

Environmental Epigenetics

Results and Opportunities for Children's Environmental Health and Disease Prevention Research

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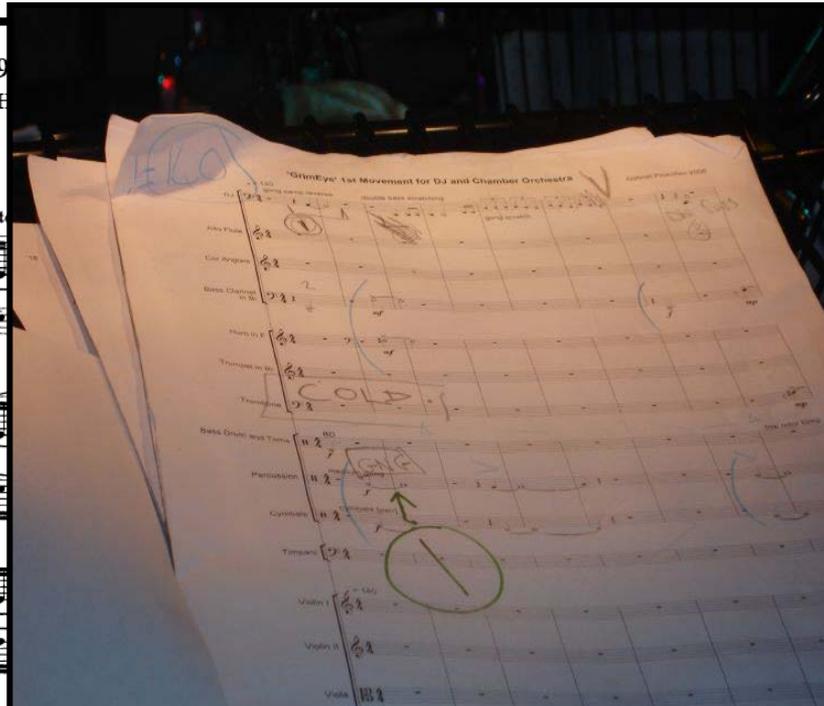
Epigenetics

- ▶ Programming of gene expression that:
 - ▶ does not depend on the DNA code
 - ▶ (relatively) stable, i.e., replicated through:
 - ▶ cell mitosis
 - ▶ meiosis, i.e. transgenerational (limited evidence in humans)
- ▶ Characteristics of Epigenetic Programming
 - ▶ Modifiable (can be reprogrammed)
 - ▶ Active or poised to be activated:
 - ▶ Potentially associated with *current* health states or predict *future* events

