Of Mice and Men: Using Animal Models to Study Gene-Environment Interactions

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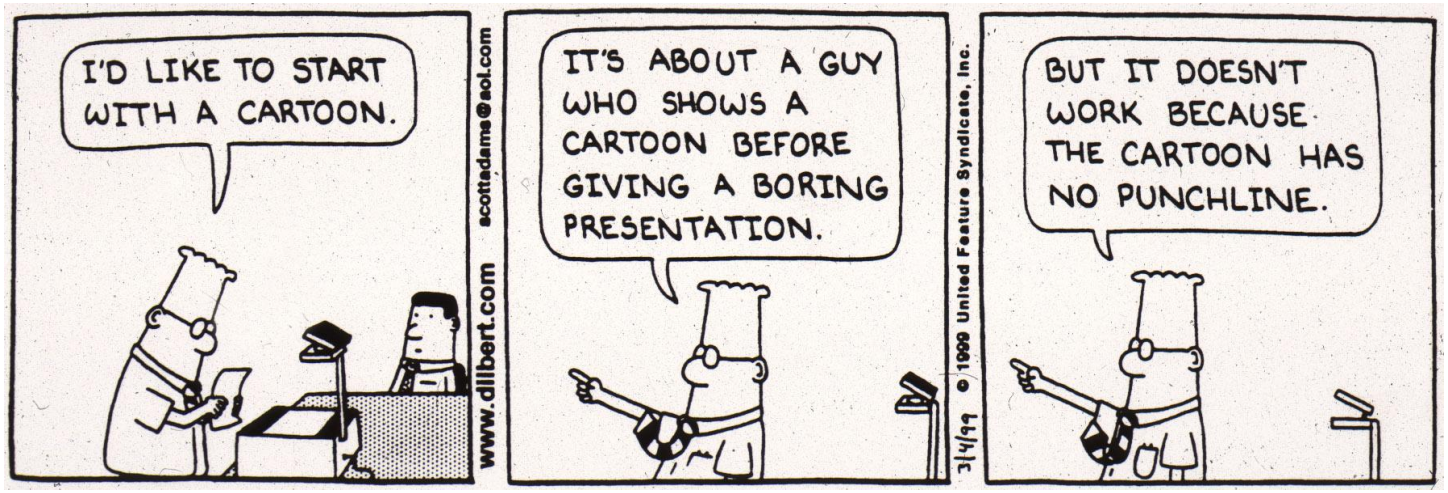
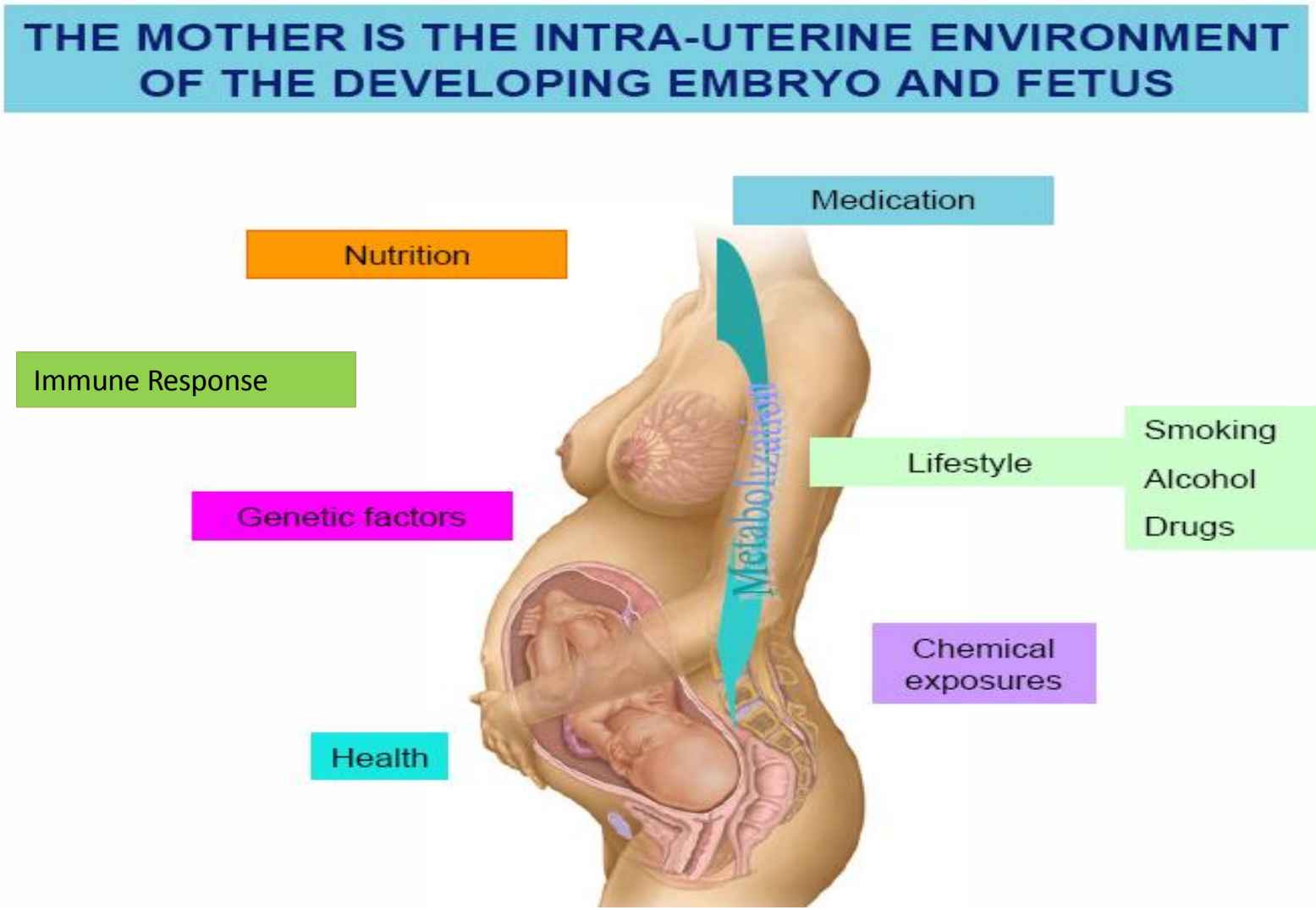


Courtesy of Jeff Murray

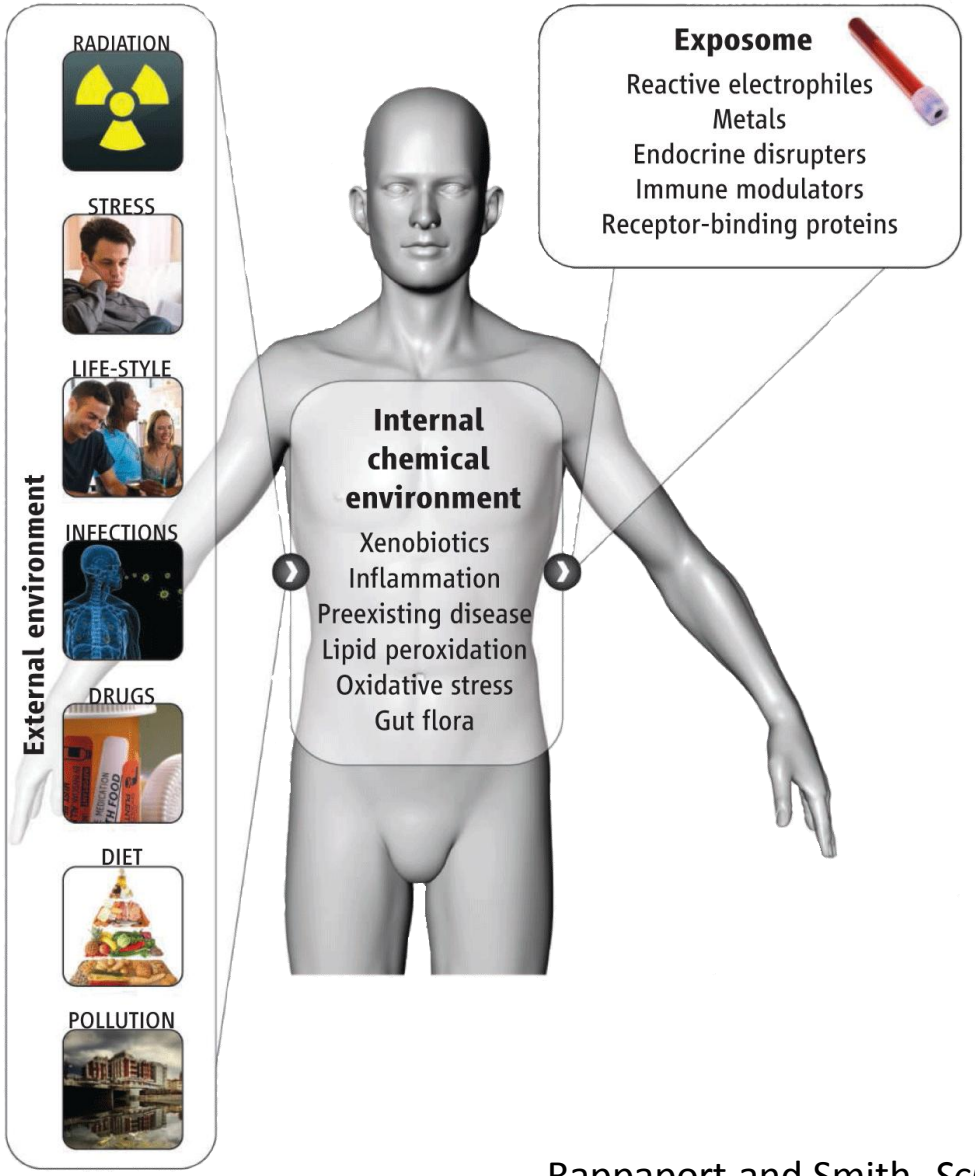
How Does One Study Gene-Environment Interactions that Governing Susceptibility to Birth Defects?

