Exposure to DES During Pregnancy and Multigenerational Neurodevelopmental Deficits

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Outline

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Endocrine Disrupting Chemicals (EDCs)

US EPA defines an EDC as an "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process."

- Several high production volume chemicals, ubiquitously present in commercial products, are known or suspected EDCs
- Due to their widespread use in consumer products, population-wide exposure to known and suspected EDCs is highly prevalent

Exposure to EDCs

Ubiquitous!









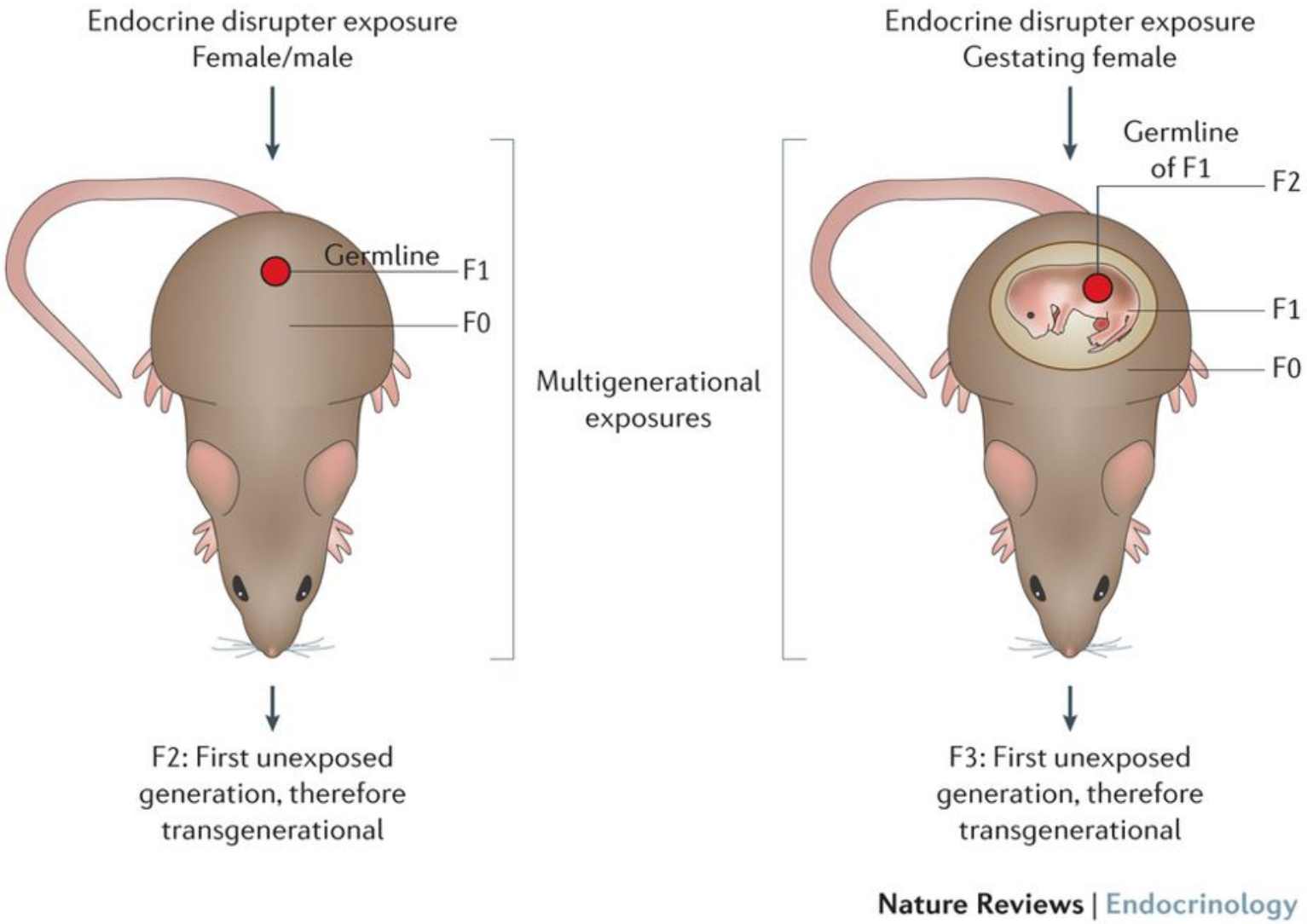
EDCs and Adverse Health

EDCs have been linked to numerous health outcomes, e.g.

