ClinGen Course, Weill Cornell Medicine
March 2021

Thinking beyond the creode: epigenomics and human disease

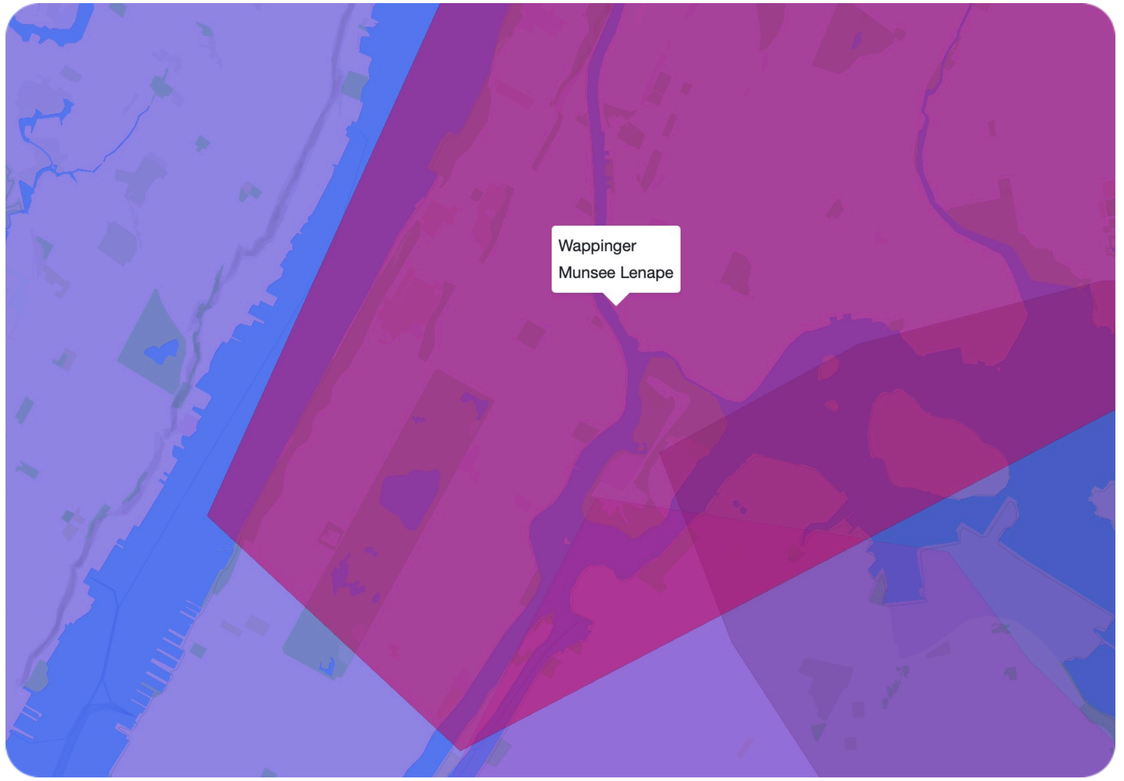
center for epigenomics | Einstein

Montefiore
THE UNIVERSITY HOSPITAL

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

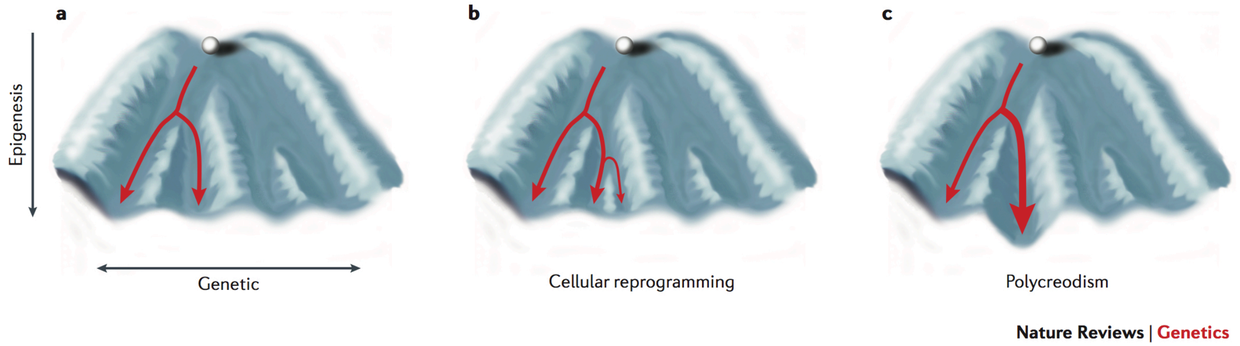
Land acknowledgement

From https://native-land.ca

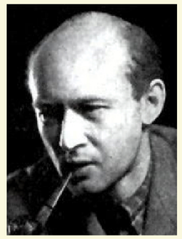
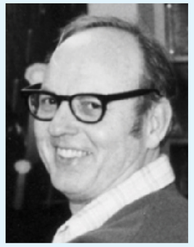
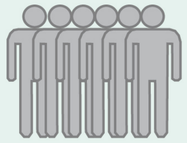
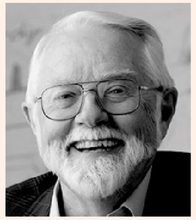


I acknowledge being on Native Land today
I acknowledge the **Munsee Lenape and Wappinger Nations**, whose lands our institutions occupy
I acknowledge that I am part of the colonisation of this land
This land was taken from the Native communities without their consent or compensation
Genocide of Native peoples is an inescapable part of this history of colonisation
I offer respect and recognition to the Native communities, to start a process of reconciliation

‘Epigenetic’

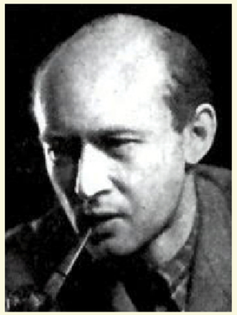

a
b
c
Epigenesis
Genetic
Cellular reprogramming
Polycreodism
Nature Reviews | Genetics

Epigenetics: five current definitions

		Epigenetics meaning:	Why this is a problem:
	Conrad Hal Waddington	The **epigenetic landscape** was proposed to resolve how epigenesis could have genetic influences.	All Waddington was trying to do was to stop the embryologists and geneticists from fighting with each other
	David Nanney	A non-nuclear heritability of long-term cellular memory	How he was persuaded, while travel-weary, not to use his favoured word ‘paragenetic’
 	John Pugh Robin Holliday	A mutational mechanism involving changes in DNA methylation, not DNA sequence. Later extended to mean gene regulation.	How John Pugh came up with the word epigenetic to describe non-genetic mutation when trying to write an abstract with a 200 word limit
	Everyone	Back-translated epi- (above, upon) -genetics (DNA sequence) to mean any biochemical process regulating the genome	Epitranscriptomics
	Arthur Riggs	Attempted to rein in the use of epi- (above) -genetics (DNA sequence) by requiring molecular process to be heritable through cell division	By including both mitotic and meiotic cell division, he generated the multigenerational definition of epigenetics

Version available on FigShare
DOI 10.6084/m9.figshare.9975359.v1

Epigenetics: the original definition



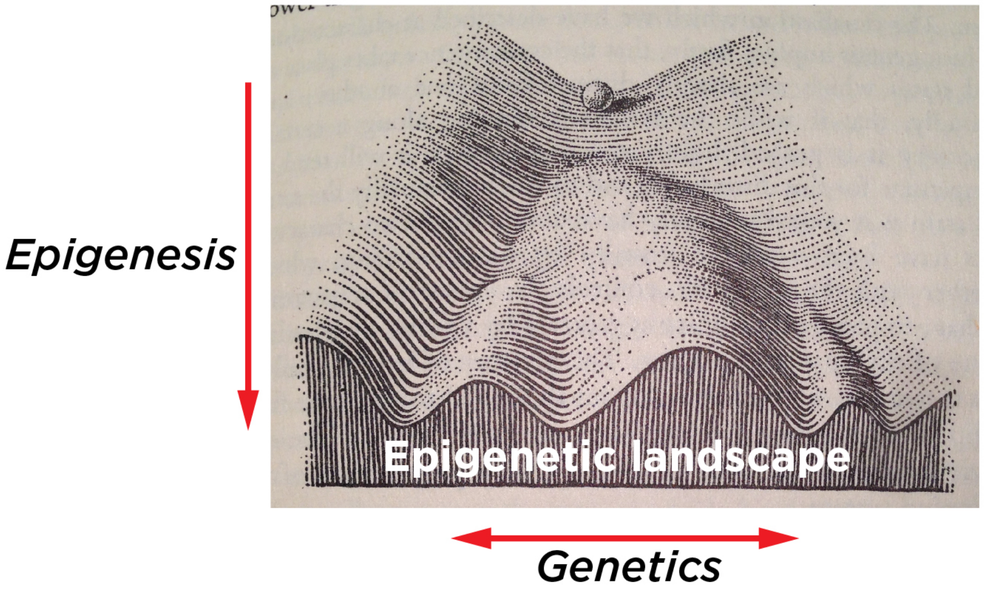
Conrad Hal Waddington

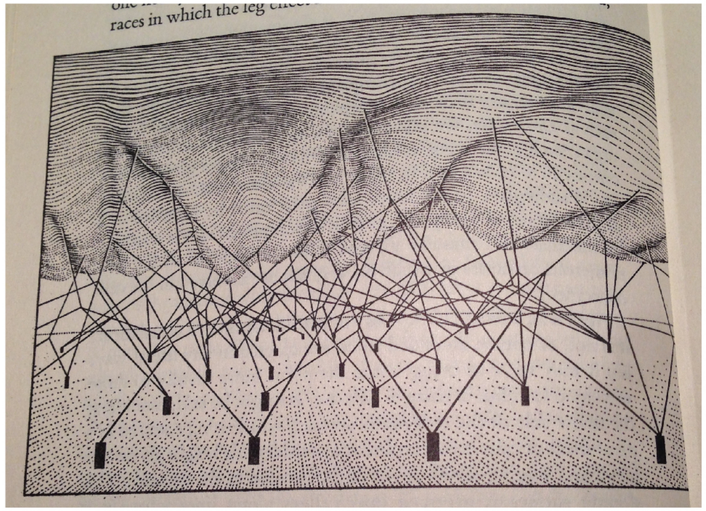
Epigenetics meaning:

The **epigenetic landscape** was proposed to resolve how epigenesis could have genetic influences.

Why this is a problem:

All Waddington was trying to do was to stop the embryologists and geneticists from fighting with each other



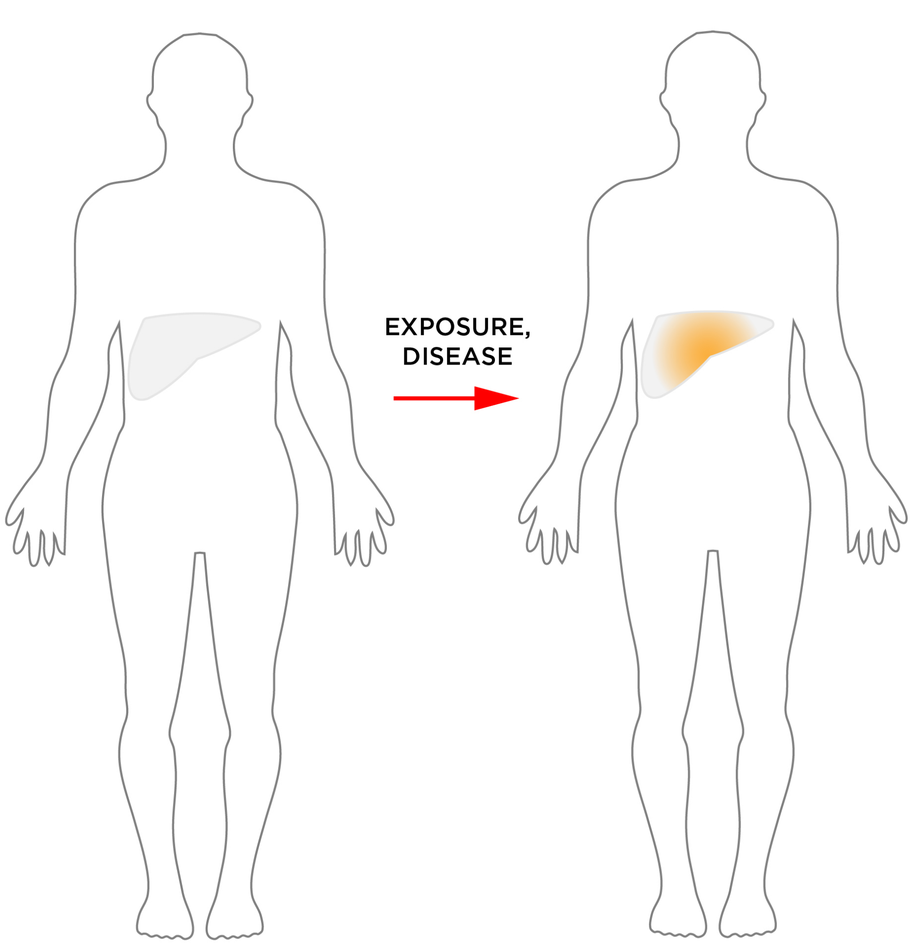


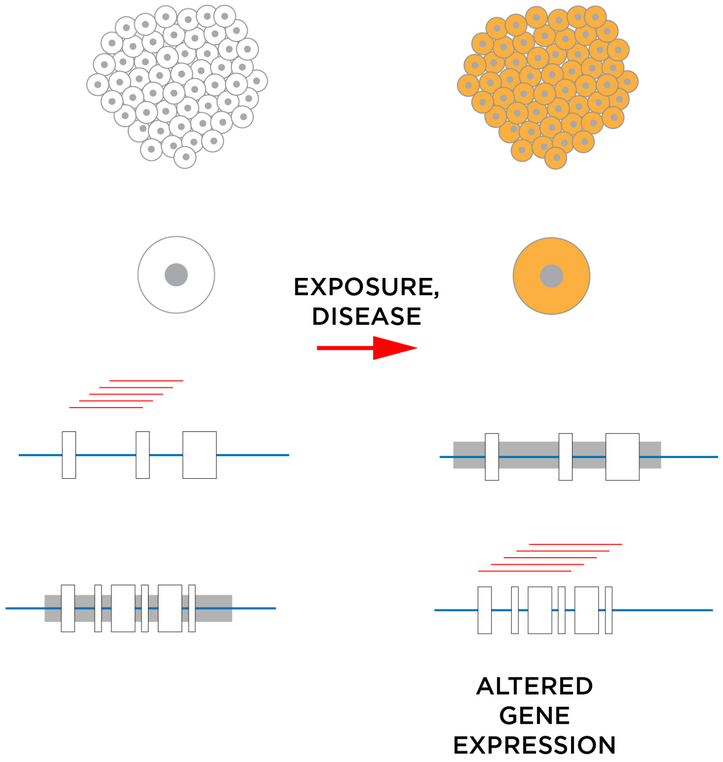
Waddington, C. H. (1957). The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology London: Ruskin House/George Allen and Unwin Ltd.

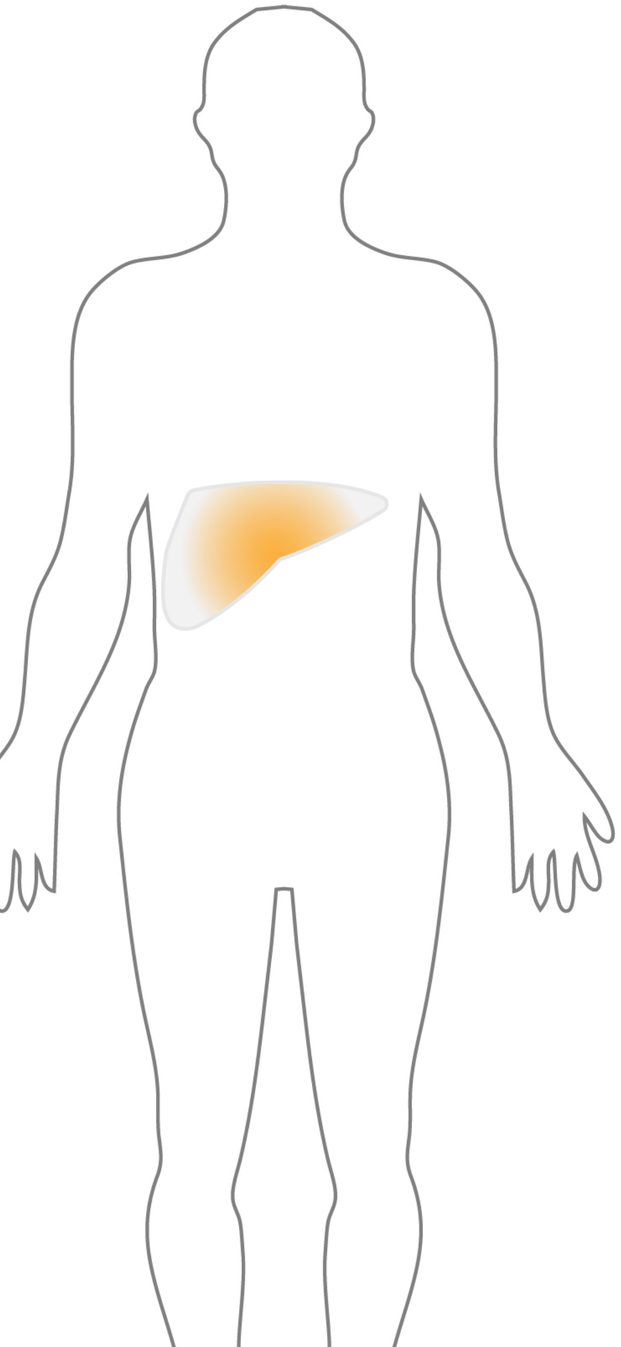
Epigenetics and human disease

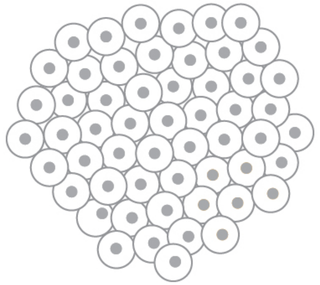
In 2021, ’epigenetic’ = non-genetic

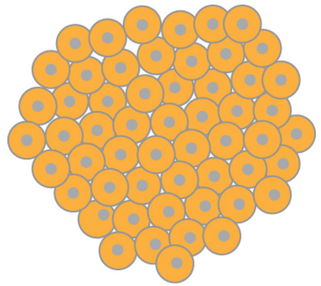
Assumption: non-mutated genes change expression to cause phenotype