Embryonic Development is Determined by Maternal Lifestyle Choices and Genetic Factors



Characterizing Environmental Exposures that Impact Human Health Outcomes

- Environment is defined as the body's internal chemical environment
- Exposure is defined as the amounts of biologically active chemicals in this internal environment



Rappaport and Smith, *Science* 330:460-1, 2010

Possible Genetic and Environmental Interactions

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

Gene-Environment Interactions-Maternal Smoking, Folate Status, and Orofacial Clefts

- population-based case-control study California, n=548,844 1987-89 births
- cases - isolated CLP n=244/318 eligible mothers interviewed and infants genotyped
- controls - randomly selected, 588/652 eligible mothers interviewed and infants genotyped
- DNA from newborn blood samples

Maternal characteristics of isolated CLP cases and nonmalformed controls

	Cases (n=244) %	Controls (n=588) %
Multivitamin Use		
No	29.5	18.5
Use -1 through +2	68.4	80.4
Cigarette Smoking		
No	66.8	76.2
Yes -1 through +2	32.8	23.3

Shaw et al., Am J Epidemiol. 2005;162(12):1207-14

Nitric Oxide Synthase

- *NOS3* variants influence (raise) homocysteine concentrations
- smoking compromises *NOS3* activity
- folate intake influences (lowers) homocysteine concentrations
- is clefting risk from *NOS3* variants modified by smoking and further modified by vitamin intake (folic acid)?

Genotyping

- 3 SNPS, A922G, C690T, and G894T
- multilocus allele-specific hybridization assay
- Roche Molecular Systems
- panel of 32 SNPs
- all 3 SNPs consistent with Hardy-Weinberg equilibrium in controls

NOS3 C690T genotypes, maternal smoking, maternal vitamin use, and CLP risks

Genotype	Smoking	Vitamin Use	Odds Ratio	95% CI
Variant	Yes	No	4.7	0.9-26.8
Variant	Yes	Yes	2.0	0.7-5.8
Wildtype	Yes	No	3.1	1.6-6.0
Wildtype	Yes	Yes	1.7	1.1-2.6
Wildtype	No	Yes	Ref	-----

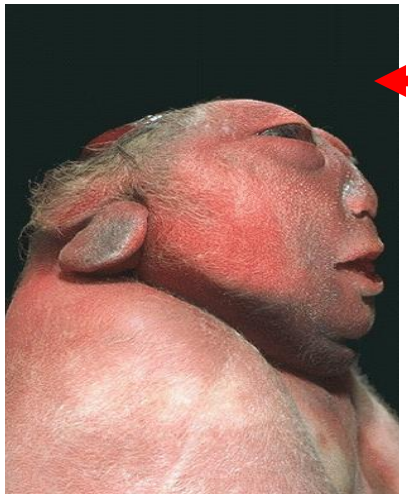
Shaw et al., *Am. J. Epidemiol*. 162:1207-14, 2005

Neural Tube Defects

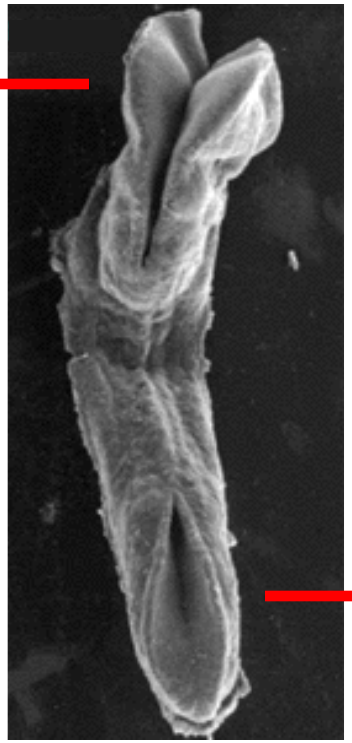
- 250-300,000 NTD births annually worldwide; 3,000 in US
- Result in lifelong disability
  - Problems with bladder, bowel, and sexual function
  - Learning and developmental problems
  - Orthopedic problems
- Some NTDs are preventable-Approx. 20% reduction since folate fortification in US

Neural Tube Defects

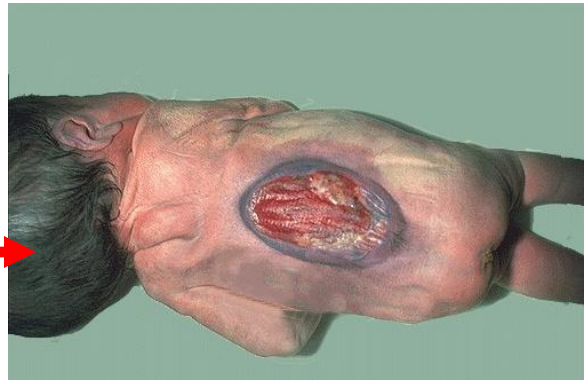
neural plate neural folds neural tube



Anencephaly



Spina Bifida



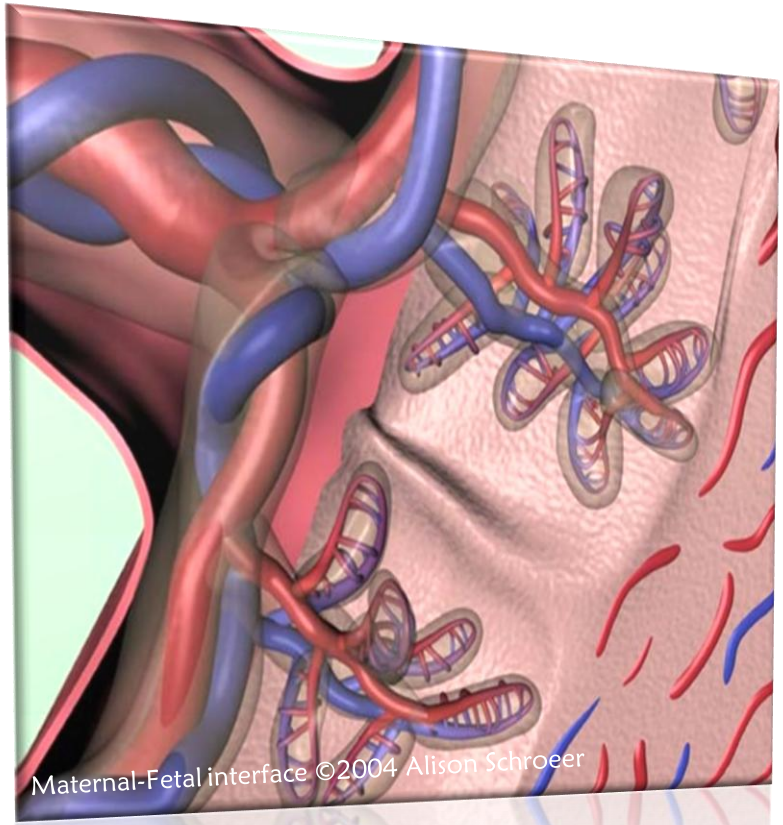
NTDs are Complex Traits

- They have a strong genetic component

- They also require a significant environmental interaction in order to express the abnormal phenotype

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

While No Doubt True….

dy of mankind is man.” Alexander Pope

Courtesy of Laura Mitchell

IN EXPERIMENTAL DESIGNS TO TEST GENETIC SUSCEPTIBILITY

ENVIRONMENT IS HELD CONSTANT WHILE MANIPULATING THE GENOTYPE OF THE EXPERIMENTAL ORGANISM

FOR EXPERIMENTS CONCERNING GENETIC SUSCEPTIBILITY TO TERATOGENESIS, THE MOUSE IS THE IDEAL EXPERIMENTAL ORGANISM

- >22,000 individual genes have been identified and mapped with the completion of the mouse genome project

