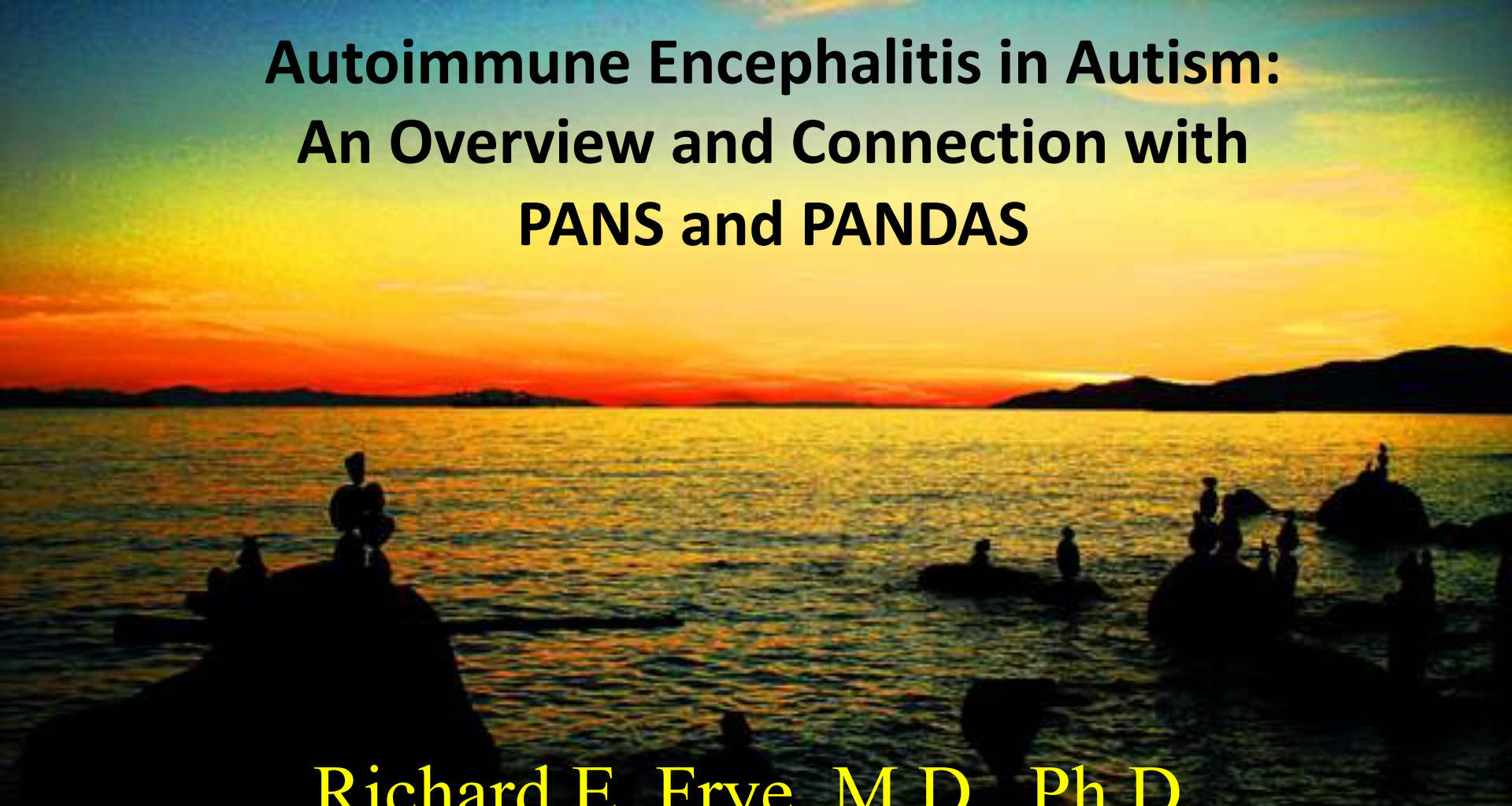
Autoimmune Encephalitis in Autism: An Overview and Connection with PANS and PANDAS



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Autoimmune Family History in Children with Autism

Table 1. *Epidemiological Studies of Autoimmunity and Immune dysfunction in Families of Children with ASD*

References	Study population, no.	Reporting	Association with ASD?	Autoimmune diseases and immune dysfunction
Comi et al.[5] (1999)	107	Self-report	Yes	Rheumatoid arthritis (mat); general autoimmunity (mat, pat)
Sweeten et al.[6] (2003)	303	Self-report	Yes	Hypothyroidism and Hashimoto's thyroiditis (mat, pat); rheumatic fever (mat, pat)
Micali et al.[7] (2004)	140	Self-report	No	—
Croen et al.[3] (2005)	2,520	Medical records	Yes	Psoriasis (mat), asthma and allergies
Molloy et al.[8] (2006)	308	Self-report	Yes	Autoimmune thyroid disease (mat, pat)*
Mouridsen et al.[9] (2007)	441	Medical records	Yes	Ulcerative colitis (mat); type 1 diabetes
Valicenti-McDermott et al.[10] (2008)	100	Self-report	Yes	Rheumatoid arthritis (mat)†; celiac disease (mat)†
Atladóttir et al.[2] (2009)	689,196	Medical records	Yes	Rheumatoid arthritis (mat); celiac disease (mat); type 1 diabetes (mat, pat)

ASD = autism spectrum disorder; mat = maternal (autoimmunity link in mothers); pat = paternal (autoimmunity link in fathers).
*Autoimmune thyroid disease was found to be associated with the families of children with regressive ASD. †Rheumatoid arthritis and celiac disease in this study were associated with language regression.

Neuroglial Activation and Neuroinflammation in the Brain of Patients with Autism

Diana L. Vargas, MD,[1,2] Caterina Nascimbene, MD,[1–3] Chitra Krishnan, MHS,[1] Andrew W. Zimmerman, MD,[1,4] and Carlos A. Pardo, MD[1,2,5]

Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. To investigate whether immune-mediated mechanisms are involved in the pathogenesis of autism, we used immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent assays to study brain tissues and cerebrospinal fluid (CSF) from autistic patients and determined the magnitude of neuroglial and inflammatory reactions and their cytokine expression profiles. Brain tissues from cerebellum, midfrontal, and cingulate gyrus obtained at autopsy from 11 patients with autism were used for morphological studies. Fresh-frozen tissues available from seven patients and CSF from six living autistic patients were used for cytokine protein profiling. We demonstrate an active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemoattractant protein (MCP)–1 and tumor growth factor–β1, derived from neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1. Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain.

Ann Neurol 2005;57:67–81

Immune Dysfunction in Autism

Neuroglial Activation and Neuroinflammation in the Brain of Patients with Autism

Diana L. Vargas, MD,[1,2] Caterina Nascimbene, MD,[1–3] Chitra Krishnan, MHS,[1]
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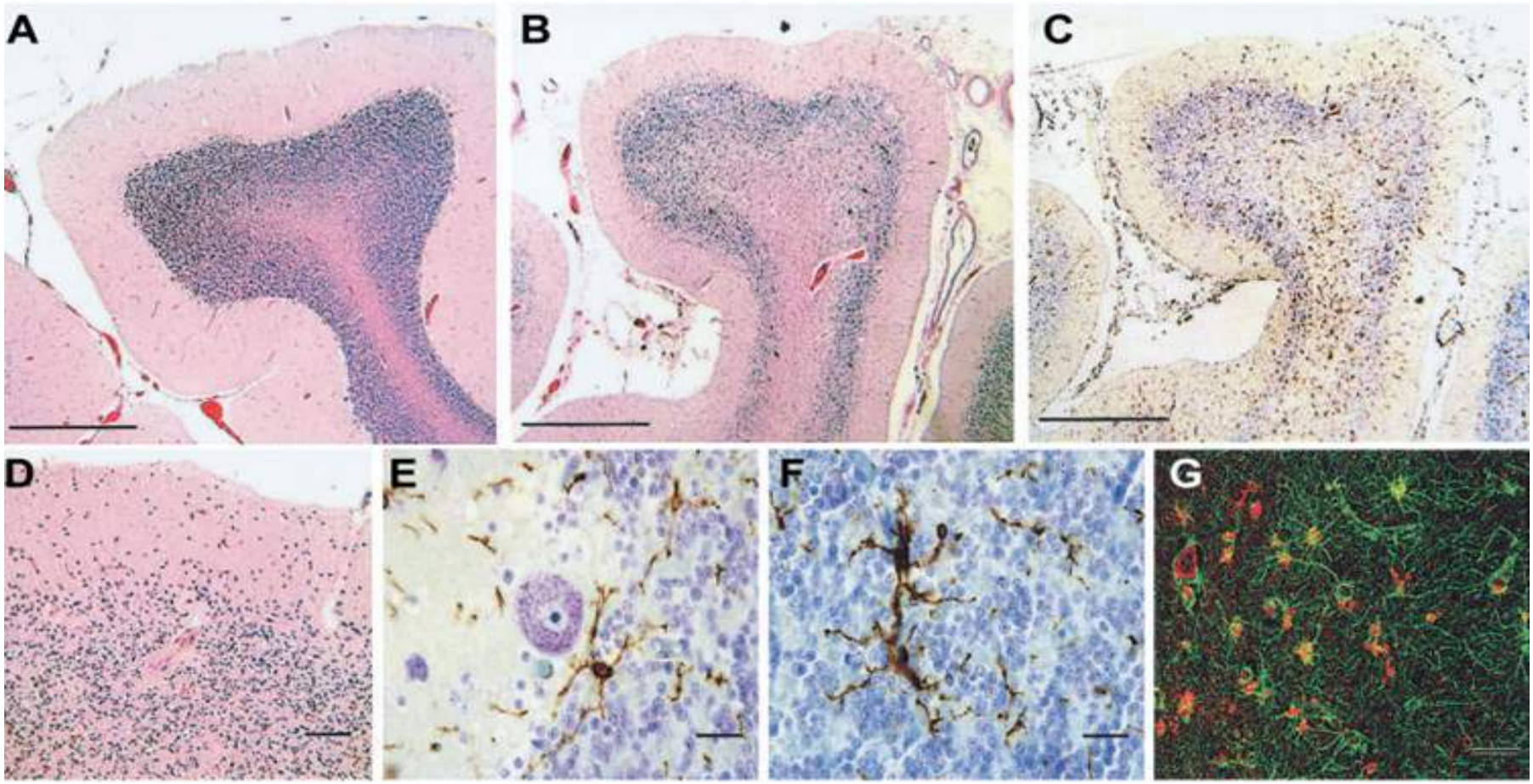
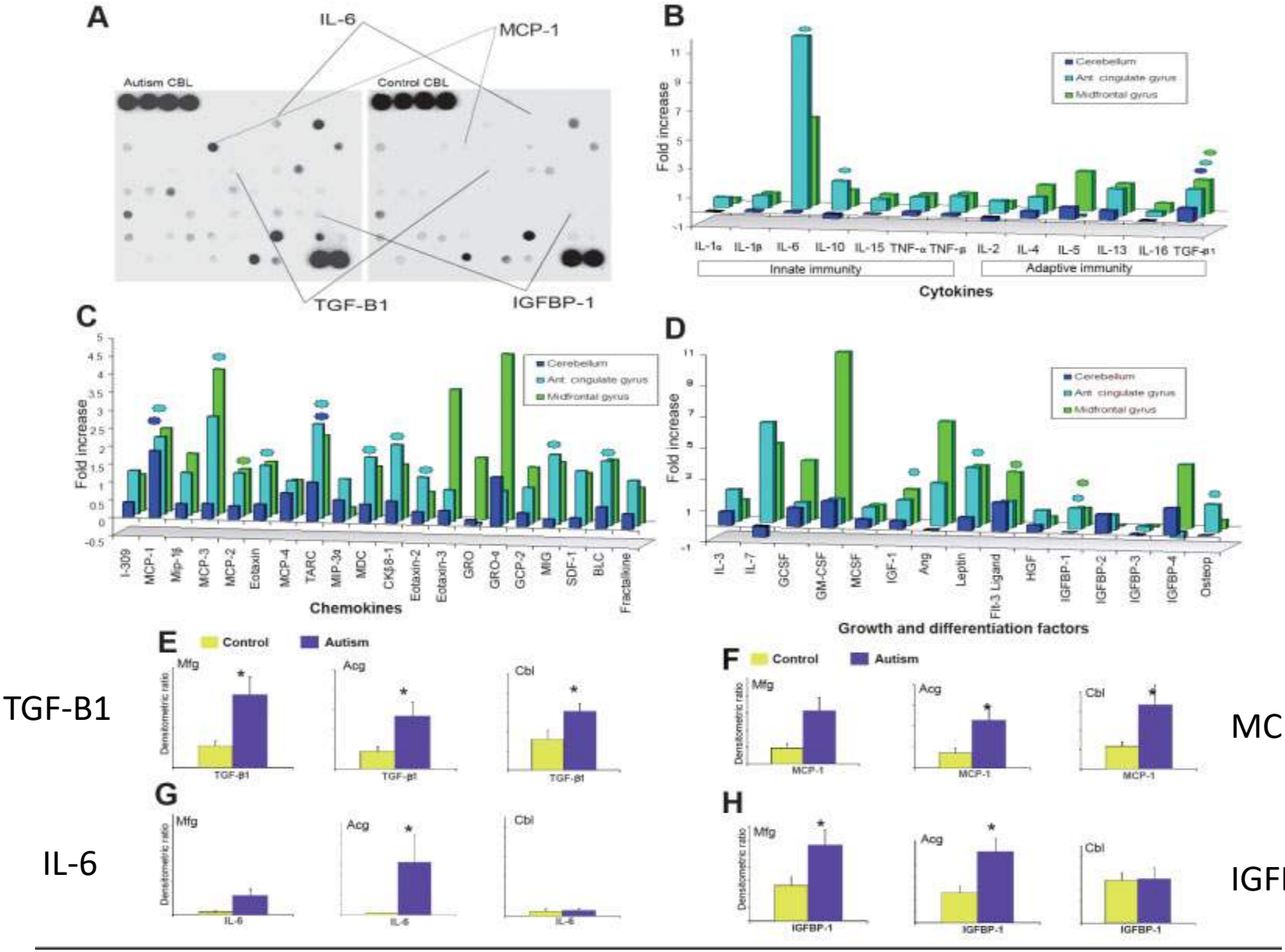


Immune Dysfunction in Autism



TGF-B1

IL-6

MCP-1

IGFBP-1

Immune Dysfunction in Autism

Table 7. Cytokines with Significant Increase in the Cerebrospinal Fluid of Patients with Autism

Cytokine	Fold Increase	p[a]
IFN-γ	232.5	0.008
TGF-β2	30.9	<0.001
MCP-1[b]	12.2	<0.001
IL-8	6.0	<0.001
IP-10	18.2	0.018
Angiogenin	3.3	0.003
VEGF	81.8	0.001
IGFBP-1[b]	0.4	0.036
IGFBP-3	26.3	<0.001
IGFBP-4	13.3	0.003
LIF	1.0	<0.001
FGF-4	0.23	0.005
FGF-9	70.0	0.012
PARC	11.3	0.002
Osteoprotegerin[b]	5.2	0.002
HGF	0.3	0.005
IGFBP-3	26.3	<0.001
IGFBP-4	13.3	0.003

[a]Mann–Whitney *U* test.
[b]Found significantly increased also in one or more brain regions in brain tissue analysis.