A Symphonic Example

DNA

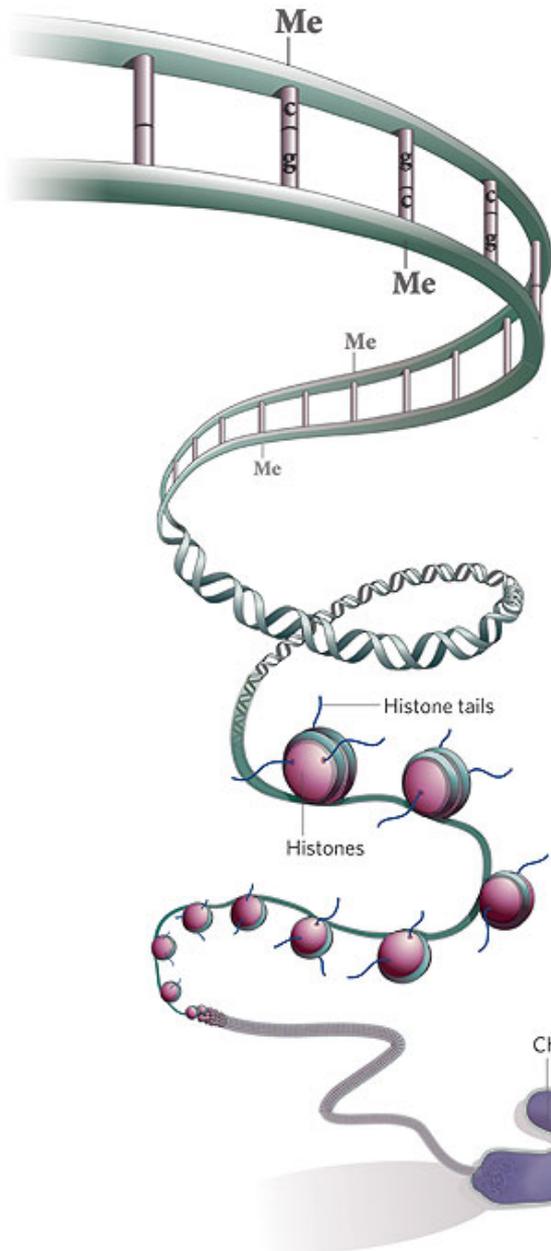
Phenotype



Epigenetics

th

Epigenetic Marks

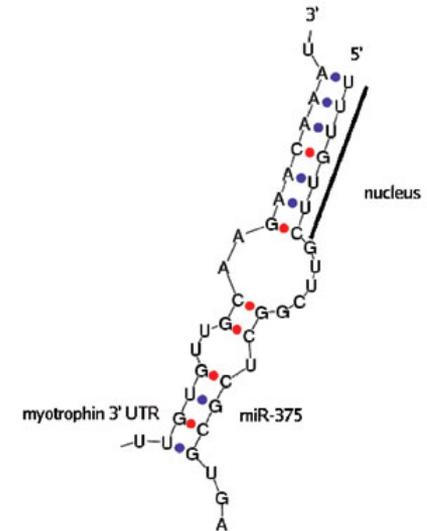


DNA methylation

Methyl marks added to certain DNA bases **repress** gene transcription

Histone modifications

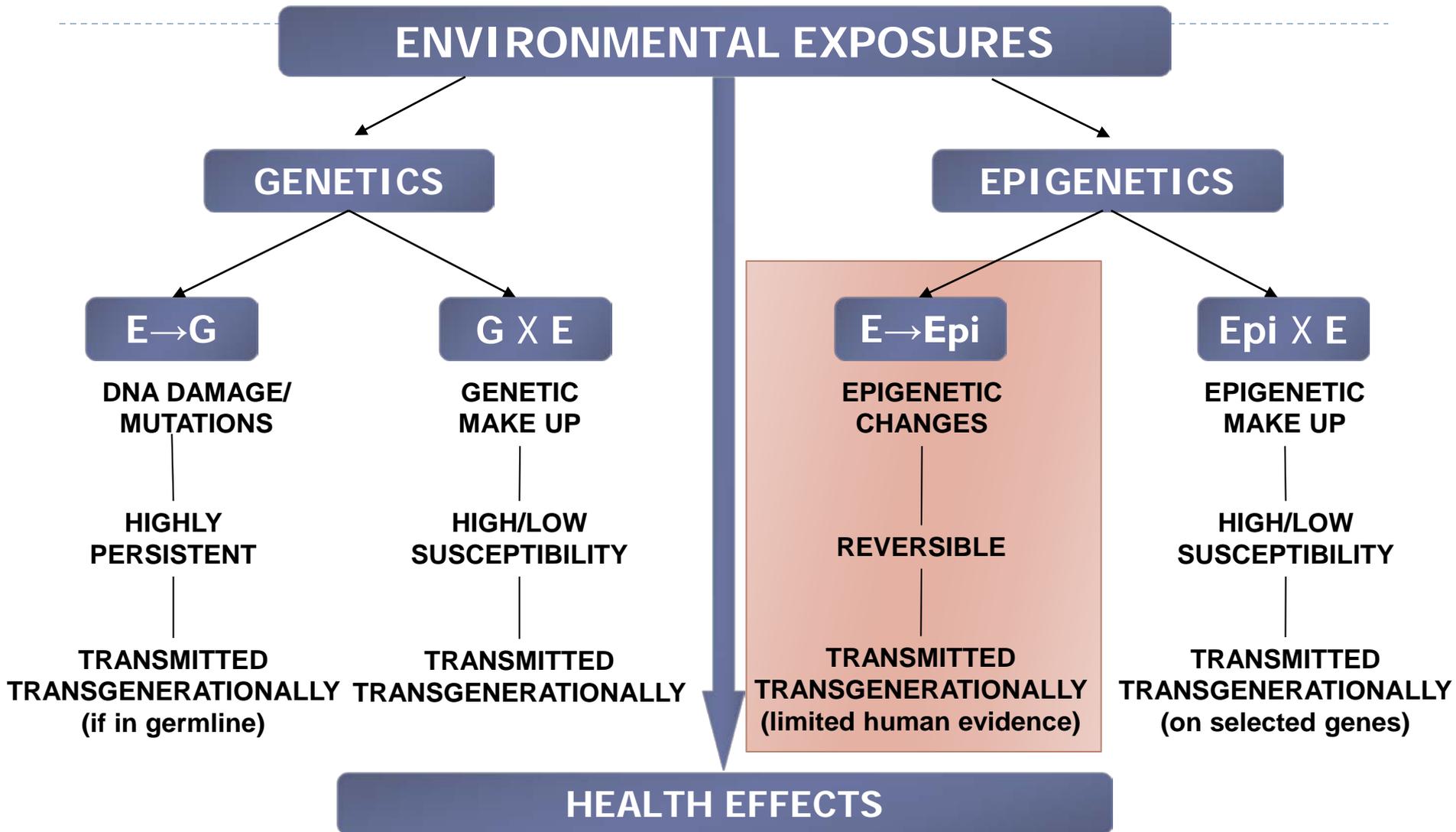
A combination of different molecules can attach to the 'tails' of proteins called histones. These **alter** the activity of the DNA wrapped around them



microRNAs

Small non-coding RNAs that **block translation** of messenger RNAs into proteins

Environment, Genetics, Epigenetics



Environmental Effects on blood DNA methylation – Results from our lab (1)

- ▶ Hypomethylation of non-coding DNA (50% of genome):
 - ▶ Air Pollution
 - ❑ Baccarelli et al., AJRCCM 2009 (elderly in Eastern Massachusetts)
 - ❑ Tarantini et al., Environ Health Perspectiv 2009 (PM in steel factory)
 - ❑ Controlled human exposure study (Bellavia et al., in preparation)
 - ▶ Bone Lead (elderly in Eastern Mass, Wright et al., EHP 2010)
 - ▶ Benzene
 - ▶ Bollati et al., Cancer Res 2007 (traffic officers, gas station attendants)
 - ▶ Seow e al., under review (petrochemical workers, Bulgaria study)
 - ▶ Pesticides and POPs (Inuit Greenlanders, Rusiecki et al, EHP 2008)
 - ▶ Psychosocial Stress (PTSD, Rusiecki et al. Epigenomics 2012)

Environmental Effects on blood DNA methylation – Results from our lab (2)

- ▶ Altered methylation of candidate genes
 - ▶ Air pollution (Particulate Matter)
 - *i*NOS methylation (Tarantini et al EHP 2009)
 - Inflammatory genes in blood
 - Ongoing work in elderly in Eastern Mass (Madrigano et al., others);
 - Steel workers study (Tarantini et al in preparation,).
 - Normative Aging Study (Bind et al IJE 2012)
 - ▶ Tumor suppressor genes (Hou L, Part Fiber Toxicology 2011)
- ▶ Arsenic and p16 hypermethylation (Kile et al, under review)
- ▶ PAHs & tumor suppressor genes
 - ▶ Polish PAH study (Pavanello et al. IJC 2010 & Carcinogenesis 2011)
 - ▶ Ma-Ta-Puth, Thailand (Peluso M et al. under review)

Why Environmental Epigenetics in Children's Research?

