Epigenetic Variability in Healthy Aging & Exceptional Longevity

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Every single individual is unique

- First cloned cat *[Shin et al. Nature. 2002]*
- Donor (left) and clone (right) are genetically identical
- Coat color is not the same

⇒ Epigenetic variability

Source: College of Veterinary Medicine and Biomedical Sciences, A&M University, Texas, USA

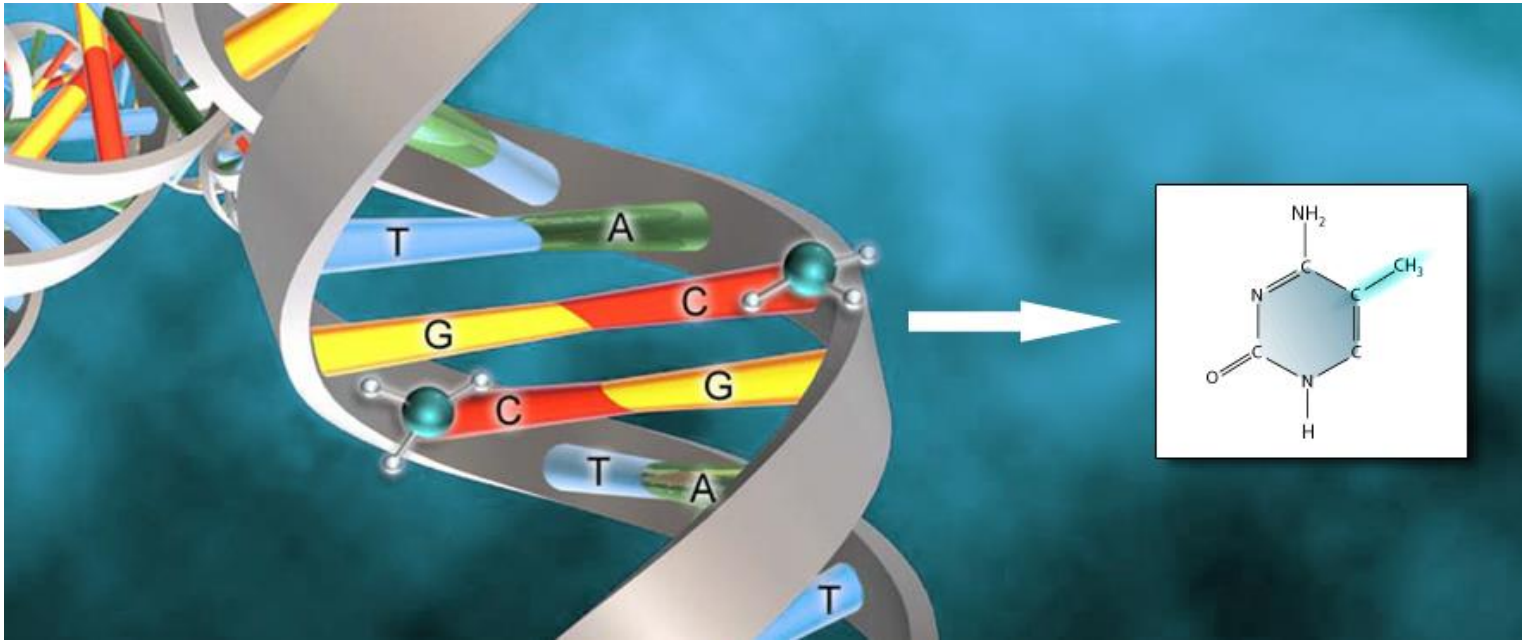
What is epigenetics?

- Epigenetics is the study of changes to the genome which are ***not*** caused by alterations in the DNA sequence
- Chemical compounds modify the genome "on top" of it
- Epigenetic modifications are highly dynamic and form the intersection between the genome and the environment
- They play an important role in controlling gene expression and genomic (in-)stability → phenotype

What do epigenetic modifications do?

- Many different cells in our body: brain cells, blood cells, bone cells…
- They all have essentially the same genome
- Epigenetic modifications influence which genes are active and which proteins are produced in a cell, giving rise to the phenotype
- **Multicellular organism has one genome but many epigenomes**

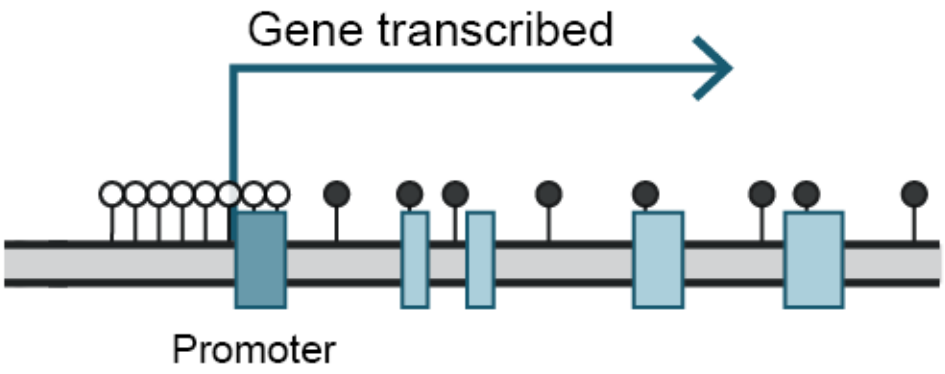
DNA methylation

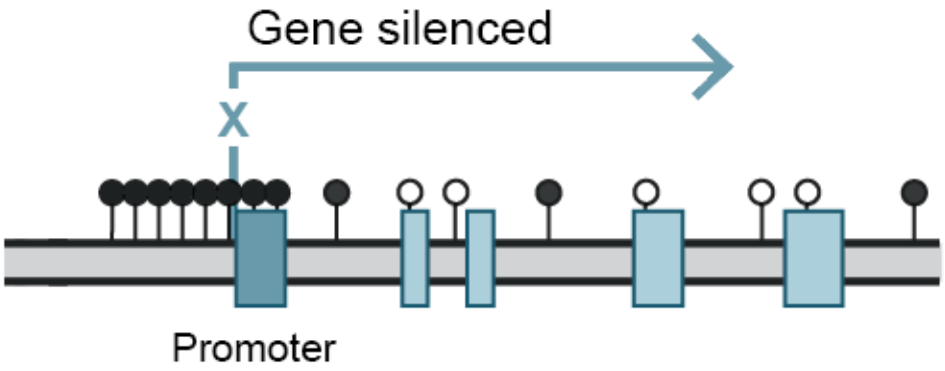


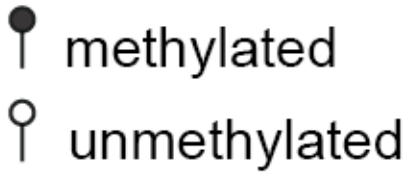
Adapted from jonlieffmd.com

- DNA methylation is the most studied epigenetic modification
- Adds methyl group to DNA base cytosine → methylcytosine
- Regulates transcription, X chromosome inactivation, etc.

DNA methylation and transcription







Adapted from commons.wikimedia.org

Example: Agouti gene variable methylation

- Agouti promoter methylated (right)
  - Gene not expressed
  - Brown healthy mouse
- Agouti promoter unmethylated (left)
  - Gene expressed
  - Yellow obese mouse with diabetes, cancer
- Depends on diet of mother during pregnancy: methyl-rich or standard diet

These two mice are genetically identical and have the same age

Source: learn.genetics.edu

- Gene can be methylated to varying degrees
- Even differs from cell to cell

Source: Morgan et al. Nat Genet. 1999

"Life is a study in contrasts between randomness and determinism"

Cell

Leading Edge
Review

Nature, Nurture, or Chance: Stochastic Gene Expression and Its Consequences

Arjun Raj[1] and Alexander van Oudenaarden[1,*]
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DOI 10.1016/j.cell.2008.09.050

Gene expression is a fundamentally stochastic process, with randomness in transcription and translation leading to cell-to-cell variations in mRNA and protein levels. This variation appears in organisms ranging from microbes to metazoans, and its characteristics depend both on the biophysical parameters governing gene expression and on gene network structure. Stochastic gene expression has important consequences for cellular function, being beneficial in some contexts and harmful in others. These situations include the stress response, metabolism, development, the cell cycle, circadian rhythms, and aging.

Introduction

Life is a study in contrasts between randomness and determinism: from the chaos of biomolecular interactions to the precise coordination of development, living organisms are able to resolve these two seemingly contradictory aspects of their internal workings. Scientists often reconcile the stochastic and the deterministic by appealing to the statistics

et al. (1990). They examined the effect of different doses of glucocorticoid on the expression of a glucocorticoid-responsive transgene encoding beta-galactosidase and found that the cell-to-cell variability in the expression of the transgene was surprising large. Moreover, increasing the dose led to an increased frequency of cells displaying a high level of expression rather than a uniform increase in expression in every cell;

Biological variability

- Genetically identically cells or organisms display an incredible variety of phenotypes, even in homogenous environments *[Gaertner, Lab Anim, 1990]*
- Arises from randomness and noise present in all biological systems and processes

- This variability
  - plays a key role in development and cellular differentiation in multicellular organisms allowing for selection and propagation of cell type specific expression
  - enables rapid adaptation to changing environmental conditions leading to benefits in survival, e.g. stress-response
  - leads to population robustness, e.g. allowing for the tight control of programmed cell death by graded responses of the population of cells

Different levels of variability

- Cell-to-cell variability in a population of cells
- Inter-individual variability of multicellular organisms
- Variability within and across populations and species
- Spatiotemporal variability

Different levels of variability are related to each other

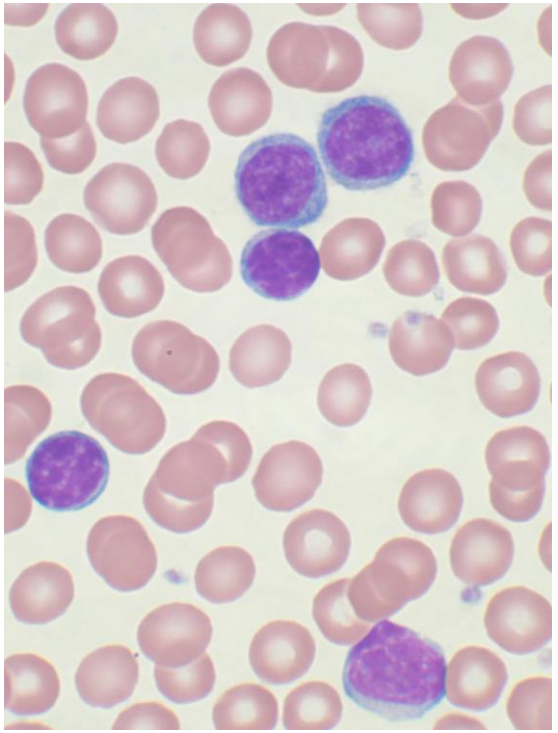
- Correspondence between measuring variability at one time point in a population of 1,000 cells and measuring the variability of one cell across 1,000 time points
- Correlation between cell-to-cell variability and variability across cell populations, and even across species to a lesser extent

Importance of variability

- **Aging**
  - Increased variability with age
- **Disease**
  - Cancer!
  - Autoimmune diseases:
    Type 1 Diabetes, Rheumatoid Arthritis…
  - …
- **Therapy**
  - Fractional killing
  - Therapeutic resistance
  - Personalized medicine!

