Emerging Patterns of Metabolic Disturbance in Autism Spectrum Disorders

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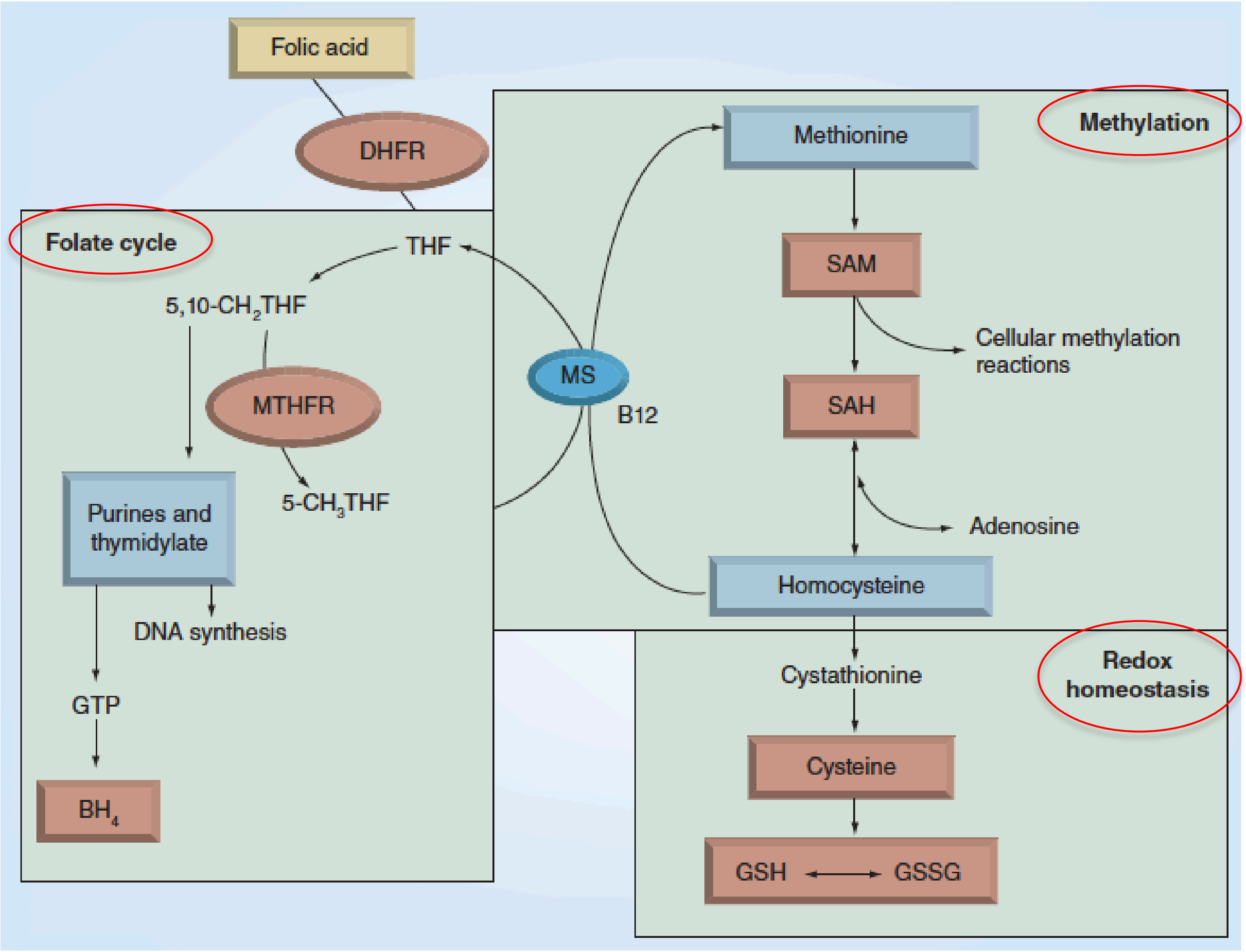
Summary

- All ASD subjects examined to date have metabolic abnormalities
- Most of the mitochondrial dysfunction found in ASD is secondary, and is not the result of single-gene Mendelian or mtDNA defects
- Redox, glutathione, and methylation disturbances are common (>50%)
  - Special Request: present some of Dr. Jill James work on ASD biochemistry and treatment (4 slides)
- The Cell Danger Hypothesis
- Autism-like behaviors, metabolism, and synaptic defects were corrected by APT in mouse models of ASD
- NextGen metabolomics identifies the disturbances
  - Mouse models and humans have the same core pathway abnormalities
  - Previously identified as the effector pathways of the CDR

Message from Dr. Jill James

- "Please do not place my work in the category of the 'oxidative stress school' (ROS cause disease)"
- We found oxidative changes in glutathione and the methionine cycle in a majority of children with ASD (2004)
- Treatment of underlying redox disturbances with methyl-B12 and folinic acid restored extracellular glutathione balance in some (2013)
- Extracellular glutathione redox improvements were correlated with behavioral benefits in our open label study (2013)



Richard Frye and Jill James. *Biomarkers in Medicine*, 2014. PMID 24712422.

Design: Open label treatment, no placebo
65 Screened, 48 Enrolled, 37 completed
75 µg/kg methyl-B12 sq 2/wk
400 µg folinic acid PO BID x 3 months

Clinical Study

Effectiveness of Methylcobalamin and Folinic Acid Treatment on Adaptive Behavior in Children with Autistic Disorder Is Related to Glutathione Redox Status

Richard E. Frye,[1] Stepan Melnyk,[1] George Fuchs,[1] Tyra Reid,[1] Stefanie Jernigan,[1] Oleksandra Pavliv,[1] Amanda Hubanks,[1] David W. Gaylor,[2] Laura Walters,[1] and S. Jill James[1]

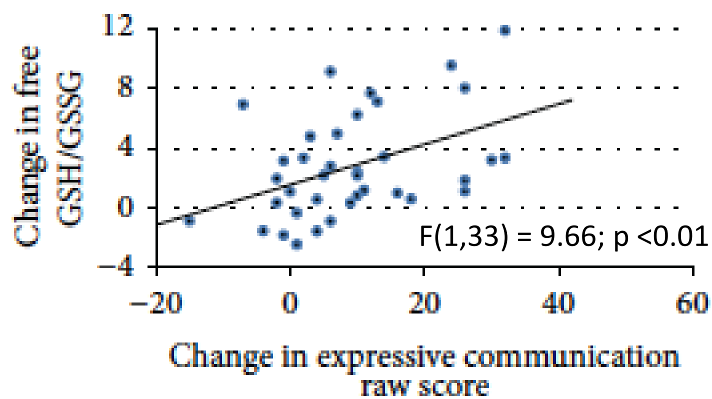
Table 2: Age equivalent scores from the Vineland Adaptive Behavior Scales at baseline before and after 3-month intervention with methylcobalamin and folinic acid. The change in age equivalent scores with 95% confidence interval (CI) is also given. The overall average of all subscales is also given in the last row of the table.

Vineland subscale	Baseline age equivalent (months) (mean ± SE)	Postintervention age equivalent (months) (mean ± SE)	Change in age equivalent (months) (mean; 95% CI)
Receptive language	23.1 ± 1.8	31.4 ± 3.4	8.3 (2.9, 13.7)
Expressive language	20.6 ± 1.9	27.5 ± 2.9	6.0 (3.3, 9.4)
Written language	40.5 ± 3.8	46.7 ± 4.0	6.2 (3.4, 9.0)
Personal skills	30.5 ± 2.3	40.5 ± 3.8	10.0 (3.8, 16.2)
Domestic skills	30.3 ± 4.1	39.3 ± 5.9	9.0 (−1.4, 19.4)
Community skills	32.9 ± 2.9	36.1 ± 3.8	2.0 (−3.0, 6.9)
Interpersonal skills	18.7 ± 2.7	24.1 ± 3.9	5.4 (0.0, 10.9)
Play/leisure skills	22.0 ± 4.5	34.0 ± 4.1	12.0 (4.1, 19.6)
Coping skills	25.8 ± 2.5	34.3 ± 4.0	11.5 (4.9, 18.0)
Overall skills	26.6 ± 2.3	34.3 ± 3.6	7.7 (3.4, 12.0)

TABLE 1
Comparison of methionine cycle and transsulfuration metabolites between autistic children and control children[1]

	Control children (n = 33)	Autistic children (n = 20)
Methionine (µmol/L)	31.5 ± 5.7 (23–48)	19.3 ± 9.7 (15–25)[2]
SAM (nmol/L)	96.9 ± 12 (77–127)	75.8 ± 16.2 (68–100)[3]
SAH (nmol/L)	19.4 ± 3.4 (16–27)	28.9 ± 7.2 (14–41)[2]
SAM:SAH	5.2 ± 1.3 (4–8)	2.9 ± 0.8 (2–4)[2]
Adenosine (µmol/L)	0.27 ± 0.1 (0.1–0.4)	0.39 ± 0.2 (0.17–0.83)[4]
Homocysteine (µmol/L)	6.4 ± 1.3 (4.3–9.0)	5.8 ± 1.0 (4.0–5.8)[3]
Cystathionine (µmol/L)	0.17 ± 0.05 (0.1–0.27)	0.14 ± 0.06 (0.04–0.2)[5]
Cysteine (µmol/L)	202 ± 17 (172–252)	163 ± 15 (133–189)[2]
tGSH (µmol/L)	7.6 ± 1.4 (3.8–9.2)	4.1 ± 0.5 (3.3–5.2)[2]
Oxidized glutathione (nmol/L)	0.32 ± 0.1 (0.11–0.43)	0.55 ± 0.2 (0.29–0.97)[2]
tGSH:GSSG	25.5 ± 8.9 (13–49)	8.6 ± 3.5 (4–11)[2]

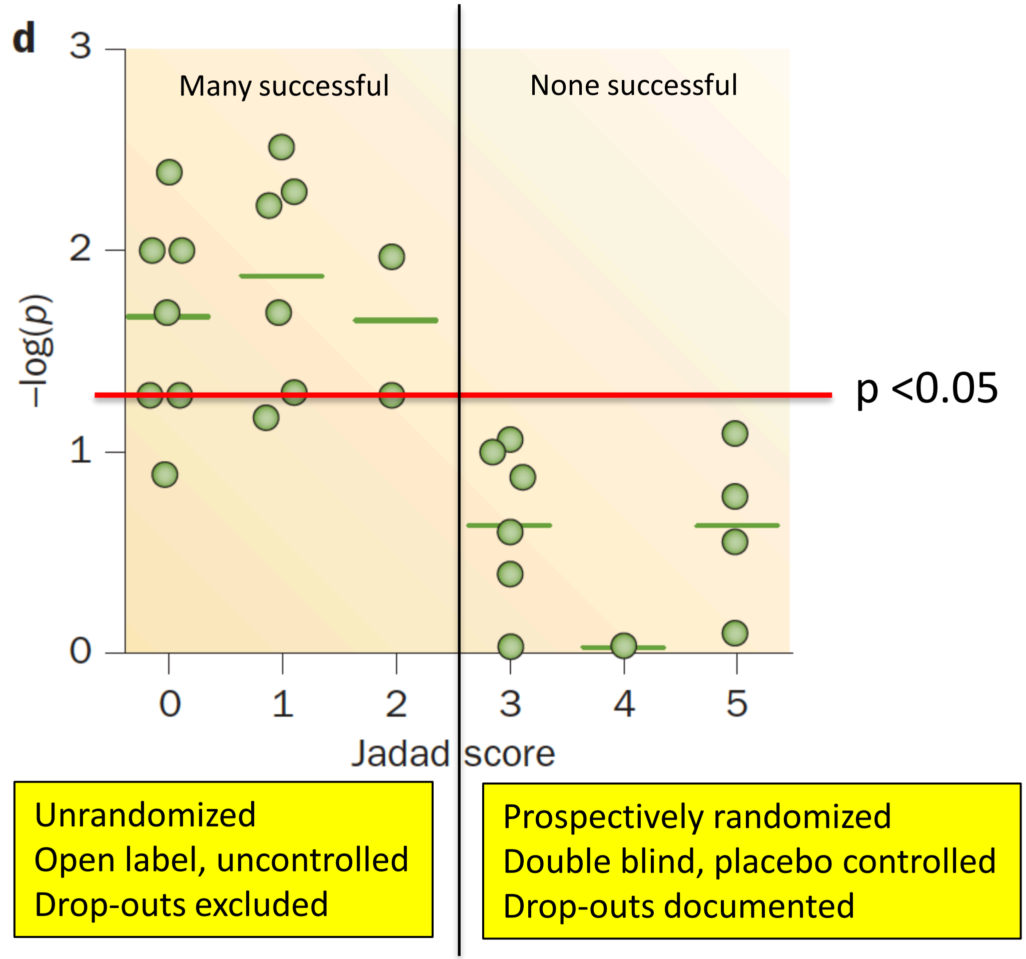
Plasma, not cells



James SJ, *et al. Am J Nutr*, 2004 PMID 15585776

Frye RE, *et al. Aut Res Treat*, 2013. PMID 24224089

Clinical Trials in Complex Disease—A Cautionary Tale from Mitochondrial Medicine



Treatment is Effective

Conclusion Threshold

Treatment is Ineffective

35 of 1,039 Clinical Trials were described in enough detail to generate a Jadad Score

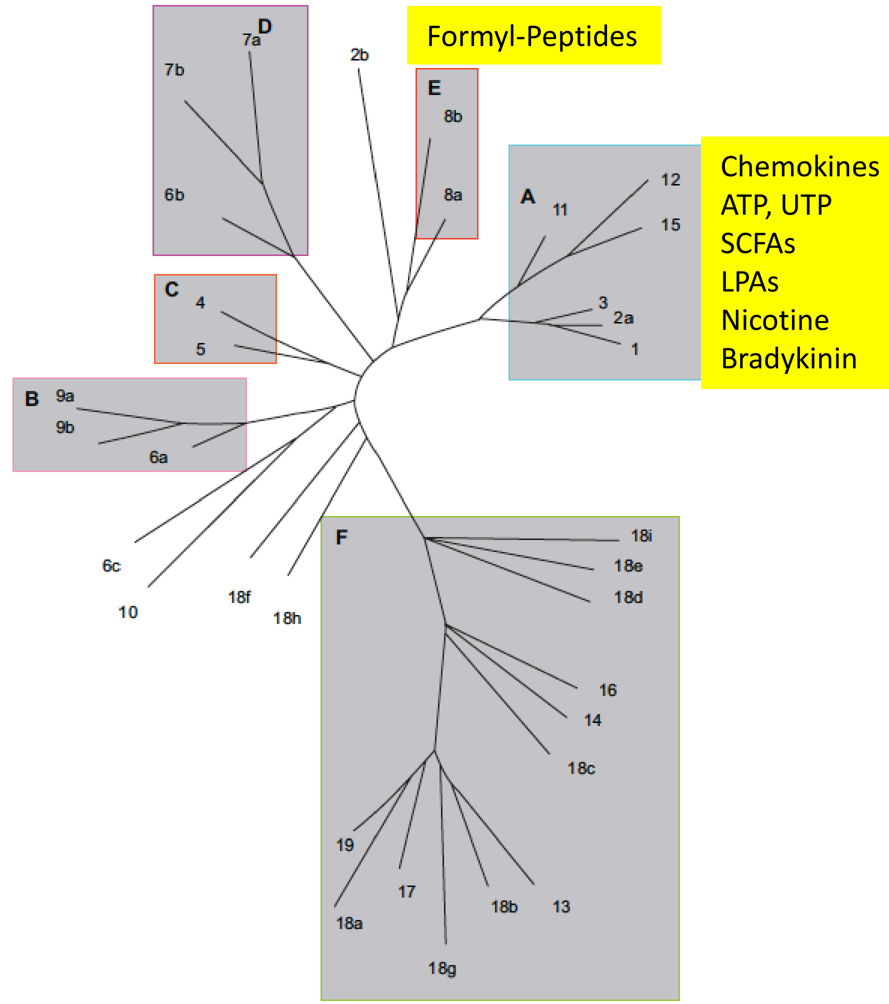
Pfeffer, *et al. Nature Rev Neurol*, 2013. PMID 23817350.

