Neurodevelopmental Consequences of Environmental Toxicants

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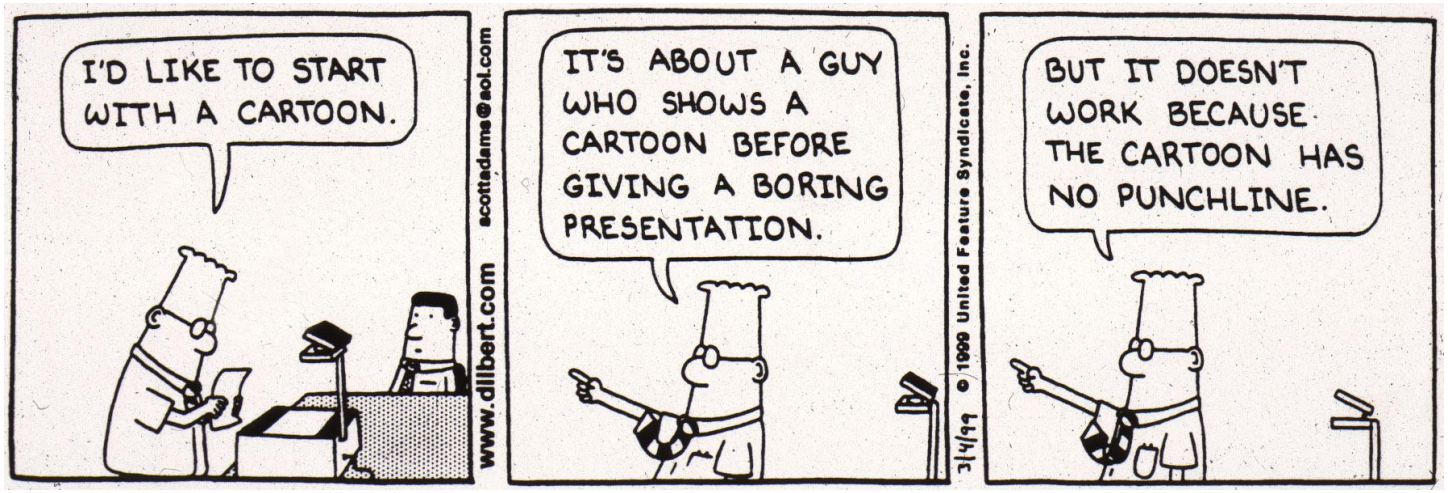


Courtesy of Jeff Murray

Pregnancy Outcomes in the United States- Not as Good as it Should Be

- U.S. Birth Defects Prevalence 2-3 per 100

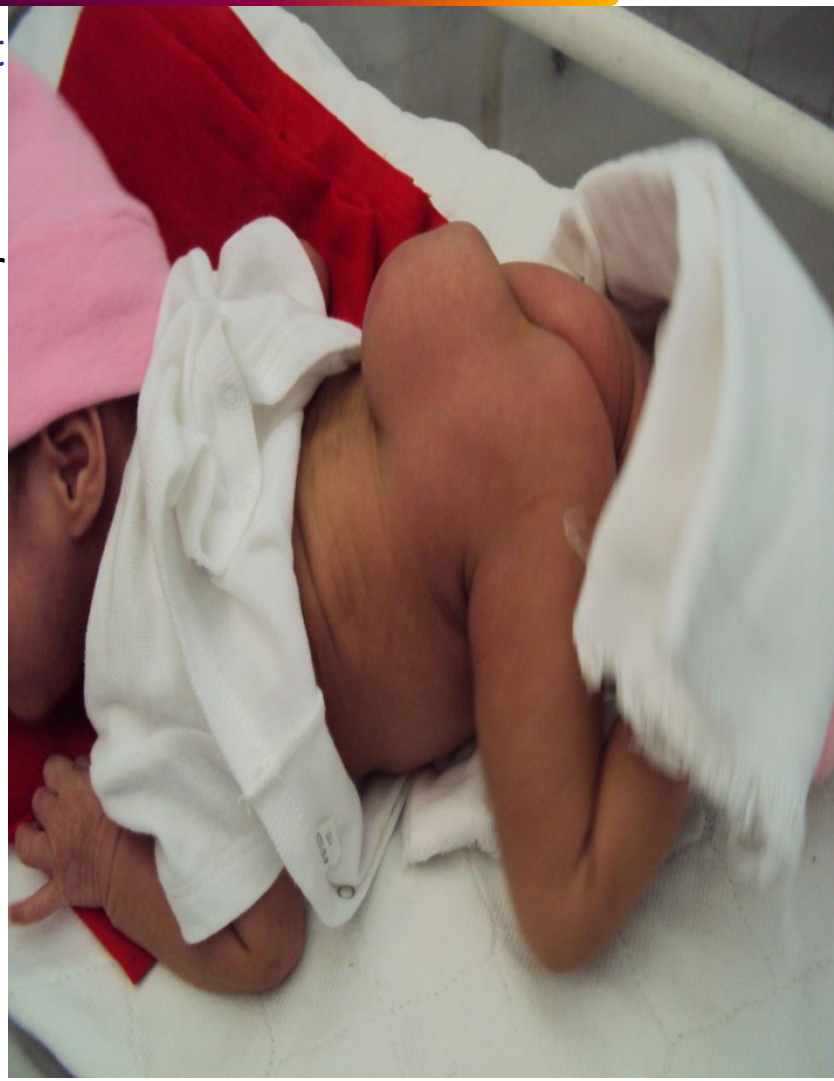
- Infant Mortality 7 per 1000 births
  - 29th in world

- Autism 1 out of 110 US children-(2.6 per 100 in So. Korea)

- ADHD 8 per 100 children

Birth Defects Are

- Birth defects are a **global problem** that affect approximately 6% of all births
- At least 7.9 million infants are born each year with a birth defect-likely under-reporting of functional and late onset defects
- At least 4.4 million children under the age of 5 die each year because of a serious birth defect. Those that survive could be mentally or physically disabled for life
- Birth defects are one, if not the leading healthcare concern for the youngest members of our societies



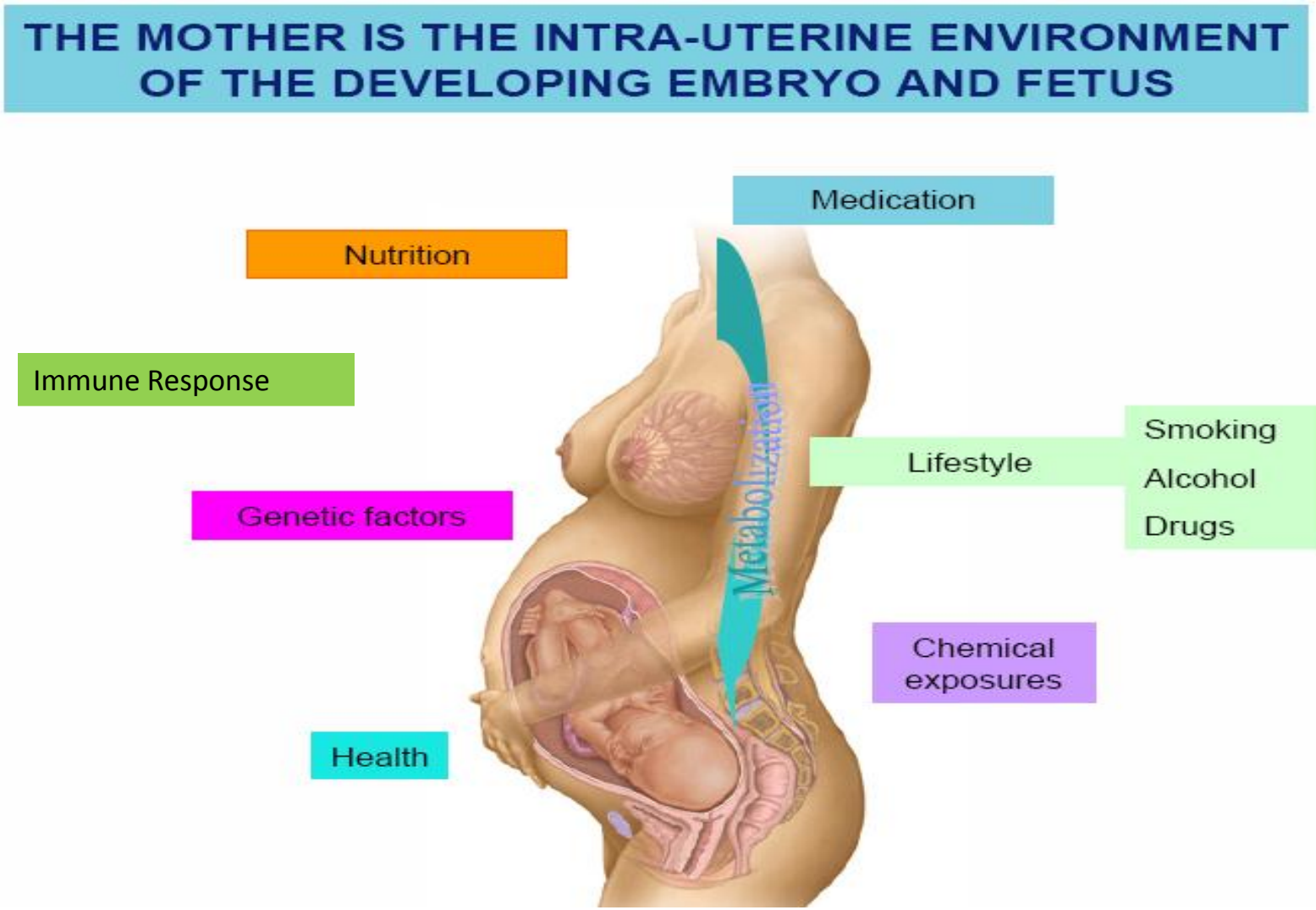
Leon, Nicaragua November 12, 2009

How Does One Study Those Gene-Environment Interactions Governing Susceptibility to Birth Defects and Neurodevelopmental Deficits?

