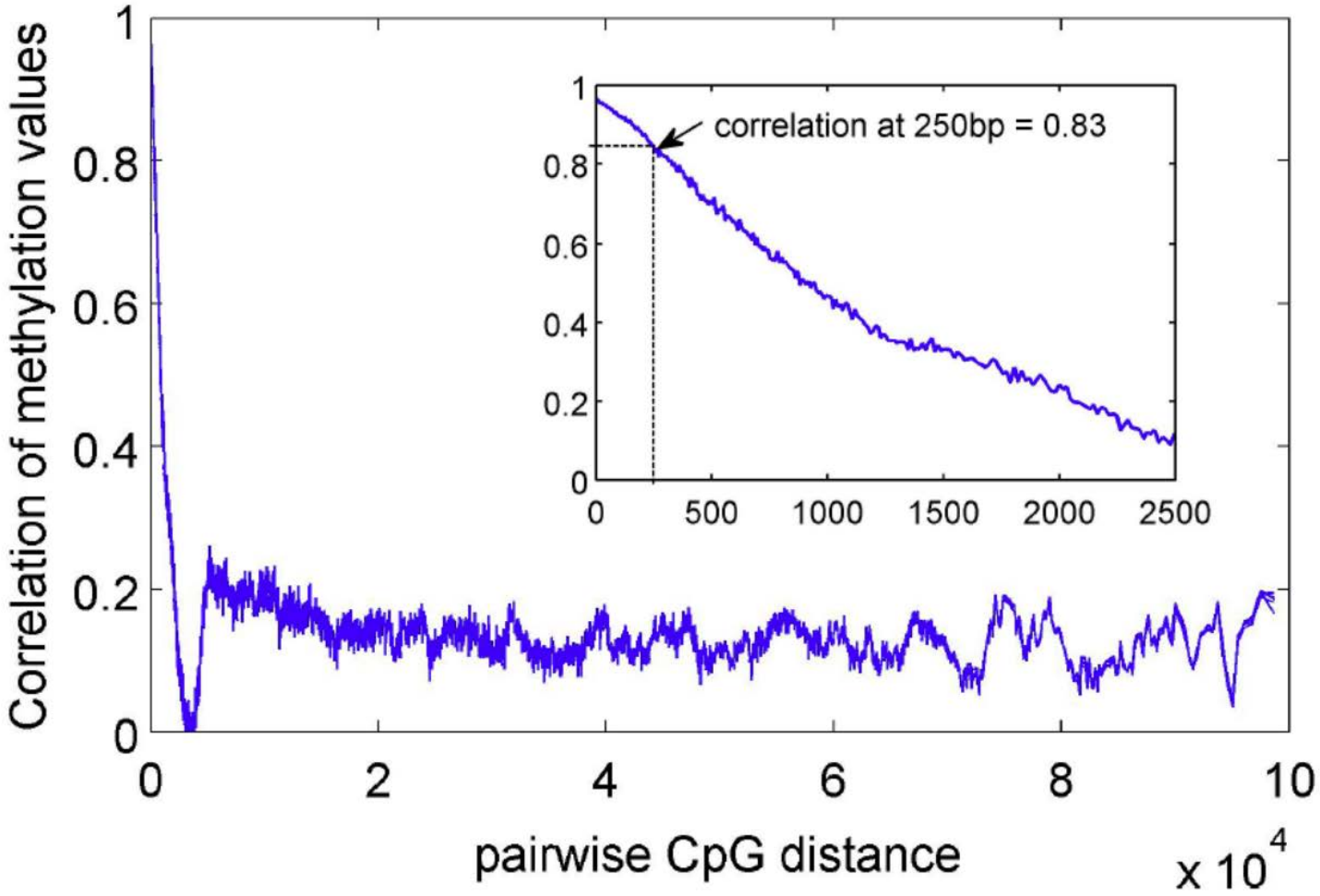
Outline for Session 4 (1.30 – 3.00pm)

- Multiple-testing
- Bumphunting
- Annotation

- Example of DNA methylation studies that are not EWAS
  - Genetic control of DNA methylation
  - Epigenetic aging

Probe correlation



Ong et al Aging Cell 2013

Multiple testing correction

- We are performing ~400,000 test per EWAS
- Expect a large number of nominally significant hits
  - 20,000 at $p < 0.05$
  - 400 at $p < 0.001$
- Need to determine an appropriate significance threshold

Multiple testing correction – “The Ugly”

- Use 5×10^{-8} because that is what we use in GWAS…

- We know the correlation structure in DNA methylation does not extend as far as SNP LD
- Probably not bad for the current generation of arrays…

Multiple testing correction – “The Bad”

- False Discovery Rate

- DNA methylation data is correlated
- Standard FDR approaches assume the data is independent

- Results in an inflation in the FDR

Multiple testing correction – “The Good”

- Bonferroni correction

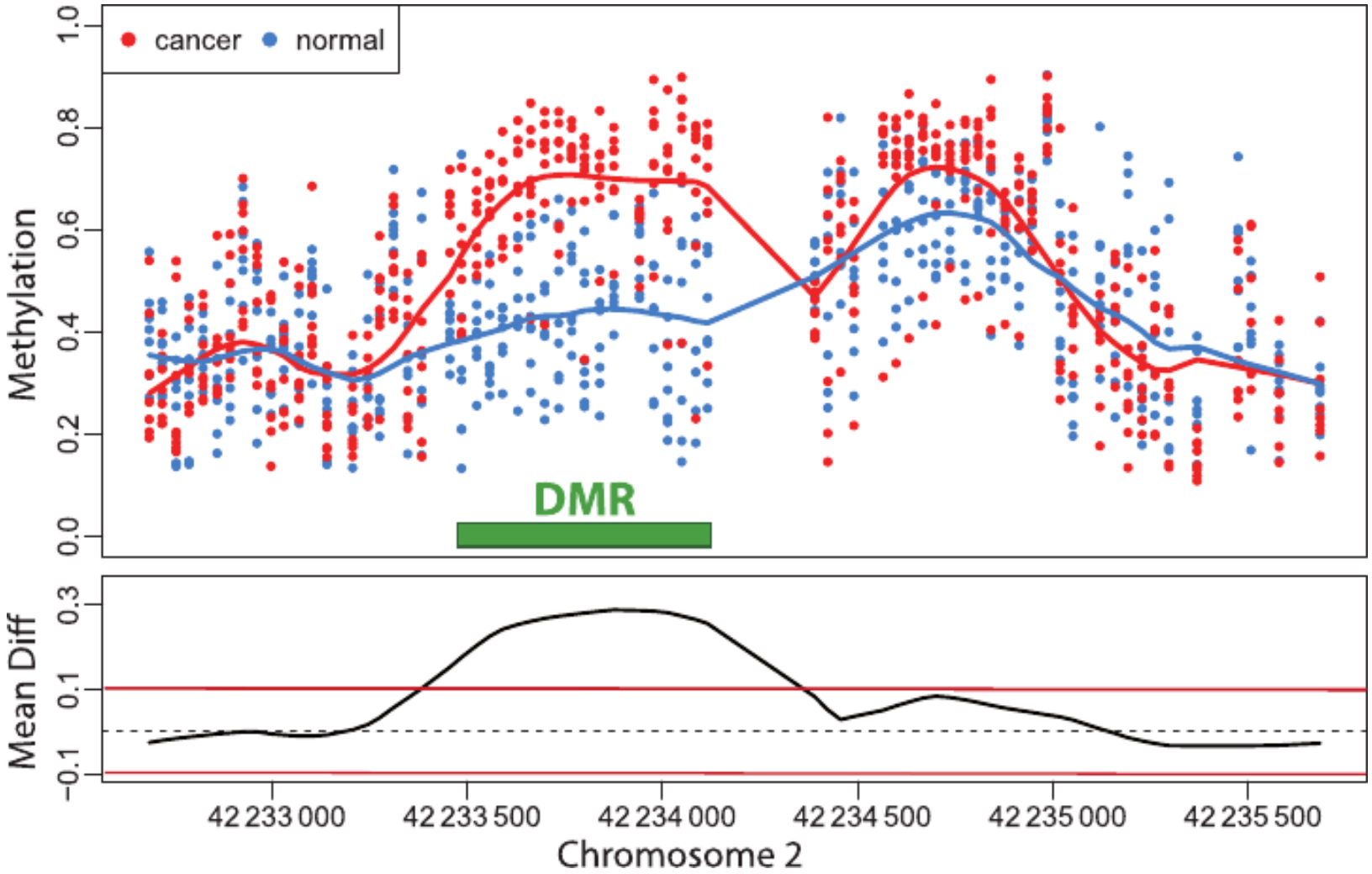
- Divide 0.05 by the number of test performed
- Assumes all tests are independent
- Results in conservative threshold (may miss true positives)

