



Persistent Epigenetic Effects of Dietary Imbalance

Rob Waterland, PhD



Pediatrics

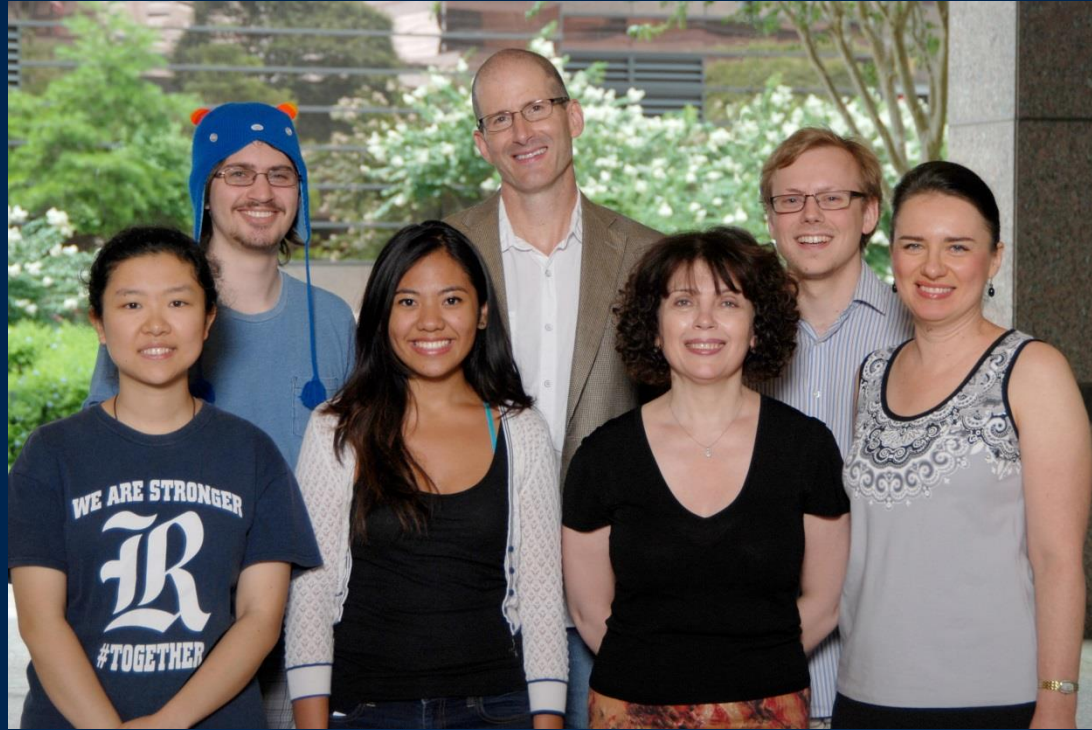
Acknowledgements

Waterland Lab

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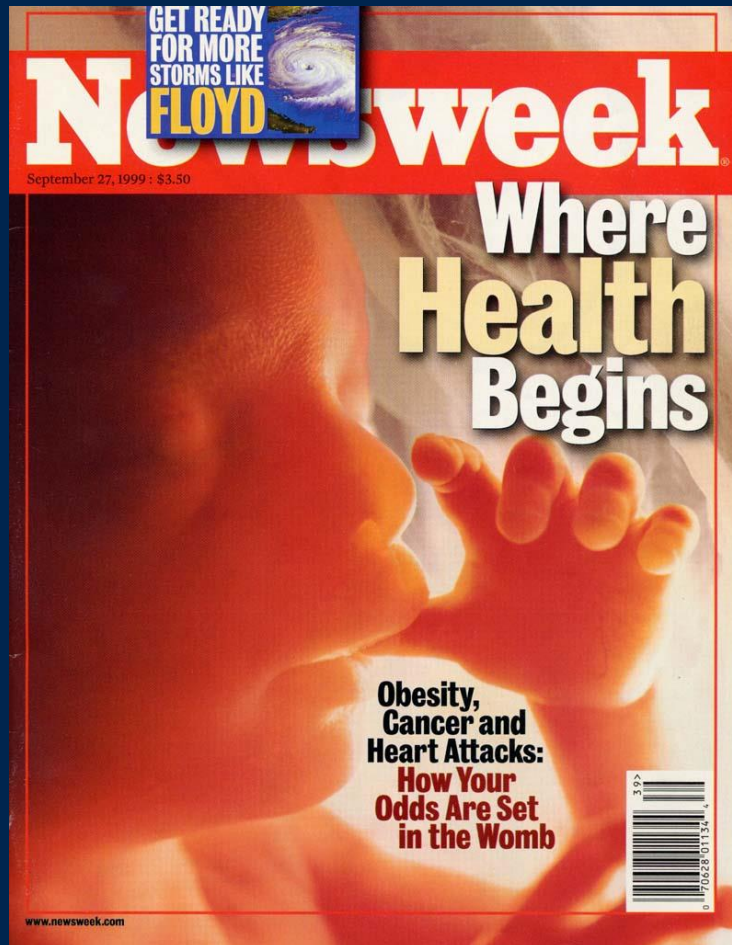
Collaborators

