Epigenetic age-acceleration effects in healthy breast tissue

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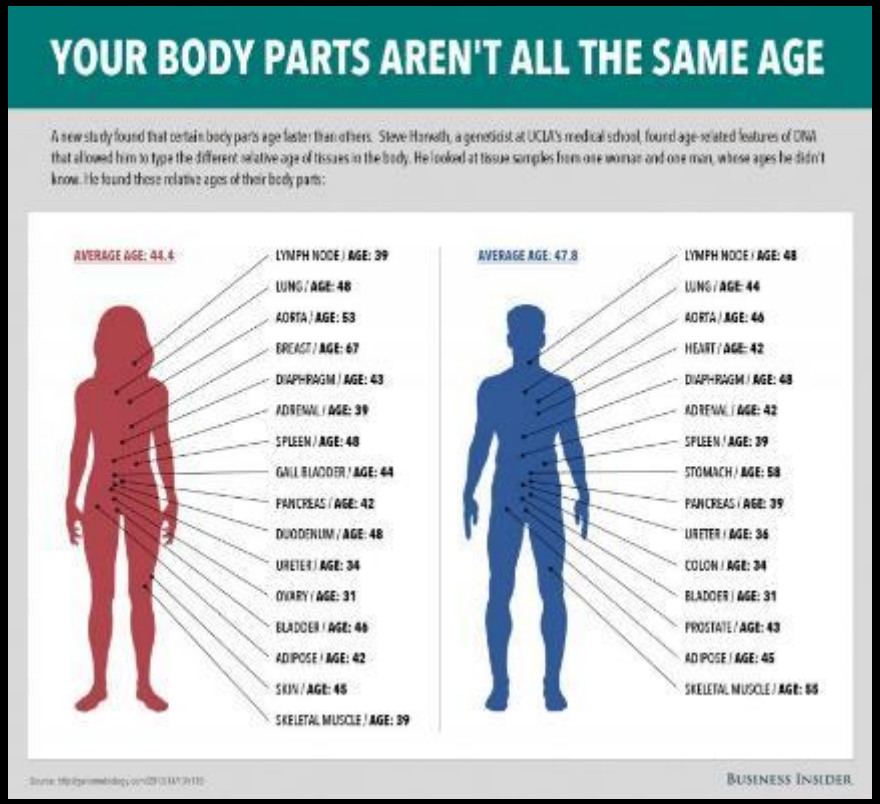
Introduction

- Age, estrogen expsoure are major risk factors for breast cancer.
- 'Epigenetic age' of female breast tissue appears older than chronological age.



- Mechanisms underlying the link between chronic estrogen stimulation, increased cell cycling, cellular aging in the breast are poorly understood.

Research questions

- Does female breast tissue appear older than peripheral blood in healthy women? Why?

- Does cumulative exposure to estrogen drive the acceleration of epigenetic aging in the female breast?

Outline of Talk

- Aging, inflammation, and cancer risk
- Epigenetic Clock
- DNA methylation (DNAm) age in healthy breast compared with peripheral blood
- Cumulative estrogen exposure and breast DNAm age

Cancer and aging

- Rival demons
- Tumor suppressor mechanisms evolved to regulate embryogenesis and protect against accumulation of oncogenic mutations with age
  - prevent/repair DNA damage (caretakers)
  - Inhibit propagation of cancer cells (gatekeepers)
  - Induce cell death (apoptosis)
  - Permanently arrest their division (senescence)
- Cancer incidence increases with age

Campisi J Cancer and Ageing: rival demons? Nature Reviews Cancer 2003;3:339-349.