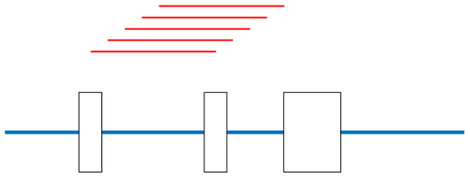


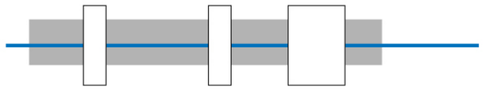


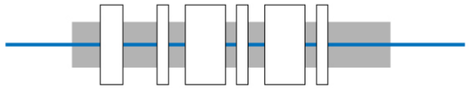


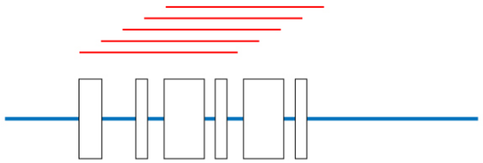


EXPOSURE,
DISEASE







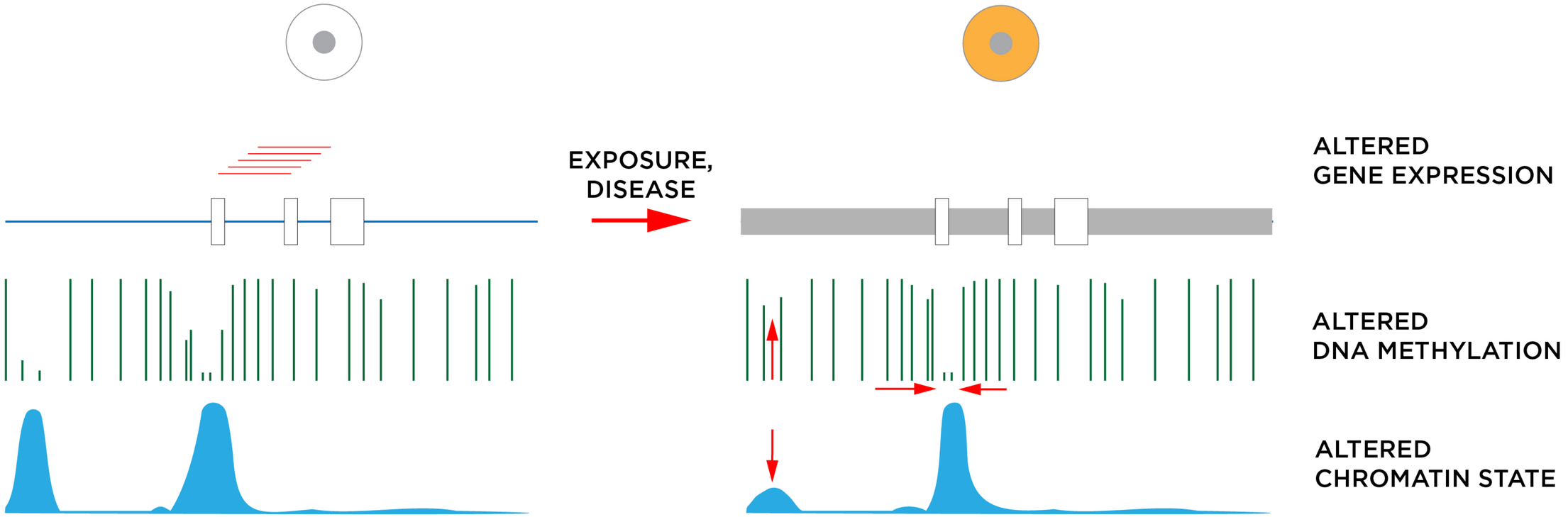


ALTERED
GENE
EXPRESSION

Epigenetics and human disease

Non-genetic but mediated by the genome

Assumption: phenotype mediated by ‘epigenetic’ transcriptional regulation

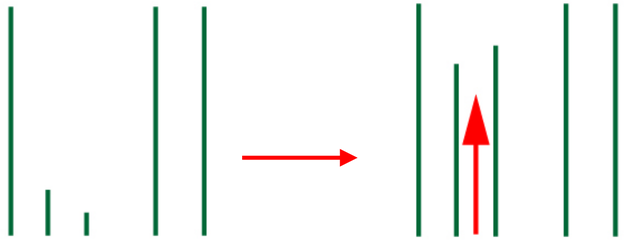


Epigenetics and human disease

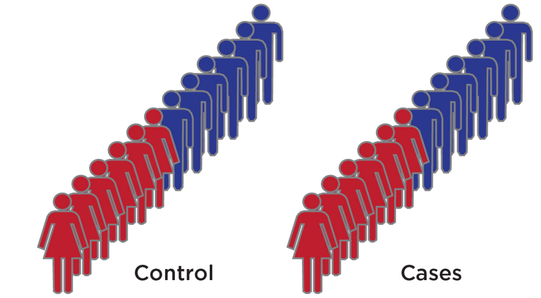
Testing epigenetic mechanisms in human diseases

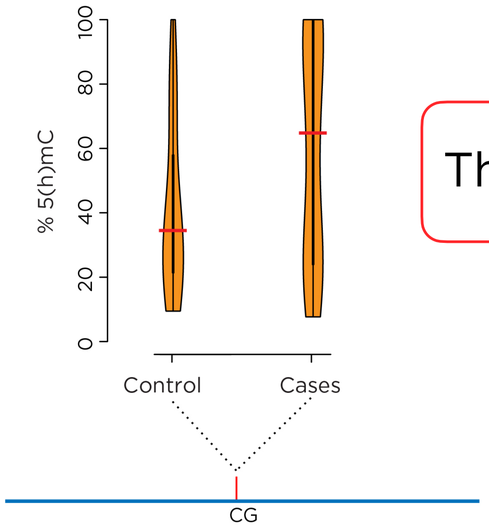
Assumption:

A positive epigenome-wide association study result is evidence supporting ‘epigenetic’ mechanisms



A significant change in DNA methylation





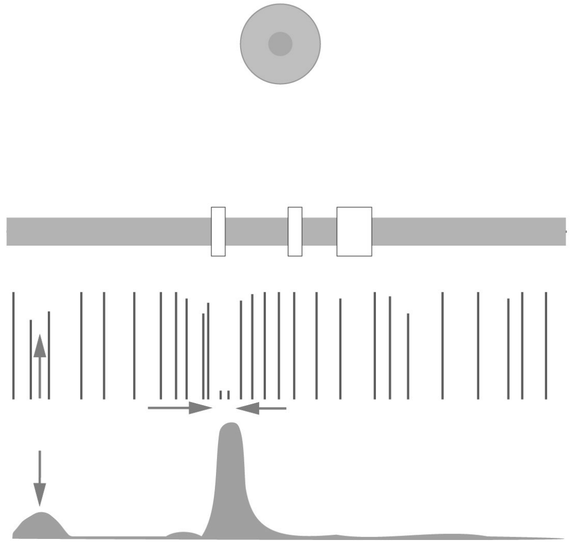
This finding is uninterpretable

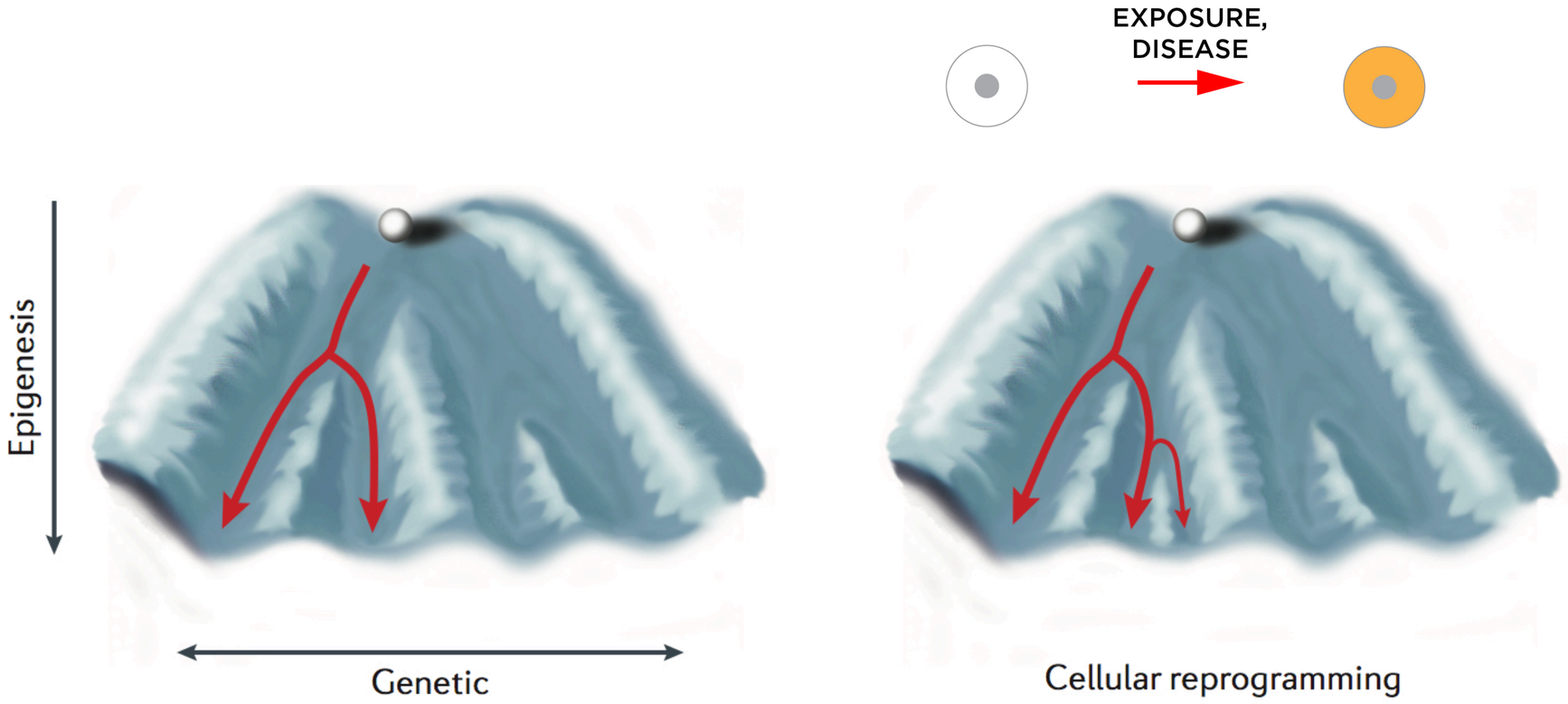
Epigenetics and human disease

The presumed cellular model

It is assumed that cells undergo **reprogramming**

A canonical cell type acquires new molecular properties, independent of DNA sequence





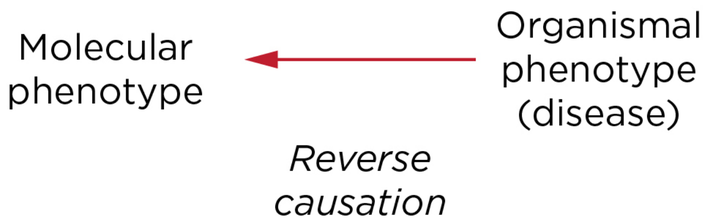
Lappalainen T, Greally JM.
Associating cellular epigenetic models with human phenotypes. Nat Rev Genet. 2017 Jul;18(7):441-451.

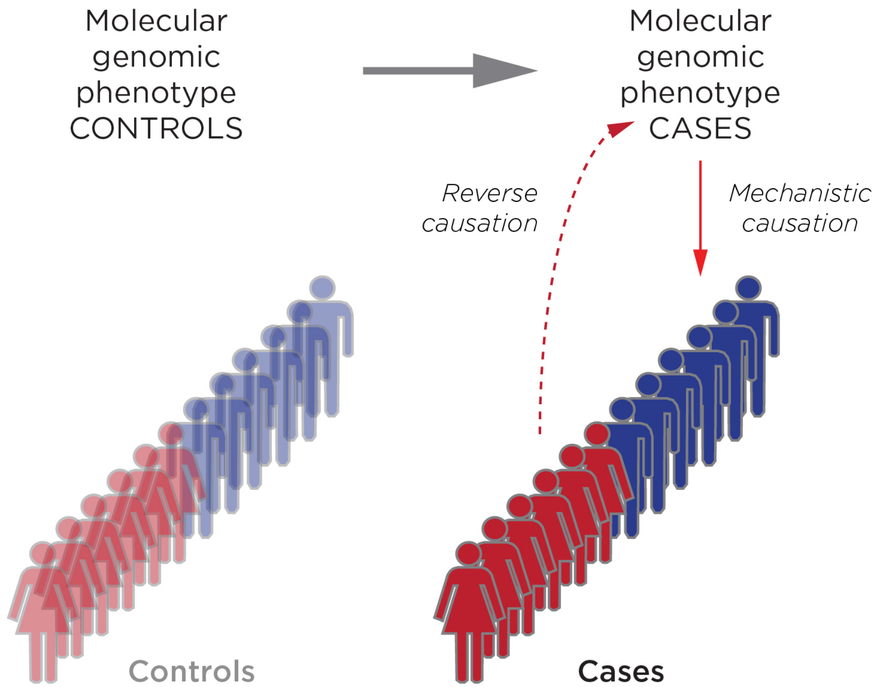
Epigenetics and human disease

Confounding effect

Reverse causation

The disease phenotype may cause the molecular phenotype





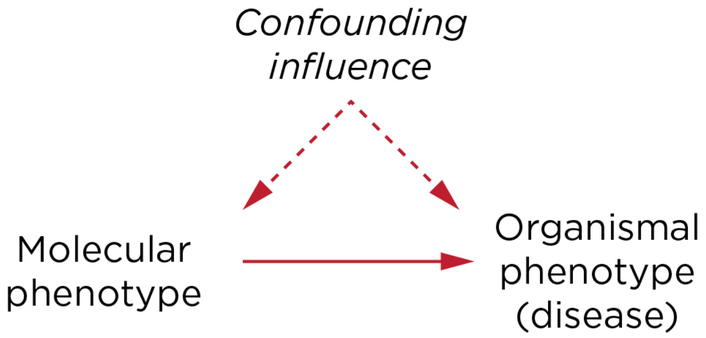
Birney E, Smith GD, Greally JM. Epigenome-wide Association Studies and the Interpretation of Disease -Omics. PLOS Genet. 2016 Jun 23;12(6):e1006105.

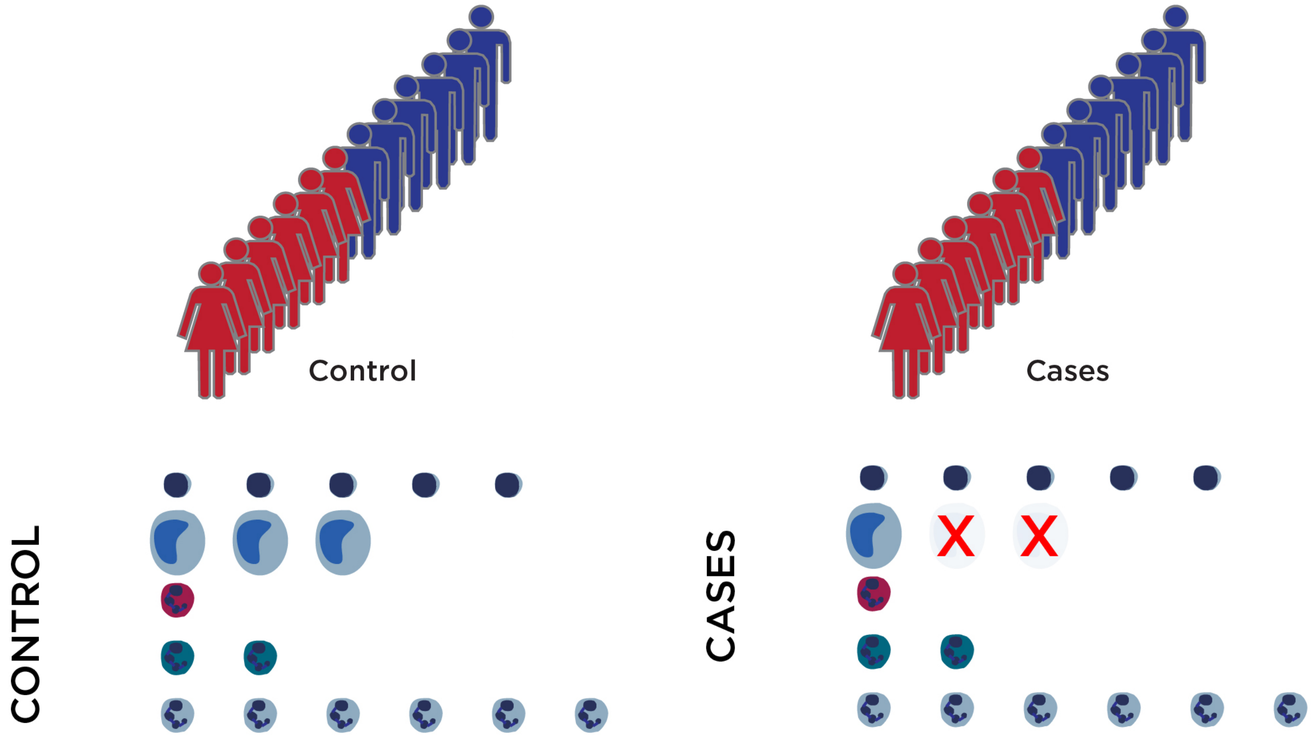
Epigenetics and human disease

Confounding effect

Cell subtype proportion changes

The transcription/regulatory changes may be due to altered cell subtype proportions in the tissue studied