IFN = interferon; TGF = tumor growth factor; MCP = macrophage chemoattractant protein; IL = interleukin; VEGF = vascular endothelial growth factor; IGFBP = insulin-like growth factor binding protein; LIF = leukemia inhibitory factor; HGF = hepatic growth factor.

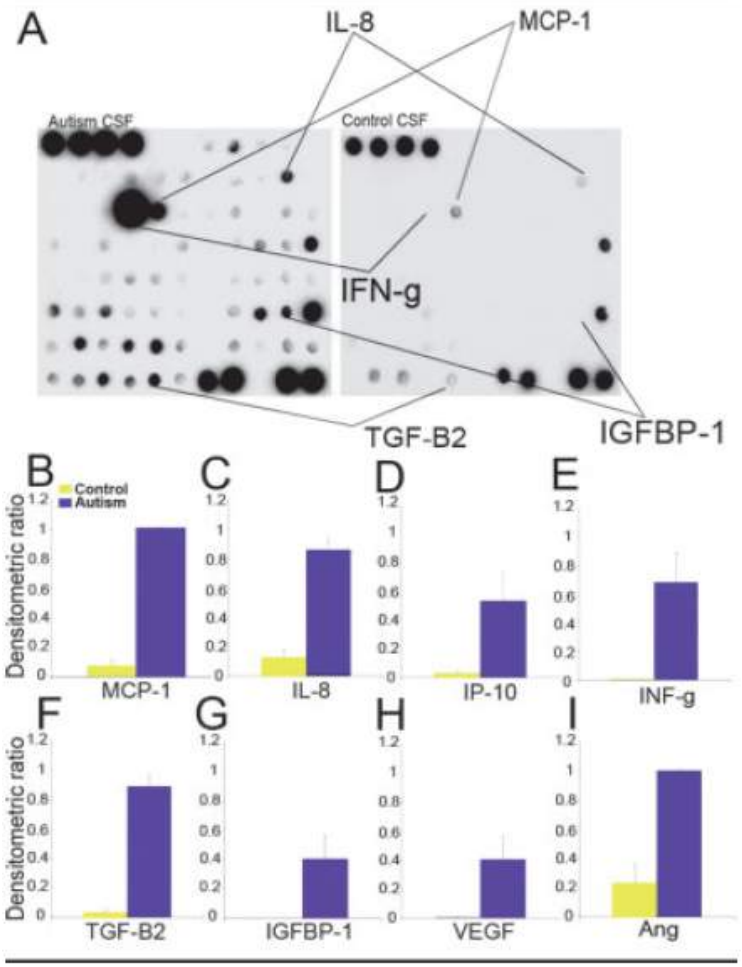


Fig 5. Cytokine profiles in cerebrospinal fluid (CSF) from autistic and control patients. (A) Cytokine protein arrays in CSF samples from an autistic patient and a control. The spots for macrophage chemoattractant protein (MCP)–1, interferon-γ, TGF-β2, interleukin-8, and IGFBP-1 showed a marked density increase as compared with the CSF control. (B–I) Profile of expression (fold increase) of cytokines that were found markedly increased in autistic patients as compared with controls. $p < 0.05$, *Mann–Whitney* U *test.*

Immune Dysfunction in Autism

Table 3. *Genetic Studies of Human Leukocyte Antigen (HLA) Haplotypes in Individuals with ASD*

References	Association with ASD?	HLA	Study population, no.	Region
Stubbs et al.[29] (1980)	No	Not available	20 families; 757 controls	Not specified
Stubbs et al.[30] (1985)	Yes	Shared HLA*	52 families; 83 families (historical)	Oregon and southern California; United Kingdom
Spence et al.[31] (1985)	No	Not available	27 families	Not specified
Warren et al.[32] (1992)	Yes	B44-SC30-DR4	21 families; 62 controls	Utah
Daniels et al.[33] (1995)	Yes	B44-SC30-DR4	44 families; 126 controls†	Utah
Warren et al.[34] (1996)	Yes	HLA-DRB1	45 subjects; 79 controls	Not specified
Rogers et al.[35] (1999)	No	Not available	90 families	Not specified
Torres et al.[36] (2002)	Yes	HLA-DR4; HLA-DR13 (protective)	103 families	Oregon and Utah
Lee et al.[37] (2006)	Yes and No‡	HLA-DR4	16 and 33 families; 475 normal controls	Tennessee; United States
Guerini et al.[38] (2009)	No§	Microsatellite regions	37 families	Sardinia
Johnson et al.[39] (2009)	Yes	HLA-DR4‖	31 families	New Jersey

*Stubbs et al.[30] found that mothers of children with ASD had similar HLA types more often than did typically developing mother–child pairs.
†Daniels et al.[33] added 23 new families to the 1992 cohort of Warren et al., and added 64 new control subjects.
‡Lee et al.[37] Differences were found between the geographical defined families and controls, but not between the geographical diverse families and controls.
§Guerini et al.[38] did not find an HLA linkage, but did find linkages to microsatellite regions in proximity to previously reported HLA linkages.
‖Johnson et al.[39] found that HLA DR4 was associated with mothers of children with ASD.

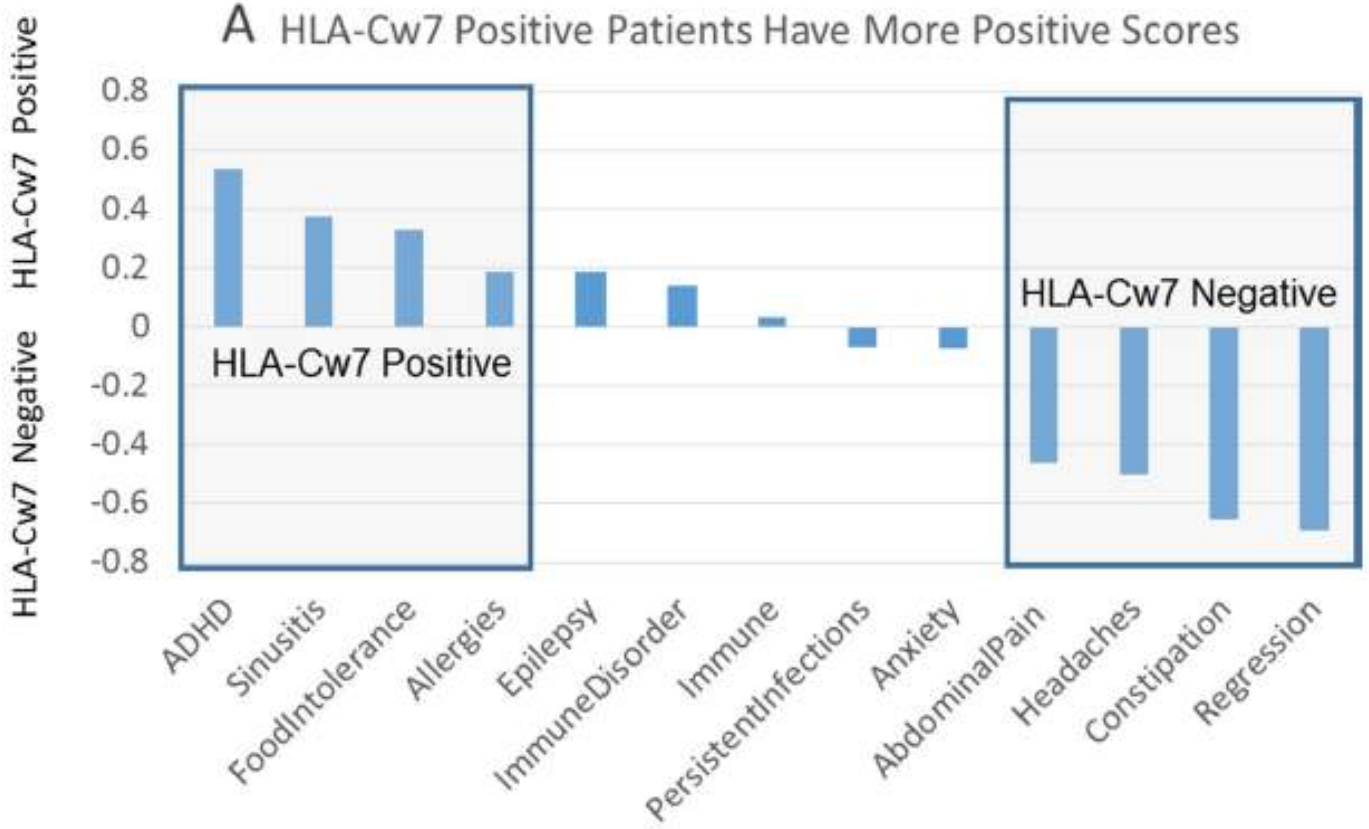
Immune Dysfunction in Autism

Inheritance of HLA-Cw7 Associated With Autism Spectrum Disorder (ASD)

ORIGINAL RESEARCH
published: xx Month 2019
doi: 10.3389/fpsyt.2019.00612
frontiers in Psychiatry

*Terry Harville[1,2], Bobbie Rhodes-Clark[1], Sirish C. Bennuri[3,5], Leanna Delhey[4,5], John Slattery[6], Marie Tippett[3,5], Rebecca Wynne[7], Shannon Rose[3,5], Stephen Kahler[3,5] and Richard E. Frye[8,9]**

143 ASD samples
HLA-Cw7 was over represented
Suggest NK Cell Activation



Variations in Mitochondrial Respiration Differ in IL-1ß/IL-10 Ratio Based Subgroups in Autism Spectrum Disorders

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 20 February 2019
doi: 10.3389/fpsyt.2019.00071

Harumi Jyonouchi[1,2*], *Lee Geng*[1], *Shannon Rose*[3,4], *Sirish C. Bennuri*[3,4] and *Richard E. Frye*[5,6]

TABLE 5 | IL-1ß/IL-10 ratios and Mitochondrial function in ASD cells and non-ASD control cells.