How do cells “smell” safety and danger in the world? (Hint: It’s all about metabolism.)

Vertebrate Chemosensory Receptor Evolution

7 Transmembrane GPCRs

	Sight	Smell	Pheremones		Taste	
					Bitter	Sweet Umami
	Opsin	OR	V1R	V2R	T2R	T1R
Mouse	3	1,037(354)	165(165)	61(148)	35(6)	3
Human	4	388(414)	2(115)	0	25(11)	3

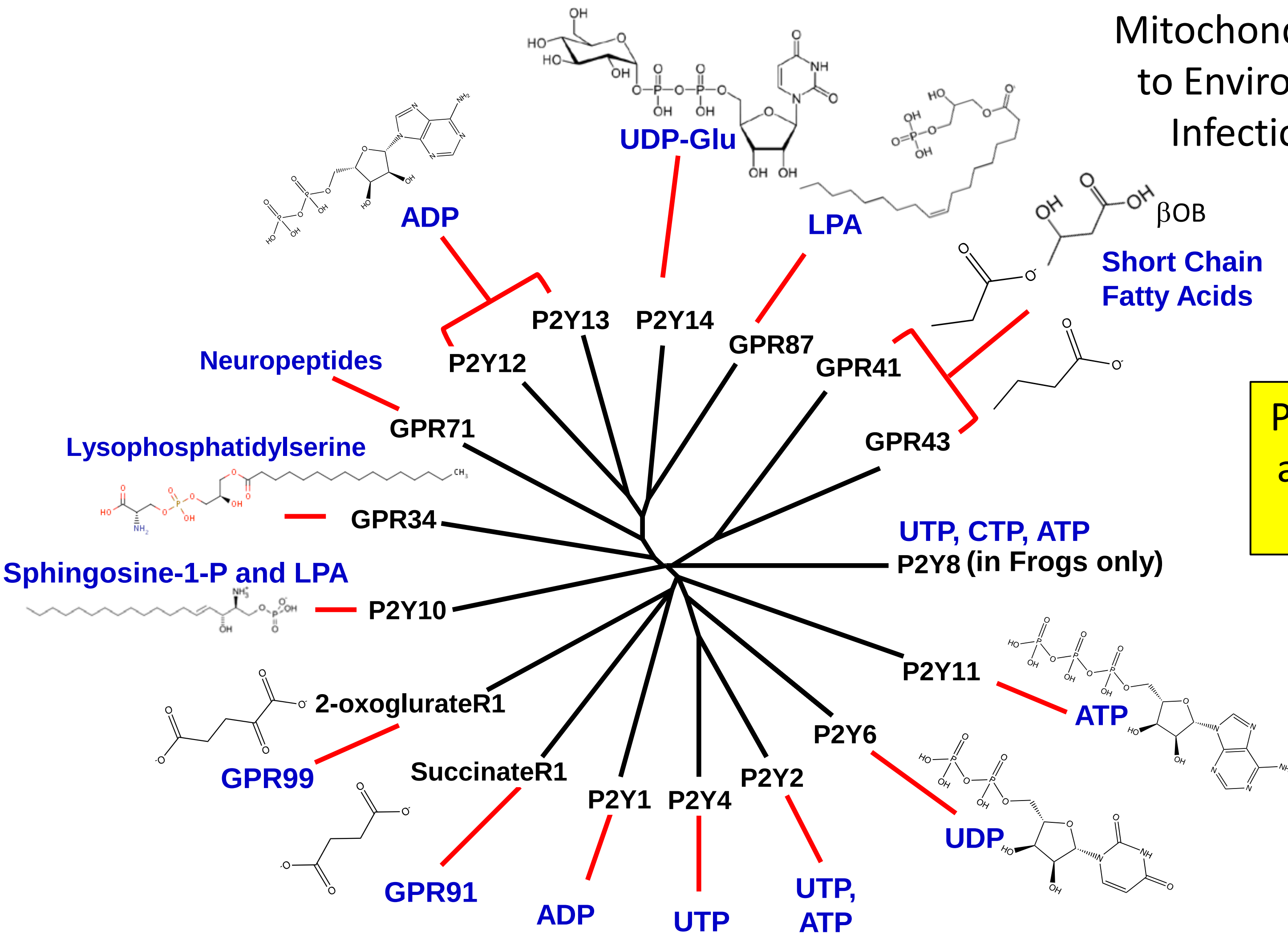


Liman ER. *Adv Exp Med Biol*, 2012. PMID 22399404

Shi/Zhang. *Results Probl Cell Diff*, 2009. PMID 19145414

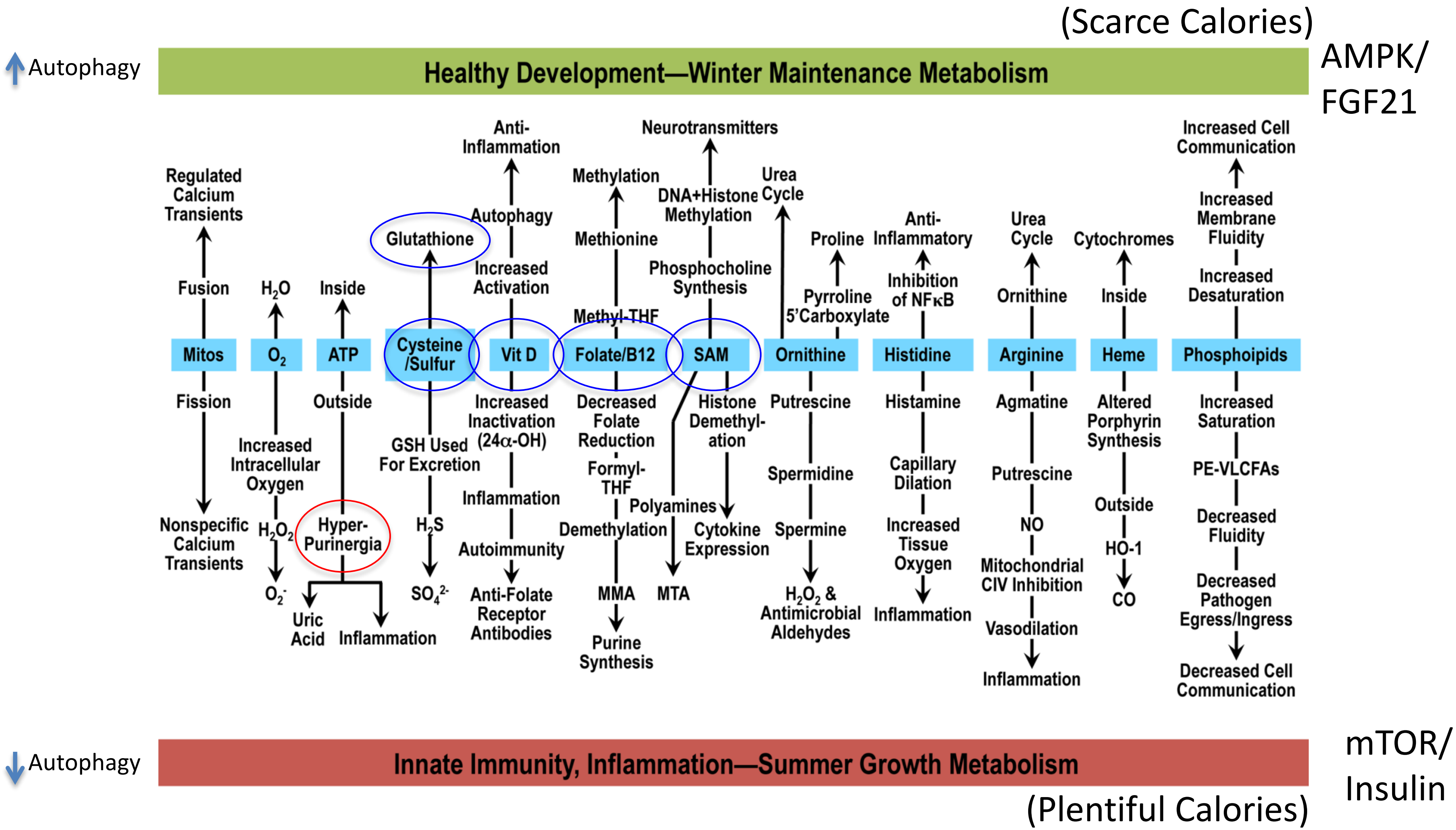
"Mitokine Receptors" are Like Extranasal Cellular Odorant Receptors—7 Transmembrane GPCRs

The Ligands are Traceable to Mitochondria and adapt Rapidly to Environmental Change, eg, Infection, Injury, or Toxins



Purinergic Receptors are the most widely expressed class

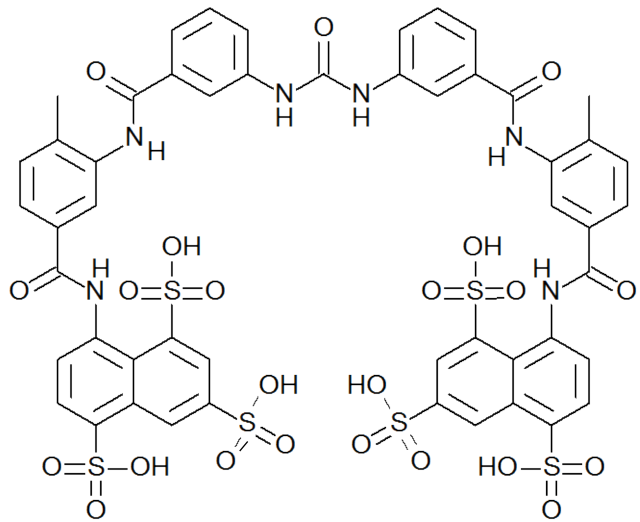
Metabolic Features of the CDR and Its Evolutionary Origins in the Seasons



From Naviaux RK. Metabolic Features of the Cell Danger Response. *Mitochondrion*, 2013.

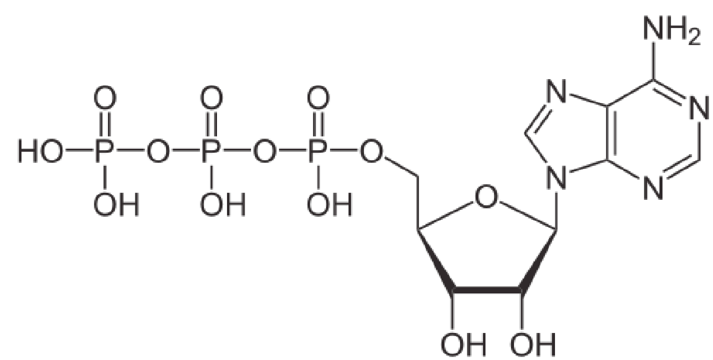
Suramin is a Competitive Antagonist of Purinergic Signaling

a



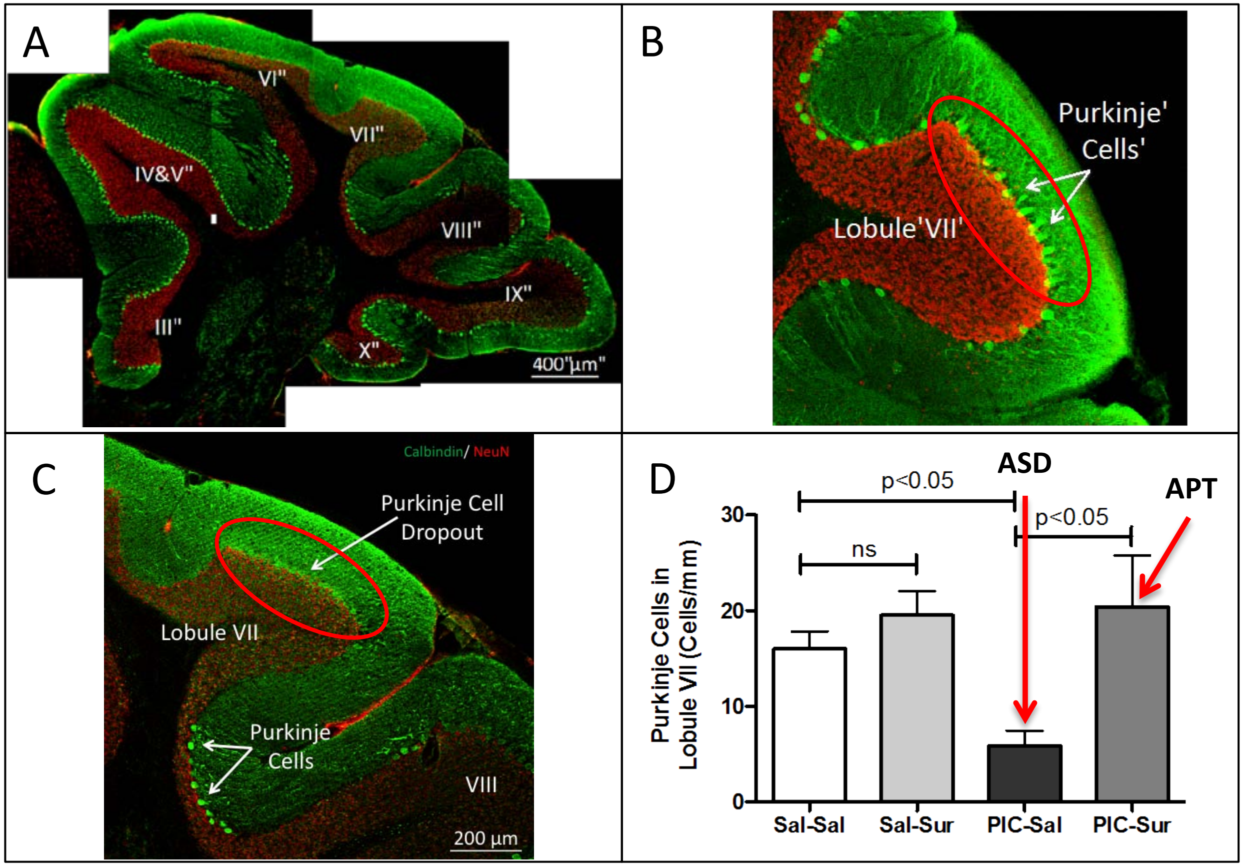
Suramin

b



ATP

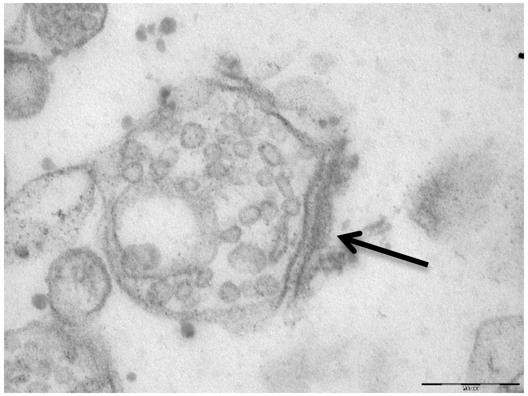
APT Prevented Loss of Cerebellar Purkinje Cells When Started by 2 Months of Age



Naviaux RK, et al. *PLOS One* 2013:8;e57380. March 13, 2013.