- Disruptions to male and female reproductive systems
- Development of cancer
- Obesity
- Neurodevelopmental disorders
  - Including ADHD
  - Especially following in utero exposures

Outline

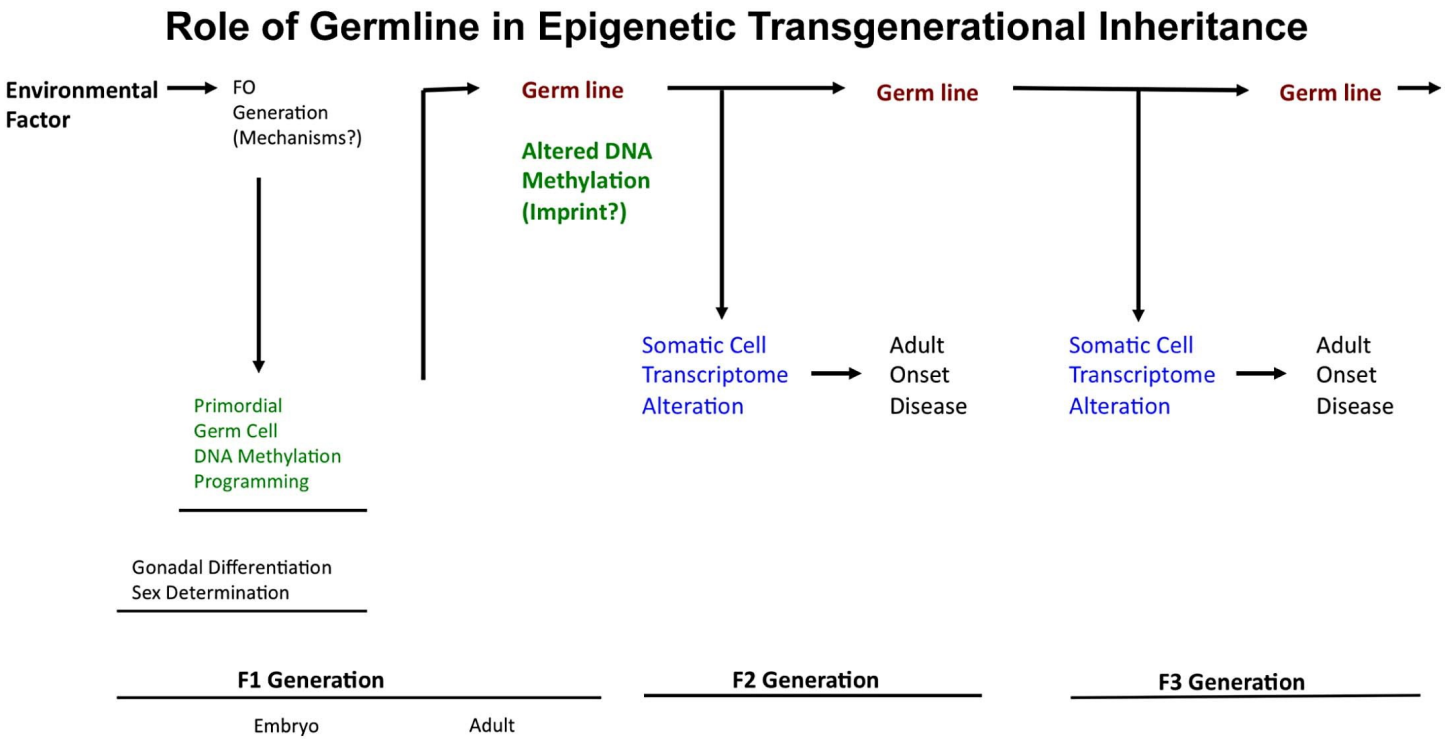
Multi- vs. Transgenerational



Skinner MK, Nature Reviews Endocrinology 2016

Multi- and Transgenerational Effects of EDCs

- Increasing interest in the potential multi- & transgenerational effects of EDC exposure
- Hypothesized biological mechanism $\rightarrow$ epigenetic reprogramming of the germline