- >4000 genetically engineered mouse lines now exist

- >250 inbred mouse strains exist

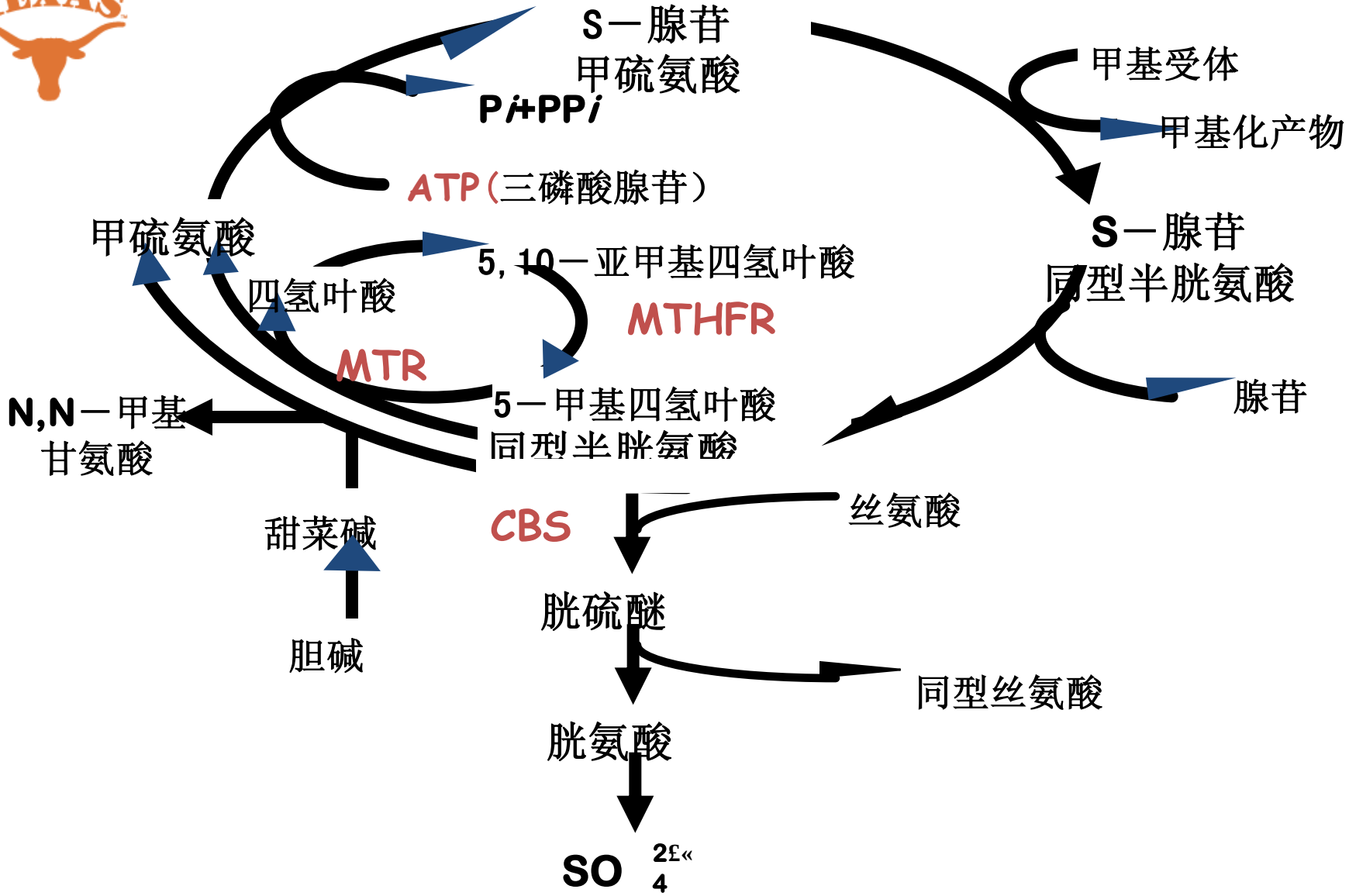


图1　同型半胱氨酸代谢

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

Modelo de ratón knockout para un gen transportador de ácido fólico.

