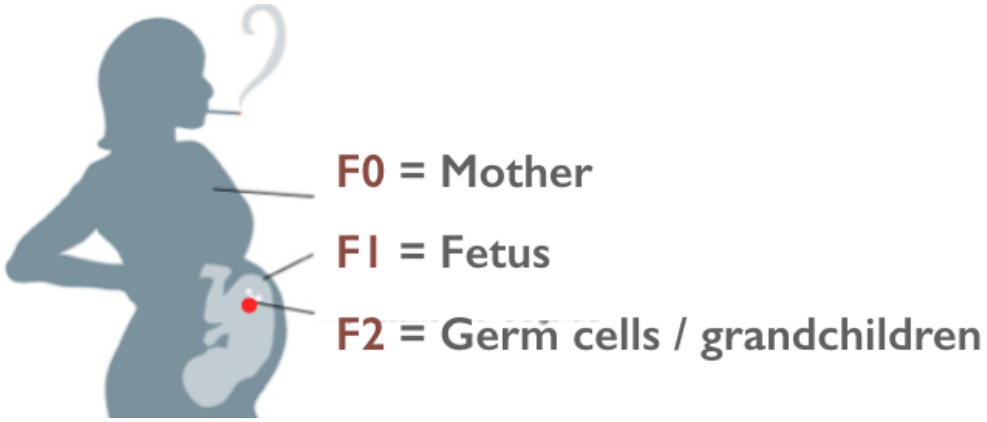


Grand-maternal smoking in pregnancy and grandchild's autistic traits and diagnosed autism

Jill Escher

Escher Fund for Autism

"No matter how obscure the subfield of science, there is bound to be some crazed egghead out there who finds it fascinating."

—Robert Sapolsky

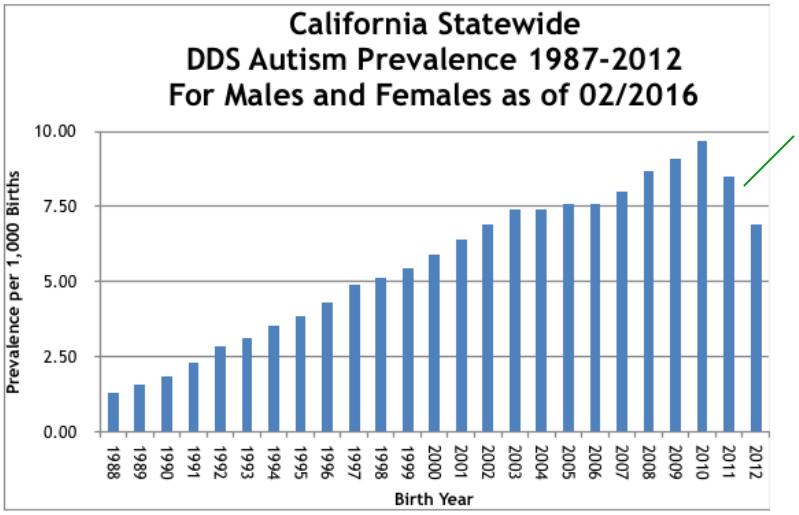
Environmental Mutagenesis and Genomics Society, September 2017

Dr. De Marini asked me to first provide some background

Autism and Autism Research Today

Autism rates have exploded.

- 1980: about 2k cases; now about 100k Calif. DDS)
- 1.5% of live-born Calif males end up as DDS autism cases (Calif. DPH)
- DD system is being crushed by growing caseload (see eg, On the Brink of Collapse, Calif. ARCA, 2016)
- Economic burden of autism may reach 3% of GDP within 10 years (Leigh et al, JADD 2015).



Note: delay entering system

Source: California Dept. of Public Health

Yet (seemingly in contradiction) autism is highly heritable.

- Heritable among siblings.
- In autism research, heritability is routinely presumed to be "genetic." Focus has been on gene hunting.

