Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

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Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

Estimated Prevalence of Genetic Abnormalities

(Schaefer and Mendelson, Genetics in Medicine, 2013: 15-399-407)

Cytogenetic Abnormalities	3%
Fragile X	1-5%
Rett Syndrome (Females only)	4% (~1% overall)
Chromosomal Microarray	10%
PTEN Mutation	<1%
Other	?? Estimated @ ~10%
Total	21-29%

This leaves 71%+ without an identified genetic diagnosis.

Inherited Metabolic Disorders – Mostly Case Reports

Mitochondrial Disease Cases (~25%)
6-*N*-trimethyllysine dioxygenase deficiency
Dihydropyrimidinase deficiency
Disorders of γ-aminobutyric acid metabolism
Carnitine Biosynthesis
Smith–Lemli–Opitz Syndrome
Adenylosuccinate lyase deficiency
Disorders of creatine metabolism
Sulfation defects
Branched Chain Ketoacid Dehydrogenase Kinase Deficiency
Phosphoribosylpyrophosphate synthetase superactivity
Succinic semialdehyde dehydrogenase deficiency

Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

Non-inherited Immune & Metabolic Conditions Associated with Autism

Mitochondrial Disorders	Redox -Folate Abnormalities	Immune Dysfunction
Mitochondrial Disease 75% no genetic abnormalities Electron Transport Chain Deficiencies in Immune Cells and Brain Tissue Electron Transport Chain Complex I and IV overactivity Acyl-carnitine Elevations	Decreased Glutathione, Methionine & Cysteine Reduced enzyme function Glutathione Peroxidase Increased oxidized Glutathione DNA, Proteins and Lipids MTHFR/DHFR Polymorphisms Cerebral Folate Abnormalities	Microglial Activation Elevated proinflammatory cytokines Autoantibodies Folate Receptor Alpha Basal Ganglia Endothelial Maternal fetal brain Ab

Genetics Disorders Associated with Autism & Metabolic Abnormalities

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

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)†

A review of 133 ASD patients evaluated for a mitochondrial disorder revealed a high prevalence of three biomarkers for mitochondrial disease after confirmation

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%

Frye. NAJMS 2012, 5:141-147

Mitochondrial Dysfunction in Autism.

Giulivi et al. JAMA 304:2389-2396

- 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

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

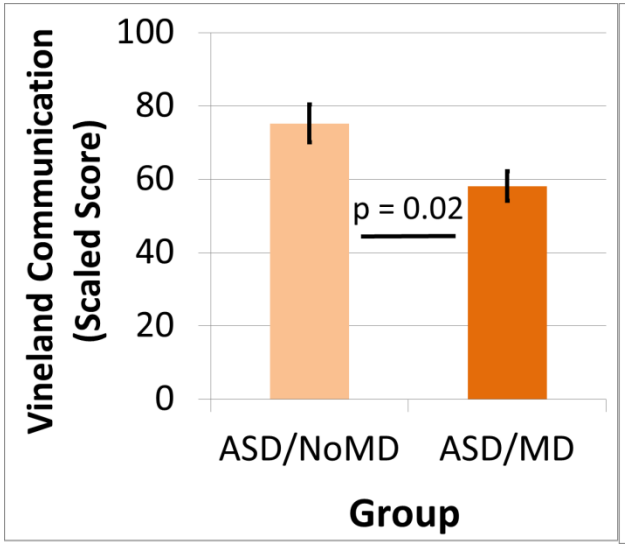
	ASD/MD		*General ASD*			*General MD*		
	%	N	%	χ^2	P	%	χ^2	P
Male	61	72	81	18.7	<0.0001	58	0.26	0.61
Developmental regression	52	83	25	32.3	<0.0001	60	2.2	0.14
Seizures	41	86	11	79.1	<0.0001	33	2.48	0.11
Hypotonia	62	55	51	2.6	0.10	67	0.62	0.43
Fatigue/lethargy	54	61				19	48.6	<0.0001
Ataxia	58	19				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

