Autism & Seizures: Impairments in Metabolism, Mitochondria, Microbiome & Personalized Nutrient Interventions

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Faculty Financial Disclosure

Commercial Interest	Nature of Relevant Financial Relationship (Include all those that apply)	
	What was received	**For what role**
• Illiad Neurosciences, Inc.	• Nothing	• Scientific Advisory Board Member

After participating in this presentation, learners should be better able to:

- Understand the connection between nutrition and metabolic systems
- Understand the impact of the microbiome on metabolic systems
- Understand the influence of nutrition on brain function
- Understand the influence of nutrition on behavior

Disclaimer

Every attempted has been made to make this presentation as accurate as possible. The information is provided without any expressed or implied warranty. This presentation should not be substituted for medical advice. Some treatments in this lecture are considered off-label and are not FDA-approved.

The Etiology of Autism: More than Genetic Disorders

Estimated Prevalence of Genetic Abnormalities	
Cytogenetic Abnormalities	5%
Fragile X	5%
Rett Syndrome (Females only)	5% (~1% overall)
Chromosomal Microarray	10%
Total	21%

This leaves about 79%+ children with ASD without an identified genetic diagnosis.

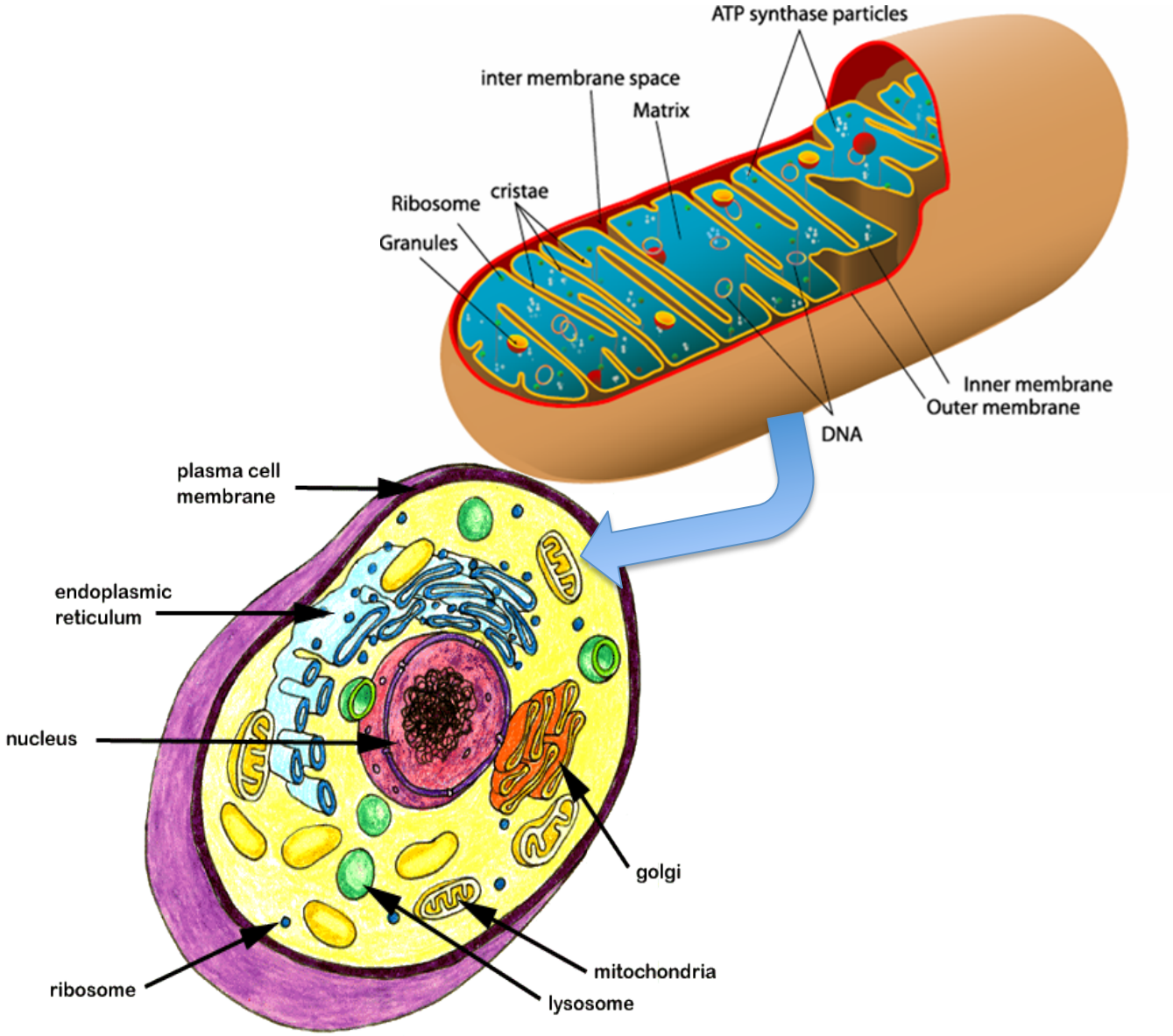
(Schaefer and Mendelson, Genetics in Medicine, 2013)

Metabolic Abnormalities Commonly Associated with Autism that are amenable to treatment

Mitochondrial Disorders
Mitochondrial Disease
Electron Transport Chain Overactivity
Unique Acyl-carnitine / Fatty Acid Oxidation Abnormalities
Redox Abnormalities
Decreased reduced Glutathione & Cysteine
Reduced Glutathione Peroxidase function
Increased oxidized Glutathione, DNA, Proteins and Lipids
Folate Abnormalities
Autoantibodies to Folate Receptor α
Mitochondrial Based Folate Receptor α Dysfunction

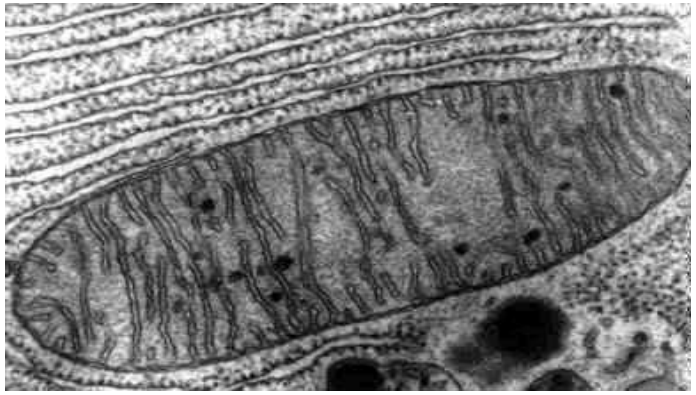
Mitochondrial Disorders and Treatments

Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures



Mitochondrial Disease

- Relatively new field
- First diseases described in 1988
  - Wallace, Leber's hereditary optic neuropathy, published in Science
  - Holt, Mitochondrial Myopathy, published in Nature
- Usually defined by extremely clinical symptoms with a progressive course
  - High energy dependent tissues
  - Neurological Disease
  - Gastrointestinal Disease
  - Immune Dysfunction
- Not just powerhouse, involved in
  - programmed (apoptotic) cell death
  - Oxygen Radical Regulation



Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

DA Rossignol[1] and RE Frye[2] Mol Psych 2012, 17:290-314

	Studies	*Total N*	*Overall prevalence*
General ASD population			
Mitochondrial disease in ASD	3	536	5.0% (3.2%, 6.9%)
Elevated lactate	6	479	31.1% (27.0%, 35.3%)
Elevated pyruvate	2	110	13.6% (7.2%, 20.1%)
Elevated lactate/pyruvate ratio	1	192	27.6% (21.2%, 33.9%)
Elevated alanine	1	36	8.3% (0.0%, 20.1%)
Low total carnitine	1	30	90.0% (81.0%, 99.0%)
Elevated creatine kinase	1	47	46.8% (32.4%, 61.2%)
Elevated ammonia	1	80	35.0% (24.5%, 45.5%)
Elevated AST	1	147	45.6% (37.5%, 53.7%)[a]
Elevated ALT	1	87	7.0% (0.5%, 13.5%)

Discrepancy between prevalence of diagnosed mitochondrial disease and prevalence of biomarkers of mitochondrial disease likely be due to criteria used to define mitochondrial disease

Biomarker	*Number of studies*	*ASD*		*Control*		*F-value*	*Hedge's g (CI)*
		Total N	*Mean (95% CI)*	*Total N*	*Mean (95% CI)*		
Lactate ($mM\,l^{-1}$)	5	114	1.73 (1.61, 1.88)	114	0.91 (0.87, 0.96)	8.72†	1.42 (0.92, 1.92)†
Pyruvate ($nM\,l^{-1}$)	1	24	0.12 (0.11, 0.14)	24	0.06 (0.06, 0.06)	20.25†	1.96 (0.85, 3.08)†
Carnitine ($mg\,ml^{-1}$)	1	30	3.83 (3.44, 4.31)	30	6.40 (6.22, 6.62)	4.61†	2.51 (1.61, 3.42)†
Ubiquinone	1	15	91.4 (81.9, 103.0)	15	144.2 (130.4,161.1)	2.13	1.90 (0.79, 3.01)†

Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

DA Rossignol[1] and RE Frye[2] Mol Psych 2012, 17:290-314

ASD children with mitochondrial disease have more medical abnormalities than idiopathic ASD children

Only 23% of ASD children with mitochondrial disease have mitochondrial DNA abnormalities

	ASD/MD		*General ASD*			*General MD*		
	%	N	%	χ^2	P	%	χ^2	P
Male	61	72	81	18.7	<0.0001	58	0.26	0.61
Developmental regression	52	83	25	32.3	<0.0001	60	2.2	0.14
Seizures	41	86	11	79.1	<0.0001	33	2.48	0.11
Hypotonia	62	55	51	2.6	0.10	67	0.62	0.43
Fatigue/lethargy	54	61				19	48.6	<0.0001
Ataxia	58	19				13	34.0	<0.0001
Growth delay	21	73						
Motor delay	51	79	9	170.1	<0.0001			
GI abnormalities	74	35	20	63.8	<0.0001	39	18.0	<0.0001
Cardiomyopathy	24	38				26	0.1	0.79
Myopathy	0	12				11	1.5	0.22
Elevated lactate	78	50	31	51.6	<0.0001	54	12.4	<0.001
Elevated pyruvate	45	22	14	17.6	<0.0001			
Elevated lactate/pyruvate ratio	43	23	28	2.6	0.11			
Abnormal organic acids	36	36						
Elevated creatine kinase	34	29	47	1.96	0.16			
Elevated alanine	32	28						
Abnormal brain imaging	23	69				70	72.6	<0.0001
Normal ETC activity	16	69				3	40.1	<0.0001
Abnormal complex I	53	96				45	2.48	0.12
Abnormal complex II	9	65				8	0.09	0.76
Abnormal complex III	30	96				31	0.04	0.83
Abnormal complex IV	20	97				34	8.47	0.004
Abnormal complex V	23	44				12	5.0	0.03
Multiple complex deficiency	36	59				27	2.43	0.12
Elevated citrate synthase	24	17				44	2.76	0.10
Abnormal light microscopy	18	49				81	126.4	<0.0001
mtDNA abnormality	23	87				16	3.17	0.08

Mitochondrial Dysfunction in Autism

Cecilia Giulivi, PhD
Yi-Fan Zhang, BS
Alicja Omanska-Klusek, MS
Catherine Ross-Inta, BS
Sarah Wong, BS
Irva Hertz-Picciotto, PhD
Flora Tassone, PhD
Isaac N. Pessah, PhD

JAMA, December 1, 2010—Vol 304, No. 21 **2389**

- Lymphocytes from 10 children with autism and 10 age and gender matched controls
- 80% demonstrated abnormal function in at least one electron transport chain complex
  - 60% complex I abnormality
  - 40% complex V abnormality
  - 50% multiple complexes
- 20% demonstrated abnormalities in cytB, a mitochondrial DNA gene

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PLOS ONE

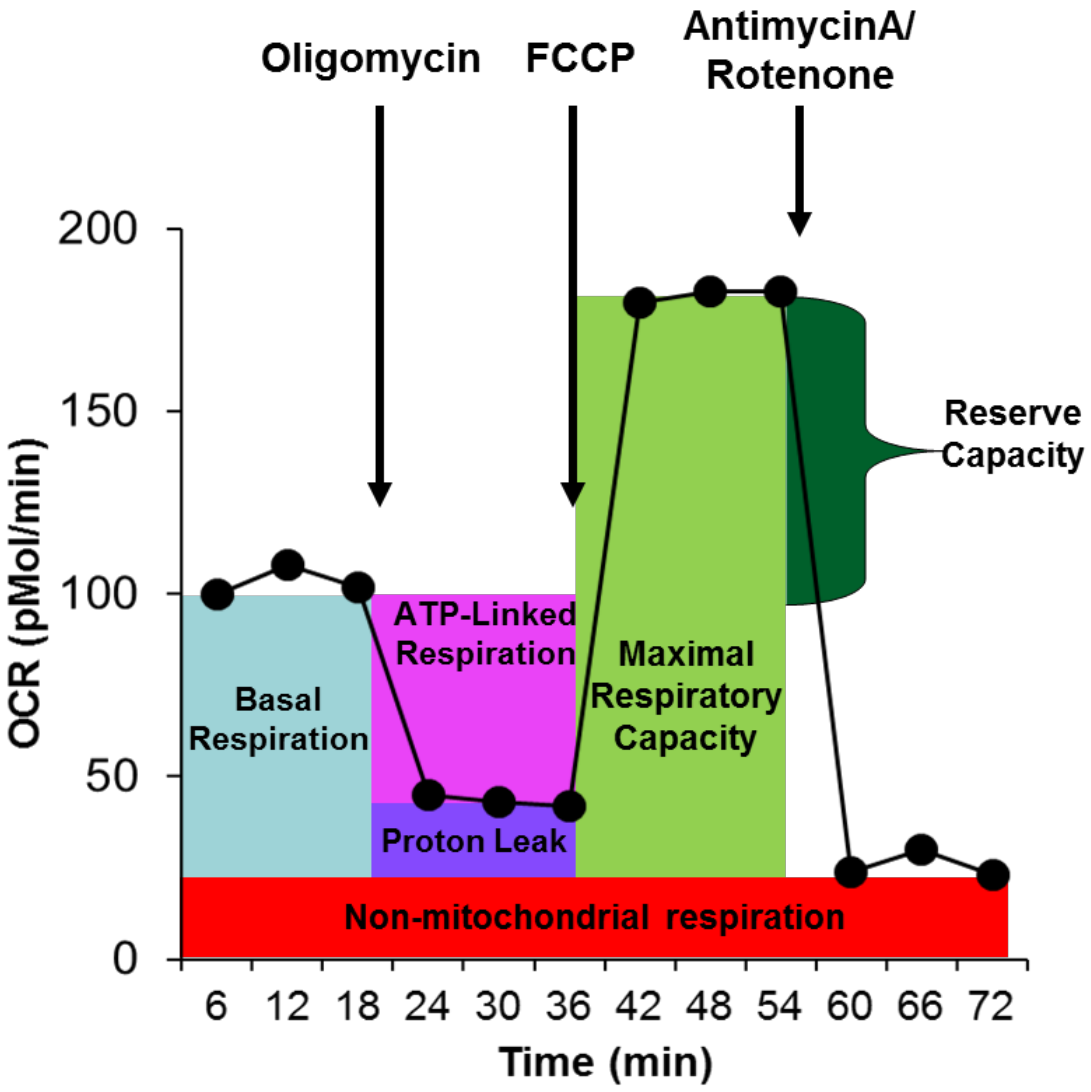
Oxidative Stress Induces Mitochondrial Dysfunction in a Subset of Autism Lymphoblastoid Cell Lines in a Well-Matched Case Control Cohort

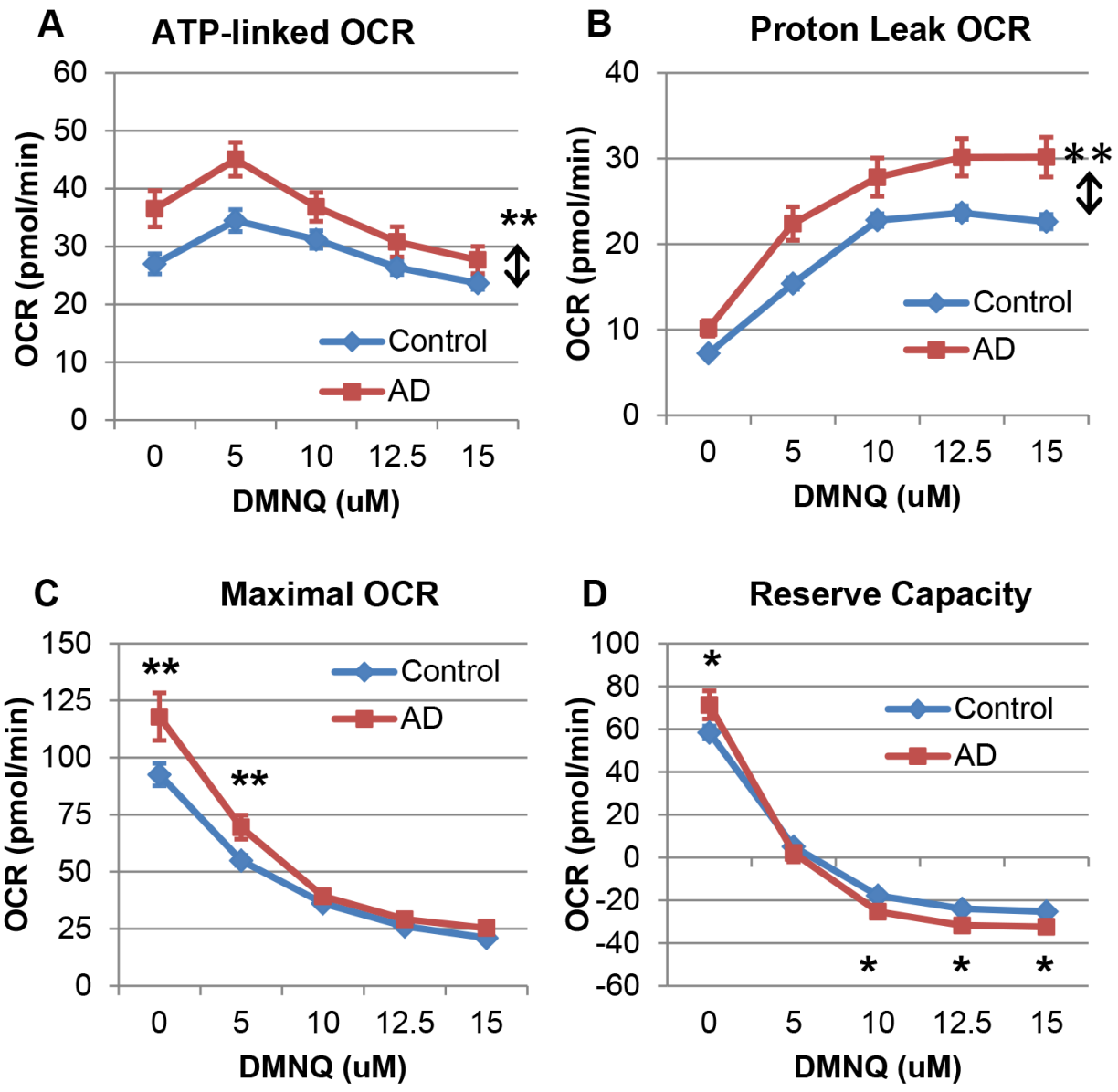
Shannon Rose, Richard E. Frye*, John Slattery, Rebecca Wynne, Marie Tippett, Oleksandra Pavliv, Stepan Melnyk, S. Jill James

- 25 pairs of Lymphoblastoid Cell Lines (LCLs)
  - Derived from children with autism
  - Derived from unaffected control children
  - Age and Gender Matched
- Mito Stress Test on Seahorse
  - 1hr DMNQ exposure (0-15µM)
  - 48hr N-acetyl cysteine (NAC) pretreatment of the autism LCLs (1mM)

Seahorse Bioscience Extracellular Flux Analyzer

- Poly D lysine coated plates
- 110k cells/well
- Plated 1hr prior to assay
- Seahorse DMEM
  - 11mM glucose
  - 2mM glutamax
  - 1mM pyruvate
- DMNQ added directly to cells in plate
- Each plate with an AD/ Control LCL pair


Oligomycin
FCCP
AntimycinA/
Rotenone
200
150
100
50
0
OCR (pMol/min)
Reserve
Capacity
ATP-Linked
Respiration
Basal
Respiration
Maximal
Respiratory
Capacity
Proton Leak
Non-mitochondrial respiration
6
12
18
24
30
36
42
48
54
60
66
72
Time (min)


