		Fed		Fasted	
Anticoagulant	EDTA		Citrate		EDTA	

- Common metabolites seen across the studies included:
  - Amino acids, uremic toxins, selected lipids, and others.
- Suggests:
  - Subtypes and a better way of detecting them
  - A single large study of highly characterized subjects with metadata



Children's Autism Metabolome Project (CAMP)

Largest study of the metabolism of children with ASD

Study Design

- A clinical study powered for successful identification of subtypes
- Children ages 18-48 months with ASD, developmental delay and typical development
- ADOS-2, DSM-V and MSEL as behavioral measures

Study Logistics

- Recruited 1,100 patients over 3 years at 8 centers
- $8M study with extensive metadata
- Designed for earlier diagnosis and to develop precise therapies for ASD

First DX Blood Test Validated in CAMP

- A first diagnostic panel of a dozen metabolic subtypes has been prospectively validated
  - Published in Biological Psychiatry on September 6, 2018
- More diagnostic panels in development

Funded By:
NIMH and Nancy Lurie Mar Family Foundation

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Dedicated Clinical Sites

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First Publication From CAMP Study

Biological Psychiatry

A Journal of Psychiatric Neuroscience and Therapeutics

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Article in Press

Amino acid dysregulation metabotypes: potential biomarkers for diagnosis and individualized treatment for subtypes of autism spectrum disorder

Alan M. Smith, Joseph J. King, Paul R. West, Michael A. Ludwig, Elizabeth L.R. Donley, Robert E. Burrier*, David G. Amaral*

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DOI: https://doi.org/10.1016/j.biopsych.2018.08.016

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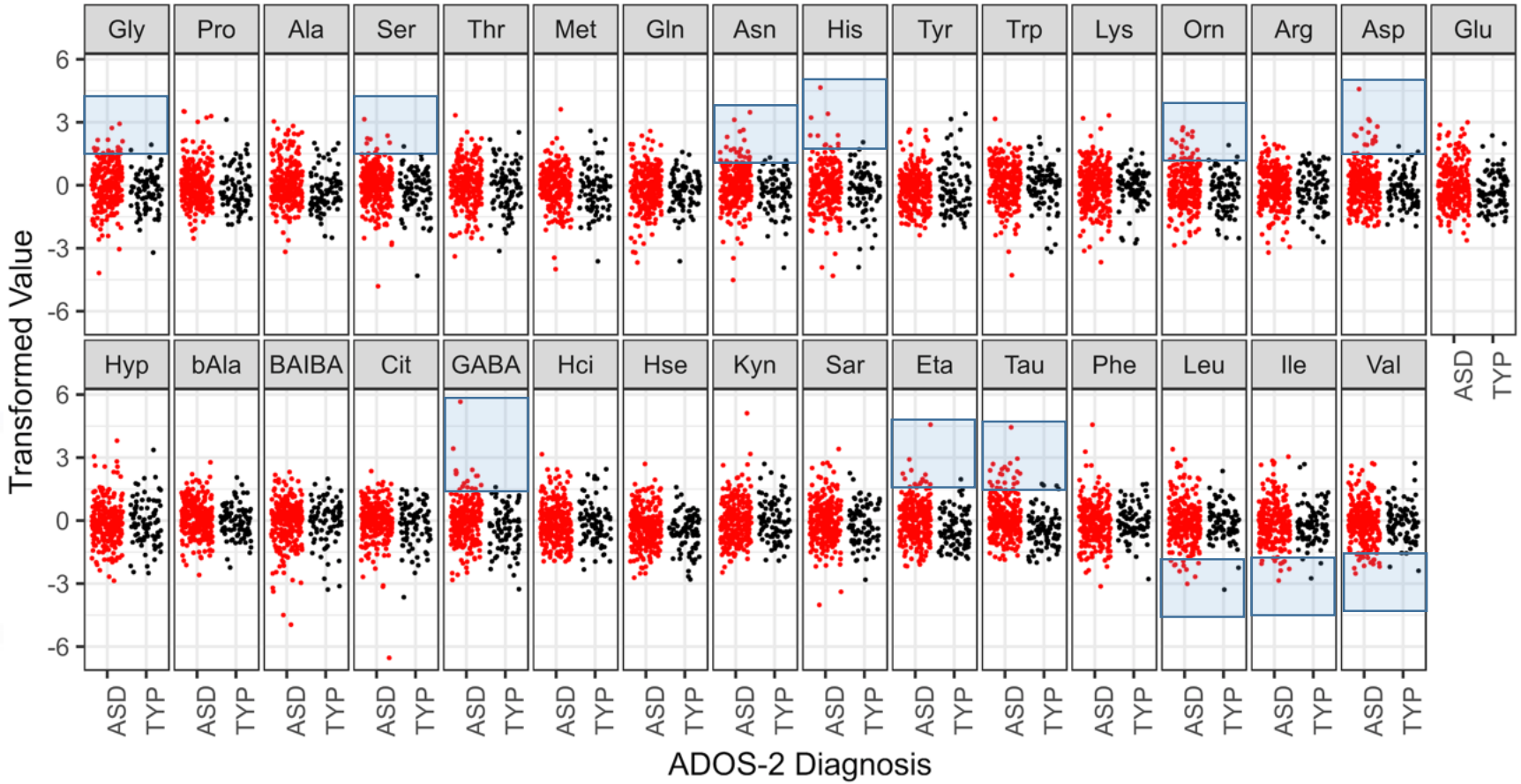
Metabolite Distributions Show Evidence of Metabotypes