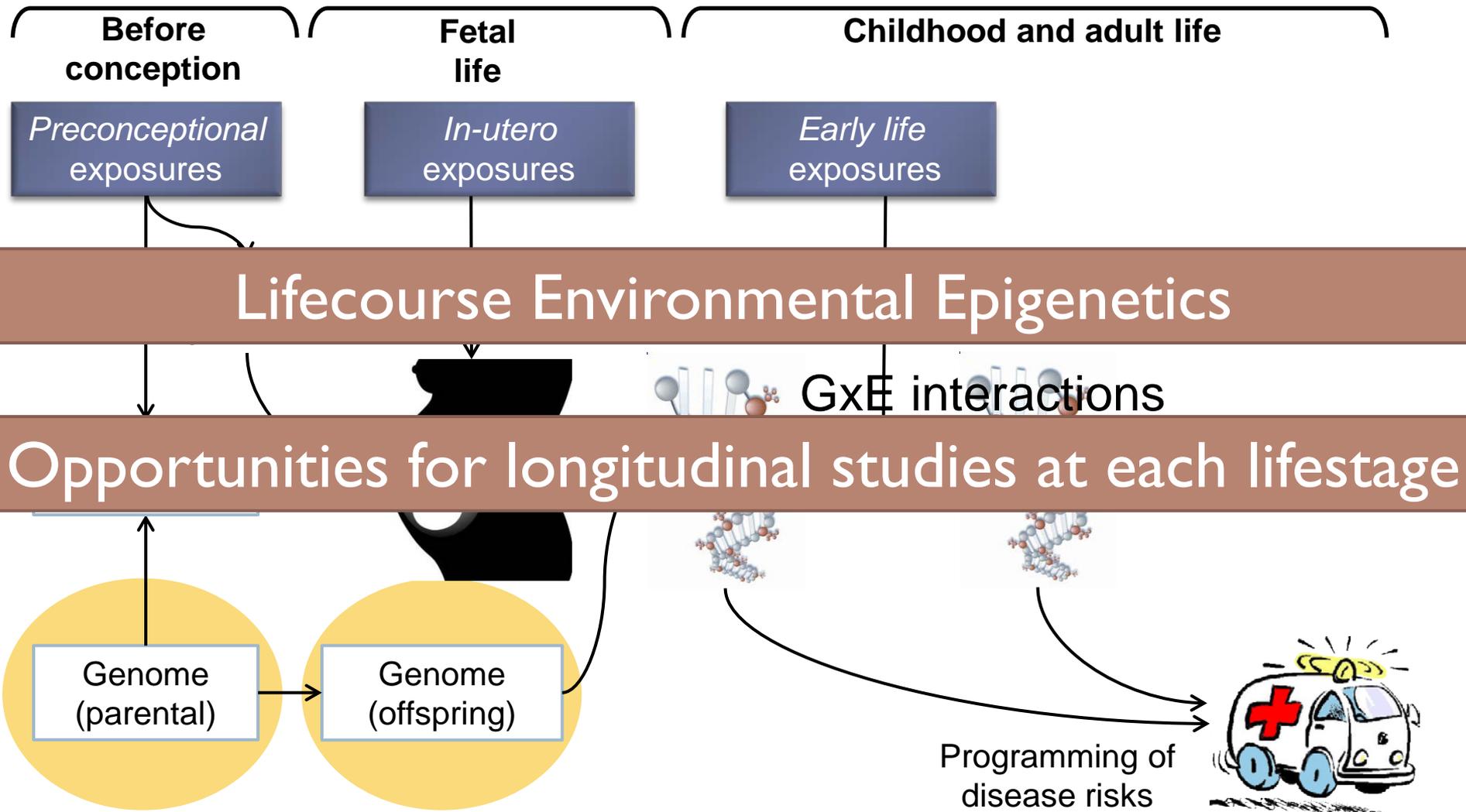
- ▶ The epigenome is erased and re-established in-utero
 - ▶ Animal models (see Dana Dolinoy's work) indicate exquisite susceptibility to in-utero exposures
- ▶ The epigenome could be used to track environmental effect on tissue and system plasticity during childhood
 - ▶ Need for prospective studies
- ▶ Fetal/early life experiences may determine lifelong trajectories of health risk
 - ▶ Epigenomics provides molecular substrate for biological memory of gene programming

‘Each living organism has two histories that determine its biology:

an evolutionary history whose duration is in the hundreds of thousands of years, and a developmental history that starts at the time of its conception.’

Ze'ev Hochberg, 2011

Developmental history of the epigenome



Children Studies and Environmental Epigenetics

▶ Environmental Epigenetics in PubMed

- ▶ 1245 papers as of March 4th 2012

- ▶ Queried for:

- ▶ (epigenetics OR DNA methylation OR epigenomics OR histone modification OR histone methylation OR histone acetylation OR miRNA OR microRNA) AND (chemicals OR toxicants OR environmental health OR environmental exposure)