Possible Genetic and Environmental Interactions

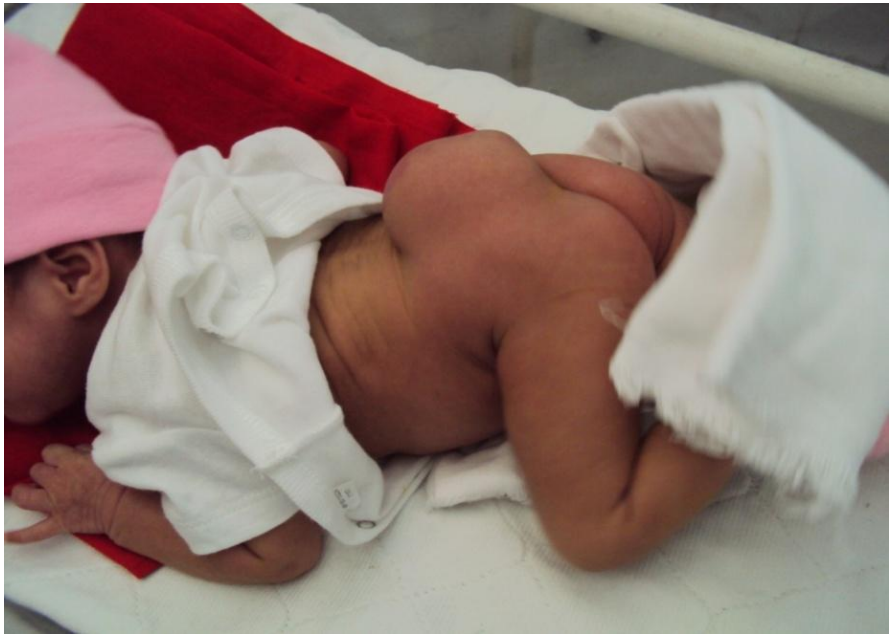
- gene-gene
- gene-environment
- environment-environment
- g x g x e x e……

Embryonic Development is Determined by Maternal Lifestyle Choices and Genetic Factors

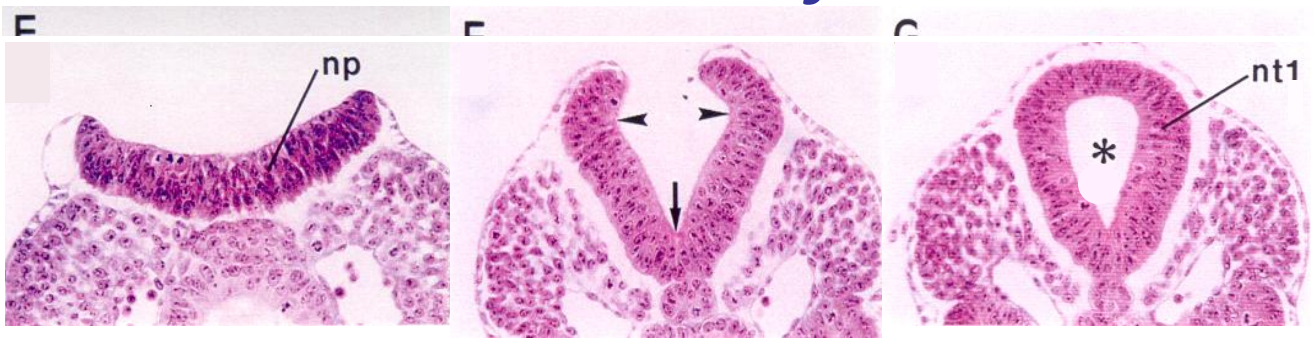


Neural Tube Defects

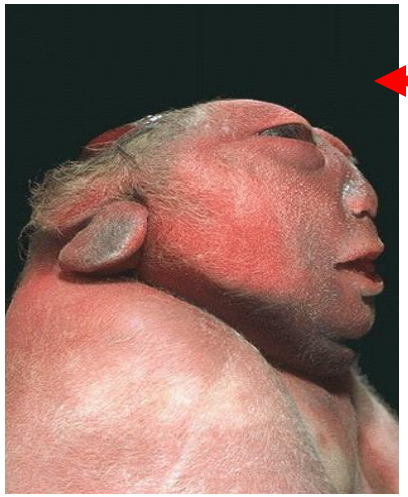
- 250-330,000 NTD births annually worldwide; 3,000 in US
- Result in lifelong disability
  - Problems with bladder, bowel, and sexual function
  - Learning and developmental problems
  - Orthopedic problems
- Some NTDs are **folate** preventable



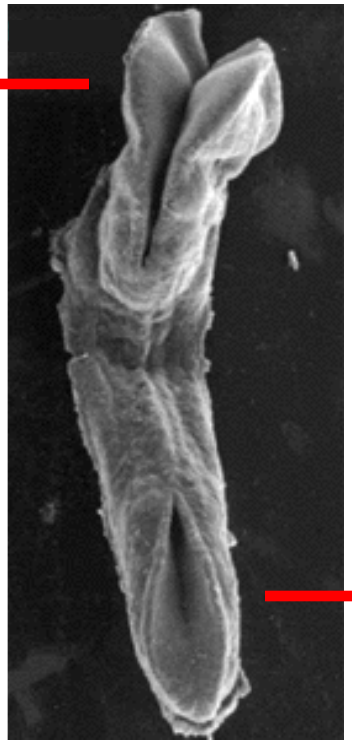
Neural Tube Defects



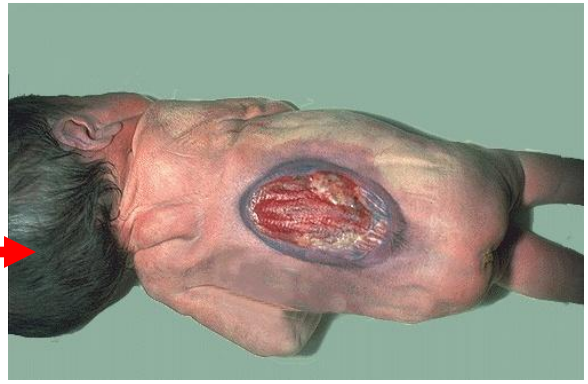
neural plate neural folds neural tube



Anencephaly



Spina Bifida



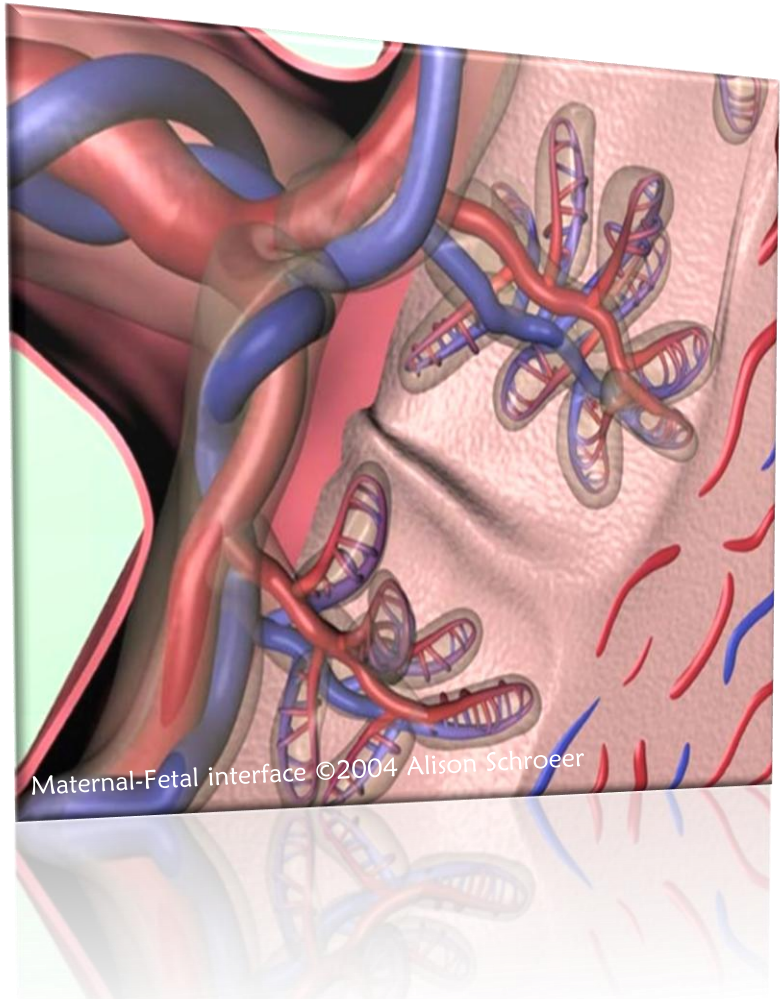
"NTDs are caused by a little bit of this and a little bit of that"

Clarke Fraser
09/12/09

F. Clarke Fraser

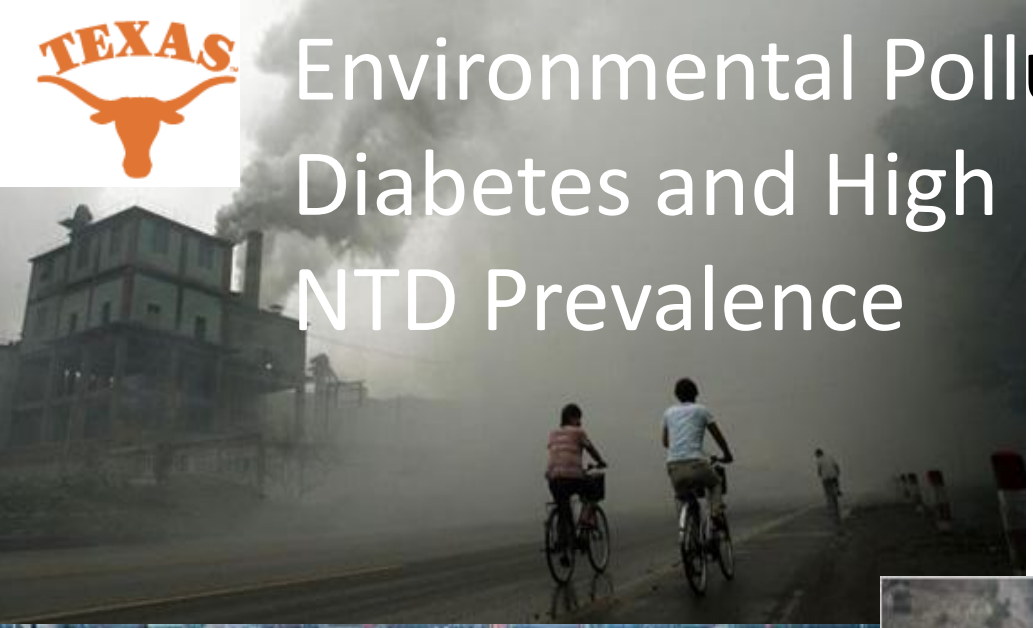
ENVIRONMENTAL RISK FACTORS FOR NTDS

maternal characteristics as well as exposures that influence the *in utero* environment of the developing embryo