A
ATP-linked OCR
OCR (pmol/min)
60
50
40
30
20
10
0
**
Control
AD
0
5
10
12.5
15
DMNQ (uM)
B
Proton Leak OCR
OCR (pmol/min)
40
30
20
10
0
**
Control
AD
0
5
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12.5
15
DMNQ (uM)
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Maximal OCR
OCR (pmol/min)
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50
25
0
**
**
Control
AD
0
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10
12.5
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DMNQ (uM)
D
Reserve Capacity
OCR (pmol/min)
100
80
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40
20
0
-20
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Control
AD
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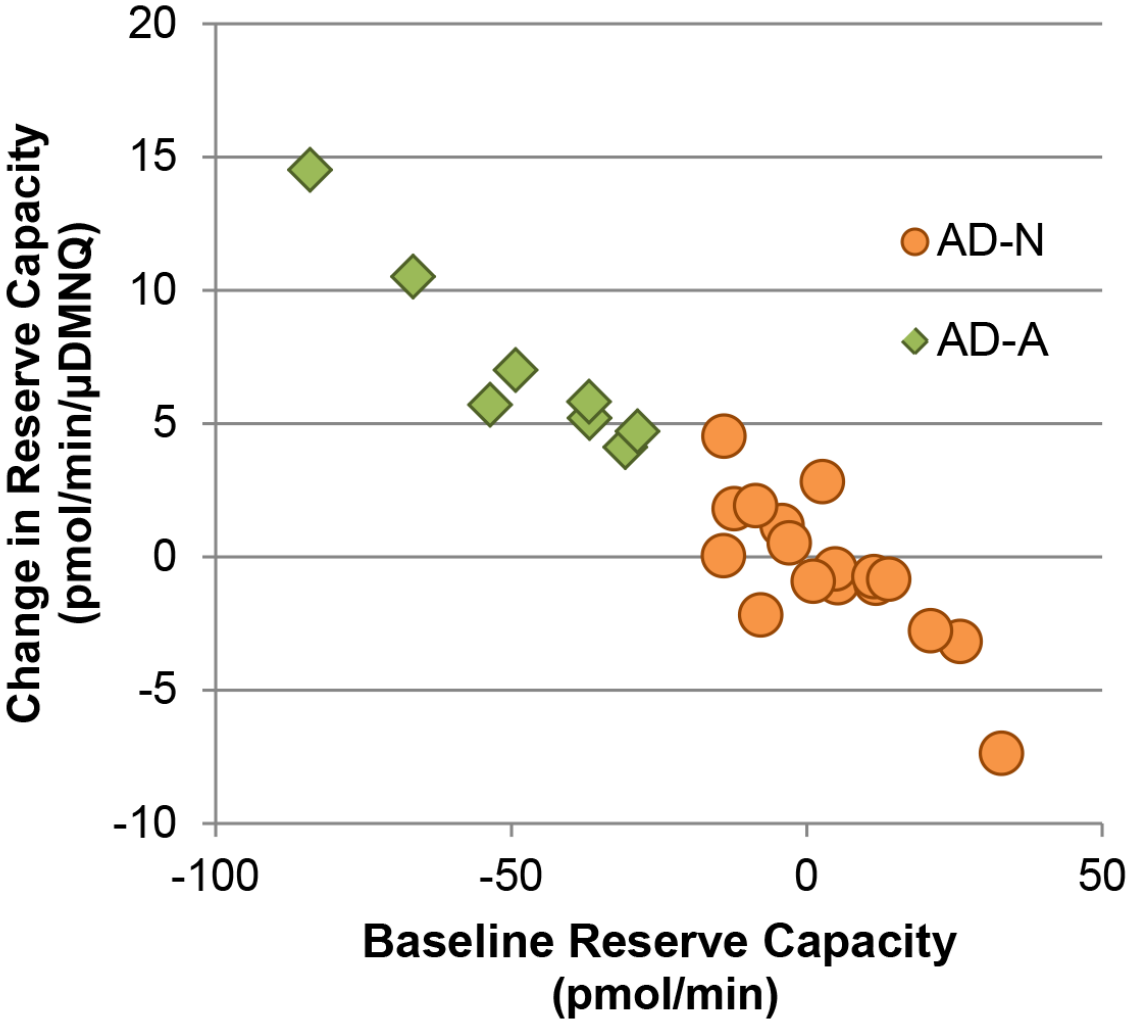
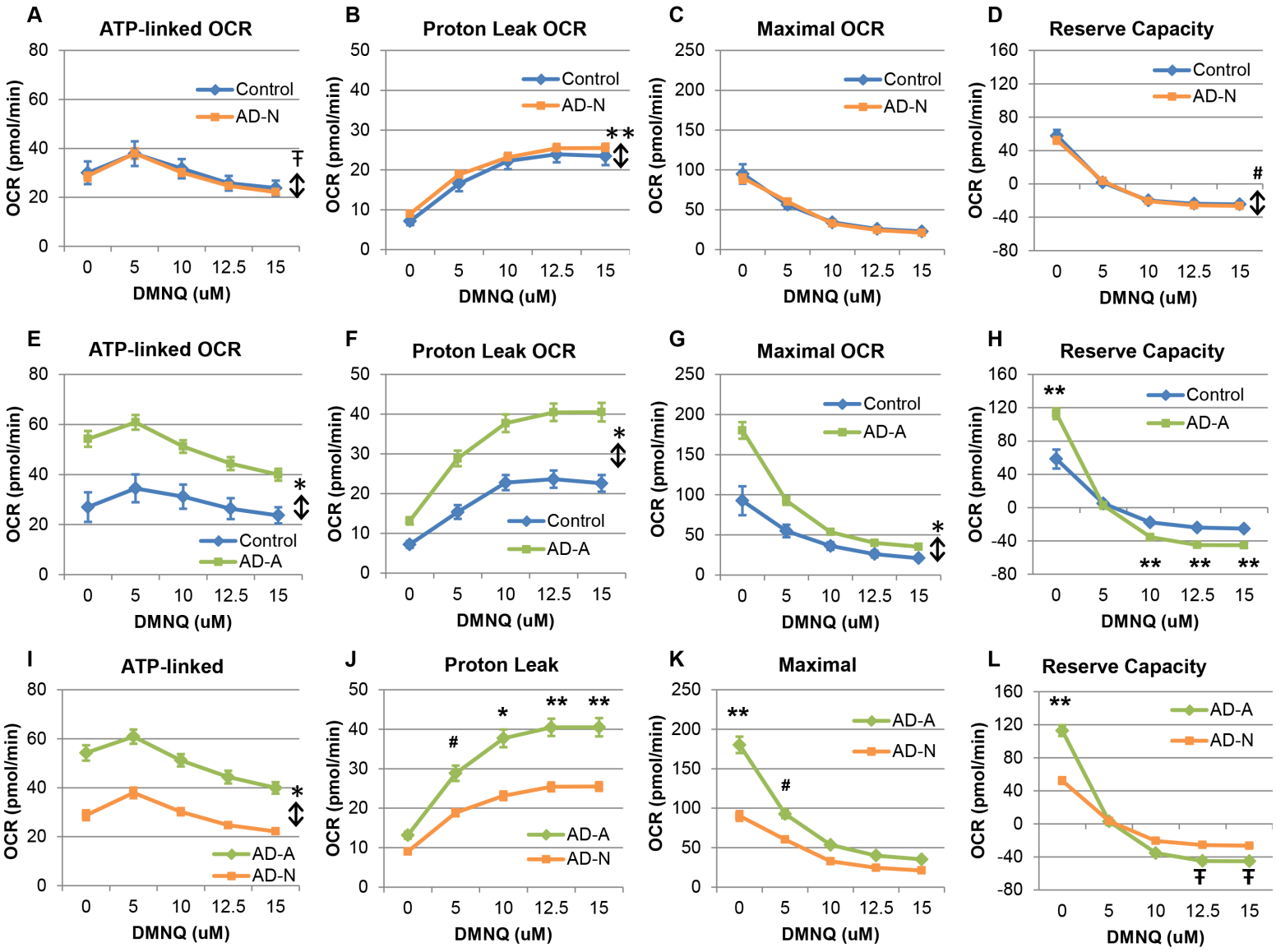
12.5
15
DMNQ (uM)



Cluster analysis reveals 2 significantly different subgroups.

- AD-N (n=17)
- AD-A (n=8)

Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures



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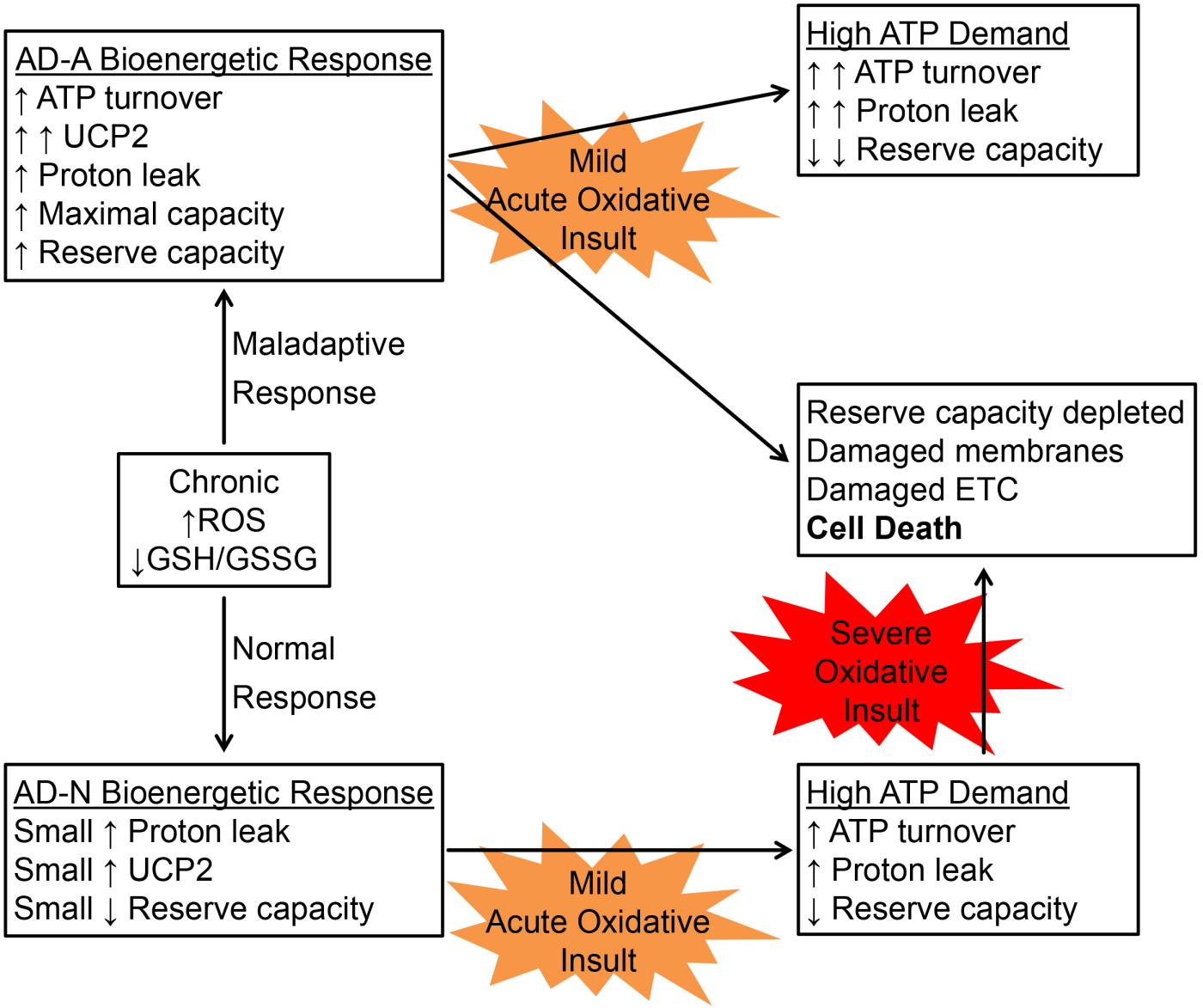

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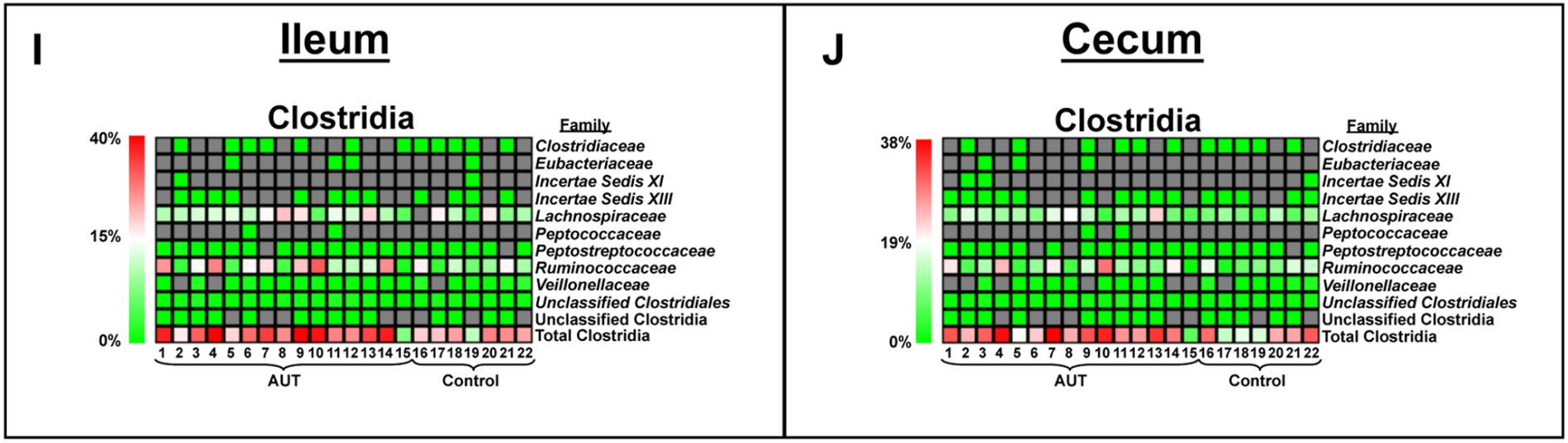
PLoS one

Impaired Carbohydrate Digestion and Transport and Mucosal Dysbiosis in the Intestines of Children with Autism and Gastrointestinal Disturbances

Brent L. Williams[1], Mady Hornig[1], Timothy Buie[2], Margaret L. Bauman[3], Myunghee Cho Paik[4], Ivan Wick[1], Ashlee Bennett[1], Omar Jabado[1], David L. Hirschberg[1], W. Ian Lipkin[1]*

1 Center for Infection and Immunity, Columbia University, New York, New York, United States of America, 2 Division of Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital, Boston, Massachusetts, United States of America, 3 Department of Neurology, Harvard Medical School and Departments of Neurology and Pediatrics and Learning and Developmental Disabilities Evaluation and Rehabilitation Services (LADDERS), Massachusetts General Hospital, Boston, Massachusetts, United States of America, 4 Department of Biostatistics, Columbia University, Mailman School of Public Health, New York, New York, United States of America



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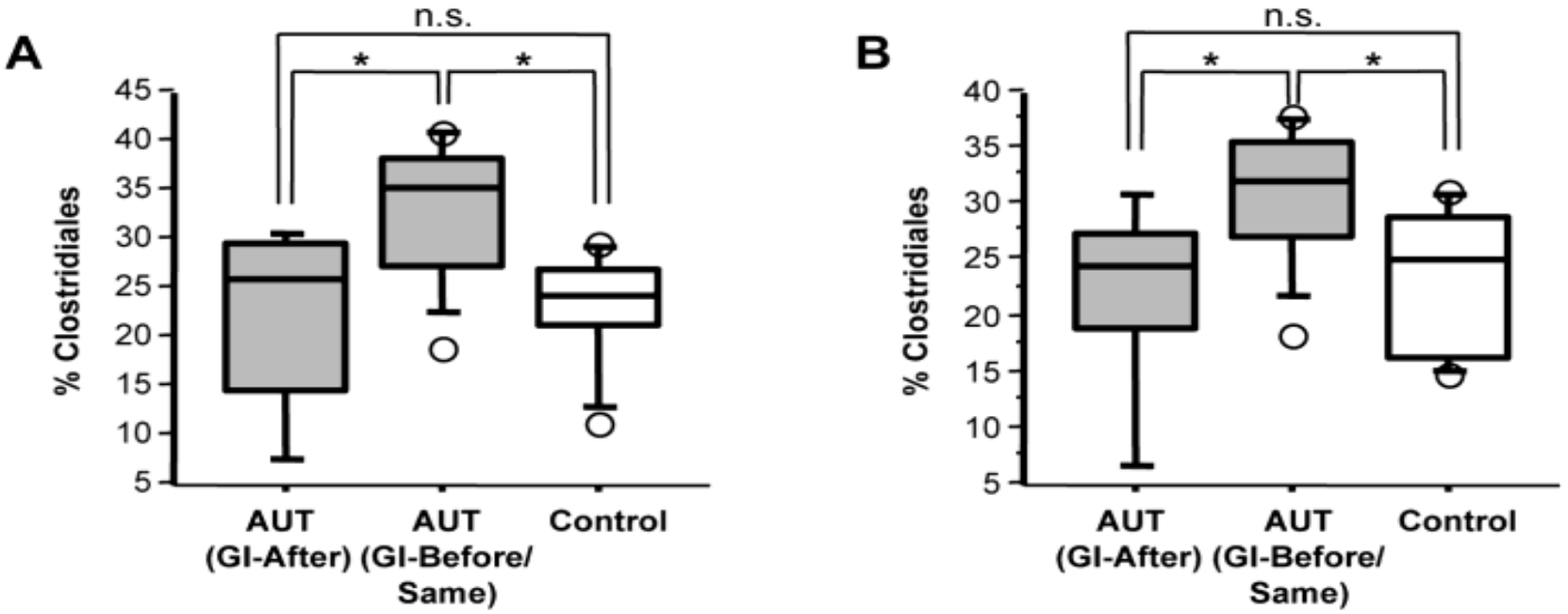
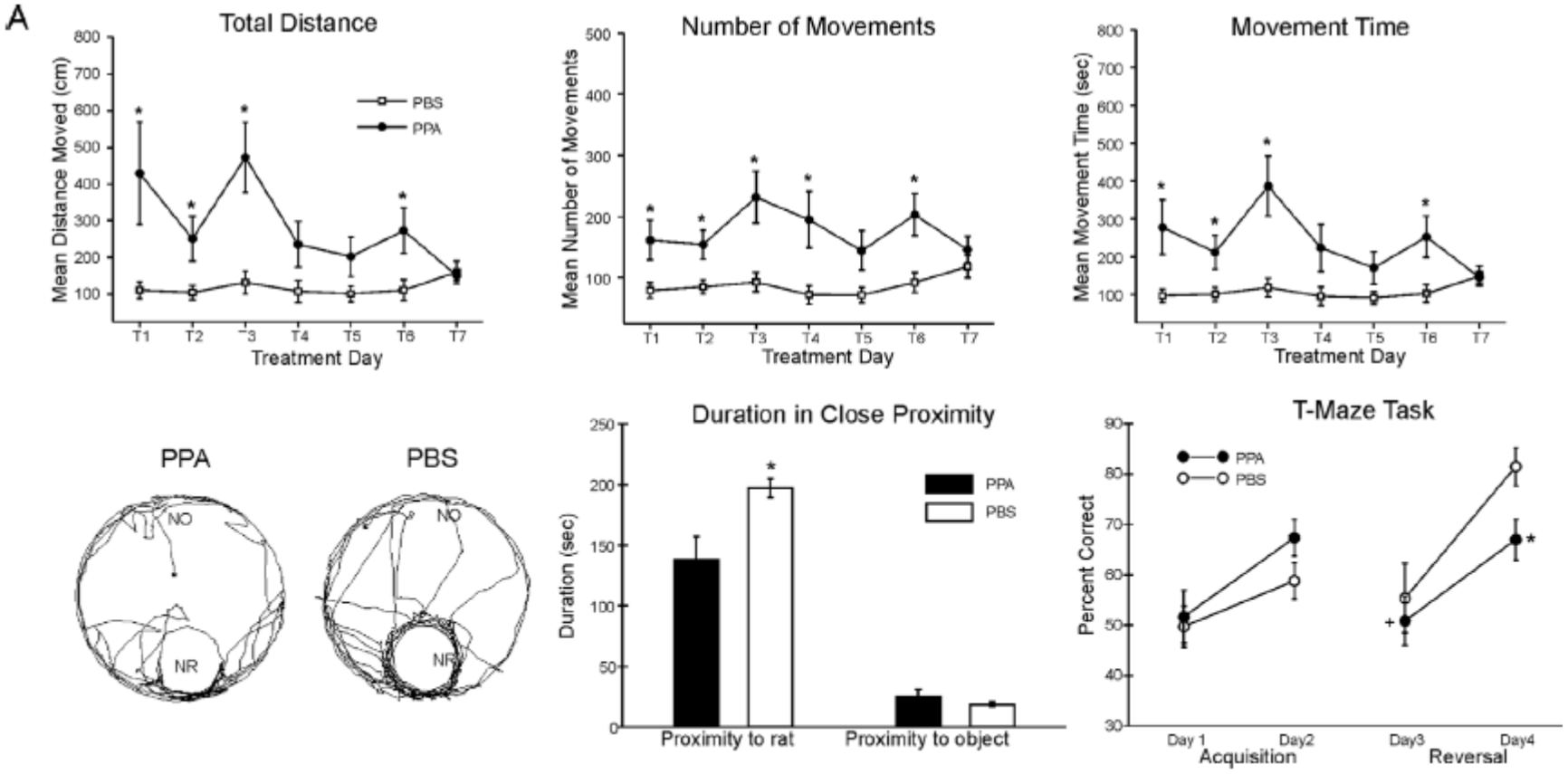


GI-After = GI Symptoms started after the onset of Autism symptoms

GI-Before/Same = GI Symptoms started before or at the same time as Autism symptoms

Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders

Derrick F. MacFabe, MD*



North American Journal of Medicine and Science Jul 2012 Vol 5 No.3

Original Research

Biomarkers of Abnormal Energy Metabolism in Children with Autism Spectrum Disorder

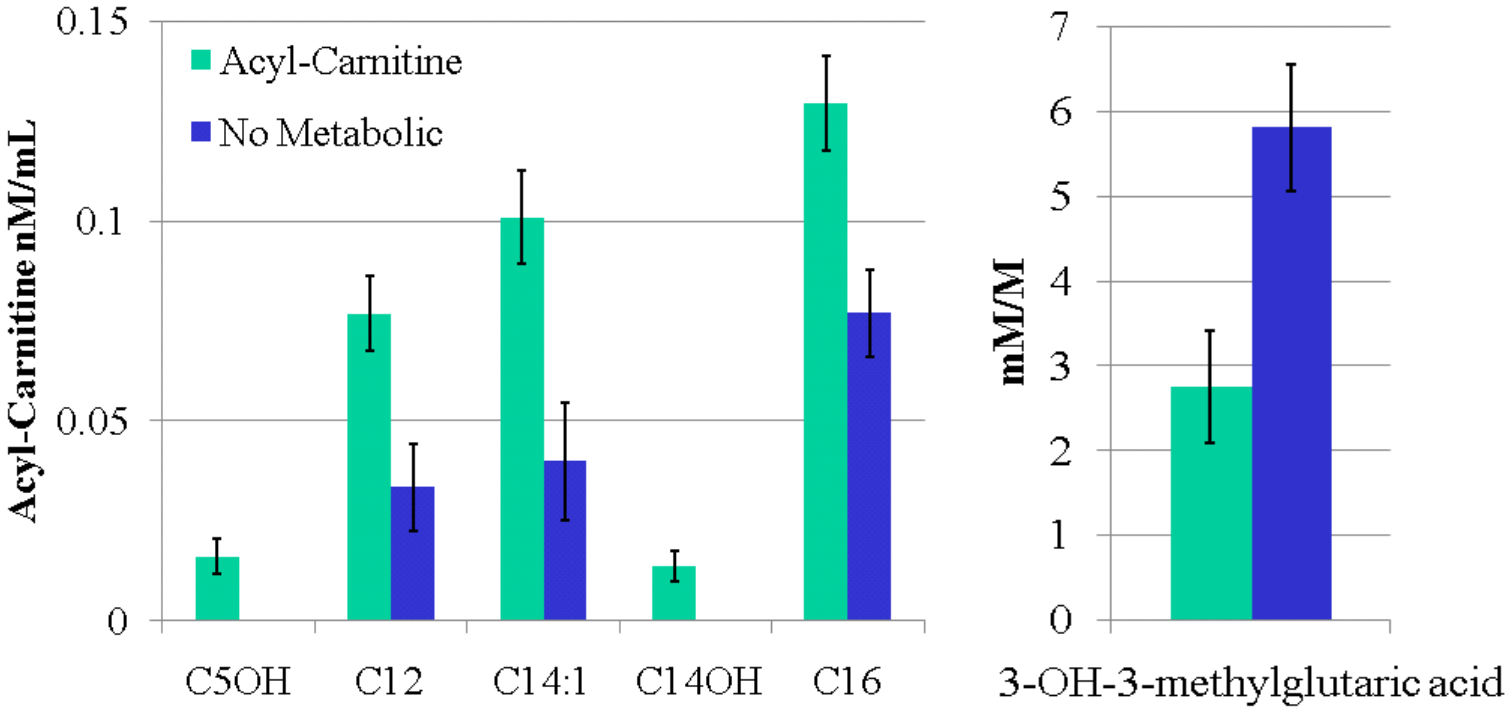
Richard E. Frye, MD, PhD*

Division of Autism Research, Department of Pediatrics Arkansas Children's Hospital Research Institute, Little Rock, AR

Biomarker	Total Tested	Abnormal at Least Once	Patients with Abnormalities Tested Twice	Abnormal Twice	Prevalence
Lactate	96	34 (35%)	20 (59%)	9 (45%)	15.9%
Alanine	94	8 (9%)	5 (63%)	1 (20%)	1.7%
AST	113	20 (18%)	14 (70%)	8 (57%)	10.1%
CK	81	11 (14%)	4 (36%)	2 (50%)	6.8%
Alanine-to-Lysine Ratio	98	39 (40%)	20 (51%)	8 (40%)	15.9%
Acyl-carnitine	58	23 (40%)	10 (44%)	6 (60%)	23.8%

Acyl-Carnitine Group Had Rate of Regression of 67% VERY HIGH

	Regression	Epilepsy
Lactate (n=9)	2 (22%)	3 (33%)
AST (n=8)	3 (38%)	1 (13%)
Alanine-to-Lysine Ratio (n=8)	2 (25%)	6 (75%)
Acyl-carnitine (n=6)	4 (67%)	1 (17%)
ASD Control (n=9)	5 (55%)	3 (33%)



3-hydroxy-3-methylglutaryl is a metabolite of Acetyl-CoA, the starting point of the citric acid cycle. Suggests that the citric acid cycle is working inefficiently.