Lanlan Shen, MD, PhD
Yongtao (Grant) Guan, PhD
Cristian Coarfa, PhD
Rui Chen, PhD
Andrew Prentice, PhD
Matt Silver, PhD
Branwen Hennig, PhD
Sophie Moore, PhD
YongSun Lee, PhD

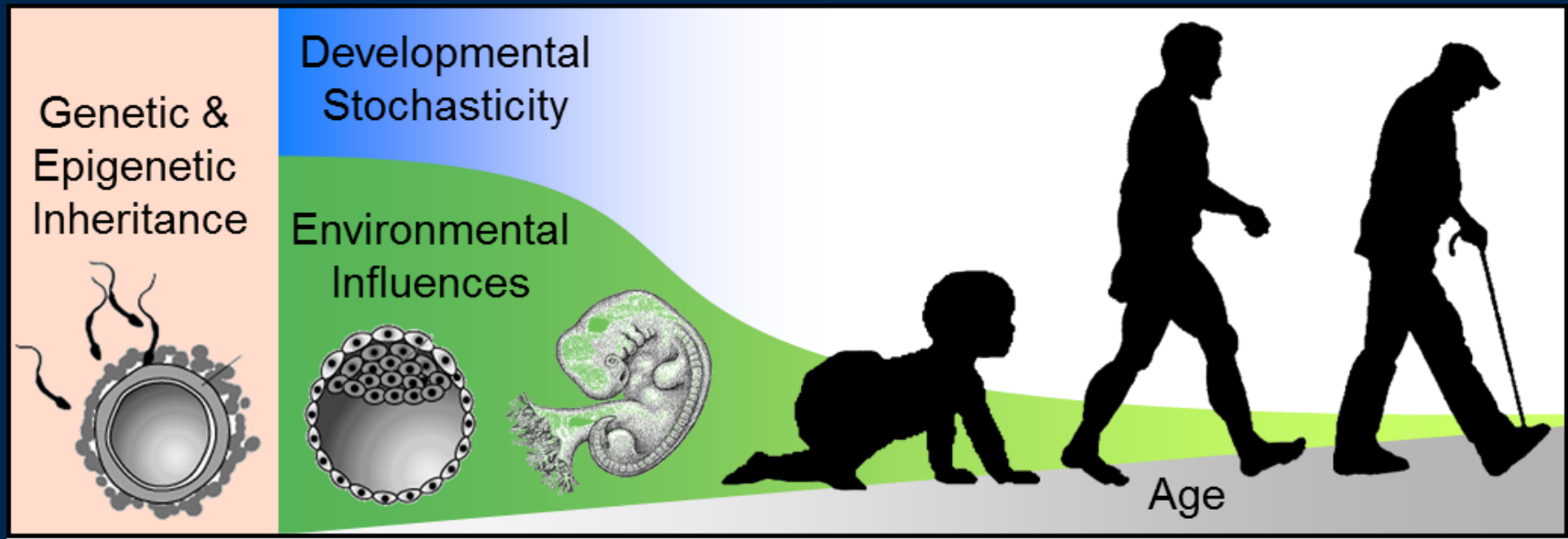


Funding

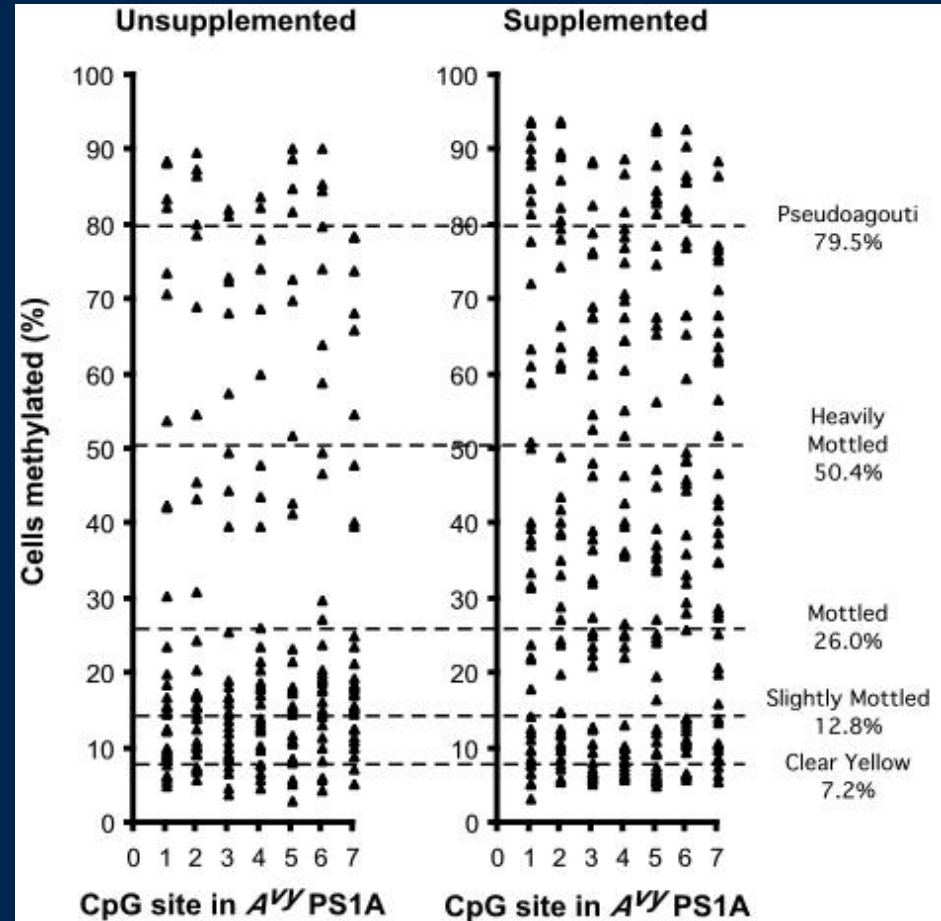
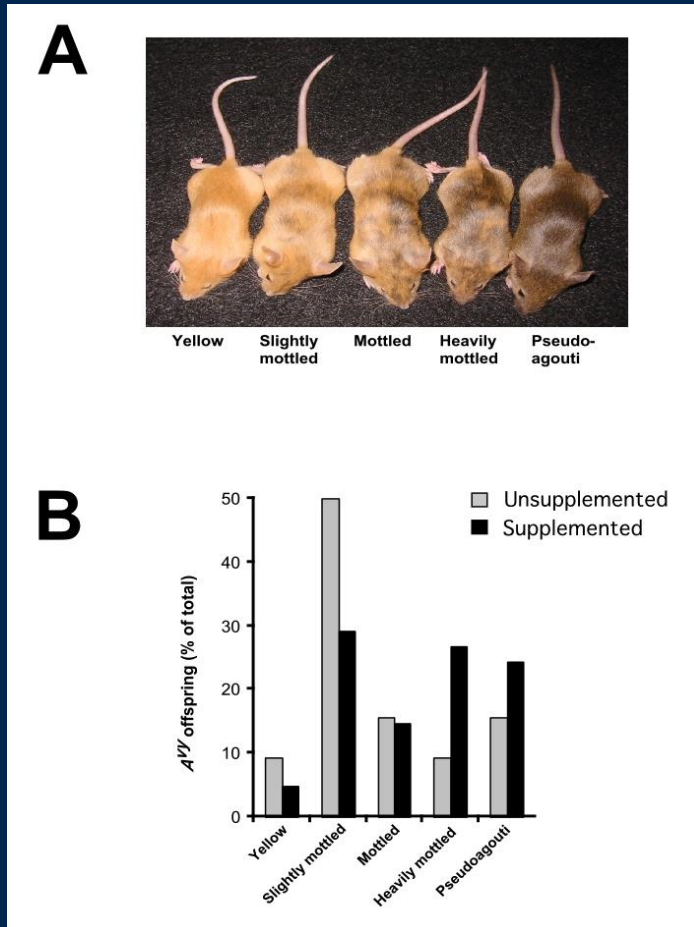
USDA, NIH-NIDDK, UK MRC



Sources of Interindividual Epigenetic Variation



Methyl Supplementation Changes Coat Color by Increasing A^y Methylation



Wolff et al 1998 *FASEB J*

Waterland & Jirtle 2003 *Mol Cell Biol*



Significance of A^{vy} Study

- Demonstrated causal pathway:
maternal nutrition – epigenetic change – phenotypic change
- Highlighted the potential involvement of metastable epialleles

Metastable Epiallele (ME)

“An allele at which the epigenetic state can switch and establishment is a probabilistic event. Once established, the state is mitotically inherited.”

Rakyan et al *Trends in Genetics* 2002

Agouti viable yellow



Axin fused



At MEs, Maternal Environment Affects Establishment of Epigenotype

- Maternal methyl supplementation increases DNA methylation