▶ Children and Environmental Epigenetics in PubMed

- ▶ 183 papers as of March 4th 2012

- ▶ 80 were reviews

- ▶ Many not actually related to environmental health

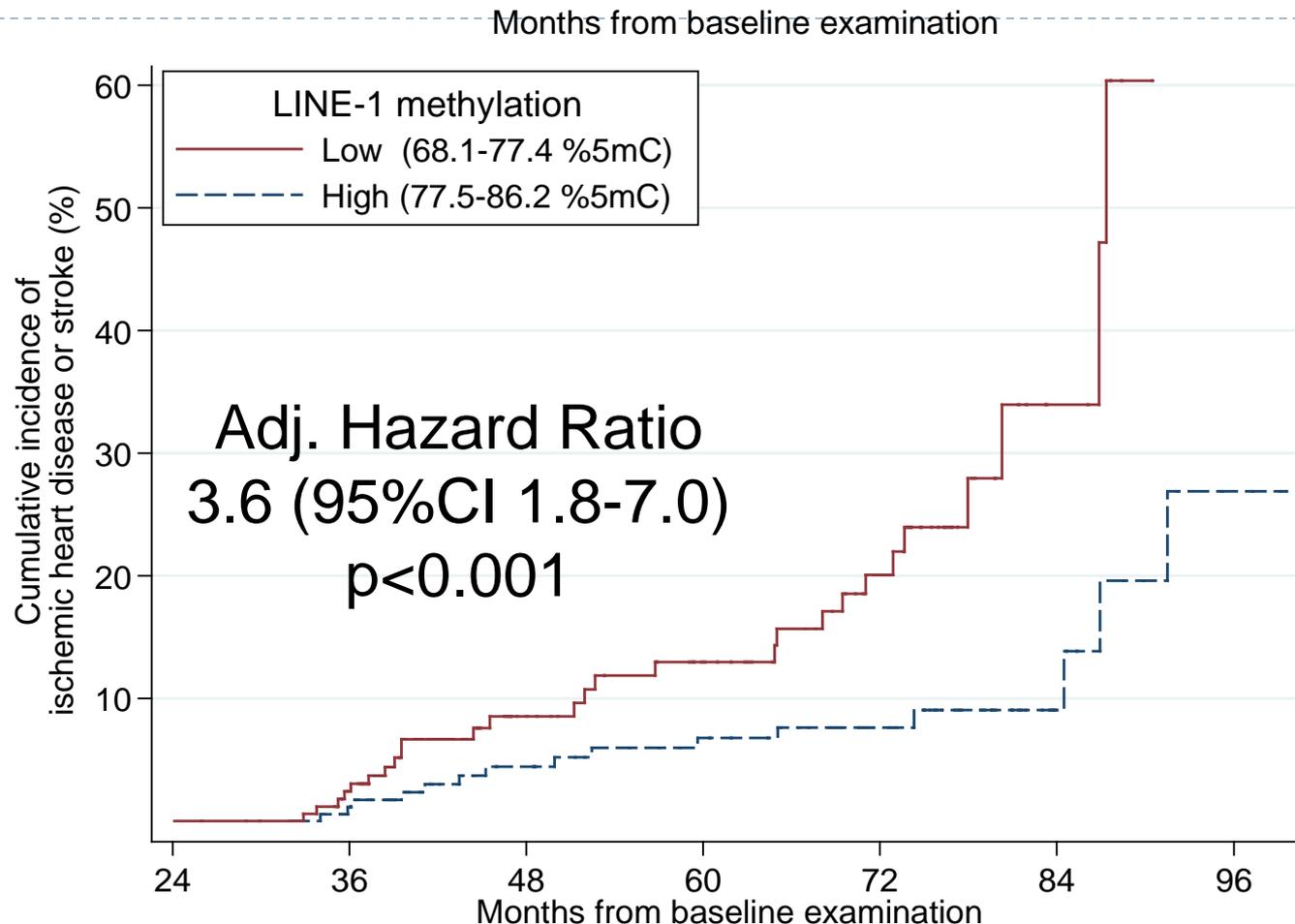
- ▶ Sub-queried for:

- ▶ child OR children OR pediatric OR pediatrics OR "in utero" OR prenatal OR perinatal

Ongoing Research & Future Trends in Environmental Epigenetics

1. Exposures can modify DNA methylation in easily obtainable tissues, such as blood (and others)
 - ▶ Do epigenetic modifications in blood predict the risk of future disease?
2. Can we move beyond surrogate tissues?
 1. Opportunities in perinatal and child research
3. Other epigenetic mechanisms are less studied
 - ▶ Histone modifications
 - ▶ MiRNAs