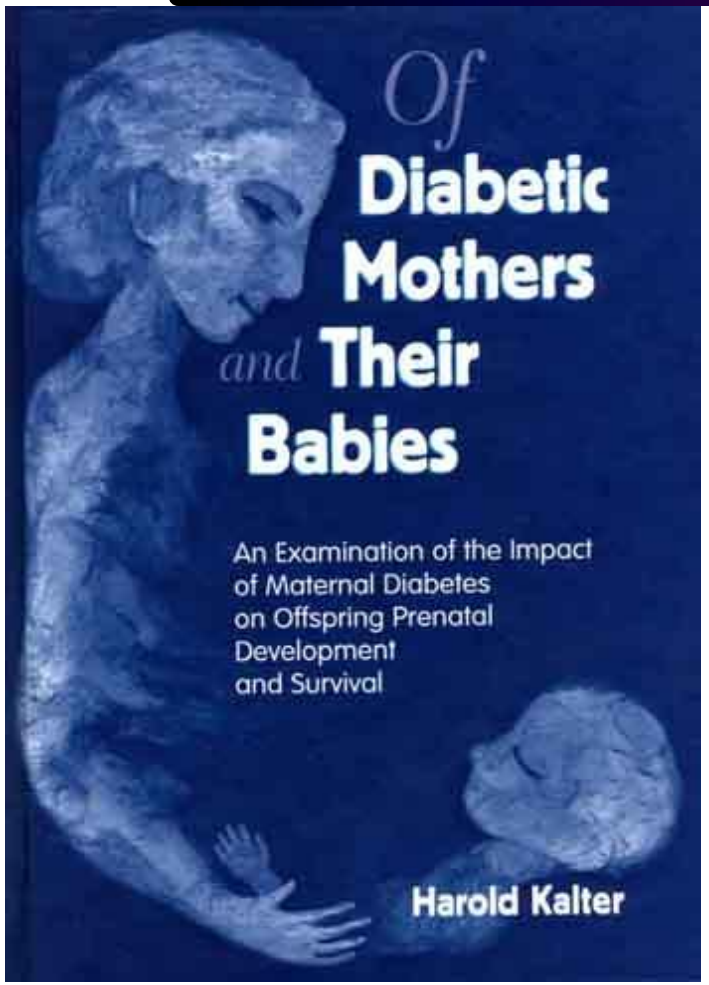
- **Established risk factors**
  - maternal folate status
  - pre-gestational diabetes
  - maternal use of anti-epileptic drugs
  - maternal obesity
- **Compelling evidence**
  - maternal vitamin B12 status
  - maternal hyperthermia
- **Proposed, but unconfirmed**
  - exposure to fumonisins
  - pesticides
  - hazardous waste sites

Environmental Pollutants Associated With Diabetes and High NTD Prevalence



- Fine particulate air pollution (<2.5 µm; $PM_{2.5}$)
- Arsenic
- Carbon disulphide
- Cadmium
- Lead
- TCDD

Diabetes and NTD Risk



BMC Genomics 2009 Jun 18;10:274
Maternal diabetes alters transcriptional programs in the developing embryo
Gabriela Pavlinkova, J Michael Salbaum and Claudia Kappen

- **Diabetes is an autoimmune disease**

- Type 2 diabetes mellitus, characterized by hyperglycemia secondary to a relative lack of insulin, is a group of disorders whose multi-factorial etiology has both environmental and genetic components

- Pre-gestational diabetes is associated with a two- to ten-fold increase in the risk of having an NTD-affected pregnancy

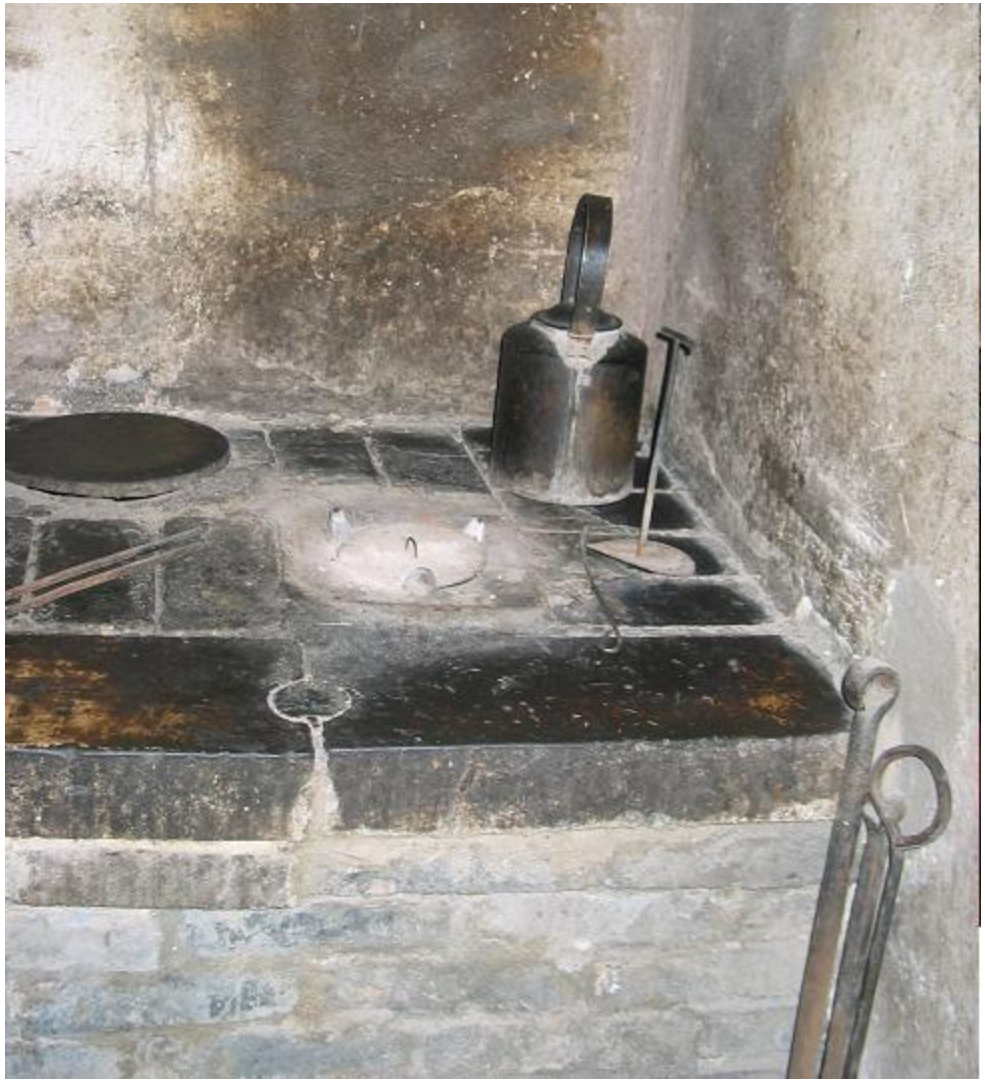
- Diabetes-induced birth defects are thought to be related to:
  - **Increased apoptosis**
  - Perturbation of prostaglandin synthesis and metabolism
  - **Altered embryonic gene expression**

Village in Eastern Shanxi Province
July, 2007

Pingding County, Shanxi Province, China

“The Coal Warehouse of China”

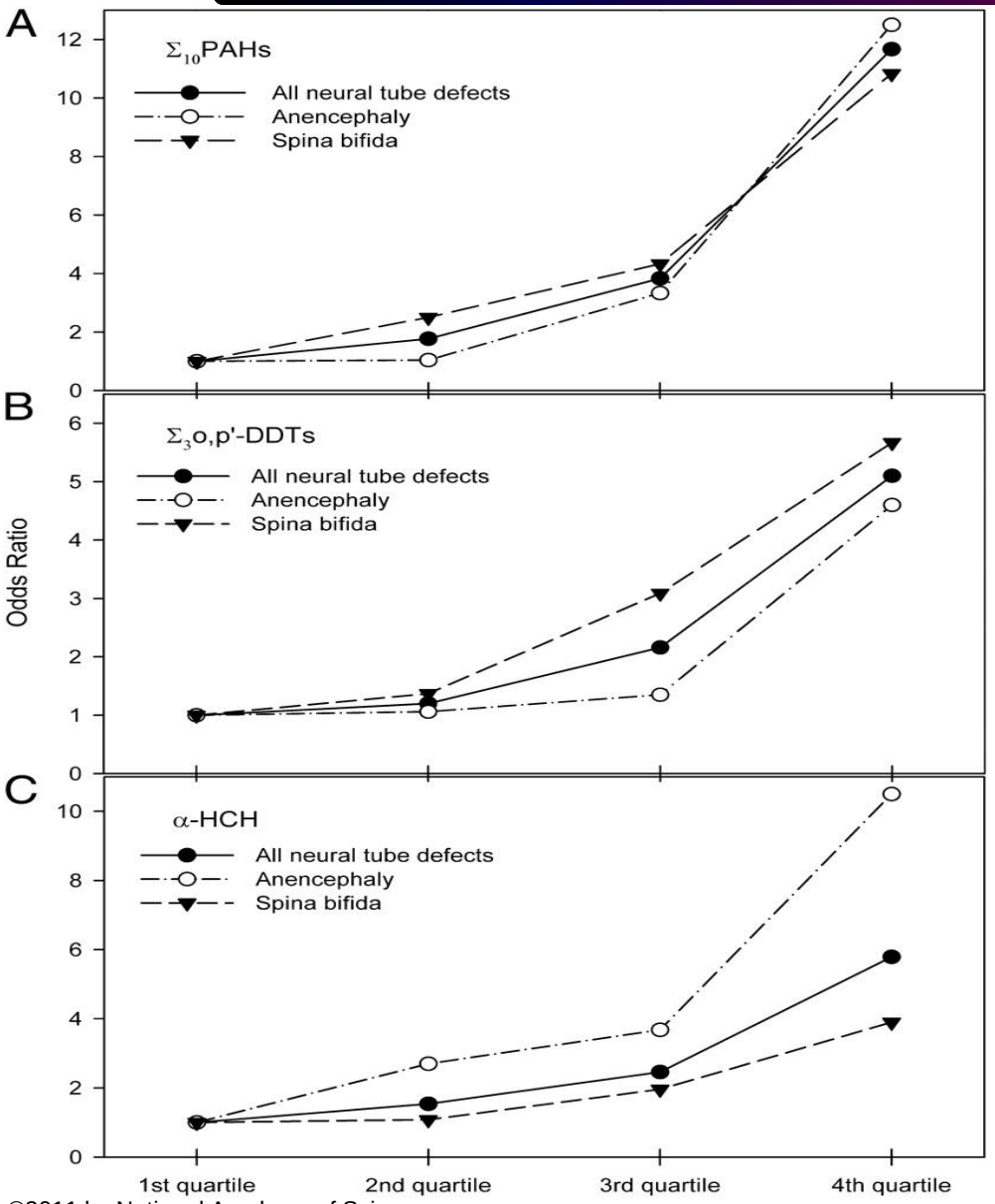
Pingding County, Shanxi Province, China July, 2004

