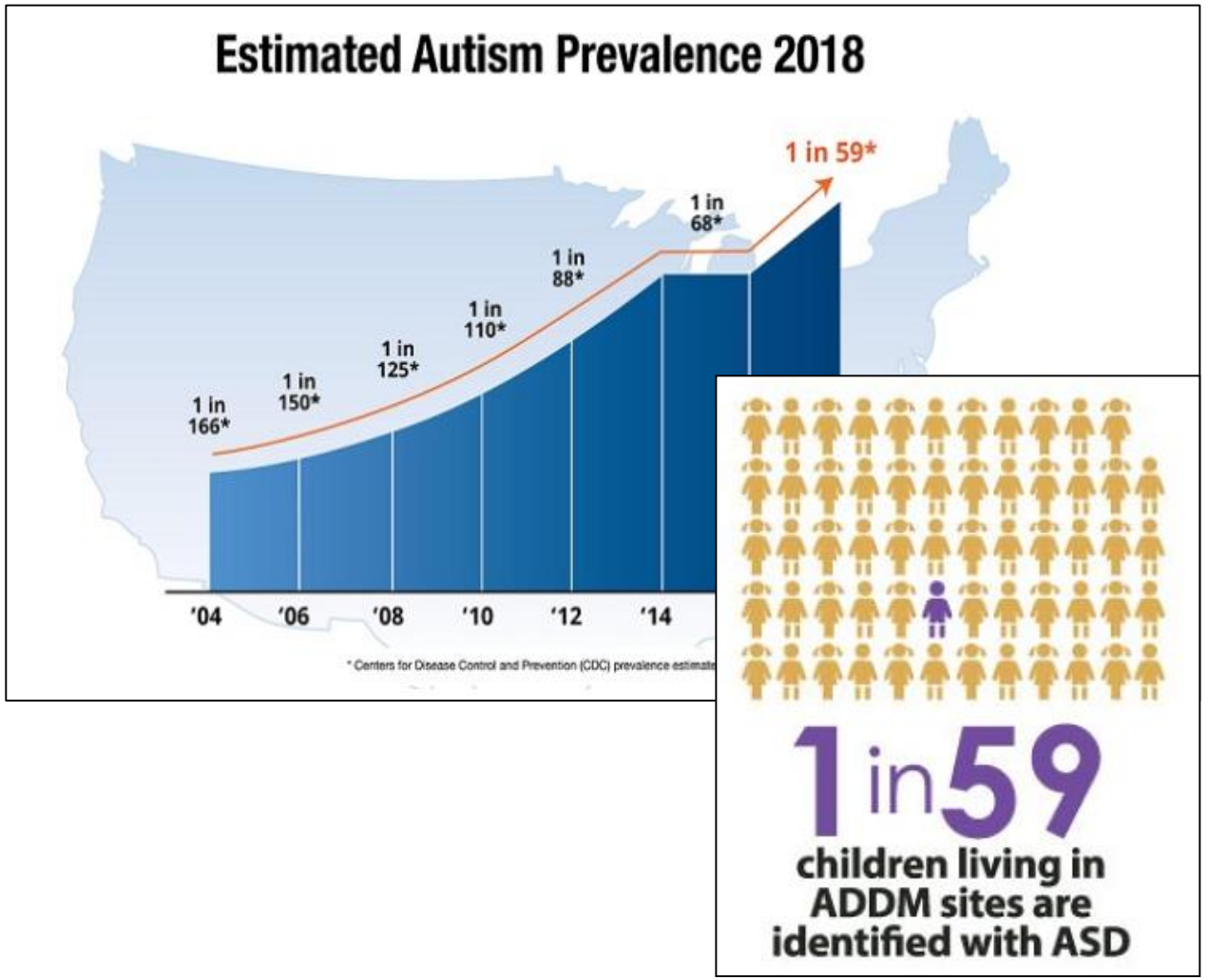
Fetal Origins of Autism Spectrum Disorders
Fetal Neuro-programming

Hind Moussa, M.D., FACOG

No relevant conflicts of interest to report

Autism Spectrum Disorders (ASD)

- Difficulties:
  - Social interaction
  - Verbal and nonverbal communication
  - Repetitive behaviors
- USA-ASD prevalence 1-2%
- ASD affects 1 in 59 children
- 4x more common in boys



Prevalence in Lebanon

J Autism Dev Disord (2016) 46:514–522
DOI 10.1007/s10803-015-2590-7

ORIGINAL PAPER

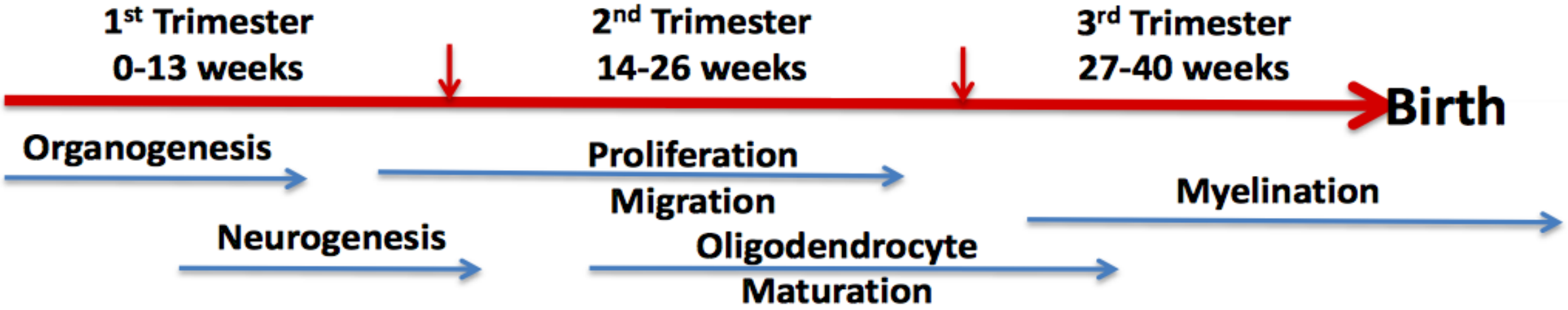
Prevalence of Autism Spectrum Disorder in Nurseries in Lebanon: A Cross Sectional Study

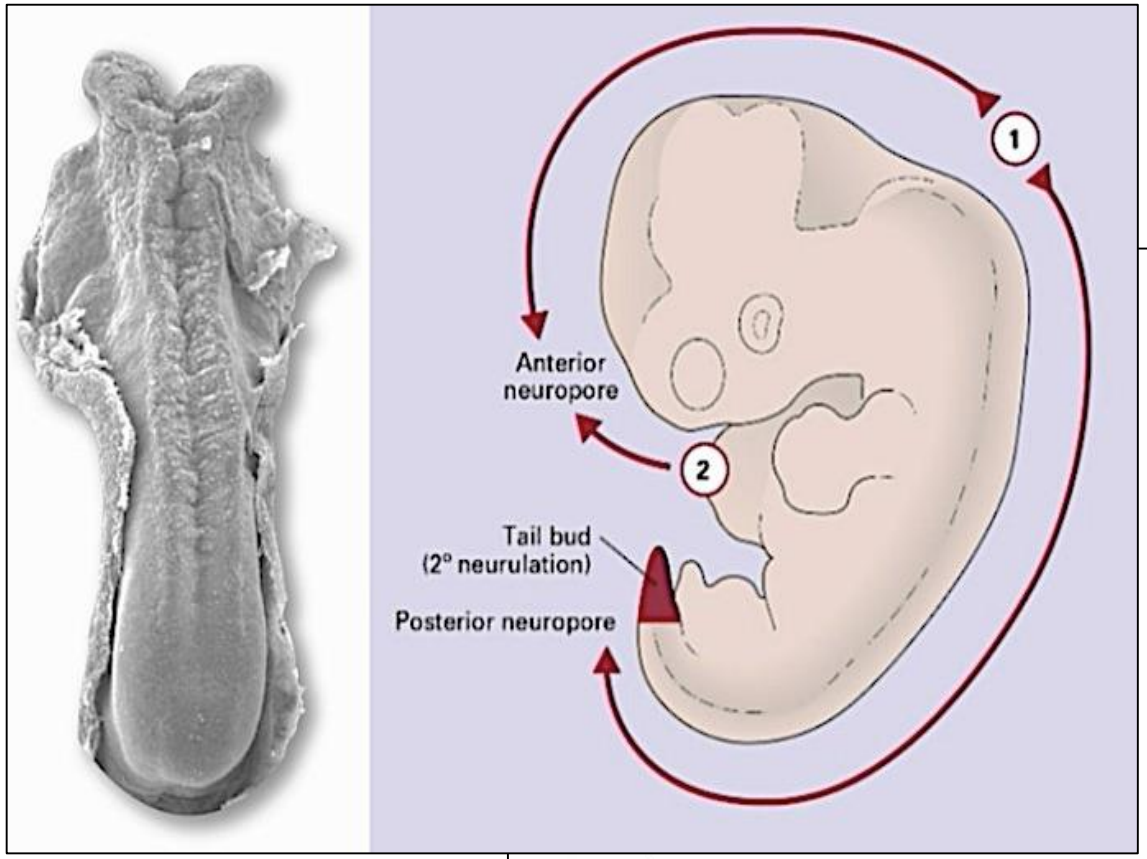
Monique Chaaya[1] · Dahlia Saab[2] · Fadi T. Maalouf[3] · Rose-Mary Boustany[2,4]

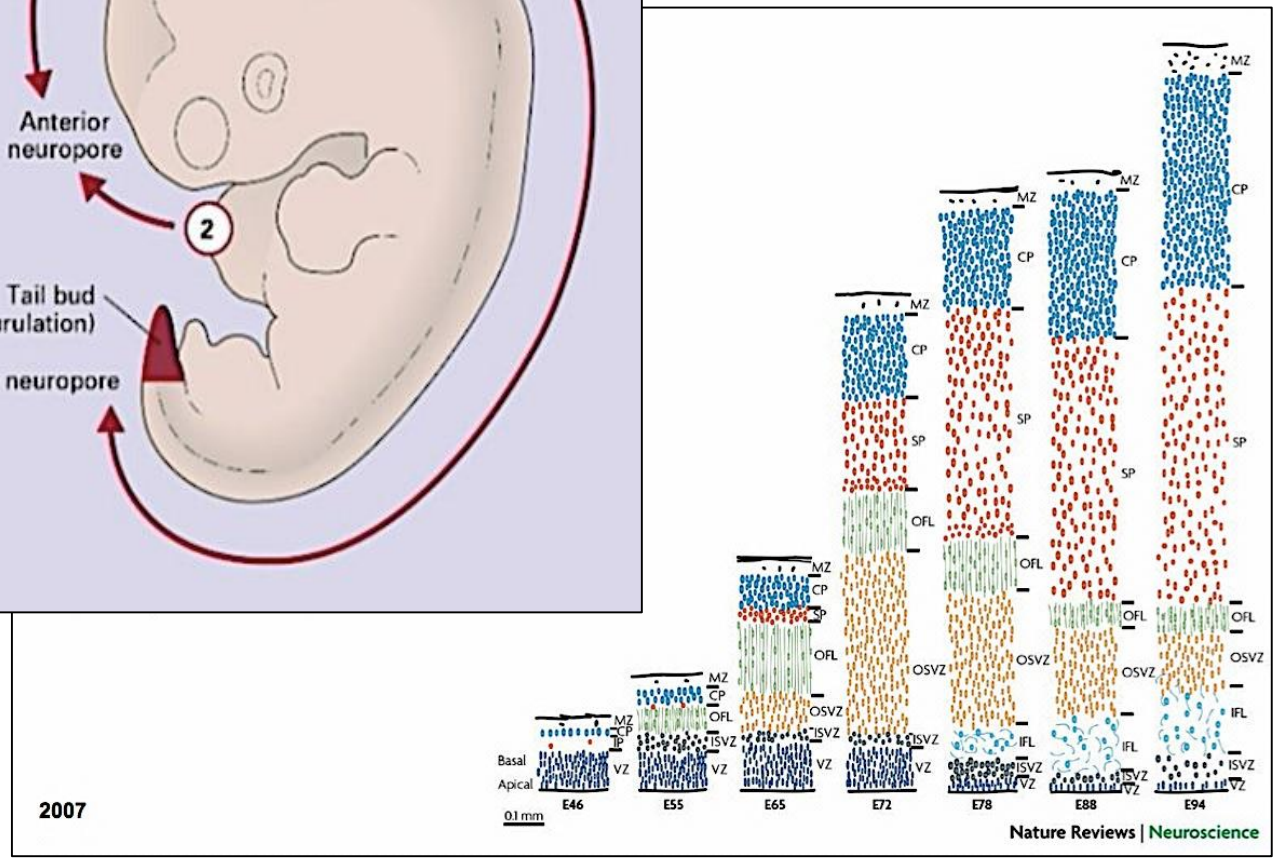
	Prevalence ASD, according to M-CHAT N (%)	Prevalence ASD corrected (M-CHAT prevalence*0.058) %	Prevalence ASD (95 % CI)
Total	263 (26.4)	1.53	0.77–2.29
Gender			
Male	144 (26.8)	1.55	0.51–2.59
Female	118 (25.7)	1.49	0.38–2.60
Governorate			
Beirut	66 (30.4)	1.76	0.01–3.51
Mount Lebanon	197 (25.2)	1.46	0.62–2.30

- **Early human brain development**
  - Sequence of intricate processes
  - Functionally operative neural circuits

- **Developmental trajectories of early brain network formation**
  - Genetically programmed
  - Epigenetic influences
  - Environmental influences




1
Anterior neuropore
2
Tail bud (2° neurulation)
Posterior neuropore


MZ
CP
SP
OFL
OSVZ
ISVZ
IFL
VZ
Basal
Apical
0.1 mm
E46
E55
E65
E72
E78
E88
E94
2007
Nature Reviews | Neuroscience

GW4-6

- GW5 nascent cerebral hemispheres can be seen
- Symmetric cell division of neuroepithelial stem cells, which become ventricular radial glia cells