Citation: Transl Psychiatry (2013) 3, e220; doi:10.1038/tp.2012.143

www.nature.com/tp

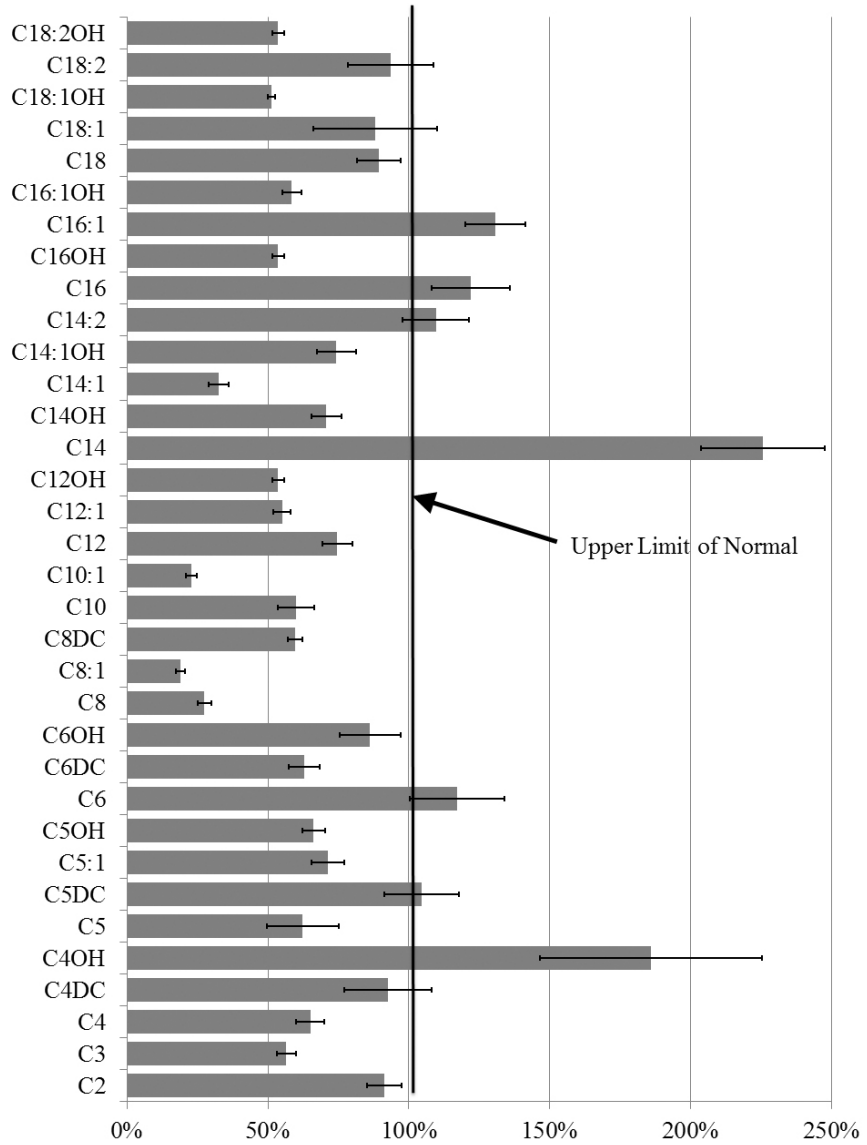
Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder

RE Frye[1], S Melnyk[1] and DF MacFabe[2]

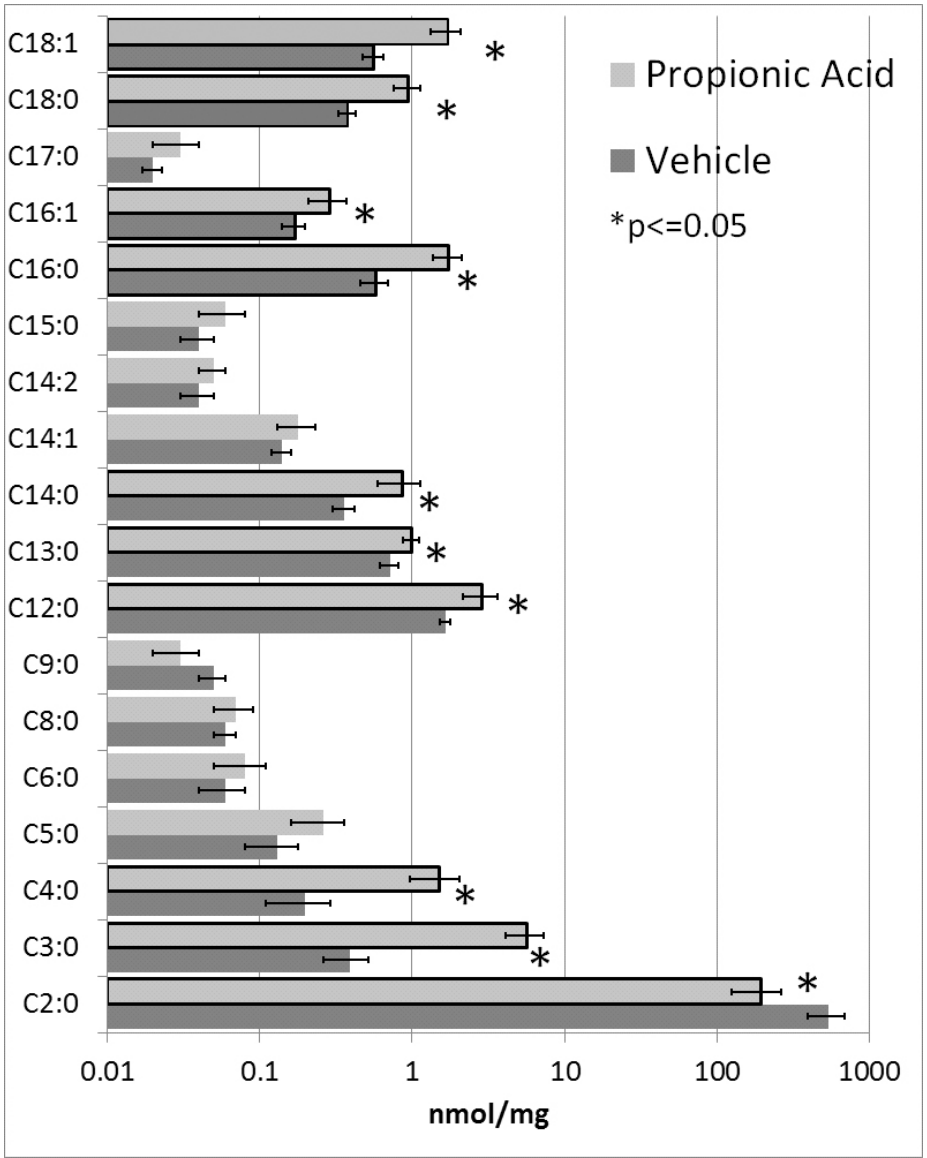
- 213 ASD patients screened with acyl-carnitine biomarkers
- 74 (35%) with >=3 fasting acyl-carnitine elevations
- Acyl-carnitine abnormalities were confirmed in 48%
- Corrected prevalence of 17% of ASD children screened.

Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures

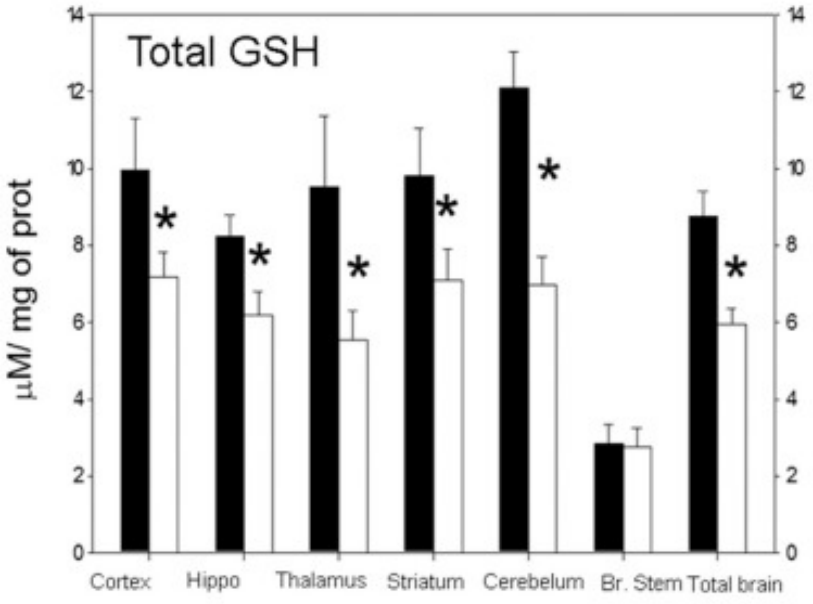
Autistic Children



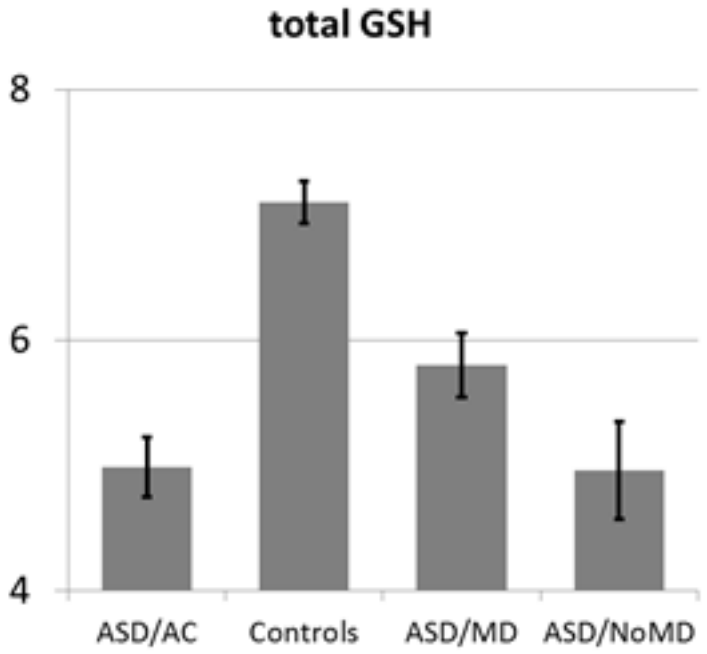
Rodents



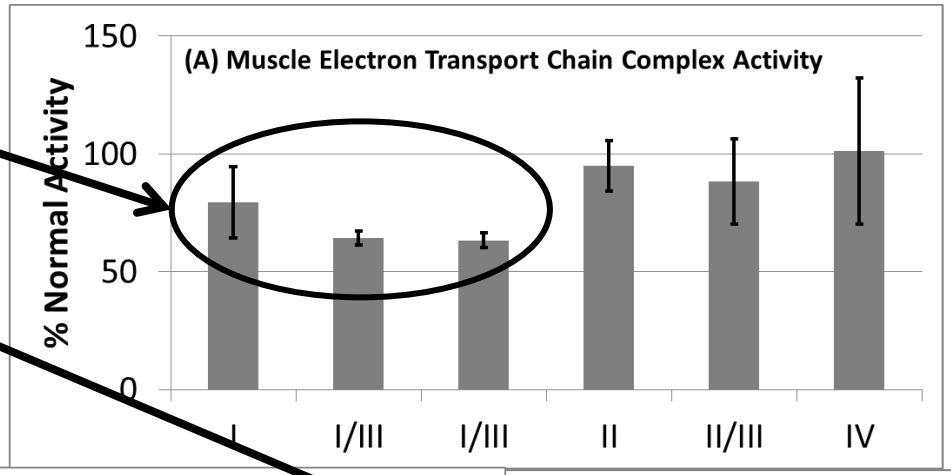
Rodents

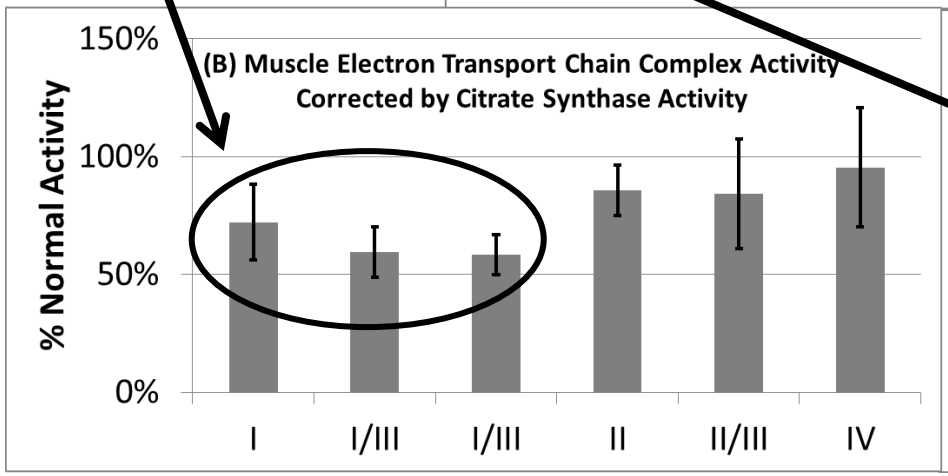


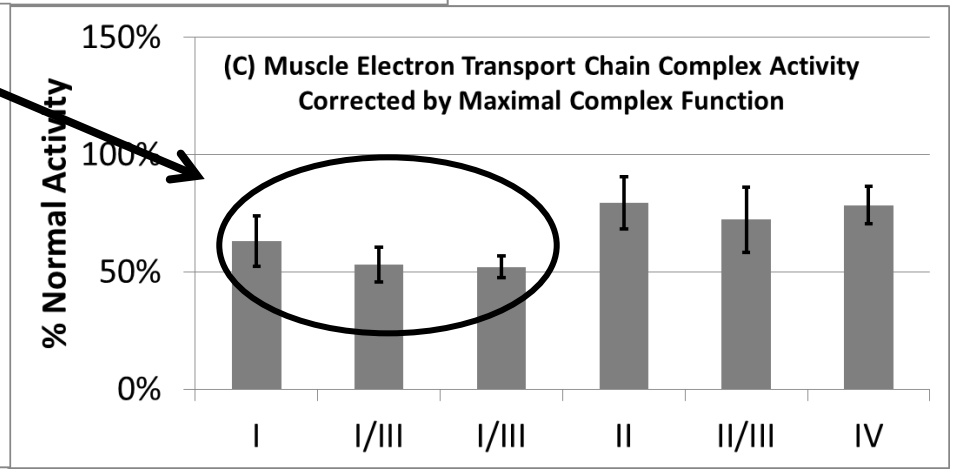
ASD Children

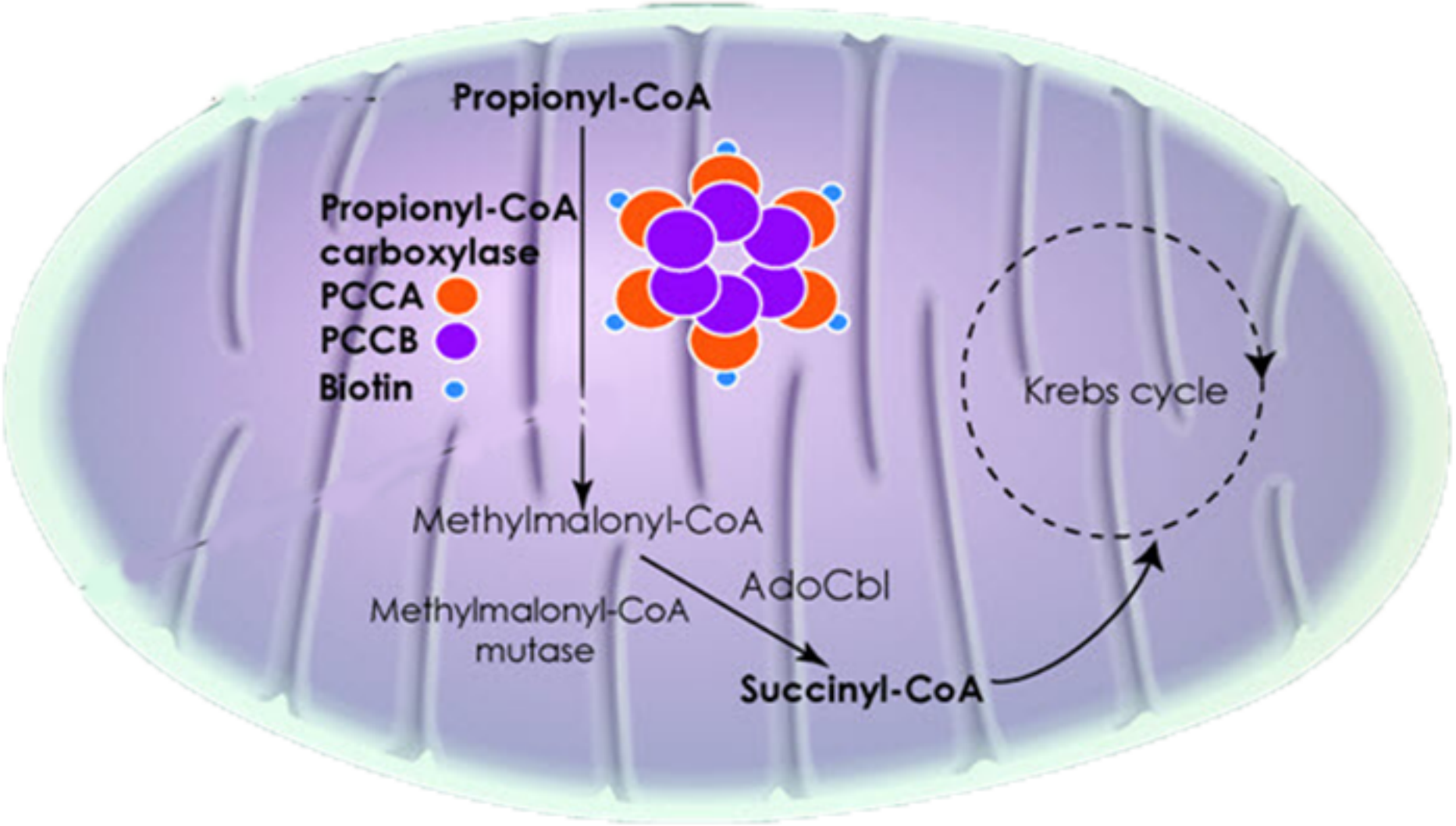


Decreased Complex I Activity








Propionyl-CoA
Propionyl-CoA carboxylase
PCCA
PCCB
Biotin
Krebs cycle
Methylmalonyl-CoA
AdoCbl
Methylmalonyl-CoA mutase
Succinyl-CoA

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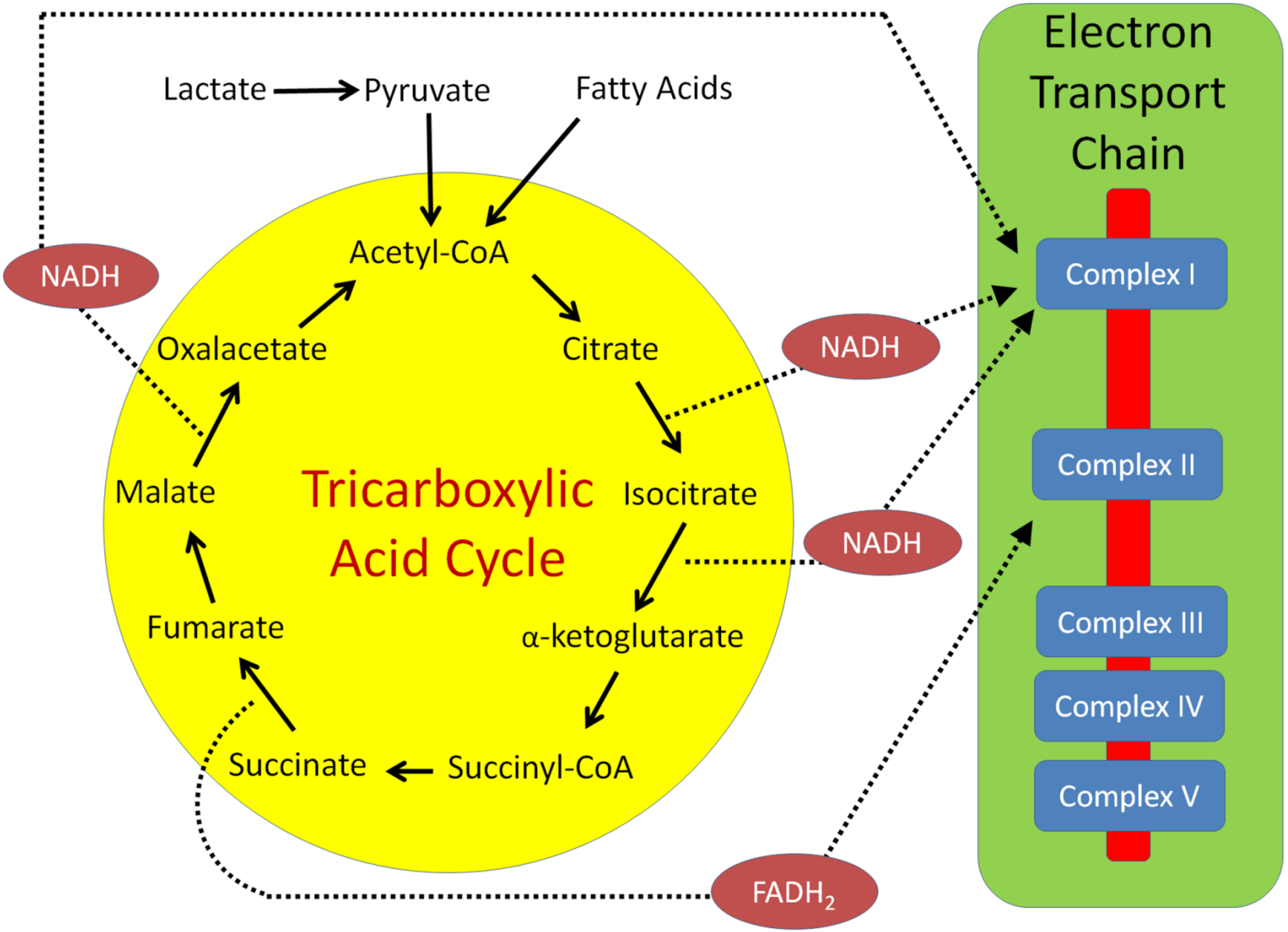