- 450K array 450,000 tests $p < 1.1 \times 10^{-7}$
- EPIC array 850,000 tests $p < 5 \times 10^{-8}$

Differentially methylated regions (DMRs)

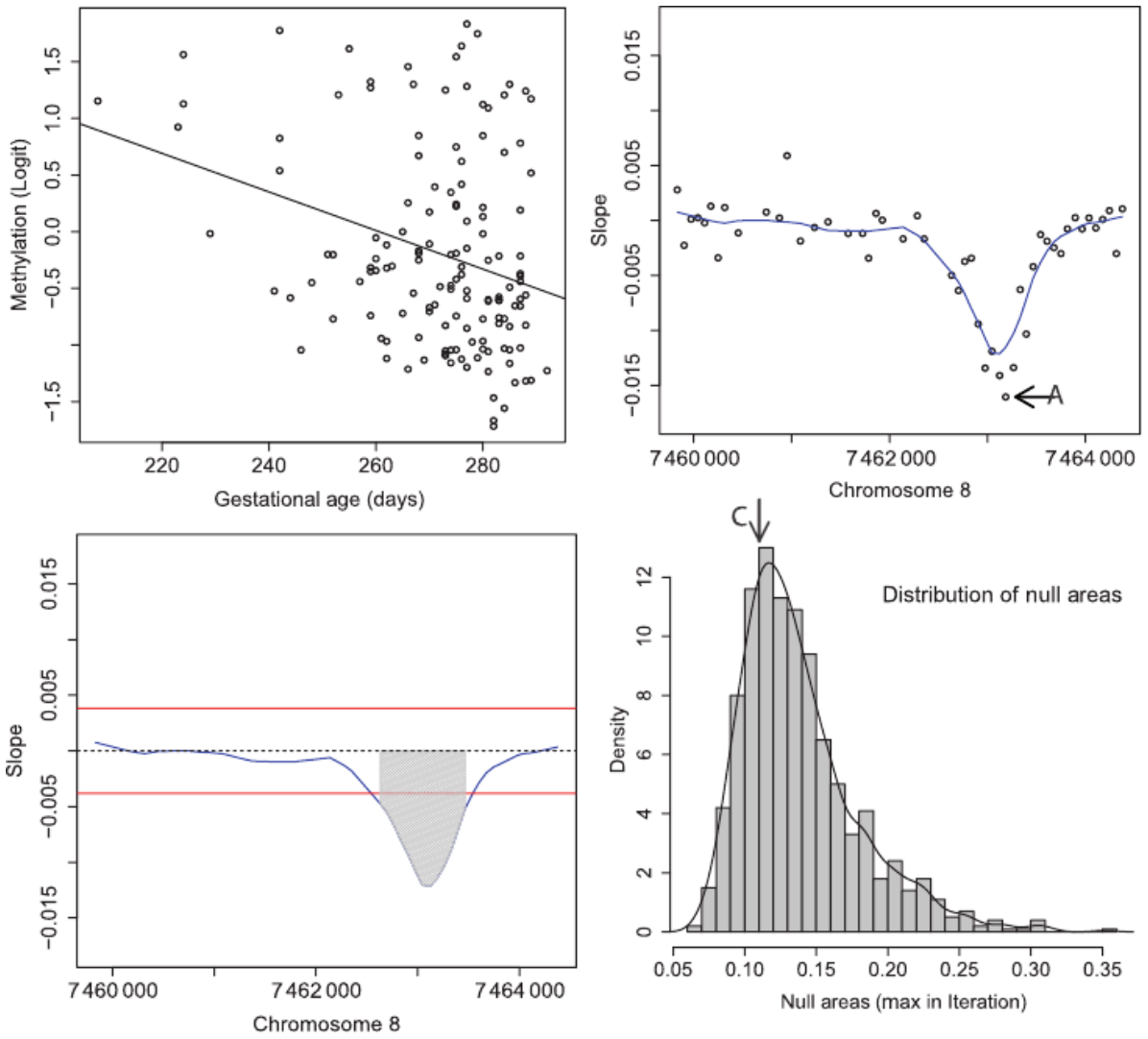
- Several methods have been proposed to look for “bumps” in EWAS results
- Look at combined evidence of association across multiple methylation sites
- May improve power if a region has multiple independent signals

Differentially methylated regions (DMRs)



Differentially methylated regions (DMRs)

- Bumphunter



Differentially methylated regions (DMRs)

