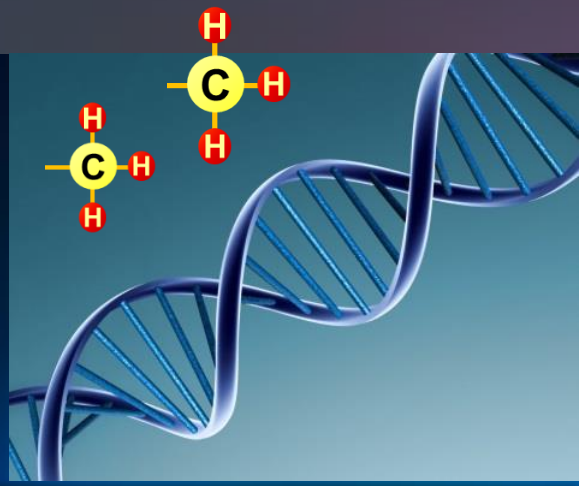
Reducing Prenatal Risk for Autism

Rebecca J. Schmidt, Ph.D.

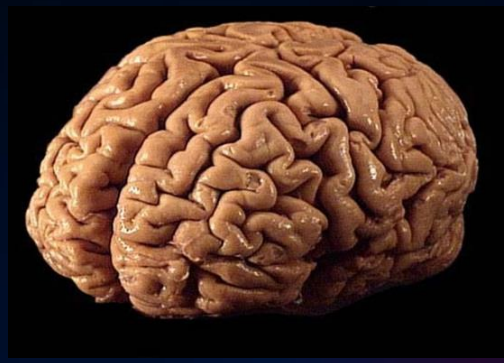
Assistant Professor of Public Health Sciences,

UC Davis School of Medicine and the MIND Institute



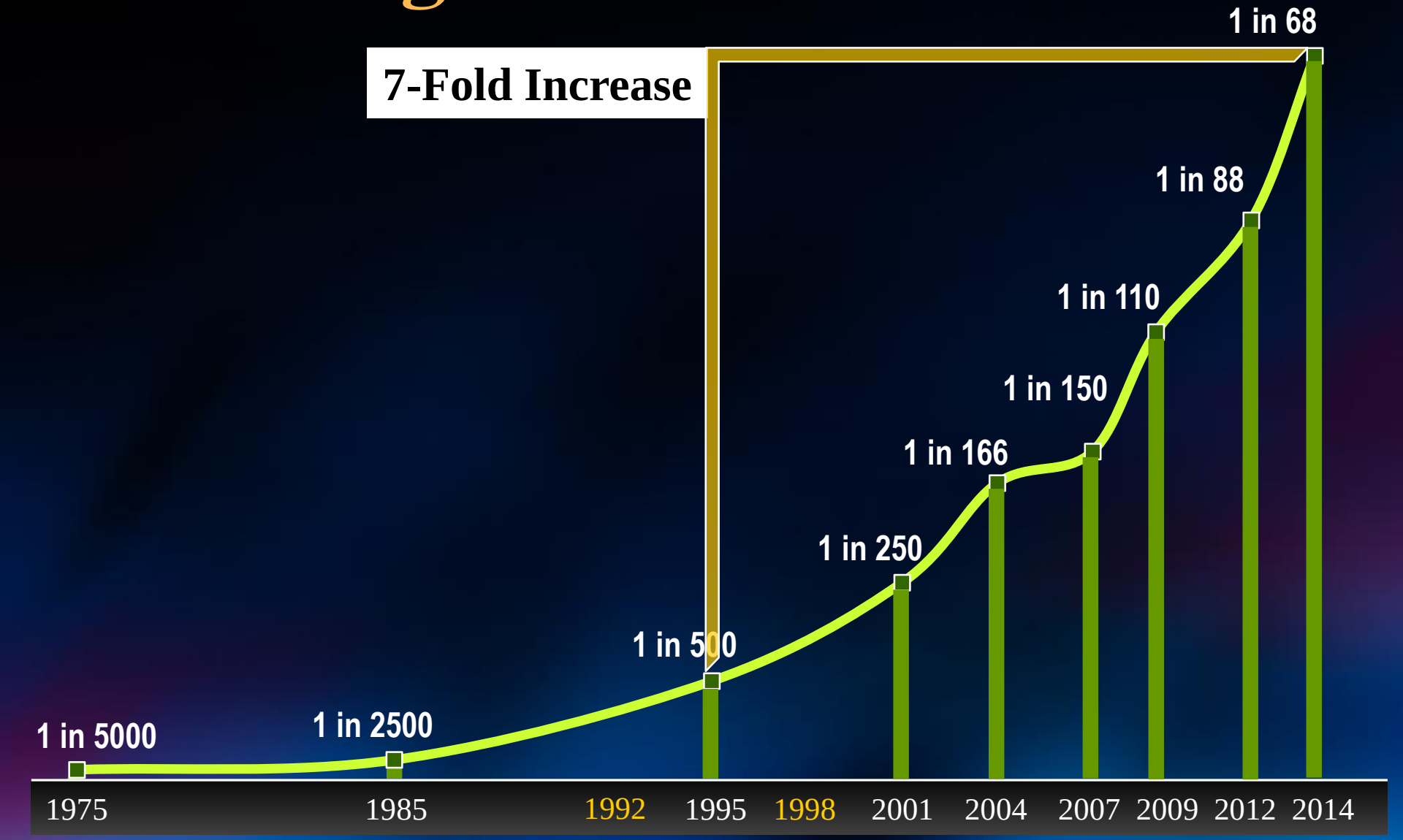
UC DAVIS
UNIVERSITY OF CALIFORNIA

Autism Spectrum Disorder



- Neurodevelopmental Disorder
  - Impairments in Social Reciprocity
  - Language / Communication Deficits
  - Repetitive Behaviors / Restricted Interests
- Neurobiologic Basis: Aberrant Brain Development
- Sex ratio 4-5 M : 1 F (CDC 2014)
- Prevalence in US: 1 in 68 (CDC 2014)

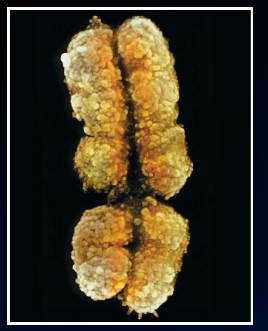
Rising Autism Prevalence



UC DAVIS
UNIVERSITY OF CALIFORNIA

Adapted Figure from Autism Speaks

ASD Etiology



Causes Unknown

Highly Heritable

Sibling Risk ~1/5

Hallmayer et al, 2011 (US)

H=38% (14%-67%) for ASD

Sandin et al, 2014 – Largest study (Sweden)

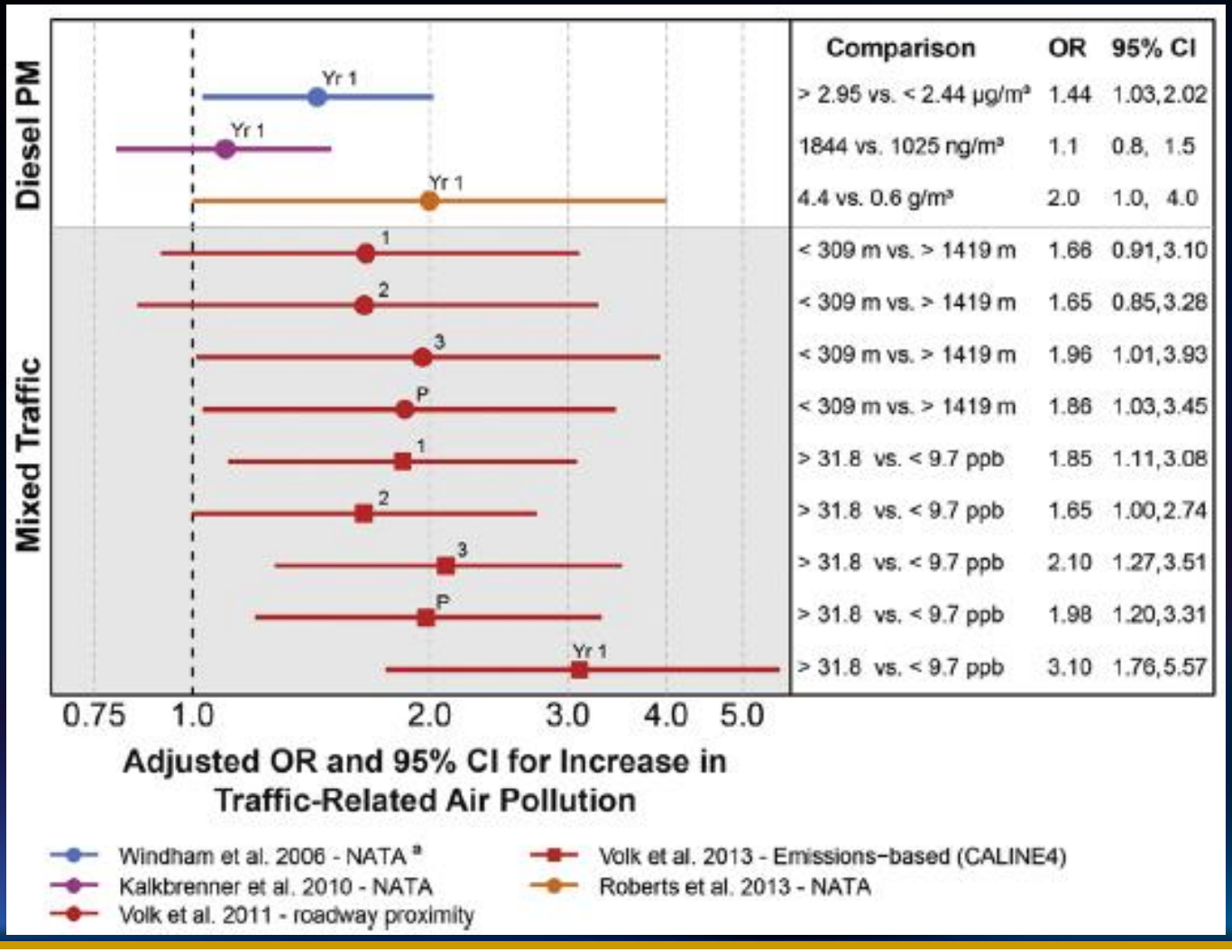
H=50% (45%-56%) for ASD

Risk Factors for ASD

- Advance Parental Age (Shelton et al 2010)
- Prenatal Infections (Rubella) (Chess 1971; Arndt et al 2005)
- Closely Spaced Pregnancies (Cheslack-Postava et al 2014)
- Month of Conception (Zerbo et al 2011)
- Medications (Deykin & MacMahon 1980; Gillberg & Gillberg 1983; Piven et al 1993)
  - Thalidomide (for morning sickness) (Stromland et al1994; Lotter 1966)
  - Valproic acid (anti-epileptic) (Moore et al 2000; Rasalam et al 2005)
  - SSRIs (antidepressant) (Croen et al 2011)
- Maternal Metabolic Conditions (Krakowiak et al 2012; Li et al 2016)