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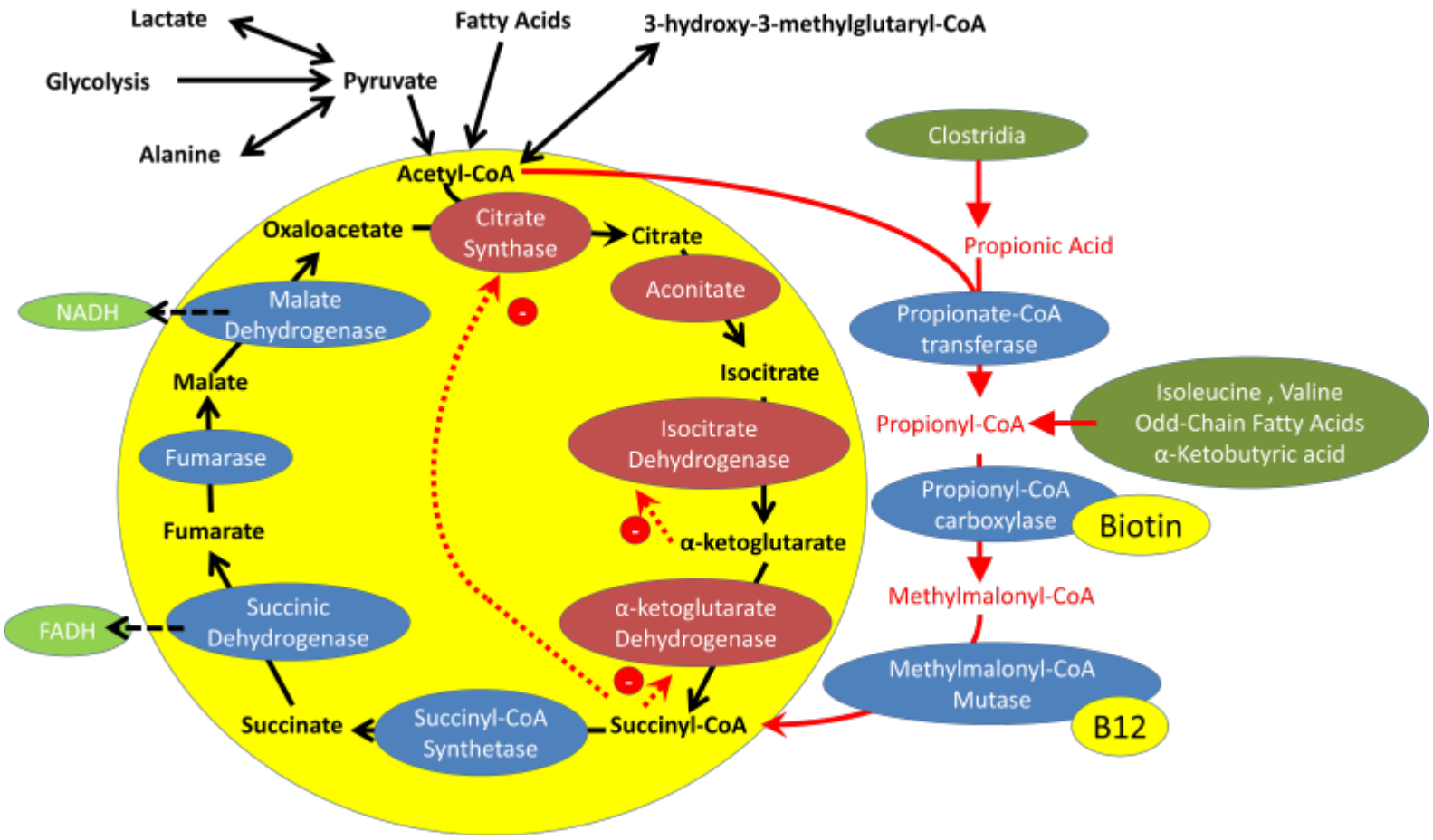


Table 3. Agents commonly used to treat PMD and SMD

Vitamin	Dose	Adverse effects	Function
Electron transport chain support			
CoQ10 (reduced): ubiquinol	5–30 mg/kg/day, 1–2×/day	appetite loss, nausea, diarrhea at high doses	energy carrier between complex I and III, and complex II and III
CoQ10 (oxidized): ubiquinone	10–30 mg/kg/day, 1–2×/day		
Electron carrier support			
Niacin (B_3)	50–100 mg given daily	flushing reaction	nicotinamide adenine dinucleotide (NAD) precursor
Riboflavin (B_2)	100–400 mg given daily	nausea at high doses	flavin adenine dinucleotide (FAD) precursor
Energy Storage			
Creatine monohydrate	100 mg/kg/day; 1–2×/day	increased urination	high-energy phosphate buffer precursor to phosphocreatine
Fatty acid oxidation support			
L-carnitine or acetyl-L-carnitine	30–120 mg/kg/day, 1–2×/day	stool loose/fishy smell	carrier of long-chain fatty acids
Biotin (B_7)	5–10 mg/day given daily	none	cofactor for carboxylase enzymes
Mitochondrial enzyme cofactors			
Thiamine (B_1)	50–100 mg given daily	none	cofactor for citric acid cycle enzymes
Pantothenic acid (B_5)	5–1,200 mg/day, 1–3×/day	diarrhea at high doses	precursor to coenzyme A
Pyridoxine (B_6)	200 mg given daily	headache, paresthesia, nausea, headache at high doses	cofactor for over 100 enzymes
Biotin (B_7)	as above	none	cofactor for carboxylase enzymes
Alpha-lipoic acid	50–200 mg given daily	headache, paresthesia, rash, muscle cramps	cofactor for citric acid cycle enzymes
Antioxidants			
CoQ10	as above	as above	targets ETC oxidative stress
L-carnitine	as above	as above	scavenger of organic acids
Vitamin E	200–400 IU given daily	bleeding at high doses	protects cell membranes
Vitamin C	100–500 mg given daily	diarrhea at high doses	protects iron and copper
Redox metabolism support			
Methylcobalamin (B_{12})	5–2,000 µg every 1–3 days	hyperactivity, sleep disruption	supports methylation and folate cycles, and glutathione production
Reduced folate (B_9)	folinic acid 400–800 µg/day	none	supports methylation and folate cycles
N-acetyl-L-cysteine (NAC)	10–70 mg/kg/day, 1–3×/day	diarrhea at high doses	precursor to glutathione
Zinc	10–40 mg daily	suppresses iron and copper absorption	supports superoxide dismutase
Central folate support			
Folinic acid/leucovorin calcium (B_9)	0.5–4 mg/kg/day, 1–3×/day	hyperactivity	supports adequate folate levels in the brain

Adams et al. BMC Pediatrics 2011, 11:111
http://www.biomedcentral.com/1471-2431/11/111

BMC Pediatrics

RESEARCH ARTICLE Open Access

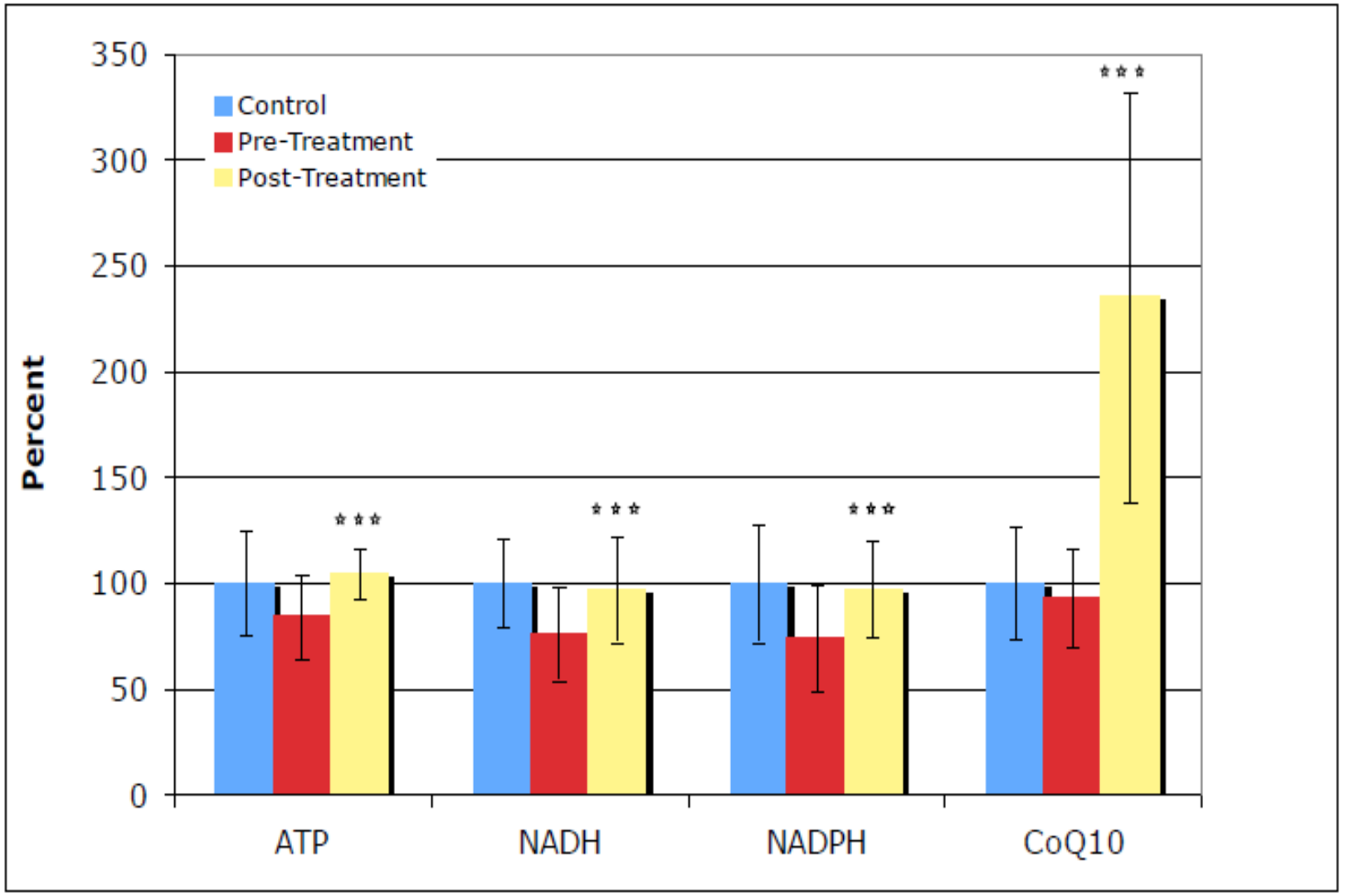
Effect of a vitamin/mineral supplement on children and adults with autism

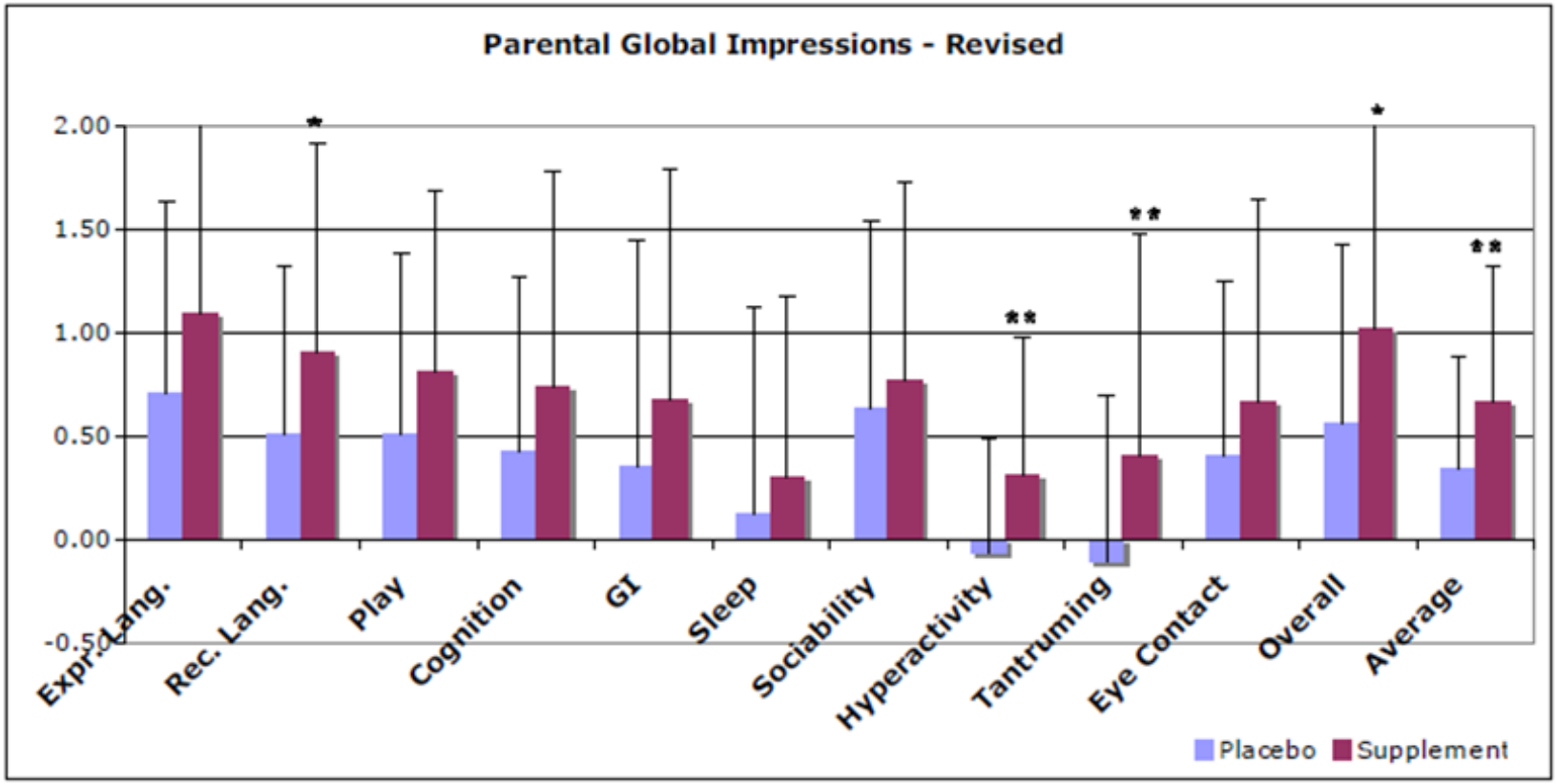
James B Adams[1*], Tapan Audhya[2], Sharon McDonough-Means[3], Robert A Rubin[4], David Quig[5], Elizabeth Geis[1], Eva Gehn[1], Melissa Loresto[1], Jessica Mitchell[6], Sharon Atwood[1], Suzanne Barnhouse[1] and Wondra Lee[1]

VITAMINS	Current Supplement (for 60 lb child)
Vitamin A (palmitate)	1000 IU
Vitamin C (calcium ascorbate)	600 mg
Vitamin D3 (cholecalciferol)	300 IU
Vitamin E	150 IU
Mixed Tocopherols	70 mg
Vitamin K	0
B1 (thiamin HCl)	20 mg
B2 (riboflavin)	20 mg
B3 (niacin/niacinamide)	15 mg niacin 10 mg niacinamide
B5 (calcium d-pantothenate)	15 mg
B6 (pyridoxine HCl)	40 mg
B12 (cyanocobalamin)	500 mcg
Folic Acid	100 mcg
Folinic Acid	550 mcg
Biotin (biotin)	150 mcg
Choline (choline chloride)	250 mg
Inositol	100 mg
Mixed Carotenoids	3.6 mg
Coenzyme Q10	50 mg
N-acetyl cysteine	50 mg

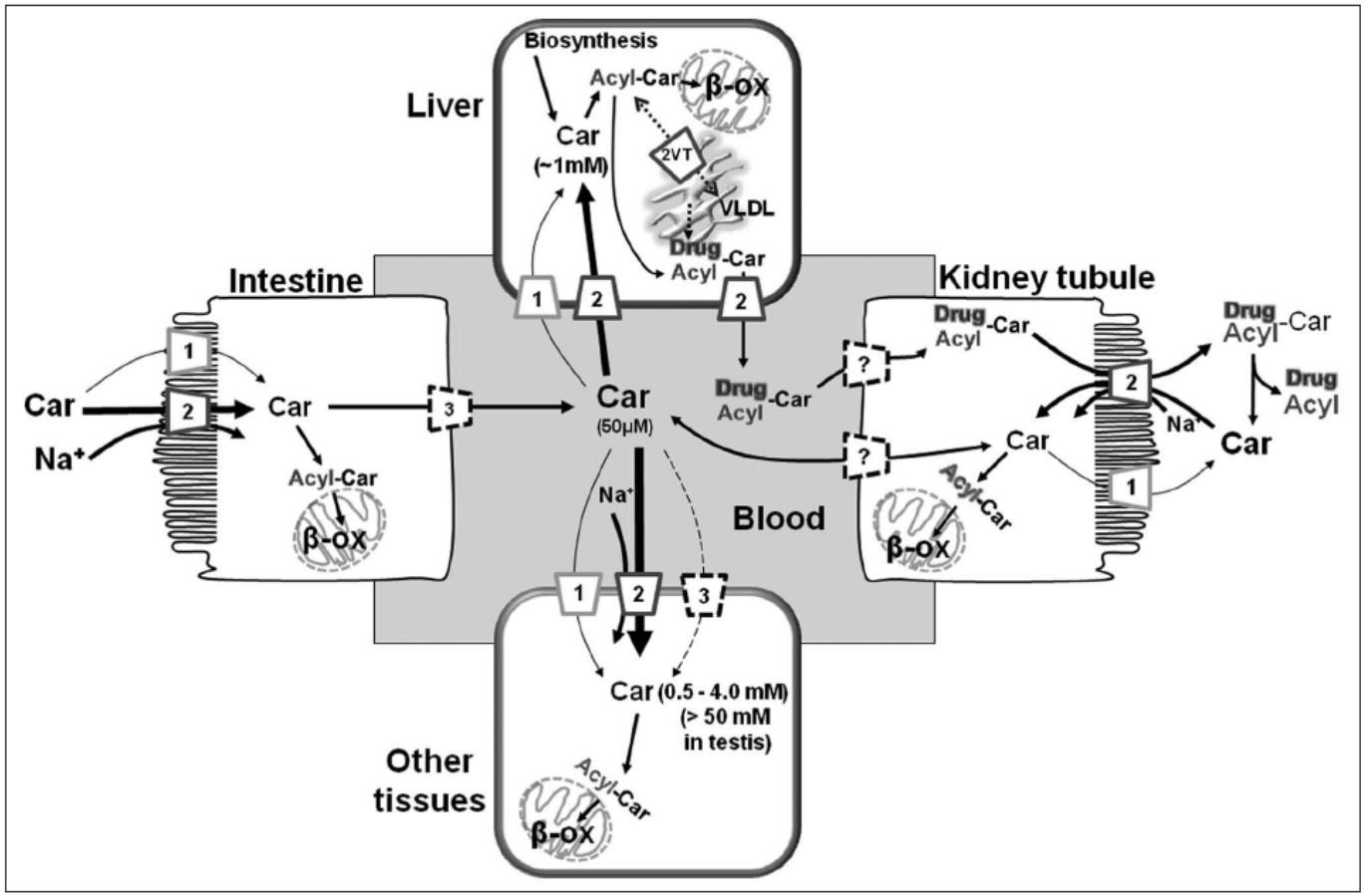
MINERALS	
Calcium (from calcium ascorbate)	100 mg
Chromium (chromium amino acid chelate)	70 mcg
Copper	0
Iodine (potassium iodide)	100 mcg
Iron	0
Lithium (lithium orotate)	500 mcg
Magnesium (magnesium chloride hexahydrate)	100 mg
Manganese (manganese amino acid chelate)	3 mg
Molybdenum (sodium molybdate dihydrate)	150 mcg
Phosphorus	0
Potassium (potassium chloride)	50 mg
Selenium (selenomethionine and sodium selenite)	22 mcg