Synaptosomal Ultrastructural Abnormalities in the MIA Model—Corrected by Antipurinergic Therapy

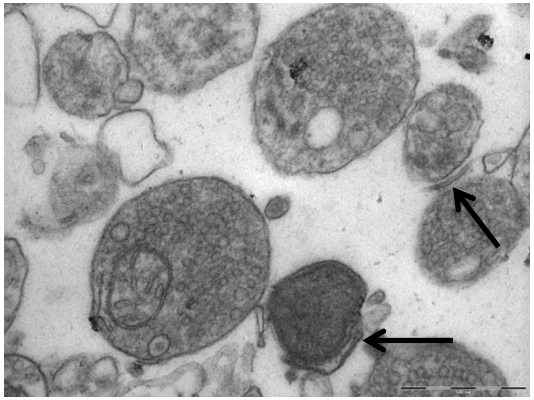
Normal Post-
Synaptic Densities



Saline-Saline

Control

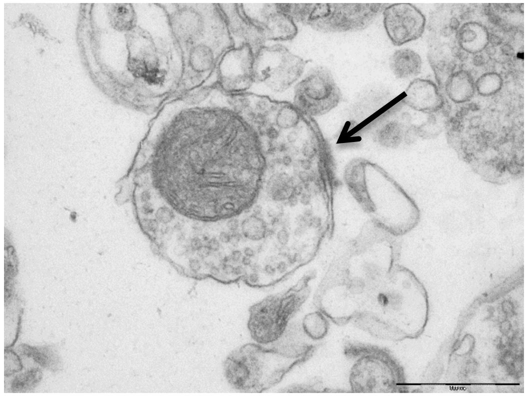
Hypomorphic
Post-Synaptic Densities;
Electron Dense
Matrix Material



Poly(IC)-Saline

ASD

Normalized Post-
Synaptic Densities
And Matrix



Poly(IC)-Suramin

Treated

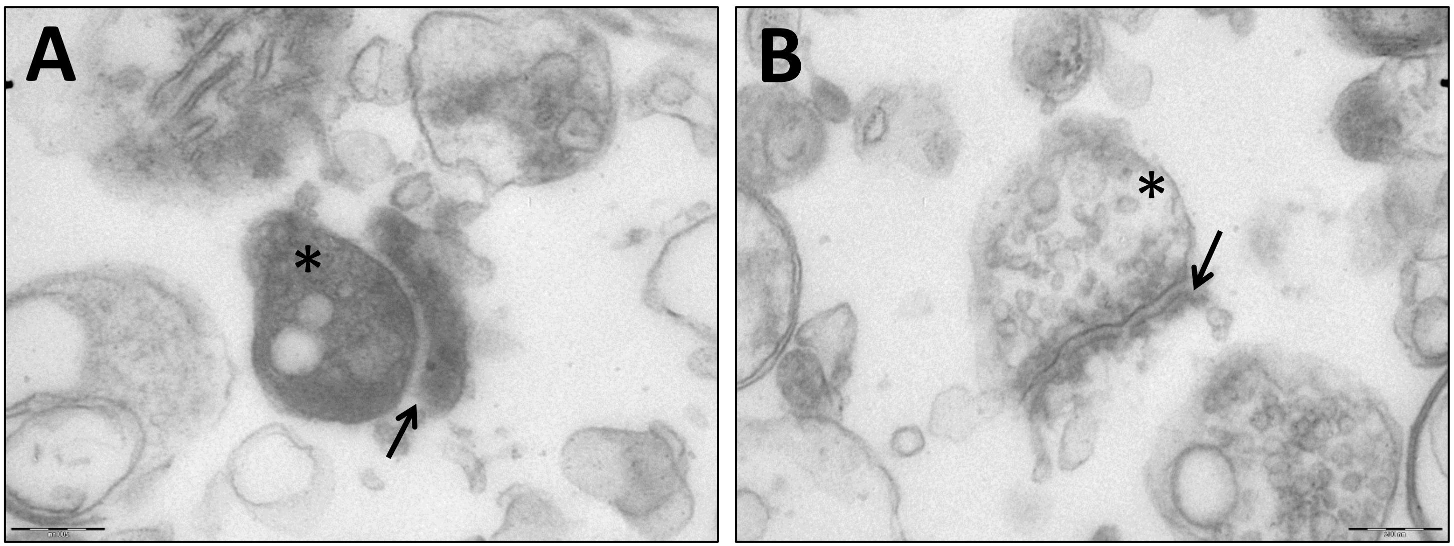
Synaptosomal Ultrastructural Abnormalities in the Fragile X Model—Corrected by Antipurinergic Therapy

Hypomorphic
Post-Synaptic Densities;
Electron Dense Matrix

ASD-Like

Normalized Post-
Synaptic Densities
And Matrix

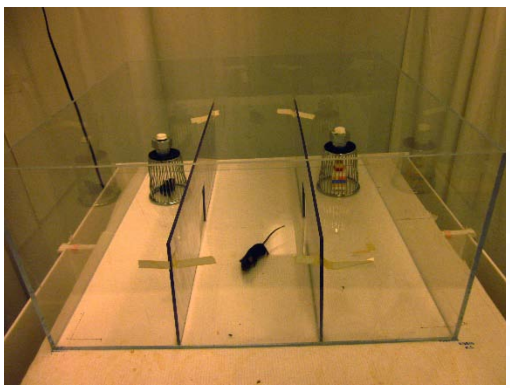
Treated



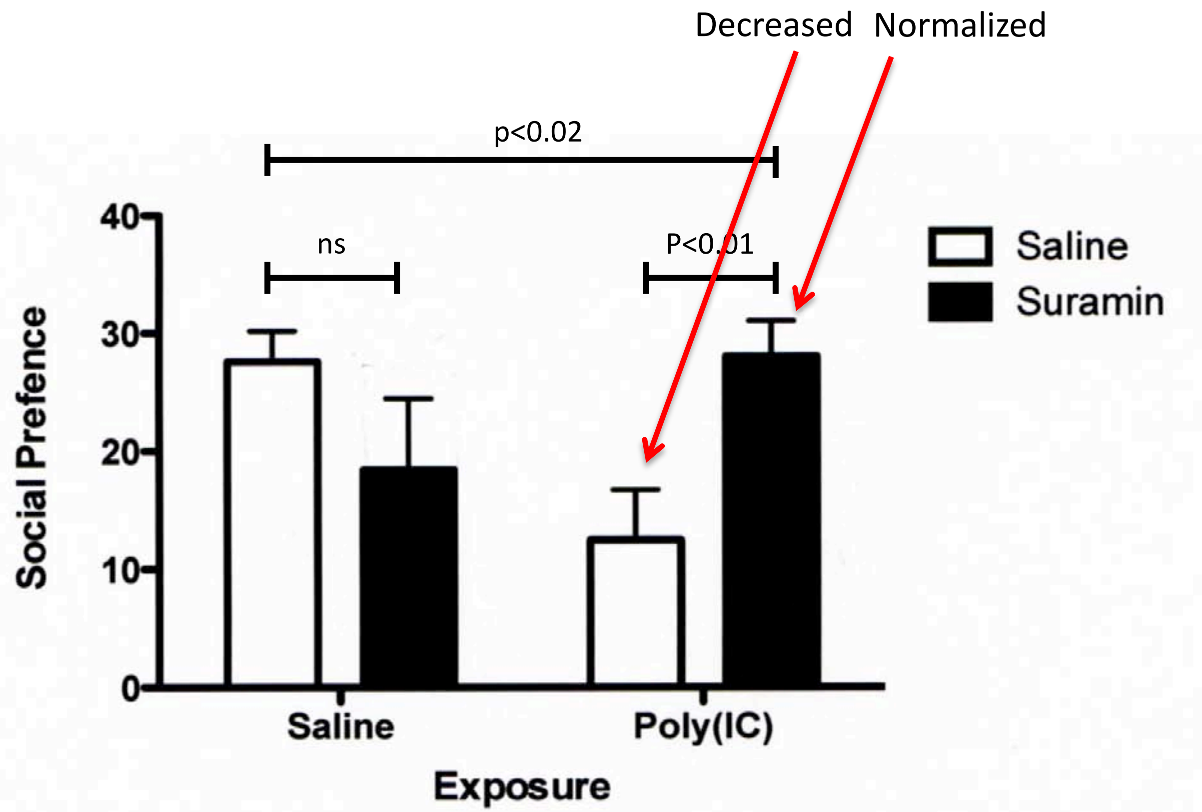
Fragile X/Saline

Fragile X/Suramin

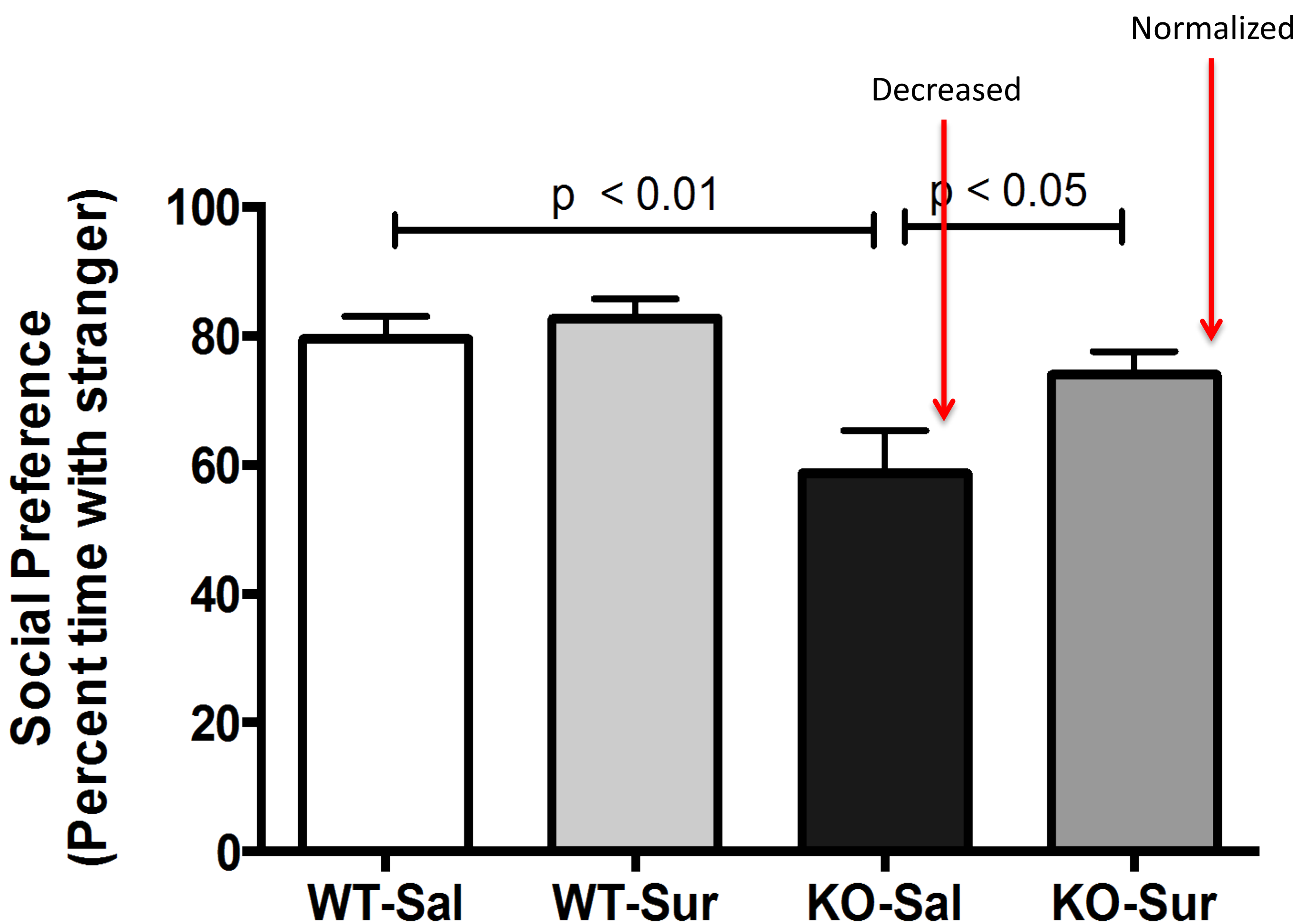
Social Approach Abnormalities in the MIA Model Were Corrected by Antipurinergic Therapy (APT)



Crawley Social Approach Chamber

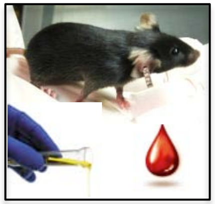


Social Approach Abnormalities in the Fragile X Model Were Corrected by Antipurinergic Therapy (APT)

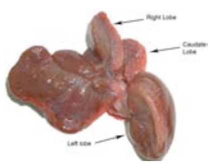


UCSD Metabolomics

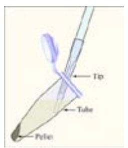
20-100 µl samples of urine and blood



Internal Standards



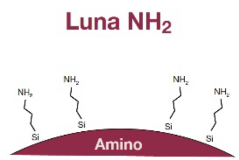
10-50 mg of Liquid Nitrogen Powdered Tissue



Sample Extraction (to remove proteins, DNA, and RNA)

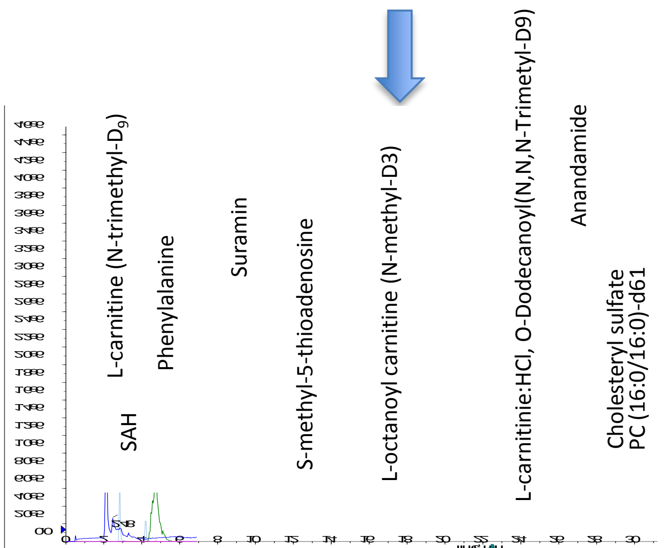
Polar + Nonpolar Metabolites

UHPLC



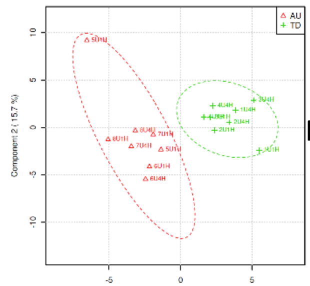
LC-MS/MS

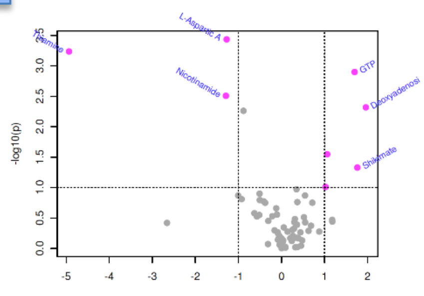
Comprehensive Metabolite Quantitation (Scheduled MRMs in Both Positive and Negative Mode)