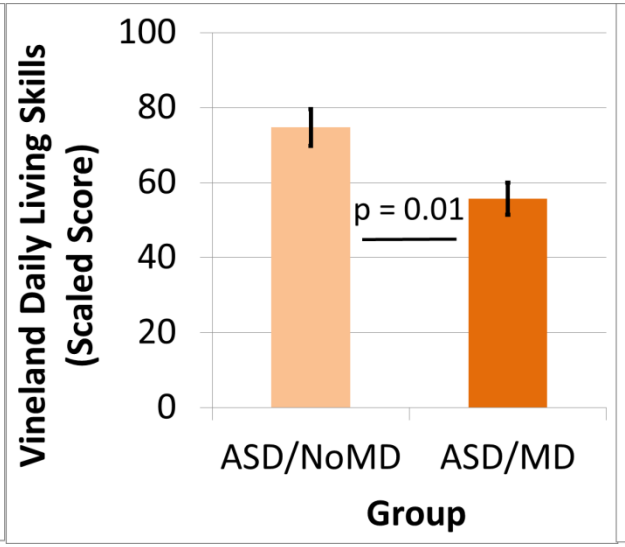
Redox metabolism abnormalities in autistic children associated with mitochondrial disease

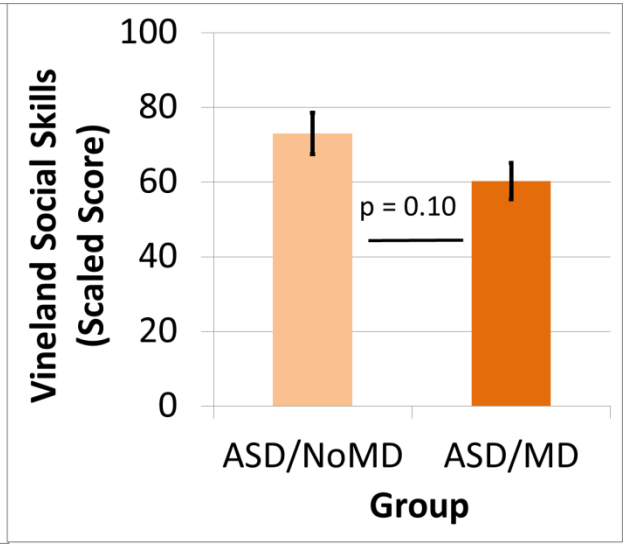
RE Frye[1,2], R DeLaTorre[3], H Taylor[4], J Slattery[1,2], S Melnyk[1,2], N Chowdhury[1] and SJ James[1,2]

Transl Psychiatry (2013) 3, e273; doi:10.1038/tp.2013.51

18 children with ASD and mitochondrial disease (ASD/MD) were compared to 18 children with ASD in which mitochondrial disease had been ruled out (ASD/NoMD). Development, as evaluated by the Vineland Adaptive Behavior Scale (2nd Edition), demonstrated significantly lower development in communication and daily living skills in children with ASD and mitochondrial disease.







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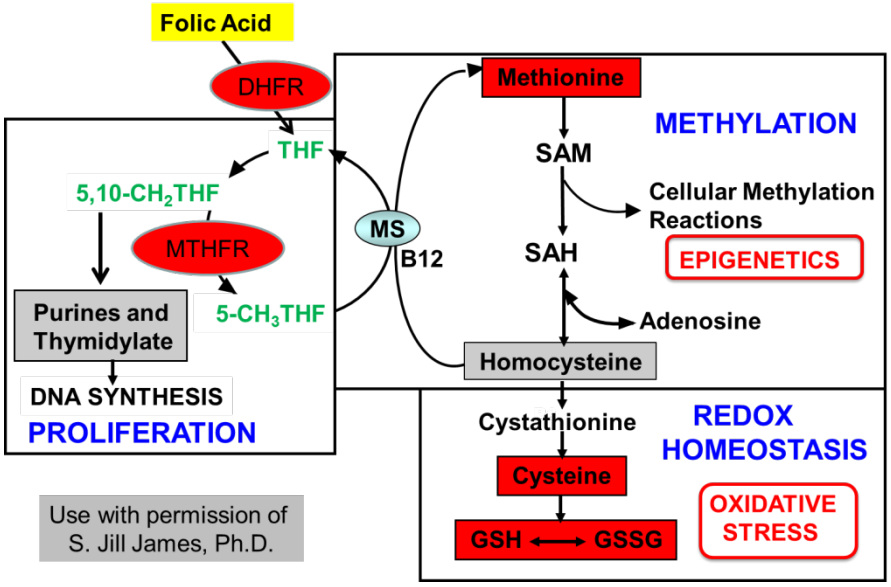
Redox metabolism is linked with methylation and antioxidant capacity (James et al., 2009)