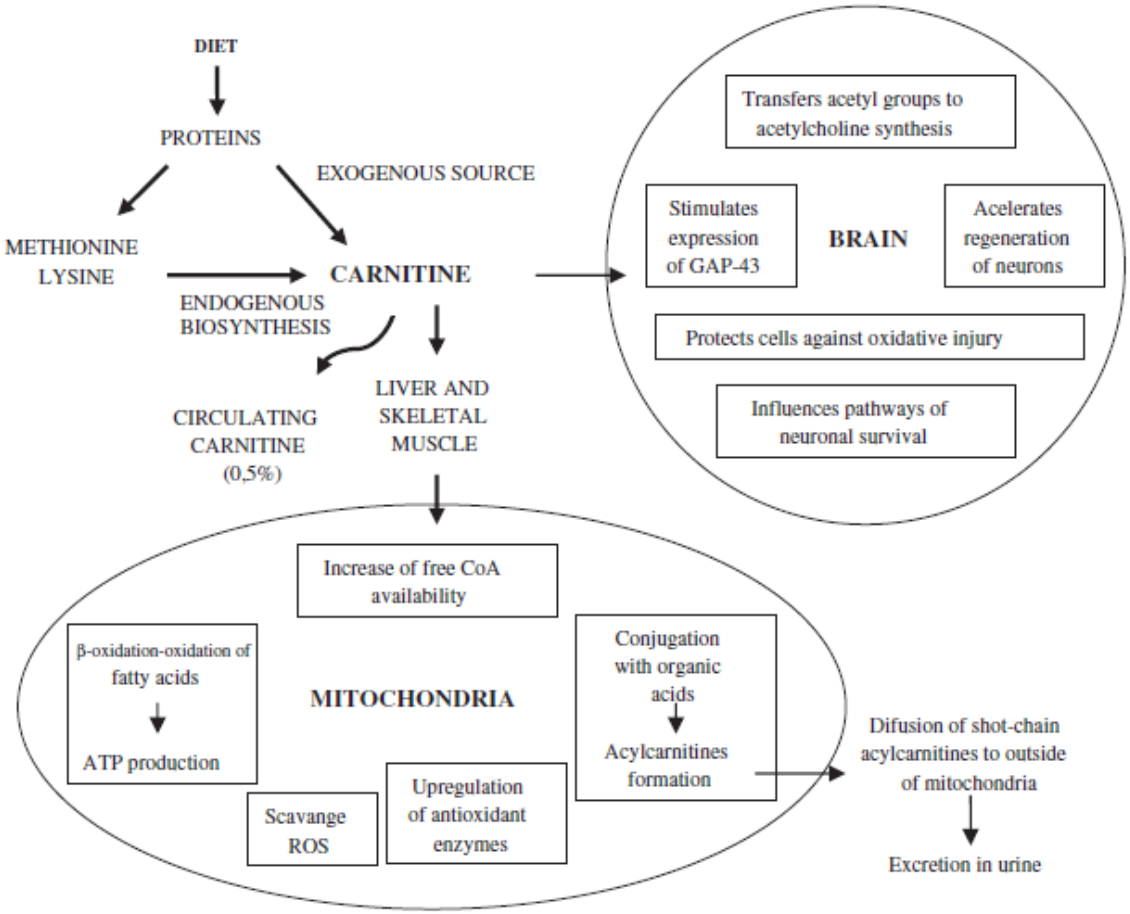
Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures




Parental Global Impressions - Revised
2.00
1.50
1.00
0.50
0.00
-0.50
*
**
**
**
*
Expr. Lang.
Rec. Lang.
Play
Cognition
GI
Sleep
Sociability
Hyperactivity
Tantruming
Eye Contact
Overall
Average
Placebo
Supplement

L-Carnitine


Biosynthesis
Liver
Acyl-Car
β-ox
Car
(~1mM)
2VT
VLDL
Drug-Car
Acyl
Intestine
Kidney tubule
Car
Na+
Car
Acyl-Car
β-ox
Car
(50μM)
Drug-Car
Acyl
Drug-Car
Acyl
Drug
Acyl-Car
Drug
Acyl
Na+
Car
Car
Acyl-Car
β-ox
Blood
Na+
Car (0.5 - 4.0 mM)
(> 50 mM
in testis)
Other
tissues
Acyl-Car
β-ox


DIET
PROTEINS
EXOGENOUS SOURCE
METHIONINE
LYSINE
CARNITINE
ENDOGENOUS
BIOSYNTHESIS
CIRCULATING
CARNITINE
(0,5%)
LIVER AND
SKELETAL
MUSCLE
Transfers acetyl groups to acetylcholine synthesis
Stimulates expression of GAP-43
BRAIN
Acelerates regeneration of neurons
Protects cells against oxidative injury
Influences pathways of neuronal survival
Increase of free CoA availability
β-oxidation-oxidation of fatty acids
ATP production
MITOCHONDRIA
Conjugation with organic acids
Acylcarnitines formation
Scavange ROS
Upregulation of antioxidant enzymes
Difusion of shot-chain acylcarnitines to outside of mitochondria
Excretion in urine

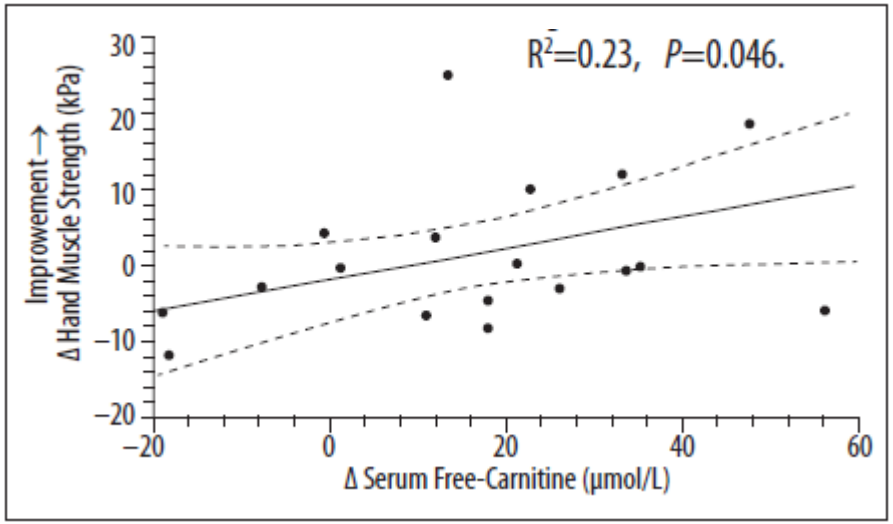
A prospective double-blind, randomized clinical trial of levocarnitine to treat autism spectrum disorders

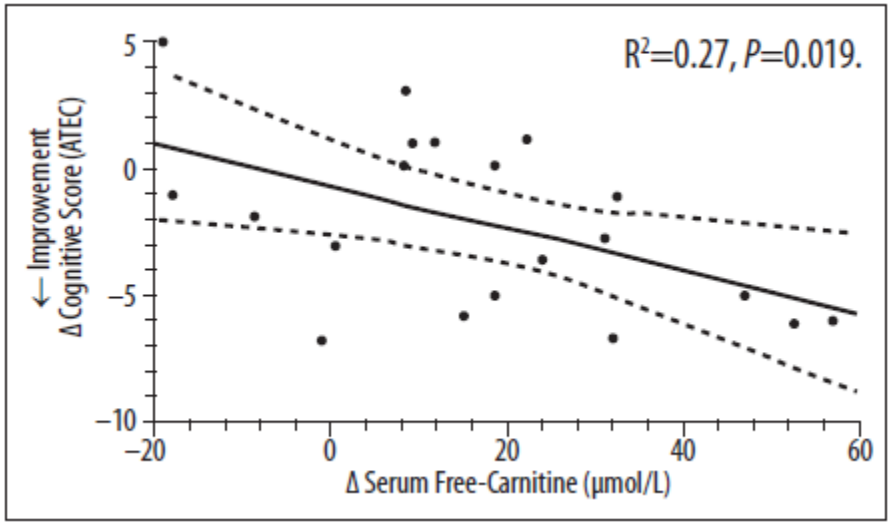
David A. Geier[1,2ABCDEFG], Janet K. Kern[3,4ABCDEFG], Georgia Davis[5BE], Paul G. King[6BE], James B. Adams[7AG], John L. Young[8BE], Mark R. Geier[9ABCDEFG]

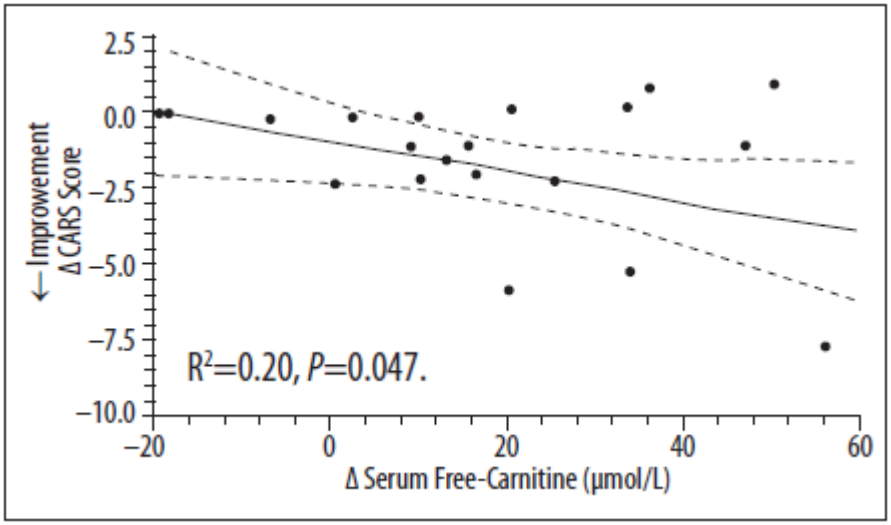
© Med Sci Monit, 2011; 17(6): PI15-23

	L-carntine baseline (*n*)	L-carnitine end (*n*)	Total Δ (%)	Placebo baseline (*n*)	Placebo end (*n*)	Total Δ (%)	Contrast between groups (95% CI)	*P*-value for contrast between groups
CARS** (professional)	35.7±5.3*** (16)	33.8±5.8 (16)	−1.94±2.5 (−5.3)	38.2±6.0 (11)	38.4±6.3 (11)	0.09±1.4 (0.5)	−2.03# (−3.7 to −0.31)	0.02
Modified** CGI (professional)	2.0 (16)	1.5±0.63 (16)	−0.5±0.63 (−25)	2.0 (11)	2.09±0.7 (11)	0.09±0.7 (4.3)	−0.69 (−1.1 to −0.06)	0.03
Cognitive	12.7±6.1 (15)	9.2±5.5 (15)	−3.5±3.4 (−27.6)	14.4±7.6 (8)	14.9±7.3 (8)	0.5±2.6 (3.4)	−4 (−6.9 to −1.1)	0.009

Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures







L-Carnitine supplementation improves the behavioral symptoms in autistic children

Sarah Farid Fahmy [a], Manal H. El-hamamsy [a], Osama K. Zaki [b], Osama A. Badary [a,*]

[a] Clinical Pharmacy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
[b] Medical Genetics unit, faculty of medicine Ain shams university hospital, Cairo, Egypt

Table 4
Childhood autism rating scale scores in L-carnitine and placebo group following 3 and 6 months therapy.

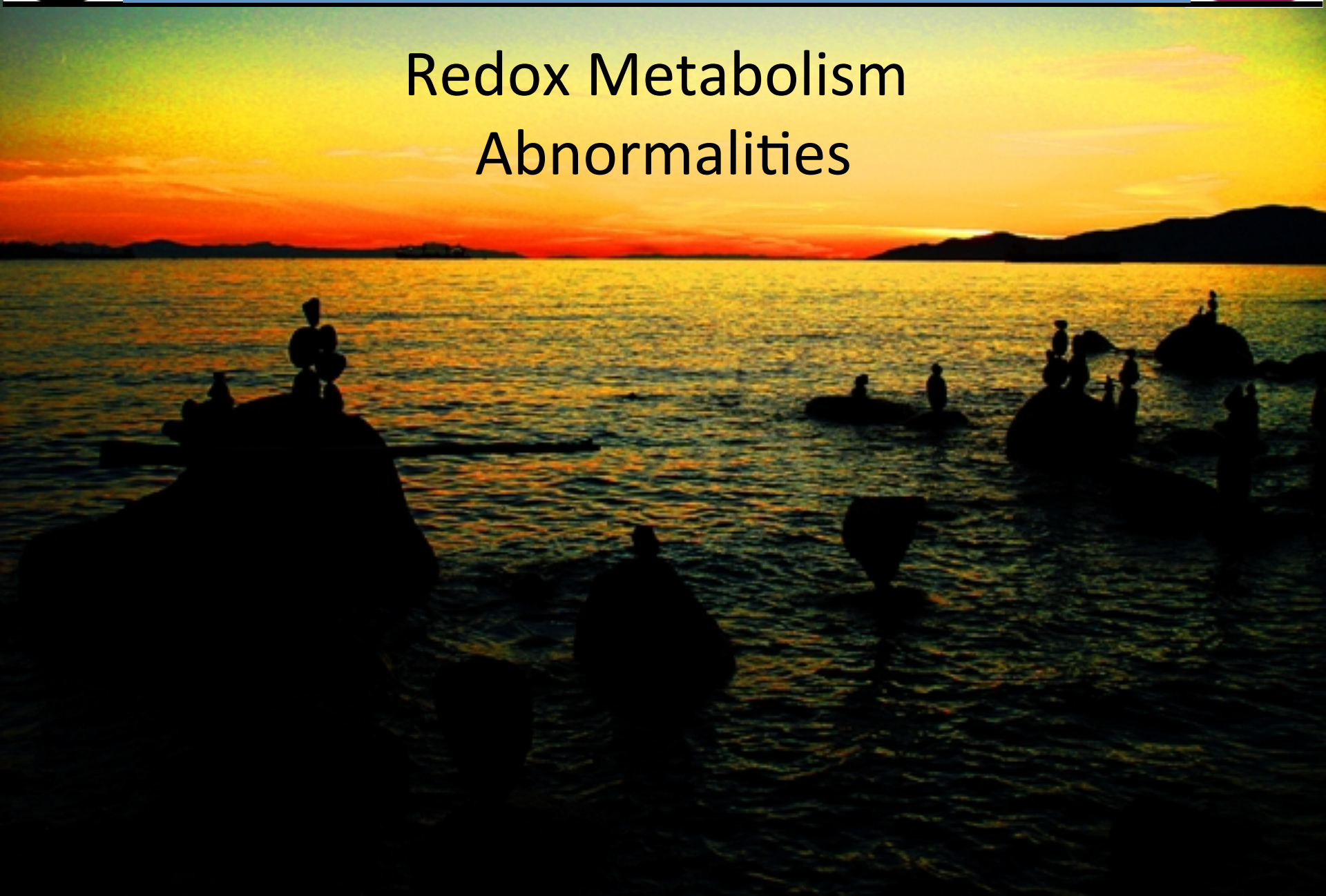
Time (months)	L-Carnitine group (mean ± SD)	Placebo group (mean ± SD)	*P*-Value		
			Groups	Overtime	Interaction
0	45.25 ± 6.191	36.71 ± 5.594	<0.001	0.006	<0.001
3	42.06 ± 6.287	35.36 ± 4.715			
6	37.06 ± 5.882	34.71 ± 4.631			

Table 5
Percentage of change in childhood autism rating scale scores in L-carnitine group following 3 and 6 months therapy.

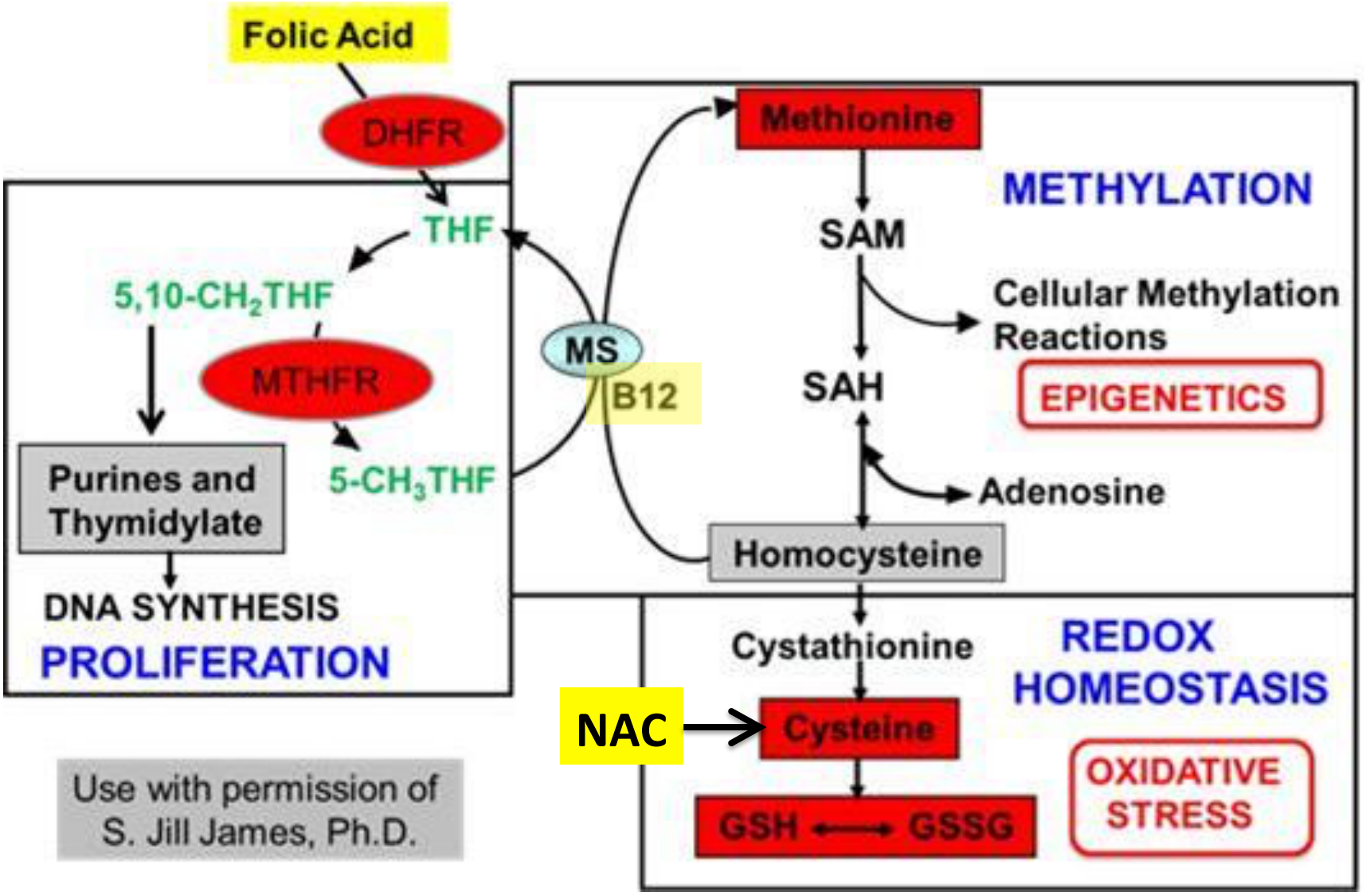
L-Carnitine group		% change from baseline to 3 months and baseline to 6 months	*P*-Values
CARS	Mean ± SD	Mean ± SD	
Baseline	45.3 ± 6.2		
3 months	42.1 ± 6.3	7.1 ± 4.5	<0.001
6 months	37.1 ± 5.9	17.9 ± 8	<0.001

SD, standard deviation; CARS, childhood autism rating scale.

Redox Metabolism Abnormalities



Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures



Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism[1–3]

S Jill James, Stepan Melnyk, George Fuchs, Tyra Reid, Stefanie Jernigan, Oleksandra Pavliv, Amanda Hubanks, and David W Gaylor

TABLE 1

Mean plasma metabolite concentrations (±SD) in age-matched control children, children who had autism at baseline before intervention, and children with autism after 3-mo intervention with methylcobalamin and folinic acid[1]

		Children with autism		
Plasma metabolite concentration	Control children ($n = 42$)	Pretreatment ($n = 40$)	Posttreatment ($n = 40$)	P value[2]
Methionine	24 ± 3	21 ± 4[3]	22 ± 3[4]	NS
SAM (nmol/L)	78 ± 22	66 ± 13[3]	69 ± 12[4]	NS
SAH (nmol/L)	14.3 ± 4.3	15.2 ± 5	14.8 ± 4	NS
SAM:SAH (μmol/L)	5.6 ± 2.0	4.7 ± 1.5[3]	5.0 ± 2.0	NS
Homocysteine (μmol/L)	5.0 ± 1.2	4.8 ± 1.8	5.3 ± 1.1	0.04
Cysteine (μmol/L)	210 ± 18	191 ± 24[3]	215 ± 19	0.001
Cysteinylglycine (μmol/L)	45 ± 6	40 ± 9[3]	46 ± 9	0.002
tGSH (μmol/L)	7.5 ± 1.8	5.4 ± 1.3[3]	6.2 ± 1.2[4]	0.001
fGSH (μmol/L)	2.8 ± 0.8	1.5 ± 0.4[3]	1.8 ± 0.4[4]	0.008
GSSG (μmol/L)	0.18 ± 0.07	0.28 ± 0.08[3]	0.22 ± 0.06[4]	0.001
tGSH:GSSG	47 ± 18	21 ± 6[3]	30 ± 9[4]	0.001
fGSH:GSSG	17 ± 6.8	6 ± 2[3]	9 ± 3[4]	0.001

[1] fGSH, free glutathione; SAH, *S*-adenosylhomocsyteine; SAM, *S*-adenosylmethionine; tGSH, total glutathione; GSSG, oxidized glutathione disulfide. NS, $P > 0.05$.

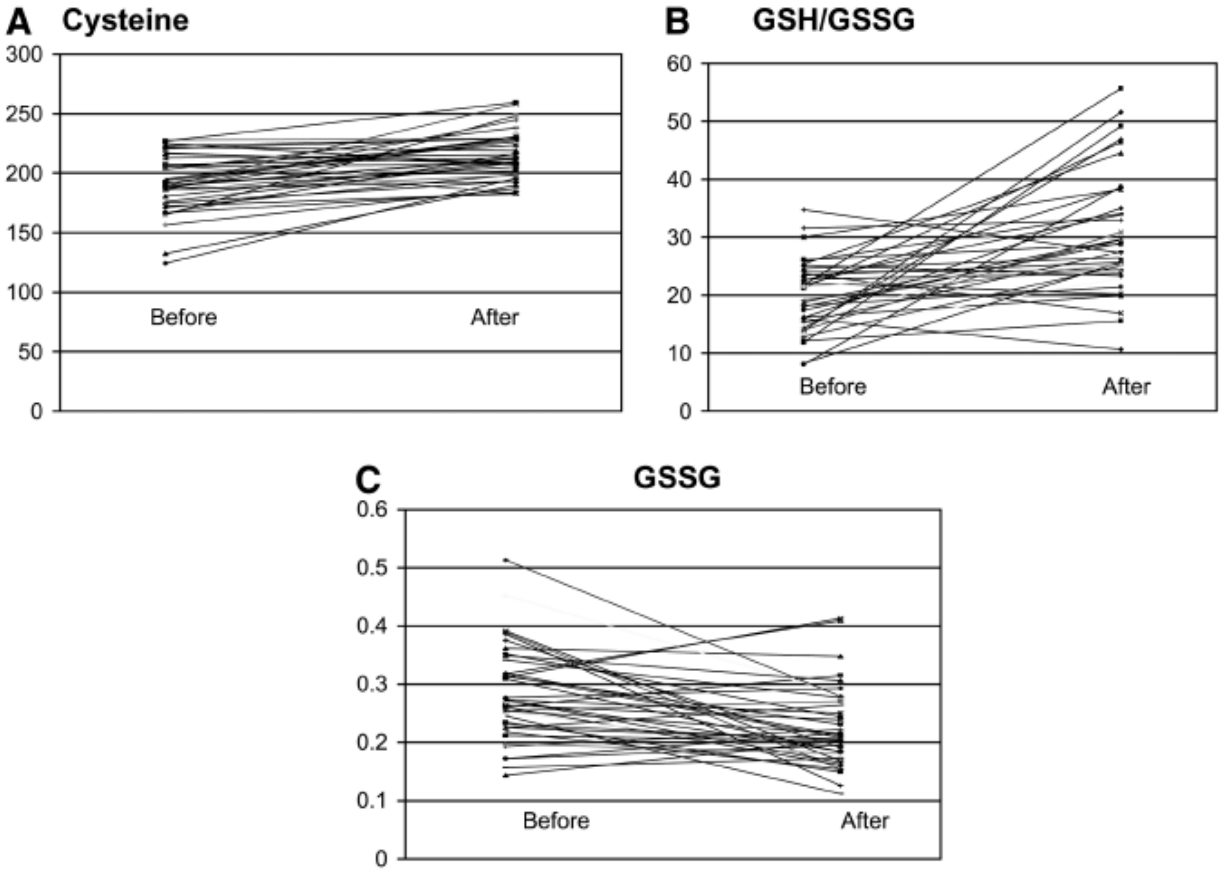
[2] Pre- and posttreatment comparison.