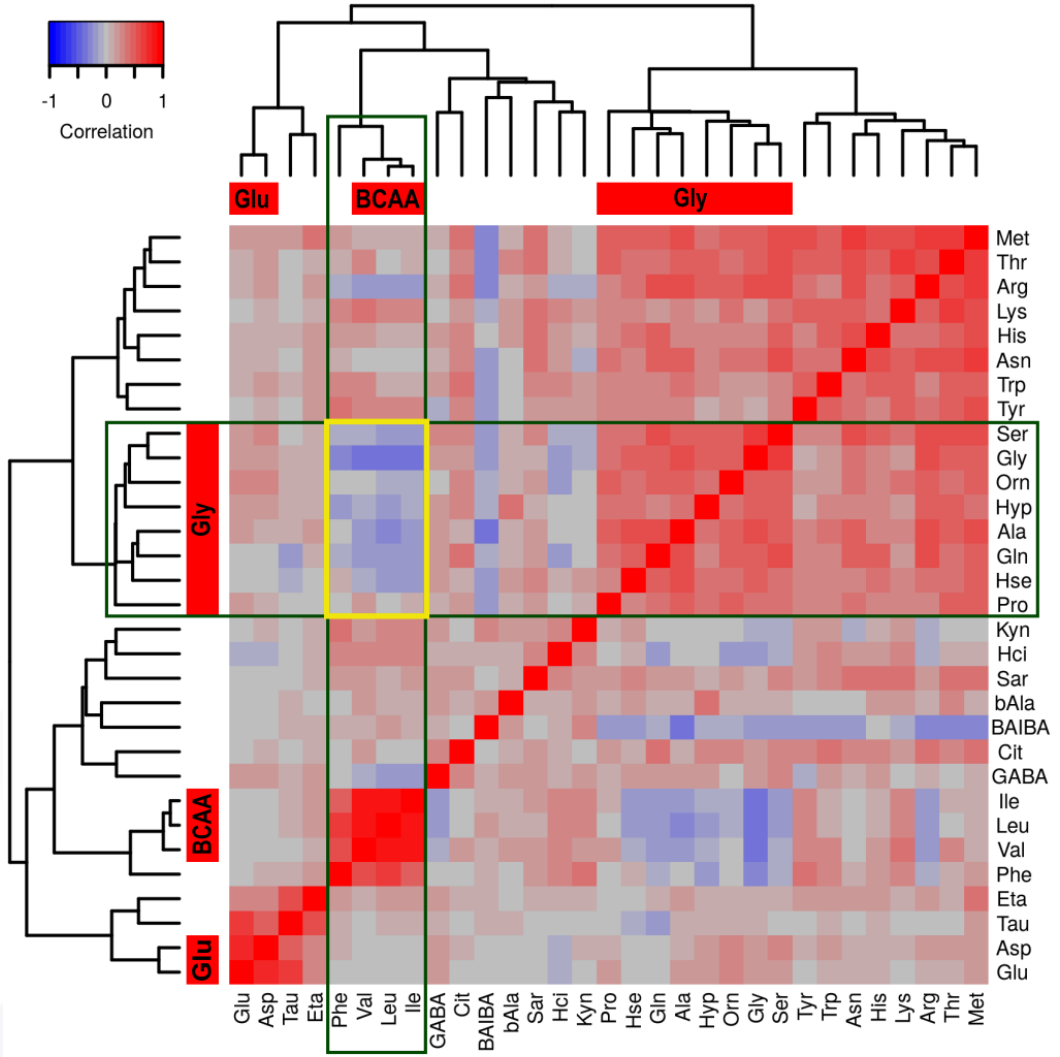


- Averages of the metabolite means are not significantly different
- Extreme values observed in ASD population indicative of subpopulations

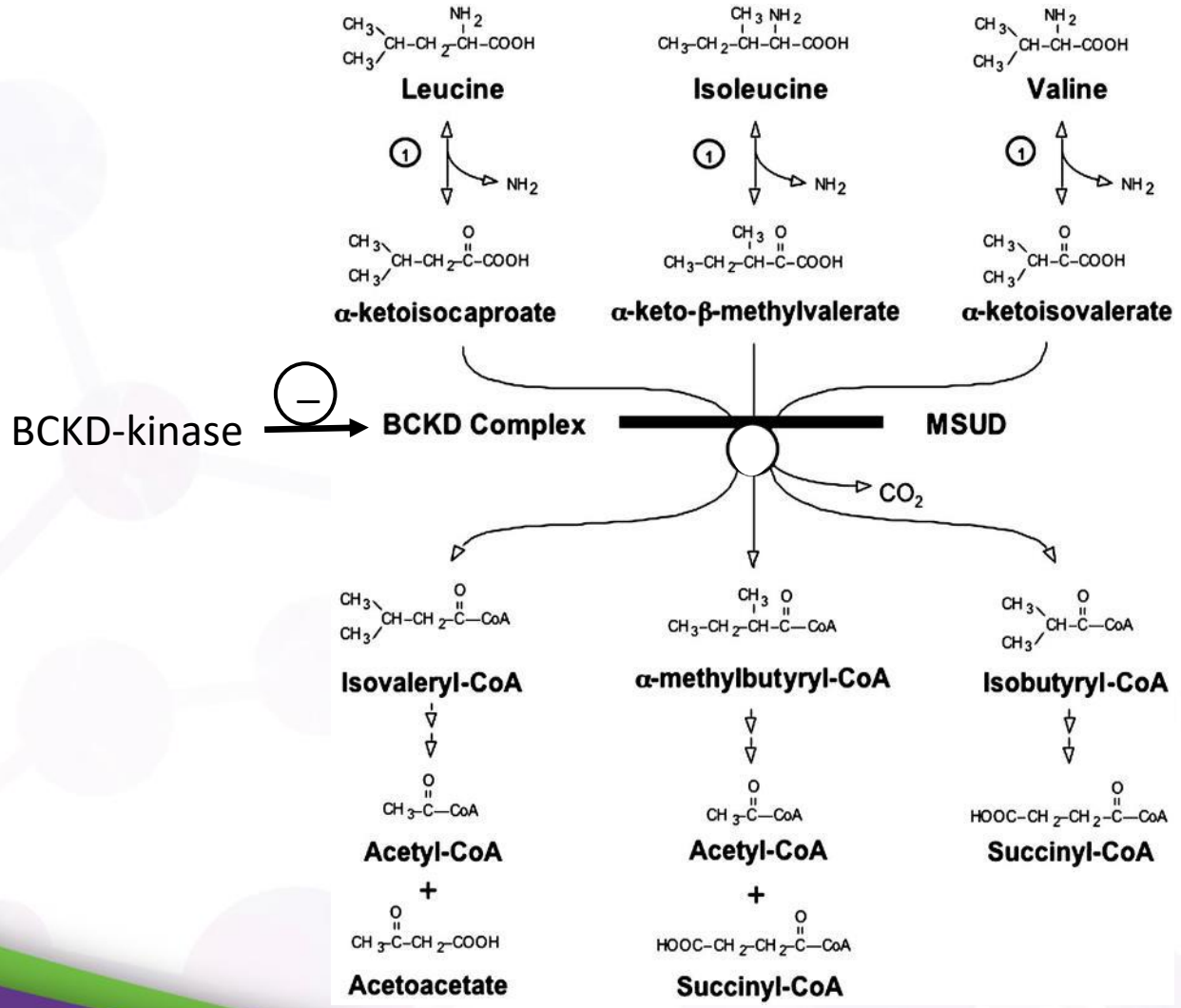
Co-regulation of Metabolism Identifies Negatively Correlated Metabolites

- A negative correlation suggests ratios may amplify signal
  - For example, similar approach used for diagnosis of PKU (ratio of Phenylalanine/Tyrosine)

- Reflects a biological relationship involving both enzymatic and transport biology