 - At A^{vy} (Waterland and Jirtle 2003 *Mol Cell Biol*)
 - At $Axin^{Fu}$ (Waterland et al 2006 *Genesis*)
- Maternal bisphenol A exposure decreases DNA methylation at $Cabp^{IAP}$
 - Dolinoy et al. 2007 *PNAS*

Does epigenetic variation at MEs contribute to human phenotypic variation?

Characteristics of Murine Metastable Epialleles

- Interindividual variation in DNA methylation that is
 - Systemic (not tissue-specific)
 - Stochastic (not due to genetic variation)
- Establishment of DNA methylation affected by maternal nutrition before/during pregnancy

Seasonal Variation in Rural Gambia



Seasonal Variation in Rural Gambia



Seasonal Variation in Rural Gambia



Rainy Season - Hungry



Dry Season - Harvest



Season of Conception in Rural Gambia Affects DNA Methylation at Putative Human Metastable Epialleles

Robert A. Waterland^{1,2*}, Richard Kellermayer¹, Eleonora Laritsky¹, Pura Rayco-Solon³, R. Alan Harris², Michael Travisano⁴, Wenjuan Zhang¹, Maria S. Torskaya¹, Jiexin Zhang⁵, Lanlan Shen¹, Mark J. Manary¹, Andrew M. Prentice³