Chronic Lymphocytic Leukemia (CLL)

- Most frequent leukemia in adults
- Two subtypes based on the mutational status of IGHV region:
  - M-CLL
    - high level of IGHV mutations
    - favorable clinical outcome
  - U-CLL
    - no or low level of IGHV mutations
    - worse clinical outcome



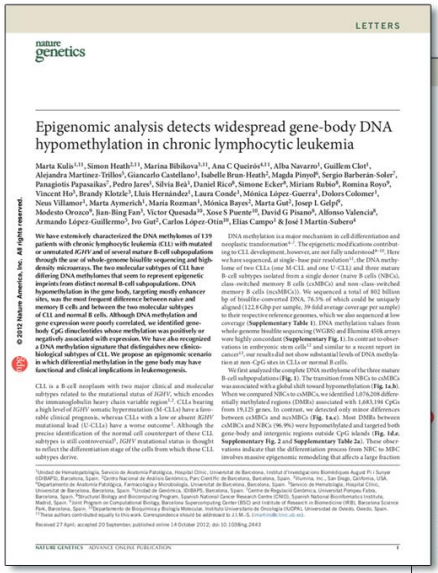
CLL cells

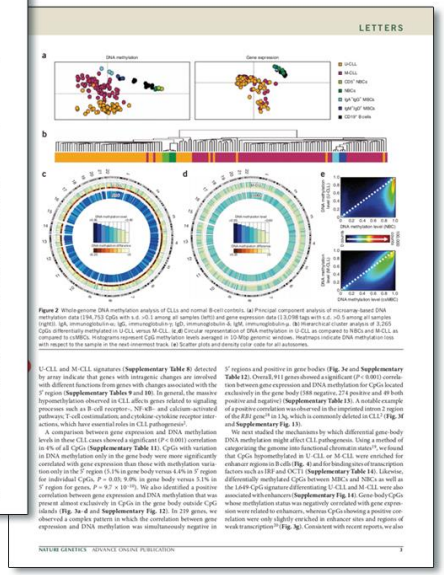
Source: Wikimedia.org

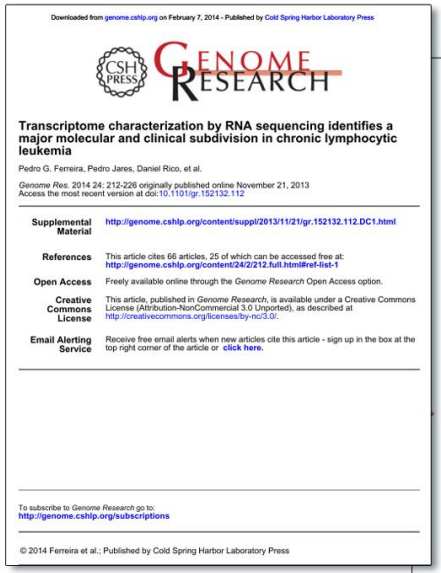
International Cancer Genome Consortium

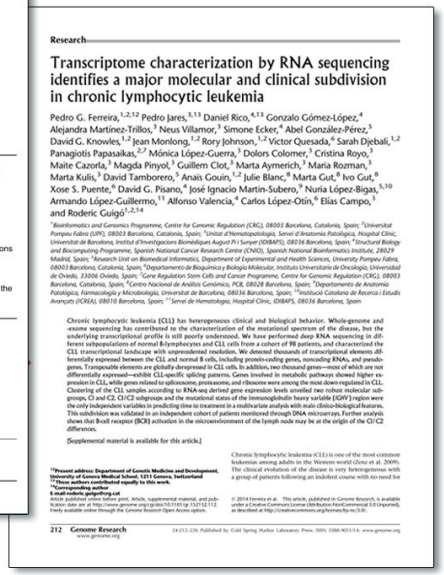
Spain: The ICGC Chronic Lymphocytic Leukemia Genome Project

- CLL methylome study *[Kulis et al. Nat Genet. 2012]*
- CLL transcriptome study *[Ferreira et al. Genome Res. 2014]*



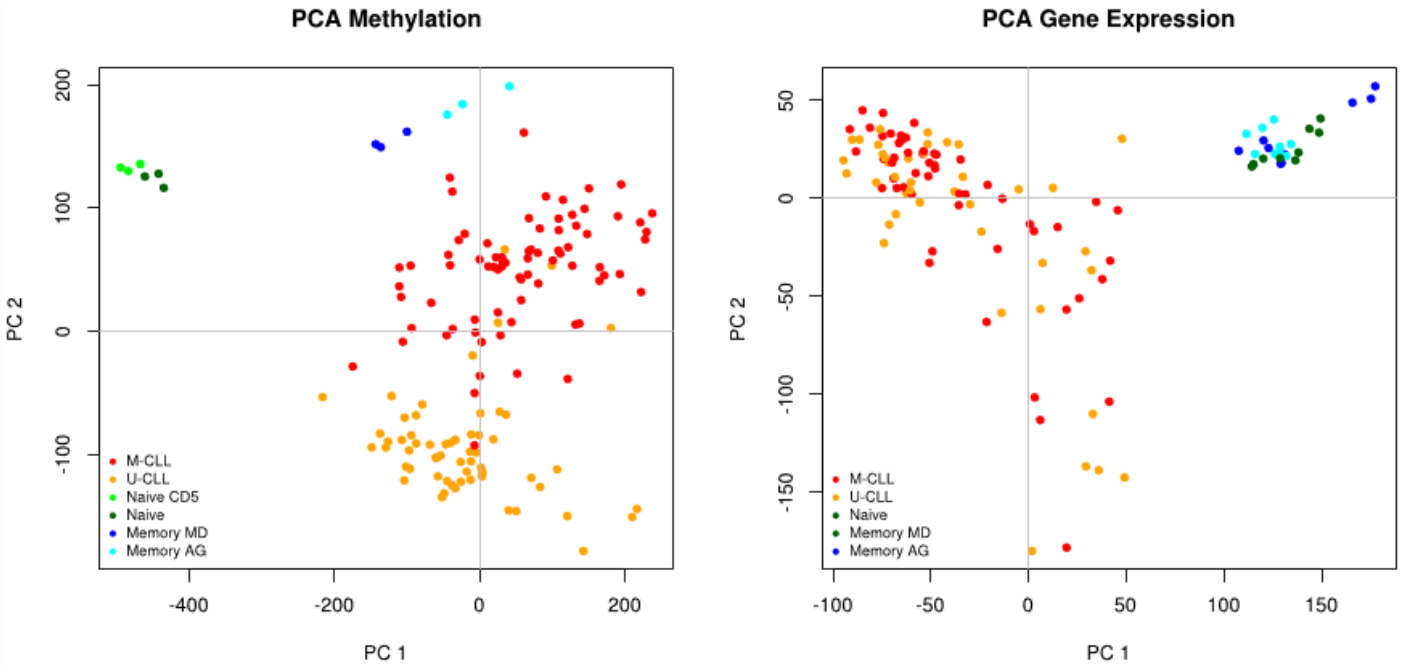






CLL differential expression variability study

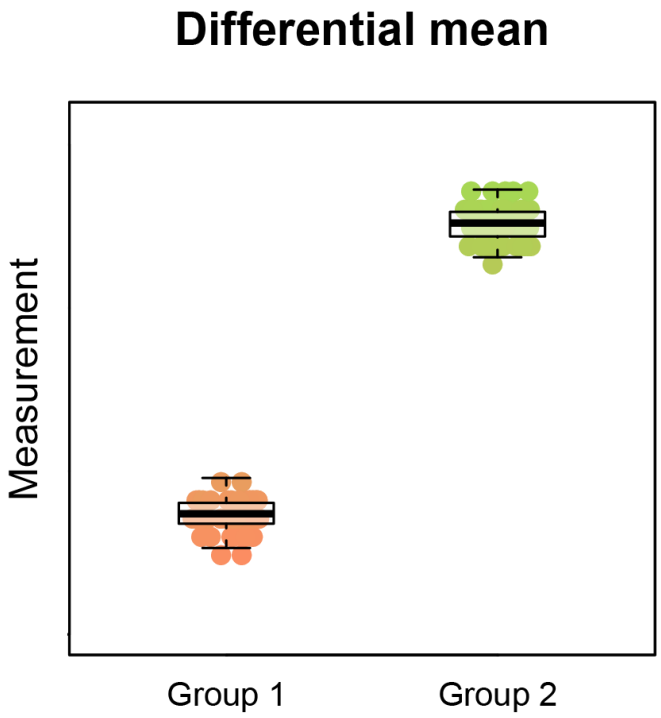
- DNA methylation patterns directly related to known subtypes
- But no separation of M-CLL and U-CLL in gene expression data

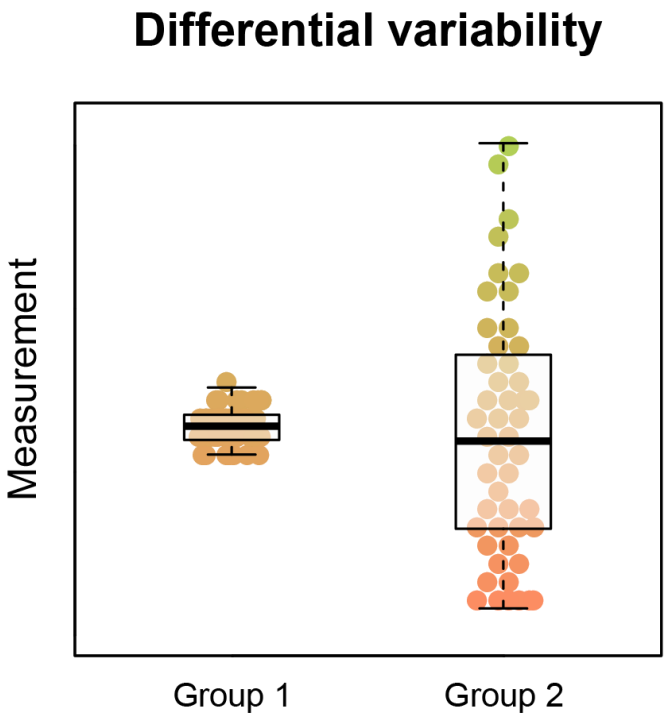


- Difference in variability between M-CLL and U-CLL?