Metabolites

THF	TetraHydroFolate
SAM	*S*-Adenosyl methionine
SAH	*S*-Adenosyl homocysteine
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione

Enzymes

DHFR	Dihydrofolate Reductase
MS	Methionine Synthase
MTHFR	Methylenetetrahydrofolate Reductase

Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses
Frustaci et al. Free Radical Biology and Medicine 2012; 52:2128-41.

- Significant reduction in blood GSH, Methionine and Cysteine
- Significant elevation in blood GSSG
- Significant reduction in blood glutathione peroxidase
- Significant association of MTHFR homozygous C677T polymorphism with ASD

Preliminary evidence for involvement of the folate gene polymorphism 19 bp deletion-DHFR in occurrence of autism. Adams et al. Neuro Letters. 2007, 422:24.
Implicates DHFR association with ASD, particular if MTHFR C677T is also present

Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

Glutathione Abnormalities are found in several tissues in children with ASD

Dr Jill James, Ph.D. and her group has demonstrated in case-control studies that glutathione antioxidant/detoxification capacity is decreased in lymphoblastoid cell lines, peripheral blood mononuclear cells and post-mortem brain from children with ASD (Rose et al. Autism Res Treat. 2012, 2012: 986519; Rose et al. Transl Psychiatry. 2012, 2:e134; James et al. FASEB J. 2009, 23:2374-83; James et al. Am J Med Genet. 2006, 141B:947)

Redox Abnormalities can lead to DNA, protein and lipid oxidative damage in ASD

Studies have demonstrated oxidative damage in children with ASD (Melnyk et al., JADD 2012, 42:367; Rose et al. Transl Psychiatry. 2012, 2:e134; Napoli et al. Mol Autism. 2013, 4:2; Meguid et al. Biol Trace Elem Res 2011, 143:58; Damodaran et al. Redox Rep 2011 16:216)

Redox Abnormalities may result in Epigenetic Changes in children with ASD

SAM, methionine and SAM/SAH ratio was decreased and DNA was hypomethylated in ASD children compared to unaffected sibling controls suggesting a reduced DNA methylation capacity which is essential for epigenetic regulation (Melnyk et al., JADD 2012, 42:367-77)

Redox abnormalities may be amendable to treatment

Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism. James et al. Am J Clin Nutr 2009; 89:425–30.

- Simple treatment of 75 µg/kg methylcobalamin every 3 days and 400mcg folinic acid every day significantly improved GSH, GSSG and GSH/GSSG ratio in ASD children

Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

Inherited Immune Abnormalities in Autism

- Studies find an increased incidence of autoimmune disease in families of ASD children
- Studies implicating Human Leukocyte Antigen Haplotypes with ASD are inconsistent (Careaga et al, Neurotheraputics 2010, 7:283)

Humoral Immune Abnormalities in Autism (and treatment)

- Studies have associated a variety of autoantibodies with ASD
  - Brain directed: Myelin basic protein, Endothelial cells, Serotonin receptor, caudate nucleus, cerebral cortex, cerebellum (Careaga et al, Neurotheraputics 2010, 7:283)
  - Folate Receptor Alpha (Frye et al., Mol Psych. 2013, 18:369)
  - Maternal Fetal Brain Antibodies (Fox et al. Dev Neurobiol. 2012, 72:1327)
- ASD is associated with reduced IgG and IgM levels and lower IgG levels correlate with higher aberrant behavior checklist scores (Heuer et al. Autism Res. 2008, 1:275-283)
- Small open-label studies have treated ASD patients with IVIG
  - Monthly treatment of 10 autism patients with underlying antibody deficiencies for 6 months resulted in improvements in behavior and autistic symptoms by subjective measures (Gupta et al. JADD. 1996, 26:439)
  - Monthly treatment of 10 autism patient with normal immune system with IVIG at 6 week intervals at a lower than standard dose resulted in symptom improvement in only one child (Plioplys. JCN. 1998, 13:79)

Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

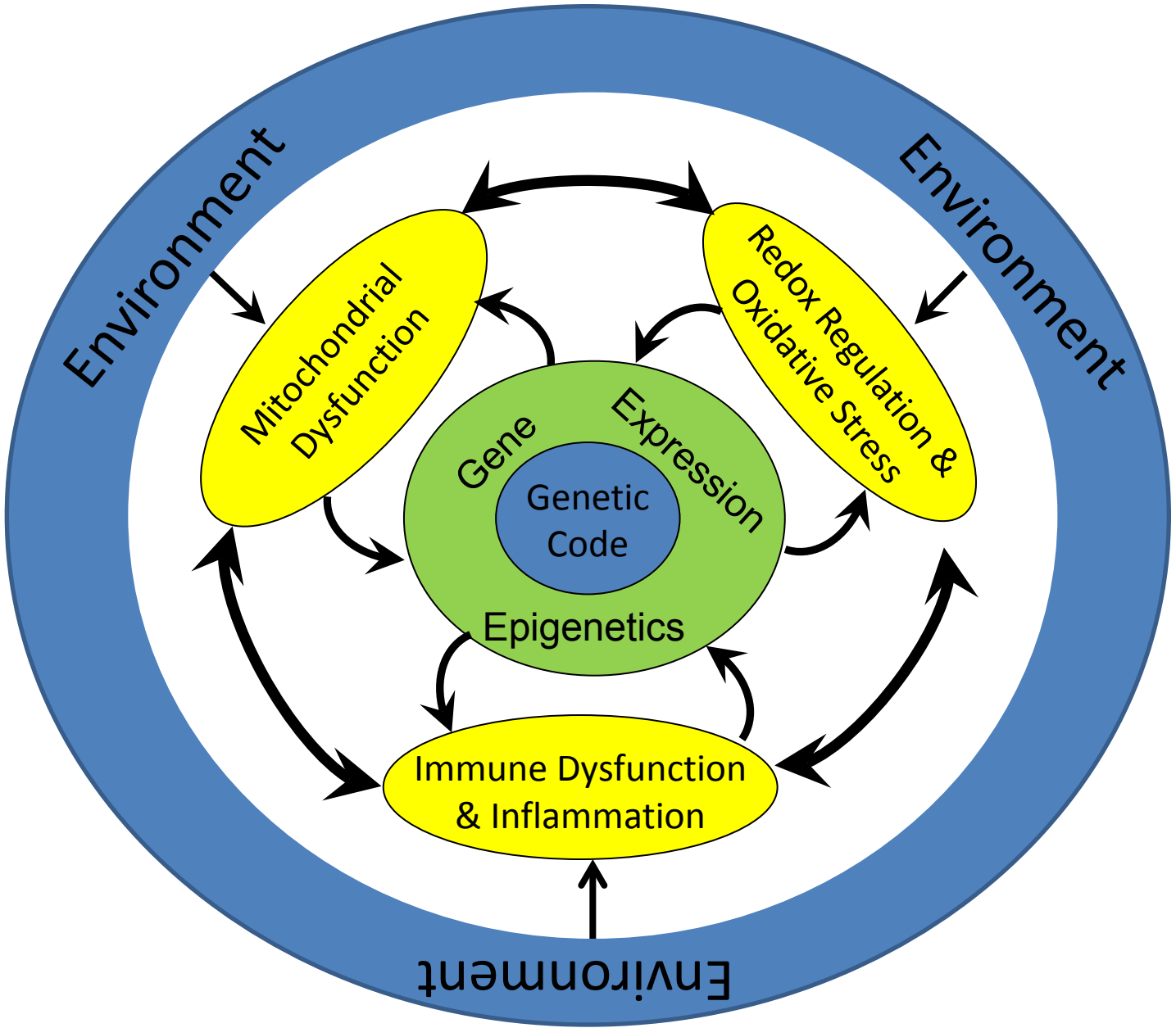
Proinflammatory cytokines are increased in the blood, brain tissue and cerebrospinal fluid