Risk Factors for ASD

Traffic-Related Air Pollution



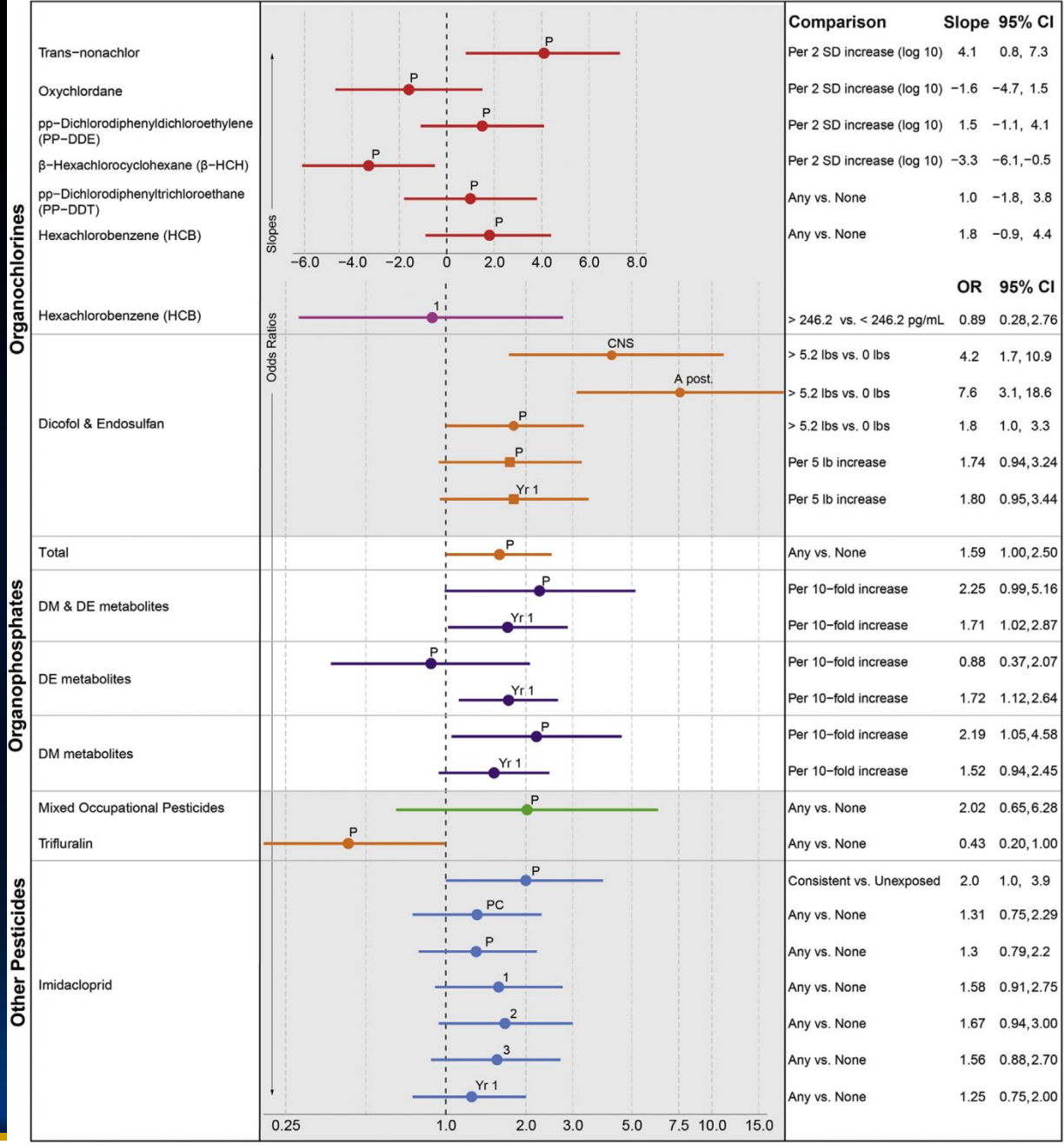
UC DAVIS
UNIVERSITY OF CALIFORNIA

Kalkbrenner, Schmidt & Penlesky 2014

Risk Factors for ASD

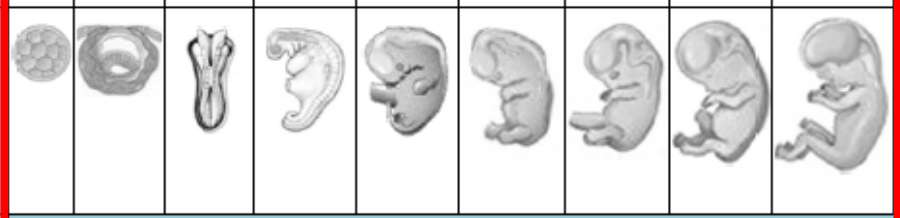
Pesticides

Kalkbrenner, Schmidt & Penlesky 2014



UC DAVIS
UNIVERSITY OF CALIFORNIA

Critical Periods of Susceptibility Indicated from Studies of ASD

Trimester	First									Second			Third	
Gestational Weeks	1	2	3	4	5	6	7	8	9	16	20	22	28	38
Brain Pathology														
Neurogenesis[1-3]	Weeks 1-20													
Neuronal Migration[1,4]	Weeks 1-16													
Neuronal Maturation[1,5]	Weeks 1-24													
Cortical Layer Formation, Organization, and Neuronal Differentiation[6]	Weeks 1-30													
Exposure														
Freeway Proximity[7]													3rd Trimester	
Traffic-related Air Pollution[8]	1st, 2nd, and 3rd Trimester													
Pesticides[9-11]				Days 26-81										
Prenatal Vitamins[12]	1st Month and 3 Months Before													
Folic Acid[13,14]	1st Month[a]													
Rubella Infection[15,16]	Weeks 1-8													
Fever[17,18]	1st and 2nd Trimester													
Thalidomide[19]				Days 20-24										
Valproic Acid[20,21]				Days 22-28										
SSRI[22, 23]	1st Trimester[b]													
Prenatal Stressors[24]												Weeks 25-28		

Days=Fetal days after conception. For exposures with more than one study, dark blue indicates overlapping period and light blue indicates timing suggested by one but not all studies

UC DAVIS UNIVERSITY OF CALIFORNIA

Schmidt RJ, Lyall K, Hertz-Picciotto I; 2014. *Cutting Edge Psychiatry in Practice*: CEPiP 2014;1:21-38

Reducing Risk for ASD

- Avoid exposures associated with increased risk for ASD:
  - Traffic-related air pollution (don't live within 100 m of a major freeway)
  - Pesticides (use alternative methods to kill pests, do not have your home sprayed/fogged regularly, do not live next to agricultural fields)
- Mechanisms unknown but could involve direct neurotoxic effects, inflammation, oxidative stress, epigenetic effects

Protective Factors for ASD

- Maternal prenatal vitamins near conception
  (Schmidt et al 2011)
- Maternal Iron during pregnancy & BF
  (Schmidt et al 2014)
- Maternal Folic Acid near conception
  (Schmidt et al 2012, Suren et al 2013)

- Might counter the effects of risk factors

CHARGE

Findings from the

CHARGE CASE-CONTROL STUDY

CHildhood Autism Risks from Genetics And Environment

PI: Irva Hertz-Picciotto

UCDAVIS
UNIVERSITY OF CALIFORNIA

3 Diagnostic Groups

California DDS

1. Autism
2. Developmental Delay

ASD

DD

California Birth files

3. General Population Frequency matched to projected distributions in cases of: age, gender & geography

TD

UCDAVIS
UNIVERSITY OF CALIFORNIA

Maternal Iron

Total Iron Intake (mg)	Maternal Age <35 Years Adjusted OR[a] (95% CI)	Maternal Age 35+ Years Adjusted OR[a] (95% CI)
Highest Quintile (86+)	Reference	0.6 (0.2 – 1.7)
Quintiles 2-4 (30 - <86)	1.5 (0.9 - 2.4)	1.9 (0.98 – 3.5)
Lowest Quintile (< 30)	1.4 (0.8 - 2.5)	**5.0** (2.0 – 12.7)

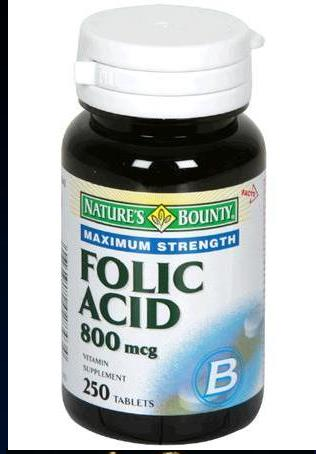
Total Iron Intake (mg)	No Metabolic Condition During Pregnancy[b] Adjusted OR[a,c] (95% CI)	Metabolic Condition During Pregnancy[b] Adjusted OR[a,c] (95% CI)
Highest Quintile (86+)	Reference	**1.5** (0.4 – 5.8)
Quintiles 2-4 (30 - <86)	**1.6** (0.98 - 2.6)	**2.2** (1.0 - 4.3)
Lowest Quintile (< 30)	**1.6** (0.9 - 2.8)	**4.7** (1.7 – 13.2)

[a] Adjusted for maternal folic acid intake, child birth year, home ownership
[b] Metabolic conditions include obesity (prepregnancy BMI≥30), hypertension, diabetes
[c] Adjusted for maternal race/ethnicity & education; child sex, type of health insurance, & regional center catchment area

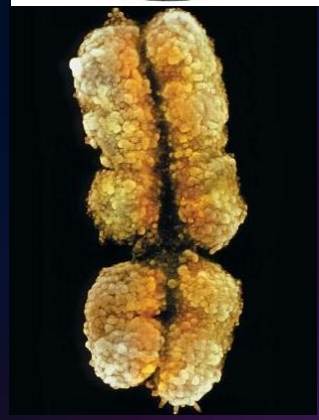
UC DAVIS UNIVERSITY OF CALIFORNIA

Schmidt et al 2014

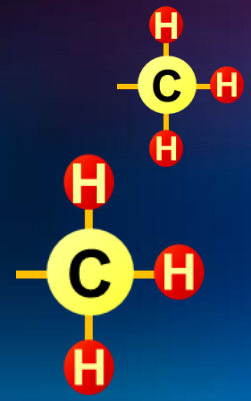
Maternal Folic Acid



- Higher folic acid linked to ~40% lower ASD risk
- Near conception
- Dose-response
- Replicated
- Especially for individuals with inefficient 1-C metabolism