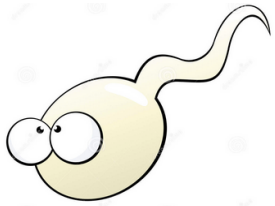
Environmental research has focused on fetal somatic.

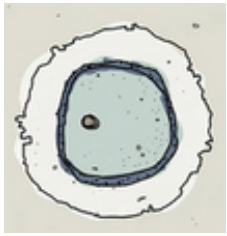
- In addition, environmental research in autism focuses almost exclusively on fetal somatic and some perinatal exposures.

Germ cell exposures (particularly PGC) = no man's land

Mysterious, Devastating Neurodevelopmental Abnormality

No mental abnormality up our family trees



Three normal, healthy pregnancies

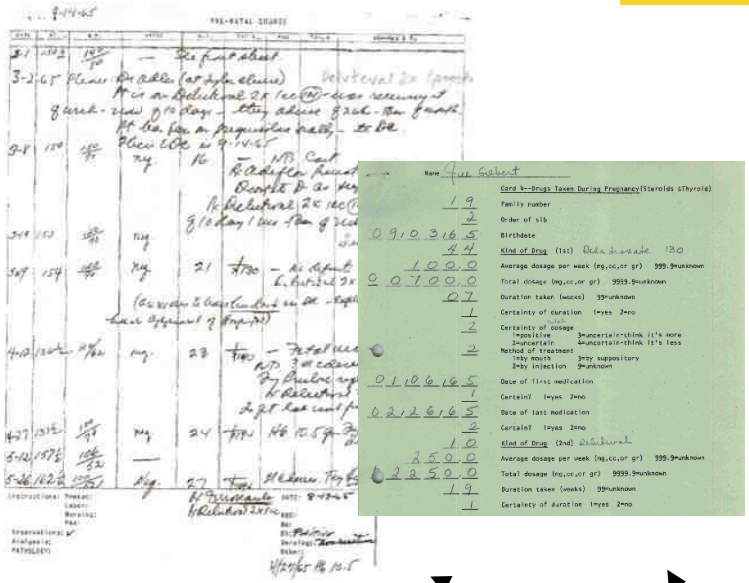
NT

Nonverbal Autism

Nonverbal Autism

Autism is highly “heritable” in my family. But why?

Holy #$%@#!



Some of the records
I discovered.

Several years ago, I discovered I (with PGCs) had been heavily, continuously prenatally exposed to synthetic steroid hormone drugs used as "anti-miscarriage" protocol, in 1965 Los Angeles:

Regular doses of synthetic corticosteroids ("Pregnisolone") through the first trimester. Regular doses of 2 synthetic progestins (Delalutin and Deladroxate) with synthetic estrogens through at least 7th month

Prenatal Exposure to Synthetic Progestins and Estrogens: Effects on Human Development

June Machover Reinisch, Ph.D.,[1] and William G. Karow, M.D.[2]

Seventy-one offspring of mothers administered combinations of synthetic progestins and estrogen for the maintenance of at-risk pregnancy were evaluated for their performance on IQ and personality tests. Siblings born of untreated pregnancies acted as controls. Hormone-exposed subjects were partitioned into three treatment subgroups dependent on the ratio of progestin to estrogen administered to their mothers during pregnancy. No difference in IQ was obtained