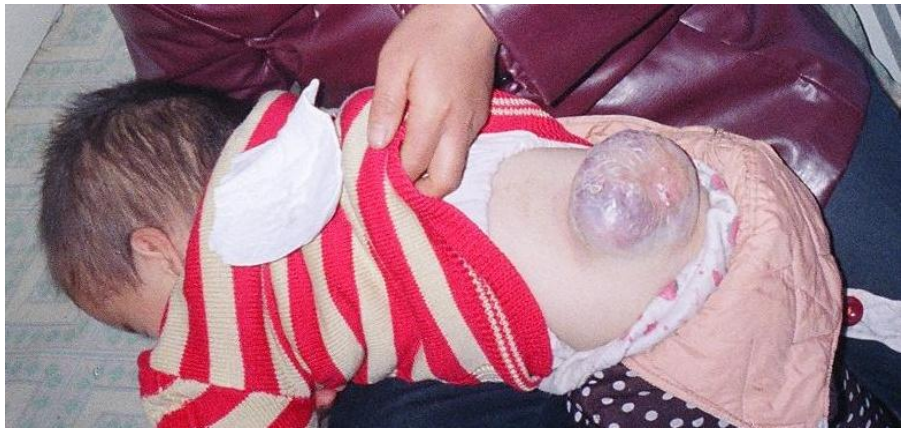


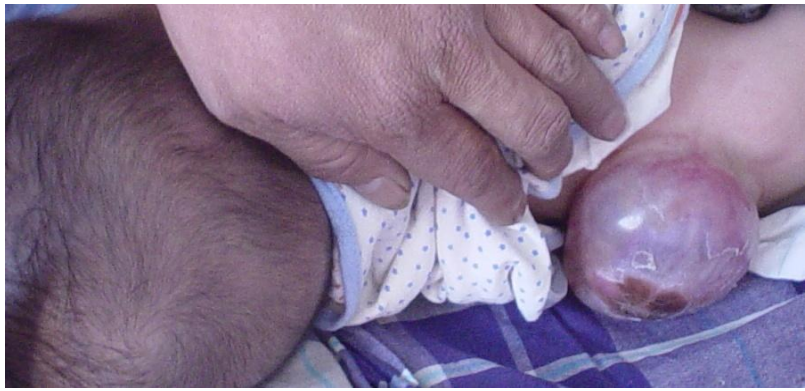
Typical Residential Kitchen:

- Coal fired
- Poorly ventilated
  - PAHs
  - Arsenic

Quartiles of Σ10PAHs (A), Σ3o,p'-DDTs (B), and α-HCH (C) in the placenta and the risks of NTDs







Ren A et al. PNAS 2011;108:12770-12775

PNAS

Antiepileptic drugs (AEDs)

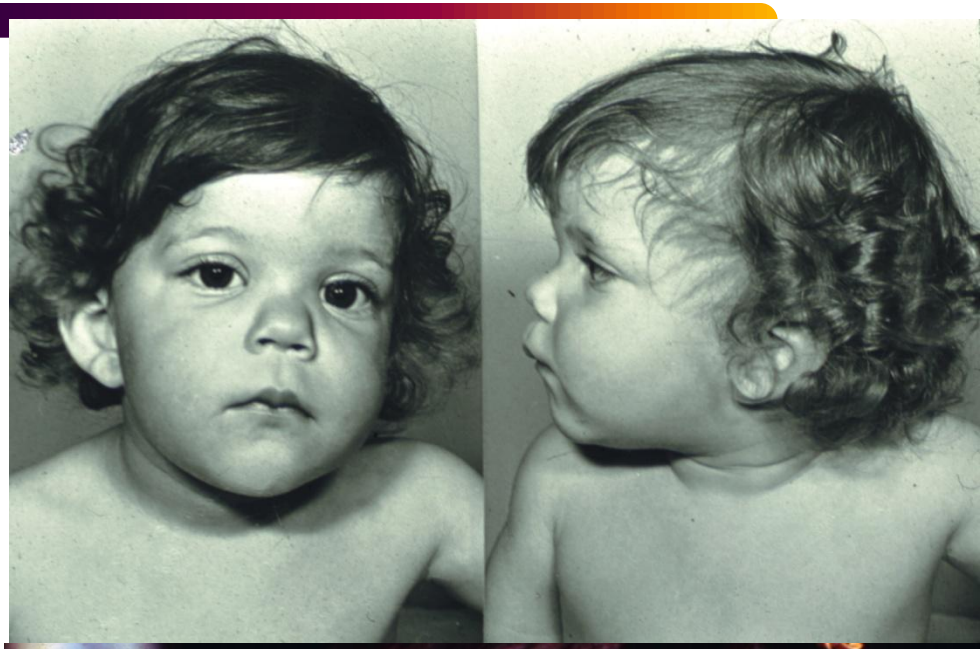
- Epilepsy, schizo-behavioral disorders, migraine headaches, cancer, obesity and more
- 1 in 251 women take AEDs during pregnancy (Holmes et al., 2001)
- 10-35% experience neurodevelopmental deficits (Moore et al., 2000)

30,000 Anticonvulsant Drug Complicated Pregnancies Annually in the United States

- 2-3% of infants are born with congenital defects

- 6-10% of AED exposed infants are born with congenital defects

- Clearly, AEDs increase the risks for an adverse outcome in some pregnancies but not in others



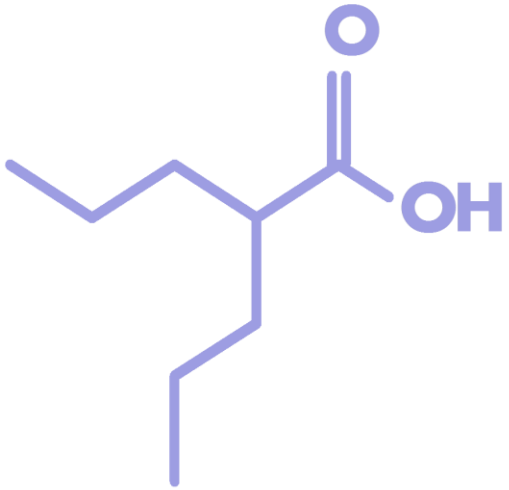
Meta-analyzed Incidence of Congenital Malformations by AED Exposure

Treatment	Malformations (total events)		Births with malformations	
	t (n)	*% [95% CI]*	*t (n)*	*% [95% CI]*
Women without epilepsy	9 (108,084)	3.27 [1.37, 5.17]	16 (315,381)	2.28 [1.46, 3.10]
Carbamazepine	24 (4,411)	4.62 [3.48, 5.76]	9 (544)	**5.68 [3.71, 7.65]**
Lamotrigine	5 (1,337)	2.91 [2.00, 3.82]	3 (600)	1.55 [0.00, 3.48]
Phenobarbital	14 (945)	4.91 [3.22, 6.59]	4 (126)	5.90 [0.00, 13.46]
Phenytoin	16 (1,198)	7.36 [3.60, 11.11]	5 (289)	5.48 [2.80, 8.16]
Valproate	19 (2,097)	**10.73 [8.16, 13.29]**	**6 (217)**	**17.64 [5.25, 30.03]**

Meador et al., *Epilepsy Res*. 81:1-13, 2008

Valproic Acid (VPA)

- Anticonvulsant activity discovered- 1963
- FDA approves use in absence seizures- 1978
- Current and exploratory indications
  - Epilepsy
  - Affective disorders
  - Migraine
  - Head trauma
  - Cancer



- VPA is a Folate Antagonist

In Utero Exposure to Anti-Epileptic Drugs

- The Neurodevelopmental Effects of Antiepileptic Drugs (NEAD) Study is an ongoing prospective study in the US and UK that enrolled pregnant women with epilepsy on AEDs

- The study seeks to determine if differential long-term neurodevelopmental effects exist across four commonly used AEDs (carbamazepine, lamotrigine, phenytoin, valproate)

- Valproate has been found to be associated with a higher risk of malformations than other AEDs, particularly NTDs.

