IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

September 23, 2014

John Edward Porter Neuroscience Research Center
35 Convent Drive, Building 35, Room 620
Bethesda, MD 20892

Conference Call Access:
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IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Morning Agenda

9:00 AM **Welcome and Introductions**

Thomas Insel, M.D.
Director, National Institute of Mental Health and Chair, IACC

Geraldine Dawson, Ph.D.
Professor, Departments of Psychiatry and Behavioral Science, Psychology, and Neuroscience, Co-Chair, IACC Basic and Translational Research Subcommittee

Susan Daniels, Ph.D.
Director, Office of Autism Research Coordination (OARC), (NIMH) and Executive Secretary, IACC

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Panel 1:
Overview of Co-Occurring Conditions in Children and Adults
–
What have We Learned about Co-Occurring Conditions from Epidemiological and Clinical Experience?

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IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Morning Agenda - continued

9:15 AM **Panel 1: Overview of Co-Occurring Conditions in Children and Adults – What have We Learned about Co-Occurring Conditions from Epidemiological and Clinical Experience?**

9:15 – 9:30 **Anjali Jain, M.D.**
Vice President
Lewin Group



HEALTH CARE AND HUMAN SERVICES POLICY, RESEARCH, AND CONSULTING - WITH REAL-WORLD PERSPECTIVE.

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Lessons from Epidemiological and Clinical Experience

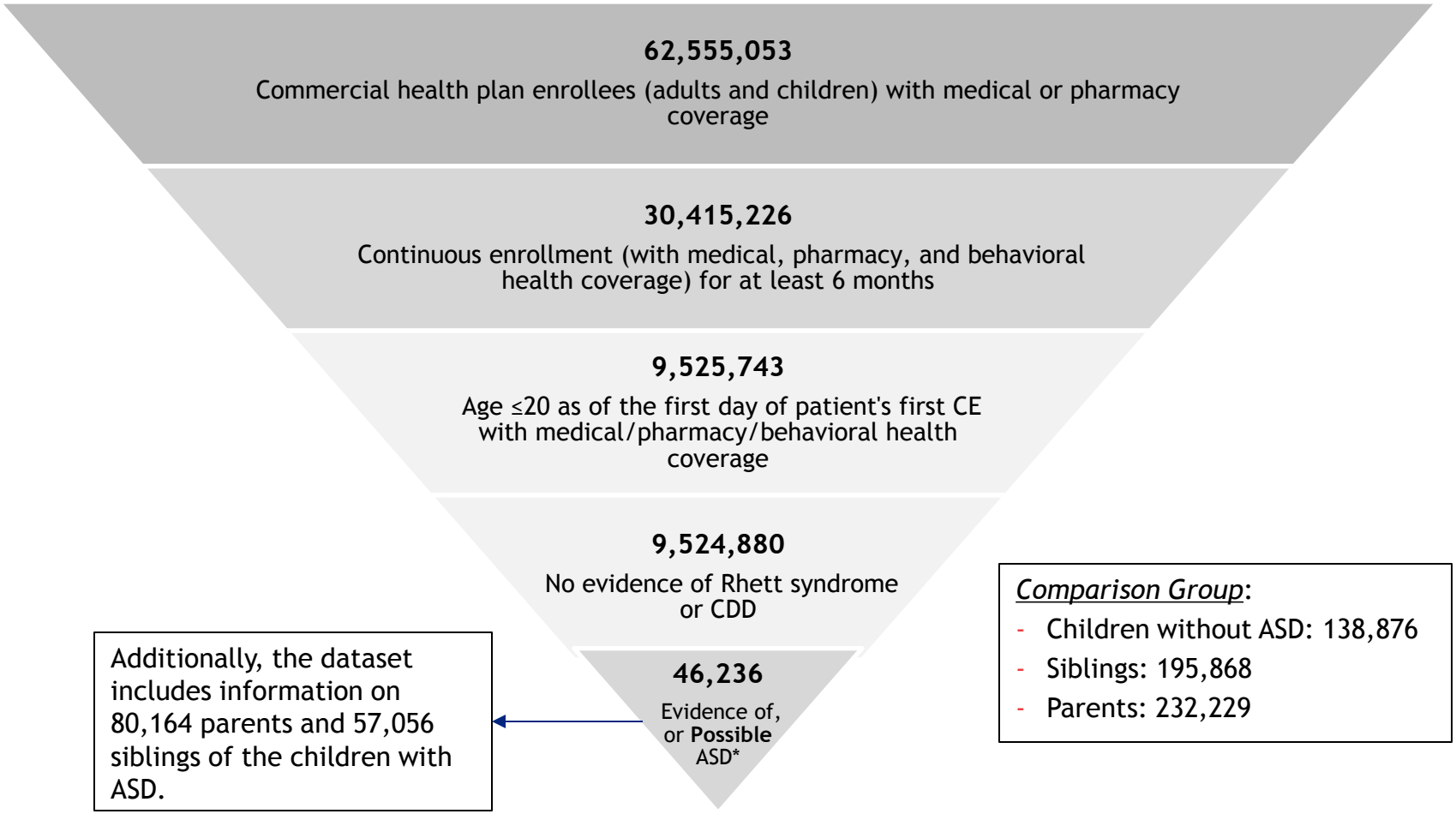
Lewin Health Outcomes Study overview – 4 tasks

- **Task A:** Identify and describe sample
  - Identify children with ASD and their families within the research claims databases and conduct initial descriptive analyses to examine children with and without ASD and their families
  - Conduct a medical chart review to validate the claims-based **ASD case identification algorithms**
- **Task B:** describe and compare the health outcomes of children with ASD and their families to similar families without a child with ASD.
- **Task C:** Describe and compare the use of health services by children with ASD and their families to similar families without a child with ASD.
- **Task D:** Propose an approach for using administrative data to identify potential risk factors for ASD for future research.

Research study overview – baseline data sources

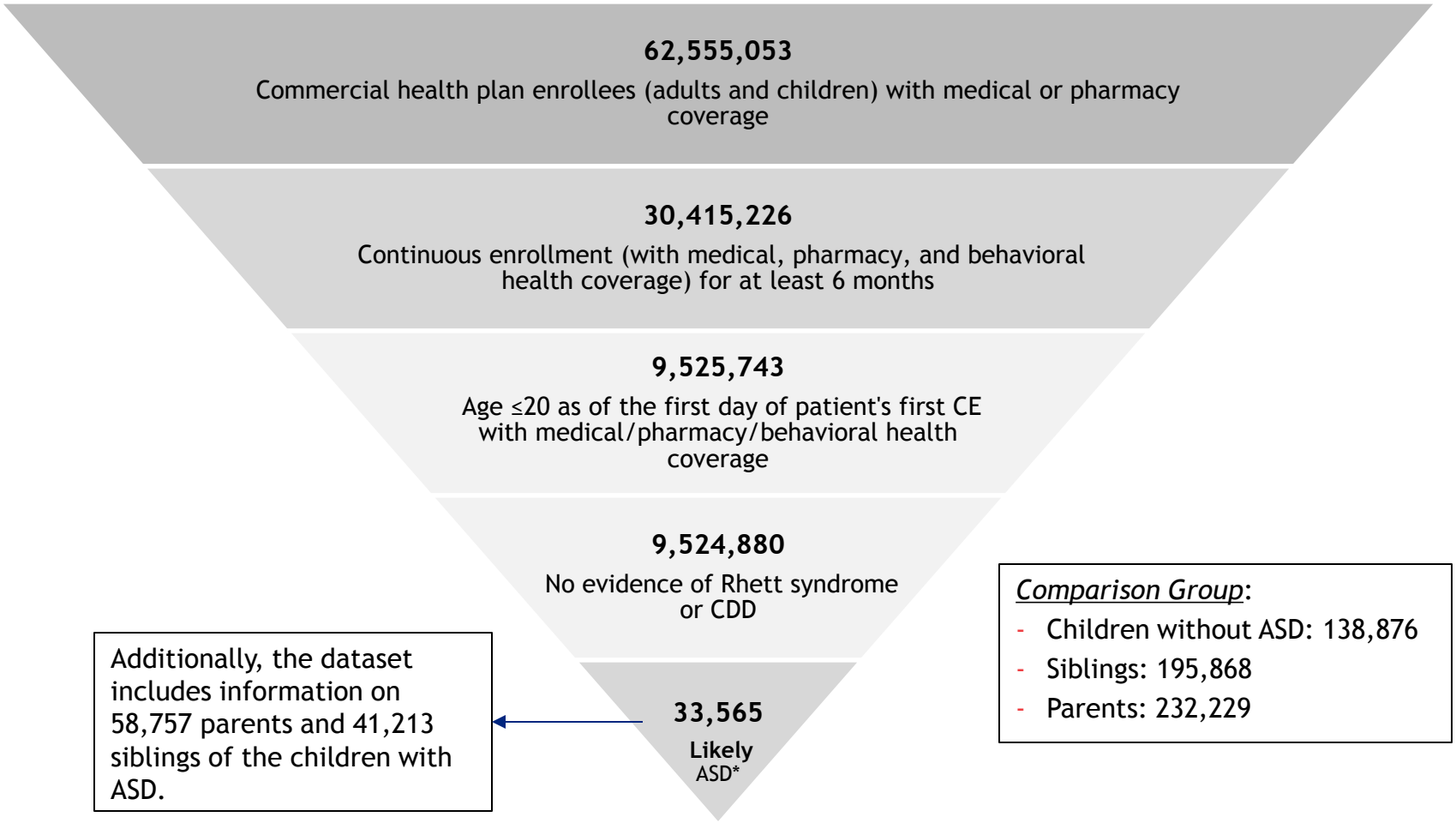
- OptumInsight Research Claims Database: proprietary research database
  - medical and pharmacy claims and linked enrollment information from 1993 to present;
  - geographically diverse across the US, fairly representative of the US population
- Sociodemographic database:
  - person and household level data on socioeconomic characteristics
  - derived through a match between health plan members and a consumer database maintained for a large segment of the US population

Research study overview – sample size



*presence of one or more claims with an ICD-9 for Asperger's, Autism, or PDD-NOS

Research study overview – sample size



*presence of two or more claims with an ICD-9 for Asperger's, Autism, or PDD-NOS

What have we learned in this research area?

- Evidence that the co-morbidity in every category is more common in persons with ASD.

Comorbidity*	Likely ASD (N=34,754)		Possible ASD (N=11,482)		Total ASD (N=46,236)		Comparison (N=138,876)	
	n	%	n	%	n	%	n	%
Disorders usually diagnosed in infancy, childhood, or adolescence	34,260	98.58	10,957	95.43	45,217	97.80	1,012	0.73
Factors influencing health care	29,680	85.40	9,353	81.46	39,033	84.42	86,890	62.57
Respiratory infections	26,251	75.53	8,161	71.08	34,412	74.43	80,142	57.71
Ear conditions	20,312	58.45	6,297	54.84	26,609	57.55	48,528	34.94
Symptoms; signs; and ill-defined conditions	20,360	58.58	6,029	52.51	26,389	57.07	54,465	39.22
Immunizations and screening for infectious disease [10.]	19,172	55.16	6,065	52.82	25,237	54.58	56,134	40.42
Attention deficit, conduct, and disruptive behavior disorders	17,711	50.96	4,155	36.19	21,866	47.29	7,894	5.68
Eye disorders	16,124	46.39	4,593	40.00	20,717	44.81	35,436	25.52
Developmental disorders	16,220	46.67	4,124	35.92	20,344	44.00	2,493	1.80
Other nervous system disorders [95.]	15,531	44.69	3,864	33.65	19,395	41.95	5,336	3.84
Other upper respiratory disease [134.]	13,920	40.05	3,942	34.33	17,862	38.63	30,306	21.82
Residual codes; unclassified; all E codes [259. and 260.]	13,901	40.00	3,738	32.56	17,639	38.15	27,536	19.83
Viral infection	12,795	36.82	3,790	33.01	16,585	35.87	33,508	24.13
Other lower respiratory disease [133.]	12,568	36.16	3,763	32.77	16,331	35.32	28,842	20.77

* Based on Clinical Classification Software managed by AHRQ. Comorbidities listed are top 10 for Total ASD sample.

What have we learned in this research area?

- Evidence that the mental and behavioral health co-morbidities are more common in persons with ASD.
- Note, these are crude rates and have not been adjusted for other variables.

Comorbidity*	Likely ASD (N=34,754)		Possible ASD (N=11,482)		Total ASD (N=46,236)		Comparison (N=138,876)	
	n	%	n	%	n	%	n	%
Anxiety	6,336	18.23	1,114	9.70	7,450	16.11	3,669	2.64
Attention Deficit (with or without hyperactivity)	12,782	36.78	2,560	22.30	15,342	33.18	5,102	3.67
Bipolar Disorder	3,786	10.89	452	3.94	4,238	9.17	971	0.70
Depression	4,880	14.04	907	7.90	5,787	12.52	5,072	3.65
Epilepsy and other seizure disorders	2,401	6.91	509	4.43	2,910	6.29	470	0.34
Intellectual Disability	1,728	4.97	231	2.01	1,959	4.24	56	0.04
Obsessive-Compulsive Disorder	1,829	5.26	208	1.81	2,037	4.41	267	0.19
Schizophrenia	313	0.90	27	0.24	340	0.74	37	0.03
Tourette Syndrome	492	1.42	63	0.55	555	1.20	66	0.05
Any of the above	19,168	55.15	3,884	33.83	23,052	49.86	10,974	7.90

What have we learned in this research area?

- To capture and control for comorbidity for the child samples in our study, we calculated a comorbidity score (ranging from 0-9) based on the presence (or absence) of diagnosis codes on medical claims (children with and without ASD and siblings).

- Modeled on a similar measure created by Feudtner, et al. (2000).

- For each subject, a dichotomous flag (0/1) was created for each of 9 categories of chronic conditions: 1) neuromuscular, 2) cardiovascular, 3) respiratory, 4) renal, 5) gastrointestinal, 6) hematologic or immunologic, 7) metabolic, 8) other congenital or genetic defect, and 9) malignant neoplasms.

What have we learned in this research area?

- Evidence that the co-morbidity is more common, with higher relative risk, in persons with ASD.
  - Our research found that children with ASD had higher *unadjusted* rates of: infectious diseases, neurodevelopmental disorders, mental health conditions, metabolic dysfunction, autoimmune conditions, congenital/genetic disorders, gastrointestinal conditions.

Health condition	Proportion with Condition*		Odds Ratio	
	ASD (N=33,565)	Comparison (N=138,876)	ASD vs Comparison	
	%	%	Odds Ratio	p value
Infectious diseases	50.0	34.8	1.88	<0.001
Neurological/neurodevelopmental disorders	70.8	9.2	24.07	<0.001
Mental health conditions	70.1	8.7	24.68	<0.001
Metabolic dysfunction	4.7	1.1	4.38	<0.001
Autoimmune disorders	6.6	3.9	1.75	<0.001
Congenital/genetic disorders	5.1	1.5	3.52	<0.001
Gastrointestinal/nutritional disorders	19.5	5.1	4.44	<0.001
	Rate	**Rate**	**Rate Ratio**	**Upper 95% CI**
Comorbidity Score	0.191	0.082	2.340	2.294
Note: Proportions adjusted for enrollment time.				

What have we learned in this research area?

- Evidence that the co-morbidity is more common in siblings of persons with ASD.
  - Siblings of children with ASD also had higher proportions of all these co-morbidities relative to siblings of children without ASD.

	Proportion with Condition*		Odds Ratio	
	ASD Siblings (N=41,213)	Comparison Siblings (N=195,868)	ASD vs Comparison	
Health condition	%	%	Odds Ratio	p-value
Infectious diseases	41.60	31.50	1.550	<0.001
Neurological/neurodevelopmental disorders	17.30	9.00	2.104	<0.001
Mental health conditions	17.90	8.60	2.321	<0.001
Metabolic dysfunction	1.30	1.10	1.231	<0.001
Autoimmune disorders	4.50	3.30	1.365	<0.001
Congenital/genetic disorders	2.10	1.40	1.515	<0.001
Gastrointestinal/nutritional disorders	7.40	4.20	1.797	<0.001
	Rate	Rate	Rate Ratio	Upper 95% CI
Comorbidity Score	0.091	0.075	1.202	1.176
Note: Proportions adjusted for enrollment time.				

What have we learned in this research area?

- Evidence that the injuries are more common in persons with ASD.
  - Among younger children (during the ages of 0-5), children with ASD had *higher* risk of injury after adjustment for co-occurring conditions and sociodemographic variables.
  - Among older children (11+ years of age), children with ASD had a lower risk of injury than the comparison group.

Independent Variables	Age Period				
	0-2 Years	3-5 Years	6-10 Years	11-20 Years	21+ Years
Sample					
Comparison	ref.	ref.	ref.	ref.	ref.
ASD	1.141*	1.282*	1.001	0.634*	0.580*

What have we learned in this research area?

- Evidence that gastrointestinal disorders are more common in persons with ASD.
  - After controlling for enrollment time and other potential confounders, children with ASD had higher odds of a GI condition than children without ASD (OR=3.94, p<0.001).
  - We also found that the odds of a GI condition were higher following, compared to the 12 months before, the child's initial ASD diagnosis (OR = 1.40, p<0.001)

Independent Variables	Gastrointestinal/Nutritional Conditions			
	Odds ratio	Lower 95% CI	Upper 95% CI	p-value
Sample				
Comparison	ref.	-	-	-
ASD	3.939	3.788	4.096	<0.001

Surveillance Bias

- A significant limitation is the extent of surveillance bias affecting our results. That is, children with ASD receive more care in general than children without ASD, thereby increasing the chance of receiving diagnoses for other conditions in general.

- Our attempts to control for bias using preventive office visits as a marker of health care users suggested this did not usually alter results for chronic or severe conditions

- Nonetheless, surveillance bias continues to be a challenge for more common conditions where care is more optional (otitis, upper respiratory infections, etc.)

Appendix Slides

Demographics

Characteristic	Total ASD (N=46,236)		Comparison (N=138,876)	
	n	%	n	%
Gender				
Male	37,419	80.93	70,321	50.64
Female	8,817	19.07	68,555	49.36
Geographic Region				
Northeast	7,278	15.74	14,537	10.47
Midwest	15,507	33.54	42,064	30.29
South	17,013	36.80	61,497	44.28
West	6,438	13.92	20,778	14.96
Race/Ethnicity*				
N available	28,084	60.74	71,503	51.49
White	24,002	85.47	56,286	78.72
African American/Black	1,002	3.57	4,883	6.83
Native Hawaiian or other Pacific Islander	5	0.02	44	0.06
American Indian or Alaskan Native	85	0.30	203	0.28
Asian	666	2.37	1,899	2.66
Hispanic	1,945	6.93	7,434	10.40
Other	379	1.35	754	1.05
	mean	SD	mean	SD
Continuous Enrollment (months)	37.31	26.42	27.48	21.84

Demographics, cont.

Characteristic	Total ASD (N=46,236)		Comparison (N=138,876)	
	n	%	n	%
Household Income*				
N available	26,457	57.22	63,092	45.43
<$15,000	146	0.55	553	0.88
$15,000 – $19,999	156	0.59	542	0.86
$20,000 - $29,999	405	1.53	1,720	2.73
$30,000 - $39,999	1,238	4.68	4,599	7.29
$40,000 - $49,999	2,437	9.21	7,779	12.33
$50,000 - $59,999	2,840	10.73	7,783	12.34
$60,000 - $74,999	4,199	15.87	10,443	16.55
$75,000 - $99,999	6,458	24.41	13,789	21.86
$100,000 - $124,999	4,800	18.14	9,030	14.31
$125,000 - $149,999	2,370	8.96	4,283	6.79
$150,000 – $249,999	1,039	3.93	1,952	3.09
$250,000 +	369	1.39	619	0.98
Age Group at Index Date				
0-1 years	5,965	12.90	20,864	15.02
2-10 years	27,592	59.68	56,435	40.64
11-17 years	11,161	24.14	43,840	31.57
18-20 years	1,518	3.28	17,737	12.77

New areas to explore

- To what degree are there common patterns of co-occurring conditions among children with ASD, their siblings or among subgroups of children with ASD?
  - In particular, our data could be used to understand the natural course of ASD during transition periods from young childhood to adolescence and from adolescence to early adulthood.
  - This could lead to more optimal timing of treatment interventions and highlight opportunities to impact adult well-being and productivity.
- Research to determine the extent to which elevated risk is a common factor within families. For example, is the increased risk for GI conditions in a child with ASD associated with elevated risk for the same GI conditions among his/her siblings?
  - Analyses of family risk patterns could inform research on etiology as well as research about potential interventions.

Panel 1: Overview of Co-Occurring Conditions in Children and Adults – What have We Learned about Co-Occurring Conditions from Epidemiological and Clinical Experience?

9:30 – 9:45 **Lisa Croen, Ph.D., M.P.H.**
Director, Kaiser Permanente Autism Research Program
Senior Research Scientist, Division of Research Kaiser Permanente Northern California

9:45 – 10:00 **Isaac Kohane, M.D., Ph.D.**
Lawrence J. Henderson Professor of Pediatrics and Health Sciences and Technology Harvard Medical School
Director, Francis A. Countway Library of Medicine Harvard University

Psychiatric and Medical Conditions Among Adults with ASD

Lisa Croen, PhD

Children with ASD have increased rates of medical and psychiatric conditions

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Children with ASD become adults with ASD

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ASD in Adults Study Objectives

- Health status of adults with ASD
- Health care utilization among adults with ASD
- Healthcare provider knowledge and experience

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Study Population

- Adults 18+ years of age
- Kaiser Permanente Northern California (KPNC) member for 9+ months per year
- 2008-2012

- **ASD CASES (N = 1,507)**
  - 2+ ASD diagnoses recorded in KPNC medical record
  - Anytime through Dec 31, 2012

- **CONTROLS (N = 15,070)**
  - No ASD diagnoses
  - Randomly sampled at 10:1 ratio
  - Matched to cases on total length of KPNC membership, sex and age

Health Status Definitions

All conditions recorded in the electronic medical record between 2008-2012

- Validated algorithms using ICD-9 codes, lab results, medications
- Linkage to cancer and diabetes registries
- ICD-9 code groupings based on PheWAS
- Body Mass Index calculated at office visits

Demographic Characteristics

	Adults with ASD (N=1,507)	Controls (N=15,070)
Age, mean (SD)	29.0 (12.2)	29.4 (12.1)
>35	26%	26%
Race/ethnicity, %		
White, non-Hispanic	65.6%	44.0%
White, Hispanic	3.9%	4.2%
Black	7.6%	7.3%
Asian	11.1%	16.8%
Other	11.7%	27.7%
Sex, %		
Male	73.5%	73.2%
Female	26.9%	26.9%

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Phenotypic Characteristics

	Adults with ASD (N=1,507)
ASD Diagnosis	
Autistic Disorder	37.2%
Asperger Syndrome	29.7%
Not specified	33.1%
Intellectual Disability	
Yes	19.2%
Mild	12.8%
Moderate	3.1%
Severe	6.2%
NOS	77.9%
No	80.8%

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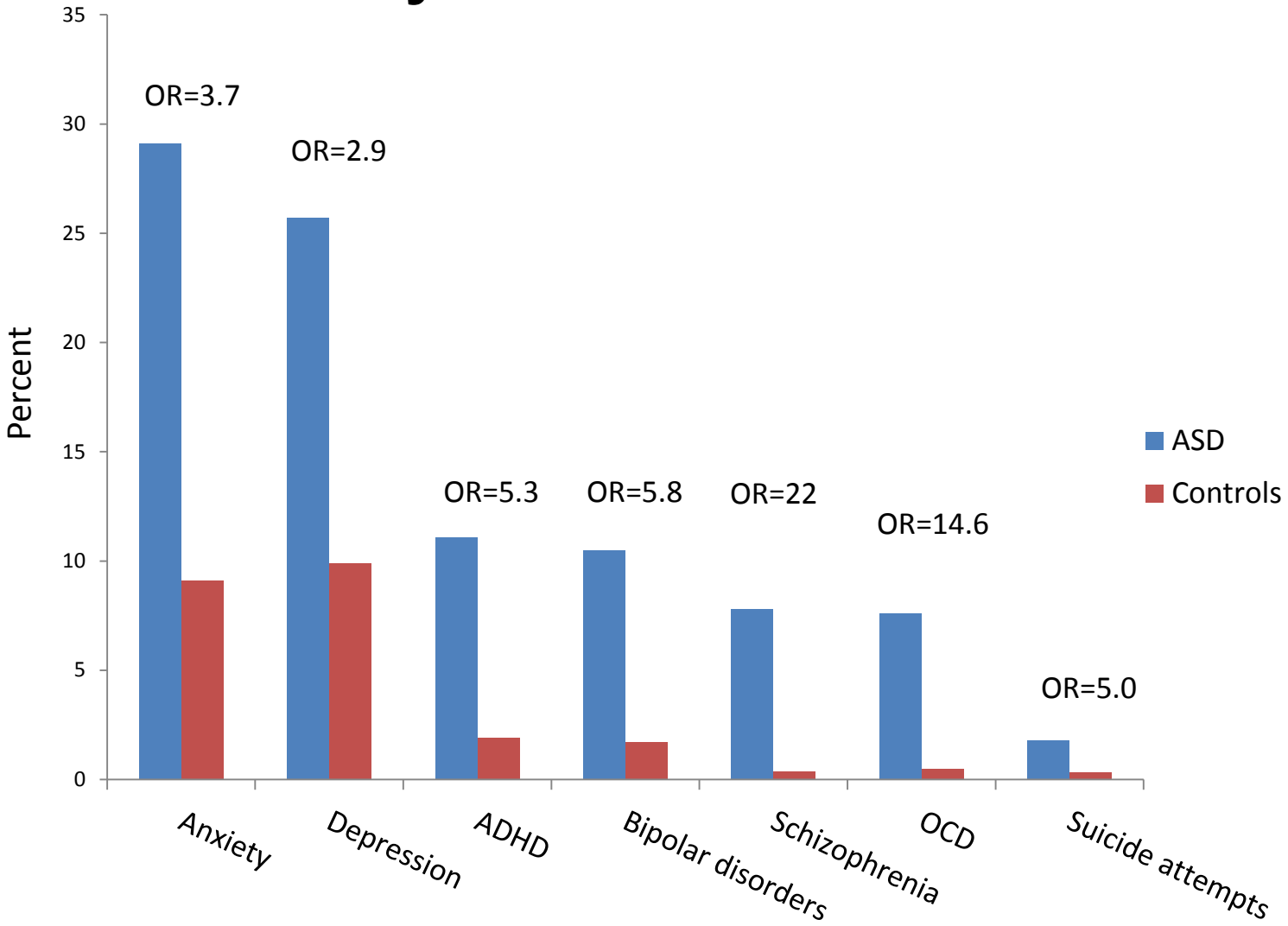
Psychiatric Conditions



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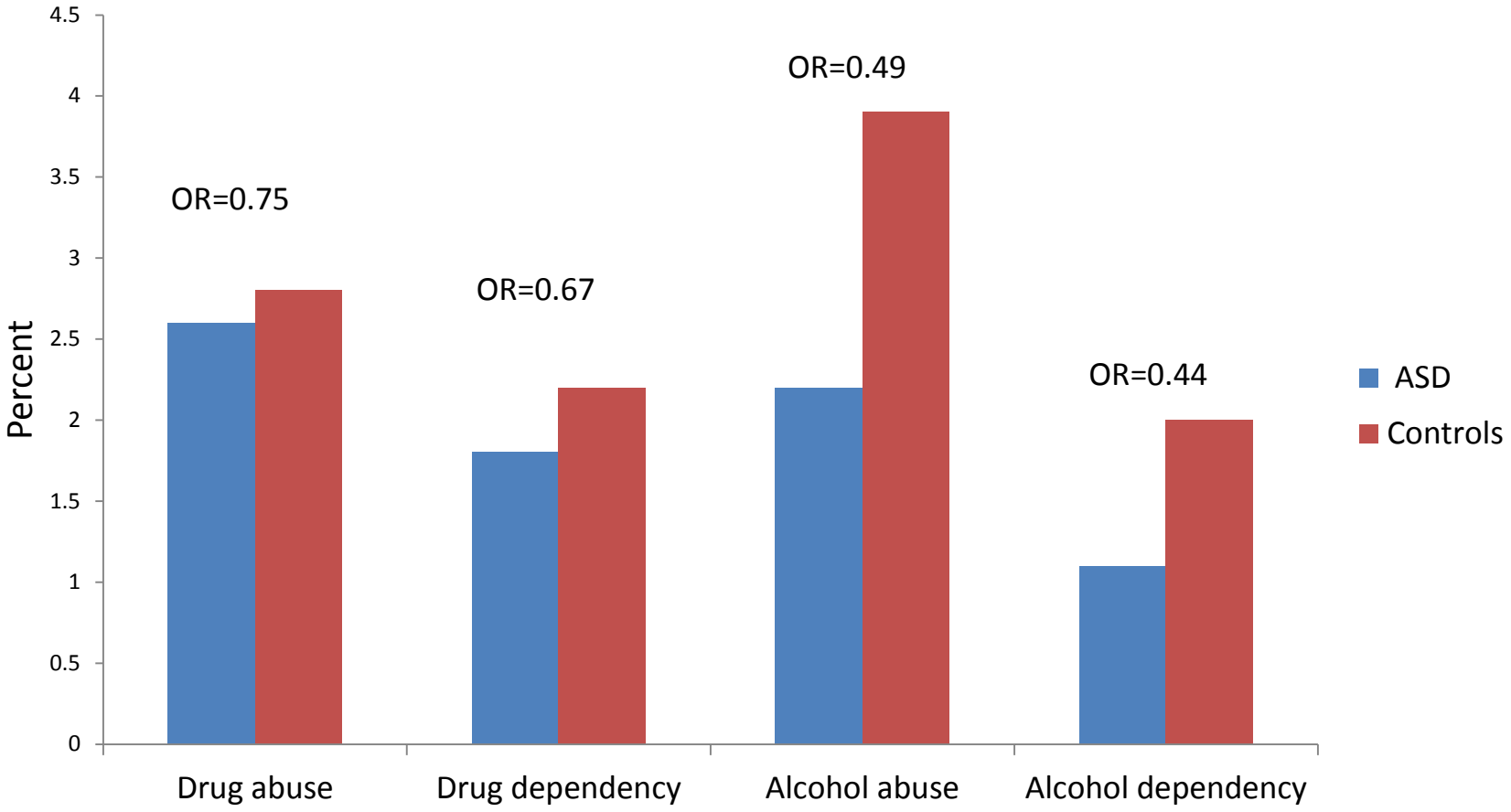
Drug and Alcohol Use



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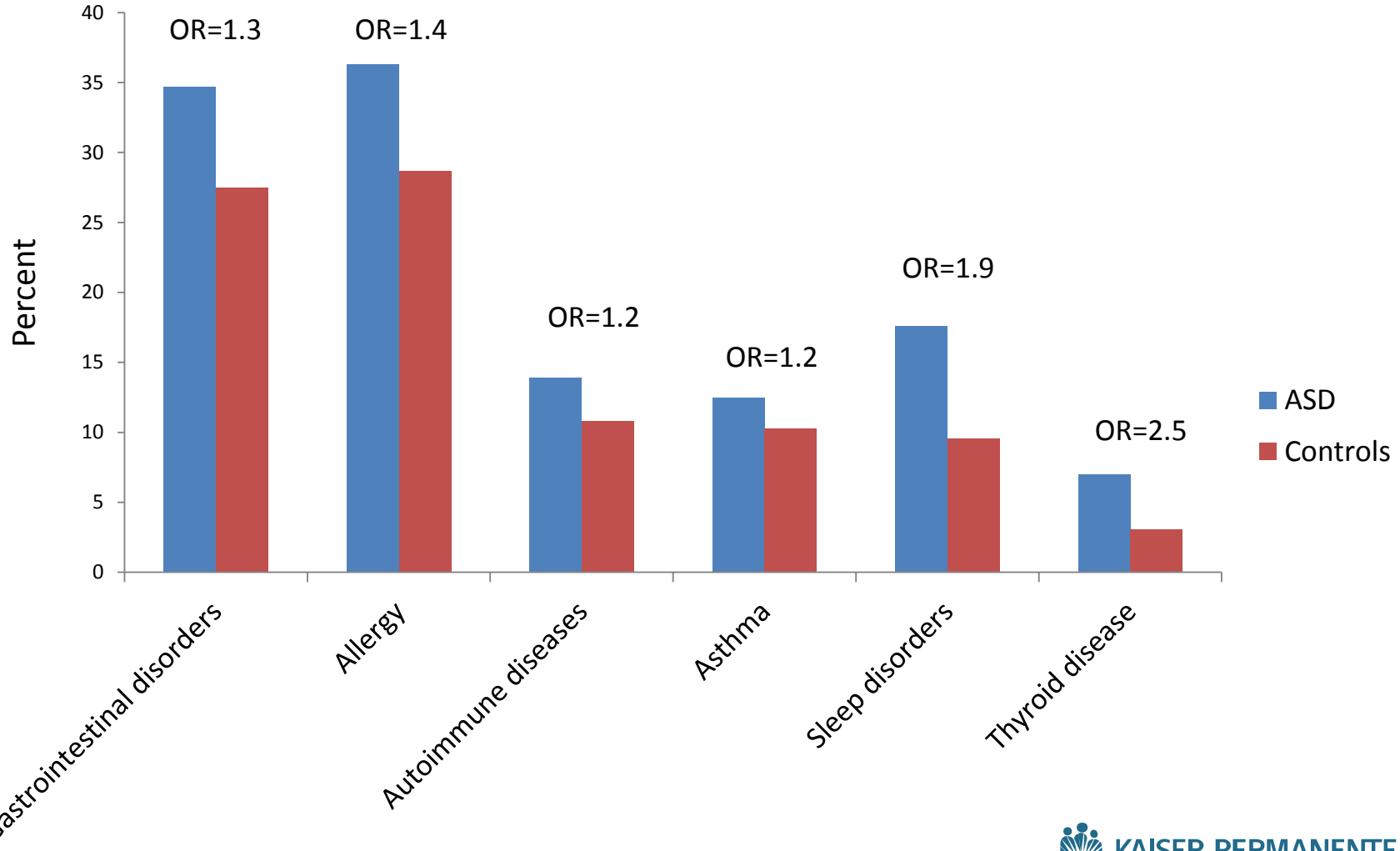
GI, Immune, Sleep, Thyroid



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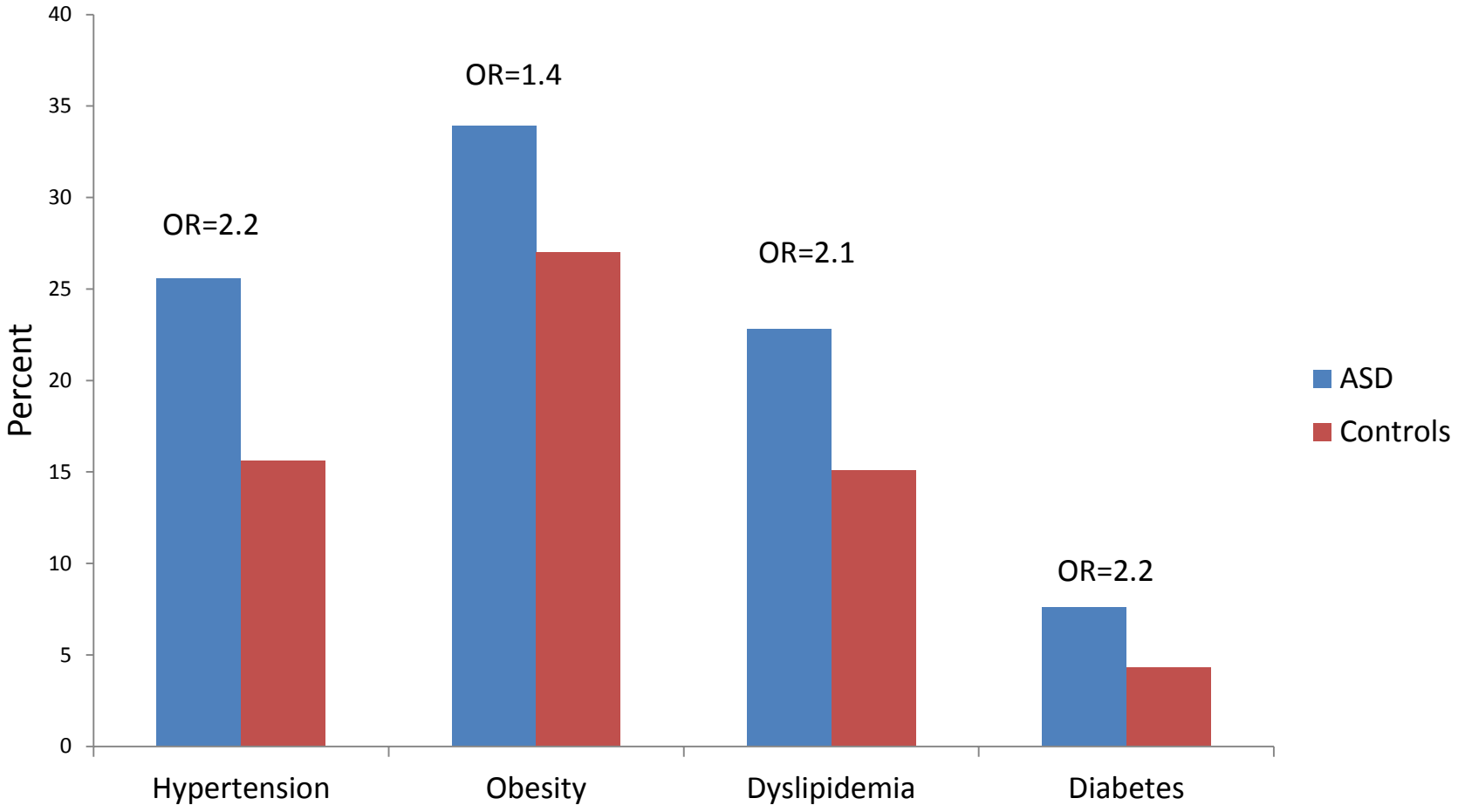
Metabolic Conditions



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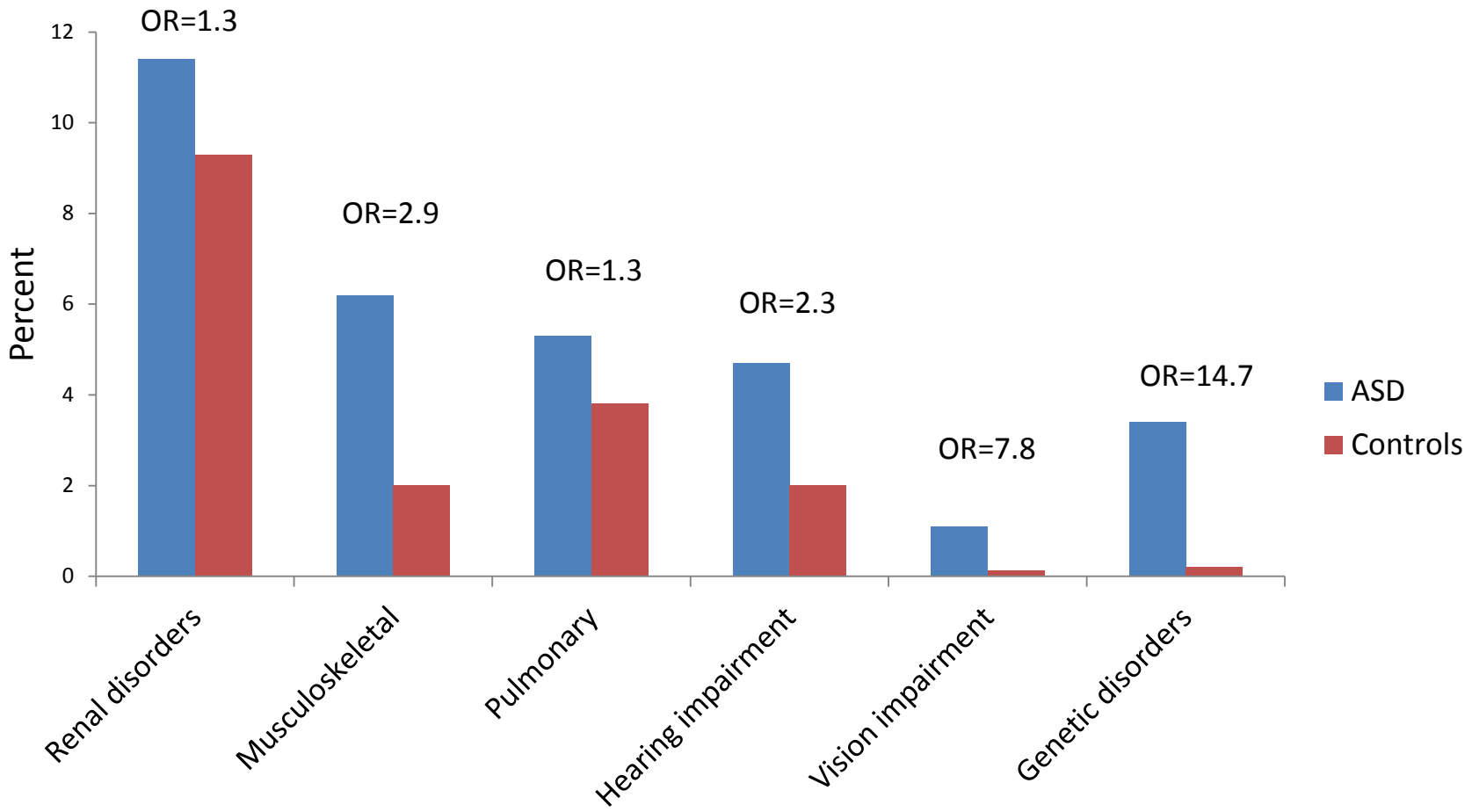
Other Medical Conditions



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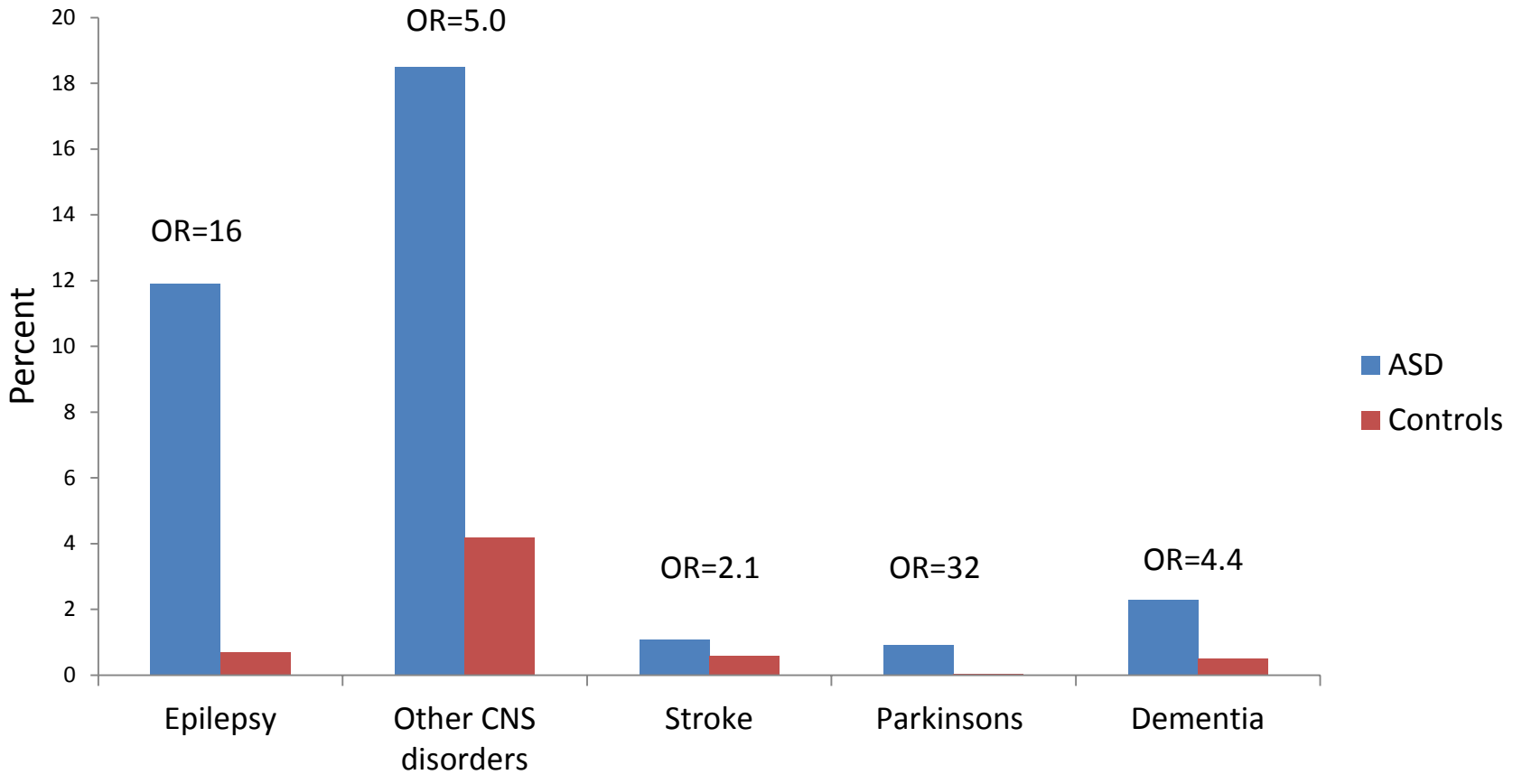
Neurologic Conditions



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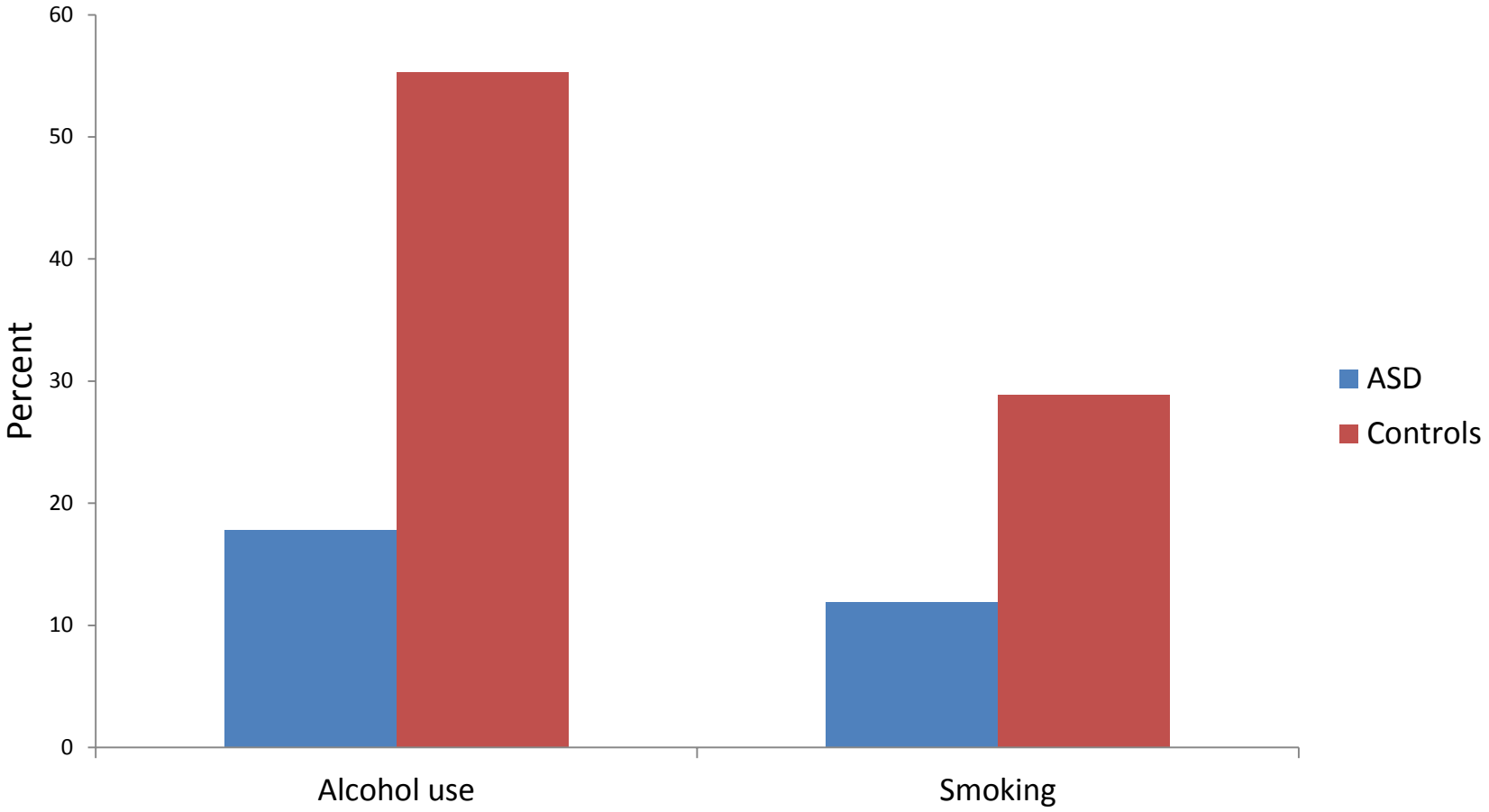
Alcohol and Tobacco Use Self-Reported



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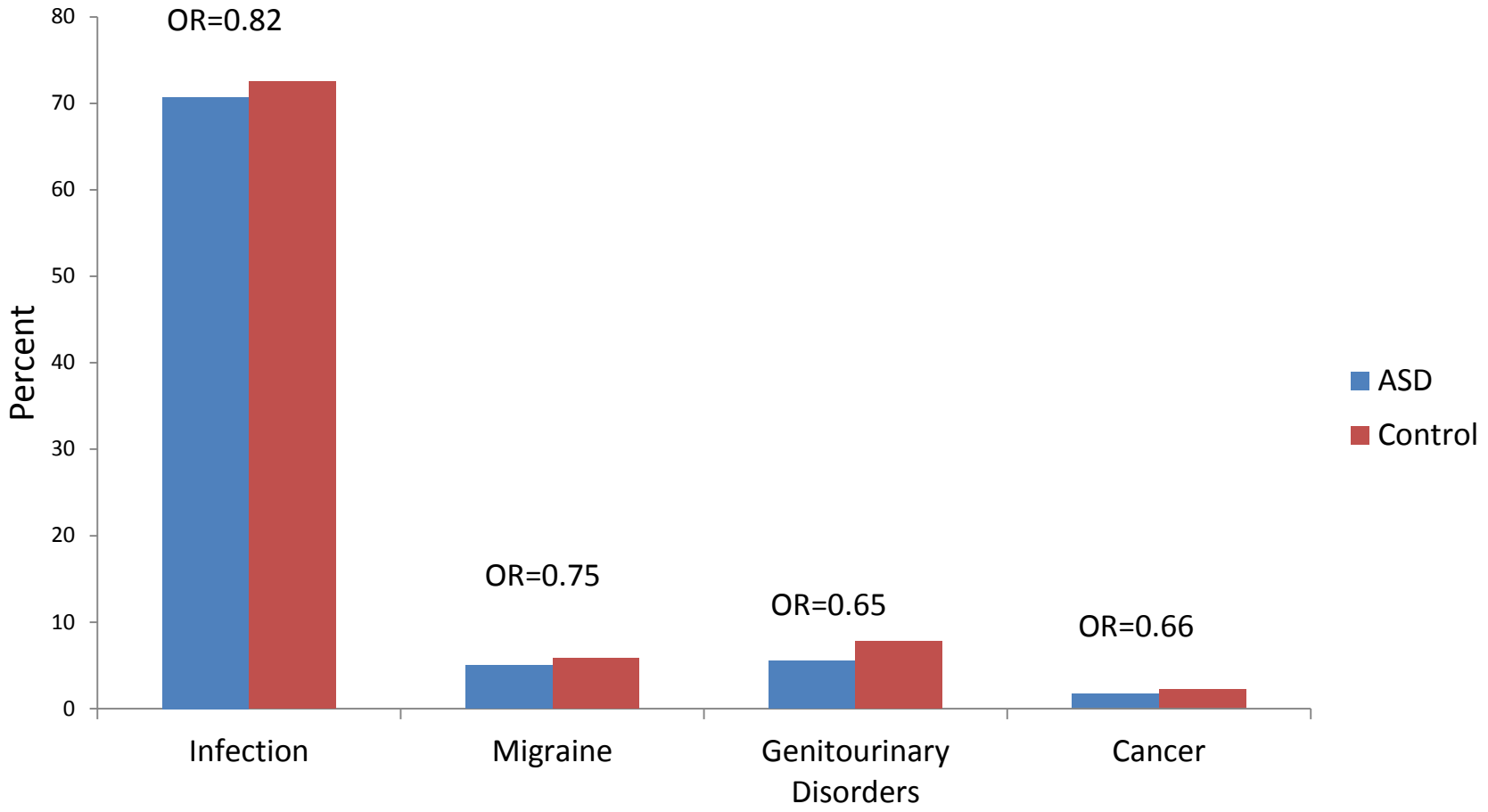
Conditions Less Common in ASD



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Summary and Conclusions

- Evidence for increased rates of many health conditions in ASD
- Some evidence for common biologic causes
  - E.g., shared genetic susceptibility to several psychiatric disorders including ASD
  - Obesity is a risk factor for several chronic conditions observed in autistic adults
- Communication and social impairments and sensory issues impede preventive health, early diagnosis, timely treatment
- Need health education and lifestyle interventions early on to improve diet, exercise, and reduce risk factors for chronic illnesses
- Need better integration of people with ASD into all aspects of society to reduce social isolation, discrimination and lower burden of disease

Research Opportunities

- Understand the social, health care access, and biologic mechanisms underlying the increased rates of medical and psychiatric conditions
- Understand how physicians investigate and manage chronic disease in adults with ASD
- Develop and test improved strategies for delivering health care to adults with ASD

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Coauthors

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Autism in Adults Workgroup

ARP Community Advisory Board KPNC

Clinical Partners

Children and Adults with ASD and Families

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Autism and Autisms.