- Post-mortem brain tissue of ASD individuals demonstrated increases in TGF-β1, MCP-1, IGFBP-1 with production from reactive astrocytes (Vargus et al., Ann Neuro. 2005, 57:67)
- CSF from individuals with ASD demonstrates a wide variety of proinflammatory cytokines: IFN-γ, TGF-β2, MCP-1, IL-8, IP-10, VEGF, IGFBP-1, IGFBP-3, IGFBP-4. LIF, FGF-4, FGF-9, PARC, HGF (Vargus et al., Ann Neuro. 2005, 57:67) and TNF-α (Chez et al. Pediatr Neurol 2007, 36:361)

Microglial activation may play a role in autism

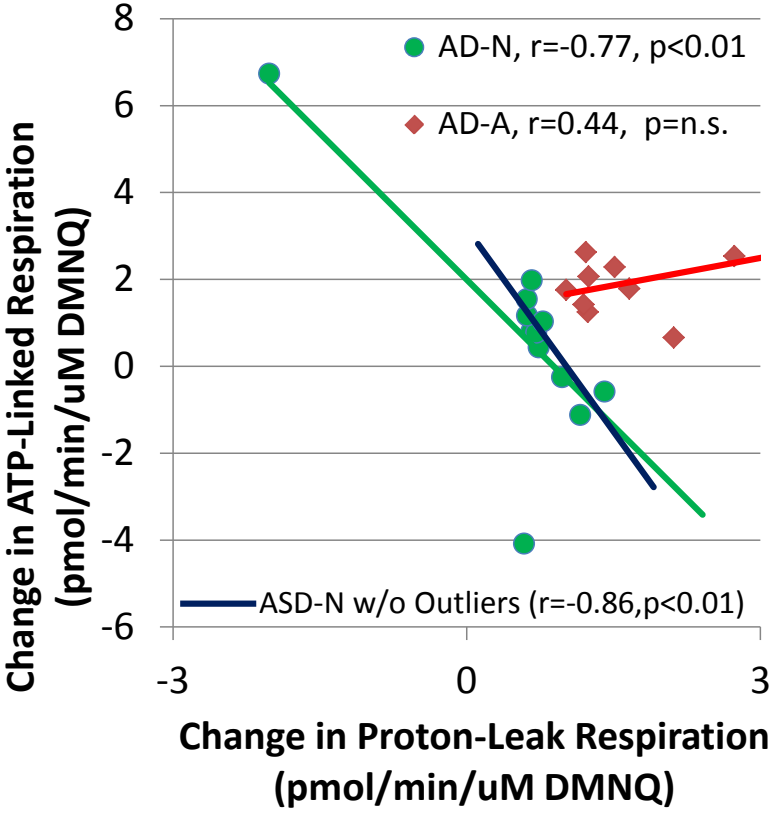
- Post-mortem histology of ASD brains showed microglial activation associated with neuron cell loss particularly in the cerebellum (Vargus et al., Ann Neuro. 2005, 57:67)
- Wild-type bone marrow transplant and targeted expression of MECP2 in myeloid cells resulted in normalization or marked attenuation of most symptoms in the Rett mouse model. Microglial phagocytic activity was key (Derecki et al., Nature 2012. 484:105)
- Other studies have supported activation of microglial in ASD using post-mortem (Morgan et al, Bio Psychiatry. 2010, 68:368; Tetreault et al, JADD 2012. 42:2569) and PET imaging (Suzuki et al, JAMA Psychiatry 2013, 70:49) techniques.
- Ten children with ASD were treated with minocycline, a drug believed to reduce neuroinflammation and microglial activation, in an open-label study for 6 months. No clinical improvement was found despite changes in BDNF, HGF and IL-8 (Pardo et al. J Neurodev Disord. 2013, 5:9).