- DMRcate

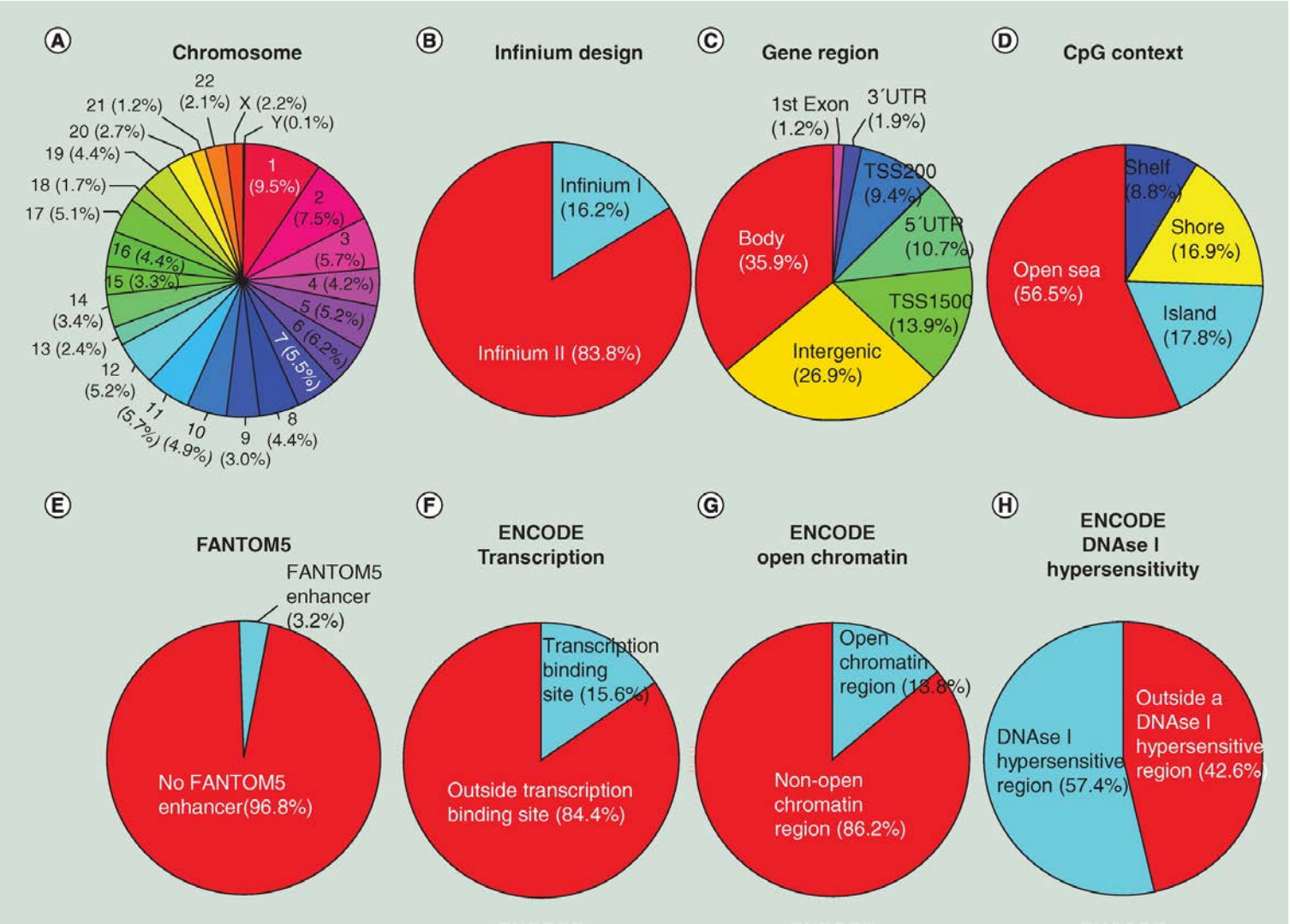
- Applies a similar approach to bumphunter
- Gives marked better results

Functional Annotation

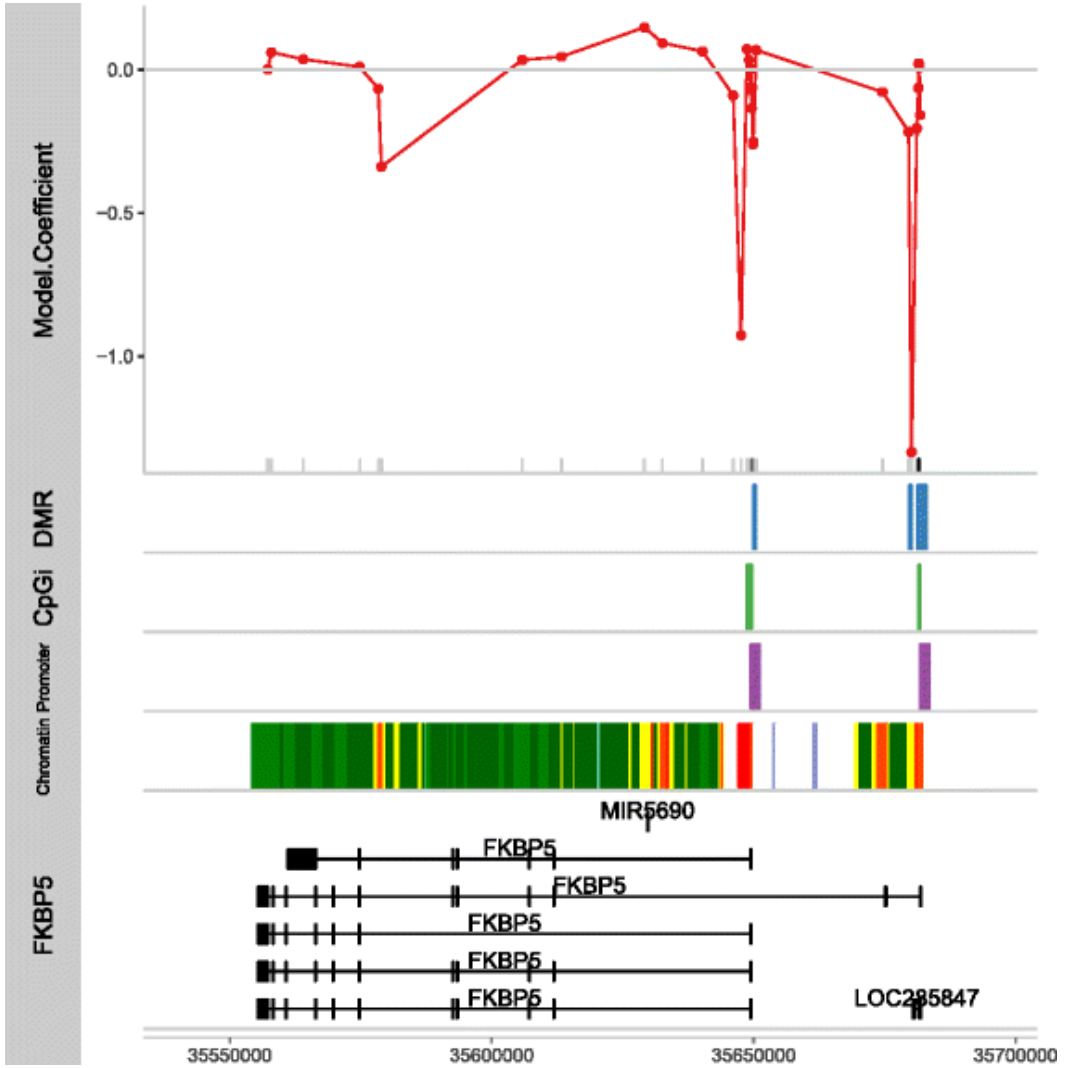
- A range of annotations have been generated for Illumina array data

- Includes
  - Chromosome, position
  - Nearest genes and distance to them
  - Position relative to CpG islands
  - Probe sequence
  - SNPs in probe binding region
  - …