Skinner MK, Molecular & Cellular Endocrinology 2014

Evidence from Toxicological Studies

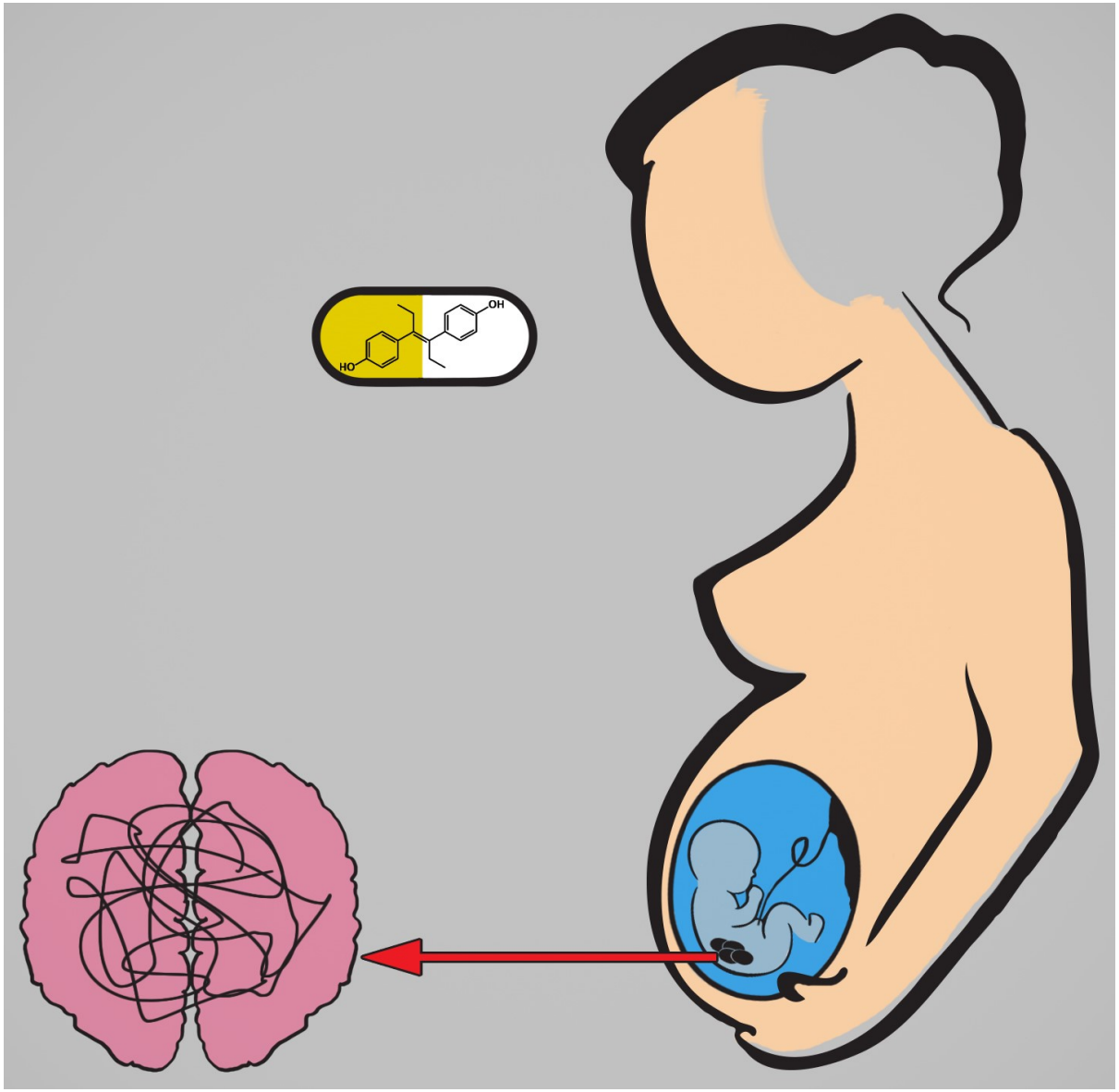
- Di(2-ethylhexyl) phthalate $\rightarrow$ alter third-generation behavior and stress responses, observed corticosterone levels, and pituitary gene expression and behavior in mice
- BPA $\rightarrow$ changes in third- to fifth-generation social interactions in mice