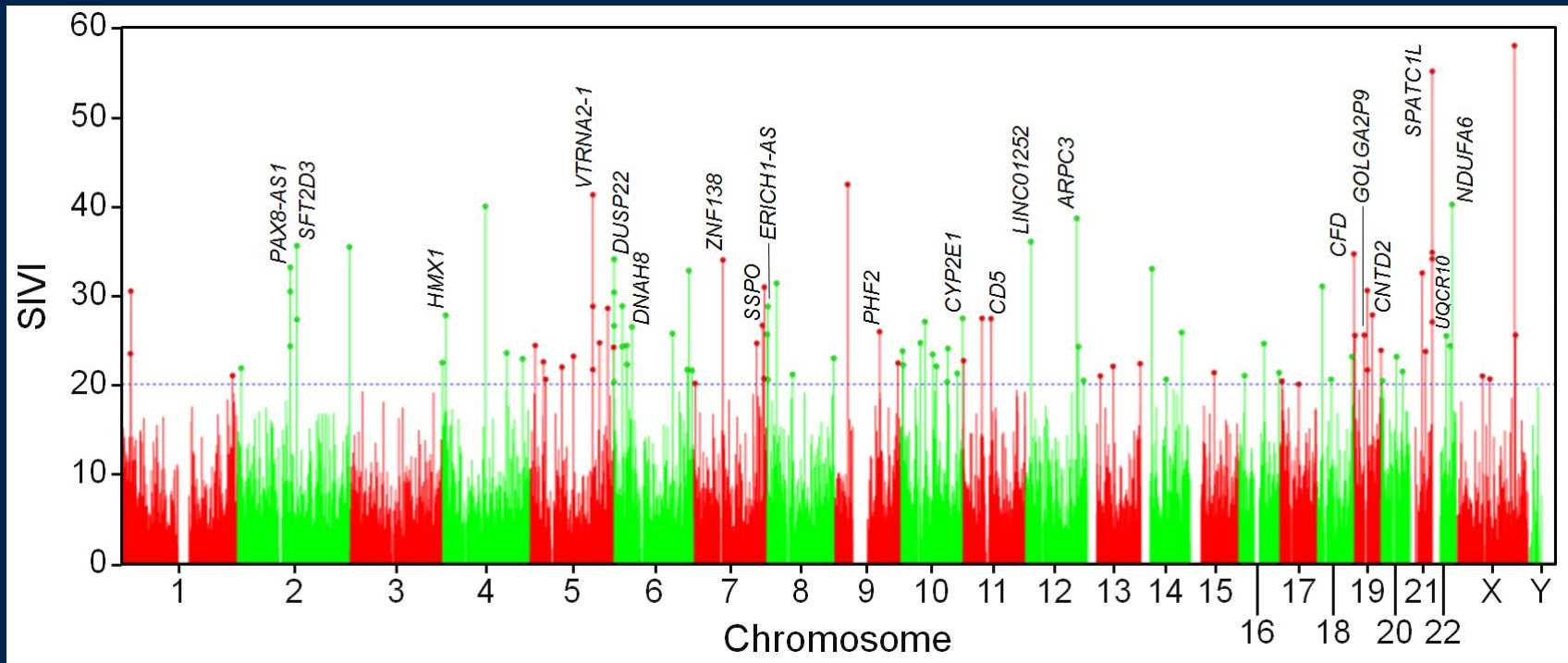
Maternal nutrition at conception modulates DNA methylation of human metastable epialleles

Paula Dominguez-Salas¹, Sophie E. Moore¹, Maria S. Baker², Andrew W. Bergen³, Sharon E. Cox¹, Roger A. Dyer⁴, Anthony J. Fulford¹, Yongtao Guan^{2,5}, Eleonora Laritsky², Matt J. Silver¹, Gary E. Swan⁶, Steven H. Zeisel⁷, Sheila M. Innis⁴, Robert A. Waterland^{2,5}, Andrew M. Prentice¹ & Branwen J. Hennig¹

Genome-Wide Screen for Human MEs

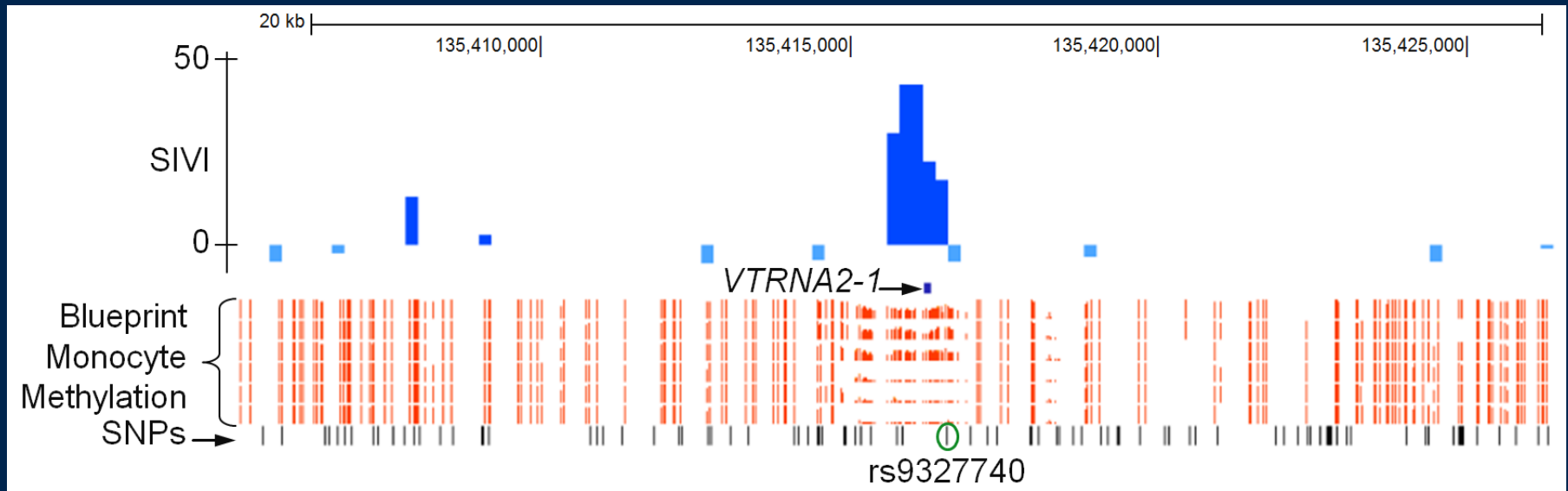
- Studied peripheral blood lymphocytes (PBL) and hair follicle (HF) from each of two Caucasian males
- Genome-wide bisulfite sequencing (Bisulfite-seq)
 - 26x average coverage per sample
 - Estimated % methylation in 6.2 million 200bp bins containing at least 2 CpG sites (85% of all CpGs)