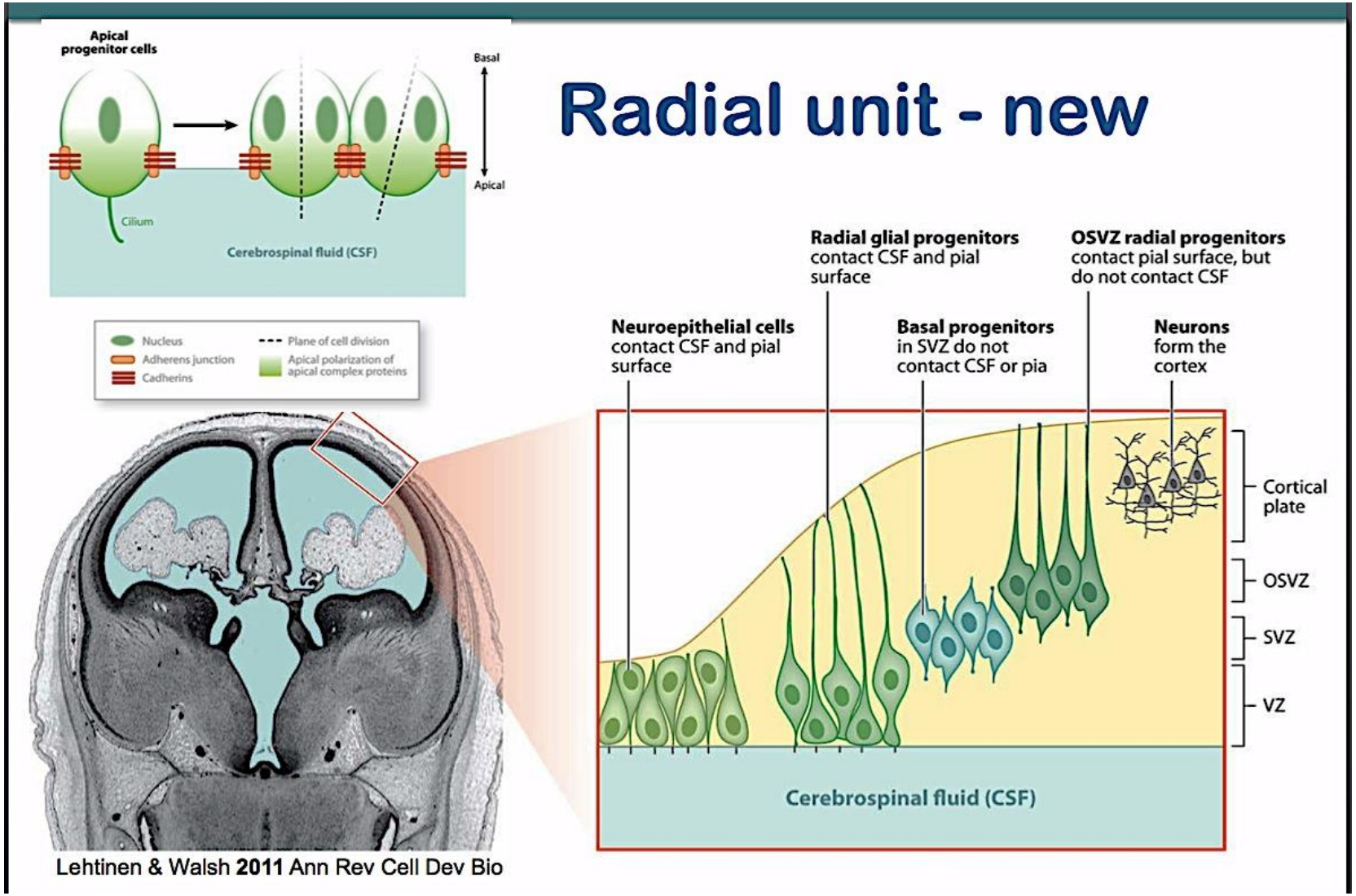
GW 6-10

- GW6 neurogenesis of first wave destined for cortical plate
- GW7-10 neurogenesis and neuronal migration to cortical plate
- Neural progenitor cells = radial glia in VZ
- Asymmetric cell division: separating proliferating cells from postmitotic neurons

Dividing cells generate pairs of daughter cells

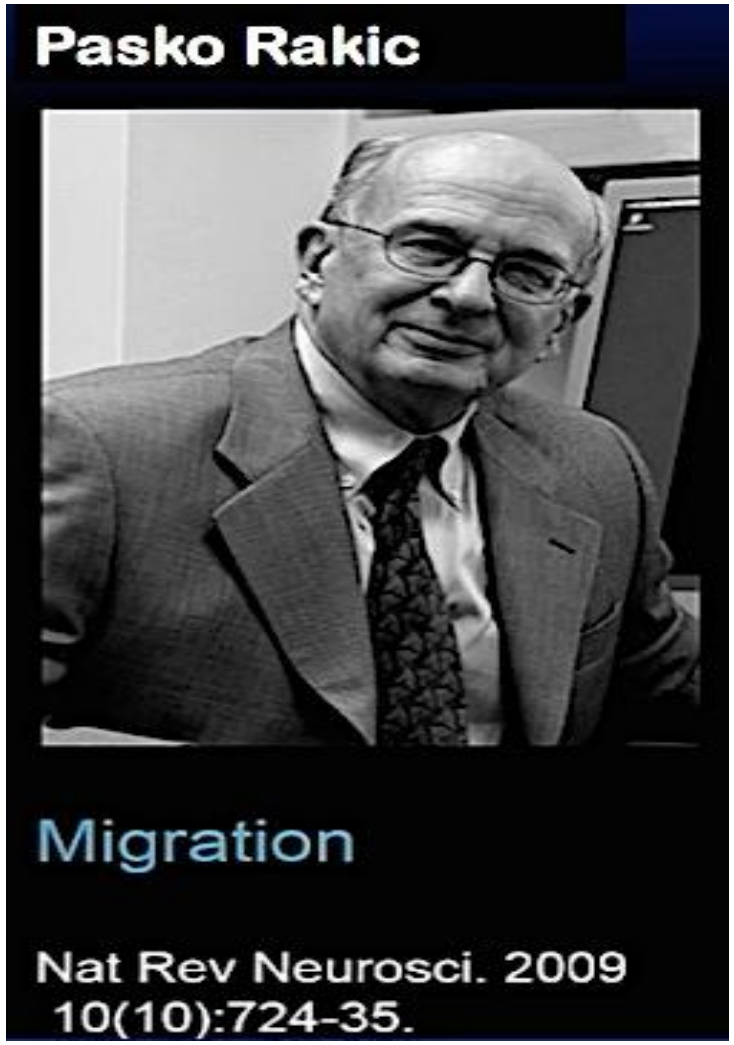
- with the same symmetric cell fate (Two progenitor cells)
- with distinct, asymmetric cell fates (one progenitor and one neuron)

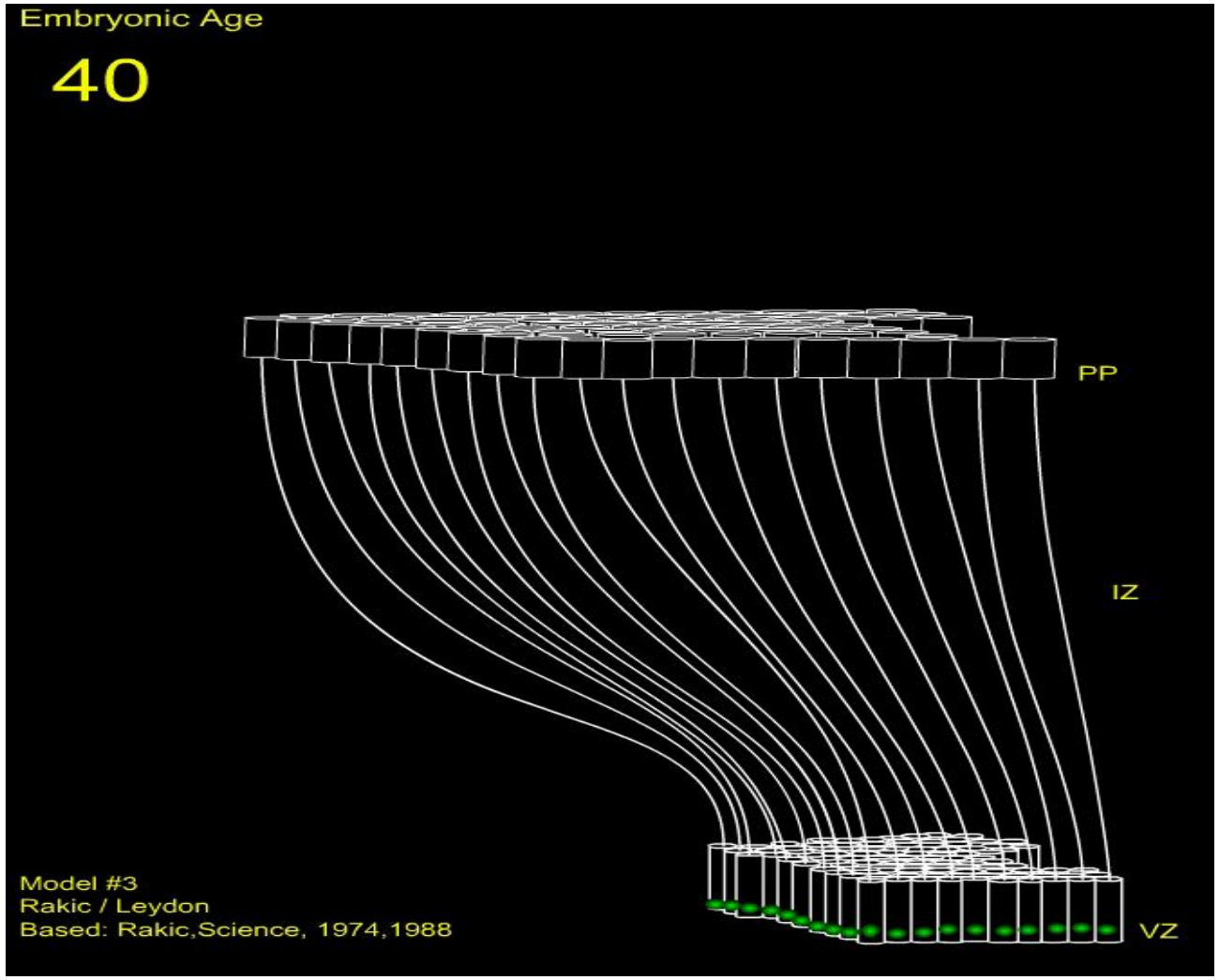
Neuronal Migration



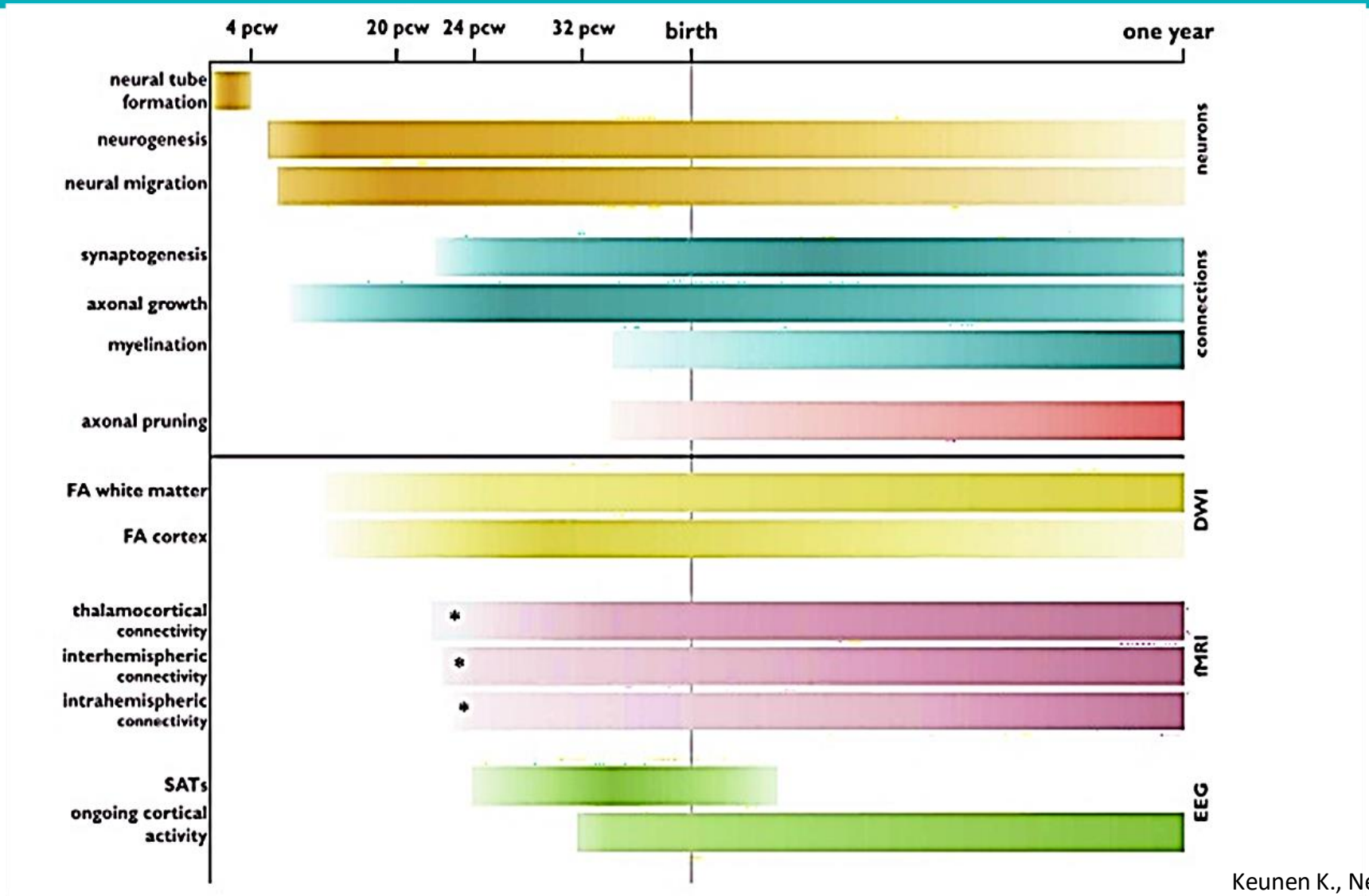
Lehtinen & Walsh **2011** Ann Rev Cell Dev Bio