- *The impact of fetal exposure to maternal AEDs on cognitive and behavioral development suggests that maternal use of some AEDs during pregnancy may negatively impact cognition in the infants of these women with epilepsy.*

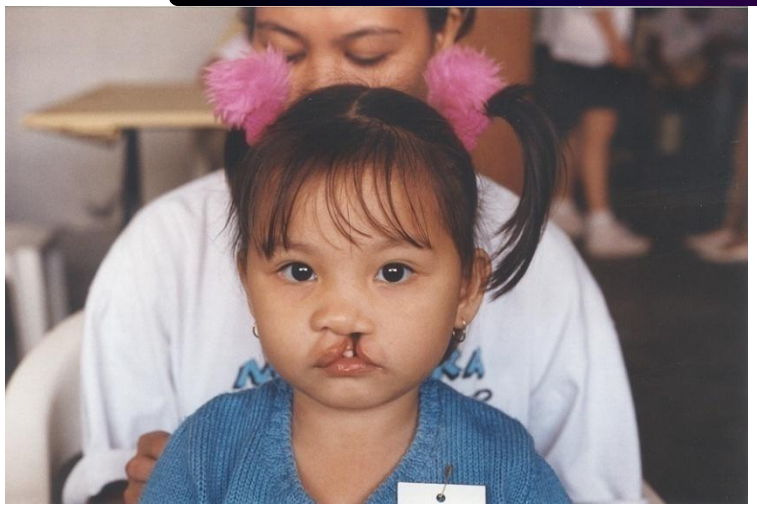
In Utero Exposure to Anti-Epileptic Drugs

- *In utero* exposure to AEDs has also been linked with autism spectrum disorder (ASD) primarily with exposure to carbamazepine or valproate, compared with other AEDs

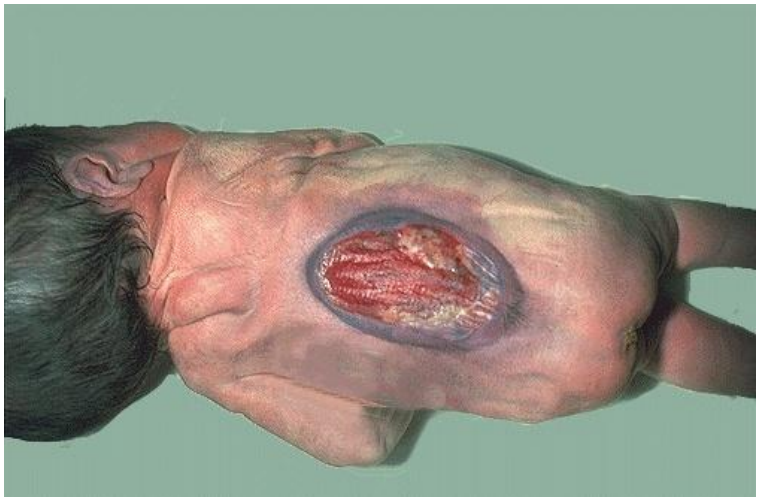
- Another study from the UK found 8.9% of children exposed *in utero* to valproate to meet Diagnostic and Statistical Manual of Mental Disorders IV criteria for ASD, with prevalence more than 8 times greater than in the general population

- The LMNDG study found that 6.3% of children exposed to VPA *in utero* had ASD, 7 times higher than the control group. The early evidence clearly supports *in utero* exposure to some AEDs, particularly VPA, is associated with adverse behavioral effects in children.
- VPA is a folate antagonist

Folate Responsive Complex Congenital Malformations



Cleft Lip and Palate

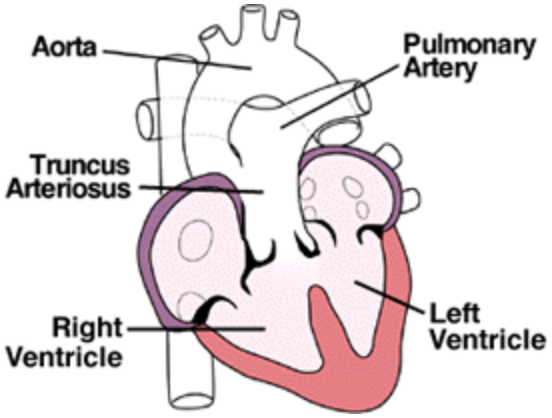


Spina Bifida

Phenotypes are often controlled by multiple genes and environmental factors

Genetic background will modify the expression of most complex and even Mendelian traits

Few genes contributing to complex traits have been identified in last 20 years given the difficulty of studying gene-environmental interactions



Conotruncal Heart Defects

It All Started with Spinach

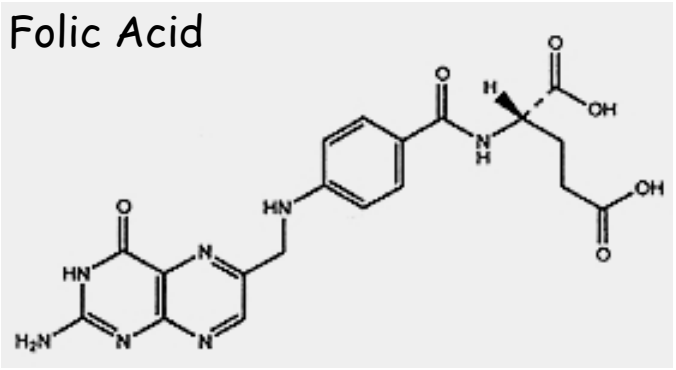
Spinacia oleracea

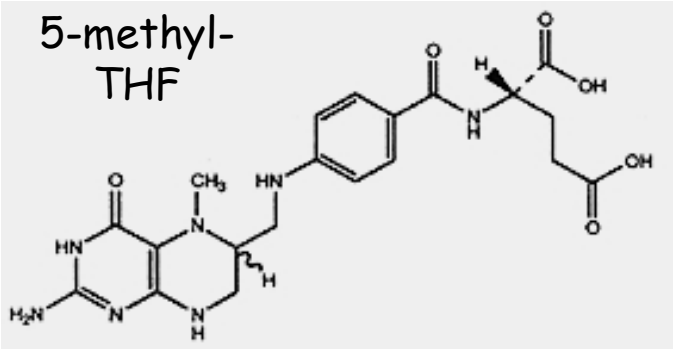
- 1940s-Start with 4 tons of spinach, lots of chemistry graduate students, and a steam kettle and filter press in the attic of Welch Hall at the Univ. of Texas

- Esmond Snell credited with the isolation of vitamin B9, which they named folic acid

Physiological Roles of Folates

- Folic acid (pteroylglutamic acid) is an essential vitamin
- *In vivo*:
  - Reduced to bioactive tetrahydrofolates (THF)
  - Polyglutamated
- Biologic role: coenzyme in one-carbon metabolism
  - Synthesis of nucleic acids, amino acids, neurotransmitters
  - Methylation
    - 5MTHF is involved in >100 different methyl transfer reactions