Differential variability

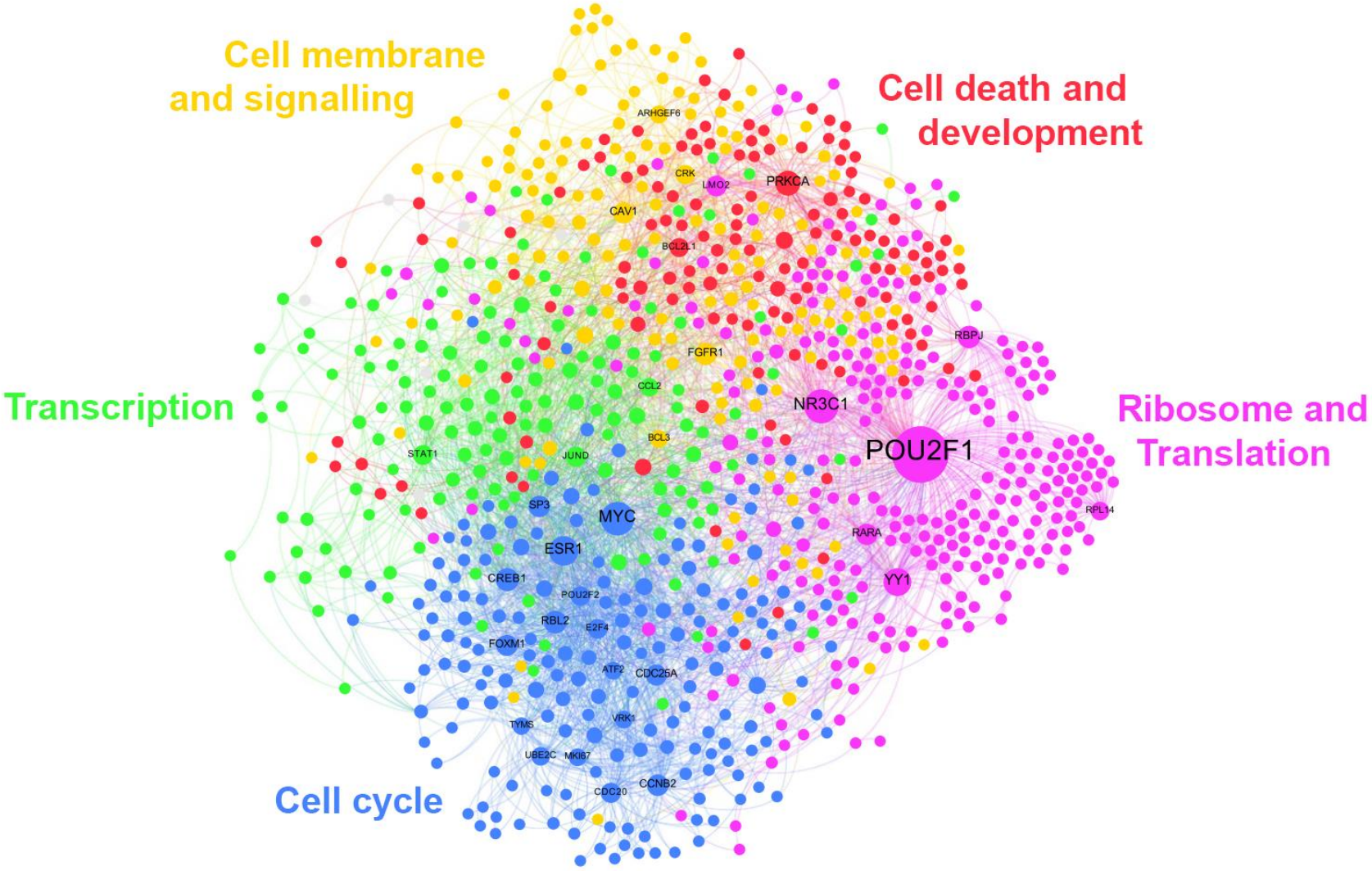
- Differential mean → different but consistent mean
- Differential variability → small vs large deviations from mean





- No difference between M-CLL and U-CLL in gene expression
- Increased variability in U-CLL!

Variability significantly increased in U-CLL



Interactions taken from Lefebvre et al, Mol Syst Biol, 2010. Figure adapted from Ecker et al, Genome Med, 2015.

BLUEPRINT

- Large-scale European research effort of the International Human Epigenome Consortium (IHEC)
- Generation of ≥ 100 reference epigenomes of distinct normal human hematopoietic cells and their malignant counterparts
- Investigation of biological processes and mechanisms systematically linking epigenetic variation with phenotypic plasticity

BLUEPRINT Human Variation Epigenome Project

- Chen *et al.* Genetic drivers of epigenetic and transcriptional variation in human immune cells. *Cell.* 2016.
- Ecker *et al.* Genome-wide analysis of differential transcriptional and epigenetic variability across human immune cell types. *Genome Biol.* 2016.

Differential variability in normal blood cells

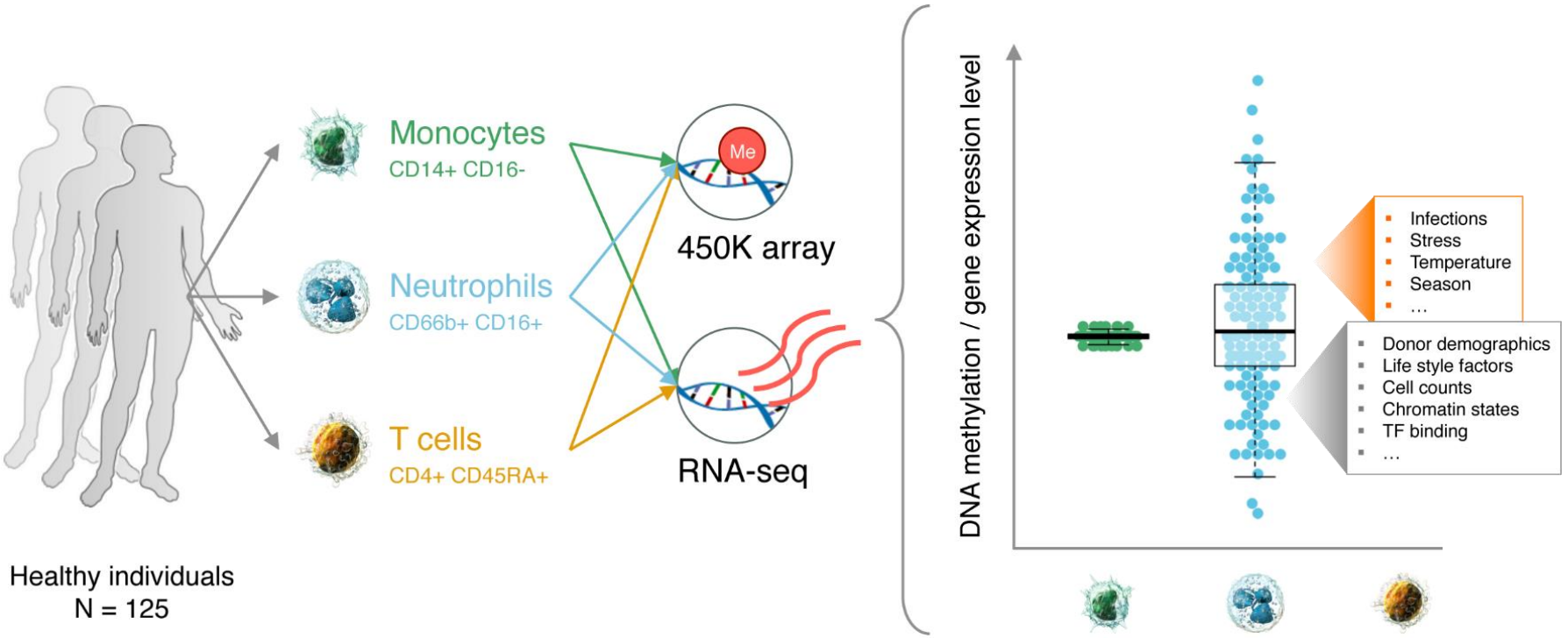


Figure adapted from Ecker et al, Genome Biol, 2016.