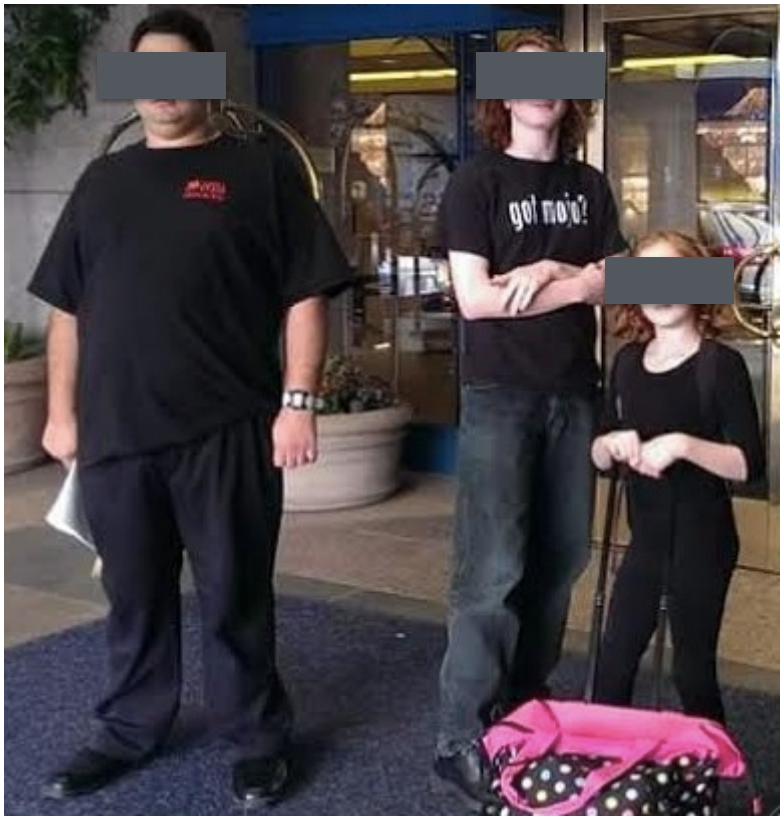
1977 paper by Reinish. I was studied when I was 8 years old.

- Did these drugs affect **my development**? **YES** (Reinsich, Nature, Arch. Sex Behav. 1977).
- Did these drugs affect **my PGCs/eggs**? **That's my hypothesis**
- Same pattern in **other autism families**? **YES**