LINE-1 Methylation and Incidence of non-fatal IHD or Stroke

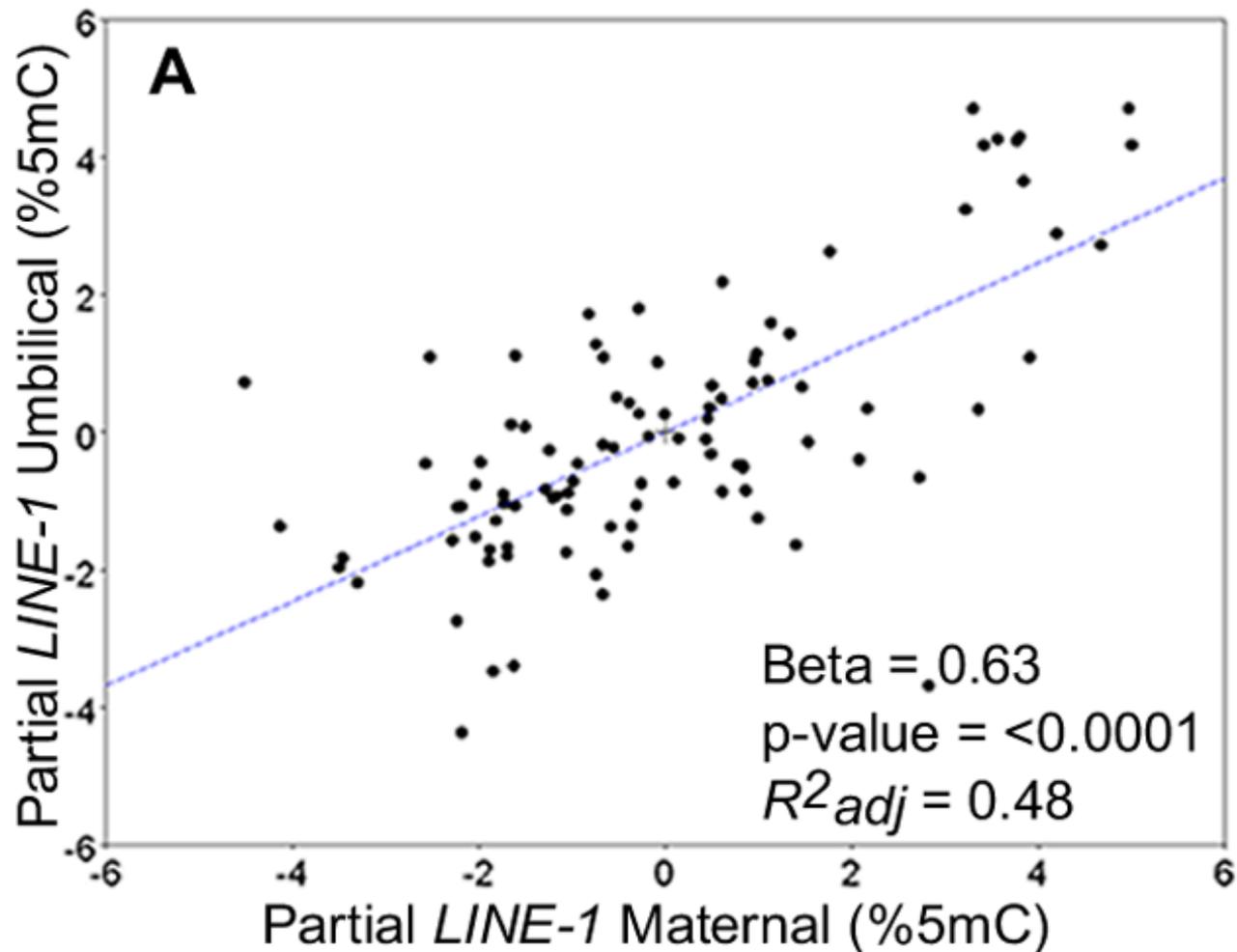


HR=2.8 (95%CI 1.3-5.9), p=0.009 for IHD

HR=4.3 (95%CI 0.7-25.8), p=0.11 for stroke

Baccarelli et al., Epidemiology 2010

Blood LINE-1 hypomethylation: When is that happening?



Kile ML et al, PlosOne 2010

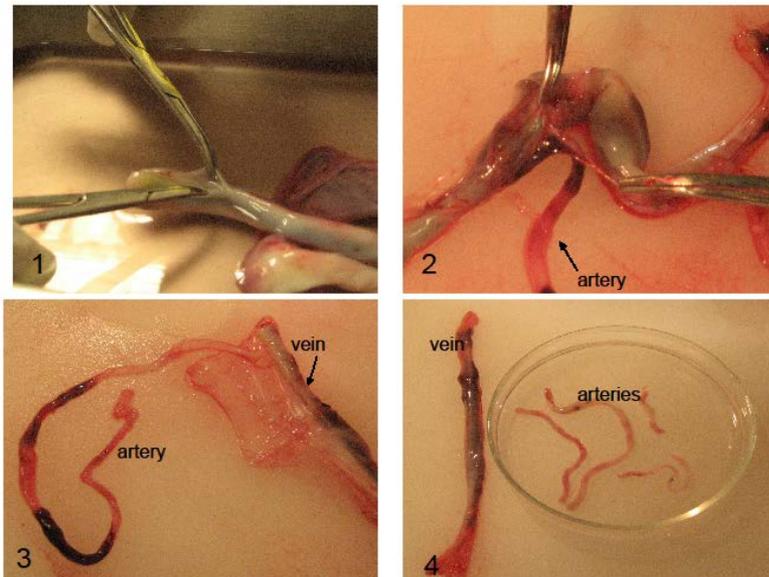
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Environmental causes of fetal growth

Ongoing Research in the ELEMENT/PROGRESS study

Figure 3: Umbilical artery and vein dissection



- ▶ Umbilical vessels and placenta are critical to maternal-fetal nutrient transfer
- ▶ Ongoing case-control study nested in the ELEMENT/PROGRESS Mexico Cohort
- ▶ Fetal growth and potential environmental determinants

Top 20 methylation sites in blood, artery, vein associated with birth weight

	Blood		Artery		Vein	
Rank	SYMBOL	p.value	SYMBOL	p.value	SYMBOL	p.value
1	KLF15	0.00005	POLR2J2	0.00003	SFT2D1	0.00006
2	EBPL	0.00018	IER5	0.00009	SLITRK4	0.00007
3	ITPKB	0.00022	LR8	0.00009	MGAT5B	0.00014
4	TOPORS	0.00023	CFHR5	0.00016	CGRRFI	0.00015
5	ZNF365	0.00026	HTATIP2	0.00018	NOX1	0.00017
6	ACOT4	0.00026	SAMD7	0.00028	IQWD1	0.00020
7	CCNJ	0.00030	ALOX15B	0.00035	NOL9	0.00026
8	DCK	0.00033	POLD2	0.00036	SFXN1	0.00030
9	DDX27	0.00048	SPIB	0.00045	RPS15A	0.00031
10	C17orf88	0.00052	MAPK9	0.00050	CAMK2D	0.00042
11	WDR37	0.00057	CER1	0.00055	PRSS27	0.00044
12	PGPEPI	0.00063	WDFY3	0.00057	GJA3	0.00048
13	RPS15A	0.00063	LETM2	0.00058	C1orf26	0.00050
14	REST	0.00065	RGSL1	0.00064	LYPLA1	0.00055
15	C19orf22	0.00071	BSDC1	0.00069	FLJ45803	0.00056
16	VPS54	0.00073	MSR1	0.00074	PAK1	0.00064
17	PDE9A	0.00074	FOXF2	0.00074	C21orf129	0.00081
18	CLDN11	0.00075	ZNF592	0.00080	SENP6	0.00091
19	SPAG1	0.00076	PTPDC1	0.00083	TNFRSF1A	0.00099
20	ARIHI	0.00076	FLJ20920	0.00089	SLC22A6	0.00106