Isaac S. Kohane, MD, PhD

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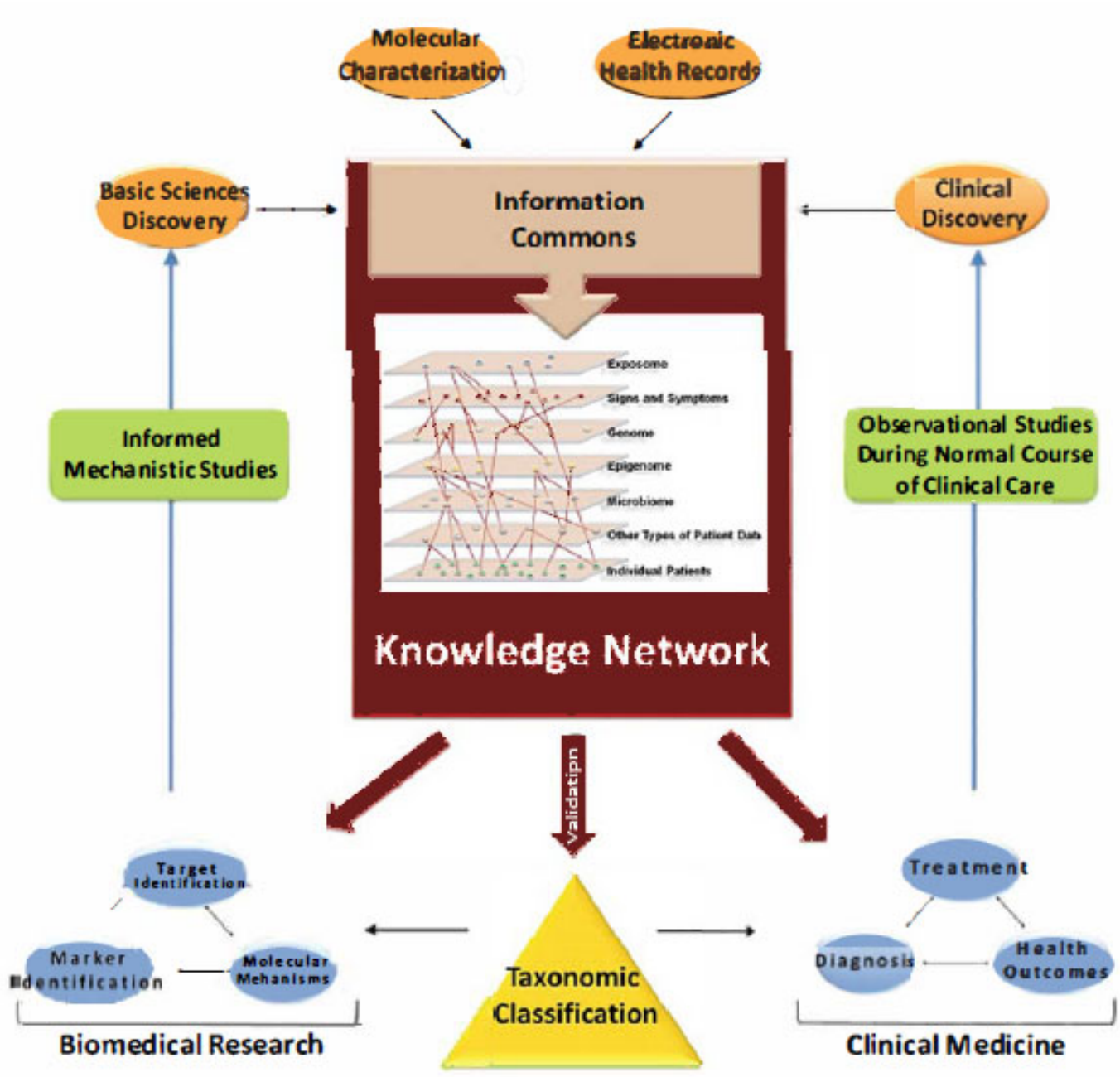
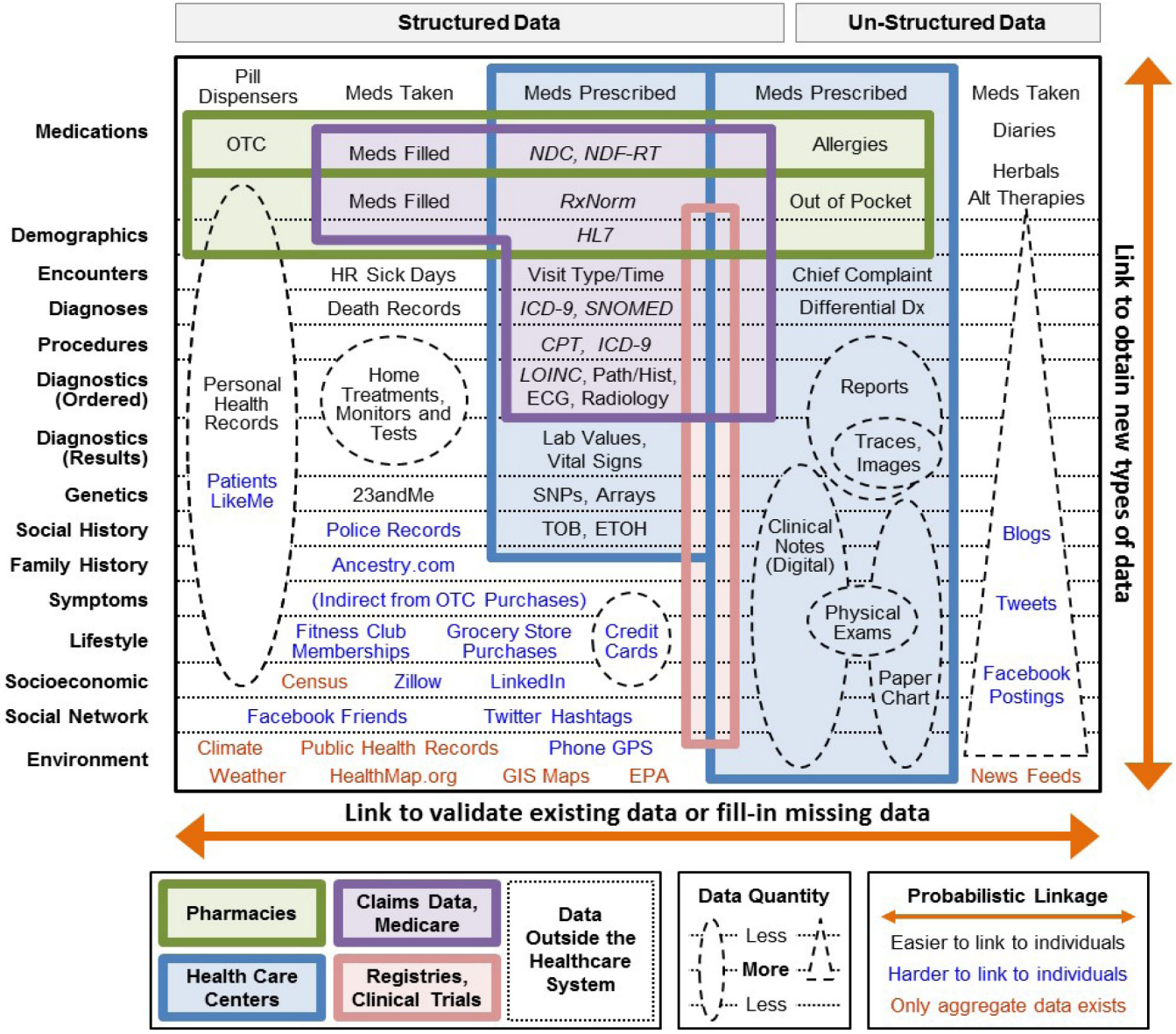
HARVARD MEDICAL SCHOOL


Molecular Characterization
Electronic Health Records
Basic Sciences Discovery
Information Commons
Clinical Discovery
Exposome
Signs and Symptoms
Genome
Epigenome
Microbiome
Other Types of Patient Data
Individual Patients
Informed Mechanistic Studies
Observational Studies During Normal Course of Clinical Care
Knowledge Network
Validatipn
Target Identification
Marker Identification
Molecular Mehanisms
Taxonomic Classification
Treatment
Diagnosis
Health Outcomes
Biomedical Research
Clinical Medicine


Structured Data
Un-Structured Data
Medications
Demographics
Encounters
Diagnoses
Procedures
Diagnostics (Ordered)
Diagnostics (Results)
Genetics
Social History
Family History
Symptoms
Lifestyle
Socioeconomic
Social Network
Environment
Pill Dispensers
Meds Taken
Meds Prescribed
Meds Prescribed
Meds Taken
Diaries
OTC
Meds Filled
NDC, NDF-RT
Allergies
Herbals
Alt Therapies
Meds Filled
RxNorm
Out of Pocket
HL7
HR Sick Days
Visit Type/Time
Chief Complaint
Death Records
ICD-9, SNOMED
Differential Dx
CPT, ICD-9
Personal Health Records
Home Treatments, Monitors and Tests
LOINC, Path/Hist, ECG, Radiology
Reports
Lab Values, Vital Signs
Traces, Images
Patients LikeMe
23andMe
SNPs, Arrays
Clinical Notes (Digital)
Blogs
Police Records
TOB, ETOH
Ancestry.com
Tweets
(Indirect from OTC Purchases)
Physical Exams
Fitness Club Memberships
Grocery Store Purchases
Credit Cards
Facebook Postings
Census
Zillow
LinkedIn
Paper Chart
Facebook Friends
Twitter Hashtags
Climate
Public Health Records
Phone GPS
Weather
HealthMap.org
GIS Maps
EPA
News Feeds
Link to obtain new types of data
Link to validate existing data or fill-in missing data
Pharmacies
Claims Data, Medicare
Health Care Centers
Registries, Clinical Trials
Data Outside the Healthcare System
Data Quantity
Less
More
Less
Probabilistic Linkage
Easier to link to individuals
Harder to link to individuals
Only aggregate data exists

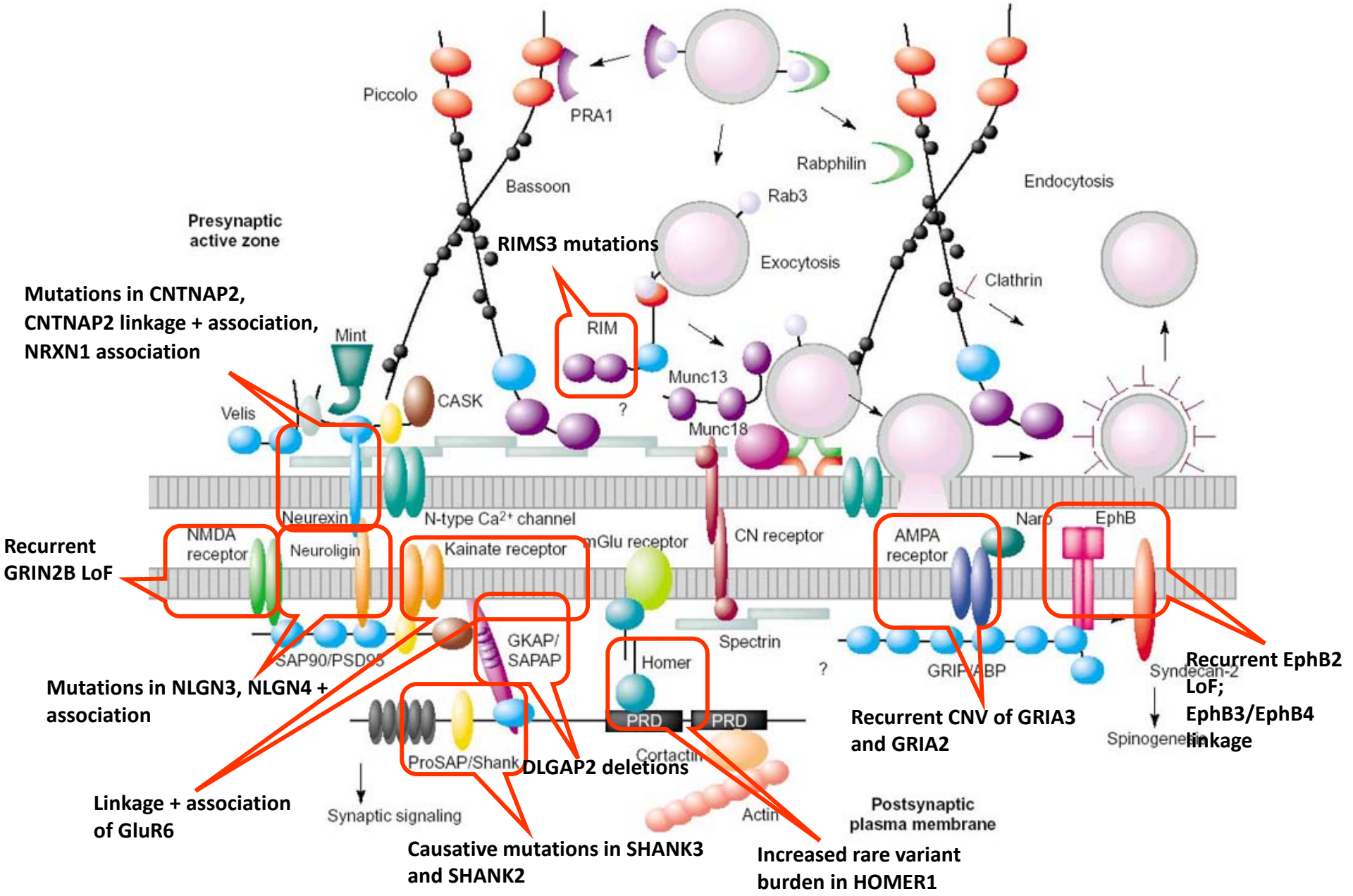
So what does precision medicine mean?

Probability of a disease *D*

Given the findings *F*

$$p(D \mid F)$$

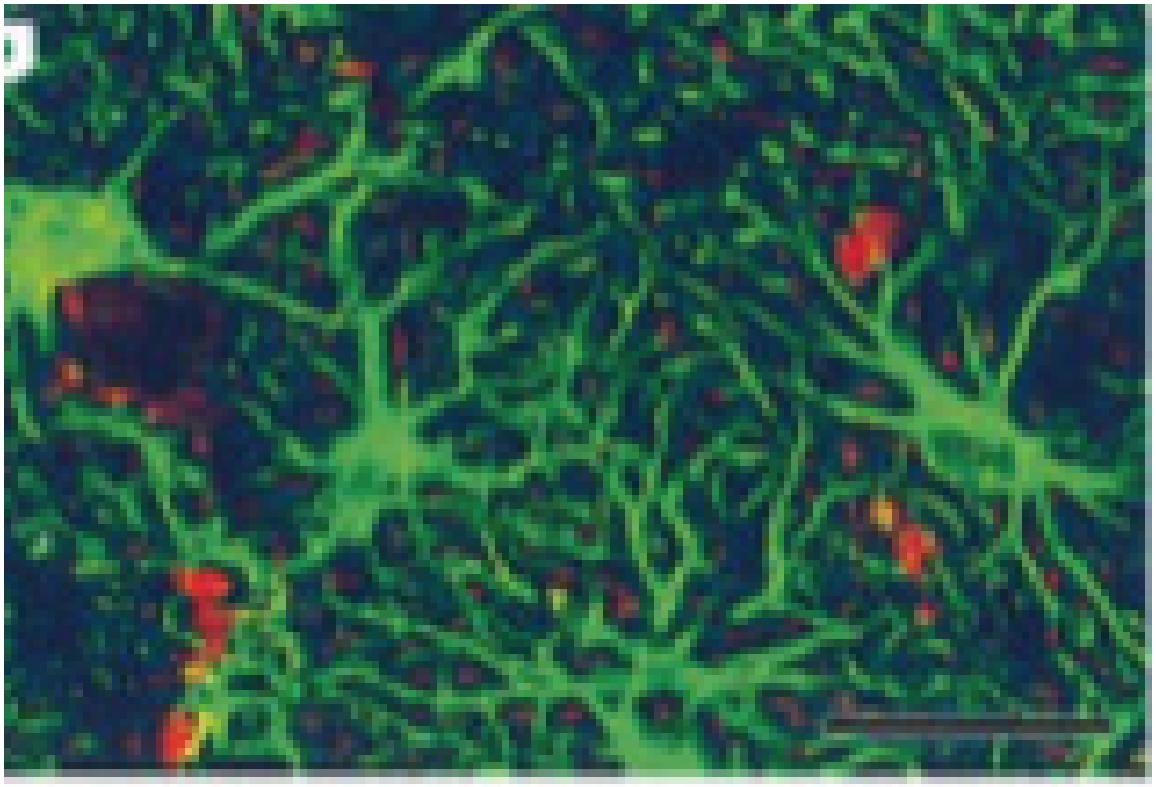
Convergence on synaptic function



Pathways from Predictor

KEGG pathways	Count	%	P-Value	FDR	Genes
Neurotrophin signaling pathway	11	4.5	0.000018	0.0018	CRK. CRKL, KIDINS220, MAP2K1, MAP3K5, MAPK8, PIK3CB, PRKCD. RPS6KA3, SH2B3, YWHAG
Fc gamma R-mediated phagocytosis	7	2.9	0.0034	0.16	CRK, CRKL, DOCK2, MAP2K1I, PIK3CB. PRKCD, PTPRC
Focal adhesion	10	4.1	0 0037	0.12	ACTN1, CRK, CRKL, IQGAP2, ITGB2, MAP2K1, PDGFC, PIK3CB, PPP1R12A, ROCK1
Renal cell carcinoma	6	2.5	0.0044	0.11	CREBBP, CRK, CRKL, EGLN1. MAP2K1, PIK3CB
Regulation of actin cytoskeleton	10	4.1	0.0057	0.11	ACTN1, CRK, CRKL, IGF IR, MAP2K1, MAPK8, PDGFC, PIK3CB, PPP1R12A, ROCK1
Vascular smooth muscle contraction	7	2.9	0.0075	0.12	GNAQ, GUCY1B3, NIAP2K1, PPP1R12A, PPP1R12B, PRKCD, ROCK1
Chemokine signaling pathway	9	3.7	0.008	0.11	CCR2, CRK, CRKL, DOCK2, JAK2, MAP2K1, PIK3CB, PRKCD, ROCK1
Long-term potentiation	5	2.1	0.021	0.23	CREBBP, GNAQ, MAP2K1, PPP1 R12A, RPS6KA3
Chronic myeloid leukemia	5	2.1	0.029	0.28	CRK, CRKL, CTBP2, MAP2K1, PIK3CB
Fc epsilon RI signaling pathway	5	2.1	0.032	0.28	LCP2, MAP2K1, MAPK8, PIK3CB, PRKCD
Notch signaling pathway	4	1.6	0.036	0.29	CREBBP, CTBP2, MAML3, NOTCH 1
Type II diabetes mellitus	4	1.6	0.036	0.29	HK2P1, MAPK8, PIK3CB, PRKCD
Progesterone-mediated oocyte maturation	5	2.1	0.044	0.31	IGF1R, MAP2K1, MAPK8, PIK3CB, RP56KA3

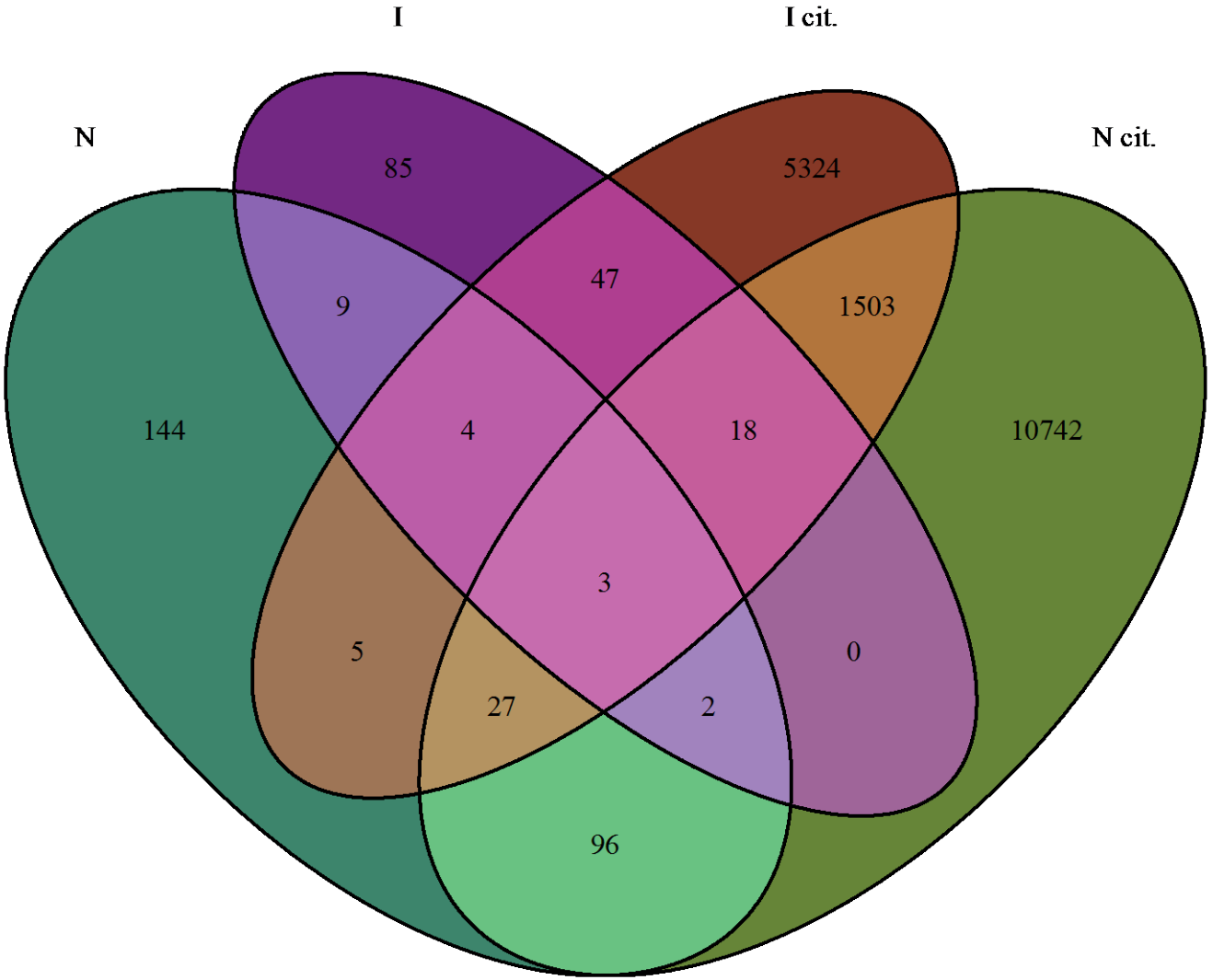
Vargas *et al. 2005*



And some interesting observations

- Mother's with RA and Father's with T1DM
- Mother's with high gestational CRP
- Intra- and peri-partum infection
- Mouse models of inflammation

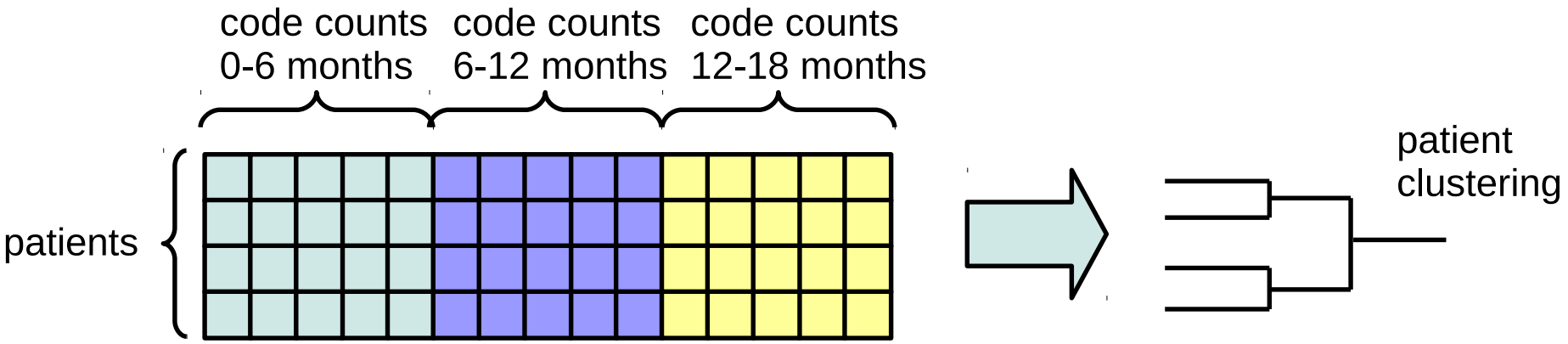
(Immune vs Synapse)+Gene



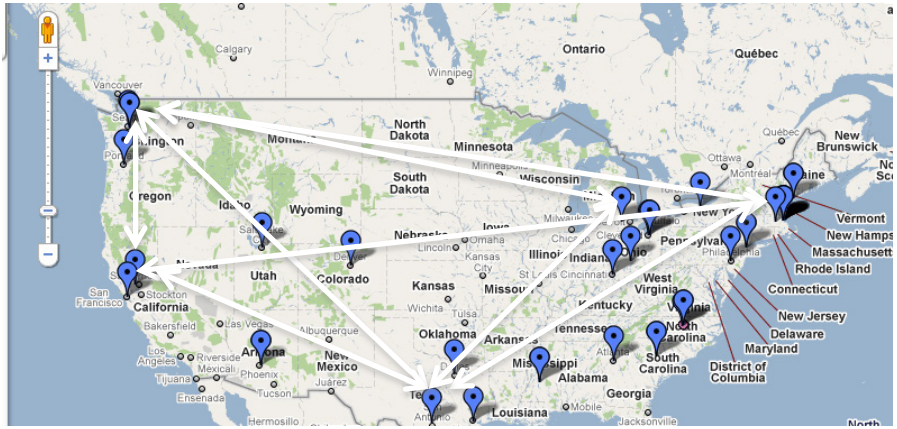
Across AHC's at a glance

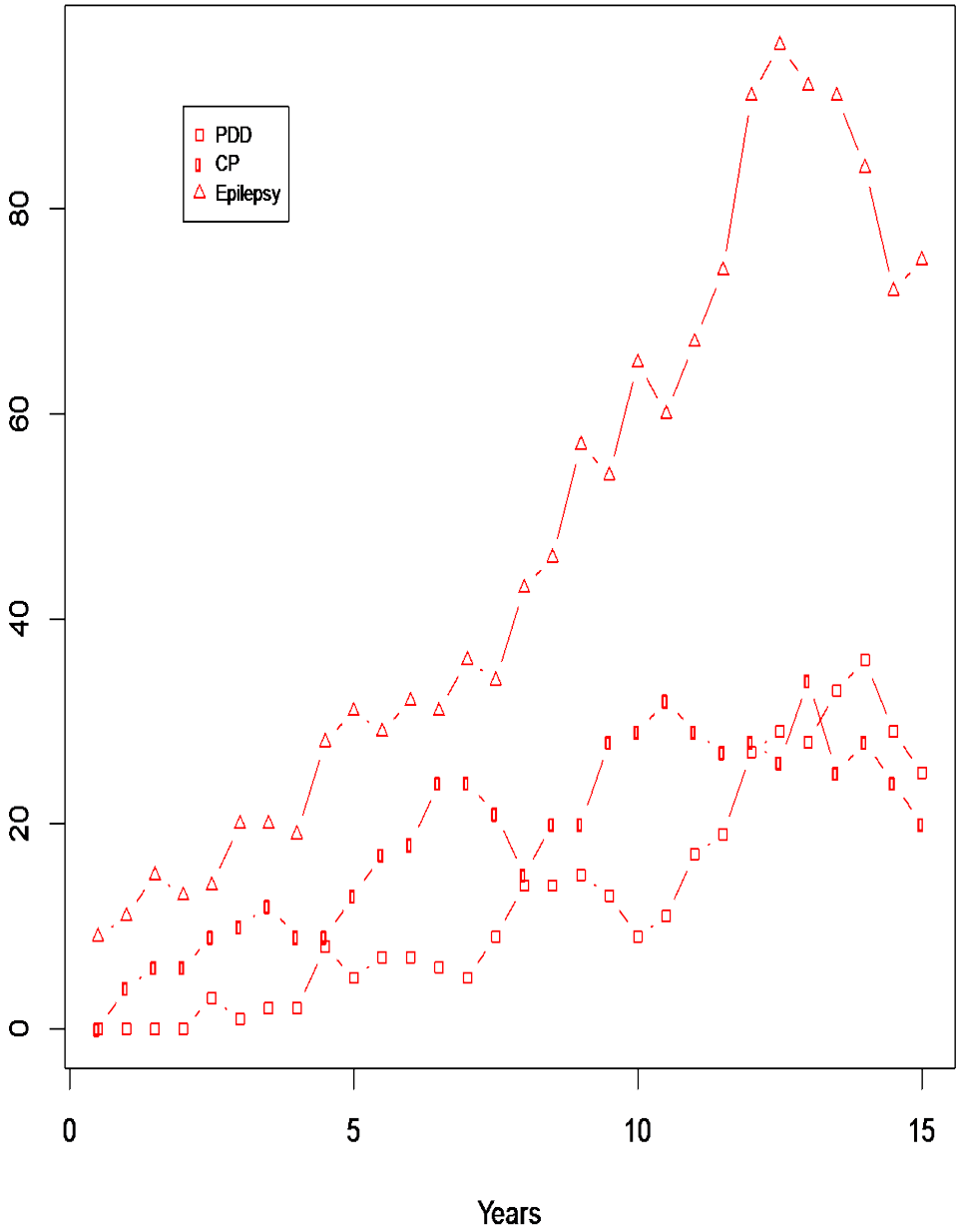
- Patients: 13750 (with basic demographics)
- ~0.5% hospital population
- M:F (5:1)
- Diagnoses: 5627
- Laboratory measurements: 3,158,234 on 3581 lab measurement types
- Medications: > 800,000 Rx's

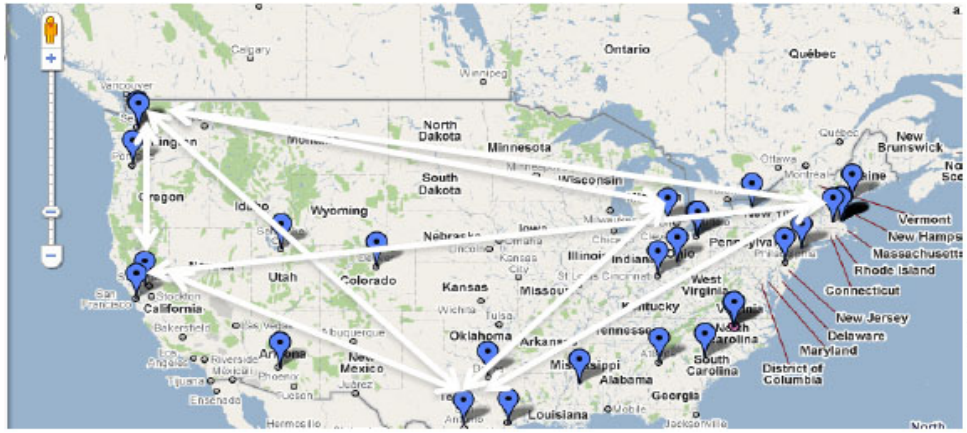
Unbiased clustering



Autism or Autisms?



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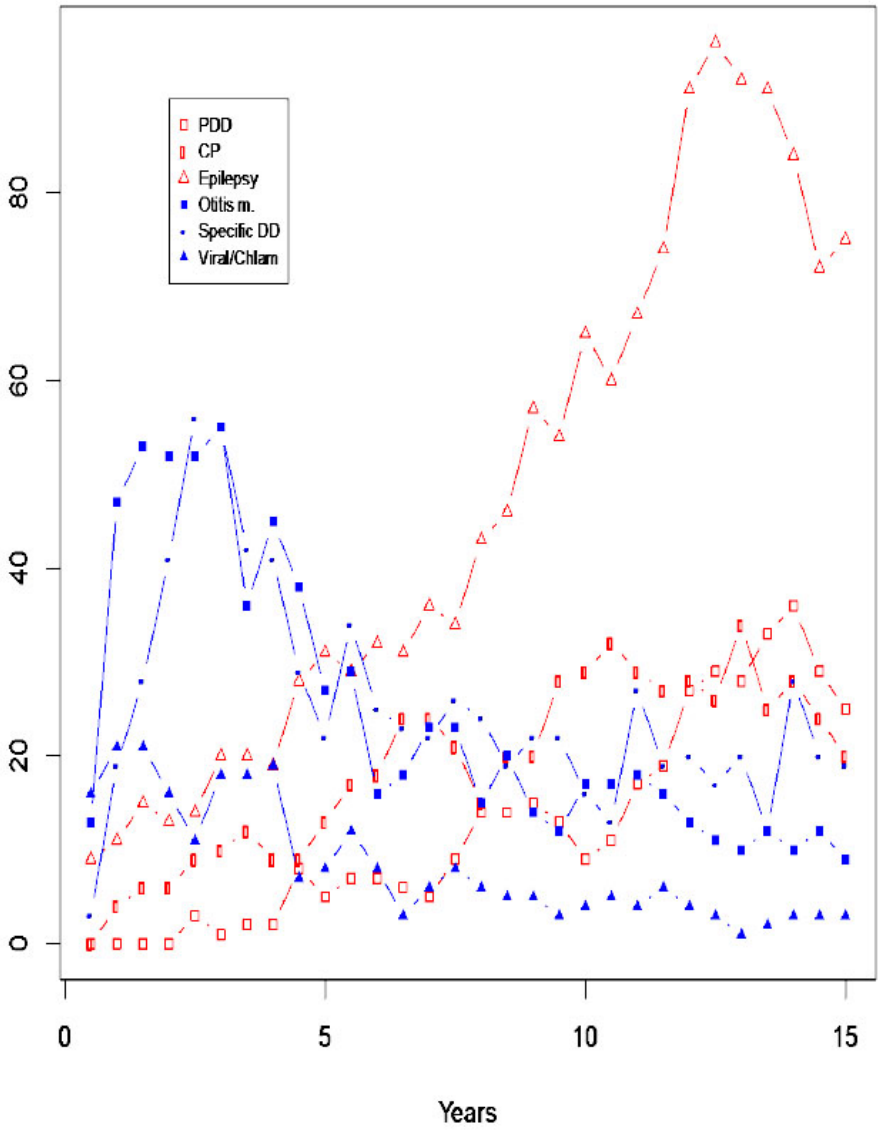
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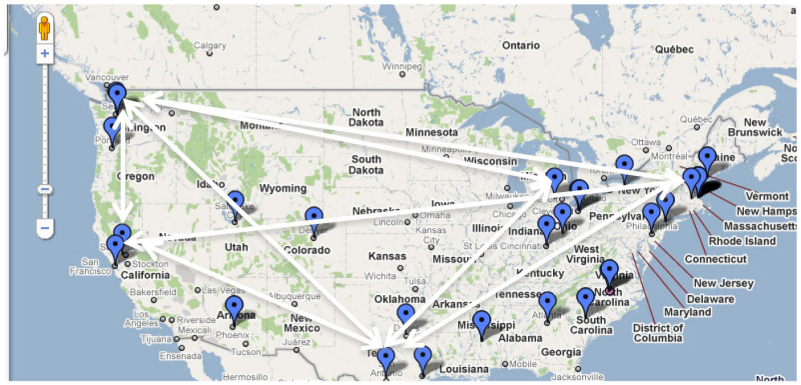
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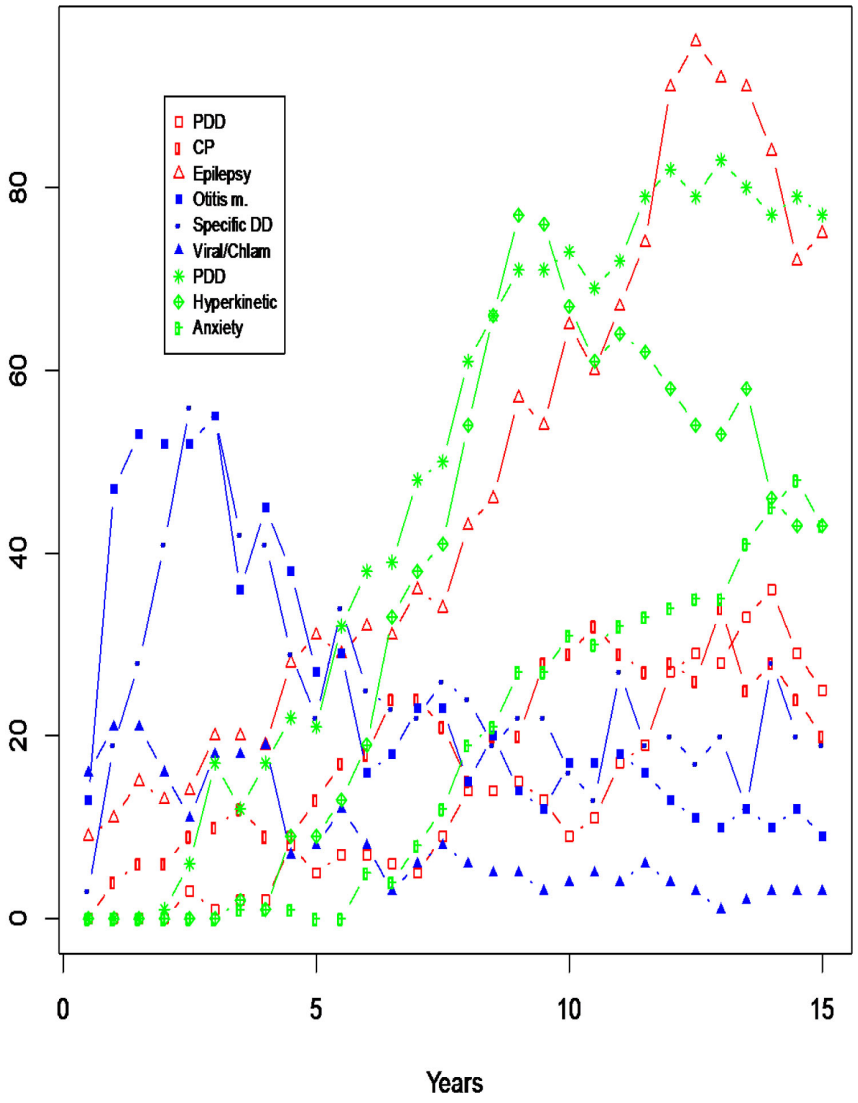
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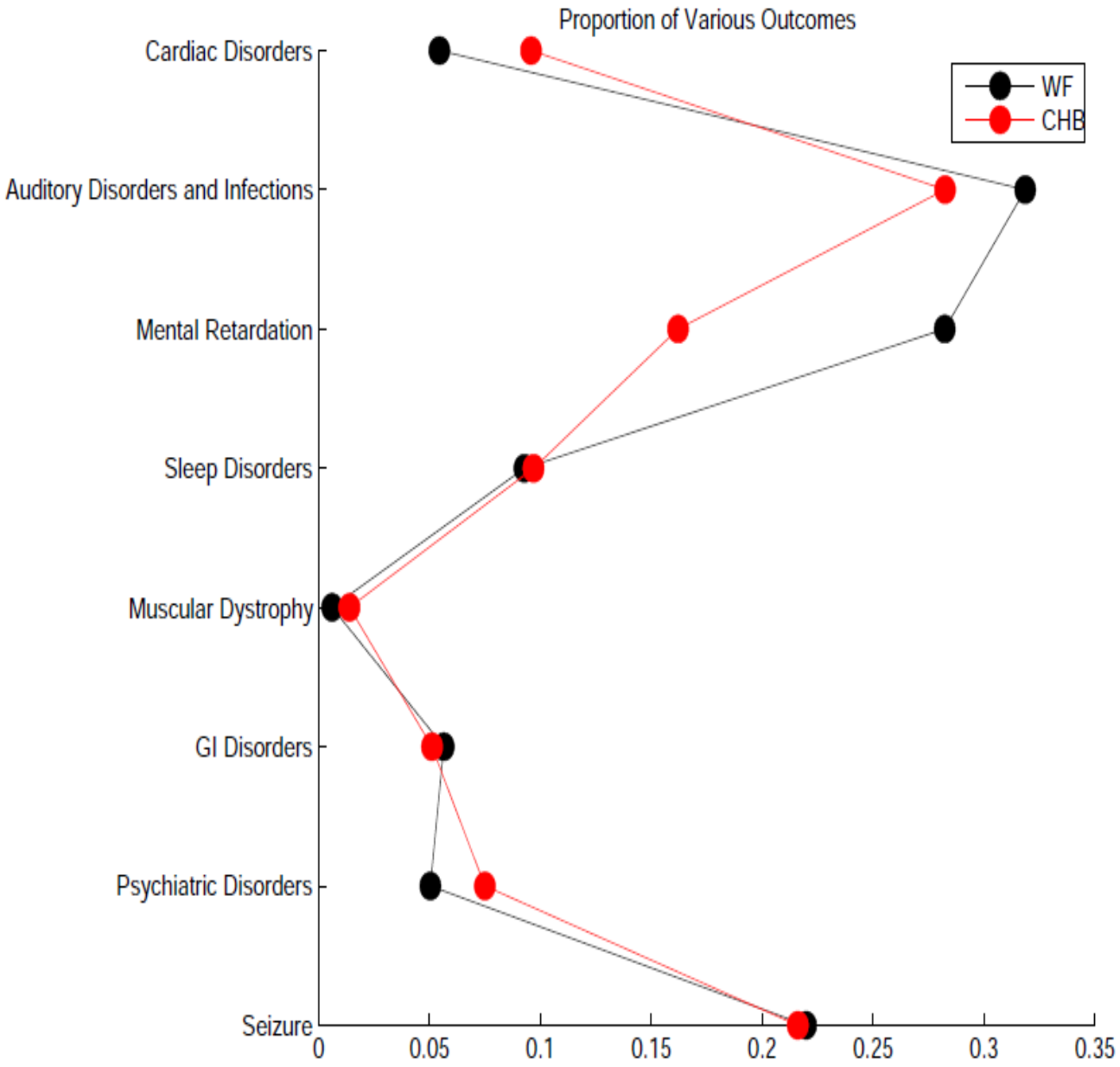
Reproducibility

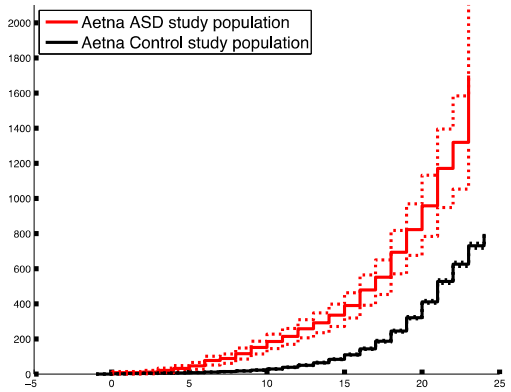


Thought experiment

- Autism, a common disease with prevalence ~1%
- Let's say causal variants ~1% frequency, RR = 2
- Then with 80% power (5% alpha) ~23,000
- What if ASD is really 10+ diseases?
  - Many reasons why it will not be clinically noticed.
- Then causal variants will have 10% frequency and with same power/alpha ~2300 subjects
- But until now phenotype-first was unaffordable.

Validating IBD Findings

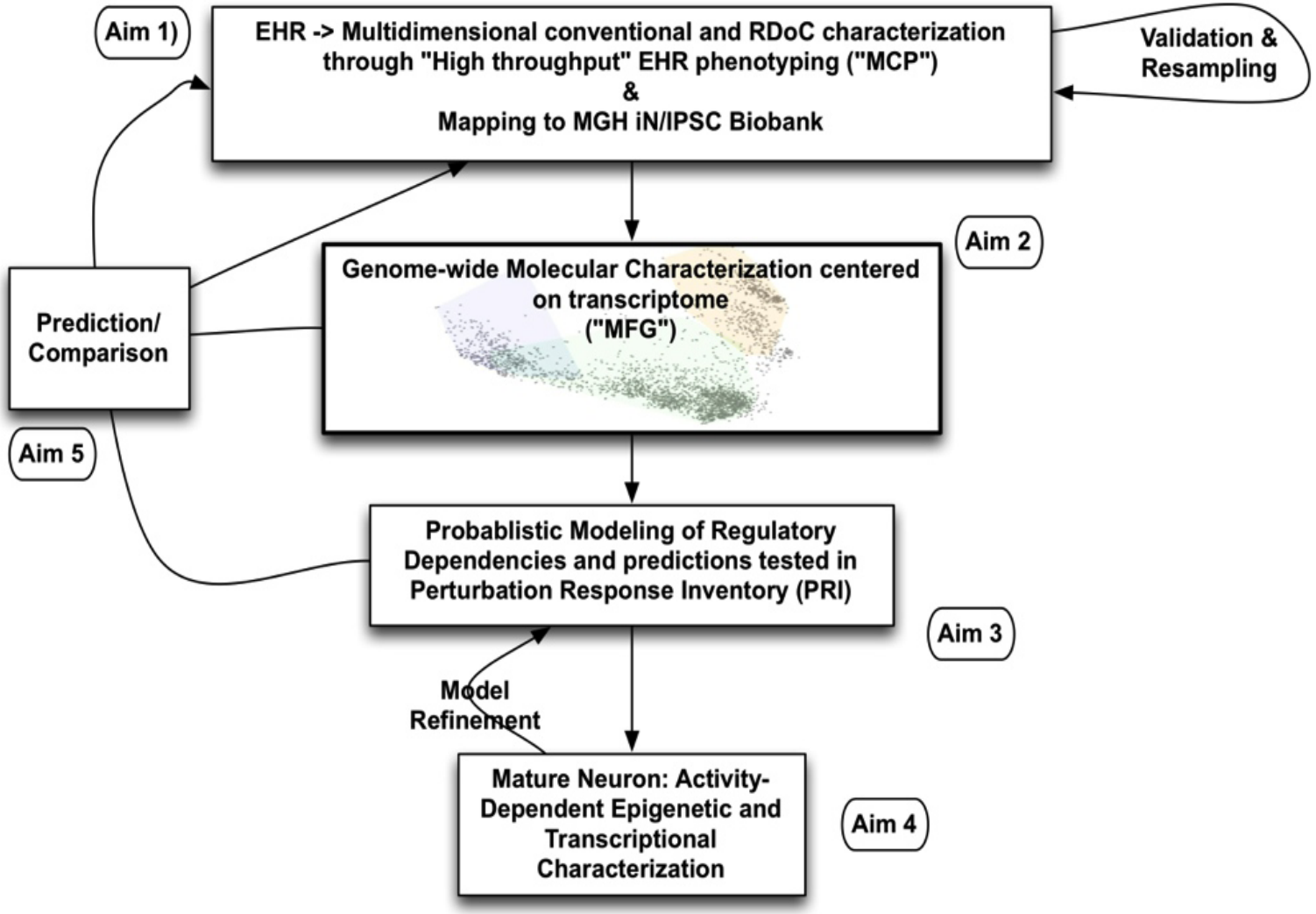
Study Population	Control	ASD
Aetna	186.4(183.3-189.5)	446.0 (376.8-529.8)
BCH	433.4(417.5-449.9)	630.6 (462.1-859.9)
WFBMC	282.9 (260.7-306.7)	361.9(140.0-1824.8)



Summary

- The conventional wisdom regarding the causes of autism is incomplete, divided and obscured.
- Phenotype-first strategies may massively accelerate discovery of genetic architecture.
- There is a lot of shared pathobiology across autism
- There is a lot of undiscovered heterogeneity and distinctive pathobiology within conventionally labeled diseases.
- Aggressively ecumenical approach to integrative data analysis will accelerate discovery.

Neuropsychiatric Genome-Scale and RDoC Individualized Domains (N-GRID)



Thank you

Psychiatric Genetics
Roy Perlis
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Alal Eran
Chirag Patel
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Stan Shaw
Peter Szolovits
Tianxi Cai
Finale Doshi
Daria Prilutsky
David Margulies

Developmental Medicine
Lenny Rappaport
Chuck Nelson
Stephanie Brewster

Genomics
Lou Kunkel

Panel 1: Overview of Co-Occurring Conditions in Children and Adults – What have We Learned about Co-Occurring Conditions from Epidemiological and Clinical Experience?

10:00 – 10:15 **Daniel L. Coury, M.D.**
Chief, Section on Developmental and Behavioral Pediatrics Chief, Child Development Center Program Director, Developmental/Behavioral Pediatrics Fellowship, Nationwide Children's Hospital
Professor of Pediatrics and Psychiatry, College of Medicine
The Ohio State University

10:15 Discussion

Autism Spectrum Disorder and Co-Occurring Conditions

Daniel L. Coury, MD, FAAP

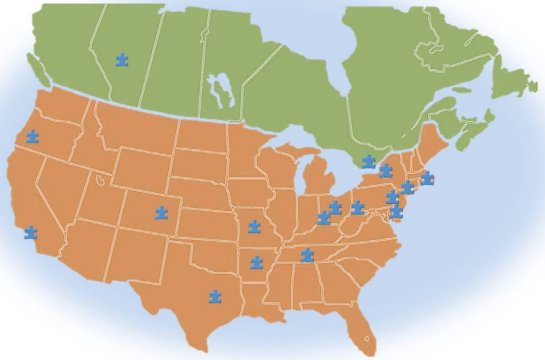
Professor of Pediatrics and Psychiatry
The Ohio State University
Nationwide Children's Hospital

Director, Clinical Coordinating Center,
Autism Speaks Autism Treatment Network

Medical Director, Autism Intervention Research Network on Physical Health (AIR-P)

These materials are the product of on-going activities of the Autism Speaks Autism Treatment Network, a funded program of Autism Speaks. It is supported in part by cooperative agreement UA3 MC 11054, Autism Intervention Research Network on Physical Health (AIR-P Network) from the Maternal and Child Health Bureau, Health Resources and Services Administration, Department of Health and Human Service to the Massachusetts General Hospital.

Autism Speaks
Autism Treatment Network



- 14 sites in North America
- Dedicated to improving care for children with ASD and their families
- Emphasis on medical conditions among children with ASD
- Serves as the HRSA funded Autism Intervention Research Network on Physical Health (AIR-P)
  - Clinical intervention research on physical health
  - Efforts to improve care and disseminate findings

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AIR-P Autism Intervention Research Network on Physical Health

AIR-P Research and AS ATN Registry Data

- Much reported in November 2012 supplement to Pediatrics (open access at www.pediatrics.org)
- Registry – currently >6,300 children with data
- Supplement also includes clinical practice guidelines and network research reports

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Co-occurring Conditions and Symptoms

- Gastrointestinal and nutrition symptoms and disorders
  - Variations in diet preferences and supplements
  - Motility
  - Microbiome
- Epilepsy
- Sleep disorders
- Immune conditions
- Other mental health conditions

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GI Disorders

GI problem	Any past 3 months	Chronic past 3 months
Constipation	34.2%	23.9%
Diarrhea	29.4%	14.7%
Abdominal pain	26.2%	13.0%
Other GI	14.9%	11.4%
Nausea	13.6%	8.8%
Bloating	11.6%	5.2%
Any GI problem	52%	

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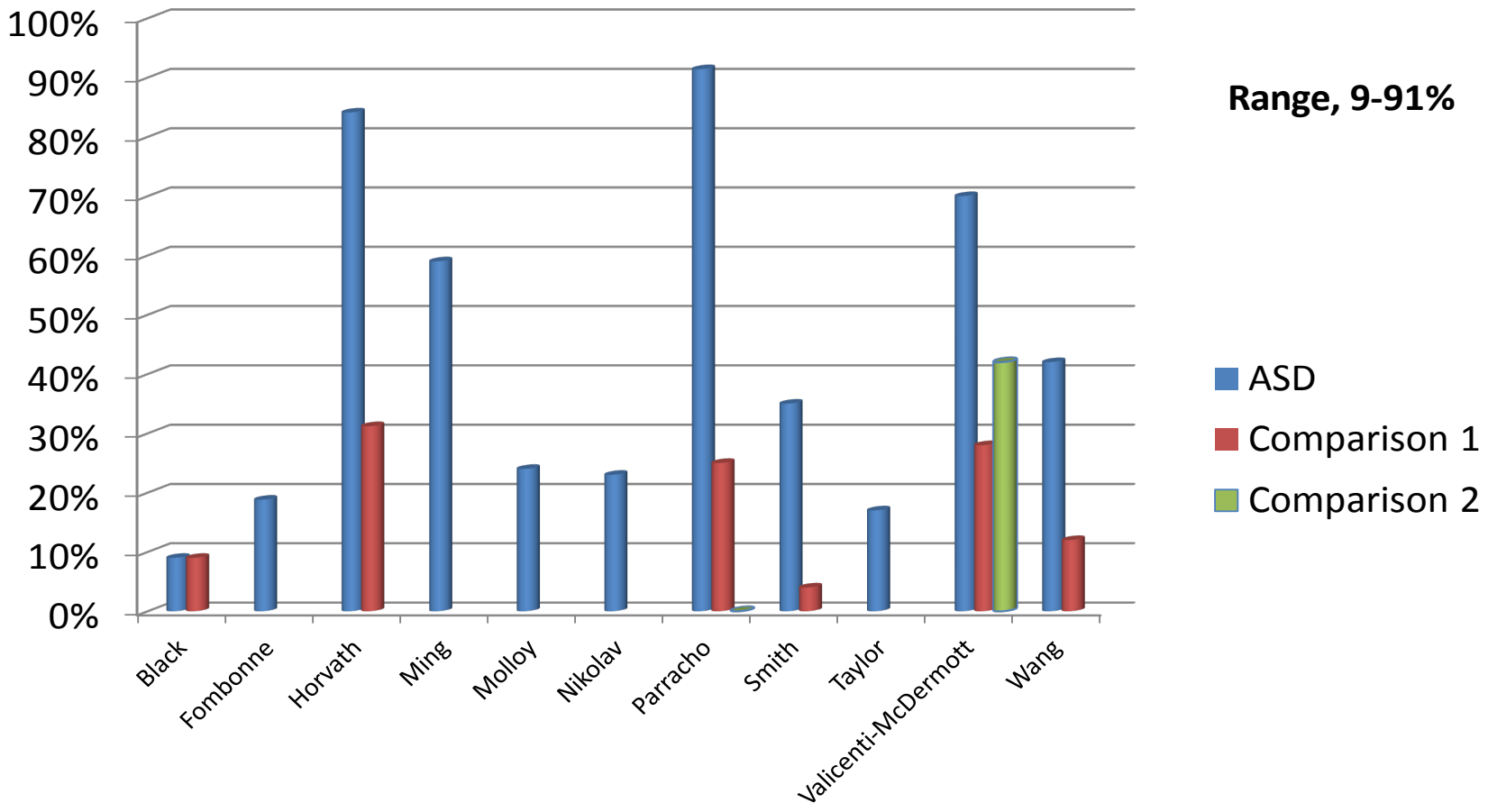
Reported Prevalence of GI Disorders in Children with ASD



ATN Autism Treatment Network

AIR-P Autism Intervention Research Network on Physical Health

Seizure Disorders in Children with ASD (n=2,569)

- 420 with seizures (16%)
- No differences by ASD diagnoses or gender
- Higher rates among White (p=0.01) and Latino populations (p=0.04)
- IQ difference (p=0.04)
  - Children with seizures: IQ <70 40%
  - Children without seizures: IQ <70 35%
- Parent report of skill loss (p<0.001)
  - Children with seizures: 21%
  - Children without seizures: 13%

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Seizure Disorders and Associated Findings

- Higher rates of GI problems ($p<0.001$) and sleep problems ($p< 0.001$) compared to those without seizures
- Lower Vineland adaptive scores and certain CBCL scales

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Sleep Disorders

- Previous reports: 53-78% of children with ASD; 26-32% in children without ASD
- Sikora et al. (Pediatrics, 2012)
  - 1,193 children ages 4-10 years
    - Good sleepers – 340 (28.5%)
    - Mild sleep problems – 638 (53.5%)
    - Moderate-severe sleep problems – 215 (18.0%)
  - Lower rates in older children
  - Sleep problems associated with problems in daytime behaviors

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Psychiatric Symptoms

- Previous reports
  - ADHD in 41-78% of children with ASD
- Symptom reports from Registry (from Child Behavior Checklist)
  - 37% high scores on attention subscale
  - 14% on aggressive subscale
  - 22% on hyperactivity subscale

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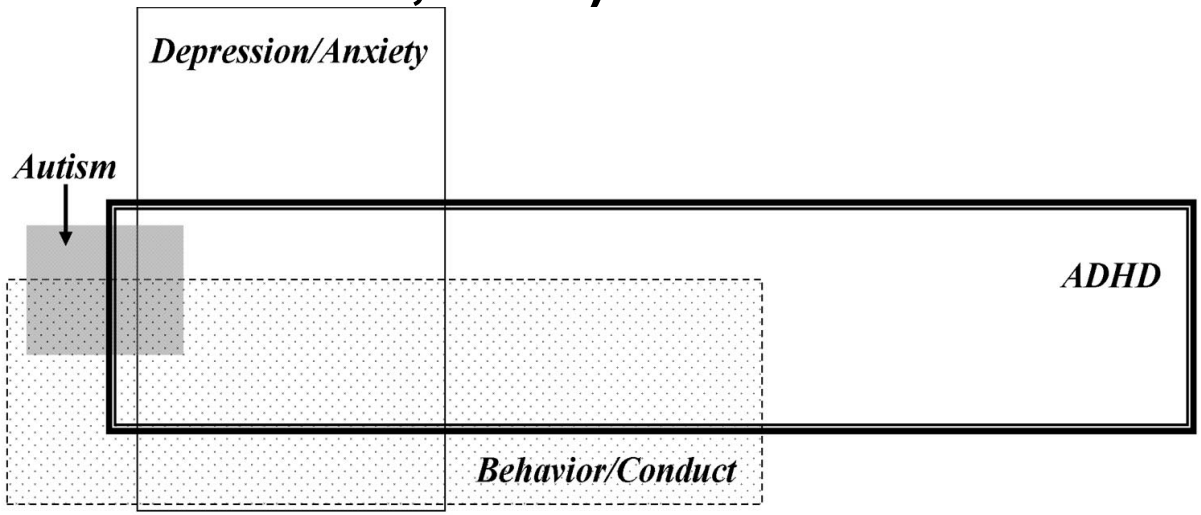
Overlapping Conditions (National Survey of Children's Health, 2007)



	Prevalence	% w/comorbid behavioral condition	% w/comorbid physical health condition
ADHD	6.8%	50.2%	31.3%
Behavior/Conduct Problem	5.3%	67.0%	36.0%
Depression/Anxiety	4.2%	63.1%	38.6%
Autism	0.5%	76.7%	39.0%

From Sheldrick and Perrin (EC), JDBP, 2010

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Psychotropic Medication Use (AS-ATN Registry, n=2741)

Age	Number of children in registry	Percent using any psychotropic
<6 yo	1514	10
6-11 yo	951	44
12-17 yo	276	66

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Medications Used

- Stimulants 35.6% of total
- SSRI 22.7%
- Atypical antipsychotics 22.5%
- Alpha-agonists 19.3%

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Co-existing Psychiatric Diagnoses

- Depression 2.5%
- Bipolar 1.5%
- ADHD 19%
- OCD 5.4%
- Anxiety 7.5%

- i.e., many children are treated without additional diagnosis

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Acknowledgments

- *ATN / AIR-P Clinical Coordinating Center*
  - James Perrin, Brian Winklosky, Kirsten Klatka, Karen Kuhlthau, Alix Nozzolillo, Jessie Figueroa
- *Autism Speaks*
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- *Funders*
  - HRSA, MCHB
  - Autism Speaks

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Workshop on Under-Recognized Co-Occurring Conditions in ASD

Presentations will address:

1. What do we know and what have we learned in each research area? (Prevalence in autism vs. developmental disability vs. typical population? Underlying biology? Challenges in diagnosing these condition in individuals with ASD?)

2. What do we need, both in the clinic and in research to decrease the burden of illnesses/extent of disability, or reduction in quality of life due to the co-occurring conditions?

3. Are there any specific gaps or barriers that need to be addressed before progress can be made in this area?

4. Has recent research created new opportunities that need to be explored?

Workshop on Under-Recognized Co-Occurring Conditions in ASD

Discussion questions:

1. What do we need in order to help those affected by these conditions now? (e.g., provider and community awareness, practice guidelines, services, policies.)

2. What kind of research is needed to support development of new and improved treatments for these conditions in the future?

3. Are there any lessons we can learn from other fields to help accelerate progress?

4. Are any of these fields ready for practice guidelines or other steps that have not yet occurred due to knowledge or service gaps or other barriers? If so, how can the IACC, federal agencies or private organizations help accelerate progress?

Panel 1: Overview of Co-Occurring Conditions in Children and Adults – What have We Learned about Co-Occurring Conditions from Epidemiological and Clinical Experience?

Discussion

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Break

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Panel 2:

Psychiatric Disorders

These slides do not reflect decisions of the IACC and are for discussion purposes only.

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

11:00 Panel 2: Psychiatric Disorders

11:00 – 11:15 **Lawrence Scahill, Ph.D., M.S.N., M.P.H.**
Professor of Psychiatry, School of Medicine Director of Clinical Trials, Marcus Autism Center
Emory University

11:15 – 11:30 **Jeffrey Wood, Ph.D.**
Clinical Child Psychologist
Associate Professor, Division of Child Psychiatry and Human Development and Psychology
University of California, Los Angeles

Toward Better Measurement of Anxiety in ASD: Outline

Lawrence Scahill, MSN, PhD
Professor of Pediatrics,
Emory School of Medicine
Director of Clinical Trials,
Marcus Autism Center

Disclosures

- Consultant
  - Roche, Coronado, Neuren, MedAvante, Shire
- Research Funding
  - NIMH
  - Roche

Toward Better Measurement of Anxiety in ASD: Outline

- General issues on outcome measurement
- Autism Speaks Task Force
- Patient (Parent) Reported Outcomes
- Building an outcome measure: Current NIMH grant

NIH Multisite Trials in Children with ASDs past 16 years				
Study	N	Target	Ages	Published, date
Risperidone vs placebo	101	Irritability	5-17	NEJM, 2002
Methylphenidate vs placebo	72	Hyperactivity	5-14	Arch Gen Psych, 2005
Citalopram vs placebo	149	Repetitive Behavior	5-17	Arch Gen Psych, 2009
Risperidone vs RIS + Parent Training	124	Irritability & Adaptive Behavior	4.5-13	J Am Acad Child Psych, 2009; 2012
Parent Training vs Parent Education	180	Irritability & Adaptive Behavior	3-7	Enrollment completed
Guanfacine vs placebo	62	Hyperactivity	5-14	Enrollment completed

Characteristics of a good outcome measure

- Relevant (clinically meaningful)
- Measures a separate & definable construct
- Orderly distribution (Mean $\pm$ SD)*
- Has “normative” data (to interpret Mean $\pm$ SD)*
- Solid Internal Consistency (a little noise is ok)
- Good test-retest
- Not: too long, too brief, too narrow, too broad
- Sensitive to change

* In ASD: consider $\geq$ 70 < 70

Autism Speaks Task Force

Lecavalier, L., Wood,J.J., Halladay,A.K., Jones, N.E., Aman, M.G., Cook, E.H., Handen, B.L., King, B.H., Pearson, D.A., Hallett, V., Sullivan, K.S., Grondhuis, S., Bishop, S.L., Horrigan, J.P., Dawson, G., Scahill, L. (2014).

Measuring Anxiety as a Treatment Endpoint in Youth with Autism Spectrum Disorder.

JAutism Developmental Disorders, 44(5): 1128-1143

Measures Dubbed *Appropriate with Conditions*

Measure	Type	Rel & Valid	Sensitivity to Change	Comment
CASI-Anxiety	parent	yes	yes (pilot)	Incomplete coverage
MASC	Parent	yes[a]	yes[a]	↑ reliance on language
Pediatric Anx Rating Scale	Clinician (interview)	yes[a]	yes[a]	↑ ↑ reliance on language
a=high functioning samples				

FDA Monograph, 2009

Guidance for Industry

Patient-Reported Outcome Measures:

Use in Medical Product Development

to Support Labeling Claims

Conceptual Problems: Co-morbidity vs Complication vs Convergent

Co-morbidity model

- ASD and Anxiety Disorders are independent
  - Anxiety disorders in ASD same as TD children

Complication model

- ASD ↑ risk of anxiety & complicates the picture
  - Anxiety symptoms blend & amplify ASD picture

Convergent Model

- Anxiety is part of ASD condition
  - Insistence on routines, social avoidance = anxiety

Practical Problems

- Disentangling anxiety from ASD
  - Social avoidance in ASD vs Social Anxiety Disorder
  - Protest on separation from mother: insistence on sameness, Separation Anxiety or both
- Cognitive & Language Delay
  - Difficulty expressing worries, interpreting physiological signals and sorting emotions
- Anxiety in ASD may be different
  - Insistence on routines: all about predictability ➔ vigilance & over-reaction

Blurry Boundaries

- In typically developing children:
  - Anxiety is dimensional and categorical
  - Boundaries between anxiety disorders are not sharply drawn
- Boundary problems are  in ASD

Three-site NIMH grant

- Aim # 1: Six focus groups with parents of children with ASD on manifestations of anxiety in ASD.