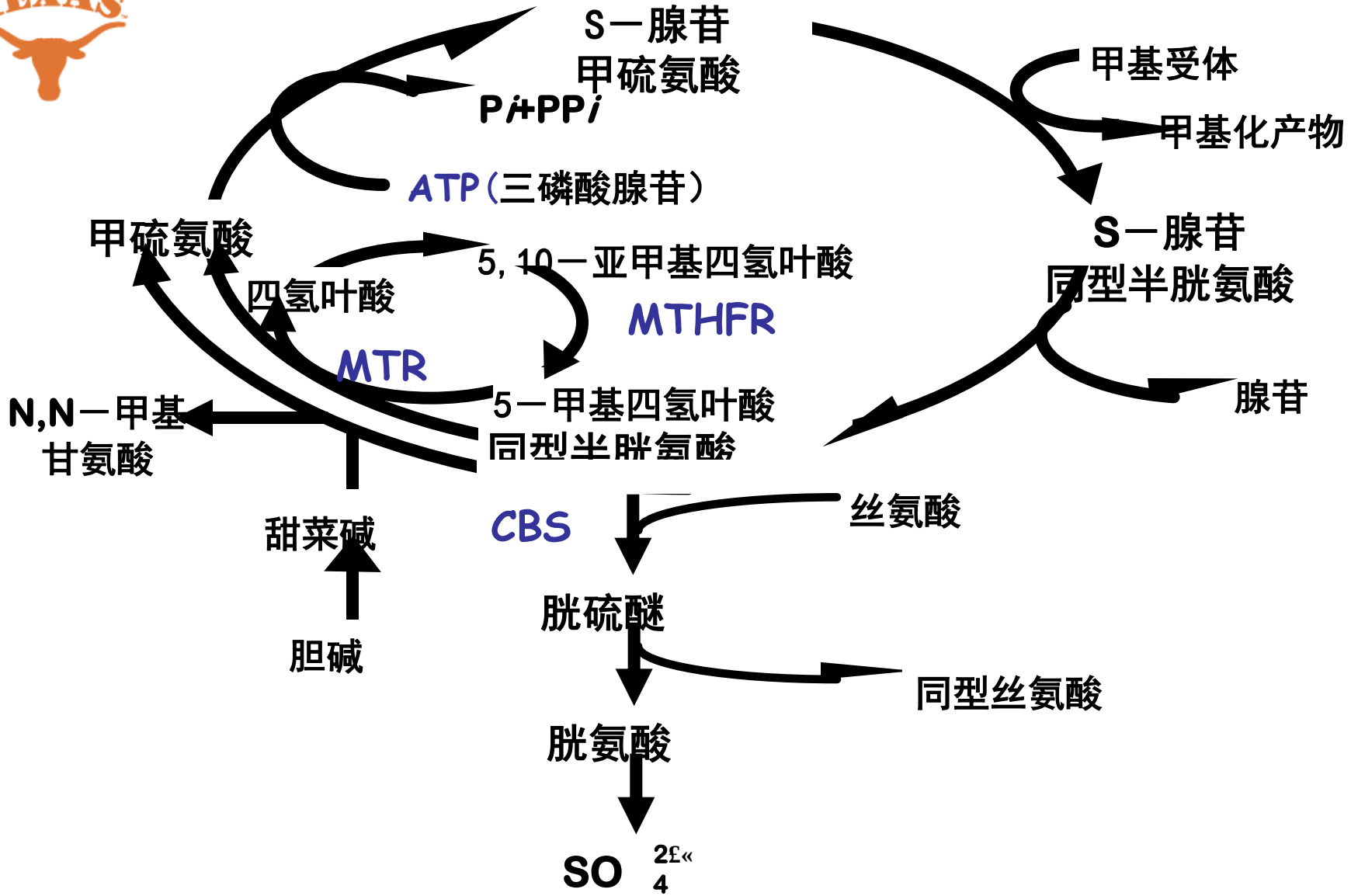


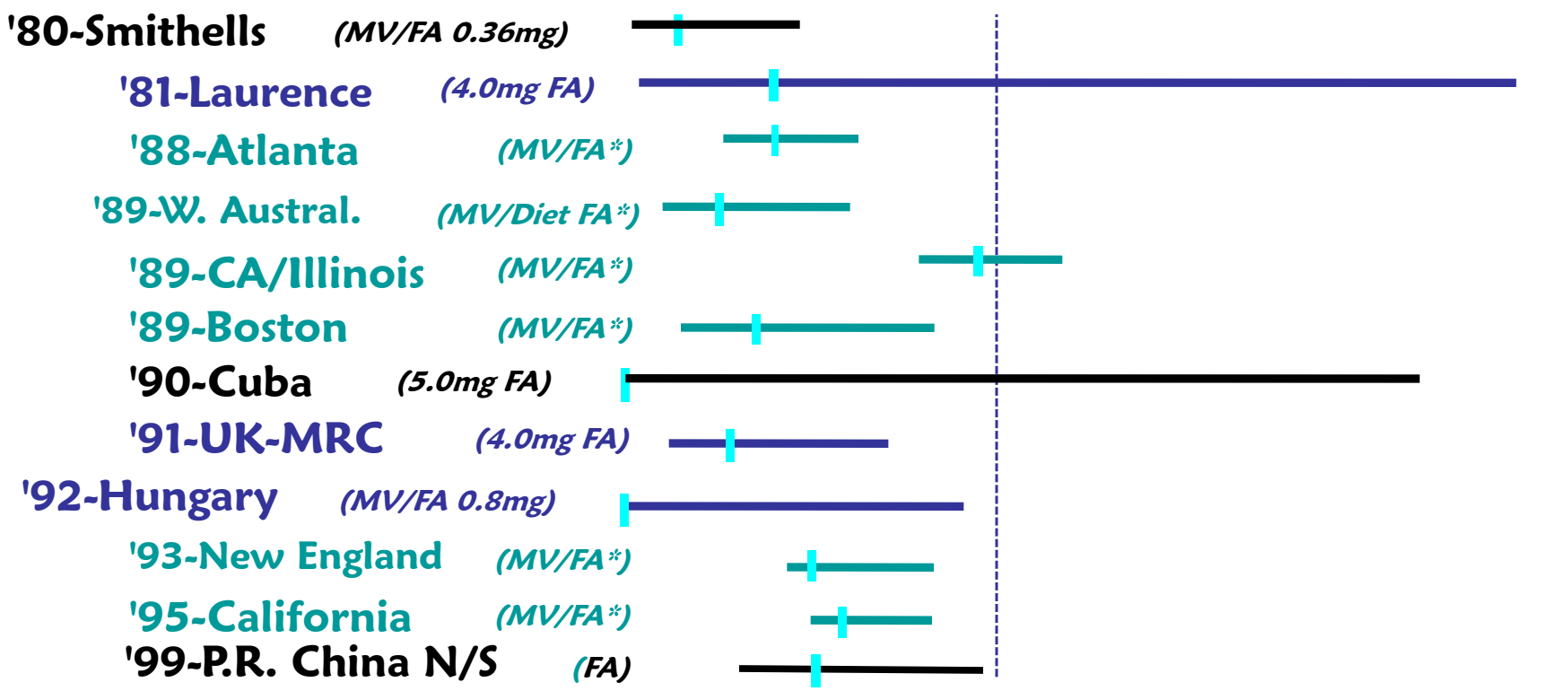
图1　同型半胱氨酸代谢

CBS：胱硫醚β合成酶

MTR：蛋氨酸合成酶

MTHFR：　亚甲基四氢叶酸还原酶

Folic Acid/Multivitamin - NTD Studies



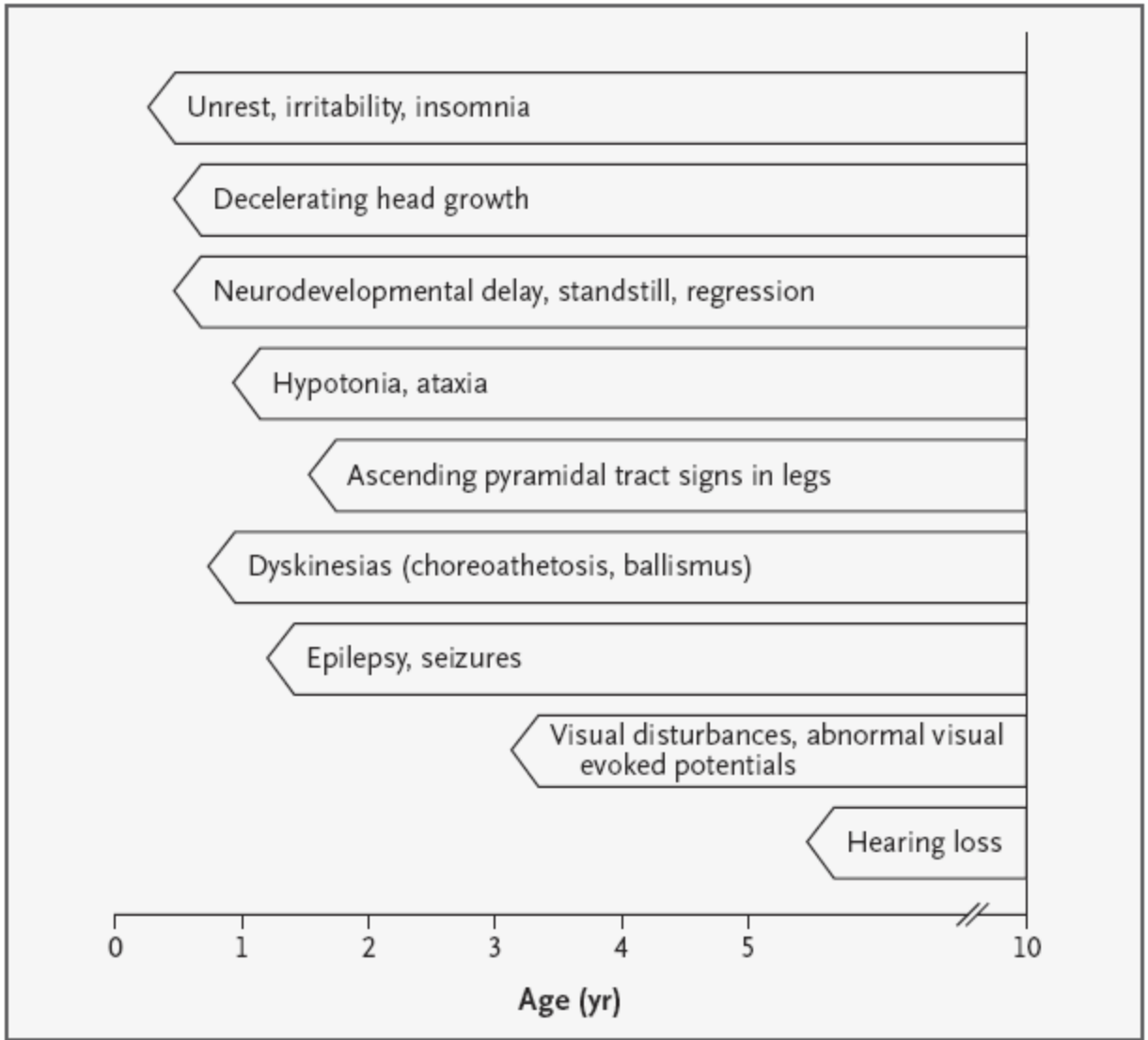
Randomized trials | Non-randomized trials | Observational studies

* *All observational studies researched lower dosage folic acid (FA) (0.1 - 1.0mg)*

Cerebral Folate Deficiency Syndrome

Photos used with permission

Major Clinical Features of the Cerebral Folate Deficiency Syndrome

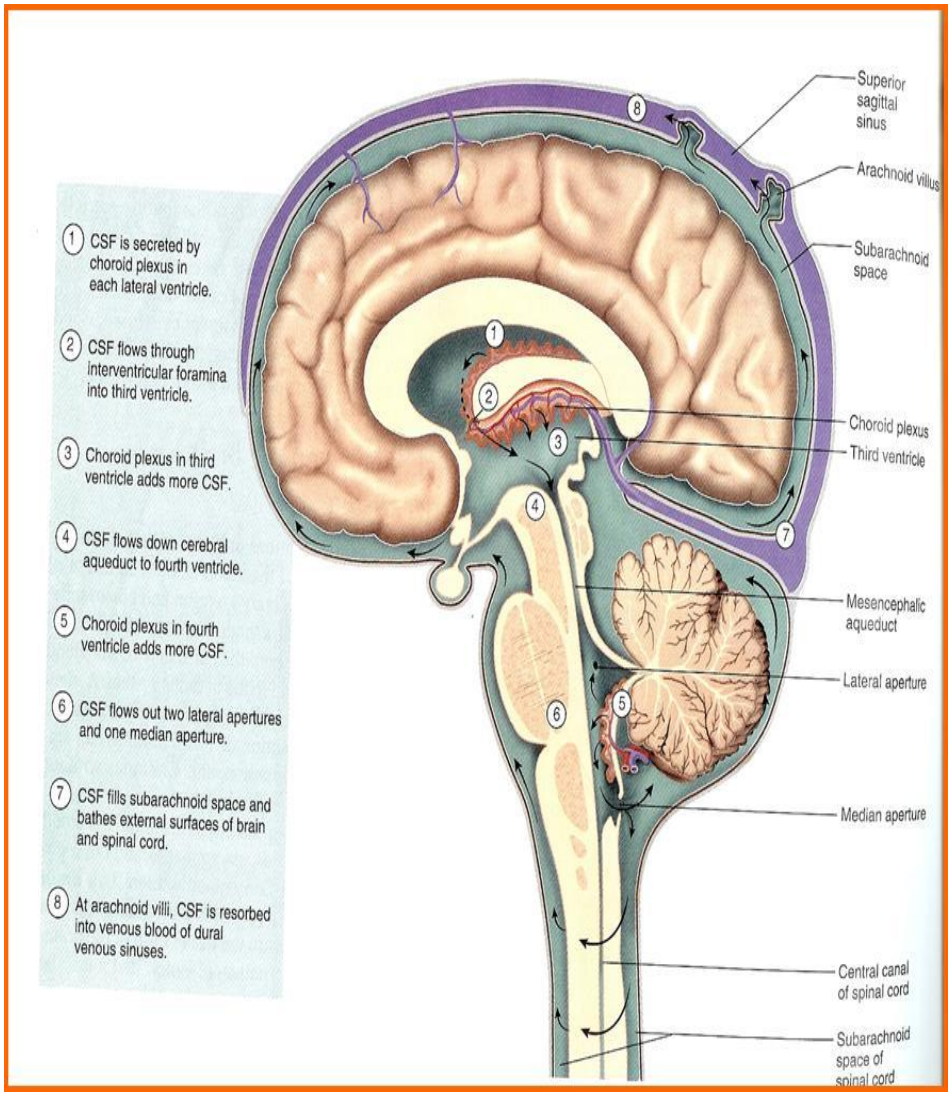


Ramaekers et al., *NEJM* 2005; 352:1985-1991

Cerebral Folate Deficiency and ASD

- Reduced folate transport to the CNS was identified in two autism spectrum disorders-Rett syndrome and Infantile Low-Functioning Autism with neurological abnormalities