Neuronal Migration





Functional Neurodevelopment



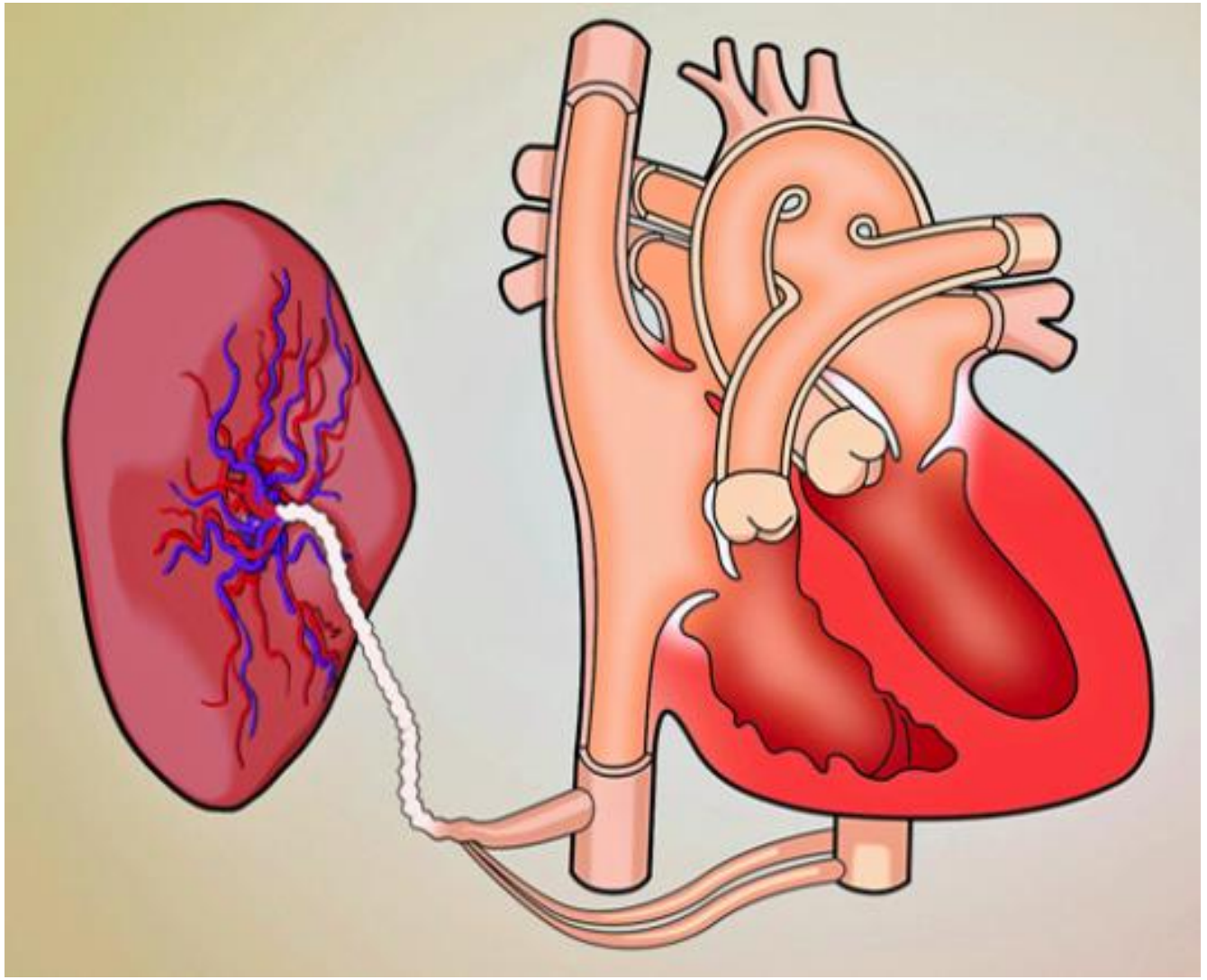
Keunen K., NeuroImage (2017)

Intrauterine Environment

1. Fetal Factors
2. Placental Factors
3. Maternal Factors

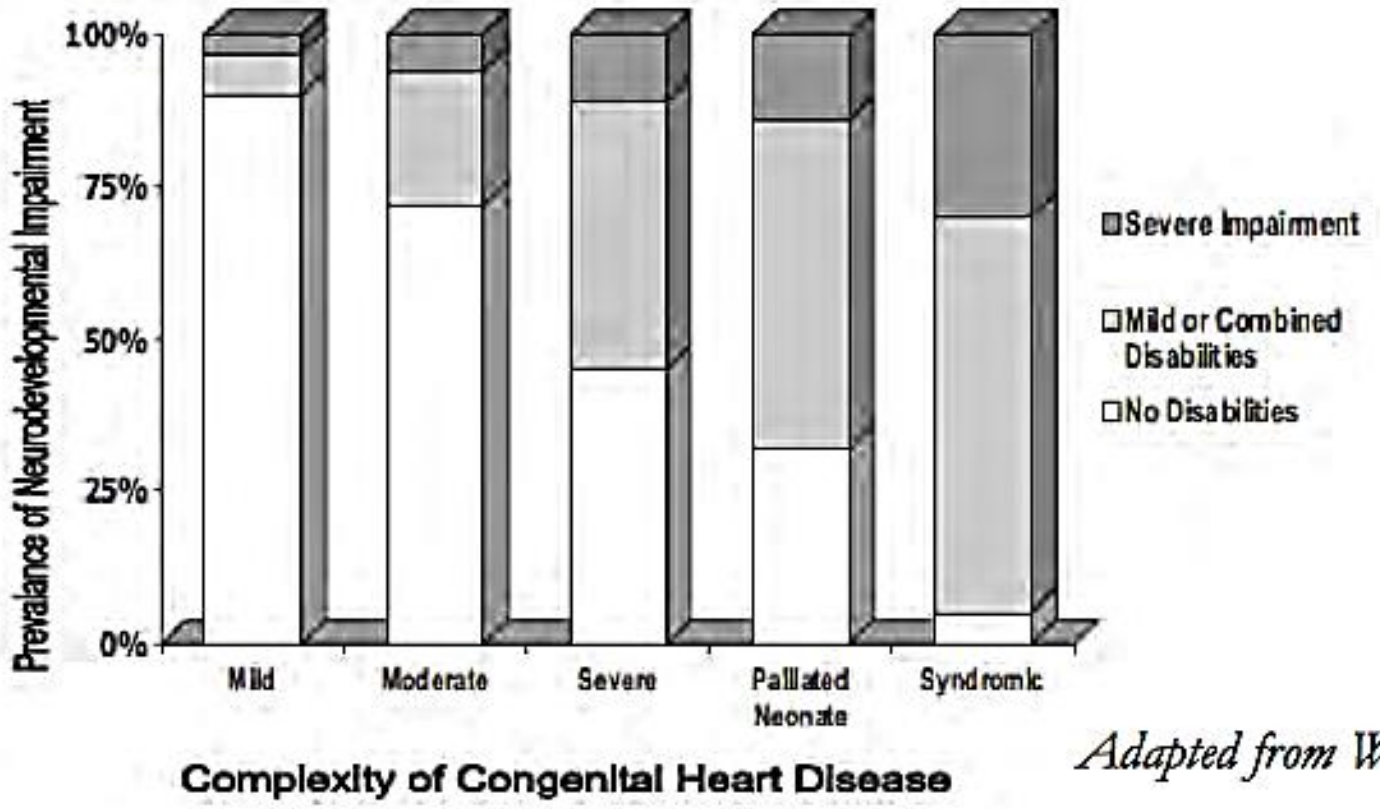
1. The Passenger
2. The Placenta
3. The Parent

Congenital Heart Disease



Fetal Brain Oxygenation and Perfusion in Congenital Heart Disease:

Impact on Neurodevelopment

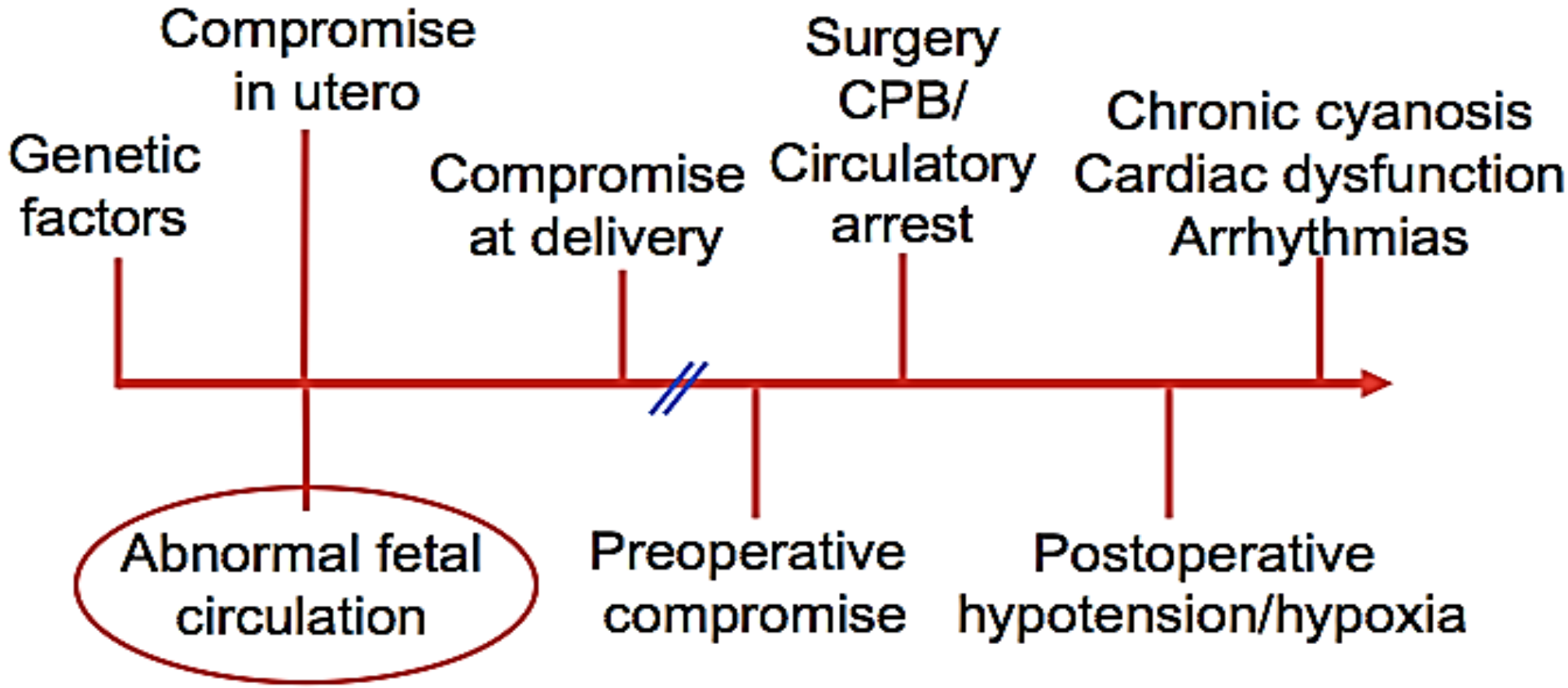


Adapted from Wernovsky, 2006

Fetal Brain Oxygenation and Perfusion in CHD

Neurodevelopmental Sequelae of CHD

Why does it happen?



Brain Development and Injury in CHD: Benefit of Prenatal Diagnosis

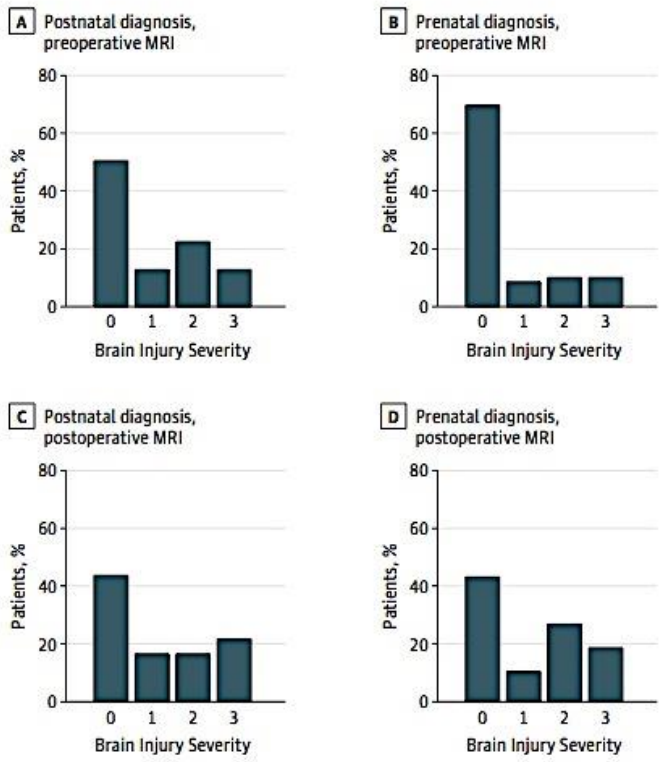
- **Preop injury and postop brain development in TGA/SV**
  - Brain injury less in those with prenatal dx (24 vs 48%)
  - More rapid brain development postop in those with a prenatal diagnosis

Table 3. Prevalence of Preoperative Brain Injury by Cardiac Diagnosis and Postnatal vs Prenatal Diagnosis of Critical Congenital Heart Disease

Preoperative Brain Injury and Cardiac Diagnosis	No. With Injury/Total No. With Cardiac Diagnosis (%)		*P* Value[a]
	Postnatal Diagnosis	Prenatal Diagnosis	
Any injury[b]			
All patients	41/86 (48)	16/67 (24)	.003
TGA	31/68 (46)	6/28 (21)	.03
SVP	10/18 (56)	10/39 (26)	.03
SVP with aortic arch obstruction	9/17 (53)	7/31 (23)	.02
White matter injury			
TGA	17/68 (25)	3/28 (11)	.09
SVP			.06
Stroke			
TGA			.09
SVP			.61
Hypoxic-ische			
TGA			.71
SVP			.32

Prenatal diagnosis, delivery room care, and fetal treatment to improve O2 delivery

Figure 1. Preoperative and Postoperative Brain Injury Severity by Postnatal vs Prenatal Diagnosis of Critical Congenital Heart Disease



Peyvandi, JAMA Pediatrics 2016

Intrauterine Environment

1. Fetal Factors
2. Placental Factors
3. Maternal Factors

1. The Passenger
2. The Placenta
3. The Parent

The Growth Restriction Intervention Trial (GRIT)

A randomised trial of timed delivery for the compromised preterm fetus: short term outcomes and Bayesian interpretation

The GRIT Study Group*

Infant wellbeing at 2 years of age in the Growth Restriction Intervention Trial (GRIT): multicentred randomised controlled trial

*The GRIT study group**

RESEARCH www.AJOG.org

OBSTETRICS

The Growth Restriction Intervention Trial: long-term outcomes in a randomized trial of timing of delivery in fetal growth restriction

Dawn-Marie Walker, PhD; Neil Marlow, DMFMedSci; Lisa Upstone, DClinPsy; Harriet Gross, PhD; Janet Hornbuckle, MD, MB, MRCOG; Andy Vail, MSc; Dieter Wolke, PhD; Jim G. Thornton, MD, FRCOG