➔ Draft parent-rated anxiety measure (based on focus group data).

- Aim # 2: Draft measure on the web, obtain 900 children with ASD.

➔ Evaluate the distribution, factors, item analysis to refine draft.

- Aim # 3: Conduct clinical assessments on 90 children with ASD.

➔ Evaluate validity and test-retest reliability of new parent-rated scale and the Pediatric Anxiety Rating Scale (PARS).

- Aim # 4: Compare heart rate variability in 30 subjects with ASD + elevated anxiety to 30 subjects with ASD + low anxiety.

➔ potential biomarker

Six Focus Groups: 600 pages of transcripts

- Triggers (specific stimulus)
  - Loud noises
  - Crowds
  - New Situations
- Observable Behaviors
  - Request for reassurance
  - Avoidance with distress
- Child Coping Behaviors
  - Withdrawal
  - Self-soothing behavior
  - Breakdown in coping → emotional outbursts

Draft Items

- Selected from new 52 items + 20 existing items

Requires frequent reassurance about upcoming events	0	1	2	3
Uneasy in noisy situations (e.g., school cafeteria, malls)	0	1	2	3
Gets upset if someone breaks the rules	0	1	2	3
Overly fearful of weather events (e.g., storms, hurricanes or tornados)	0	1	2	3
Uncomfortable in social situations	0	1	2	3
Gets stuck on what might go wrong	0	1	2	3
Compares self to others in a negative manner	0	1	2	3
Over-reacts when things do not go as planned	0	1	2	3
Needs a lot of reassurance that things will work out	0	1	2	3
Gets upset when routine is not followed	0	1	2	3
On the look-out for any change in routine	0	1	2	3
Worries about being left home alone or with a sitter	0	1	2	3
Anxious about upcoming events	0	1	2	3

Plan

- Put 72 items on the web; factor/item analysis

  Goal: dimensional parent measure

- Assess 90 subjects (30 per site) in person: new parent measure, revised PARS, CYBOCS-ASD

  Goal: establish reliability & validity

- 30 Hi ANX vs 30 Low ANX on HRV

  Goal: validate HRV as a biomarker

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  Karen Bearss, Opal Ousley (Emory)

  Michael Aman, Luc Lecavalier, Michael Edwards (OSU)

  Robert Schultz, John Herrington (Penn)

Thank you

Psychiatric Comorbidity in Individuals with ASD

Jeffrey J. Wood, Ph. D.
University of California, Los Angeles

Psychiatric Comorbidity in ASD

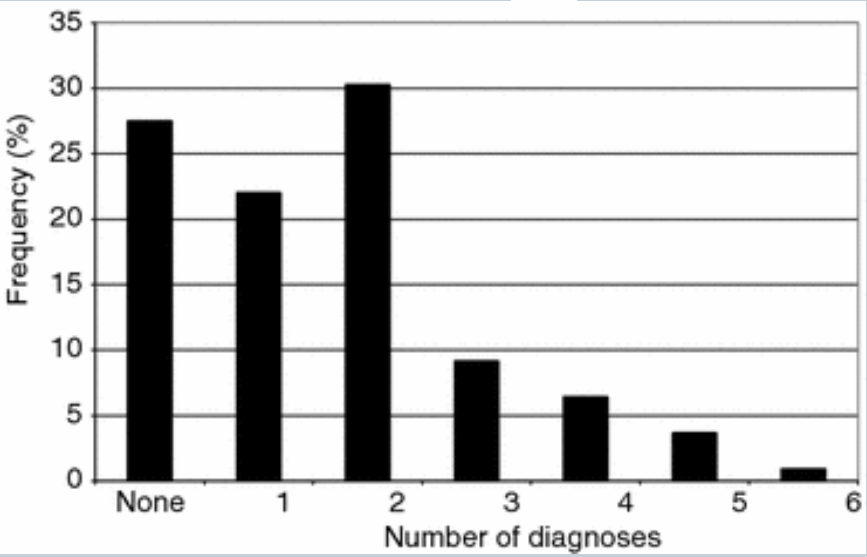


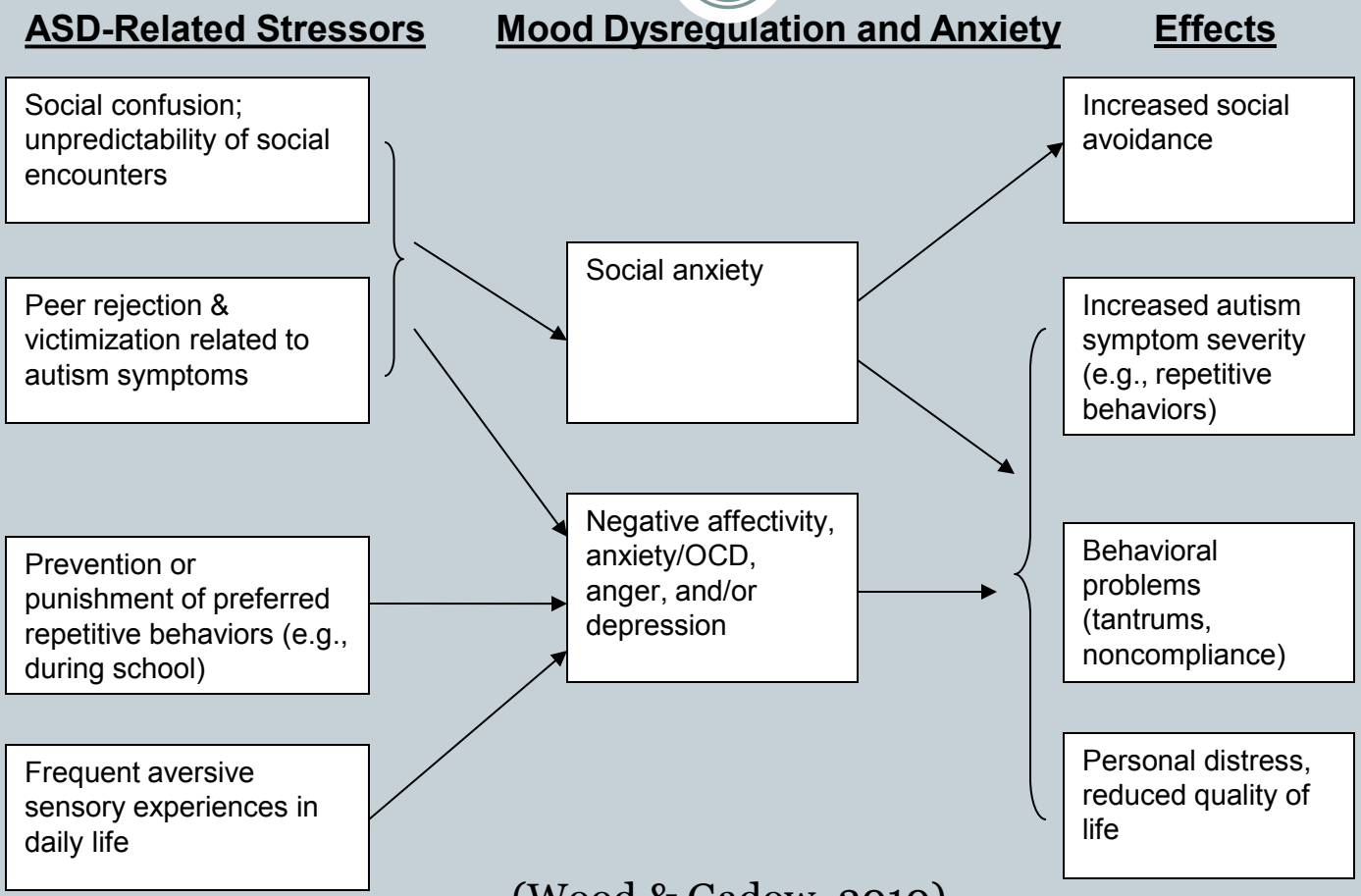
Fig. 1 Frequency of the number of comorbid lifetime psychiatric diagnoses per child with autism. Only DSM-IV diagnoses are included (Leyfer et al. 2006)

Understanding the Linkage

- Common neurocognitive mechanisms.
  - Executive functioning deficits are characteristics of autism and a number of psychiatric disorders (anxiety, ADHD, etc.) (Geurts et al., 2004)
  - Poor attention shifting and executive dysfunction underlies both prolonged negative emotion (anxiety, anger) and perseverative thought. Link with illogical thought (e.g. Solomon et al., 2008)

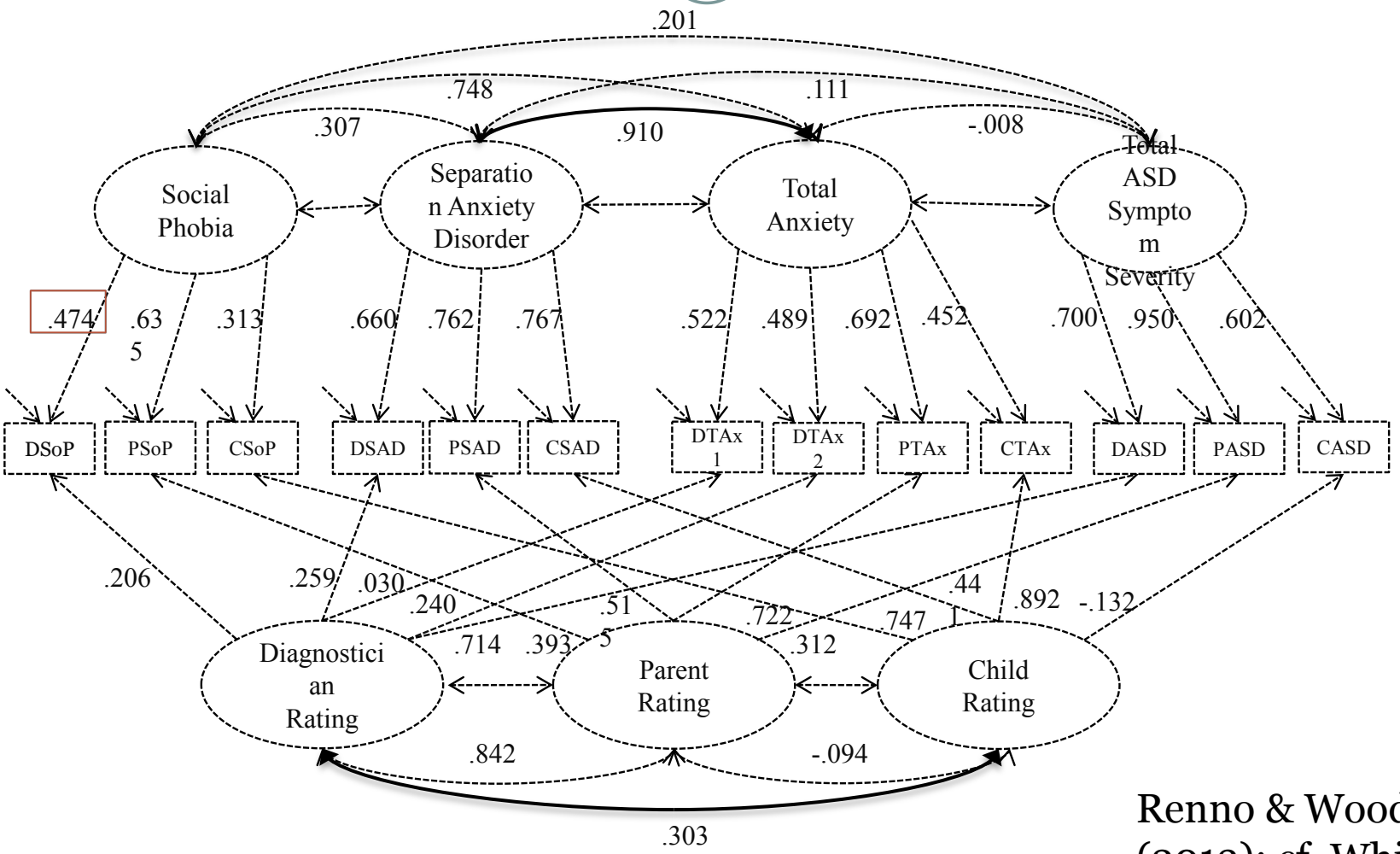
- Other traits and their biological substrates that serve as vulnerabilities for psychiatric disorder may be more common in ASD, too.
  - For example, genetic factors that are markers of negative affectivity/anxiety in typical youth are also present in children with ASD and anxiety; e.g. dopaminergic gene polymorphisms such as DAT1 intron8; serotonin transporter 5-HTTLPR.
    (Cohen et al., 2003; Gadow et al., 2014, 2008, 2009, 2010; Roohi et al., 2009)

Hypothetical Model



(Wood & Gadow, 2010)

Discriminant Validity



Renno & Wood (2013); cf. White et al. (2014)

Future Directions

- Evidence based treatment approaches, possibly modified for individuals with ASD, require further research.
  - EG: Does CBT and/or SSRIs reduce clinical anxiety, OCD, and depression in individuals with ASD?
- Investigations of the genetic, neurologic, psychophysiological, neuropsychological, and personality substrates of comorbid psychiatric disorders in ASD.
  - EG: Functional neuroimaging of people with and without high anxiety in the context of ASD
  - EG: Do executive functioning deficits and stress predict greater psychiatric comorbidity concurrently and over time?

Panel 2: Psychiatric Disorders

11:30 – 11:45 **Evdokia Anagnostou, M.D.**
Senior Clinician Scientist
Associate Professor
Bloorview Research Institute,
University of Toronto
Canada Research Chair in
Translational therapeutics in ASD
University of Toronto

11:45 Discussion

Psychiatric comorbidities in ASD

Evdokia Anagnostou, MD

University of Toronto

Bloorview RESEARCH INSTITUTE

autism RESEARCH CENTRE

UNIVERSITY of TORONTO

Holland Bloorview Kids Rehabilitation Hospital

Prevalence

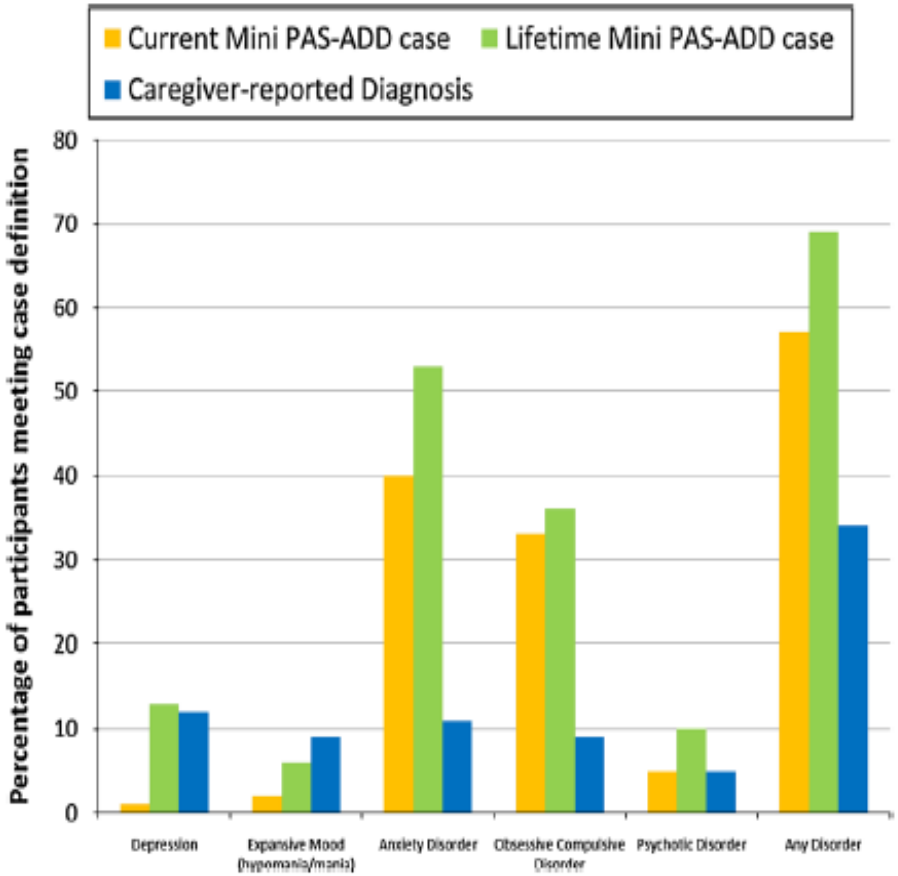


Fig.1 Percentages of participants with a psychiatric disorder based on caregiver-report current and lifetime Mini PAS-ADD criteria

However, mush lower rates of both anxiety and depression in those with ID

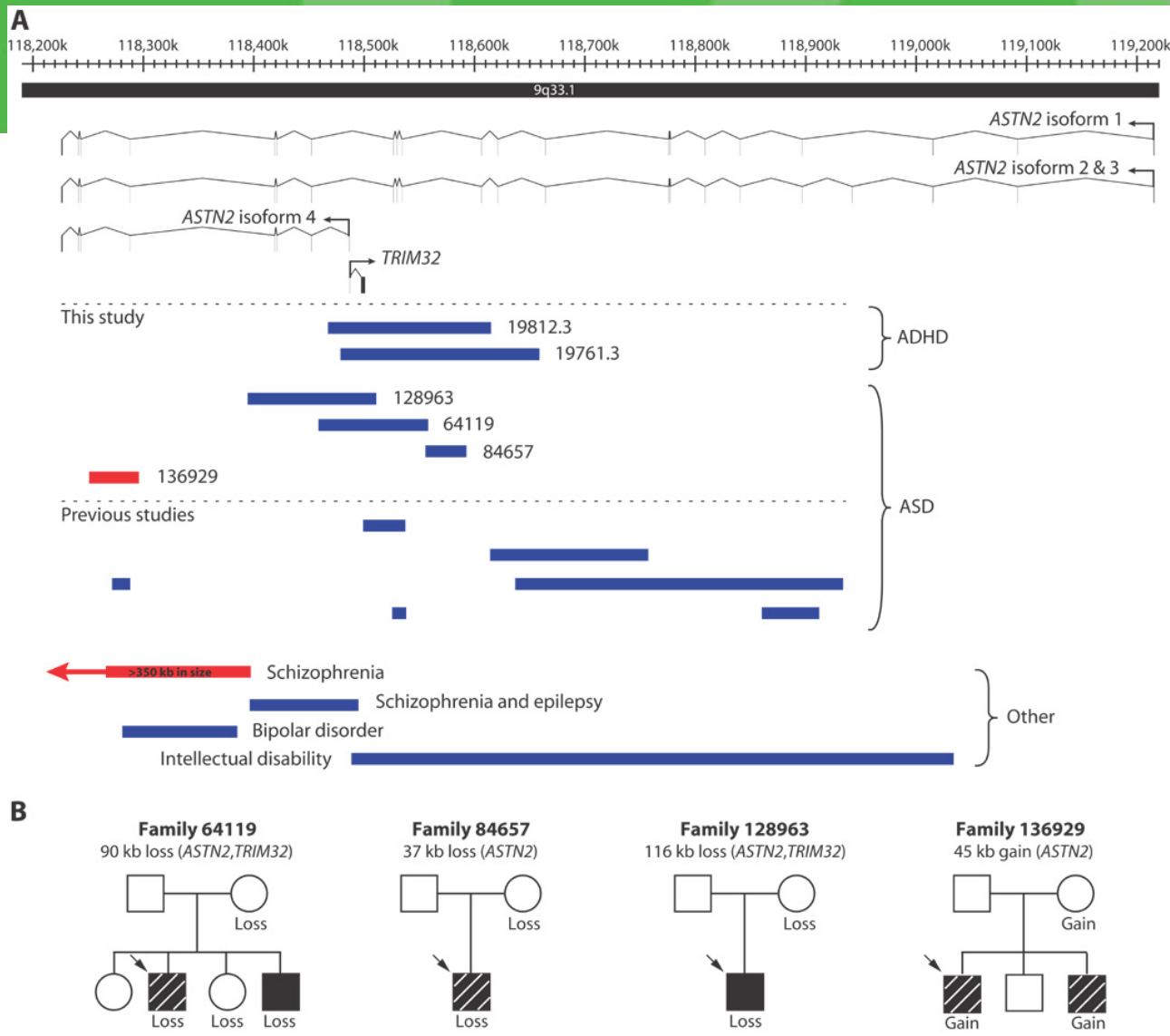
Buck et al, 2014

Questions raised by high prevalence

- High co-occurrence of several neuropsychiatric conditions, more than expected from general population rates

- Do our diagnostic constructs map onto distinct biologic constructs
- Is there construct confusion, from measurement point of view
- What does it mean of treatment development

- Do our diagnostic constructs map onto distinct biological constructs?



Most common phenotypes: ASD, ADHD, ID, language delay, anxiety, OCD

Lionel A C et al. Sci Transl Med 2011;3:95ra75-95ra75

Is there construct confusion, from measurement point of view

- How do we make diagnoses about internalizing disorders in the context of limited reporting of subjective experiences
  - E.g. is a diagnosis of anxiety made based on observable behavior the same construct as a diagnosis of anxiety made based on self report of internal states
  - Can biology clarify that question

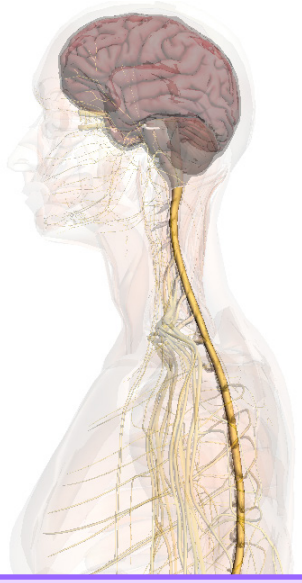
ANS Response To Anxiety

Well-documented in typically-developing individuals

Parasympathetic branch

"rest & digest" response

Decreases sweating
Decreases heart rate
Cutaneous vasodilation



Sympathetic branch

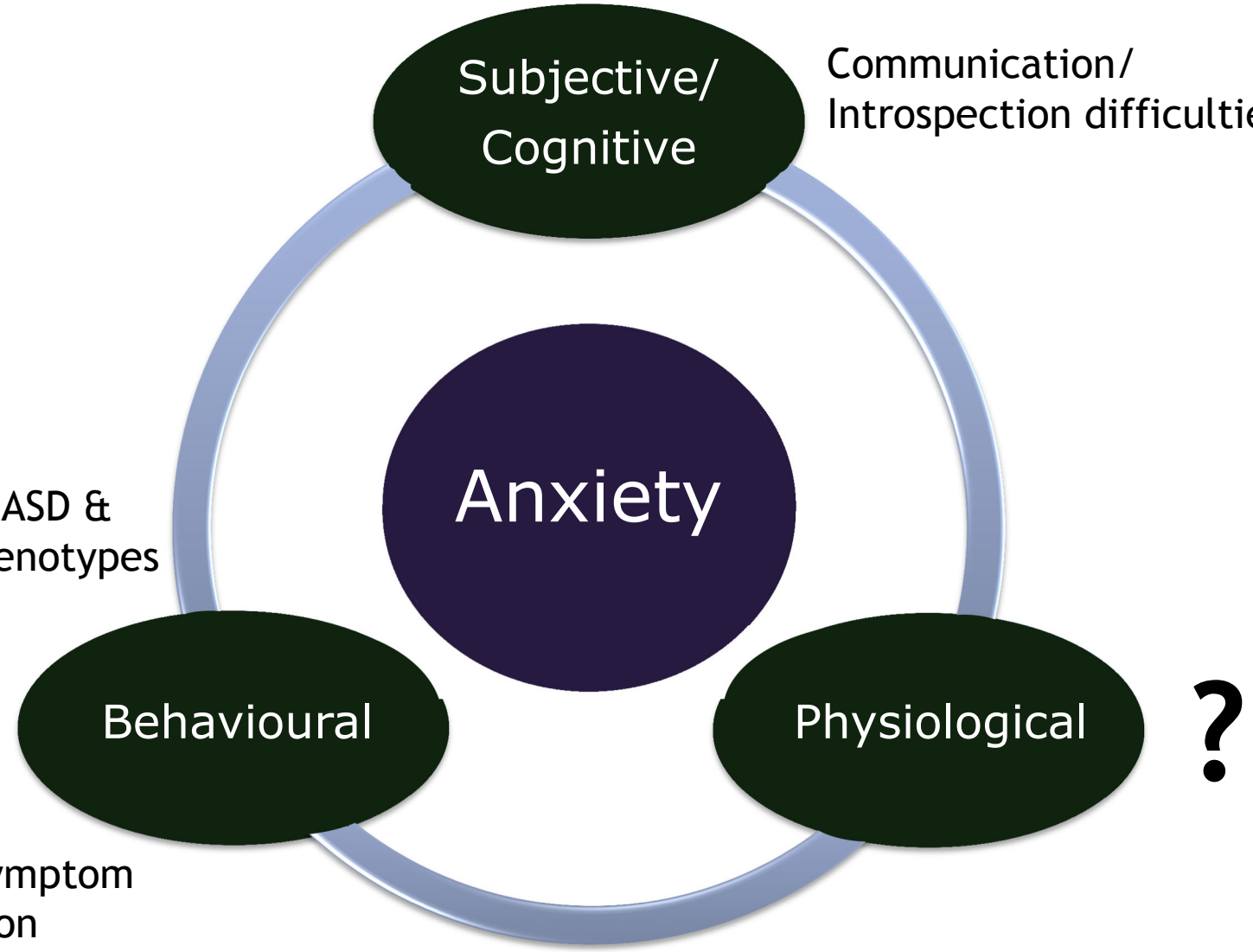
"fight or flight" response

Increases sweating
Increases heart rate
Cutaneous vasoconstriction

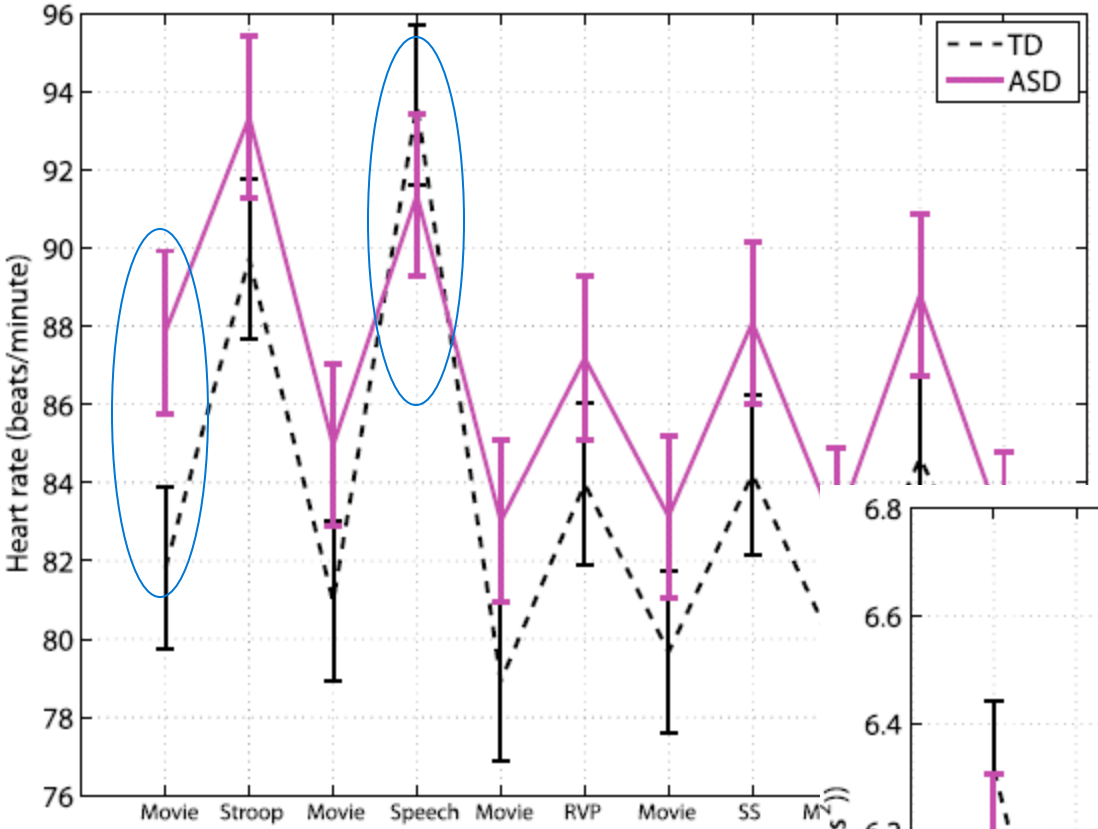
- Electrodermal activity
- Cardiac activity

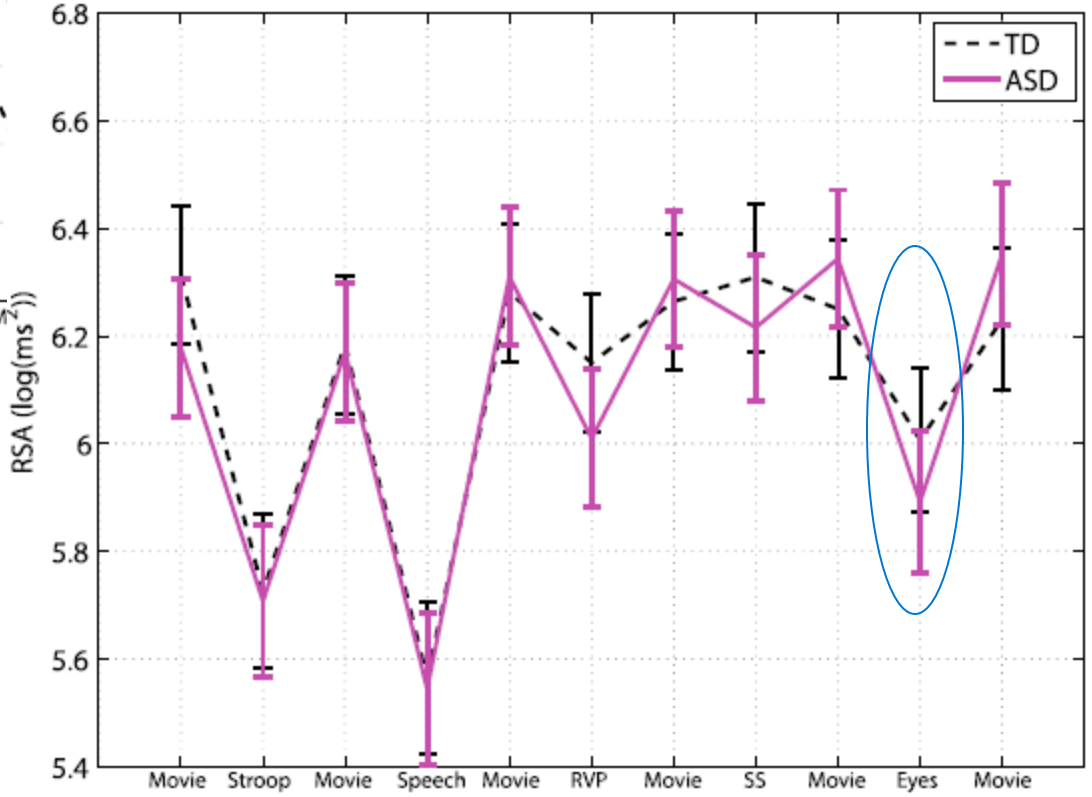
Tripartite Model of Anxiety (Lang, 1968)

Implications for assessment & treatment



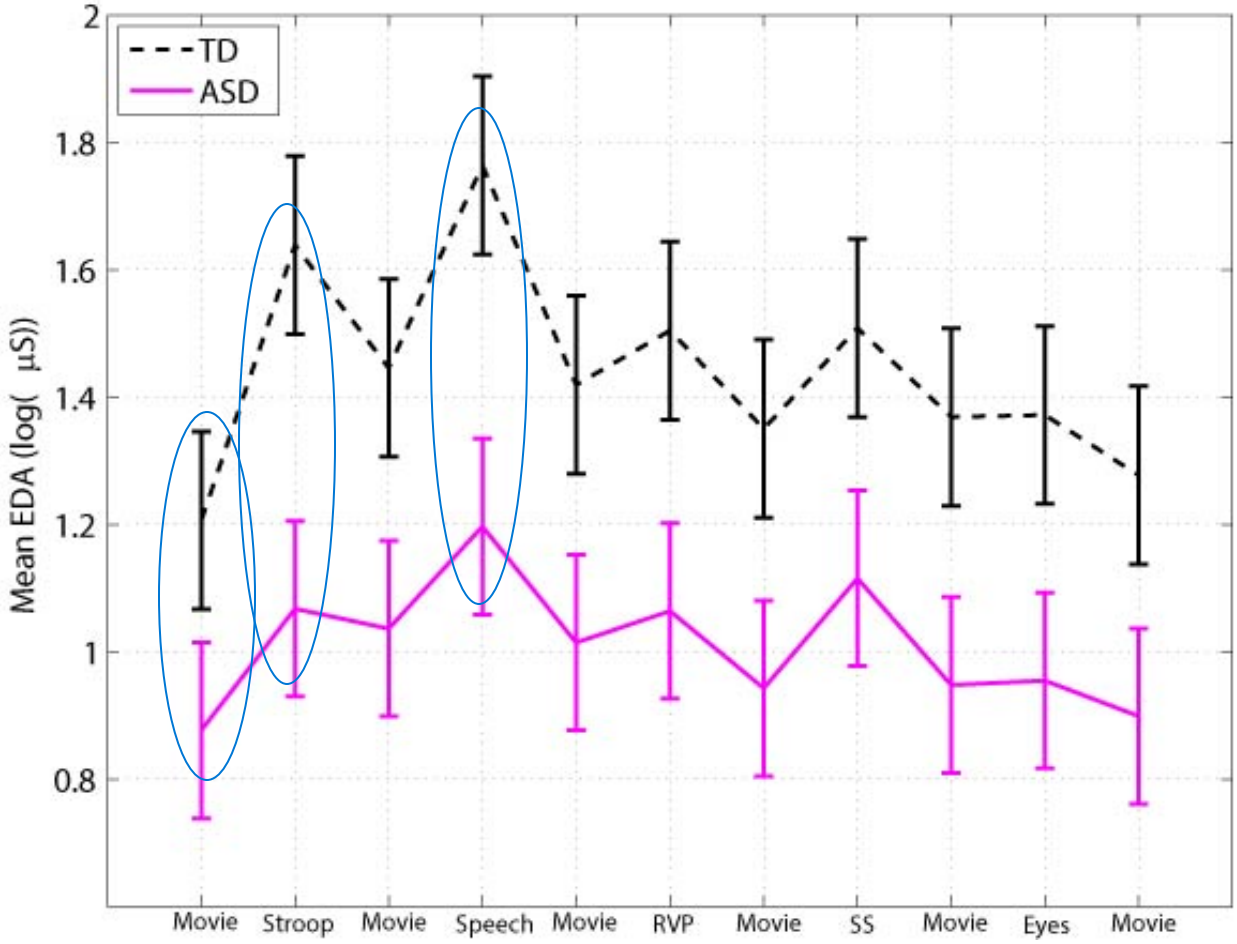
Results: Cardiac activity



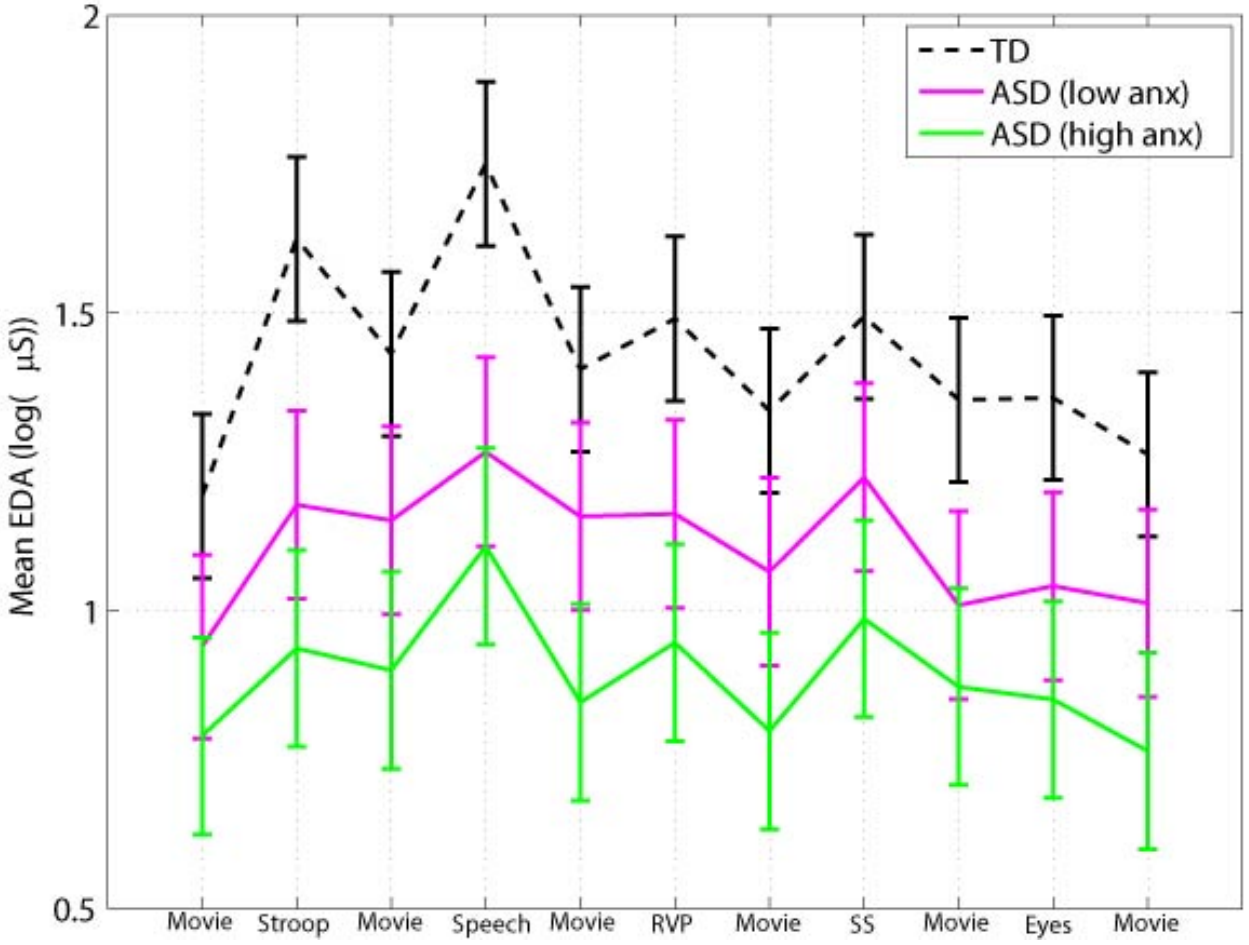


- Kushki et al 2014

Results: Electrodermal activity (EDA)



Results: Effect of anxiety



What does it mean of treatment development

- Construct confusion is a critical barrier to drug development
  - E.g. is anxiety in the general population the same biologic construct as in ASD
  - E.g. are the attention deficits seen in ADHD in the general population the same as those seen in children with ASD+ADHD
    - *If yes, treatments are available and treatment development in the general population is of relevance to ASD*
    - *If no, distinct drug development pathways needed to target the biological construct of such co-occurring conditions in ASD*
- How does lack of specificity of genomic findings for co-occurring neuropsychiatric conditions affect drug development

From disability to possibility

Workshop on Under-Recognized Co-Occurring Conditions in ASD

Presentations will address:

1. What do we know and what have we learned in each research area? (Prevalence in autism vs. developmental disability vs. typical population? Underlying biology? Challenges in diagnosing these condition in individuals with ASD?)

2. What do we need, both in the clinic and in research to decrease the burden of illnesses/extent of disability, or reduction in quality of life due to the co-occurring conditions?

3. Are there any specific gaps or barriers that need to be addressed before progress can be made in this area?

4. Has recent research created new opportunities that need to be explored?

Workshop on Under-Recognized Co-Occurring Conditions in ASD

Discussion questions:

1. What do we need in order to help those affected by these conditions now? (e.g., provider and community awareness, practice guidelines, services, policies.)

2. What kind of research is needed to support development of new and improved treatments for these conditions in the future?

3. Are there any lessons we can learn from other fields to help accelerate progress?

4. Are any of these fields ready for practice guidelines or other steps that have not yet occurred due to knowledge or service gaps or other barriers? If so, how can the IACC, federal agencies or private organizations help accelerate progress?

Panel 2: Psychiatric Disorders

Discussion

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Lunch

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Public Comments and Discussion

These slides do not reflect decisions of the IACC and are for discussion purposes only.

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Panel 3:

Sleep and Neurological Disorders

These slides do not reflect decisions of the IACC and are for discussion purposes only.

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

1:15 Panel 3: Sleep and Neurological Disorders

1:15 – 1:30 **Beth Malow, M.D., M.S.**
Professor of Neurology and Pediatrics
Department of Neurology and Pediatrics School of Medicine
Vanderbilt University

1:30 – 1:45 **Ashura Buckley, M.D.**
Clinical Investigator
National Institute of Mental Health
National Institutes of Health

Sleep in Autism

IACC Workshop on Under-Recognized Co-Occurring Conditions

Beth A. Malow, M.D., M.S.
Burry Chair in Cognitive Childhood Development
Professor of Neurology and Pediatrics
Director, Sleep Disorders Division
Vanderbilt University

Have you seen this child?

- Alex is a 6-year-old boy with autism spectrum disorder (ASD). He takes hours to fall asleep. His parents state that "he can't shut his brain down." He drinks Mountain Dew with dinner, and plays video games after dinner. He can't settle down to go to sleep and leaves his room repeatedly to find his parents.
- Once asleep, he awakens multiple times during the night. Sometimes he awakens his parents. Other times he wanders around the house, goes to the kitchen to eat, and falls asleep in a different room.
- It is "nearly impossible" to awaken Alex in the morning for school. His parents are exhausted and very overwhelmed. Alex's teacher describes him as being hyperactive and disruptive in class.

Framing Questions

- What do we know about sleep in autism?
- What is the evidence linking biological causes of sleep disturbance with features of ASD?
- What do we need to learn in order to treat sleep disturbance in ASD? What are the gaps? What are the opportunities?
- What autism-specific features affect proper diagnosis and treatment?

What Have We Learned?

- High prevalence of parent–reported sleep concerns in ASD, across cognitive levels.
  - Courturier (2005): **78% ASD** (TD 26%)
  - Krakowiak (2008): **53% ASD** (DD 46%, TD 32%)
  - Souders (2009): **66% ASD** (TD 45%)
- Sleep disturbance associated with child behavior/family functioning
- Insomnia is the most prevalent sleep disturbance
  - ✓ Prolonged time to fall asleep
  - ✓ Preference for delayed bedtime (older children)
  - ✓ Bedtime resistance (younger children)
  - ✓ Increased arousals and awakenings
  - ✓ Decreased sleep duration

Reynolds AM & Malow BA. (2011), Richdale AL & Schreck KA. (2009)

What Have We Learned?

- ✓ Multiple causes of insomnia; many are treatable.
- ✓ Medical (GI) & Neurological (epilepsy)
- ✓ Psychiatric: Anxiety, bipolar disorder, depression, obsessive compulsive or ADHD symptomatology
- ✓ Medications: Serotonin reuptake inhibitors, stimulants, some antiepileptic drugs
- ✓ Other Sleep Disorders: Obstructive sleep apnea, parasomnias, restless legs syndrome
- ✓ Behavioral: poor sleep habits…and also features related to ASD such as difficulty with transitions and sensory sensitivities. Exhausted parents may think poor sleep is part of autism and be unaware that behavioral approaches can help.
- ✓ Other causes related to ASD: Neurotransmitter abnormalities, including in melatonin pathway, possibly GABA and serotonin

Framing Questions

- What do we know and what have we learned about sleep in autism?
- What is evidence linking biological causes of sleep disturbance with features of ASD?
- What do we need to learn in order to treat sleep disturbance in ASD? What are the gaps? What are the opportunities?
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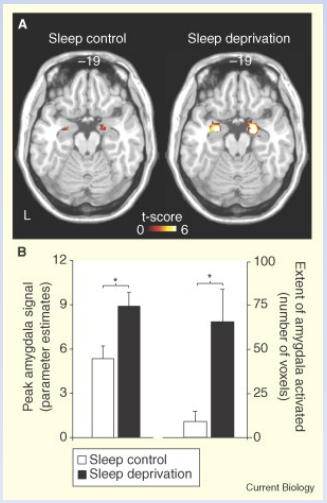
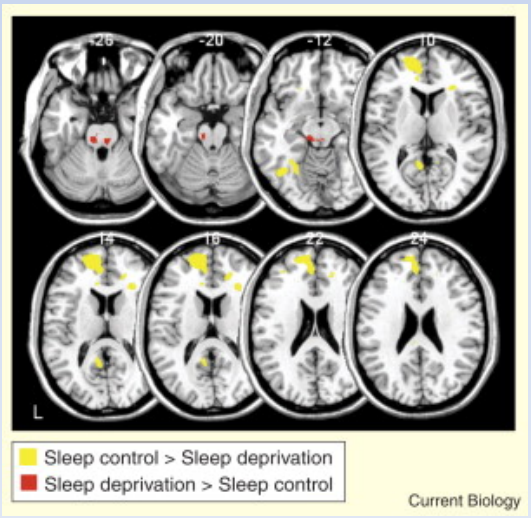
Biology of Sleep Disturbance and ASD: Emotional Regulation

Sleep deprivation affects the neural circuitry underlying emotional regulation, including connectivity of the amygdala and prefrontal cortex. (reviewed in Maski, 2013). This abnormal connectivity also exists in ASD.

- ✓An fMRI study in which sleep-deprived healthy adult participants were compared with those who had slept showed increased amygdala activation after viewing images that were emotionally adversive. (Yoo, 2007)
- ✓In addition, the functional connectivity was stronger between the medial-prefrontal cortex and the amygdala in the sleep control group, and the autonomic brainstem regions and the amygdala in the sleep deprived group.

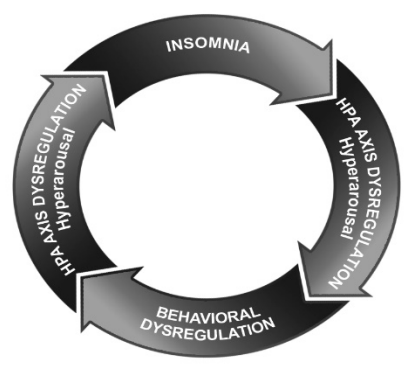




Biology of Sleep Disturbance and ASD: Arousal Dysregulation

- Arousal dysregulation (hyperarousal) may tie together several features of ASD (Mazurek, 2013)
  - ✓Anxiety
  - ✓Sensory over-responsivity
  - ✓Functional GI problems

  Insomnia?



- Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis occurs in both insomnia and ASD, in association with daytime stressors (Buckley, 2005 and Corbett, 2008 and 2009).
- Studies of autonomic function provide additional evidence for hyperarousal (elevated baseline heart rate; Kushki, 2013)
- Insomnia treatment studies designed to target hyperarousal provide an opportunity to measure biological markers of autonomic and HPA dysfunction

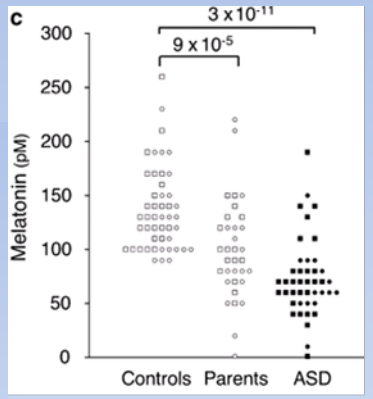
Biology of Sleep Disturbance and ASD: Melatonin

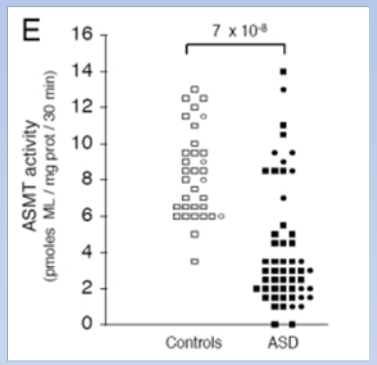
- Endogenous melatonin, produced by the pineal gland, promotes sleep and stabilizes circadian rhythms through actions on receptors of the SCN. (Pandi-Perumal, 2006)
- Apart from hypnotic and circadian properties, melatonin inhibits ACTH responses in the human adrenal gland. (Campino, 2011)
- Melatonin processing appears to be altered in ASD.

Biology of Sleep Disturbance and ASD: Melatonin

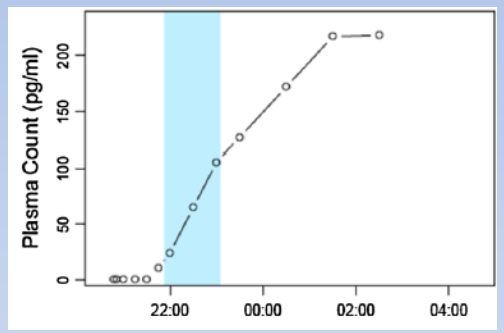
Examine melatonin synthesis and degradation pathways with both biochemical and molecular approaches

Tryptophan
↓
serotonin
↓ AA-NAT
N-acetylserotonin
↓ ASMT
Melatonin
↓ CYP1A2
6-sulfoxymelatonin
(major metabolite)

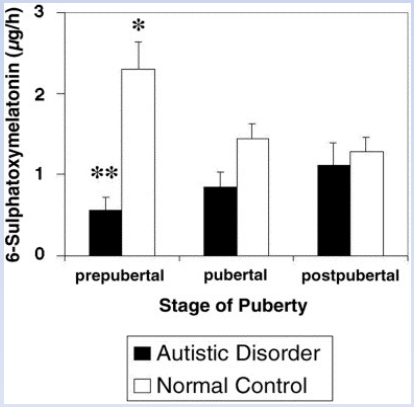




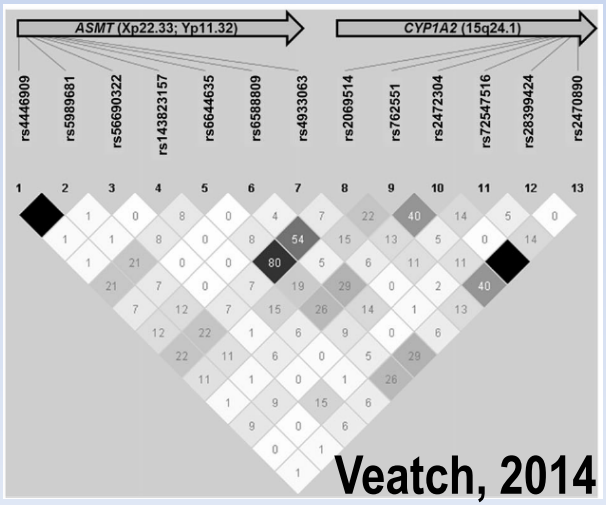
Melke, 2008



Goldman, 2014



Tordjman, 2005



Veatch, 2014

Framing Questions

- What do we know about sleep in autism?
- What is evidence linking biological causes of sleep disturbance with features of ASD?
- What do we need to learn in order to treat sleep disturbance in ASD? What are the gaps? What are the opportunities?
- What autism-specific features affect proper diagnosis and treatment?

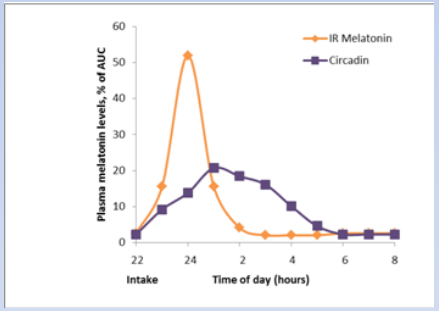
Gaps and Opportunities in Treatment

Autism Speaks Autism Treatment Network (AS ATN) Practice Pathway for Management of Insomnia– Supplement in *Pediatrics* (Malow, 2012)

- ✓ Clinicians need to ask about sleep.
- ✓ Medical co-occurring conditions need to be identified and treated.
- ✓ Behavioral sleep education works (Vriend, 2011; Malow, 2014)
  (and also improves child behavior and family functioning)
- ✓ Limited studies of sleep medications for ASD; none FDA-approved.

Under study:

- ✓ Melatonin agonists
  (Tasimelteon®, Vanda Pharmaceuticals)
- ✓ Prolonged release melatonin minitablet preparations that children can easily swallow
  (Circadin®, Neurim Pharmaceuticals)

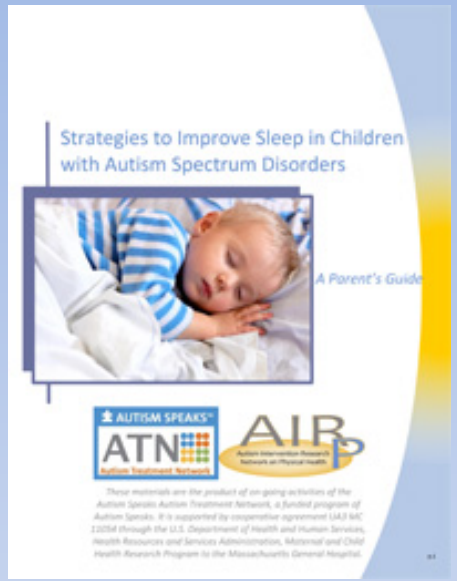


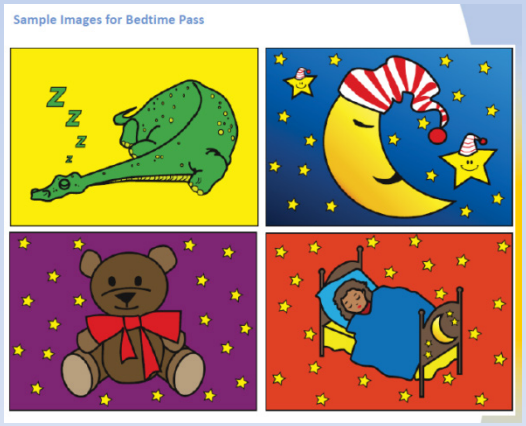
Zisapel, 2010

Measures of baseline sleep status and treatment response

- ✓ Polysomnography
- ✓ Actigraphy
- ✓ Sleep Questionnaires and Diaries

Autism Speaks Autism Treatment Network Toolkits





http://www.autismspeaks.org/science/resources-programs/autism-treatment-network/tools-you-can-use/sleep-tool-kit
Or search for Autism Speaks Sleep Toolkit

Summary and Future Directions

What we know:

✓ Sleep disturbance, especially insomnia, is common in ASD (50-80%)

✓ Sleep disturbance associated with child behavior/family functioning

✓ There are many treatable causes of insomnia

What we think we know (more data are needed):

✓ Improving sleep impacts favorably on children and families

What we still need to know:

✓ How do we best measure sleep and improvements in sleep?

✓ What treatments are effective? And in which children?

✓ Which children need medications for sleep? (vs. sleep education alone)

✓ Whether biological markers of sleep disturbance (e.g., cortisol, melatonin, genetic variability) predict treatment response.

2014 IACC Workshop on Under-Recognized Co-Occurring Conditions

Epilepsy and ASD

Ashura Williams Buckley, MD
Staff Physician
National Institute of Mental Health
Pediatrics and Developmental Neuroscience Branch

objectives

Who are we talking about?

What are the clinical and biological relationships between ASD and epilepsy?

How do we address research and treatment in this population?

VIEWS & REVIEWS

NINDS epilepsy and autism spectrum disorders workshop report

Roberto Tuchman, MD
Deborah Hirtz, MD Laura A. Mamounas, PhD

Correspondence to Dr. **Tuchman:** roberto.tuchman@gmail.com

ABSTRACT

The association of epilepsy and autism spectrum disorders (ASD), although well-recognized, is poorly understood. The purpose of this report is to summarize the discussion of a workshop sponsored by the National Institute of Neurological Disorders and Stroke, with support from the National Institute of Child Health and Human Development. Autism Speaks, and Citizens United for Research in Epilepsy, that took place in Bethesda, Maryland, on May 29 and 30, 2012. The goals of this workshop were to highlight the clinical and biological relationships between ASD and epilepsy, to determine both short- and long-term goals that address research and treatment conundrums in individuals with both ASD and epilepsy, and to identify resources that can further both clinical and basic research. Topics discussed included epidemiology, genetics, environmental factors, common mechanisms, neuroimaging, neuropathology, neurophysiology, treatment, and research gaps and challenges in this unique population. ***Neurology*® 2013;81:1630-1636**

Individuals with both ASD & epilepsy

These are common disorders with extensive overlap

Prevalence of epilepsy in ASD is affected by ID and age

21.5% if ID is present, 8% if IQ> 70 *(Amiet et al Biol Psych, 2008)*

Increase in epilepsy in adolescence *(Woolfenden et al,* Dev Med Child Neurol, *2012)*

Highest risk in people with ID

Prevalence of ASD in epilepsy

Pediatric population: 4- 5% (Geerts et al. Epilepsia 2011, Berg et al, J Child Neurology 2011)

Sevenfold increase in odds of having ASD if you have epilepsy (Rai et al, Epilepsia, 2012)

Highest risk in people with ID

What causes them to occur together?