109 Top Candidate MEs Show No Evidence of Genetic Variation, and High CpG Density



Silver & Kessler, et al *Genome Biol* 2015

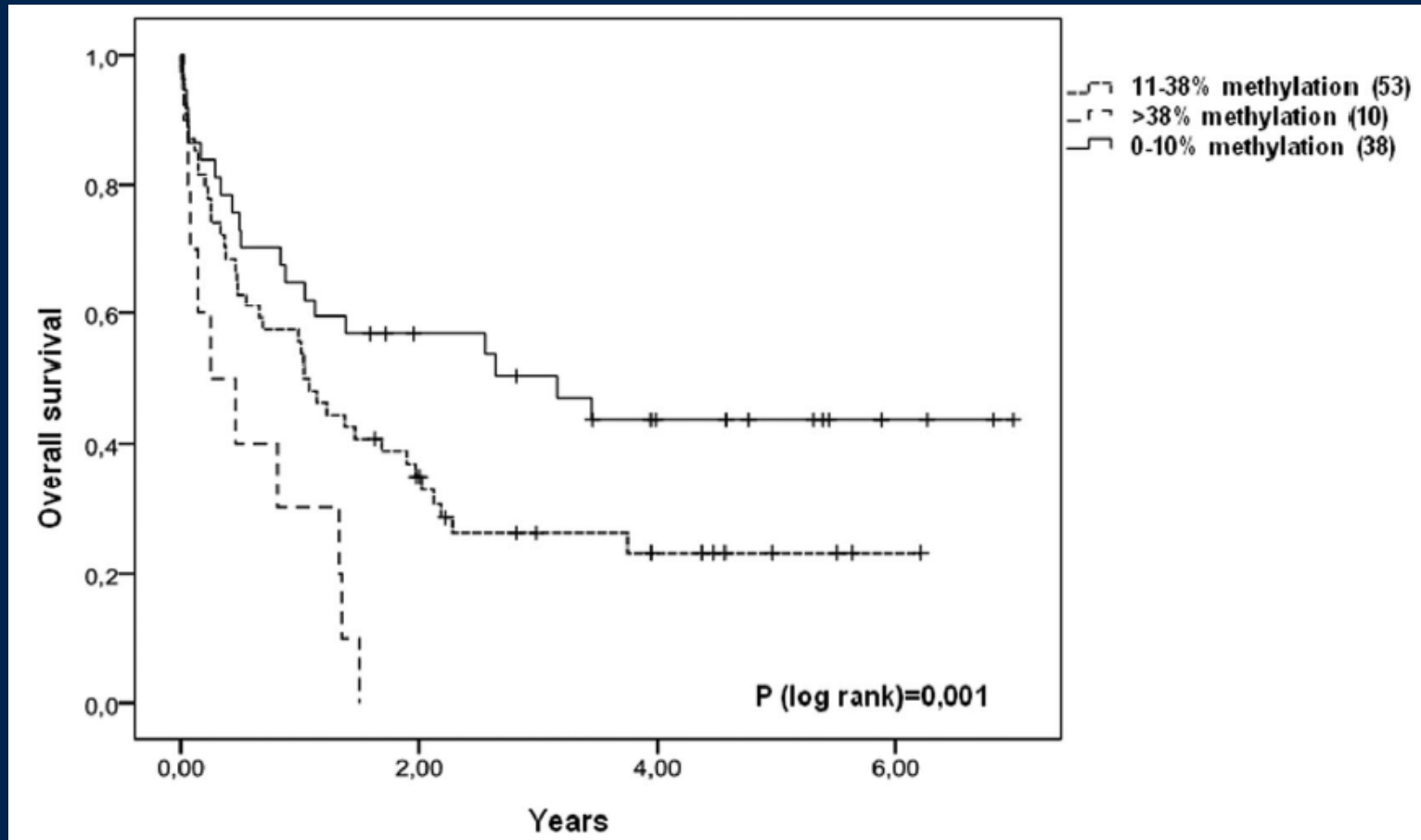
We looked for genes associated with multiple ME bins. *VTRNA2-1* was a top hit.