Pilot Study
 20 children with
 small birth
 weight
 vs.
 20 children with
 normal birth
 weight

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Metal-rich air particles exposure of steel workers

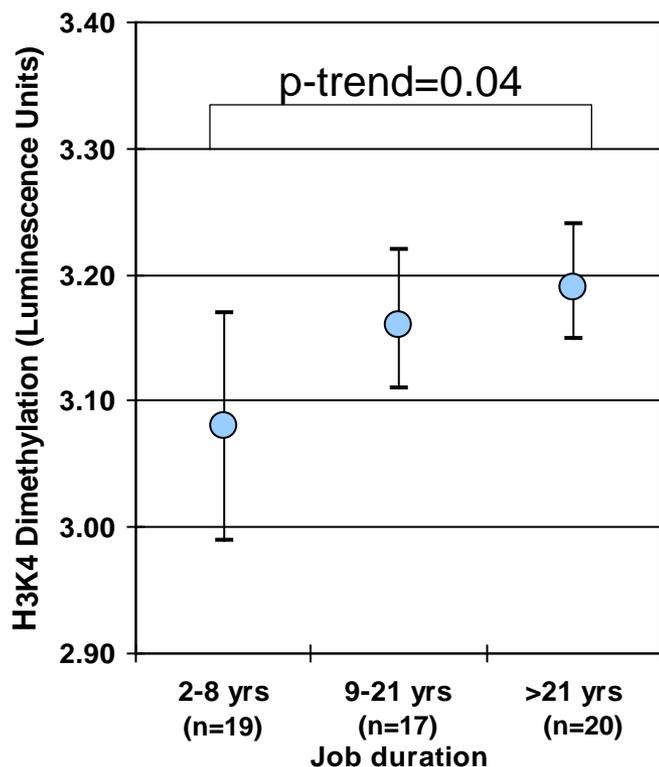


- ▶ Foundry work has been associated with cardiovascular disease and lung cancer
- ▶ Chemical exposures are low in modern foundry facilities
- ▶ Particulate Matter (PM) Rich in metals 10+ fold higher than ambient levels

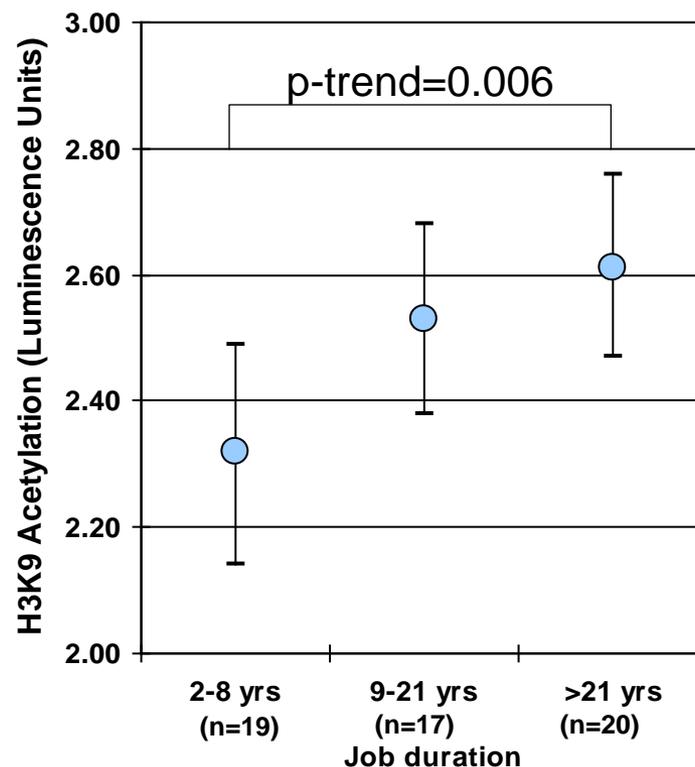


Years of Employment and Blood H3 activating modifications

H3K4 Dimethylation



H3K9 Acetylation



-Associations were not due to age effects

-Associations with Nickel and Arsenic contained in particles

Cantone et al., EHP 2011

MicroRNA expression & Metal-rich PM (Motta et al., in preparation)