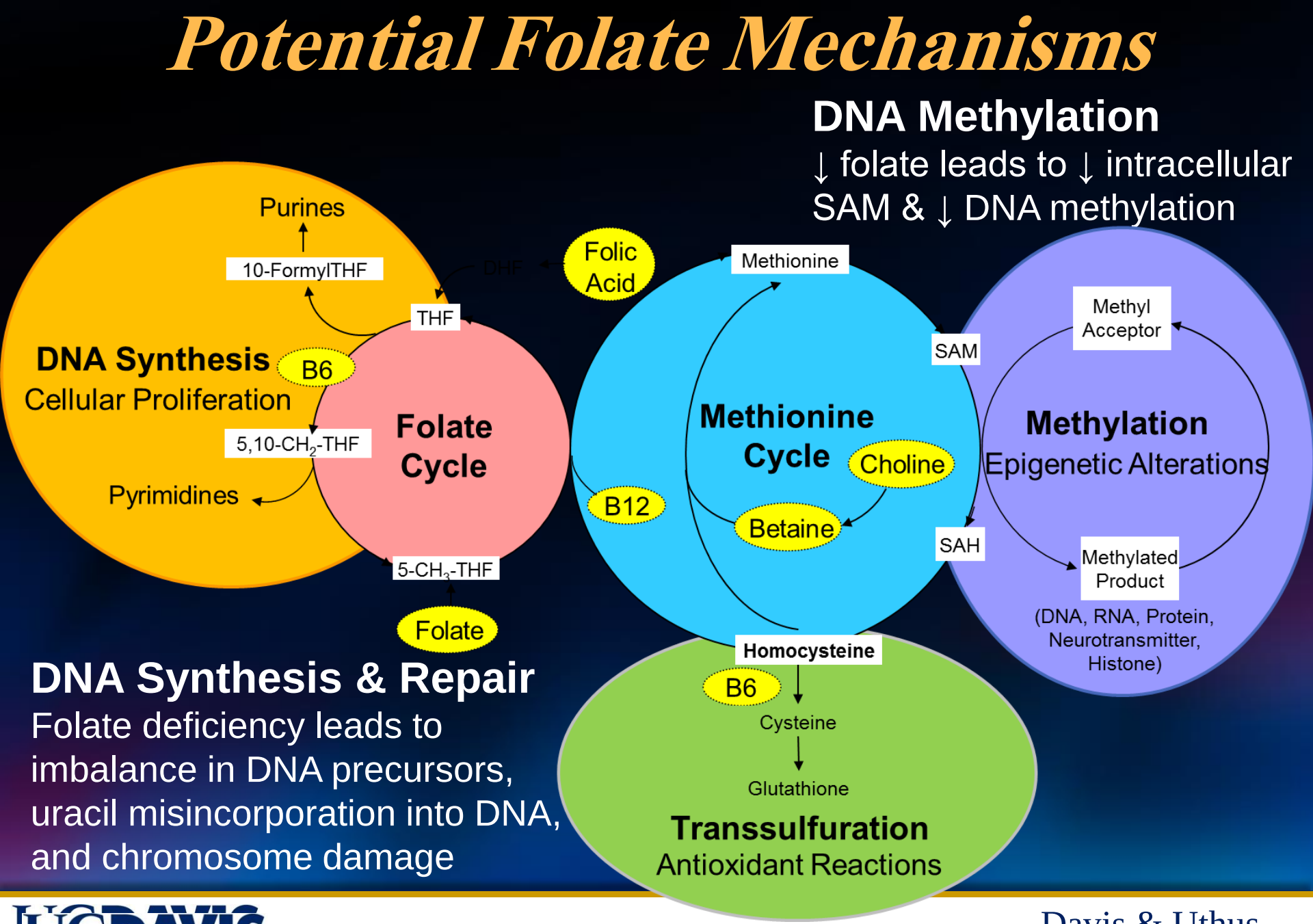
- Mechanisms



UC DAVIS
UNIVERSITY OF CALIFORNIA

Schmidt et al, 2011; Schmidt et al, 2012; Suren et al 2013, Braun et al 2014, Steenweg-de Graaff et al 2014, Virk et al 2015