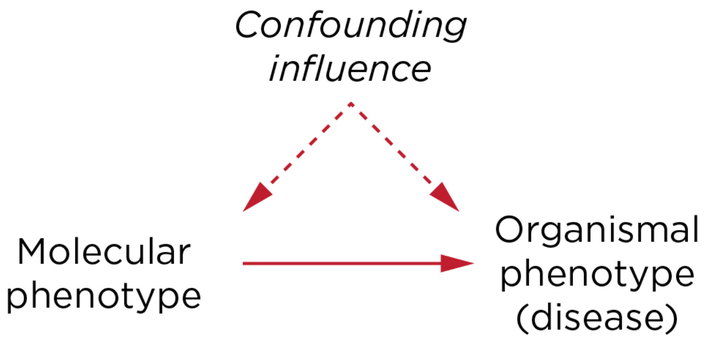
Epigenetics and human disease

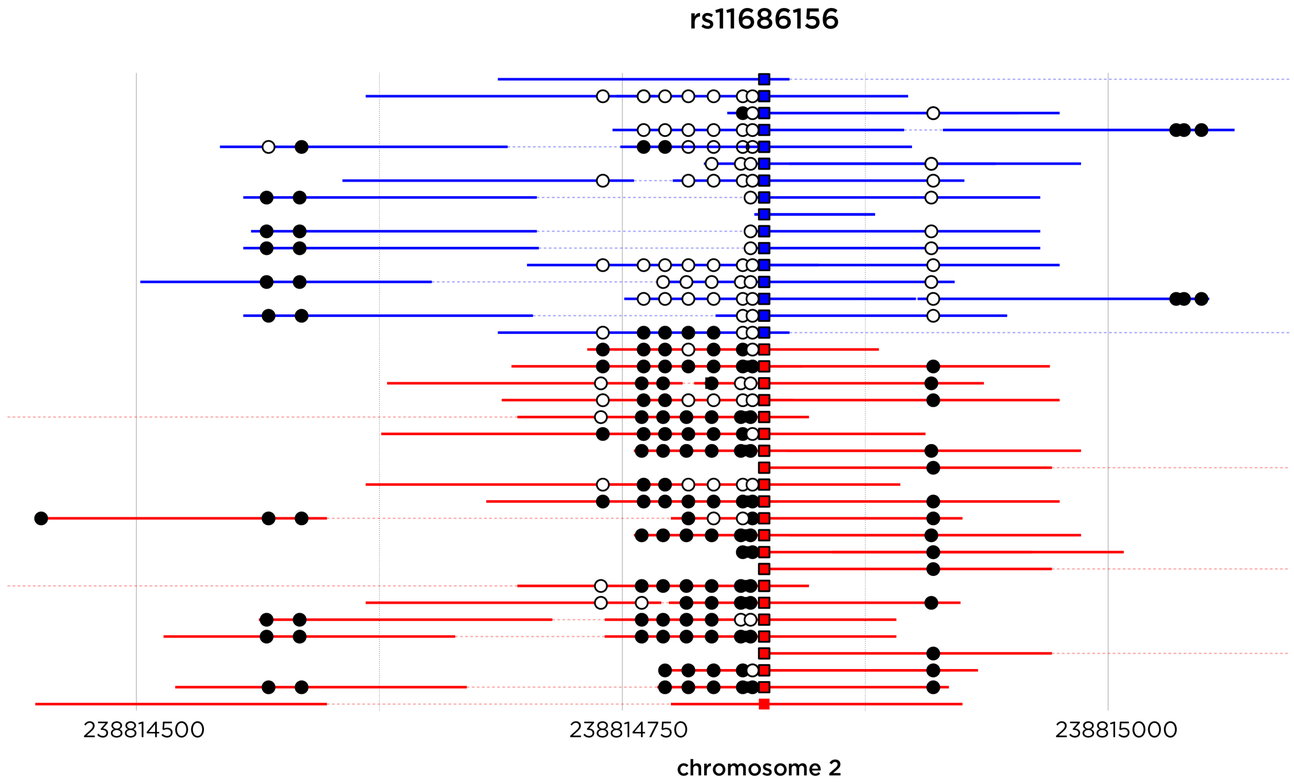
Confounding effect

DNA sequence variability

DNA sequence can alter molecular genomic patterns

Functional variants, methylation quantitative trait loci (meQTLs)





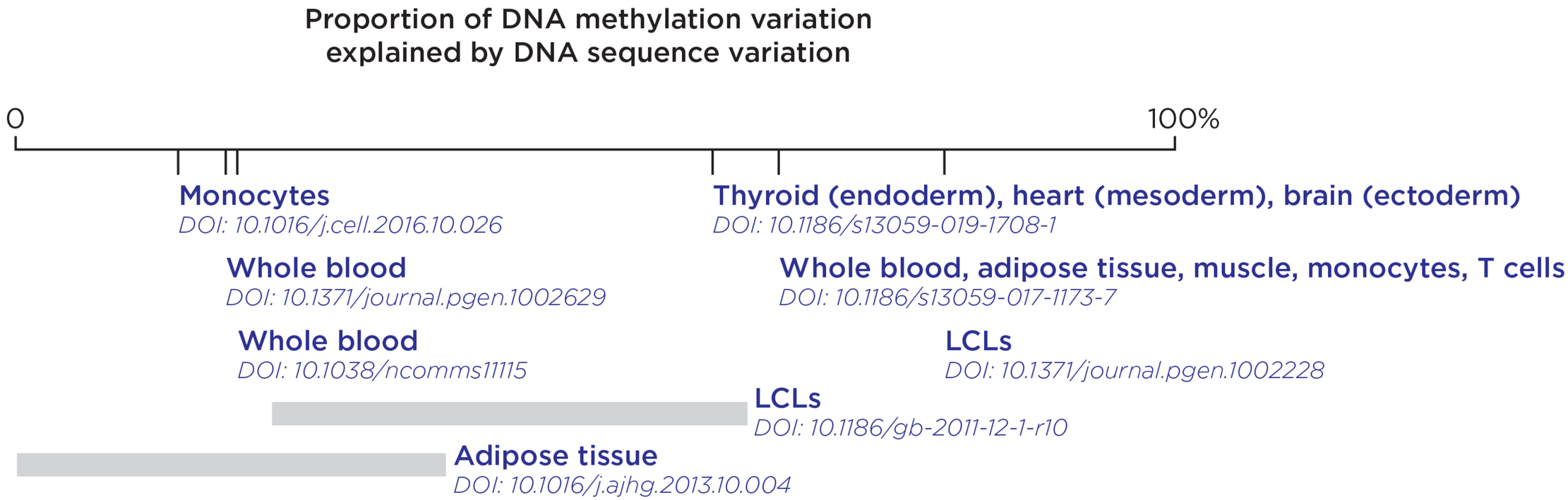
Suzuki M, Liao W, Wos F, Johnston AD, DeGrazia J, Ishii J, Bloom T, Zody MC, Germer S, Greally JM. Whole-genome bisulfite sequencing with improved accuracy and cost. Genome Res. 2018 Sep;28(9):1364-1371

Epigenetics and human disease

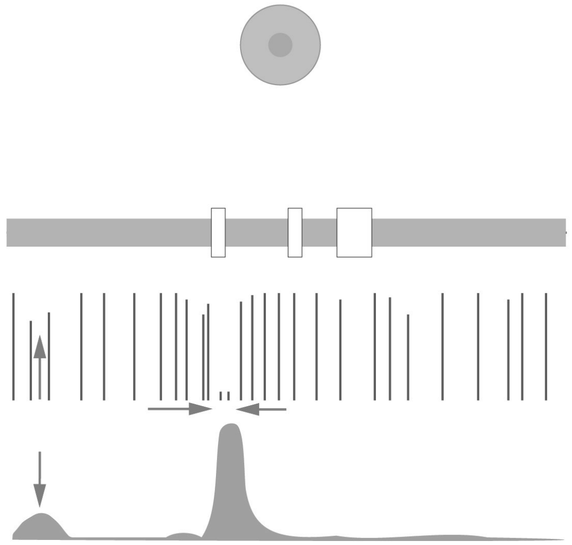
Confounding effect

DNA sequence variability

~14-80% of DNA methylation variation due to DNA sequence variation



Epigenetics and human disease

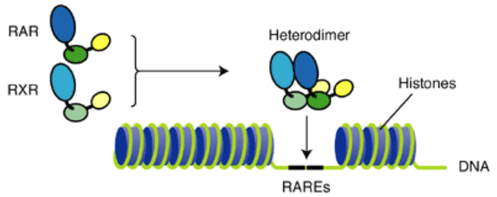


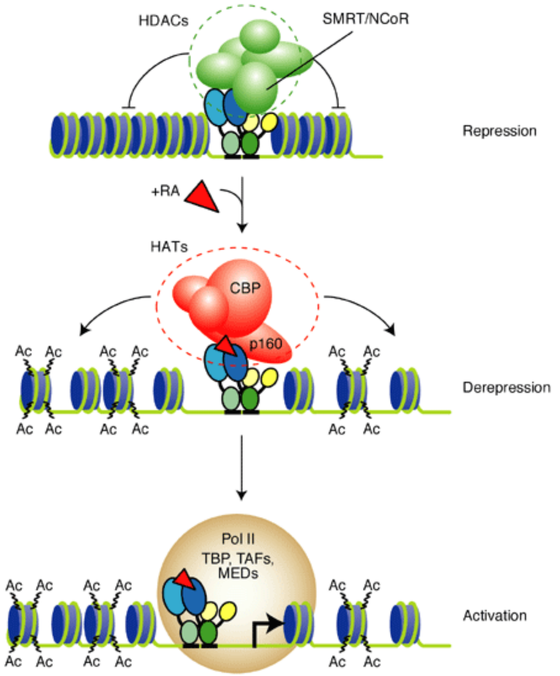
Are we studying transcription factor biology?

The transcriptional regulators we call epigenetic have no ability to target specific sequences

- DNA modifications
- Chromatin remodellers
- Histone modification enzymes

They are targeted to loci by **transcription factors**



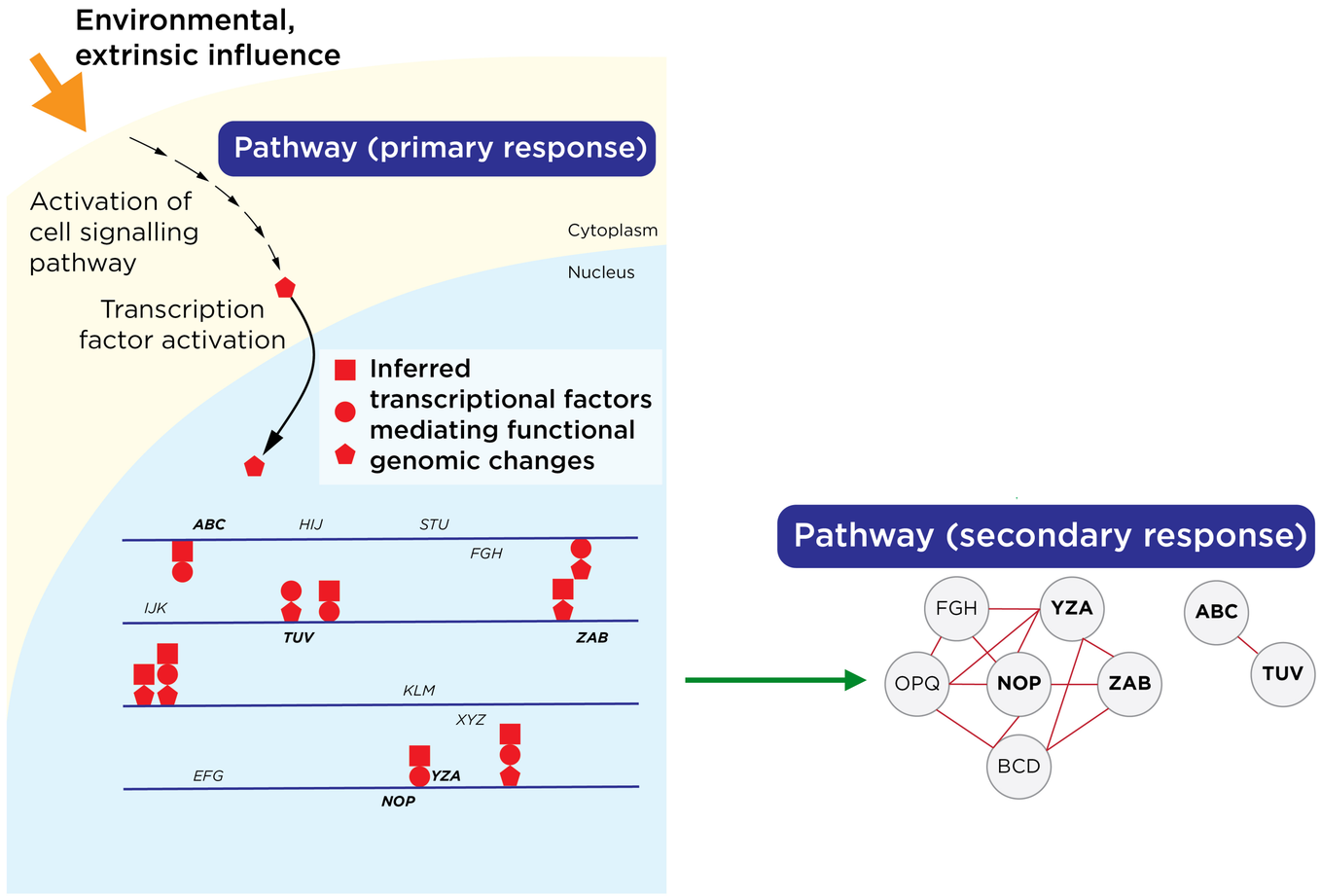


Clarke N, Germain P, Altucci L, Gronemeyer H. Retinoids: potential in cancer prevention and therapy. Expert Rev Mol Med. 2004 Nov 30;6(25):1-23.

Epigenetics and human disease

What regulates transcription factors?

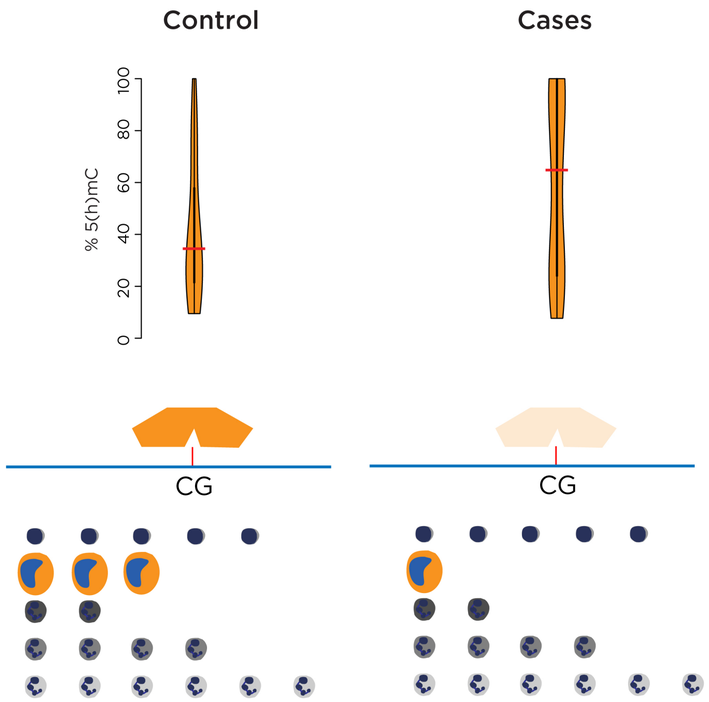
Insights into cell signalling pathways



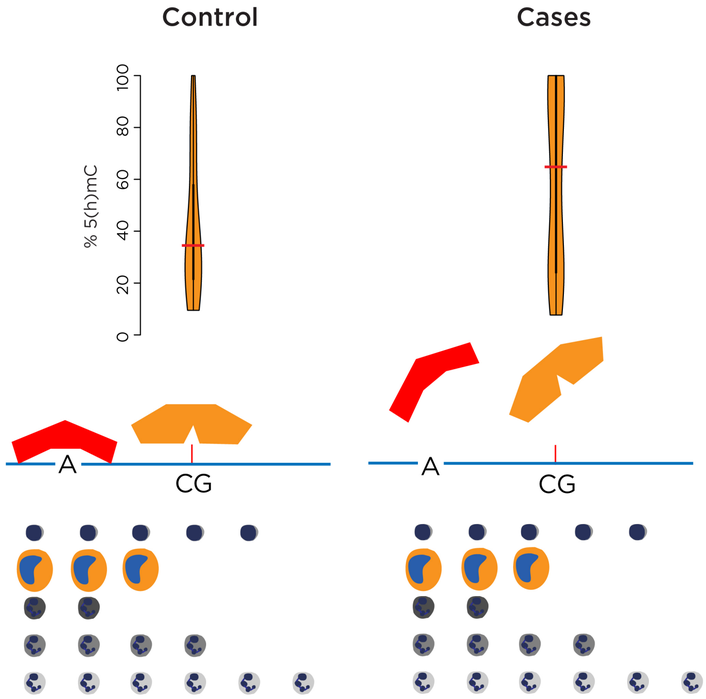
Epigenetics and human disease

Embracing the confounders

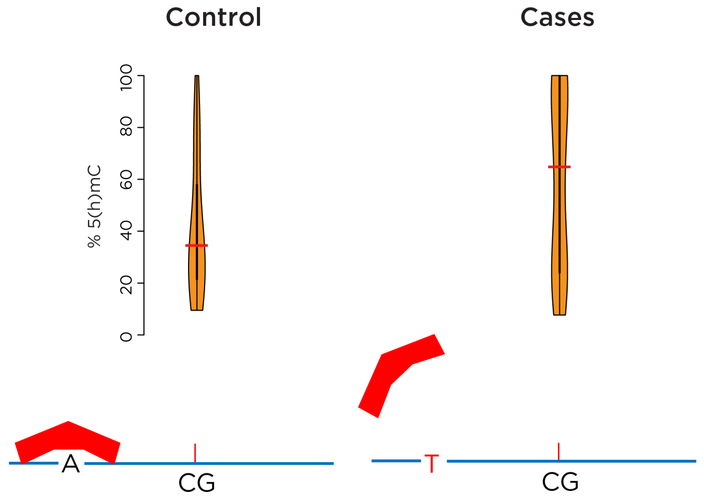
Gaining insights from the influences on molecular genomic assays