[3] Significantly different from control children, $P < 0.005$.

[4] Significantly different from control children, $P < 0.01$.

Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism[1–3]

S Jill James, Stepan Melnyk, George Fuchs, Tyra Reid, Stefanie Jernigan, Oleksandra Pavliv, Amanda Hubanks, and David W Gaylor



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

Autism Research and Treatment
Volume 2013, Article ID 609705, 9 pages
http://dx.doi.org/10.1155/2013/609705

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]

Vineland Subscale	Baseline Age Equivalent Months (mean ± SE)	Post-Intervention Age Equivalent Months (mean ± SE)	Change (months) (mean; 95% C I)
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)**

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

Autism Research and Treatment
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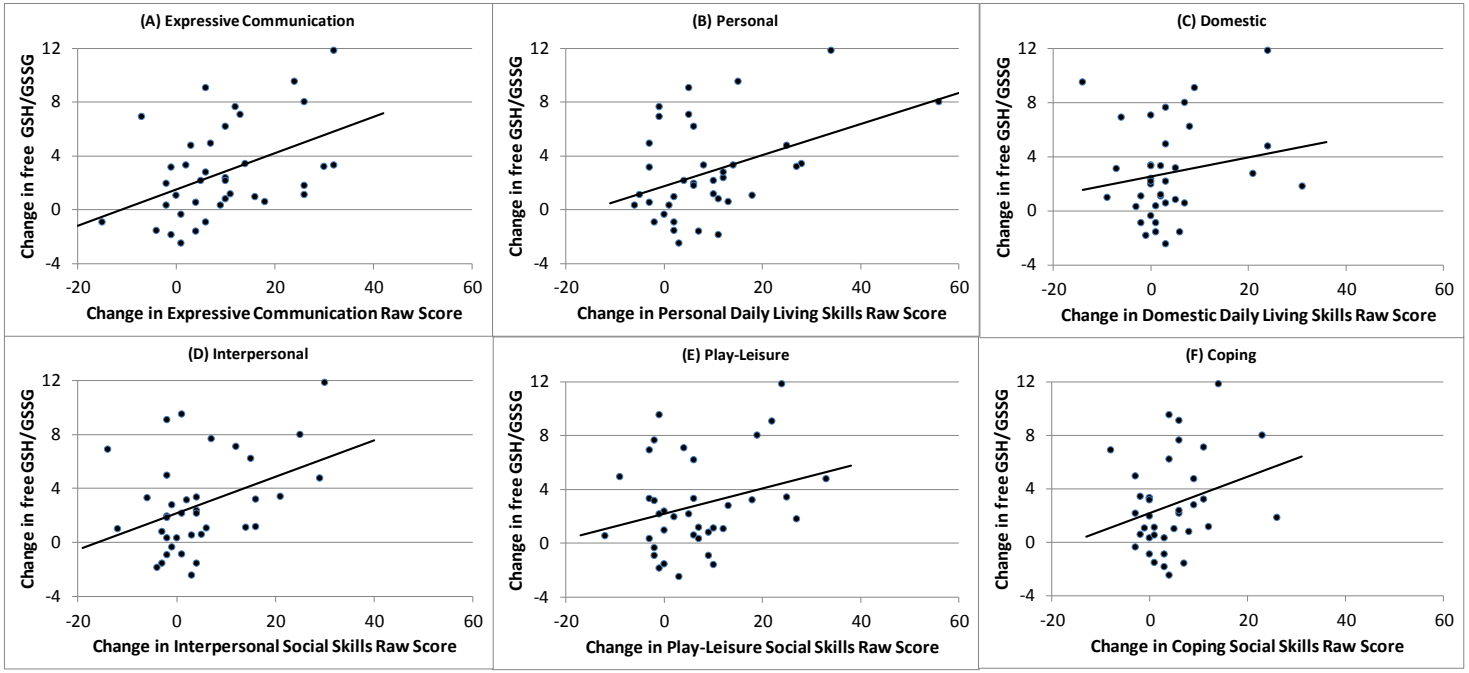

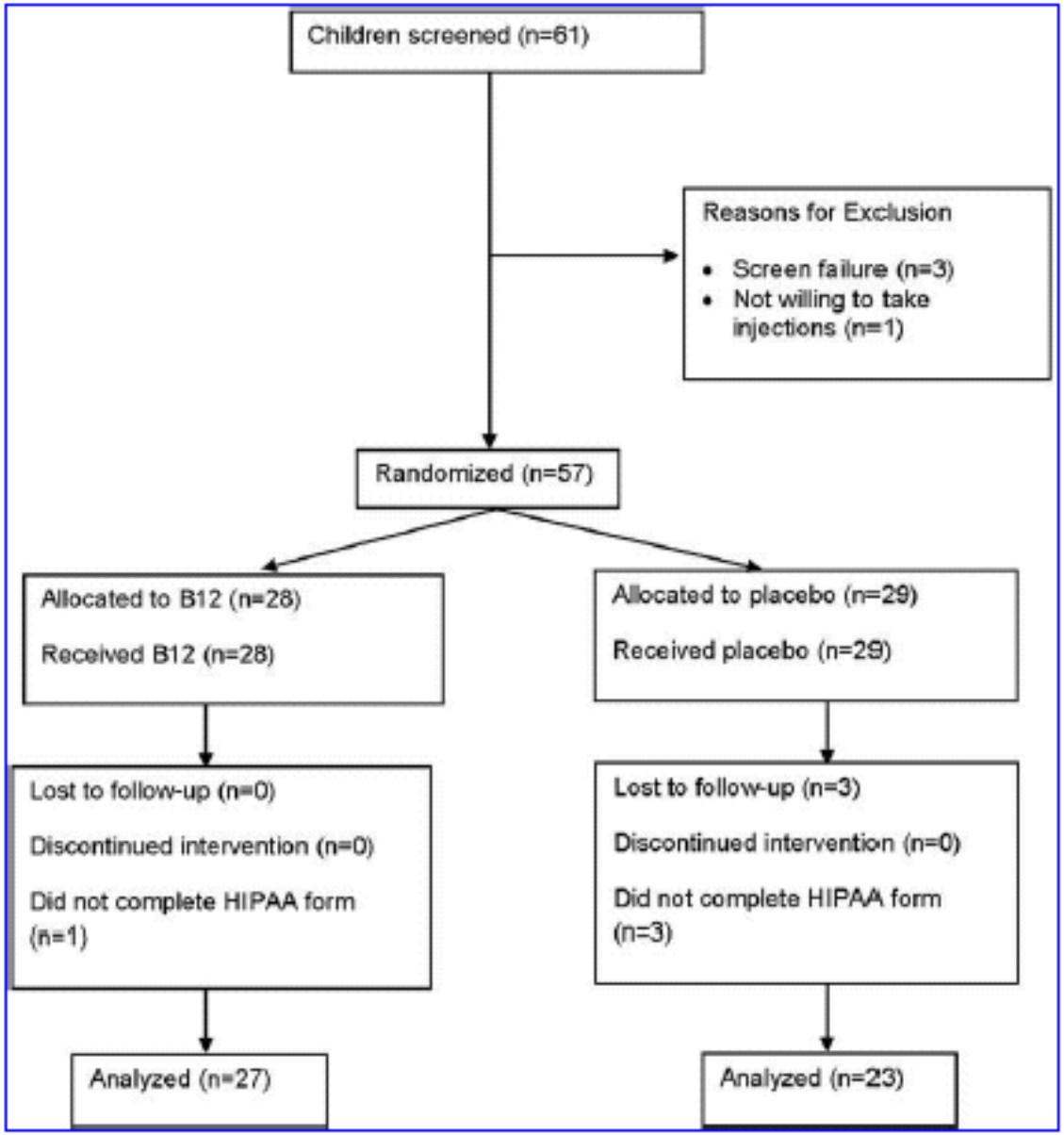
Children screened (n=61)
Reasons for Exclusion
Screen failure (n=3)
Not willing to take injections (n=1)
Randomized (n=57)
Allocated to B12 (n=28)
Received B12 (n=28)
Allocated to placebo (n=29)
Received placebo (n=29)
Lost to follow-up (n=0)
Discontinued intervention (n=0)
Did not complete HIPAA form (n=1)
Lost to follow-up (n=3)
Discontinued intervention (n=0)
Did not complete HIPAA form (n=3)
Analyzed (n=27)
Analyzed (n=23)

Randomized, Placebo-Controlled Trial of Methyl B12 for Children with Autism

Robert L. Hendren, DO[1], S. Jill James, PhD[2], Felicia Widjaja, MPH[1], Brittany Lawton, BS[1], Abram Rosenblatt, PhD[1], and Stephen Bent, MD[1]

JOURNAL OF CHILD AND ADOLESCENT PSYCHOPHARMACOLOGY
Volume X, Number x, 2016

Variable	*Mean change placebo (SD)*	*Mean change methyl B12 (SD)*	*Effect size*	*Difference in mean change*	*95% CI*	p *value*
CGI-Improvement	3.1 (0.8)	2.4 (0.8)	0.84	0.7 (0.8)	−1.2 to −0.2	0.005*
ABC Hyperactivity	−3.9 (7.1)	−0.9 (4.8)	−0.48	−3.0 (6.2)	−7.0 to 1.1	0.11
ABC Inappropriate Speech	−0.3 (1.6)	0.3 (1.4)	−0.43	−0.6 (1.5)	−1.6 to 0.3	0.17
ABC Irritability	−2.6 (4.3)	−0.1 (3.7)	−0.61	−2.5 (4.0)	−5.1 to 0.2	0.08
ABC Lethargy	−1.2 (7.1)	−1.9 (5.8)	0.12	0.8 (6.6)	−3.5 to 5.0	1.00
ABC Stereotypy	0.3 (3.2)	−0.3 (2.2)	0.23	0.6 (2.8)	−1.2 to 2.5	0.58
ABC Total Score	−7.6 (17.4)	−2.9 (12.1)	−0.30	−4.7 (15.4)	−14.6 to 5.3	0.23
SRS Social Awareness	0.0 (18.3)	−3.9 (10.0)	0.26	3.9 (15.0)	−5.9 to 13.7	0.18
SRS Social Cognition	−3.6 (10.5)	−2.7 (6.9)	−0.10	−0.9 (9.0)	−6.8 to 5.0	0.87
SRS Social Communication	−3.6 (8.8)	0.1 (15.0)	−0.30	−3.6 (12.1)	−11.5 to 4.2	0.22
SRS Social Mannerisms	−1.6 (14.3)	−0.8 (10.7)	−0.06	−0.8 (12.8)	−9.1 to 7.5	0.53
SRS Social Motivation	−6.1 (10.0)	0.2 (6.6)	−0.73	−6.3 (8.6)	−11.9 to −0.7	0.02*
SRS Total Score	−4.1 (7.7)	−1.6 (7.7)	−0.32	−2.5 (7.7)	−7.5 to 2.5	0.21

ARCHIVAL REPORTS

A Randomized Controlled Pilot Trial of Oral *N*-Acetylcysteine in Children with Autism

Antonio Y. Hardan, Lawrence K. Fung, Robin A. Libove, Tetyana V. Obukhanych, Surekha Nair, Leonore A. Herzenberg, Thomas W. Frazier, and Rabindra Tirouvanziam

NAC –900mg up to Three Times Per Day

Table 1. Baseline Comparison of Participants with Autism Assigned to Receive NAC or Placebo

	Placebo	NAC
Number in Group	15	14
Male/Female	15/0	12/2
Age (Years)	7.2 (2.2) [3.2–10.7]	7.0 (2.1) [4.4–10.4]
ABC Irritability Score	14.8 (9.6) [5–41]	16.9 (7.9) [1–27]
CGI Severity Score	5.3 (.8) [4–6]	5.1 (.7) [4–6]
SRS Total	104.7 (28.1) [48–158]	111.9 (28.3) [64–150]
RBS-R Total	38.2 (24.0) [16–115]	33.1 (16.2) [8–66]

ARCHIVAL REPORTS

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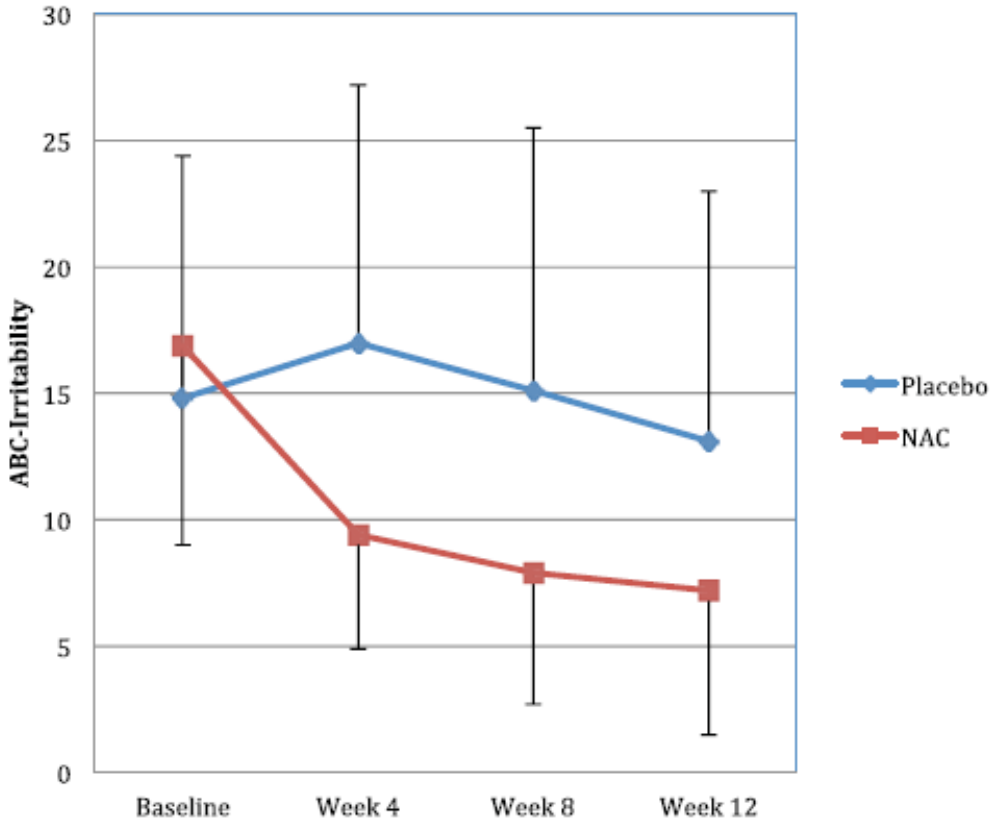
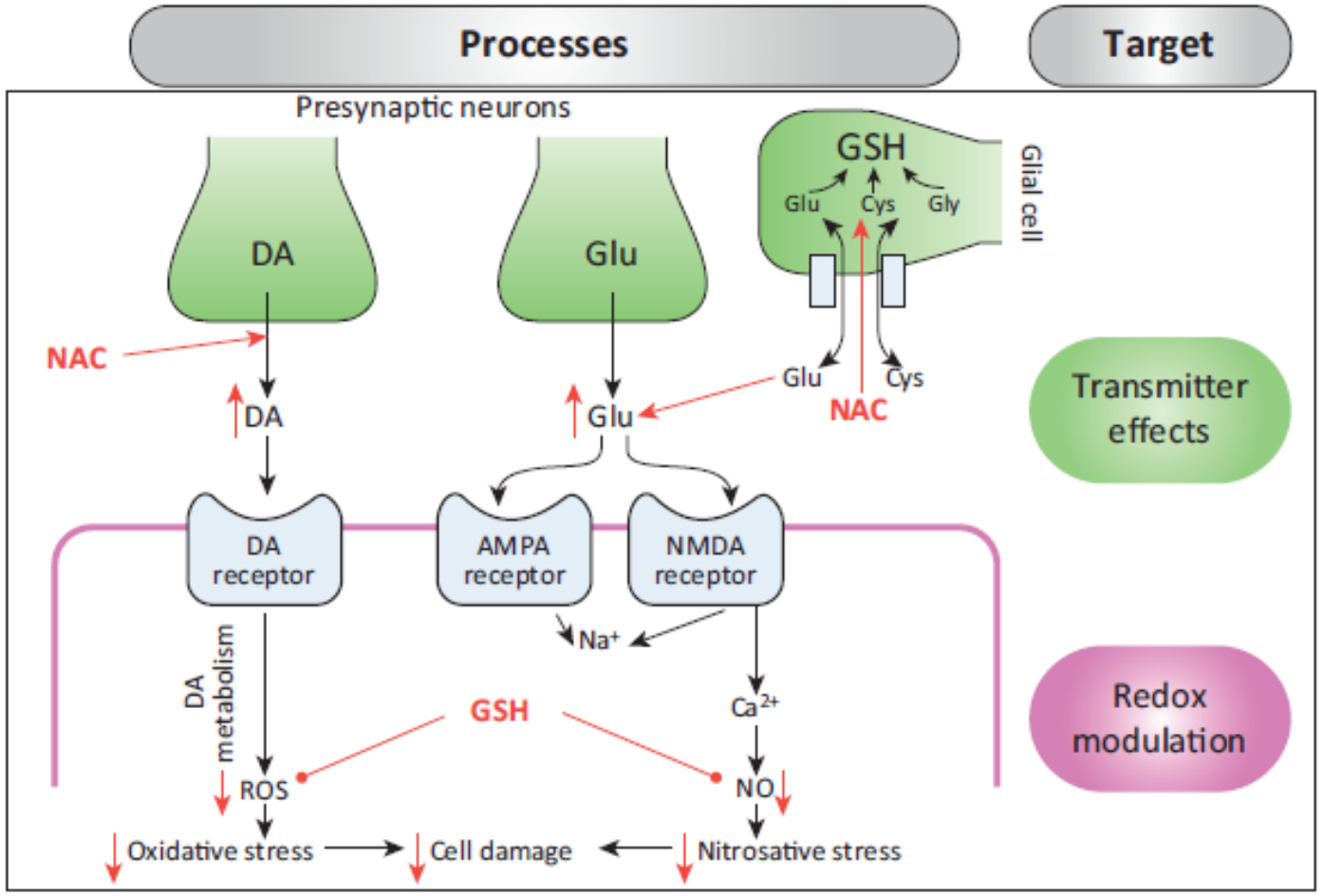


	F	*p*	Cohen's *d*
ABC			
ABC-irritability	6.80	<.001	.96
ABC-lethargy	1.93	.134	−.30
ABC-stereotypy	2.21	.096	.72
ABC-hyperactivity	1.97	.130	.72
ABC-inappropriate speech	1.25	.297	.28
RBS-R			
RBS-stereotypies	7.07	.014	.90
RBS-self-injurious behavior	2.47	.129	.63
RBS-compulsions	2.48	.128	.70
RBS-rituals	.24	.631	.17
RBS-sameness	1.26	.273	.46
RBS-restricted	3.77	.064	.73
SRS Total	2.36	.141	.44
SRS social awareness	.34	.565	.26
SRS social cognition	4.99	.037	.99
SRS social communication	.01	.998	.04
SRS social motivation	.29	.597	.24
SRS autism mannerisms	4.56	.045	.95
CGI Severity	1.73	.170	.57
CGI Improvement	.81	.449	.30

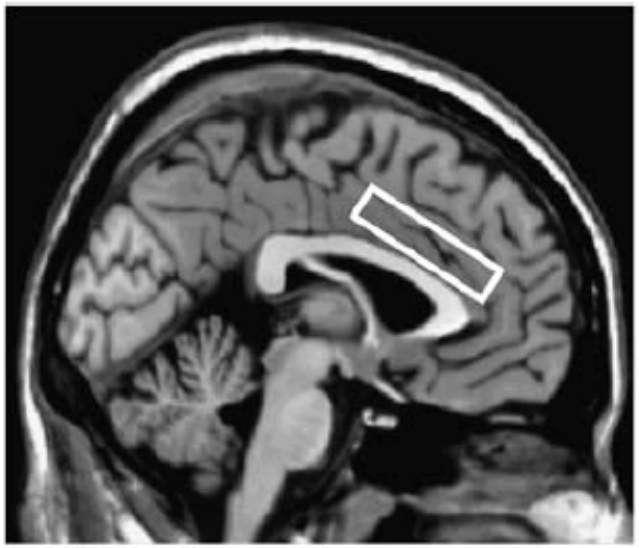


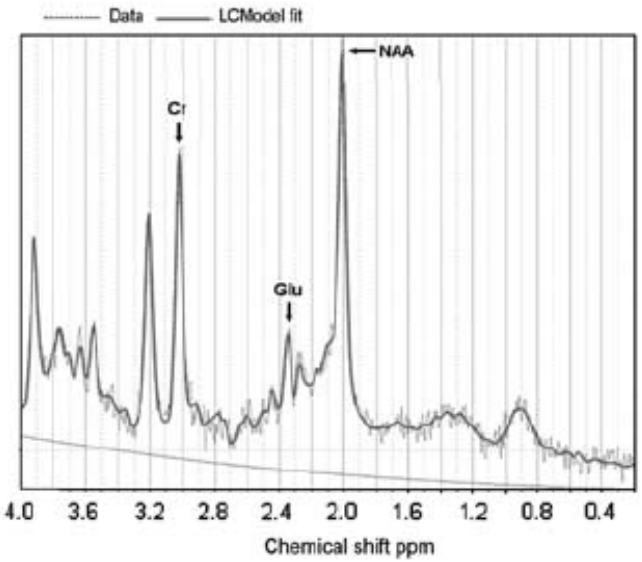
(Berk et al, Trends in Pharmacological Science, 2013)

N-Acetylcysteine Normalizes Glutamate Levels in Cocaine-Dependent Patients: A Randomized Crossover Magnetic Resonance Spectroscopy Study