JH Family

Synthetic steroid hormones

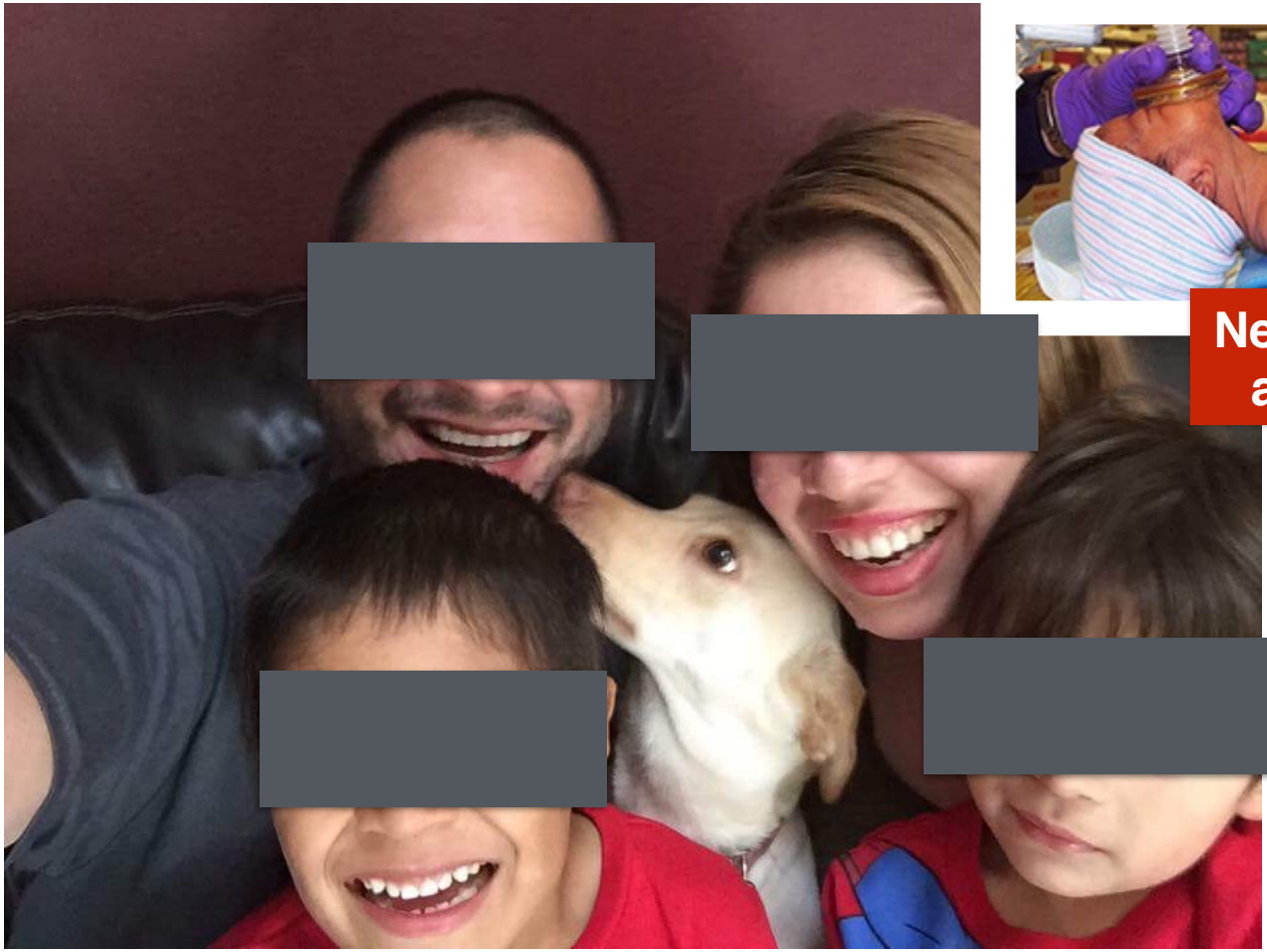
NT NT



Autism Autism Autism

+ many similar families

KR Family



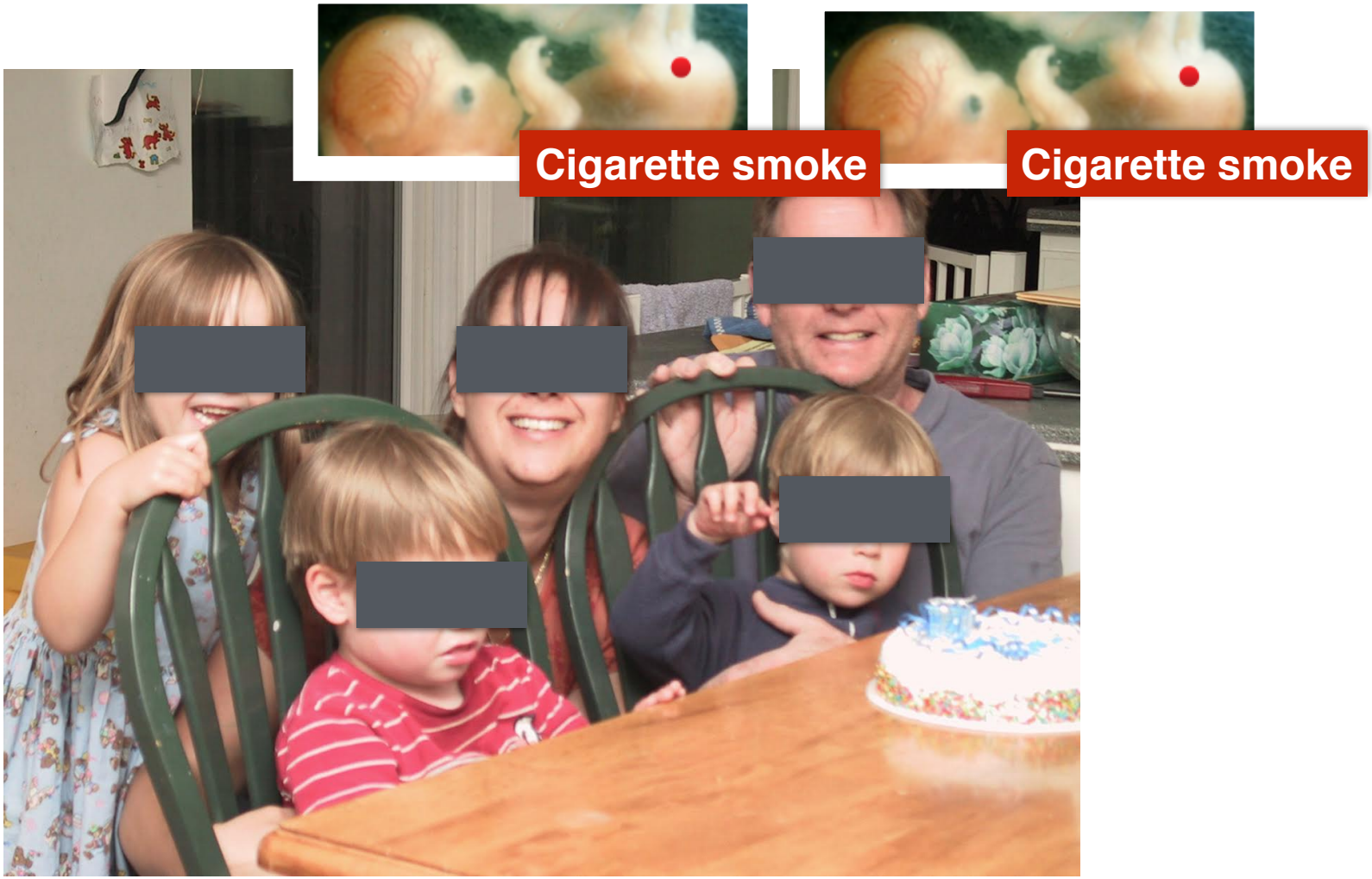
Neonatal general anesthesia 2x

Autism

Autism

+ many similar families

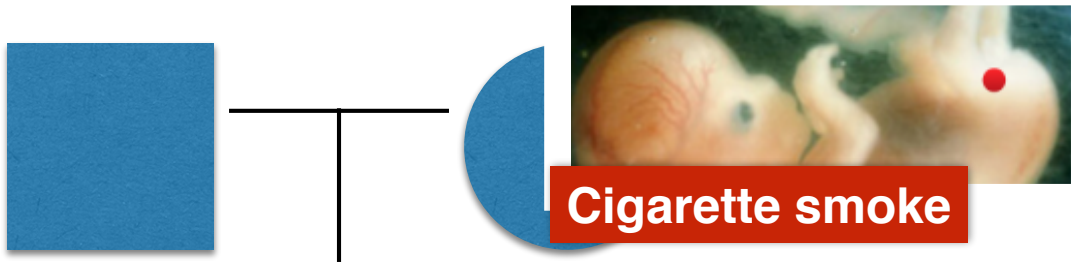
BH Family

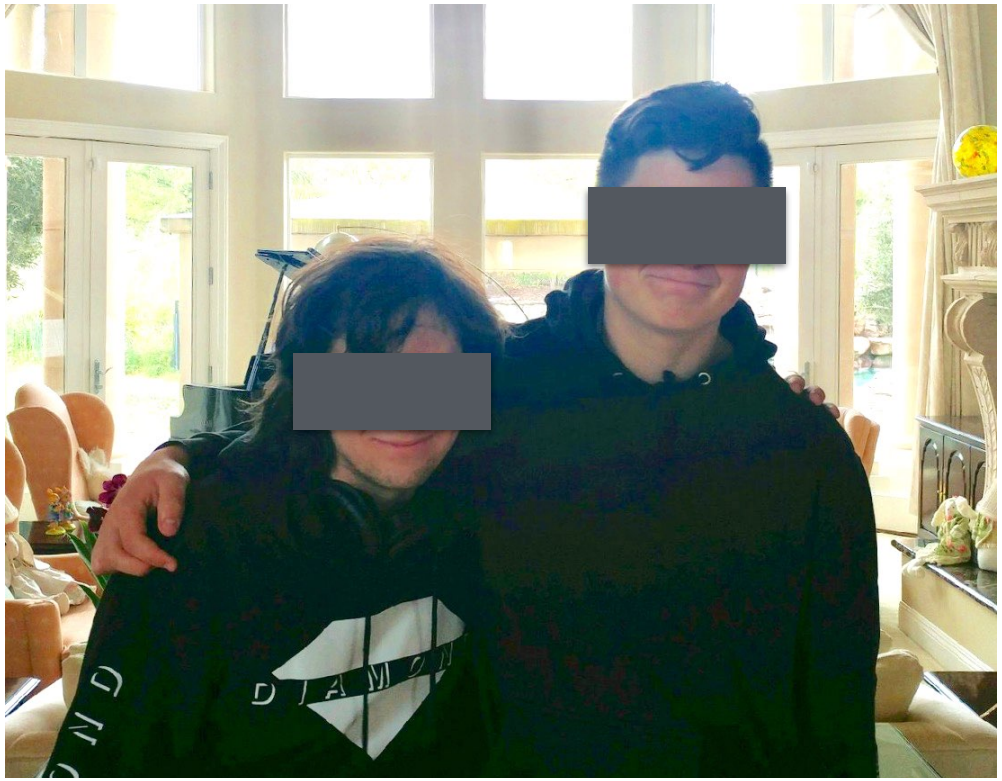


NT **Autism** **Autism**

+ many similar families

DT Family





Autism ADHD

+ many similar families

Plausible link?

Anecdotes alone prove nothing. But **animal models/mechanistic studies** suggest a biological plausibility. Some examples:

Hormone disrupting chemicals/drugs—

- F1 mouse *in utero* exposure to hormone disrupting chemicals (eg **BPA, vinclozolin**) increases abnormal behaviors and changes in gene expression in the brain, in F2 mice (eg, Wolstenholme et al. Endocrinology, 2012, Crews et al, PNAS, 2012)
- Let's not forget F2 diethylstilbestrol (**DES**) studies in humans

General anesthesia—

- F1 mouse *in utero* exposure to **GA agents** resulted in mental retardation in F2 offspring. (Chalon et al, Anesthesia and Analgesia, 1981)

