Why genetics, environment, and epigenetic regulation all matter to breast cancer risk

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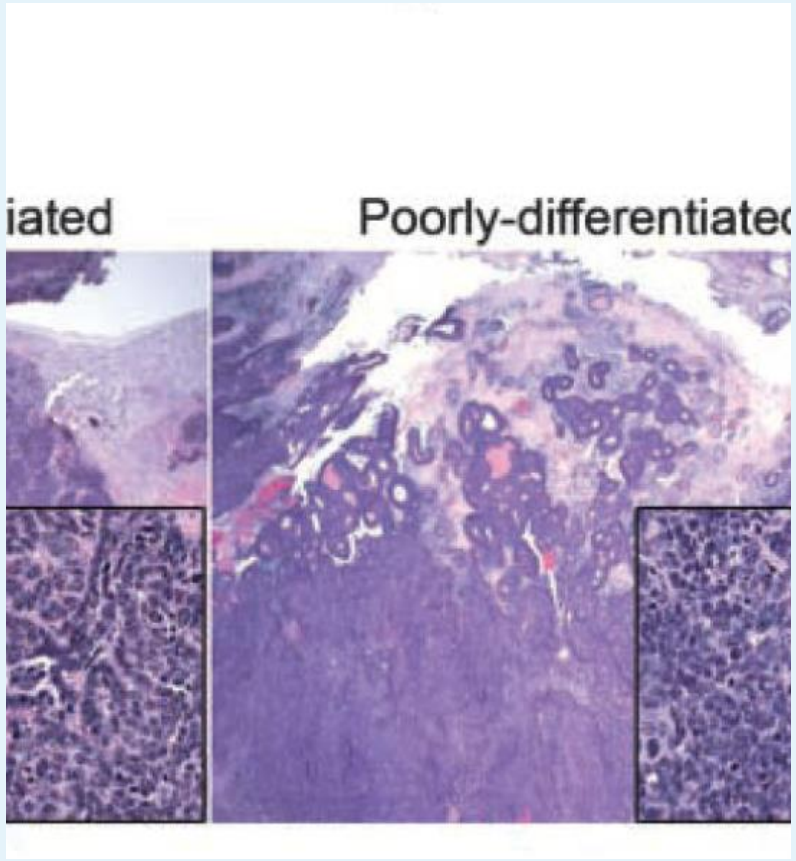
Definitions

Susceptibility	Definition	Eg
Genetic (G)	Mutations and other genetic variants	BRCA mutation carriers
Environment (E)	Exposures, life styles including during time windows of susceptibility	Cigarettes; Int. Agency for Cancer Research carcinogens
Epigenetic regulation (Epig)	DNA methylation, post translational modifications of histone, noncoding RNAs	Methylation of cell cycle regulation, detoxification, adhesion and invasion, apoptosis, DNA repair, hormone receptors genes

Why all matter?
Evidence of G X E in breast cancer



- Transgenic mice driving a viral oncogene that inactivates tumor suppression.
  - C3(1)/SV40 T-antigen; p53 and retinoblastoma inactivated
- Socially isolated (single-housed) vs group housing since weaning.
- Found:
  - Increased expression of metabolic/ inflammatory genes in premalignant mammary gland at 15 weeks.
  - Larger mammary tumors at 21 weeks.

Williams Cancer Prev Res 2009

Furthering our understanding of G X E in breast cancer

- Experimental approaches
  - Genetically diverse outbred mouse stock
  - Genetic manipulation
    - Transgenic and knock out genes
    - Comparisons across mouse strains
  - Experimentally controlled exposures during specific time windows of susceptibility (WOS)
- Epidemiological approaches
  - Population studies
  - Assessment of high susceptibility by genetic risk during WOS to environment exposures

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Environmental epigenetic regulation