Functional Annotation



Functional Annotation



Functional annotation

GREAT improves functional interpretation of *cis*-regulatory regions

Cory Y McLean[1], Dave Bristor[1,2], Michael Hiller[2], Shoa L Clarke[3], Bruce T Schaar[2], Craig B Lowe[4], Aaron M Wenger[1] & Gill Bejerano[1,2]

http://bejerano.stanford.edu/great/public/html/index.php

GO Biological Process (20+ terms) — Global controls

Table controls: Export | Shown top rows in this table: 20 Set | Term annotation count: Min: 1 Max: Inf Set | Visualize this table: [select one]

Term Name	Binom Rank	Binom Raw P-Value	*Binom FDR Q-Val*	Binom Fold Enrichment	Binom Observed Region Hits	Binom Region Set Coverage	Hyper Rank	*Hyper FDR Q-Val*	Hyper Fold Enrichment	Hyper Observed Gene Hits	Hyper Total Genes	Hyper Gene Set Coverage
regulation of cellular senescence	1	1.1180e-26	1.1672e-22	44.9592	20	8.51%	13	2.2239e-2	21.0268	4	12	1.40%
skeletal system development	58	1.5432e-9	2.7778e-7	2.8964	40	17.02%	1	1.3446e-3	3.6001	23	403	8.04%
connective tissue development	82	5.4523e-8	6.9418e-6	3.5668	25	10.64%	4	4.6218e-3	4.7226	14	187	4.90%
cartilage development	86	8.9515e-8	1.08									

Human Phenotype (20+ terms) — Global controls

Table controls: Export | Shown top rows in this table: 20 Set | Term annotation count: Min: 1 Max: Inf Set | Visualize this table: [select one]

Term Name	Binom Rank	Binom Raw P-Value	*Binom FDR Q-Val*	Binom Fold Enrichment	Binom Observed Region Hits	Binom Region Set Coverage	Hyper Rank	*Hyper FDR Q-Val*	Hyper Fold Enrichment	Hyper Observed Gene Hits	Hyper Total Genes	Hyper Gene Set Coverage
Abnormality of body height	5	1.2462e-9	1.5318e-6	2.7300	44	18.72%	1	6.8587e-5	3.2110	31	609	10.84%
Growth delay	6	2.5444e-9	2.6063e-6	2.5543	47	20.00%	5	6.7258e-4	2.6972	31	725	10.84%
Short stature	7	1.1038e-8	9.6914e-6	2.6986	40	17.02%	2	9.8619e-5	3.2207	29	568	10.14%
Aplasia/Hypoplasia involving the skeleton	8	1.3466e-8	1.0345e-5	2.7244	39	16.60%	4	5.6439e-4	3.1859	25	495	8.74%
Aplasia/Hypoplasia affecting bones of the axial skeleton	9	3.8574e-8	2.6342e-5	2.9947	32	13.62%	31	6.6900e-3	3.0198	18	376	6.29%

Genetic Control of DNA methylation

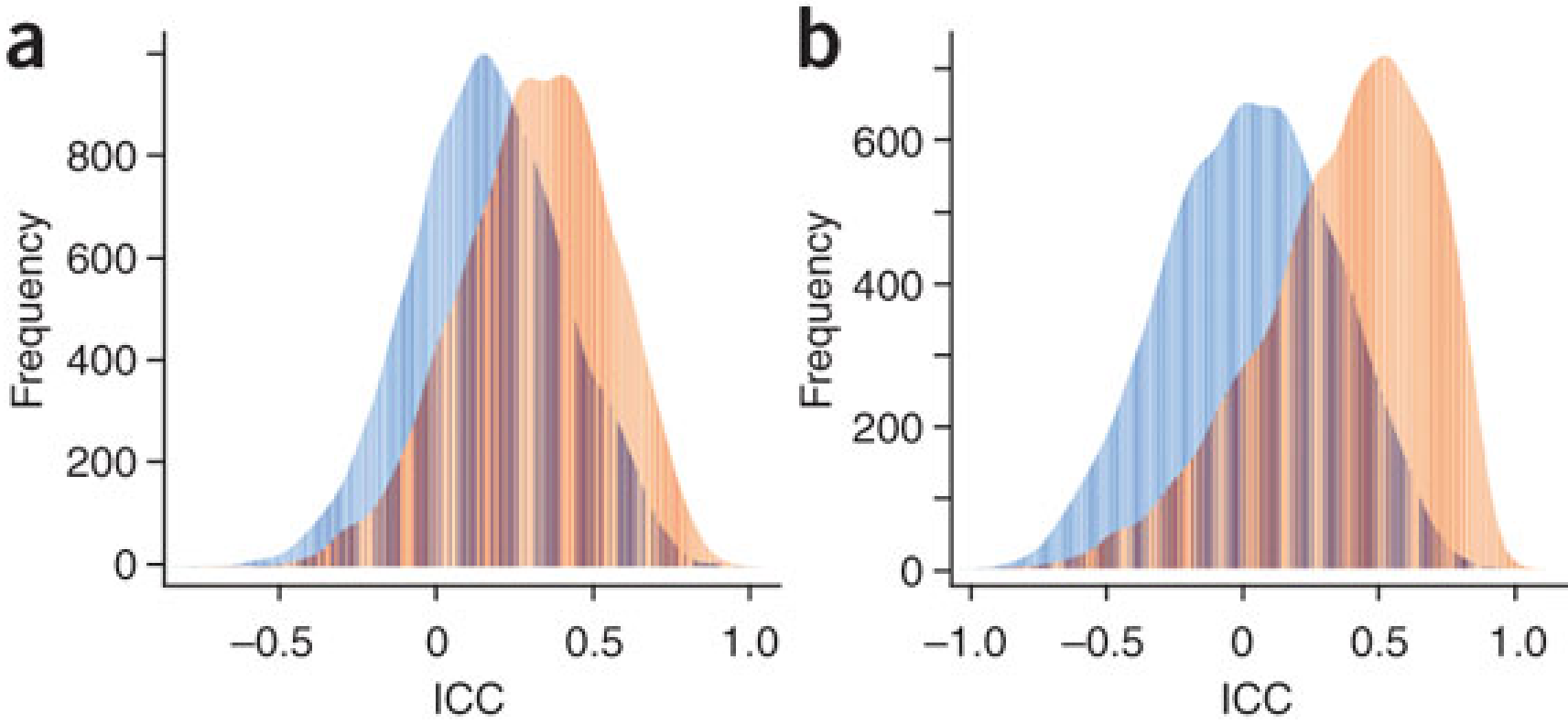
- There is much interest in the transmission of DNA methylation across generations

- Potential to pass on environmental insults across generations?
- Epigenetic inheritance?

- Genetic influences on DNA methylation?