Discovery: profiling of 847 miRNAs on
10 pre-post exposure blood sample pairs

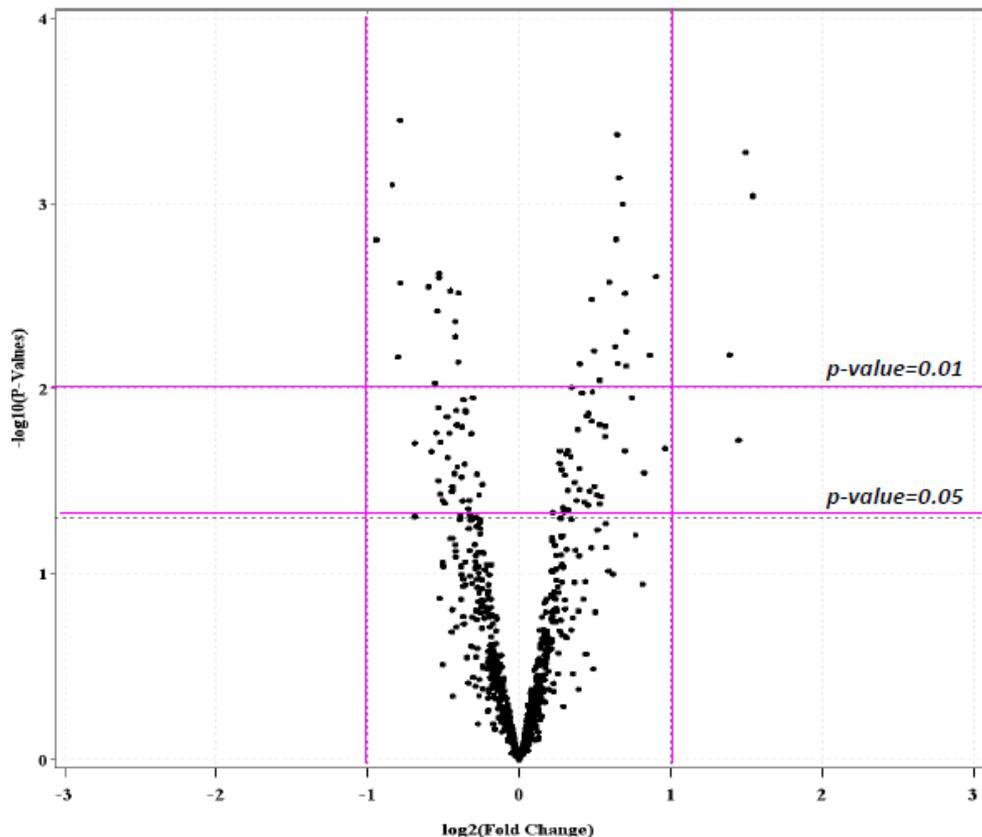


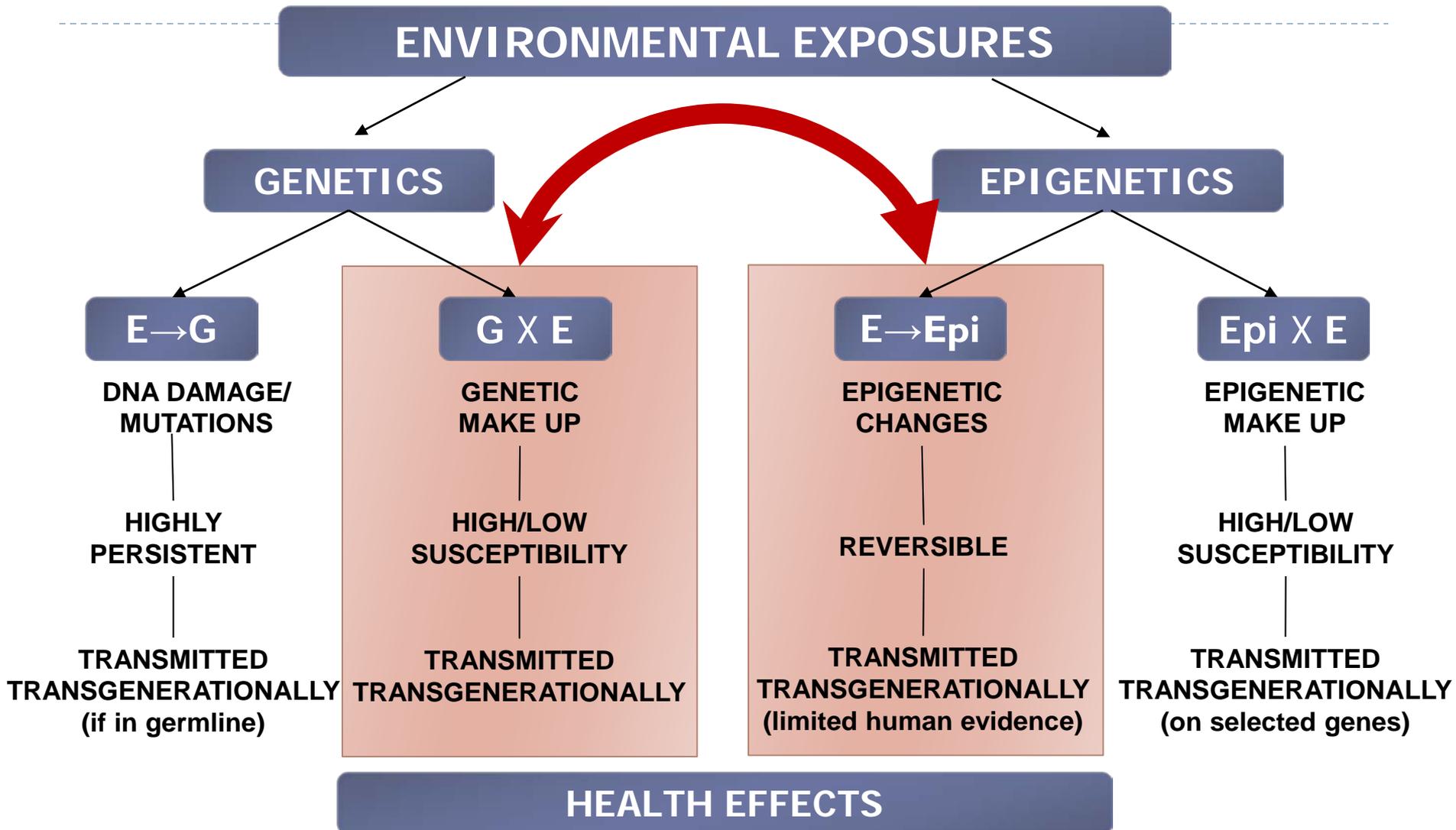
Figure1: Volcano-plot representing differential miRNA expression in blood leukocyte RNA of foundry workers pre-and post-exposure.