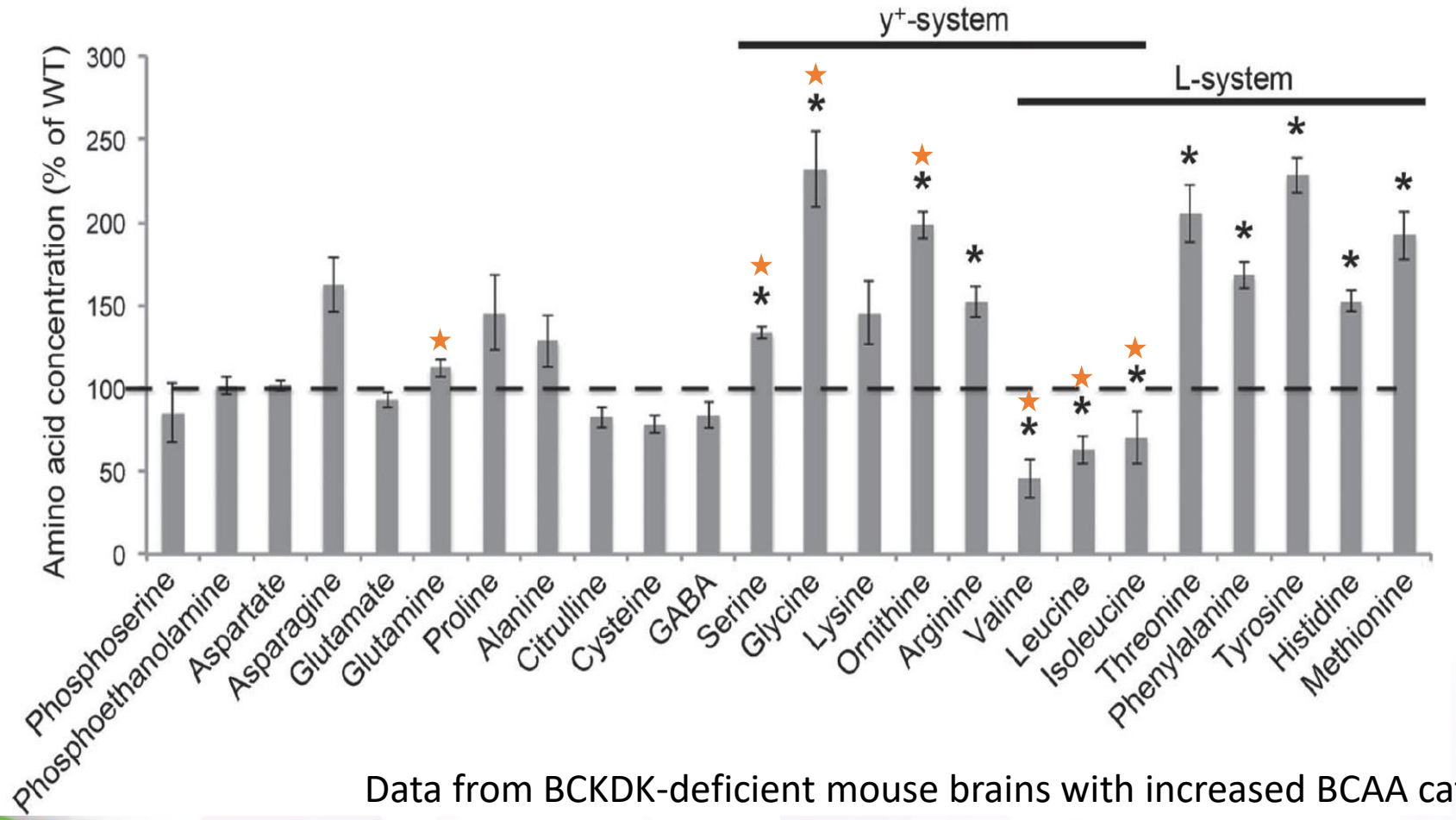
Branched Chain Amino Acid Catabolism

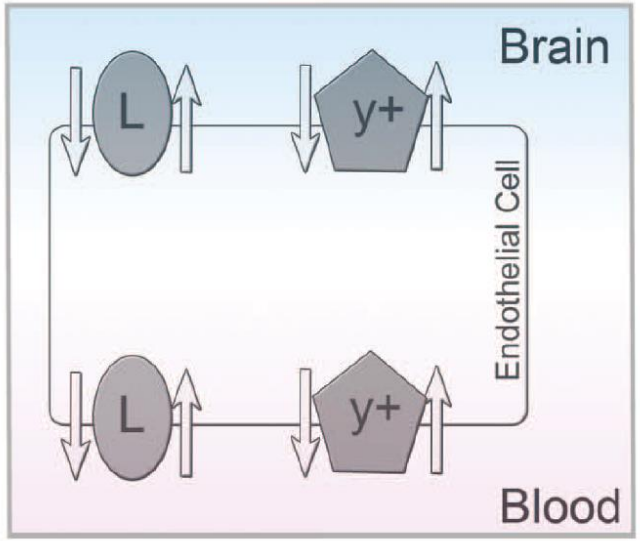


Amino acid levels are tightly controlled

- Defects in BCKDH cause elevated BCAA: Maple Syrup Urine Disease
- BCKDH-kinase cause lower BCAA: ASD, DD, and epilepsy

BCAA Catabolism Defects Cause Brain Amino Acid Imbalances





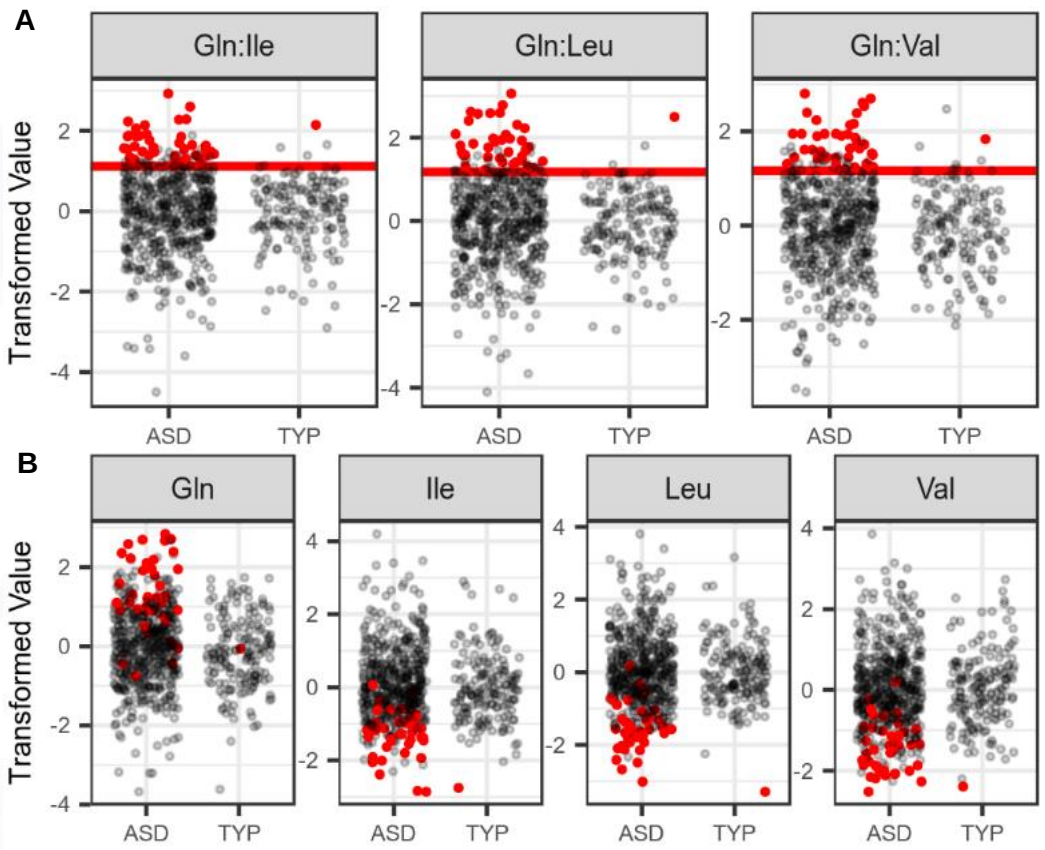
Data from BCKDK-deficient mouse brains with increased BCAA catabolism+