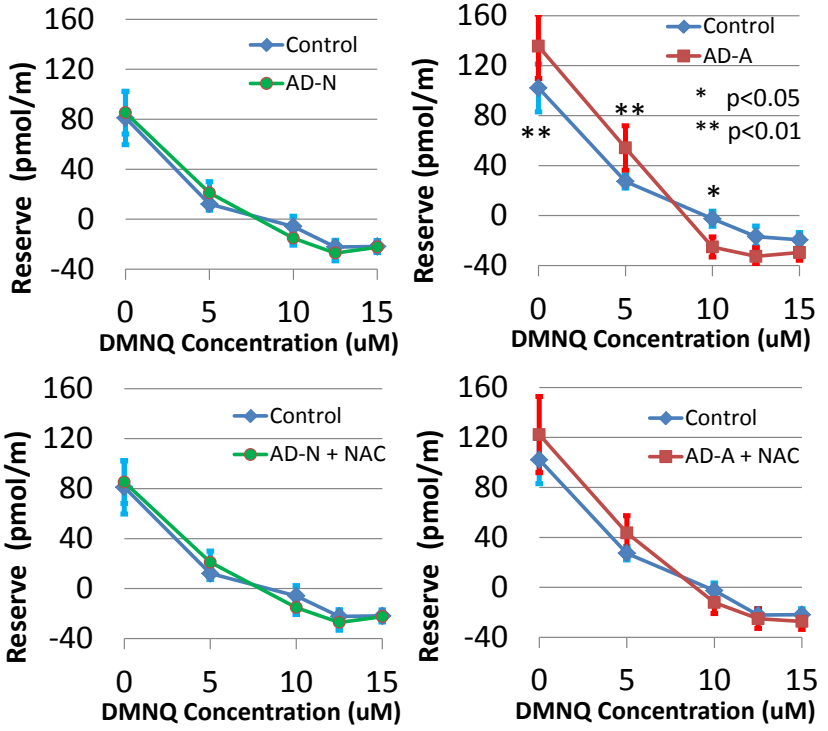
Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder



Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

Lymphoblastic cell lines (LCLs) were challenged with DMNQ (known to increase intracellular superoxide). Change in mitochondrial function in 22 LCLs derived from children with autistic disorder (AD) was compared to paired control LCLs. Two different patterns of change in mitochondrial function were found in the AD LCLs, but not in the control LCLs. **45%** of the AD LCLs demonstrated a unique change in mitochondrial function that was associated with a sharp reduction in reserve capacity suggesting vulnerability to oxidative stress. Incubating AD LCLs in N-acetyl-cysteine prior to the challenge normalized the reserve capacity in the abnormal group.