GRIT study group, 2003, 2004, 2010

Intrauterine Environment -Placenta

GRIT Studies, 2003, 2004, 2010

547 singleton / Twins - 24-36 GA

Unsure about delivery timing

	Immediate delivery	+4.9 days (*6.9 > 31 SSW)	Deliver when no longer in doubt	
BJOG 2003				
C-section	91%	<0.05	76%	
FDIU	2	<0.05	9	
neonatal Death	27	0.06	18	
Perinatal Mortality	29	n.s.	27	
Prematurity-related	17	<0.05	8	<30 weeks
Lancet 2004	*"2 year Follow-up: Griffith DQ, CP, Mortality"*			
Dev. delay	14	<0.05	5	<30 weeks
Cerebral palsy	8	<0.01	0	
AJOG 2010	*"6-13 year Follow-up: Kaufmann ABC, Cognitive Outcome"*			

Identical Long-term outcomes

Trial Of Randomized Umbilical And Fetal Flow In Europe (Truffle)

Perinatal morbidity and mortality in early-onset fetal growth restriction: cohort outcomes of the trial of randomized umbilical and fetal flow in Europe (TRUFFLE)

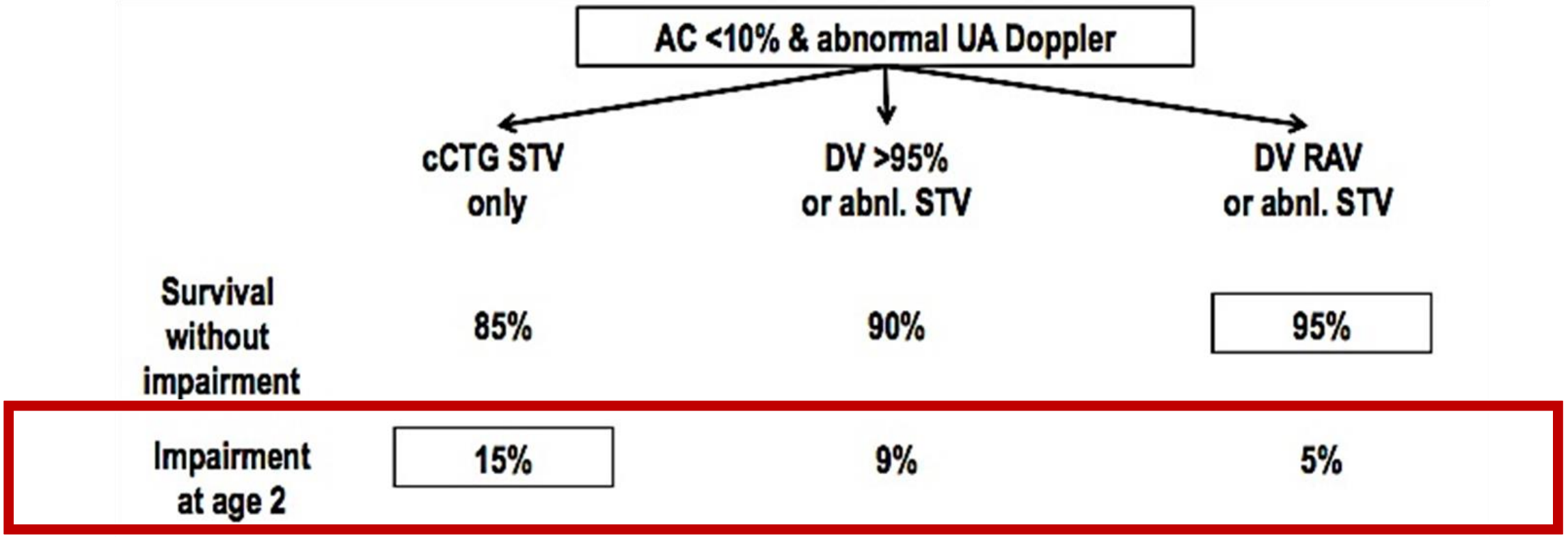
C. LEES[1], N. MARLOW[2], B. ARABIN[3], C. M. BILARDO[4], C. BREZINKA[5], J. B. DERKS[6], J. DUVEKOT[7], T. FRUSCA[8], A. DIEMERT[9], E. FERRAZZI[10], W. GANZEVOORT[11], K. HECHER[9], P. MARTINELLI[12], E. OSTERMAYER[13], A. T. PAPAGEORGHIOU[14], D. SCHLEMBACH[15], K. T. M. SCHNEIDER[13], B. THILAGANATHAN[14], T. TODROS[16], A. VAN WASSENAER-LEEMHUIS[17], A. VALCAMONICO[8], G. H. A. VISSER[18] and H. WOLF[11], on behalf of the TRUFFLE Group#

2 year neurodevelopmental and intermediate perinatal outcomes in infants with very preterm fetal growth restriction (TRUFFLE): a randomised trial

*Christoph C Lees, Neil Marlow, Aleid van Wassenaer-Leemhuis, Birgit Arabin, Caterina M Bilardo, Christoph Brezinka, Sandra Calvert, Jan B Derks, Anke Diemert, Johannes J Duvekot, Enrico Ferrazzi, Tiziana Frusca, Wessel Ganzevoort, Kurt Hecher, Pasquale Martinelli, Eva Ostermayer, Aris T Papageorghiou, Dietmar Schlembach, K T M Schneider, Baskaran Thilaganathan, Tullia Todros, Adriana Valcamonico, Gerard H A Visser, Hans Wolf, for the TRUFFLE study group**

Lees et al (2013), (2015)

Trial Of Randomized Umbilical And Fetal Flow In Europe (Truffle)



A conservative approach to timing delivery in waiting for late Ductus Venosus changes, unless severe CTG changes occur first, was associated with a more favorable 2 year outcome in early onset fetal growth restriction

Intrauterine Environment

1. Fetal Factors
2. Placental Factors
3. Maternal Factors

1. The Passenger
2. The Placenta
3. The Parent

KETTERING Health Network

Maternal Factors

Maternal Conditions	OR	Confidence Interval	Study	Reference
Obesity (BMI≥ 30)	**3.2 ****	1.10-2.56	CHARGE Population Study	Pediatrics. 2012;129:e1121–e1128.
Pre-Eclampsia	**1.5****	1.18-4.68	CHARGE Population Study	JAMA Pediatr. 2014 Dec 8.
Severe Pre-Eclampsia with Placental Insufficiency	**3.39**	1.06-3.50	CHARGE Population Study	JAMA Pediatr. 2014 Dec 8.
Maternal thyroid peroxidase antibody positivity (TPO-Ab+)	**2.6**	1.16–2.75	Nested case-control design of the Finnish Prenatal Study of Autism (FiPS-A)	Prog Neuropsychopharmacol Biol Psychiatry. 2015 Mar 3;57:86-92.
Rheumatoid Arthritis	**2.36**	1.07–2.54	The study cohort consisted of all of the children born in Denmark from 1993 through 2004 (689 196 children).	Pediatrics 124: 687–694
Celiac Disease	**2.12**	1.27–5.75	The study cohort consisted of all of the children born in Denmark from 1993 through 2004 (689 196 children).	Pediatrics 124: 687–694
Type I Diabetes	**1.86**	1.07–3.77	The study cohort consisted of all of the children born in Denmark from 1993 through 2004 (689 196 children).	Pediatrics 124: 687–694
Febrile Episode	**1.84**	1.1-2	Danish Cohort	Pediatrics. 2012 Dec;130(6):e1447-54.

Journal of Autism and Developmental Disorders (2018) 48:2010–2021
https://doi.org/10.1007/s10803-017-3449-x

ORIGINAL PAPER

Association of Autism with Maternal Infections, Perinatal and Other Risk Factors: A Case-Control Study

Dikran Richard Guisso[1] · Fadi S. Saadeh[1] · Dahlia Saab[2] · Joud El Deek[1] · Sarah Chamseddine[1] · Hadi Abou El Hassan[1] · Ghidaa Majari[1] · Rose-Mary Boustany[2,3]

Parental Inheritance of Endothelial Nitric Oxide Synthase (eNOS) Gene and Abnormal Uterine Environment Contribute to Autism Spectrum Disorders in a Hypertensive Murine Model

Hind Moussa, Baha Sibai, Sean Blackwell, Mateo Leon, John Redell, Yin Liu, Pramod Dash, Monica Longo

Division of Maternal Fetal Medicine

Department of Obstetrics, Gynecology and Reproductive Sciences

UT Health- University of Texas Medical School at Houston

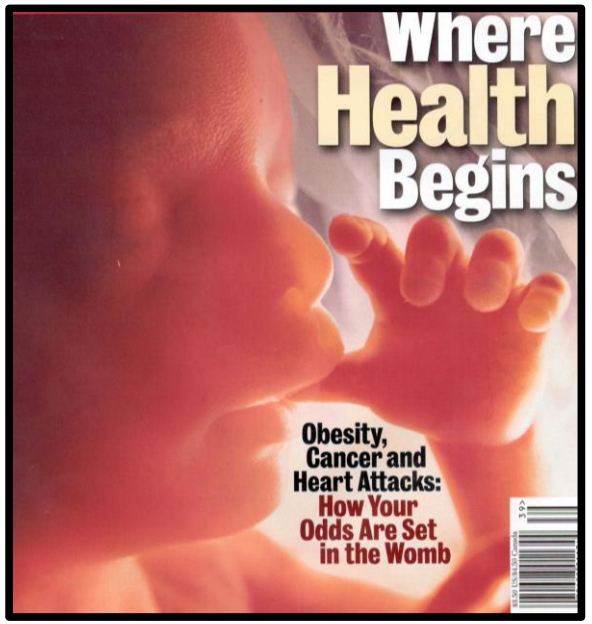
Genetic and Uterine Environment Factors

Maternal Factors → Uterine Environment

HYPERTENSION

Maternal and Paternal Genes
20%

ZYGOTE



PHENOTYPE

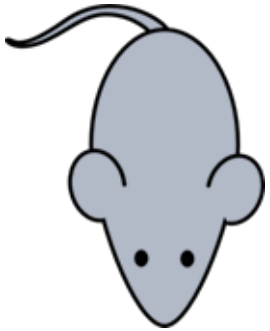
Mice Offspring Developing in an Abnormal Uterine Environment, Secondary to Maternal Hypertension, Will Have an ASD-Like Phenotype

Aims

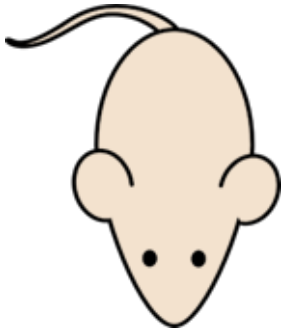
1. To evaluate the contribution of maternal hypertension to ASD-like phenotype

2. To identify novel ASD related genes and their biological processes in the cerebellum

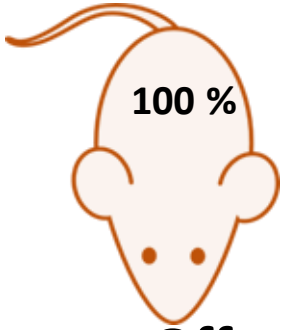
Endothelial Nitric Oxide Synthase (eNOS) Hypertension Mouse Model