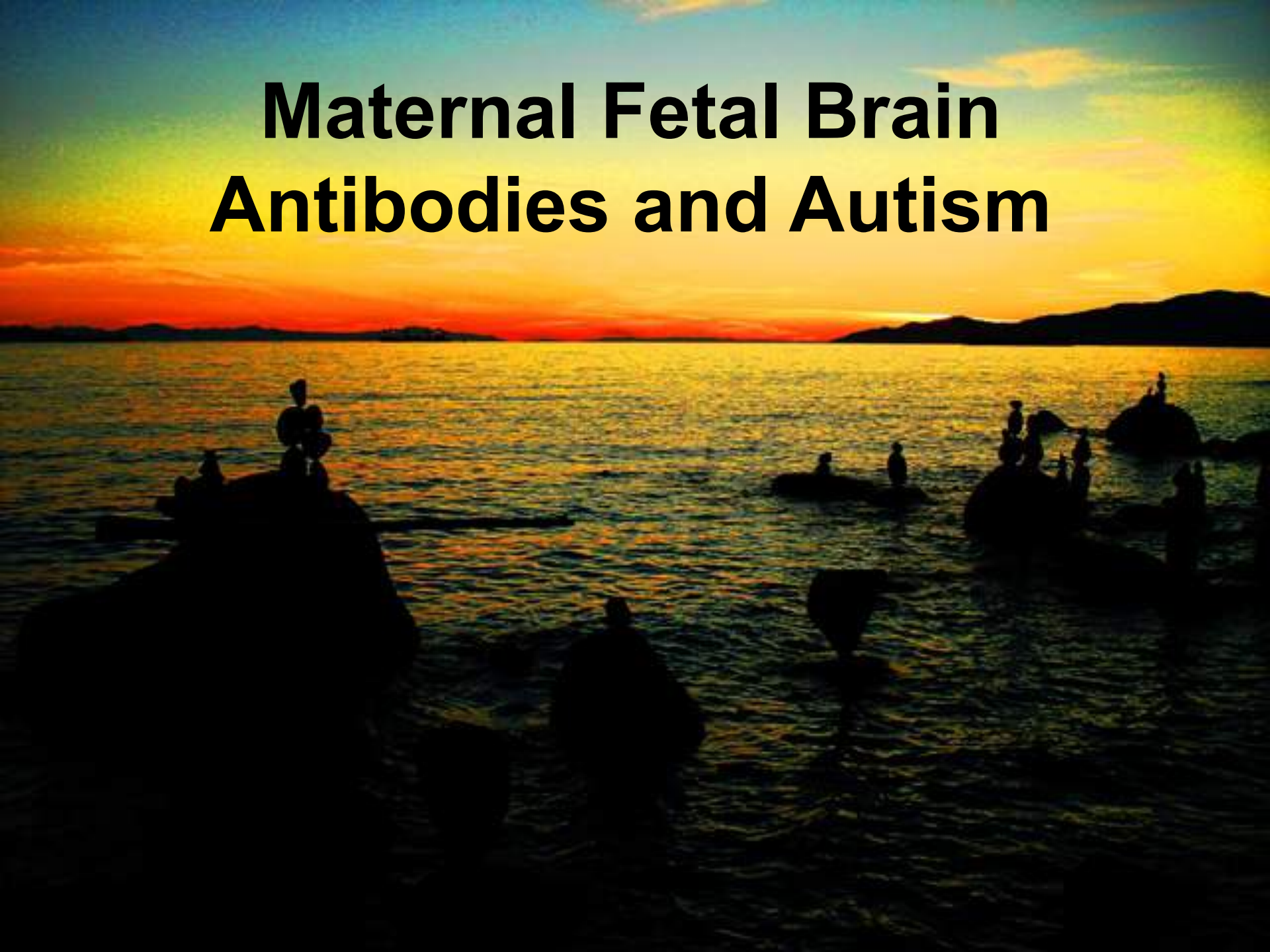
	IL-1ß/IL-10 ratio[a] based ASD cell subgroups			Non-ASD controls ($N = 38$)	Krushkal wallis test
	High ($N = 56$)[e]	Normal ($N = 59$)	Low ($N = 22$)		
IL-1ß/IL-10 RATIOS CULTURED WITH *P*-VALUE					
medium	1.54 ± 2.03[d]	0.79 ± 1.12	0.32 ± 0.30	0.88 ± 0.92	<0.00001
LPS[c]	2.03 ± 1.43	1.19 ± 0.51	0.77 ± 0.56	1.81 ± 2.02	<0.00001
Zymosan	5.86 ± 3.94	2.53 ± 0.87	1.42 ± 0.74	3.25 ± 1.98	<0.00001
CL097	9.40 ± 16.84	2.89 ± 2.84	2.72 ± 2.19	3.95 ± 2.77	0.00061
MITOCHONDRIAL RESPIRATION[b] *P*-VALUE					
PLR	6.2 ± 5.2	8.3 ± 5.2	7.5 ± 3.8	7.9 ± 5.8	0.06771
ALR	27.2 ± 10.8	31.2 ± 14.0	27.3 ± 15.7	27.7 ± 13.2	0.5153
MRC[f]	93.3 ± 57.2	104.7 ± 59.0	84.6 ± 59.3	70.8 ± 44.4	0.0269
RC	59.9 ± 49.5	64.6 ± 48.5	49.7 ± 47.9	35.1 ± 33.6	0.00788

Immune Dysfunction in Autism

Table 4. *The Presence of Antibodies Directed Against Adult Brain or CNS Tissue in Children with ASD*

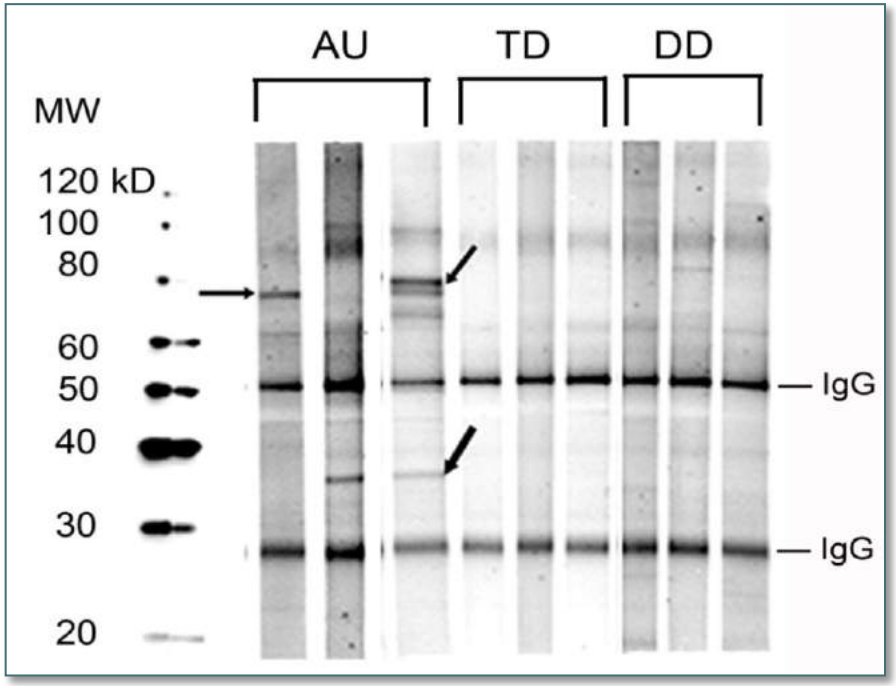
Study investigators (year)	Antibody Directed Toward	Positive or Negative?
Todd et al.[62] (1985)	Serotonin receptor	Positive
Singh et al.[63] (1993)	Myelin basic protein (MBP)	Positive
Singh et al.[64] (1997)	Neuron-axon acidic protein (NAFP); glial fibrillary acidic protein (GFAP)	Positive
Singh et al.[65] (1998)	Myelin basic protein (MBP); neuron-axon filament	Positive
Evers et al.[66] (2002)	Heat shock protein 90 (HSP90)	Positive
Vodjani et al.[67] (2004)	Gliadin; cerebellar peptides; heat shock protein 60 (HSP60)	Positive
Singh et al.[68] (2004)	Caudate nucleus; cerebral cortex; cerebellum	Positive
Singh et al.[69] (2004)	Nucleus and laminin	Negative
Silva et al.[20] (2004)	Unknown ~20 kDa protein	Positive*
Connolly et al.[70] (2006)	Brain-derived neurotrophic factor (BDNF); endothelial cells (EC); myelin basic protein	Positive
Singer et al.[71] (2006)	Unknown 73 and 100 kDa proteins	Positive
Libbey et al.[72] (2008)	Myelin basic protein	Negative
Kirkman et al.[73] (2008)	Glial fibrillary acidic protein (GFAP)	Negative
Wills et al.[74] (2009)	Unknown 52 kDa protein	Positive

*Silva et al.[69] found a positive finding for a ~20 kDa protein, but determined it not to be MBP.


Maternal Fetal Brain
Antibodies and Autism

Results of First Study

Study population	Number 37 kDa & 73 kDa band positive (%)
AU (n=61)	7 (12%)
TD (n=62)	0 (0%)
DD (n=40)	0 (0%)

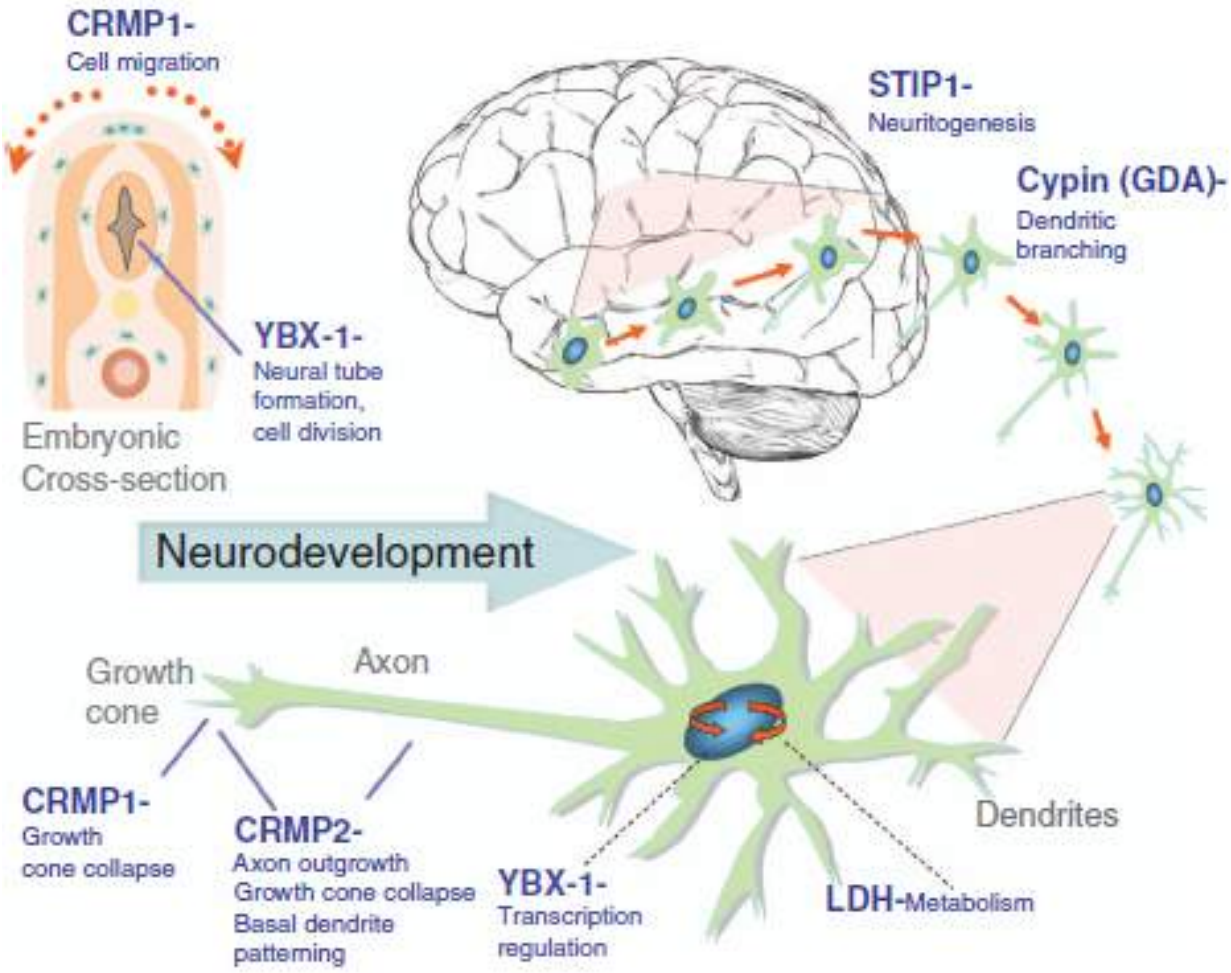


Neurotoxicology 29:226-231, 2008 E-Pub ahead of print (November 2007)

Abbreviations: AU = Autism; TD = Typically developing; DD = Developmental delays

Autism-specific maternal autoantibodies recognize critical proteins in developing brain

D Braunschweig[1,2,3], P Krakowiak[4], P Duncanson[1,2,3], R Boyce[1,2,3], RL Hansen[2,3,5], P Ashwood[2,3,6], I Hertz-Picciotto[2,3,4], IN Pessah[2,3,7] and J Van de Water[1,2,3]




Immune Abnormalities
Disrupt Folate Metabolism
in Autism

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.

Immune Dysfunction in Autism

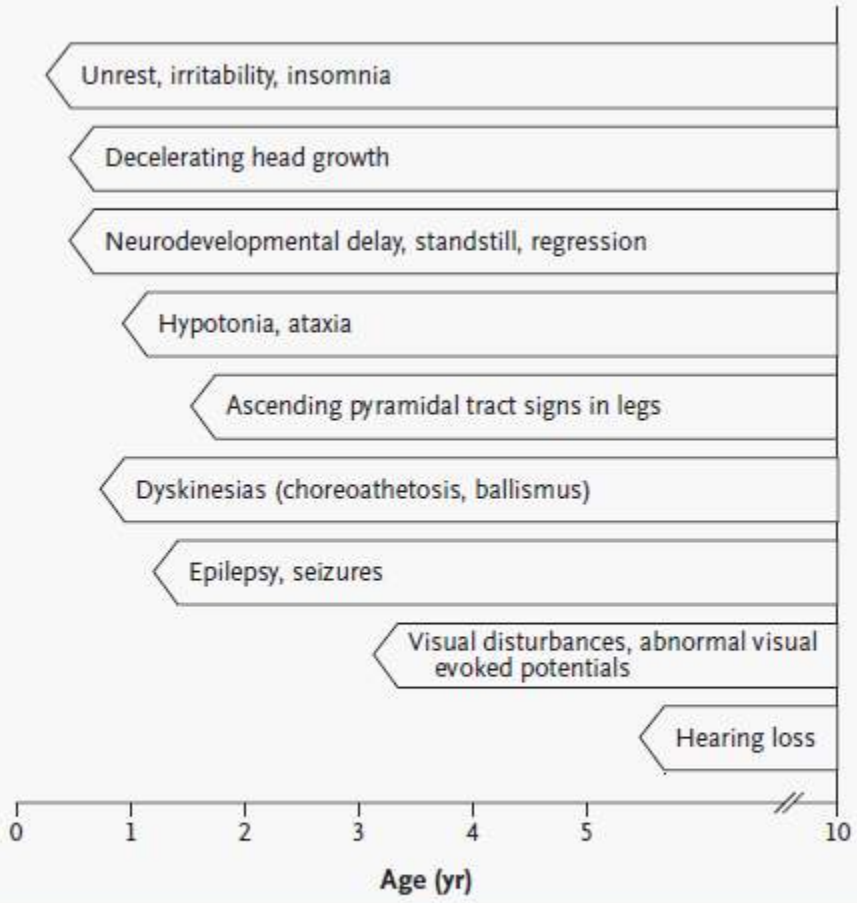


Figure 1. Age at Onset of Major Clinical Symptoms Associated with Cerebral Folate Deficiency.