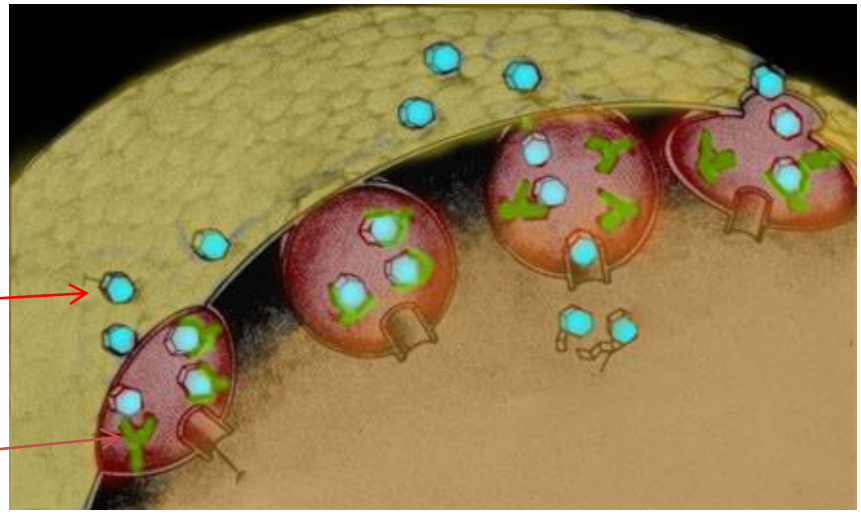
Receptor de folatos

Folr1

Folr2

RFC1

PCFT

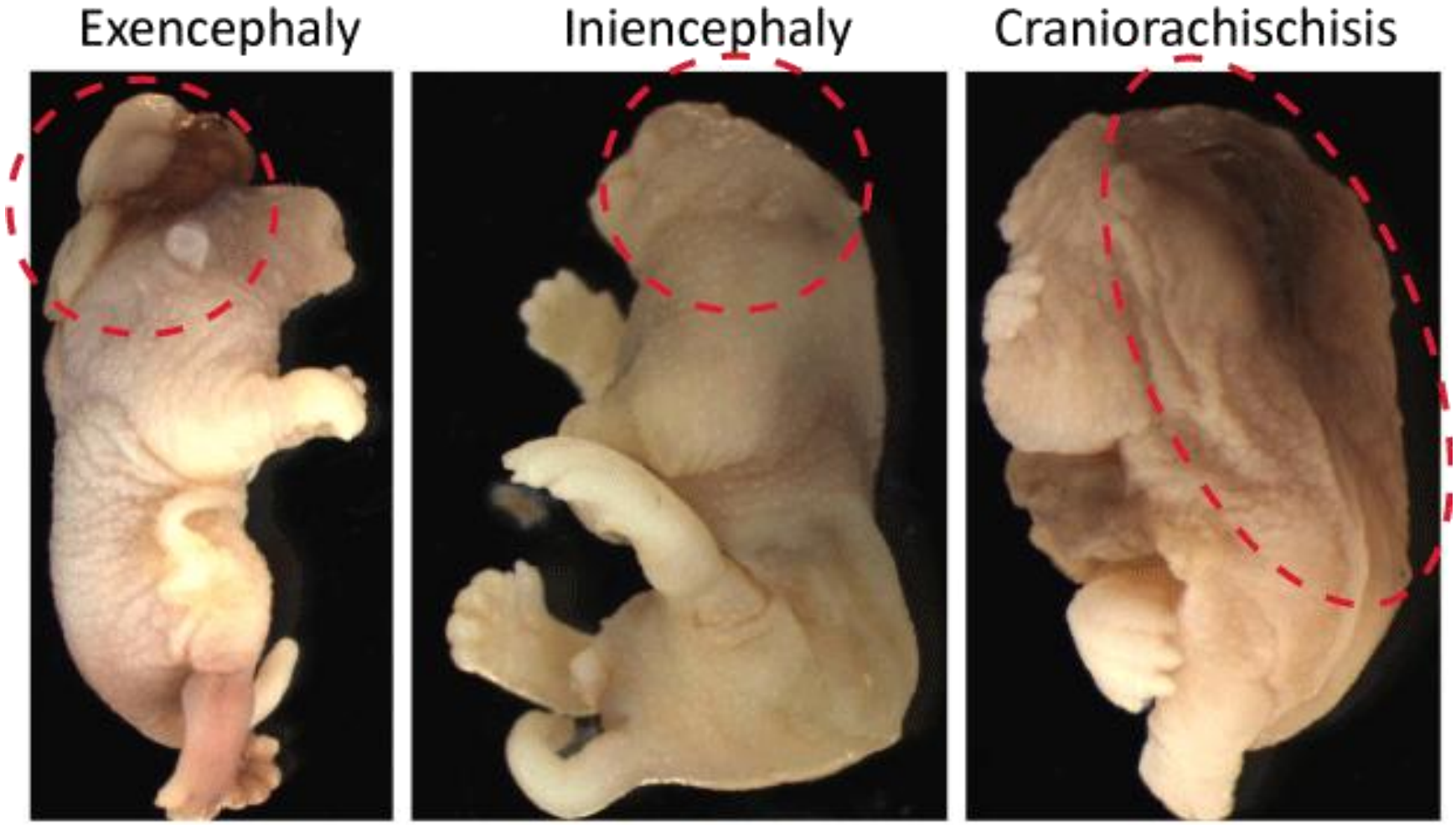


Ácido Fólico

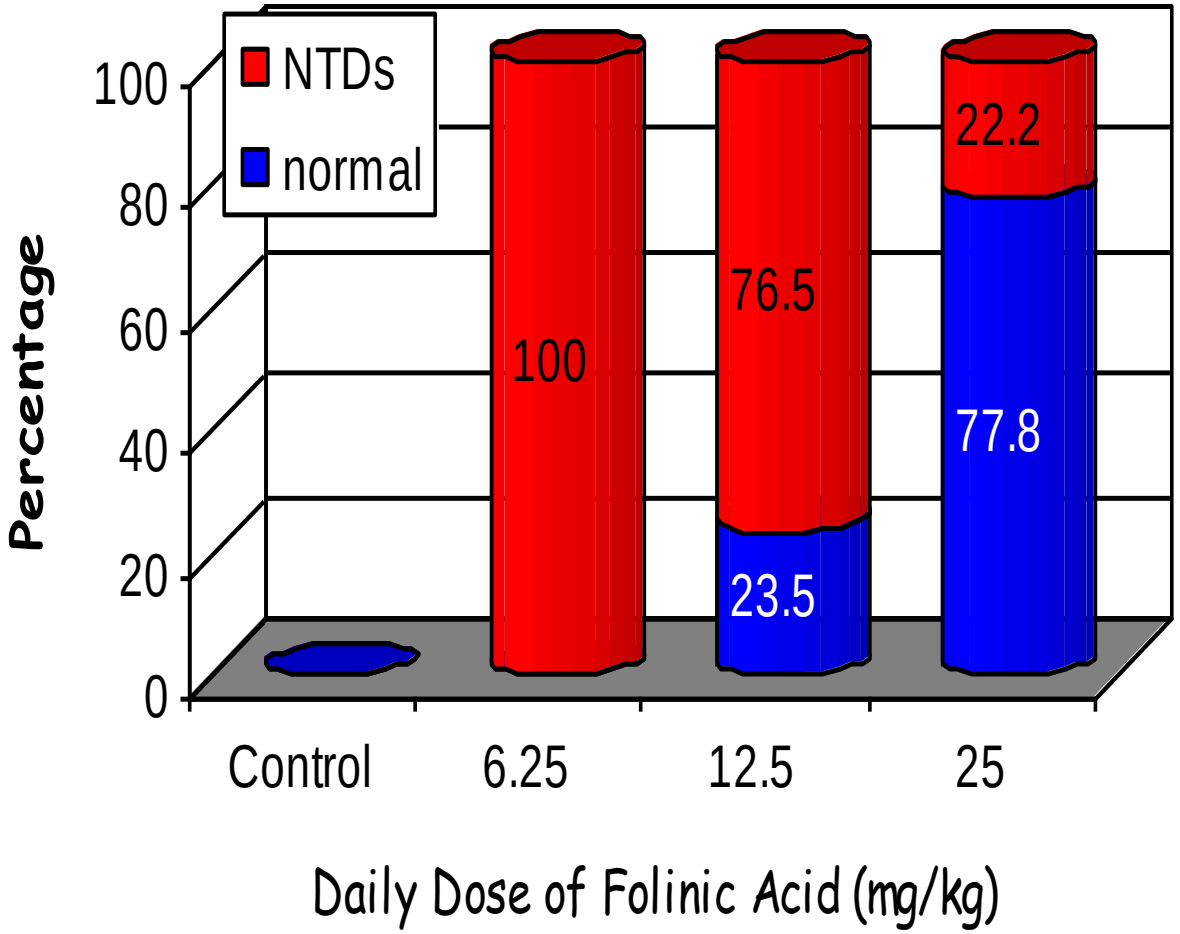
(Folr1) Receptor de Folatos

NTDs With Folate Supplementation

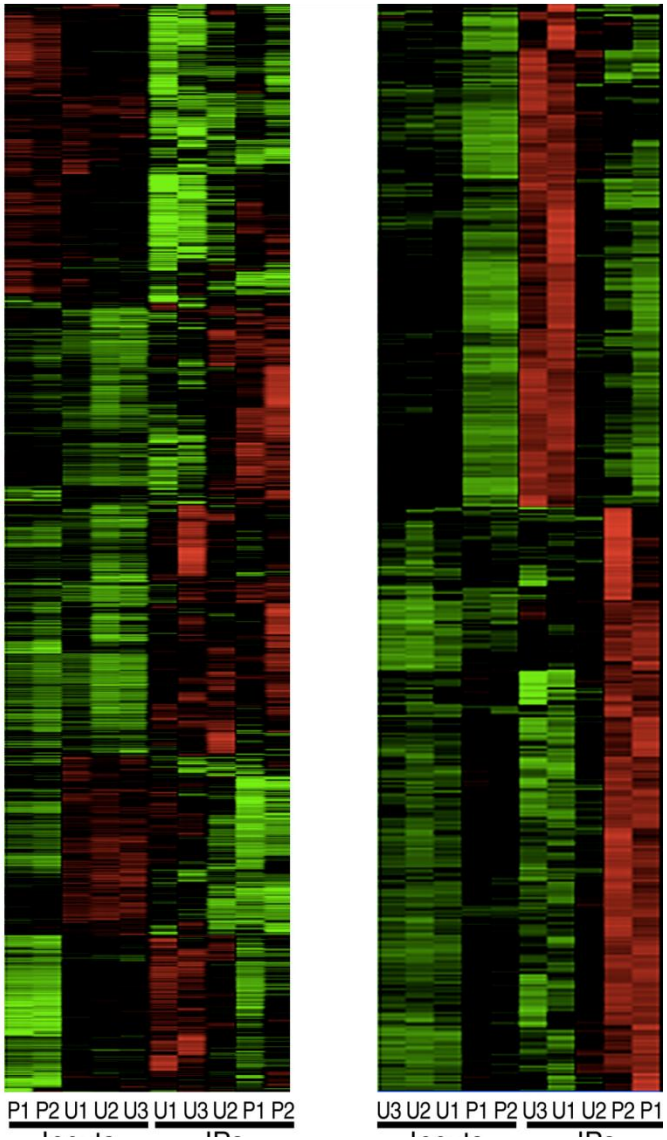
(*Folr1*$^{-/-}$, E18, 5M-THF, 12.5mg/kg)

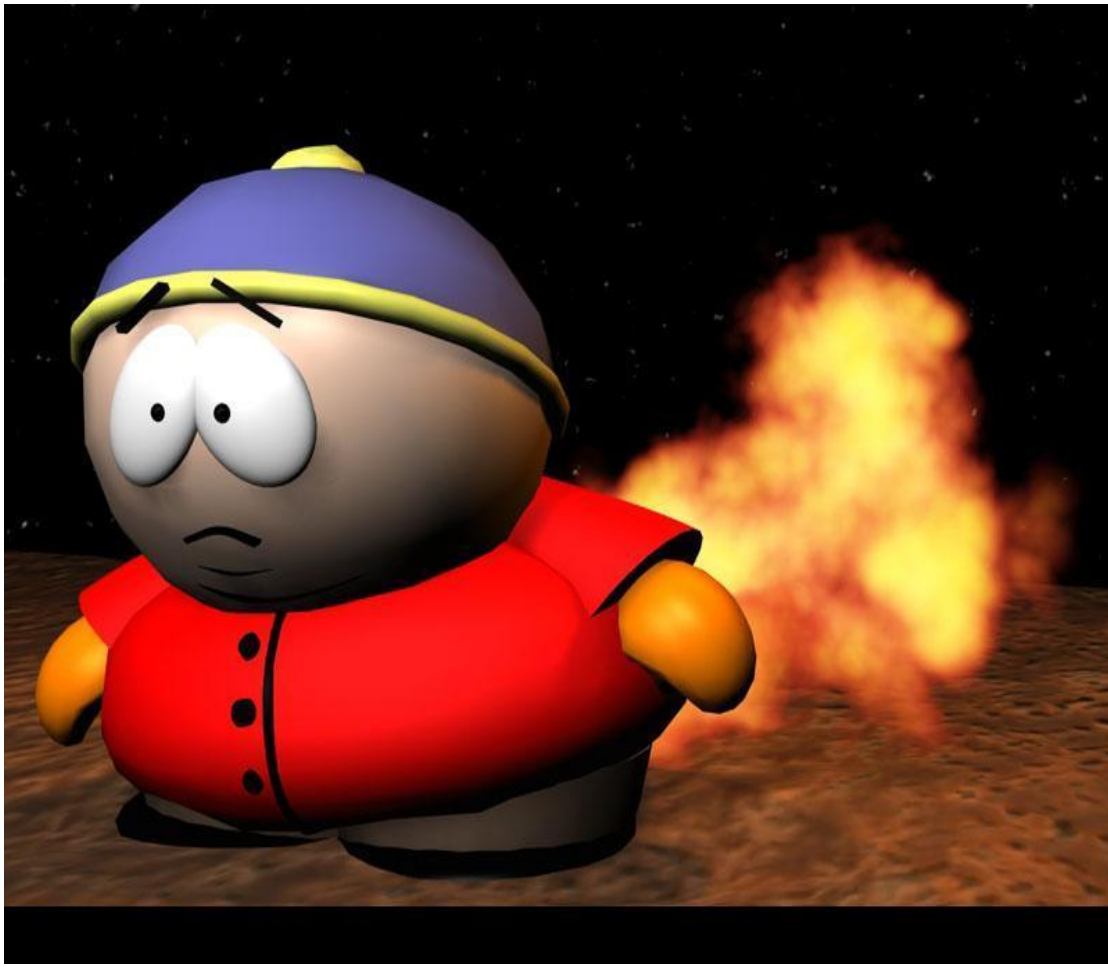


Restoring Folate Concentrations in Light of Gene Defect Restores Normal Phenotype



Folic Acid Responsive Targets (FARTs)





Pollutants in Areas Associated With High NTD Prevalence

Fine particulate air pollution (<2.5 µm; $PM_{2.5}$)