Environmental exposures

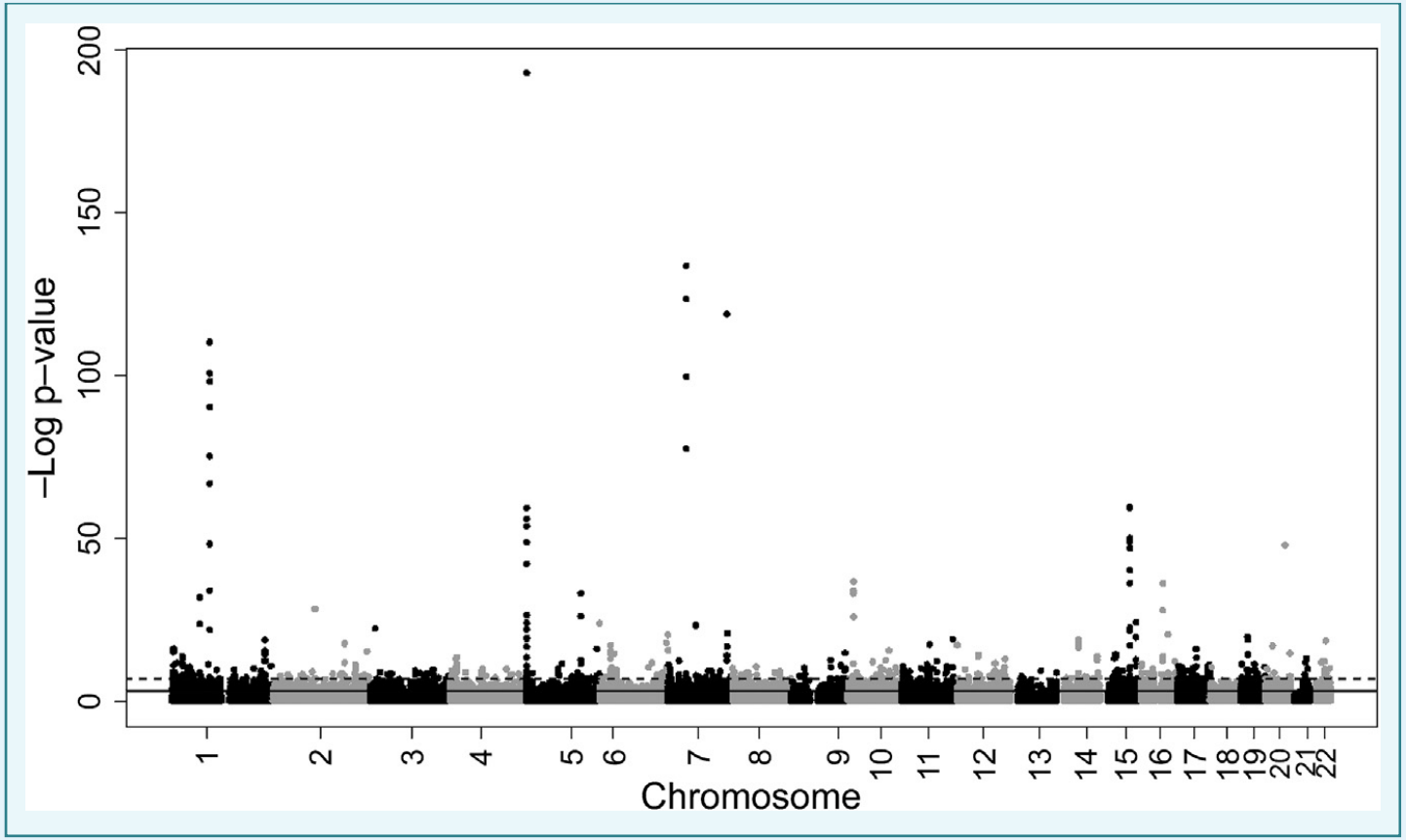
Air pollution diet

cigarettes stress

Epigenetic modifications

- **DNA methylation**
- **Posttranslational modifications of histones**
- **Noncoding RNA**
- **RNA methylation**
- **DNA hydroxymethylation**