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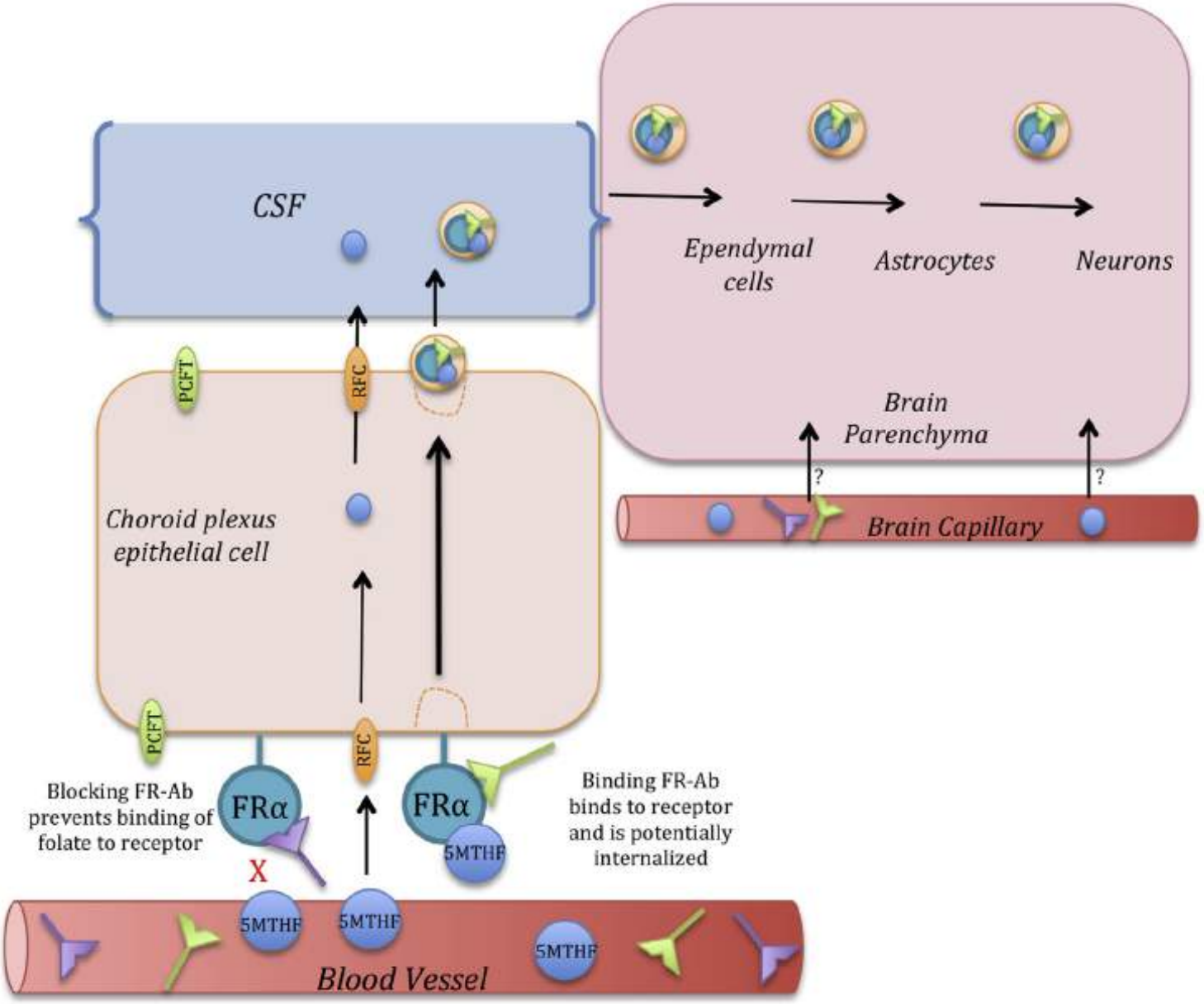
Immune Dysfunction in Autism



The Expanding Association between Autism and Cerebral Folate Deficiency

Open

Molecular Psychiatry (2012), 1–13

www.nature.com/mp

ORIGINAL ARTICLE

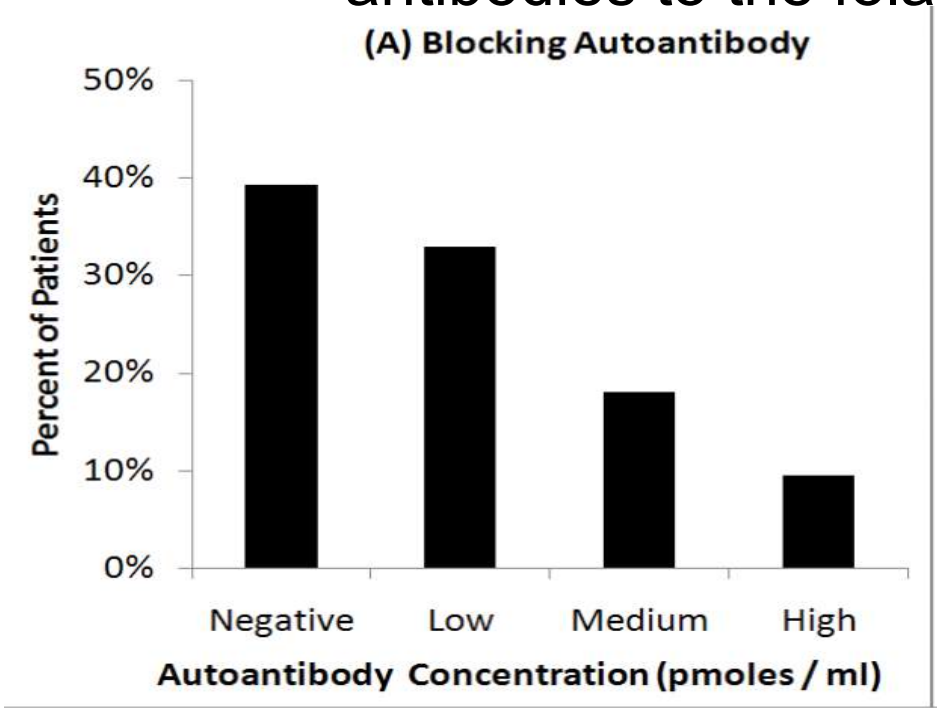
Cerebral folate receptor autoantibodies in autism spectrum disorder

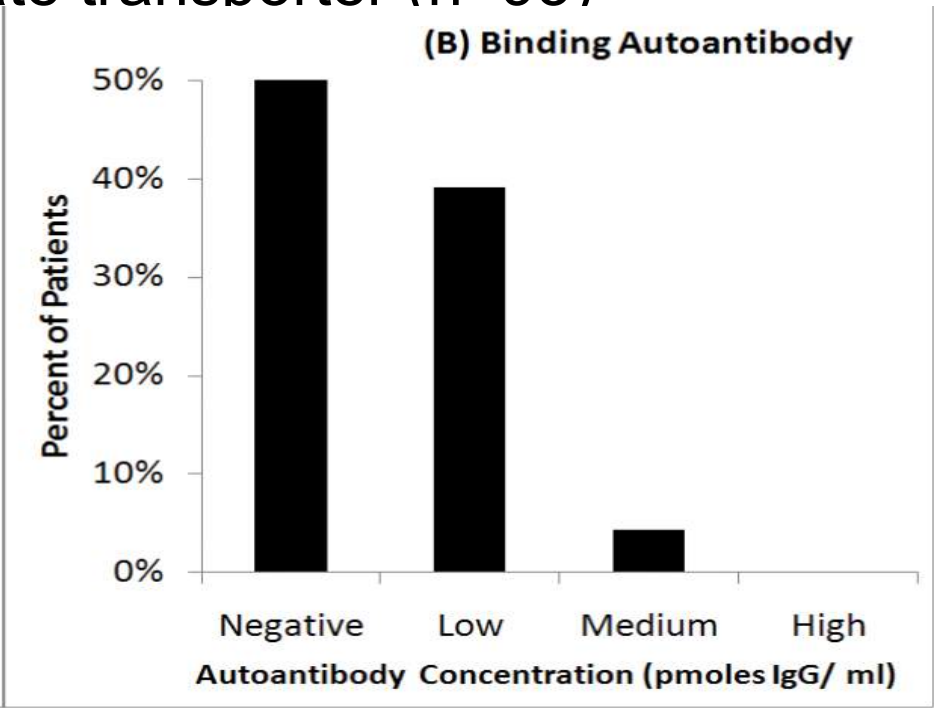
RE Frye[1], JM Sequeira[2], E Quadros[2], SJ James[1] and DA Rossignol[3]

[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

Immune Dysfunction in Autism

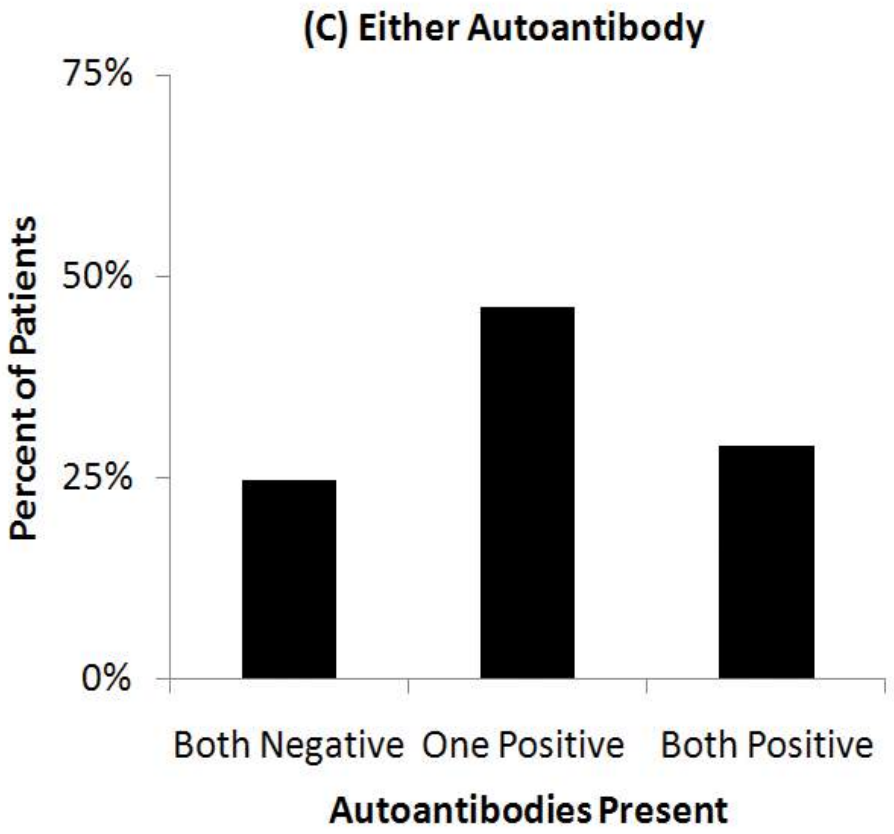
More than half of children with Autism Spectrum Disorder referred to two autism specialty clinics test positive for antibodies to the folate transporter (n=93)