Early Neurodevelopment

High rates of synaptogenesis

Rapid maturation of synaptic plasticity mechanisms

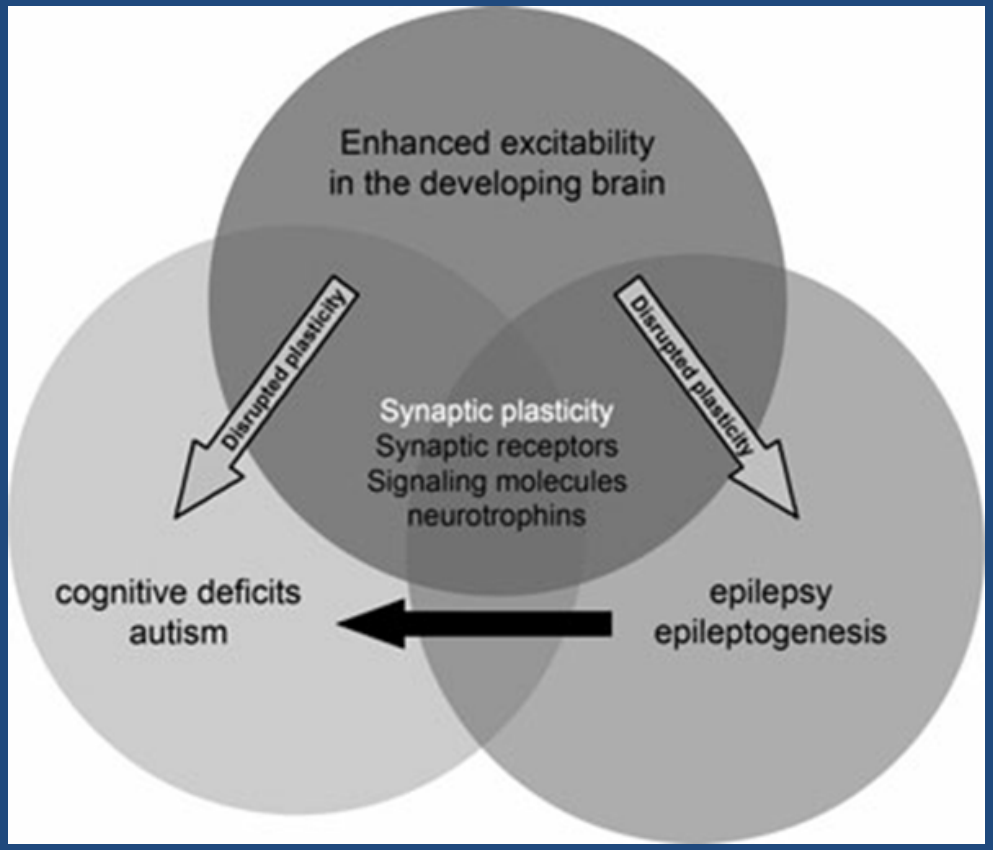
Excitatory mechanisms>>inhibitory *(Rakhade & Jensen et al, 2009)*

ASD, Epilepsy *PLUS* Intellectual Disability

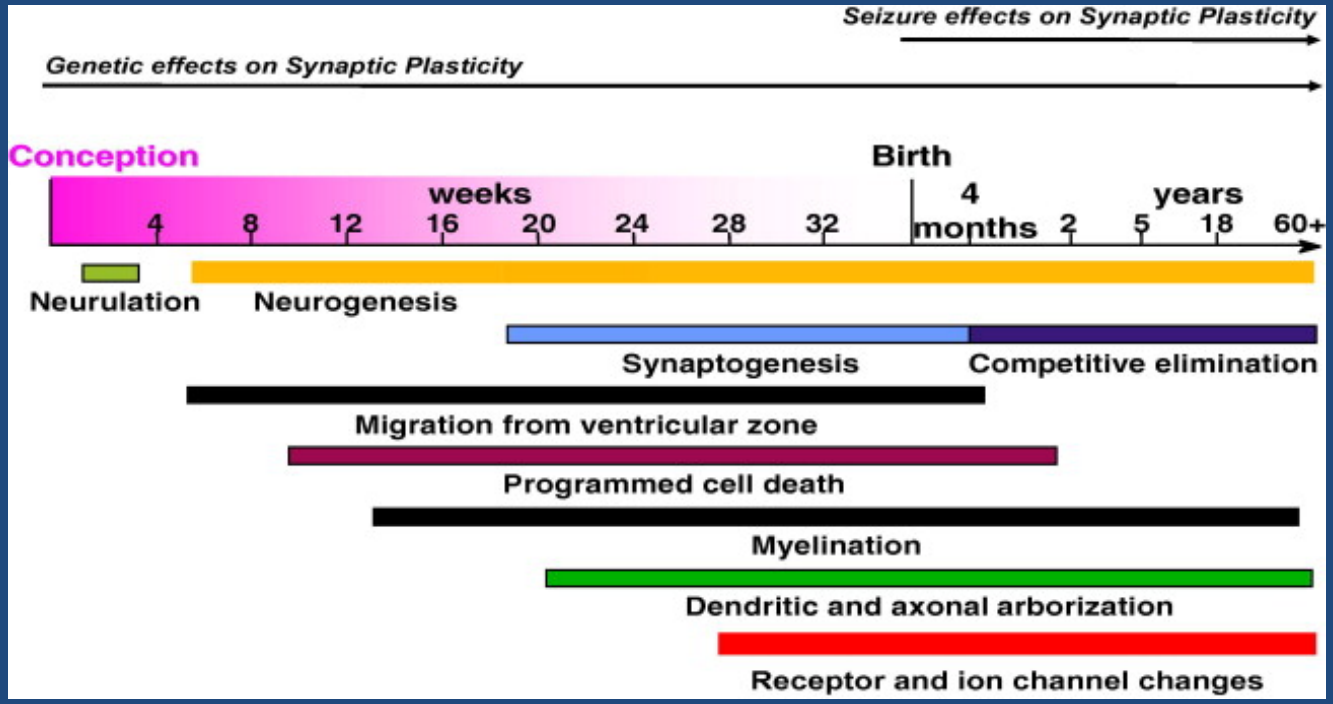
- When in same person may represent a primary disruption (genetic, early insult) in synaptogenesis causing both ASD and seizures
- Seizures themselves may be the primary disruption resulting in both cognitive impairment and ASD
- Seizures may further exacerbate cognitive impairment in ASD

Autism Spectrum Disorders and Epilepsy

The association between epilepsy and autism spectrum disorders is both well recognized and not well understood



Epilepsia ILAE



Amy Brooks Kayal

Epilepsy and autism spectrum disorders: Are there common developmental mechanisms?

Brain and Development, Volume 32, Issue 9, 2010, 731 738

http://dx.doi.org/10.1016/j.braindev.2010.04.010

What Can Research on Syndromes tell us?

Single gene disorders with high occurrence of ASD and epilepsy

TSC

FXS

RTT

Through various pathways, the end common result may be a marked imbalance in the excitatory/inhibitory brain circuits in the developing brain predisposing to ASD, epilepsy *and* ID (Fu et al, Stafstrom , 2012, Mcleod 2013)

Summary
What We Know

Both ASD and Epilepsy are spectrum disorders

Both may be conceptualized as disorders of neural connectivity resulting from dysregulation of synaptic plasticity

Comorbidity with ID increases the risk of having the other

Summary
What we want to know more about

Better characterization of the seizure patterns in ASD

What is the role of ID in outcomes in ASD, in epilepsy and in ASD-epilepsy phenotypes?

Is there a critical window for intervention that can arrest or reverse the dysfunction in neural circuitry?

Summary
What we need or next steps

Put newly identified ASD genes in context with what is known about molecular pathways and brain circuitry

Better animal models

Effective collaborations across labs

Models to identify and correct neural dysfunction in populations with both ASD/epilepsy and include people with ID

Panel 3: Sleep and Neurological Disorders

1:45 – 2:00 **Mustafa Sahin, M.D., Ph.D.**
Director of Translational Neuroscience Center, Boston Children's Hospital
Associate Professor of Neurology
Harvard Medical School
Harvard University

2:00 Discussion

Dissecting the Neural Circuitry of ASD with Tuberous Sclerosis as a Model

Mustafa Sahin, MD, PhD

Associate Professor of Neurology

Director, Translational Neuroscience Center

Boston Children's Hospital

[Name of minor withheld] Story

- At 20 week *in utero* ultrasound showed “growths” in his heart
- Fetal MRI showed tubers in the brain
- Born at full term
- Started having seizures at 3 months

[Photo redacted]

[NMW] Story

[Photo redacted]

Today, [PII redacted] seizures are under good control.

He has significant sleep problems.

Adam was diagnosed with autism spectrum disorder.

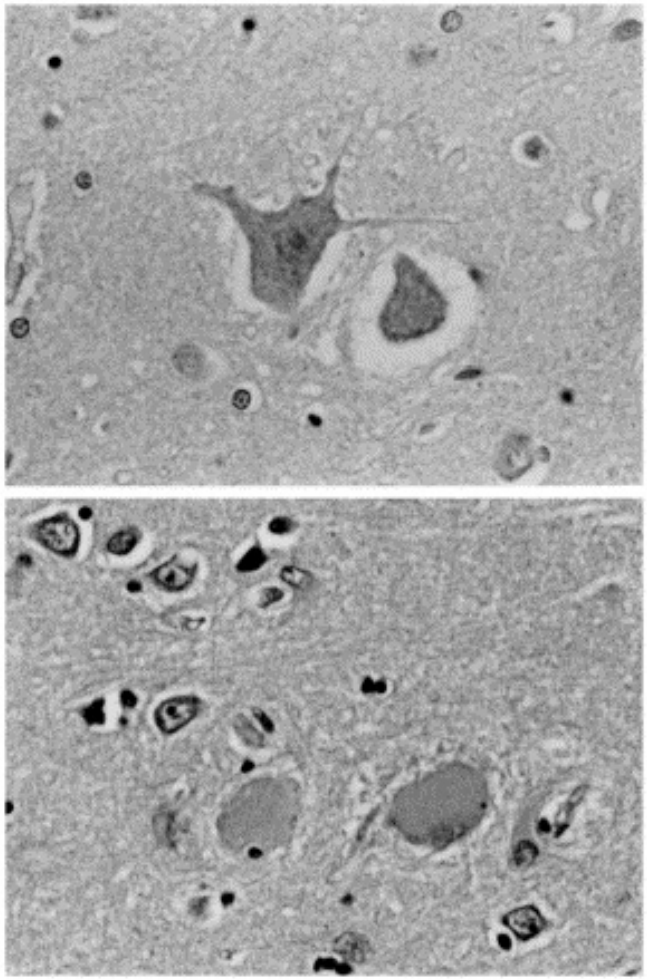
Why is TSC a good model to study autism?

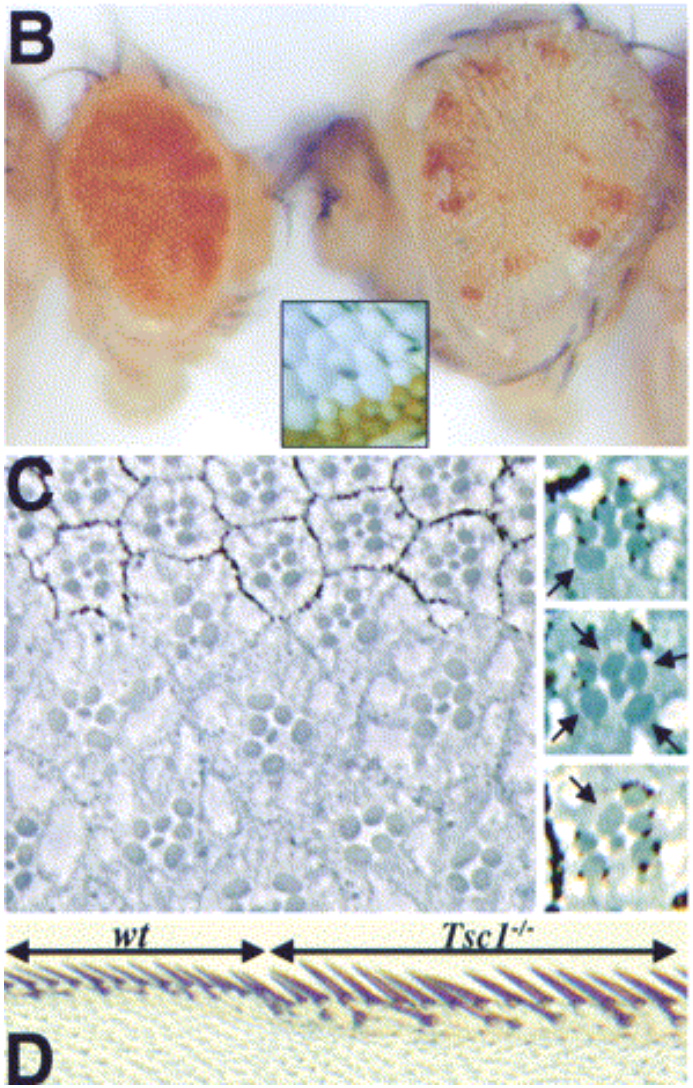
- ~ 50% of TSC patients are affected with Autism Spectrum Disorder.
- Many of the TSC patients may be diagnosed before birth or at the time of birth.
- Cellular mechanisms aberrant in TSC are beginning to be understood.
- FDA-approved specific inhibitors are available.

What is Tuberous Sclerosis Complex?

- Causes benign tumors in brain, eye, skin, kidneys, and heart
- Usually presents with seizures, intellectual disability, autism
- Incidence: 1:6,000-10,000
- Genes: *TSC1* and *TSC2*

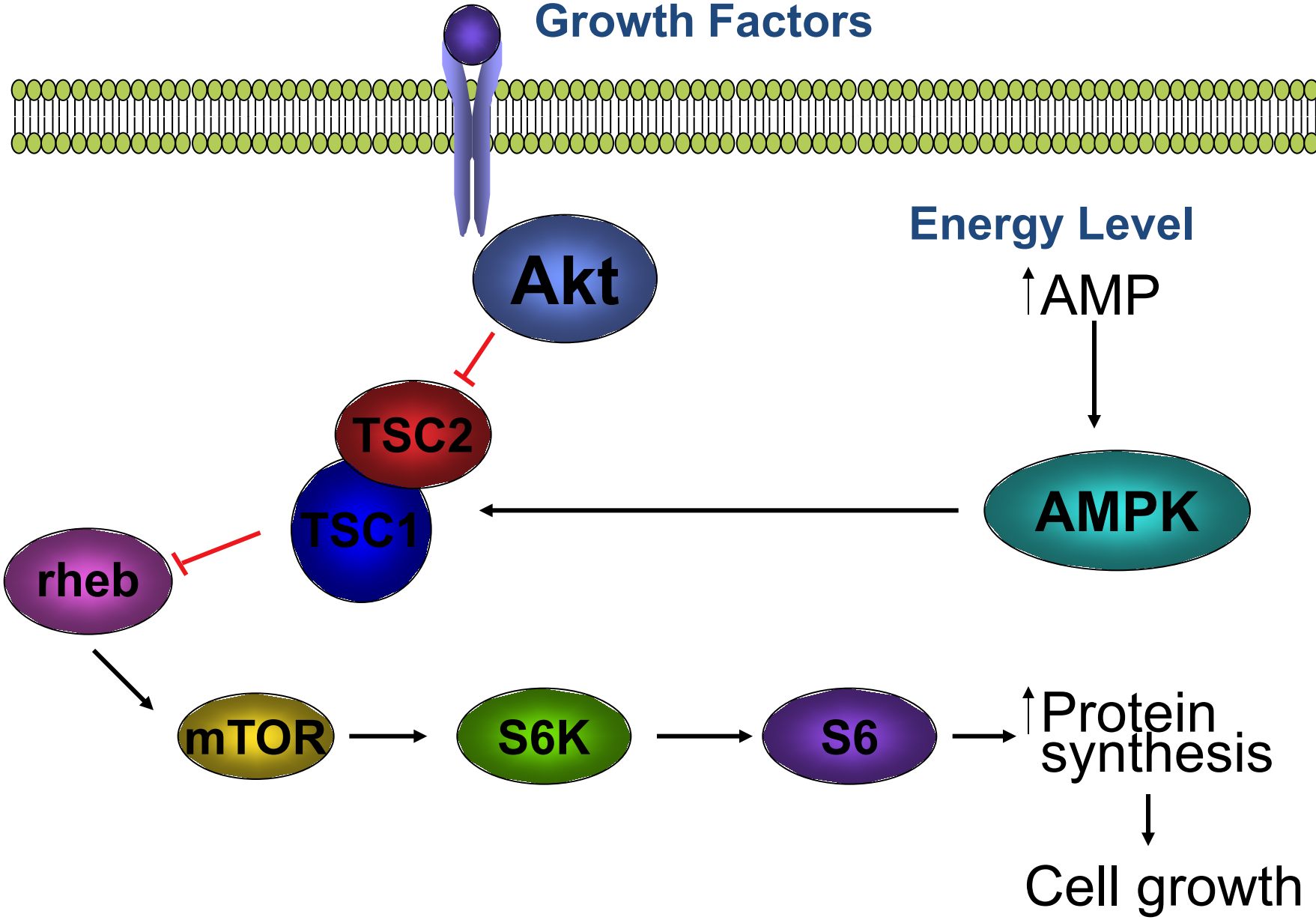
TSC is a disease of cell size…





Tapon et al., 2001

TSC is a disease of cell size



Cortical Tubers and Autism

- Cortical tubers in temporal lobes necessary, but not sufficient (Bolton et al., *Brain* 2002).
- Several studies have failed to show a similar correlation, and others have implicated tubers in the cerebellum as a correlate of autism.
  - Walz et al., *J Child Neurol* 2002
  - Weber et al., *J Autism Dev Disord* 2000
  - Wang et al., *J Child Neurol* 2006
  - Eluvathingal et al., *J Child Neurol* 2006

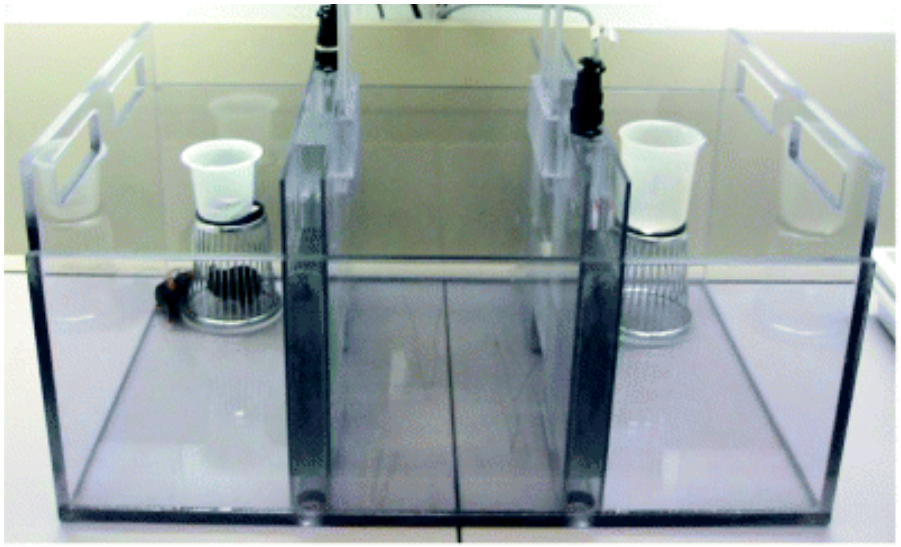
Hypothesis:

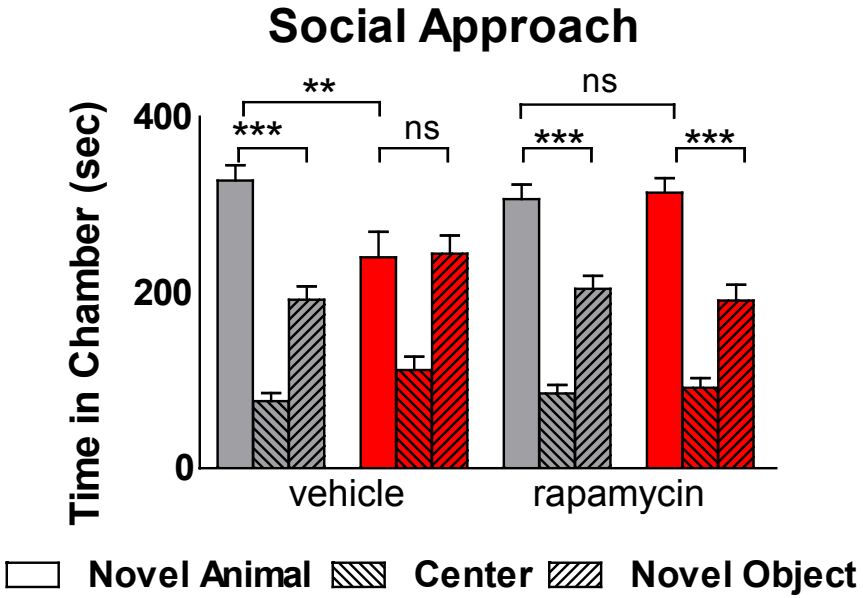
Miswiring of neuronal connections may contribute to the pathogenesis of TSC

Cerebellum in Autism and TSC

- Most consistent finding on brain pathology in ASD: reduced Purkinje cell numbers
- 37% of newborns with isolated cerebellar hemorrhage are diagnosed with subsequent ASD *(Limperopoulos et al., 2007)*
- Hypermetabolism in deep cerebellar nuclei (PET imaging) in TSC patients with ASD vs No ASD *(Asano et al., 2001)*

Autistic-like Behaviors in TSC Mice



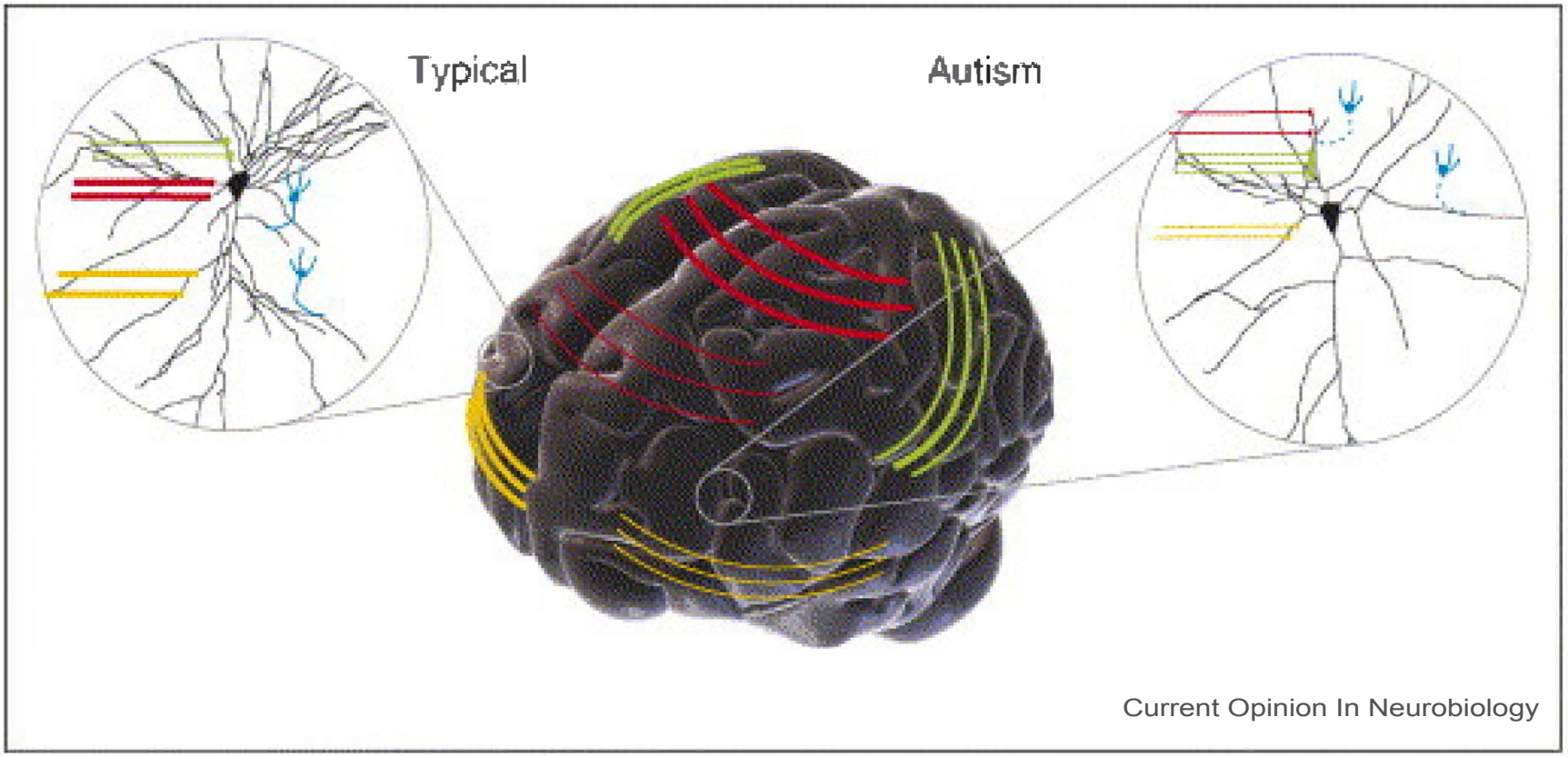


Treatment with rapamycin prevents autistic-like features

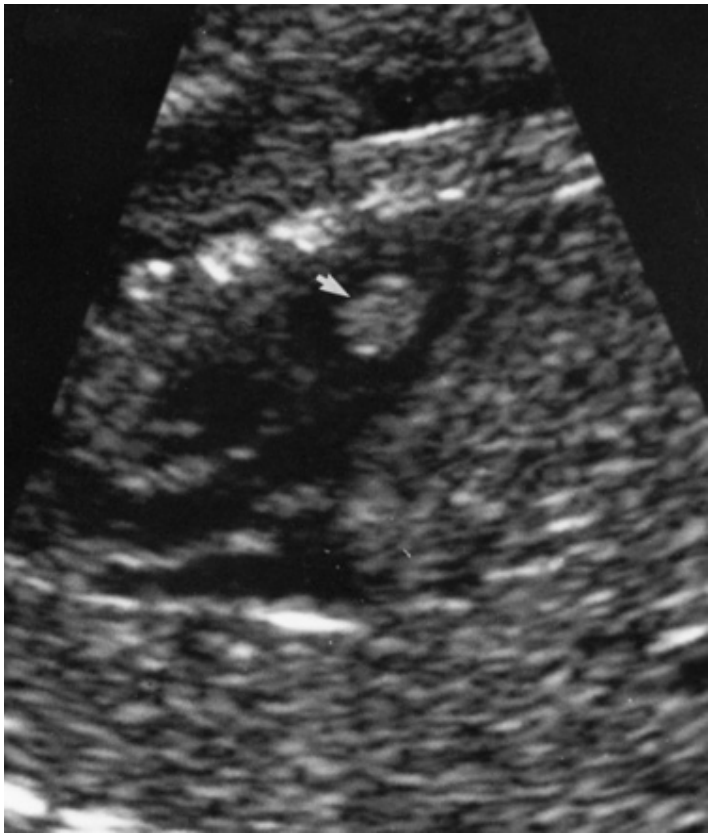
Tsai et al., *Nature*, 2012

Autism spectrum disorders: developmental disconnection syndromes

Daniel H Geschwind[1] and Pat Levitt[2]



Many of the TSC patients are diagnosed pre- or neo-natally



Among fetuses or newborns with multiple cardiac tumors, the chances of having TSC is 95%.

Tworetzky et al.,
Am J Cardio (2003)

Early Detection of Autism in TSC

Can we detect which infants with TSC will develop autism?

[Photo redacted]

1. Neurocognitive assessment of infants

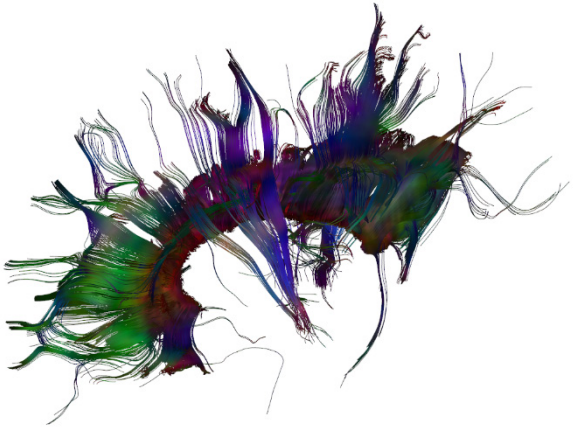
2. Diffusion tensor imaging (MRI)

3. Neurophysiological assessment of face processing and other visual paradigms

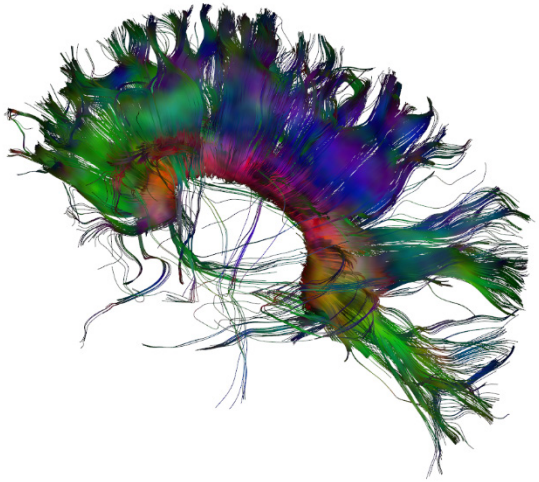
In collaboration with Simon Warfield, Shafali Jeste and Chuck Nelson

Diffusion Tensor Imaging

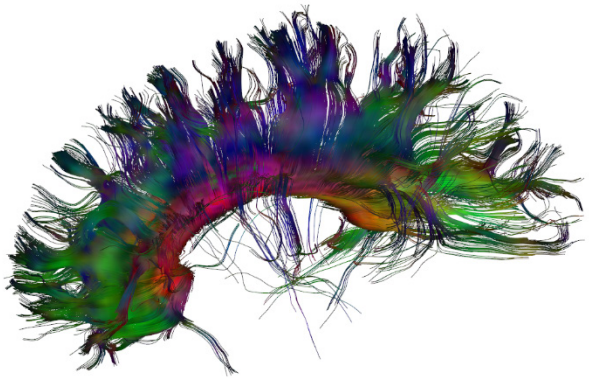
(Peters et al. 2012)



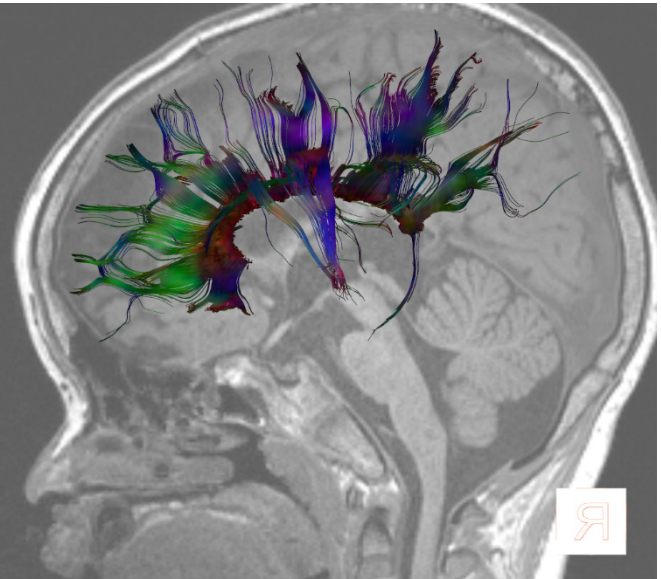
TSC ASD+ 4.9 yo AFA 0.34

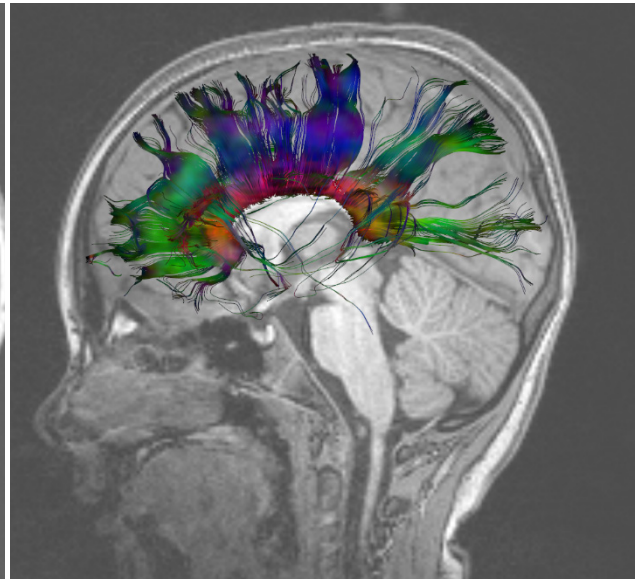


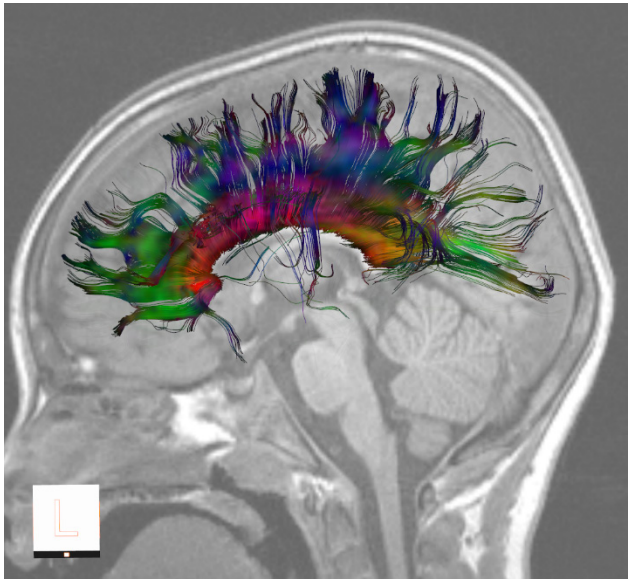
TSC ASD- 4.7 yo AFA 0.54



Control 5.3 yo AFA 0.53







TACERN

(*TSC Autism Center of Excellence Research Network)*

Boston Children's Hospital

Cincinnati Children's

UTHealth
The University of Texas
Health Science Center at Houston

UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

U01 NS082320 PIs: Sahin and Krueger
P20 NS080199 PI: Bebin

mTOR inhibitors in TSC mouse models

1. Improves myelination and seizures (Meikle et al., 2008)
2. Prevents or stops seizures (Zeng et al., 2008)
3. Improves learning (Ehninger et al., 2008)
4. Prevents autistic-features (Tsai et al., 2012)

Randomized Phase II Trial of an mTOR inhibitor in TSC: ***Neurocognition***

- 6-21 year olds with TSC, IQ>60
- Randomized placebo controlled, double blind
- 50 patients from 2 sites (Cincinnati & Boston)
- Neurocognitive testing at baseline, 3 months, 6 months
- Secondary endpoints: autism, seizures, sleep

Developmental Synaptopathies Consortium (NIH)

Scientific Advisory Board

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Project Leader
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PTEN Longitudinal Study

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Joyce Wu / Julian A. Martinez-Agosto, MD (UCLA)

PTEN Clinical Pilot Study

Project Leader
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Co PIs
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Thomas Frazier, PhD (CC)
Charis Eng, MD, PhD (CC)

U54 NS092090

Summary

- TSC is a model for understanding the early biomarkers associated with ASD.
- Mutations in TSC genes affect neuronal connectivity.
- Mouse models of TSC provide insight into autistic-like behaviors and enable pre-clinical trials.
- Treatment trials in TSC may shed light on related neurodevelopmental disorders.

Workshop on Under-Recognized Co-Occurring Conditions in ASD

Presentations will address:

1. What do we know and what have we learned in each research area? (Prevalence in autism vs. developmental disability vs. typical population? Underlying biology? Challenges in diagnosing these condition in individuals with ASD?)

2. What do we need, both in the clinic and in research to decrease the burden of illnesses/extent of disability, or reduction in quality of life due to the co-occurring conditions?

3. Are there any specific gaps or barriers that need to be addressed before progress can be made in this area?

4. Has recent research created new opportunities that need to be explored?

Workshop on Under-Recognized Co-Occurring Conditions in ASD

Discussion questions:

1. What do we need in order to help those affected by these conditions now? (e.g., provider and community awareness, practice guidelines, services, policies.)

2. What kind of research is needed to support development of new and improved treatments for these conditions in the future?

3. Are there any lessons we can learn from other fields to help accelerate progress?

4. Are any of these fields ready for practice guidelines or other steps that have not yet occurred due to knowledge or service gaps or other barriers? If so, how can the IACC, federal agencies or private organizations help accelerate progress?

Panel 3: Sleep and Neurological Disorders

Discussion

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Break

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Panel 4:

Metabolic and Immune Disorders

These slides do not reflect decisions of the IACC and are for discussion purposes only.

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

2:45 Panel 4: Metabolic and Immune Disorders

2:45 – 3:00 **Carlos Pardo-Villamizar, M.D.**
Associate Professor of Neurology and Pathology School of Medicine
Johns Hopkins University

3:00 – 3:15 **Judy Van de Water, Ph.D.**
Professor
Department of Internal Medicine, School of Medicine
Director, Center for Children's Environmental Health
Co-Director, Intellectual and Developmental Disabilities Research Center (IDDRC), MIND Institute
University of California, Davis

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IACC Workshop 2014:

Under-Recognized Co-Occurring Conditions in Autism Spectrum Disorder (ASD)

Immune system and Autism Spectrum Disorders

Carlos A. Pardo, MD

Johns Hopkins University School of Medicine

Baltimore, Maryland

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IACC WORKSHOP 2014:

Immune Disorders

Judy Van de Water, Ph.D.
Professor of Medicine
Director, UC Davis Center for Children's Environmental Health
Department of Internal Medicine and the M.I.N.D. Institute
University of California Davis

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Disclosures:

Conflict of Interest: None

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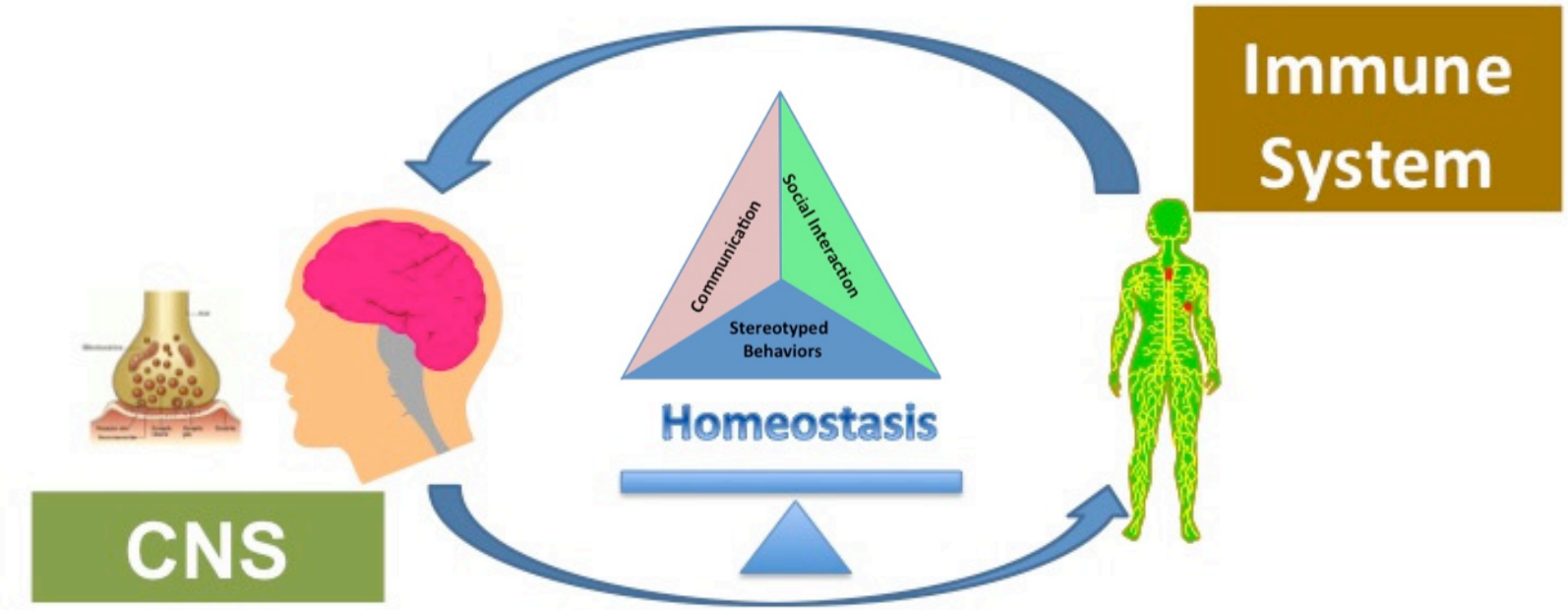
- ***The Bart McLean Fund for Neuroimmunology Research***
- ***Peter Emch Fund for Autism Research***
- ***Cure Autism Now/Autism Speaks***
- ***National Institutes of Health, National Institutes of Mental Health (HHSN271200700179)***



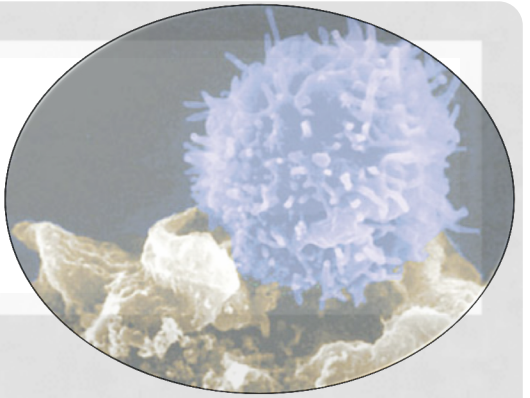
Gene-Environment Interaction:
Etiologic Model of Autism Spectrum Disorders

Genetic Factors

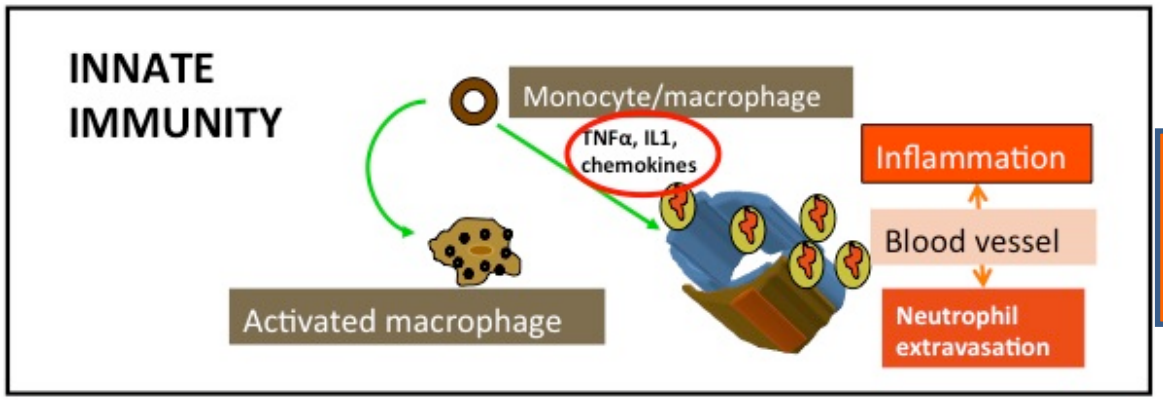
Immunity

Environment

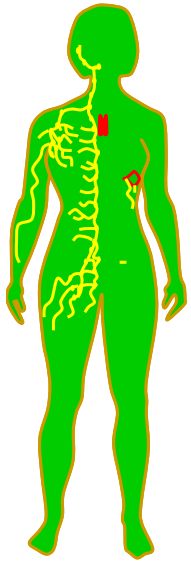
THE FIRST QUESTIONS:



- What is the level of evidence for immune system involvement in ASD?
- Is there evidence for a common biologic cause of the co-morbidity?
- What are the autism-specific features that affect proper diagnosis and treatment of the co-morbidity?

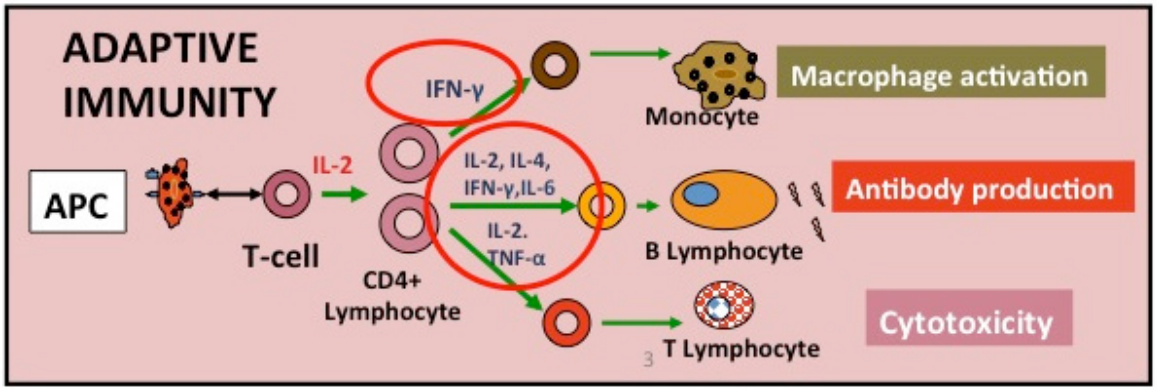


Non-specific Response



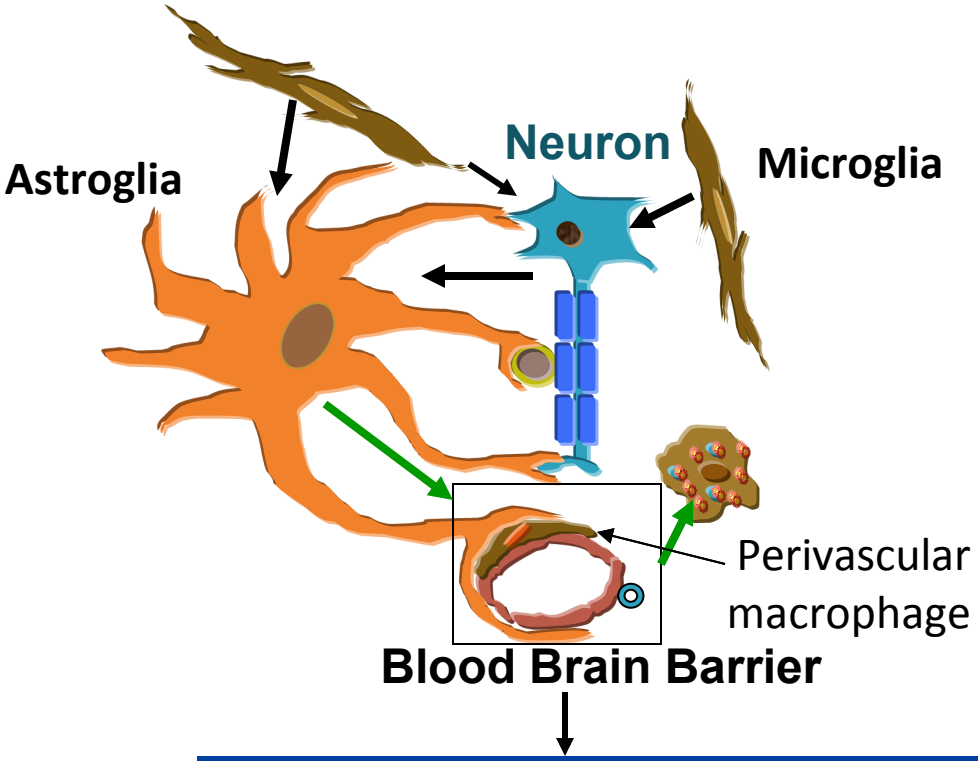
Homeostasis

Genetic factors
Trauma
Infections
Malignancy
Autoimmunity
Metabolic
others



Highly-specific Response

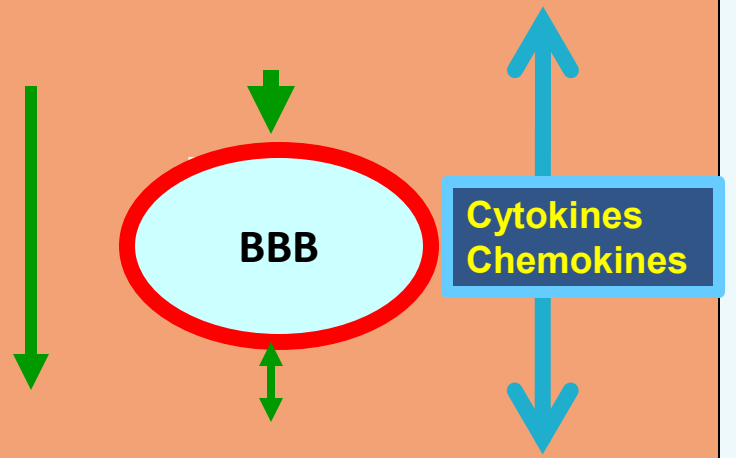
The Neuroimmune System



Modulation of immune responses
Lymphocyte/monocyte trafficking

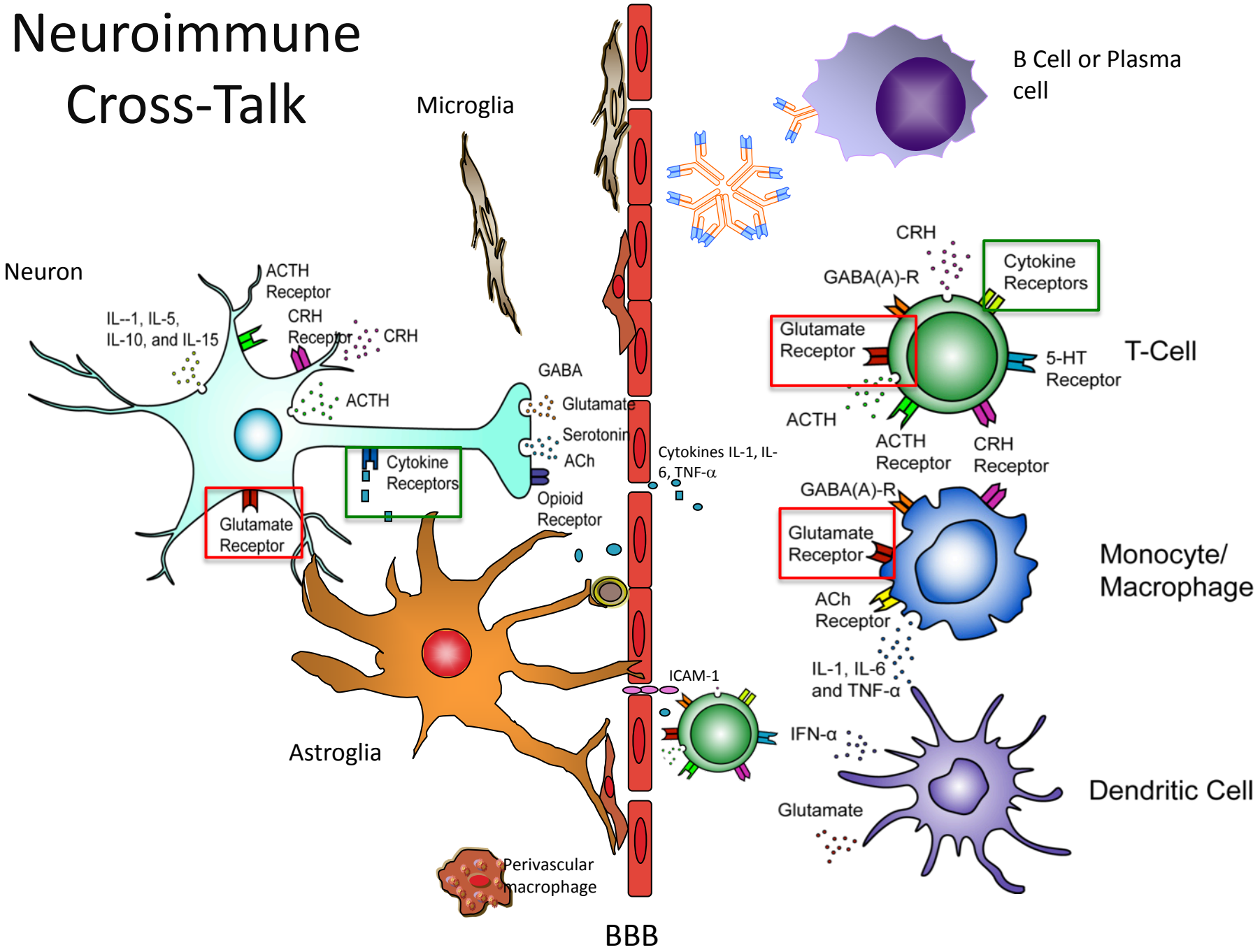
CENTRAL NERVOUS SYSTEM

Innate immunity
Microglia & astroglia



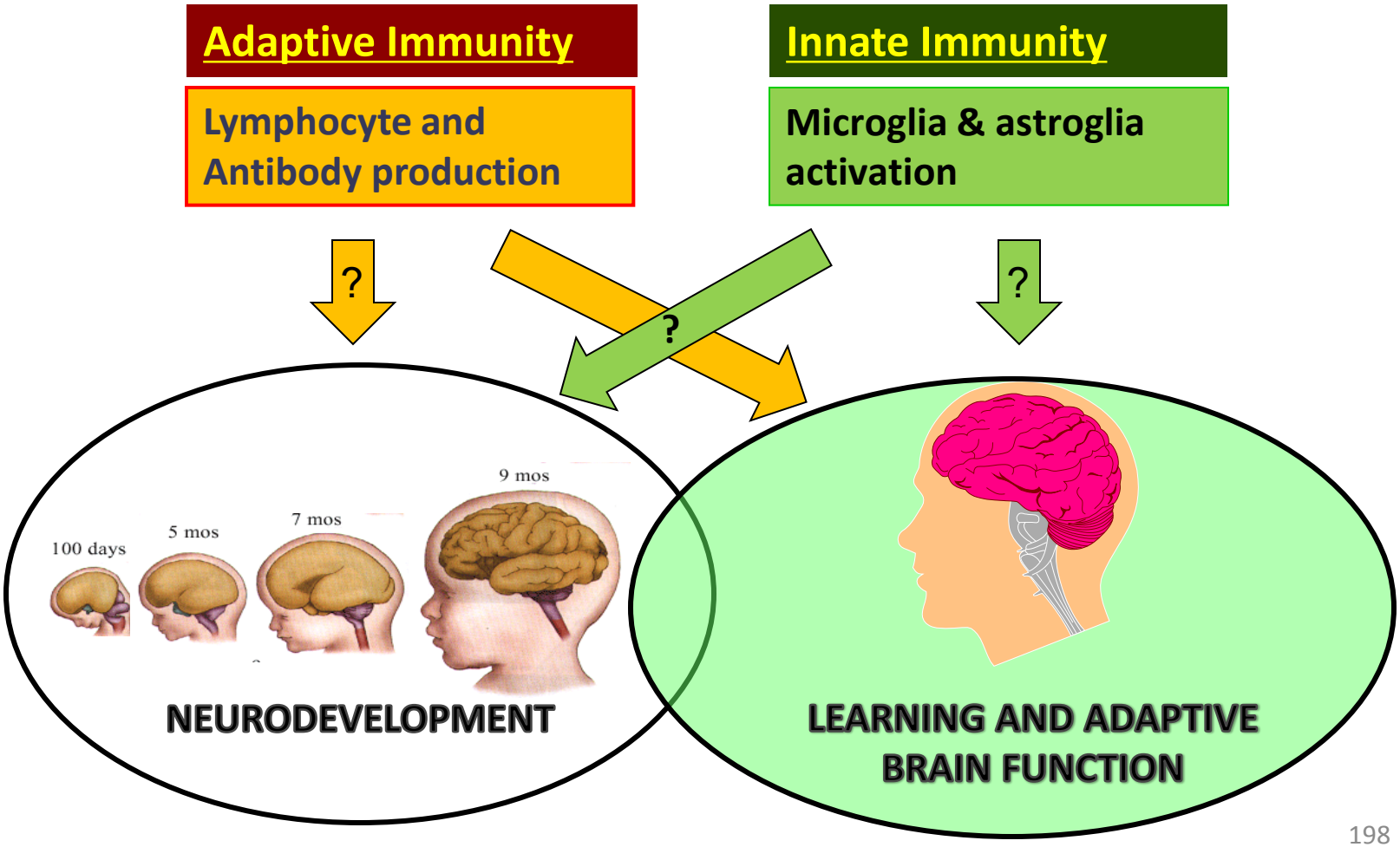
Adaptive immunity
Specific T cell and antibody responses

Neuroimmune Cross-Talk

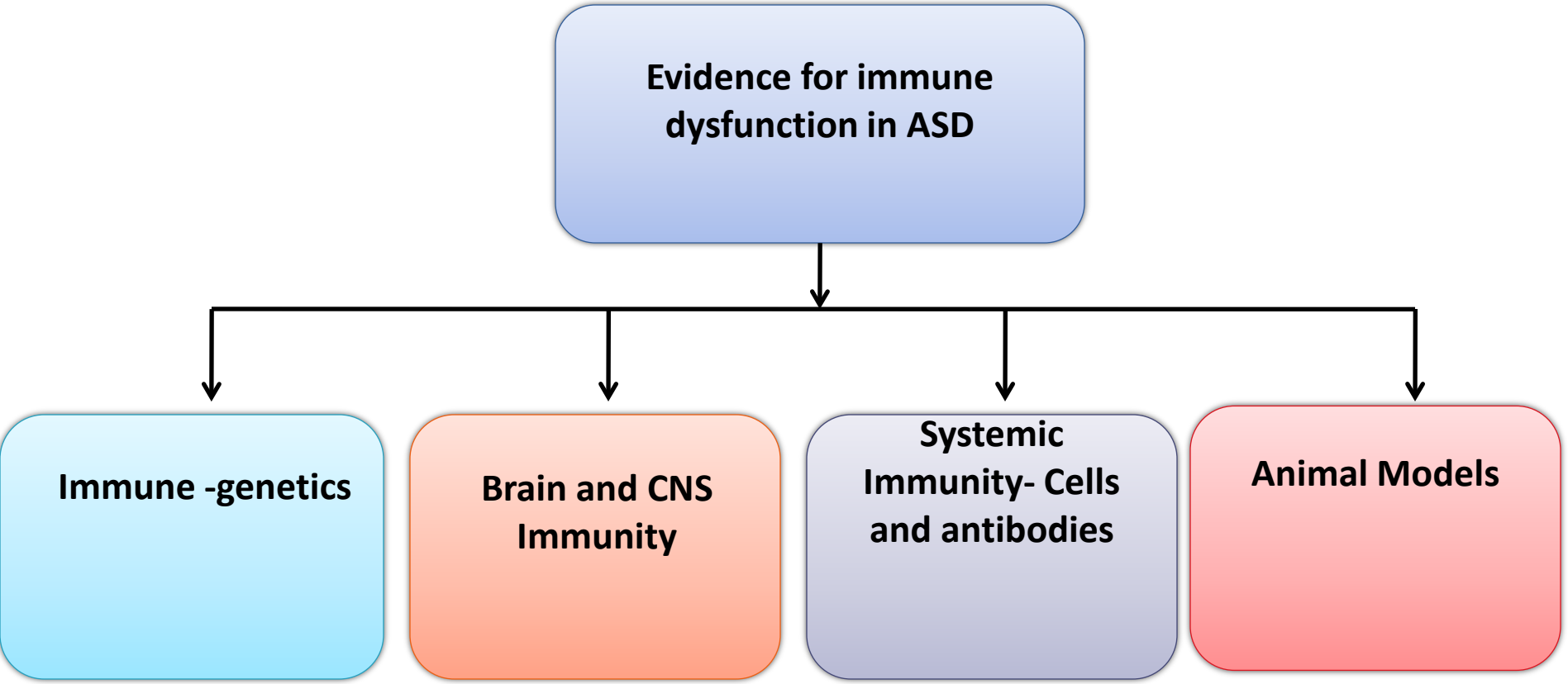


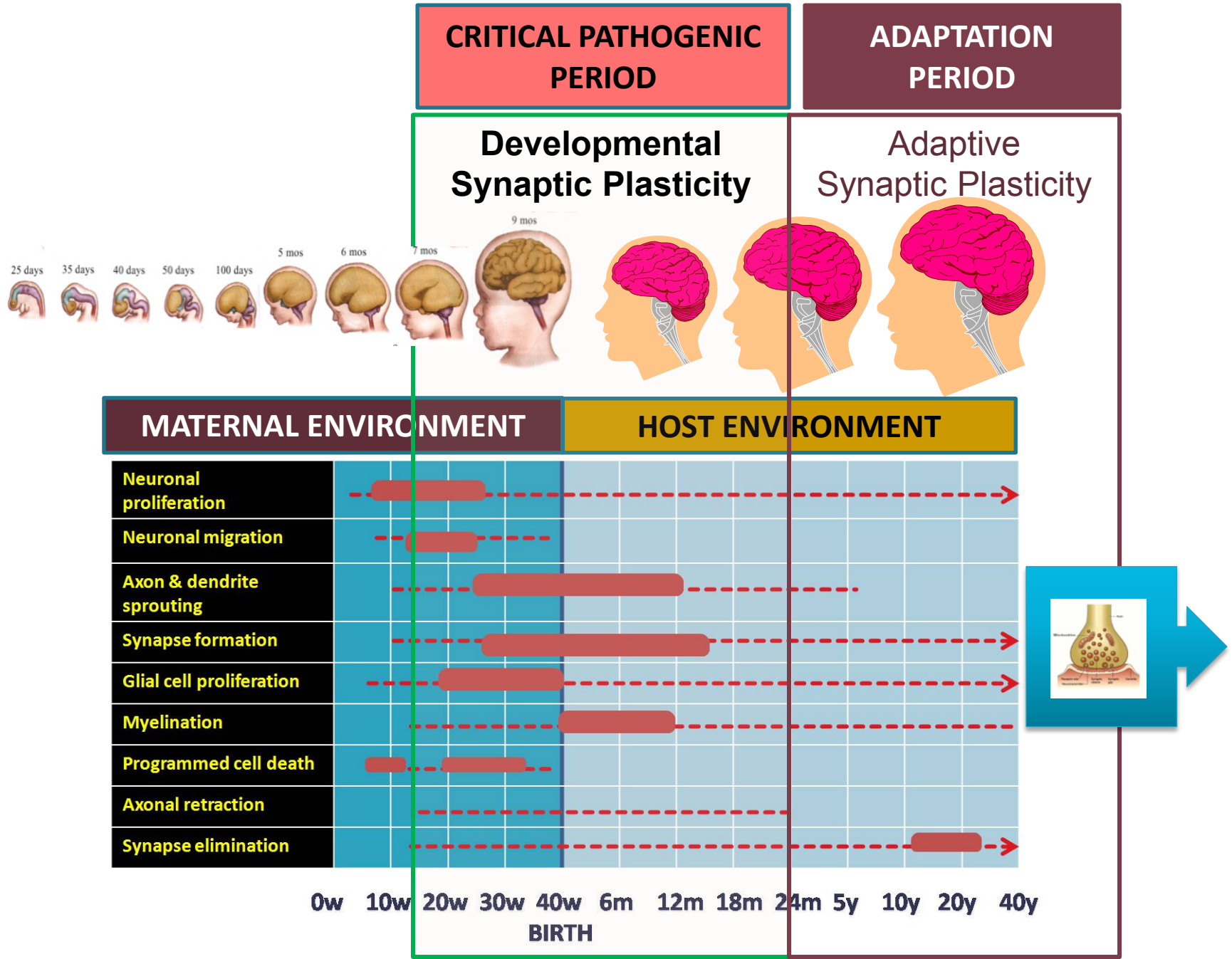
Key Questions:

Are immune or neuroimmune mechanisms involved in pathogenesis of ASD? What is the evidence?