Cell subtype effect



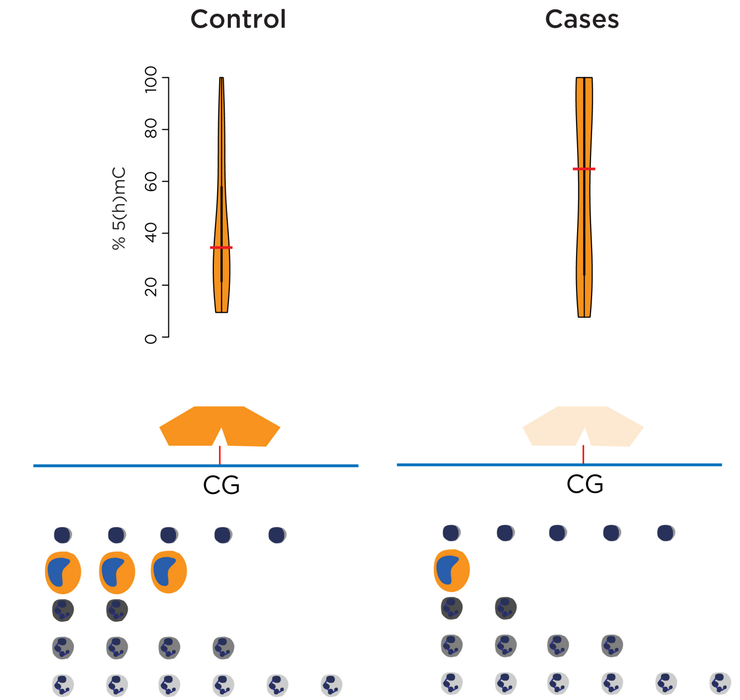
Transcription factor effects



Insights into DNA sequence variants associated with phenotypes

DNA sequence polymorphism effect

Revealing cell subtype effects



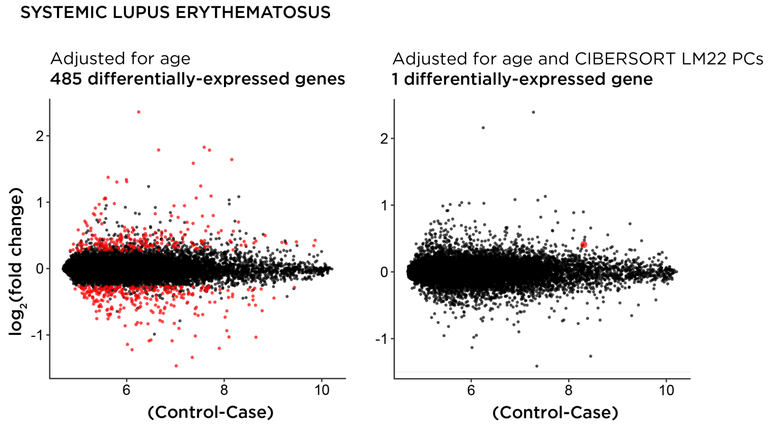
Epigenetics and human disease

When cell subtype effects dominate epigenomic assay results

Blood leukocyte study of systemic lupus erythematosis
Estimated and adjusted for cell subtype composition (CIBERSORT)

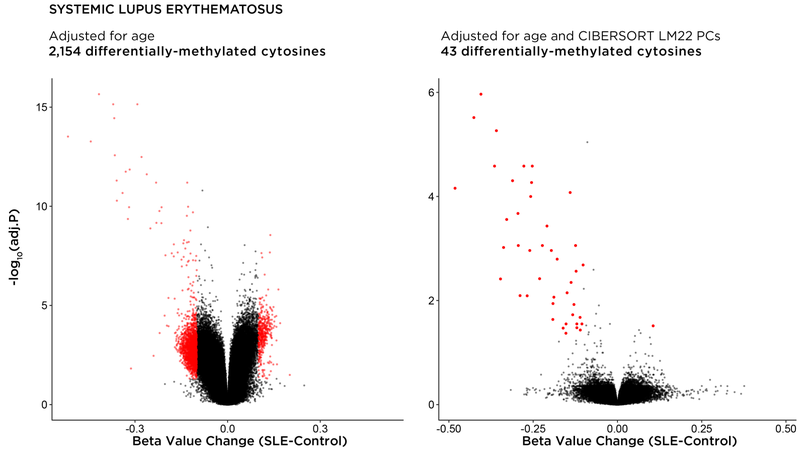
Masako Suzuki
Center for Epigenomics
Einstein

Gene expression



Before: **485**
After: **1**

DNA methylation



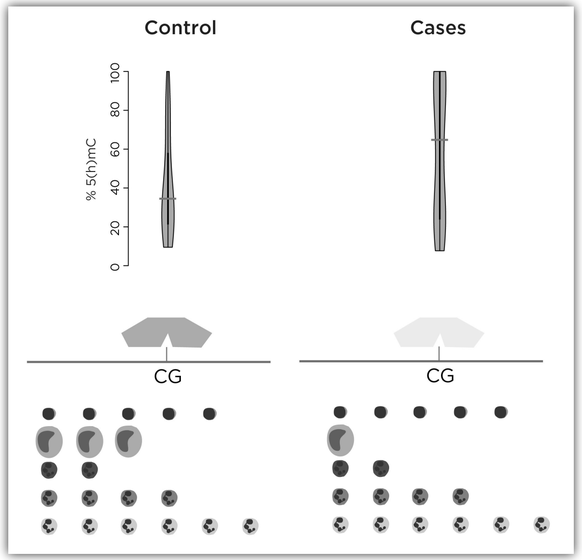
Before: **2,154**
After: **43**

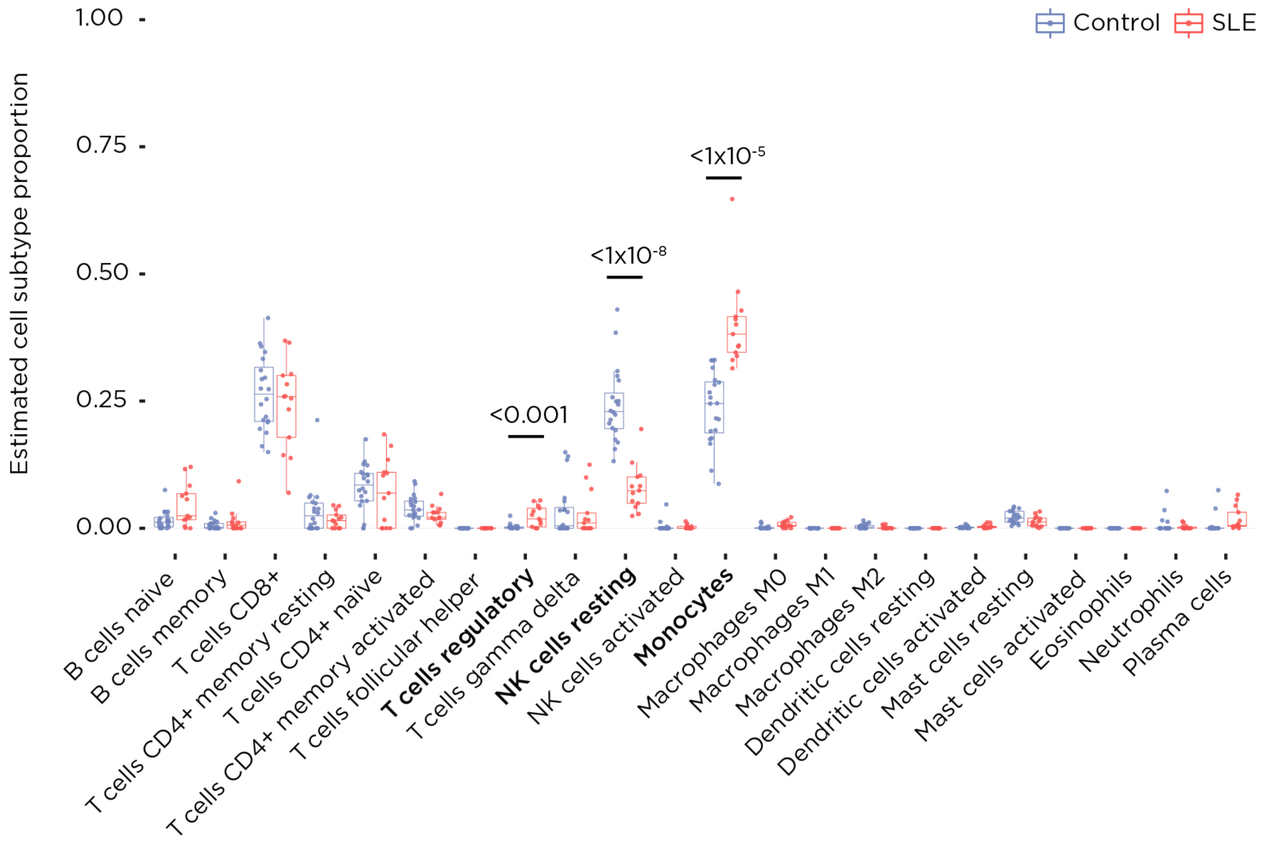
Kong Y, Rastogi D, Seoighe C, Greally JM, Suzuki M.
Insights from deconvolution of cell subtype proportions enhance the interpretation of functional genomic data. PLOS One. 2019 Apr 25;14(4):e0215987.

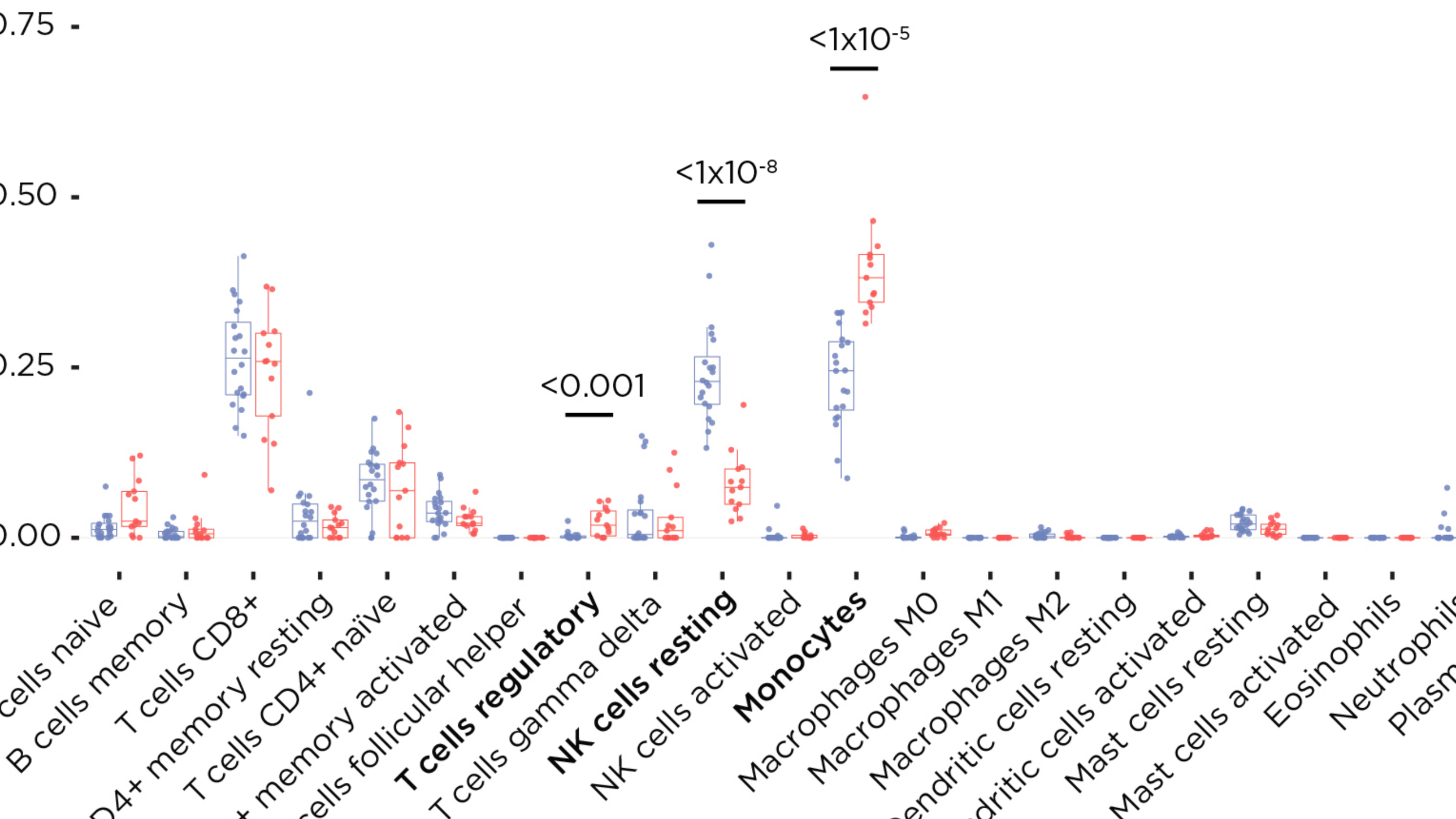
Epigenetics and human disease

When cell subtype effects dominate epigenomic assay results

To be a confounder, it needs to be over-represented in cases or controls
Cell subtype proportions in blood distinctive in lupus patients





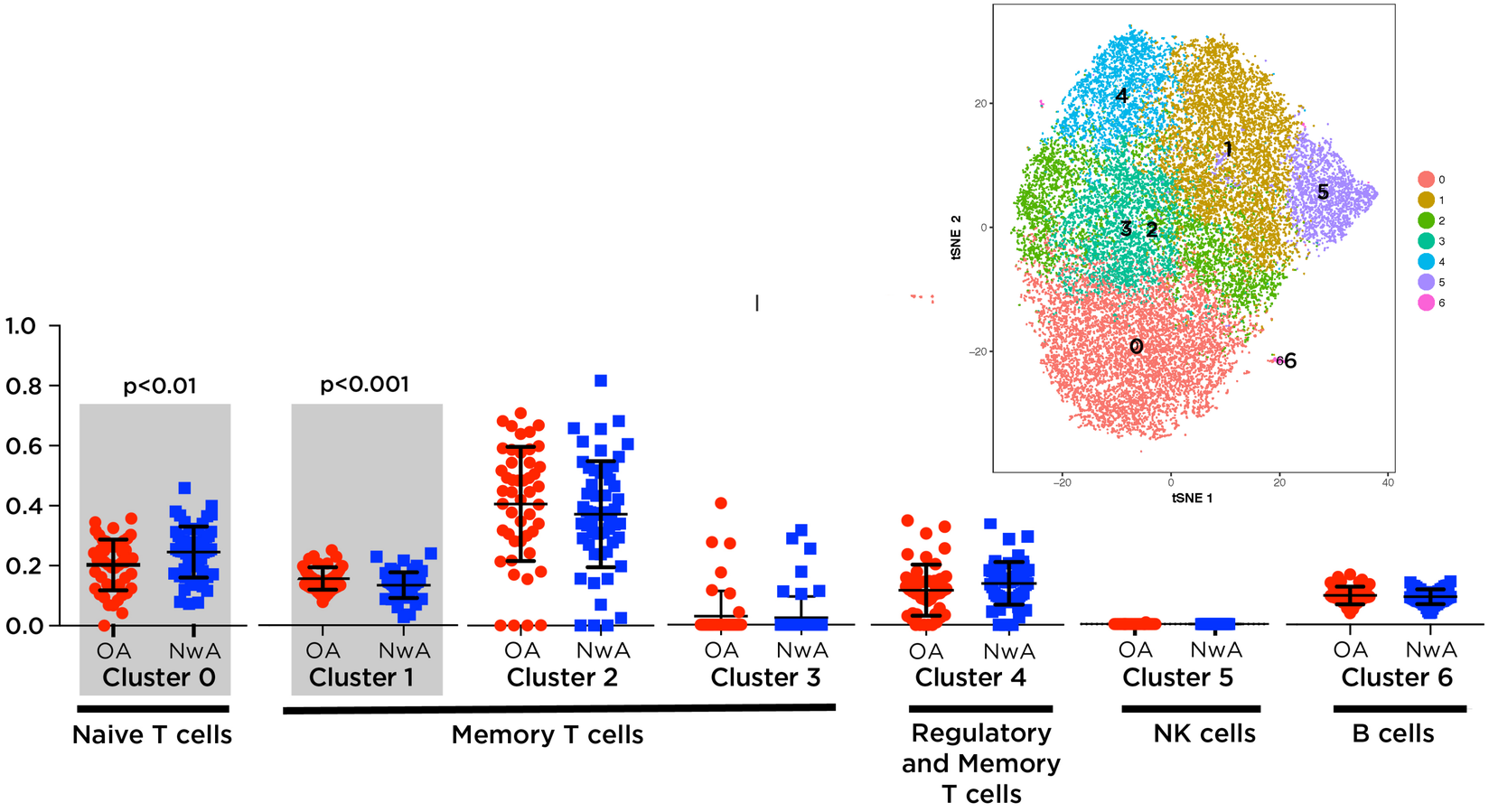

0.75
0.50
0.25
0.00
<1x10-5
<1x10-8
<0.001
cells naive
B cells memory
T cells CD8+
D4+ memory resting
T cells CD4+ naïve
memory activated
cells follicular helper
T cells regulatory
T cells gamma delta
NK cells resting
NK cells activated
Monocytes
Macrophages M0
Macrophages M1
Macrophages M2
Macrophages resting
endritic cells activated
Mast cells resting
Mast cells activated
Eosinophils
Neutrophils
Plasm