Arsenic

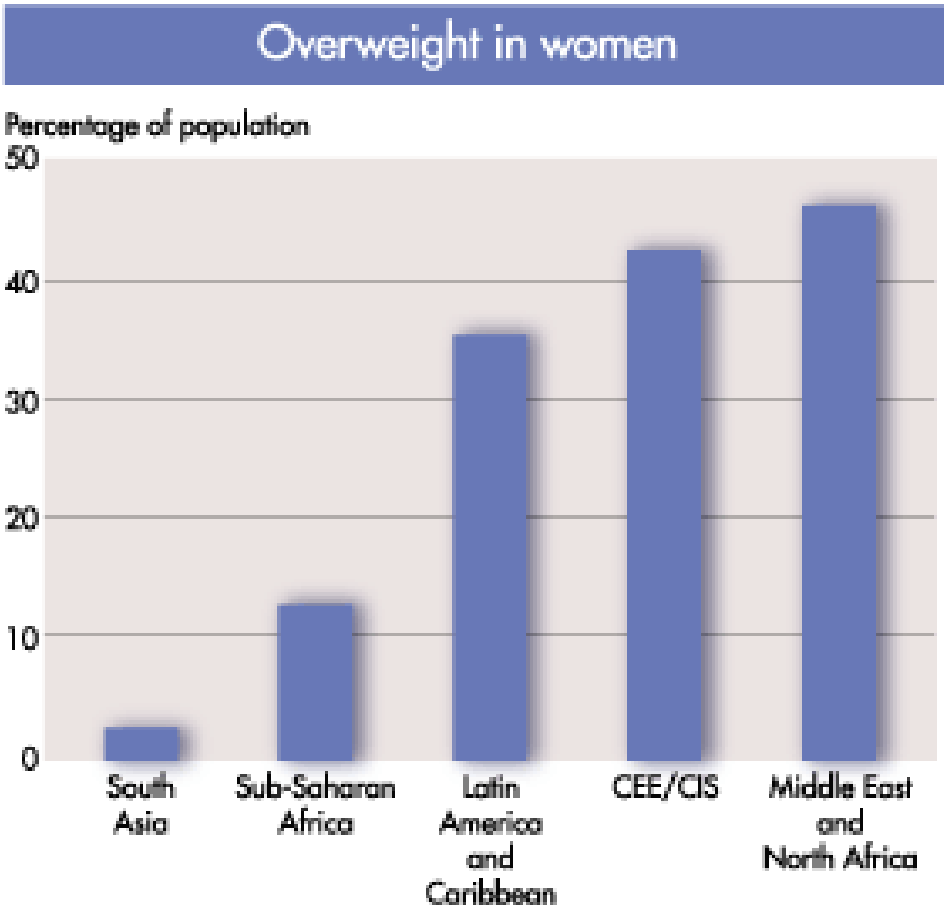
Carbon disulphide

Cadmium

Lead

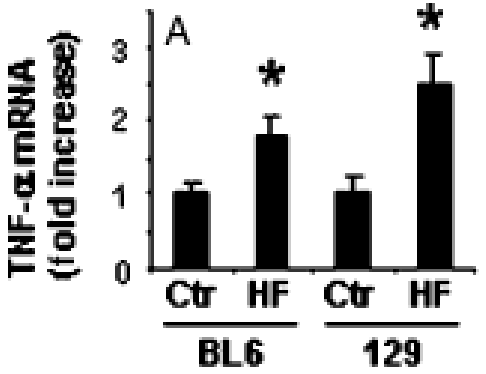
TCDD

Maternal Obesity is an NTD Risk Factor



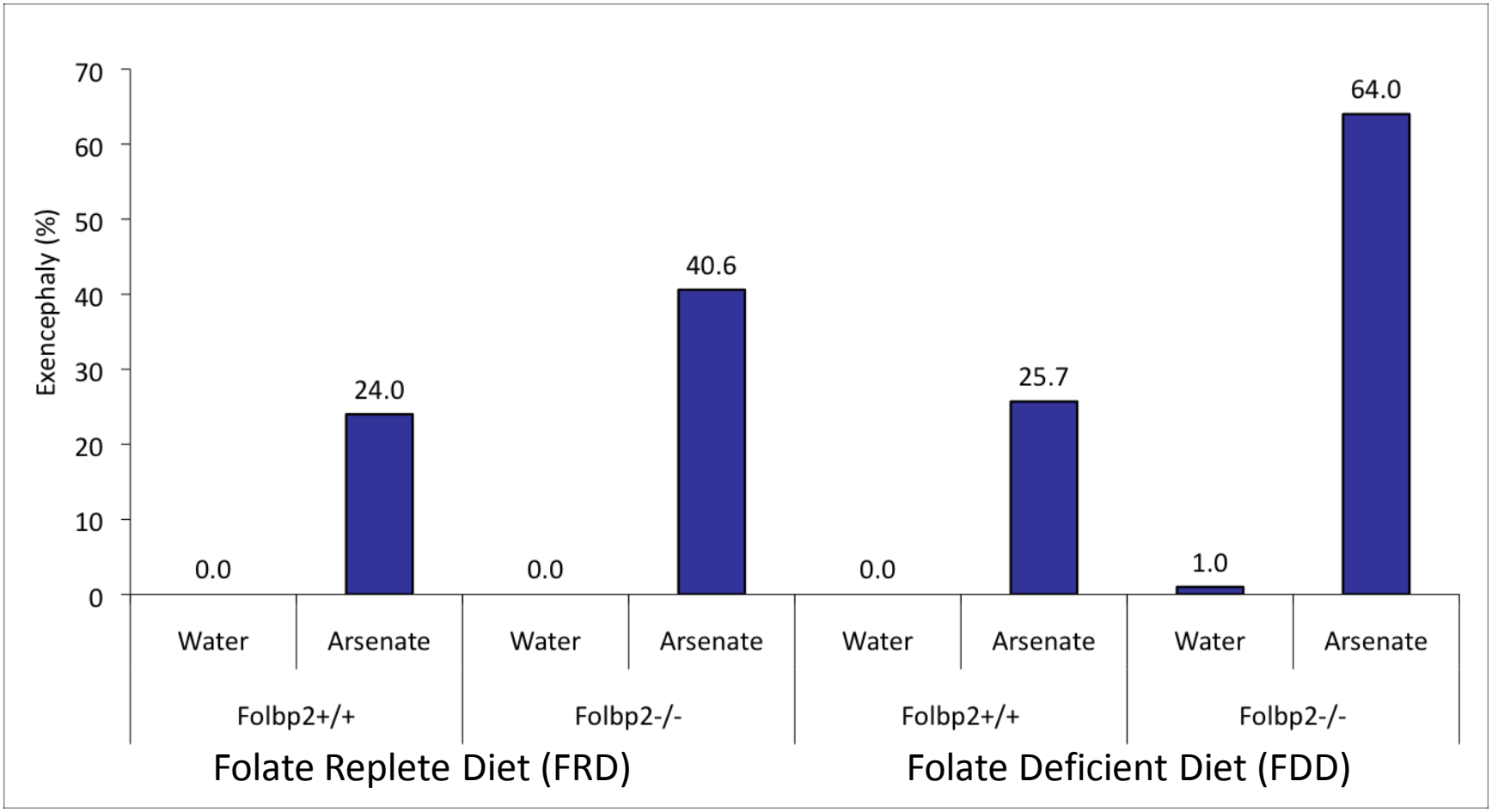
2/3rds of US women overweight=1.22 OR
1/3rd of US women are obese = 1.7 OR
7% of US women are morbidly obese=3.1 OR

- **Obesity is an Inflammatory Disease**



Inflammatory markers increase on high fat diets in mice

Arsenic-Induced NTDs in Folr2 Mice



Folr2 Nulls are Highly Susceptible to Arsenic-Induced NTDs

Gene X Environment X Environment Interaction

MATERNAL HYPERTHERMIA AS A NEURAL TUBE DEFECT CAUSING TERATOGEN

- Risks associated with increase in core temperature above 38.9° C

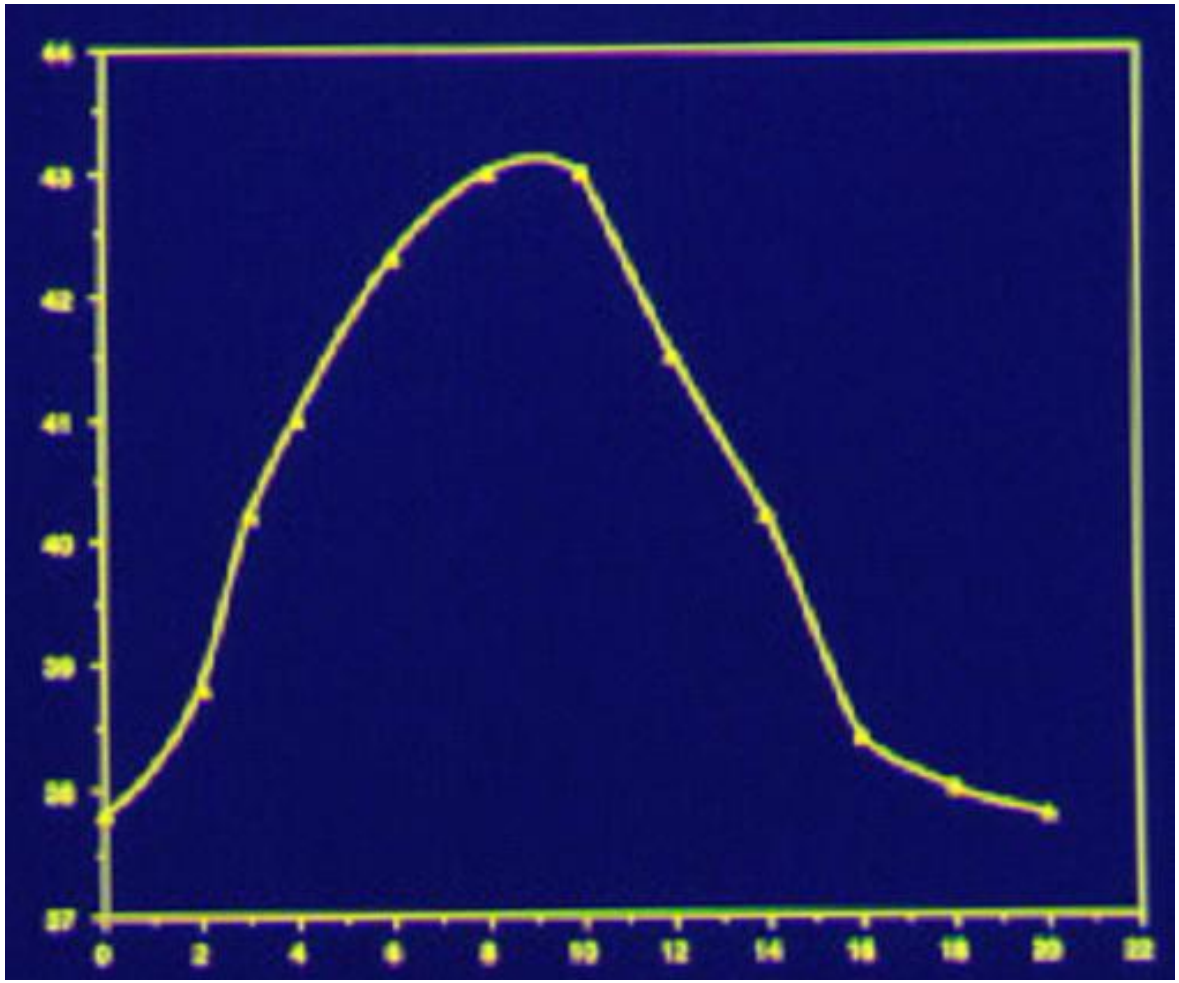
- Could be occupational or secondary to a disease process