Quantitation of About 700 Metabolites, 300 Drugs, Toxins, and Xenobiotics

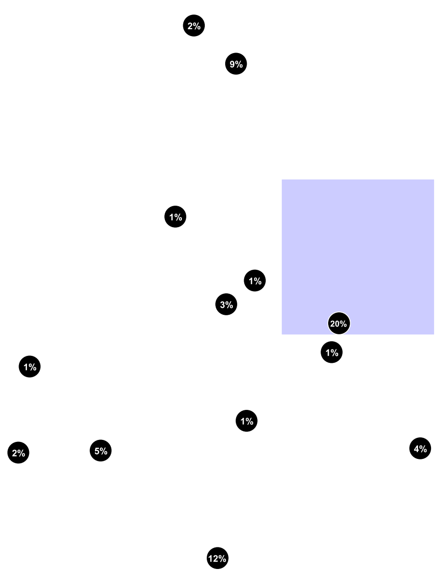
PLS-DA and PC Analysis



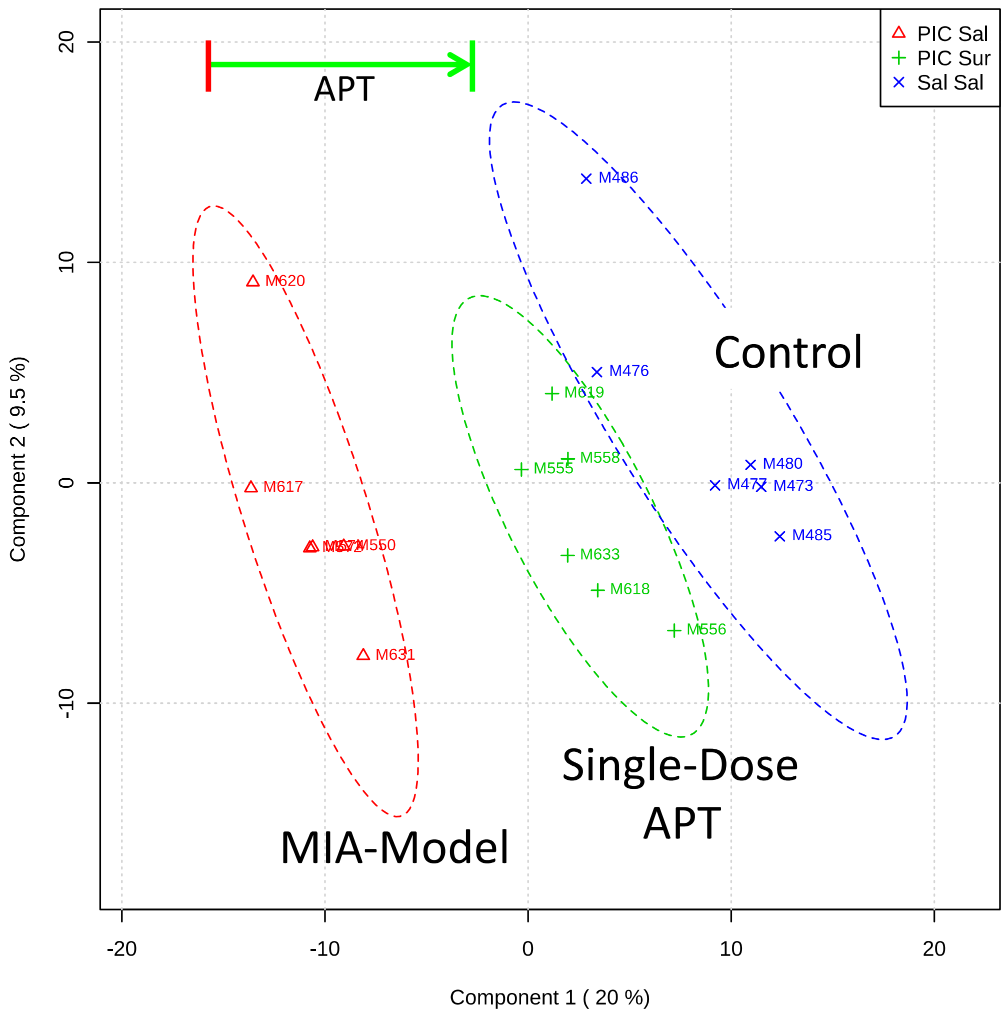


Volcano Plots

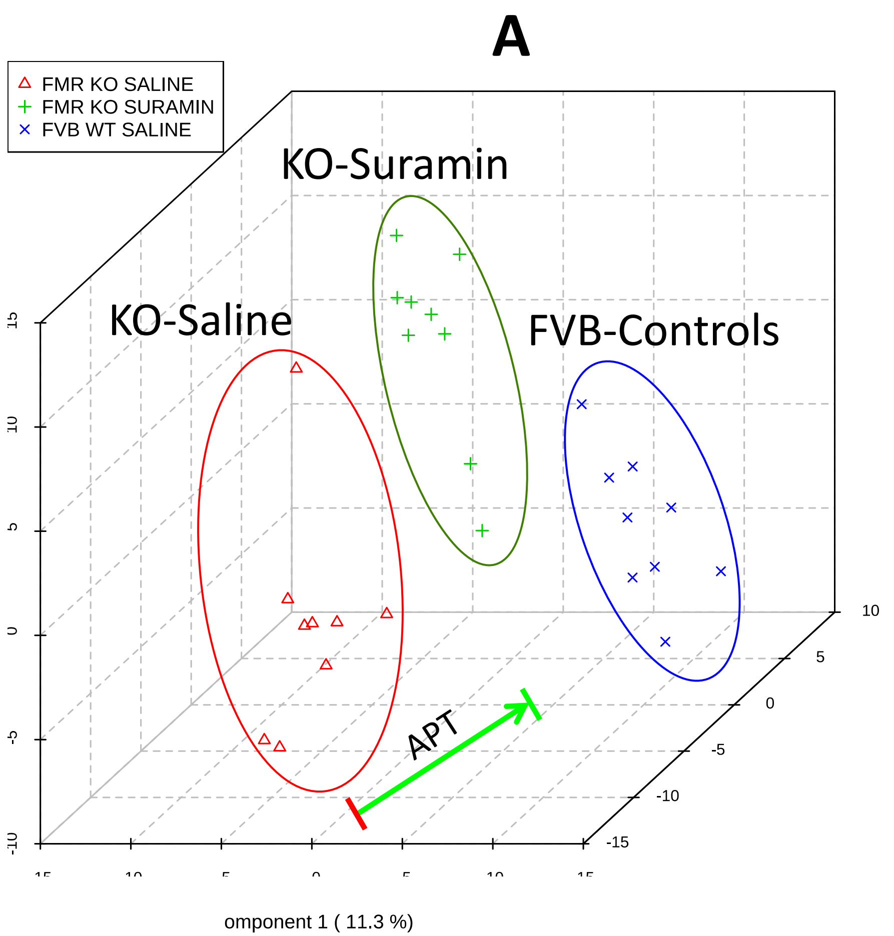
Biochemical Pathway Visualization



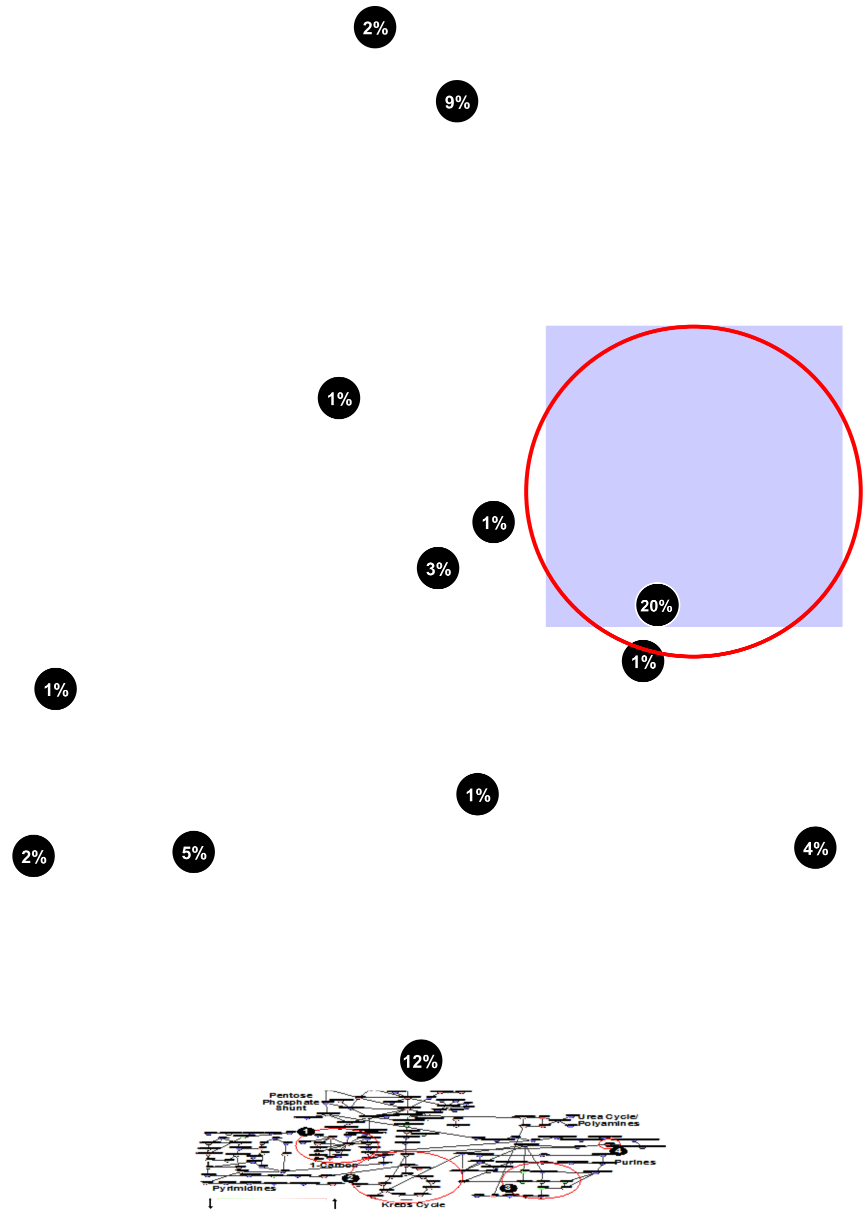
Metabolic Abnormalities in the MIA Model were Improved by Antipurinergic Therapy



Metabolic Abnormalities in the Fragile X Model Were Improved by Antipurinergic therapy



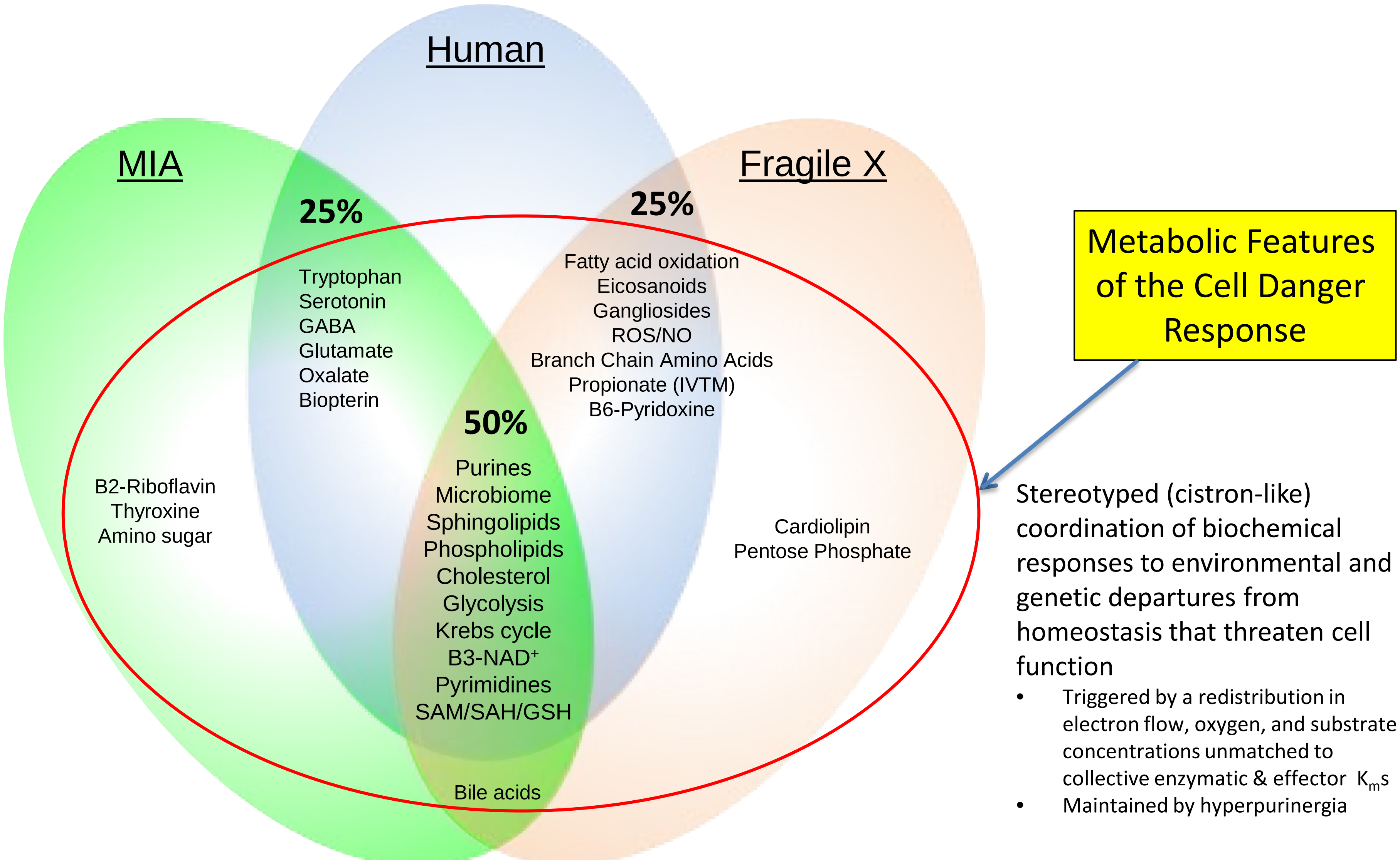
20 Different Metabolic Pathways Were Improved by APT in the Fragile X Model



Purine Metabolism Was the Top Pathway Associated With Restoration of Social Behavior

Also found in a gene expression study of human ASD by Ginsberg/Natowicz PMID 22984548—Thank you Sophia Colamarino

Metabolomics of Autism Spectrum Disorders— Pathway Abnormalities Known in Humans Were Also Seen and Corrected by Suramin in Two Animal Models



Stereotyped (cistron-like) coordination of biochemical responses to environmental and genetic departures from homeostasis that threaten cell function

- Triggered by a redistribution in electron flow, oxygen, and substrate concentrations unmatched to collective enzymatic & effector K_ms
- Maintained by hyperpurinergia

Take Home Messages

- The brain controls metabolism
  - Corollary: All brain disorders have metabolic disturbances
- Cells "smell" the world through conserved chemosensory receptors that continuously monitor metabolism
- Purinergic signaling and mitokines control the cellular response to safety and danger
  - "Safety" and "Danger" are not anthropomorphic constructs
  - "Danger" is the graded mismatch between the instantaneous concentrations of substrates and effectors, and the collective Kms and Kds of the enzymes and receptors evolved by natural selection in past environments and passed on to us by our ancestors
- About a dozen core metabolic pathway disturbances were shared by the environmental MIA, the genetic Fragile X mouse model, and human ASD

Thank You

- Jane Botsford Johnson Foundation
- The UCSD Christini Fund
- Autism Speaks Trailblazer Award
- Wright Family Foundation
- Lennox Foundation
- MRSII Demonstration Grant Program

[PII redacted]
1996-1998

Species and Cellular Survival and Persistence States, Hypometabolism, and the Cell Danger Response (CDR)