Epigenetics and human disease

Cell subtype effects revealed by scRNA-seq: obese asthma

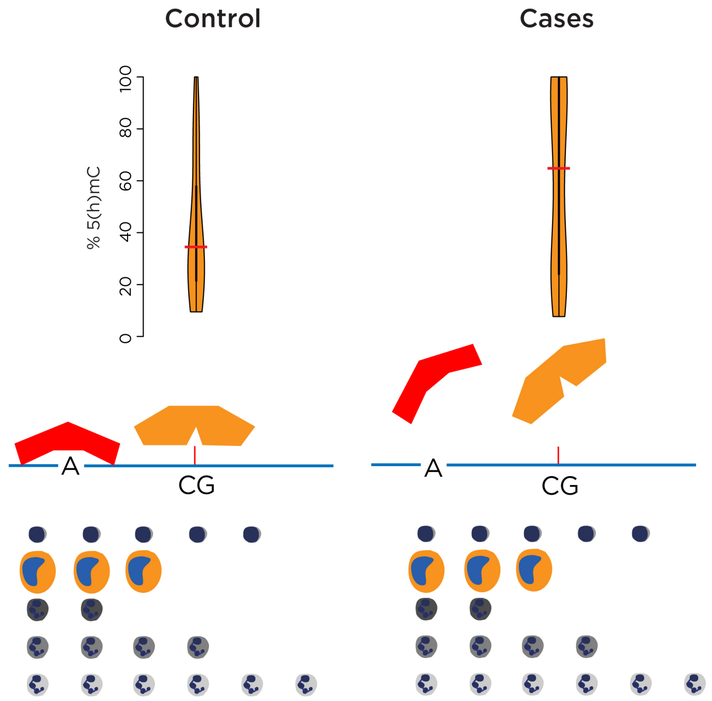
CD4+ T lymphocyte subsets distinctive in obese asthmatic children

Deepa Rastogi
Children's National, DC
(formerly
Center for Epigenomics)



Rastogi D, Nico J, Johnston AD, Tobias TAM, Jorge Y, Macian F, Greally JM. CDC42-related genes are upregulated in helper T cells from obese asthmatic children. J Allergy Clin Immunol. 2018 Feb;141(2):539-548.e7.

Revealing transcription factor effects

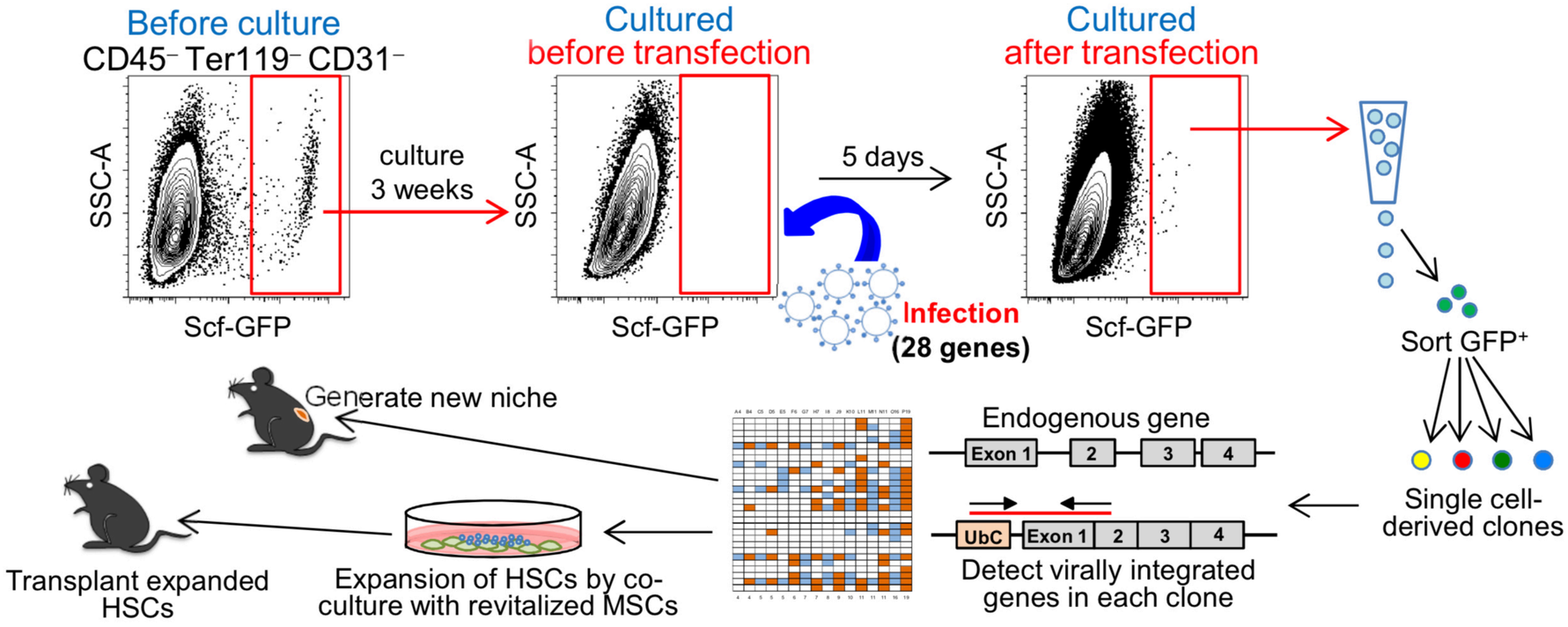


Transcription factors

Paul Frenette
Einstein

TFs and cellular reprogramming

Cellular reprogramming to revitalise bone marrow MSCs



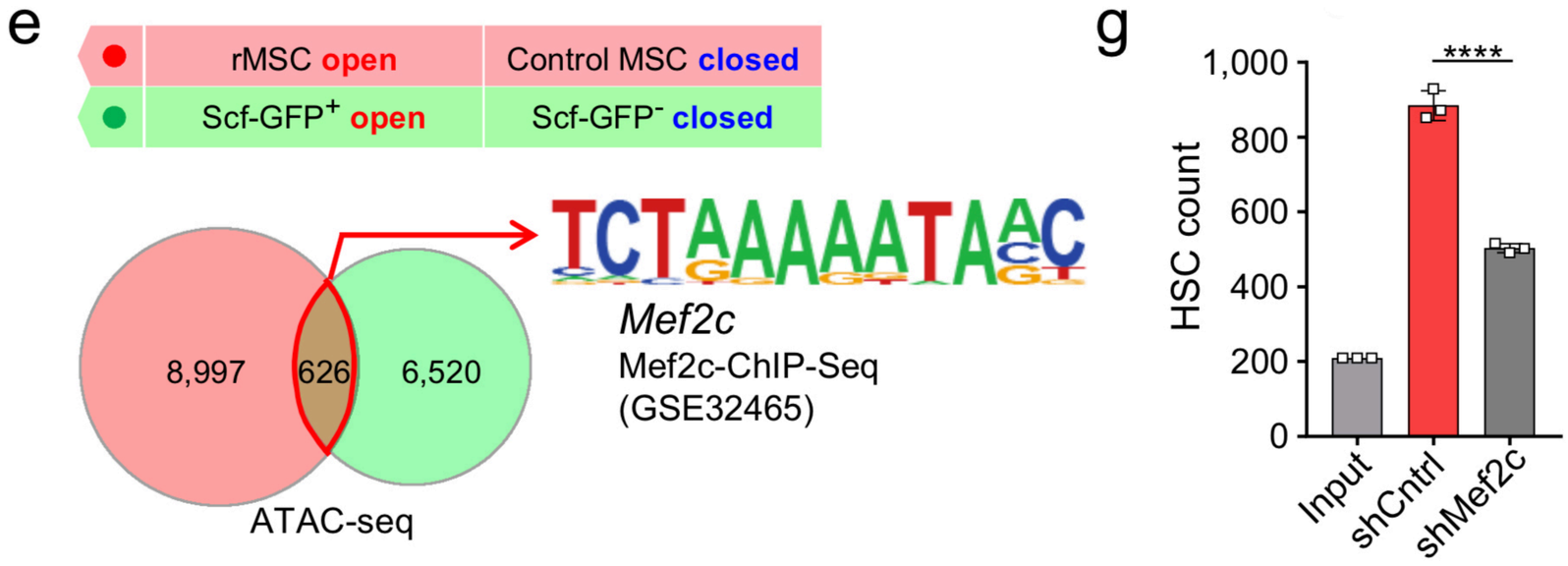
Implicated *Ostf1*, *Xbp1*, *Irf3* and *Irf7* (OXII) reprogramming factors

Nakahara F, Borger DK, Wei Q, Pinho S, Maryanovich M, Zahalka AH, Suzuki M, Cruz CD, Wang Z, Xu C, Boulais PE, Ma'ayan A, Greally JM, Frenette PS. Engineering a haematopoietic stem cell niche by revitalizing mesenchymal stromal cells. Nat Cell Biol. 2019 May;21(5):560-567.

Transcription factors

TFs and cellular reprogramming

Complementary approach:
ATAC-seq to find loci with open chromatin in revitalised MSCs



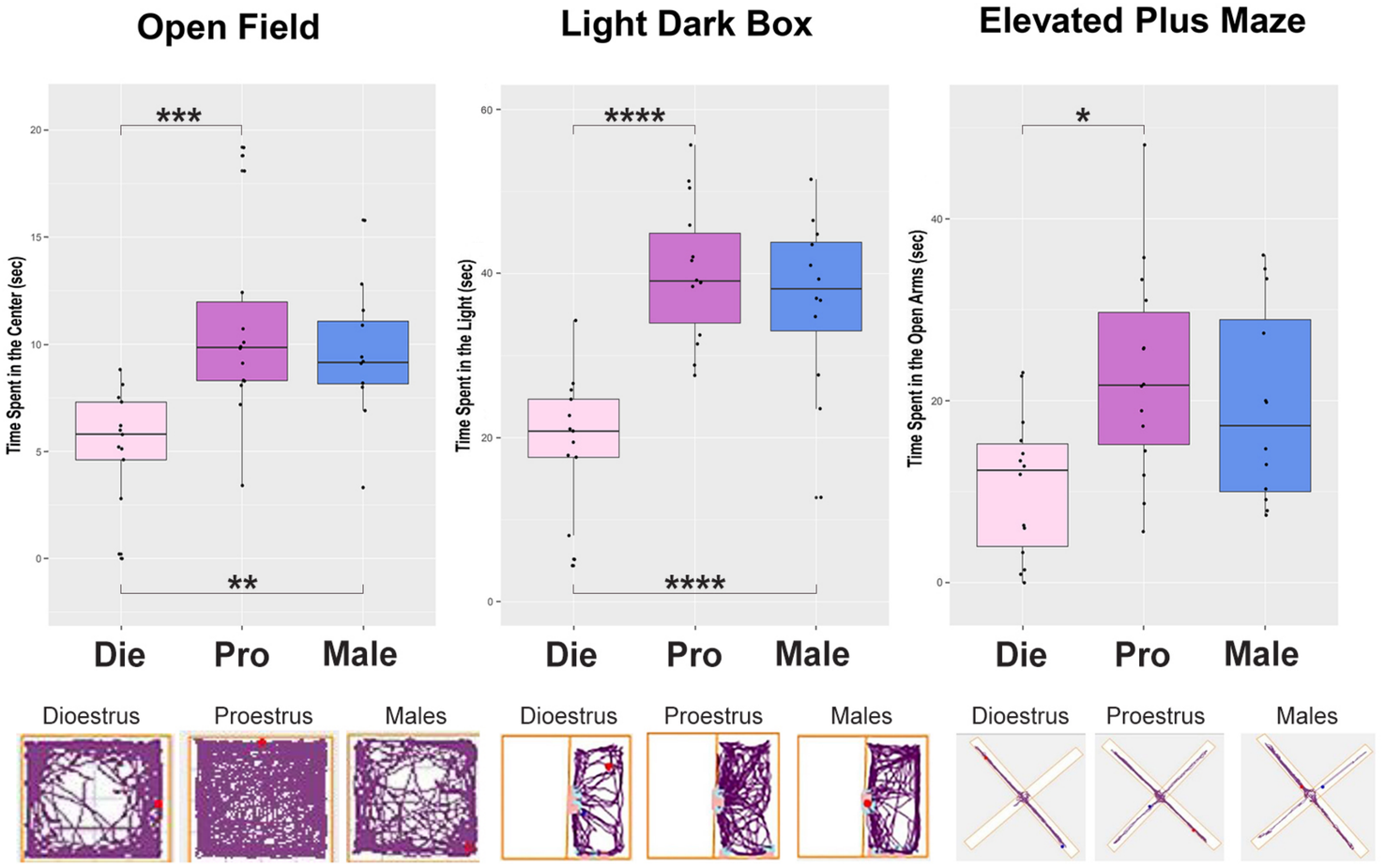
Motif analysis implicated *Mef2c* as an additional factor
Confirmed with knock-down assay

Epigenetics and human disease

Marija Kundakovic
Fordham University

The cellular reprogramming model

Low oestradiol stage of oestrus cycle associated with increased anxiety

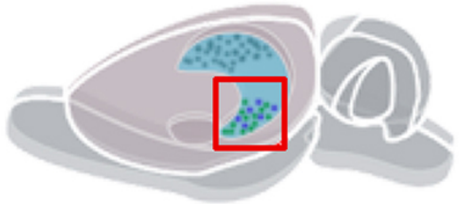


Jaric I, Rocks D, Greally JM, Suzuki M, Kundakovic M. Chromatin organization in the female mouse brain fluctuates across the oestrous cycle. Nat Commun. 2019 Jun 28;10(1):2851.

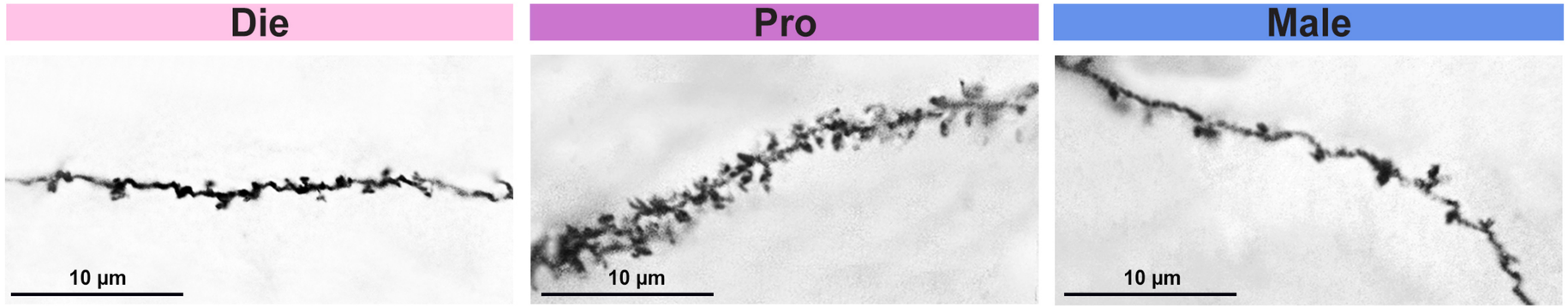
Epigenetics and human disease

The cellular reprogramming model

Cycling of dentritogenesis in ventral hippocampus



Ventral Hippocampus



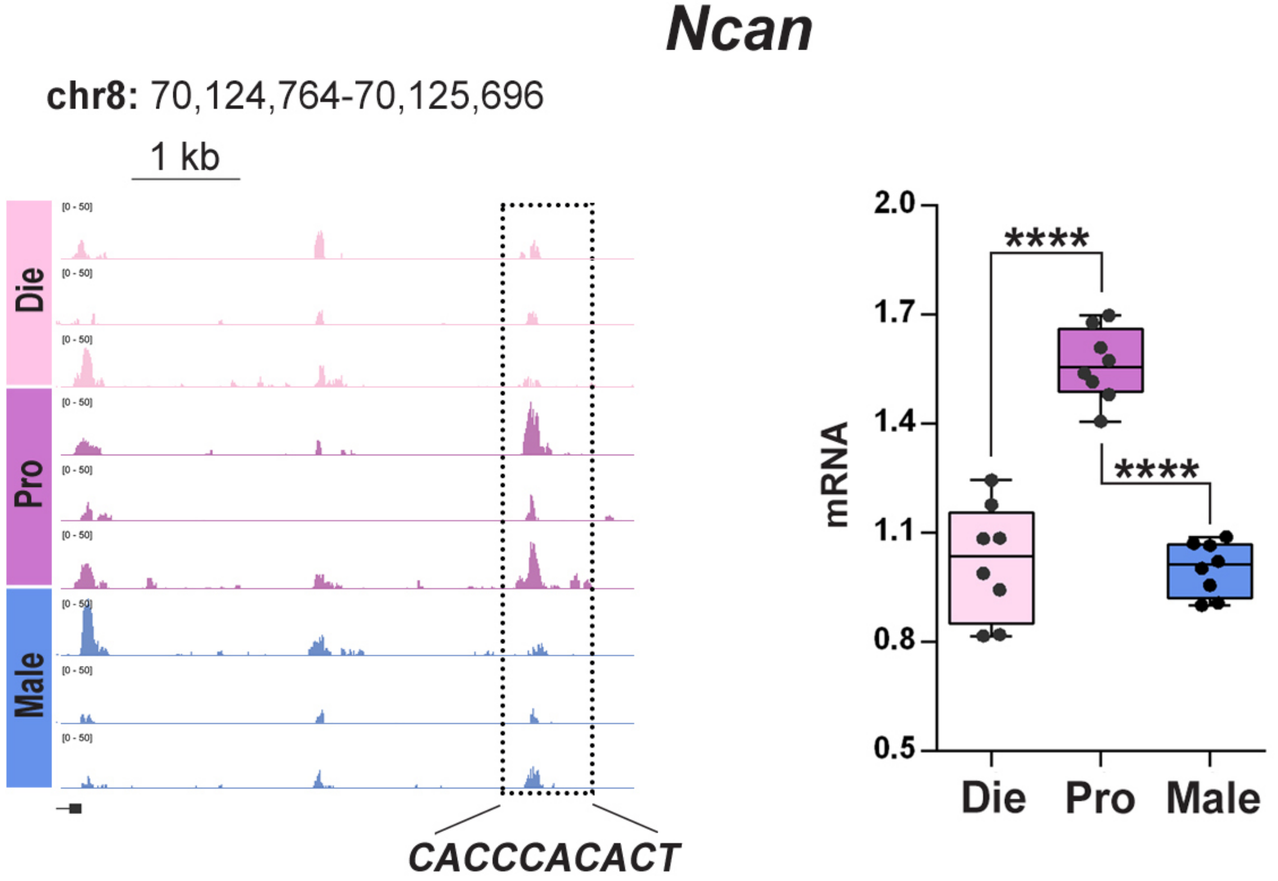
Epigenetics and human disease

The cellular reprogramming model

Neuronal nuclear ATAC-seq

Dynamic patterns of chromatin accessibility over 4-5 day cycle

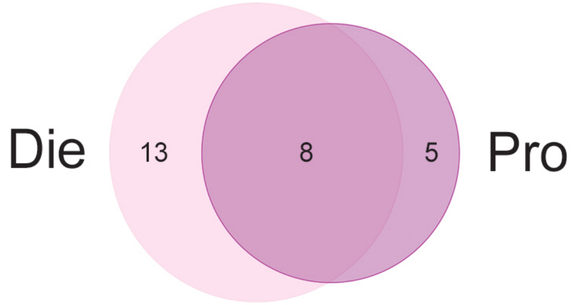
Associated local changes in gene expression