Tobacco components—

- F1 mouse *in utero* exposure to **Benzo[a]Pyrene** increases mutation burden in soma and sperm of adult mice. (Meier et al. Environ Health Perspect., 2017) Major effects seen on ovaries as well, but could not measure mutations in eggs (too few eggs). (Luderer, Marchetti et al (paper submitted))
- F1 mouse *in utero* exposure to **nicotine** causes hyperactivity in F2 mice. (Zhu et al. J Neurosci, 2014)

Though suggestive, this still proves nothing. How to fill in the gaps?

Escher Fund: Questions we ask	Current interests
Are fetal/early germ cells vulnerable to certain toxicants?	Synthetic hormones, cigarette smoke, GA agents, other drugs
In what windows of susceptibility?	Primordial germ cell, neonatal
Via what mechanisms/phenomena? Eg, mutagenesis, methylation, transcription factor binding/de-silencing, imprinting/ monoallelic expression, mosaicism, ncRNAs/lncRNAs, histones/ chromatin, long genes, noncoding regions, transposons, mitochondria and other cytoplasmic, hormone receptors	Agnostic
What aspects of development or traits?	Pathologies of early neurodevelopment incl. autism, ADHD
If neurodevelopment, might programming for **circuits underpinning higher levels of cognition** be particularly vulnerable to disruption?	Some lines of research suggest this may be true
If this phenomenon exists, **how pervasive / deleterious** might it be?	Who knows?

We ask novel questions and fund pilot projects.

Avon Longitudinal Study of Parents and Children (ALSPAC)

Aka "Children of the 90s"

- 14,500 families in the Bristol area
- F2 births during 1991 and 1992 (now about 26 years old) (most F1 *in utero* exposures would have been 1950s-60s)
- Most detailed study of its kind in the world

University of BRISTOL

Avon Longitudinal Study of Parents and Children

Jean Golding

Marcus Pembrey

ALSPAC Studies on Generational Effects of Smoking

ALSPAC studies of parental exposure in utero (due to either grandmother smoking in pregnancy) and child's development – when the mother also smokes in pregnancy or not.