* Significant AA concentration differences from control

★ Prominent amino acids in AADM Panels

+Novarino et al. Cell 2012

Gln-BCAA Test Combination



Plots contain all subjects

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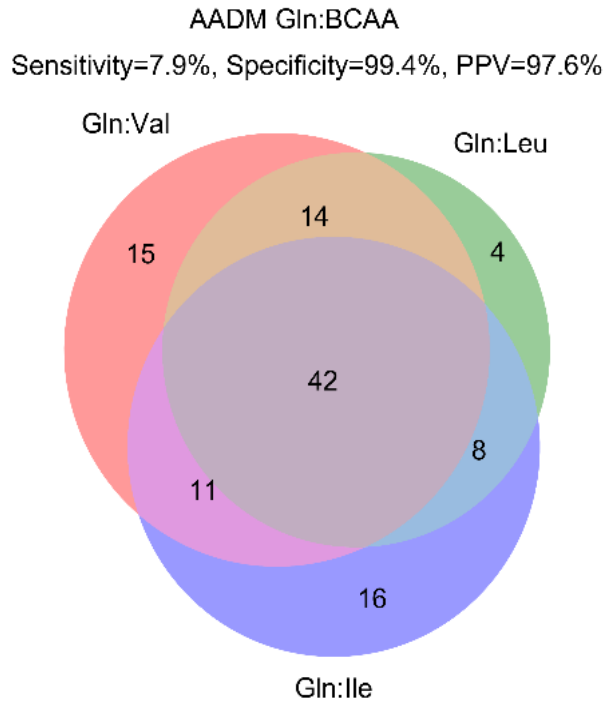
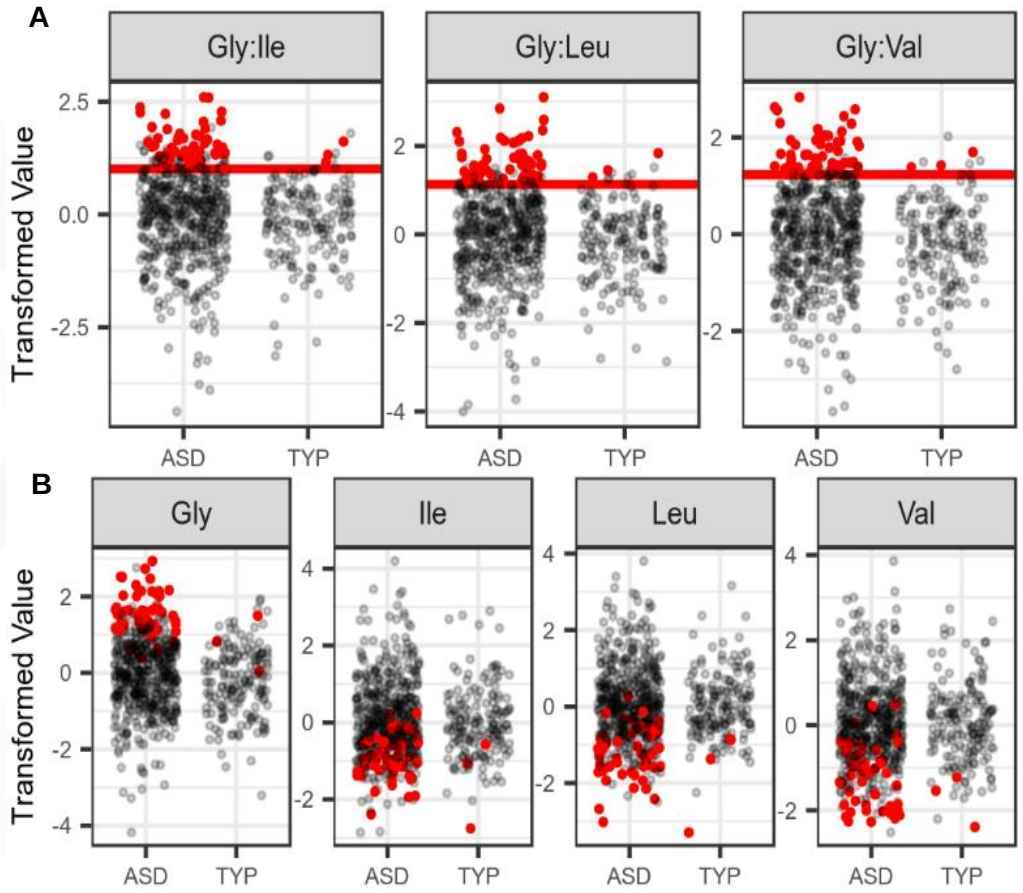


Venn All Subjects

Gly-BCAA Test Combination



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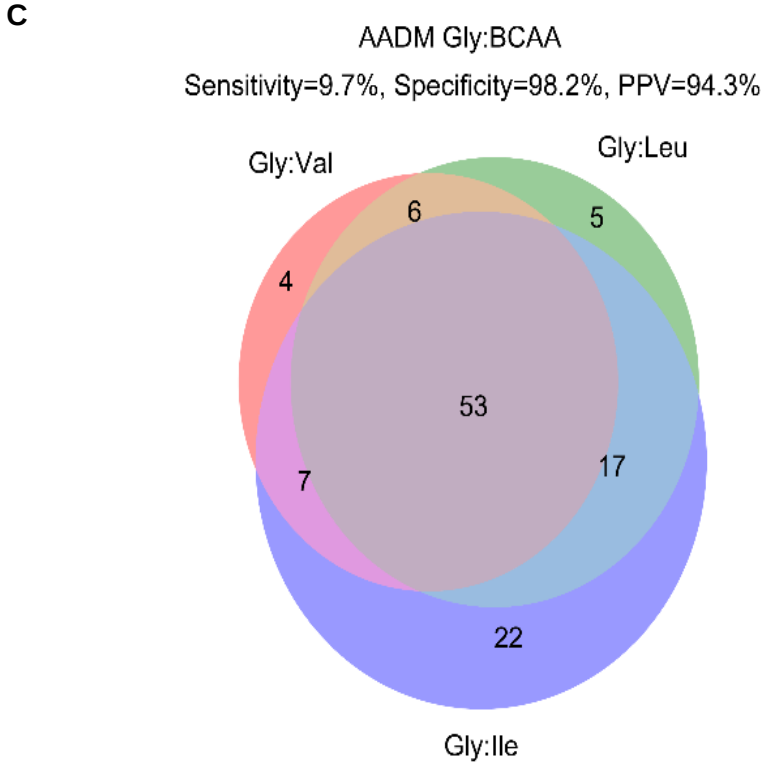
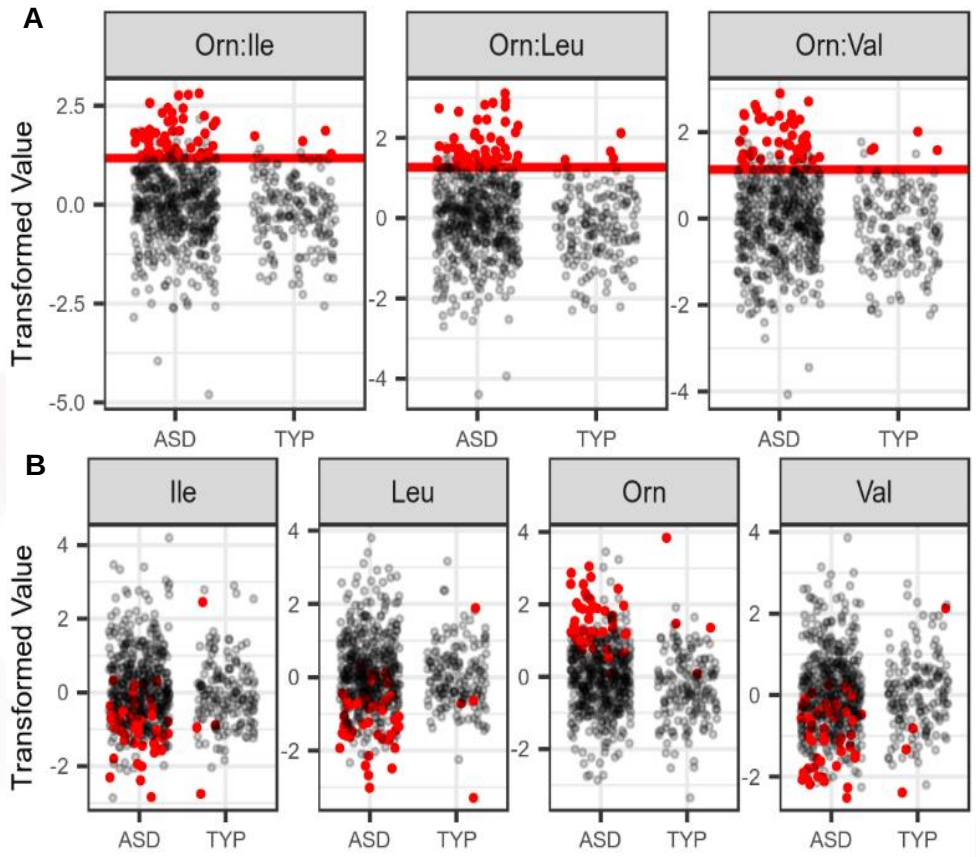