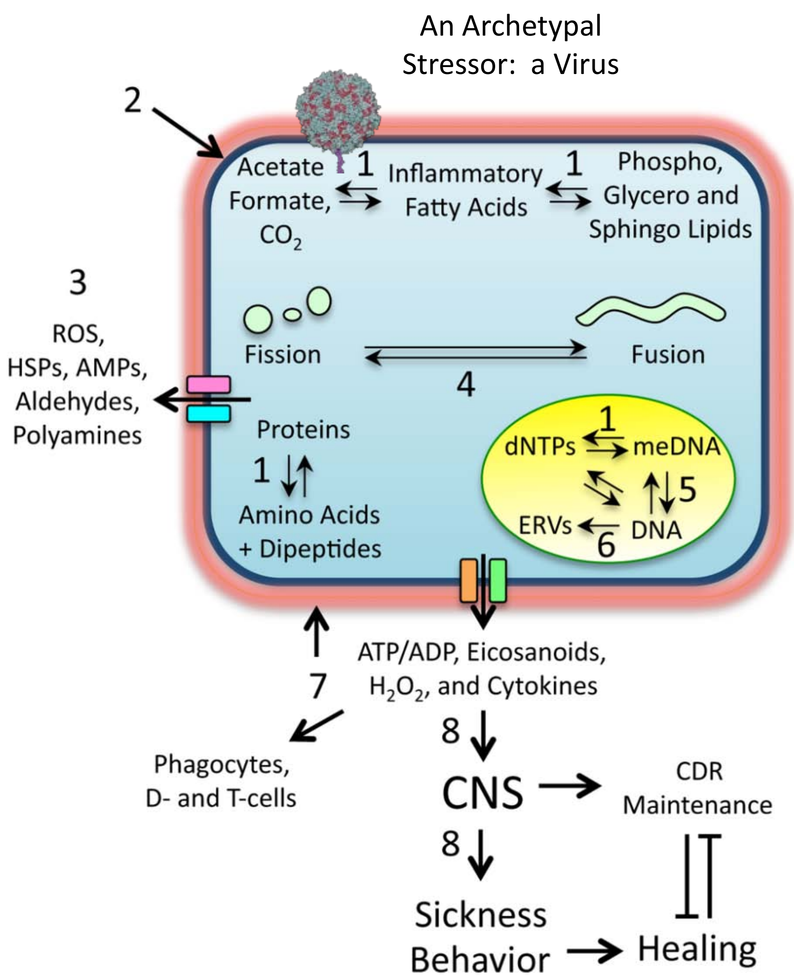
Stress Conditions

- Tardigrade tun state
- Nematode dauer & diapause
- Memory T-cells
- Mammalian embryo diapause
- Oocyte and egg cell metabolism
- Plant seed metabolism
- Hummingbird torpor
- Hibernation
- Estivation
- ?Autism

Shared Metabolic Features

- Decreased basal oxygen consumption
- Increased glycolysis
- Oxidize glutathione
- Decreased heat production
- Decreased fatty acid oxidation
- Intracellular lipid accumulation
- Increased mitochondrial coupling
- Increased mitochondrial reserve capacity
- Increased vitamin-independent methionine synthesis
  - Increased Betaine-Homocysteine Methyltransferase (BHMT) expression
- Increased capacity for ROS production
  - Increased SOD, GSH-peroxidase
- Increased ATP turnover
  - Hypothesis: Hyperpurinergia maintains the abnormal metabolism and behavior

Understanding the Cell Danger Response: Follow the Electrons.....



Electron Steal Drives Rapid Mitochondrial Redox Change

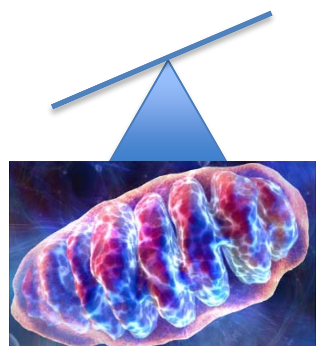
0. Decrease oxygen consumption → increase dissolved O_2 concentration
1. Shift from polymer to monomer synthesis (ΔG)
2. Stiffen cell membranes
3. Release anti-viral and anti-microbial chemicals
4. Increase mitochondrial fission and autophagy
5. Change DNA methylation
6. Mobilize endogenous retroviruses and LINEs
7. Warn neighboring cells and call in effector cells—the "purinergic halo"
8. Alter host **behavior** to prevent spread of disease to kin

From Naviaux RK. Metabolic Features of the Cell Danger Response. *Mitochondrion*, 2013.

The Long Road to Purinergic Signaling in Autism Spectrum Disorders—1929-Present

Mitochondrial Disease

$\neq$



Autism Spectrum Disorders

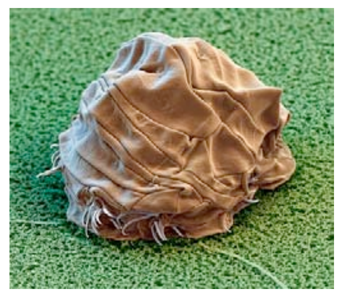
Cell Persistence Strategies Across Species—Thinking "Analogically"

<u>Stress Response</u>

- Stop eating
- Mitochondrial oxphos declines
- Oxygen consumption declines
- Heat production declines
- Lipid droplets accumulate

Tardigrades
("Water Bears")

Desiccation,
heat stress, etc



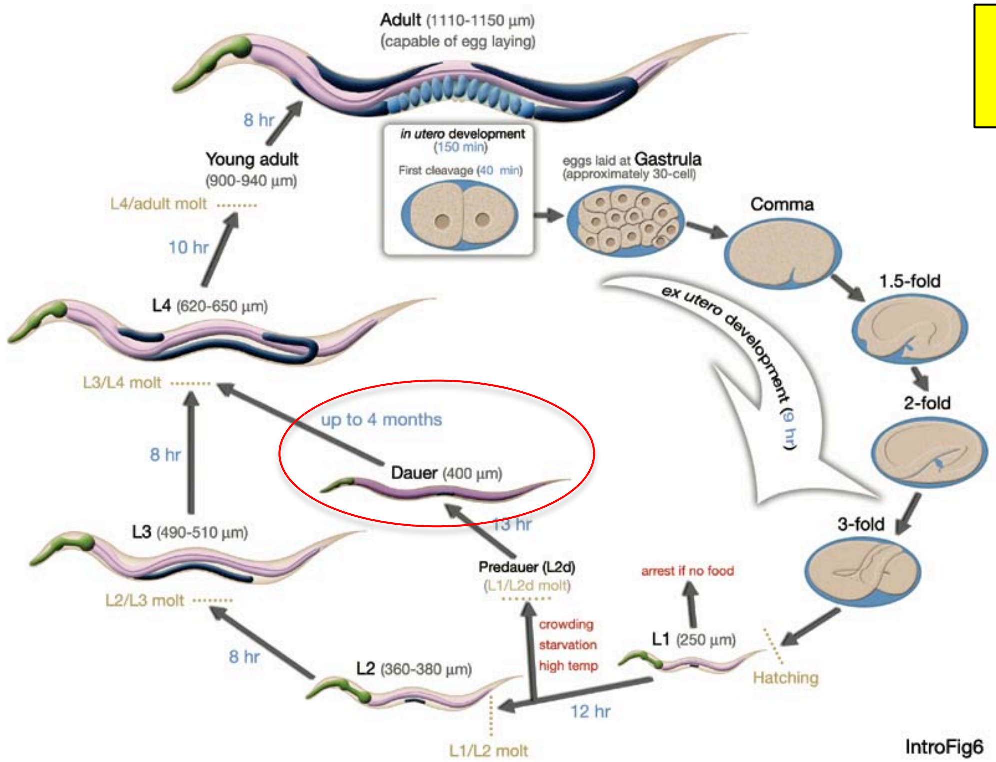
Tun State

Add water + carbs

Resistant to:
Drying, heating,
Freezing, radiation,
Toxins

Reproductive Cycle = 2.5 days
Menopause After 6 days
Normal life span = 13 days

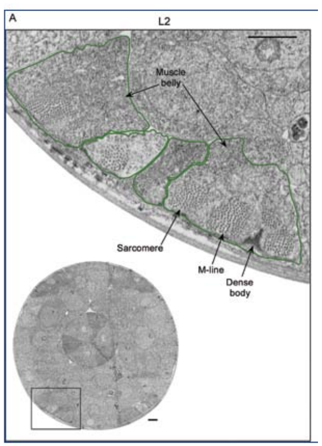
C. elegans



Normal

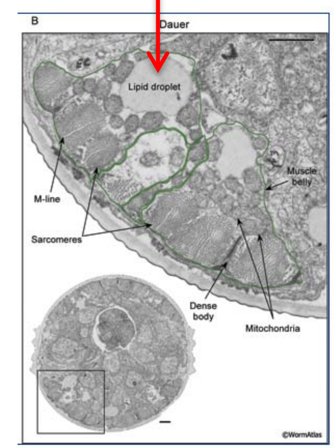
Dauer

Lipid Droplets

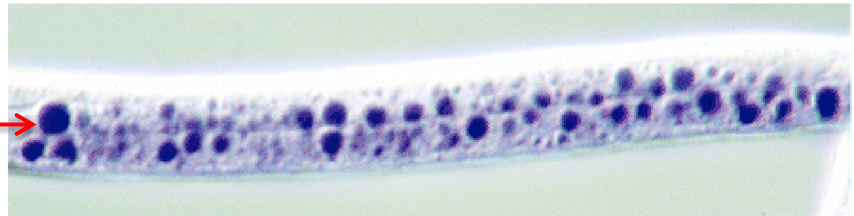


Dauer Shifts

- Stop eating
- Mitochondrial oxphos declines
- Oxygen consumption declines
- Lipid droplets accumulate
- Glycolysis increases
- Glyoxylate shunt increases to increase OAA and gluconeogenesis



Lipid Droplets



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Perspectives in Pharmacology

June 2012

Oxidative Shielding or Oxidative Stress?

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ABSTRACT

In this review I report evidence that the mainstream field of oxidative damage biology has been running fast in the wrong direction for more than 50 years……..

“Oxidative stress is not the prime cause of chronic disease. The prime cause of chronic disease may be the pathological persistence of the cell danger response--the evolutionarily conserved process that generates the metabolic features (biochemical symptoms) that protect the cell acutely from viral attack and homeostatic threats, but can persist chronically, causing disease.”

OPEN ACCESS Freely available online

PLOS ONE

Antipurinergic Therapy Corrects the Autism-Like Features in the Poly(IC) Mouse Model

Robert K. Naviaux[1,2,3,4*], Zarazuela Zolkipli[1,5], Lin Wang[1,2], Tomohiro Nakayama[1,5], Jane C. Naviaux[1,6], Thuy P. Le[1,3], Michael A. Schuchbauer[6], Mihael Rogac[1,2¤], Qingbo Tang[2], Laura L. Dugan[2], Susan B. Powell[6]

March 2013

OPEN

Citation: Transl Psychiatry (2014) **4**, e400; doi:10.1038/tp.2014.33

www.nature.com/tp

ORIGINAL ARTICLE

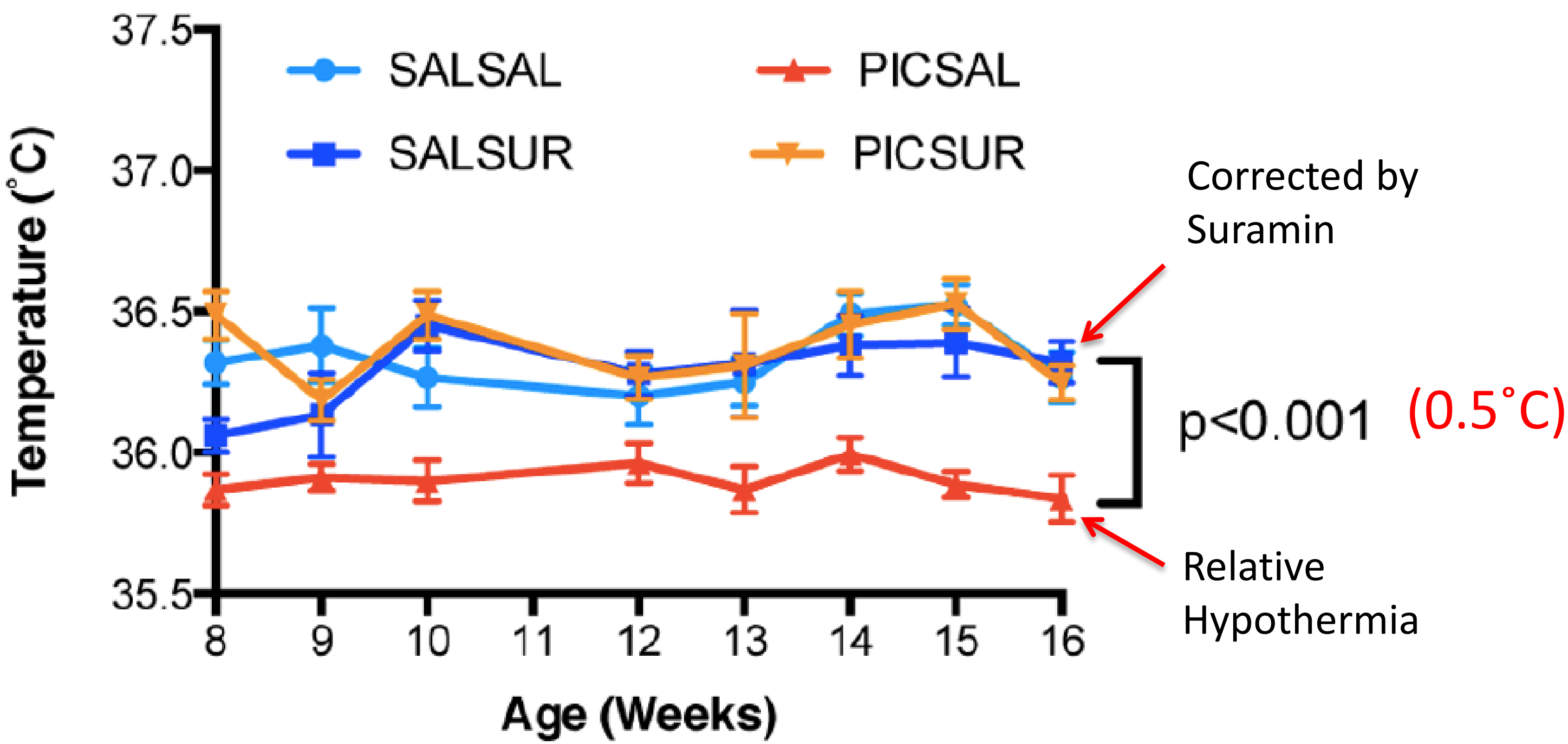
Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy

JC Naviaux[1], MA Schuchbauer[1], K Li[2,3], L Wang[2,3], VB Risbrough[1,4], SB Powell[1] and RK Naviaux[2,3,4,5,6]