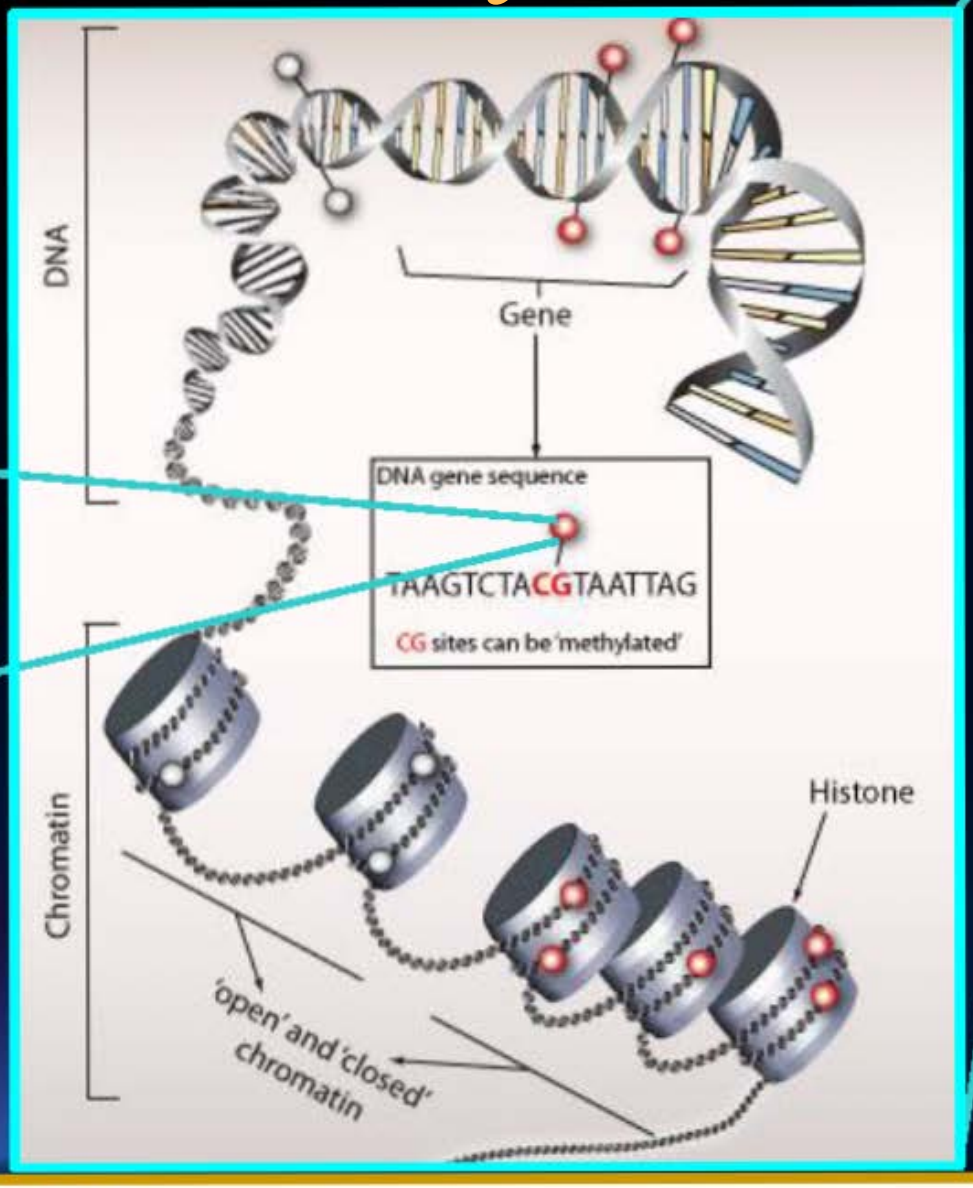
Potential Folate Mechanisms

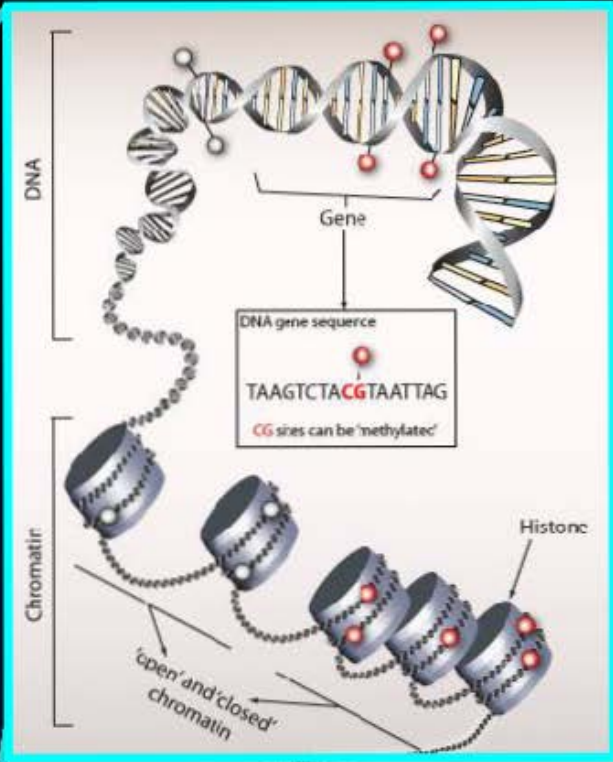


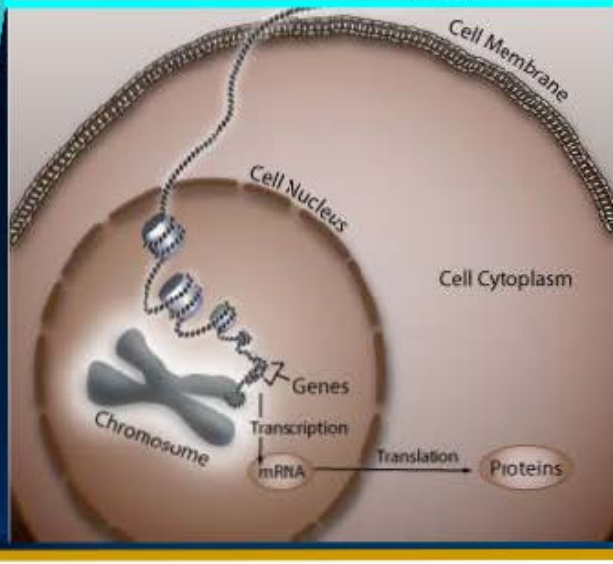
UC DAVIS
UNIVERSITY OF CALIFORNIA

Davis & Uthus 2004; Duthie 1999

DNA Methylation

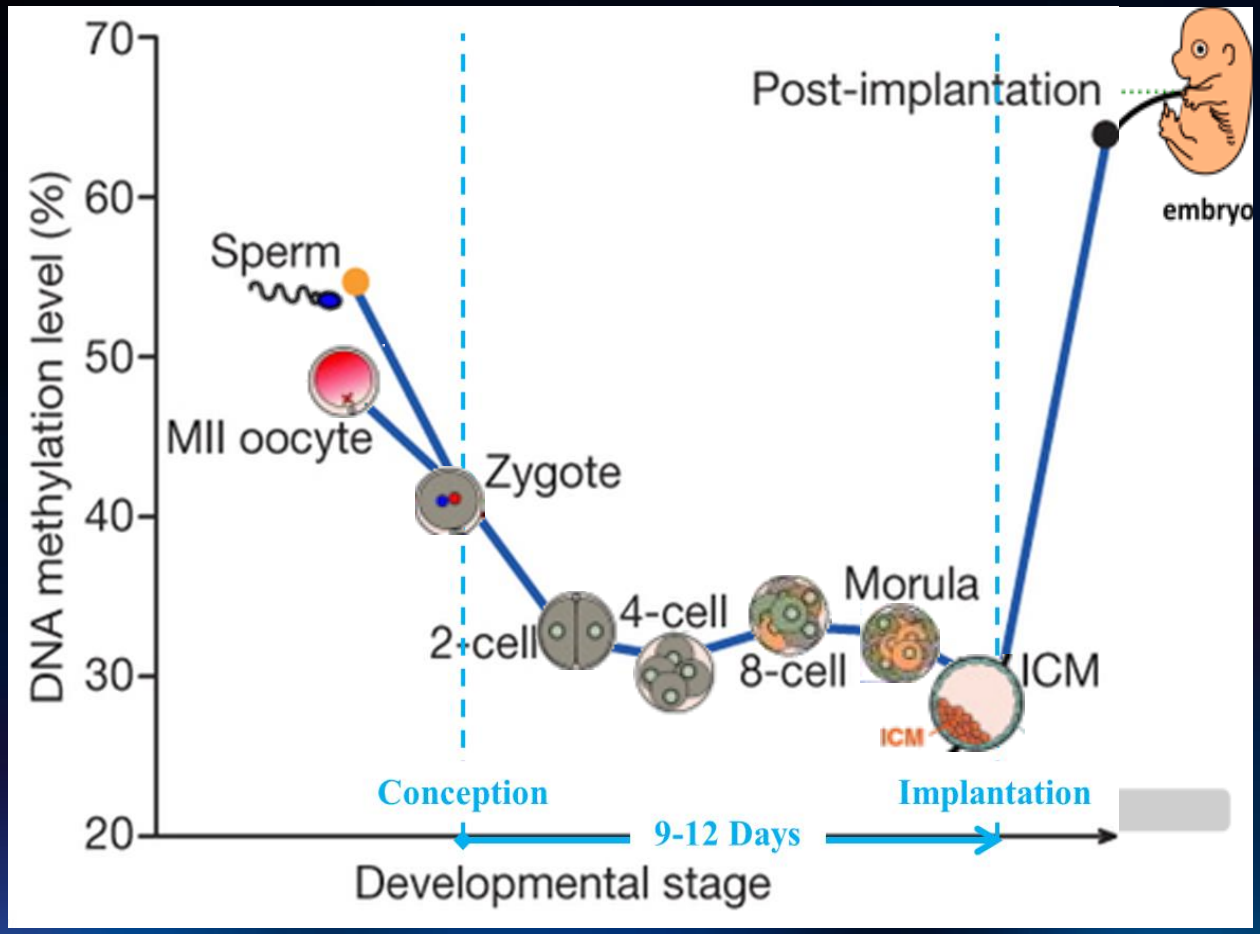






UC DAVIS
UNIVERSITY OF CALIFORNIA

The DNA Methylation Life Cycle

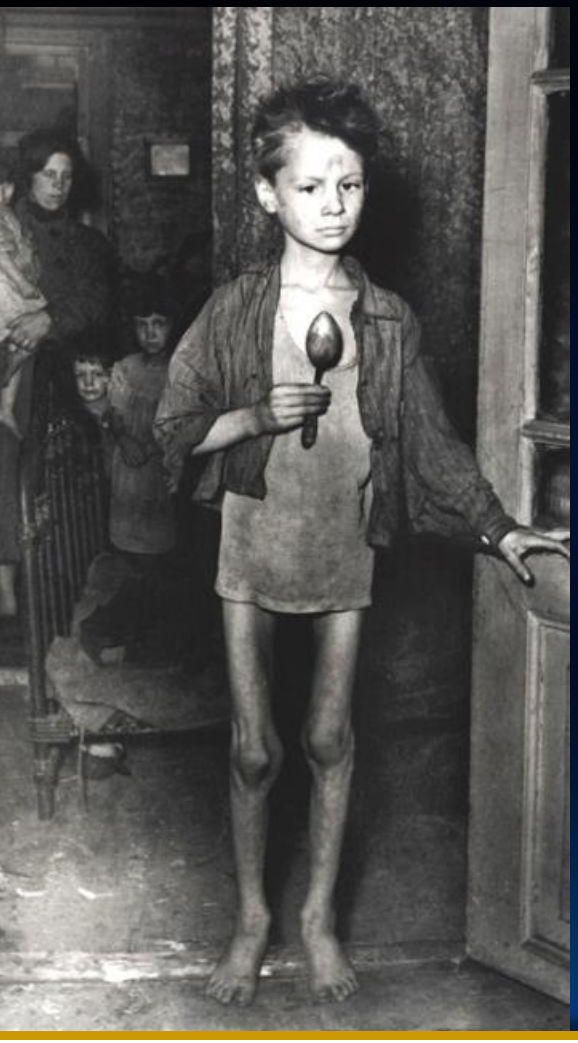


UCDAVIS UNIVERSITY OF CALIFORNIA

http://www.sciencedirect.com/science/article/pii/S0168952511001582

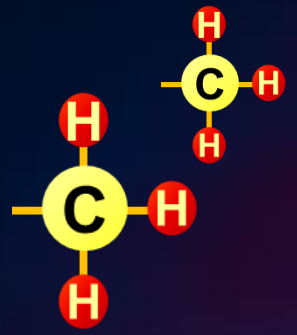
Guo et al, 2014 (July 31) *Nature* 511:606-610, Smith et al, 2014

Folate and Methylation



Maternal dietary methyl donors affect offspring's:

- DNA methylation
- Gene expression
- Health outcomes



UC DAVIS
UNIVERSITY OF CALIFORNIA

Tobi et al 2009 Hum Molec Genet 18(21):4046–4053
Wolf et al 1998 FASEB J 12(11):949-957
Cooney et al 2002 J Nutr 132(8):2393S-2400S

Methyl Pool

Dietary Inputs

Folate
Vitamin B12

One-carbon Cycle

SAM

DNA Methylation

Oxidative Stress
Glutathione Reactions

Genomic Instability

Cellular Proliferation
DNA Synthesis

Environmental Toxicants
SAM Inhibitors (Hcy)

CNV Duplication
DNA Repair

Growth
Inflammation
Immune Reactions

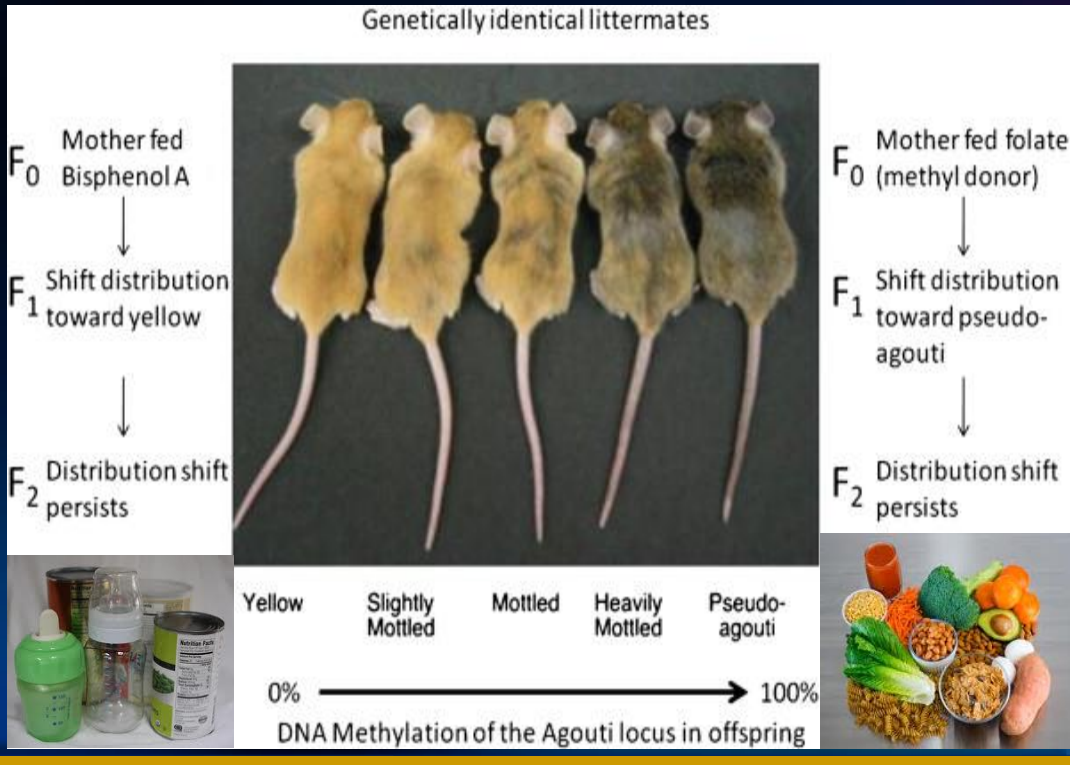
UC DAVIS
UNIVERSITY OF CALIFORNIA

Animal Evidence: Agouti Mice

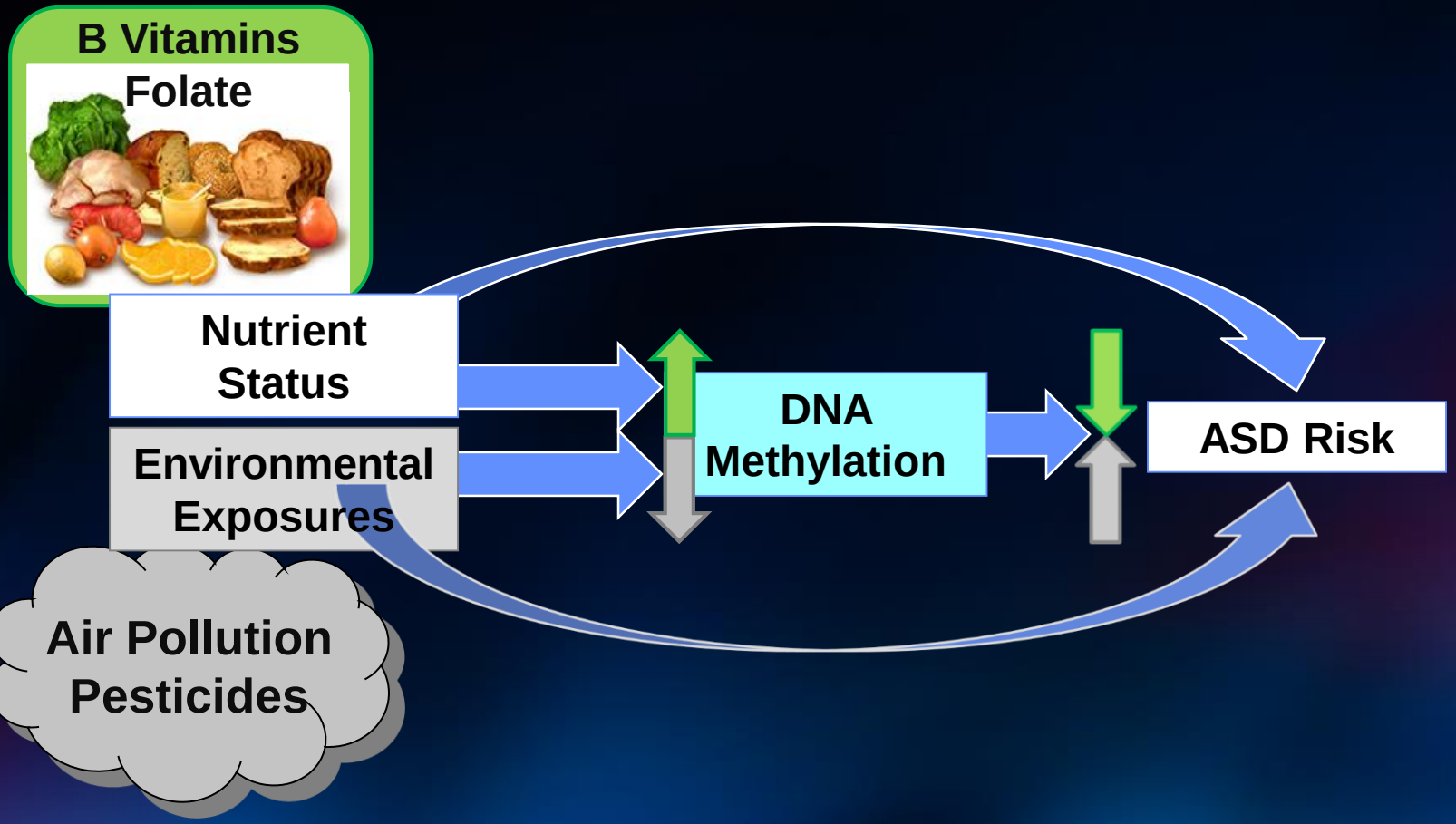
Bisphenol A (BPA)