Increased variability in neutrophils

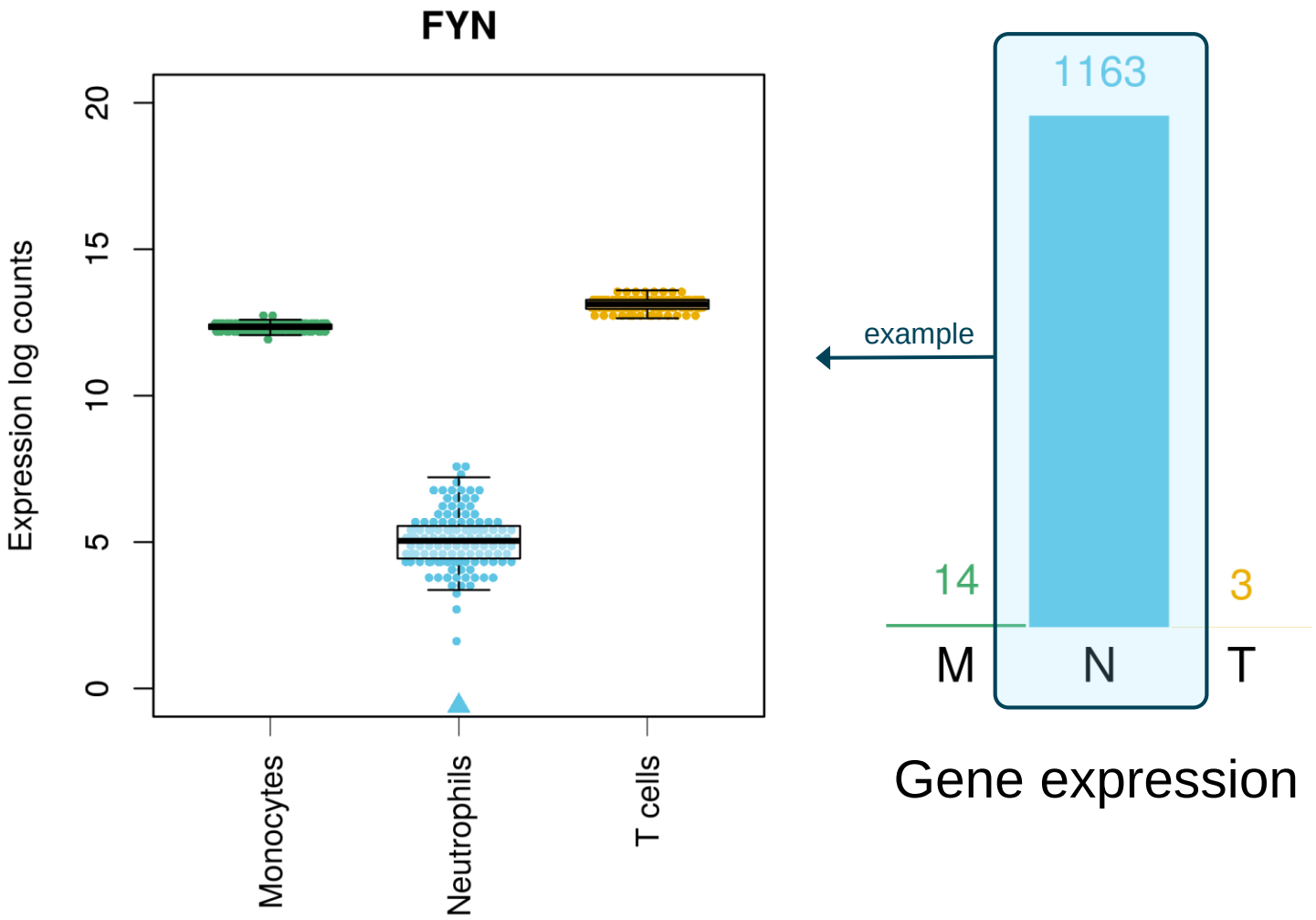


Figure adapted from Ecker et al, Genome Biol, 2016.

Sex-specific differential expression

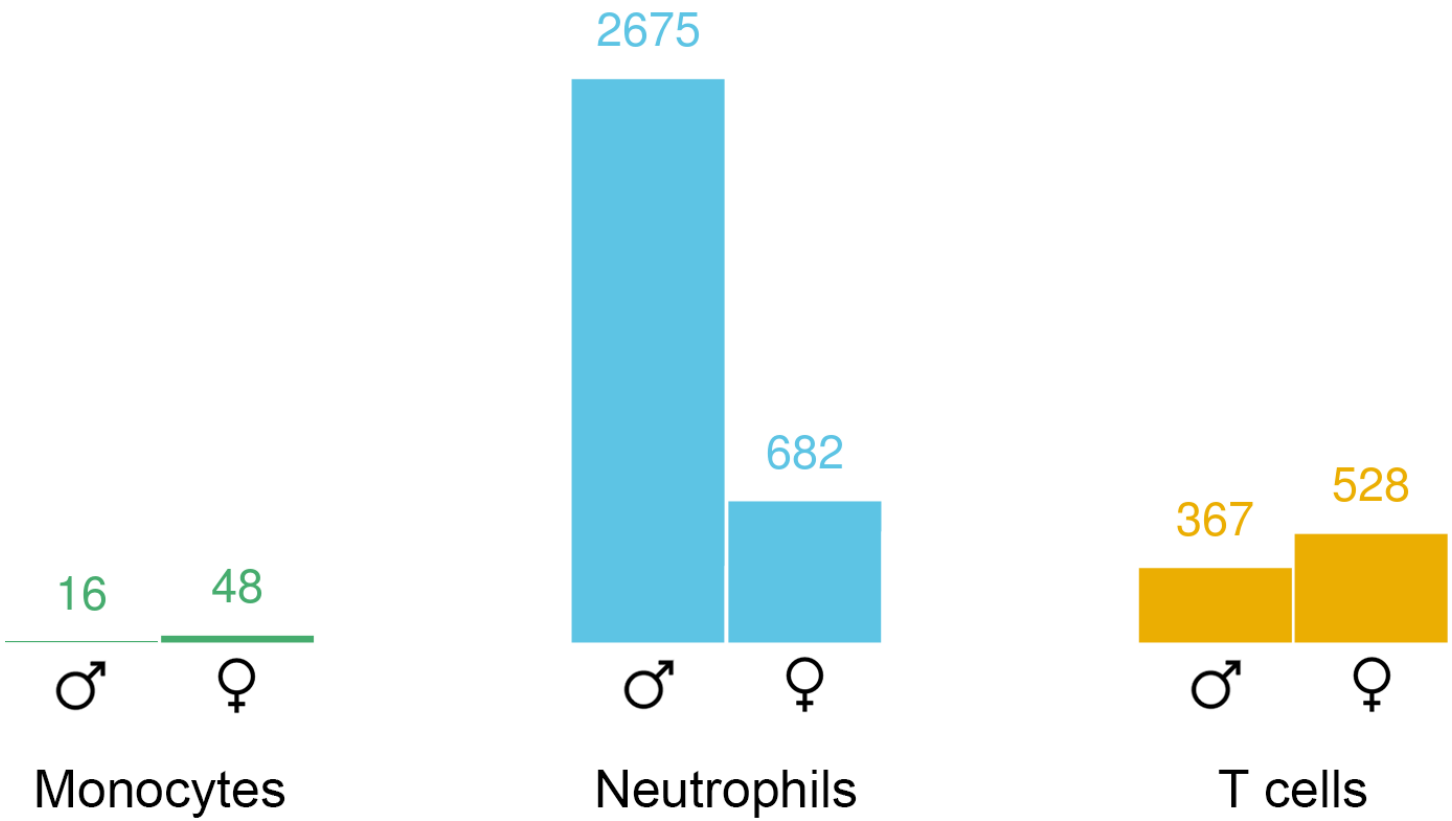


Figure adapted from Ecker et al, Genome Biol, 2016.

Blue Zones: Highest longevity in the world

Image adapted from www.bluezones.com and www.ezilon.com

Blue Zone of Nicoya

- Nicoya peninsula is the largest Blue Zone in the world
- Nicoyans 60 years old have seven times the probability of reaching 100 years compared to the rest of Costa Rica

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The Nicoya region of Costa Rica: a high longevity island for elderly males

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Abstract

Reliable data show that the Nicoyan region of Costa Rica is a hot spot of high longevity. A survival follow-up of 16,300 elderly Costa Ricans estimated a Nicoya death rate ratio (DRR) for males 1990–2011 of 0.80 (0.69–0.93 CI). For a 60-year-old Nicoyan male, the probability of becoming centenarian is seven times that of a Japanese male, and his life expectancy is 2.2 years greater. This Nicoya advantage does not occur in females, is independent of socio-economic conditions, disappears in out-migrants and comes from lower cardiovascular (CV) mortality (DRR = 0.65). Nicoyans have lower levels of biomarkers of CV risk; they are also leaner, taller and suffer fewer disabilities. Two markers of ageing and stress—telomere length and dehydroepiandrosterone sulphate—are also more favourable. The Nicoya diet is prosaic and abundant in traditional foods like rice, beans and animal protein, with low glycemic index and high fibre content.

1 Introduction

A book published by the National Geographic Society identified the region of Nicoya in Costa Rica as one of a very small number of locations in the world with exceptional longevity—regions that were referred to as *blue zones* (Buettner 2010). Other locations noted as having high longevity were the islands of Sardinia in Italy and Okinawa in Japan. Although the selection of Nicoya was mostly based on unpublished evidence, more than a century earlier a Swiss geographer and botanist had observed: "in no other place people blessed with such long lives" (Pittier 1904). Recently, this high longevity has been examined in a population-based sample of elderly Costa Ricans, finding that Nicoya indeed has a significantly lower death rate ratio of 0.71 compared to the rest of the country (Rosero-Bixby and Dow 2012), which is an extraordinary result given the already high life expectancy of elderly Costa Ricans in general (Rosero-Bixby 2008).

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Longer leukocyte telomere length in Costa Rica's Nicoya Peninsula: A population-based study

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ABSTRACT

Studies in humans suggest that leukocyte telomere length may act as a marker of b
ed whether individuals in the Nicoya region of Costa Rica, known for exceptional l
length than those in other parts of the country. After controlling for age, age squa
gender, the mean leukocyte telomere length in Nicoya was substantially longer (
in other areas of Costa Rica, providing evidence of a biological pathway to which
related. This relationship remains unchanged (79 base pairs, p < 0.05) after statist
potential biological, dietary and social and demographic mediators. Thus the diffe
telomere length that characterizes this unique region does not appear to be explain
and biological risk factors. More detailed examination of mean leukocyte telomere
regional telomere length difference declines at older ages.

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1. Introduction

Telomeres are repetitive canonical sequences of DNA at the ends of chromosomes that, together with associated proteins, protect chromosome ends and also prevent the degradation of coding regions of DNA that would otherwise result from the inability of DNA replication enzymes to copy the end of a DNA strand. Although the functional importance of telomeres has been understood for decades, their implication in the human aging process has begun to emerge more recently. Shorter telomeres were first found to occur at older ages (Lee et al., 2002), and more recent work has shown associations with chronic disease (Brouilette et al., 2007) and mortality, independent of age (Bakaysa et al., 2007; Cawthon et al., 2003; Honig et al., 2006). However, associations with mortality have not been consistent, especially for individuals at older ages (Boonekamp et al., 2013; Mather et al., 2011). Furthermore, the social and economic determinants of telomere length are not yet clear. There is some evidence that shorter telomeres are associated with chronic stress (Epel et al., 2004; nomic position, less educational attainment et al., 2009; Cherkas et al., 2006; Steptoe et a that associations are stronger with earlier life position, such as education (Needham et al., Surtees et al., 2012).