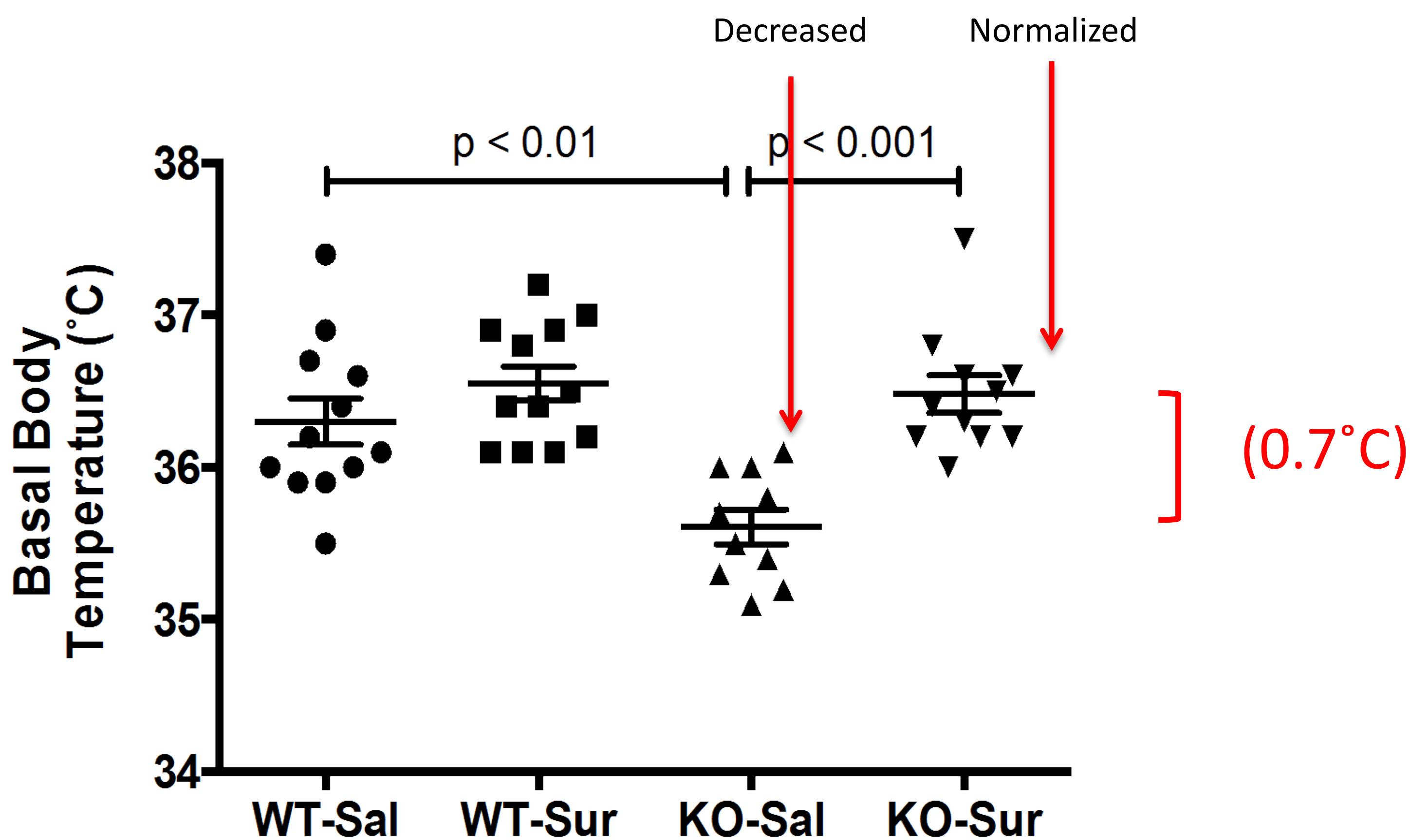
June 2014

The CDR →

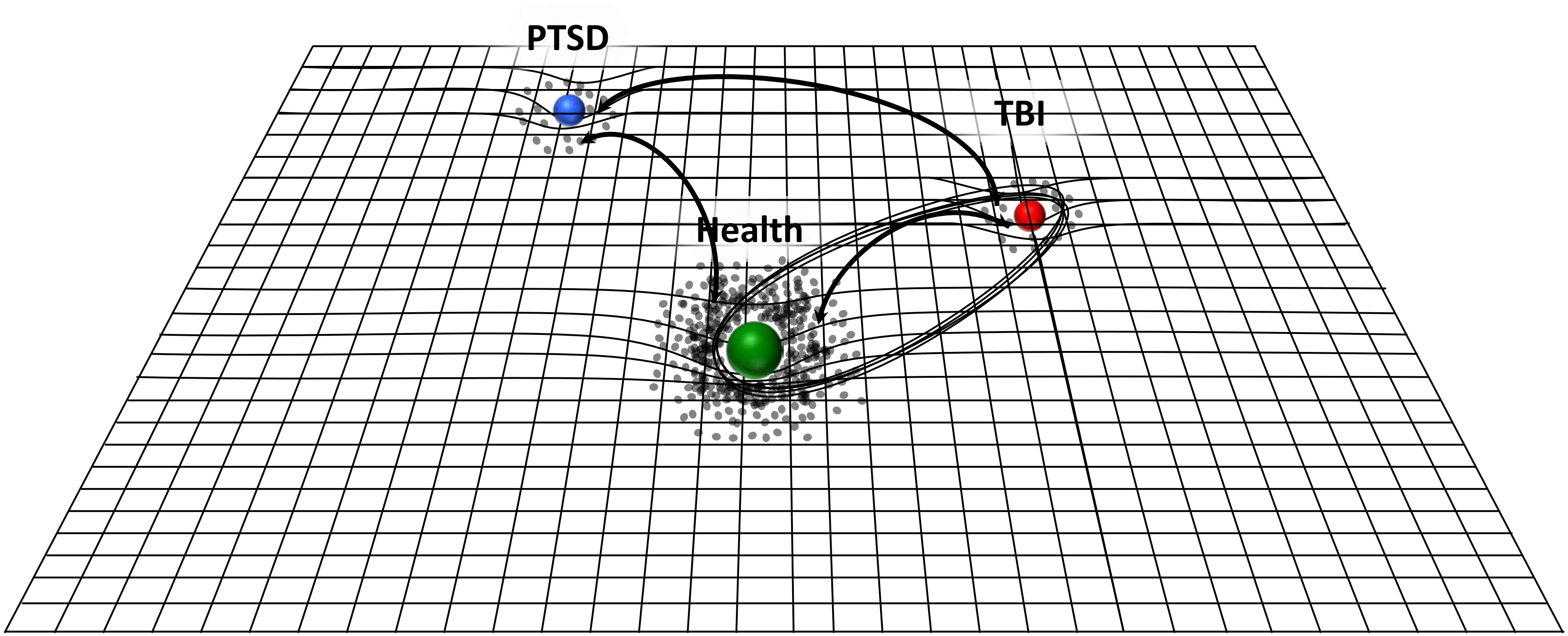
The MIA Mouse Model of ASD has Relative Hypothermia—Corrected by Antipurinergic Therapy



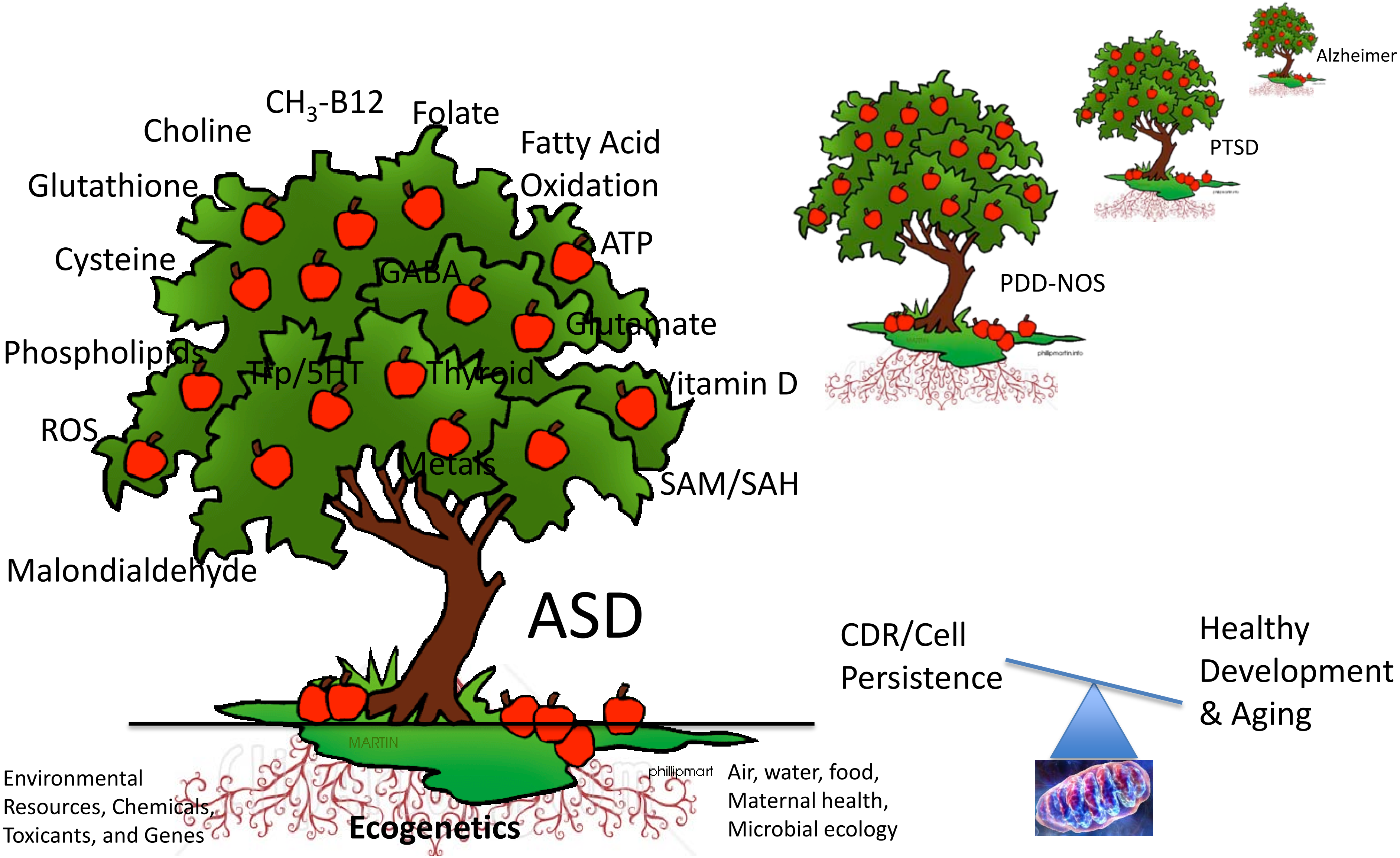
The Fragile X Mouse Model of ASD has Relative Hypothermia—Corrected by Antipurinergic Therapy

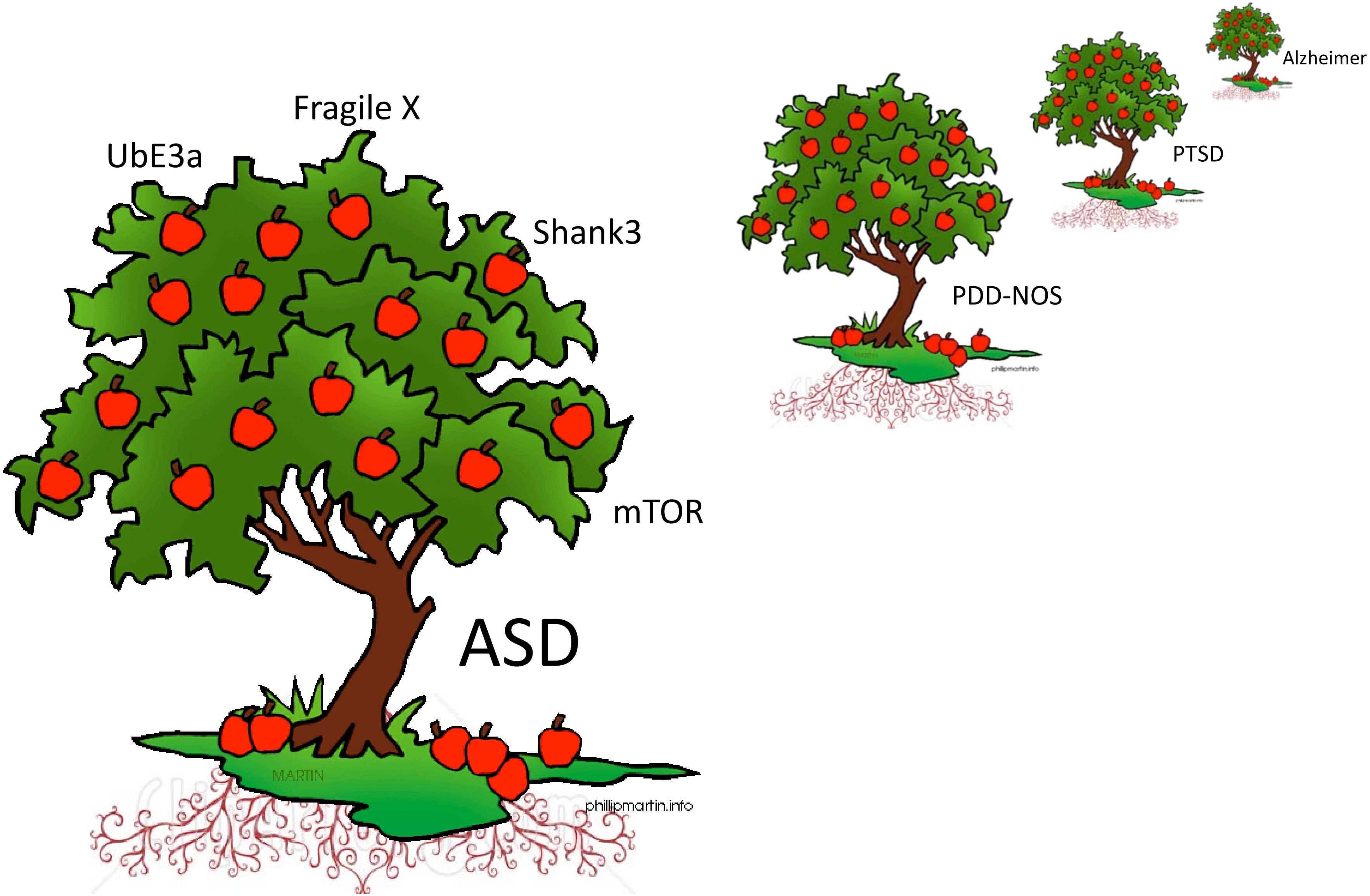


Metabolic ‘Landscape’



Modified from Will Alaynick, 2014


Alzheimer
PTSD
PDD-NOS
CH_3-B12
Choline
Folate
Fatty Acid
Oxidation
Glutathione
ATP
Cysteine
GABA
Glutamate
Phospholipids
Trp/5HT
Thyroid
Vitamin D
ROS
Metals
SAM/SAH
Malondialdehyde
ASD
CDR/Cell
Persistence
Healthy
Development
& Aging
MARTIN
phillipmart
Environmental
Resources, Chemicals,
Toxicants, and Genes
Ecogenetics
Air, water, food,
Maternal health,
Microbial ecology


UbE3a
Fragile X
Shank3
mTOR
ASD
PDD-NOS
PTSD
Alzheimer

Metabolic Features of Cellular Persistence and the Cell Danger Response

Dauer

- Decreased oxygen consumption
- Decreased heat production
- Decreased Fatty acid oxidation
- Intracellular lipid accumulation
- Increased mitochondrial coupling
- Increase mitochondrial reserve capacity
- Increased ATP turnover

Autism

- Decreased oxygen consumption
- Decreased heat production
- Decreased Fatty acid oxidation
- Intracellular lipid accumulation
- Increased mitochondrial coupling
- Increase mitochondrial reserve capacity
- Increased ATP turnover

Richard Haas
UCSD 2012

William Graf

2000—First Hints of a Mitochondrial DNA Connection to Autism—*Compensatory Overfunction*

Autism Associated With the Mitochondrial DNA G8363A Transfer RNA^{Lys} Mutation

William D. Graf, MD; Jose Marin-Garcia, MD; H.G. Gao, MD; Senia Pizzo, PhD; Robert K. Naviaux, MD, PhD; David Markusic, BS; Bruce A. Barshop, MD, PhD; Eric Courchesne, PhD; Richard H. Haas, MB, BChir

J Child Neurol, 2000

Proband
Leigh Syndrome → "Definite"
Mitochondrial Disease

"Unlikely"
Mitochondrial Disease
(Without mtDNA)

Table 1. Summary of Clinical, Morphologic, Biochemical, and Genetic Findings in the Family Described

	II-1	II-2	I-1	II-3	II-4
Irritable bowel	–	–	+	–	–
Epilepsy	++	–	–	+	+
Learning disability	++	–	–	–	–
Cognitive regression	–	–		+	+++
Leigh syndrome	–	–	–	+++	–
Autism	–	–	–	–	+++
Brain Magnetic Resonance Imaging	Normal	Normal	ND	Abnormal	Normal
Muscle analysis	ND	ND	ND		
Histology				Normal	↑ Lipid
Histochemistry				COX–	Subsarcolemmal+
Biochemistry				↓ CIV & V	↑ CI
G3863A mitochondrial DNA mutation					
PCR analysis in blood	+	+	+	+	+
Percent in blood	ND	ND	28%	82%	60%
Percent in muscle	ND	ND	ND	86%	61%

– = absent; + = mild; ++ = moderate; +++ = severe; ND = not determined; ↑ = increased, ↓ = decreased; C = citrate synthase corrected respiratory chain complex activity; COX– = absence of cytochrome *c* oxidase staining; PCR = polymerase chain reaction.