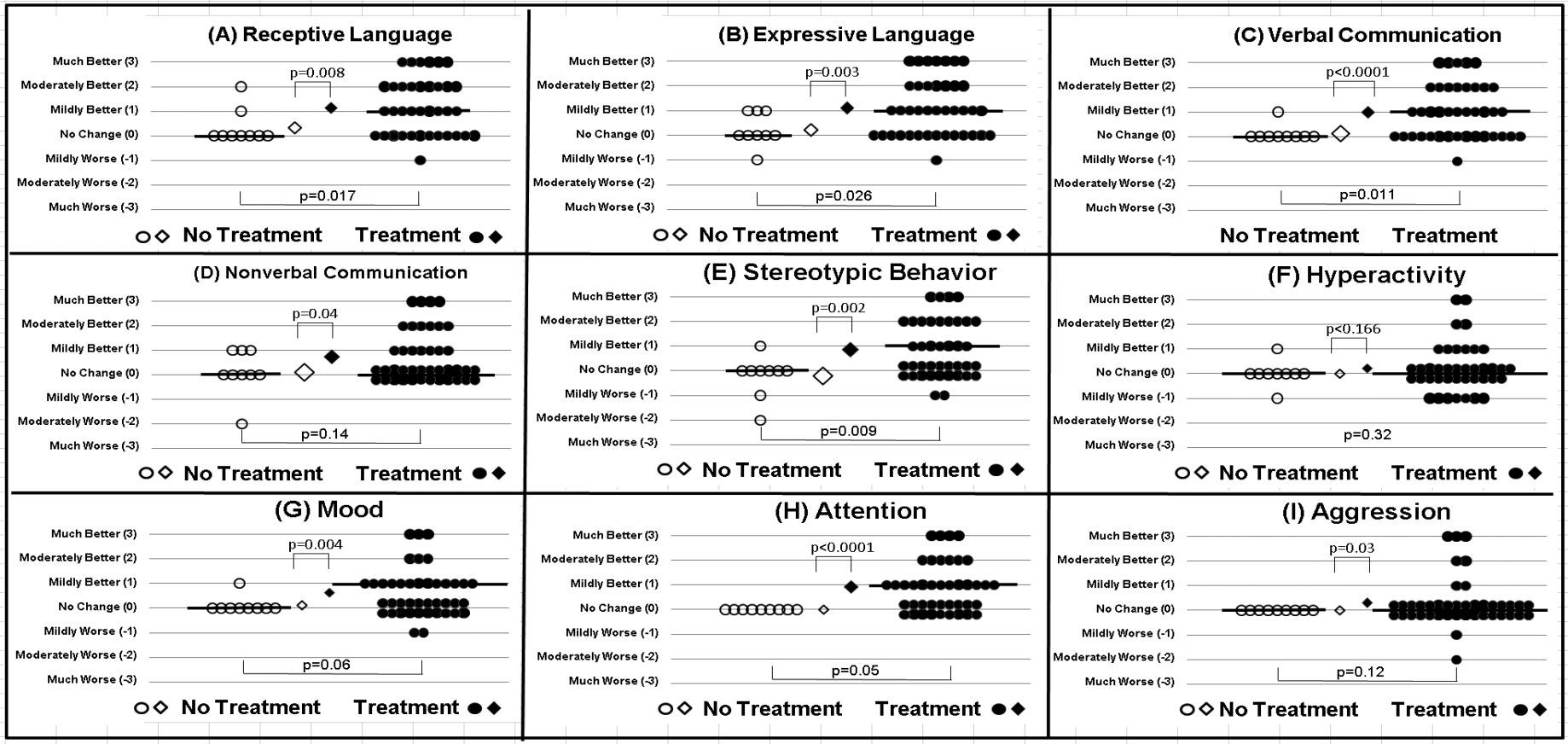
Immune Dysfunction in Autism

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



Immune Dysfunction in Autism

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



Immune Dysfunction in Autism

Molecular Psychiatry (2016) 00, 1–10

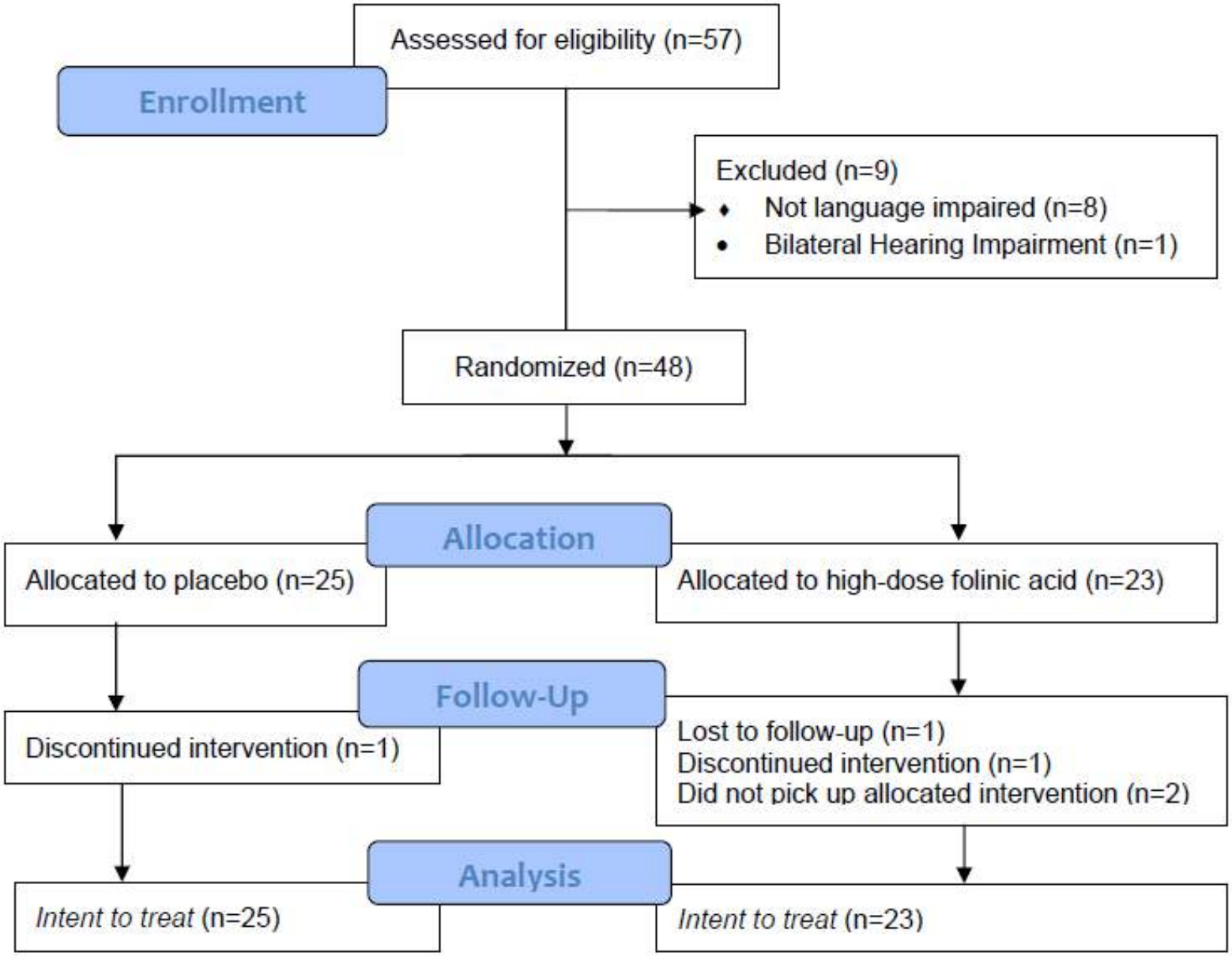
ORIGINAL ARTICLE

Folinic acid improves verbal communication in children with autism and language impairment: a randomized double-blind placebo-controlled trial

RE Frye[1,2,3], J Slattery[2,3], L Delhey[2,3], B Furgerson[1], T Strickland[1], M Tippett[1,2], A Sailey[2,3], R Wynne[2,3], S Rose[2,3], S Melnyk[2,3], S Jill James[2,3], JM Sequeira[4] and EV Quadros[4]

[1]Arkansas Children's Hospital, Little Rock, AR, USA; [2]Arkansas Children's Research Institute, Little Rock, AR, USA;

Immune Dysfunction in Autism



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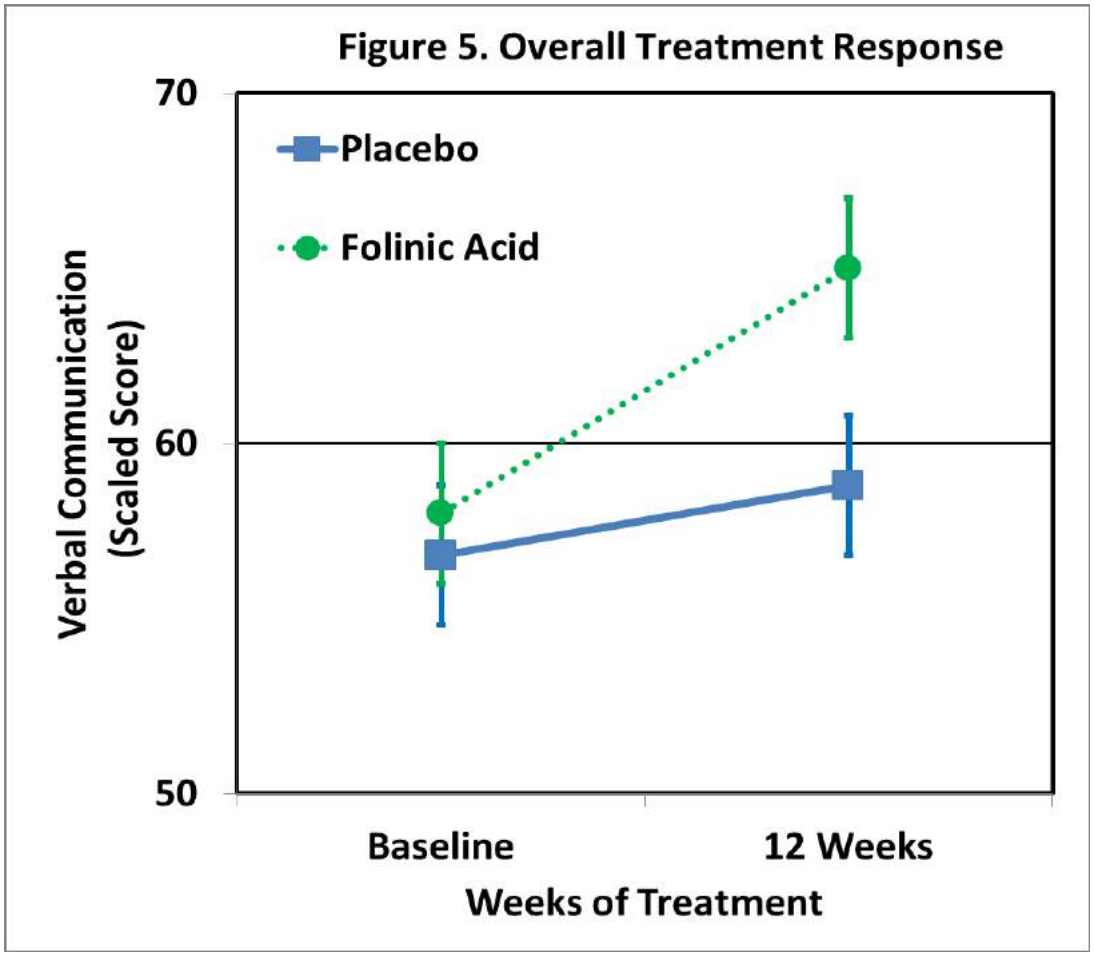
Immune Dysfunction in Autism



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Immune Dysfunction in Autism

	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


Autoimmune Encephalopathy
In Autism

Immune Dysfunction in Autism

Intravenous immunoglobulin for the treatment of autoimmune encephalopathy in children with autism

Kathleen Connery[1], Marie Tippett[1], Leanna M. Delhey[1], Shannon Rose[1], John C. Slattery[2], Stephen G. Kahler[1], Juergen Hahn[3,4], Uwe Kruger[3], Madeleine W. Cunningham[5], Craig Shimasaki[6] and Richard E. Frye[7,8]

82 Patients Evaluated For Autoimmune Encephalopathy

60%

IVIG Recommended (N=49)
- 32 Cunningham Panel Positive
- 4 Elevated CamKinase II and Severe Behavior
- 3 Elevated Antibodies and Refractory Epilepsy
- 3 Refractory Epilepsy
- 2 Elevated Antibodies and Severe Behavior
- 1 Elevated CamKinase II and Immune Abnormality
- 1 Elevated CamKinase II and Refractory Epilepsy
- 1 N-Type Calcium Channel Autoantibody
- 1 Folate Autoantibody and Elevated CSF Cytokines
- 1 Folate Autoantibody and Immune Deficiency

47 IVIG Ordered

77%

3 Insurance Denied
8 Insurance Appeal

36 IVIG Received Treatment

31 Received IVIG Under Authors' Care Team

5 Receiving IVIG under Outside Care Team

Immune Dysfunction in Autism

Table 1 Starting and Most Recent Dosing Schedule for Intravenous Immunoglobulin (IVIG) Treatment

Starting dosing schedule	Monthly dose	Number of patients	Most recent dosing schedule[a]	Monthly dose	Number of patients
2.0 g/kg × 1 days monthly	2.0 g/kg	10% (3/31)	1.0 g/kg × 2 days every 3 wks	2.7 g/kg	6% (2/30)
1.0 g/kg × 2 days monthly	2.0 g/kg	74% (23/31)	0.8 g/kg × 3 days monthly	2.4 g/kg	3% (1/30)
0.75 g/kg × 2 days monthly	1.5 g/kg	3% (1/31)	2.0 g/kg × 1 days monthly	2.0 g/kg	10% (3/30)
1.0 g/kg × 1 days monthly	1.0 g/kg	3% (1/31)	1.0 g/kg × 2 days monthly	2.0 g/kg	37% (11/30)
0.8 g/kg × 1 days monthly	0.8 g/kg	7% (2/31)	1.0 g/kg × 1 days every 2 wks	2.0 g/kg	3% (1/30)
1.0 g/kg × 2 days once		3% (1/31)	0.8 g/kg × 1 days every 2 wks	1.6 g/kg	3% (1/30)
			0.75 g/kg × 2 days monthly	1.5 g/kg	3% (1/30)
			2.0 g/kg × 1 day every	1.3 g/kg	3% (1/30)
			1.0 g/kg × 1 day every 3 wks	1.3 g/kg	10% (3/30)
			1.3 g/kg × 1 day monthly	1.3 g/kg	3% (1/30)
			1.0 g/kg × 1 day monthly	1.0 g/kg	3% (1/30)
			0.8 g/kg × 1 day monthly	0.8 g/kg	6% (2/30)
			1.0 g/kg × 1 day every 6 wks	0.7 g/kg	3% (1/30)
			0.4 g/kg × 1 day monthly	0.4 g/kg	3% (1/30)