- Industrial chemical used to make certain resins and plastics often used in containers that store food and beverages
- Maternal exposure induces DNA hypomethylation in offspring

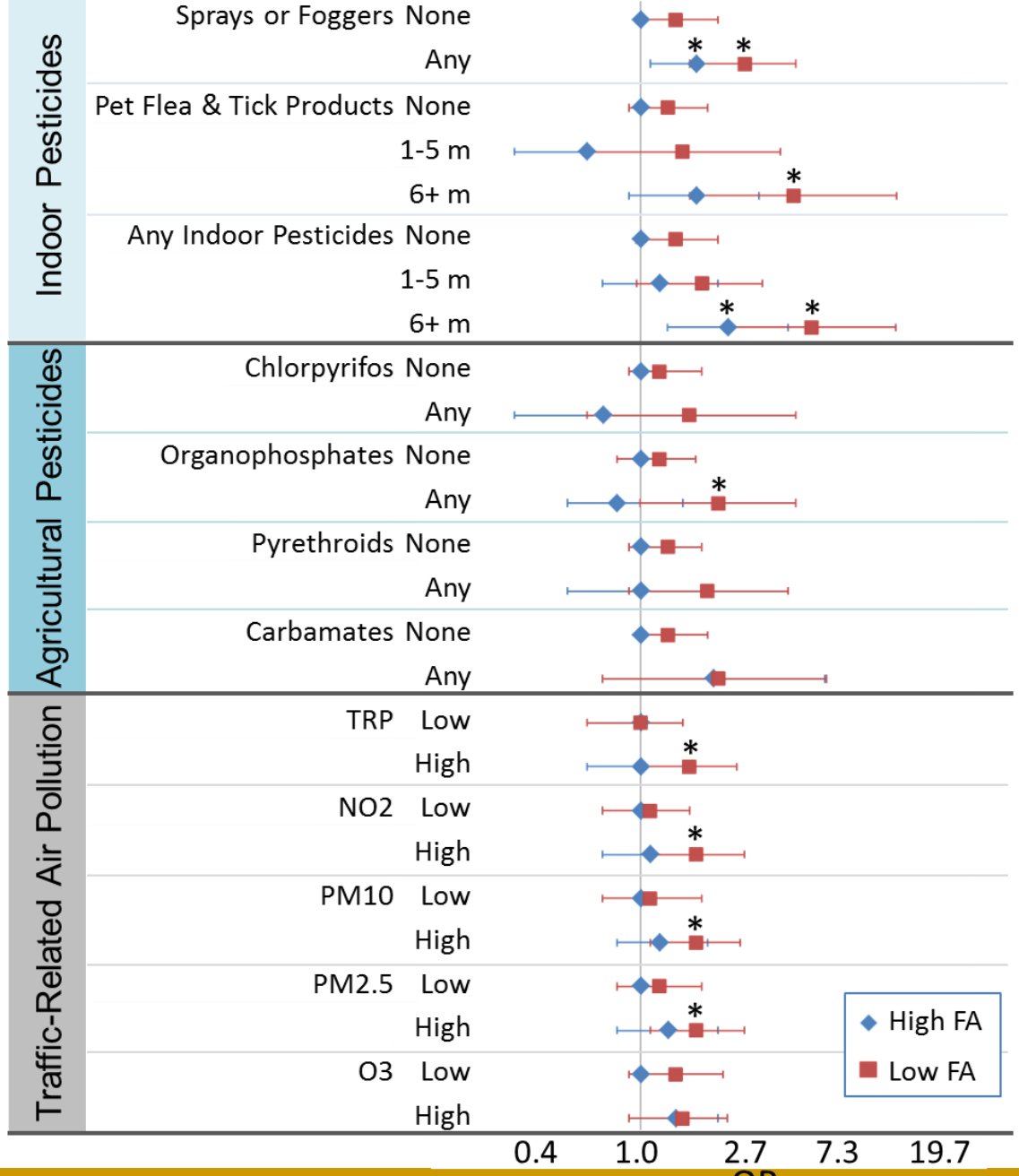
Maternal high-methyl (FA-supplemented) diet counteracts BPA-induced DNA hypomethylation in early development **(Dolinoy et al, 2007)**



Model To Investigate : CHARGE



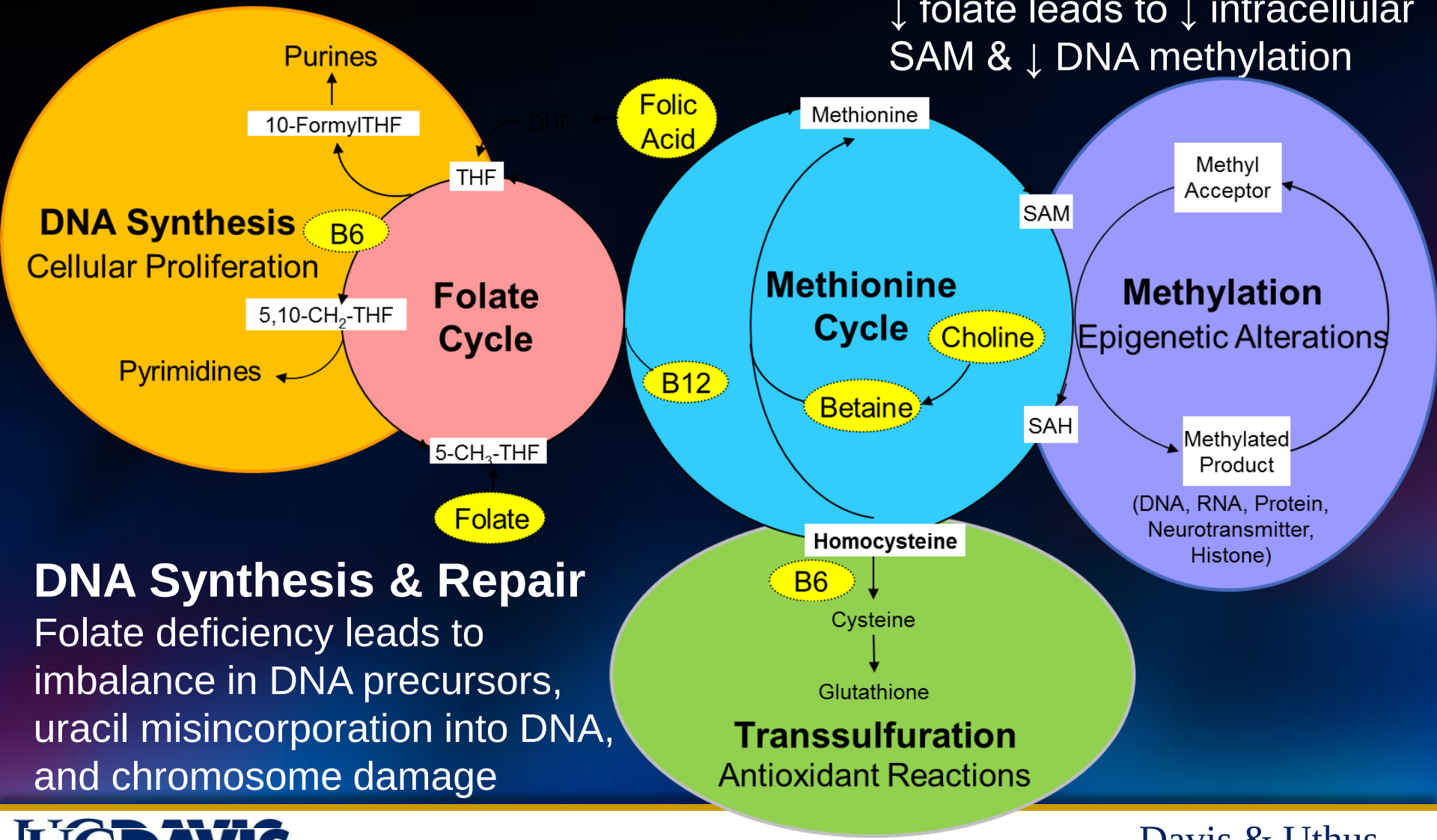
ASD ORs for Environment & FA Combinations



Comments

- Nutrient x environmental exposure combined effects in expected directions
- Congruent with animal models, but more complex
- Could work for other contaminants
- Epigenetic mechanisms, or others

Potential Folate Mechanisms



DNA Synthesis & Repair

Folate deficiency leads to imbalance in DNA precursors, uracil misincorporation into DNA, and chromosome damage

UC DAVIS
UNIVERSITY OF CALIFORNIA

Davis & Uthus 2004; Duthie 1999

Acknowledgements

CHARGE, MARBLES

Irva Hertz-Picciotto (PI, UCD)
Cheryl Walker (UCD)
Sally Ozonoff (UCD)
Robin Hansen (UCD)

DNA Methylation

Janine LaSalle (UCD)
Florence Crary (UCD)
Rima Woods (UCD)
Dani Fallin (JHU)
Kelly Bakulski (JHU)

Air Pollution

Heather Volk (USC)

Pesticides

Janie Shelton (US Embassy)
Irva Hertz-Picciotto (UCD)
Lora Delwiche (UCD)

Biostatistics

Daniel Tancredi (UCD)
Ana-Maria Iosif (UCD)
J. Erin Dienes (CDC)
Heather Volk (USC)
Amanda Goodrich (USC)
Vladimir Kogan (USC)
Jin Yao (USC)