Genetic Control of DNA methylation

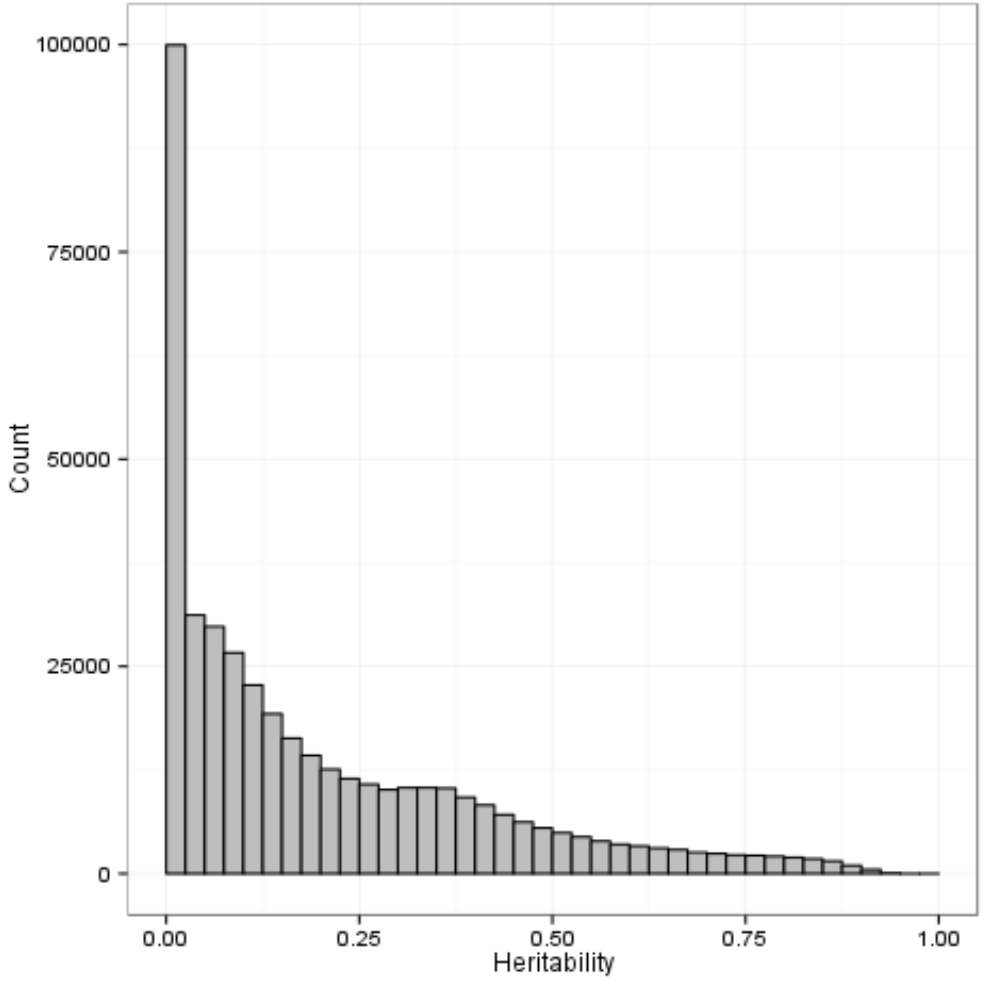
- Kamisky et al 2007
- MZ twins have have more similar DNA methylation than DZ twins
- Chorionicity has an effect