Epigenetics and human disease

The cellular reprogramming model

Loci with chromatin accessibility changes enriched for TF binding motifs, including Egr1



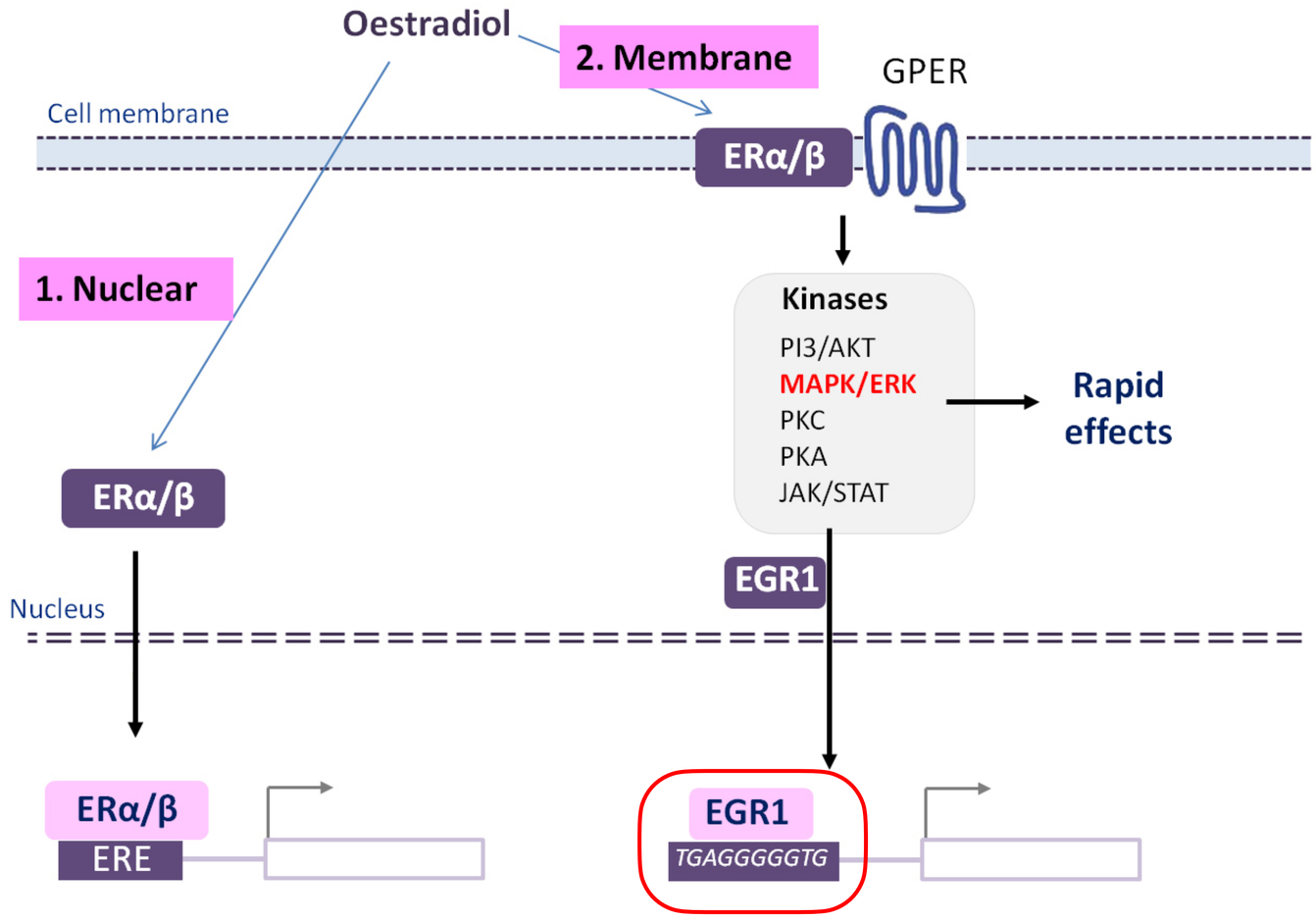
Proestrus Specific Motifs	P value	% Targets	% Bkgd
Mef2c	1e-148	25.95%	16.42%
Mef2d	1e-148	13.17%	6.47%
Mef2b	1e-126	38.84%	28.49%
Egr1	1e-104	26.48%	18.29%
Mef2a	1e-101	22.02%	14.56%

Epigenetics and human disease

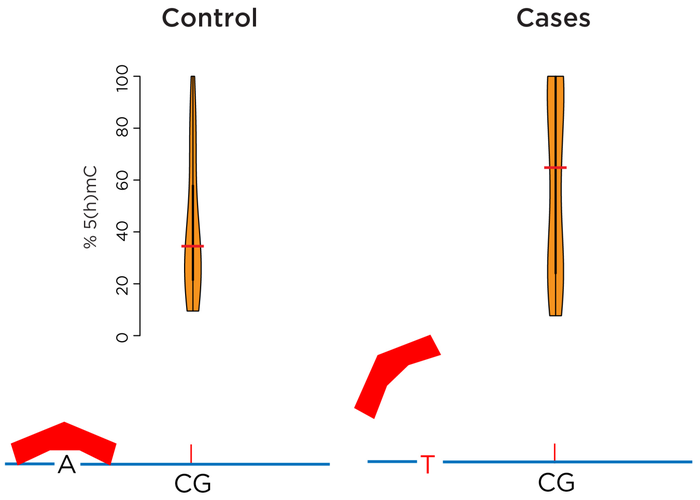
TF insights revealing primary pathway regulation

Egr1 identified as TF ultimately mediating oestradiol response

Allows insights into regulation of TF signalling



Revealing sequence variation effects



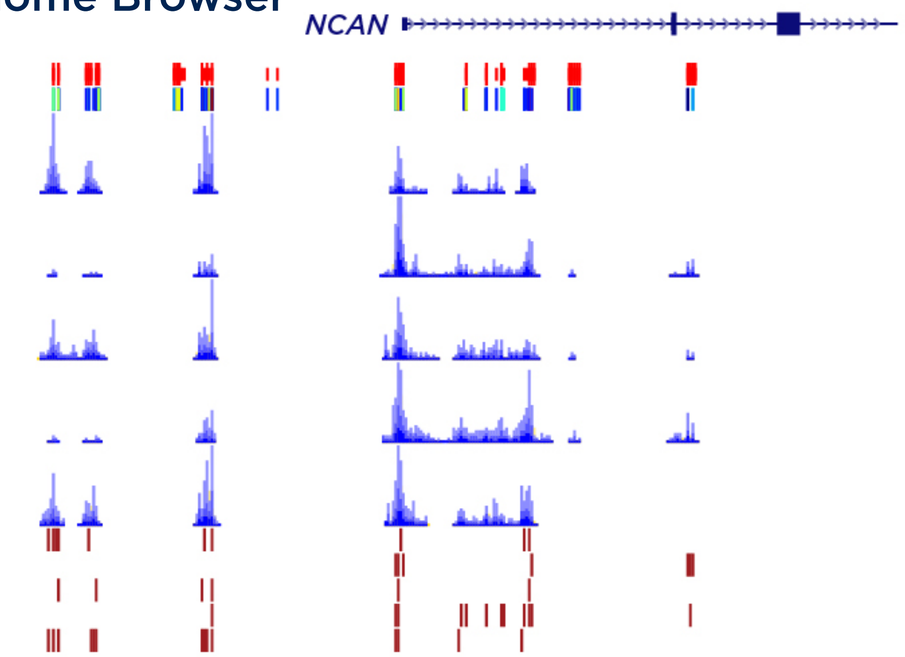
Epigenomics and sequence polymorphism

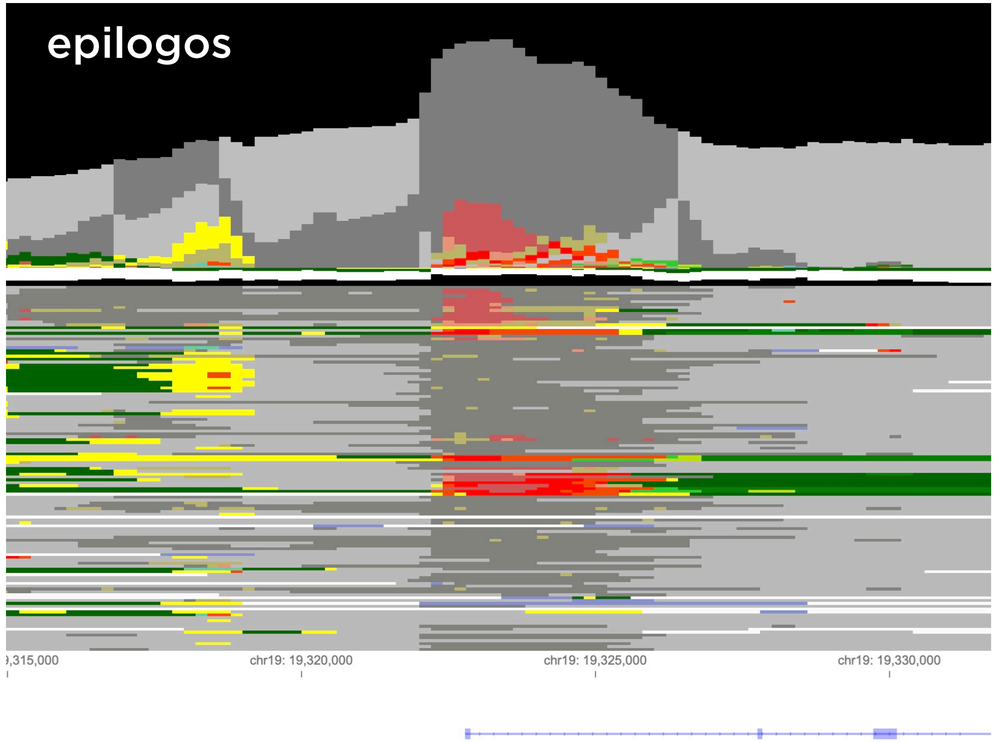
Transcription factors: act at non-coding DNA

Regulatory loci for gene expression

Sequence polymorphism at these loci: functional variants

UCSC Genome Browser





Vierstra J, Lazar J, Sandstrom R, ... Meuleman W, Stamatoyannopoulos JA. Global reference mapping of human transcription factor footprints. Nature. 2020 Jul;583(7818):729-736.

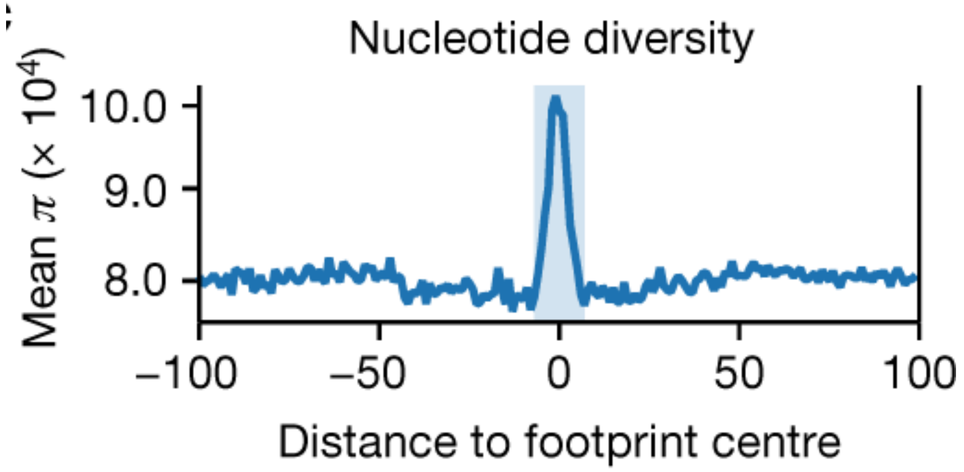
Epigenomics and sequence polymorphism

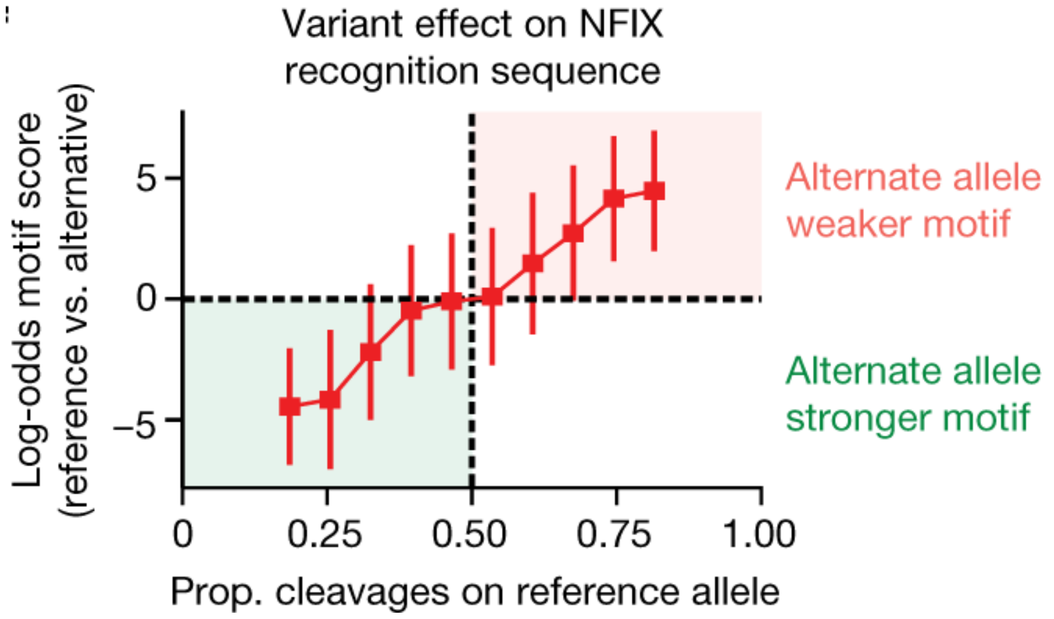
Transcription factors: act at non-coding DNA

Unexpected finding:

TF binding sites have locally **increased** polymorphism

Half the time the DNA sequence variant **increases** TF affinity

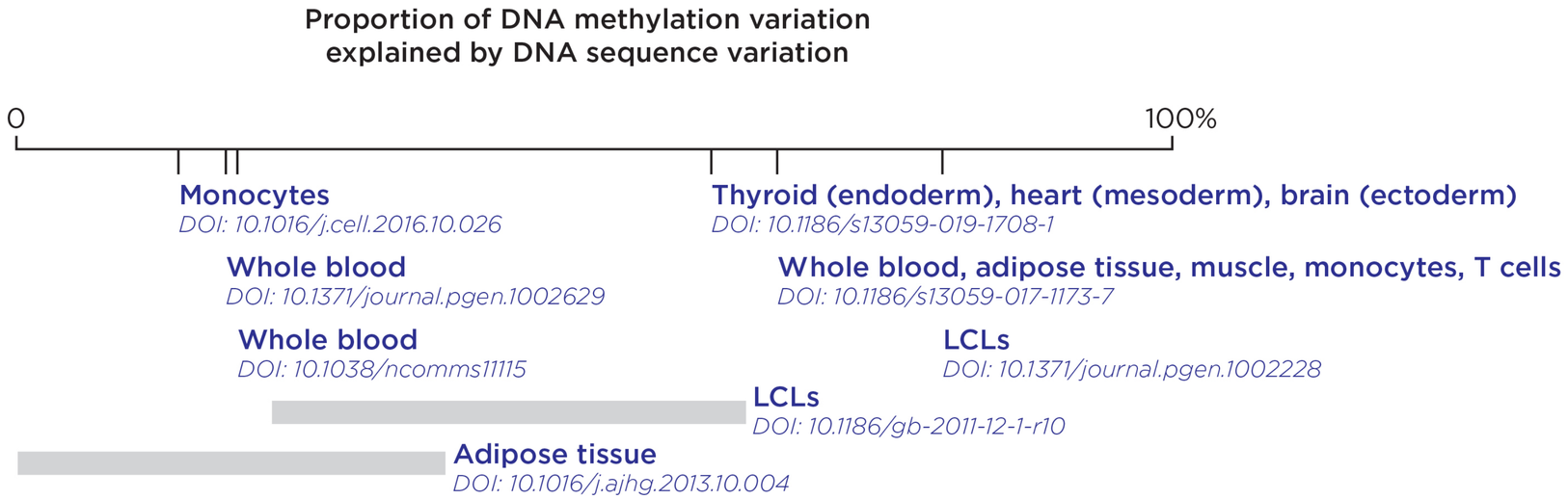




Epigenomics and sequence polymorphism

Polymorphic TF binding sites

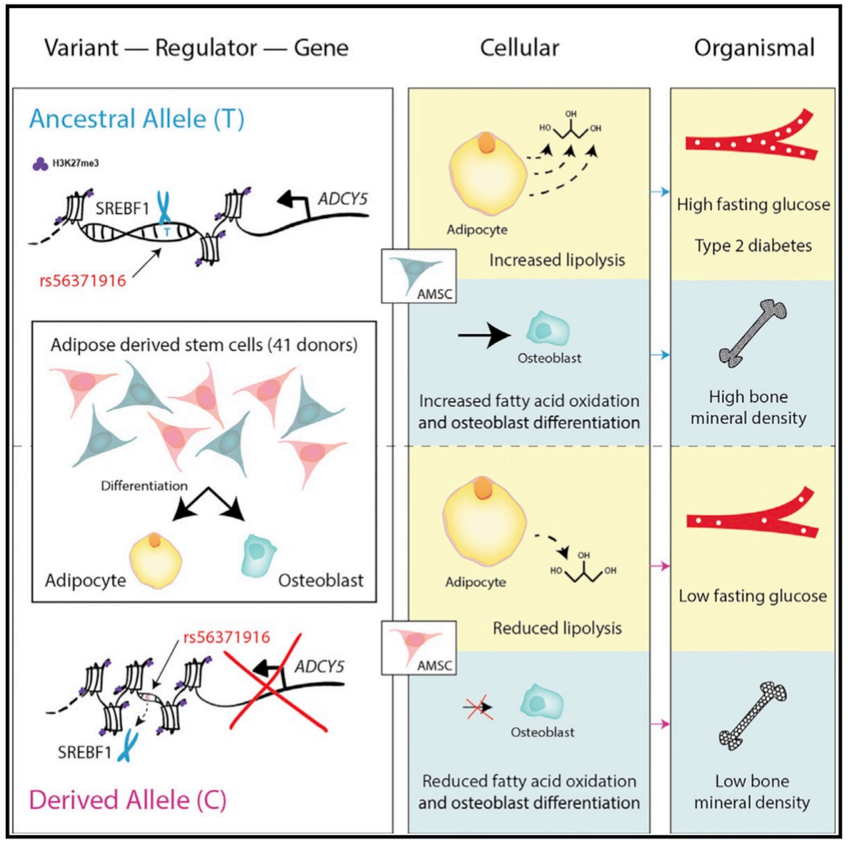
Likely mediators of genetic influences on epigenomic assays



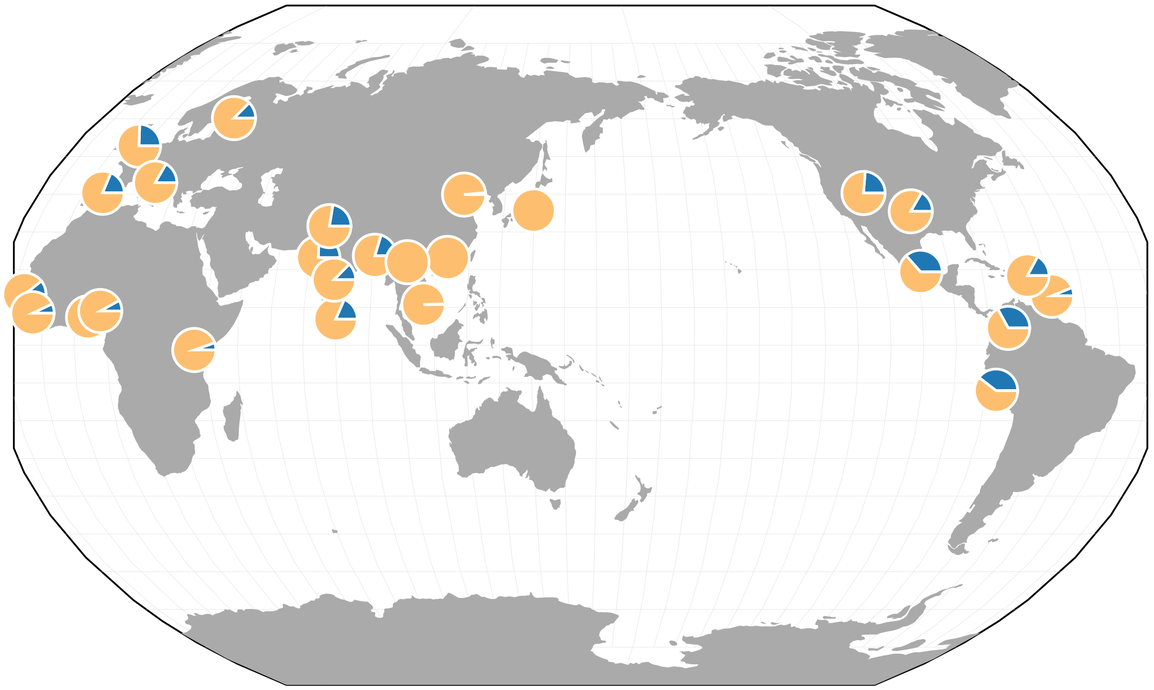
Epigenomics and sequence polymorphism

The *ADCY5* intronic variant rs56371916

Implicated by GWAS in both type 2 diabetes mellitus and osteoporosis



chr3:123095543 C/T

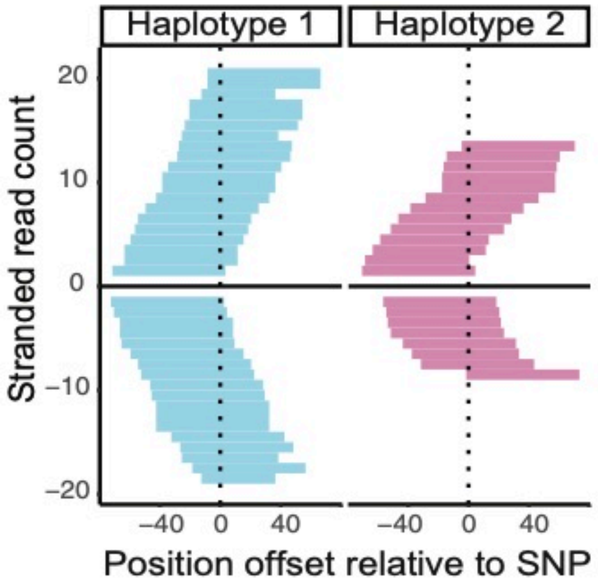


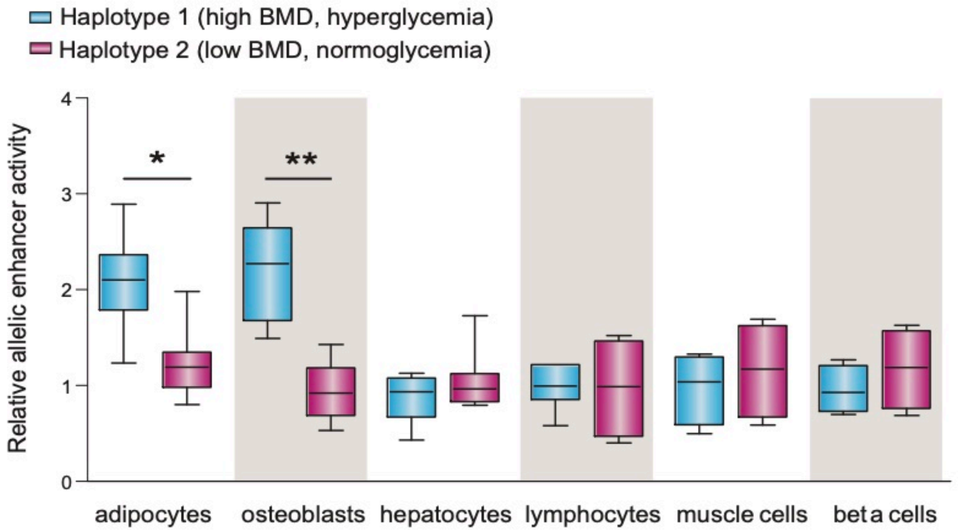
Sinnott-Armstrong N, Sousa IS, Laber S, ... Lander ES, Kiel DP, Claussnitzer M. A regulatory variant at 3q21.1 confers an increased pleiotropic risk for hyperglycemia and altered bone mineral density. Cell Metab. 2021 Jan 28:S1550-4131(21)00001-2.

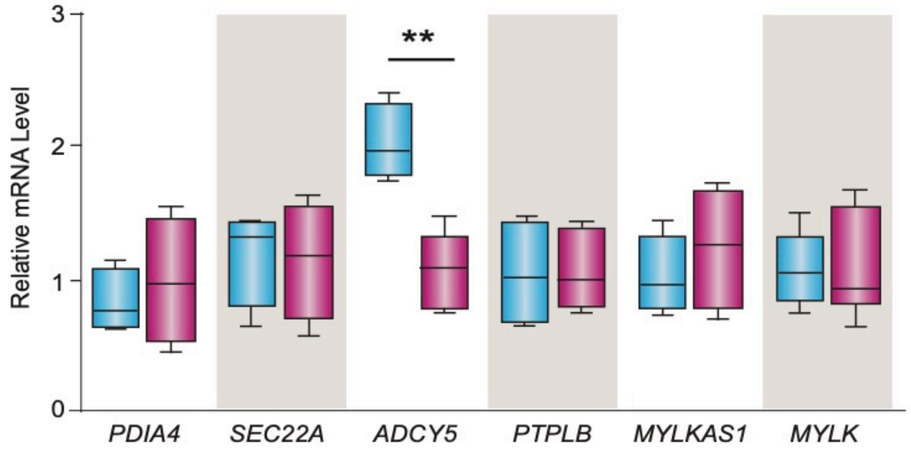
Epigenomics and sequence polymorphism

rs56371916 is a functional variant

Influences overlying chromatin accessibility
Influences *ADCY5* expression
Ancestral allele binds SREBP1 transcription factor, but not derived allele







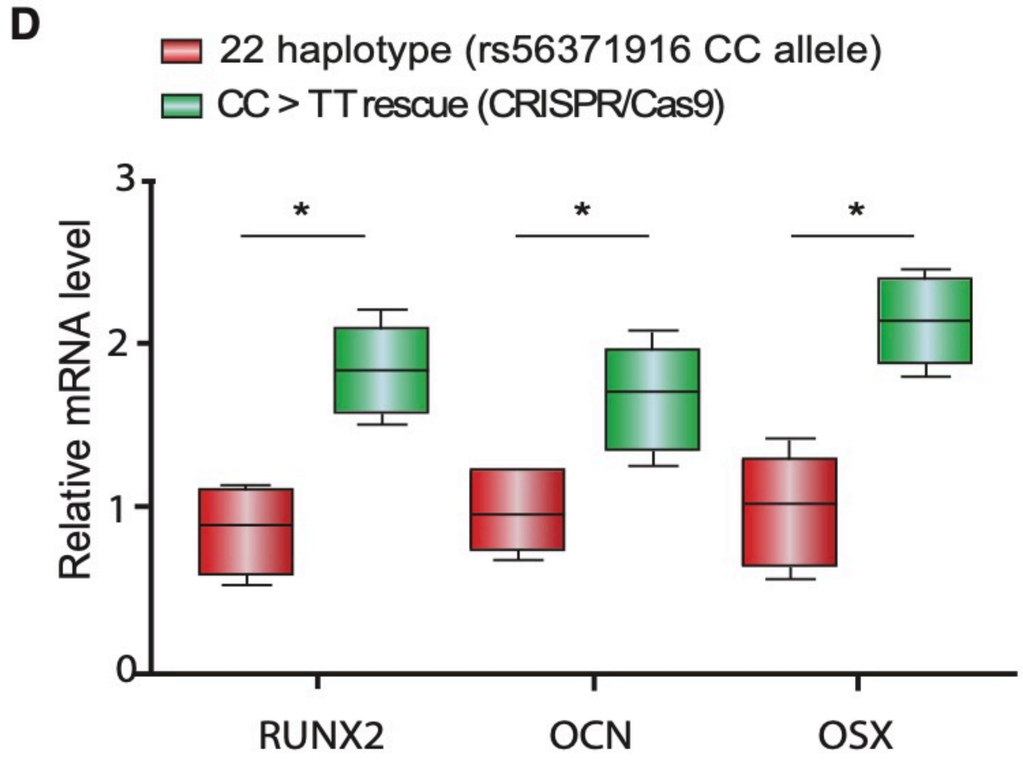
ATAC-seq

RNA-seq

Epigenomics and sequence polymorphism

rs56371916 may influence mesenchymal lineage decisions

CRISPR editing from derived (CC) to ancestral (TT) state
Osteoblast induction leads to higher expression of osteoblast differentiation marker genes/increased osteogenesis

