DNA methylation clock and drift in aging

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DNA methylation

Cytosine (in DNA)

5-Methylcytosine

DNMTs

SAM

SAH

Maintenance

DNMTs
Typical mammalian DNA methylation landscape
Why study DNA methylation?

- Cell-type-, stage-specific
- Mutable, can be influenced by external factors
- Changes that occur over time
- Medium-term indicator, not as acute as transcription
- Regulates transcription and more....
Why study DNA methylation in aging?

Aging is characterized by global HYPOmethylation!

Key:
- Unmethylated CpG
- Methylated CpG
- Transposable elements

Pal and Tyler, Science advances 2016
Caloric restriction (CR) and epigenetic drift

Pal and Tyler, Science Advances 2016
Hahn et al, Genome Biology 2017
Increased endogenous bilirubin mimics CR
Gilbert’s syndrome

Heme $\rightarrow$ Biliverdin $\rightarrow$ BLVRA $\rightarrow$ Unconjugated Bilirubin $\rightarrow$ Bilirubin glucuronosides $\rightarrow$ Stercobilin

$\text{HMOX1/2}$

NADPH/NADP$^+$

UDP glucuronate/UDP

$\text{UGT1A1}$

$\text{UGT1A1*1 (TA)$_6$}$

$\text{UGT1A1*28 (TA)$_7$}$

$\text{O}_2, \text{Fe}^{2+}, \text{CO}$
Bilirubin: the key to longevity?
Study design

UGT1A1*1 / Control

UGT1A1*28 / GS

20-34 y.o.
(n = 22)

35-70 y.o.
(n = 20)
Reduced Representation Bisulfite Sequencing

1. Purification of genomic DNA
2. Restriction enzyme digest
3. End repair
4. Adapter Ligation
5. A-Tailing
6. Bisulfite Conversion
7. PCR Amplification
8. Sequencing

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Calculating DNA methylation

\[
\% \text{Methylation} = \frac{M}{M + U} \times 100
\]

In the above example, the methylation level of the locus is 40%
The Horvath epigenetic aging clock

Based on Illumina 27K or 450K bead arrays; overlap with RRBS poor: 5628 and 66224 sites, respectively, out of more than 1 million sites in RRBS.
How to find CpG sites from RRBS data that can predict age?
Prediction models with elastic net regularisation
Linear model for age prediction
Logistic model for age prediction
How to find CpG sites that act as age clocks in healthy individuals but drift with age in GS?
Epigenetic clock vs. epigenetic drift

Differentially methylated sites with > 10% diff, $p < 0.05$

Jones et. al., 2015, *Aging cell*
Hypomethylated sites with age

Clock

Control

Drift

Gilbert’s
Hypomethylated sites with age

In healthy controls:
- 0.37% per year
p = 0.00169
Hypermethylated sites with age
In healthy controls:

0.47% per year

p = 0.000629
Overlap between clock CpG sites

Clock CpG sites hypermethylated with age

Clock CpG sites hypomethylated with age
Where are these sites located and what are their functions?

- Annotation in progress!!!
- No overlap with changes in RNA expression
- Only a very small fraction in gene promoter regions
- Many associate with gene bodies or IncRNAs
- High representation in transposable elements, especially Alu elements
Thanks!!!!