Funders

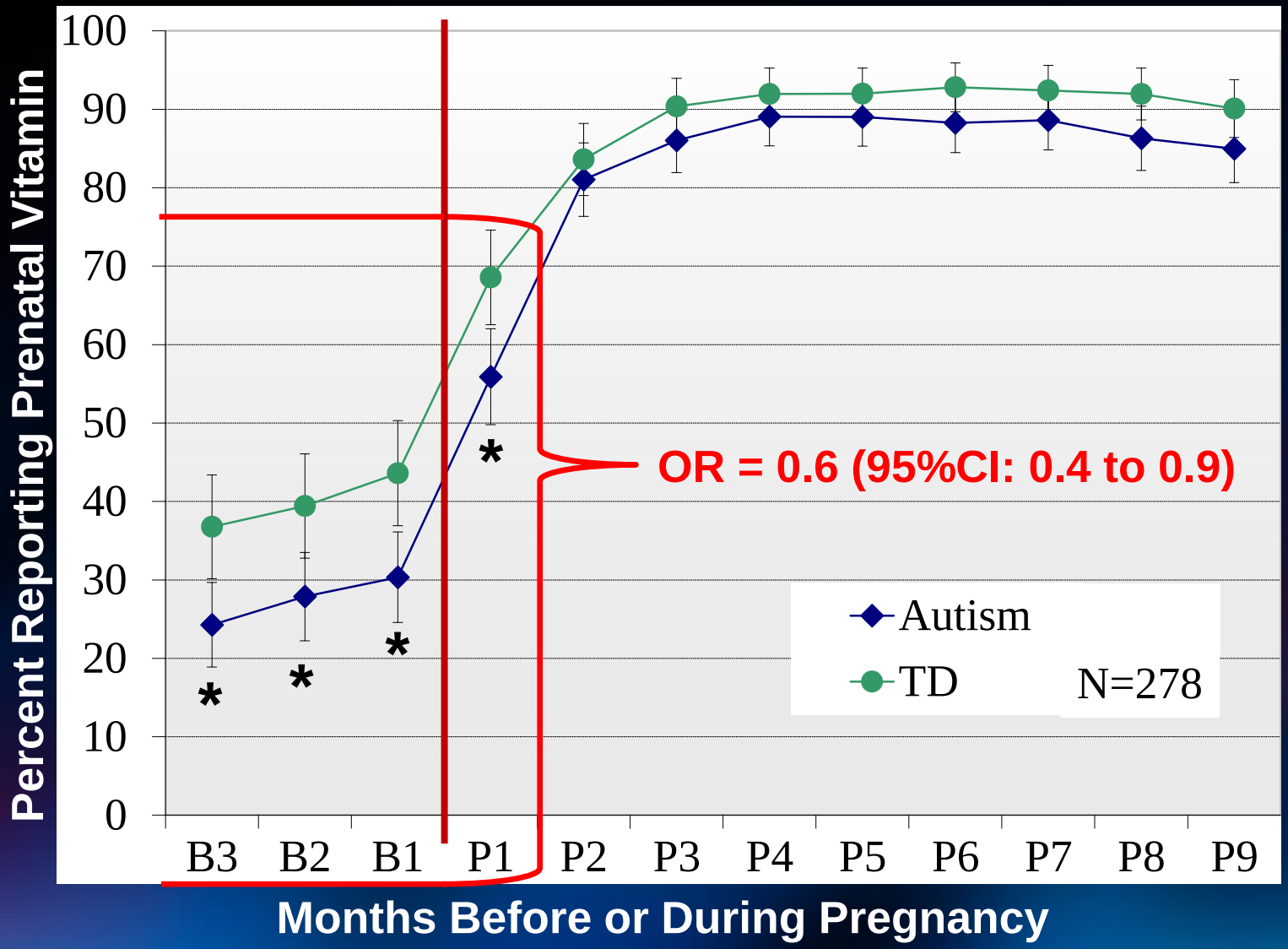
NIH R21ES021330, R01ES020392, R01ES015359, P0111269, T32 MH073124,
BIRCWH K12HD051958, **EPA** P01ES011269, R-829388 & R-833292, **DOD** AR110194
Allen Foundation 2008.604, **Autism Speaks** 9038, **MIND Institute** Pilot, **IDDRC** Pilot

UC DAVIS
UNIVERSITY OF CALIFORNIA

Folic Acid

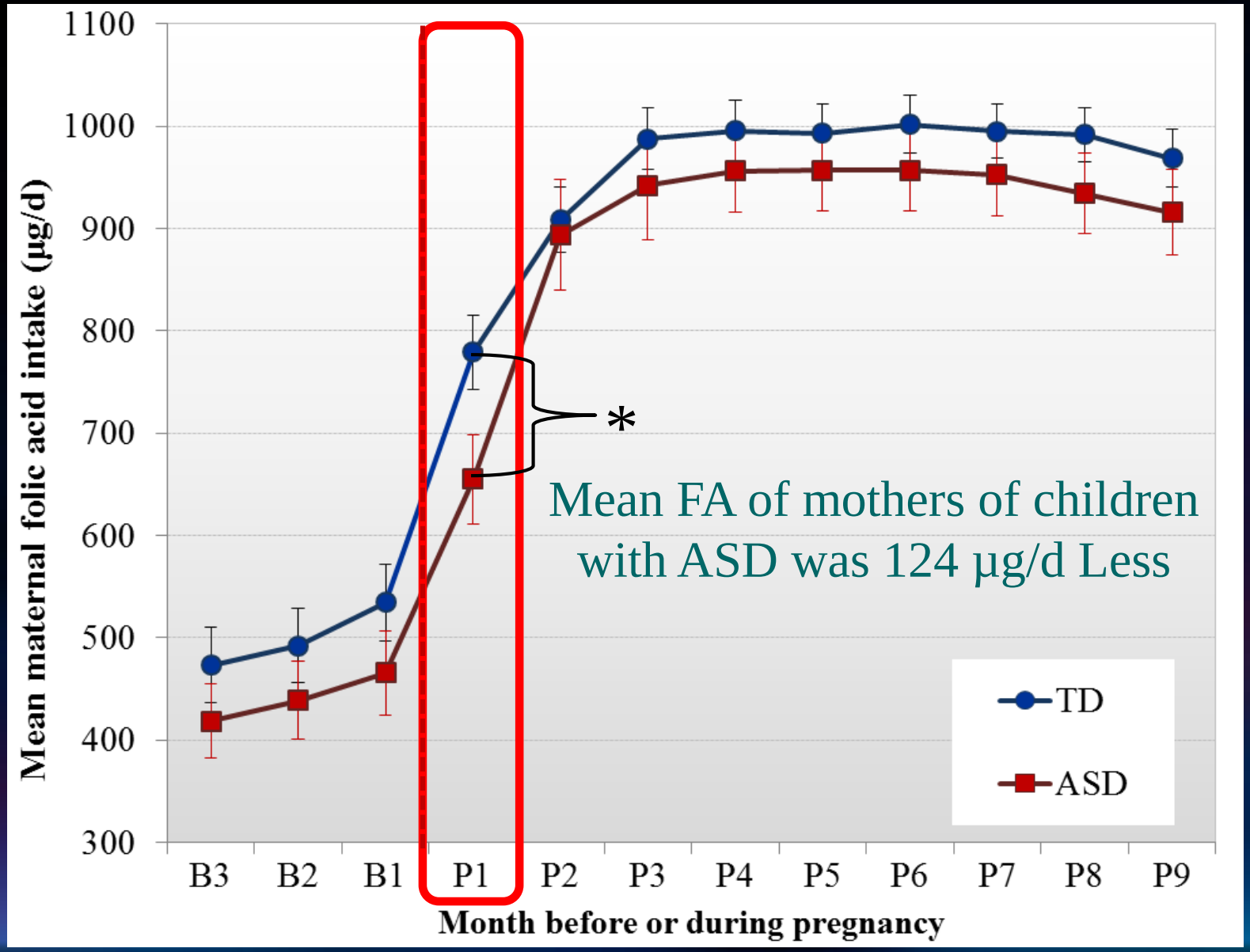
- Synthetic folate: essential vitamin B9
- Critical for development of the brain and nervous system
- Decreases risk for NTDs by 50-70%
  MRC 1991, Czeizel and Dudas 1992
- Also associated with decreased risk for behavioral, social, and peer problems and language delays & improved attention, verbal, verbal-executive function, and social competence
  Roza et al 2009, Julvez et al 2009, Scholtz et al 2010, Roth et al 2011