The Nicoya Peninsula region in Costa Ri acterized as a region with exceptionally l 2010). Mortality rates of elderly people lower than in the rest of Costa Rica accor up of a population-based sample of close t 60 years and over (Rosero-Bixby and De two or three years of additional life expec traordinary result given the already high l Costa Ricans in general (Rosero-Bixby, 20 Nicoyan mortality advantage – and even t and morbidity levels in Nicoya – are not k the recentness of the discovery of this are of high longevity. The present article aims gap about health in Nicoya by studying a an indicator of biological aging.

In the current study we examine leuko in a nationally representative population-based study of Costa Ricans. Understanding whether LTL in Nicoya differs from other Costa Rican

Abbreviations: LTL, leukocyte telomere length; CRELES, Costa Rican Study on Longevity and Healthy Aging; BMI, body mass index; GWAS, genome wide association study; bp, blood pressure.

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NATURE | NEWS

Poorest Costa Ricans live longest

Biological markers confirm unusually slow ageing regardless of wealth.

Jo Marchant
03 September 2013

Costa Rica's Nicoya Peninsula is considered one of the world's most beautiful places — a 120-kilometre sliver of land where tropical rainforest meets the Pacific Ocean. Now it has another claim to fame: its residents, despite living in poverty, seem to be ageing more slowly than wealthier people elsewhere.[1]

DNA methylation pilot study

McEwen *et al. Epigenetics & Chromatin (2017) 10:21*
DOI 10.1186/s13072-017-0128-2

Epigenetics & Chromatin

RESEARCH Open Access

Differential DNA methylation and lymphocyte proportions in a Costa Rican high longevity region

Lisa M. McEwen[1], Alexander M. Morin[1], Rachel D. Edgar[1], Julia L. MacIsaac[1], Meaghan J. Jones[1], William H. Dow[2], Luis Rosero-Bixby[3], Michael S. Kobor[1] and David H. Rehkopf[4*]

Abstract

Background: The Nicoya Peninsula in Costa Rica has one of the highest old-age life expectancies in the world, but the underlying biological mechanisms of this longevity are not well understood. As DNA methylation is hypothesized

Pilot data set

- Cross-sectional subsample (n=95) of Costa Rican Longevity and Healthy Aging Study (CRELES, n>2500)
- Age-matched whole blood DNA methylation (450K array) of Nicoyans and non-Nicoyan Costa Ricans in two age groups

Table 1 Cohort characteristics (means and percents), Nicoyans and non-Nicoyans

Characteristics	Nicoya ($n = 48$)	Non-Nicoya ($n = 47$)
Age (mean in years)	83 (14)	85 (16)
Female (%)	57	55
Low education (%)	80	68
Low wealth (%)	35	21
Currently smoke (%)	4	6
Systolic blood pressure (mean mmHg)	139 (23)	140 (25)
Diastolic blood pressure (mean mmHg)	78 (12)	78 (13)
Body mass index (mean)	24 (7.1)	25 (5.8)

McEwen et al., Epigenetics Chromatin, 2017.

The epigenetic drift

- Epigenetic variability increases with age
- First shown in monozygotic twins *[Fraga et al., PNAS, 2005]*
- Also happens in the general population
- Increased epigenetic variability is associated with disease

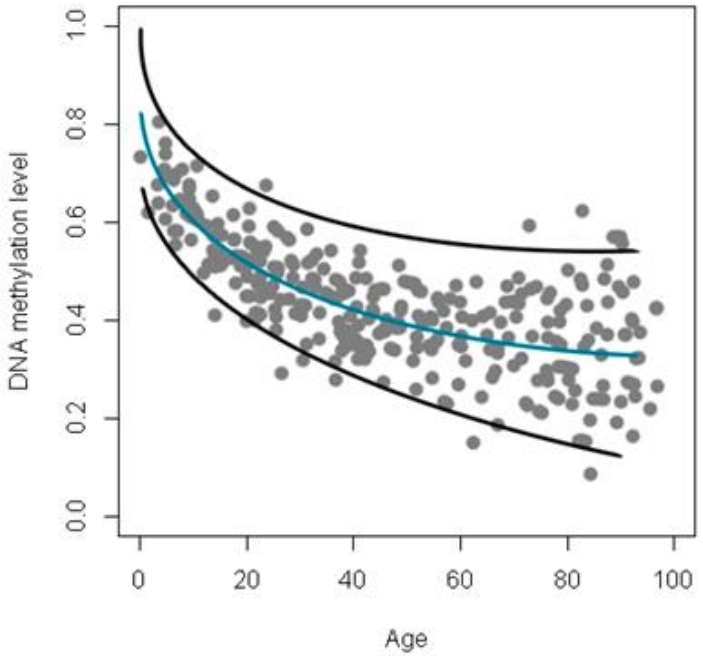
Epigenetic differences arise during the lifetime of monozygotic twins

Research

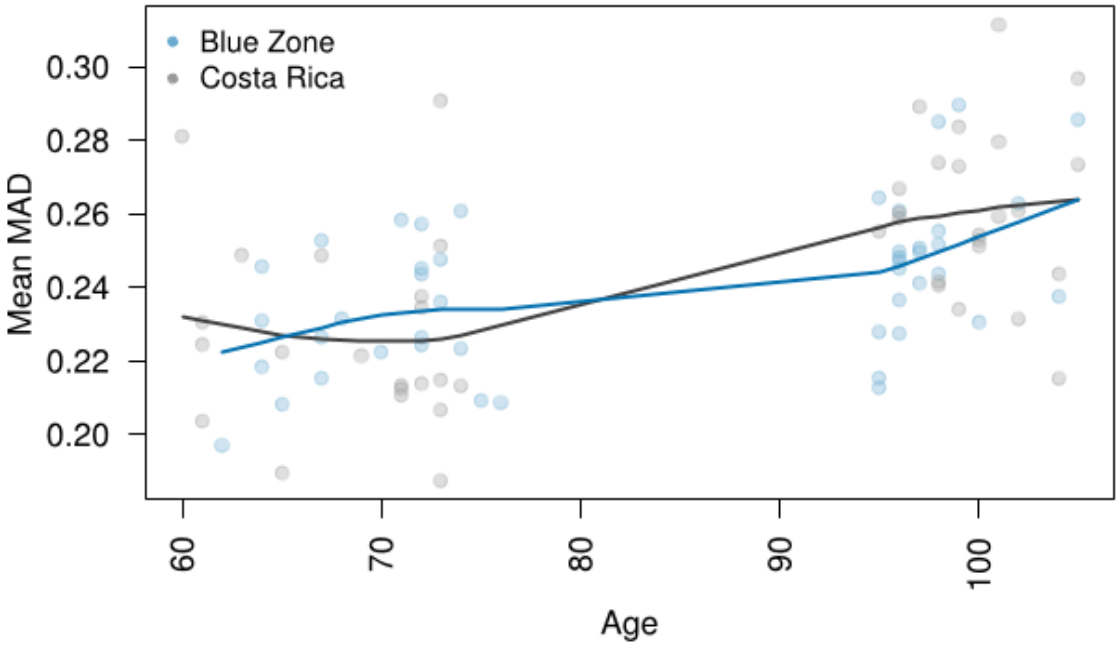
Genetic and environmental exposures constrain epigenetic drift over the human life course

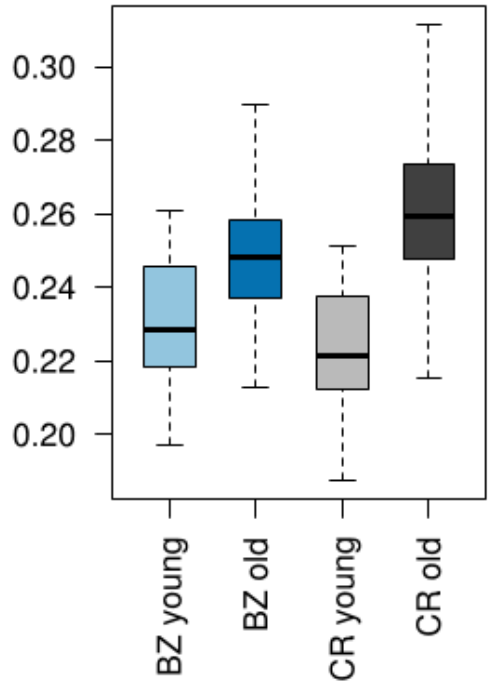
Commentary

Epigenetic Drift Is a Determinant of Mammalian Lifespan

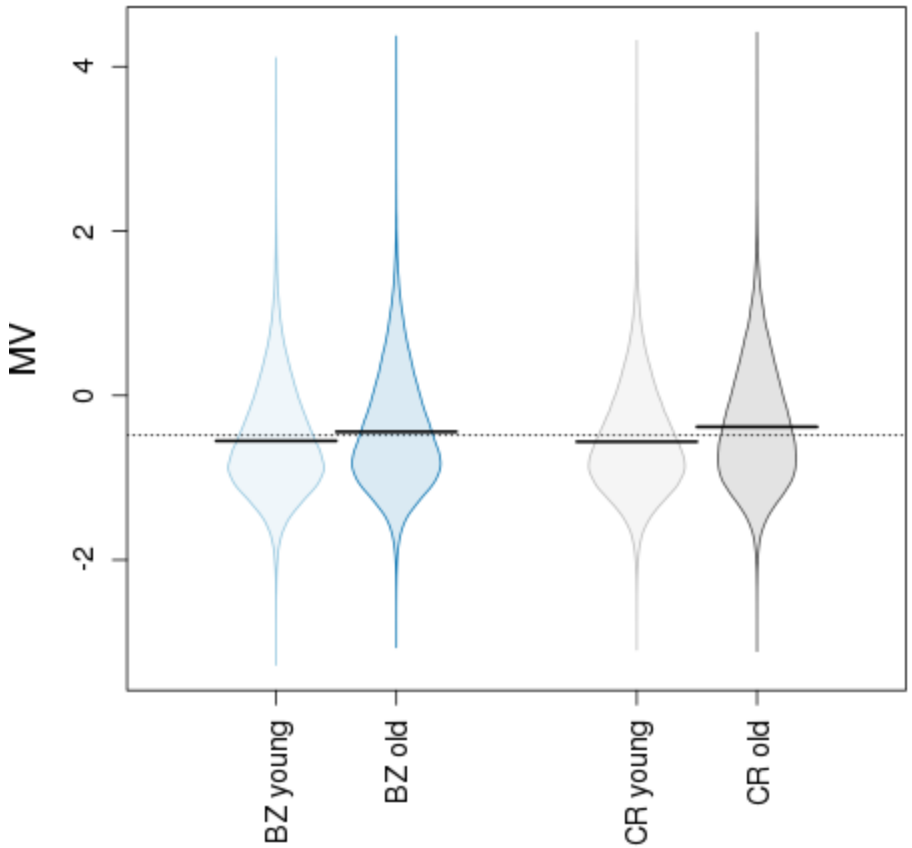


Global patterns of variability (sample-wise)