24 mos
rs

↑250%

Original Investigation

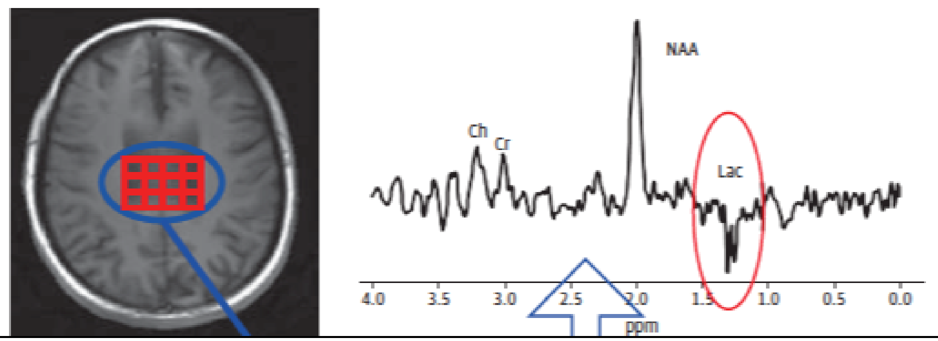
Mitochondrial Dysfunction as a Neurobiological Subtype of Autism Spectrum Disorder
Evidence From Brain Imaging

Suzanne Goh, MD; Zhengchao Dong, PhD; Yudong Zhang, PhD; Salvatore DiMauro, MD; Bradley S. Peterson, MD

JAMA Psychiatry, 2014

Suzanne Goh

Figure 1. An Example of Spectra From a Participant With Lactate-Positive Autism Spectrum Disorder



Billi DiMauro

Brad Peterson

Elevated Brain Lactate

8 of 41 Adults (19-60 yrs) with ASD = 20% (95% CI = 9-35%)

2 of 34 Children (5-18 yrs) with ASD = 6% (95% CI = 0.7-20%)

Affected Voxels: Variable between subjects; Cingulate gyrus most commonly.
Pertinent Negative: Rarely in the basal ganglia
Unifying Observation: Brain lactate elevation can be either genetic or environmental

1969—The First Reported Case of Purine-Associated Autism

William Nyhan
UCSD 2012

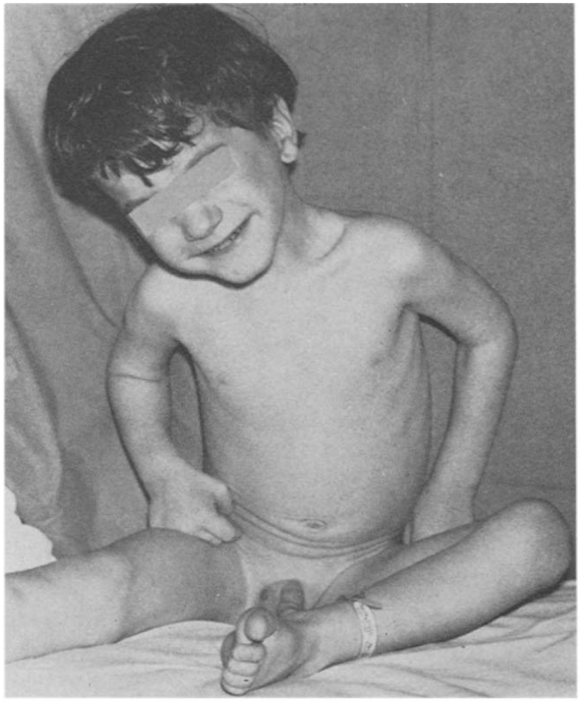


Fig. 1. S. M. at 3 years of age. His general appearance and characteristic odd grin are illustrated.

- Nyhan WL, et al. J Peds 74:20-27, 1969.
  - Disorder of *de novo* purine synthesis
- 3 year old boy with autism, hyperuricemia, hypospadius, and hearing loss
- Parasympathetic defect/Dysautonomia
  - Congenitally absent tear glands & ducts
  - Methacholine-insensitive pupillary response
- Phosphoribosyl Pyrophosphate Synthase (PRPPS) Superactivity
  - G547C/D182H—resistant to feedback inhibition by ADP and ATP
  - Increased ca 600%
- Resulted in excess ATP, ADP, GTP, and IMP synthesis by *de novo* purine synthesis
- Treated with allopurinol and hearing aids with reduction in autistic symptoms

Hamlin J, *et al*. *Aut Res Treat*, 2013. PMID: 24396597

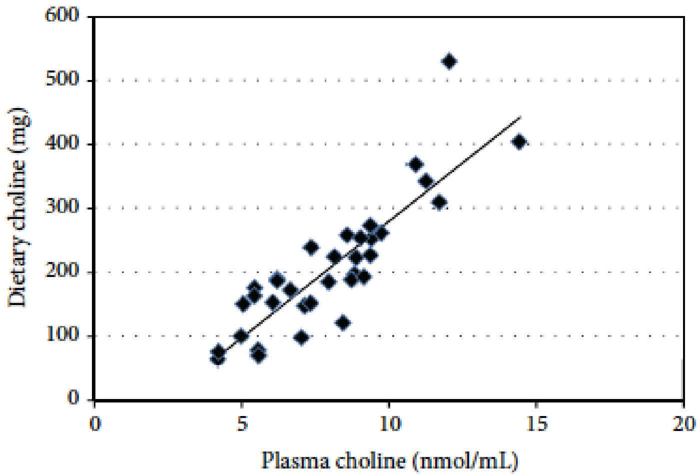


FIGURE 2: Correlation between dietary intake and plasma choline concentrations in children with ASD ($n = 35$). $r = 0.86$ and $P \leq 0.001$ using Pearson's product-moment correlation coefficient. ASD: autism spectrum disorder.

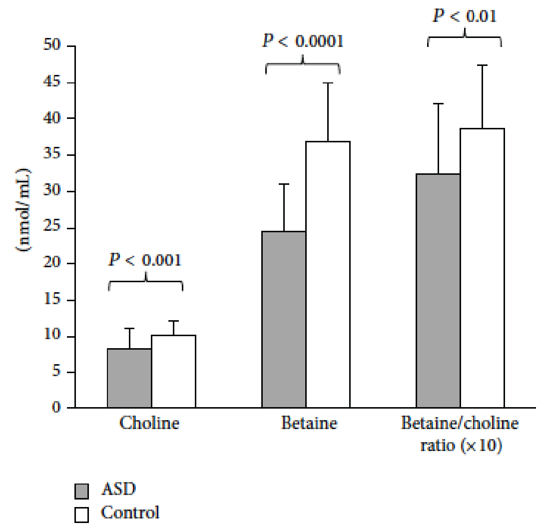


FIGURE 4: Plasma levels of choline, betaine, and the betaine/choline ratio in children with autism compared to age-matched controls.

Ho: Poor dietary choline consumption → low choline and betaine (TMG) in ASD → worsened oxidative stress.

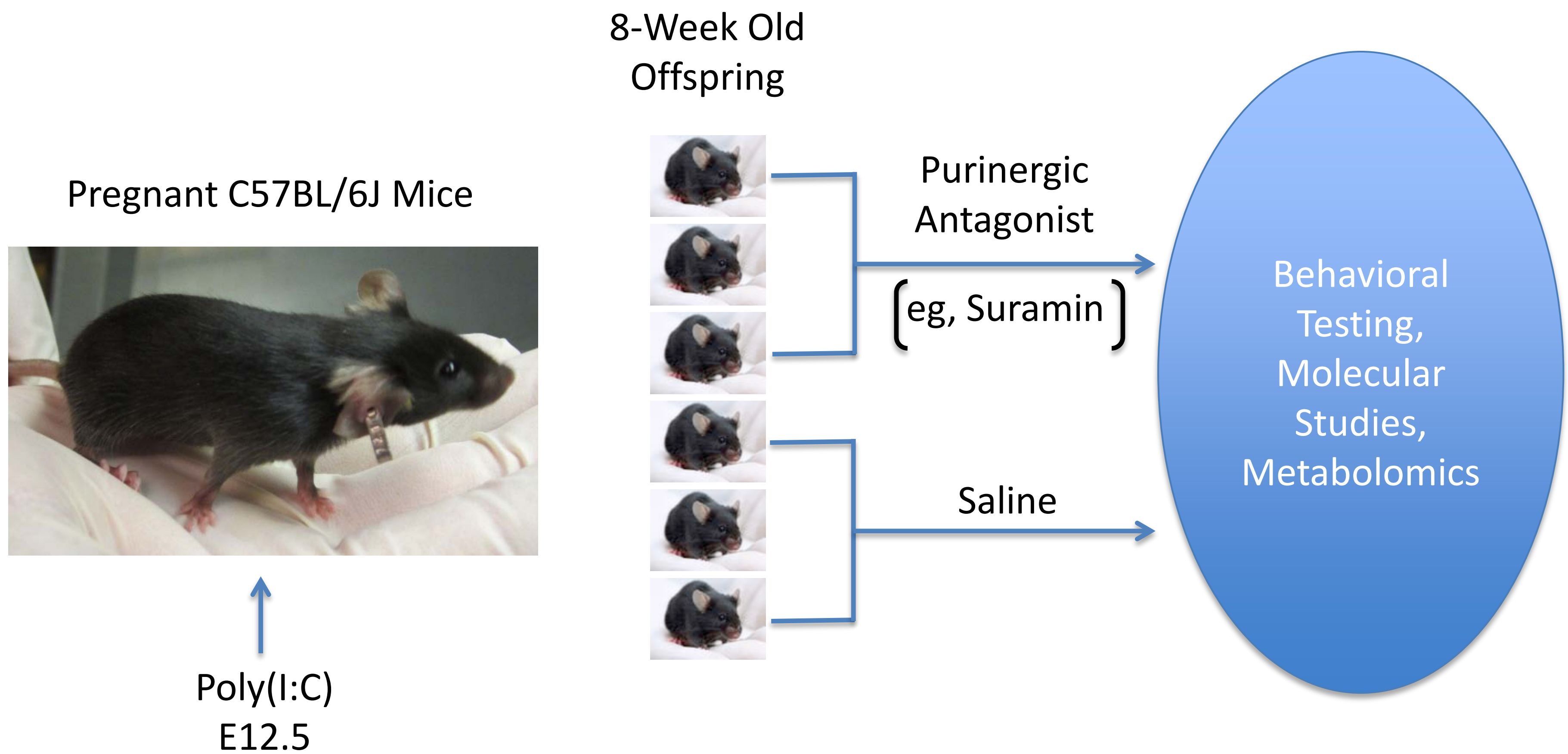
H1: The Cell Danger Response lowers choline and betaine to prevent DNA and RNA synthesis, increase the ratio of sphingo/phospholipid synthesis to stiffen membranes, and alters behavior and gut absorption to decrease dietary choline intake.

Treatment Implications

Ho: choline supplementation

H1: choline returns spontaneously after turning off the CDR

The Maternal Immune Activation (MIA) Model—
The Autism-Schizophrenia Spectrum



What About the Microbiome?

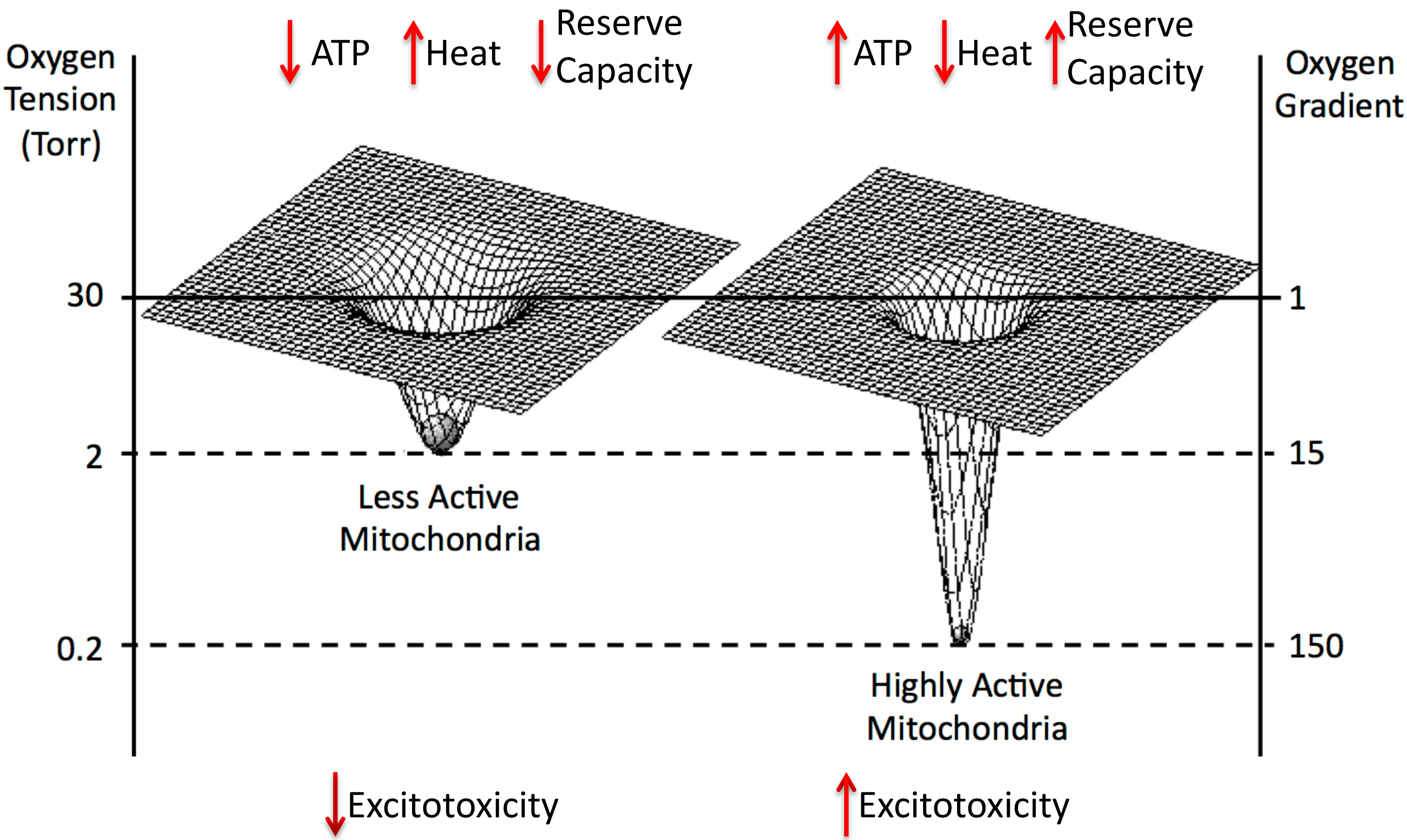
Why Does Metabolomics Work for Autism and Neurologic Disease?