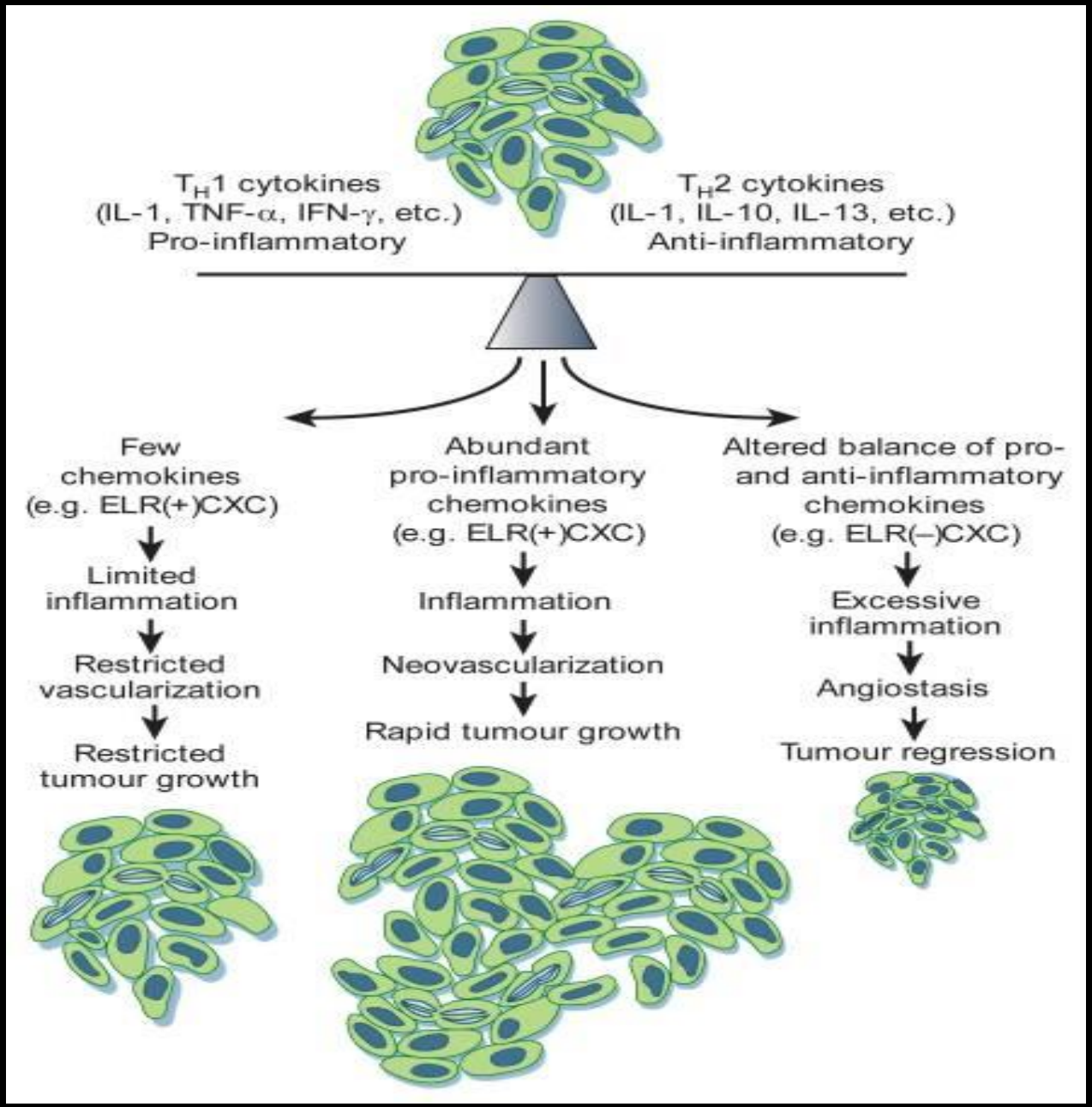
Cellular senescence

- Shortened telomeres
- Increased p16^{INK4a}
- Limit stem cell lifespan
- Reduced stress tolerance
- Increased production of pro-inflammatory cytokines

Inflammation and cancer

- Origin of cancer at sites of chronic inflammation (Virchow 1863)
- Sustained proliferation of cells in an environment rich in inflammatory cells, growth factors, activated stroma
- DNA damage-promoting agents

Balkwill F, Mantovani A. Inflammation and cancer: back to Vircho? Lancet 2001;357:539-545.
Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-867.



Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-867.

Inflammaging

- Flow cytometry markers of cellular senescence
- Chronic immune activation
- Increased secretion of pro-inflammatory biomarkers

Markers of immune activation

- IL-6, IL-10, CRP
- Activation-induced cytidine deaminase
- Soluble CD23, soluble CD27, soluble CD30
- Chemokine CXCL13 and its receptor CXCR5
- microRNA-21
- *These markers are elevated in the peripheral blood several years prior to cancer diagnosis*

Epigenetic changes with age

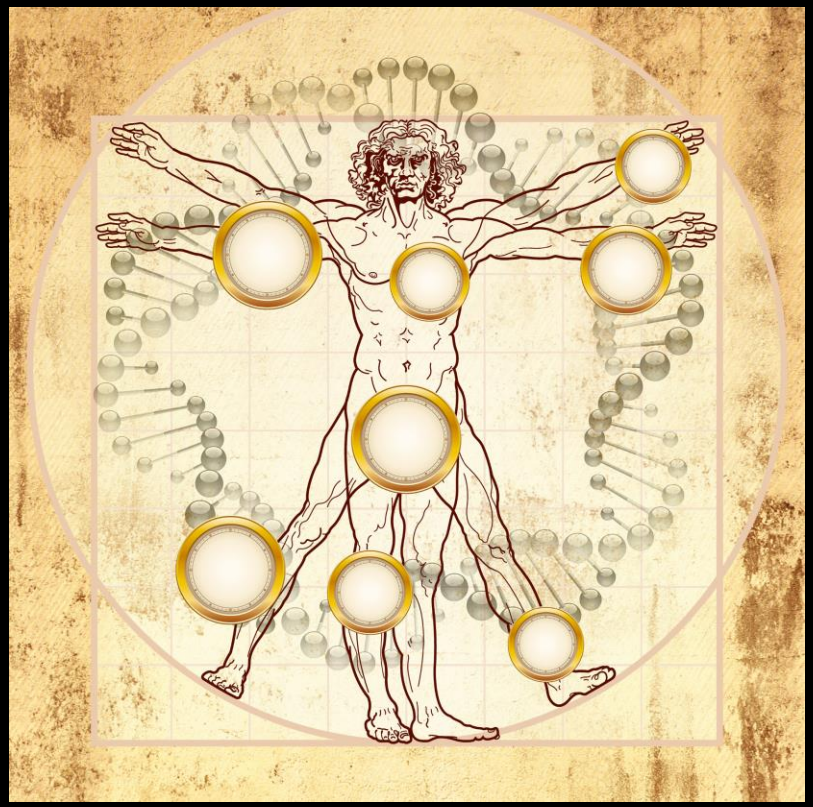
- Is there an epigenetic mechanism underlying inflammaging in peripheral blood?
- Global methylation analyses
- Search for epigenetic biomarker of aging

Bocklandt S, et al. Epigenetic predictor of age. PLoS One 2011;6:e14821

Methylation changes

- Polycomb group target proteins (Teschendorff 2013)
  - Stem cell self-renewal and differentiation
  - Aging
  - Dysplasia
  - Malignancy

Epigenetic clock



-derived from methylation levels at 353 CpGs

-Cell cycling, self-renewal

-Highly correlated with chronological age across tissues and species

Horvath S. DNA methylation age of human tissues and cell types. Genome Biology 2013;14:3156.

Development of the epigenetic clock

- Multi-tissue predictor of age using DNA methylation levels
- Developed using 8,000 samples from 82 Illumina DNA methylation array datasets
- 51 healthy tissues and cell types
- accurately predict age across broad spectrum o ftissues and cell types

Horvath S. DNA methylation age of human tissues and cell types. Genome Biology 2013;14:3156.