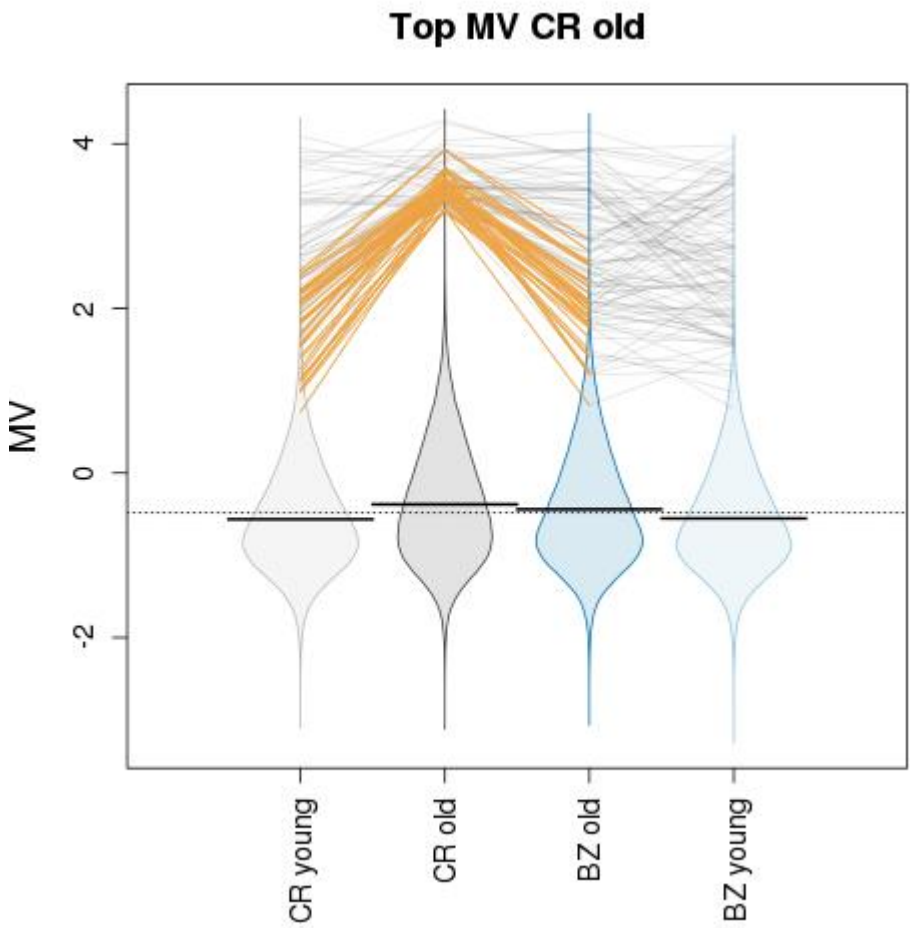


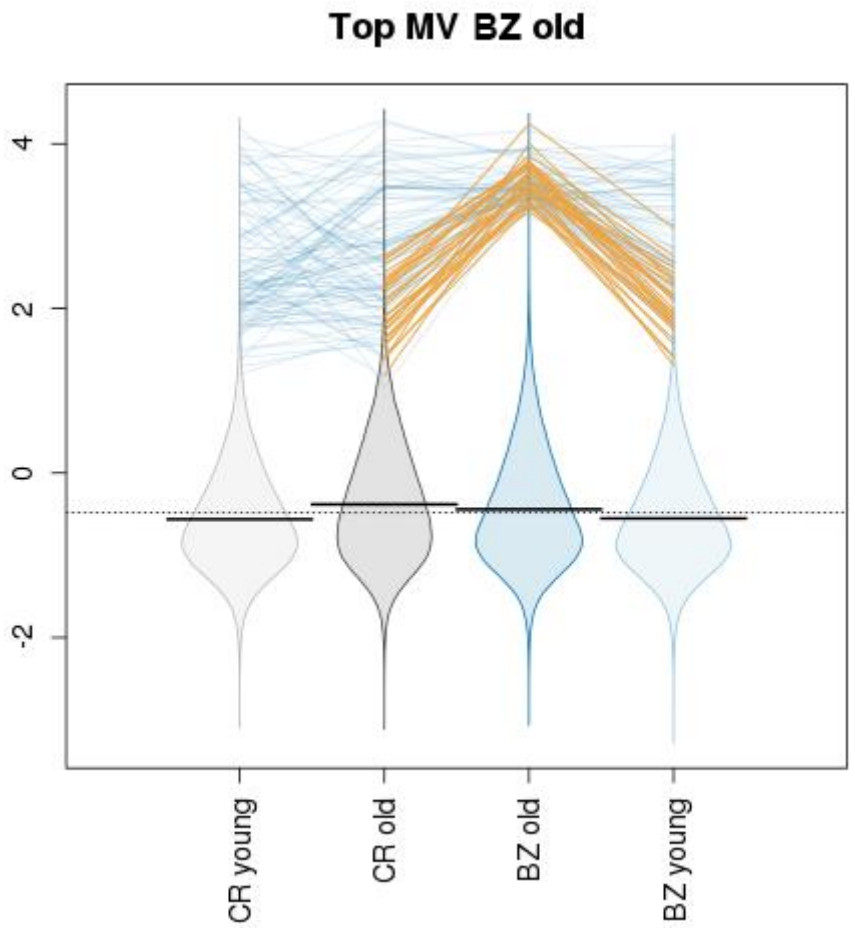
Global patterns of variability (CpG-wise)



MV-score derived by algorithm adapted from expression variability measurement developed by Alemu et al., NAR, 2014.

Top 100 variable CpGs





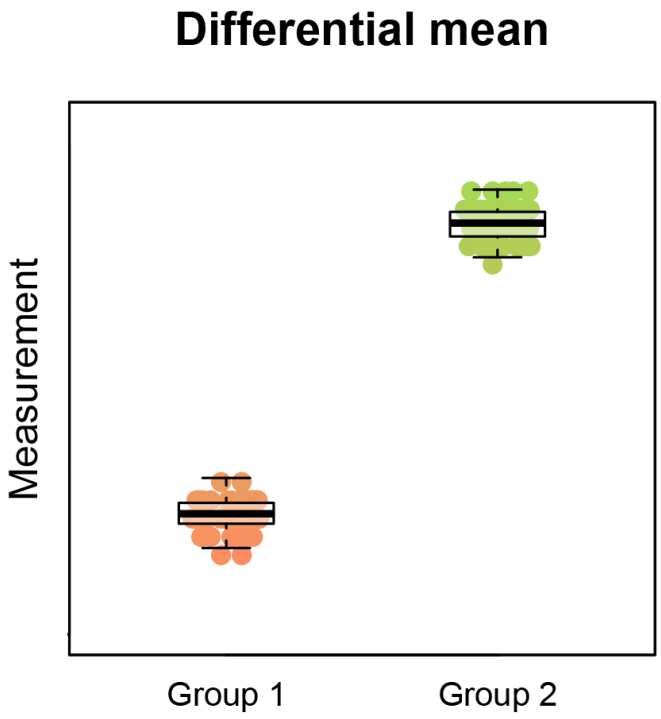
Top 100 CpGs with high variability in BZ

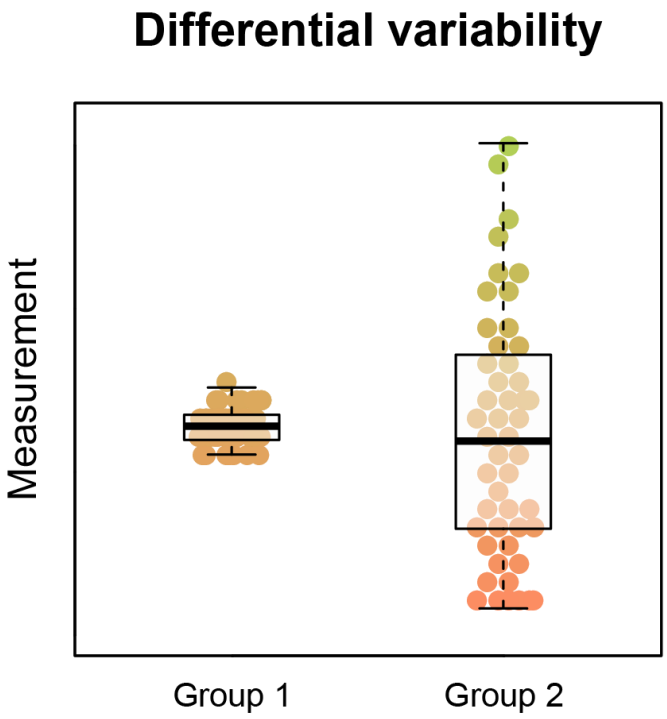
- **32 CpGs in 22 genes (9 in promoters, 17 in bodies, 6 in IGRs)**
  - ***GFPT2* (4 CpGs):** Glucose flux in hexosamine pathway
  - *MIR885* (3 CpGs)
  - ***RPTOR:*** Control of **rapamycin complex** activity in response to nutrient and hormonal signals
  - *KCNG2:* Potassium channel activity
  - *ALOXE3:* Lipoxygenase
  - *FN3K:* Deglycation of proteins and fructoselysine
  - *CCS:* Copper delivery, copper chaperone
  - …
- **GREAT functional enrichment (> 20 significant results), e.g.:**
  - Glutamine metabolic process
  - Sugar biosynthetic process
  - Regulation of fatty acid biosynthetic process

GREAT: McLean et al., Nat Biotechnol, 2010.