Venn All Subjects

Orn-BCAA Test Combination



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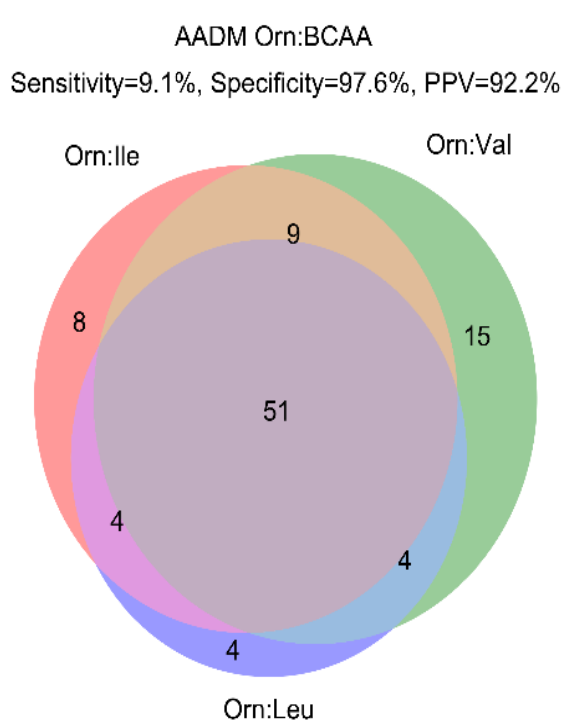
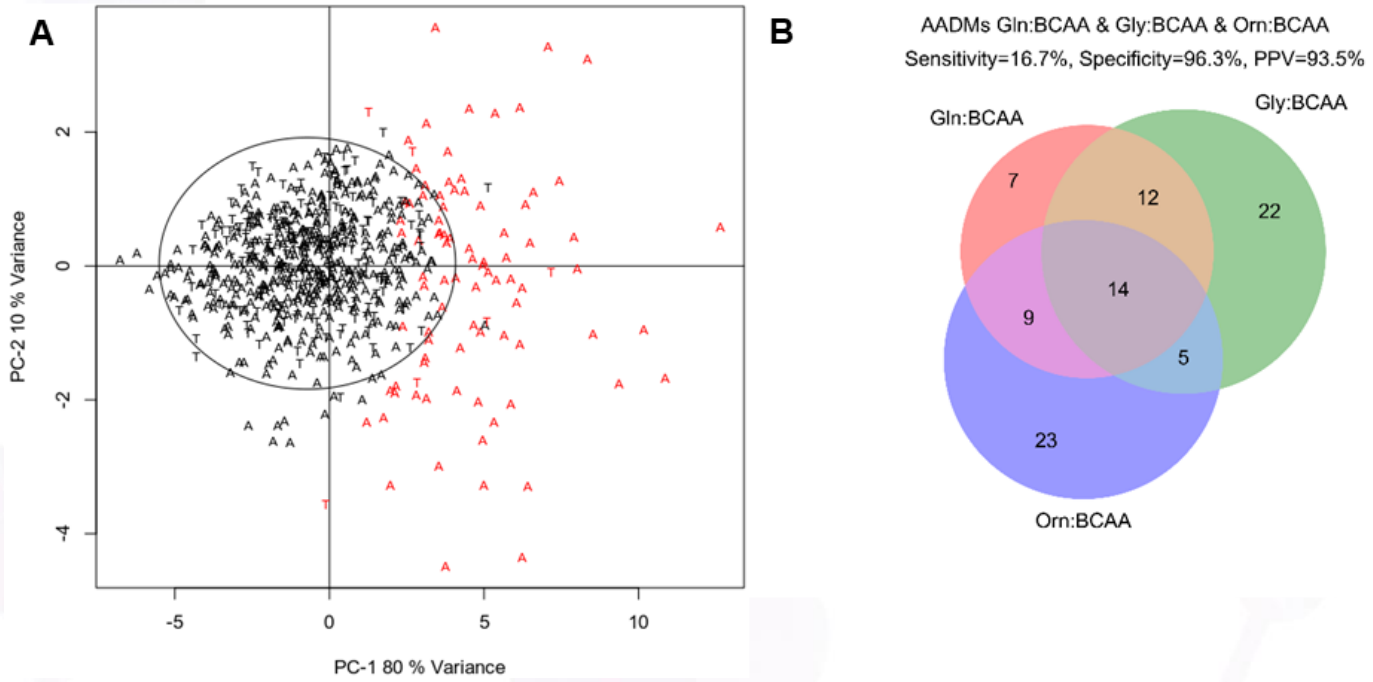


Venn All Subjects

First ASD Metabotypes in Biological Psychiatry September 2018



- Linking BCAA test combinations into a single diagnostic creates a test for a metabotype of BCAA dysregulation associated with ASD that offers early diagnosis and more precise treatment

- 16.7 % of the CAMP Study Population
- Specificity of 96.3%
- PPV of 93.5%

- Additional proprietary subtypes included in NPDX ASD identifies a total of 30% of children in CAMP

NeuroPointDX's Autism DX: NPDX ASD Test

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- Currently ASD is diagnosed through behavioral testing beginning at age 24 months, yet the average age of diagnosis is 4.5 years
  - Behavioral therapy is the current standard of care for treatment
- NeuroPointDX has validated a blood test panel in its clinical study that can identify 30% of ASD as young as 18 months based on the metabolism of the child
- NeuroPointDX's Autism diagnostic NPDX ASD identifies specific differences in amino acids associated with autism in children
  - NPDX ASD assesses the individual metabolite levels based on proprietary thresholds and ratios established in our clinical study CAMP
  - Helps inform individualized intervention based on child's metabolism

Who Should Have the NPDX ASD Diagnostic Test?

- The test is a precise amino acid profile based on clinical thresholds set in the CAMP study. It is appropriate for:
  - A child over 18 months who has been flagged ”at risk” through M-CHAT (Modified Checklist for Autism in Toddlers) screen or other missed developmental milestone
  - Any child over 18 months with a sibling diagnosed with autism
  - A child diagnosed with autism for which physicians and parents may want metabolic information about the underlying biology of the child

Test Available in Early Access Program

- Test has regulatory approval in 48 states through the Clinical Laboratory Improvement Amendment (CLIA)
  - California will approve in Q1 2019, New York in 2020
- First DX panel commercialized Q4 2018 in early access program at limited sites across the country
- Requires a order from physician or clinician
  - Requisition form available: https://neuropointdx.com/provider-order/
- Protocol for blood draw and sample preparation protocol requires -80 freezer capabilities and shipping on dry ice
- Must be first of the morning blood draw with nothing to eat or drink other than water for 12 hours prior to testing https://neuropointdx.com/parents/prepare-for-test/

Example of Treatable Metabolism based Cause of Disruptions in Neuro-Development

Phenylketonuria (PKU) is a disorder that causes an amino acid called phenylalanine to build up in the body because of a defect in the gene that helps create the enzyme needed to break down phenylalanine.

Can be successfully treated by changes in diet to eliminate phenylalanine. Untreated PKU can lead to:

- Irreversible brain damage and marked intellectual disability beginning within the first few months of life
- Neurological problems such as seizures and tremors
- Behavioral, emotional and social problems in older children and adults
- Major health and developmental problems

Proof of Concept: Nutritional Intervention

RESEARCH ARTICLE

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY

Two Novel Mutations in the *BCKDK* (Branched-Chain Keto-Acid Dehydrogenase Kinase) Gene Are Responsible for a Neurobehavioral Deficit in Two Pediatric Unrelated Patients

Angels Garcia-Cazorla,[1†] Alfonso Oyarzabal,[2†] Joana Fort,[3†] Concepción Robles,[4] Esperanza Castejón,[5] Pedro Ruiz-Sala,[2] Susanna Bodoy,[3] Begoña Merinero,[2] Anna Lopez-Sala,[1] Joaquin Dopazo,[6,7,8] Virginia Nunes,[9] Magdalena Ugarte,[2] Rafael Artuch,[10‡] Manuel Palacín,[3‡] Pilar Rodriguez-Pombo,[2*‡] and Working Group: (Patricia Alcaide[2], Rosa Navarrete[2], Paloma Sanz[2], Mariona Font-Llitjós[7], Mª Antonia Vilaseca[8], Aida Ormazabal[8], Anna Pristoupilova[11], Sergi Beltran Agulló[11])

BCAA supplementation plus a protein-rich diet was shown to:

- improve growth
- reduce hyperactivity
- reduced irritability
- improve communication and socialization

CAMP BCAA Dysregulation Subtype Study

- Pilot Study:
  - A precision medicine/therapeutic targeted to improve an imbalance of BCAA for a specific metabolomic subtype will be readily accepted as a beverage and significantly change the balance of BCAA after one month of supplementation.