Is the Growth of the Fetus of a Non-Smoking Mother Influenced by the Smoking of Either Grandmother while Pregnant? (Miller et al. PLoS One. 2014;9:e86781)

Do Grandmaternal Smoking Patterns Influence the Etiology of Childhood Asthma? (Miller et al. Chest. 2014;145:1213)

Is the growth of the child of a smoking mother influenced by the father's prenatal exposure to tobacco? A hypothesis generating longitudinal study. (Pembrey et al. BMJ Open. 2014; 4(7):e005030)

The anthropometry of children and adolescents may be influenced by the prenatal smoking habits of their grandmothers: a longitudinal cohort study. (Golding et al. Am J Hum Biol. 2014;26:731-9)

! First study to examine F1 *in utero* exposure to smoking association with F2 neurodevelopment. →

Grand-Maternal Smoking in Pregnancy and Grandchild's Autistic Traits and Diagnosed Autism. (Golding et al. Sci Rep. 2017;7:46179)

Background About the Study

F2 studied [born approx 1991]
(n=12,523; 177 dx'd autism)

- Also examined four **F2 developmental traits associated with autism**: Social Communication, Speech Coherence, Repetitive Behaviour, and Sociability Temperament (>7,000 analyzed).

- Information on F1 prenatal smoking exposure was linked with F2 child information (education and health records, questionnaires completed by mothers and teachers).

- Multivariable logistic regression models used the most extreme 15% of the trait measures as well as diagnosed ASD.

- All models were **adjusted for factors concerning the grandparents** including their years of birth, ages when the study parent was born, social group and education levels.

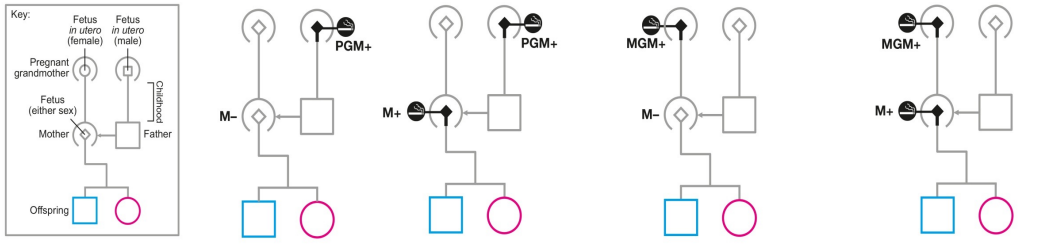
Summary of Results

- If the maternal grandmother smoked in pregnancy, this increased by **53%** the risk of her grandchildren having **diagnosed autism** (both sexes analyzed together, n too small for separate analysis).

- If a girl's maternal grandmother smoked during pregnancy, the girl is **67%** more likely to display **2 of the 4 traits linked to autism**, poor social communication skills and repetitive behaviors. The strongest association is when the study mother did not smoke.

- No associations found with paternal grandmothers smoking.

Adjusted associations of each autistic trait (by sex) and ASD diagnosis by exposure category of the study child



	PGM+M- (vs PGM-M-)		PGM+M+ (vs PGM-M+)		MGM+M- (vs MGM-M-)		MGM+M+ (vs MGM-M+)	
Autistic Traits	Boy	Girl	Boy	Girl	Boy	Girl	Boy	Girl
Social Communication (AOR)	–	–	–	–	1.04 [0.81, 1.34]	**1.67 [1.25, 2.25]*** **	–	–
Speech Coherence (AOR)	–	–	–	–	1.15 [0.93, 1.43]	1.02 [0.78, 1.32]	–	–
Repetitive Behavior (AOR)	–	–	–	–	1.11 [0.88, 1.41]	**1.48 [1.12, 1.94]*** **	–	–
Sociability Temperatment (AOR)	–	–	–	–	–	–	–	–
ASD Diagnosis	All children		All children		All children		All children	
Adjusted Odds Ratio (AOR)	–		–		**1.53 [1.06, 2.20]***		–	

MGM = Maternal Grandmother; PGM = Paternal Grandmother; M = Mother; + = smoking in pregnancy; – = not smoking in pregnancy

Golding et al 2017. Grand-maternal smoking in pregnancy and grandchild's autistic traits and diagnosed autism. Scientific Reports | 7:46179 | DOI: 10.1038/srep46179

Causal or Confounded?