Differential variability

- Differential mean → different but consistent mean
- Differential variability → small vs large deviations from mean



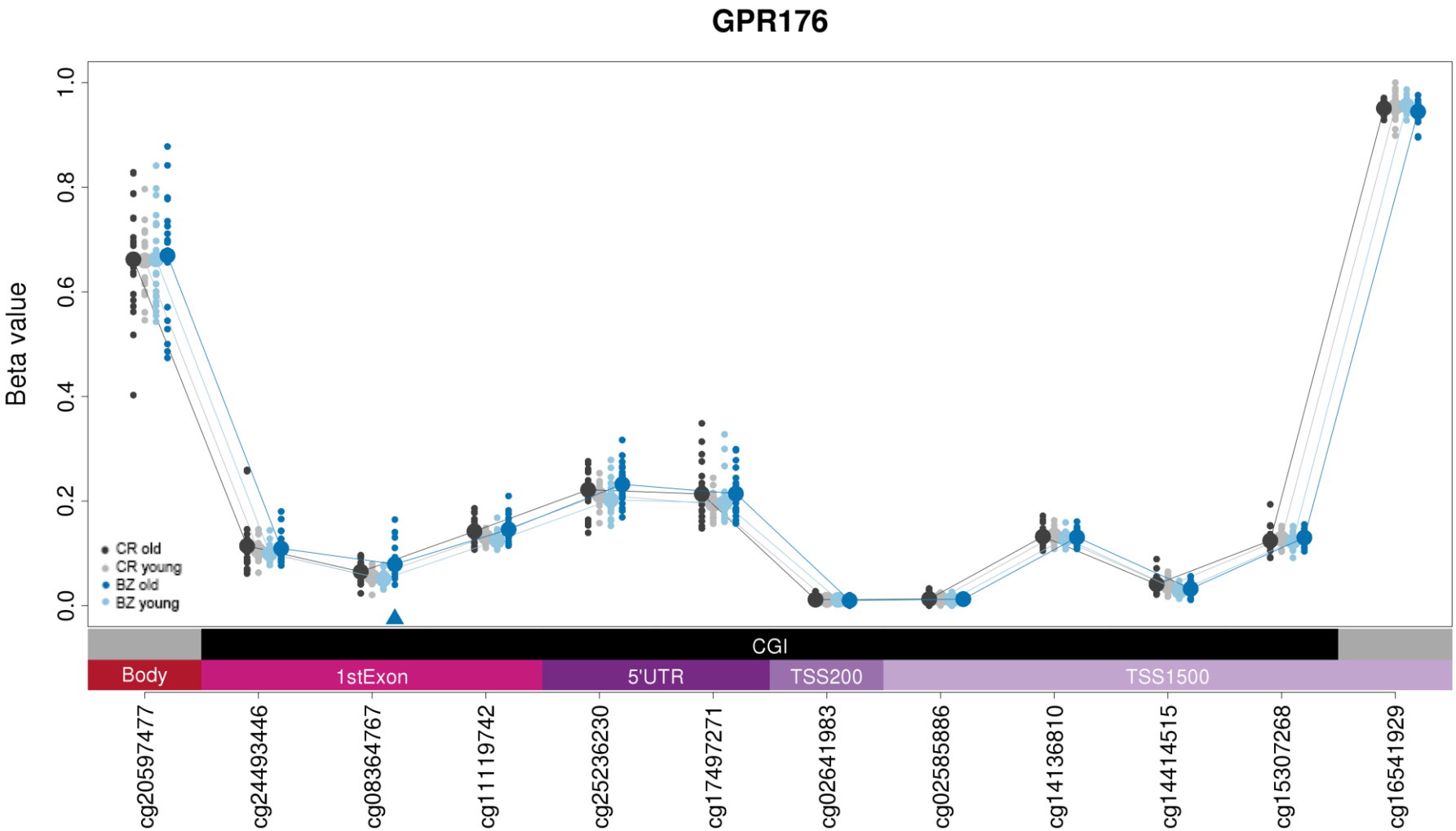


Statistically significant differential variability

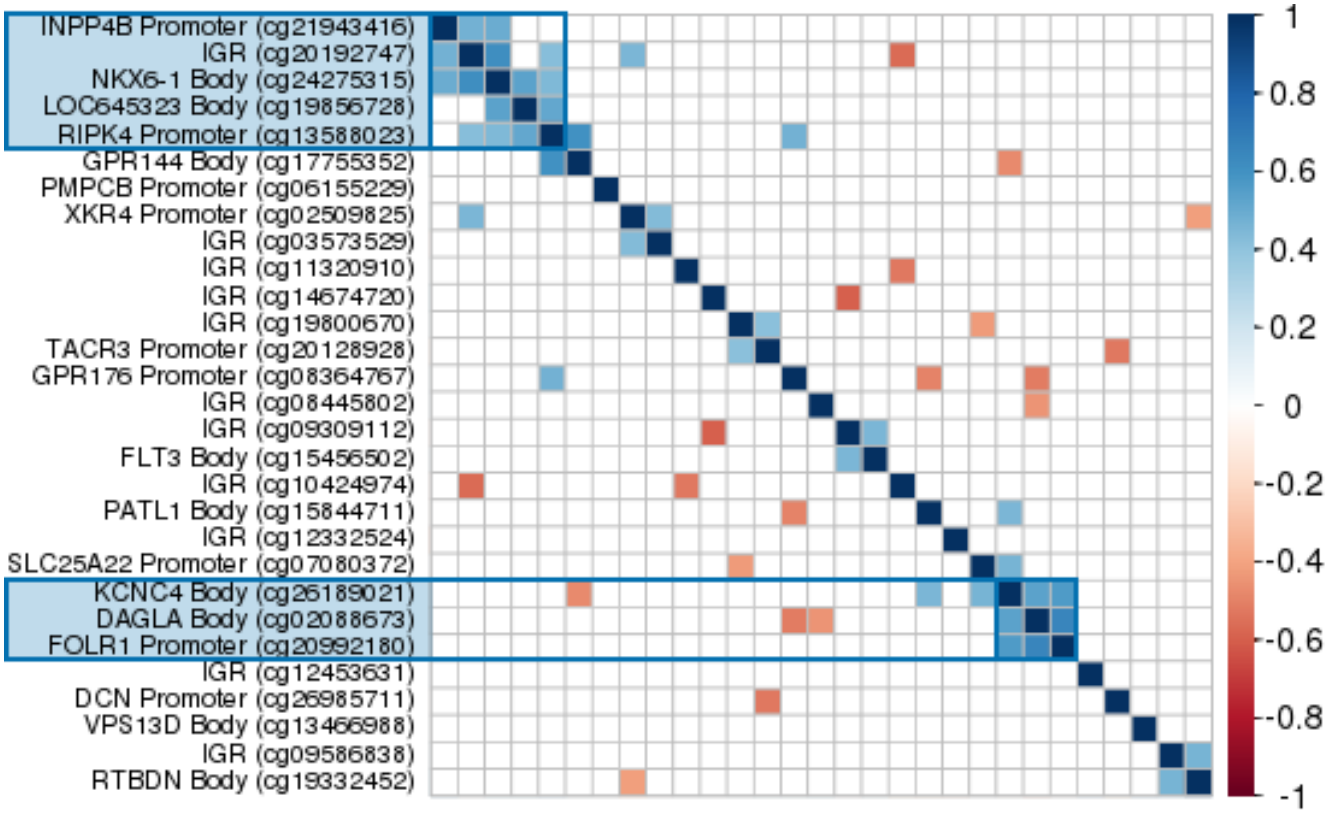
- **Applied combination of DiffVar and MV-score**
  Adjusted p-value (BH) < 0.05 and MV-difference ≥ 10%
- CR old versus young: 0 CpGs ↑ and 0 CpGs ↓
- BZ old versus young: **29 CpGs** ↑ and 5 CpGs ↓
  18 genes (9 in promoter, 9 in body, 11 in IGR), e.g.:
  - *NKX6-1:* Insulin secretion, glucose detection, response to nicotine
  - *FOLR1:* Folic acid receptor, drug binding
  - *GPR176:* G-prot coupled receptor, response to hormones
  - *DAGLA:* Lipid metabolism, metal binding, G-prot coupled receptor
  - *FLT3:* ATP binding, glucocorticoid receptor binding
  - *RIPK4:* Serine/Threonine Kinase, ATP binding
  - *KCNC4:* Ion and potassium channel activity
  - *TACR3:* Regulation of heart rate, blood pressure

DiffVar: Phipson & Oshlack, Genome Biol, 2014. MV: Alemu et al., NAR, 2014. Ecker et al., Genome Biol, 2017.

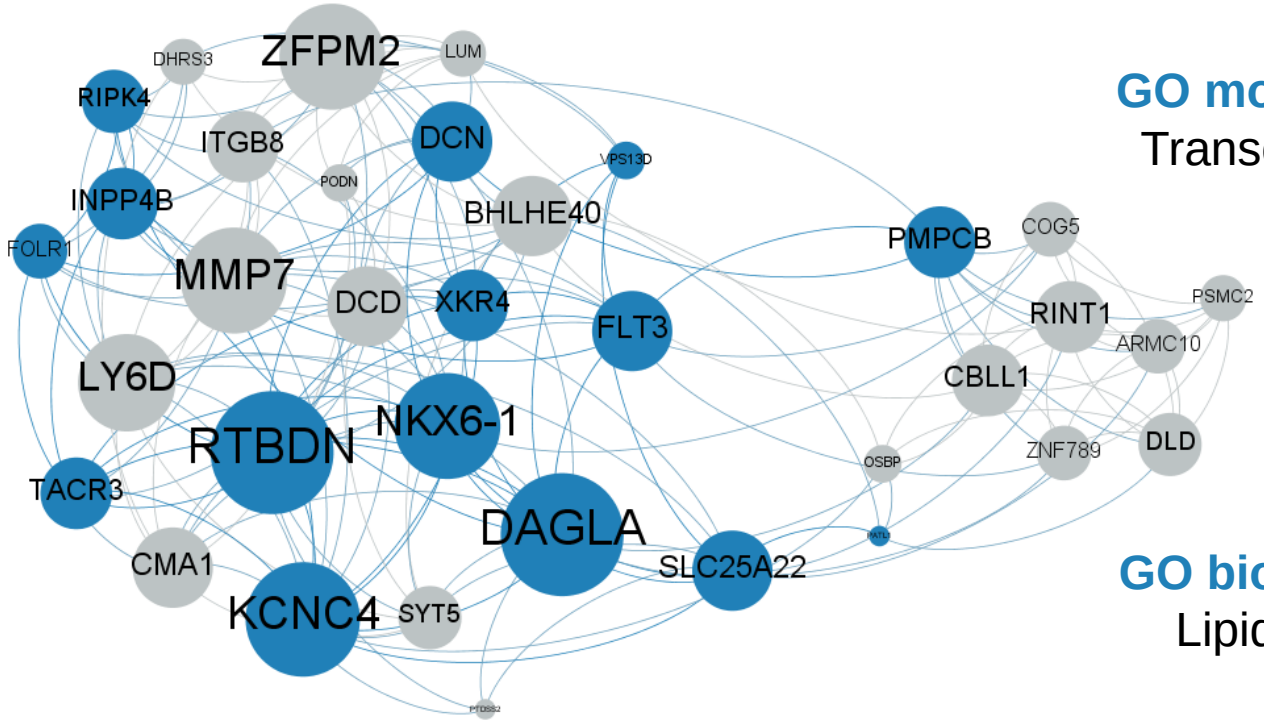
Increased variability in BZ old versus young