Identification of key probes

- Elastic net (penalized regression) model of chronologic age on thousands of markers selected 353 CpGs
- Two sets:
  - 193 positively correlated CpGs get hypermethylated with age
  - 160 negatively correlated CpGs get hypomethylated with age
- Weighted average formed by regression coefficients = epigenetic clock

Horvath S. DNA methylation age of human tissues and cell types. Genome Biology 2013;14:3156

Properties of the Epigenetic Clock

- Close to zero for embryonic stem cells and iPSCs
- Correlates with cell passage number
- Highly herital measure of age-acceleration
- Applicable to chmpanzee tissue

Horvath S. DNA methylation age of human tissues and cell types. Genome Biology 2013;14:3156.

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Pathway analysis of 353 clock CpGs

- Cell death and survival
- Cellular growth andproliferation
- Organism and tissue development
- Cancer

Horvath S. DNA methylation age of human tissues and cell types. Genome Biology 2013;14:3156.

Epigenetic clock as a measure of biologic aging

- Accelerated in disease states
  - HIV (T cells)
  - Steatohepatosis (hepatocytes)
  - Parkinson's, Huntington's disease (neurons)

Horvath S et al. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. Aging (Albany NY). 2015;7:1130-1142
Horvath S et al. Huntingont's disease accelerates epigenetic aging of human brain and disrupts dNA methylation levels. Aging (Albany NY) 2016;8:1485-1512.
Rickabaugh T et al. Acceleration of age-associated methylation patterns in HIV-1-infected adults. PLoS One 2015;10:e0119201
Horvath S et al. Obestiy accelerates epigenetic agingg of human liver. Proc Natl Acad Sci USA 2014;111:15538-15543.

DNAm aging ≈ Biological aging

- Predicts frailty
- Predicts all-cause mortality
  - After adjusting for known risk factors

Marioni RE et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol 2015;16:25.
Christiansen L et al. DNA methylation age is associated with mortality in a longitudinal Danish twin study. Aging Cell 2016;15:149-154.
Perna L et al. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clin Epigenet 2016;8:1-7
Chen BH et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging (Albany NY) 2016;8:1844-1865.

Intrinisc vs. Extrinsic epigenetic age

- Intrinsic is adjusted for:
  - cell composition
  - Chronologic age
- Extrinsic adjusted for:
  - Cell composition
  - Chronologic age
  - Estimated counts of senescent and naïve cytotoxic CD8 T lymphocytes

Chen BH et al. DNA methylation-based measures of biologic age: meta-analysis. Aging (Albany NY). 2016;8:1844-1859.

DNAm Phenotypic age

- Multi-tissue biomarker of aging
- Incorporates composite clinical measures
- Predicts:
  - All-cause mortality
  - Cancers
  - Healthspan
  - Physical functioning
  - Alzheimer's disease

Levine M et al. An epigneetic biomarker of aging for lifespan and healthspan. Aging (Albany NY) 2018;10:573-591.

DNAm Phenotypic age

- Associated with increased activation of pro-inflammatory markers
- Activated interferon pathways
- Decreased activation of transcriptional/translational machinery
- Decreased DNA damage response
- Decreased mitochondrial signatures