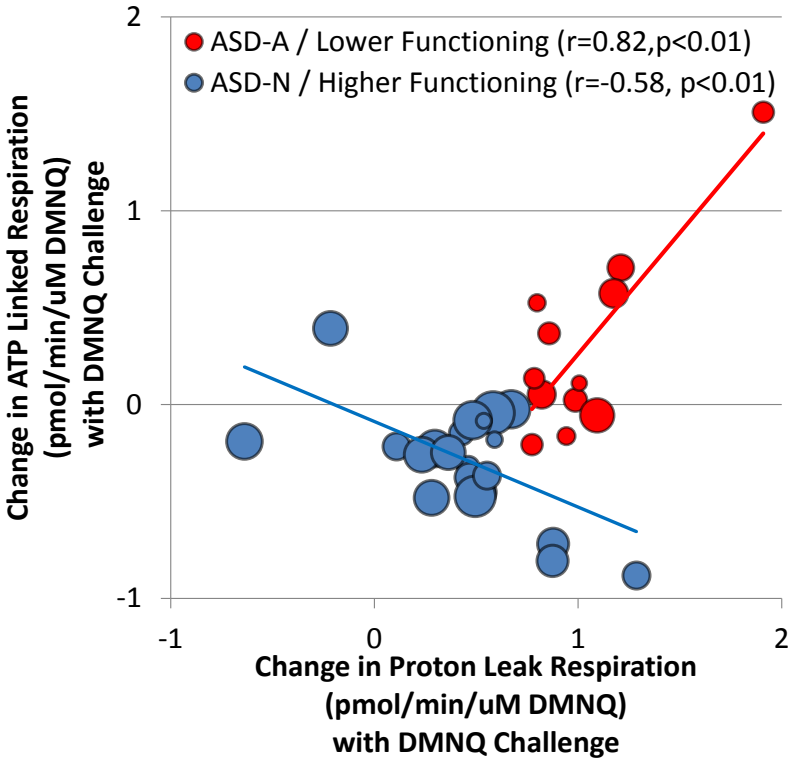


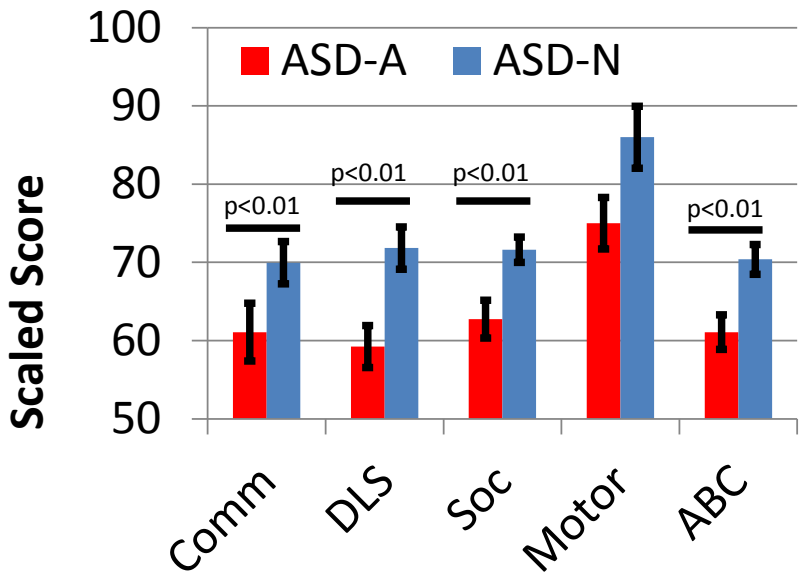


(Rose et al, Frye et al, IMFAR, 2013, Frye et al, UMDF 2013)

Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

The lymphoblastic cell line data was verified in children with autism spectrum disorder (ASD). We measured mitochondrial function in peripheral blood mononuclear cells (PBMCs) derived from 35 ASD children using the same DMNQ challenge. Again, two different patterns of change in mitochondrial function were found in ASD PBMCs (graph left). **34%** of the ASD PBMCs demonstrated the unique change in mitochondrial function associated with a reduction in reserve capacity. The Vineland Adaptive Behavior Scale demonstrated that these ASD children with atypical mitochondrial function had significantly poorer development (graph right).





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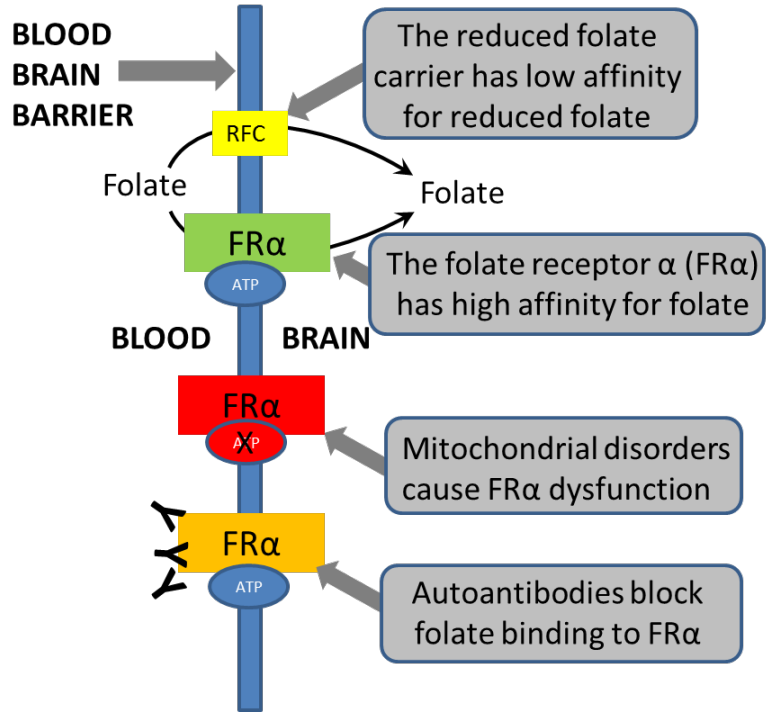
Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder



Autoantibodies have been linked to cerebral folate abnormalities in children with autism spectrum disorder in case studies and case series. (Moretti et al., Neurology 2005, 64:1088; JADD 2008, 38:1170; Ramaekers et al. NEJM 2005, 352:1985; Neuropediatrics 2007, 38:276; Dev Med Child Neurol 2008, 50:346)