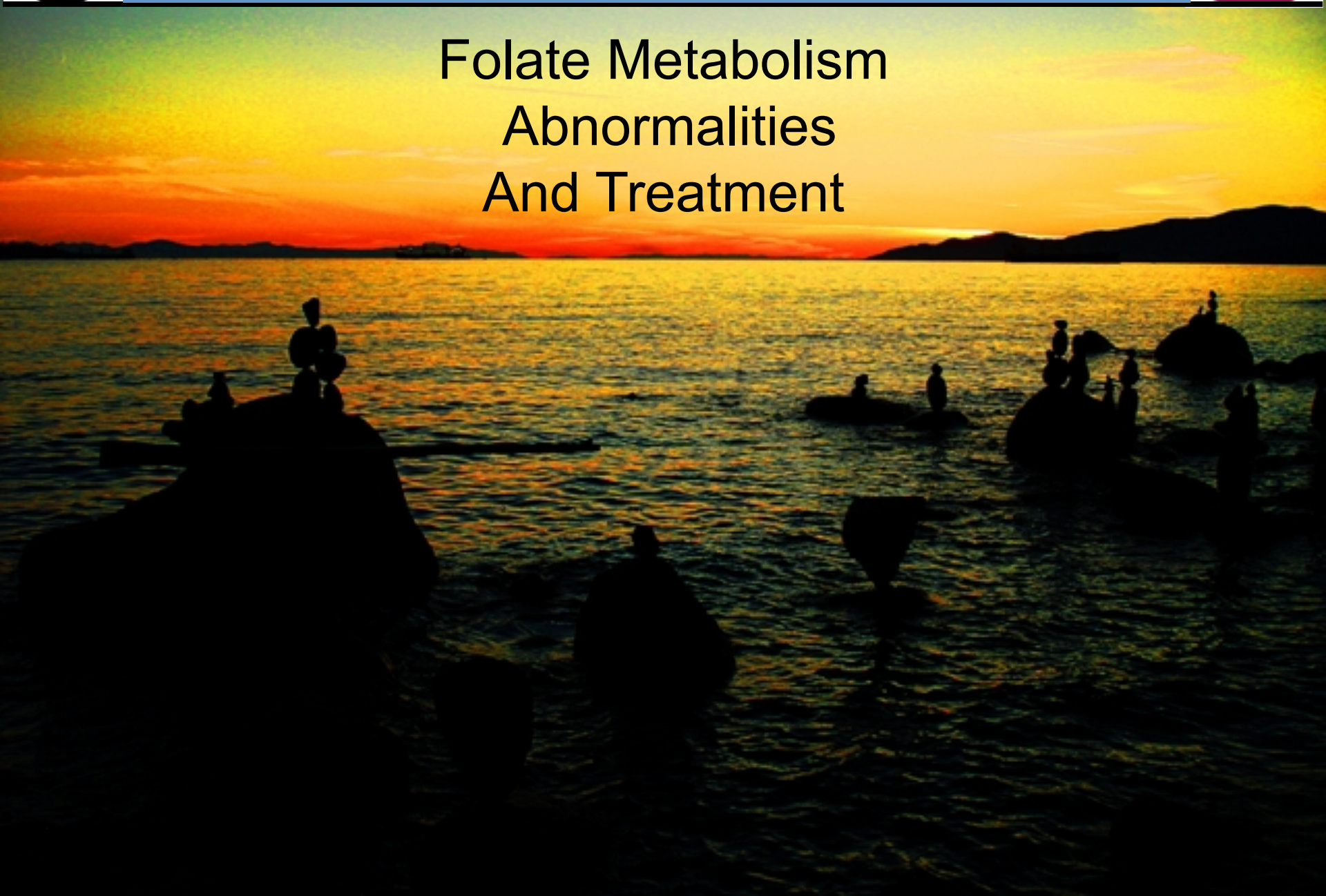
Lianne Schmaal*[,1], Dick J Veltman[1,2], Aart Nederveen[3], Wim van den Brink[1] and Anna E Goudriaan[1]

[1]Amsterdam Institute for Addiction Research, Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Psychiatry, VU University Medical Center, Amsterdam, The Netherlands; [3]Department of Radiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands





Folate Metabolism Abnormalities And Treatment



The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Autoantibodies to Folate Receptors in the Cerebral Folate Deficiency Syndrome

Vincent T. Ramaekers, M.D., Sheldon P. Rothenberg, M.D., Jeffrey M. Sequeira, M.S., Thomas Opladen, M.D., Nenad Blau, Ph.D., Edward V. Quadros, Ph.D., and Jacob Selhub, Ph.D.

N Engl J Med 2005;352:1985-91.

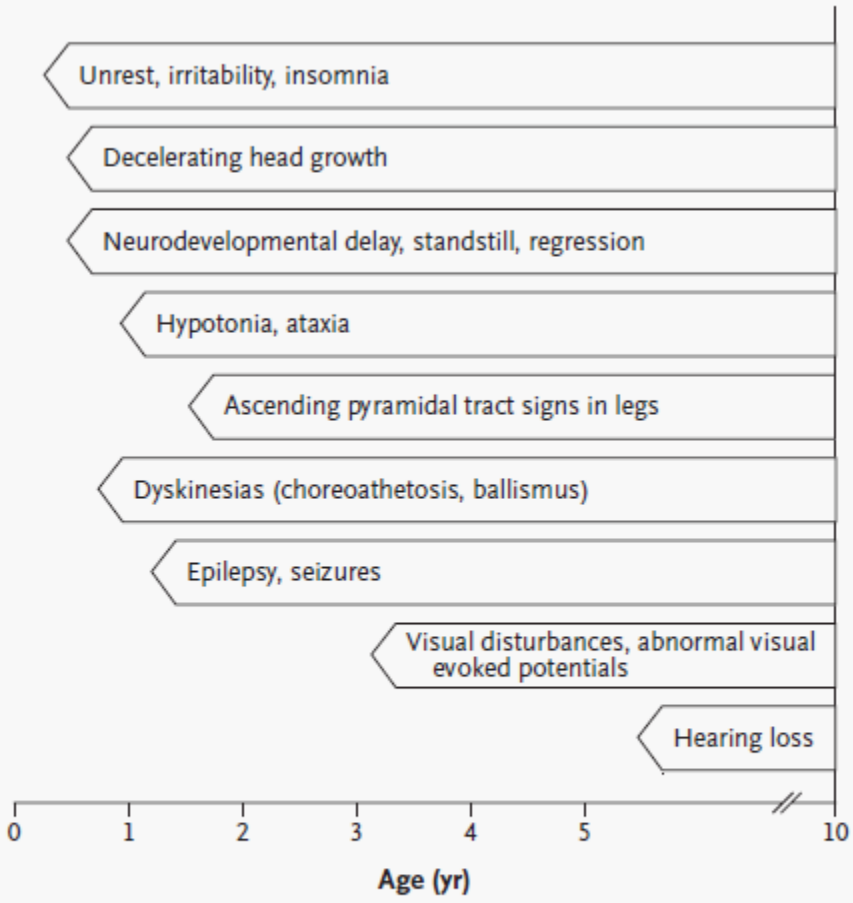
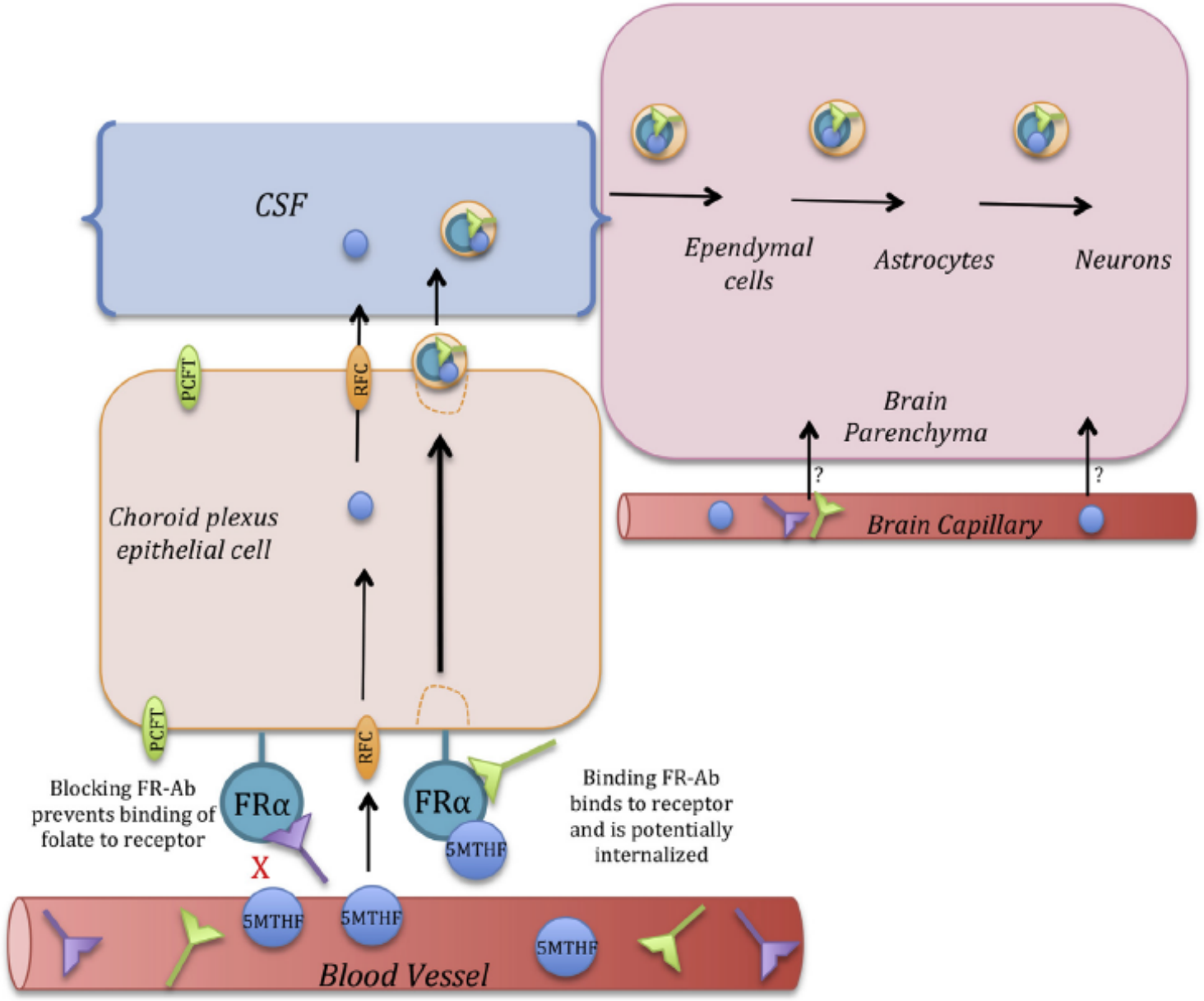


Figure 1. Age at Onset of Major Clinical Symptoms Associated with Cerebral Folate Deficiency.

Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures



The Expanding Association between Autism and Cerebral Folate Deficiency

Open

Molecular Psychiatry (2012), 1–13

www.nature.com/mp

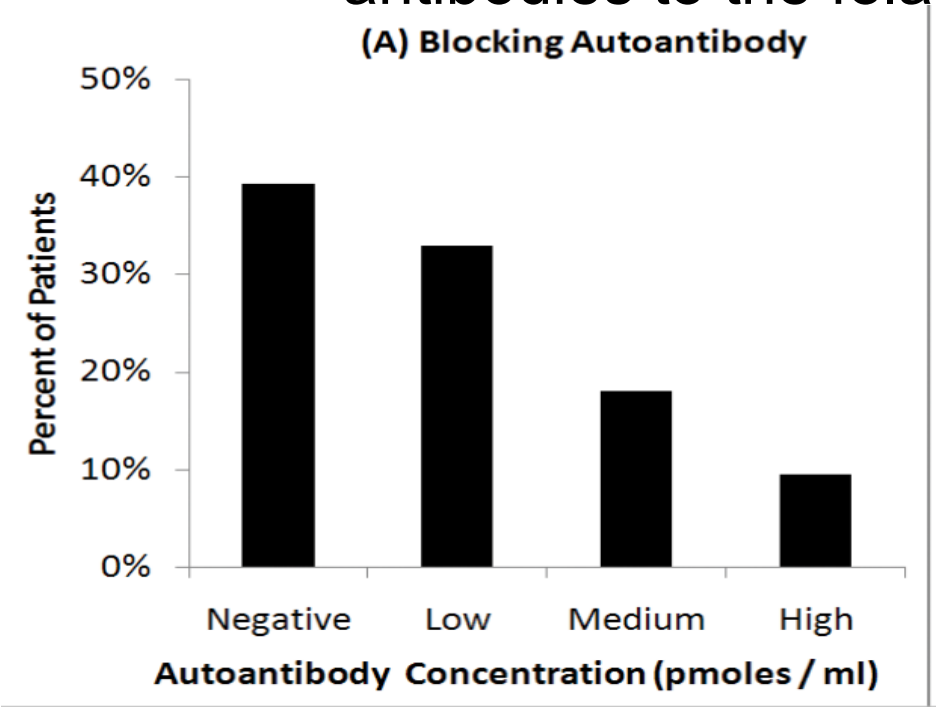
ORIGINAL ARTICLE

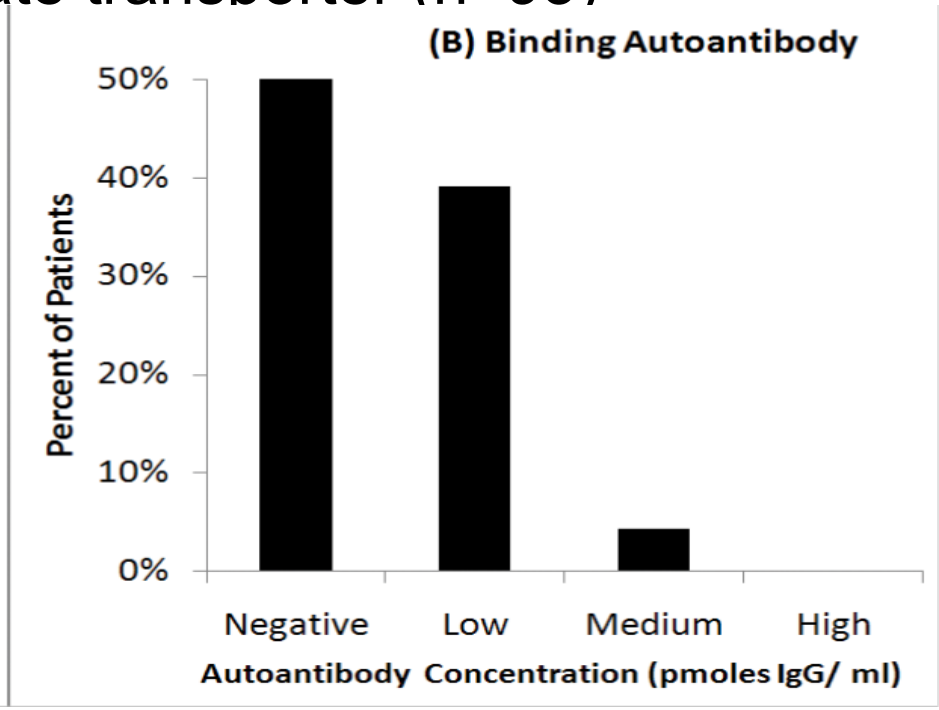
Cerebral folate receptor autoantibodies in autism spectrum disorder

RE Frye[1], JM Sequeira[2], E Quadros[2], SJ James[1] and DA Rossignol[3]

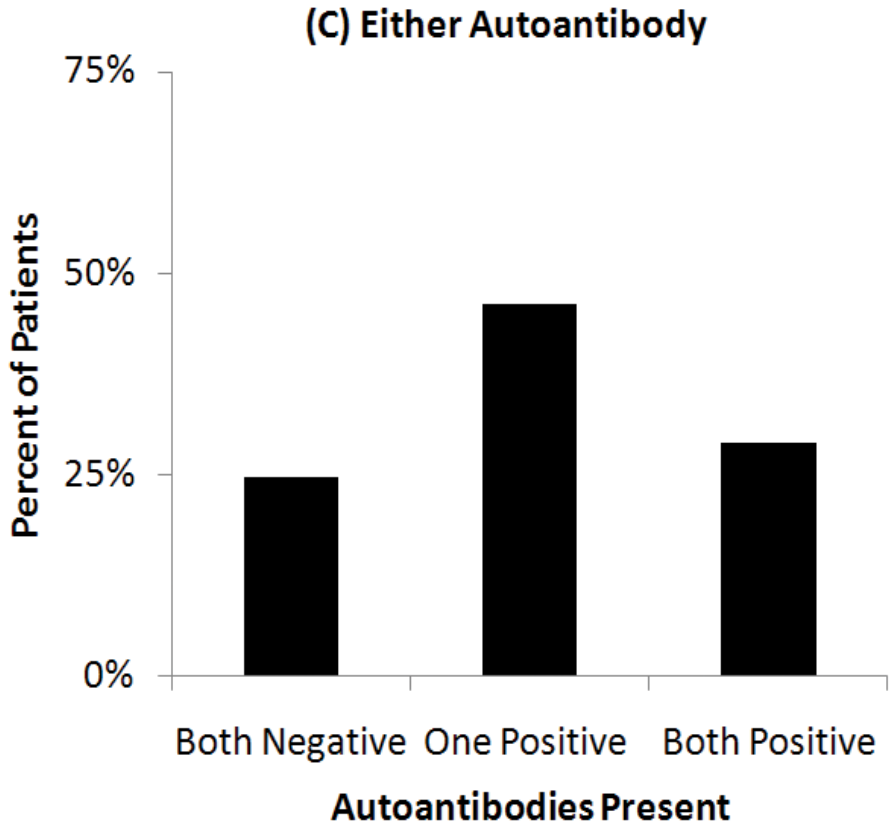
[1]Department of Pediatrics, Arkansas Children's Hospital Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA; [2]Department of Medicine, State University of New York—Downstate Medical Center, Brooklyn, NY, USA and [3]International Child Development Resource Center, Melbourne, FL, USA

More than half of children with Autism Spectrum Disorder referred to two autism specialty clinics test positive for antibodies to the folate transporter (n=93)

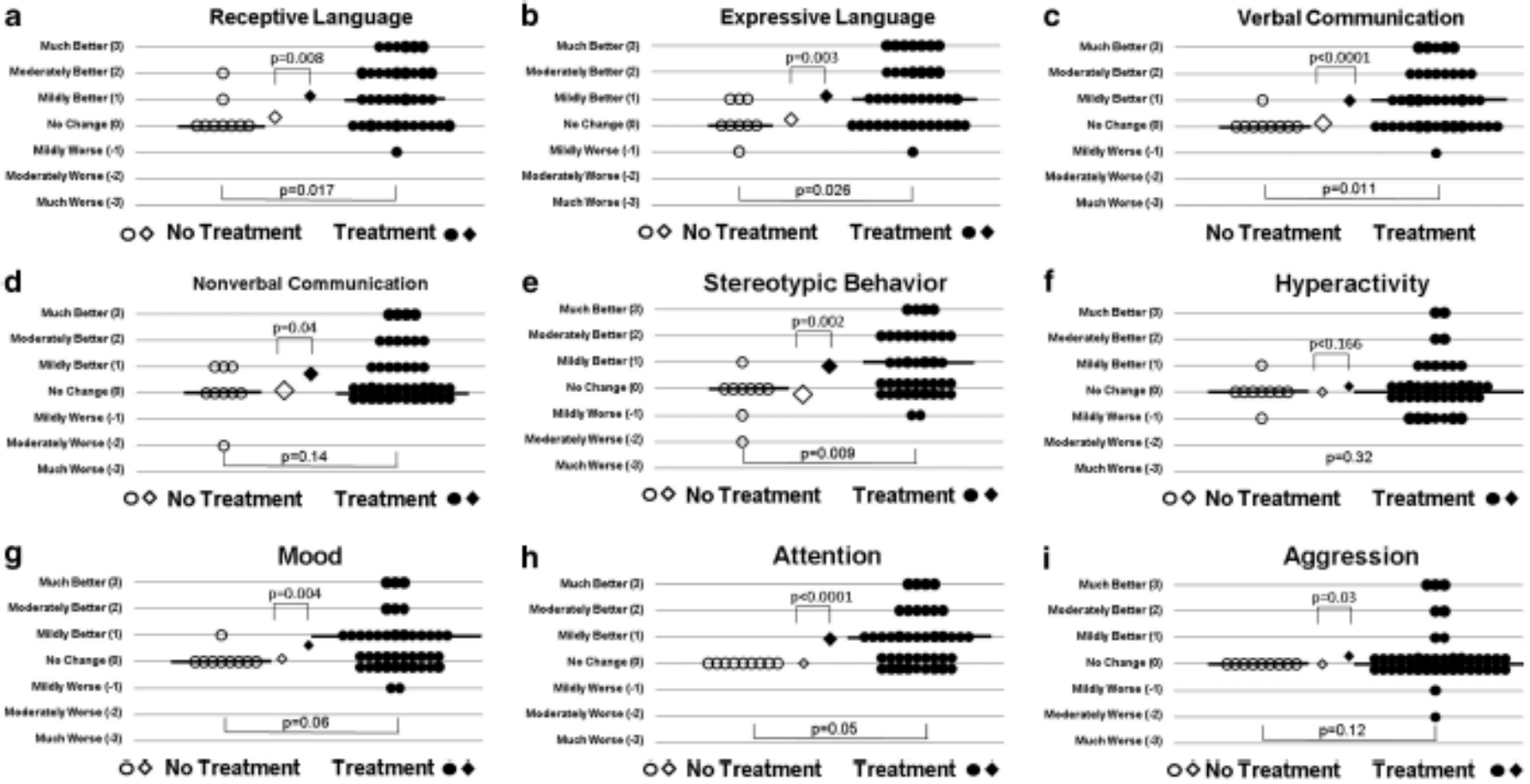


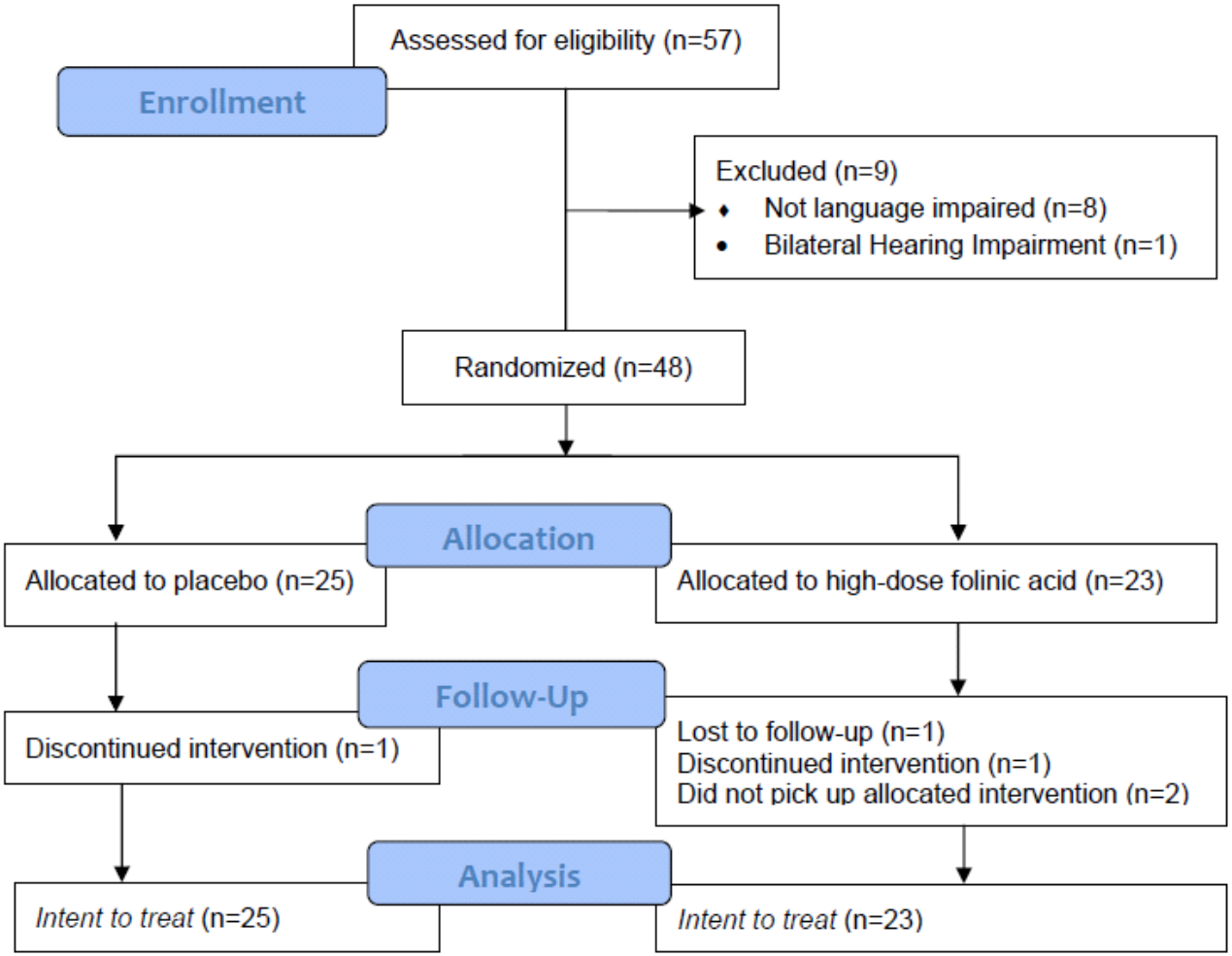


75% of children with Autism Spectrum Disorder tested positive for one of the two antibodies to the folate transporter