Evidence of prenatal E X Epigenetic in cord blood of cancer risk genes

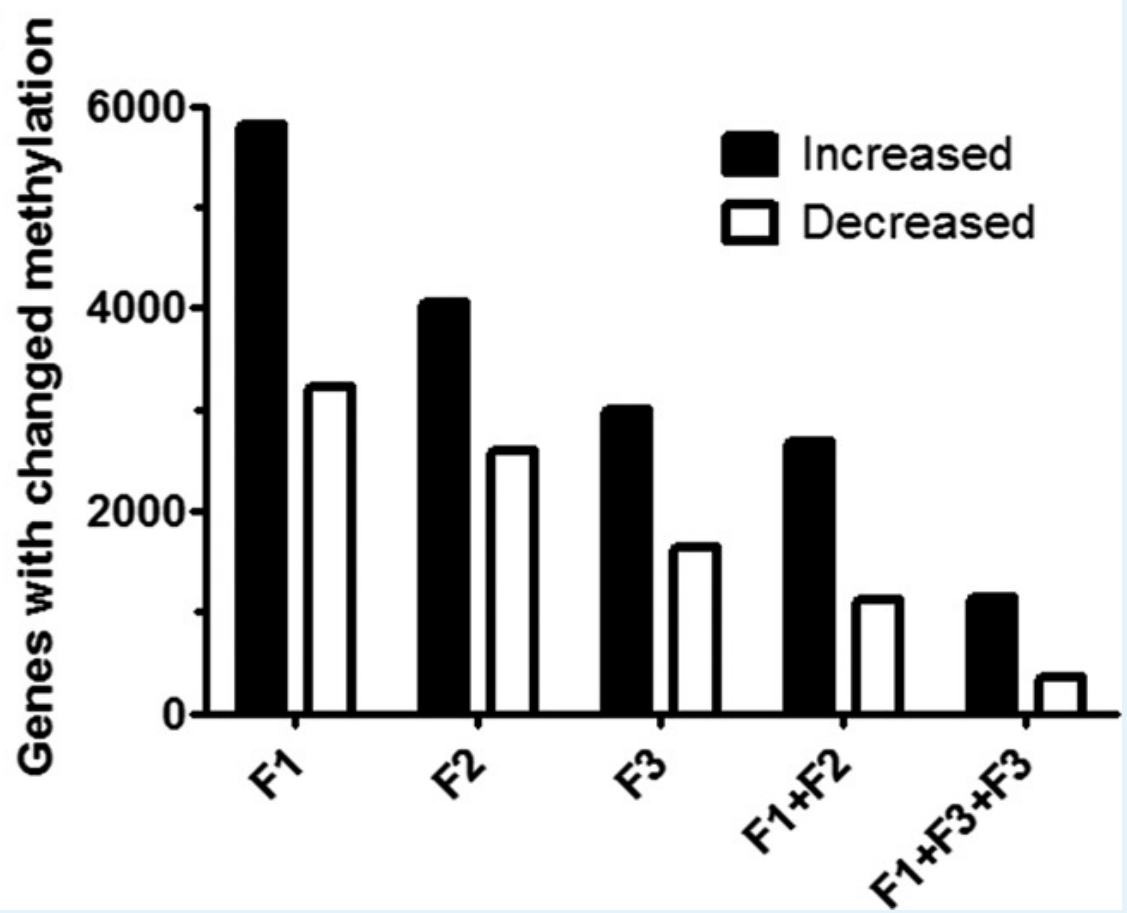


- E: Cigarette smoking during pregnancy
- Pregnancy And Childhood Epigenetics (PACE) consortium, 13 cohorts (n = 6,685 children)
- Aryl hydrocarbon receptor repressor (AhRR, chromosome 5, p=-1.64 x 10^{-193})
- Altered DNA methylation (Epig) at 568 CpG sites enriched for CpG island shores, enhancers, DNAse hypersensitivity sites

Manhattan plots using Illumina 450K BeadChip and epigenome wide analyses

Joubert AJHG 2016

Prenatal intranasal diesel (E) altered dendritic cell methylation (Epig) across 3 generations of offspring mice