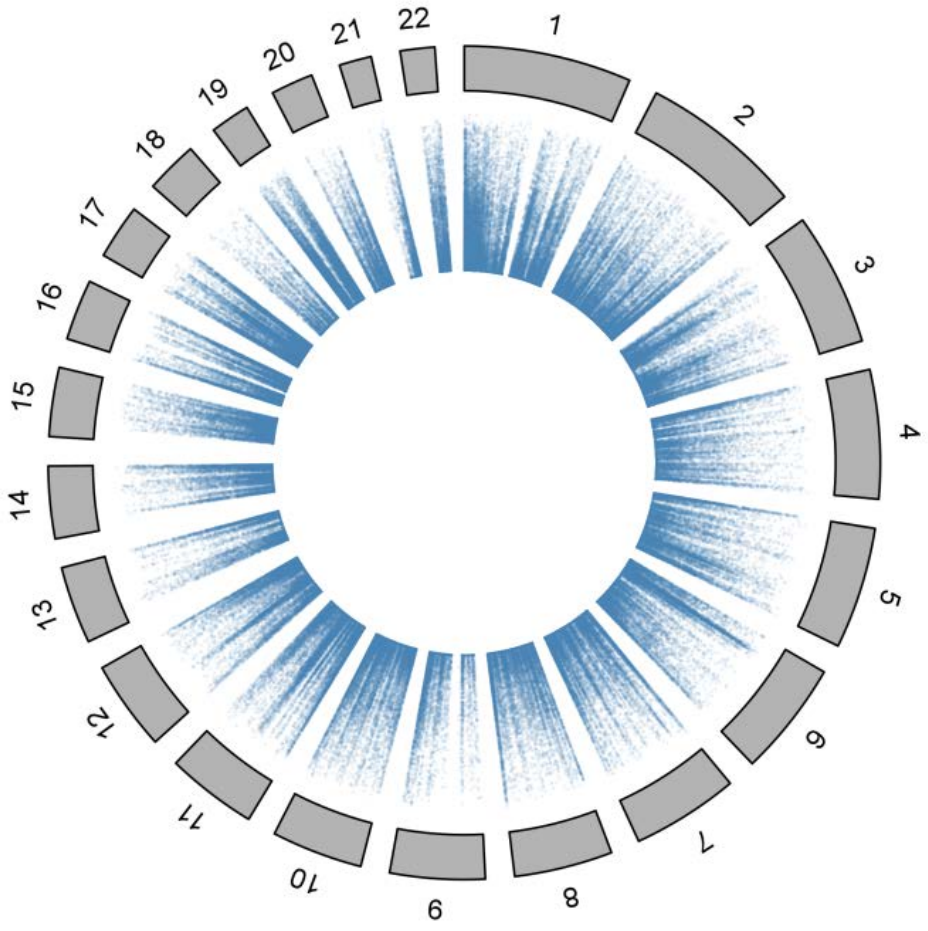


Familial Correlations of DNA methylation

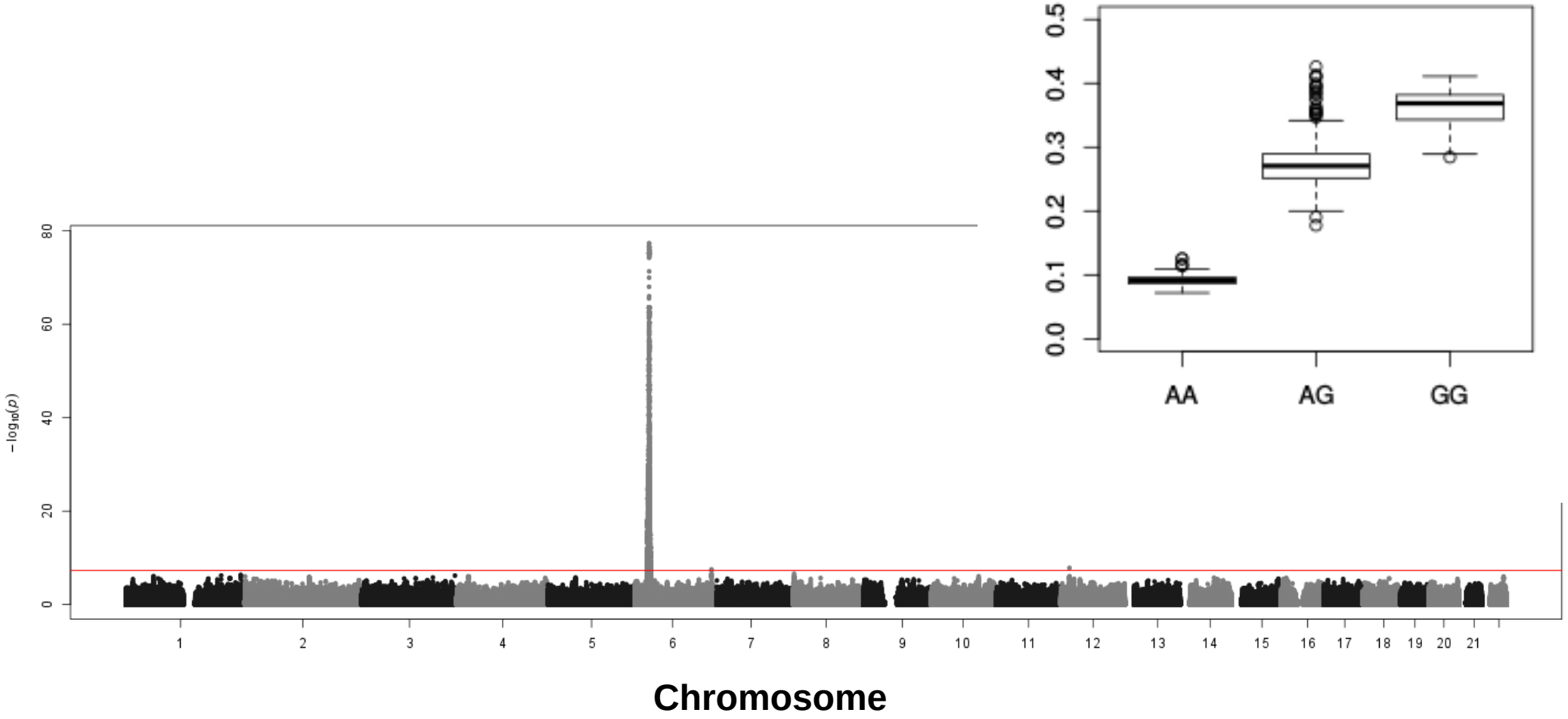
Relationship	Pairs (n)	Correlation	Expected[a]
MZ twins	67	0.200	h^2
DZ twins	111	0.109	$h^2/2$
Siblings	262[b]	0.090	$h^2/2$
Parent-Offspring	362[b]	0.089	$h^2/2$
Mother-Offspring	190	0.097	$h^2/2$
Father-Offspring	172	0.085	$h^2/2$
Parent-Parent	58	0.023	0
Unrelated	187,331[b]	-0.002	0