- Among 28 patients affected by the infantile-onset cerebral folate deficiency (CFD) syndrome, 5 patients were found to manifest low-functioning autism with neurological deficits

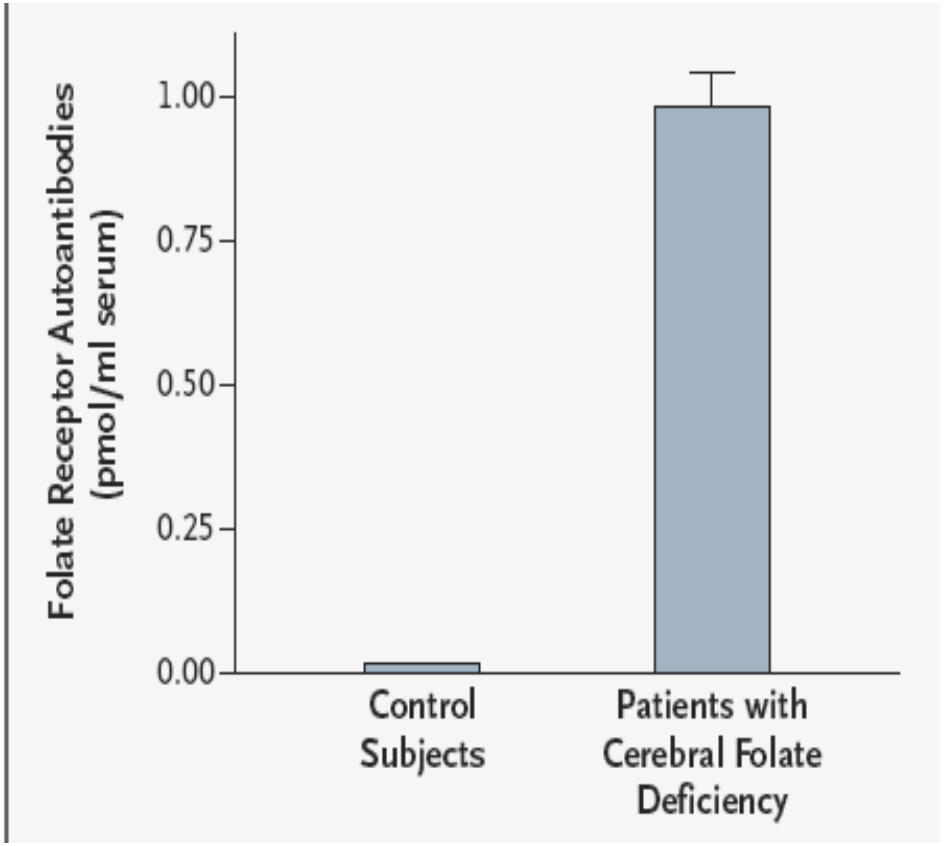


Ramaekers et al., *Neuropediatrics* 2007; 38(6): 276-281

Blocking Antibody Titers to the FR in the Cerebral Folate Deficiency Syndrome

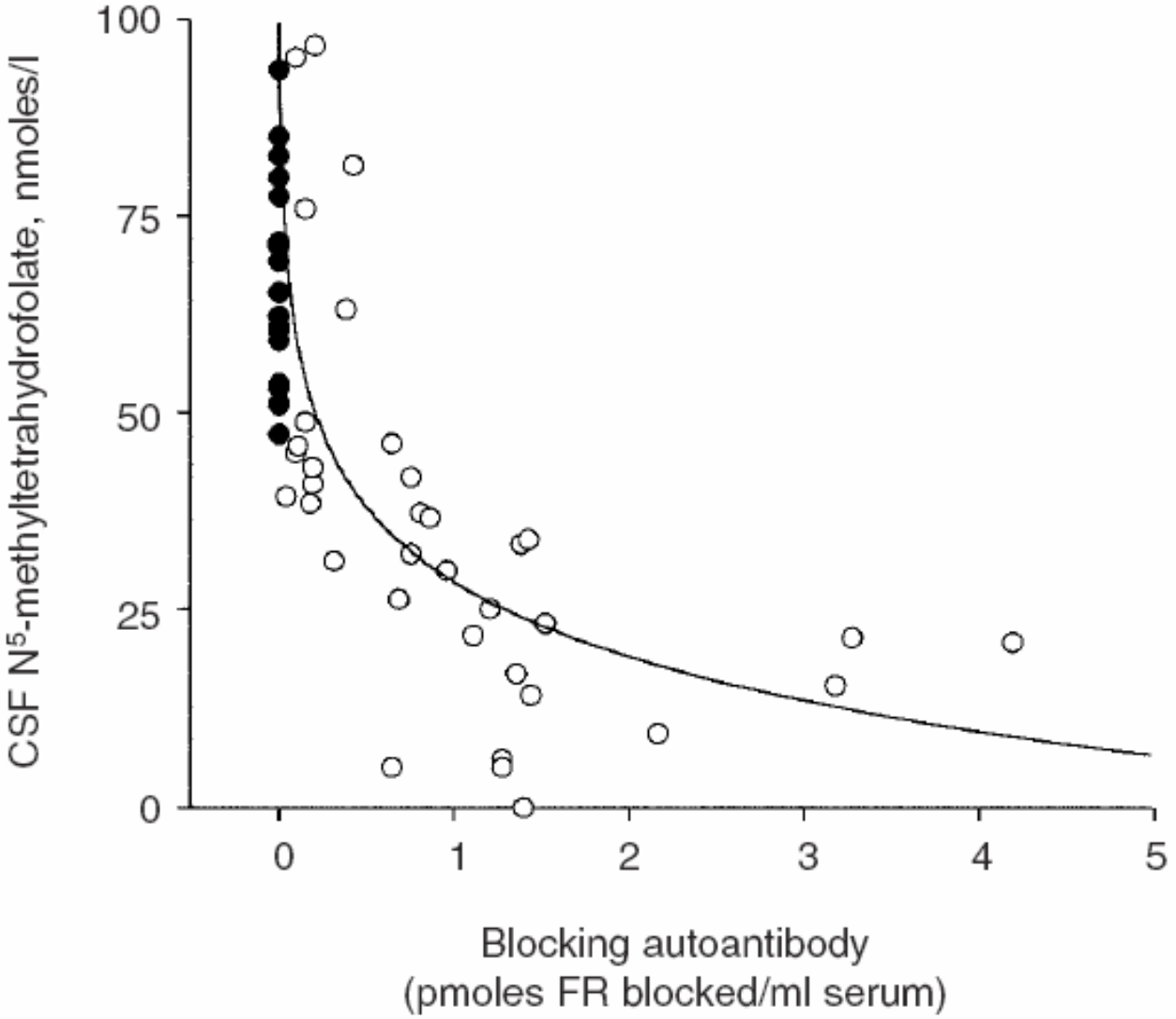
- In 4 of these 5 autistic patients with low 5MTHF in their cerebrospinal fluid (CSF), **the etiology was attributed to circulating autoantibodies in their serum directed against the folate receptors (FR).**

- In patients with CFD, these FR autoantibodies bind to the FR expressed on the choroid plexus and block the folate binding site, thereby **inhibiting the transport of folate into the CSF**

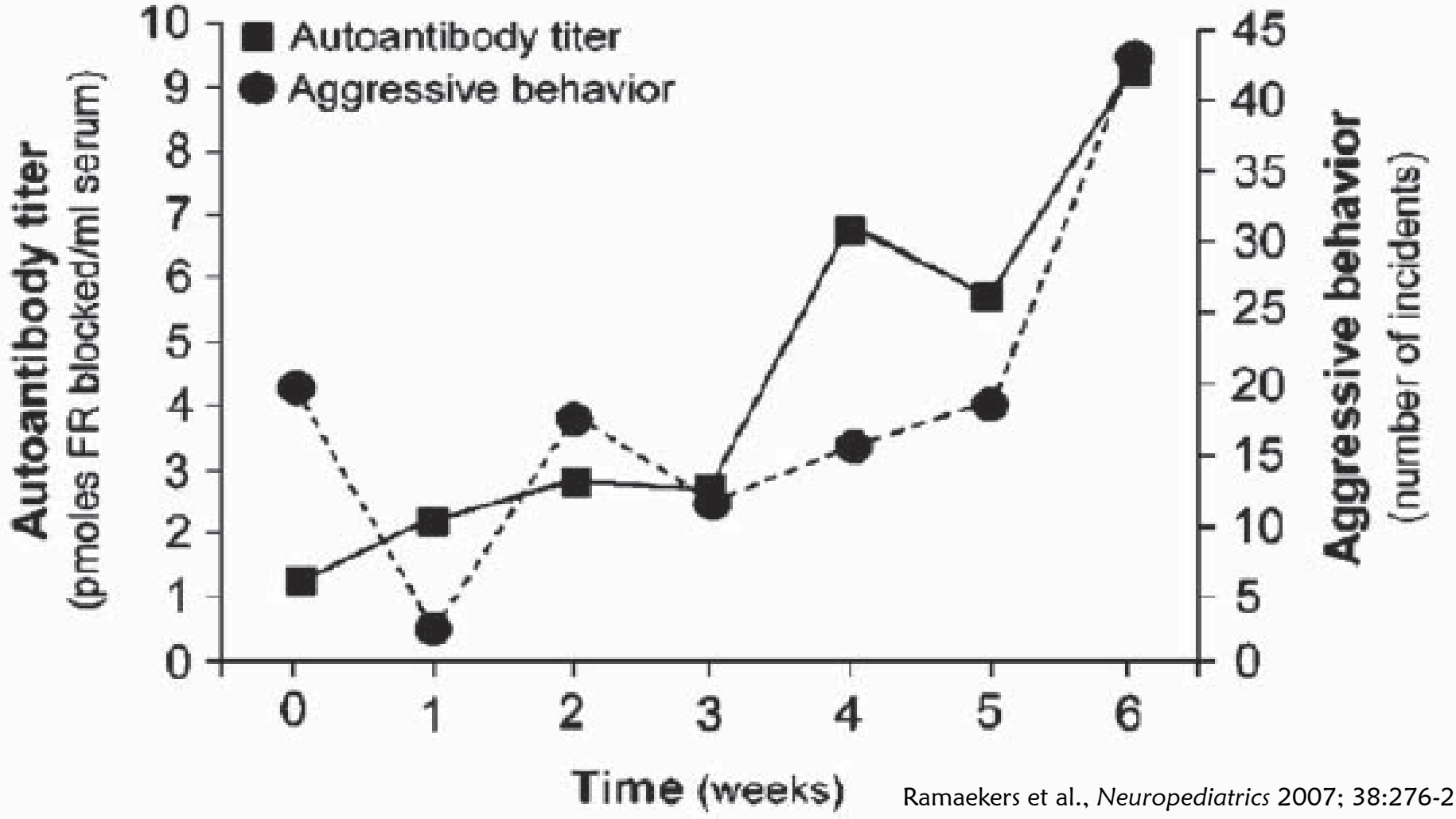


Ramaekers et al., *NEJM* 2005; 352:1985-1991

Blocking Folate Receptor Antibodies and Relationship to CSF 5MTHF Concentrations



Relationship Between FR Antibody Titers and Neurobehavioral Endpoints



Ramaekers et al., *Neuropediatrics* 2007; 38:276-281

The NEW ENGLAND JOURNAL of MEDICINE

Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect.