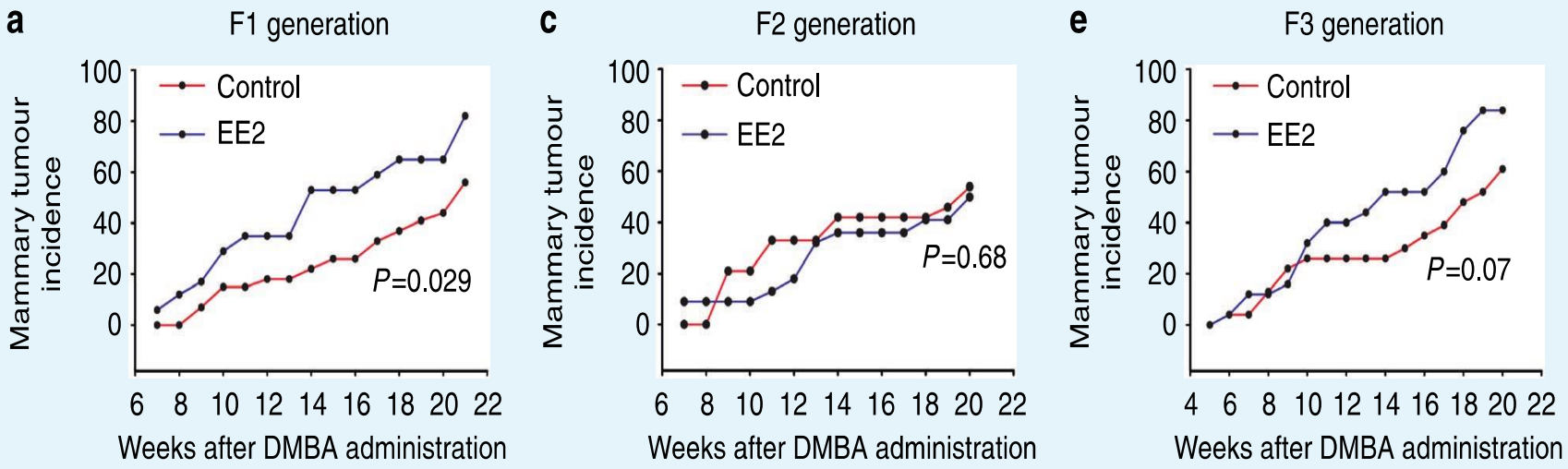


F1-offspring; F2-grandoffspring; F3-great grandoffspring

Including sustained interactions among:
IL-4 signaling genes
Chromatin remodeling genes

Gregory. Am J Physiol Cell Mol Physiol 2017

Prenatal oral ethinyl-oestradiol (EE2) altered methylation and increased mammary cancer risk across 3 generations of offspring rats