Heritability

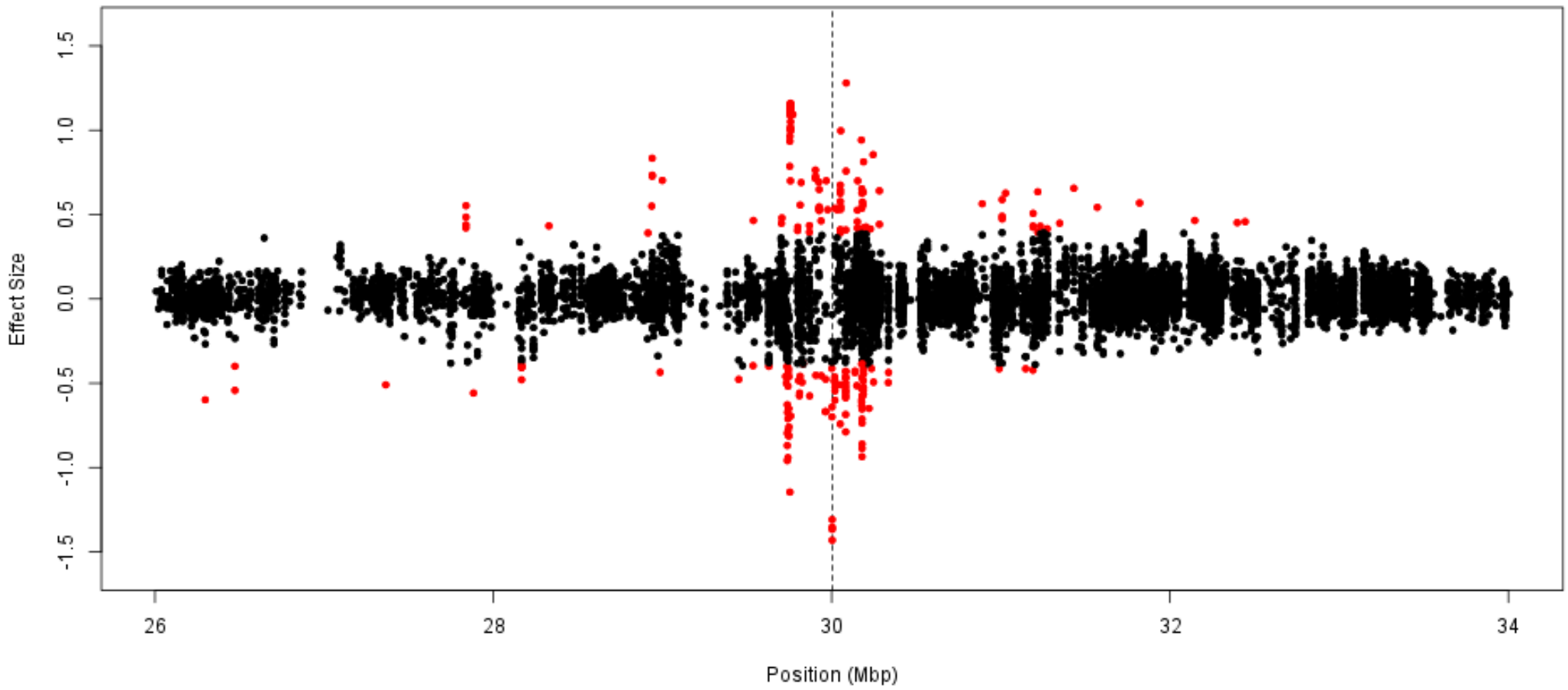




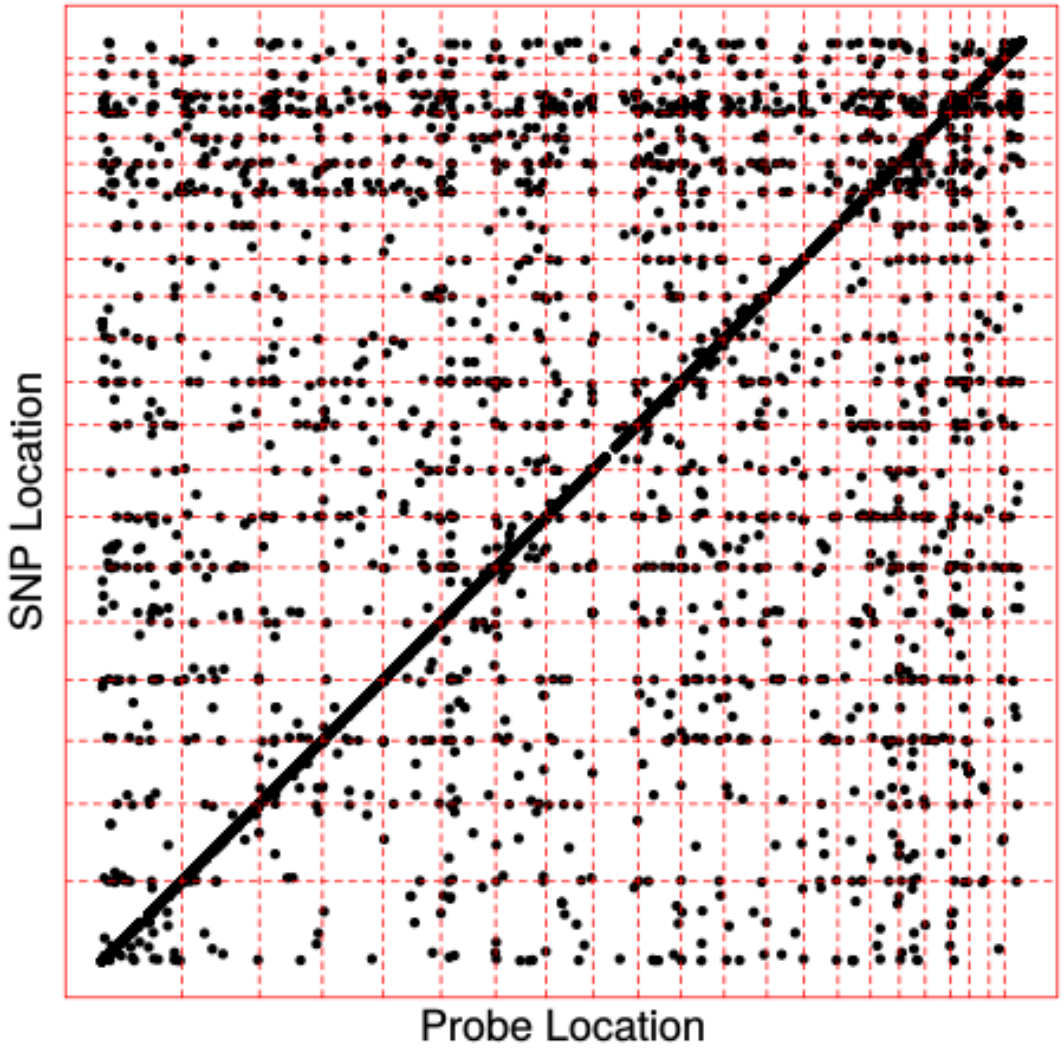
Methylation QTL (mQTL)



Methylation QTL



Methylation QTL Everywhere!



Epigenetic Clock

- DNA methylation is correlated with age
  - Global change to high DNA methylation
  - Individual loci have varying amounts of change with age

- Several methods have been presented to use DNA methylation data to make a predictor of age

DNA methylation age of human tissues and cell types

Steve Horvath

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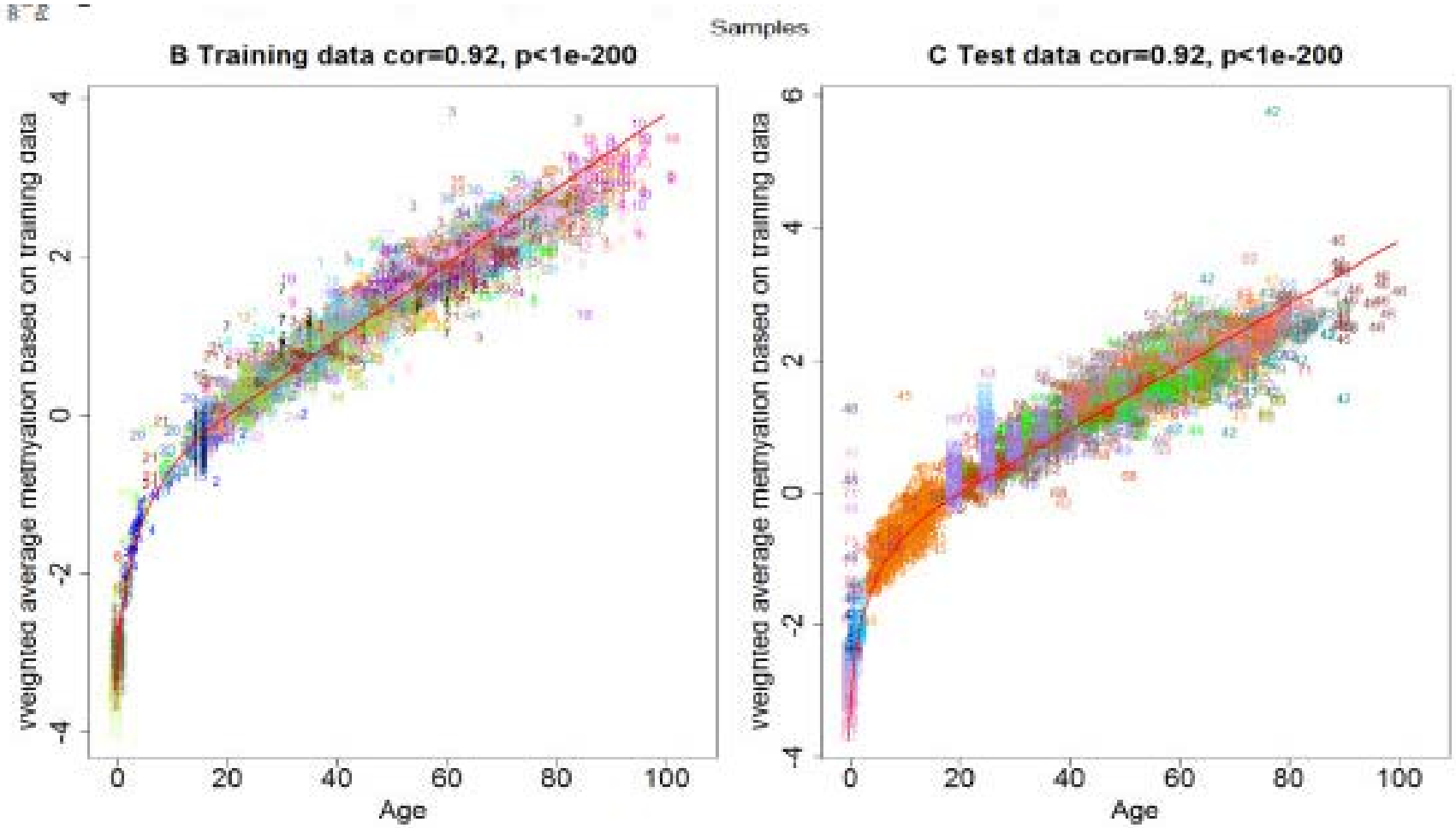
DNA Methylation Age