Knock Out eNOS-/-



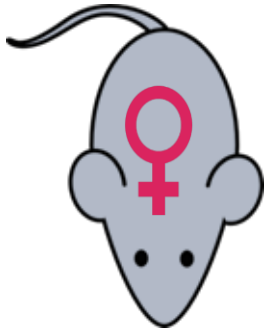
Wild Type eNOS+/+



Heterozygous Offspring eNOS-/+

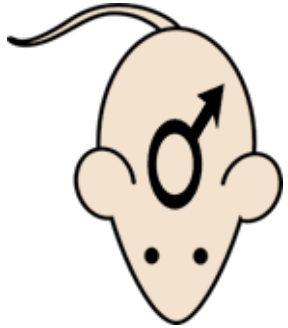
Fetal Programming Animal Model

Cross Breeding Scheme

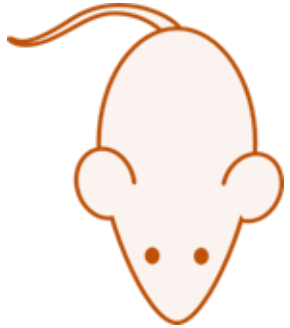


Knock Out eNOS-/-

Hypertensive Mother



Wild Type eNOS+/+

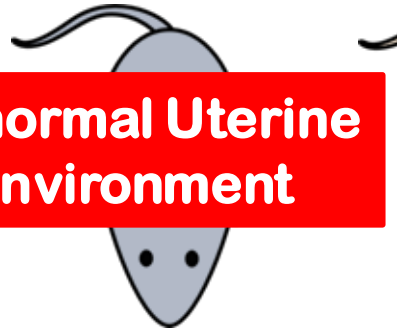


Heterozygous

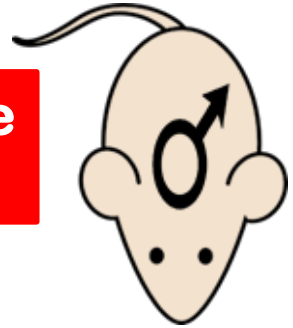
Mat-eNOS-/+

Fetal Programming Animal Model

Cross Breeding Scheme



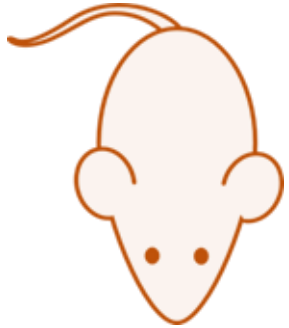
Abnormal Uterine Environment



Knock Out eNOS-/-

Wild Type eNOS+/+

Hypertensive Mother

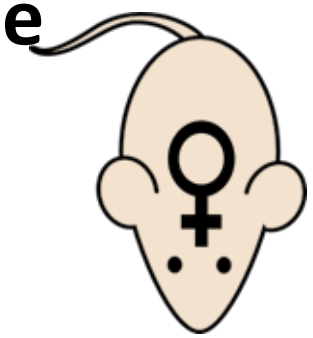


Heterozygous

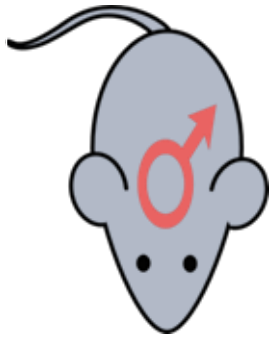
Mat-eNOS-/+

Fetal Programming Animal Model

Cross Breeding Scheme

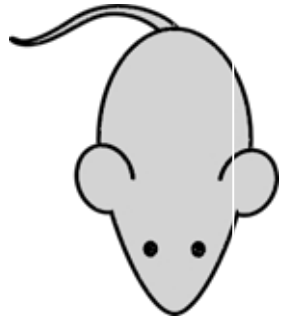


Wild Type eNOS+/+



Knock Out eNOS-/-

Normotensive Mother

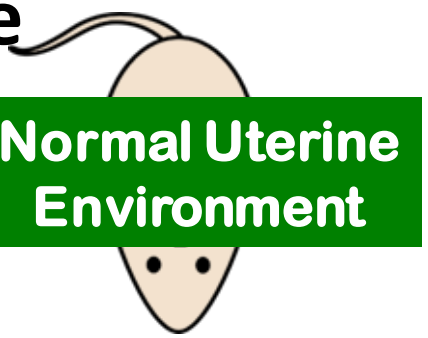


Heterozygous

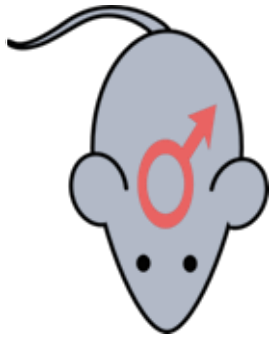
Pat-eNOS-/+

Fetal Programming Animal Model

Cross Breeding Scheme

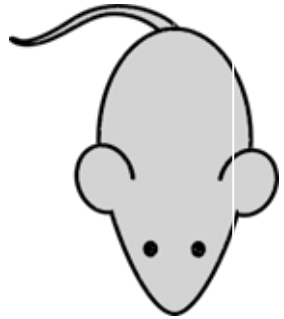


Normal Uterine Environment



Wild Type eNOS+/+ **Knock Out eNOS-/-**

Normotensive Mother



Heterozygous

Pat-eNOS-/+

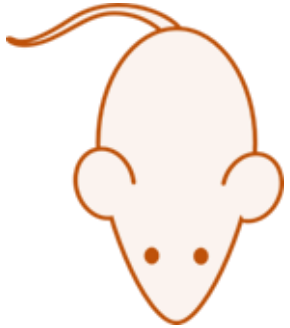
Heterozygous Offspring

Developed in an Abnormal Uterine Environment

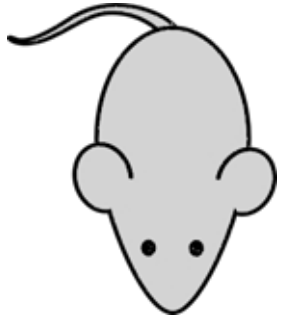
Hypertensive Mother

Developed in a Normal Uterine Environment

Normotensive Mother



Genetically Identical Offspring



Heterozygous
Mat-eNOS-/+

Heterozygous
Pat-eNOS-/+

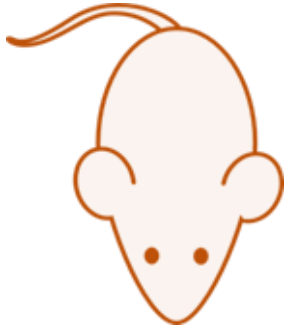
Heterozygous Offspring

Developed in an Abnormal Uterine Environment

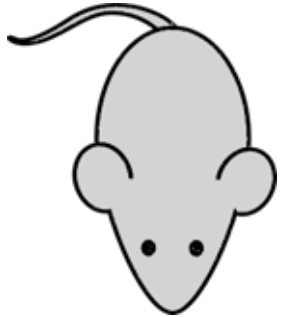
Hypertensive Mother

Developed in a Normal Uterine Environment

Normotensive Mother



ASD Features



Heterozygous Mat-eNOS-/+

Heterozygous Pat-eNOS-/+

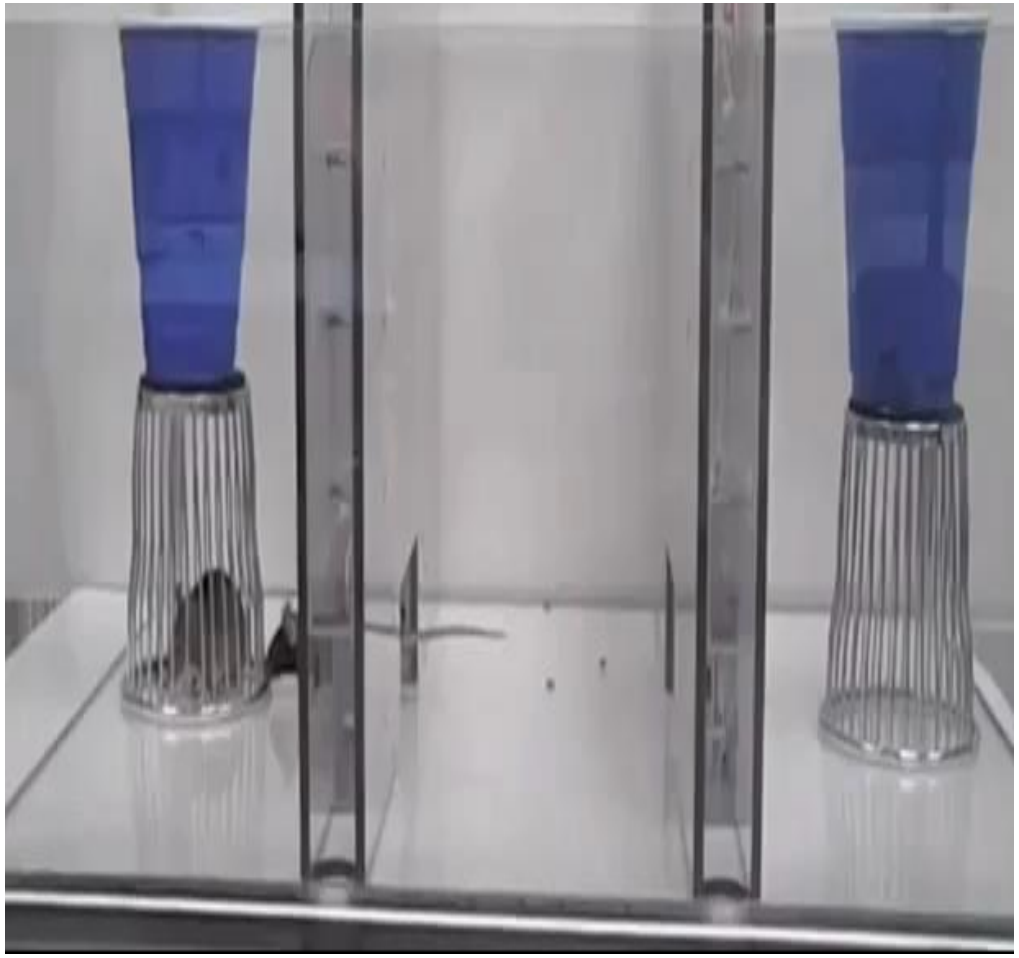
Behavioral Phenotype Characterization

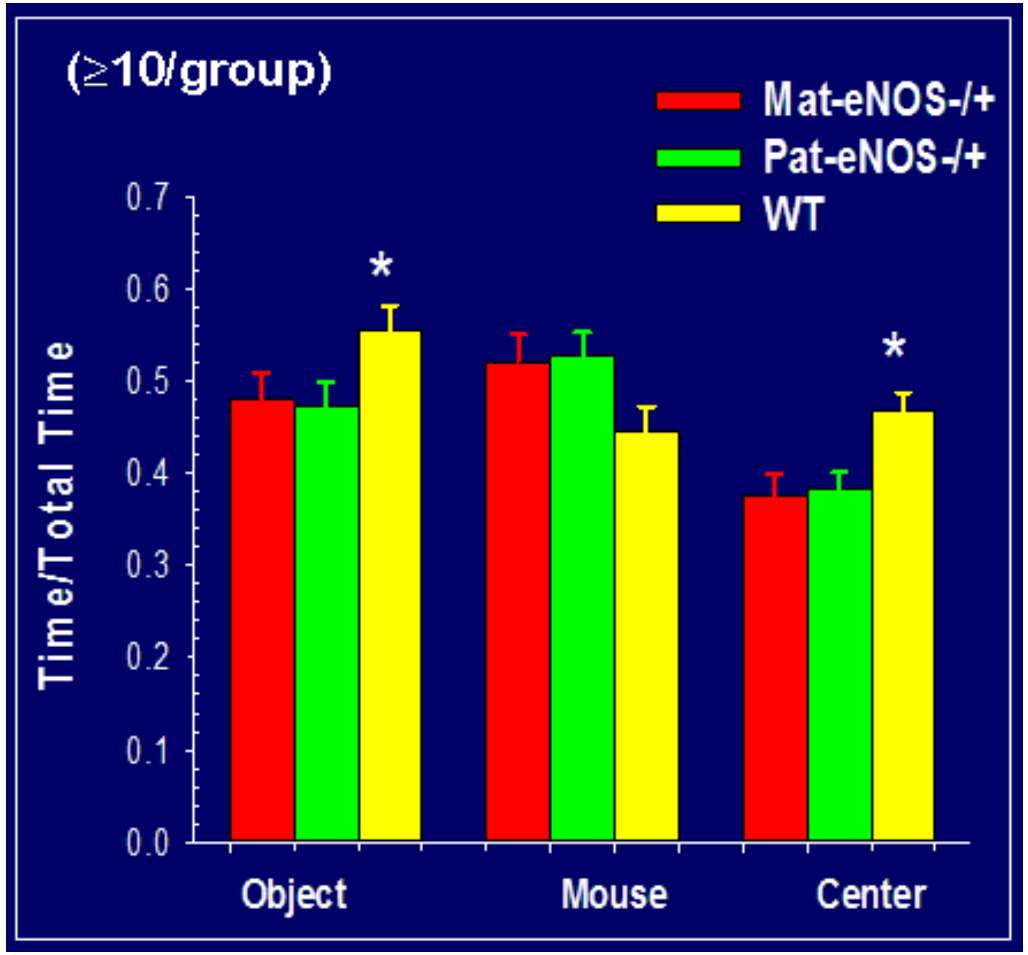
ASD Behavioral Tests

Social Behavior	Repetitive Behavior
- Sociability - Preference For Social Novelty	- Open Field - Marble Burying
Anxiety Behavior	**Motor Function**
- Light/Dark Box - Elevated Plus Maze - Open Field	- Beam Balance - Rotor Rod
Spatial Learning and Memory	
- Morris Water Maze	