What is known about *VTRNA2-1* ? (aka *pre-miR-886*, aka *nc886*)

- 102-nucleotide, ubiquitously expressed, cytoplasmic RNA
- Neither a vault RNA nor a canonical pre-microRNA
- Genomically imprinted
- Inhibits cell proliferation by inhibiting phosphorylation of protein-kinase – RNA inducible (PKR)

Methylation of *VTRNA2-1* Predicts Survival in Acute Myeloid Leukemia Patients



Treppendahl et al 2012 *Blood*

Validation of *VTRNA2-1* Metastable Epiallele

- Systemic interindividual variation confirmed in post-mortem liver, kidney, brain
- No effects of genetic variation on DNA methylation at *VTRNA2-1* (Zhang et al 2014 *Hum Mol Genet*)
- Effects of periconceptional nutrition confirmed in rural Gambians

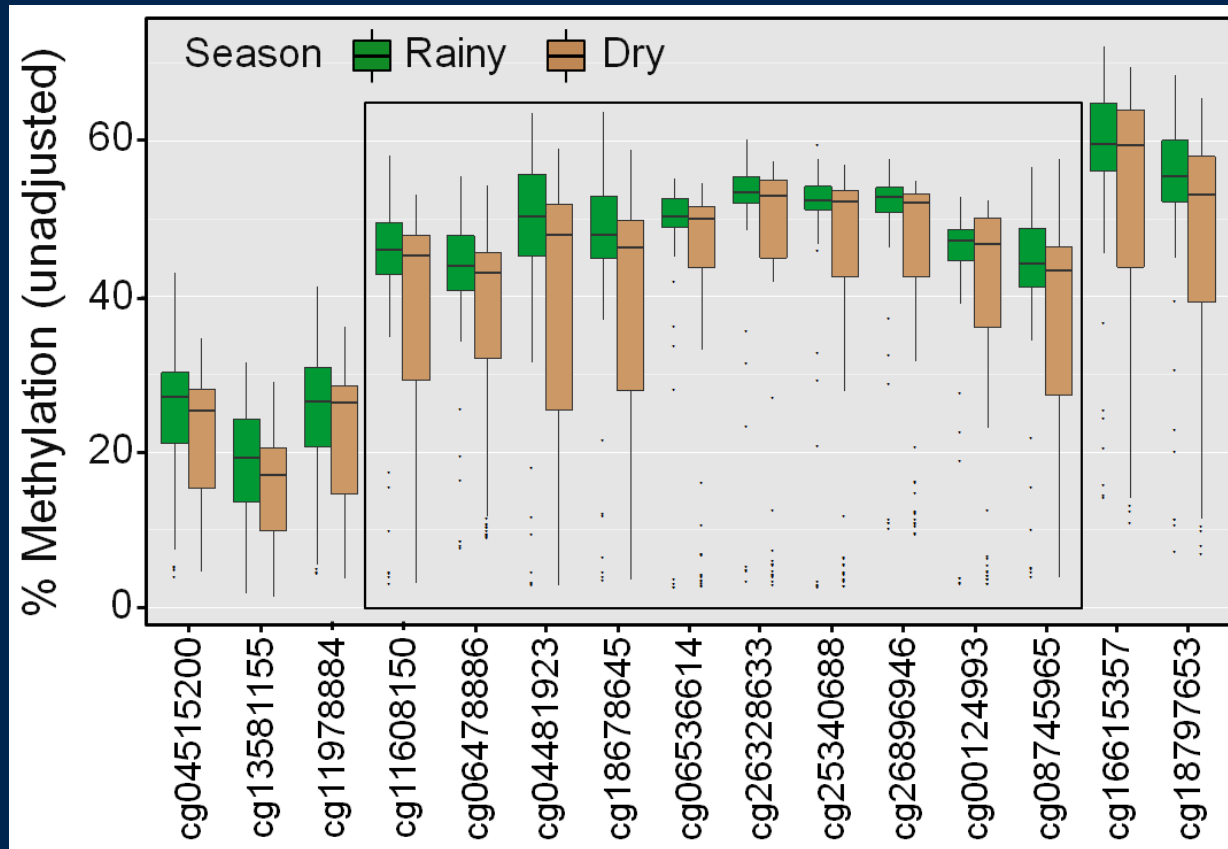
In an Independent Genome-Scale Screen (450k array) for Differentially Methylated Regions (DMRs) According to Season of Conception, *VTRNA2-1* was THE Top Hit!