- Multi-site, Randomized Clinical Trial
  - A precision medicine/therapeutic targeted to improve an imbalance of BCAA for a specific metabolomic subtype will significantly improve behavioral signs associated with ASD within 3 months

Nutritional Study Team Members

Denise Ney, PhD

- Billings-Bascom Professor of Nutritional Sciences; UW-Madison
- Expert on development of nutritional supplements with whey protein and glycol-macropeptides for treatment of PKU

Jacqueline Hind, MS

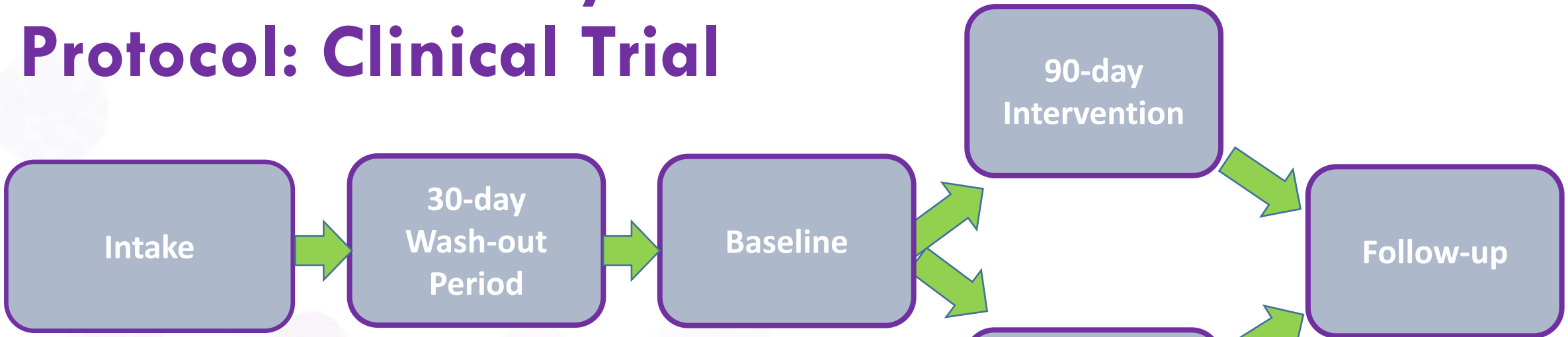
- 18+ years of experience managing clinical trials with focus on nutritional supplementation
- Experience successfully translating research into better patient care

Product Development

- Objective: Develop a novel BCAA/protein powder beverage blend to add to user's favorite beverage
  - Unique to ASD metabolic subtypes identified in CAMP
  - Formulated for easy dose based on weight of the child
  - Emphasis on optimizing sensory experience (i.e., taste, mouth feel)

- Timeline:
  - 2 months for development
  - 2-3 months for production of 8,000 packets for study

CAMP Sister Study Protocol: Clinical Trial



- Behavioral testing
- Dietitian consult
- Bloodwork: BCAA Concentration
- Urine
- 3-day diet log

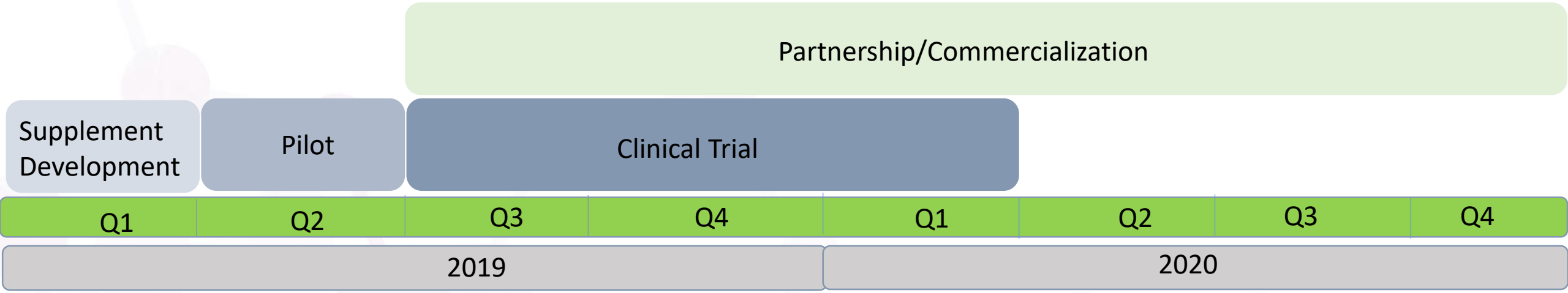
- No dietary supplements

- Behavioral testing
- Bloodwork: BCAA Concentration
- Urinalysis

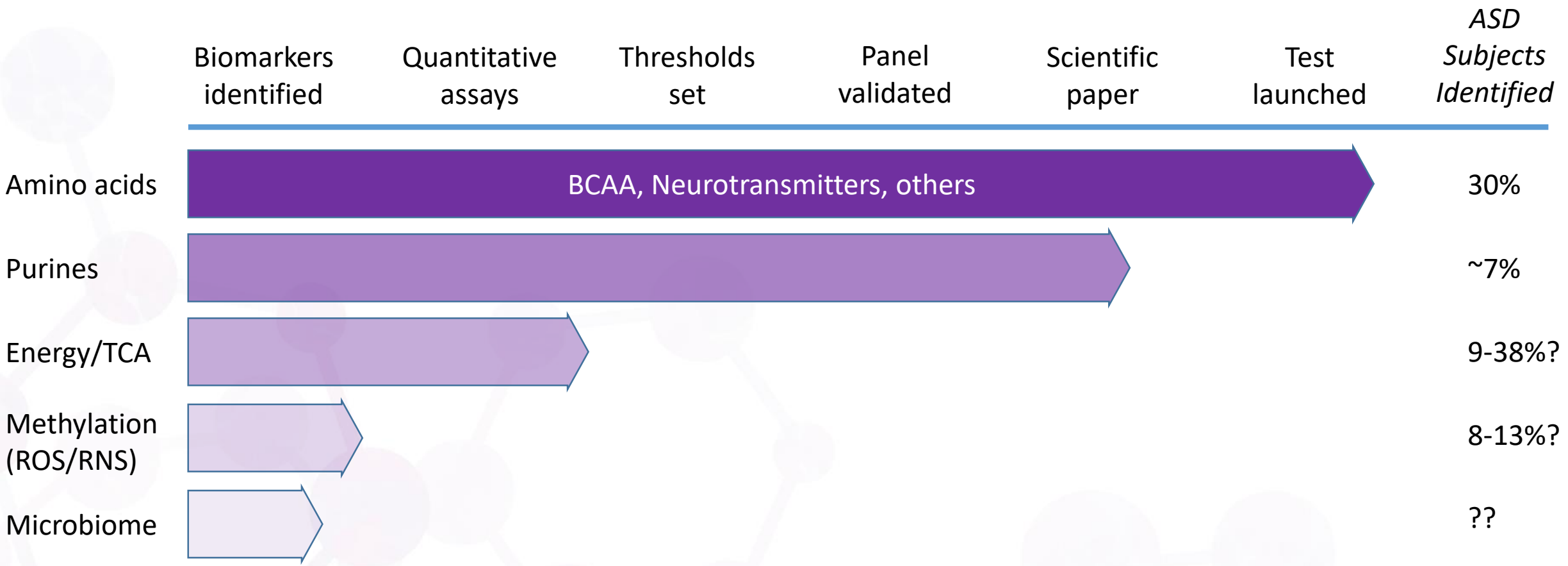
- Supplements 3x/day
- Bloodwork: BCAA Concentration
- Urinalysis
- 3-day diet log