[a]Only the 31 patients that received IVIG under the care of the authors are included in this section

Connery et al. *Translational Psychiatry* (2018)8:148
DOI 10.1038/s41398-018-0214-7

Translational Psychiatry

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Immune Dysfunction in Autism

Symptoms Improvement	Patients Reporting improvement
Communication & Language	58% (18/31)
Aberrant Behavior	35% (11/31)
Repetitive Behavior	23% (7/31)
Academics	23% (7/31)
Social Interactions	23% (7/31)
Tics	16% (5/31)
Motor	16% (5/31)
Other	16% (5/31)
Seizures	10% (3/31)
None	10% (3/31)

Number of Patients	Number of Symptoms Improved
10% (3/31)	0
19% (6/31)	1
35% (11/31)	2
13% (4/31)	3
13% (4/31)	4
6% (2/31)	5
3% (1/31)	6

Connery et al. *Translational Psychiatry* (2018)8:148
DOI 10.1038/s41398-018-0214-7

Translational Psychiatry

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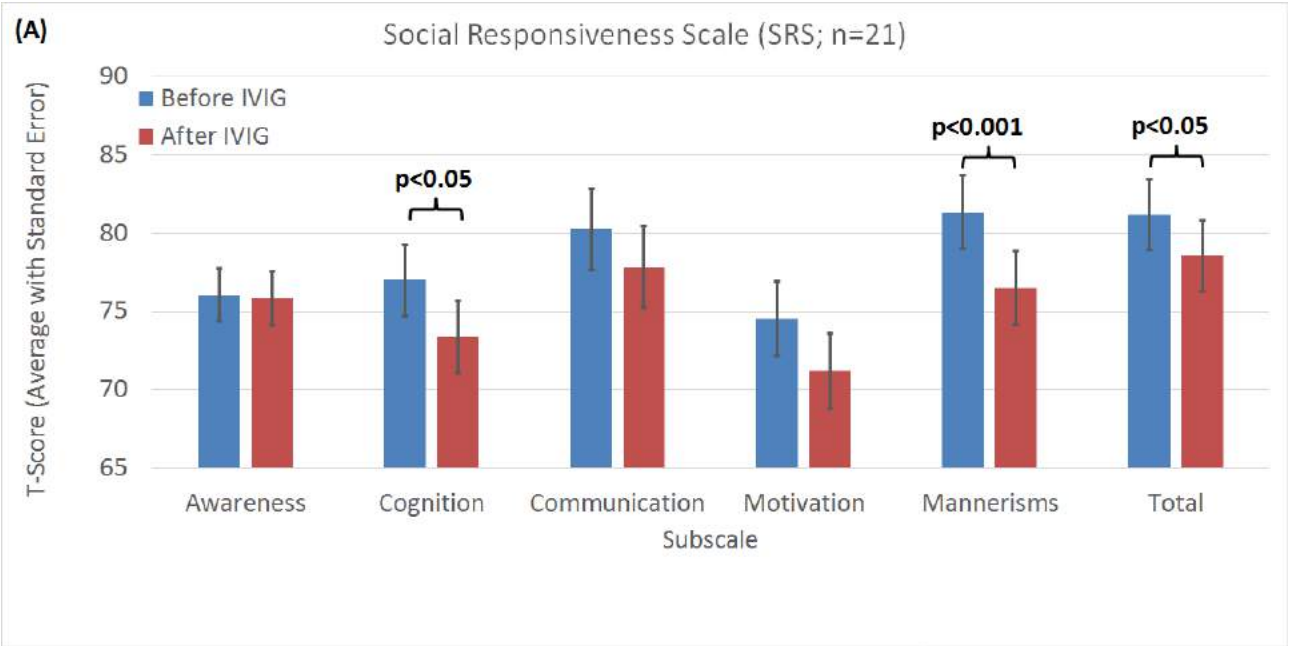
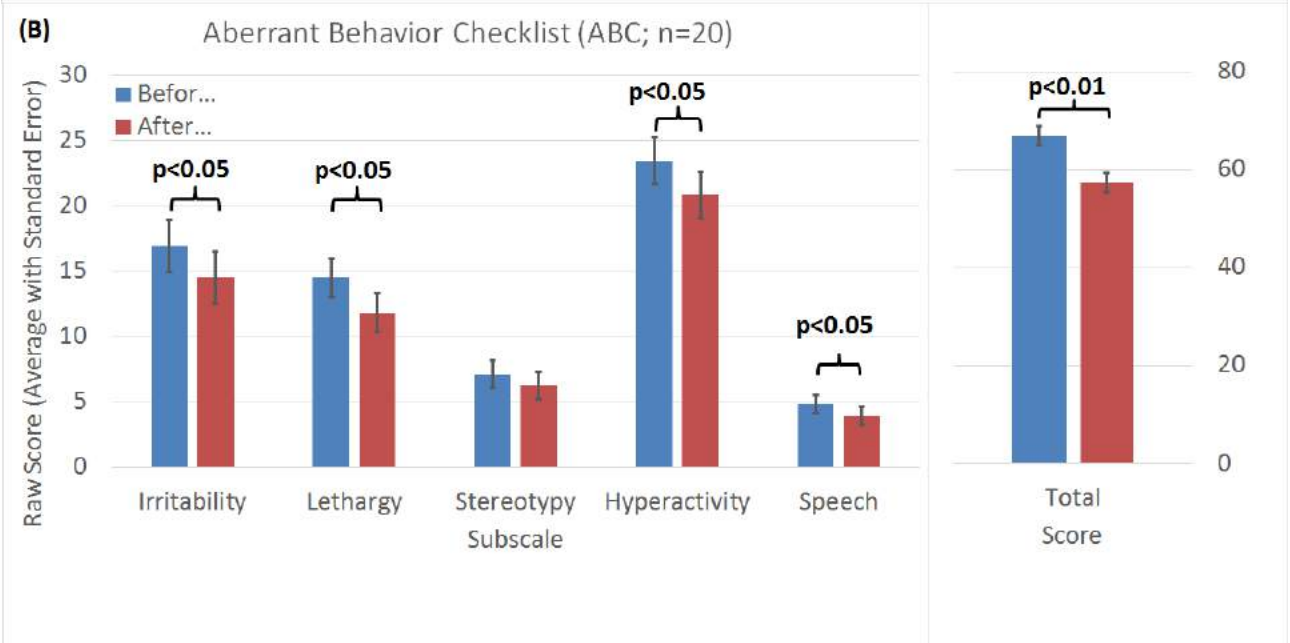
Immune Dysfunction in Autism





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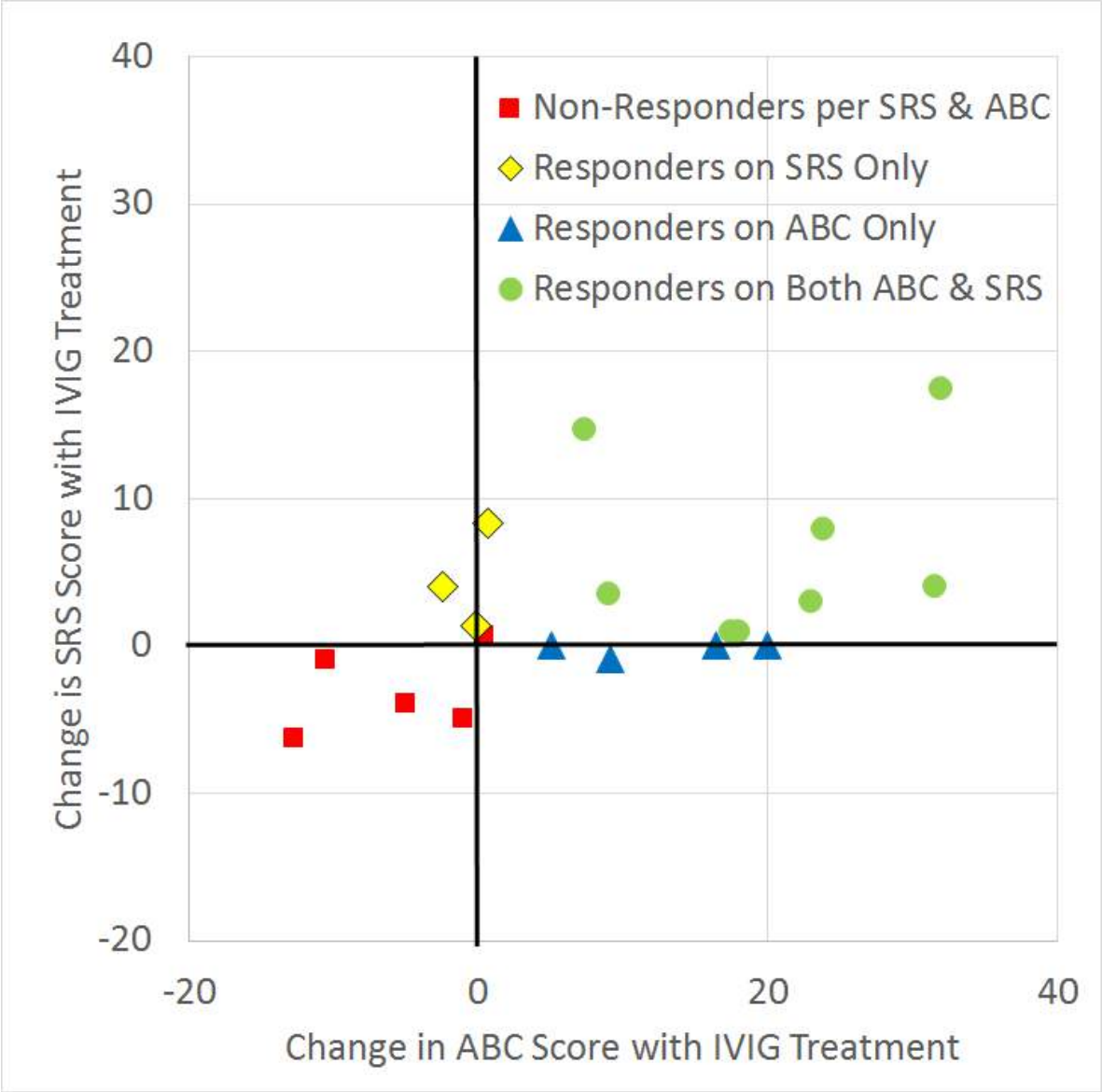
Immune Dysfunction in Autism



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DOI 10.1038/s41398-018-0214-7

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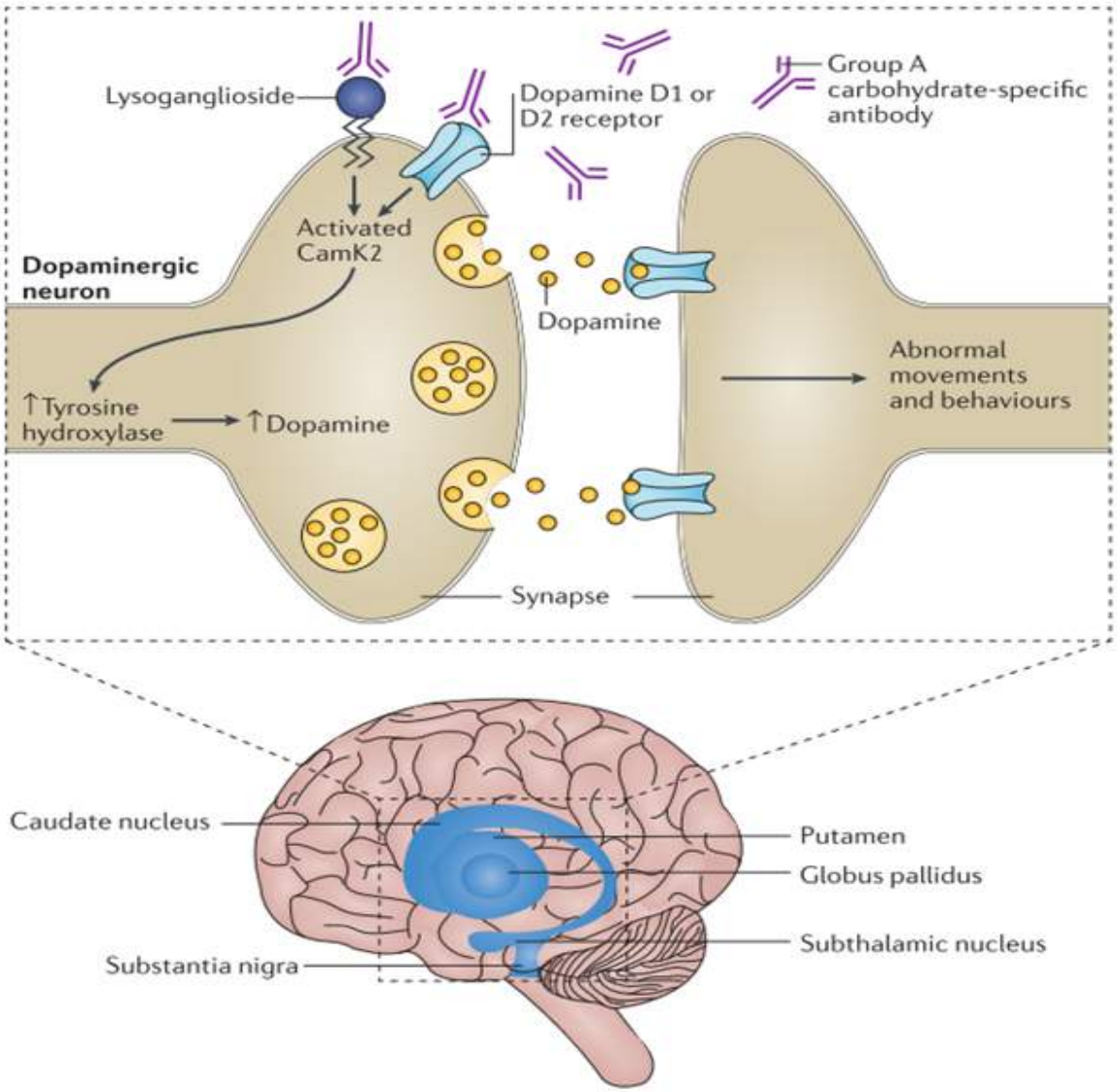
Immune Dysfunction in Autism