2-way-RM-ANOVA, 1-way-ANOVA, t-test, $P<0.05$ significant

ASD Behavioral Tests -Sociability





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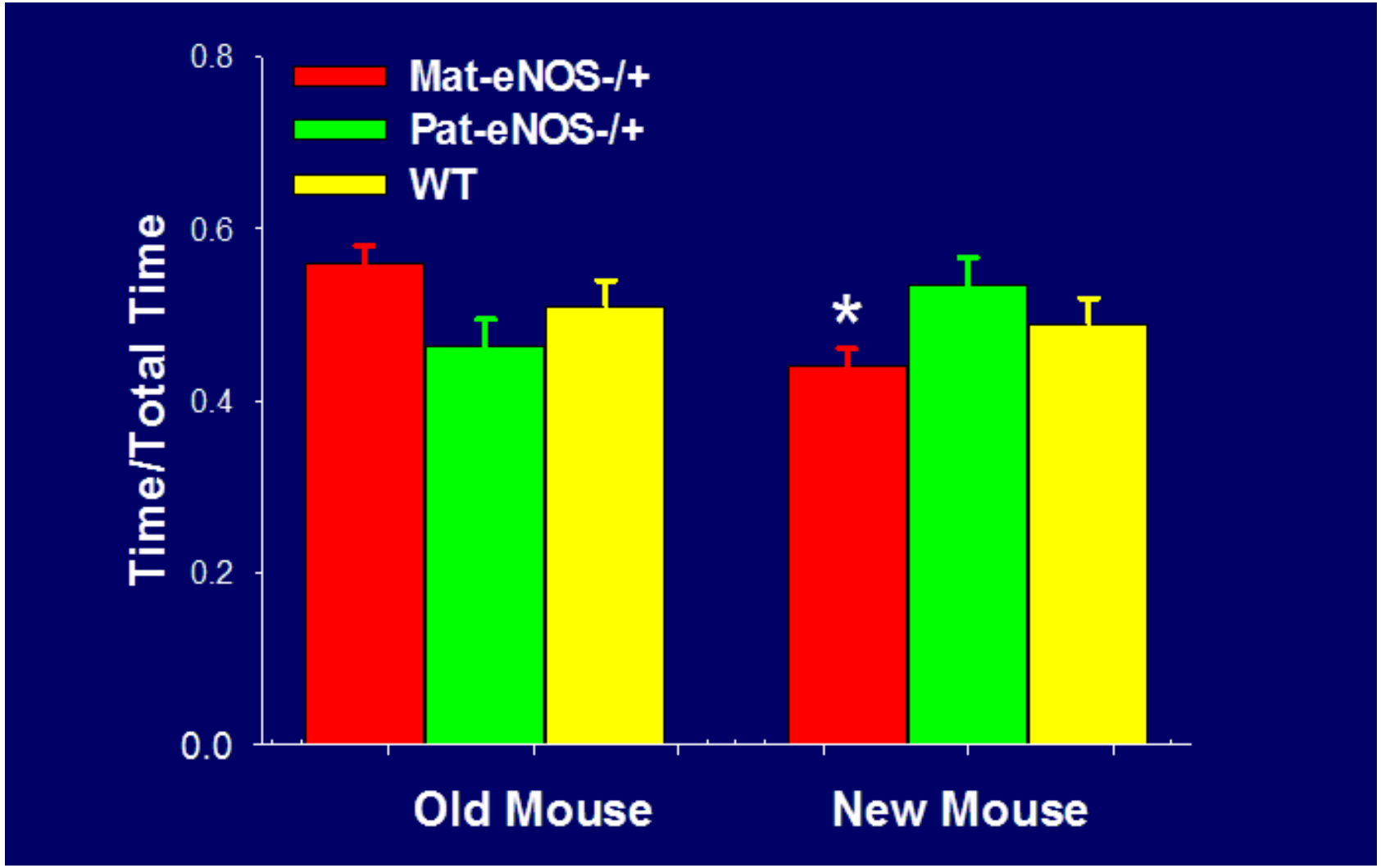
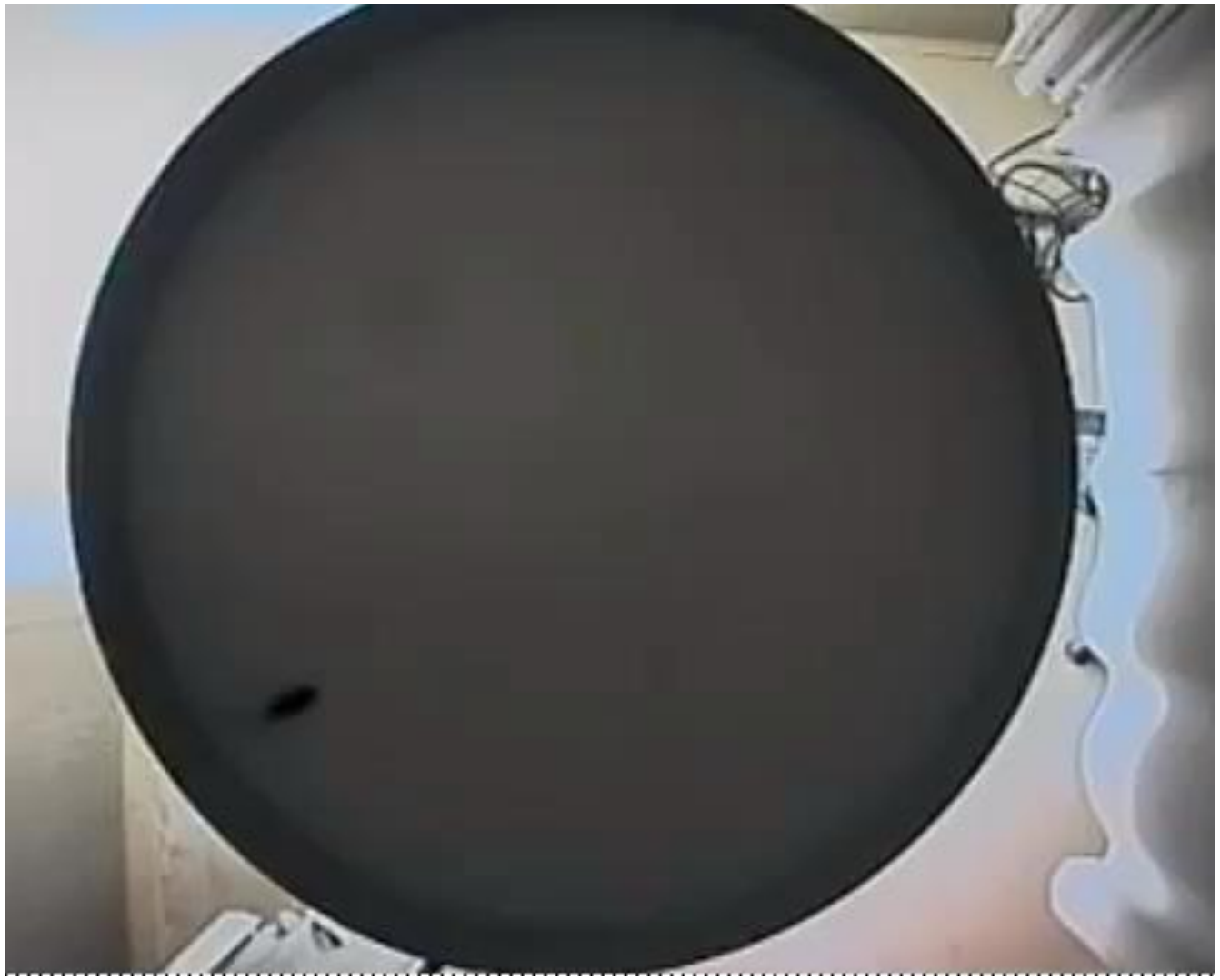
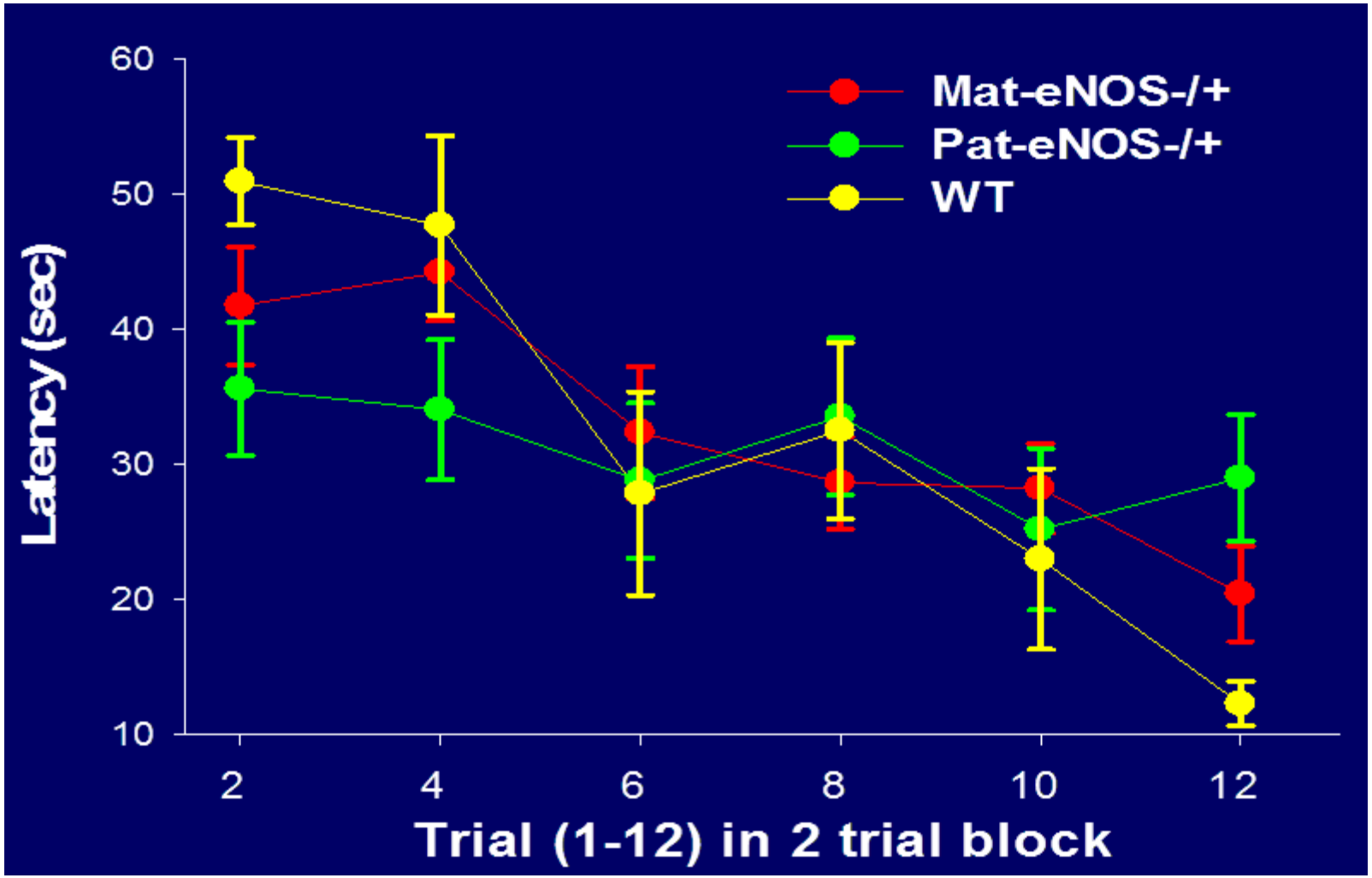
ASD Behavioral Tests
Preference for Social Novelty



Morris Water Maze
Spatial Learning and Memory



Morris Water Maze
Spatial Learning and Memory



ASD Behavioral Tests

		Wild-Type	Pat-eNOS-/+	Mat-Enos-/+
Social Behavior	Preference For Novelty	↔	↔	↓
Repetitive Behavior	Open Field	↔	↓	↓
Anxiety Behavior	Light Dark Box	↔	↓	↓↓
Spatial Learning And Memory	Morris Water Maze	↑↑	↓↓	↓
Motor Function	Beam Balance/ Rotor Rod	↔	↔	↔

ASD Behavioral Tests

		Wild-Type	Pat-eNOS-/+	Mat-Enos-/+
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Spatial Learning And Memory	Morris Water Maze	↑↑	↓↓	↓
Motor Function	Beam Balance/ Rotor Rod	↔	↔	↔

Identification of ASD Related Genes in the Cerebellum

The Cerebellum

- **Highly conserved structure and function**
  - Foliation conserved across evolution
- **Structure is “simple”**
  - Only 9 principle types of neurons
  - All morphologically distinct
  - Layers and circuitry are stereotyped
- **Contains more neurons than rest of brain**
  - In mouse, 59/71 million neurons (83%)
  - In human, 69/86 billion neurons (80%)

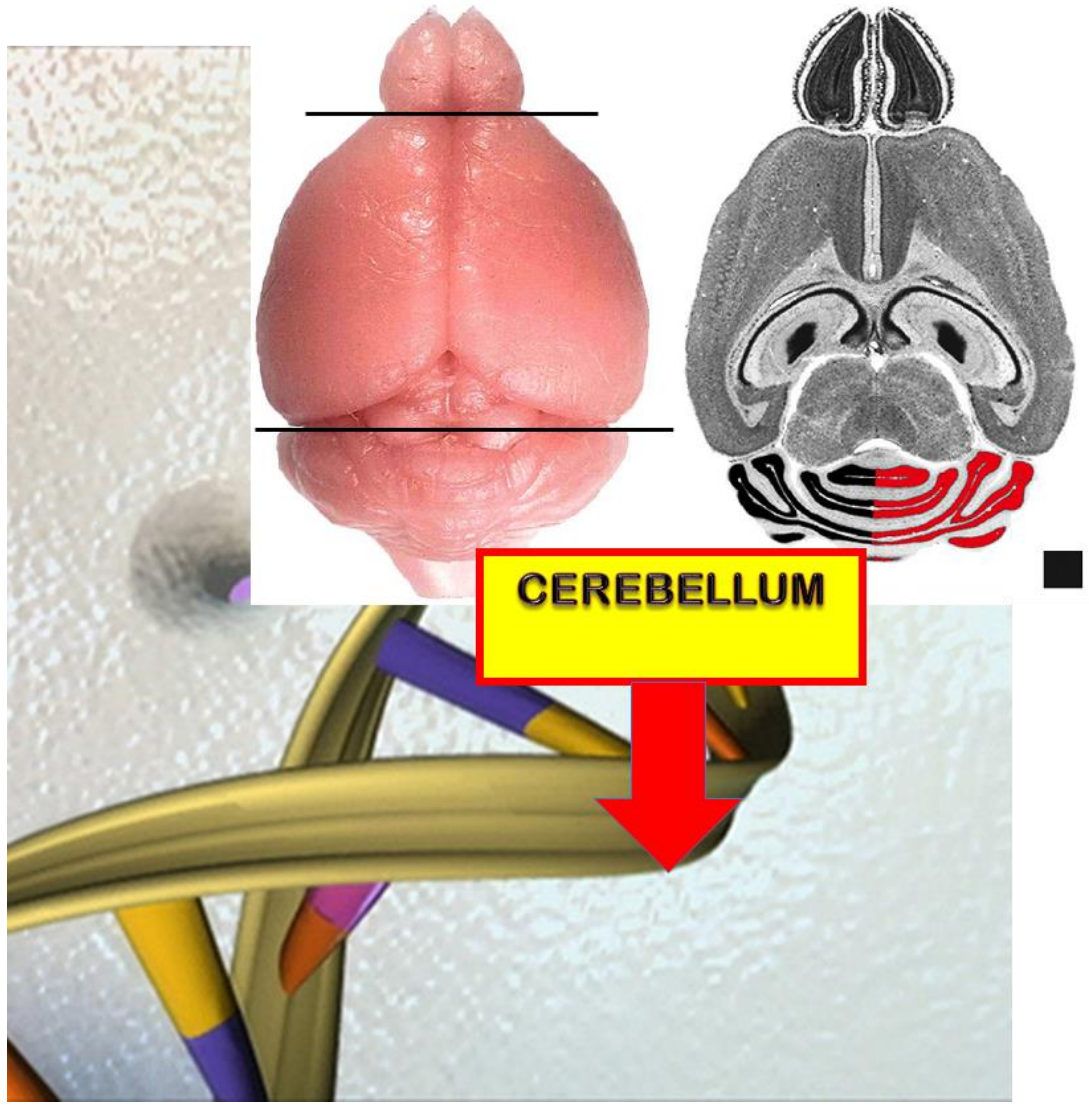
Cerebellar Disorders

- **Incidence**
  - Relatively common ~1/5000 live births
  - Can occur in isolation or part of syndrome
  - Genes identified for only a few rare forms
- **Outcome**
  - Most cause DEV delay ± ID ± motor abnormalities
  - ID and ID syndromes, autism, early life epilepsy
- **Prenatal Issues**
  - Most (not all) are visible by GW20
  - Difficult to distinguish by fetal ultrasound/MRI