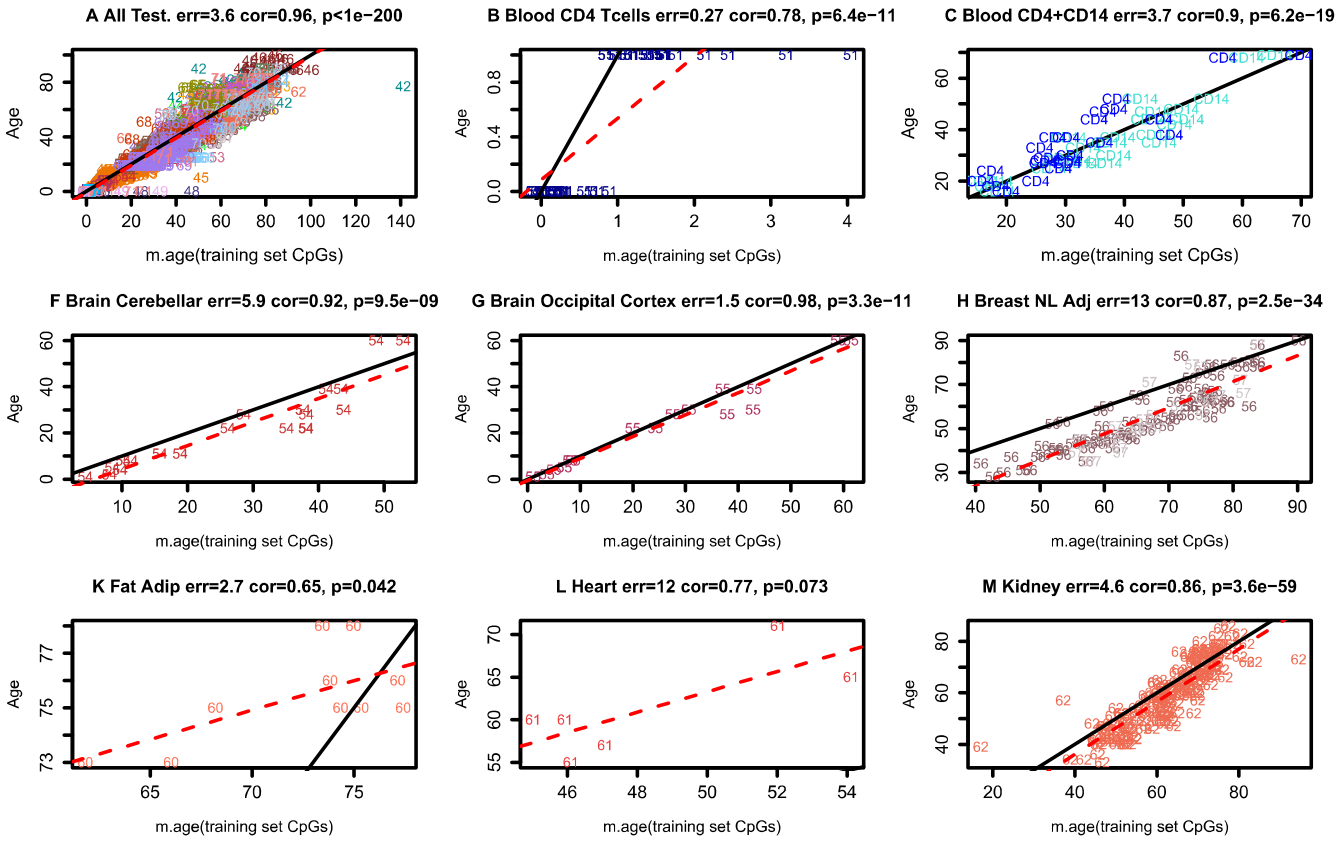
Levine M et al. An epigneetic biomarker of aging for lifespan and healthspan. Aging (Albany NY) 2018;10:573-591.

Epigenetic clock as a predictor of cancer risk

- Peripheral blood epigenetic age increased prior to cancer diagnoses:
  - Lung cancer
  - Breast cancer susceptibility
- Weak signal – need to look directly at tissues at risk

Levine ME et al. DNA methylation age predicts future onset of lung cancer in the women's health initiative. Aging (Albany NY) 2015;7:690-700.
Perna L et al. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clin Epigenet 2016;8:1-7
Joo JE et al. Heritable DNA methylation marks associated with susceptibility to breast cancer. Nat Commun 2018;9:867.