"The brain controls metabolism."

(Through the autonomic nervous and endocrine systems.)

--All brain disorders produce a signature of abnormalities that can be detected in the blood and other biofluids.

The Work Capacity of the Cell is Set by the Thermodynamic Gradients Created by Mitochondrial Oxygen Consumption



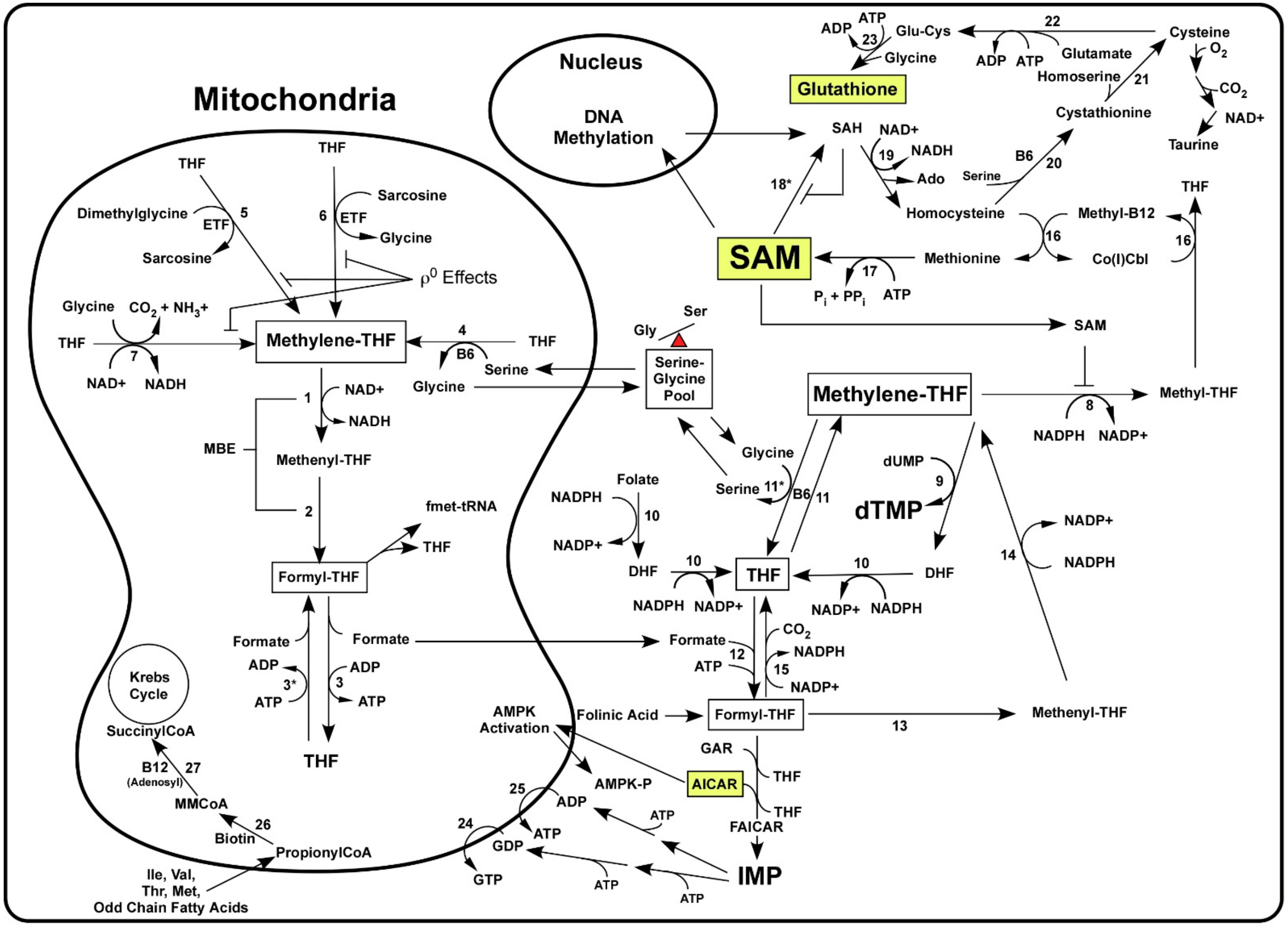
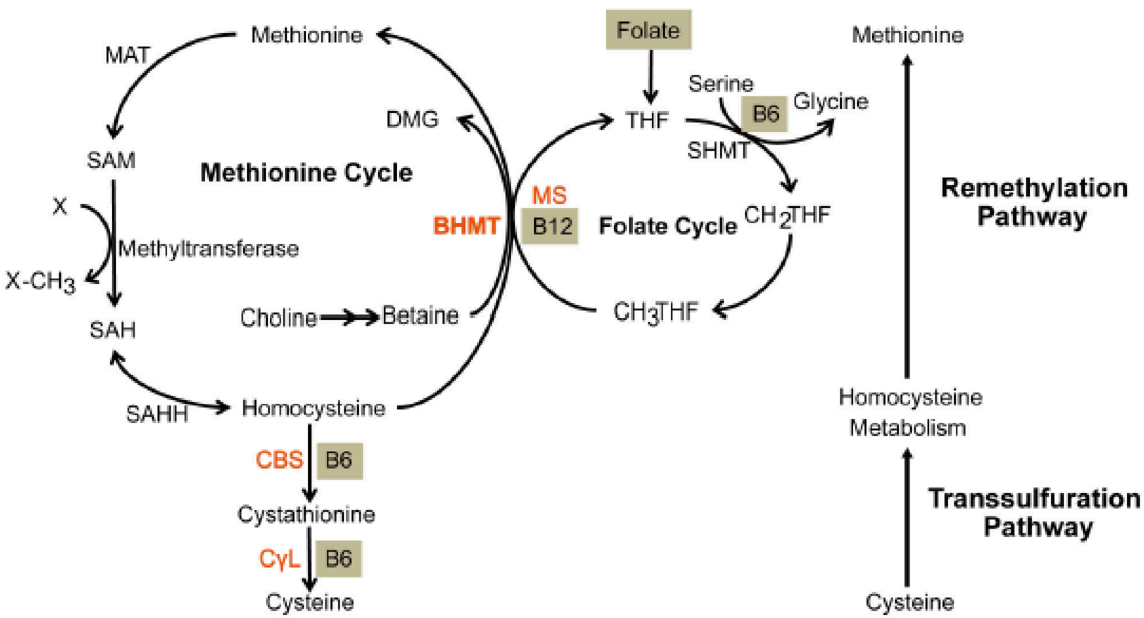
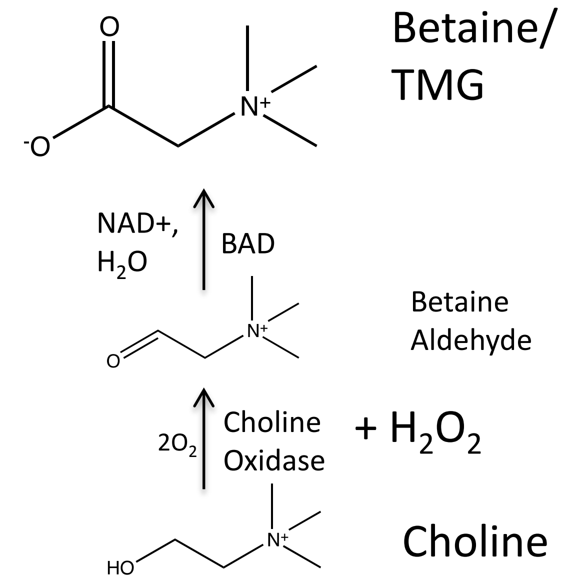
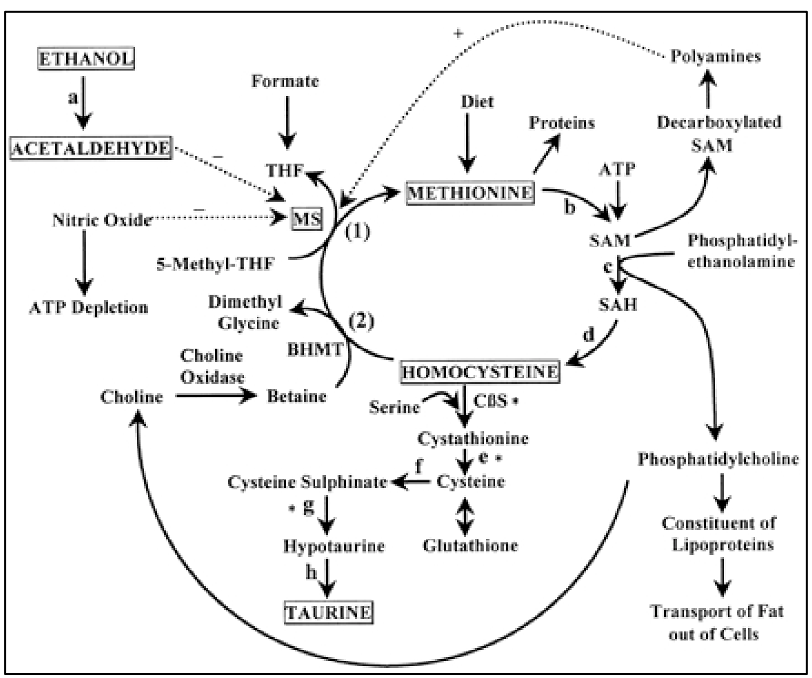


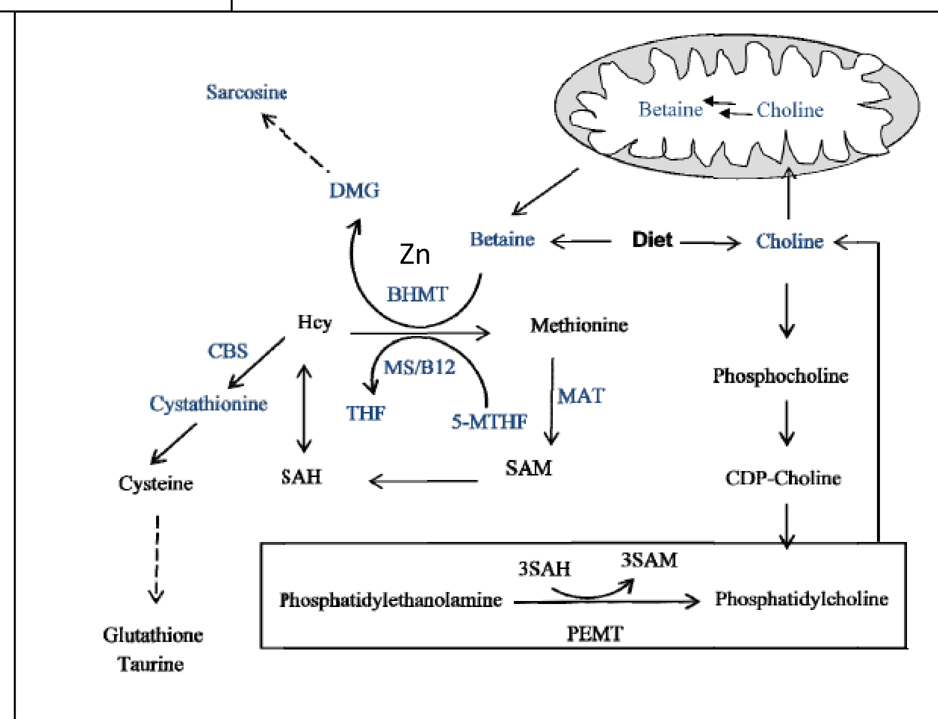
Figure 1. The Connection Between Mitochondrial Folate Metabolism and Nuclear DNA Methylation. In embryonic cells and cancer, MBE is expressed and one-carbon units are efficiently converted to Formyl-THF and formate for cytosolic nucleotide synthesis. Under these conditions, fewer one-carbon units are available for SAM synthesis and DNA methylation. When MBE is turned off in differentiated cells, less mitochondrial formate is produced and one-carbon units are directed through Methylene-THF toward increased SAM synthesis and increased DNA methylation. 1/2--Mitochondrial Bifunctional Enzyme (MBE): 1--NAD+ Dependent Methylene Tetrahydrofolate Reductase, 2--Methenyl-THF Cyclohydrolase. 3--Formyl-THF Synthase (FTS), 3*--FTS can reverse directions in differentiated cells when MBE is turned off, 4--Mitochondrial Serine Hydroxymethyl Transferase (mSHMT), 5--Dimethylglycine Dehydrogenase, ETF--Electron Transfer Flavoprotein, 6--Sarcosine Dehydrogenase, 7--Glycine Cleavage System, 8--Methylene-THF Reductase (MTFR). 9--Thymidylate Synthase, 10--Dihydrofolate Reductase (DHFR). 11--Cytosolic Serine Hydroxymethyl Transferase (cSHMT). 11*--cSHMT reverse reaction, 12/13/14--Cytosolic Trifunctional Enzyme: 12--Formyl-THF Synthase, 13--Methenyl-THF Cyclohydrolase, 14--NADPH-dependent Methylene-THF Dehydrogenase, 15--Formyl-THF Dehydrogenase, 16--Homocysteine Methyl Transferase (Methionine Synthase). 17--Methionine Adenosyl Transferase (MAT). 18*--Multiple DNA-, RNA-, Protein--, and Other Methyltransferase reactions in the nucleus, cytosol, and mitochondria. 19--S-Adenosyl Homocysteine Hydrolase (SAHH), 20--Cystathionine β-Synthase (CBS), 21--Cystathionase, 22--γ-Glutamylcysteine Synthase (GCS), 23--Glutathione Synthase, 24--Nucleoside Diphosphate Kinase, 25--ATP Synthase (Complex V), 26--Propionyl CoA Carboxylase, 27--Methylmalonyl CoA Mutase, GAR--Glycinamide Ribonucleotide, AICAR--Aminoimidazole Carboxamide Ribonucleotide, FAICAR--Formaminoimidazole Carboxamide Ribonucleotide, Ado--Adenosine.

Naviaux RK. *Cancer Biol Ther,* 2008. PMID 18719362


Methionine
MAT
SAM
Methionine Cycle
DMG
Folate
THF
Serine
B6
Glycine
SHMT
X
X-CH3
Methyltransferase
MS
BHMT
B12
Folate Cycle
CH2THF
SAH
Choline
Betaine
CH3THF
SAHH
Homocysteine
CBS
B6
Cystathionine
CγL
B6
Cysteine
Methionine
Remethylation Pathway
Homocysteine Metabolism
Transsulfuration Pathway
Cysteine


Betaine/ TMG
NAD+, H2O
BAD
Betaine Aldehyde
2O2
Choline Oxidase
+ H2O2
Choline


ETHANOL
a
ACETALDEHYDE
Formate
THF
Diet
Proteins
Polyamines
Decarboxylated SAM
ATP
MS
METHIONINE
Nitric Oxide
(1)
b
SAM
5-Methyl-THF
Phosphatidyl-ethanolamine
c
ATP Depletion
Dimethyl Glycine
(2)
SAH
d
Choline Oxidase
BHMT
HOMOCYSTEINE
Choline
Betaine
Serine
CβS
Cystathionine
e
f
Cysteine Sulphinate
Cysteine
Phosphatidylcholine
g
Hypotaurine
Glutathione
Constituent of Lipoproteins
h
TAURINE
Transport of Fat out of Cells


Sarcosine
Betaine
Choline
DMG
Zn
Betaine
Diet
Choline
BHMT
Hcy
Methionine
CBS
MS/B12
Phosphocholine
Cystathionine
THF
5-MTHF
MAT
Cysteine
SAH
SAM
CDP-Choline
3SAH
3SAM
Phosphatidylethanolamine
Phosphatidylcholine
PEMT
Glutathione
Taurine