Evidence for immune dysfunction in autism from many avenues




CRITICAL PATHOGENIC PERIOD
ADAPTATION PERIOD
Developmental Synaptic Plasticity
Adaptive Synaptic Plasticity
25 days
35 days
40 days
50 days
100 days
5 mos
6 mos
7 mos
9 mos
MATERNAL ENVIRONMENT
HOST ENVIRONMENT
Neuronal proliferation
Neuronal migration
Axon & dendrite sprouting
Synapse formation
Glial cell proliferation
Myelination
Programmed cell death
Axonal retraction
Synapse elimination
0w
10w
20w
30w
40w
6m
12m
18m
24m
5y
10y
20y
40y
BIRTH

Question:

Are maternal immune mechanisms involved in pathogenesis of ASD?

Adaptive Immunity

Lymphocyte and Antibody production

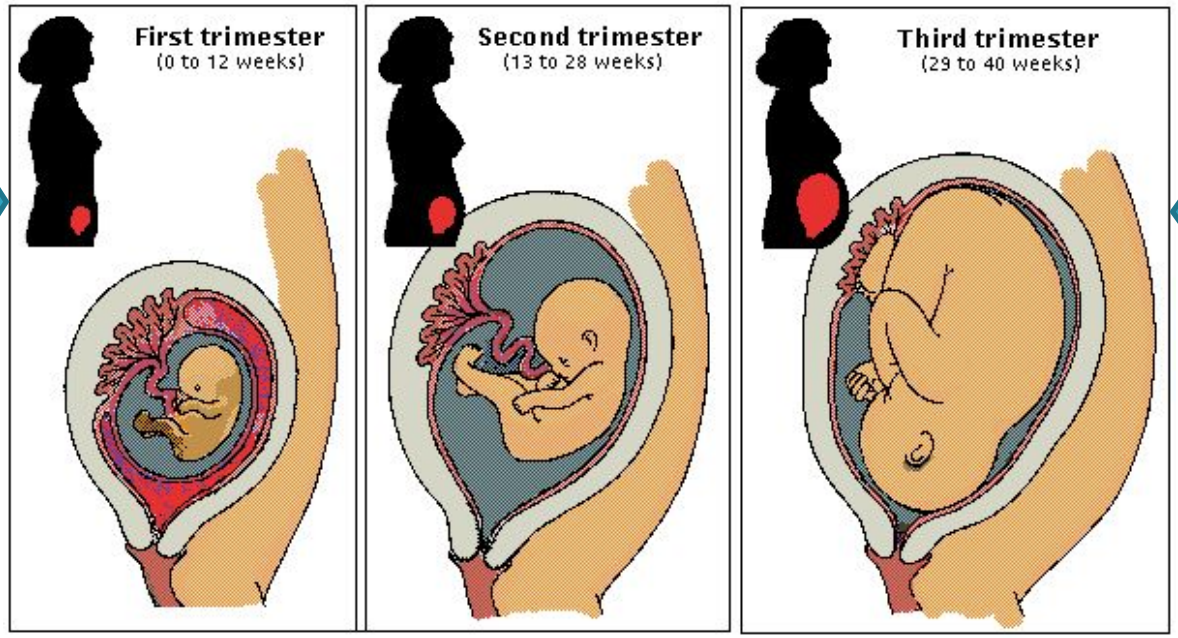
Innate Immunity

Microglia & astroglia activation

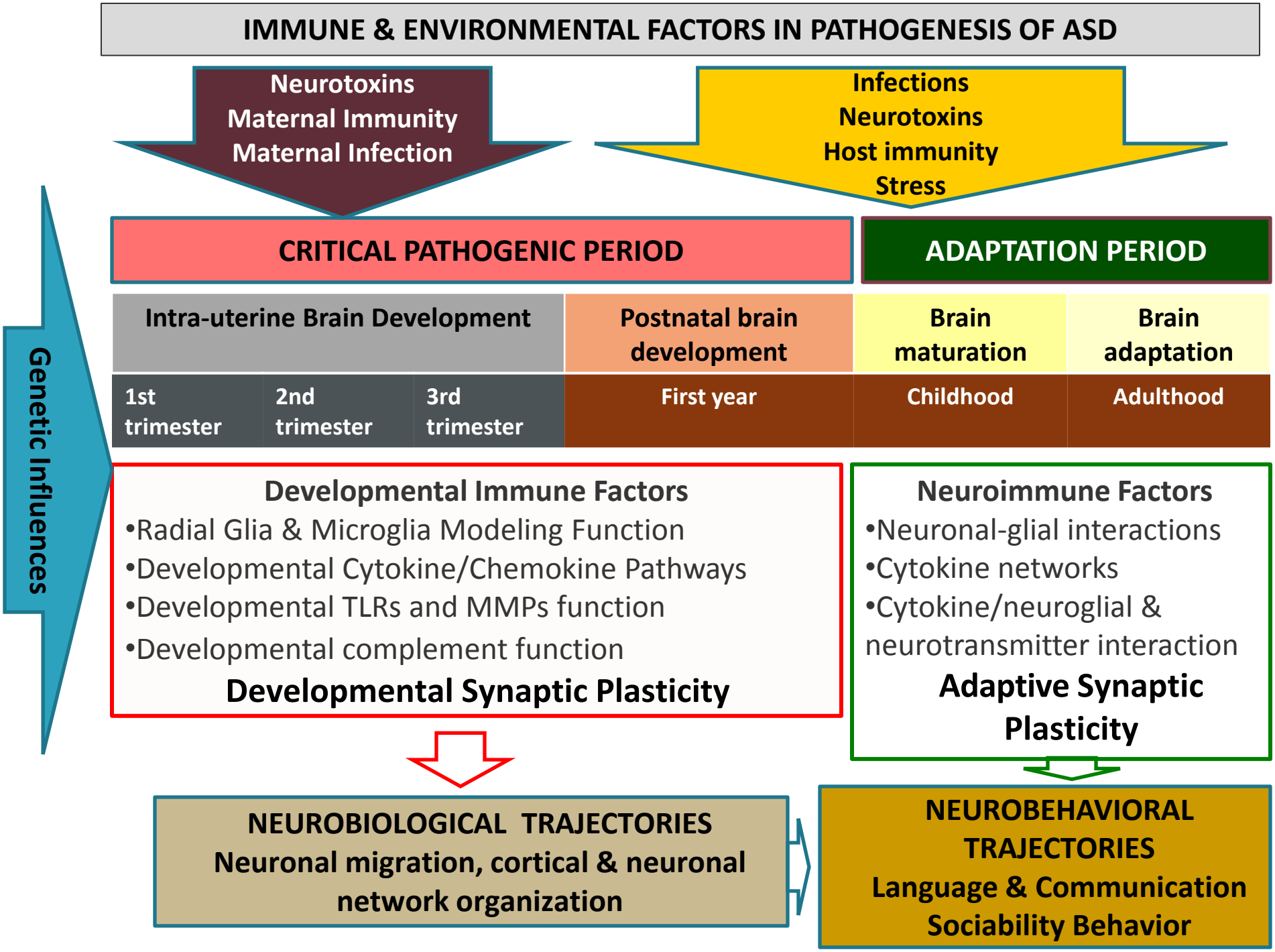
?

?

Maternal Infection



Auto-immunity


IMMUNE & ENVIRONMENTAL FACTORS IN PATHOGENESIS OF ASD
Neurotoxins
Maternal Immunity
Maternal Infection
Infections
Neurotoxins
Host immunity
Stress
Genetic Influences
CRITICAL PATHOGENIC PERIOD
ADAPTATION PERIOD
Intra-uterine Brain Development
Postnatal brain development
Brain maturation
Brain adaptation
1st trimester
2nd trimester
3rd trimester
First year
Childhood
Adulthood
Developmental Immune Factors
•Radial Glia & Microglia Modeling Function
•Developmental Cytokine/Chemokine Pathways
•Developmental TLRs and MMPs function
•Developmental complement function
Developmental Synaptic Plasticity
Neuroimmune Factors
•Neuronal-glial interactions
•Cytokine networks
•Cytokine/neuroglial & neurotransmitter interaction
Adaptive Synaptic Plasticity
NEUROBIOLOGICAL TRAJECTORIES
Neuronal migration, cortical & neuronal network organization
NEUROBEHAVIORAL TRAJECTORIES
Language & Communication
Sociability Behavior

What are the known immune mediated factors in ASD pathogenesis?

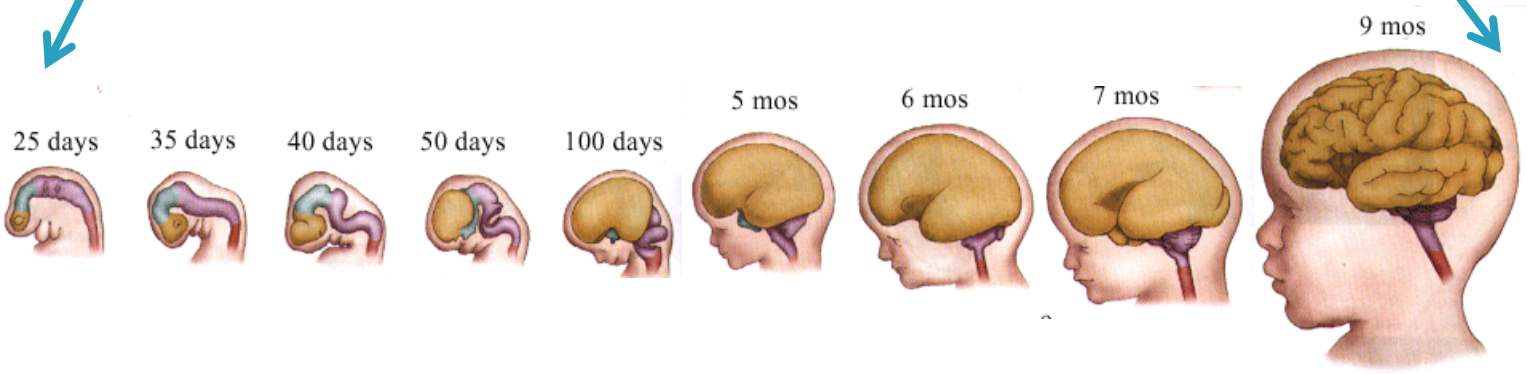
"Maternal autoimmunity in autism"

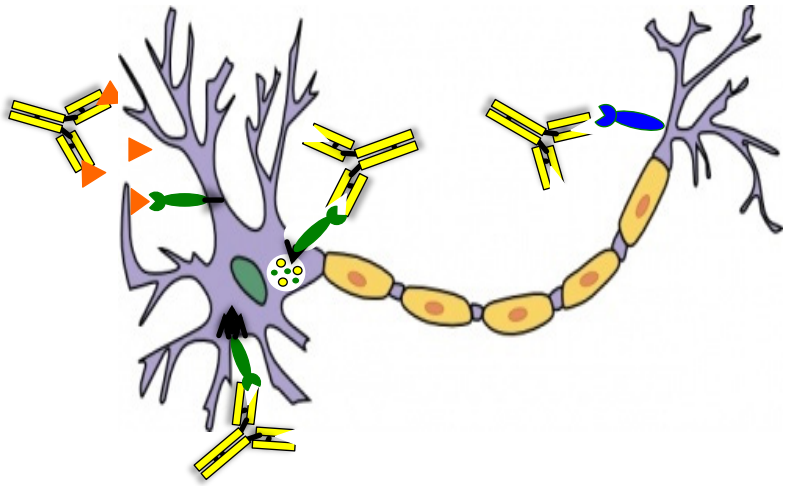
Judy Van de Water et. Al.
University of California at Davis,
Davis, California

Maternal Immunity

MATERNAL ENVIRONMENT | **ENVIRONMENT**

CRITICAL PATHOGENIC PERIOD





Maternal Autoantibody Related (MAR) Autism

Autoantibodies present in the circulation of mothers during pregnancy that recognize proteins in the developing fetal brain

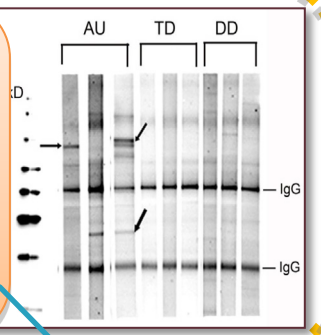
Maternal Anti-Brain Antibodies and ASD:

The studies behind the novel immune biomarker for autism risk

Epidemiology
Large population studies to identify potential risk factors for ASD

+

Initial finding: Immunology
Some mothers who have children with autism produce anti-brain antibodies



ASD Behavior

Antibodies associated with regression and language deficits, stereotypic behavior

Genetics

MET genetic variant associated with production of the anti-brain antibodies

MRI

Enlarged brain volume in male children prenatally exposed the antibodies

Basic Science

The antigenic targets of the antibodies have been identified

Animal Models

Animal models (2 monkey, 2 mouse) show behavioral changes after prenatal exposure to the antibodies; monkey model has also reported increased brain volume

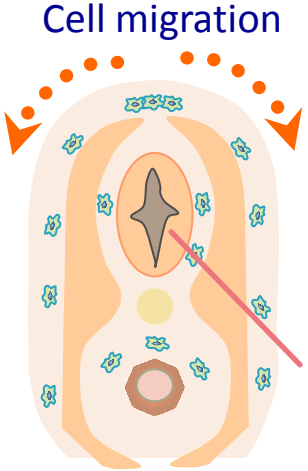
Brain Tissue Studies

Animal models provide tissue to explore brain pathology (ongoing)

Translational Potential:

- Identify kids with this sub phenotype and develop tailored behavioral treatment
- Screen women at risk and develop preventative strategies
- Define pathophysiology associated with these antibodies and develop therapeutic interventions

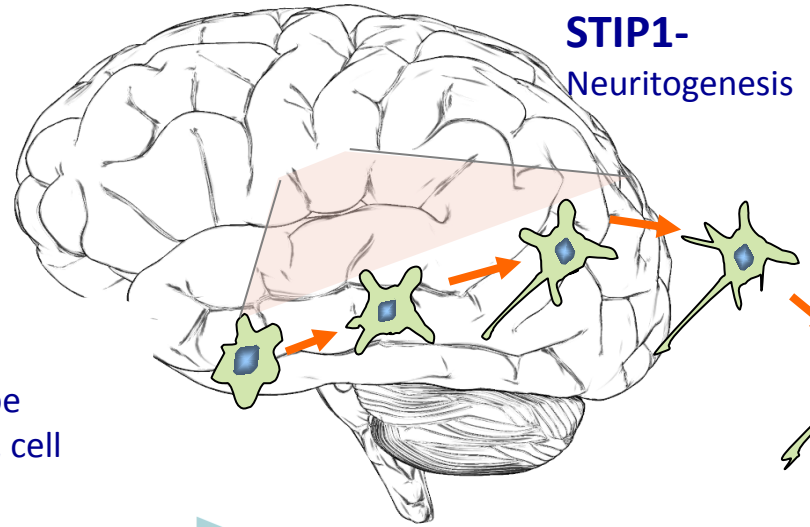
We Have Identified 7 Maternal Antibodies That Bind to Protein Targets Critical to Normal Brain Development*



CRMP1-
Cell migration

YBX-1-
Neural tube formation, cell division

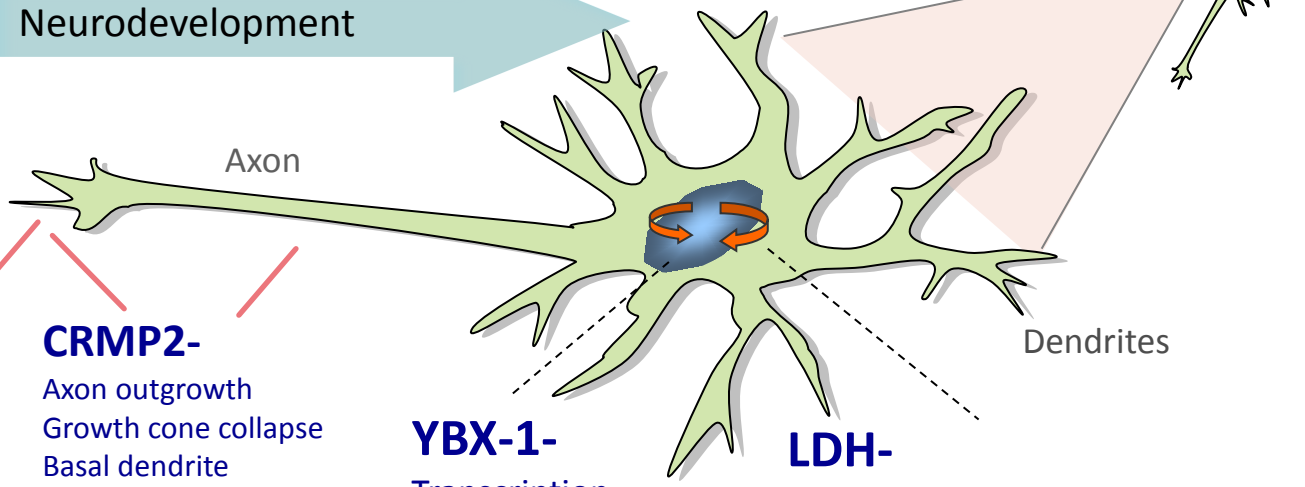
Embryonic Cross-section



STIP1-
Neuritogenesis

Cypin (GDA)-
Dendritic branching

Neurodevelopment



CRMP1-
Growth cone collapse

CRMP2-
Axon outgrowth
Growth cone collapse
Basal dendrite patterning

YBX-1-
Transcription regulation

LDH-
Metabolism

*Braunschweig, et al. ***Translational Psychiatry", July 9, 2013**

Specific MAR Antigens

# of Antigens	Antigen	% ASD (N = 241)	N	% TD (N = 147)	N	P Value
2	LDH + CRMP2	7%	17	0%	0	0.0004
2	STIP1 + CRMP2	10%	24	1%	1	0.0001
2	CRMP1 + CRMP2	7%	16	0%	0	0.0008
3	LDH + STIP1 + CRMP1	5%	12	0%	0	0.0045
3	Cypin + YBX1 + CRMP1	2%	5	0%	0	0.1615
3	Cypin + STIP1 + CRMP1	7%	18	1%	1	0.0025
3	Cypin + STIP1 + CRMP2	3%	8	0%	0	0.0267
3	YBX1 + STIP1 + CRMP2	3%	7	0%	0	0.0478
4	LDH + Cypin + YBX1 + STIP1	2%	5	0%	0	0.1615
4	LDH + Cypin + STIP1 + CRMP1	2%	5	0%	0	0.1615

Several Ab combinations are only found in mothers of ASD children

All specific combinations combined identify an association of MAR antibodies in ~23% of mothers with children on the ASD spectrum

What are the effects of these specific maternal autoantibodies?

-Do they have pathologic significance?
-do they affect how the brain develops?

Brain, Behavior, and Immunity xxx (2013) xxx–xxx

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Brain, Behavior, and Immunity

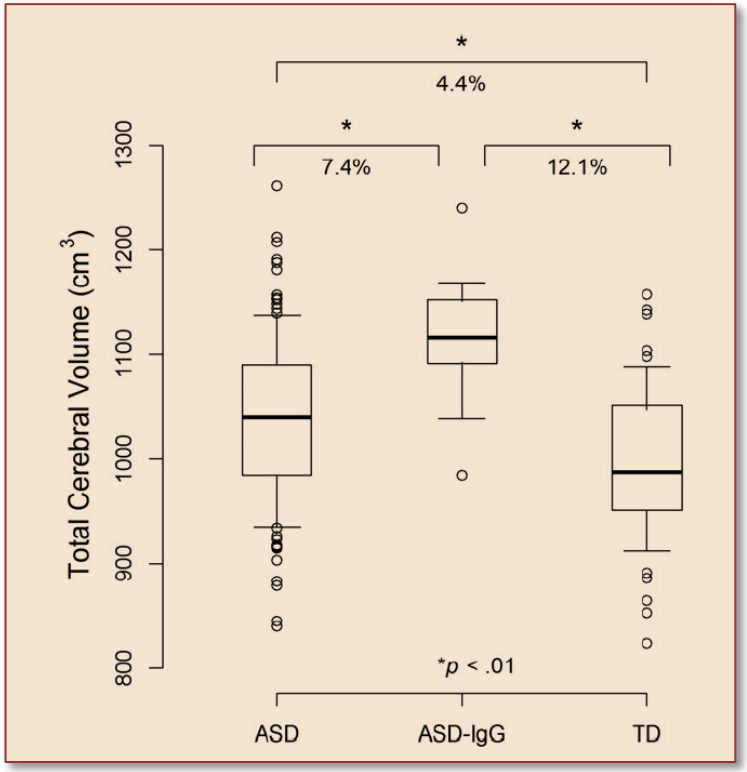
journal homepage: www.elsevier.com/locate/ybrbi

Short Communication

Maternal autoantibodies are associated with abnormal brain enlargement in a subgroup of children with autism spectrum disorder

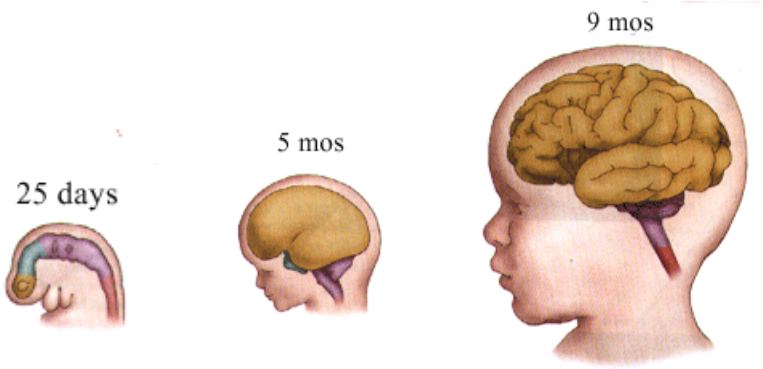
Christine Wu Nordahl [a,b,*], Daniel Braunschweig [c], Ana-Maria Iosif [d], Aaron Lee [b], Sally Rogers [a,b], Paul Ashwood [a,c], David G. Amaral [a,b], Judy Van de Water [a,c]

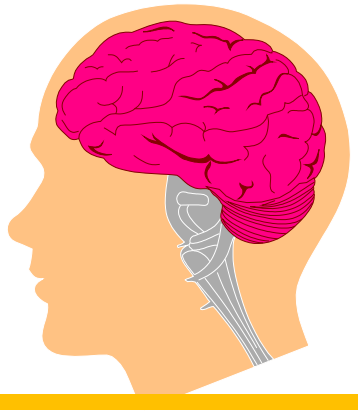
- Studied 181 2-4 YO male children (131 ASD, 50 typically developing (TD) controls) and evaluated total brain volume using structural magnetic resonance imaging (MRI).
- The ASD MAR group exhibited a **more extreme 12.1% abnormal brain enlargement** relative to TD controls.
- The remaining ASD children had a smaller 4.4% abnormal brain enlargement relative to TD controls.
- Lobar and tissue type analyses revealed that the frontal lobe is selectively enlarged
- **MAR autoantibodies may impact brain development leading to abnormal enlargement**.



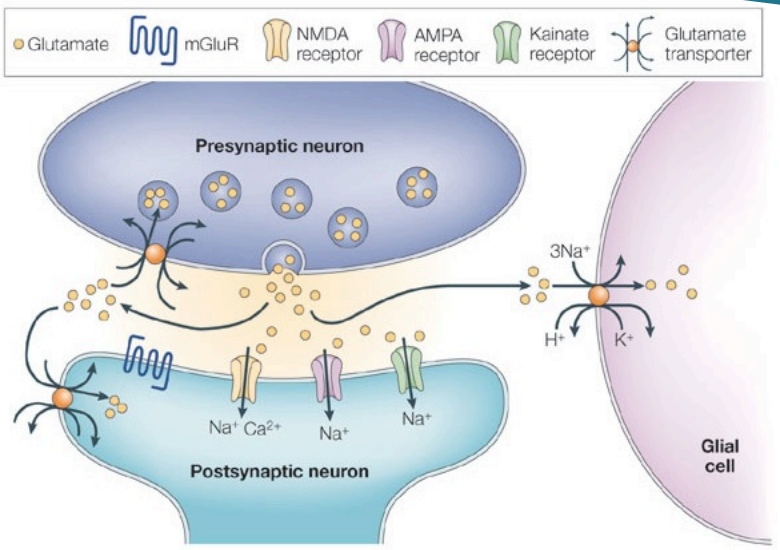
What is the pathology associated with these fetal brain specific auto-antibodies?

- Several passive transfer studies in mice and monkeys show evidence of MAR effects on behavior.
- Our first monkey model pilot study demonstrated behavioral changes in offspring following passive transfer of maternal IgG. Martin, L. A., et al 2008
- Our second monkey study by Bauman et al, Translational Psychiatry 2013, also demonstrated clear differences in social interaction.
- Maternal autism-associated IgG antibodies delay development, reduce social interaction, and produce anxiety in a mouse gestational transfer model (Braunschweig, et al. JNI, 2012).
- Increase in spontaneous grooming behaviors in response to a novel environment in mice exposed intraventricularly to MAR IgG. Comacho et al, Brain behav res. 2014.





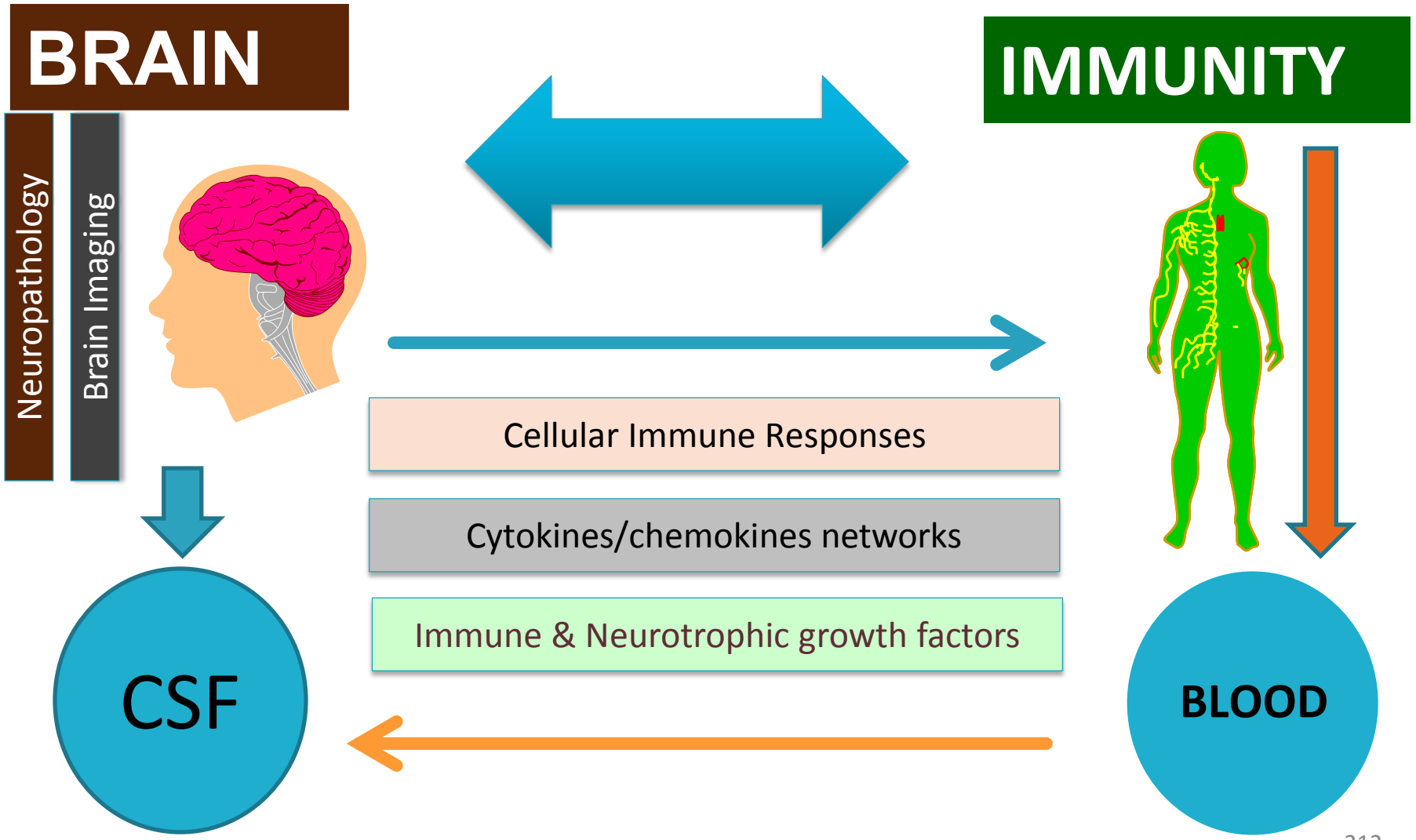
CRITICAL PATHOGENIC PERIOD				ADAPTATION PERIOD	
Intra-uterine Brain Development			Postnatal brain development	Brain maturation	Brain adaptation
1st trimester	2nd trimester	3rd trimester	First year	Childhood	Adulthood



Synaptic Plasticity
- Synaptic pruning
- Neurotransmitter homeostasis
- Synaptic stripping

Microglia
Astrocytes
Neuronal-neuroglia interaction
Cytokine/chemokine pathways

HOW TO STUDY NEURAL AND IMMUNE INTERACTIONS IN ASD?



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]

Ann Neurol 2005;57:67—81

Microglial Activation and Increased Microglial Density Observed in the Dorsolateral Prefrontal Cortex in Autism

John T. Morgan, Gursharan Chana, Carlos A. Pardo, Cristian Achim, Katerina Semendeferi, Jody Buckwalter, Eric Courchesne, and lan P. Everall

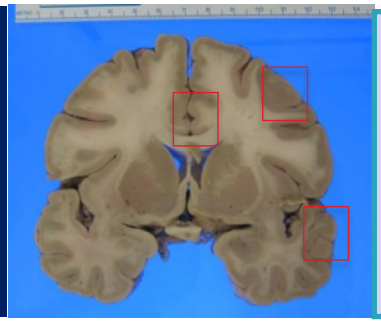
BIOL PSYCHIATRY 2010;68:368-376

Transcriptomic analysis of autistic brain reveals convergent molecular pathology

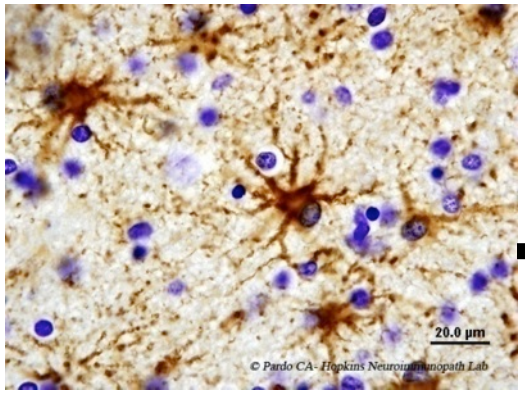
IrinaVoineagu[1],Xinchen Wang[2], Patrick Johnston[3], Jennifer K. Lowe[1], Yuan Tian[1], Steve Horvath[4], Jonathan Mill[3], Rita M.Cantor[4], Benjamin J. Blertcowe[2] & Daniel H. Geschwind[1,4]

380 | NATURE | VOL 474 | 16 JUNE 2011

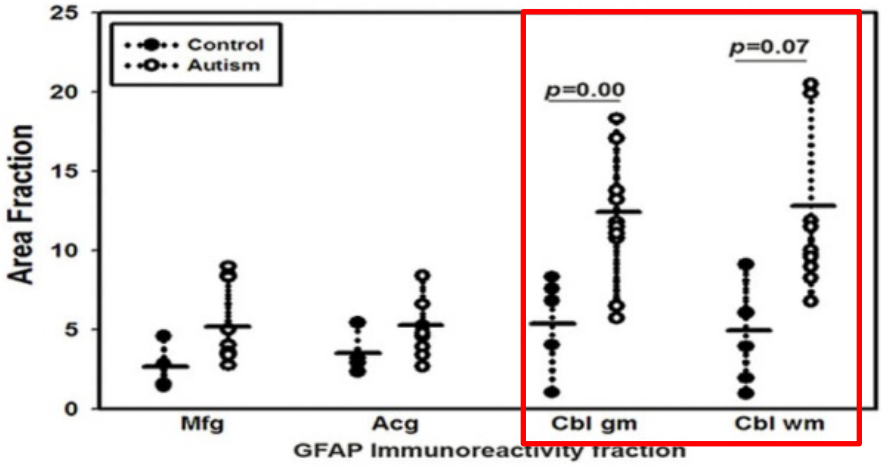
Assessment of Neuroglial reactions: Quantification of microglia and astroglia activation

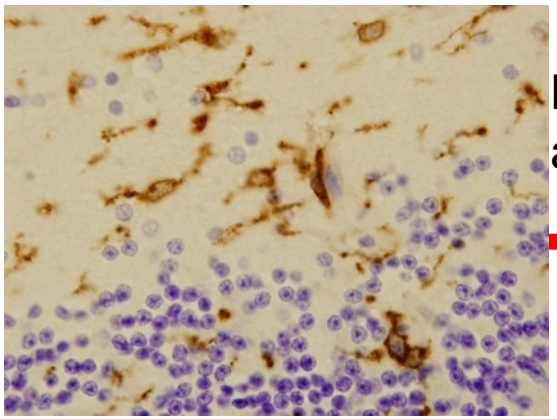


11 cases of autism
(age 5-44)
12 controls
Brain regions:
Frontal (MFG), cingulate (ACG), and cerebellum

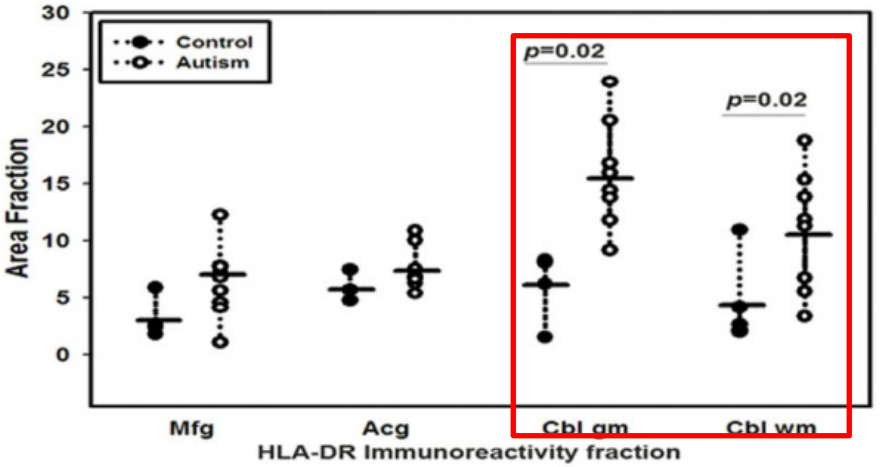


Astroglia activation



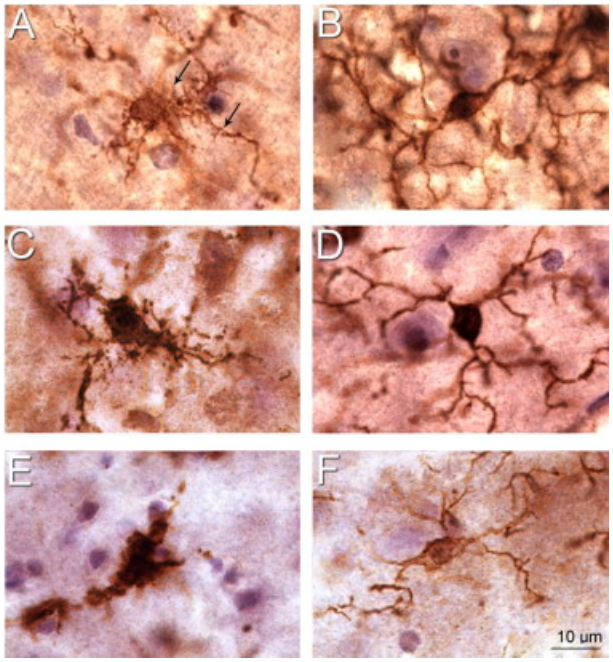


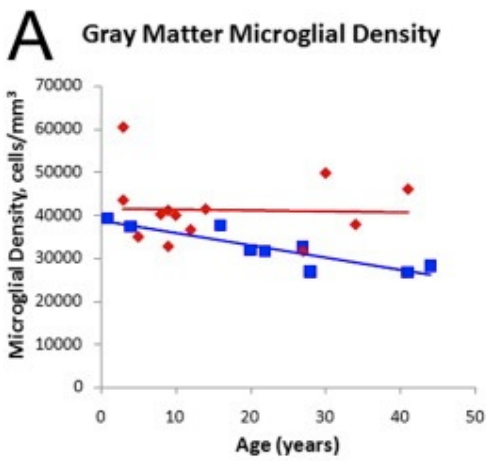
Microglia activation

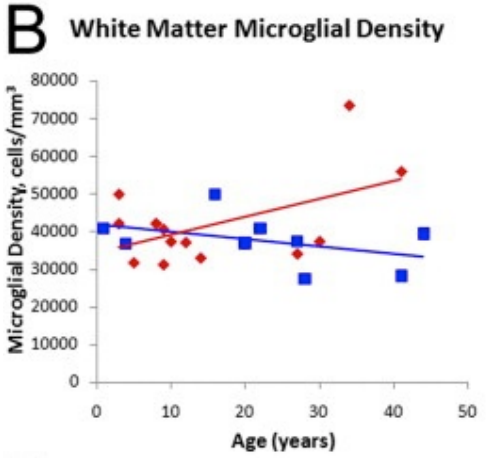


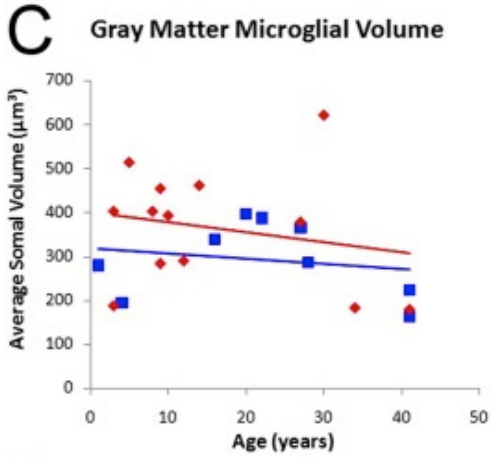
Vargas DL et al. Ann Neurol 2005

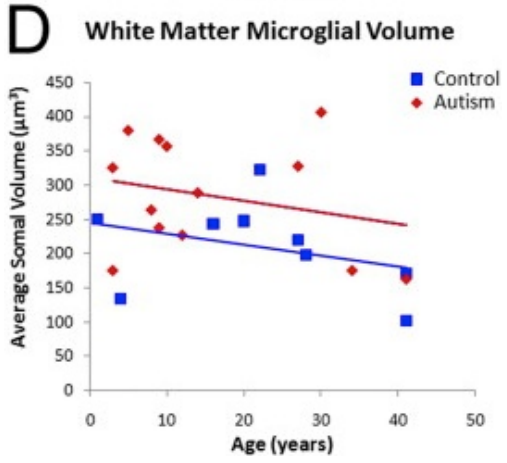
Microglia cell density increases in the cerebral cortex in ASD





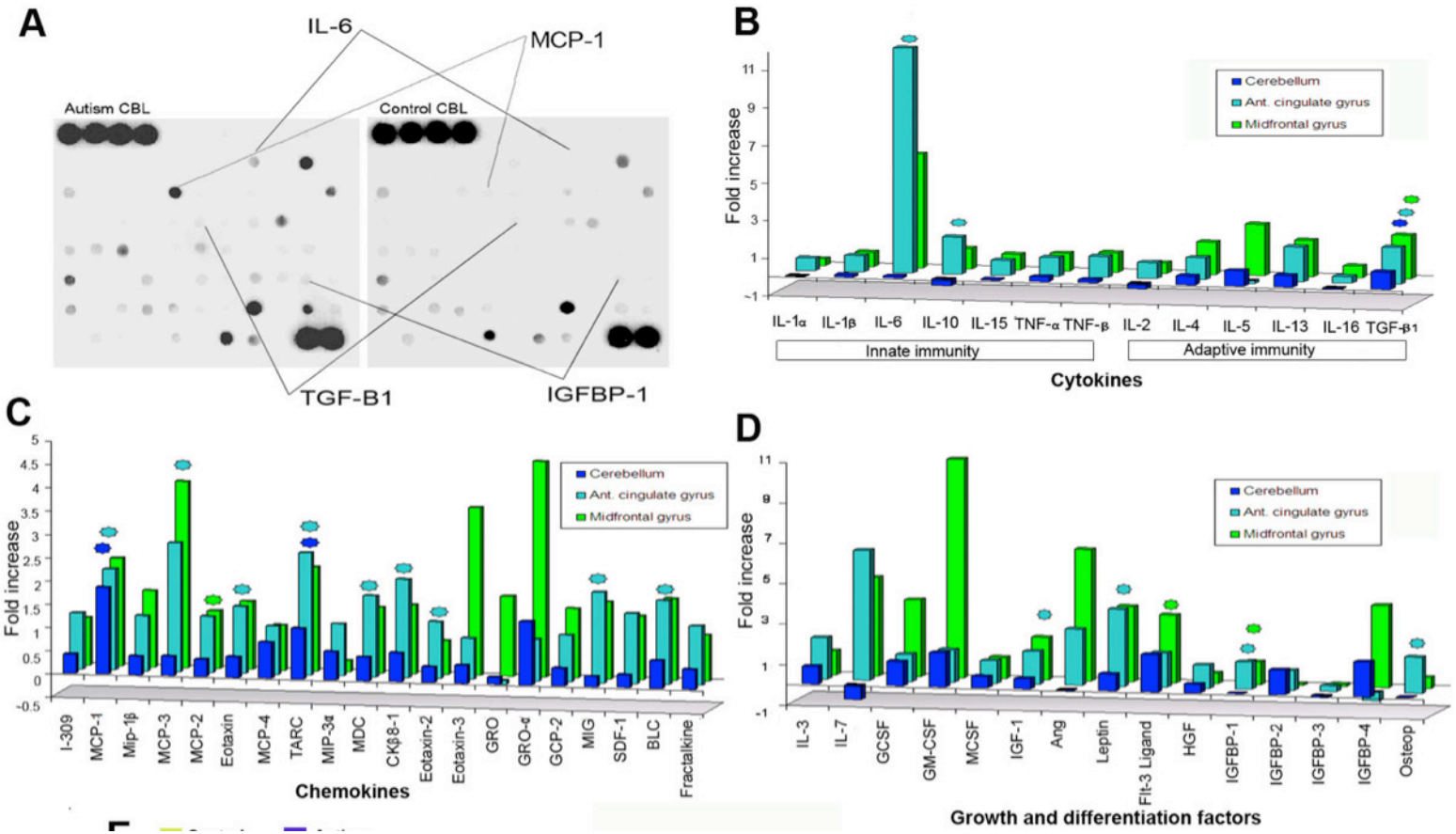






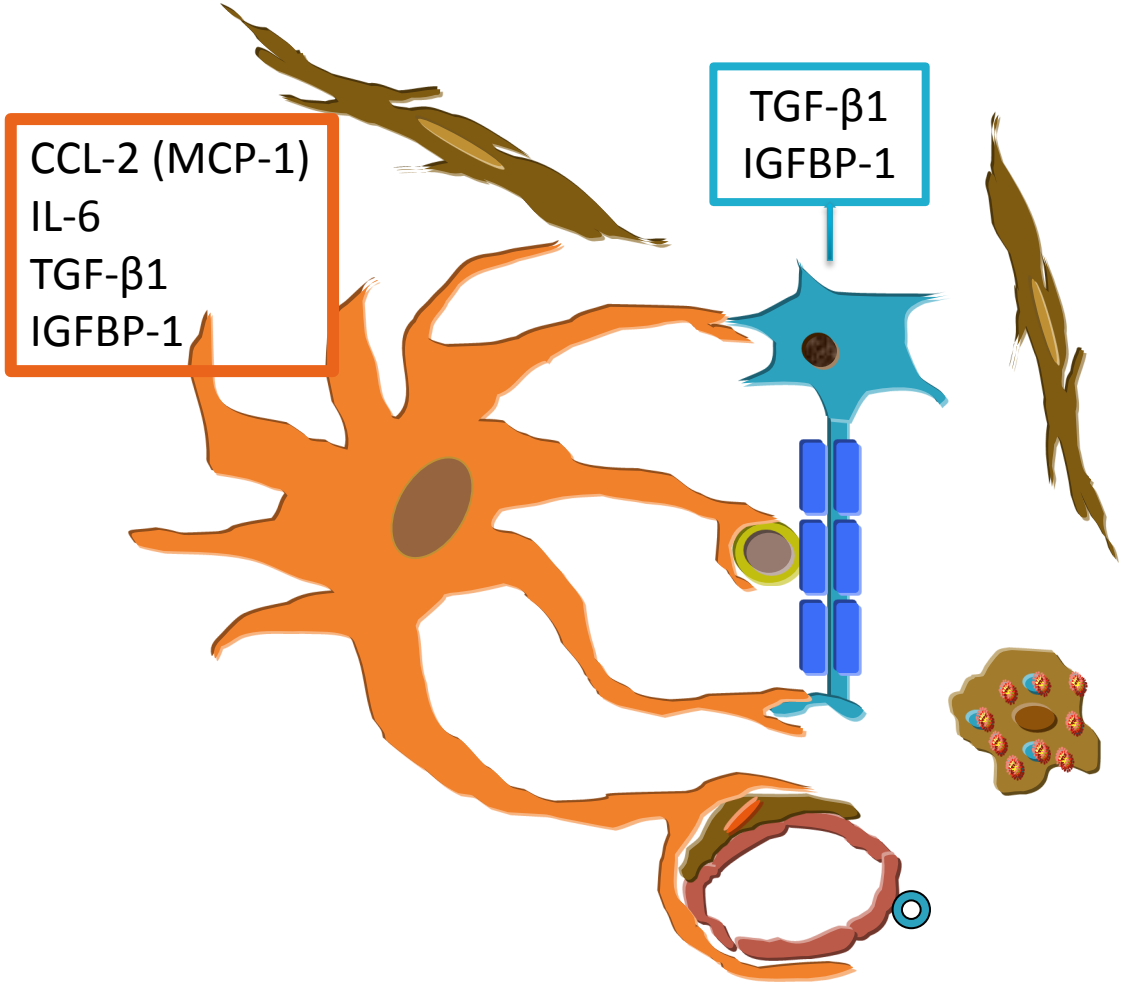
Morgan JT et al. Biological Psychiatry 2010

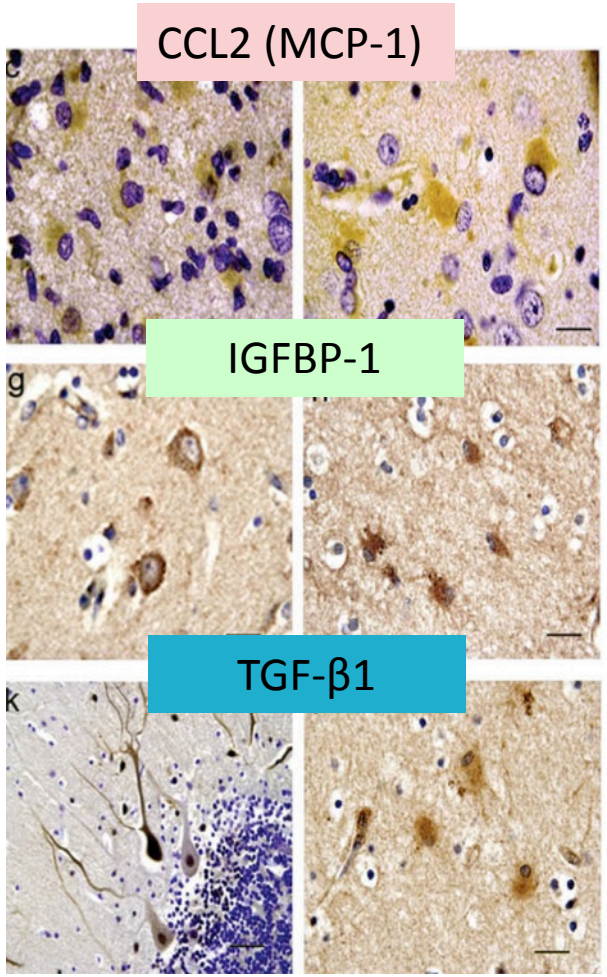
Autism: Profiles of Cytokine/Chemokine in the Brain



Vargas DL et al. Ann Neurol 2005

Brain Cytokine-Chemokine-Growth Factors in ASD

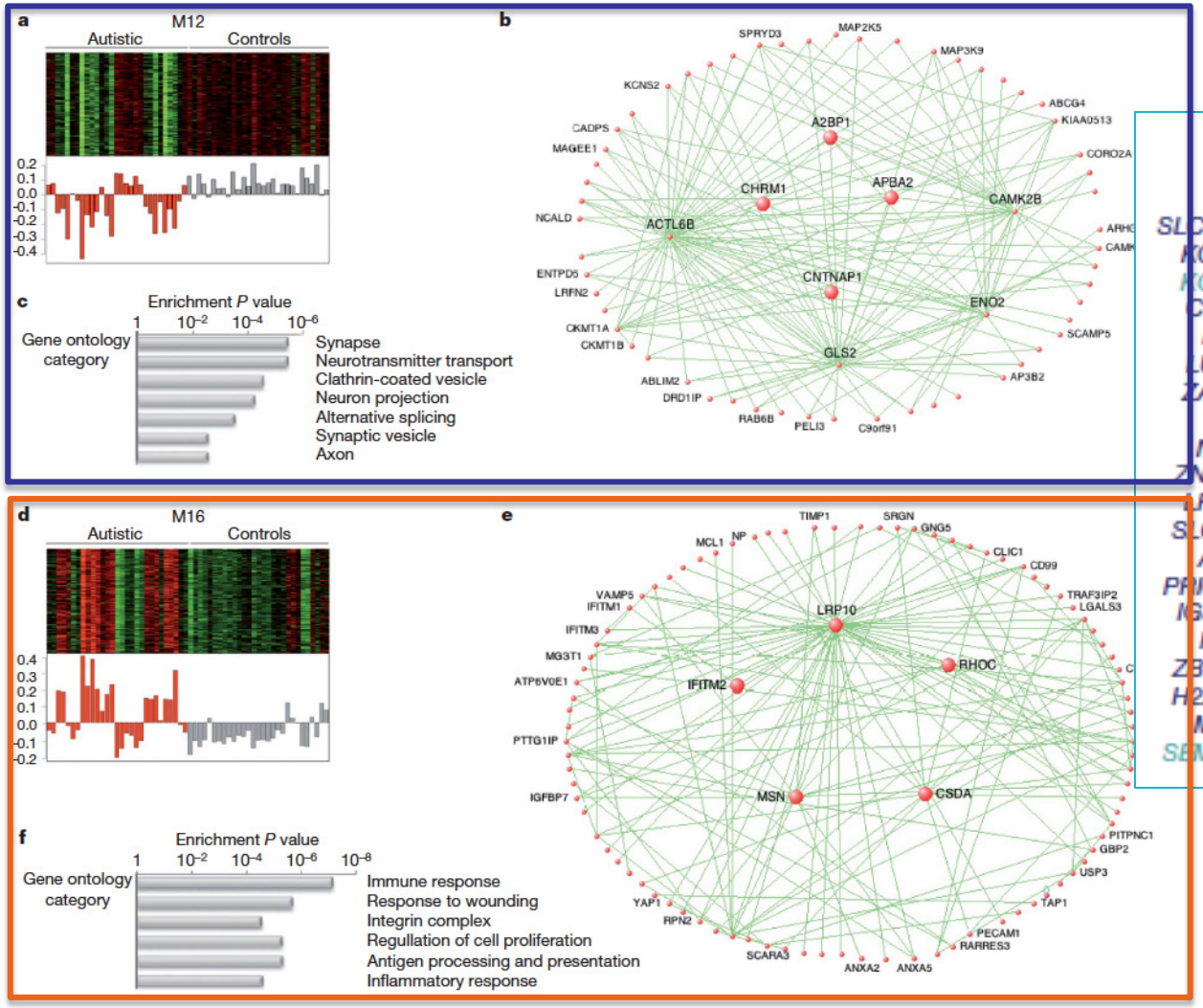


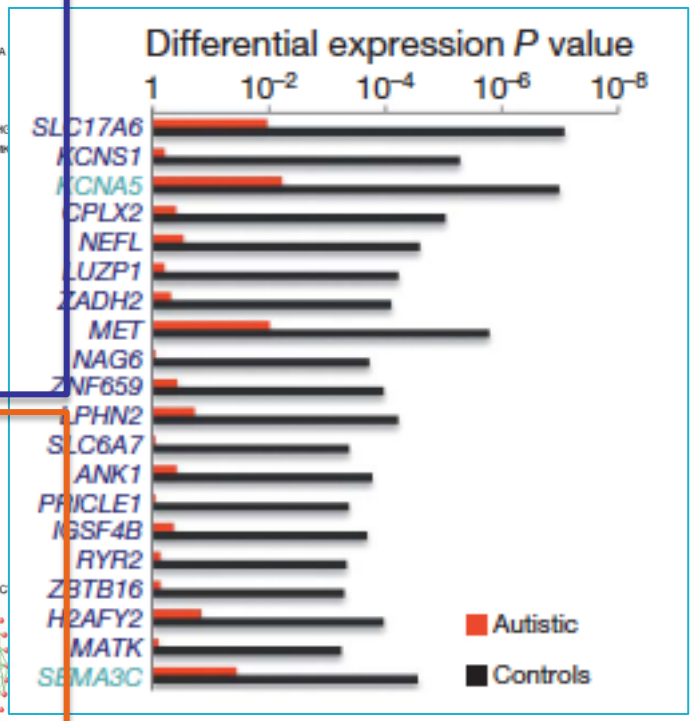


Based on Vargas DL et al. Ann Neurol 2005

Transcriptomic analysis of autistic brain reveals convergent molecular pathology

Irina Voineagu[1], Xinchen Wang[2], Patrick Johnston[3], Jennifer K.Lowe[1], Yuan Tian[1] Steve Horvath[4], Jonathan Mill[3], Rita M. Cantor[4], Benjamin J. Blenoowe[2] & Daniel H. Geschwind[1,4] 3 8 0 | NA T U R E | V O L 4 7 4 | 1 6 J U N E 2 0 1 1





ONLINE FIRST

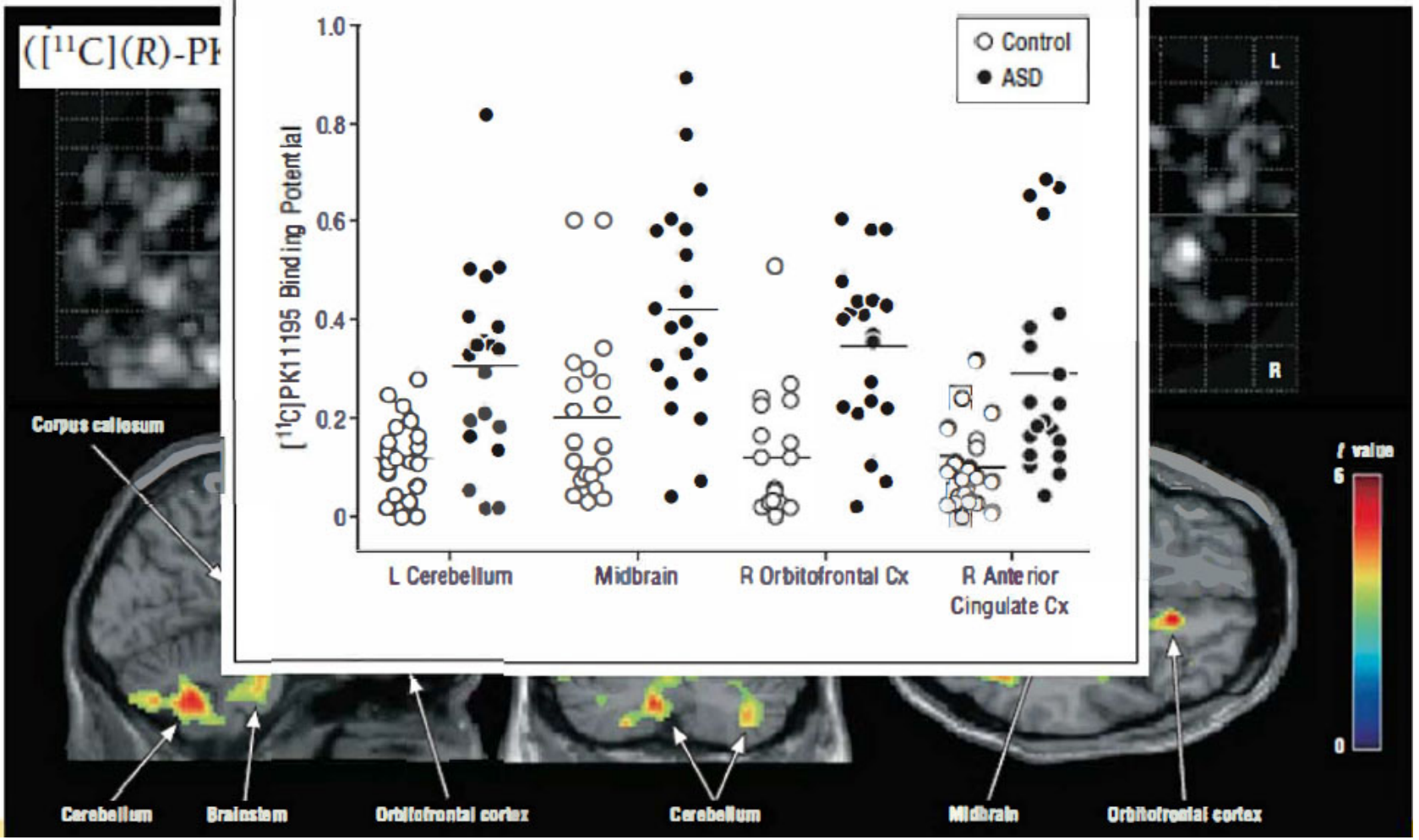
Microglial Activation in Young Adults With Autism Spectrum Disorder

JOHNS HOPKINS MEDICINE

Katsuaki Suzuki, MD, PhD; Genichi Sugihara, MD, PhD; Yasuomi Ouchi, MD, PhD; Kazuhiko Nakamura, MD, PhD; Masami Futatsubashi, BS; Kiyokazu Takebayashi, MD, PhD; Yujiro Yoshihara, MD, PhD; Kei Omata, PhD; Kaori Matsumoto, MA; Kenji J. Tsuchiya, MD, PhD; Yasuhide Iwata, MD, PhD; Masatsugu Tsuji, MA; Toshirou Sugiyama, MD, PhD; Norio Mori, MD, PhD

Arch Gen Psychiatry.