Limited level of technology needed to study hyperthermia*

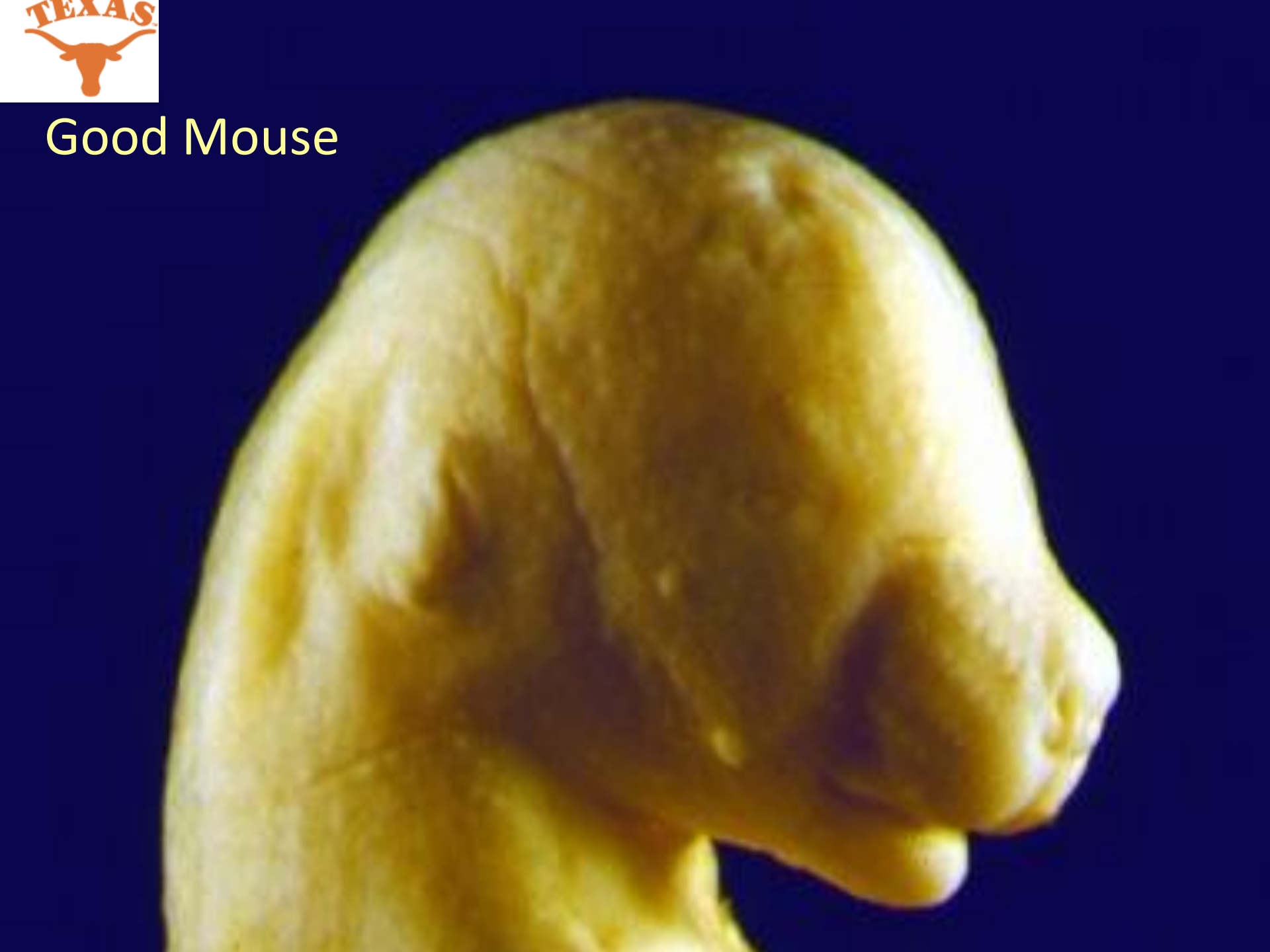
- Water bath
- Ring stand and clamp
- Thermometer
- 50ml centrifuge tube
  - Redwood decking optional