44 Fab+ children with Autism were treated with 2mg/kg of folinic acid in an open-label fashion compared to a wait list control group of Fab+ children with autism




Assessed for eligibility (n=57)
Enrollment
Excluded (n=9)
Not language impaired (n=8)
Bilateral Hearing Impairment (n=1)
Randomized (n=48)
Allocation
Allocated to placebo (n=25)
Allocated to high-dose folinic acid (n=23)
Follow-Up
Discontinued intervention (n=1)
Lost to follow-up (n=1)
Discontinued intervention (n=1)
Did not pick up allocated intervention (n=2)
Analysis
Intent to treat (n=25)
Intent to treat (n=23)

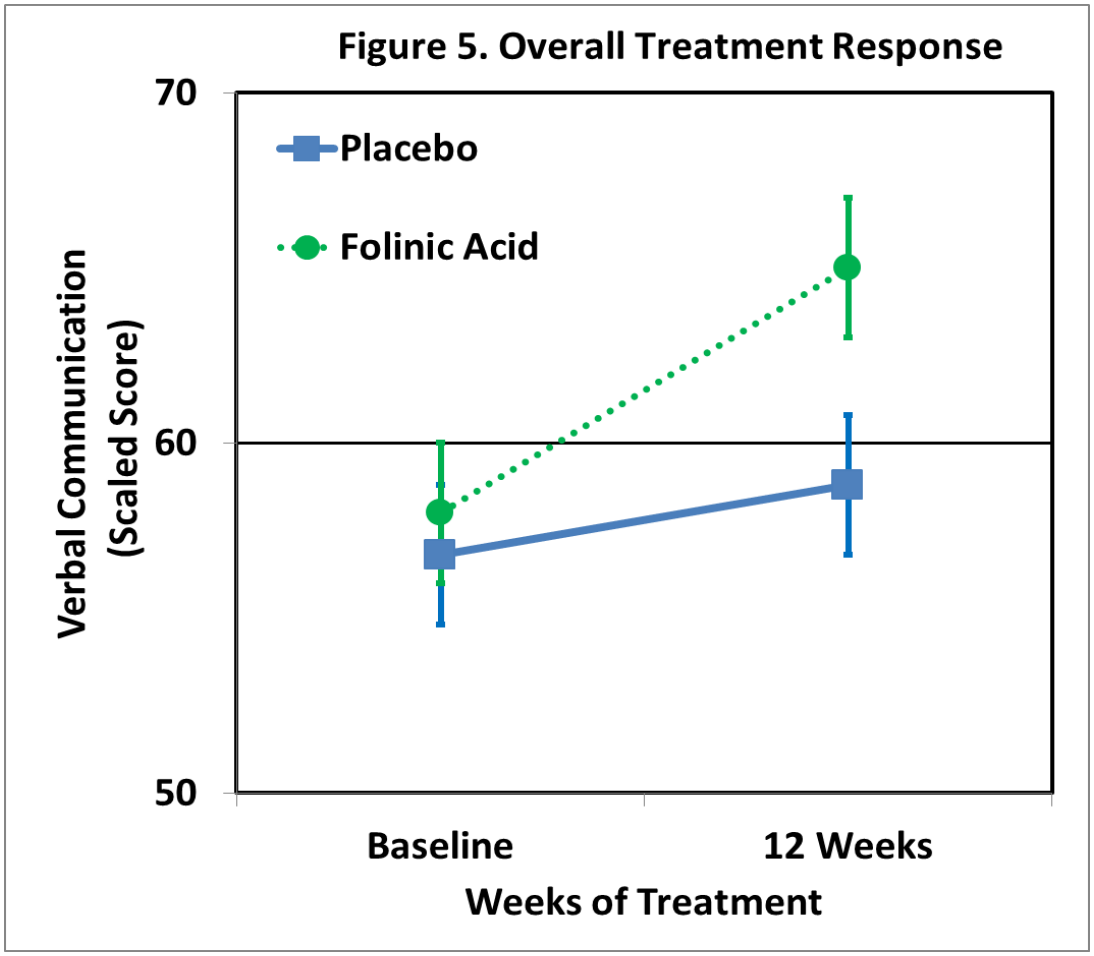


Figure 5. Overall Treatment Response
70
60
50
Placebo
Folinic Acid
Verbal Communication (Scaled Score)
Baseline
12 Weeks
Weeks of Treatment

	Effect Size	Responders			Folinic Acid Equivalent of Speech Therapy	
		Placebo	Folinic	NNT	Hours	Cost
Overall	0.70	24%	65%	2.4	185	$7,400
Negative	0.35	29%	50%	4.7	3	$120
Positive	0.91	22%	77%	1.8	177	$7,098

REVIEW ARTICLE
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Treatments for biomedical abnormalities associated with autism spectrum disorder

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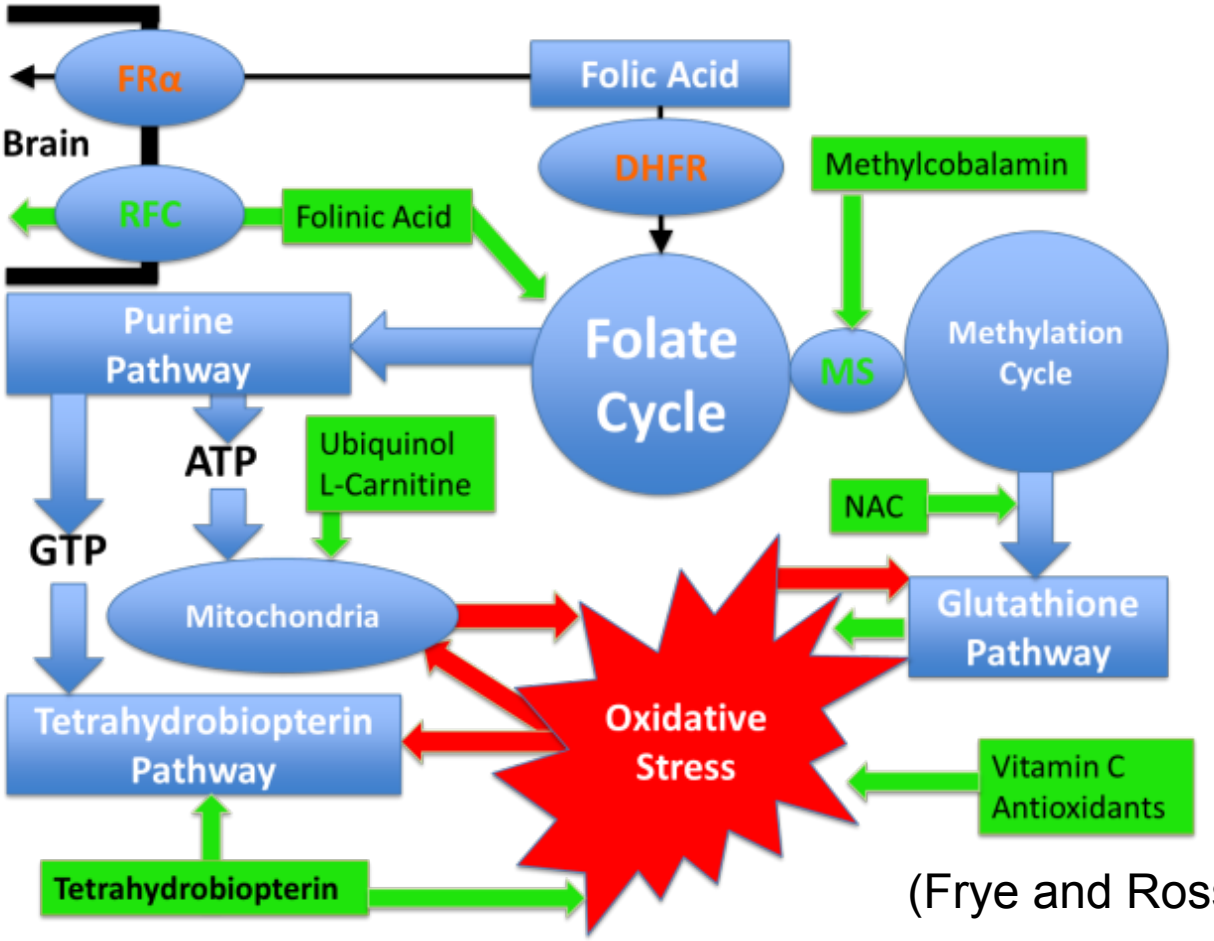
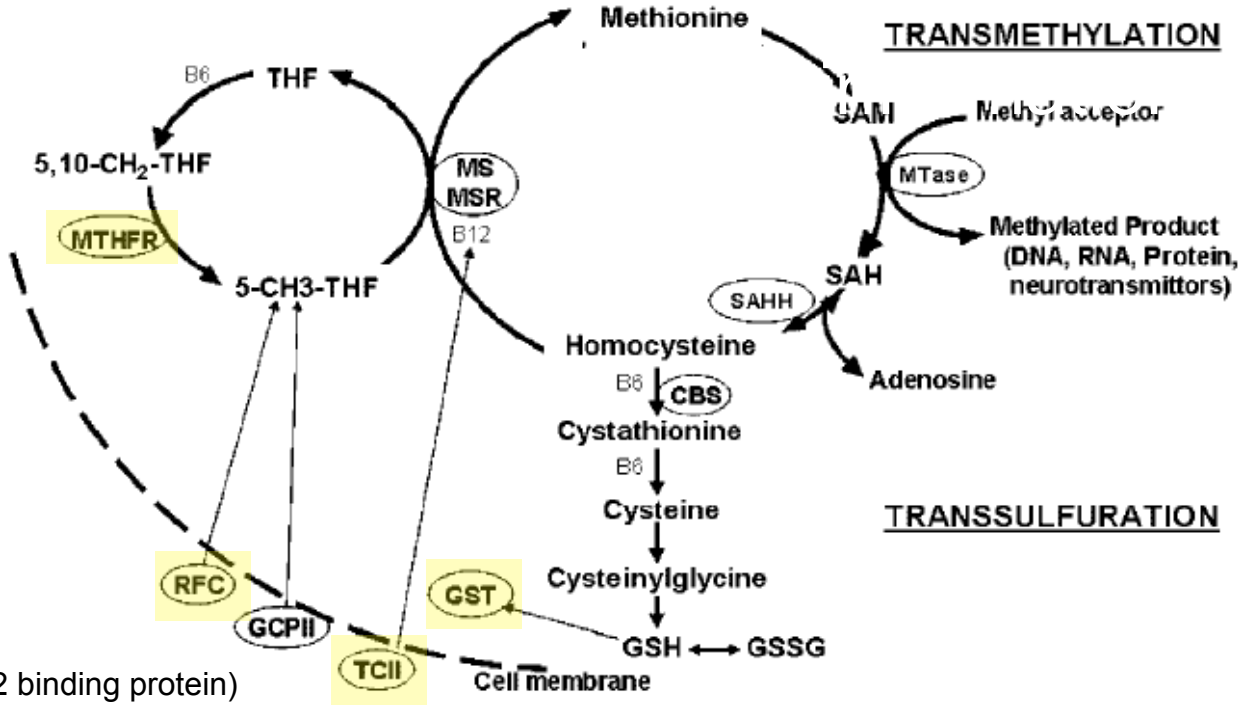


(Frye and Rossignol, 2014)


What about the Genetics?

Metabolic Endophenotype and Related Genotypes are Associated With Oxidative Stress in Children With Autism

S. Jill James,[1]* Stepan Melnyk,[1] Stefanie Jernigan,[1] Mario A. Cleves,[1] Charles H. Halsted,[2] Donna H. Wong,[2] Paul Cutler,[3] Kenneth Bock,[4] Marvin Boris,[5] J. Jeffrey Bradstreet,[6] Sidney M. Baker,[7] and David W. Gaylor[8]



RFC Reduced folate carrier
TCN2 Transcobalamin II (B12 binding protein)
MTHFR Methylenetetrahydrofolate reductase
GST Gluthathione S-Transferase

Metabolic Endophenotype and Related Genotypes are Associated With Oxidative Stress in Children With Autism

S. Jill James,[1]* Stepan Melnyk,[1] Stefanie Jernigan,[1] Mario A. Cleves,[1] Charles H. Halsted,[2] Donna H. Wong,[2] Paul Cutler,[3] Kenneth Bock,[4] Marvin Boris,[5] J. Jeffrey Bradstreet,[6] Sidney M. Baker,[7] and David W. Gaylor[8]

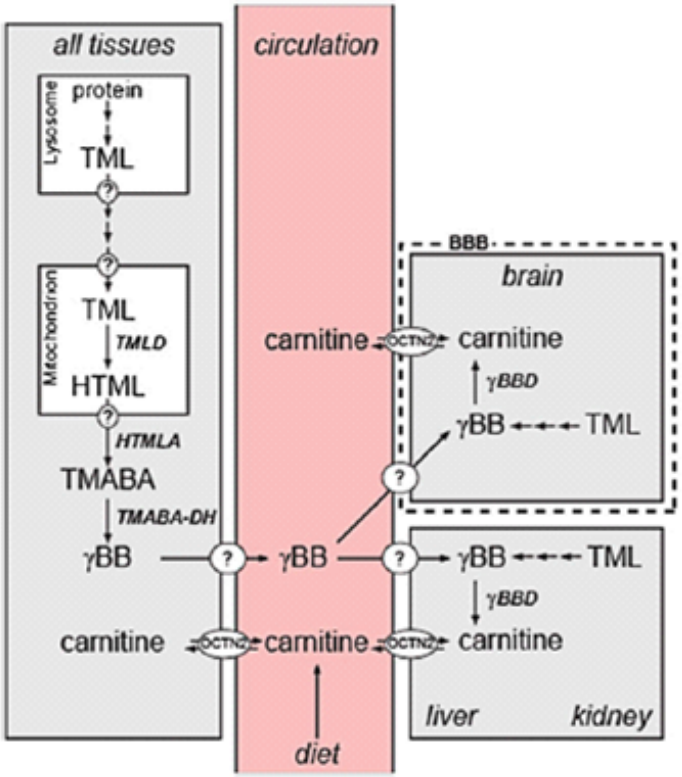
SNP	Genotype	OR (95% CI)
TCN2 776C > G/*COMT* 472G > A	CC/CC	Reference
	GG/GG	7.0 (2.32, 21.2)
RFC-1 80A > G/*MTHFR677C* > *T*	AA/CC	Reference
	GA/CT	3.24 (1.55, 6.78)
	GA/TT	4.40 (1.45, 14.0)
	GG/CT	3.10 (1.39, 6.84)
RFC-1 80A > G/*GSTM1* Null	AA/ ++	Reference
	GA/null	3.78 (1.80, 7.95)
	GG/null	2.67 (1.22, 5.89)
MTHFR 677 CT/*MTHFR* 1298AC	CT/AC	1.78 (0.97, 3.26)
MTHFR 677CT/1298AC/RFC 80G	(CT/AC)/GA	1.33 (1.33, 15.81)
	(CT/AC)/GG	3.57 (0.97, 13.49)

A common X-linked inborn error of carnitine biosynthesis may be a risk factor for nondysmorphic autism

Patrícia B. S. Celestino-Soper[a,1], Sara Violante[b,c,1], Emily L. Crawford[d], Rui Luo[e], Anath C. Lionel[f], Elsa Delaby[g], Guiqing Cai[h], Bekim Sadikovic[a], Kwanghyuk Lee[a], Charlene Lo[a], Kun Gao[e], Richard E. Person[a], Timothy J. Moss[a], Jennifer R. German[a], Ni Huang[i], Marwan Shinawi[a,j,2], Diane Treadwell-Deering[j,k], Peter Szatmari[l], Wendy Roberts[m], Bridget Fernandez[n], Richard J. Schroer[o], Roger E. Stevenson[o], Joseph D. Buxbaum[h], Catalina Betancur[g], Stephen W. Scherer[f,m], Stephan J. Sanders[p], Daniel H. Geschwind[e], James S. Sutcliffe[d], Matthew E. Hurles[i], Ronald J. A. Wanders[b], Chad A. Shaw[a], Suzanne M. Leal[a], Edwin H. Cook, Jr.[q], Robin P. Goin-Kochel[a,j,r], Frédéric M. Vaz[b,1], and Arthur L. Beaudet[a,j,r,1,3]

7974–7981 | PNAS | May 22, 2012 | vol. 109 | no. 21

Trimethyllysine hydroxylase epsilon (TMLHE) encodes the first enzyme in carnitine biosynthesis. TMLHE deficiency is common in control males (1 in 366) and not significantly increased in probands from simplex autism families (1 in 323). However, it was **2.82-fold more frequent in probands from male-male multiplex autism families (1 in 130; P = 0.023)**, suggesting that **TMLHE deficiency is a risk factor for autism although with low penetrance (2–4%)**. These data suggest that dysregulation of carnitine metabolism may be important in nondysmorphic autism



Reports of Children with Autism and Inborn Errors Of Metabolism are mostly Case Reports

Mitochondrial Disease (~25%)

Pyrimidine and Purine metabolism:

- Dihydropyrimidinase deficiency, Adenylosuccinate lyase deficiency
- Phosphoribosylpyrophosphate synthetase superactivity

Disorders of γ-aminobutyric acid metabolism:

- Succinic semialdehyde dehydrogenase deficiency

Carnitine Biosynthesis:

- 6-*N*-trimethyllysine dioxygenase deficiency

Disorders of amino acid metabolism:

- Phenylketonuria, Histidinemia, Branched Chain Ketoacid Dehydrogenase Kinase Deficiency

Disorders of Cholesterol Metabolism:

- Smith–Lemli–Opitz Syndrome

Disorders of creatine metabolism

Sulfation defects

Biotinidase deficiency

Urea Cycle Defects

- Ornithine transcarbamylase deficiency, Citrullinemia, Argininosuccinic aciduria,
- Carbamoyl phosphate synthetase deficiency

Lysosomal Storage Disease

- Sanfilippo syndrome, Infantile ceroid lipofuscinosis

Many Metabolic Abnormalities are Common in Both Genetics Disorders and Autism

Mitochondrial Disorders	
• Rett syndrome • Down syndrome • PTEN mutations	• 15q11-q13 duplication • Angelman syndrome • Septo-optic dysplasia
Redox Abnormalities	
• Rett syndrome • Down syndrome • Phenylketonuria	
Central Folate Abnormalities	
• Rett syndrome • Down syndrome	

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GENERAL PSYCHIATRY

ONLINE FIRST July 2011

Genetic Heritability and Shared Environmental Factors Among Twin Pairs With Autism

Joachim Hallmayer, MD; Sue Cleveland, BS; Andrea Torres, MA; Jennifer Phillips, PhD; Brianne Cohen, BA; Tiffany Torigoe, BA; Janet Miller, PhD; Angie Fedele, BA; Jack Collins, MBA; Karen Smith, BS; Linda Lotspeich, MD; Lisa A. Croen, PhD; Sally Ozonoff, PhD; Clara Lajonchere, PhD; Judith K. Grether, PhD; Neil Risch, PhD

Objective: To provide rigorous quantitative estimates of genetic heritability and effects of shared environment

Conclusion: Susceptibility to ASD has moderate genetic heritability (38%) and a substantial shared twin environmental component (58%)

Conclusions

- Autism is Associated with Several Metabolic Conditions, including those that affect Mitochondrial, Redox and Folate metabolism.
- Regardless of etiology of these metabolic abnormalities, there appears to be promising effective treatments that are safe with favorable adverse effects profiles.
- The etiology of many of these metabolic disorders is not clear but is most likely an interaction of polygenetic factors with environmental factors

- Many external environmental factors influences metabolic systems, including variations in the microbiome, dietary intake and toxicants.
- Metabolic systems are also influenced by abnormalities in the internal environment, including inflammation and oxidative stress.
- One of the most influential environment a child is exposed to is the fetal maternal environment, so diet, nutrition and health of the mother can have profound effects on the fetus, particularly through metabolic and immune influences

After participating in this presentation, clinicians should be better able to:

- Recommend changes to diet and nutrition to improve autism symptoms and seizures
- Discuss the profound influence of diet and nutrition can have on metabolic systems

Metabolism, Mitochondria, Microbiome and Personalized Nutrition in Autism & Seizures