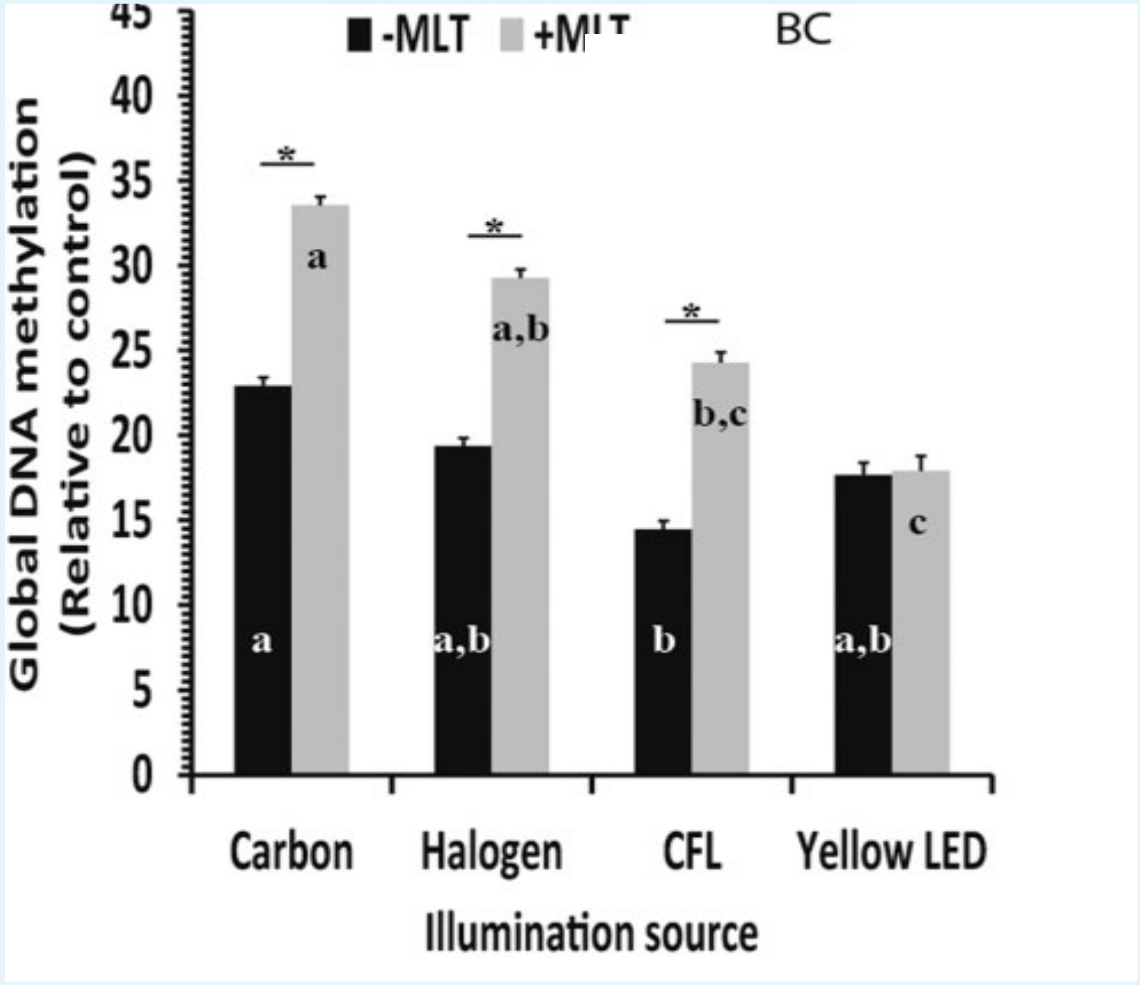


F1-offspring; F2-grandoffspring; F3-great grandoffspring

- Mammary tumors induced by administration of oral gavage of 7,12-dimethylbenz[a]anthracene (DMBA)
- EE2 led to:
  - Higher incidence and greater size of mammary tumors
  - 375 differentially methylated gene promoter regions in F1, F2 & F3 generations

De Assis Nature Communications 2012

E X Epigenetic in breast cancer cell (4TI)-inoculated BALB/c mice



CFL, compact fluorescent lamp; LED, light-emitting diode; MLT, melatonin

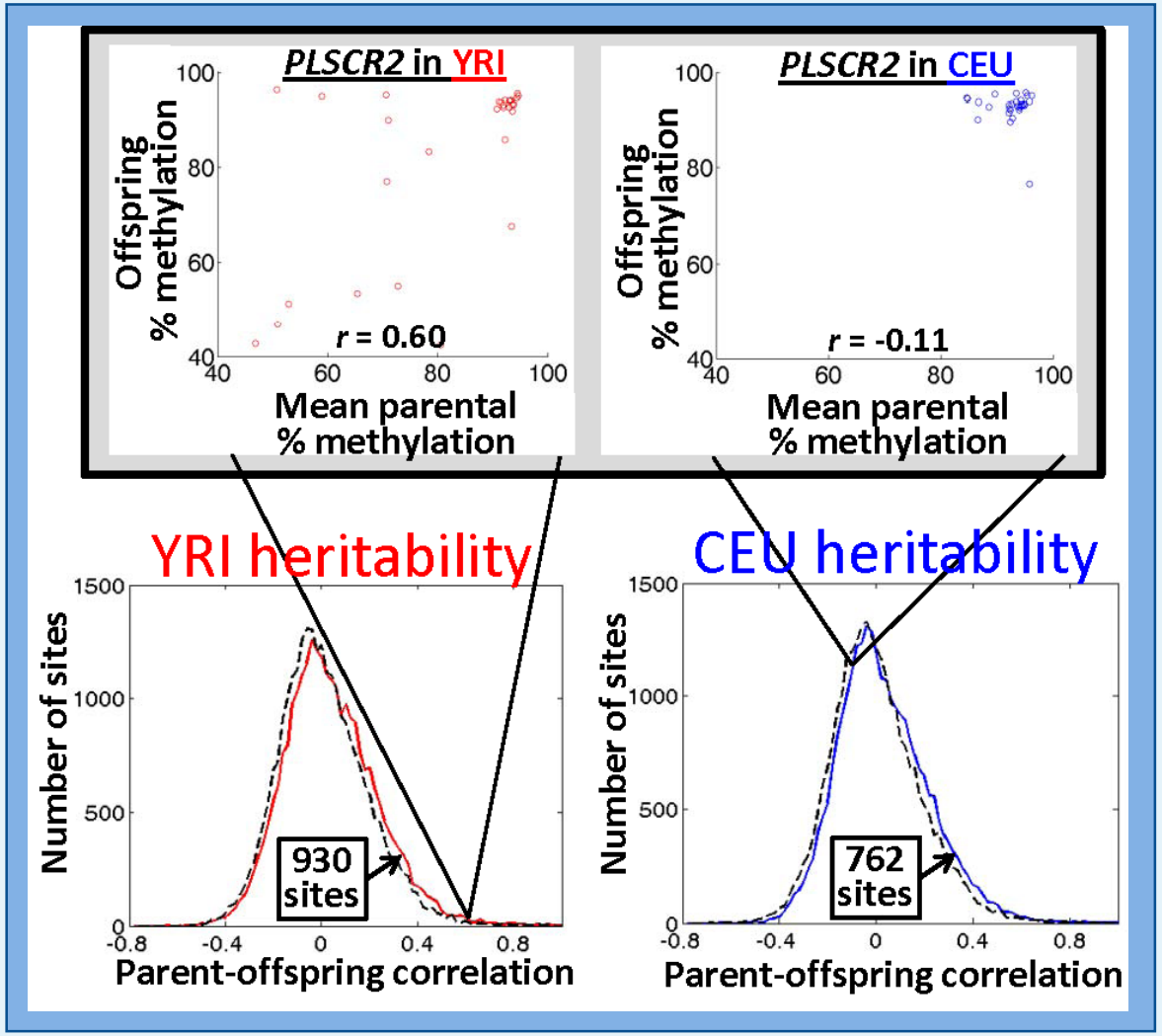
Effects of spectral composition and daily melatonin on global DNA methylation in mammary tumor tissue after 4 weeks.