Prenatal Vitamin Use by Perinatal Month



Schmidt et al, 2011 *Epidemiology*

Mean Folic Acid Intake Associated with Reduced ASD



UC DAVIS
UNIVERSITY OF CALIFORNIA

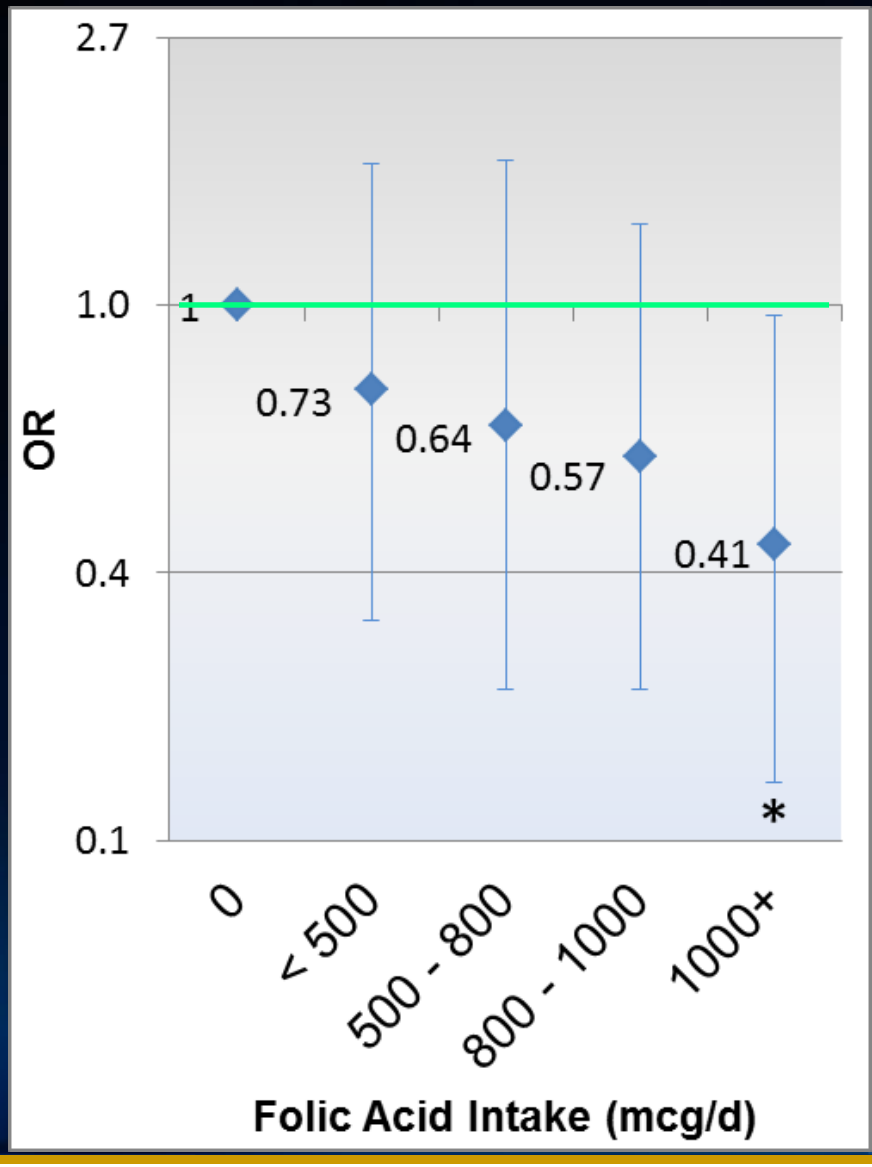
Schmidt et al, 2012 *Am J Clin Nutr*

Folic Acid and ASD: Dose

Increased Folic Acid, Decreased ASD Risk

***P* for trend = 0.001**

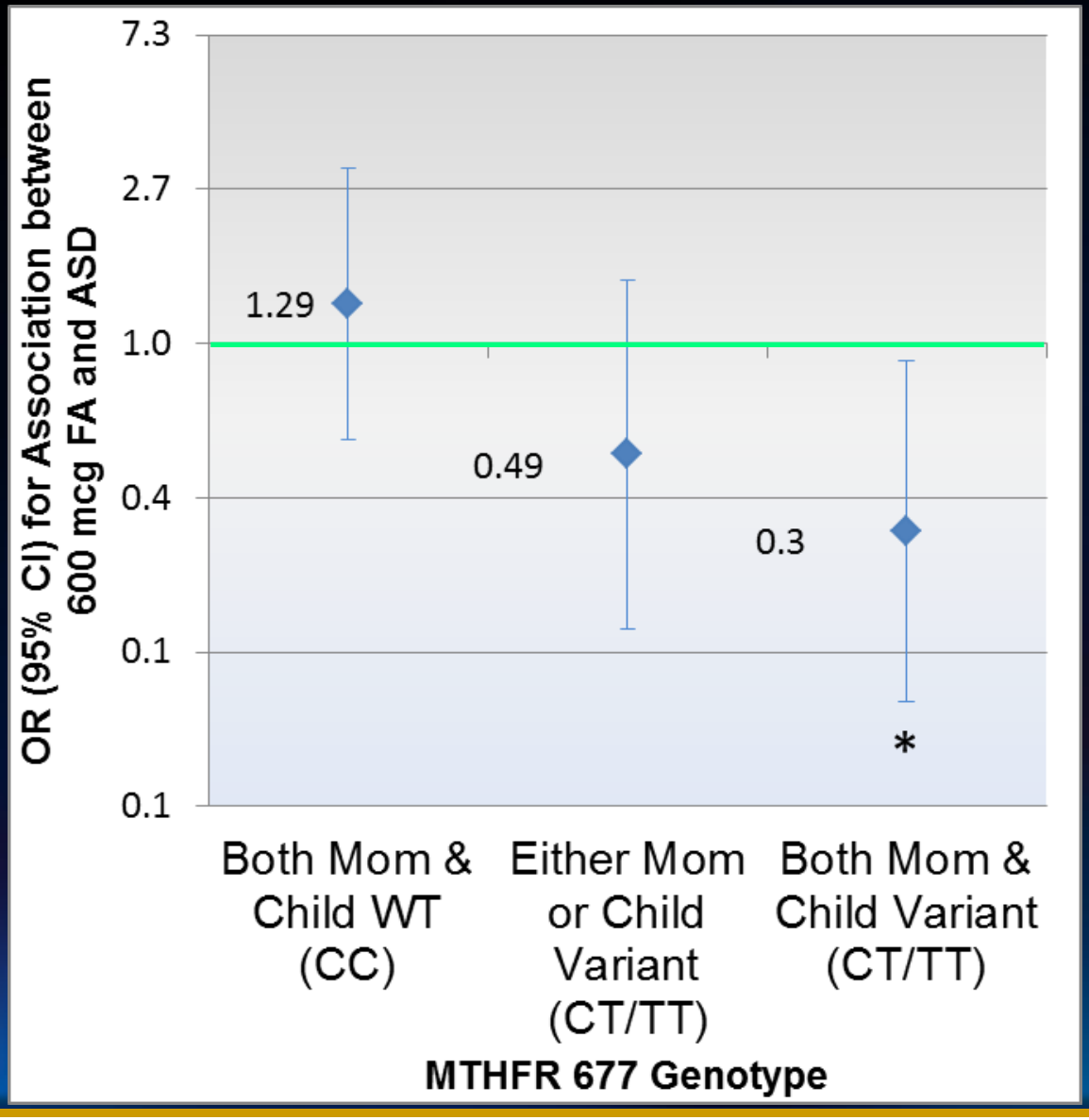
Adjusted for: maternal education level and child's birth year (similar trend, reduced ORs when adjusted for other nutrients)



UC DAVIS UNIVERSITY OF CALIFORNIA

Schmidt et al, 2012 *Am J Clin Nutr*

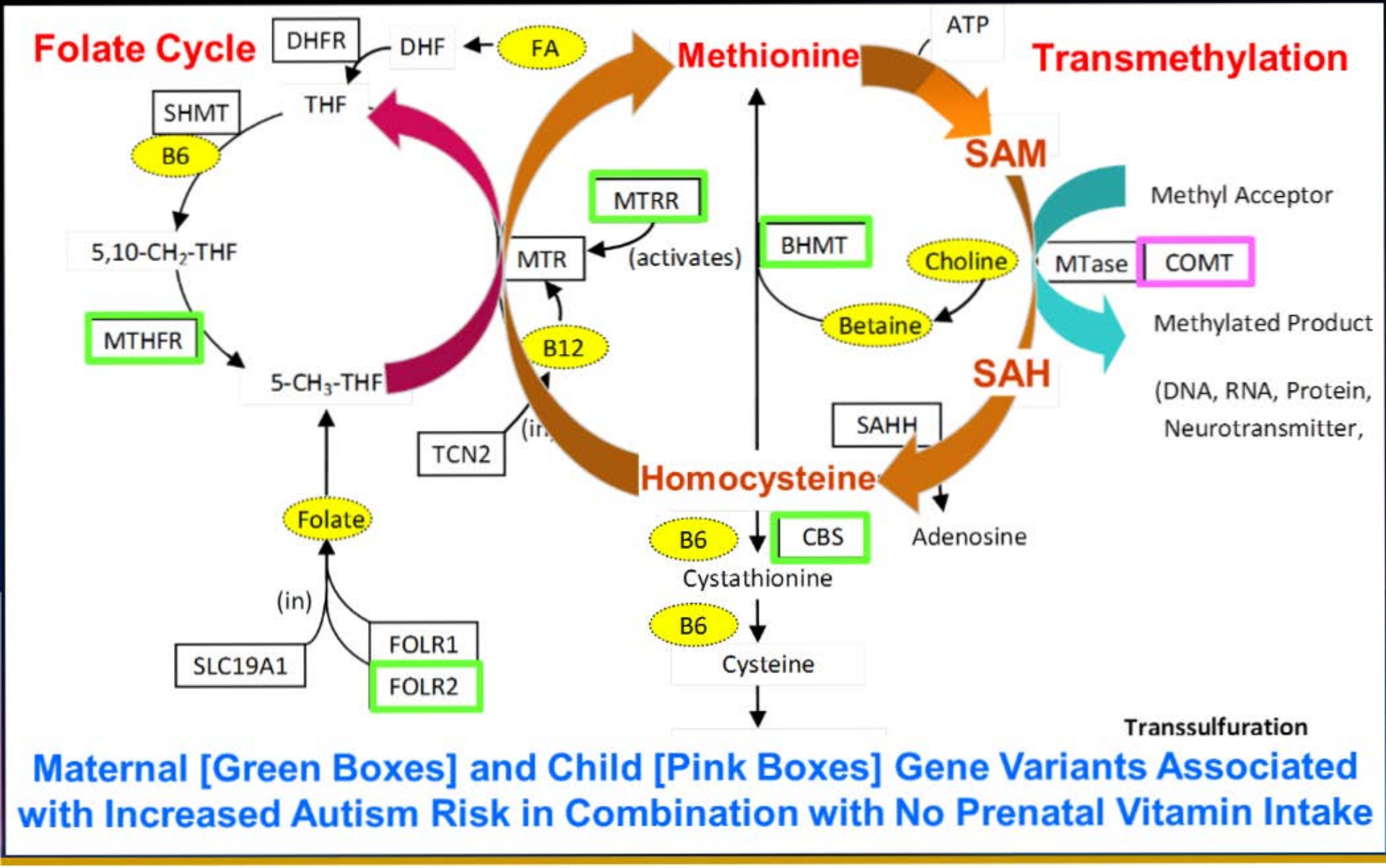
Folic Acid Stratified by MTHFR 677



UC DAVIS UNIVERSITY OF CALIFORNIA

Schmidt et al, 2012 *Am J Clin Nutr*

Folate, Methionine, and Transmethylation Pathways



Maternal [Green Boxes] and Child [Pink Boxes] Gene Variants Associated with Increased Autism Risk in Combination with No Prenatal Vitamin Intake