Epidemiological evidence on multigenerational EDC – neurodevelopment in humans is currently unavailable

Quinnies et al, Endocrinology 2015; Wolstenholme et al, Endocrinology 2012

Outline

Multigenerational DES Effects on ADHD

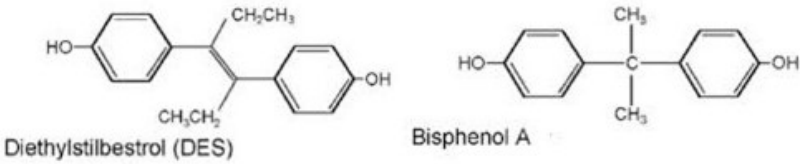


by Iordanis Kioumourtzoglou, @iq_artwork

Outline

Diethylstilbestrol (DES)

- DES is a potent perinatal EDC
- Structurally and functionally similar to BPA (more potent)



- 1938–1971: Prescribed to pregnant women to prevent pregnancy complications (e.g. miscarriages)
- Exact number of women who used DES is unknown; estimated 5-10M in the US

Giusti et al, Ann Intern Med 1995

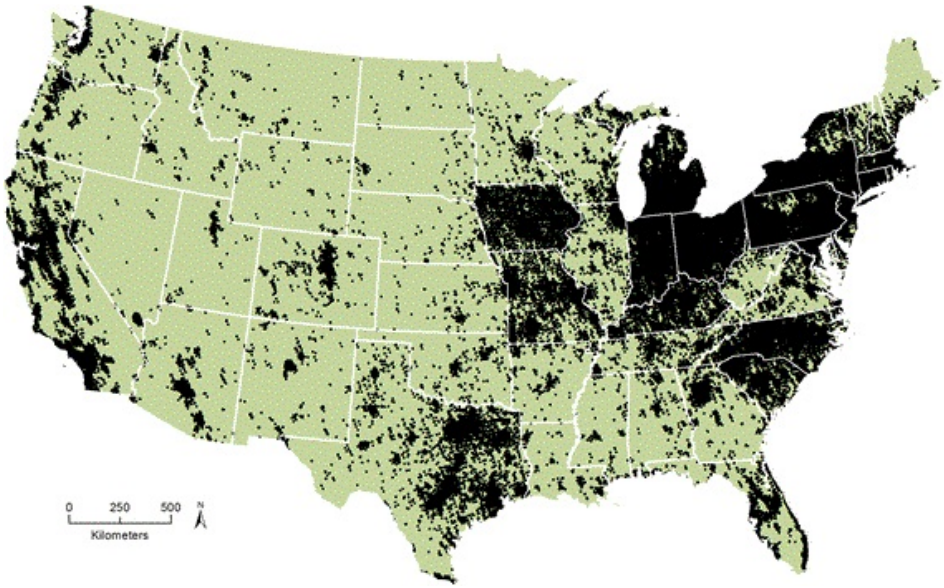
DES (cont'd)

- 1953: Study shows no actual treatment value → phase out starts
- 1971: Study links DES to rare vaginal adenocarcinomas in DES daughters → DES ban
- Since then it has been linked to multiple reproductive outcomes in DES daughters
- Multigenerational DES impacts:
  - Hypospadias
  - Delayed menstrual regularization
  - Birth defects

Dieckmann et al, Am J Obstet Gynecol 1953; Herbst et al, NEJM 1971; Giusti et al, Ann Intern Med 1995; Klip et al, Lancet 2002; Titus-Ernstoff et al, IJE 2006; Titus-Ernstoff et al, Int J Androl 2010