ıber 26, 2012.



Neuroinflammatory responses in the brain in ASD: what means as co-morbidity ?

The current evidence:

- **Increased innate immunity responses in the brain**
  - Microglial & astroglial responses
  - Cytokine & chemokine increases or disregulation
- **Differential transcriptome expression for synaptic and immune related genes**
- **In-vivo evidence of microglial "activation" (PET scanning)**

Trigger

?

❑ ***Brain pathology***

Abnormal brain development?

Response to intrauterine injury ? (e.g. infection, maternal antibodies)

Abnormal cortical function ? (e.g. epilepsy)

❑ ***Peripheral Immune responses?***

Host autoimmunity?

Systemic inflammation?

Microbial translocation?

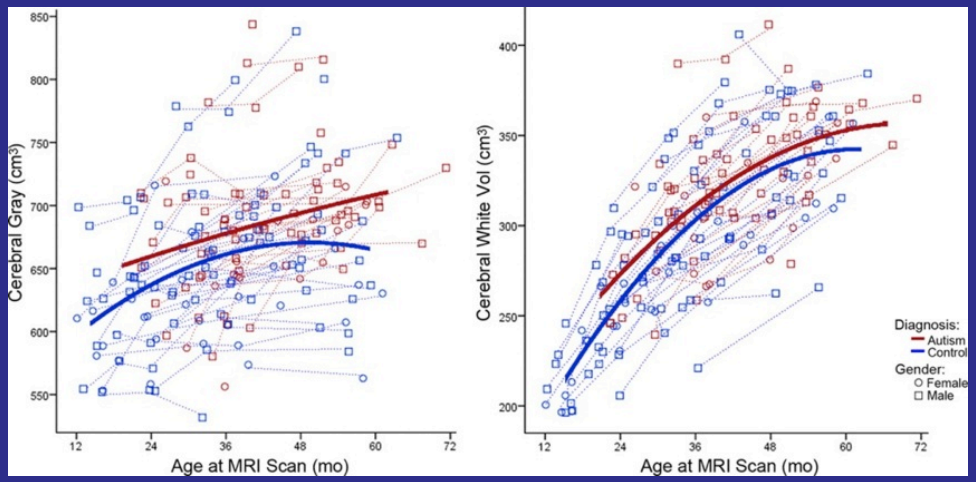
What are the triggering factors in neuroinflammation in the brain of ASD patients ?

Observations in the neuropathology of ASD:

Excess neurons, white matter and enlarged frontal lobe

What is abnormal in the white matter?

Filipek et al. 1991
Piven et al.1995,1996
Courchesne E et al. 2001-2004
Sparks et al. 2002
Aylward et al 2002
Herbert et al 2003-2005
Hardan A, 2006
Others…

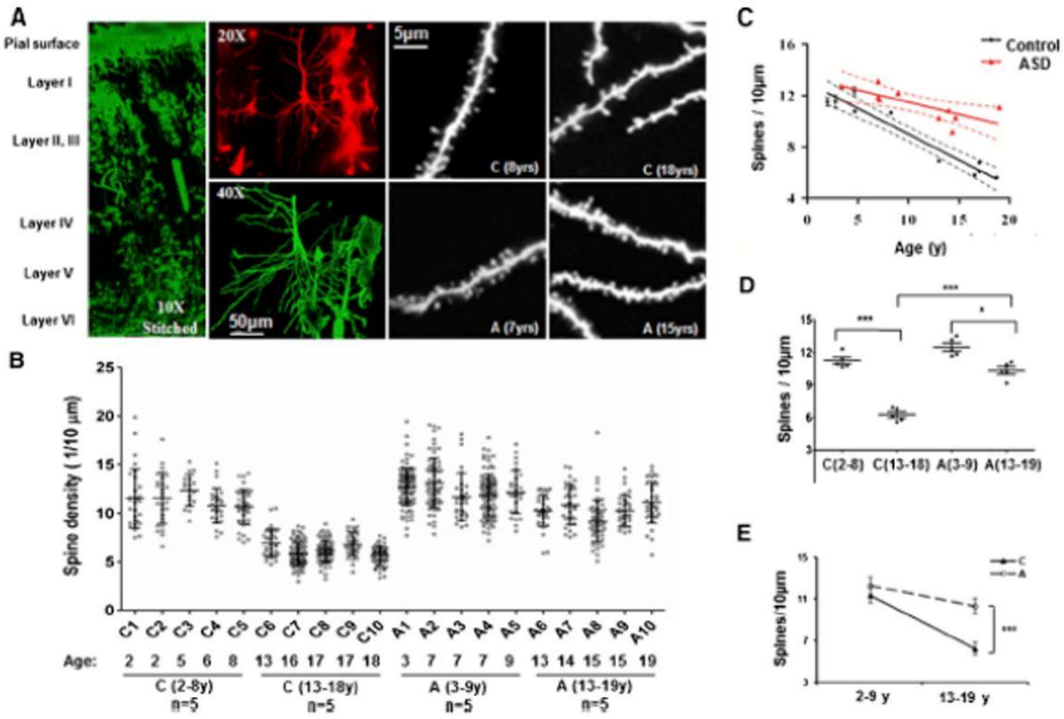


Schumann CM,
J Neurosci 2010

Loss of mTOR-Dependent Macroautophagy Causes Autistic-like Synaptic Pruning Deficits

Guomei Tang,[1] Kathryn Gudsnuk,[2] Sheng-Ha, Kuo,[1] Marisa L. Cotrina,[3,7] Gorazd Rosoldija,[4,8] Alexander Sosunov,[3] Mark S. Sonders,1 Ellen Kanter,[1] Candace Castagna,[1] Ai Yamanoto,[1] Zhenyu Yue,[6] Ottavio Arancio,[3] Bradley S. Peterson,[4,8] Frances Champagne,[2] Andrew J. Dwork,[3,4,8] James Goldman,[3] and David Sulzer[1,4,5,8,*]

Neuon *83*, 1131-1143, September 3, 2014

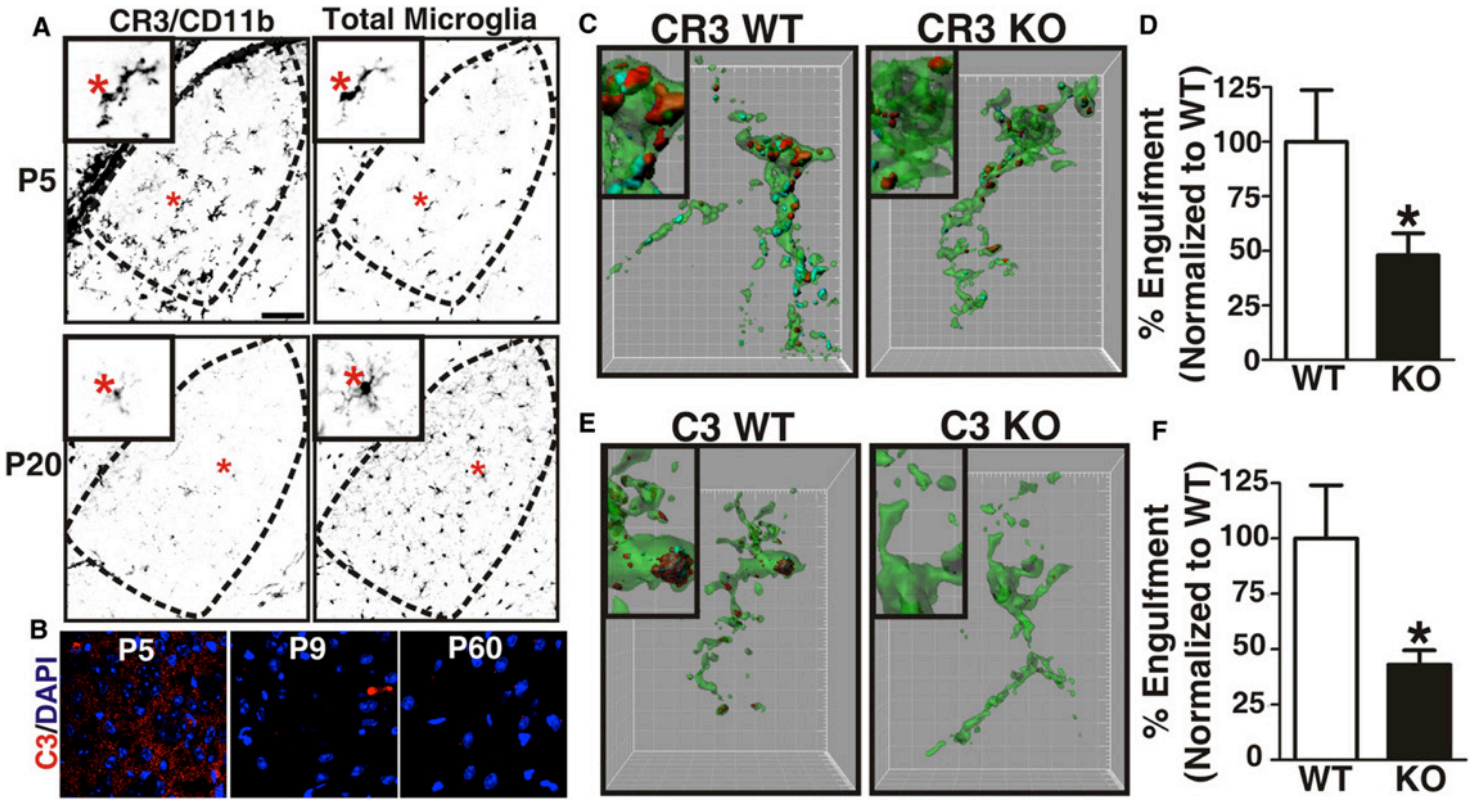


Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner

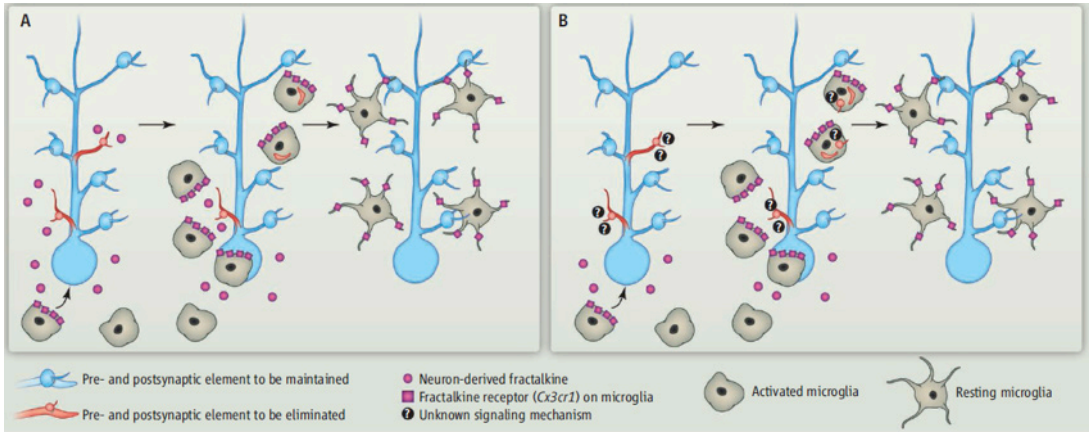
Dorothy P. Schafer,[1] Emily K. Lehrman,[1,5] Amanda G. Kautzman,[1,5] Ryuta Koyama,[1] Alan R. Mardinly,[3] Ryo Yamasaki,[4] Richard M. Ransohoff,[4] Michael E. Greenberg,[3] Ben A. Barres,[2] and Beth Stevens[1,*]

[1]Dapartment of Neunrology, F.M. Kirby Neurobiology Center, Children's Hospital, Harvard Medical School, Boston. MA 02115, USA
[2]Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA
[3]Department of Neurobiology, Harvard Medical School. Boston. MA 02115, USA
[4]Neuroinflammation Research Center, Department of Neurosciences, Lemer Research Institute and Mellen Center for MS Treatment and Research. Neurological Institute, Cleveland Clinic, Cleveland. OH 44195, USA

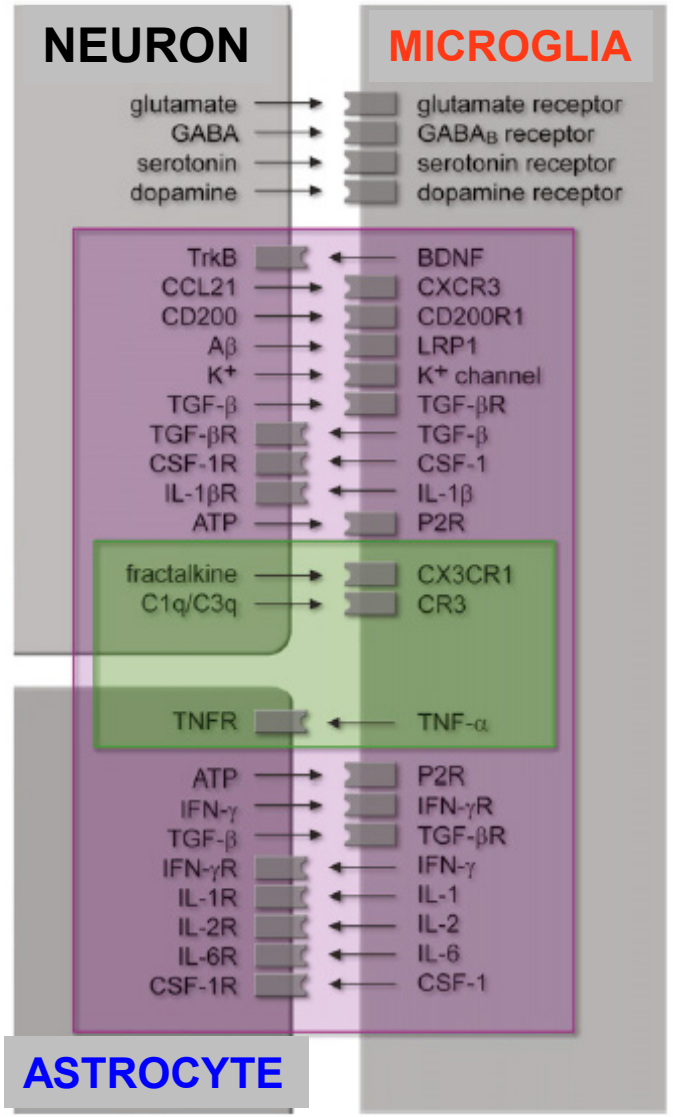
Neuron 74: 691-705, 2012



Microglia is a critical cell from the innate immune system for modeling neuronal networks and circuits during development and adaptive brain plasticity

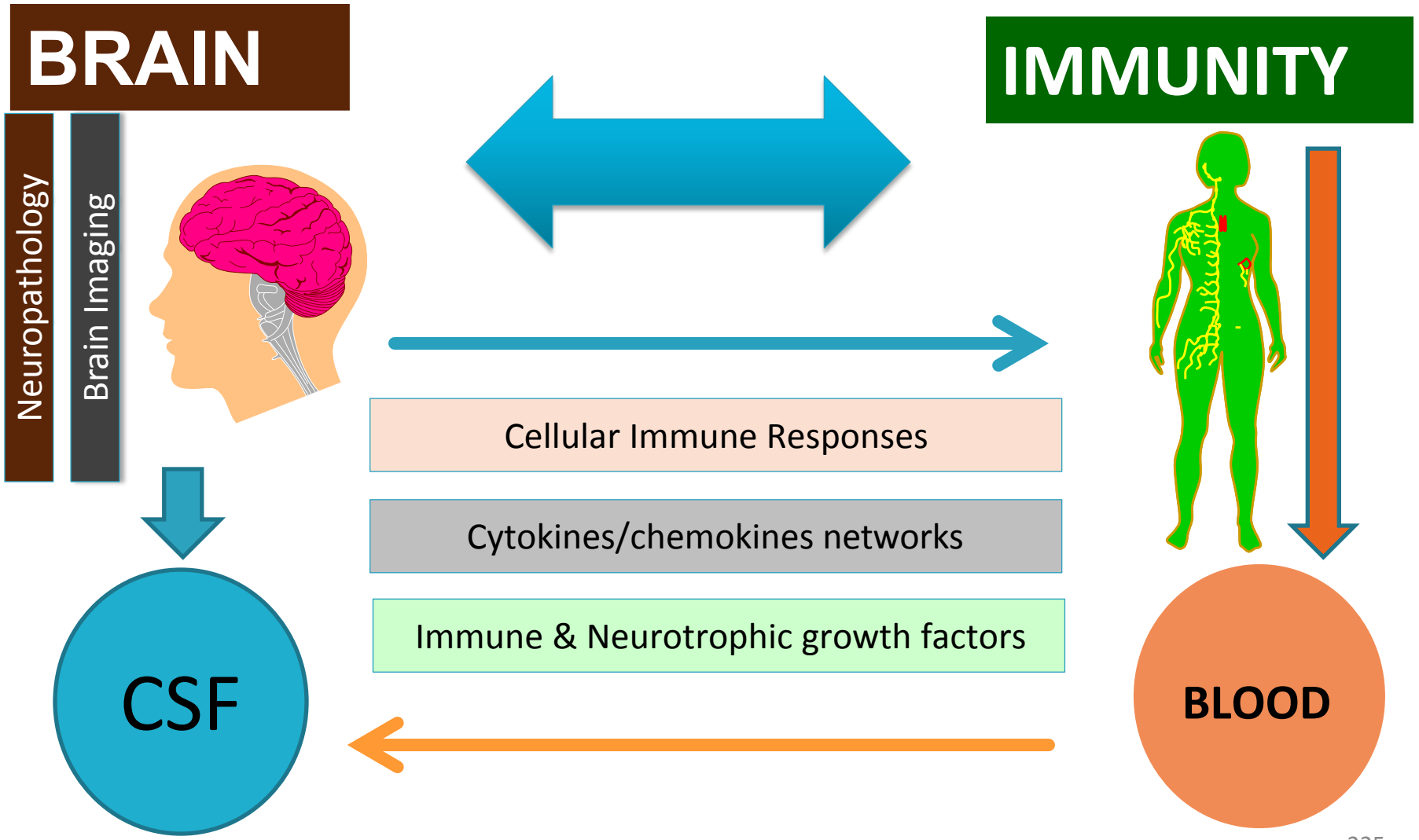


How Many Cell Types Does It Take to Wire a Brain?
Richard M. Ransohoff and Beth Stevens
Science 333, 1391 (2011);



Neuronal-Microglia-Astrocyte Interactions. Kettenmann H, Neuron 2103

HOW TO STUDY NEURAL AND IMMUNE INTERACTIONS IN ASD?

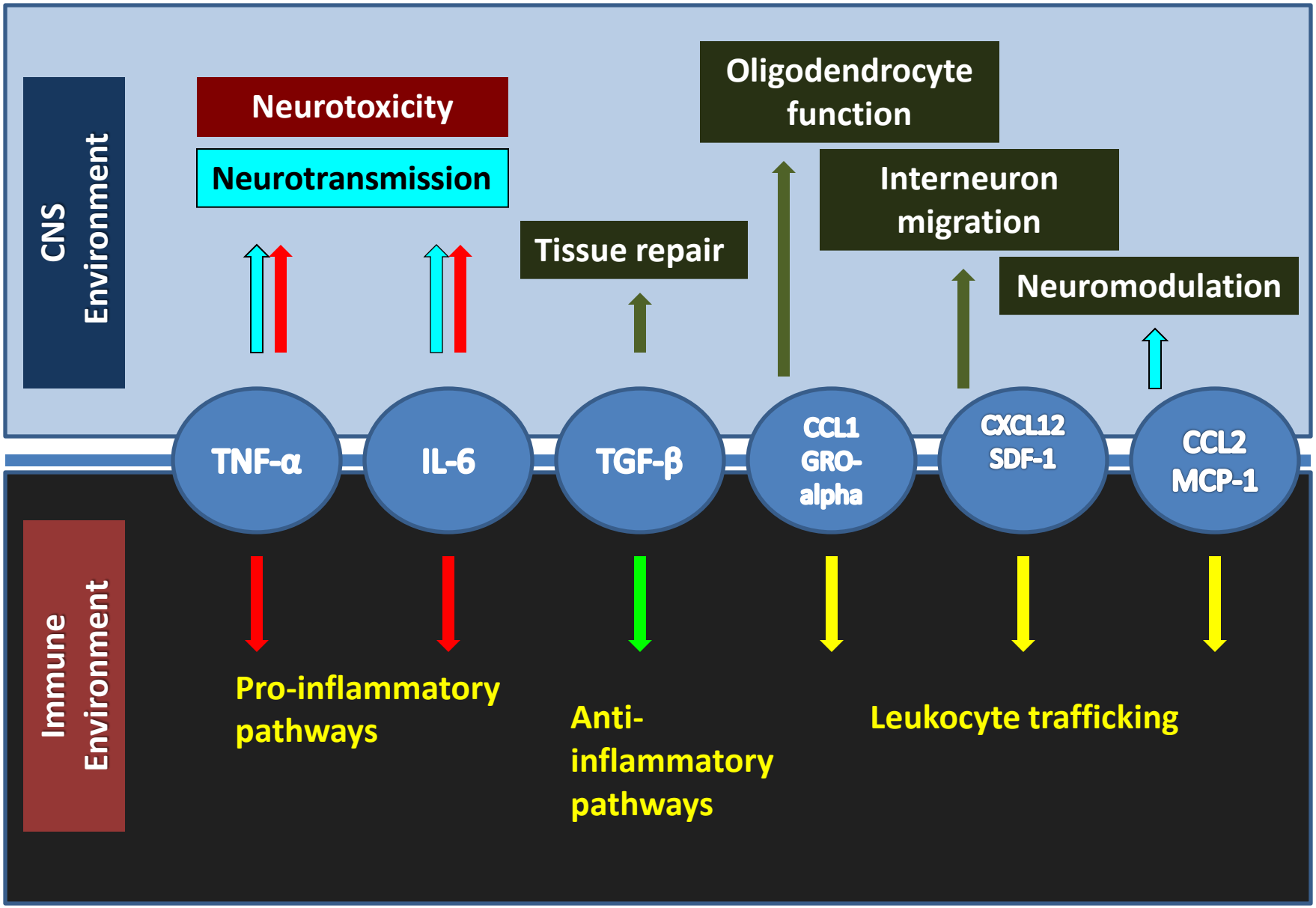


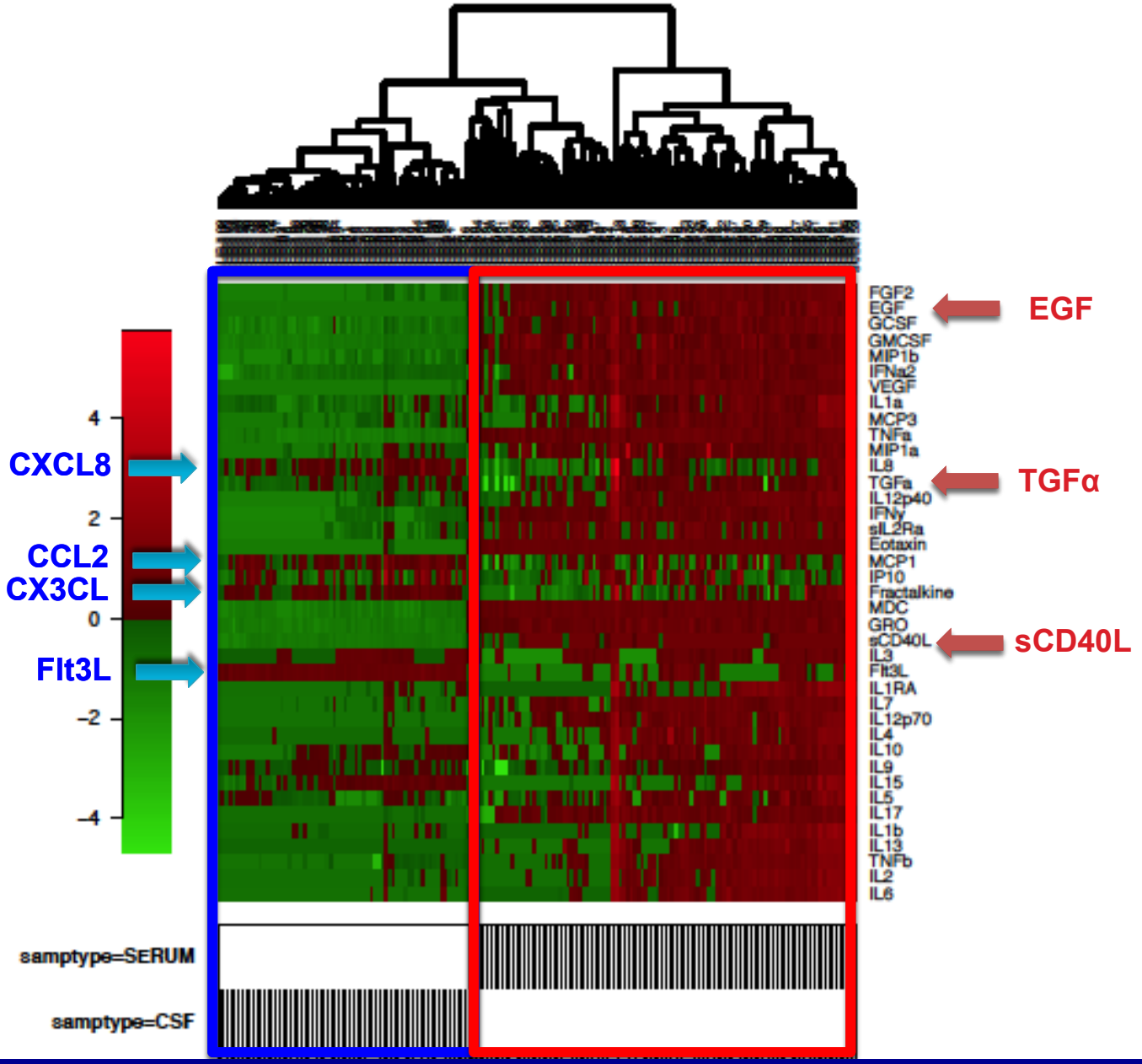
PEDIATRICS + DEVELOPMENTAL
NEUROSCIENCE BRANCH

Autism Intramural Program

S. Swedo, A. Thurm & others

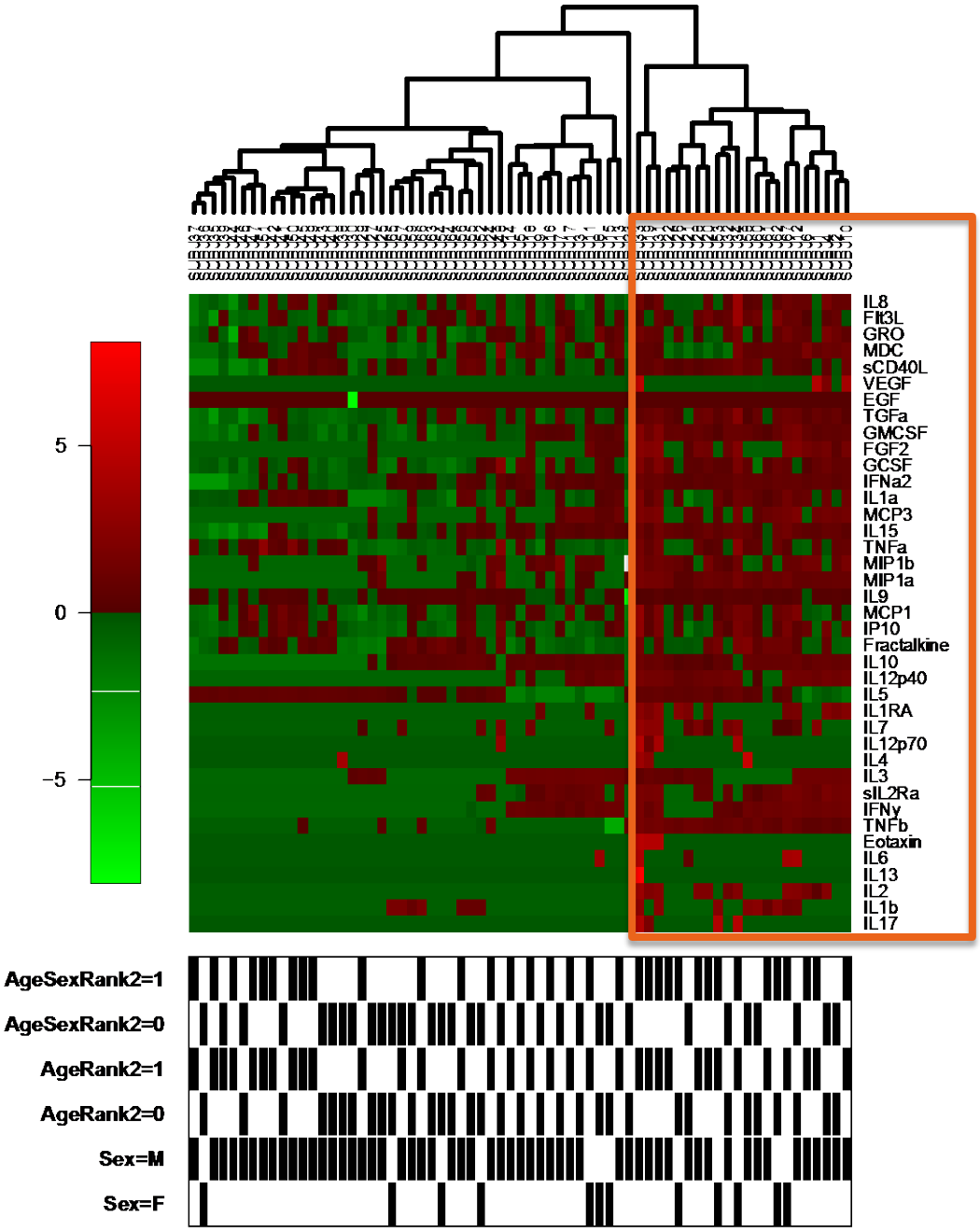
- Prospective study of patients with ASD
  - Non-regressive vs. “regressive”
  - Clinical, behavioral and cognitive assessment
  - Neuroimaging
  - **Blood/CSF studies for evaluation of immune activation**

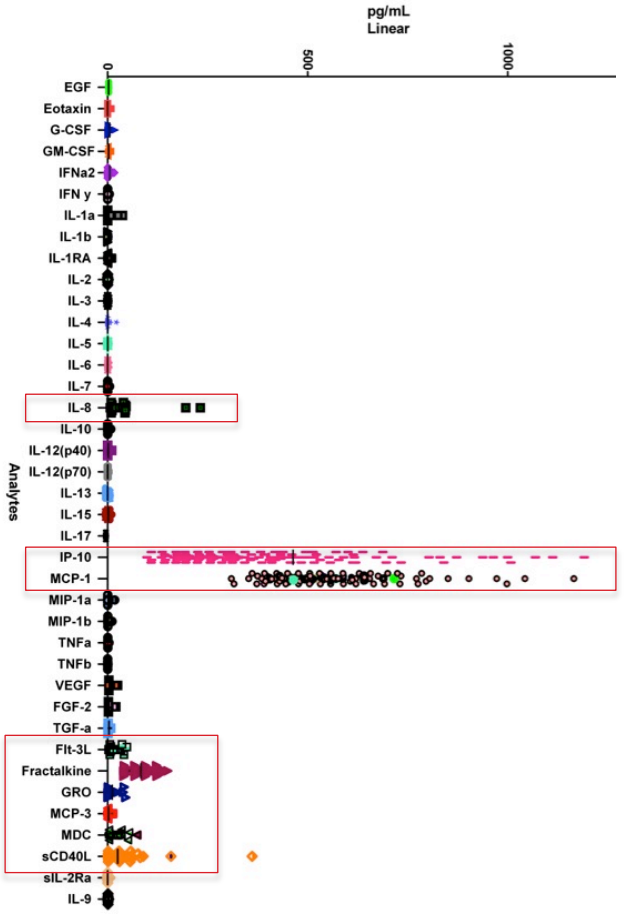

CNS Environment
Neurotoxicity
Neurotransmission
Tissue repair
Oligodendrocyte function
Interneuron migration
Neuromodulation
TNF-α
IL-6
TGF-β
CCL1 GRO-alpha
CXCL12 SDF-1
CCL2 MCP-1
Immune Environment
Pro-inflammatory pathways
Anti-inflammatory pathways
Leukocyte trafficking



NIMH Study of Immunological Factors in Autism:

Profile of cytokines and chemokines in serum & CSF





Prevalence of Immune Related Proteins in the CSF of patients with ASD

Autism and the Immune Response

What we know:

- Various immune system abnormalities have been reported in children with autistic disorders by a number of different laboratories.
- Both enhanced autoimmunity and reduced immune function have been shown.
- Development of ‘autism’ animal models with immune basis

Studies on Plasma cytokines

Study Description	Reference
Elevated levels of IL-1b, IL-6, IL-8 and IL-12p40. Associated with regression	(Ashwood, et al., 2011b)
Increase in chemokine **MCP-1, Rantes** and **Eotaxin** levels in ASD subjects compared to age-matched typically developing controls. An association between increases chemokines levels with aberrant behaviors.	(Ashwood et al., 2011c)
In male ASD subjects, an increase in cytokines **IL-1beta, IL-1RA, IL-5, IL-8, IL-12(p70), IL-13, IL-17** and **GRO-alpha.**	(Suzuki et al., 2011)
Increase in **leptin** levels in ASD subjects compared to age-matched controls.	(Ashwood et al., 2008b)
Increase in **macrophage migration inhibitory factor (MIF)** in ASD subjects compared to age-matched controls.	(Grigorenko et al., 2008)
Decrease in **TGF-beta** in subjects with ASD compared to controls.	(Ashwood et al., 2008a; Okada et al., 2007)
Increase in **IL-12** and **IFN-gamma** in ASD subjects compared to age-matched controls.	(Singh, 1996)

Serum Cytokines/Chemokines

Studies in chronological order	Immune Measure	Behavior Measure
Ashwood et al. (2008)	Low serum levels of TGFβ1	Stereotypy, Irritabilty, Lethargy, and Hyperactivity
Grigorenko et al. (2008)	High serum levels of MIF	Impaired Sociability
Kajizuka et al. (2010)	High serum levels of PDGF	Stereotypy
Ashwood et al. (2011a)	High serum chemokines CCL2/MCP-1, CCL5/RANTES and eotaxin	Lethargy, Stereotypy, Hyperactivity, Impaired Communication and Socialization
Ashwood et al. (2011b)	High serum levels of cytokines IL-1β, IL-6, IL-8 and IL-12p40	Lethargy, Stereotypy, and Hyperactivity, and Impaired Communication

Cytokine/Chemokines- activated cells

Study Description	Reference
In isolated PBMCs stimulated with PHA, increase in **GM-CSF, TNF-alpha** and **IL-13**. A decrease in **IL-12(p40)** in ASD subjects vs. controls.	(Ashwood et al., 2011d)
Stimulation of TLR on monocytes - ASD vs. to age-matched controls. Increase in **IL-1beta, IL-6, TNF-alpha**, with stimulation of TLR2. Increase in **IL-1beta**, with stimulation of TLR4. Decrease in **IL-1beta, IL-6, GMCSF, TNF-alpha** with TLR9.	(Enstrom et al., 2010)
Increase in **IFN-gamma** in NK cells from subjects with ASD.	(Enstrom et al., 2009b)
Increase production of cytokines from Th1 and Th2 cytokines in ASD subjects vs age-matched controls.	(Molloy et al., 2006)
Increase in **IL-12** and **TNF-alpha** in ASD subject with GI symptoms.	(Jyonouchi et al., 2005)
Increase in **IFN-gamma** and **TNF-alpha** in isolated PBMCs from ASD subjects compared to age-matched controls stimulated with LPS.	(Jyonouchi et al., 2002)
Unstimulated whole blood from ASD vs. age-matched controls – increase in **IFN-gamma** and **IL-1RA** with -higher **IL-6** and **TNF-alpha.**	(Croonenberghs et al., 2002)
Unstimulated PBMC- ASD subjects: higher levels of **TNF-alpha, IL-1beta,** and **IL-6** vs. controls. PBMCs stimulated with LPS, PHA and tetanus produced increase levels of **IL-12** and **IL-1beta**.	(Jyonouchi et al., 2002)

Dynamic Cellular Responses

Studies in chronological order	Immune Measure	Behavior Measure
Onore et al. (2009)	PHA induced IL-23 production	Impaired Sociability
Enstrom et al. (2010)	High LPS induced IL-1B production in purified monocytes	Imparied Sociability and Communication
Ashwood et al. (2011)	Higher T lymphocyte production of IFNγ, IL-8, TNFα lower IL-13, IL-10, IL-5	Hyperactivity, Stereoptypy, Lethargy, and Impaired Communication and Sociability
Han et al. (2011)	Higher T lymphocyte, cytotoxic lymphocyte and total lymphocyte numbers	Stereotypy, executive function
Breece et al. (2012)	Increased frequency of myeloid dendritic cells	Stereotypy, Regression, GI symptoms, amygdala volume

Immune dysregulation in ASD- evidence from response to environmental toxicant

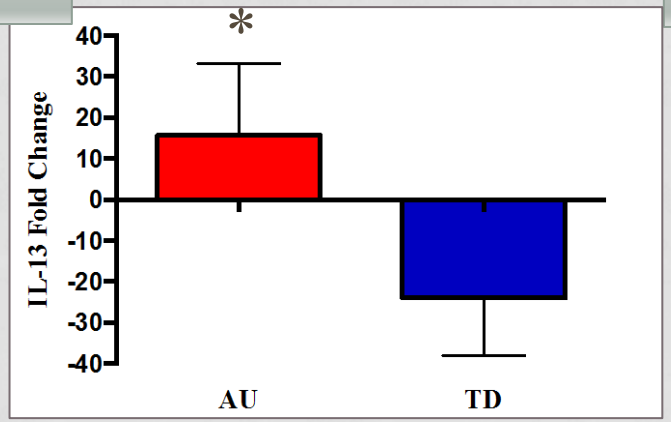
- **Differential Immune response in the presence of the toxicant BDE-49**
- ASD vs. TD Subjects demonstrate opposite response to co-culture with polybrominated diphenyl ether (BDE)-49
- Is this an example of genetic susceptibility for immune dysregulation in the context of environment?

Non-activated immune cells- Differential response to BDE-49

250nM BDE-49

IL-13

Th2 Cytokine



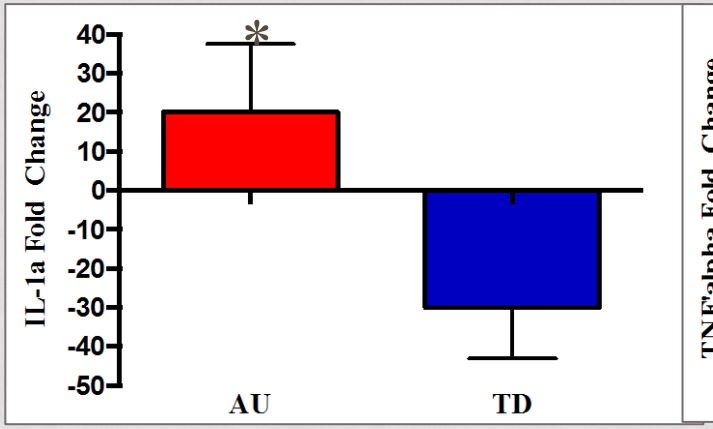
50nM BDE-49

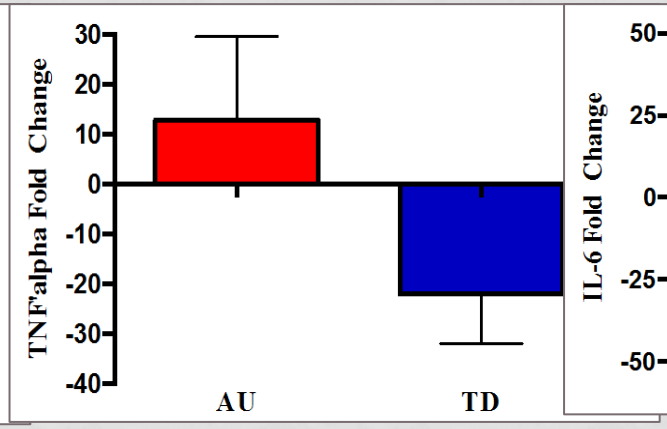
Inflammatory cytokines

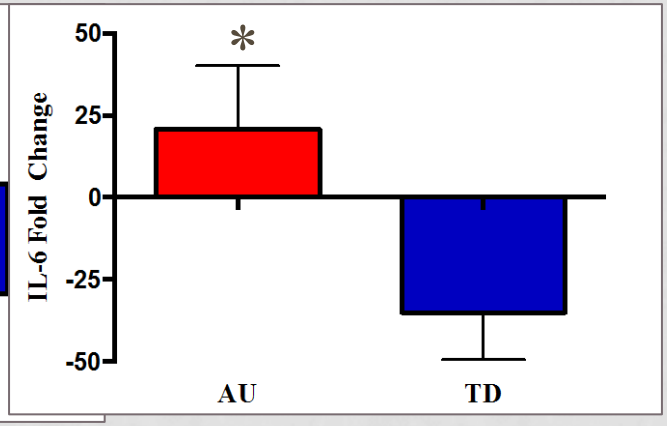
IL-1α

TNFα

IL-6





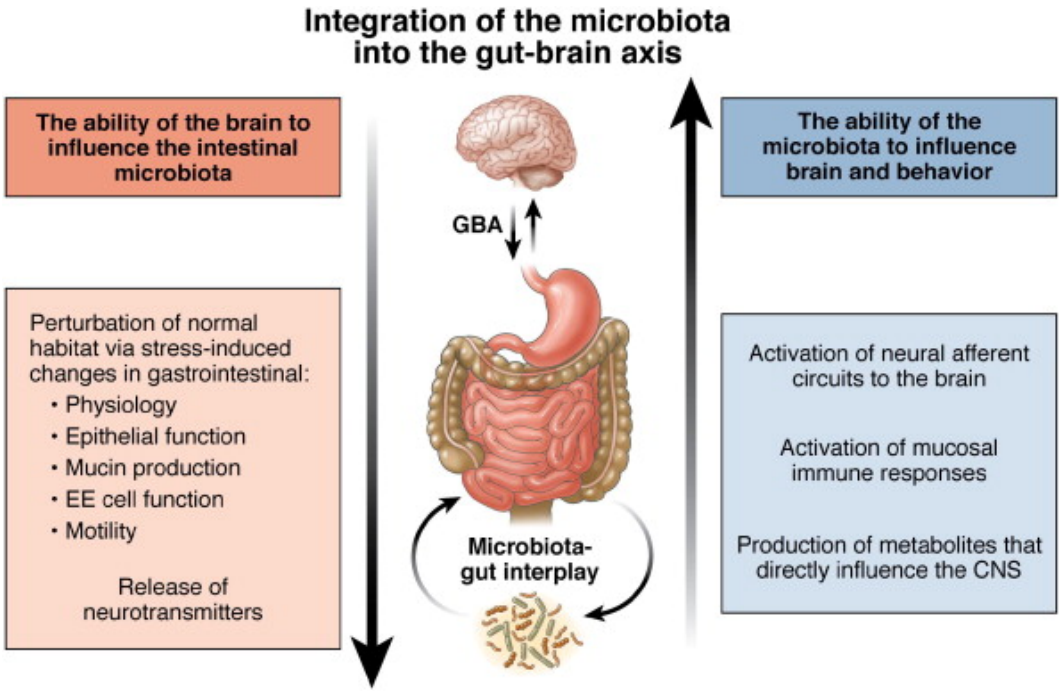


Mean fold change was derived from logn transformed pg/ml cytokine production (Treatment-Baseline/Baseline). (t-test,*p=<0.05)

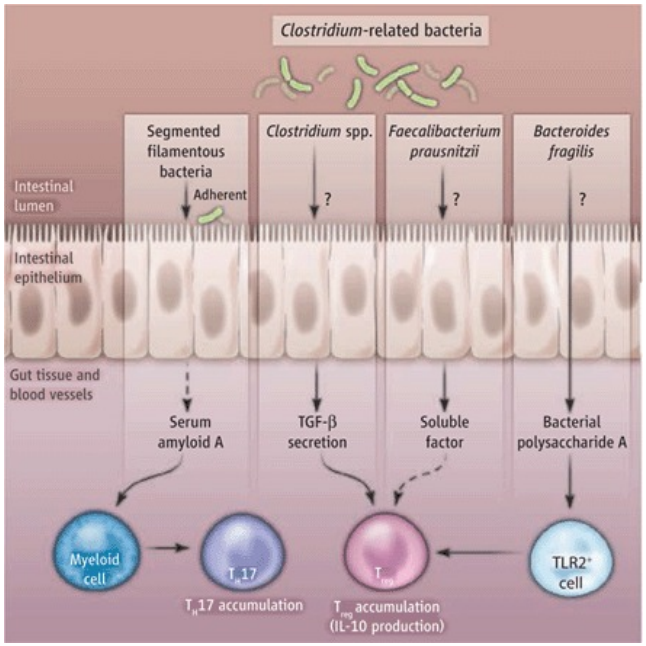
Are there systemic factors such as GI pathology involved in ASD immune abnormalities?

The Gut Microbiota Influence the CNS Function

The Gut Microbiota Modulate the Immune System

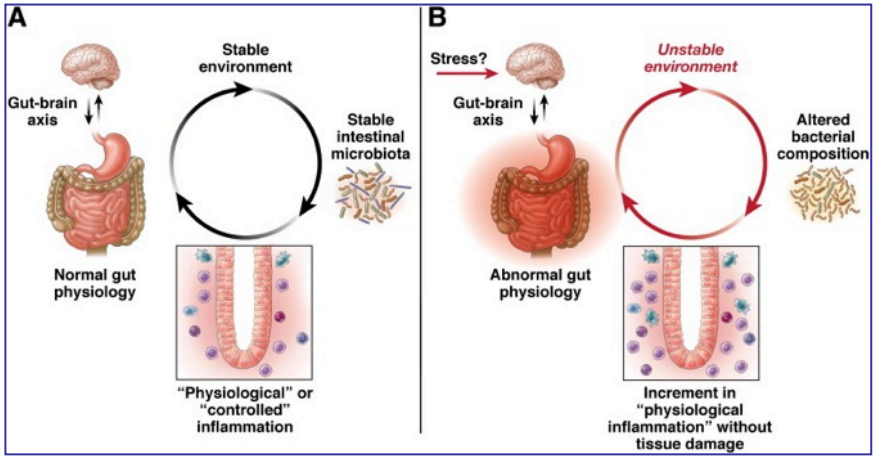


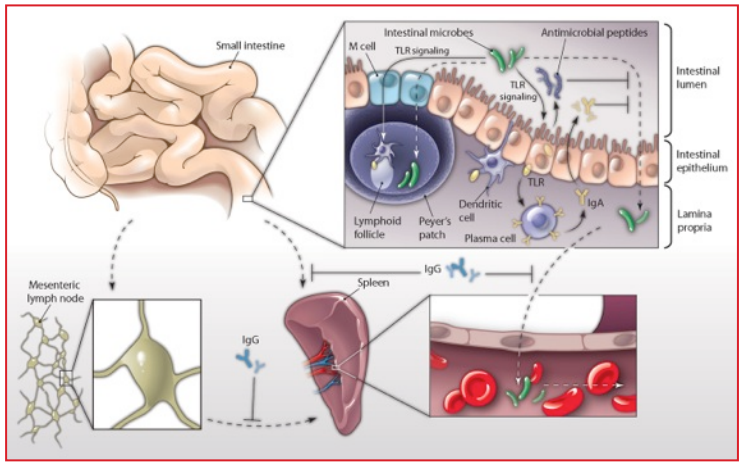
Collins SM & Bercik P.
Gastroenterology136, 2009: 2003-2014



M J Barnes, F Powrie
Science 2011;331:289-290

Collins SM & Bercik P. Gastroenterology 2009: 2003-2014

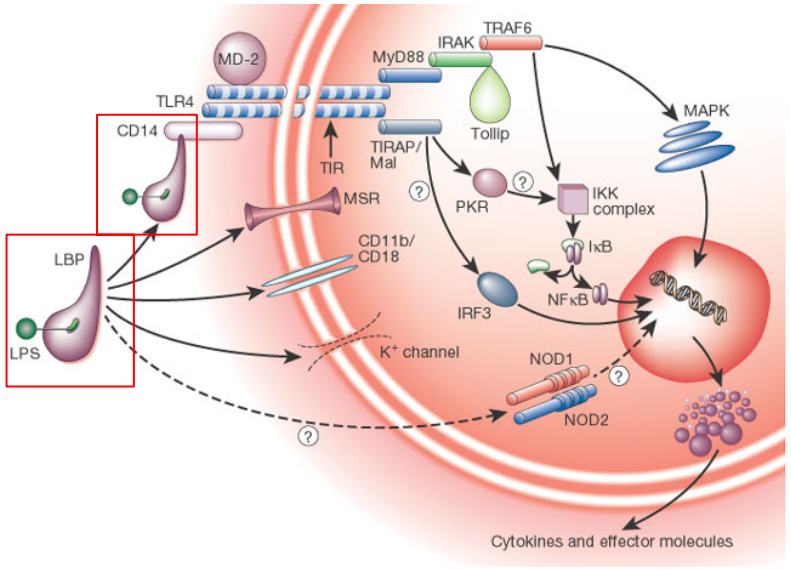




Microbial Translocation:

The innate and adaptive immune systems coordinate containment of intestinal microbes

Lipopolysacharide (LPS), a component of Gram negative bacteria cell walls, **LPS-binding protein (LBP**), and **anti-endotoxin core immunoglobulin** levels have been shown to be reliable biomarkers of MT along with markers of immune activation such as sCD14 and proinflammatory cytokines .



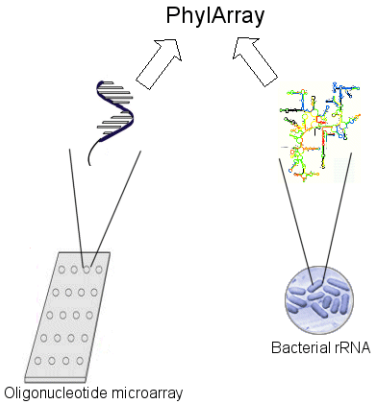
D. A. Hill et al., Sci. Signal. 2, pe77 (2009)

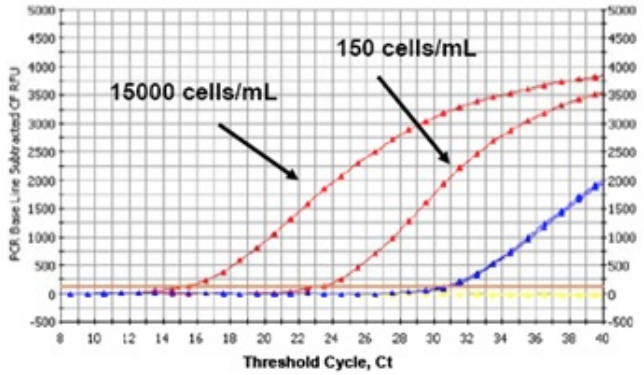
MT markers in ASD

Subjects	LPS (mean rank)	LBP (mean rank)	IgM (mean rank)	IgG (mean rank)
Autism (n=57)	42.56	40.57	45.27	43.40
Typical (n=33)	50.58	51.38	45.89	49.12
P	**0.161**	**0.056**	**0.913**	**0.418**

Subjects	LPS (mean rank)	LBP (mean rank)	IgM (mean rank)	IgG (mean rank)
Autism – Regressors (n=23)	29.46	26.36	29.79	32.84
Autism- Non Regressors (n=34)	28.91	31.57	27.83	23.33
P	**0.974**	**0.240**	**0.661**	**0.034***

Analysis of the 16S rRNA in plasma of ASD vs. control patients





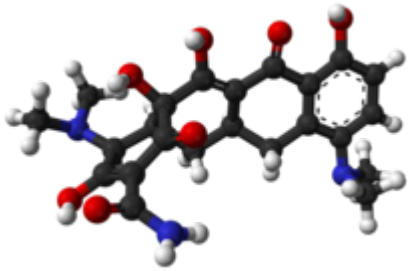
	Group	Mean	St.Dev.	N	Sig
Ct1	Autism	27.87	1.92	55	
	Typical	27.47	1.80	34	.183
Ct2	Autism	27.29	2.01	55	
	Typical	27.00	1.81	34	.366
Ct3	Autism	27.53	1.98	55	
	Typical	27.2	1.61	34	.366

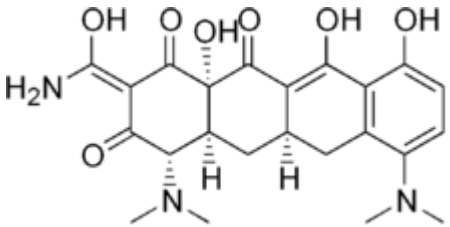
MT in ASD: Conclusions

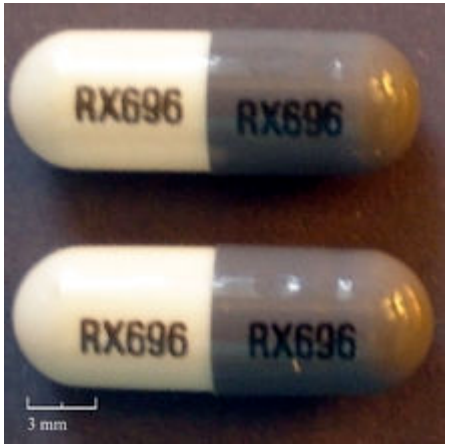
Circulating levels of MT markers, LPS, LPB, or anti-endotoxins, ***did not differ significantly*** between children with autism and age-matched typical controls, nor did a history of regression correspond to evidence of circulating MT markers.

There is not a valid rationale for the empiric use of antibiotics in the treatment of hypothetical "leaky gut" or "occult" infections in children with autism.

How to modify microglial activation?







- **Minocycline hydrochloride**, is a broad spectrum tetracycline antibiotic.
- It is the most lipid-soluble of the tetracycline-class antibiotics with greatest penetration into brain.
- Minocycline has been used in many neurological disorders:

Neuroprotective and anti-human immunodeficiency virus activity of minocycline. Zink MC, et al. JAMA. 2005; 293(16):2003-11.

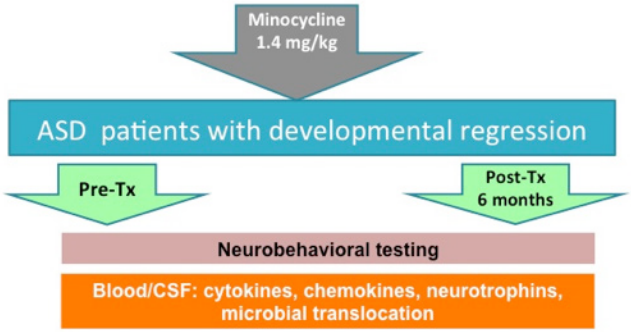
Minocycline attenuates HIV infection and reactivation by suppressing cellular activation in human CD4+ T cells. Szeto GL, et al J Infect Dis. 2010;201:1132-40

Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. Kriz J, Nguyen MD, Julien JP. Neurobiol Dis. 2002 ;10:268-78.

Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis. Gordon PH, et al. Neurology. 2004; 62:1845-7.

Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. Gordon PH, and the Western ALS Study Group. Lancet Neurol. 2007 (12):1045-53.

Pardo er al Journal of Neurodevelopmental Disorders 2013, 5:9 http://www.jneurodevdisorders.com/content/5/1/9



RESEARCH Open Access

A pilot open-label trial of minocycline in patients with autism and regressive features

Carlos A Pardo[1], Ashura Buckley[2], Audrey Thurm[2], Li-Ching Lee[3], Arun Azhagiri[1], David M Neville[2] and Susan E Swedo[2]

Table 1 Patient sample description

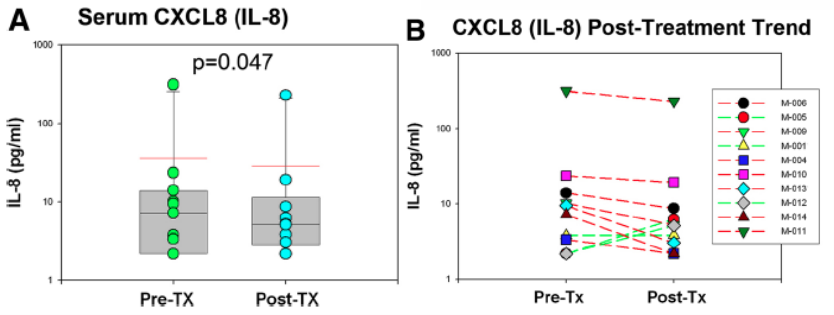
Subject	Pretreatment age (months)	Sex	Age at onset of regression (months)	Clinical assessment				
				Non verbal IQ/DQ	CGI- baseline	CGI- post treatment	Vineland-ABC baseline	Vineland-ABC post-treatment
1	99	F	27	30	6	5	55	54
2[a]	64	M	13	60	5	4[a]	68	73[a]
3	9	M	12	33	5	5	45	44
4	153	M	19	77	5	5	56	55
5	91	M	8	58	3	3	60	56
6	66	M	18	103	4	4	70	71
7	128	F	15	25	5	5	55	46
8	112	M	14	33	5	5	53	56
9	49	M	18	59	4	4	66	63
10	38	M	22	90	4	3	70	66
11	83	M	18	32	5	5	48	57
				M±SD	4.6 ± 08	44 ± 08	58.7 + 88	58.3 ± 93
				Effect Size	-	-03	-	-0.1

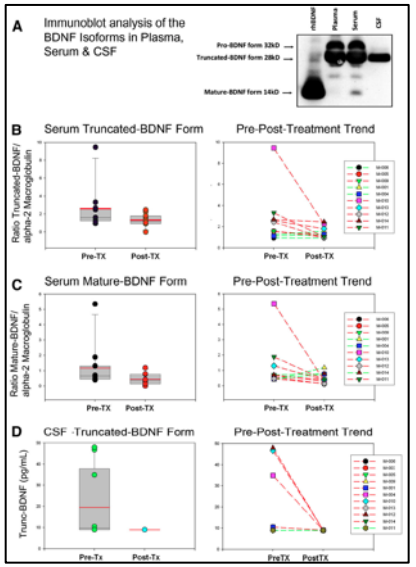
[a]Denotes child that discontinued the study at 3 months.
Note: Effect size was calculated for the within-subjects effect using the following formula: ES = (M_{BL})/SD_{BL}). CGI, Clinical Gtobal Impression.