Cerebellum

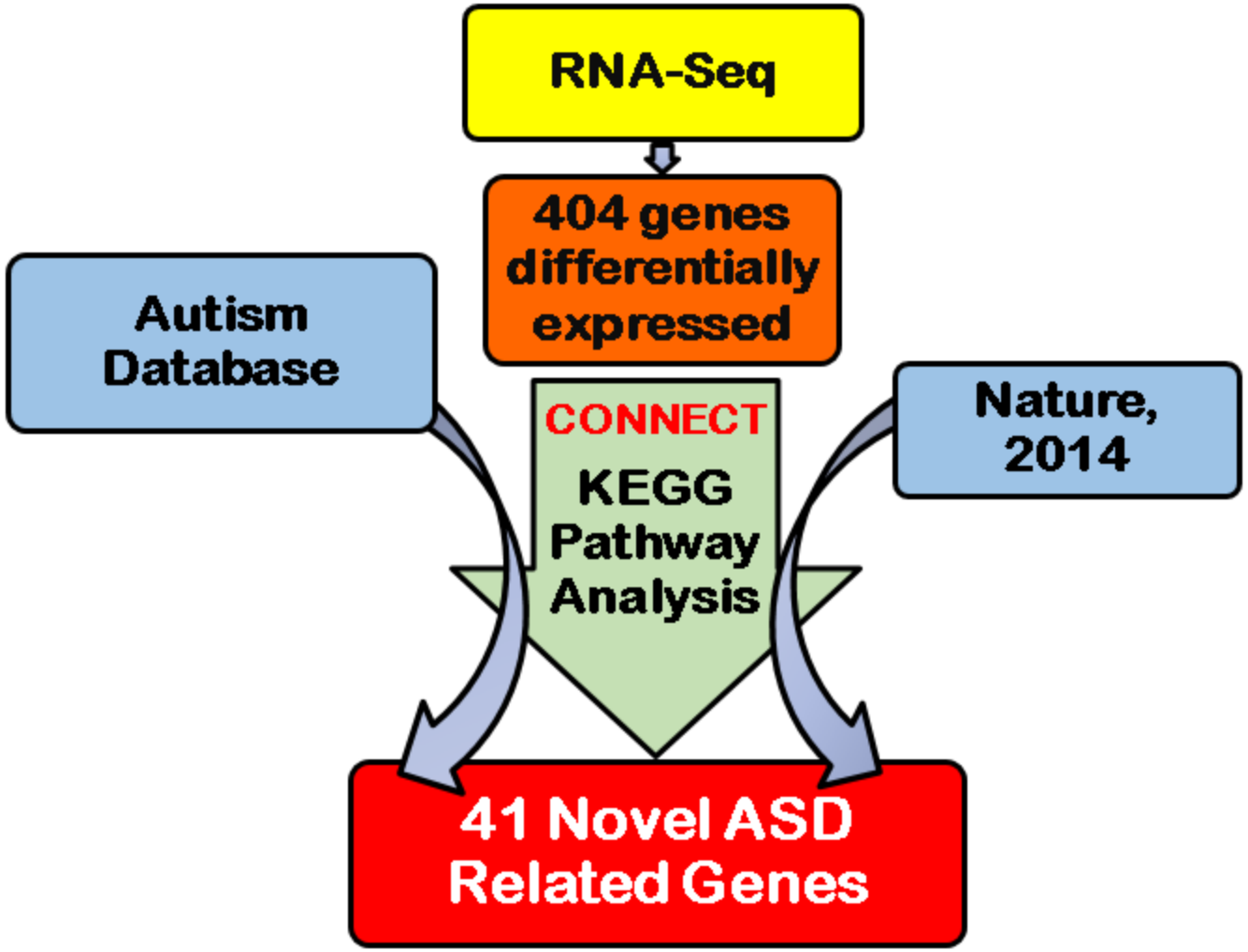
- **Historically**
  - Balance
  - Posture
  - Motor control
- **Recent**
  - External sensory
  - Neocortical circuit refinement

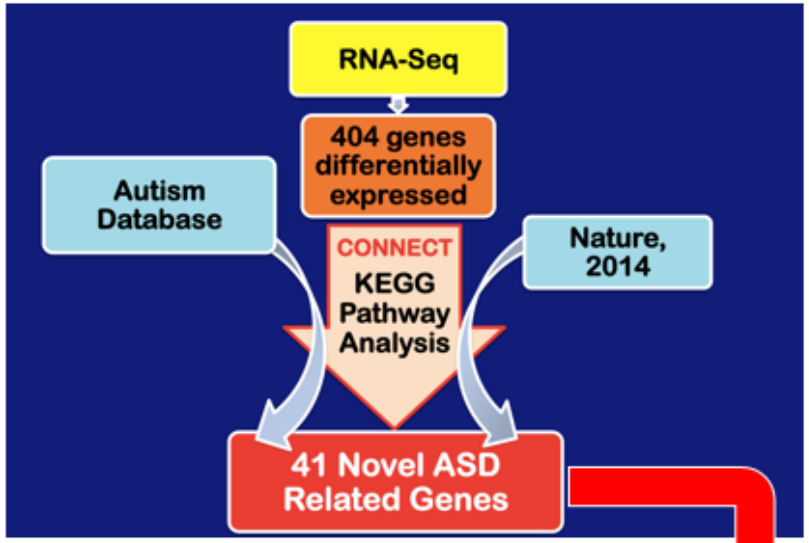
Shaping Higher Function Early In Neurodevelopment



Identification of ASD Related Genes

- RNAs from Mat-eNOS-/+ and Pat-eNOS-/+ cerebella underwent whole transcriptome shotgun sequencing using RNA-Seq
- Differentially expressed genes were examined for pathway analyses to obtain novel ASD related genes
- Gene Ontology enrichment was performed on these novel genes to identify their biologic processes


RNA-Seq
404 genes differentially expressed
Autism Database
CONNECT
KEGG Pathway Analysis
Nature, 2014
41 Novel ASD Related Genes



Gene Ontology Term

Embryo Development
Anatomical Structure Development
Cell Differentiation
Signal Transduction
Autophagy
Carbohydrate Metabolic Process
Catabolic Process

CONCLUSION

- The altered uterine environment, secondary to maternal hypertension, contributes to ASD like features in eNOS heterozygous offspring
- A social deficit, the hallmark of ASD, is differentially present in the offspring of hypertensive mothers
- Novel ASD related genes are differentially expressed between both groups
- ASD etiology has a cerebellar component

Effect of Programmed Maternal Hypertension and Metabolic-like Syndrome during Pregnancy on Offspring Neurodevelopment

F. Lu, A. E. Ontiveros, H. Moussa, M. Saade, S.C. Blackwell,
P. Dash and M. Longo

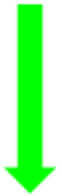
Department of Obstetrics, Gynecology and Reproductive Sciences
UTHealth-McGovern Medical School at Houston

1st Generation Pregnant Female

Wild Type (CTR)

Moderate Hypertension (HTN)

Metabolic Like Syndrome (MLS)



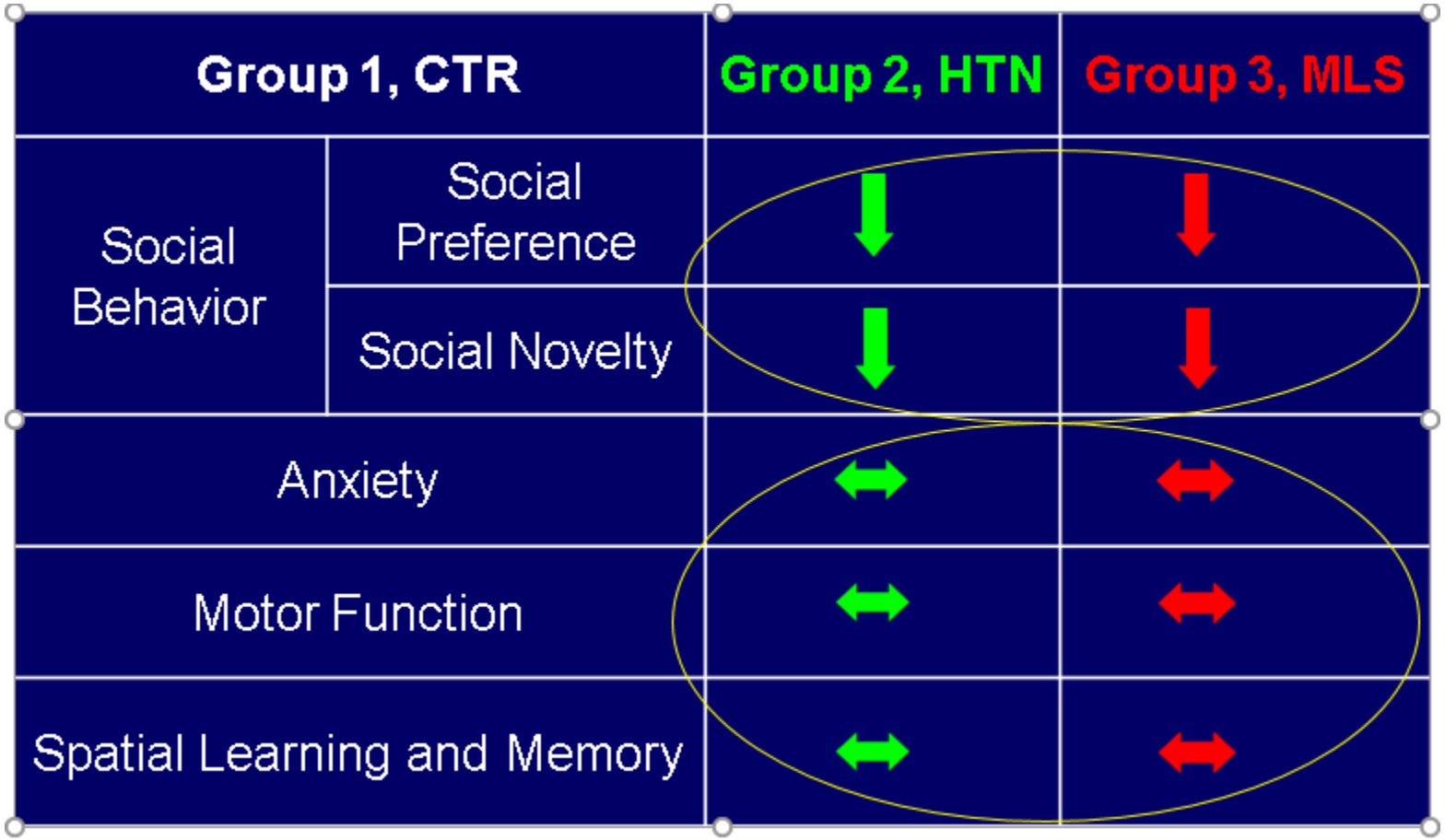
Second Generation Male Wild Type Offspring

Group 1, CTR

Group 2, HTN

Group 3, MLS

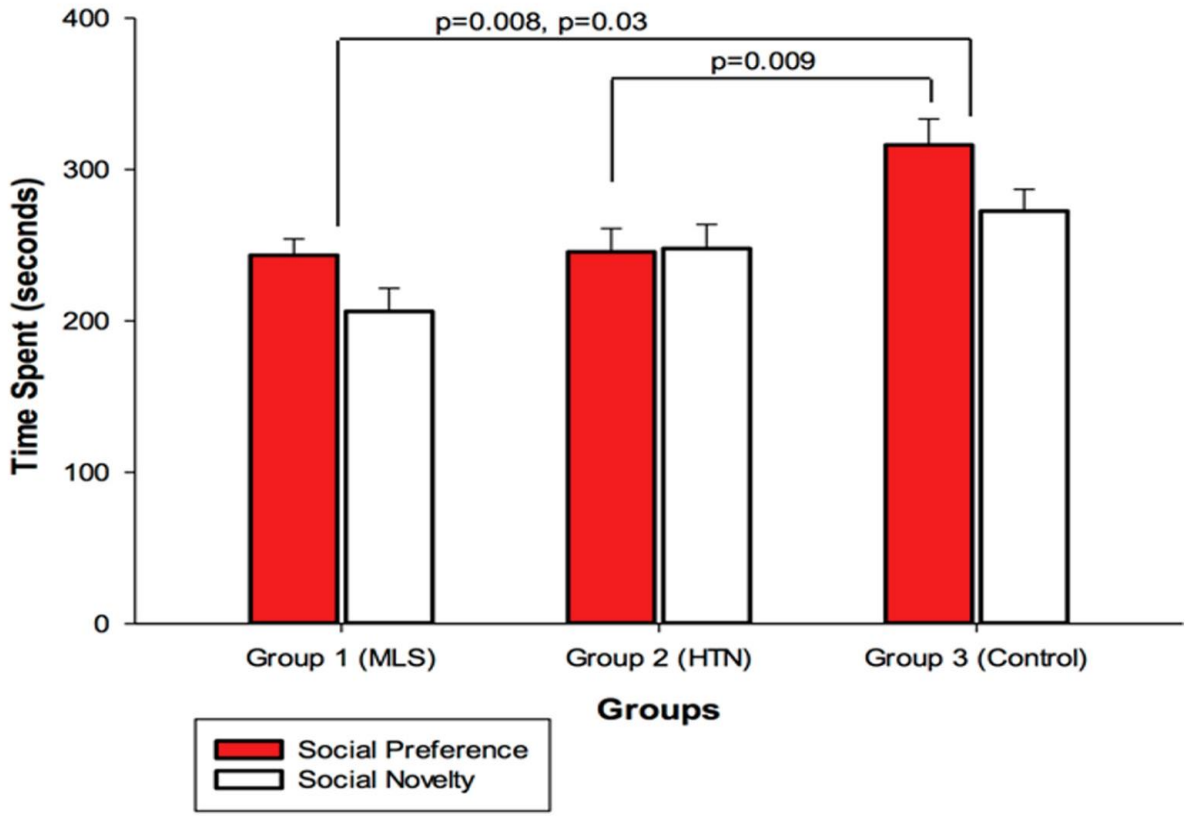
Second Generation Wild Type Offspring



62: Effect of programmed maternal hypertension and metabolic-like syndrome during pregnancy in offspring neuro-development

Fangxian Lu, Alejandra E. Ontiveros, Hind N. Moussa, Mia M. Saade, Sean C. Blackwell, Pramod Dash, Monica Longo

Figure 2: Social Interaction Tasks (Social Preference and Social Novelty)
Three groups of WT offspring were studied: born to heterozygous eNOS-KO$^{+/-}$ females fed a high fat diet (HFD) manifesting MLS (Group 1), born to heterozygous eNOS-KO$^{+/-}$ females fed a control diet (CD) manifesting HTN (Group 2), and born to WT female fed control diet (CD) use as control (Group 3).