chr	SoC-DMR (bp)	start	SoC-DMR (bp)	end	mean coeff	probes in SoC-DMR	probes in cluster	p-value	p-value (fwer)	gene
5	135,415,762		135,416,613		0.61	15	15	2.0E-05	0.01	VTRNA2-1
2	113,992,762		113,993,313		0.47	8	8	5.5E-04	0.23	PAX8*
5	23,507,030		23,507,752		0.36	12	13	8.7E-04	0.35	PRDM9
6	32,729,442		32,729,847		0.14	20	36	1.6E-03	0.58	HLA-DQB2
17	17,109,570		17,110,120		0.38	8	11	1.8E-03	0.58	PLD6
6	29,648,345		29,649,024		0.27	14	18	1.8E-03	0.60	ZFP57*
6	151,646,312		151,647,133		0.30	9	9	2.8E-03	0.73	AKAP12
12	57,040,045		57,040,204		0.36	4	9	3.2E-03	0.78	ATP5B*
5	191,242		192,103		0.26	10	11	3.8E-03	0.85	LRRC14B
13	36,944,640		36,944,649		0.36	2	8	4.4E-03	0.86	SPG20

PBL DNA from 120 children studied by Illumina 450k arrays

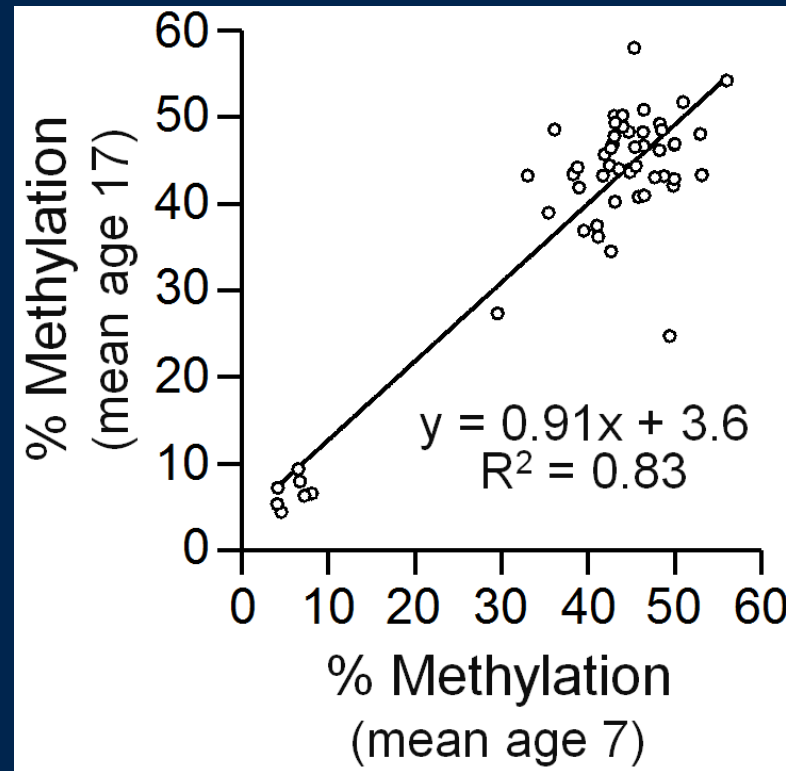
Silver & Kessler, et al *Genome Biol* 2015

450k Data – Season of Conception Effect Spans the Entire *VTRNA2-1* Imprinted DMR



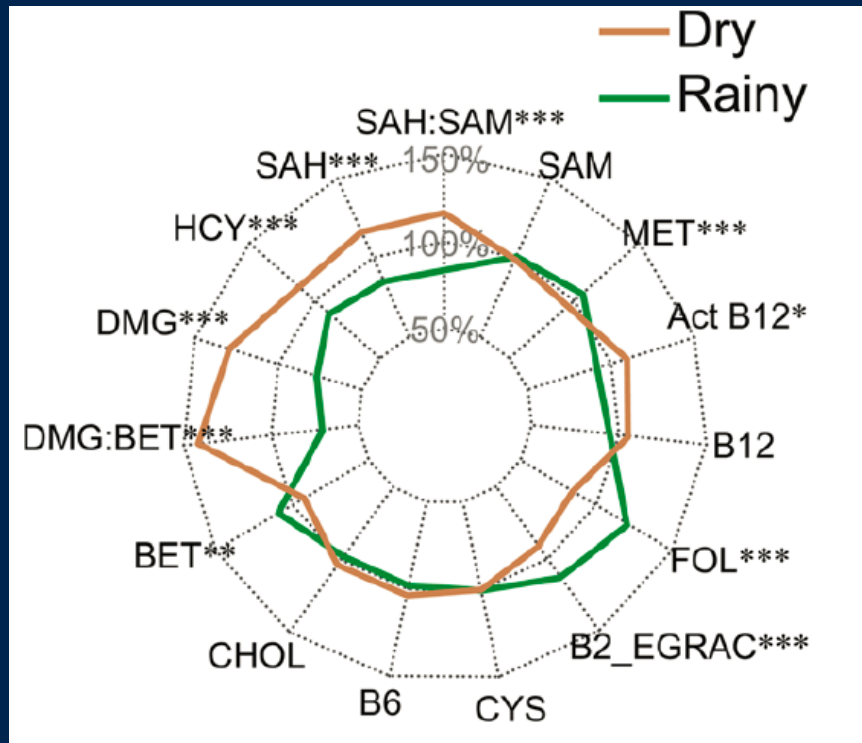
PBL DNA from 120 children studied by Illumina 450k arrays