Shorter wavelength may increase cancer burden by inducing aberrant methylation?
Suppressed by melatonin?

Zubidat Cancer Control 2018

G X Epigenetic in population-specific divergent methylation patterns

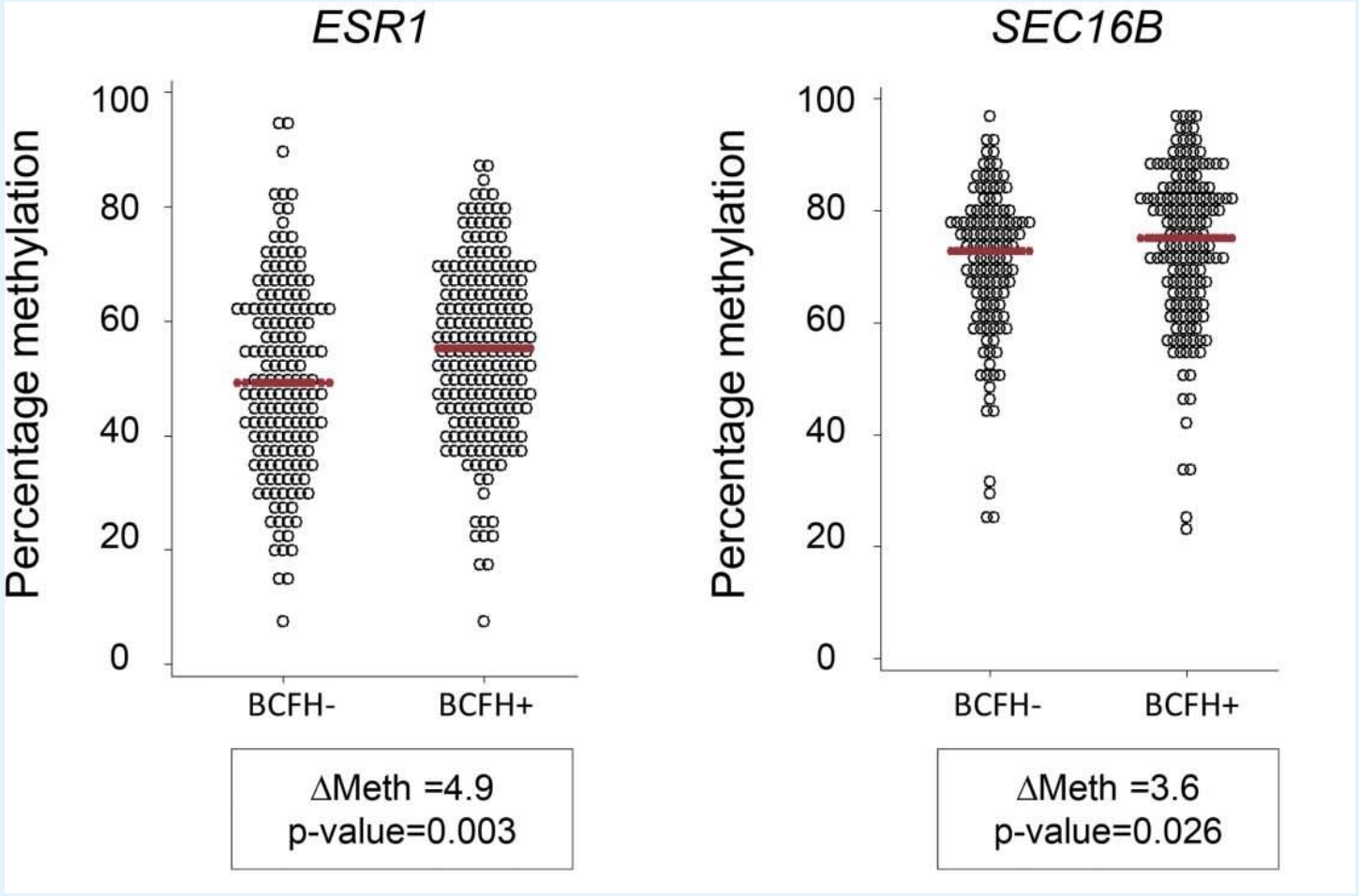
Example of a CpG site (near PLSCR2: chromosome 3)