Correlation between hypervariable CpGs



Network of genes with sig increased variability



GO cellular compartments:
Extracellular matrix

GO molecular functions:
Transcriptional regulation

GO biological functions:
Lipid metabolic process
Aging
Cardiovascular and circulatory system
JAK-STAT cascade
Organ morphogenesis
Response to cytokine stimulus

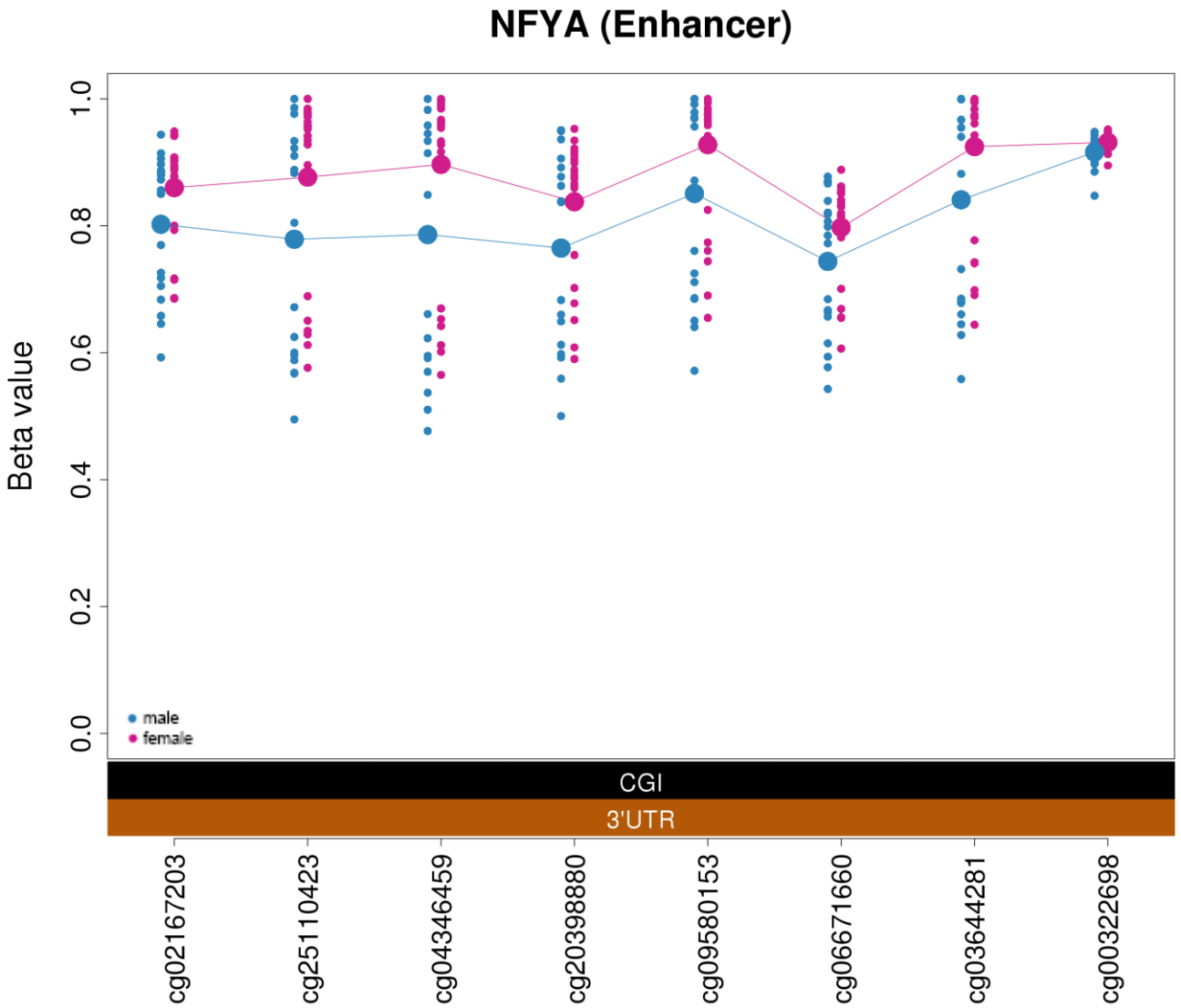
- Significantly increased DNAm variability
- Neighboring genes

Sex-specific differential methylation

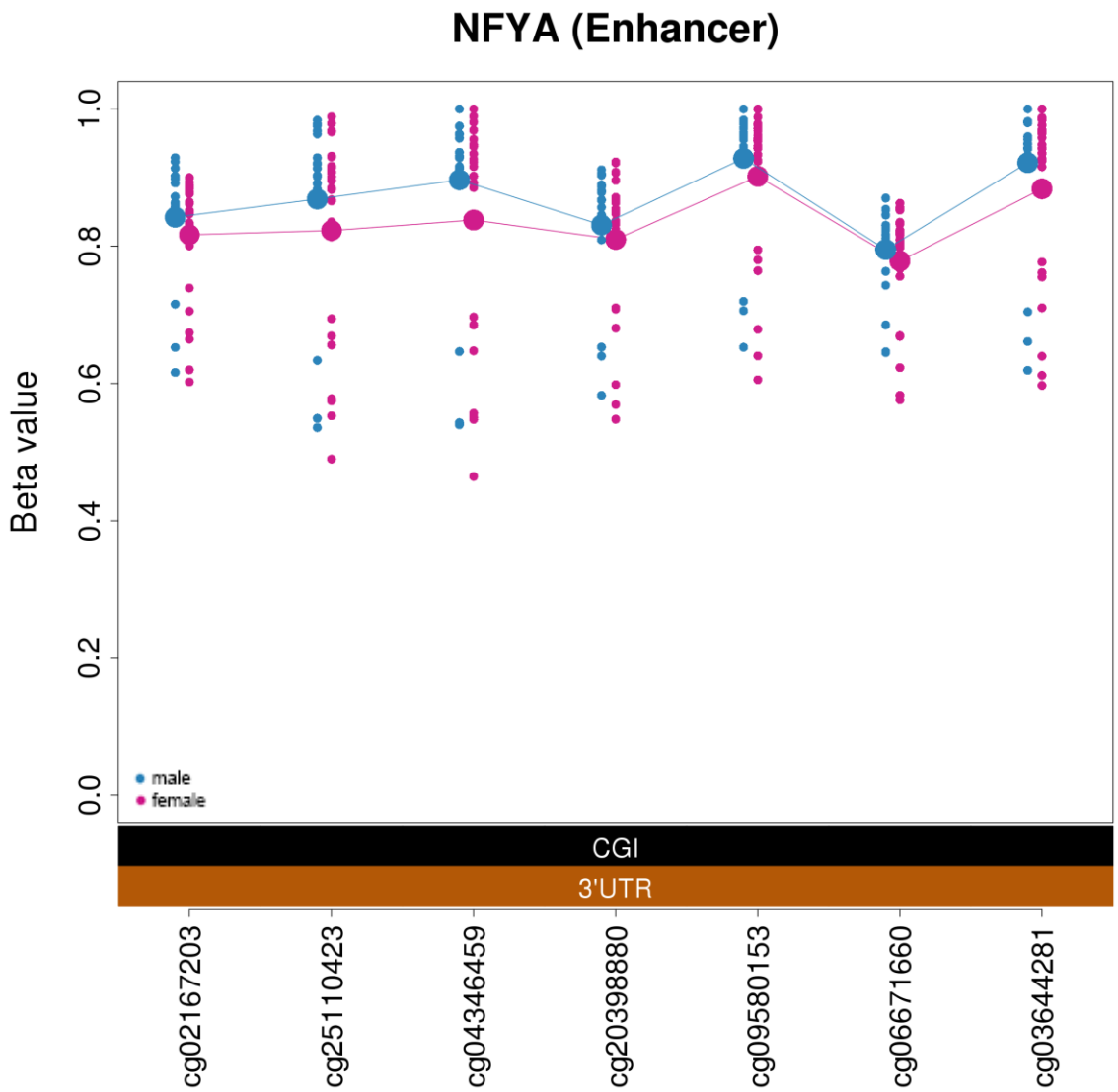
- **Applied Bumphunter and DMRCate**
  Adjusted p-value (BH) < 0.05 and region ≥ 3 neighboring CpGs
- BZ male versus female: 2 CpGs ↑ and **10 CpGs** ↓
  8 genes (5 in promoter, 3 in body, 2 in IGR), e.g.:
  - ***NFYA:*** TF subunit, enhancer, regulation of cholesterol biosynthesis
  - *OR2L12:* Olfactory receptor, G-prot coupled receptor activity
  - *DOX43:* RNA binding, ATP binding
  - *PSM4A8:* Proteasome subunit, hydrolase activity, spermatogenesis
  - *PRRT1:* Proline rich transmembrane protein, response to stimulus
  - *ASCL2:* TF activity, multicellular organism development
- CR male versus female: **3 CpGs** ↑ and 6 CpGs ↓
  3 genes (2 in promoter, 1 in body), e.g.:
  - ***NFYA:*** TF subunit, enhancer, regulation of cholesterol biosynthesis

Bumphunter: Jaffe et al., Int J Epidemiol, 2012. DMRCate: Peters et al., Epigenetics Chromatin, 2015.

NFYA hypomethylation in males of BZ



NFYA hypermethylation in males of CR



Conclusion and discussion

- Pathways and functions involved in all BZ-specific patterns:
  - Nutritional factors, metabolism, hormone signalling *(GFPT2, RPTOR, FOLR1, NKX6-1, NFYA)*
  - Serine/threonine phosphatase
  - G-protein coupled receptor activity
- Discussion:
  - Possible technical and biological confounders
  - Cell subpopulations
  - Relation single cell variability and interindividual variability
  - Larger dataset necessary
- Future plan:
  - 1000 methylomes and matched genotypes currently processed
  - Integration of further healthy aging cohorts
  - Longitudinal follow-up of CRELES participants

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