Outline

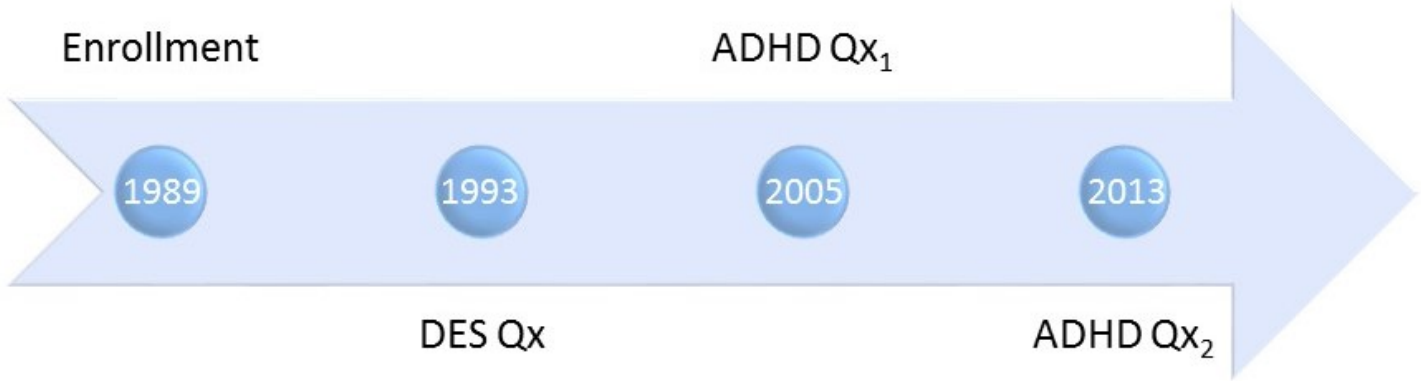
Study Population



Nurses' Health Study II

- Enrollment in 1989
- 116,686 registered nurses (25 – 42 years old)
- Mailed questionnaires every two years
- Lifestyle, risk factors, medication use, major illness occurrence
- Retention rate $> 90\%$
- All NHS-II (F1) participants born between 1946 – 1964

Study Population (cont'd)



Exclusion Criteria

1. No return of 1993, 2005 or 2013 Qx
2. No report of any live-born children
3. Multiple pregnancies (e.g. twins etc) or same-year births (from different pregnancies)
   - B/c ADHD children (F2) only identified by birth year

F0/F1: 47,540 & F2: 106,198

DES Assessment

F1-reported F0 DES use during pregnancy in 1993 Qx

Also, supplementary 1993 Qx:

- 2,742 F1 who had reported "Yes" to F0 DES use
- Response rate: 84.5%

2,032	(87.7%):	Certain or somewhat certain of F0 use
123	(5.3%):	Not certain
162	(7.0%):	No exposure

- Only used "Certain or somewhat certain" for further analyses
- This Qx also included information on the trimester of DES use

ADHD Assessment

- 2005 Qx: "Has any of your children received a doctor's diagnosis of ADHD?"
  - No question related to how many and which children
- 2013 Qx: question repeated, further requesting information on the birth year(s) of the F2 with an ADHD diagnosis
- We included information only when the 2005 and 2013 responses were concordant (92.6% concordance) to minimize potential outcome misclassification
- Used the 2013 response to identify the number of F2 per F1 with ADHD

Potential Confounders

- Only variables preceding F0 DES use
- 1999 Qx: F1 were asked if their mothers smoked during pregnancy
- 2005 Qx: F1 reported their family SES at birth, about F0 lifestyle, education and occupation
- All analyses were adjusted for:
  - F1 race and ethnicity
  - F1 year of birth (linear & squared) – time trends
  - F0 smoking during pregnancy
  - F0 home ownership at F1 birth
  - F0 & F1's father's education
  - F0 & F1's father's occupation

Statistical Analysis

- In utero F1 DES exposure may affect
  1. # of F2 within F0/F1
  2. The likelihood that any F2 has ADHD
     - The distribution of ADHD given DES may depend on the number of F2 within F1

→ *Informative clustering*

- Standard GEE no longer appropriate
  - May lead to invalid estimates and inferences
- We used cluster-weighted GEE with a logit link to account for multiple F2 within F0/F1
  - Weights: the inverse of the cluster size
  - I.e. the number of F2 per F1
- Adjusted for potential confounders
- Assessed effect modification by F2 sex