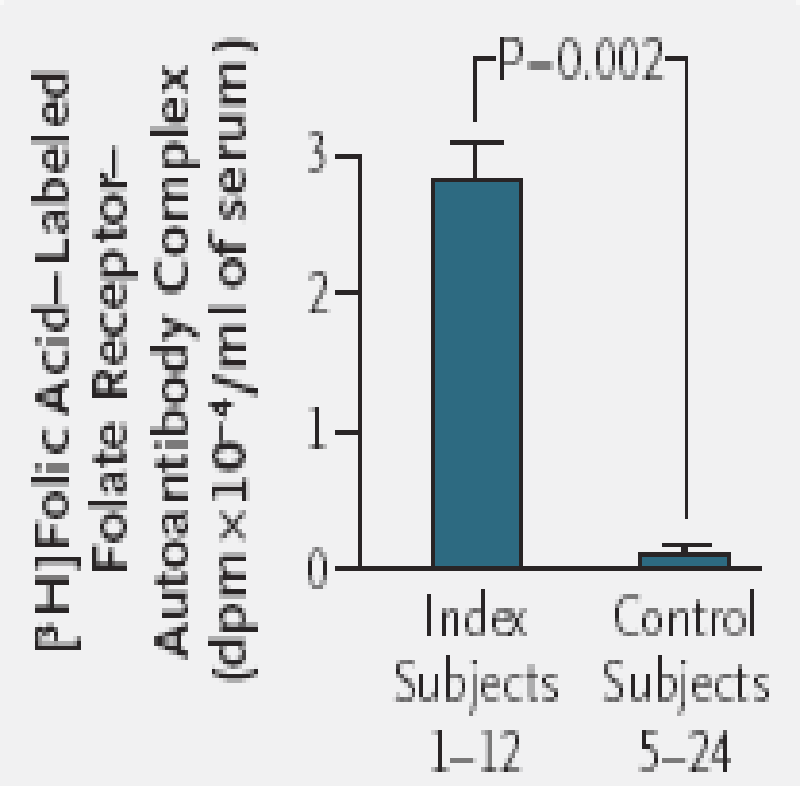
Rothenberg SP, daCosta MP, **Sequeira JM**, Cracco J, Roberts JL, Weedon J and **Quadros EV**

N Engl J Med. **2004 Jan** 8;350(2):134-42

Folate Receptor Auto-Antibodies:

An NTD Risk Factor?

Autoantibodies against Folate Receptors in Women with a Pregnancy Complicated by a Neural-Tube Defect (Rothenberg *et al. 2004)*



N Engl J Med 2004;350:134-42.

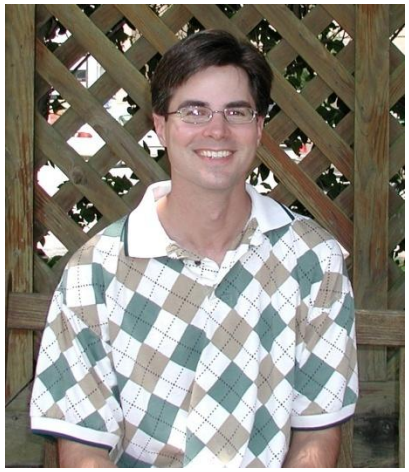
A Friendly Rivalry in Folate Receptor Antibody Studies

Finnell Laboratory vs. Quadros Laboratory

Folate Auto Antibody Titers

	Controls	NTD Cases	P-Values
		Mean Assay Values	
• FR (IgG)	5.7	12.5	0.02
• FR (IgM)	59	79.5	<0.001
• FR (FA blocked)	2.6	8.2	0.002
• Paraoxonase (U/mL)	129.7	102.2	0.26

Dr. Liz Gorman



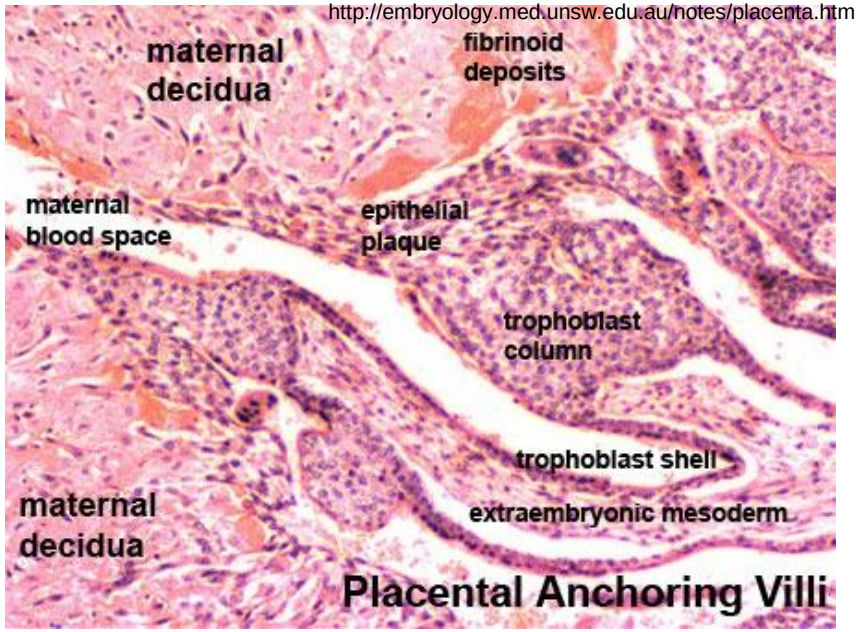
Dr. Johnathan Ballard

Cabrera et al., *Repro. Immunol*. 79:85-92, 2008

PLACENTAS

Collected placentas between 2004 to 2005.

22 NTD affected pregnancies and 29 controls. (n=51)

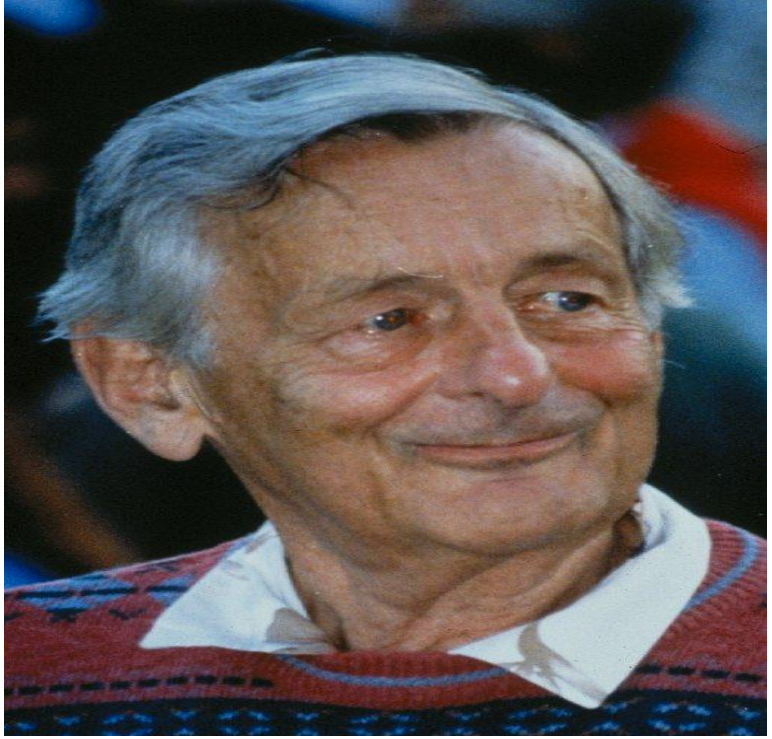


	mean CONTROLS (n=29)	mean CASES (n=22)	p-value
Anti FRα autoantibody titers (ng/mL)	**2.47** CI 95% (2.06 – 2.89)	**5,11** CI 95% (2.88-7.25)	**0.01**
FRα **blocking** autoantibodies (ng/mL)	**12.81** IC 95% (9.25–16.36)	**22.36** IC 95% (11.58 – 33.14**)**	**0.05**

Are There Gene X Environment X Nutritional Status Interactions Known to Affect Maternal Immune Responses that Affect Susceptibility to Neurodevelopmental Disorders?

"NTDs are caused by a little bit of this and a little bit of that"

Clarke Fraser
09/12/09



F. Clarke Fraser

Maybe ASDs too?

R. Finnell
11/11/11

Finnell Laboratory
Dell Pediatric Research Institute

Research Support

National Institutes of Health
HD067244 National Institute of Maternal and Child Health
NS076465 National Institute of Neurological Diseases and Stroke
HL085859 National Heart, Lung and Blood Institute

Centers for Disease Control and Prevention
Centers of Excellence for Surveillance, Research, Service and Evaluation of Birth Defects-UO1/DD000491, U01/DD000493 and UO1/DD000494

US Environmental Protection Agency
RD-83428901 Texas-Indiana Virtual STAR Center