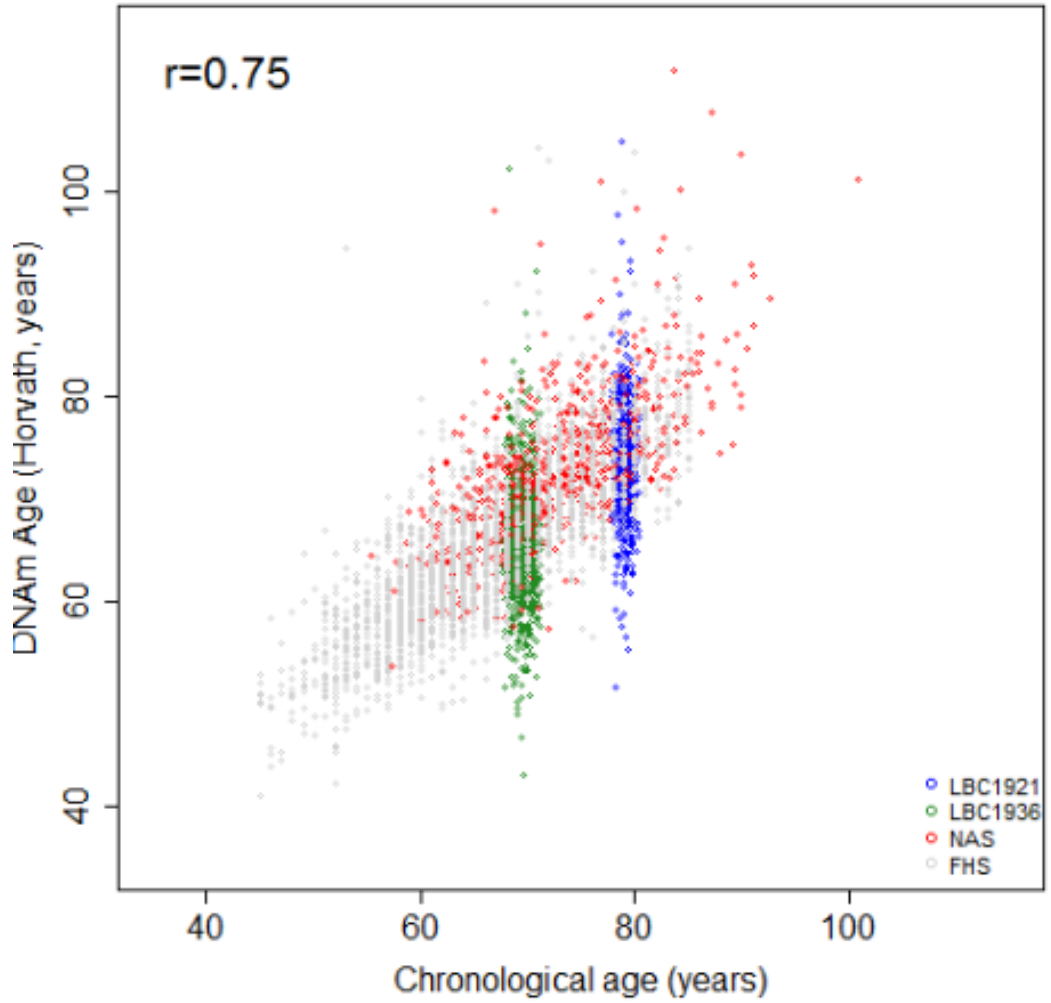


DNA methylation age

- Horvath demonstrates his measure:
  - Is applicable to a wide range of tissues
  - Works in chimpanzees
  - Stem cells have a DNA methylation age close to zero
  - Is negatively associated with number of mutations found in cancer cells

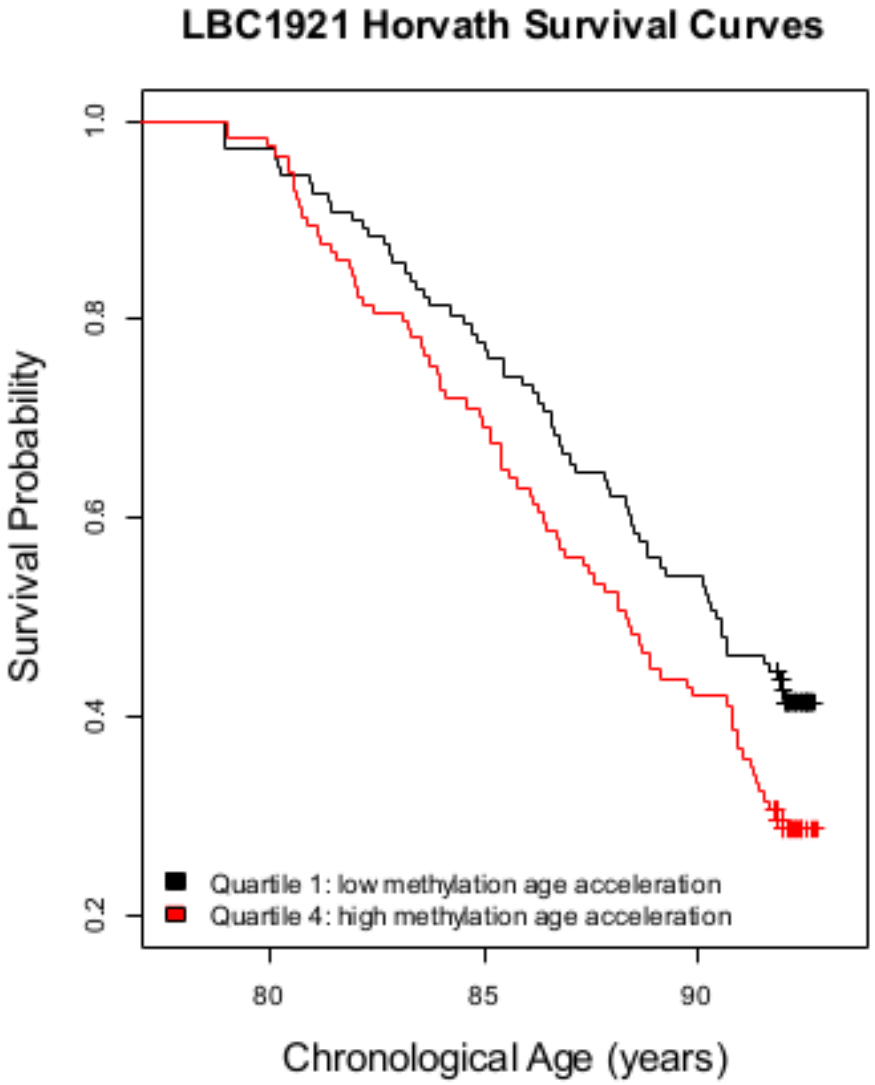
Age Acceleration

- There is variation in DNA methylation age for people with the same chronological age
- This is referred to as age acceleration
- Is this variation important?



Age Acceleration

- People with higher age acceleration have a higher rate of mortality than those with low age acceleration
- Effect still present after correcting for
  - Smoking
  - BMI
  - CVD
  - Removing people who died within five years of measurement
- Effect on mortality is independent of telomere length



Age Acceleration

- Has been associated with
  - Lung function
  - Grip strength
  - Cognition
  - Cardiovascular disease
  - ….

Age Acceleration is Heritable