Outline

Some Descriptive Characteristics

Variable	N	(%)
DES	861	1.8
F0 Education		
$<$ 9 yr	3,256	6.9
1-3 yr HS	5,422	11.4
4 yr HS	23,315	49.1
1-3 yr college	10,507	22.1
4+ yr college	4,294	9.0
F1 did not know	746	1.6
F0 Smoking during pregnancy		
Yes	11,139	23.4
No	29,918	63.1
F1 did not know	4,281	9.0
F1 race: White	45,160	95.2
F1 ethnicity: Hispanic	588	1.2

- 106,198 F2 children
  - F2 median birth year: 1983 (IQR: 1978 – 1988)
  - 5,587 (5.3%) diagnosed with ADHD

Results

Exposure	# F2	# ADHD (%)	OR (95% CI)
Any DES			
Unexposed	104,414	5,450 (5.2)	*ref*
Exposed	1,784	137 (7.7)	1.36 (1.10 – 1.67)
By Trimester			
Unexposed	104,414	5,450 (5.2)	*ref*
First	950	82 (8.6)	1.63 (1.18 – 2.25)
Second	519	33 (6.4)	0.68 (0.35 – 1.34)
Third	338	27 (8.0)	1.41 (0.72 – 2.82)
F1 did not know	625	42 (6.7)	1.15 (0.80 – 1.65)

- Completely crude model (also ignoring any clustering):
  OR = 1.51 (95%CI: 1.27–1.80)

Outline

Discussion

- Strong, harmful effect estimates of DES use on third-generation ADHD
- Robust to sensitivity analyses
- Potential biological mechanism: epigenetic transgenerational inheritance
  - EDCs $\rightarrow$ molecular alterations to the germline, mediated through epigenetic mechanisms, to promote outcomes to subsequent generations
- But not the *only* potential mechanism
  - If DES$\rightarrow$F1 ADHD – assortative mating?

Discussion (cont'd)

- DES use during the 1st trimester seems to be particularly harmful
- Use during 2nd and 3rd trimester were weaker and not significant
- Attenuation and wider CIs could be due to smaller numbers
  - 33 exposed cases for the 2nd and 27 for the 3rd trimester vs 82 for the 1st trimester
- Or our results could suggest that the 1st trimester is a critical window of vulnerability to DES exposure
- Early gestation $\rightarrow$ especially sensitive to maternal influences, resulting in embryonic and germ cell reprogramming
  - During this period a wave of genome demethylation followed by de novo remethylation occurs together with the establishment of imprints and determination of sex

Bale, Nat Rev Neurosci 2015; Weaver et al, Mamm Genome 2009

Conclusion

Our findings have important implications for exposures to other environmental endocrine disruptors (e.g. ubiquitous chemicals, such as BPA, phthalates etc.) during pregnancy and third generation adverse health effects

Acknowledgments

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Questions?

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DES use validation

- 2001: a Qx was mailed directly to 29,070 F0
  - With questions on their pregnancy with F1
- Very good agreement with the 1993 F1 responses
- $\kappa = 0.74$ for DES use
- κ did not vary by F2 ADHD status

ADHD Validation

- Maternal reports of ADHD have been found highly reliable
- Validation study:
- 92 F1 who had responded "yes" in the 2005 Qx
- ADHD Rating Scale-IV
- All F2 girls scored above 90%
- 81.1% of F2 boys scored above 80%; 63.8% of F2 boys scored above 90%

Faraone et al, J Am Acad Child Adolesc Phychiatry 1995; DuPaul et al 1998; Gao et al, Sleep Med 2011

Additional Analyses

- Main analysis:
  OR = 1.36 (95%CI: 1.10–1.67)
- No effect modification by F2 sex ($p\text{-}value = 0.62$)
- When also adjusted for F0 depression (10.8%)
  OR = 1.33 (95% CI: 1.08 – 1.63)
- Additionally adjusting for F0 birth year
  $N_{F_0} = 45{,}612$; $N_{F_2} = 101{,}830$
  OR = 1.35 (95% CI: 1.09 – 1.66)
- In validation subsample with F0-reported DES information
  $N_{F_0} = 18{,}792$; $N_{F_2} = 42{,}097$
  OR = 1.31 (95% CI: 1.00 – 1.71)