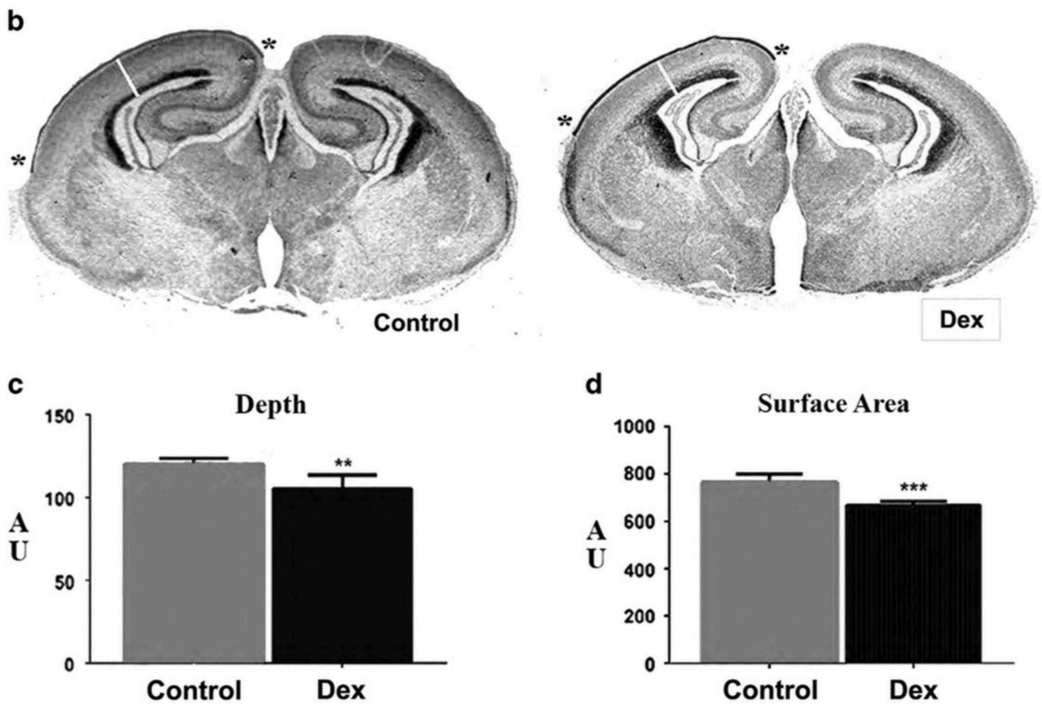


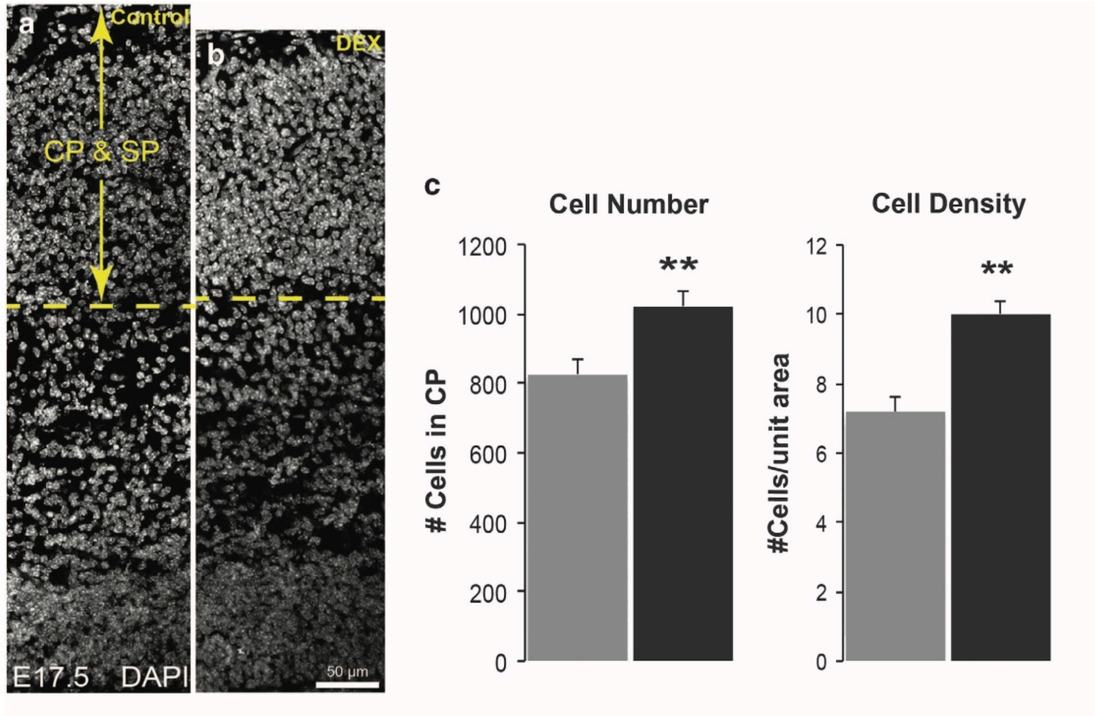
Cell fate
and tissue effects

The effect of functional variants may be on cell fate

Typically, we think of effects in terms of cellular reprogramming

Exposures/genetic polymorphisms can also influence tissue composition



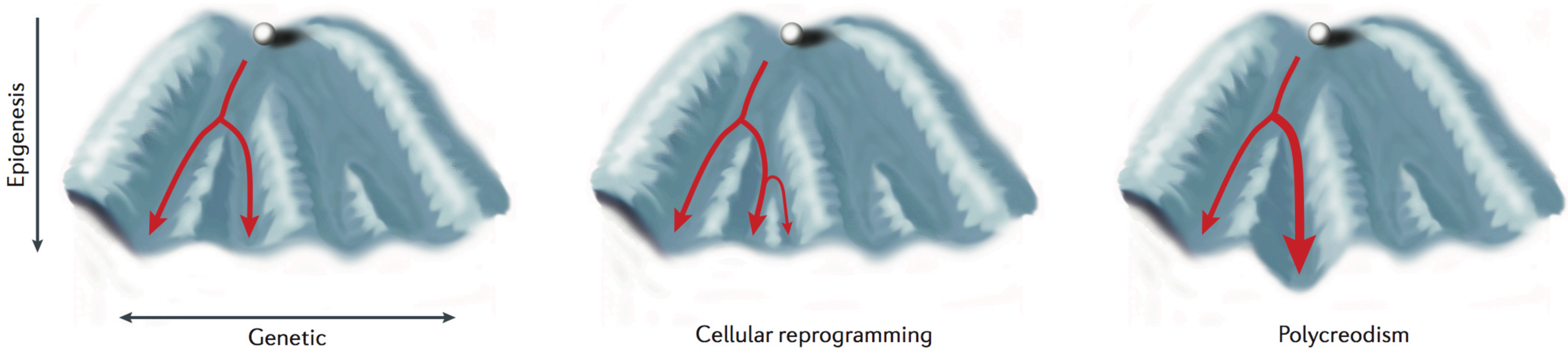


Tsiarli MA, Rudine A, Kendall N, Pratt MO, Krall R, Thiels E, DeFranco DB, Monaghan AP. Antenatal dexamethasone exposure differentially affects distinct cortical neural progenitor cells and triggers long-term changes in murine cerebral architecture and behavior. Transl Psychiatry. 2017 Jun 13;7(6):e1153.

Epigenomics and sequence polymorphism

The effect of functional variants may be on cell fate

Typically, we think of effects in terms of cellular reprogramming
Exposures/genetic polymorphisms can also influence tissue composition



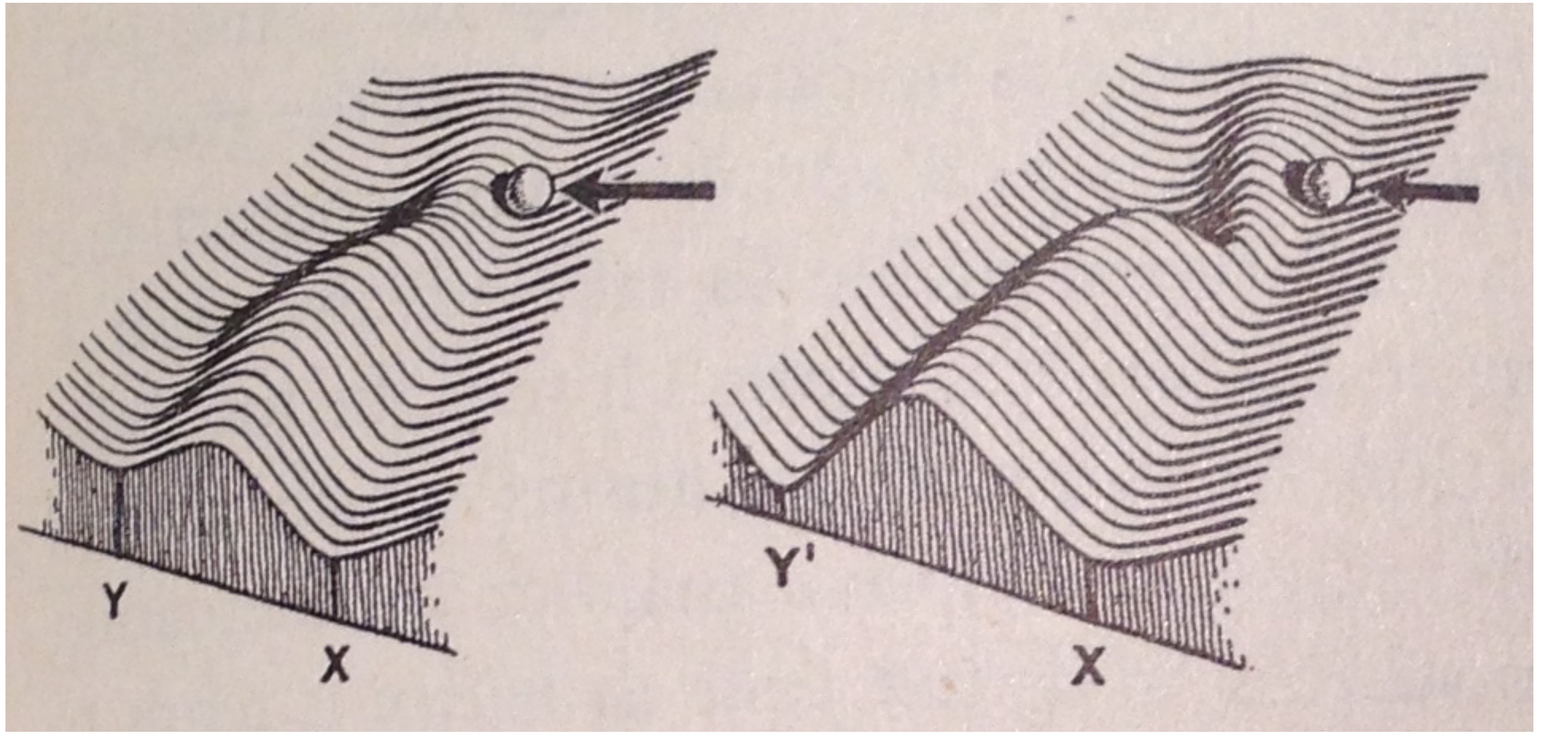
Lappalainen T, Greally JM.
Associating cellular epigenetic models with human phenotypes. Nat Rev Genet. 2017 Jul;18(7):441-451.

Waddington redux

A neo-Waddingtonian model for human disease

DNA influencing phenotypes through cell fate decisions
Detectable at *cis* regulatory elements with epigenomic assays

Conrad Hal Waddington

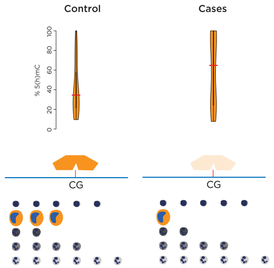


Waddington, C. H. (1957). The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology London: Ruskin House/George Allen and Unwin Ltd.

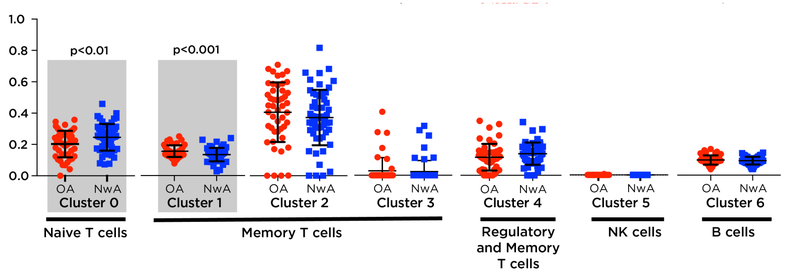
Epigenetics and human disease

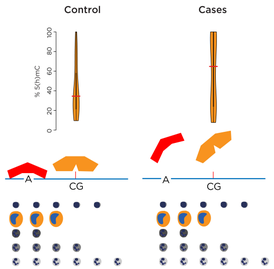
Epigenomics and human disease 2021

Harvesting confounders to reveal pathogenesis

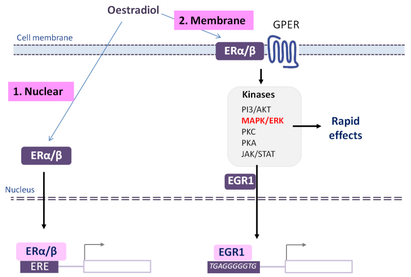


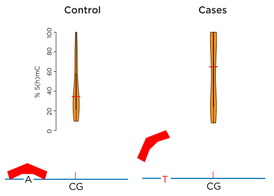
Cell subtype proportion effects



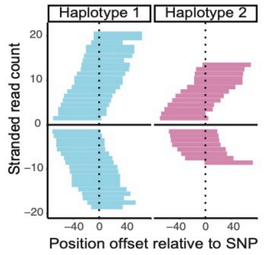


Transcription factor effects





DNA sequence polymorphism effects

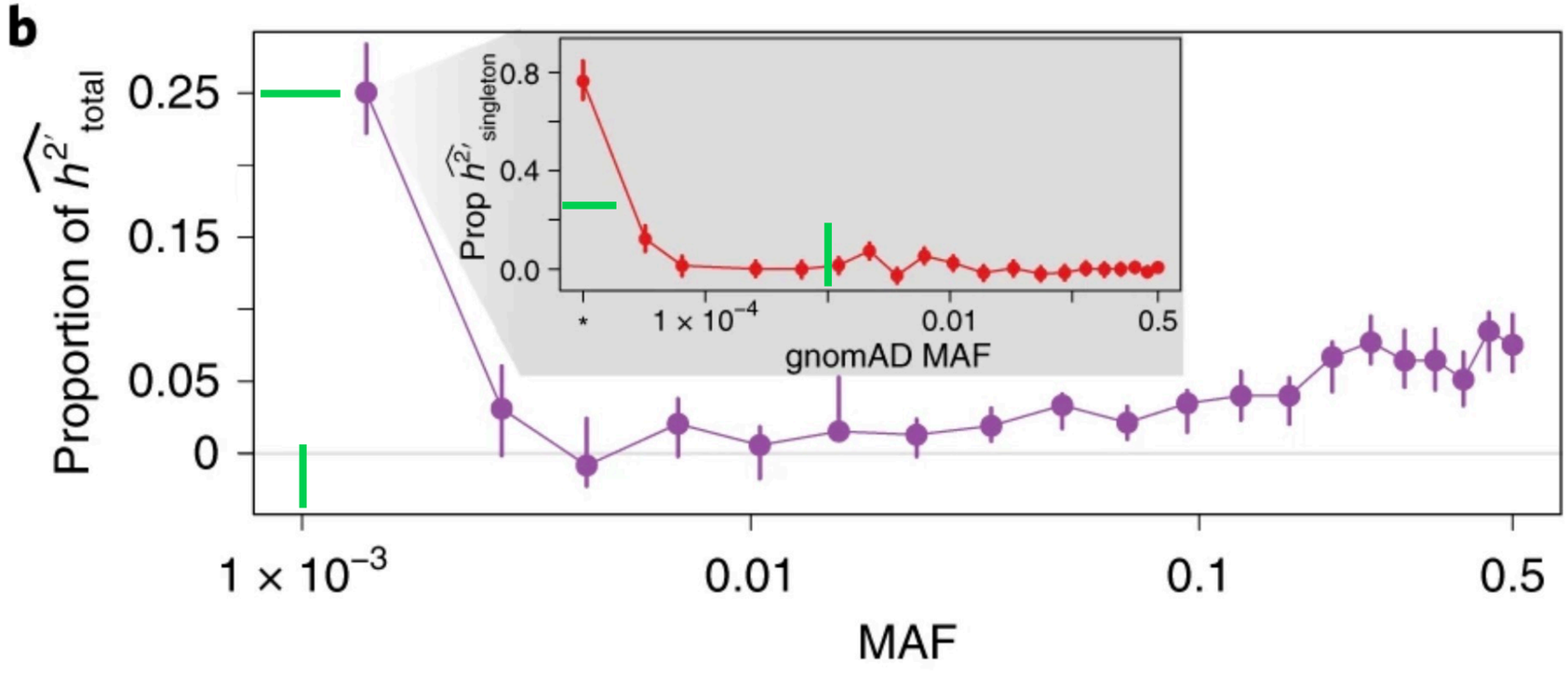


Epigenomics and ultra-rare functional variants

Ultra-rare non-coding functional variants

The loci with the greatest effects on heritability of gene expression differences occur extremely infrequently in populations (<1/10,000)

In any individual, most likely to be heterozygous



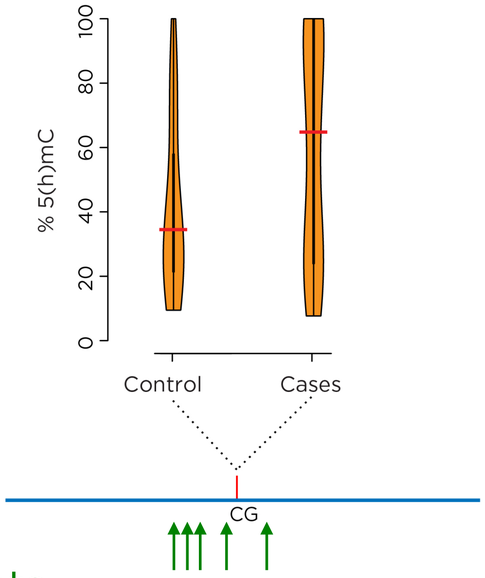
Hernandez RD, Uricchio LH, Hartman K, Ye C, Dahl A, Zaitlen N. Ultrarare variants drive substantial cis heritability of human gene expression. Nat Genet. 2019 Sep;51(9):1349-1355.

Epigenomics and ultra-rare functional variants

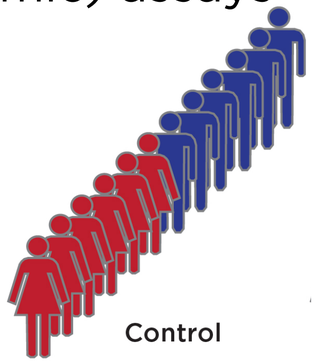
Harvesting ultra-rare functional variant confounders

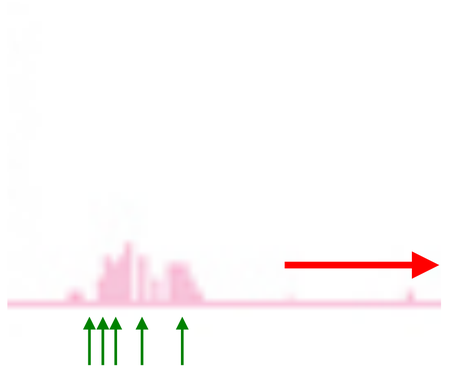
Ultra-rare functional variants difficult to define using typical statistical genetics approaches

Convergent outcomes make them detectable using molecular genomic (epigenomic) assays



Multiple rare sequence variants





Convergent (monoallelic) molecular genomic changes

Research group

GREALLY GROUP

Postdoctoral fellow
- Katherine Crocker

Clinical fellow
- Kaye Brathwaite

Graduate students
- Taylor Thompson
- Reanna Doña
- Kassidy Lundy
- Jacob Stauber
- Marliette Matos
- Eric Sosa

Study coordinator
- Kathleen Rosales

center for epigenomics | Einstein

Epigenomics Shared Facility
- Shahina Maqbool
- Raul Olea
- Yongmei Zhao

Computational Genomics group
- Rob Dubin
- Xusheng Zhang

Genome Diver
- Christian Stolte
- Kevin Shi
- Nathan Pearson
- Charles Gagnon


Katherine Crocker


Kaye Brathwaite


Taylor Thompson


Reanna Doña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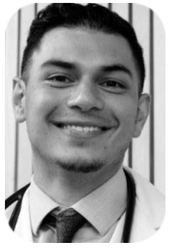
Eric Sosa


Kathleen Rosales

COLLABORATORS

Masako Suzuki, Einstein
Tuuli Lappalainen, NY Genome Center

FUNDING

NIH (NHGRI, NIMHD, NIA, NIAID, NIDCD, NICHD, NCI)

NIH National Human Genome Research Institute

NIH National Institute on Minority Health and Health Disparities

NIH National Institute on Aging

NYCKIDSEQ

FigShare DOI 10.6084/m9.figshare.9975359.v1

Waddington, C. H. (1957). London: Ruskin House/George Allen and Unwin Ltd.

Lappalainen T, Greally JM. Nat Rev Genet. 2017 Jul;18(7):441-451.

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Kong Y, Rastogi D, Seoighe C, Greally JM, Suzuki M. PLOS One. 2019 Apr 25;14(4):e0215987.

Rastogi D, Nico J, Johnston AD, Tobias TAM, Jorge Y, Macian F, Greally JM. J Allergy Clin Immunol. 2018 Feb;141(2):539-548.e7.

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Hernandez RD, Uricchio LH, Hartman K, Ye C, Dahl A, Zaitlen N. Nat Genet. 2019 Sep;51(9):1349-1355.

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