Cunningham Panel Autoantibodies and calcium/calmodulin-dependent protein kinase II (CaMKII)



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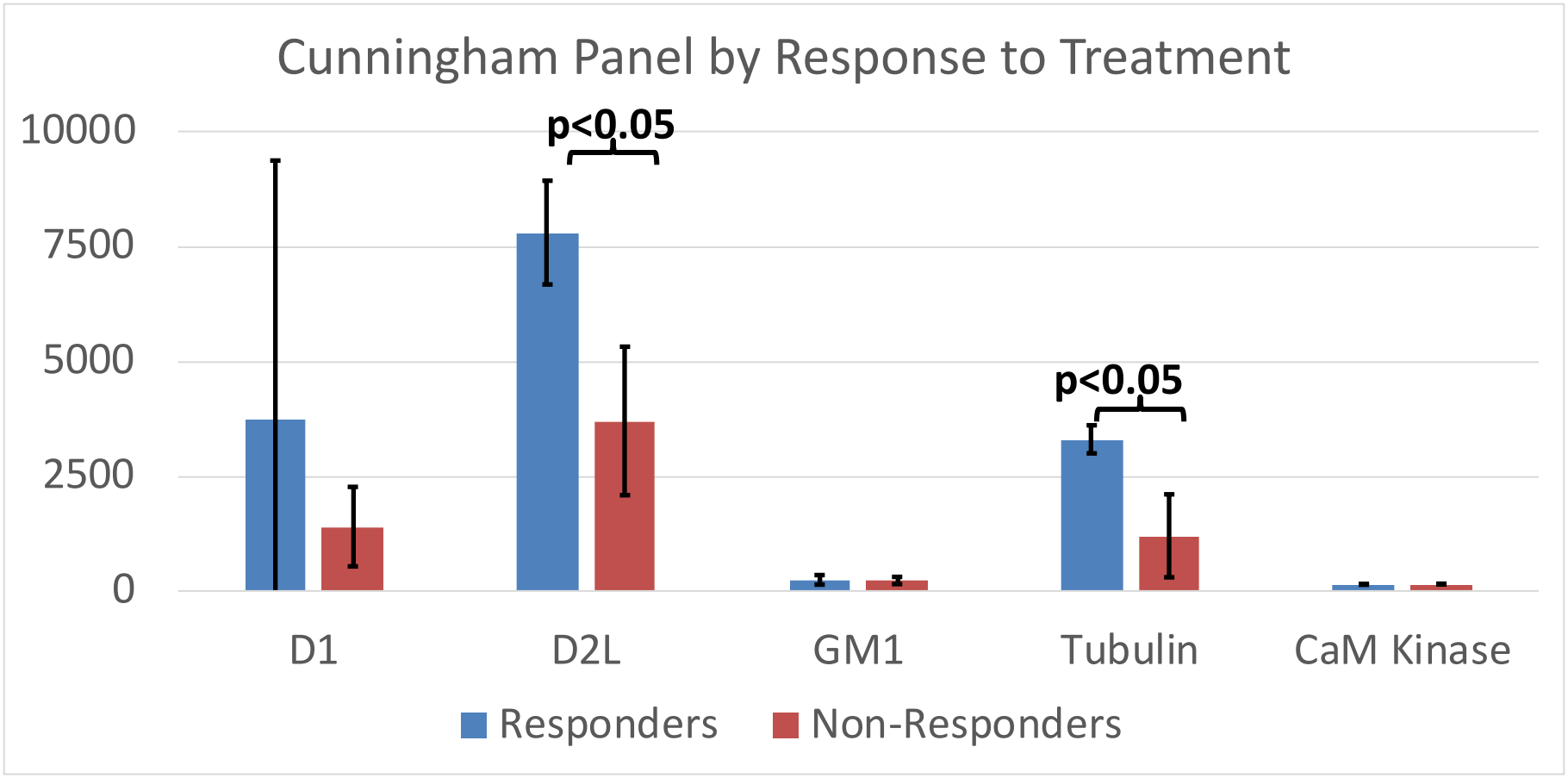

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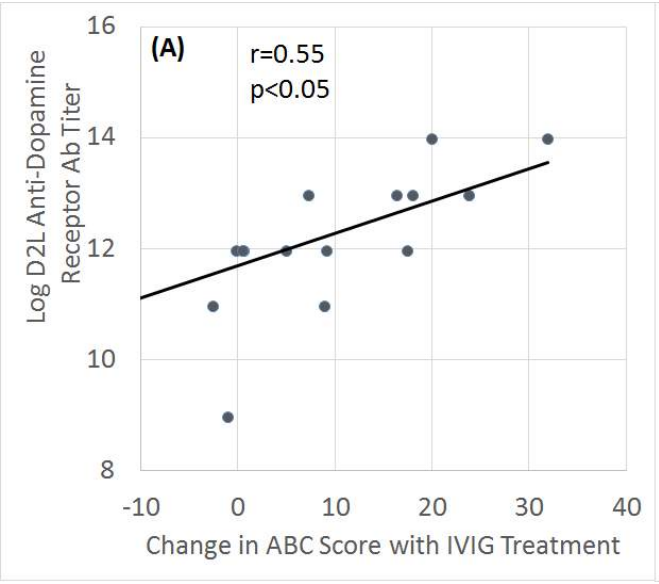

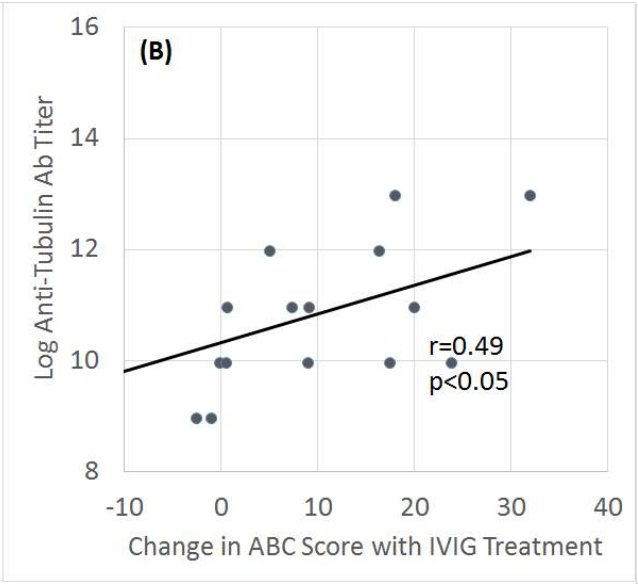

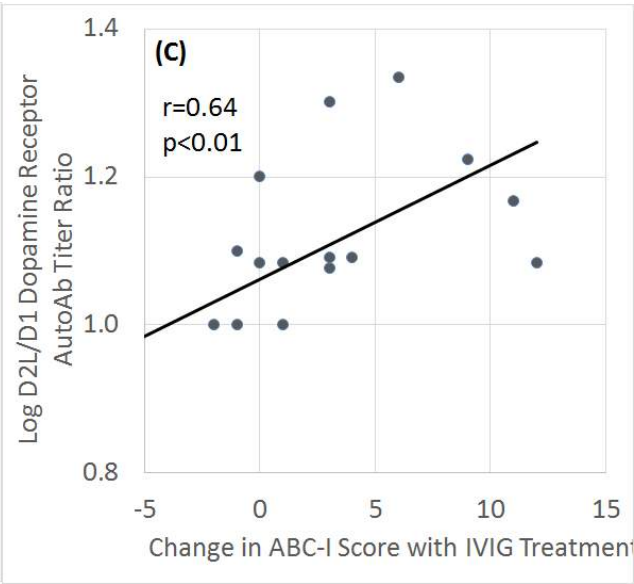

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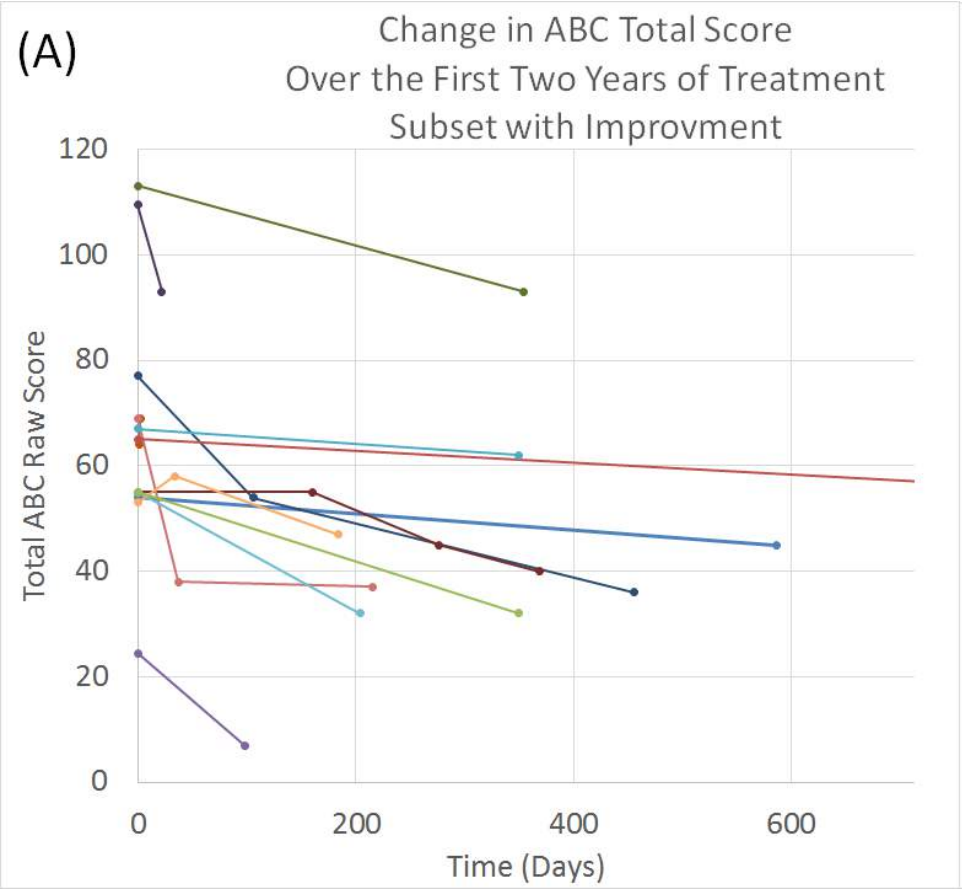