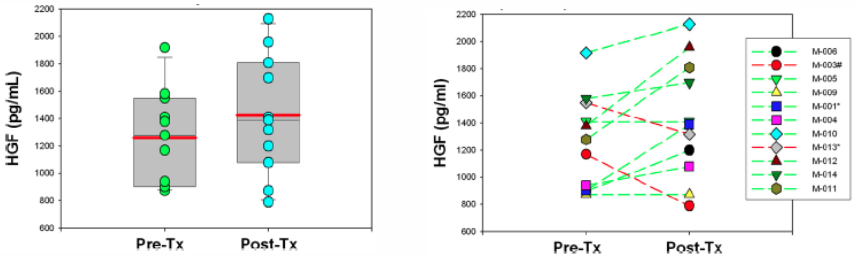
Table 3 Treatment effect on cytokines, chemokines, metalloproteinases and growth factors[a]

Analyte	Cerebrospinal fluid (CSF)		Serum		Plasma	
	Z	P	Z	P	Z	P
TNFα	−0.45	0.66	−1.78	0.074		
IL-6	−0.36	0.72	−1.75	0.08		
CCL2 (MCP-1)	−1.78	0.075	−1.27	0.24		
CCL3 (MIP-1α)	−0.18	0.86	−0.76	0.45		
CCL5 (RANTES)	−0.73	0.47	0.66	0.61		
CXCL8 (IL-8)	−0.45	0.66	−1.99	**0.047**		
BDNF[b]	−2.03	**0.042**			−0.76	0.45
Truncated-BDNF/α-2 M[c]			−2.19	**0.028**		
Mature-BDNF/α-2 M[c]			−0.87	0.386		
CD40L	−0.89	0.37			−1.33	0.18
GDNF	−0.37	0.71			−1.83	0.07
HGF	−2.19	**0.028**			−1.25	0.21
Leptin	−1.48	0.14			−0.27	0.79
MMP-1					−1.07	0.29
MMP-3					−0.15	0.88
MMP-7					−1.89	0.05
MMP-8					−0.05	0.96
MMP-9					−1.38	0.17

[a]Selected analytes, Mann–Whitney *U*-test.
[b]Quantified by Luminex technique. In CSF only the truncated-BDNF form appears to be detectable in immunobotting experiments.
[c]Quantified by immunoblotting and densitometry analysis. Significance was calculated based on ratio BDNF isoform/α-2 M.







Pardo et al. Journal of Neurodevelopmental Disorders 2013, 5:9

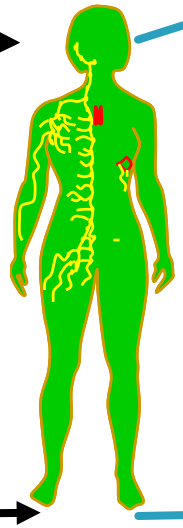
IMMUNOTHERAPIES IN AUTISM: Are these good options??

Minocycline, COX2 inhibitors , NSAIDs

IV Immunoglobulins (IVIG)

Immunomodulators
e.g. Steroids (eg. Prednisone)

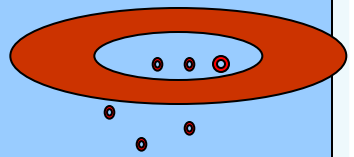
Immunosupressants
e.g. Cyclophosphamide Methotrexate, tacrolymus, etc



CNS:

- **Innate immunity**
  Microglia, astroglia, cytokines, chemokines

BBB



- **Adaptive immunity**
  Specific T cell and antibody responses

Peripheral Immune System

Brain NeuroImmunity

Autism and the Immune Response Overall Conclusions

- Various neuroimmune system abnormalities have been reported in children with autistic disorders:
  - Neuroglial and innate immune responses may reflect homeostatic mechanisms rather than pathogenic immunological reactions
- Maternal antibodies have been demonstrated in subsets of mother of children with autism, a finding that suggest a potential role of maternal-immune mediated mechanisms in neurodevelopmental disarrangements

Autism and the Immune Response Overall Conclusions

- Systemic immune abnormalities such as variable expression of cytokines and chemokines, increase of NK cells and decreased immunoglobulin G levels may reflect a dysfunctional immune response rather than an autoimmune or immunologically pathogenic process.
- This dysregulation may inform as to dysfunction in pathways common to both the immune and nervous systems.
- Gastrointestinal symptoms are not associated with microbial translocation or represent inducers of immune activation.
- **There is no role at this time for the use of immunological therapies, immmunosupressants or antibiotics to "modify" neurological or behavioral abnormalities in autism.**

Panel 4: Metabolic and Immune Disorders

3:15 – 3:30 **Robert K. Naviaux, M.D., Ph.D., M.S.**
Professor
Departments of Medicine, Pediatrics, and Pathology
Co-Director, Mitochondrial and Metabolic Disease Center
Director of Metabolomics Core, Veterans Affairs
Center for Excellence in Stress and Mental Health
School of Medicine
University of California, San Diego

3:30 Discussion

Emerging Patterns of Metabolic Disturbance in Autism Spectrum Disorders

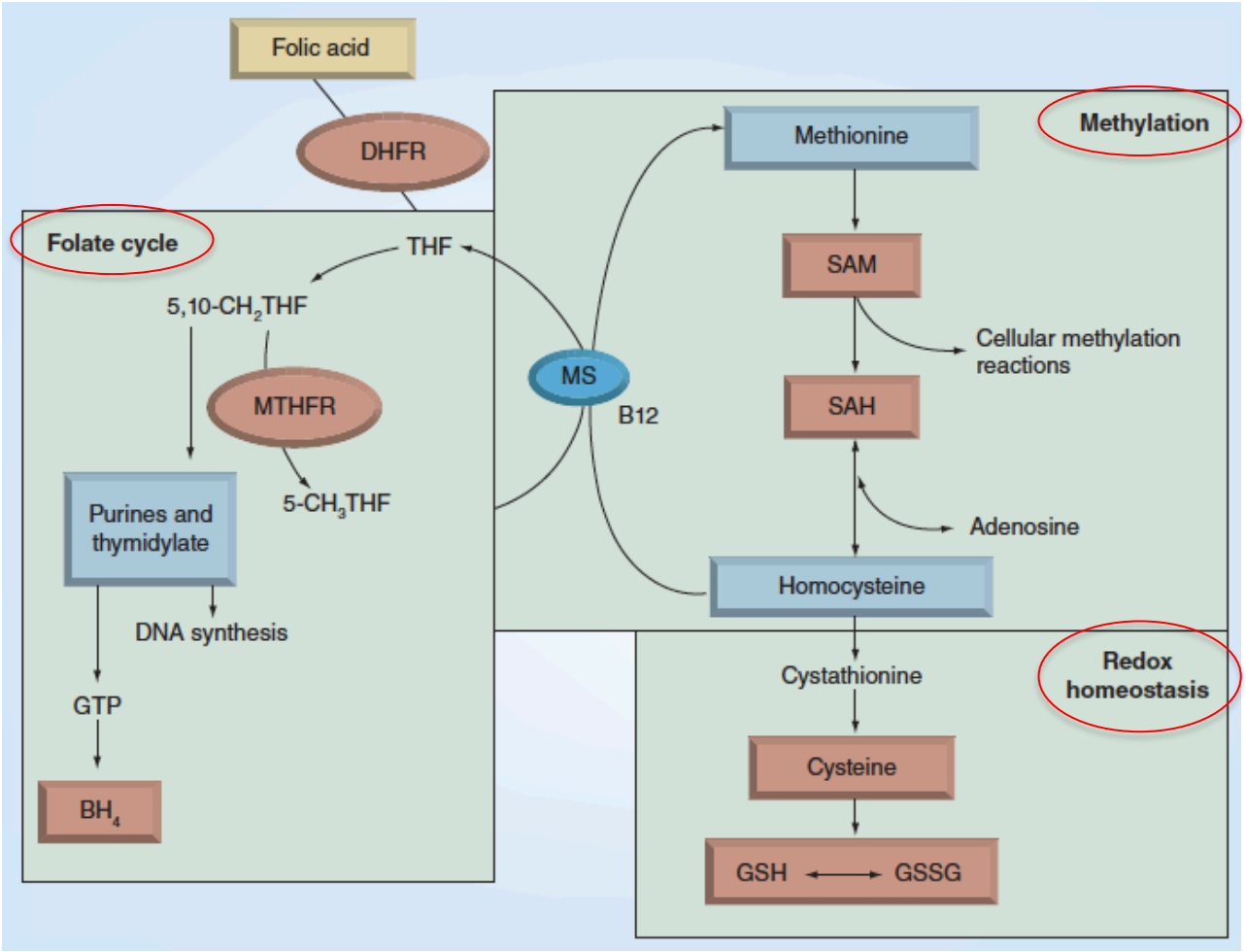
Robert K. Naviaux, MD, PhD
Professor of Genetics, Medicine, Pediatrics, and Pathology
Co-Director, The Mitochondrial and Metabolic Disease Center University of California, San Diego School of Medicine
September 23, 2014

Summary

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

Message from Dr. Jill James

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



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

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

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

Richard E. Frye,[1] Stepan Melnyh,[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 James1

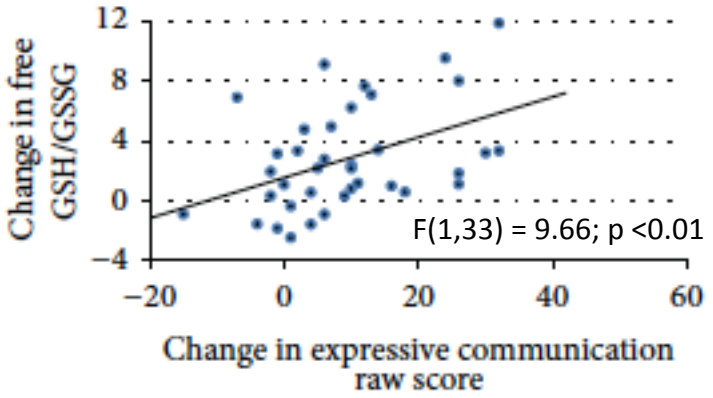
TABLE 2; Age equivalent scores from the Vineland Adaptive Behavior Scales at baseline before and after 3 month intervention with methylcobalamin and folinic acid. The change In age equivalent scores with 95% confidence Interval (CI) is also given. The overall average of all subscales is also given in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

Vineland subscale	Baseline age equivalent (months) (mean ± SE)	Postintervention age equivalent (months) (mean ± SE)	Change in age equivalent (months) (mean; 93% Cl)
Receptive language	33.1 ± 1.8	31.4 ± 3.4	8.3 (3.9. 13.7)
Expressive language	30.6 ± 1.9	37.5 ± 3.9	6.0 (3.3, 9.4)
Written language	40.5 + 3.8	46.7 ± 4.0	6.3 (3.4, 9.0)
Personal skills	30.5 ± 3.3	40.3 ± 3.8	10.0 (3.8,16.3)
Domestic skills	30.3 ± 4.1	39.3 ± 5.9	9.0 (-1.4,19.4)
Community skills	33.9 ± 3.9	36.1 ± 3.8	3.0 (-3.0,6.9)
Interpersonal skills	18.7 ± 3.7	34.1 ± 3.9	5.4 (0.0.10.9)
Play/leisure skills	33.0 ± 4.3	34.0 ± 4.1	12.0 (4.1.19.6)
Coping skills	25.8 ± 2.5	34.3 ± 4.0	11.5 (4.9,13.0)
Overall skills	36.6 ± 3.3	34.3 ± 3.6	7.7(3 4. 12.0)

TABLE 1

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

	Control children (n=22)	Autistic children (n = 20)
Methionine (µmol/L)	31.3 ±5.7 (23-48)	19.3 ± 9.7 (15-25)[2]
SAM (nmol/L)	96.9 ± 12 (77-127)	75.8 ± 16.2 (68-100)[3]
SAH (nmol/L)	19.4 ± 3.4 (16-27)	28.9 ± 7.2 (14-41)[2]
SAM: SAH	5.2 ± 1.3 (4-8)	2.9 ± 0.8.(2-4)[2]
Adenosine (µmol/L)	0.27 ± 0.1 (0.1-0.4)	0.39 ± 0.2 (0.17-0.83)[4]
Homocysteine (µmol/L)	6 4 ± 1.3 (4.3-9.0)	5.S ± 10 (4.0-5.8)[3]
Cystathionine (µmol/L)	0.17 ± 17 (172-252)	0.14 ± 0.06 (0.04-0.2)[3]
Cysteine (µmol/L)	202 ± 17 (172-252)	163 ± 15 (133-189)[2]
tGSH (µmol/L)	7.6 ± 1.4 (3.8-9.2)	4.1 ± 0.5 (3.3-5.2)[2]
Oxidized glutathione (nmol/L.)	0.32 ± 0.1 (0.11-0.43)	0.55 ± 0.2 (0.29-0.97)[2]
tGSH: GSSG	23.3 ±8.9 (13-49)	8 6 ± 3 3 (4-11)[2]

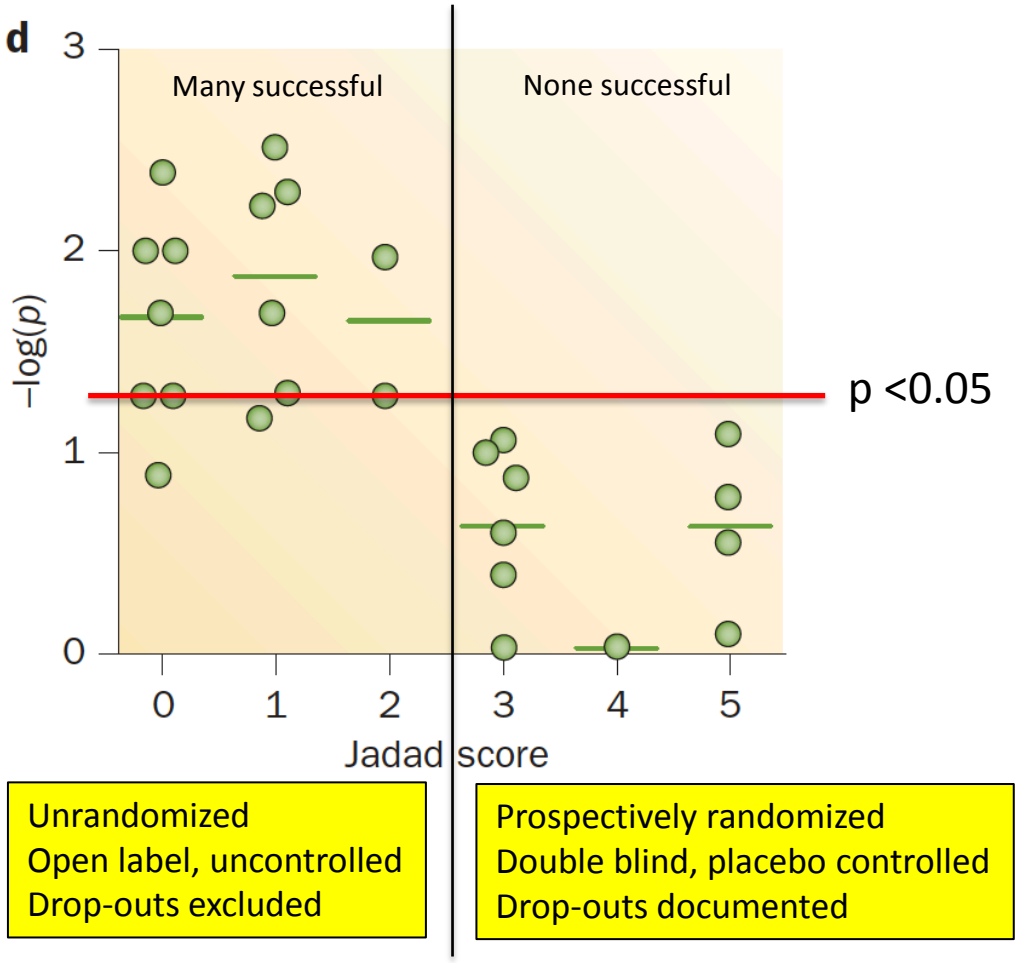


Plasma, not cells

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

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

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



Treatment is Effective

p <0.05 **Conclusion Threshold**

Treatment is Ineffective

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

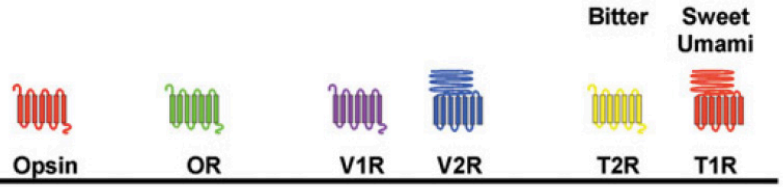
Pfeffer, *et al. Nature Rev Neurol*, 2013. PMID 23817350.

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

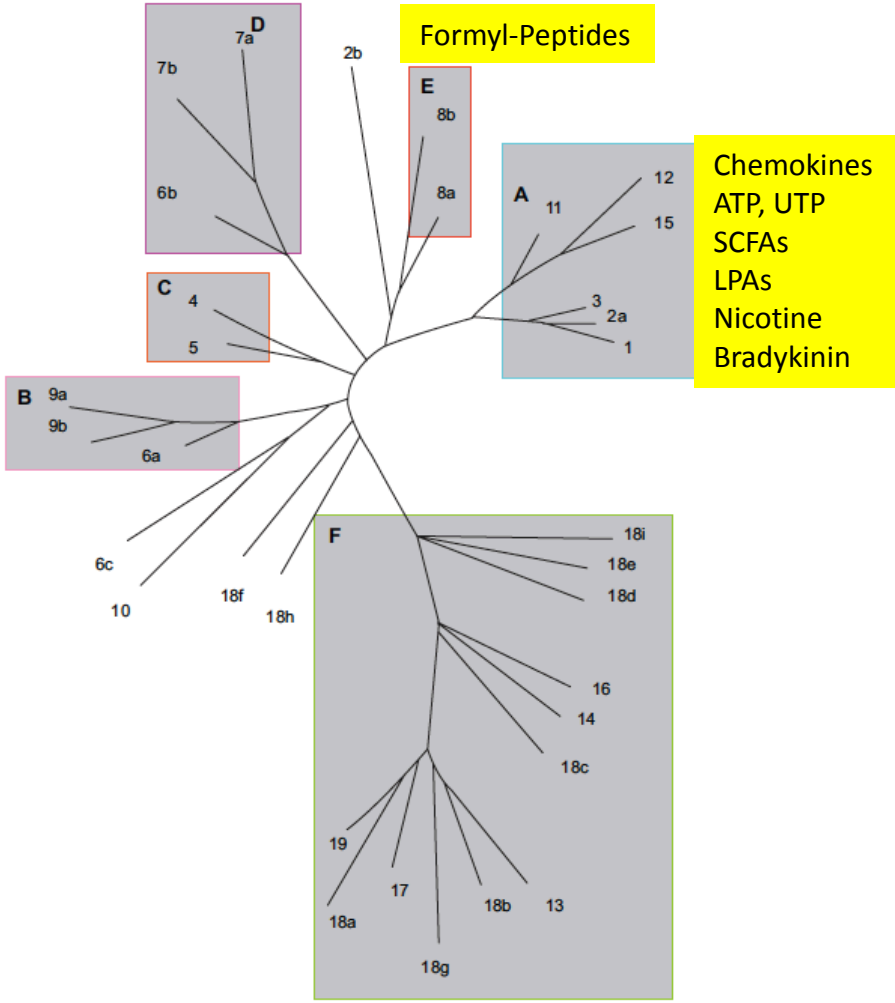
Vertebrate Chemosensory Receptor Evolution

7 Transmembrane GPCRs

Sight | Smell | Pheremones | Taste



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

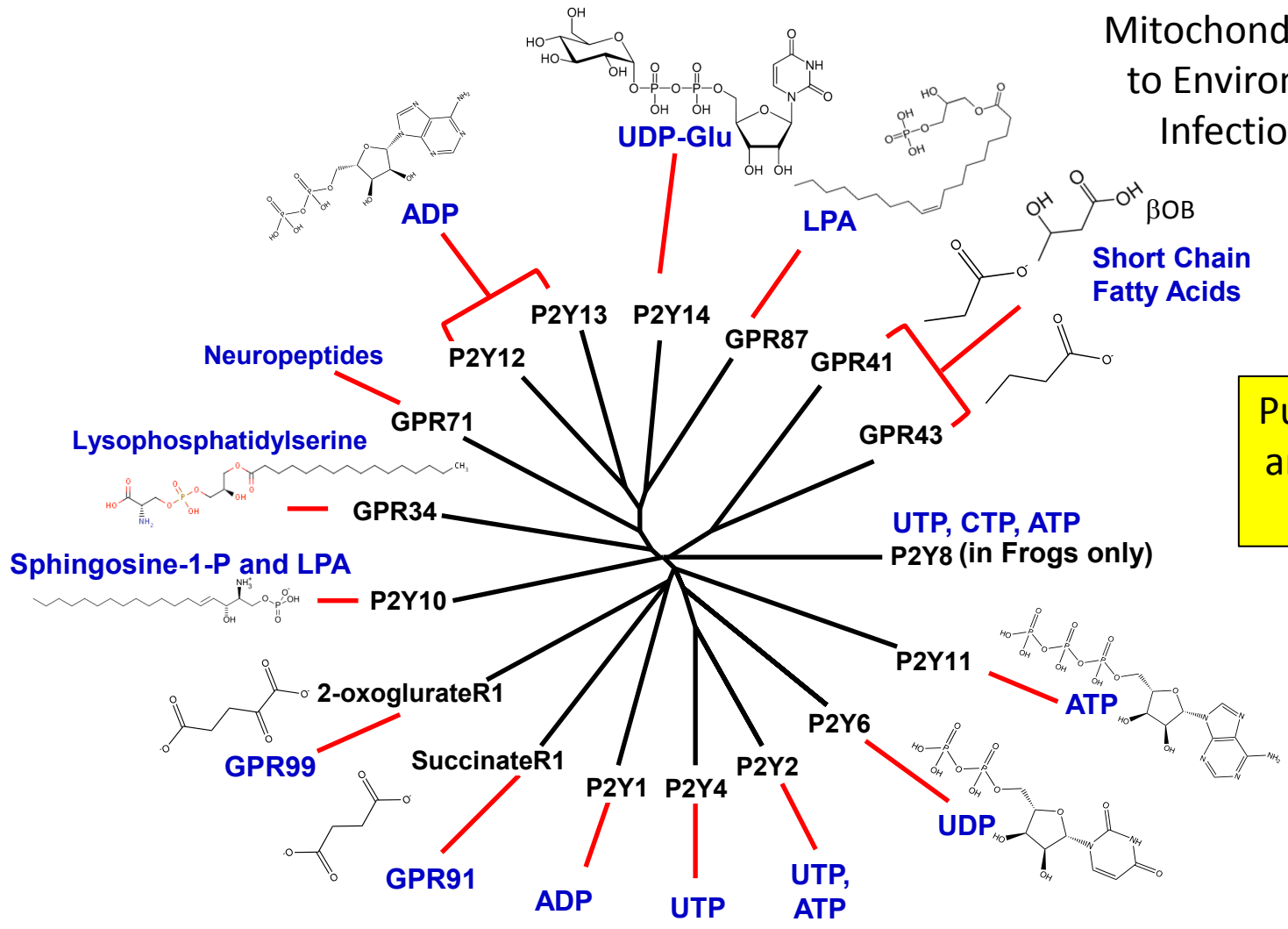


Liman ER. *Adv Exp Med Biol*, 2012. PMID 22399404

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

“Mitokine Receptors” are Like Extranasal Cellular Odorant Receptors—7 Transmembrane GPCRs

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



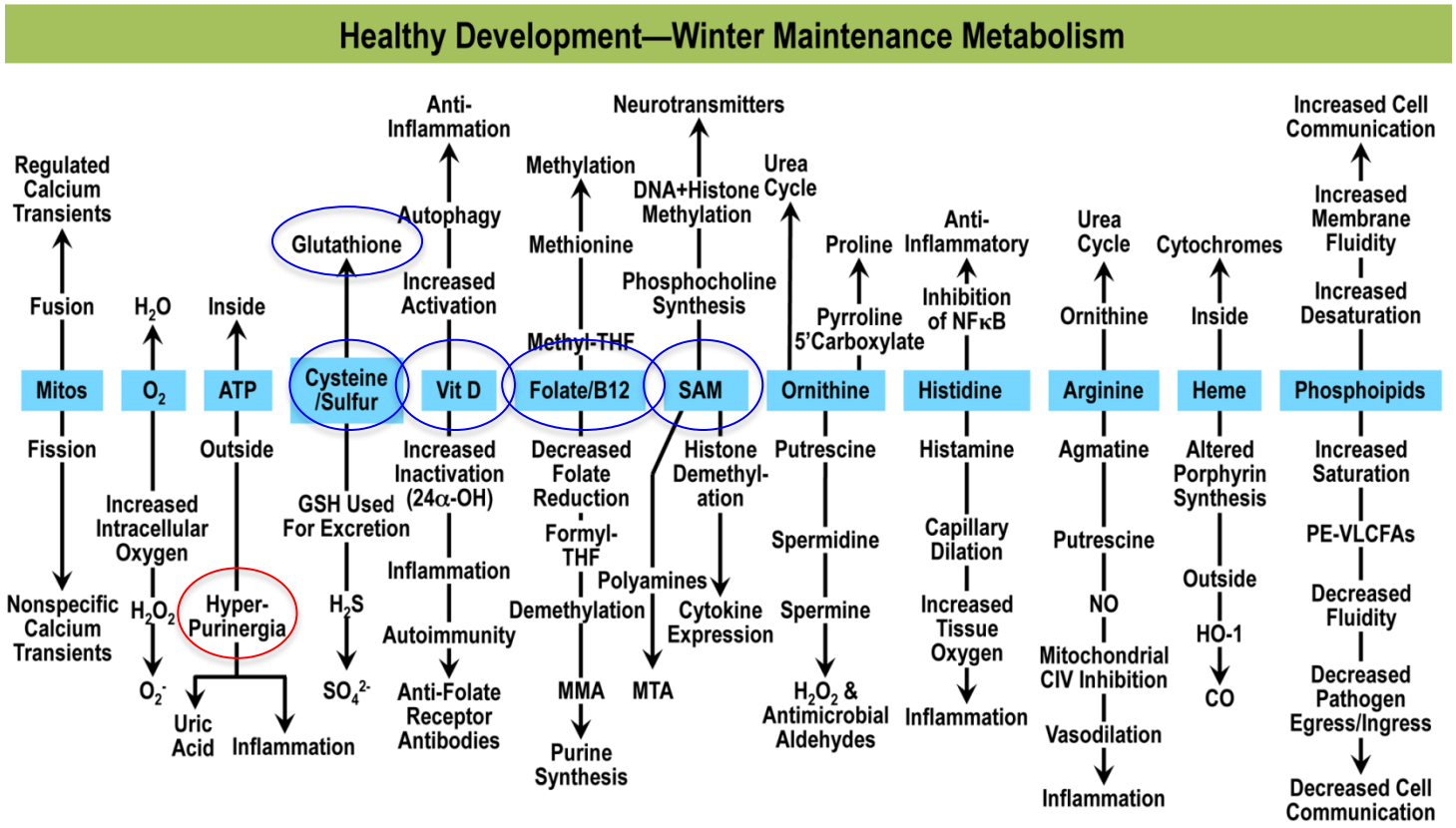
Purinergic Receptors are the most widely expressed class

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

(Scarce Calories)

Autophagy

AMPK/
FGF21



Autophagy

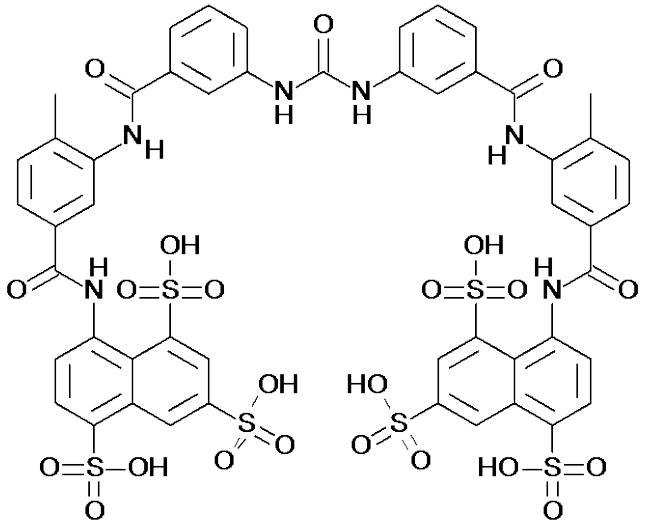
mTOR/
Insulin

(Plentiful Calories)

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

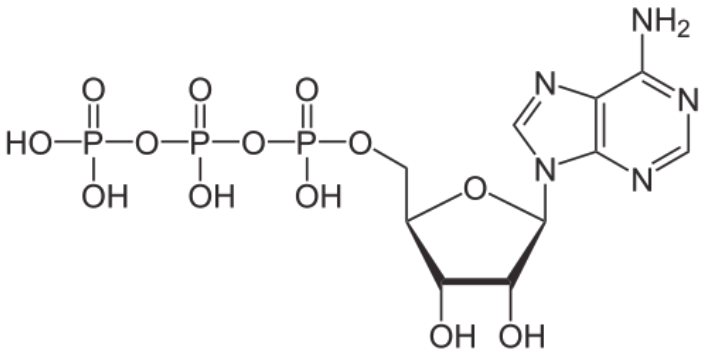
Suramin is a Competitive Antagonist of Purinergic Signaling

a



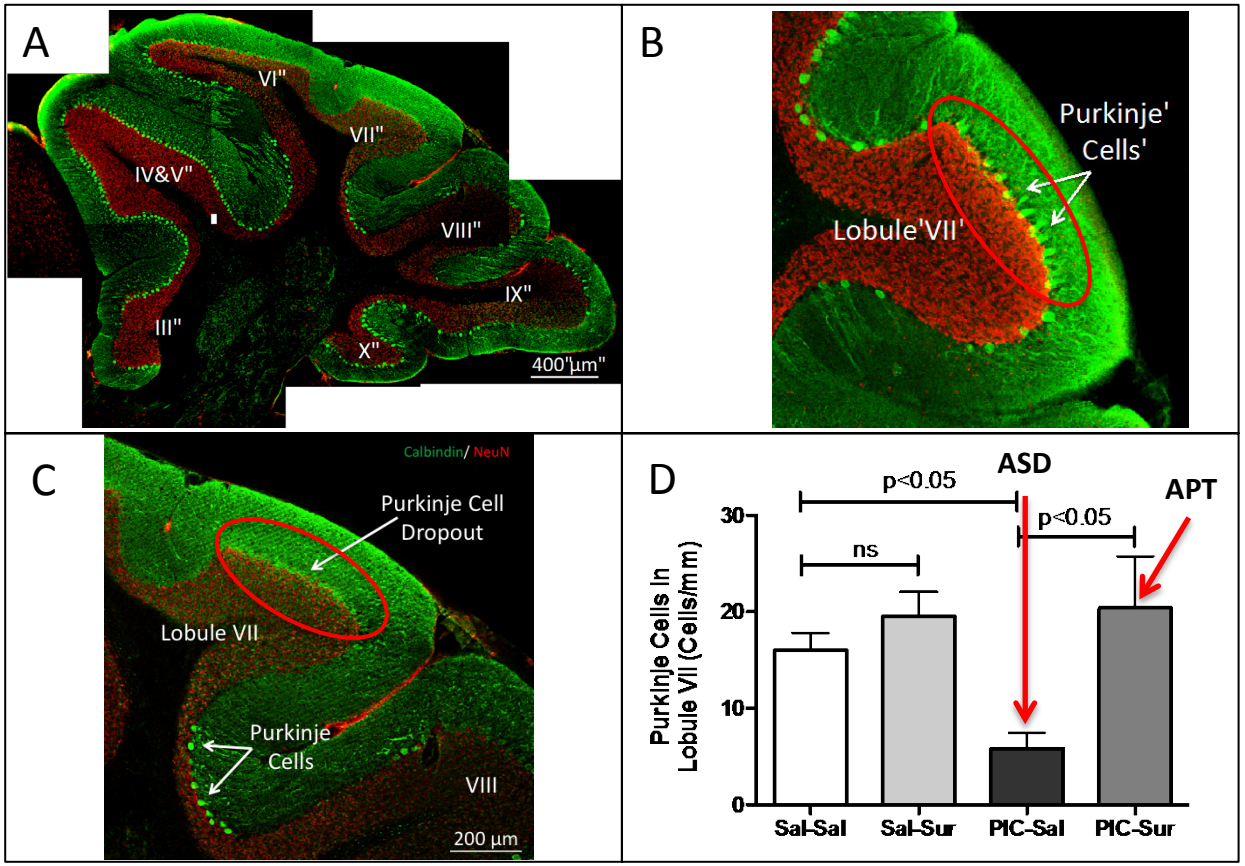
Suramin

b



ATP

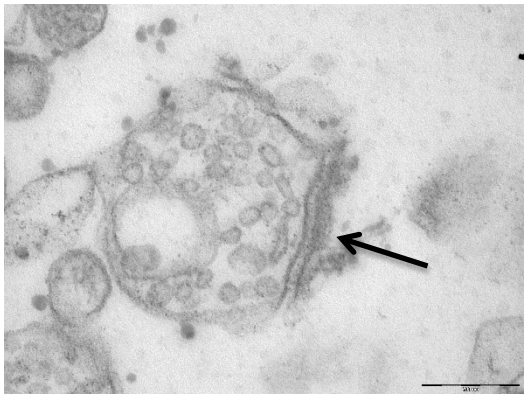
APT Prevented Loss of Cerebellar Purkinje Cells When Started by 2 Months of Age



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

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

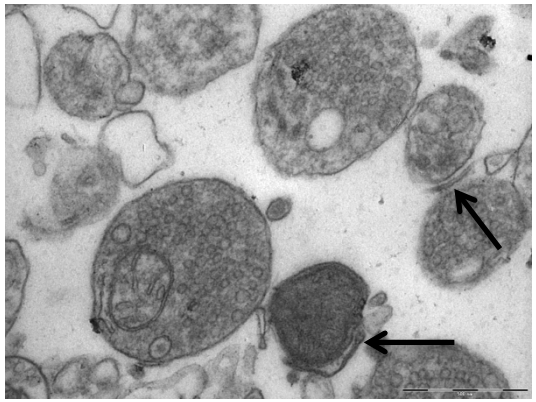
Normal Post-Synaptic Densities



Saline-Saline

Control

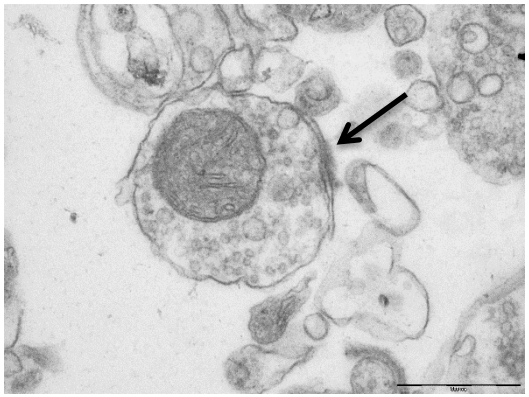
Hypomorphic Post-Synaptic Densities; Electron Dense Matrix Material



Poly(IC)-Saline

ASD

Normalized Post-Synaptic Densities And Matrix



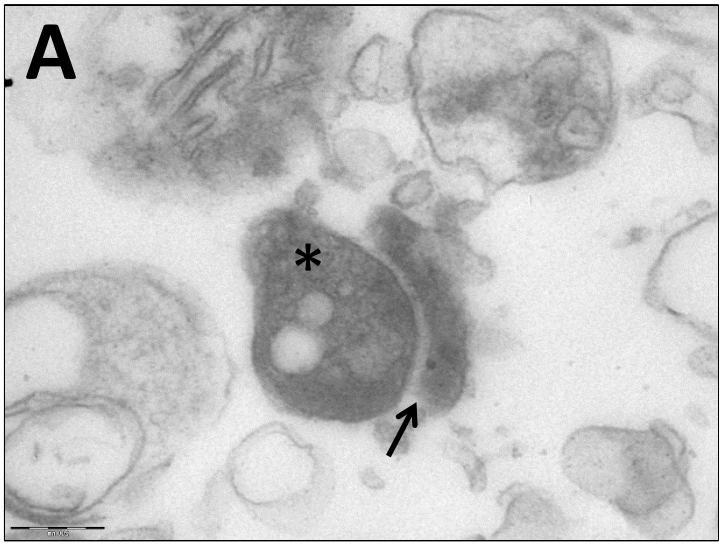
Poly(IC)-Suramin

Treated

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

Hypomorphic
Post-Synaptic Densities;
Electron Dense Matrix

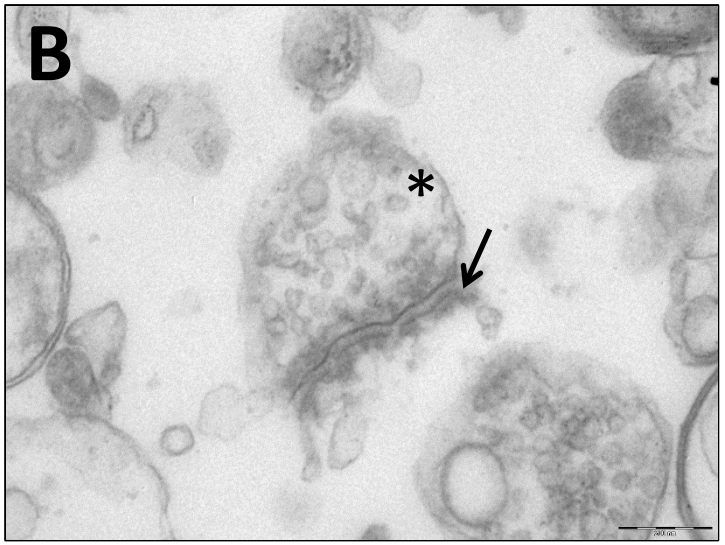
ASD-Like



Fragile X/Saline

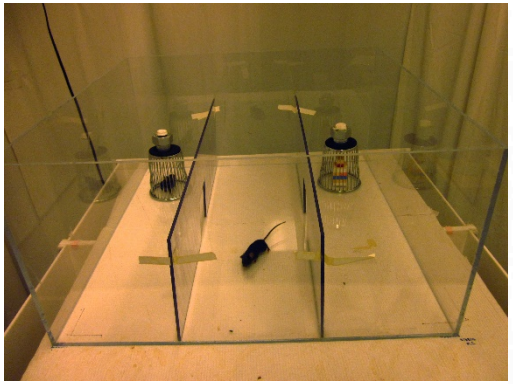
Normalized Post-
Synaptic Densities
And Matrix

Treated

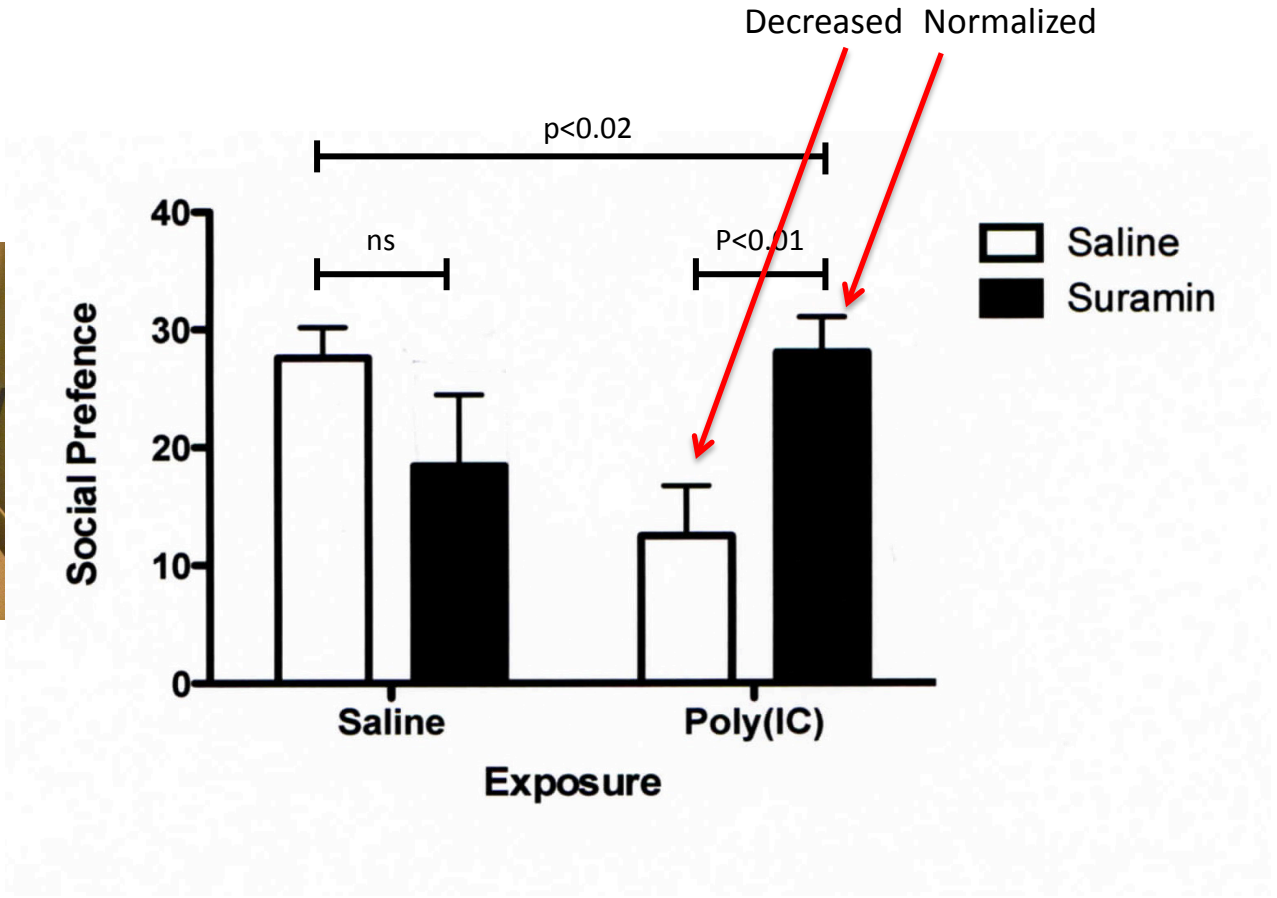


Fragile X/Suramin

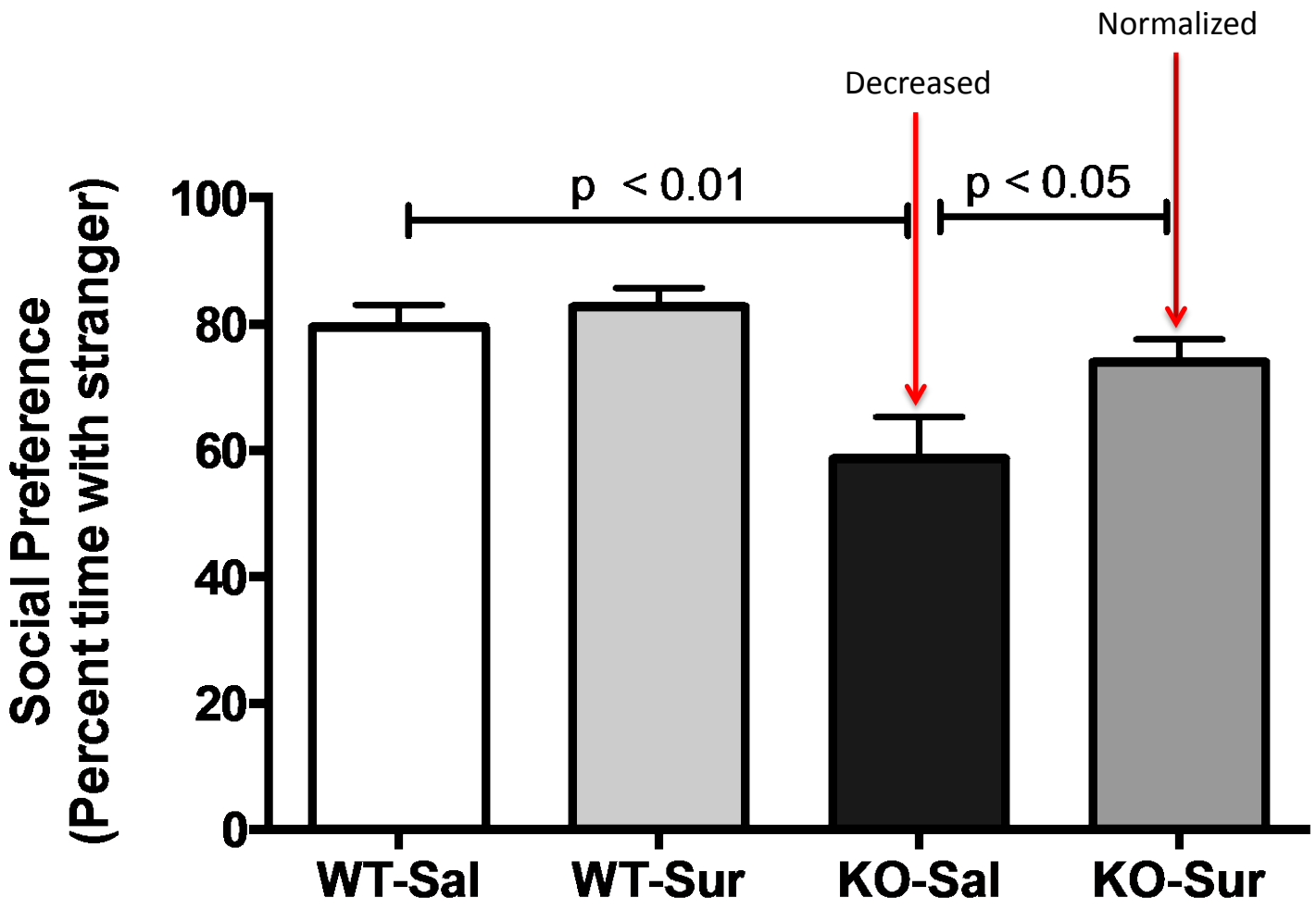
Social Approach Abnormalities in the MIA Model Were Corrected by Antipurinergic Therapy (APT)



Crawley Social Approach Chamber

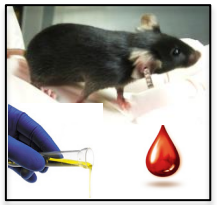


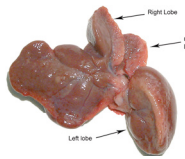
Social Approach Abnormalities in the Fragile X Model Were Corrected by Antipurinergic Therapy (APT)



UCSD Metabolomics

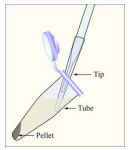
20-100 µl samples of urine and blood





10-50 mg of Liquid Nitrogen Powdered Tissue

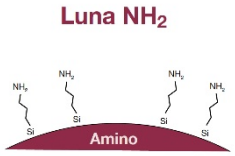
Internal Standards



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

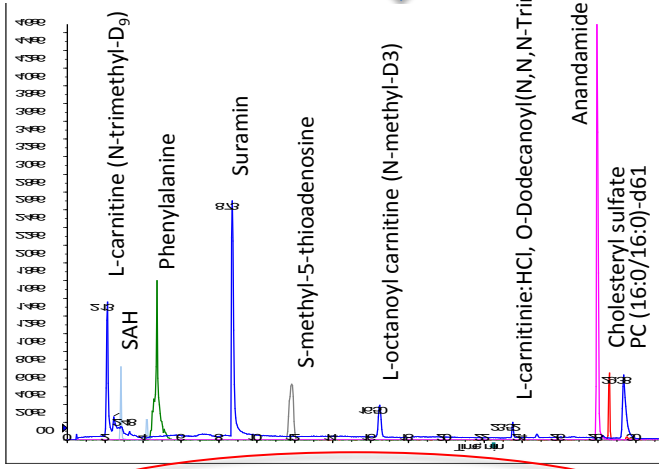
Polar + Nonpolar Metabolites

UHPLC

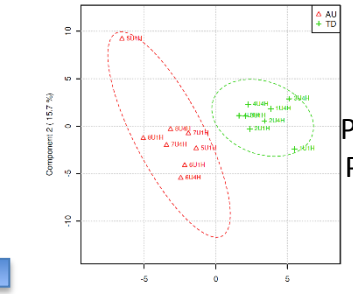


LC-MS/MS

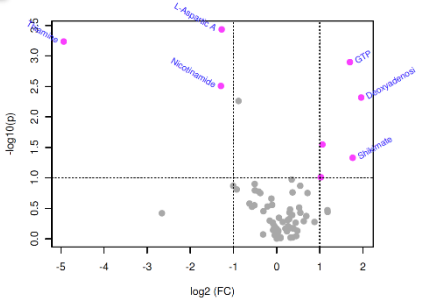
Comprehensive Metabolite Quantitation (Scheduled MRMs in Both Positive and Negative Mode)



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

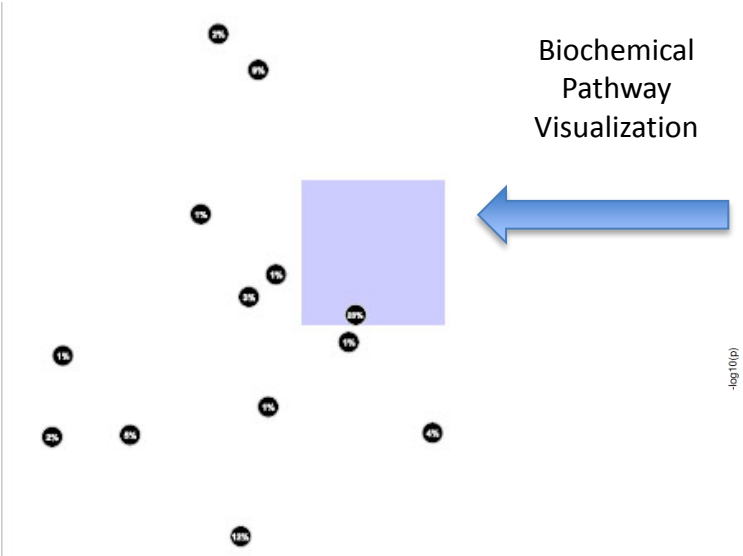


PLS-DA and PC Analysis

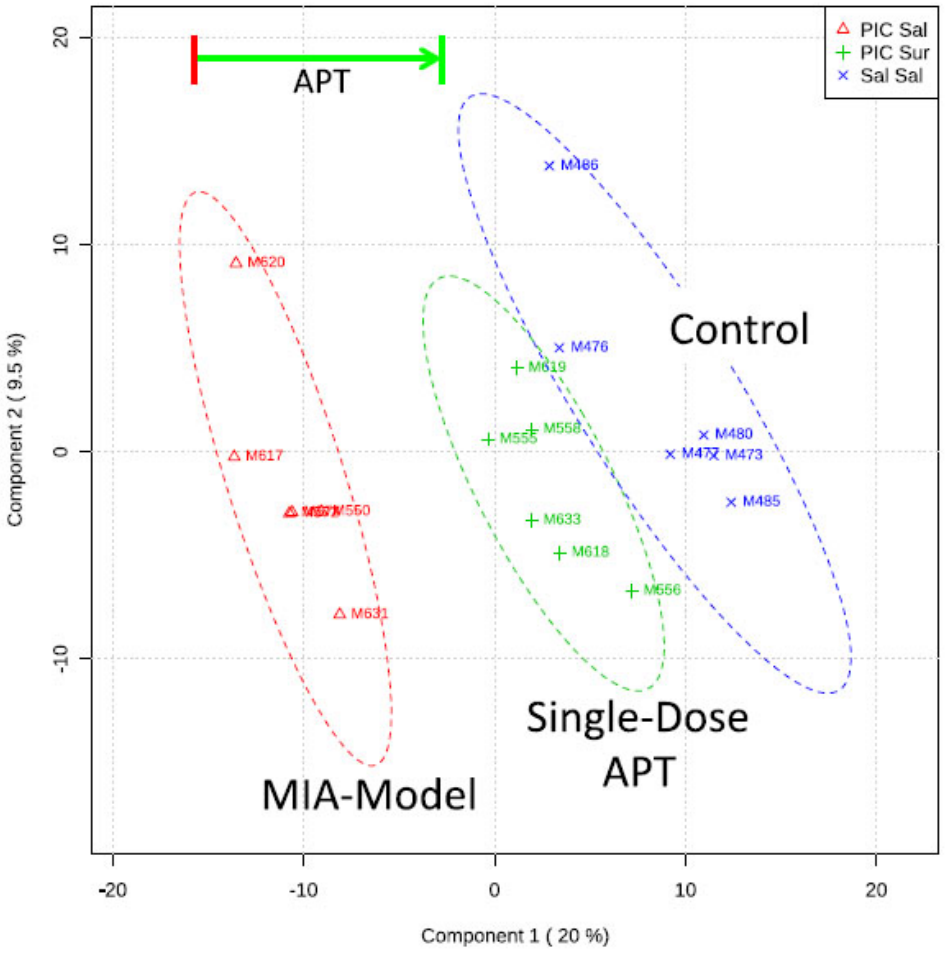


Volcano Plots

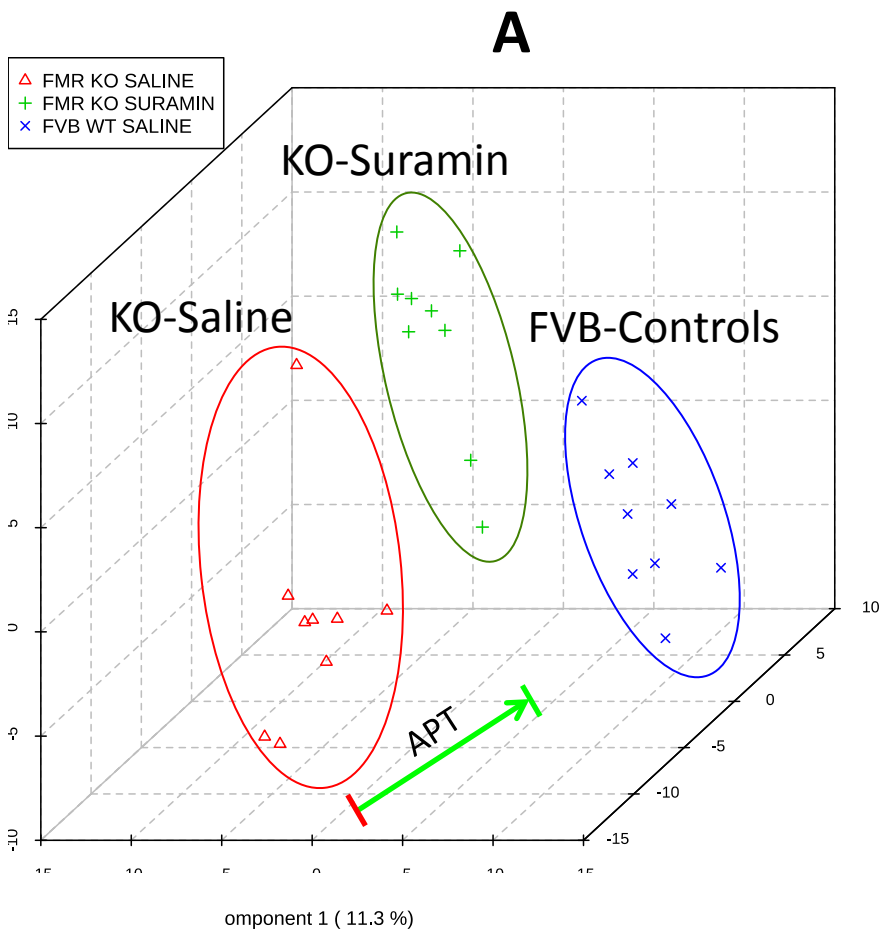
Biochemical Pathway Visualization



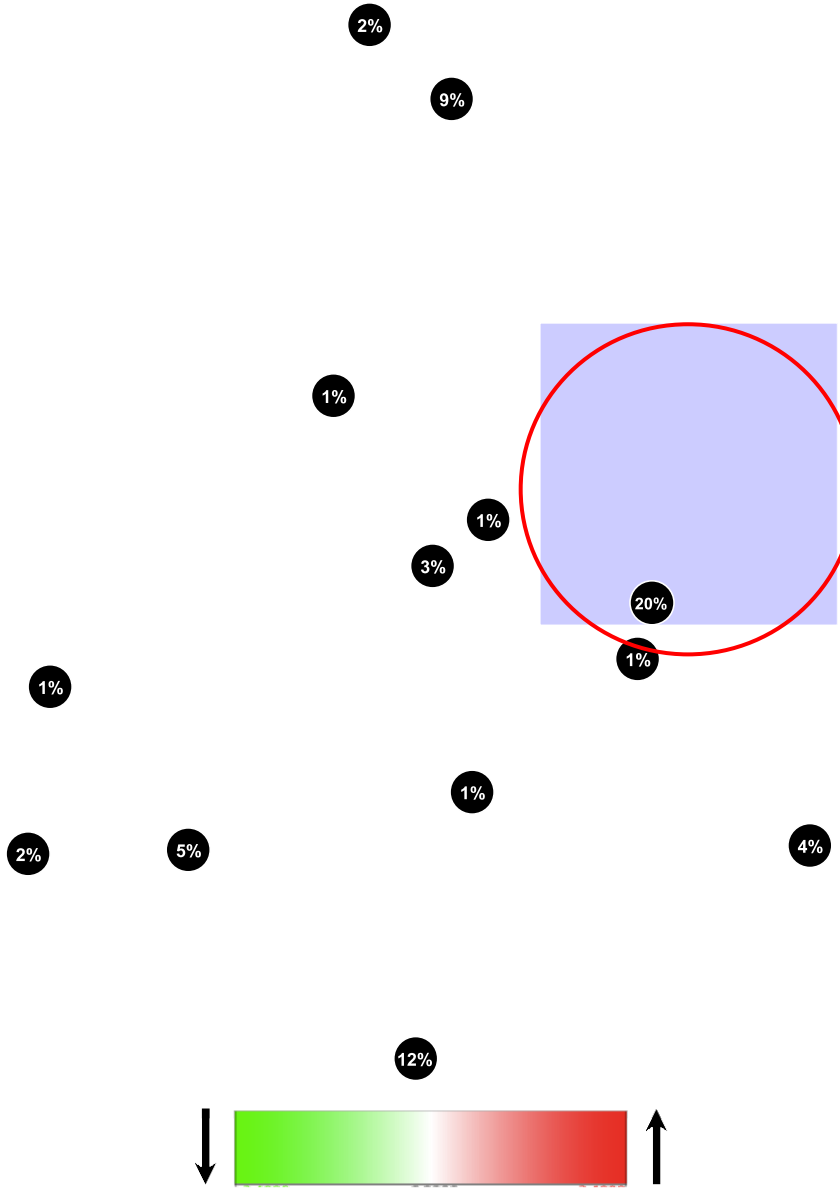
Metabolic Abnormalities in the MIA Model were Improved by Antipurinergic Therapy