- Behavioral testing
- Bloodwork: BCAA Concentration
- Urinalysis
- 3-day diet log

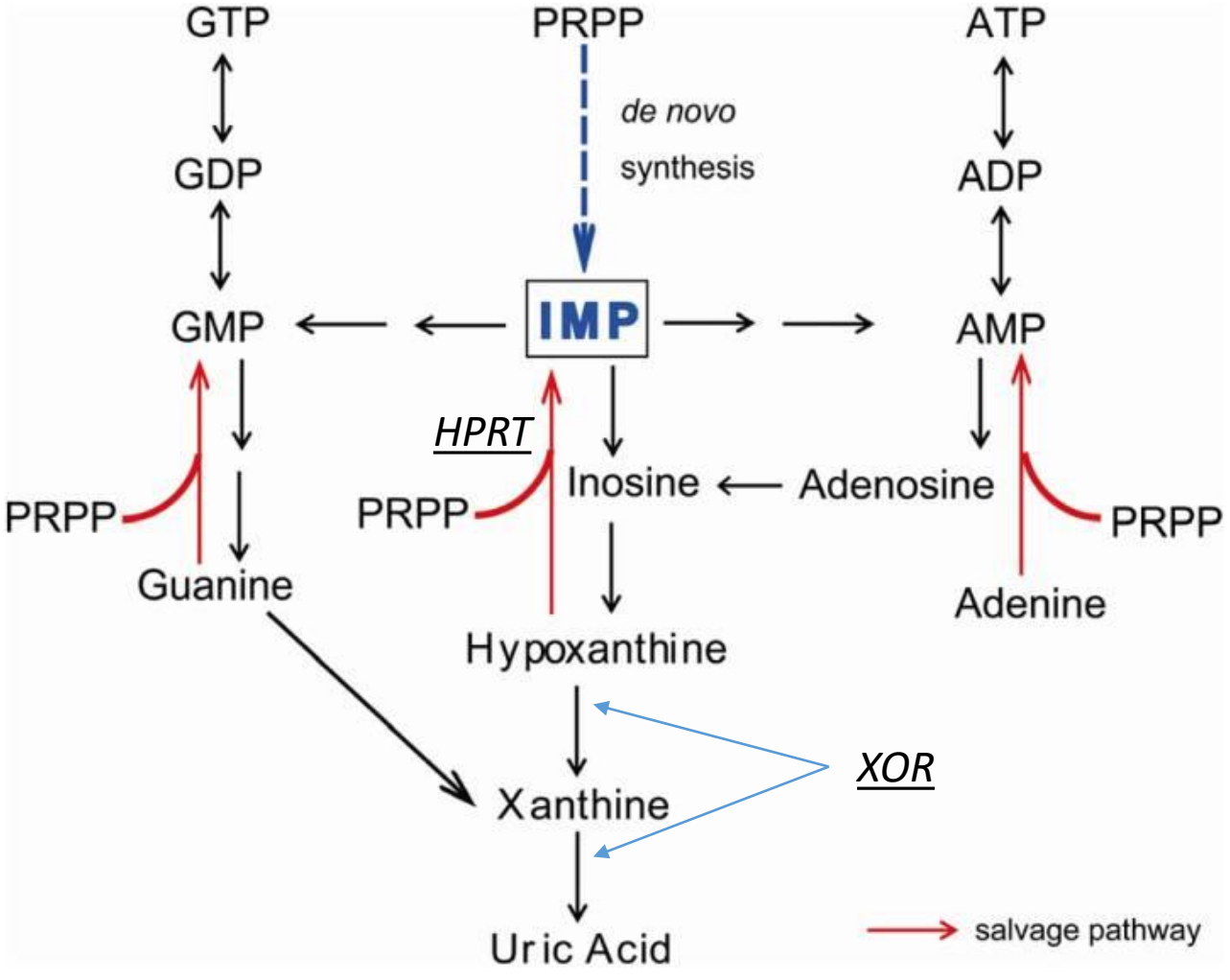
Timeline


Partnership/Commercialization
Supplement Development
Pilot
Clinical Trial
Q1
Q2
Q3
Q4
Q1
Q2
Q3
Q4
2019
2020

NeuroPointDx Autism Pipeline from CAMP



Purine Pathways and Autism



Defects in purine metabolism are related to neurodevelopment

Xanthine Story

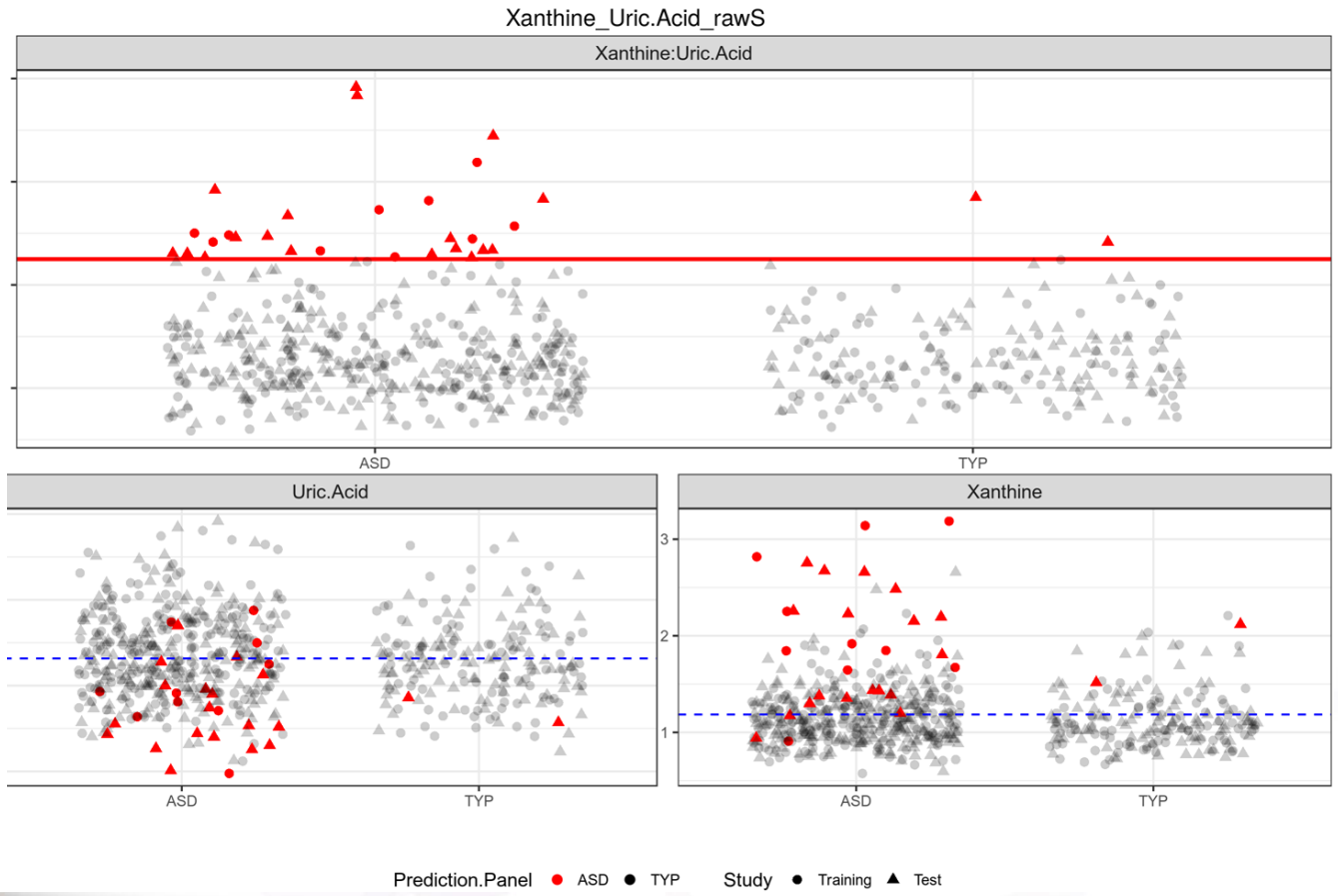
- Xanthine oxidoreductase (XOR, 2-forms)
  - Xanthine dehydrogenase (NAD cofactor)
  - Xanthine oxidase (O_2 cofactor)
- Xanthinurias
  - Xanthinuria type I: XOR defect
  - Xanthinuria type II: MOCOS cofactor defect
- MOCOS responsible for both XOR isoform maturation
  - Mutations associated with autism
  - Related to neurodevelopment via oxidative stress, neurotransmission
  - Can possibly explain GI symptoms