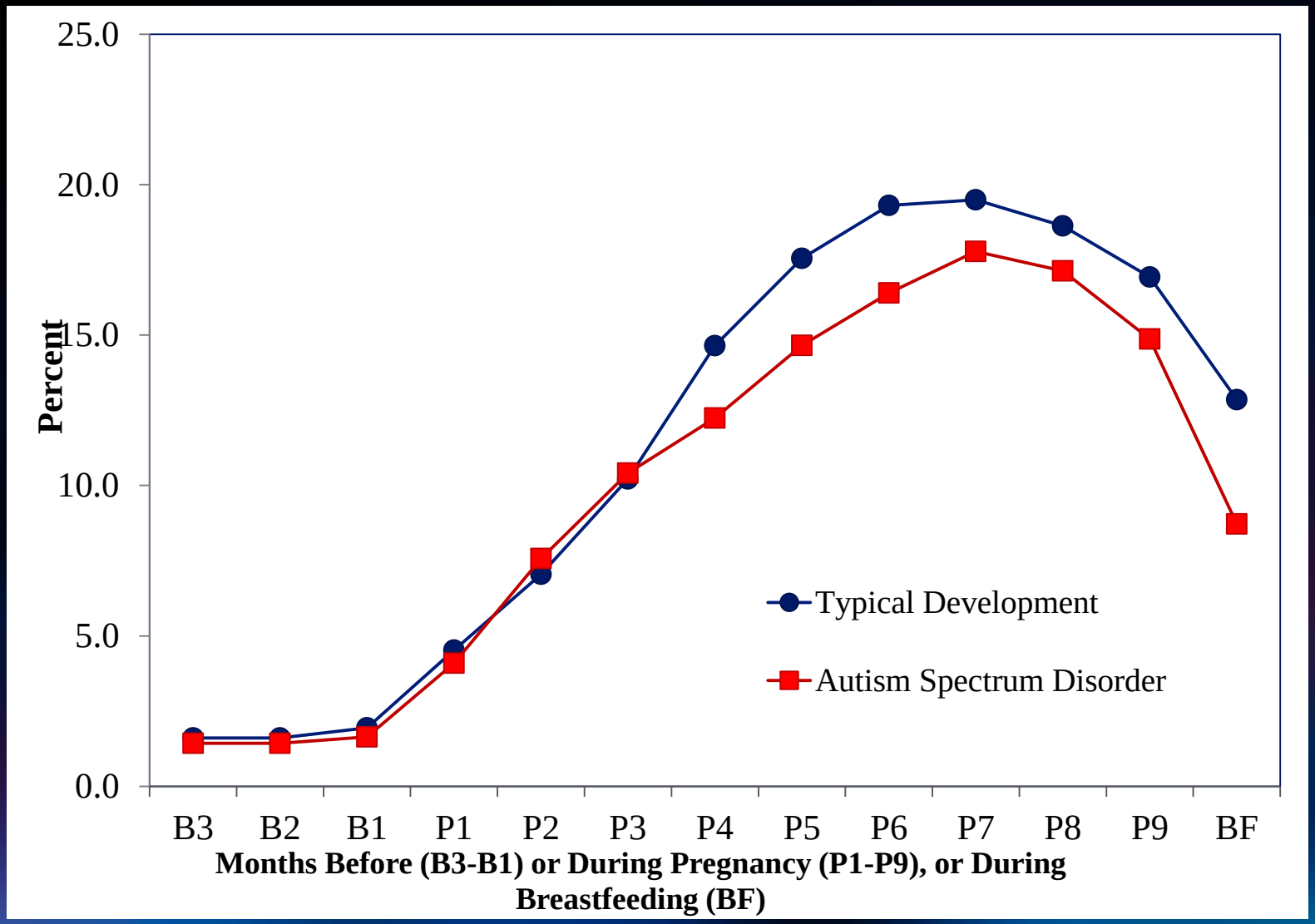
UC DAVIS
UNIVERSITY OF CALIFORNIA

Findings Replicated

- Norwegian Mother & Child Cohort (MoBa) Study (85,176 children, 114 Autistic Disorder)
- Ohio HOME Study (n=209)
  - Regular prenatal vitamin use & lower odds of clinically elevated SRS scores (autistic traits) OR=0.3 (0.1-0.9)
  - 2nd Trimester whole blood folate not associated
- Netherlands Generation R Study (n=3893)
  - FA supplement & lower SRS autistic traits
  - No significant association for maternal plasma folate concentrations at ~13 weeks gestation
- Denmark Study (n=35,059, 198 Autistic Disorder)

Surén et al, 2013; Braun et al 2014; Steenweg-de Graaff et al, 2014; Virk et al, 2015

% Reporting Taking Iron Supplements



UC DAVIS
UNIVERSITY OF CALIFORNIA

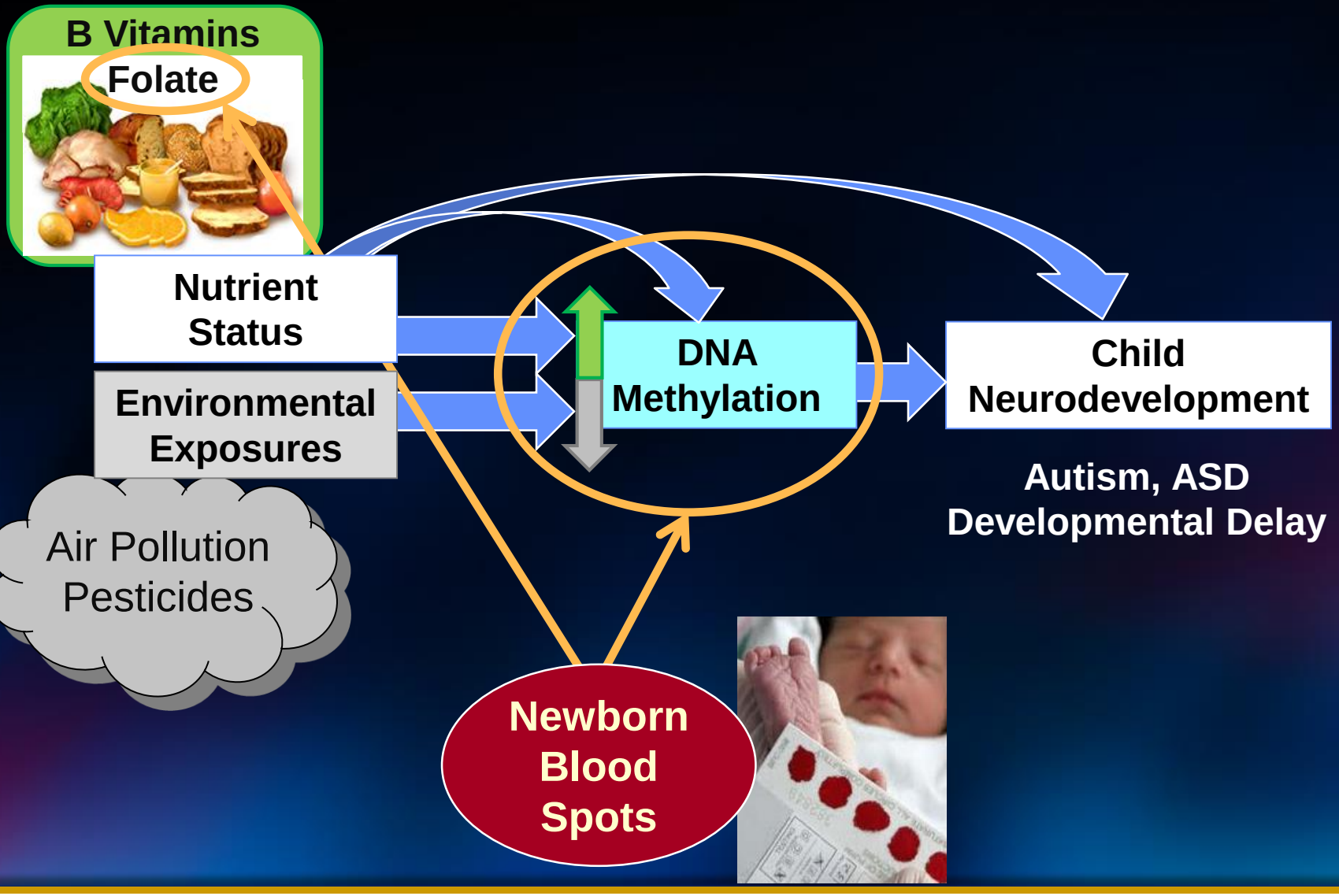
Mean Iron Intake from All Supplements

Mean Iron (mg/day)	OR[1] (95% CI)	*P*	OR[1,2] (95% CI)	*P*
< 30				
30 - < 36	0.92 (0.54 – 1.57)	0.76	0.91 (0.56 – 1.47)	0.70
36 - < 52	0.87 (0.51 – 1.47)	0.60	0.86 (0.53 – 1.39)	0.53
52 - < 86	0.78 (0.45 – 1.33)	0.35	0.80 (0.50 – 1.26)	0.33
86+	0.55 (0.31 – 0.96)	**0.04**	0.55 (0.34 – 0.89)	**0.02**

[1] **Adjusted for supplemental periconceptional folic acid intake, child's year of birth, and home ownership**

[2] **Missing values imputed**

CHARGE


B Vitamins
Folate
Nutrient Status
Environmental Exposures
Air Pollution
Pesticides
DNA Methylation
Child Neurodevelopment
Autism, ASD
Developmental Delay
Newborn Blood Spots

Air Pollution

- Self-reported residential history

- CALINE4 line-source air quality dispersion model used to obtain model-based estimates of exposure to traffic-related air pollution

FA x Air Pollution

Air Pollution

Environmental Exposure During Pregnancy		800+ mcg FA Preg Month 1 aOR (95% CI)	<800 mcg FA Preg Month 1 aOR (95% CI)
Traffic-Related Pollution (Total)	< Median	Reference	1.0 (0.6-1.5)
	≥ Median	1.0 (0.6-1.5)	**1.6 (1.3-3.3)**
NO_2 (Total)	< Median	Reference	1.1 (0.7, 1.6)
	≥ Median	1.1 (0.7, 1.8)	**1.7 (1.1, 2.7)**
PM_{10} (Total)	< Median	Reference	1.1 (0.7, 1.8)
	≥ Median	1.2 (0.8, 1.9)	**1.7 (1.1, 2.6)**
$PM_{2.5}$ (Total)	< Median	Reference	1.2 (0.8, 1.8)
	≥ Median	1.3 (0.8, 2.1)	**1.7 (1.1, 2.7)**
Ozone (Total)	< Median	Reference	1.4 (0.9, 2.2)
	≥ Median	1.4 (0.9, 2.1)	1.5 (0.9, 2.3)

UC DAVIS
UNIVERSITY OF CALIFORNIA

Pesticide Use

- Self-reported use of products to control ants, flies, cockroaches or pet pests such as fleas and ticks

- Asked product type (spray, bait, etc.), brand name, whether application was indoors, outdoors, or on pet, and about use of professional pest control services

FA x Indoor Pesticides

Indoor Pesticides

Environmental Exposure During Pregnancy		800+ mcg FA Preg Month 1 aOR (95% CI)	<800 mcg FA Preg Month 1 aOR (95% CI)
Sprays or Foggers	None	Reference	1.4 (0.96 – 2.1)
	Any	**1.7 (1.1 – 2.6)**	**2.7 (1.6 – 4.4)**
Pet Flea and Tick Products	None	Reference	1.3 (0.9 – 1.9)
	Some (1-5 Months)	0.6 (0.3 – 1.5)	1.5 (0.6 – 3.8)
	Regular (6+ Months)	1.7 (0.9 – 3.1)	**4.3 (1.6 – 11.5)**
Any Indoor Pesticides	None	Reference	1.4 (0.96 - 2.1)
	Some (1-5 Months)	1.2 (0.7 – 2.1)	1.8 (0.97 - 3.2)
	Regular (6+ Months)	**2.3 (1.3 - 4.1)**	**5.1 (2.3 - 11.4)**

UC DAVIS UNIVERSITY OF CALIFORNIA

Adjusted for home ownership

Agricultural Pesticides: Pesticide Use Report (PUR)

Geocode CHARGE addresses by month (-6 months to birth) (ArcGIS)

→ **Download PUR data (1998-2008)**

→ **Map CHARGE addresses to PUR square mile grid (ArcGIS)**

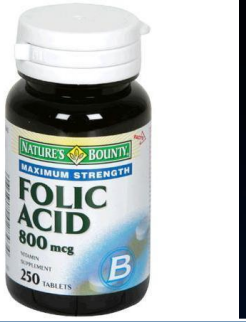
→ **Sum pesticides applied to grid within buffer by day (SAS)**

→ **Model in linear and logistic regression (SAS)**

UC DAVIS UNIVERSITY OF CALIFORNIA

J. Shelton et al, 2014

FA x Agricultural Pesticides



Ag Pesticides

Environmental Exposure (3 Months Before Pregnancy until Pregnancy Month 3)		800+ mcg FA Preg Month 1 aOR (95% CI)	<800 mcg FA Preg Month 1 aOR (95% CI)
Chlorpyrifos	None	Reference	1.2 (0.9 – 1.8)
	Any	0.7 (0.3 – 1.7)	1.6 (0.6 – 4.4)
Organophosphates	None	Reference	1.2 (0.8 – 1.7)
	Any	0.8 (0.5 – 1.5)	**2.1 (1.0 - 4.4)**
Pyrethroids	None	Reference	1.3 (0.9 – 1.8)
	Any	1.0 (0.5 – 2.0)	1.9 (0.9 - 4.1)
Carbamates	None	Reference	1.3 (0.97 – 1.9)
	Any	2.0 (0.7 – 5.8)	2.1 (0.7 – 5.9)

UC DAVIS UNIVERSITY OF CALIFORNIA

Adjusted for home ownership