Cerebral folate receptor autoantibodies in autism spectrum disorder. Frye et al. Mol Psych 2013, 18:369. Studied 93 Patients

- 75% had at least one FRα autoantibody
- **60%** had blocking FRα autoantibody
- 44% had binding FRα autoantibody

Mitochondrial disorders have been linked to cerebral folate deficiency. (Allen et al. Ann Neurol 1983, 13:679; Pineda et al. Ann Neurol 2006, 59:394; Ramaekers et al Neuroped 2007, 38:184; Garcia-Cazorla et al Neurol 2008; 70:1360; Frye, Naviaux JPN 2011. 9:427)

Role of folate receptor autoantibodies in infantile autism. Ramaekers et al. Mol Psych 2013, 18:270 Blocking autoantibody was found in **47%** of ASD children as compared to 3% of developmentally delayed non-autistic controls.

Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

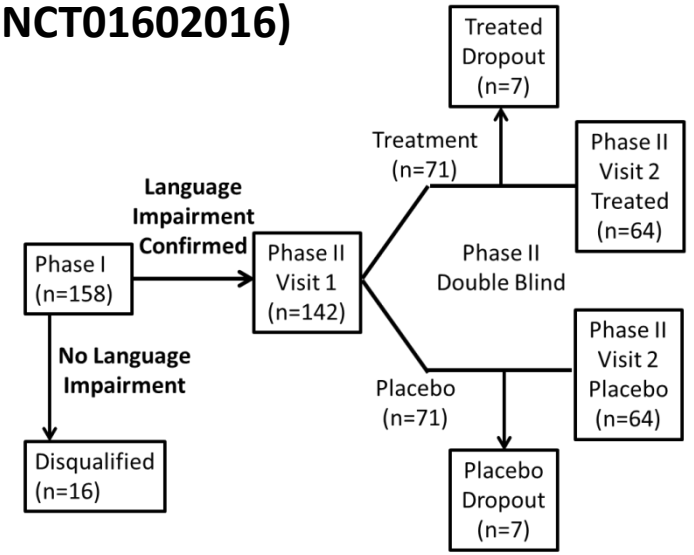
High-dose folinic acid (leucovorin calcium) treatment in ASD children with the folate receptor alpha autoantibody or cerebral folate deficiency have good outcomes in preliminary studies

Five Children with low-functioning ASD with neurological deficits. Ramaekers et al. NEJM 2005, 352:1985
20% complete recovery
40% marked improvement in communication

18 low-functioning regressive ASD children treated. Ramaekers et al. Neuropediatrics 2007, 38:276
11% amelioration of all ASD and neurologic symptoms
17% amelioration of all neurological symptoms
Others (72%) partial improvement
31% amelioration of social symptoms
69% amelioration of communication symptoms
46% amelioration of repetitive behavior and restricted interest

Cerebral folate receptor autoantibodies in autism spectrum disorder. Frye et al. Mol Psych 2013, 18:369. 44 patients with FRα autoantibody treated and compared to controls. Significant improvement in expressive and receptive language, stereotyped behavior and attention.

A DBPC study on folinic acid for ASD (NCT01602016)



Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

Major Importance of studying metabolic and immune disorders in ASD

Understanding these disorders provides a pathway for treatment with existing medications and protocols and the potential for prevention

Key Research Issues for Metabolic and Immune Disorders in Autism

- How are they defined and what is the diagnostic criteria ?
- What biomarkers can accurately reflect underlying abnormalities ?
- How can we screening children with ASD for these disorders ?
- What is the prevalence of each disorder in autism ?
- What is the significance of each disorder for autism ?
- What treatments are effective to both correct physiological abnormalities and substantially improve development ?

Keys Questions for Each Major Metabolic Disorder

Mitochondrial Disorders	Disease vs Dysfunction
Oxidative Stress	Acute vs Chronic
Immune Dysfunction	Inflammation vs Dysregulation
	Role of Antibodies, Microglial and Cytokines

Immune and Metabolic Conditions in Patients with Autism Spectrum Disorder

Open

Molecular Psychiatry (2011), 1–13

www.nature.com/mp

REVIEW

A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures

DA Rossignol[1] and RE Frye[2]

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