SWV HYPERTHERMIA TREATMENT

HEAT CURVE



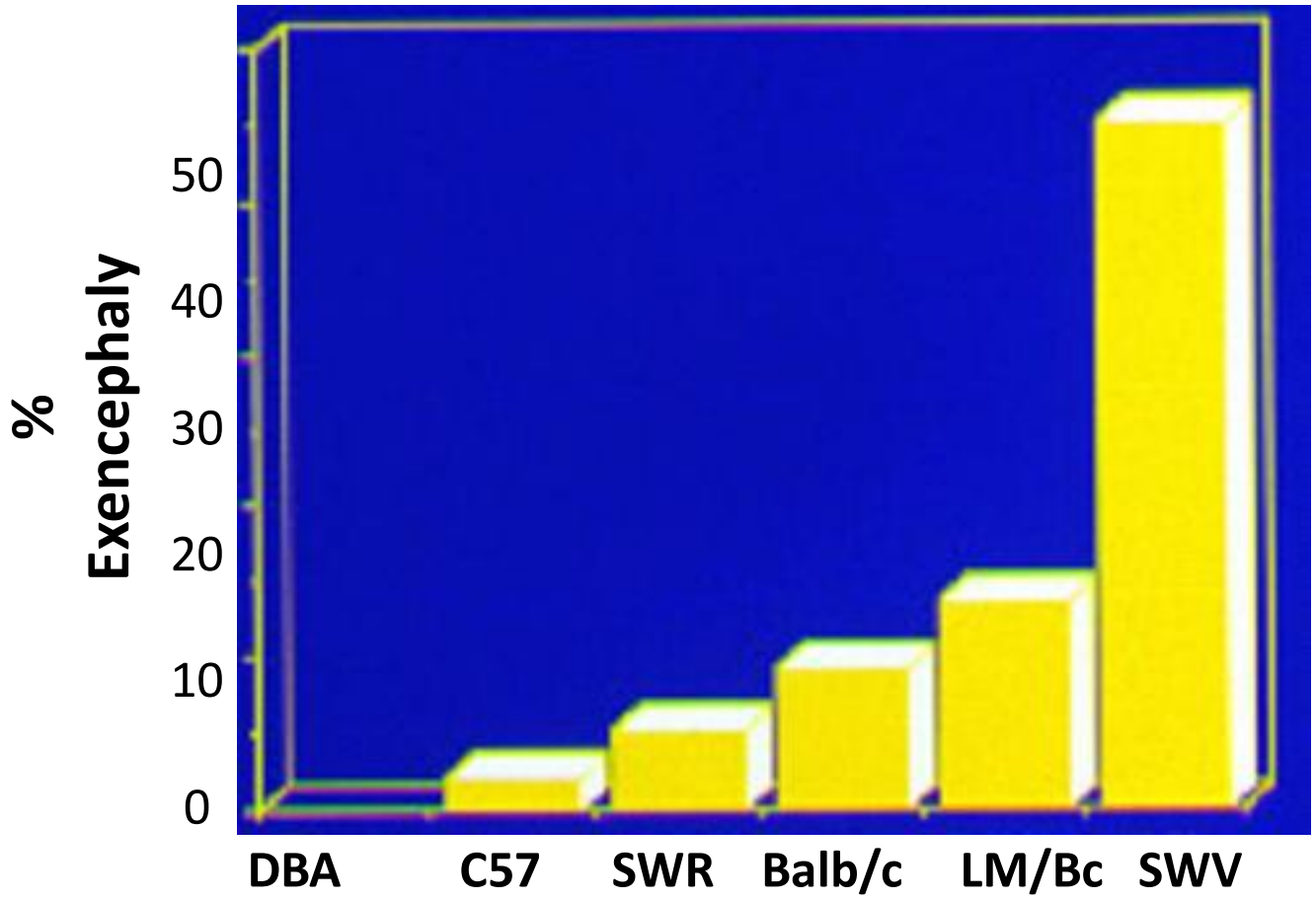
Good Mouse



Bad Mouse

Exencephaly,
cleft face

Hyperthermia-Induced Exencephaly



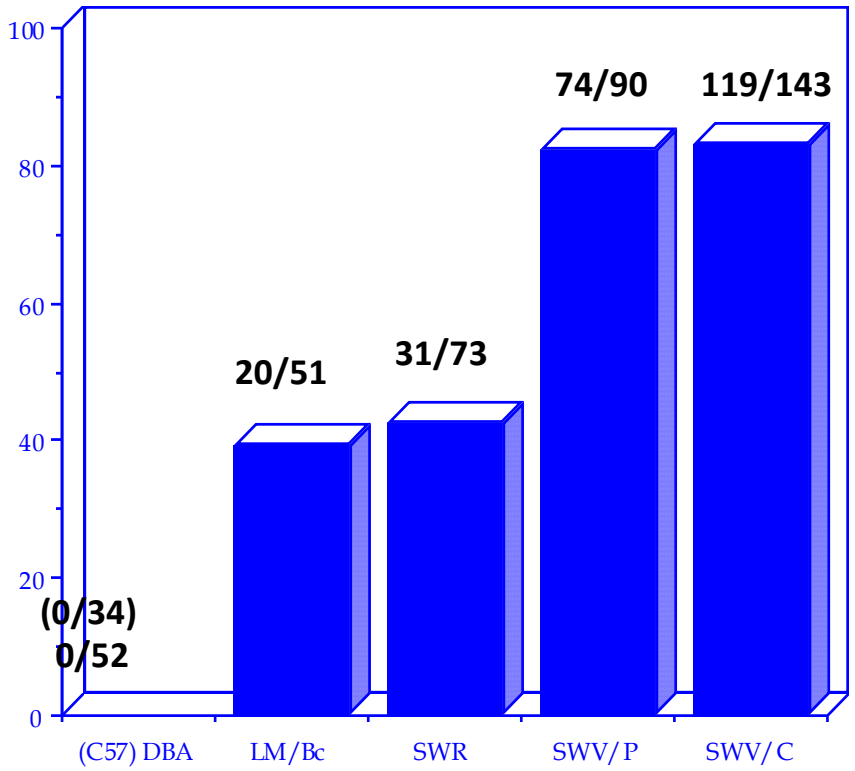
Mouse Models of Valproic Acid-Induced Neural Tube Defects

Treat Pregnant Dams From Multiple Inbred Mouse Strains at E8.5 with 600 mg/kg VPA

Collect Fetuses at E15.5 and Examine for Presence of NTDs

VALPROIC ACID TREATMENT

% VPA induced Exencephaly



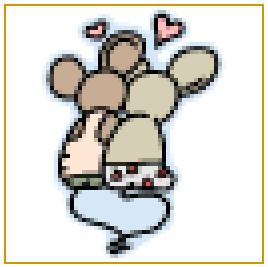
Strain Comparison

How Does One Find Modifying Genes to Explain Genetic Susceptibility to VPA-Induced NTDs?

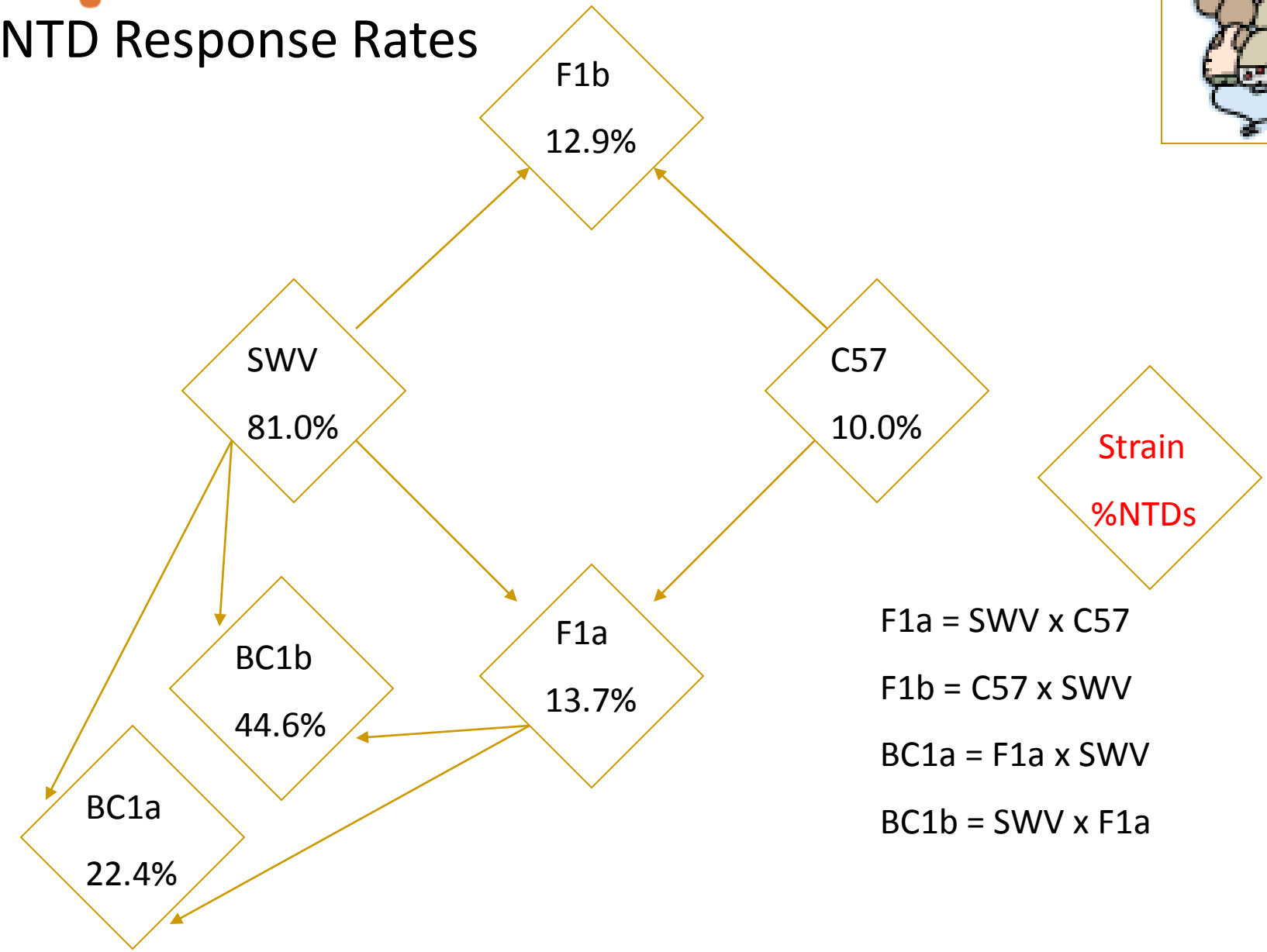
- Genetic Linkage Analyses

- Whole Genome Wide Analyses for Modifying Genes
  - Completed for SWV and C57
  - Located 1CM region on Chromosome 7
  - Multiple candidate genes localized to this region

Breeding Scheme



NTD Response Rates



F1a = SWV x C57

F1b = C57 x SWV

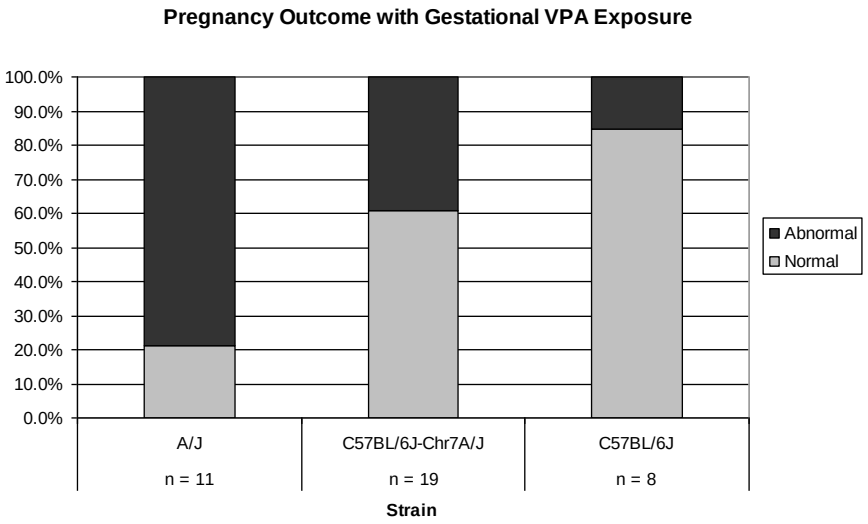
BC1a = F1a x SWV

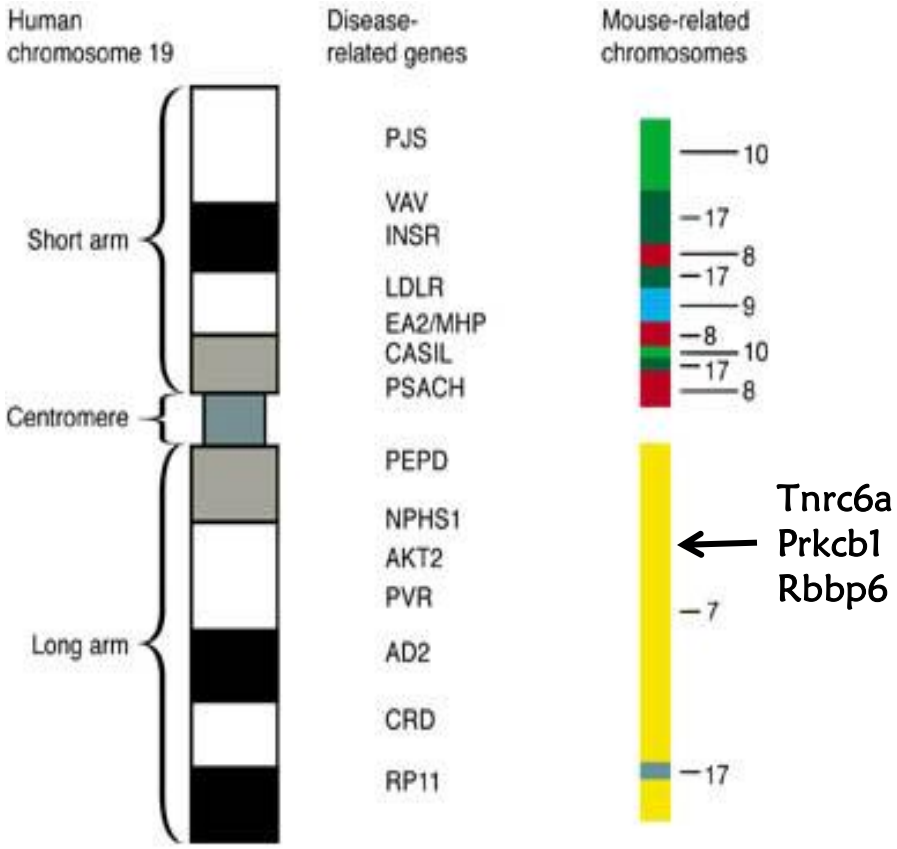
BC1b = SWV x F1a

Using Mouse Genetics to Find Modifying Genes to Explain Genetic Susceptibility to VPA-Induced NTDs?