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



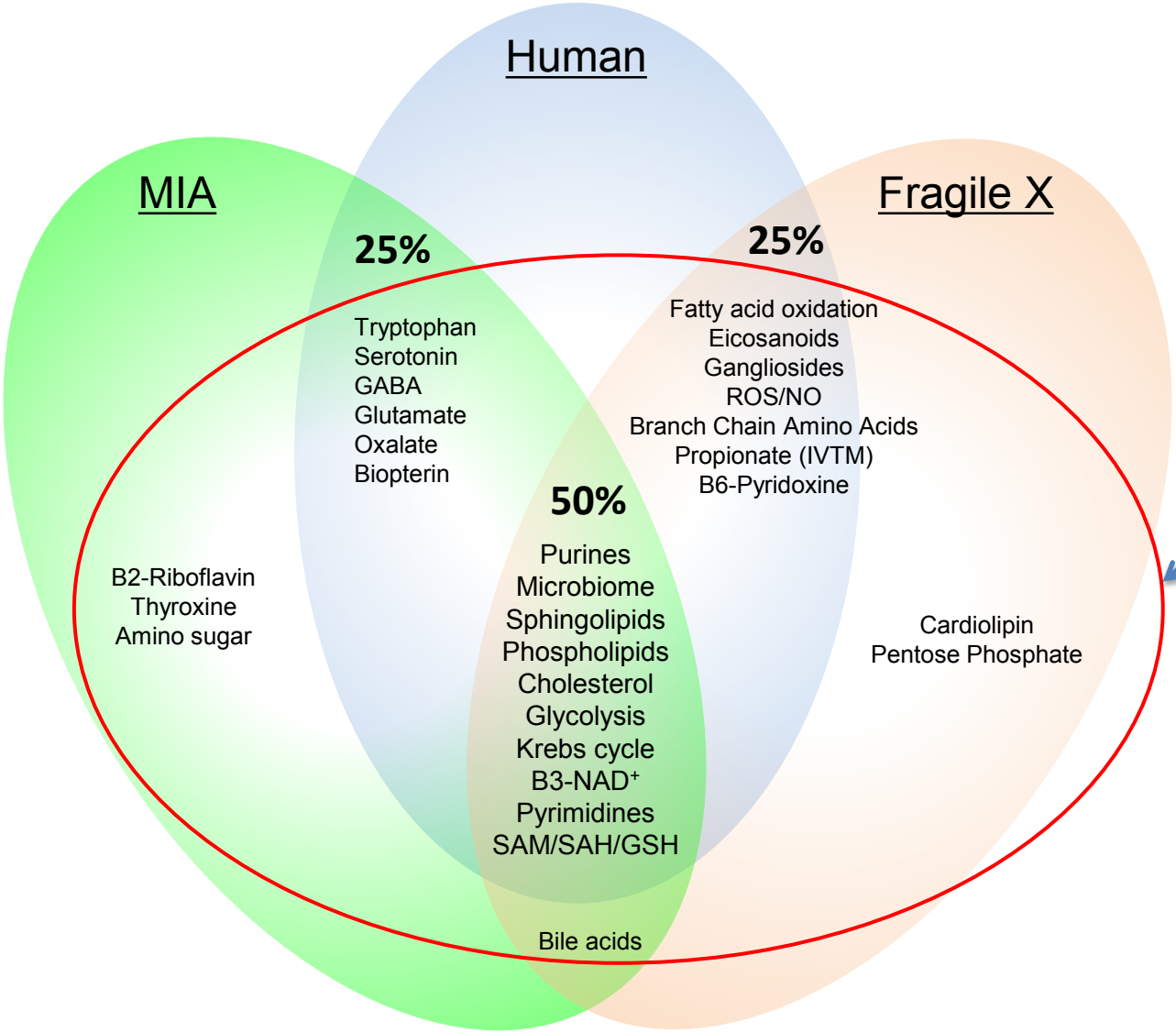
20 Different Metabolic Pathways Were Improved by APT in the Fragile X Model



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

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

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



Metabolic Features of the Cell Danger Response

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

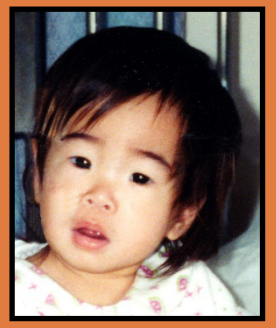
- Triggered by a redistribution in electron flow, oxygen, and substrate concentrations unmatched to collective enzymatic & effector K_ms
- Maintained by hyperpurinergia

Take Home Messages

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

Thank You

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



Christine Shimizu
1996-1998

...Give a Child Hope and Smiles

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

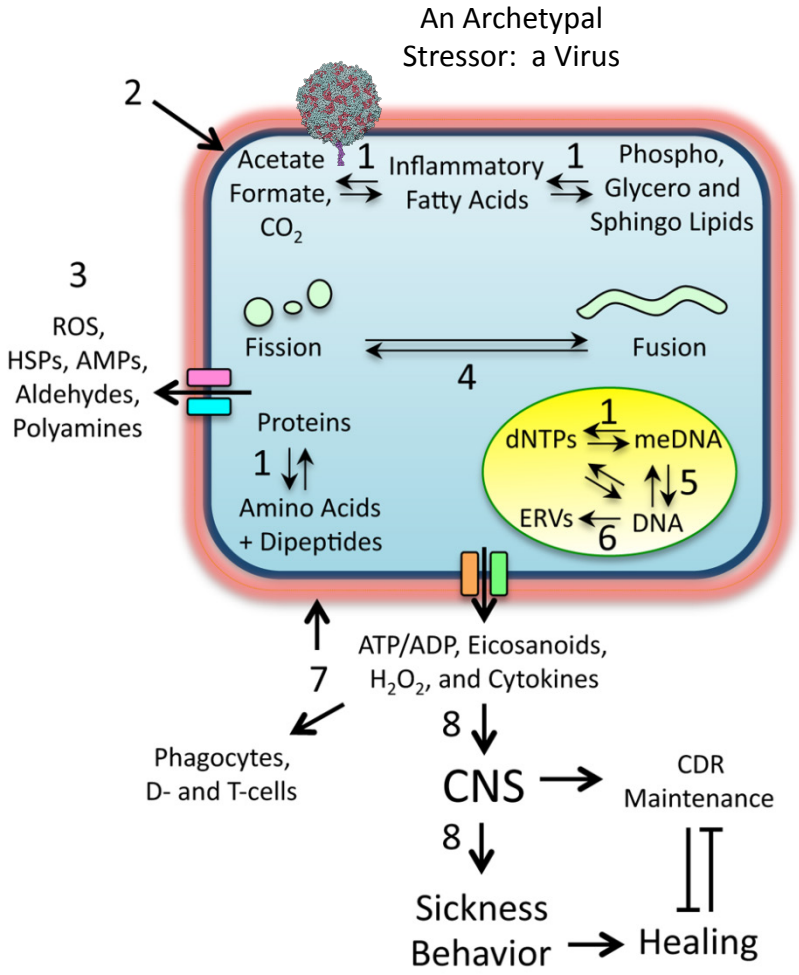
Stress Conditions

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

Shared Metabolic Features

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

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

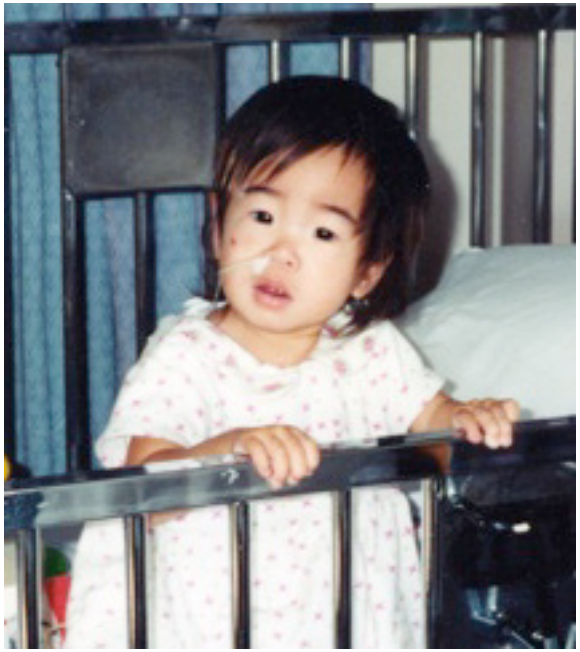


Electron Steal Drives Rapid Mitochondrial Redox Change

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

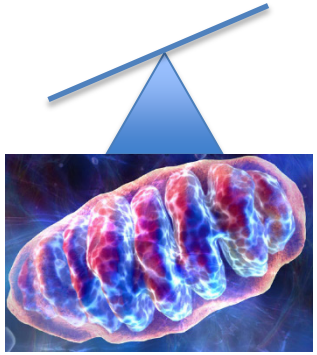
From Naviaux RK. Metabolic Features of the Cell Danger Response. *Mitochondrion*, 2013.

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



Mitochondrial Disease

≠



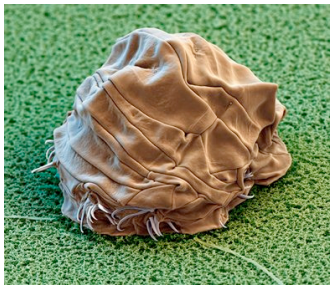
Autism Spectrum Disorders

Cell Persistence Strategies Across Species—Thinking "Analogically"

Tardigrades
("Water Bears")

Desiccation,
heat stress, etc

→



Tun State

Add water + carbs

→

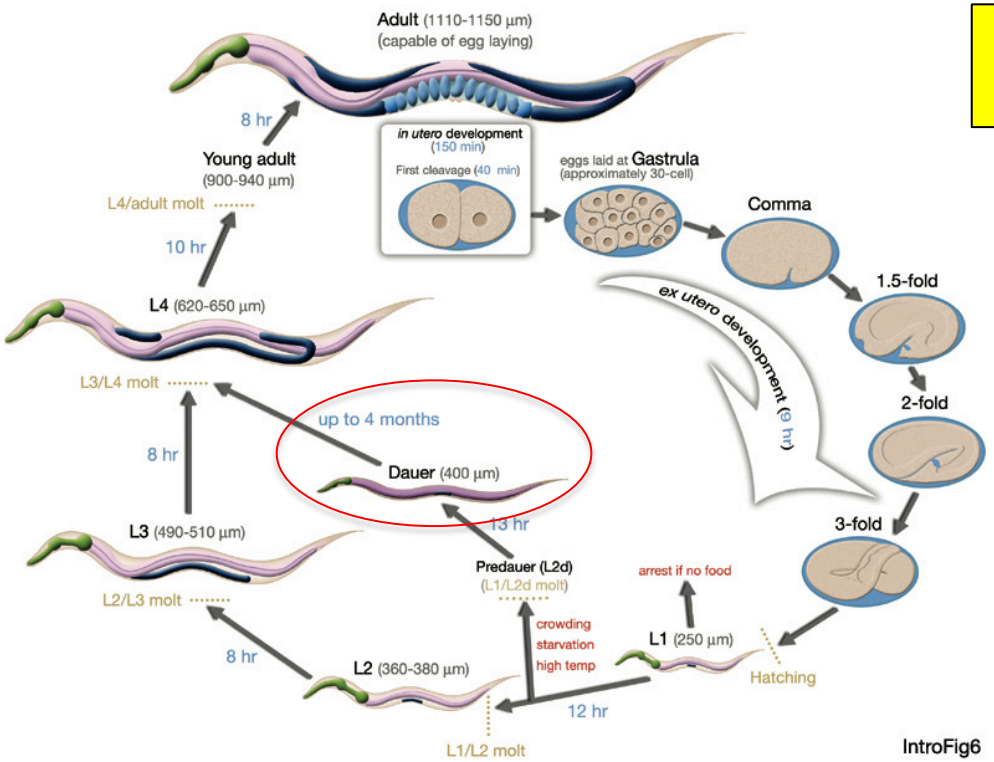
<u>Stress Response</u>

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

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

C. elegans

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

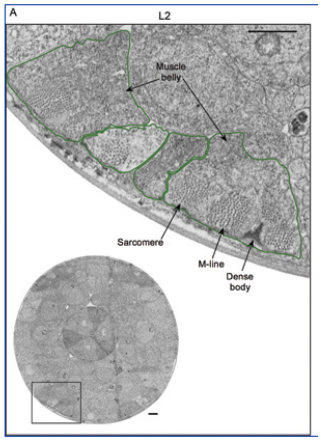


Normal

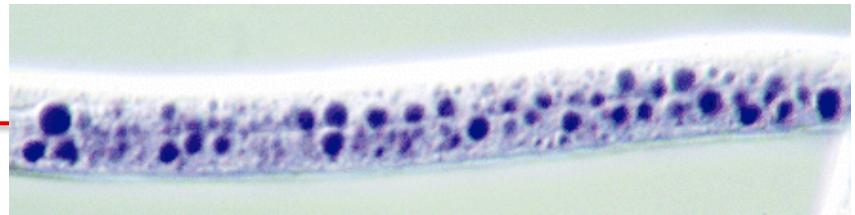
Dauer

Dauer Shifts

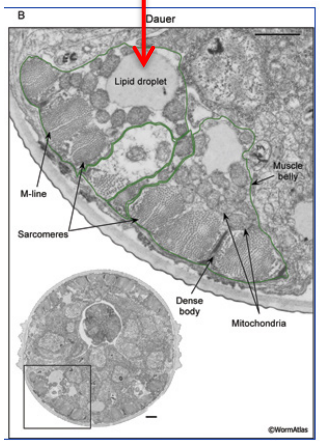
- Stop eating
- Mitochondrial oxphos declines
- Oxygen consumption declines
- Lipid droplets accumulate
- Glycolysis increases
- Glyoxylate shunt increases to increase OAA and gluconeogenesis



Lipid Droplets



Lipid Droplets



www.wormatlas.org

1521-0103/12/3423-608-618$2S.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

JPET 342:608-618, 2012

Perspectives in Pharmacology

June 2012

Oxidative Shielding or Oxidative Stress?

Robert K. Naviaux

The Mitochondrial and Metabolic Disease Center, Departments of Medicine, Pediatrics, and Pathology, University of California San Diego School of Medicine, San Diego, California

Received March 2, 2012; accepted June 8, 2012

ABSTRACT

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

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

OPEN ACCESS Freely available online

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

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

March 2013

OPEN

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

www.nature.com/tp

ORIGINAL ARTICLE

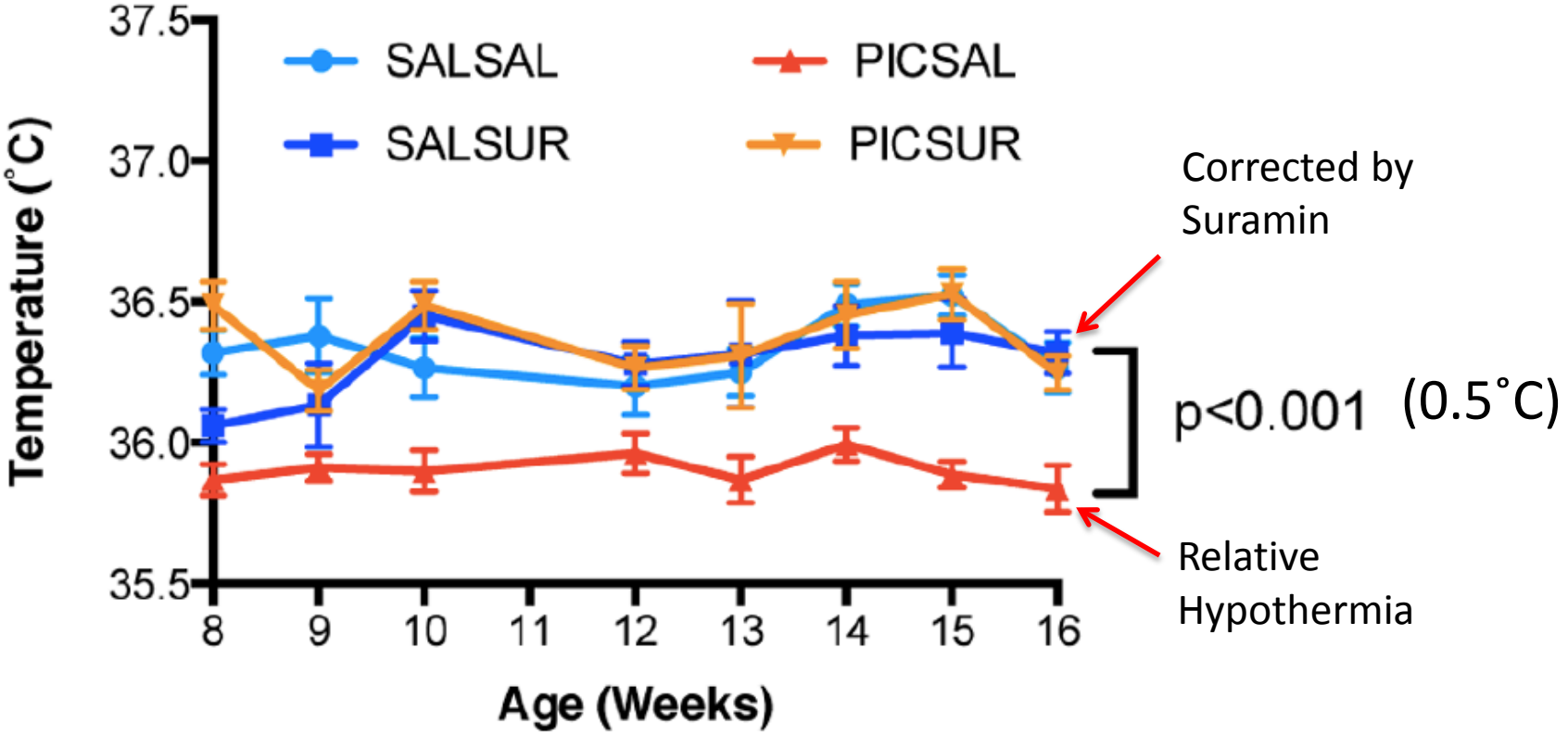
Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy

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

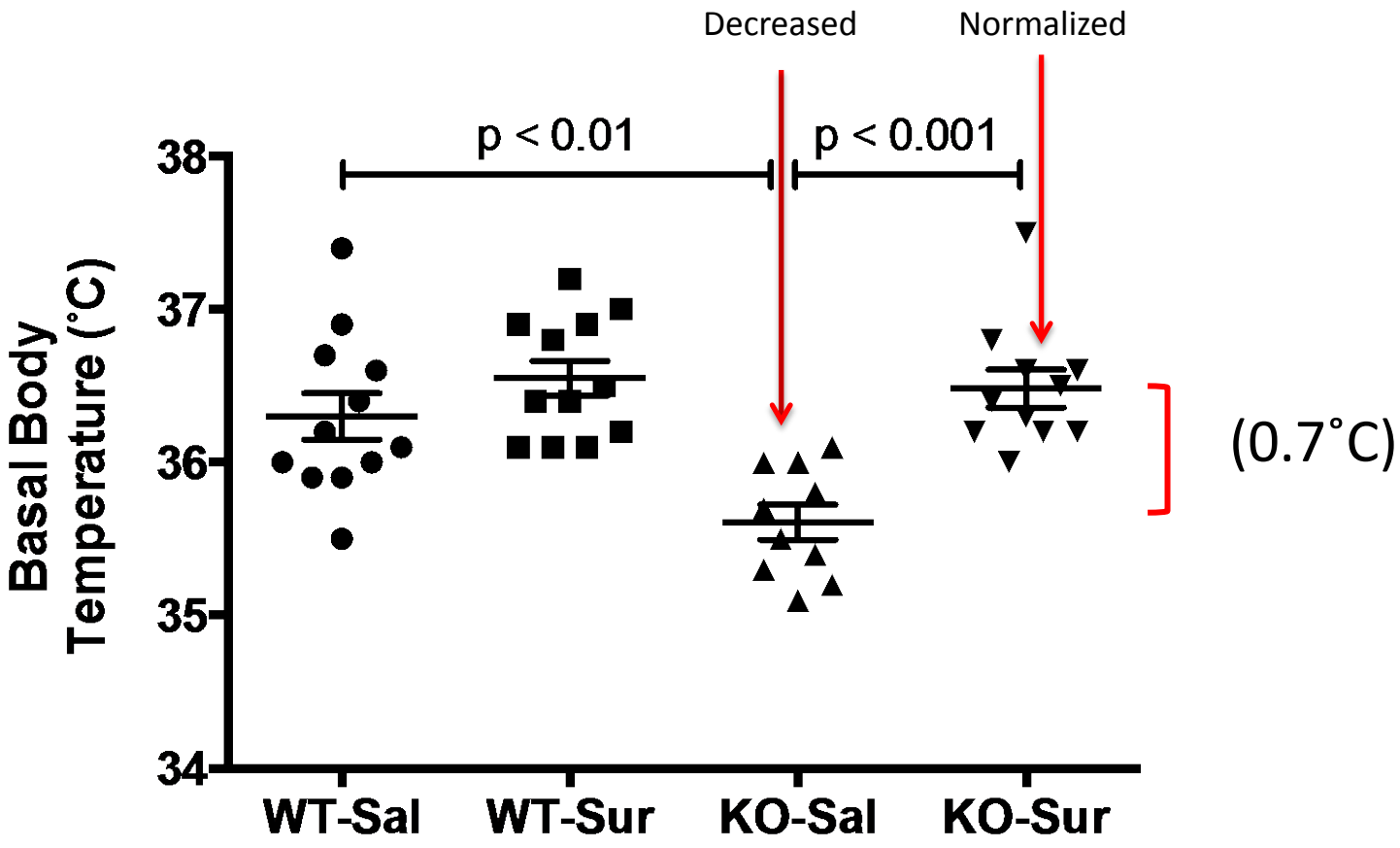
June 2014

The CDR →

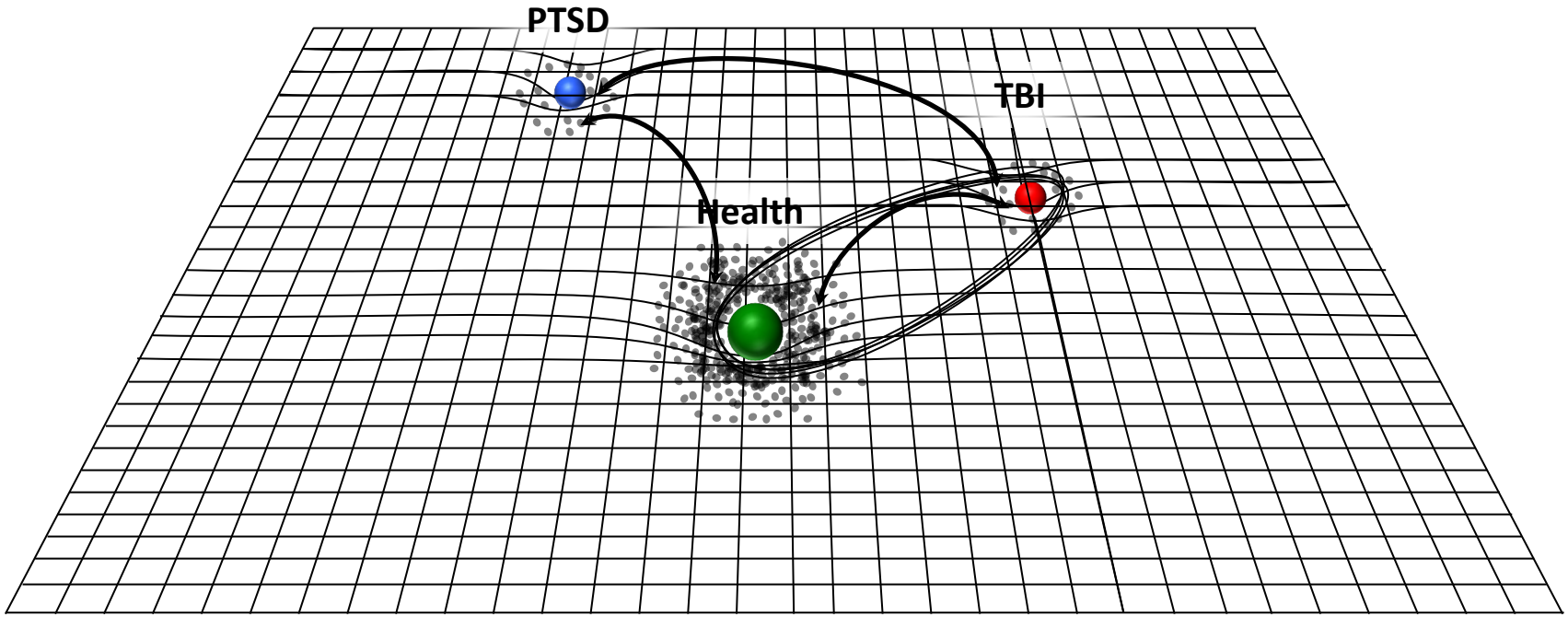
The MIA Mouse Model of ASD has Relative Hypothermia—Corrected by Antipurinergic Therapy



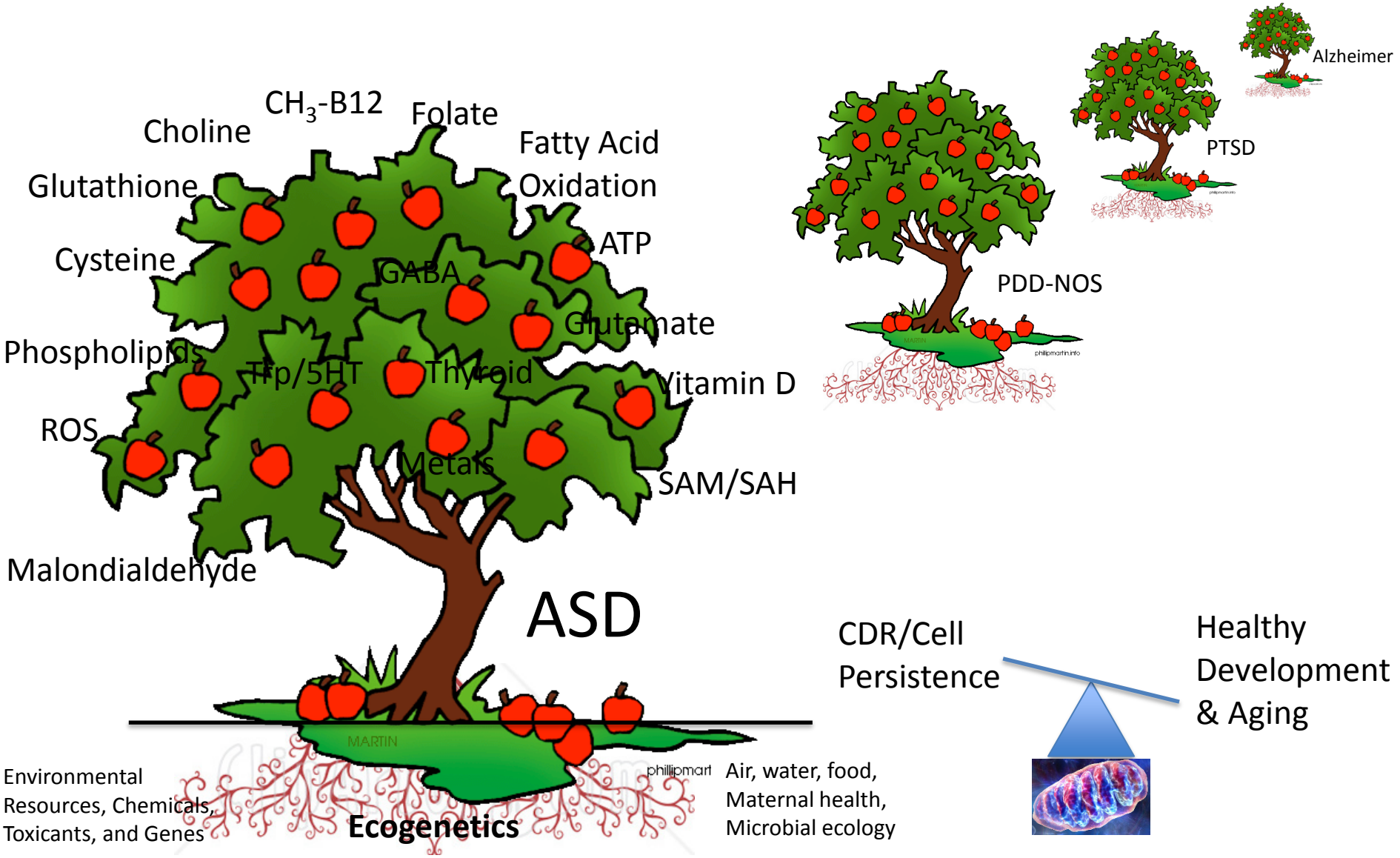
The Fragile X Mouse Model of ASD has Relative Hypothermia—Corrected by Antipurinergic Therapy

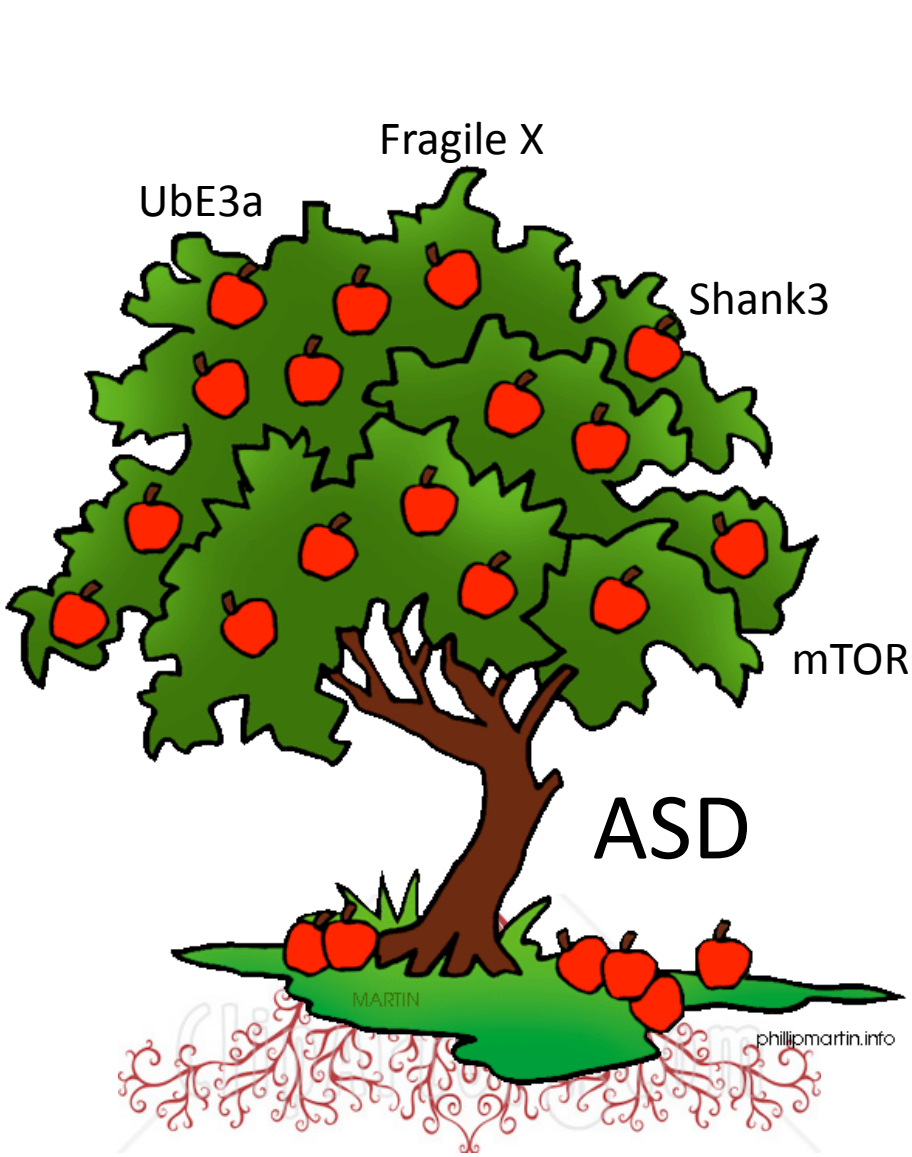


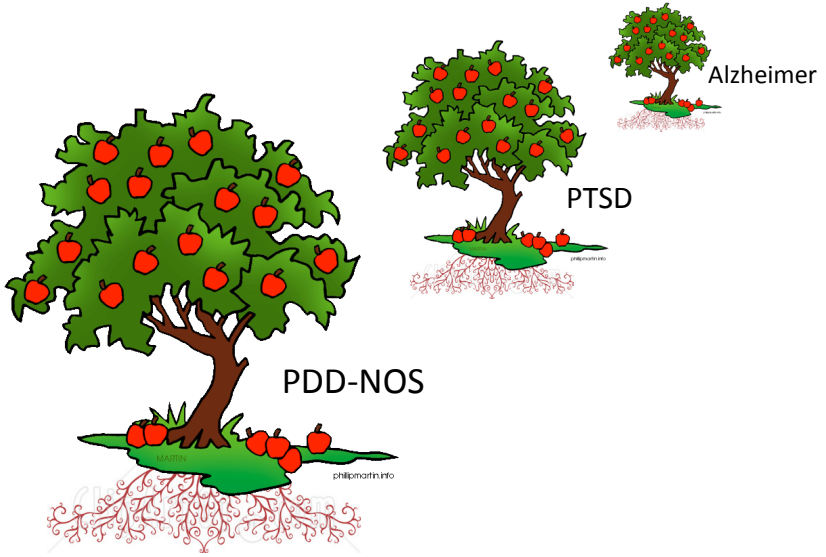
Metabolic ‘Landscape’



Modified from Will Alaynick, 2014


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


Fragile X
UbE3a
Shank3
mTOR
ASD
MARTIN
phillipmartin.info


Alzheimer
PTSD
PDD-NOS

Metabolic Features of Cellular Persistence and the Cell Danger Response

Dauer

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

Autism

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

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

Richard Haas
UCSD 2012

William Graf

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

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

J Child Neurol, 2000

Proband
Leigh Syndrome → "Definite"
Mitochondrial Disease

"Unlikely"
Mitochondrial Disease
(Without mtDNA)

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

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

31 mg/dl 12 mg/dl CSF Lactate

Regression: 18-24 mos
ASD Dx at 4.3 yrs
ADOS = 20
CARS = 51.5

↑250%

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

Original Investigation

Mitochondrial Dysfunction as a Neurobiological Subtype of Autism Spectrum Disorder

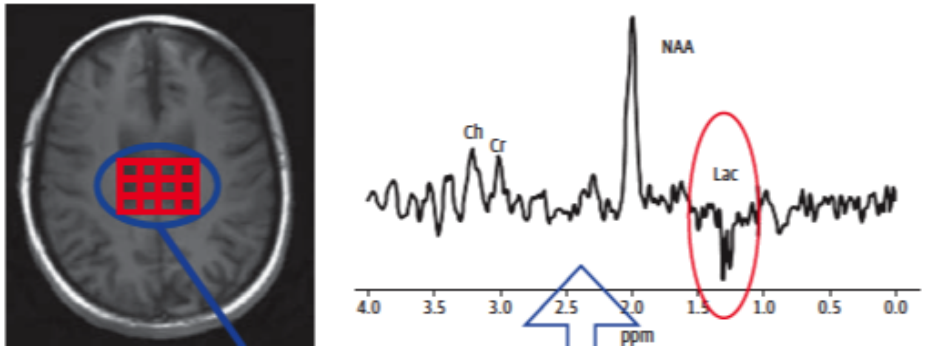
Evidence From Brain Imaging

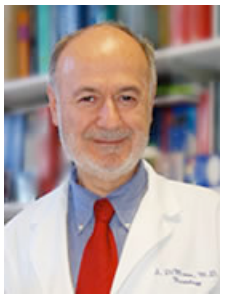
Suzanne Goh, MD; Zhengchao Dong, PhD; Yudong Zhang, PhD; Salvatore DiMauro, MD; Bradley S. Peterson, MD

JAMA Psychiatry, 2014

Suzanne Goh

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





Billi DiMauro

Brad Peterson

Elevated Brain Lactate

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

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

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

William Nyhan
UCSD 2012

1969—The First Reported Case of Purine-Associated Autism

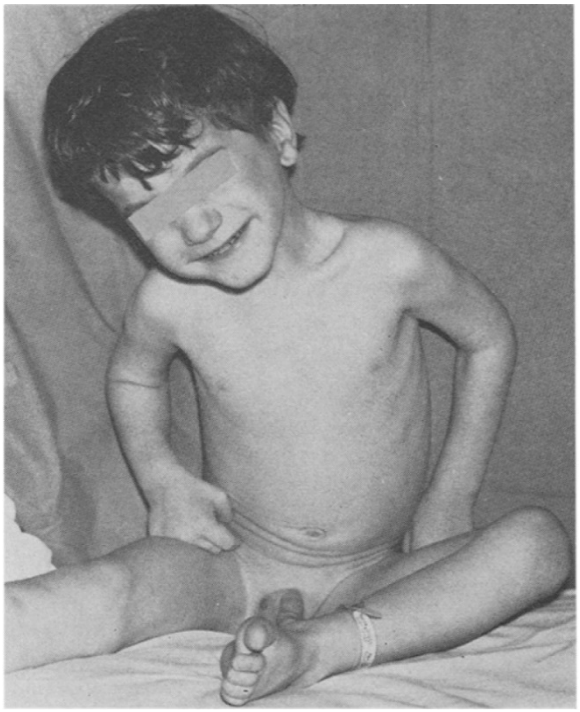


Fig. 1. S. M. at 3 years of age. His general appearance and characteristic odd grin are illustrated

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

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

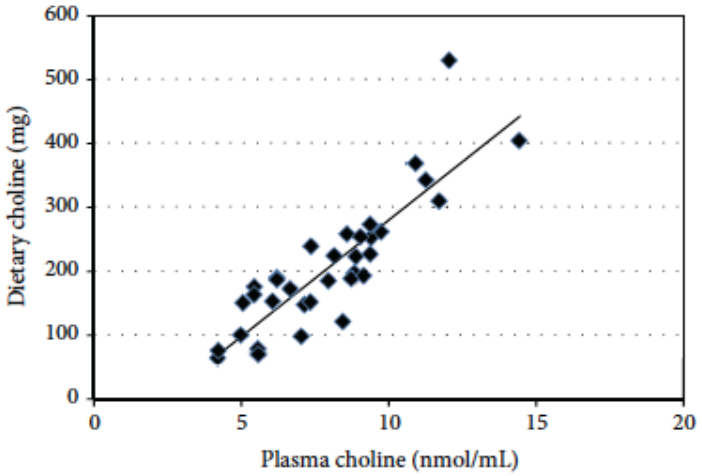


FIGURE 2: Correlation between dietary intake and plasma choline concentrations in children with ASD (n = 35). r = 0.86 and P ≤ 0.001 using Pearson's product-moment correlation coefficient. ASD: autism spectrum disorder.

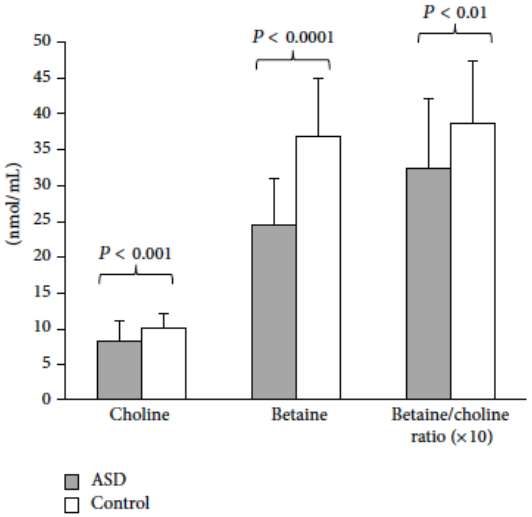


FIGURE 4: Plasma levels of choline, betaine, and the betaine/choiline
ratio in children with autism compared to age-matched controls.

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

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

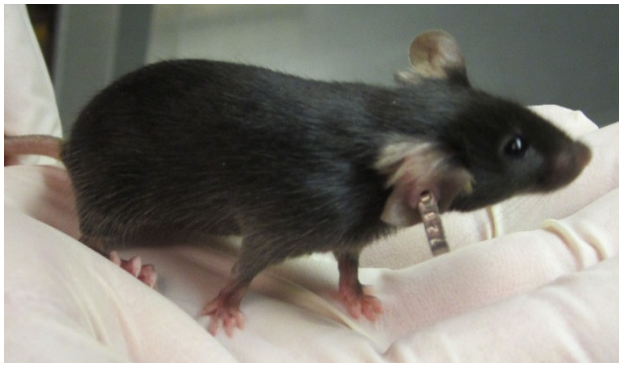
Treatment Implications

Ho: choline supplementation

H1: choline returns spontaneously after turning off the CDR

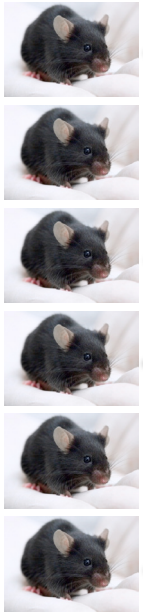
The Maternal Immune Activation (MIA) Model—
The Autism-Schizophrenia Spectrum

Pregnant C57BL/6J Mice



Poly(I:C)
E12.5

8-Week Old
Offspring



Purinergic
Antagonist

(eg, Suramin)

Saline

Behavioral
Testing,
Molecular
Studies,
Metabolomics

What About the Microbiome?

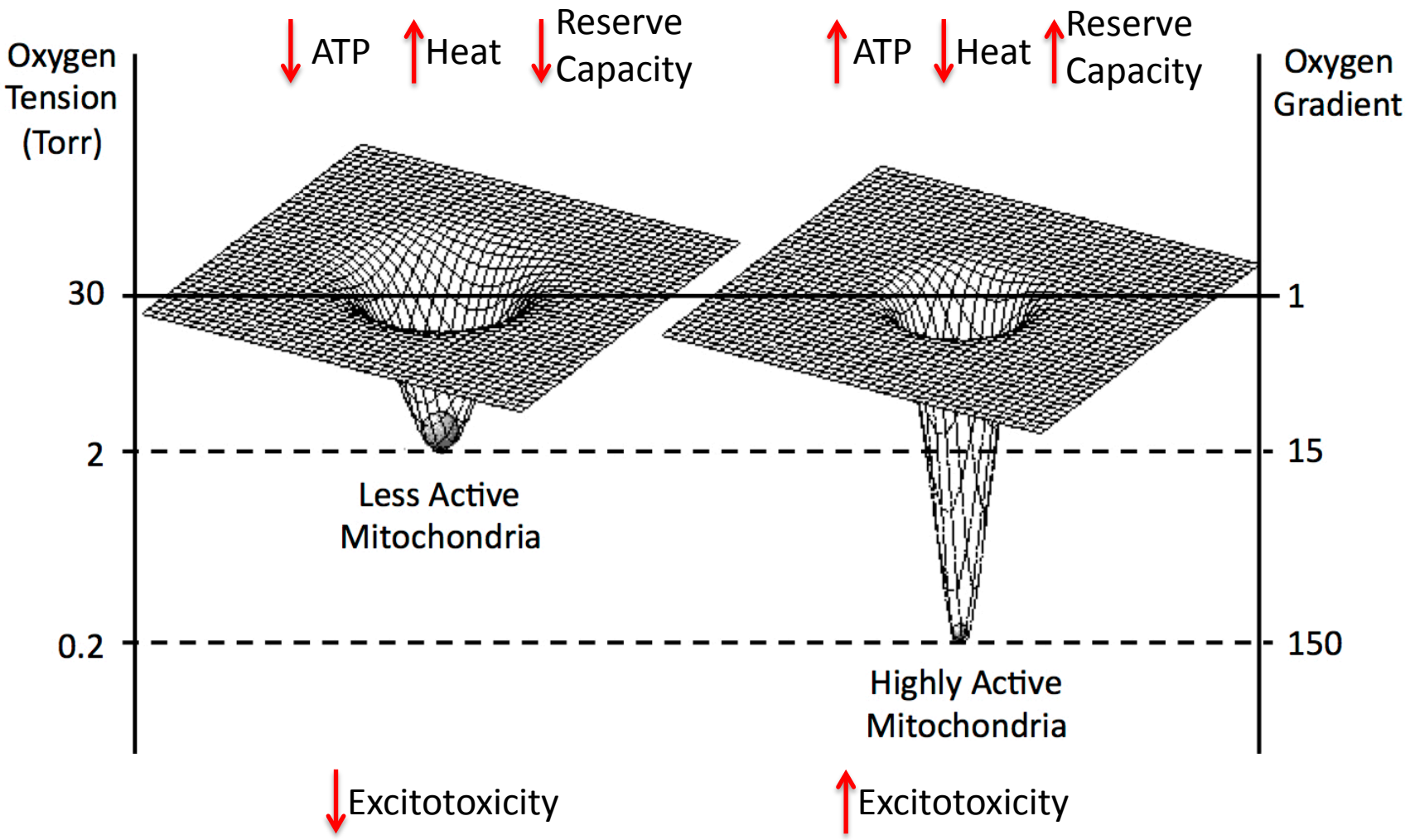
Why Does Metabolomics Work for Autism and Neurologic Disease?

"The brain controls metabolism."

(Through the autonomic nervous and endocrine systems.)

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

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



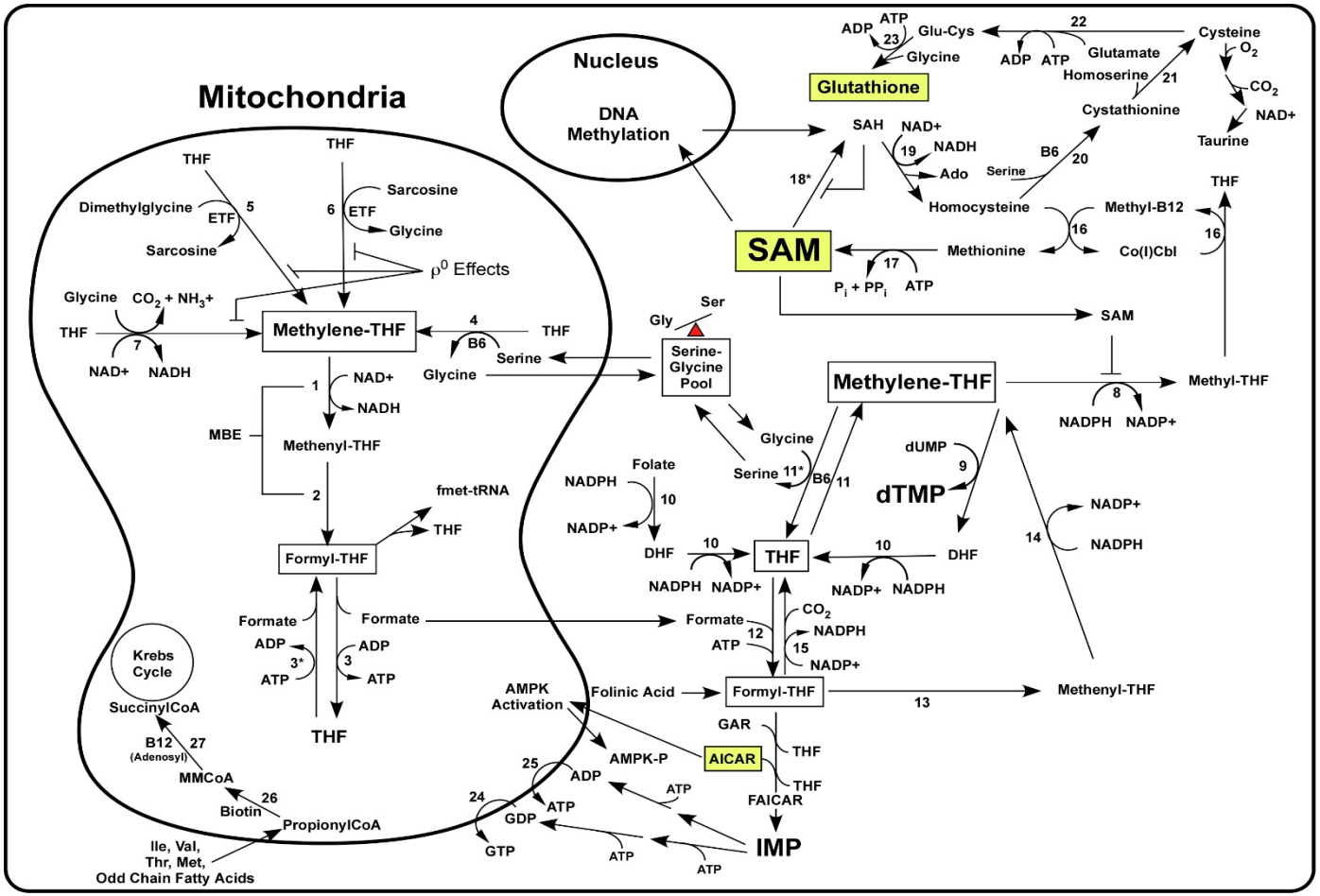
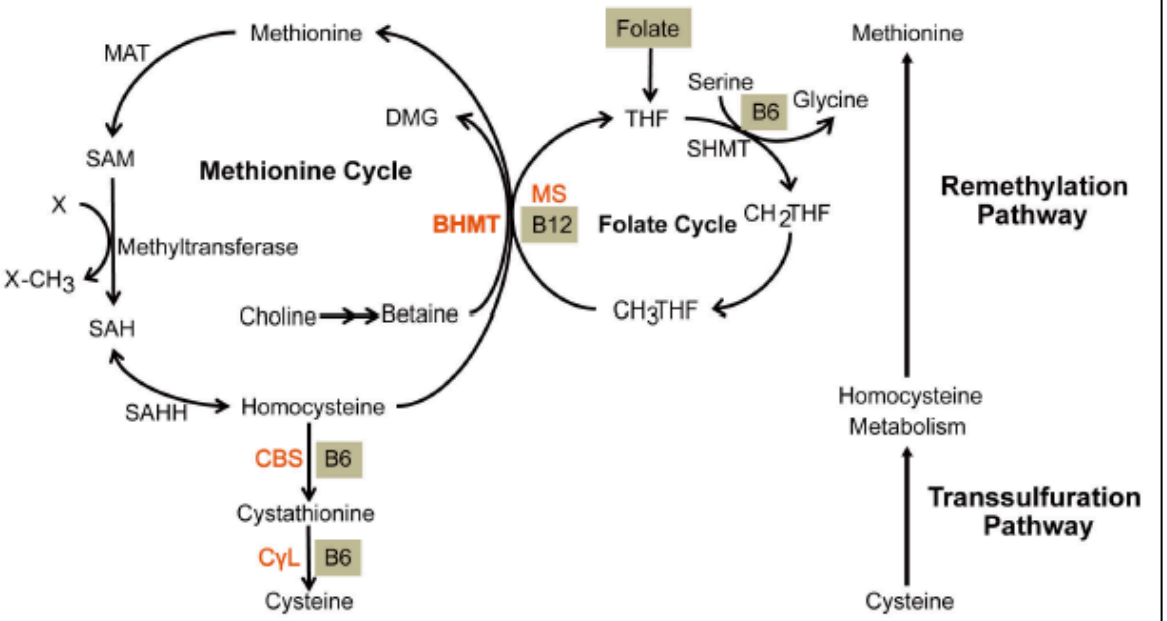
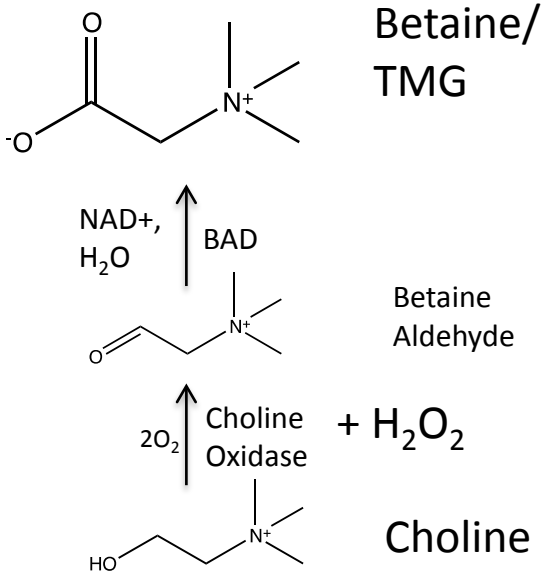
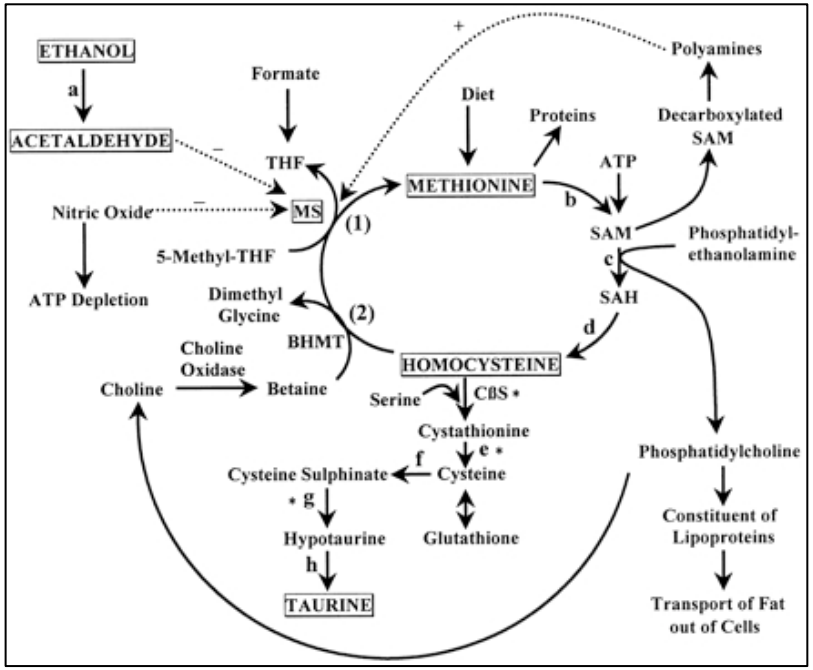


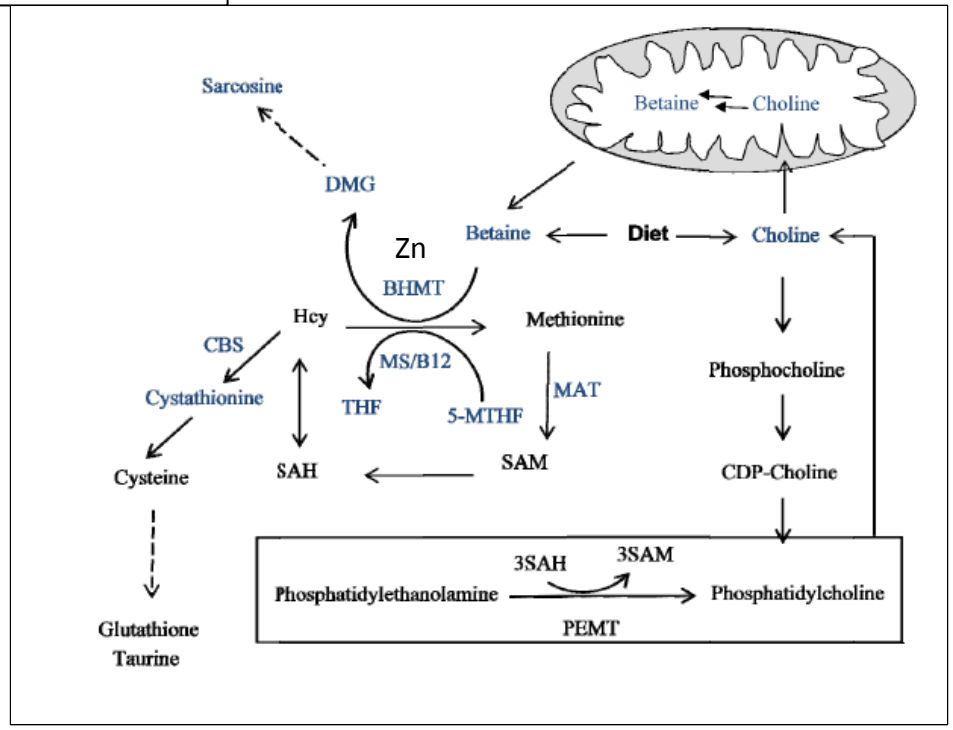
Figure 1. The Connection Between Mitochondrial Folate Metabolism and Nuclear DNA Methylation. In embryonic cells and cancer. MBE is expressed and one-carbon units are efficiently converted
to Formyl-THF and formate for cytosolic nucleotide synthesis. Under these conditions, fewer one-carbon units are available for SAM synthesis and DNA methylation. When MBE is turned off in differentiated
cells. less mitochondrial formate is produced and one-carbon units are directed through Methylene-THF toward increased SAM synthesis and increased DNA methylation. 1/2--Mitochondrial
Bifunctional Enzyme (MBE): 1-NAD+ Dependent Methylene Tetrahydrofolate Reductase, 2--Methenyl-THF Cyclohydrolase. 3--Formyl-THF Synthase (FTS), 3*--FTS can reverse directions in differentiated
cells when MBE is turned off, 4--Mitochondrial Serine Hydroxymethyl Transferase (mSHMT), 5--Dimethylglycine Dehydrogenase, ETF-Electron Transfer Flavoprotein, 6-Sarcosine Dehydrogenase,
7--Glycine Cleavage System, 8--Methylene-THF Reductase (MTFR). 9--Thymidylate Synthase, 10--Dihydrofolate Reductase (DHFR). 11--Cytosolic Serine Hydroxymethyl Transferase (cSHMT).
11*--cSHMT reverse reaction, 12/13/14--Cytosolic Trifunctional Enzyme: 12--Formyl-THF Synthase, 13-Methenyl-THF Cyclohydrolase, 14--NADPH-dependent Methylene-THF Dehydrogenase, 15-Formyl-

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


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


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


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


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

Workshop on Under-Recognized Co-Occurring Conditions in ASD

Presentations will address:

1. What do we know and what have we learned in each research area? (Prevalence in autism vs. developmental disability vs. typical population? Underlying biology? Challenges in diagnosing these condition in individuals with ASD?)

2. What do we need, both in the clinic and in research to decrease the burden of illnesses/extent of disability, or reduction in quality of life due to the co-occurring conditions?

3. Are there any specific gaps or barriers that need to be addressed before progress can be made in this area?

4. Has recent research created new opportunities that need to be explored?

Workshop on Under-Recognized Co-Occurring Conditions in ASD

Discussion questions:

1. What do we need in order to help those affected by these conditions now? (e.g., provider and community awareness, practice guidelines, services, policies.)

2. What kind of research is needed to support development of new and improved treatments for these conditions in the future?

3. Are there any lessons we can learn from other fields to help accelerate progress?

4. Are any of these fields ready for practice guidelines or other steps that have not yet occurred due to knowledge or service gaps or other barriers? If so, how can the IACC, federal agencies or private organizations help accelerate progress?

Panel 4: Metabolic and Immune Disorders

Discussion

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Group Discussion & Wrap Up

IACC Workshop on Under-Recognized Co-Occurring Conditions in ASD

Adjournment

These slides do not reflect decisions of the IACC and are for discussion purposes only.