In support of the possibility of these associations being **causal**, rather than the result of unaccounted for confounding:

- **Effect sizes (odds ratios) increase** rather than decrease when grandparental demographic and biological factors are taken into account.

- **Sex specific**: there are no comparable associations with paternal grandmothers' smoking in pregnancy. There are sex-specific autistic trait associations with poor Social Communication and Repetitive Behavior.

- Smoking data was gathered long **before** knowledge of autistic traits.

However the analyses were undertaken to ascertain whether there might be sex-specific associations between autistic traits and parental exposure to smoking in utero, but without prior hypotheses as to which grandmother or child's sex might be involved; consequently it is particularly **important that these associations be confirmed in other studies**.

Limitations of the ALSPAC Study

(1) Relies on the **accuracy of reports** by the F1 parents concerning their F0 parents.

(2) **Heavy v light smoking in F0 unknown**; other F0 medications/drugs unknown.

(3) The results are mainly relevant to white grandparents living in **Britain**, the numbers were too small to subdivide the analysis into different minority ethnic backgrounds.

(4) The study was **not originally planned to look at autism** as, at the time of planning (1988) the prevalence was thought to be so low as to suggest that no more than 10 F2 cases might be included in the study. However, by the time the children were at school age the researchers deliberately included the social communication trait and the pragmatic speech scales as indicators of autistic traits.

(5) Trait questions were not designed as measures of autistic traits but rather to identify the **child's performance in regard to a large number of attributes** at different ages; regression analyses had identified those related to social communication, coherent speech, repetitive behavior and sociability as being independently predictive of ASD within the ALSPAC study. Similarly the questions on abnormal and repetitive behavior were used post hoc to define an autistic trait, and could be criticized for this.

Next Steps

Other cohorts

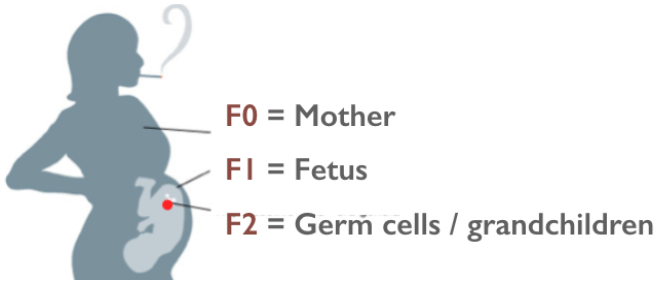
- We have funded other pilot projects on tobacco and other exposures
- Eg, CHDS (1960s Kaiser), Harvard (Nurses' Health), UCSD, Denmark (Prenatal Development Project), Finland, Israel, Sweden.
- Unclear how much smoking data we will get

Molecular mechanisms and animal models

- No one is looking at F2 effects of pregnancy drugs

For epidemiology generally. Marcus Pembrey:

- "Potential confounder of single generation studies."
- "Cohort studies have to become multigenerational."



Three generations at once

For autism research

- Autism genomics now devoted to massive hunt for "common variation," no effort re early germline stressors. My work has had little influence.

50 Years Ago


Joshua Lederberg


James Neel


Samuel Epstein


James Crow

Half-century ago environmental mutagenesis pioneers asked, "**Are novel chemicals and drugs creating a 'genetic emergency**' via exposed germline?"

Given all we have learned in the intervening decades about germ cell vulnerability, and the rise of mysterious heritable developmental pathologies, this question is more important than ever, but remains largely ignored.

Thank you.