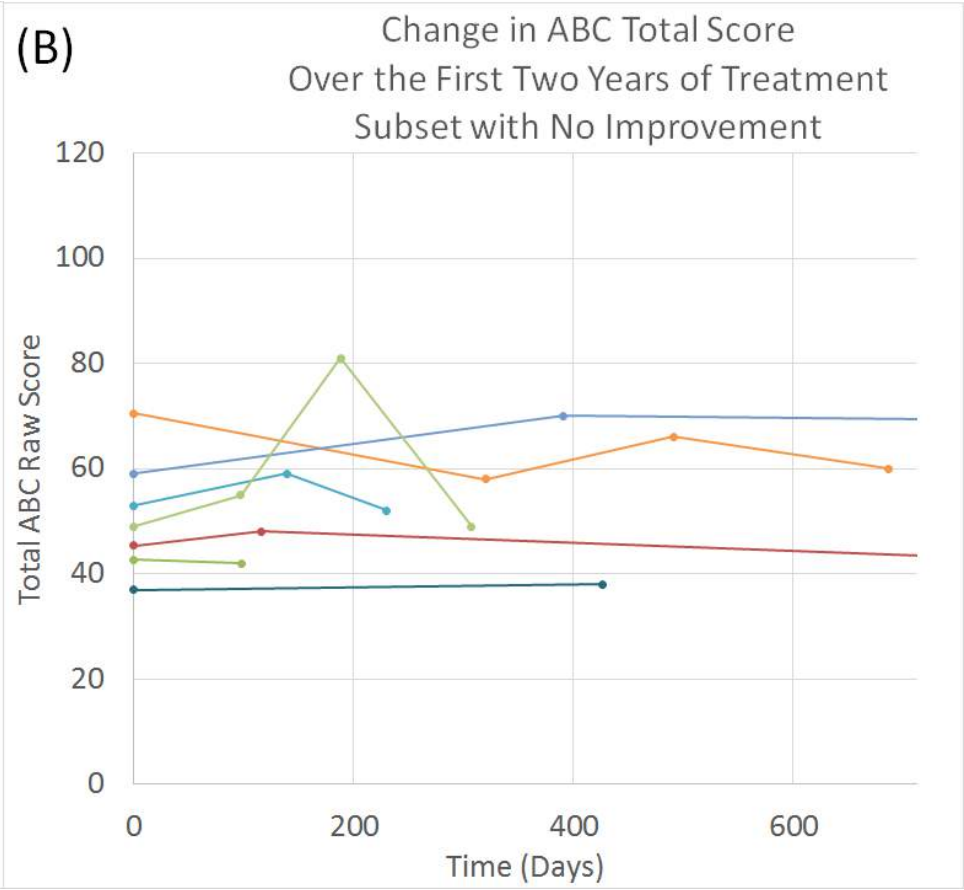

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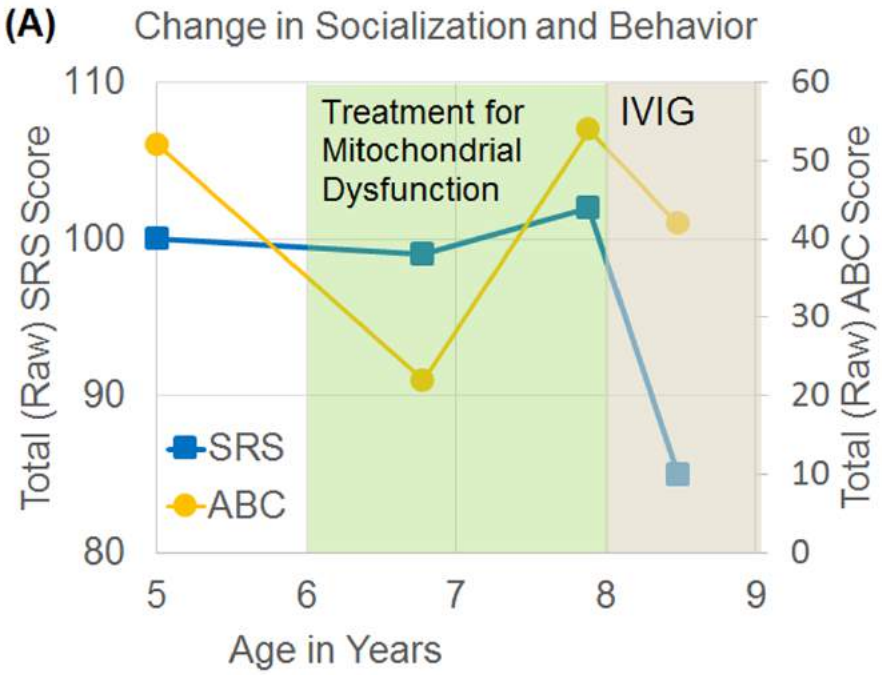
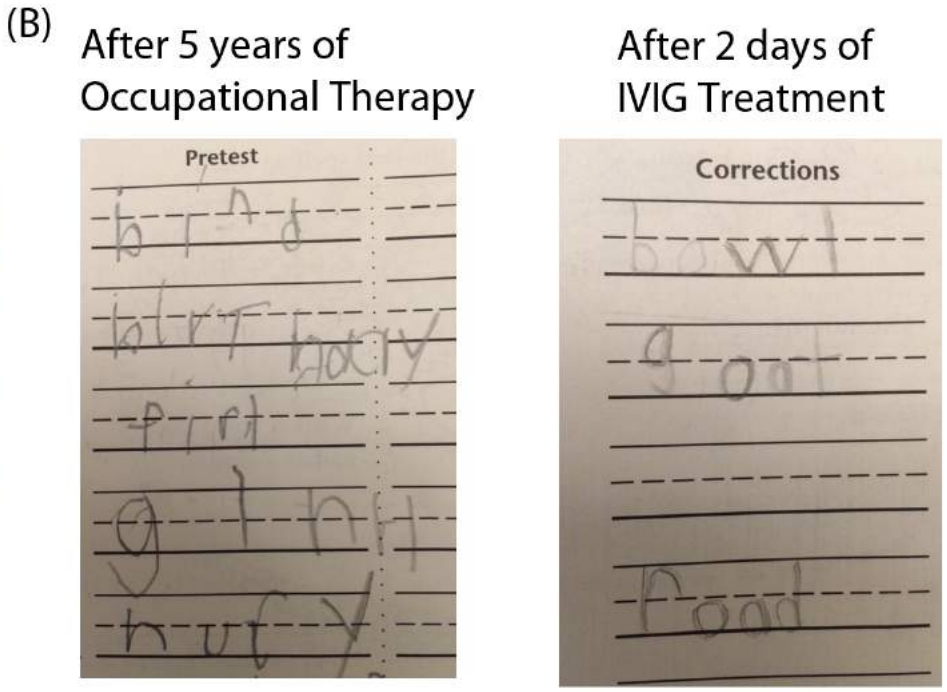
Case #1 – Mitochondrial Dysfunction and Autoimmune Encephalopathy





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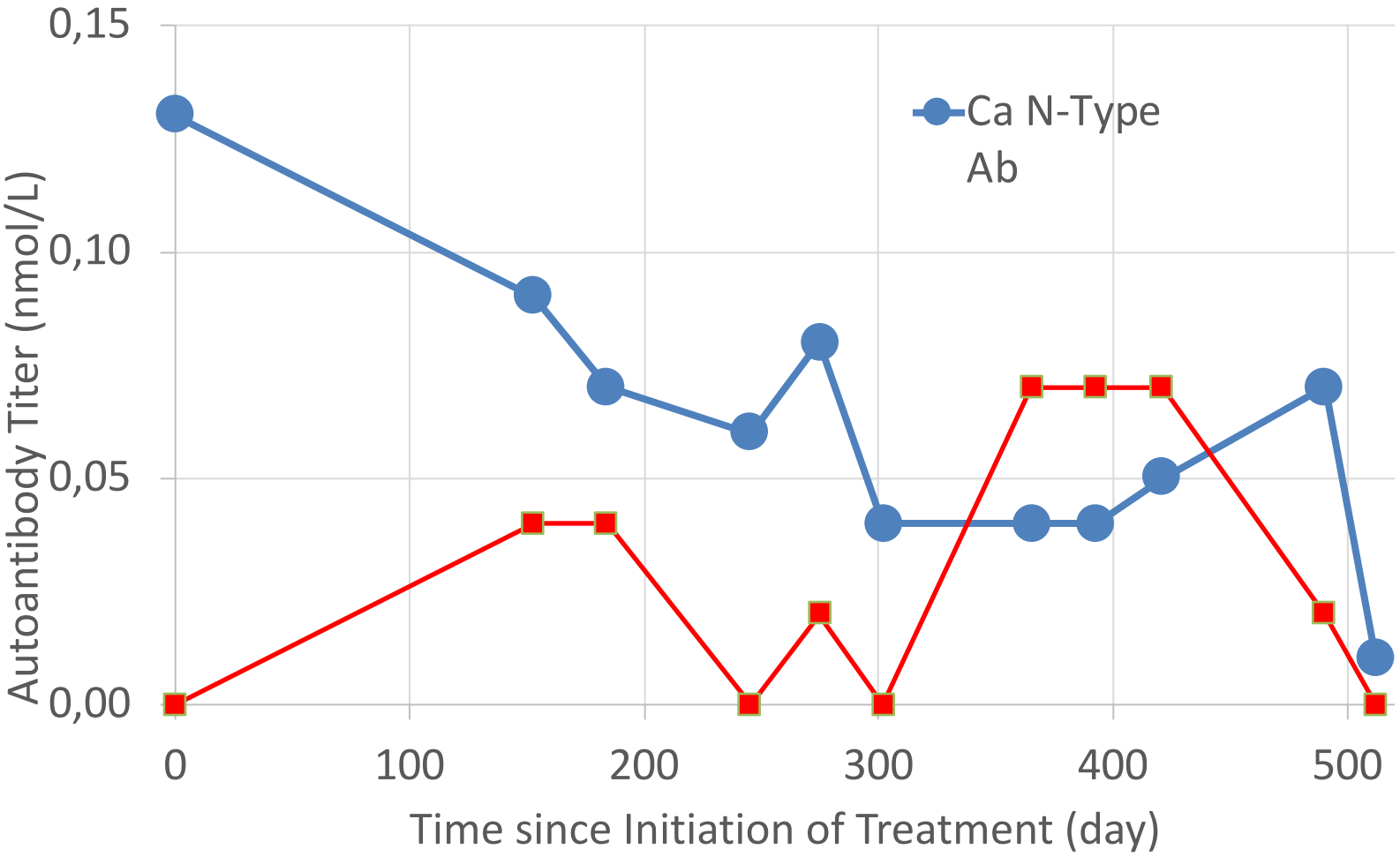
Case #2 – N-Type Calcium Channel Antibody



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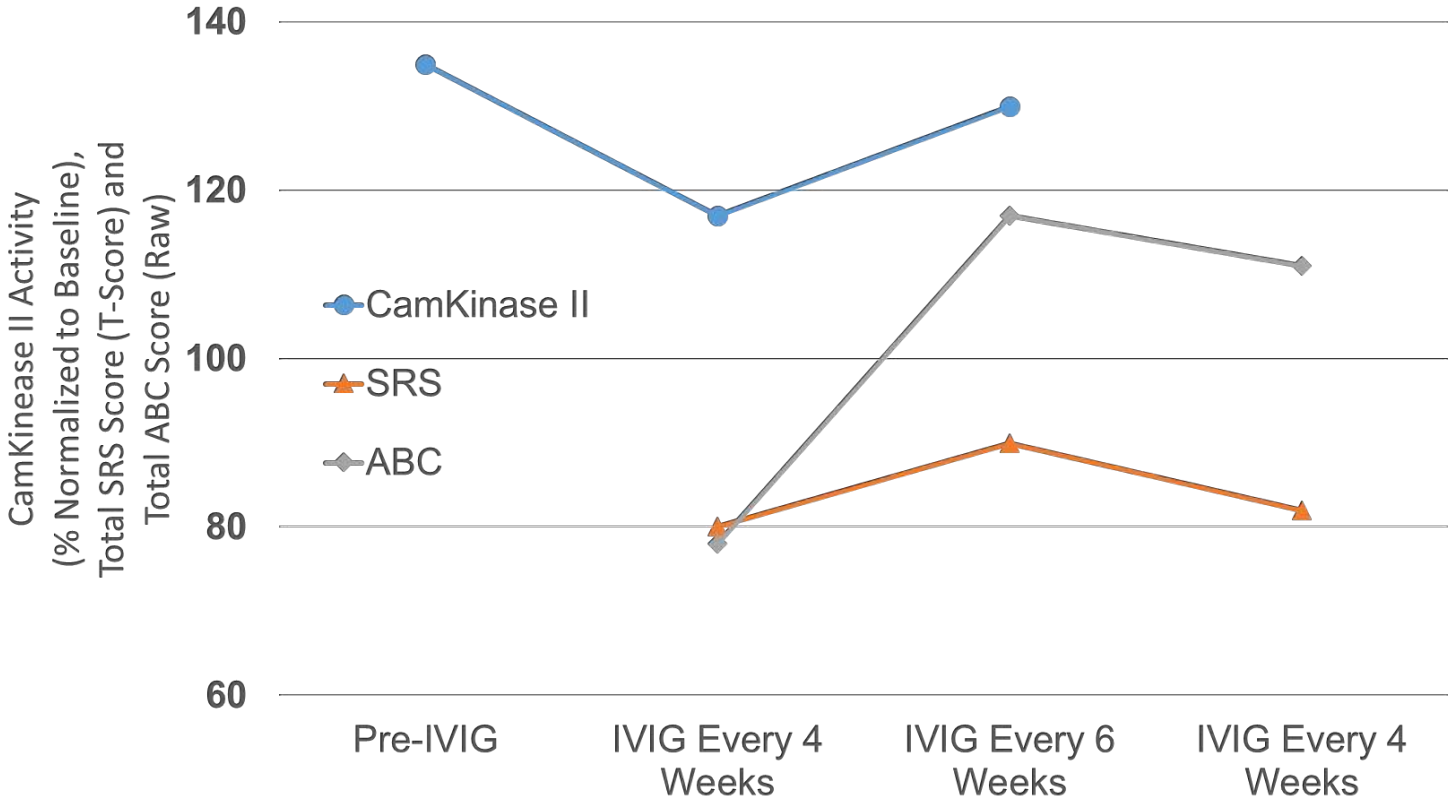
Case #3 – Behavioral Relapse with Change in Treatment Interval



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Hesselmark & Bejerot (2017)
Criticism of the Cunningham Panel

Immune Dysfunction in Autism

Two Major Limitations of Hesselmark and Bejerot Study

1. Laboratory (Wieslab) Collected in the samples in Serum Separator Tubes (Gold Top) contrary to Moleculera's Standards and Instructions which Specially Indicates that **GLASS TUBES WITHOUT ANY ADDITIVES** should be used for collection

2. For Health Controls
    1. Did not ask about Family History of Psychiatric or Autoimmune disorders
    2. Did not ask about recent, recurrent or chronic infections.
    3. Although asked about a Diagnosis of an Psychiatric or Autoimmune disorders, it is not clear that Symptoms of Psychiatric or Autoimmune disorders were investigated and to what extend (many times symptoms are only revealed on repeat and extensive questioning)

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