Hypoxanthine-Guanine PhosporibosylTransferase (HPGT) Story

- Mutations cause Lesch-Nyhan syndrome which is severe but has autism like symptoms
- Subjects overproduce uric acid which causes gout and kidney disease
- X-linked, predominantly a male disorder

Purine Related Metabotype Xanthine/Uric Acid

- Overall performance: SEN=6.3%, SPEC = 99.0%, PPV = 93.3%
- Adds ~ 3.5% sensitivity to overall metabotype identification of CAMP ASD subjects



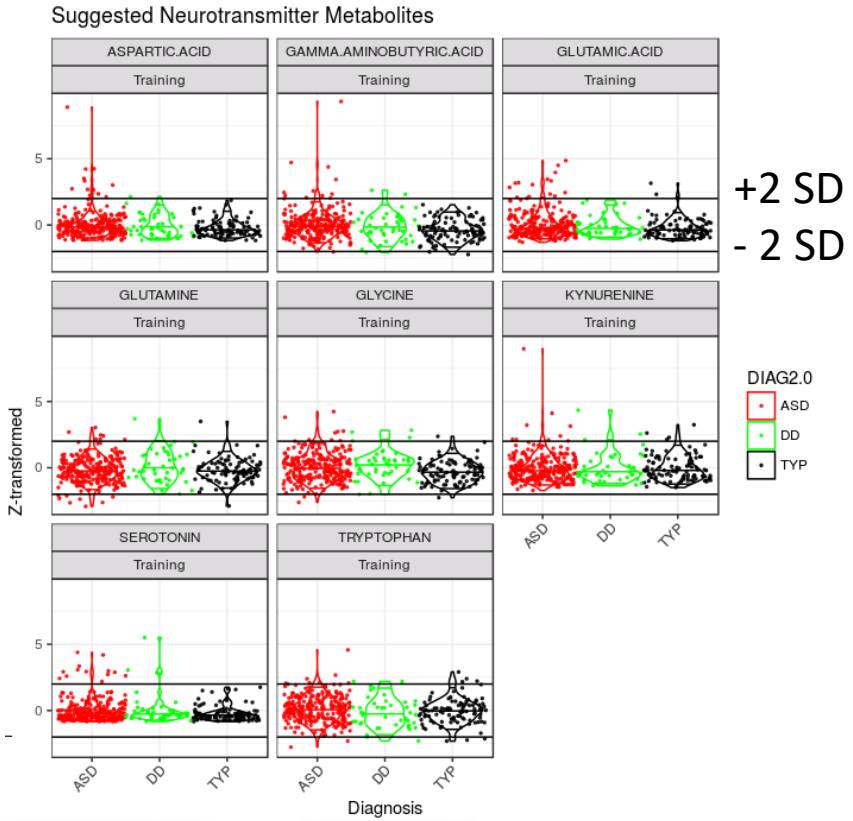
Biological Stories and Biomarkers: Publication in Progress

- Three key results:
  - Purine Metabolism
    - Xanthine, Hypoxanthine, Uric Acid
    - 6.3% ASD in metabotype
  - Energy Metabolism
    - α-Ketoglutarate, Lactate, Malate, Pyruvate, and Succinate
    - 9-38% ASD range of likely metabotype performance
  - Amino Acid Metabolism
    - BCAA dysregulation 16.7% reported in Biological Psychiatry
    - Neurotransmitters on Amine Panel being offered in Early Access Program (Glutamine, Asparagine, Kynurenine, Glycine, Serotonin, GABA) ~ 12.6%

‘Neurotransmitter’ AAs

Ratio	direction	SEN.Training	SEN.Test	SPEC.Training	SPEC.Test	PPV.Training	PPV.Test
ASPARAGINE_GLYCINE	<	0.07	0.07	0.99	0.99	0.95	0.95
GLYCINE_LYSINE	>	0.08	0.08	0.99	0.98	0.95	0.91
GLYCINE_PHENYLALANINE	>	0.06	0.06	1.00	0.99	1.00	0.94
HISTIDINE_LEUCINE	>	0.09	0.08	0.98	0.98	0.92	0.92
KYNURENINE_ORNITHINE	<	0.05	0.06	0.99	0.99	0.93	0.94
LYSINE_ORNITHINE	<	0.07	0.05	0.99	0.99	0.94	0.93

- Additional metabotypes from amine panel dataset
- Glycine and Kynurenine related ratios not reported in the Biological Psychiatry paper
- Serotonin correlated with ASP, Glu in ASD, but not TYP
- Currently exploring potential metabotypes of neurotransmitter associated metabolites



Quantitative Amine Panel CAMP Training

Case Study: Twin brothers take NPDX Test

Twins evaluated using NPDX AA test at age 74 months

- Twin A was diagnosed with behavioral testing with moderate functioning ASD
  - Behavioral therapy was did not result in improvement of ASD behaviors and symptoms
  - Physician (ASD specialist) ordered NPDX test to learn more about the underlying biology
  - NPDX Test gave a positive result for the Gln/(Ile, Leu) metabotype panel:
  - Physician requested BCAA supplements using adult BCAA formulations at reduced doses
  - Patient also received IVIg therapy
  - Subject now showing significant gains
- Twin B tested with high functioning ASD and has improved with therapy.
  - Same physician ordered NPDX test to help understand brother's biology
  - NPDX Test gave a positive result for the Gly/Asn metabotype
  - No follow-up recommended for this patient
  - Patient is improving with behavioral therapy

BOARD members	SAB members
Heiner Dreismann, PhD Former CEO of Roche Molecular Diagnostics Myriad Board of Directors	**David G. Amaral, PhD** Research Director Autism Phenome Project, MIND Institute, UC-Davis
Kevin Krenitsky, MD Former COO and CCO Foundation Medicine	**Marvin Natowicz, MD, PhD** Cleveland Clinic Specialist: ASD, clinical neurogenetics and neuro-metabolism
Andrew Ferrara CEO, Chairman, Boston Healthcare	**Oliver Fiehn, PhD** West Coast Center for Metabolomics, UC-Davis
Marc Schneebaum, MBA SVP & Chief Financial Officer, Madrigal Pharmaceuticals	**Steven Fisher, PhD** Senior Scientist Metabolomic, Agilent, Inc.
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