Individual Methylation at *VTRNA2-1* is Highly Stable



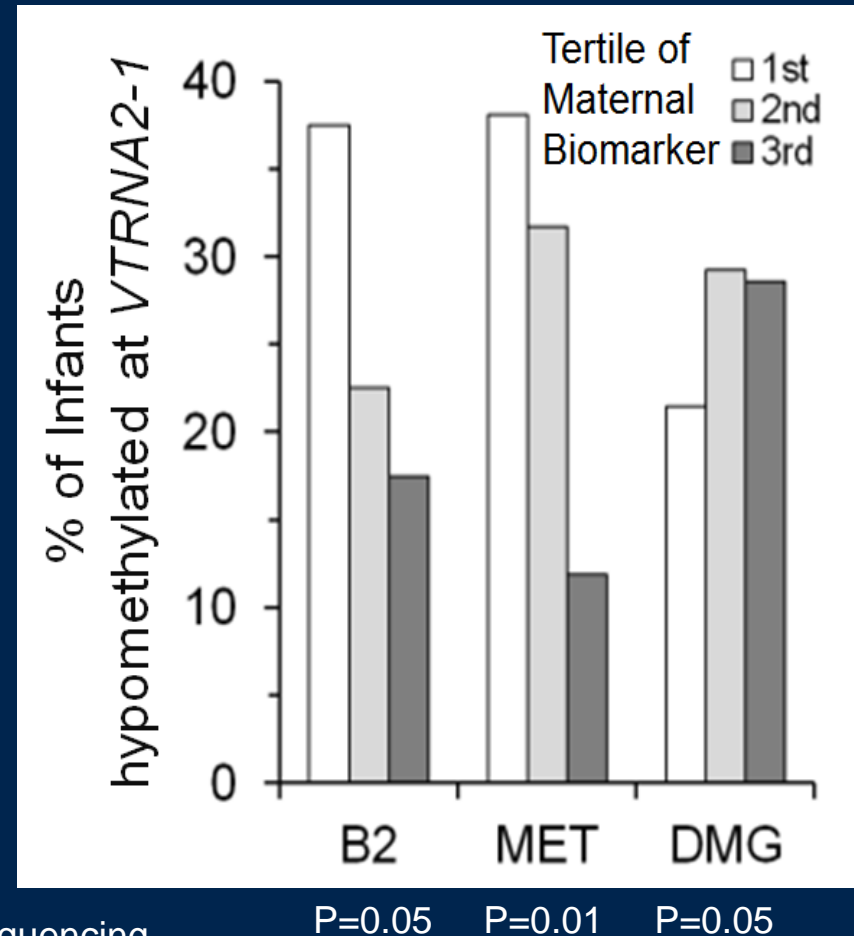
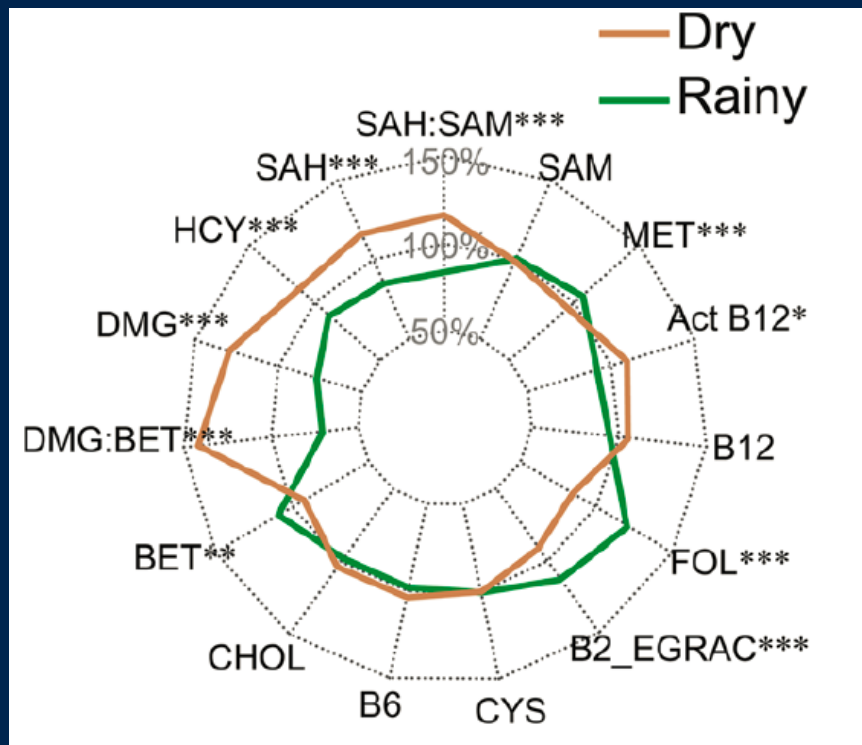
VTRNA2-1 methylation in PBL by pyrosequencing; n=55 Gambian children.

Maternal Periconceptional Nutrition Predicts *VTRNA2-1* Hypomethylation in Her Infant



Silver & Kessler, et al *Genome Biol* 2015

Maternal Periconceptional Nutrition Predicts *VTRNA2-1* Hypomethylation in Her Infant



Hypomethylation defined as <40% methylation by pyrosequencing.
 Complete data on ~120 infants.

Silver & Kessler, et al *Genome Biol* 2015

Sources of Interindividual Epigenetic Variation