American Journal of Obstetrics & Gynecology 2016 214, S44-S45DOI: (10.1016/j.ajog.2015.10.080)

478: Parental inheritance of NOS3 and uterine environment alter cytokine levels in a murine model of autism like disorder

Hind Moussa, Baha Sibai, Sean Blackwell, Mateo Leon, Anthony Moore, Alissa R. Carver, Maged Costantine, Pramod Dash, Monica Longo

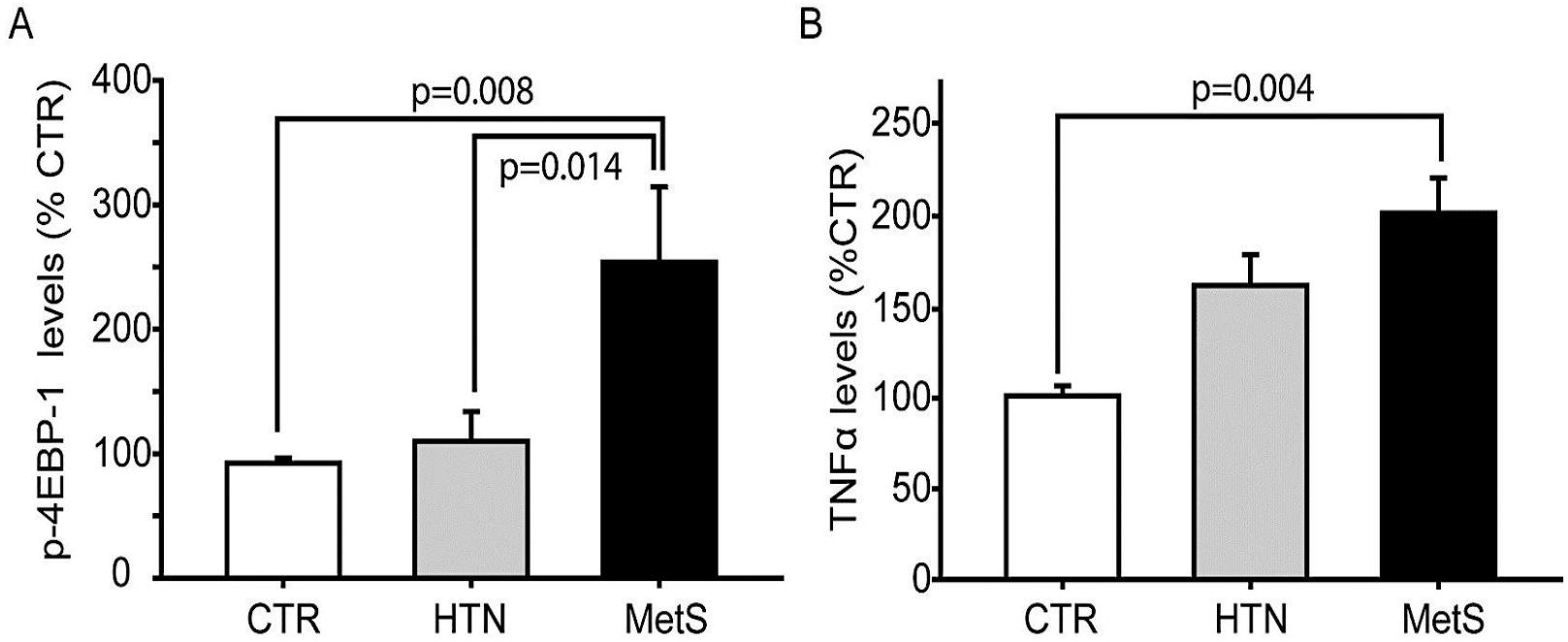
- Blood and brain were collected from KO, KOM, KOP and WT offspring
- N=7-10/group at 12 wks.
- Bio-Plex Mouse Cytokine Assay was run on
  - Serum
  - Cerebellum
  - Hippocampus
- 1-way-ANOVA and *t*-test were used for statistical analysis.

Pro-Inflammatory Cytokines
IL-1β, IL-6, IL-17A TNF-α, IFN γ
Anti-Inflammatory Cytokines
IL-10

851: Maternal metabolic syndrome and hypertension altered TNFα and mTOR1 activity in the cerebellum of adult offspring: implications for autism-spectrum disorder

Fangxian Lu, Anthony N. Moore, Danielle Hamrick, Jerrie S. Refuerzo, Baha M. Sibai, Sean C. Blackwell, Pramod Dash, Monica Longo

American Journal of Obstetrics & Gynecology
Volume 216, Issue 1, Pages S487-S488 (January 2017)
DOI: 10.1016/j.ajog.2016.11.760

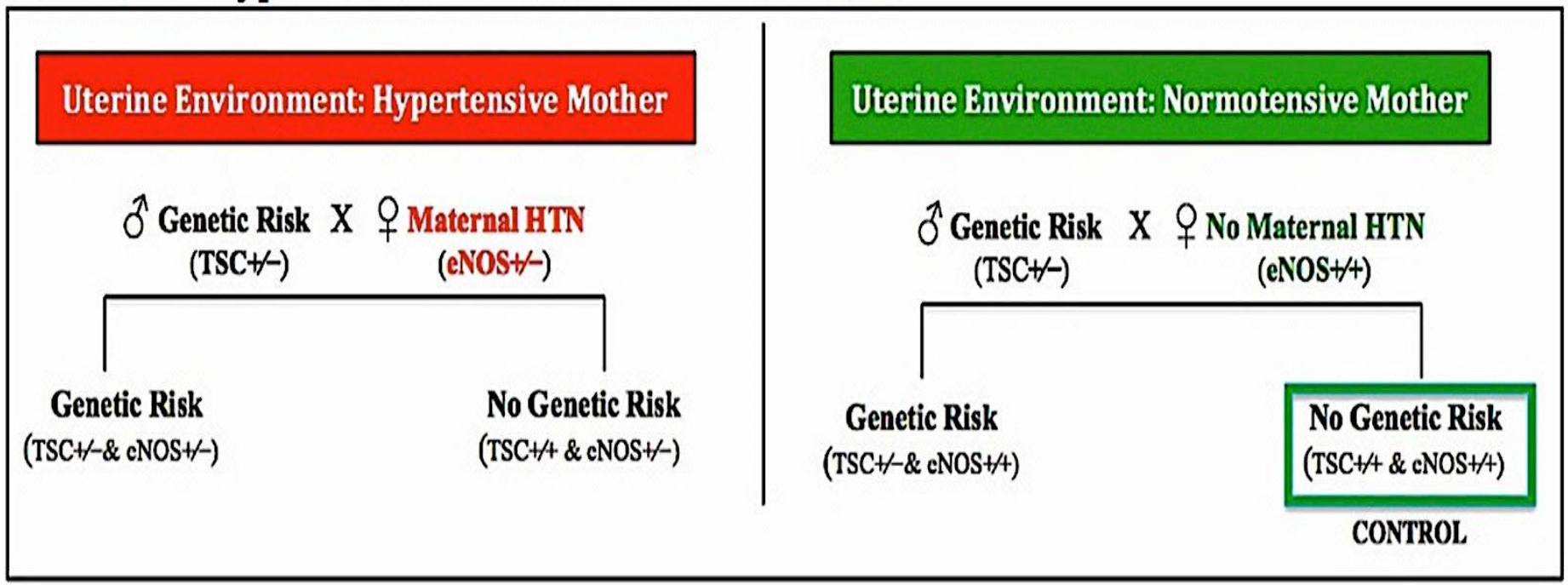


American Journal of Obstetrics & Gynecology 2017 216, S487-S488DOI: (10.1016/j.ajog.2016.11.760)

226: Genes or environment? A novel double knockout mouse model for fetal origins of autism study

Hind N. Moussa, Baha M. Sibai, Sean C. Blackwell, David A. Fournie, Alejandra E. Ontiveros, Fangxian Lu, John Redell, Pramod Dash, Monica Longo

Figure 1: Breeding scheme to obtain offspring with and without TSC2 genetic risk born to hypertensive vs. normotensive mothers.



American Journal of Obstetrics & Gynecology 2016 214, DOI: (10.1016/j.ajog.2015.10.264)

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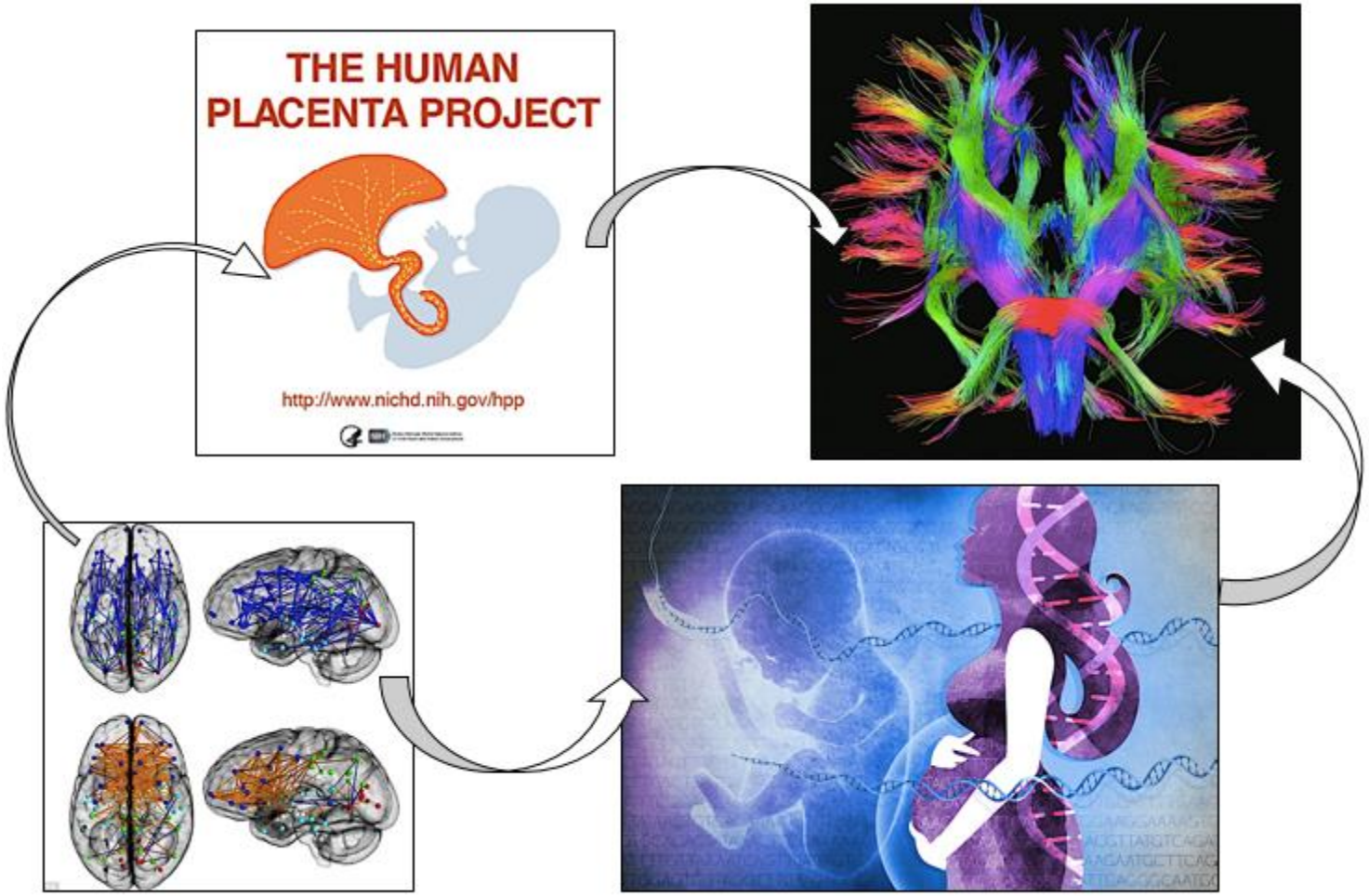
226: Genes or environment? A novel double knockout mouse model for fetal origins of autism study

Hind N. Moussa, Baha M. Sibai, Sean C. Blackwell, David A. Fournie, Alejandra E. Ontiveros, Fangxian Lu, John Redell, Pramod Dash, Monica Longo

- Offspring with and without genetic (TSC2) and environmental risk (HTN) factors performed similarly in behavioral tasks assessing motor function, spatial learning, memory, and anxiety
- Significant interaction between the genetic & environmental risk factors in a social behavior task (P=0.048)
- After adjusting for gender, there was a social deficit in males as compared to females, and that deficit was driven by the HTN environmental factor and not the TSC2 genetic risk (Sociability task, male gender P=0.014, $eNOS^{+/-}$ P=0.013, and $TSC2^{+/-}$ P=0.135, interaction of male gender X environmental factor P=0.009)


THE HUMAN PLACENTA PROJECT
http://www.nichd.nih.gov/hpp

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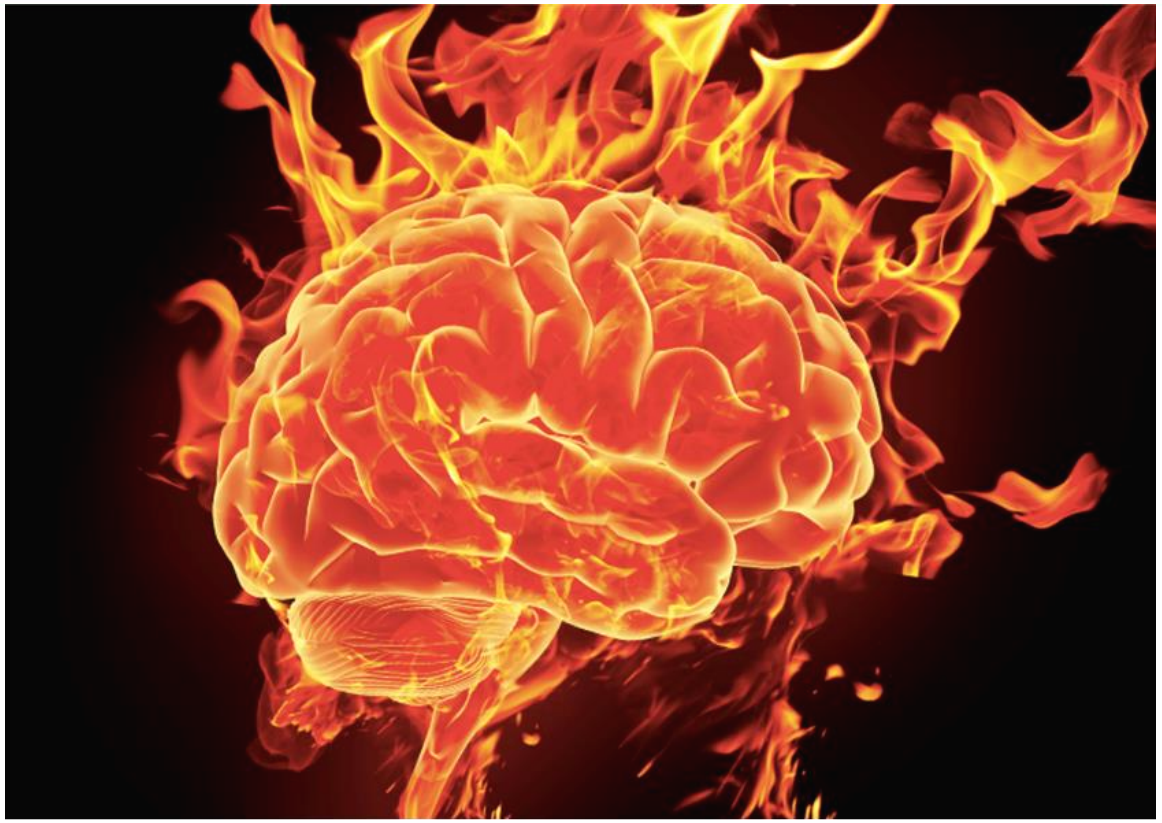

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