Use of Consomic (Chr. Substitution) Mouse Strains

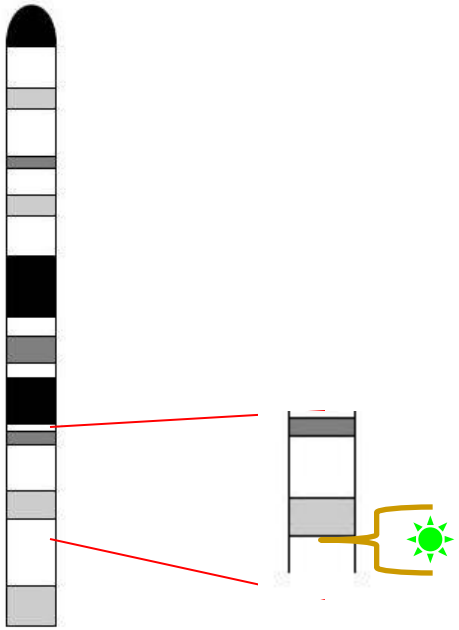
- Treat pregnant mice with Chr. 7 from VPA sensitive strain (A/J) placed on resistant background (C57) with VPA and collect fetuses
- Confirms Chr. 7 as site of sensitivity genes
- Screen candidate genes for SNPs associated with increased susceptibility



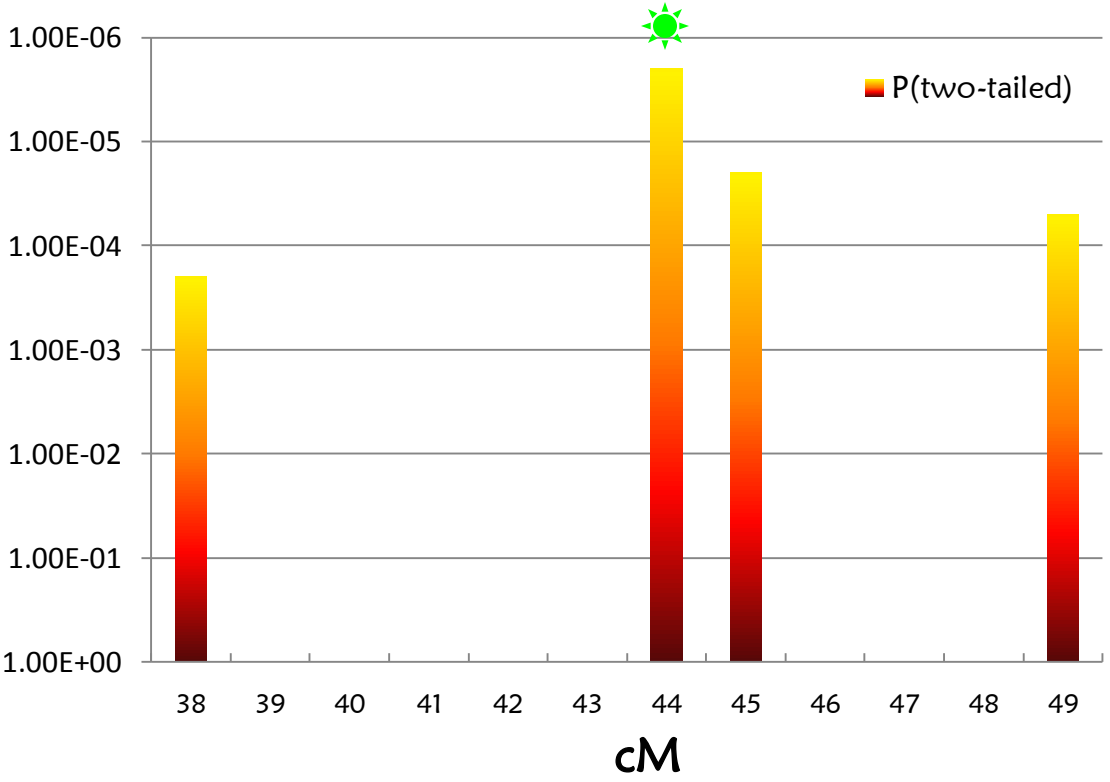


Linkage Region

	Location	P(two-tailed)	Chi^2	Genotype	Chrm	Bp	-bp
D7Mit220	38.3	2.00E-04	15.47	88/43	7	111543239	111543373
D7Mit285	44.8	2.00E-06	23.1	93/38	7	129629838	129629943
D7Mit101	45.9	2.00E-05	18.34	90/41	7	132776553	132776641
D7Mit105	49.2	5.00E-05	16.87	89/42	7	135707912	135708169

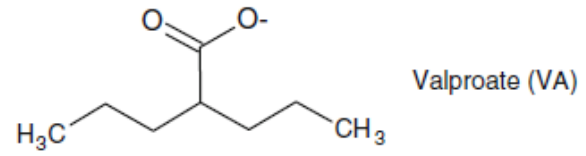


Chrm 7



ACSMs

Acyl-CoA Synthetase Medium-Chain Family Members

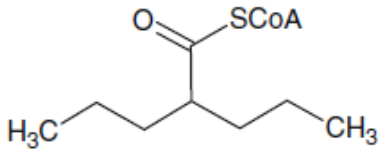


Valproate (VA)

ACSMs

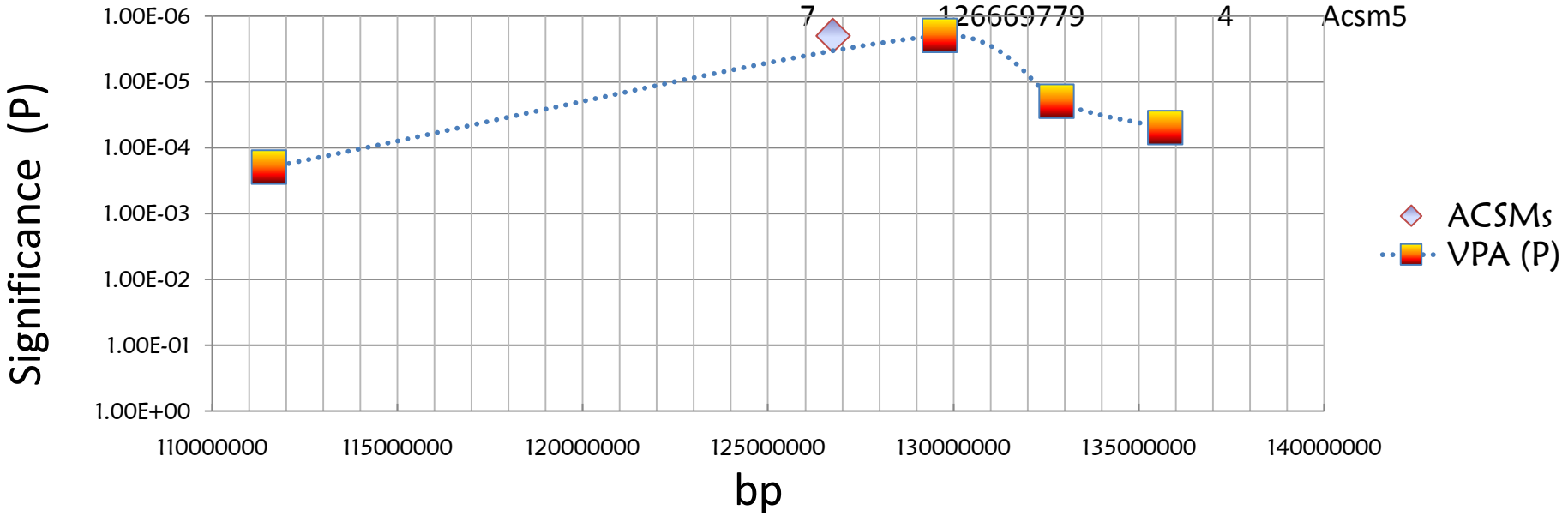
CoASH, ATP

AMP, PPi



Valproyl-CoA (VA-CoA)

Chrm	bp	-bp	Sym
7	126750540	126806029	Acsm1
7	126705007	126740115	Acsm2
7	126904437	126931027	Acsm3
7	126833540	126858081	Acsm4
7	126669779	126686874	Acsm5



Next Steps to Understanding Genetic Basis of Susceptibility to VPA-Induced NTDs

- Deep DNA Resequencing of Relevant Regions of the ACSM gene family in SWV and C57 Mice

- Development of ACSM Knockout Mouse Models to be Challenged with VPA Treatment
  - Have Obtained ES clones for ACSM4 and Blastocyst Injections are in Progress
- Human Patients From NEAD Study Exposed to VPA *in utero* with Variable Outcomes will be sequenced for variants in the ACSM gene family
  - Multiple candidate genes localized to this region

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

Clarke Fraser
09/12/09

F. Clarke Fraser

Finnell Laboratory
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