- 180 Lymphoblastoid cell lines
- Quantitative bead- array-based DNA methylation analysis
- CEU: 30 family trios of Northern European ancestry
- YRI: 30 trios of Yoruban (West African) ancestry

Fraser. Genome Biol 2012

From LEGACY: G X Epigenetic in girls with positive breast cancer family history (BCFH +)



WBC DNA methylation differences between BCFH+ and BCFH- girls.

Wu Epigenetics 2018

Prenatal polycyclic aromatic hydrocarbons (E) X Epig on mammary pathology across 2 generations of mice

- Current research gaps:
  - Can prenatal PAH exposure induce breast cancer or heighten its risk?
  - Pathways involved?
    - Epigenetically mediated?

Hypotheses

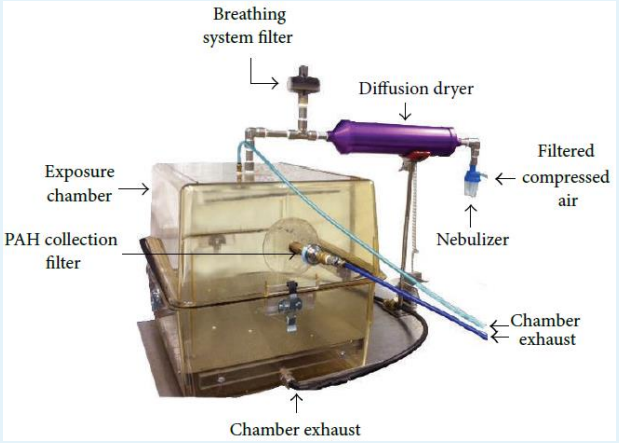
1. Prenatal PAH exposure induces mammary epithelial cell proliferation and hyperplasia in mammary glands of adult female offspring (F1) and grand-offspring mice (F2).

2. Effects may be due to ERα and downstream events.

    -altered expression and DNA methylation of the ERα pathway.

3. Effects may occur early and be sustained.

Our approach



- Assess G
  - Balb/c mouse strain (high affinity allele for AhR).
  - Replicating in 2nd strain/genetic makeup with low affinity AhR allele (129SVj).
- Assess E
  - Delivered controlled physiologic ambient PAH vs control air throughout pregnancy.
- Assess Epig
  - Measured differences in methylation, gene regulation (AhRR, BRCA1, ERα, p53, PR) and their effects on functional and cellular events (i.e. hyperplasia, proliferation) across multiple generations of mice.

Still needed

More assessment of G:

Effects by mouse strain; among outbreds

More assessment of E:

Controlled experimental exposures that focus on timing of exposure

- Cumulative exposures, lags, time windows of susceptibility

Effects of mixed components, sequential exposures

More assessment of Epig:

Duration of effects by WOS

- Within, across generations

Mechanisms of exposure

Concluding remarks

- Genetic, environment, and epigenetic regulation *matter* to breast cancer risk.
- Demonstrated in some experimental systems, epidemiological studies with careful measures of exposures that assess by WOS, genetic risk and intervening epigenetic regulation.
- Substantial, but solvable research gaps.
  - Dependent on careful research design
- Visit us! Poster 15-T (Dr. Debashish Sahay)