Validation: Real time PCR of 12
miRNAs on 62 blood sample pairs

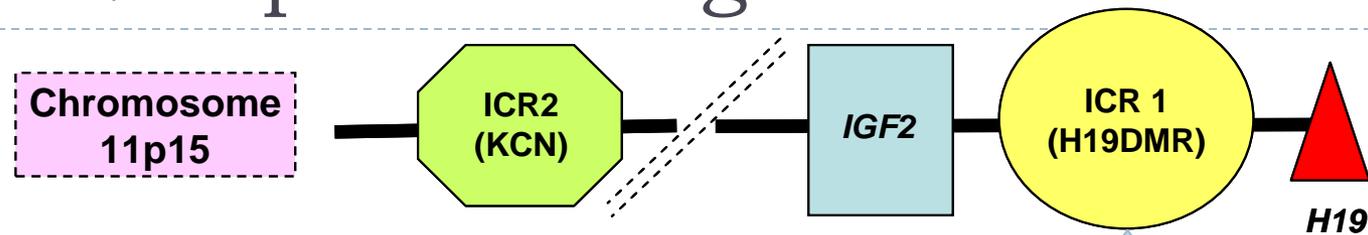
Real Time Results					
miRNA	logFC	FC	t	p-value	Confirmed
hsa-miR-421	-1.15	0.45	1.10	0.2768	No
hsa-miR-29a	3.02	8.10	-4.15	<0.001	Yes
hsa-miR-146a	1.65	3.15	1.33	0.192	No
hsa-let-7g	-0.22	0.86	1.02	0.312	No
hsa-miR-130a	0.00	1.00	-0.00	0.997	No
hsa-miR-150	-0.51	0.70	0.50	0.618	No
hsa-miR-17	-0.12	0.92	0.41	0.682	No
hsa-miR-186	-3.30	0.10	9.71	<0.001	No
hsa-miR-210	-1.88	0.27	3.29	0.002	No
hsa-miR-222	7.12	139.14	-5.66	<0.001	Yes
hsa-miR-320a	-4.67	0.04	4.75	<0.001	Yes
hsa-miR-155	-0.08	0.94	0.43	0.669	No

Table 2: MicroRNA expression measured by RT-PCR.

Environment, Genetics, Epigenetics



IGF2 Imprinted Region



Paternal
(Favors *IGF2* expression)

Unmethylated

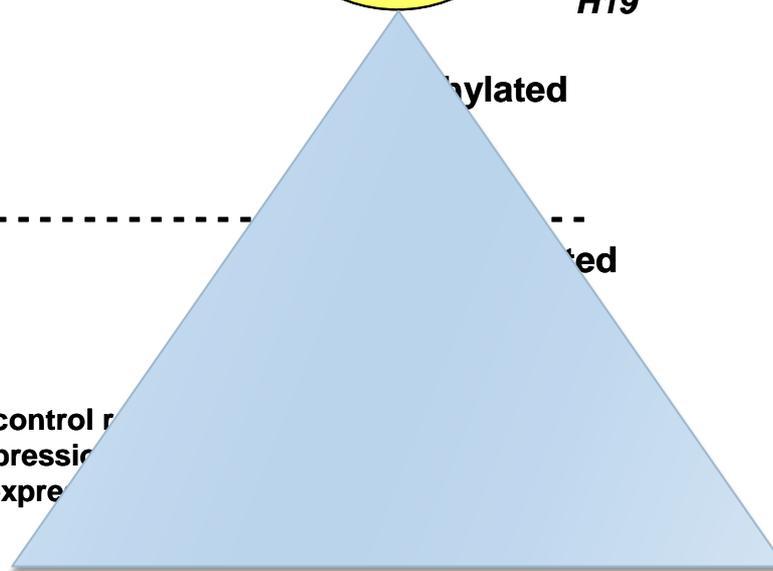
Methylated

Maternal
(Inhibits *IGF2* expression)

Methylated

Unmethylated

IGF2, Insulin-like growth factor 2; ICR, imprinting control region; *H19*, a non-coding RNA thought to inhibit *IGF2* expression; KCN, KCNQ10T1, an upstream regulator of *IGF2* expression



CpG sites analyzed

1 2 3 4

TGTGATGTGTGAGCCTGCACTGCCGCC[G/A]CGCGGCCAC
ACACTACACACTCGGACGTGACGGCGG[C/T]GCGCCGGTG

Sequencing Primer

SNP, RS10732516

Epigene X gene interactions and birthweight

Effect of ICR1 methylation on birth weight stratified by
ICR1 SNP rs10732516

	n	Birthweight difference (β) in grams (95% CI)	Interaction P value
Minor T allele (ht or hm)	77	-187 (-389, 15)	
Major C allele	136	65 (-61, 190)	0.04

Change in birth weight per an SD increase in DNA methylation

Challenges & Opportunities

- ▶ **How many epigenomes?**
 - ▶ Tissue specificity
 - ▶ Most studies in humans are on blood DNA
 - ▶ Need to investigate tissues relevant for the exposure-disease of interest (challenging in epidemiology)
 - ▶ The epigenome changes over time
 - ▶ Reverse causation is always a potential issue
 - ▶ Need for longitudinal studies
- ▶ **Current focus on DNA methylation**
 - ▶ Opportunities for investigating other mechanisms
- ▶ **Can we keep our epigenome healthy?**
 - ▶ Opportunities for research leading to primordial, primary, secondary interventions

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