DNAm age of breast appears older than other tissues

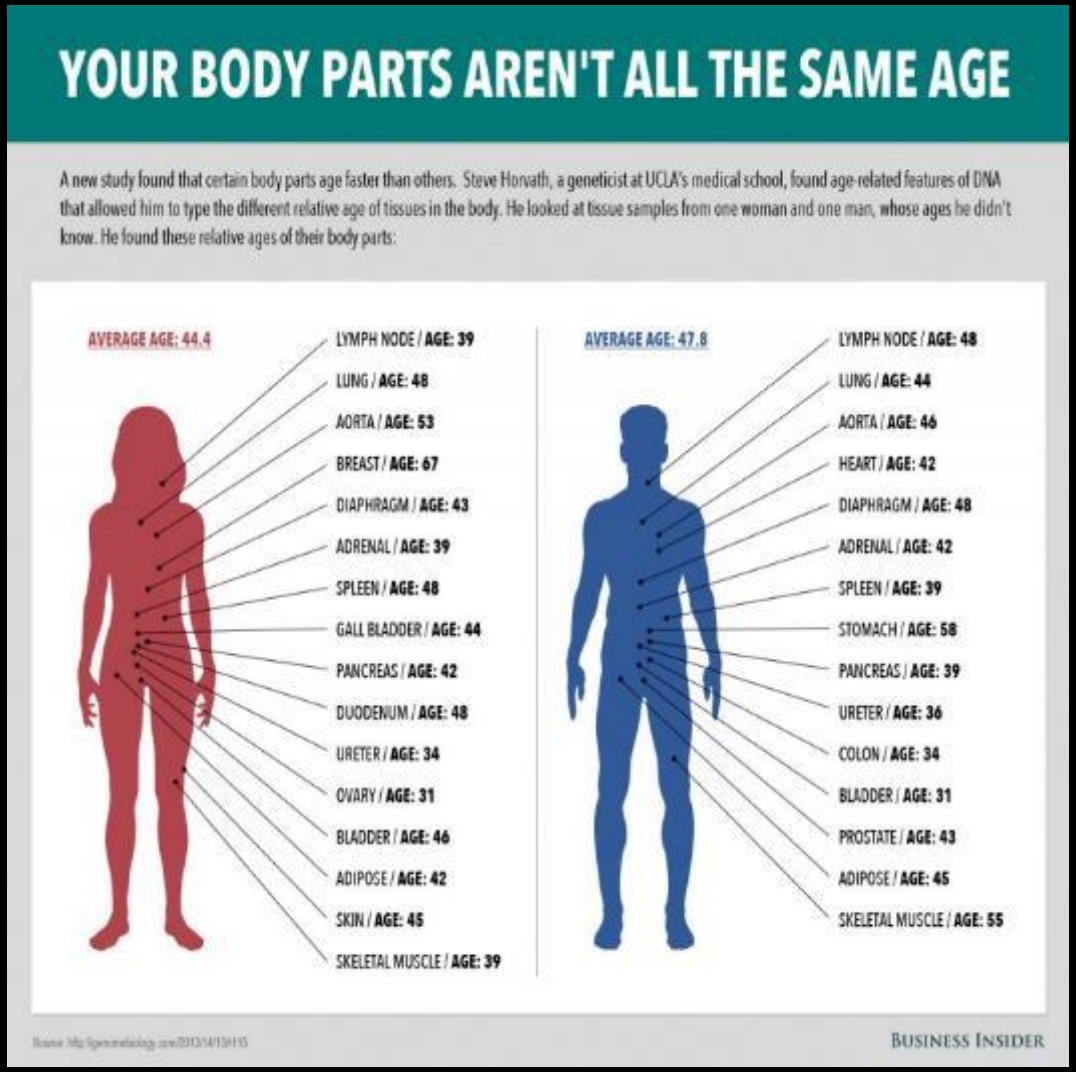


Horvath S. DNA methylation age of human tissues and cell types. Genome Biology 2013;14:3156.

Caveats

- Methodologic limitation
- Normal adjacent breast tissue datasets
- Breast cancer patients

Comparing tissues using pooled data



Horvath S. DNA methylation age of human tissues and cell types. Genome Biology 2013;14:3156.

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Why does breast tissue appear older other tissues?

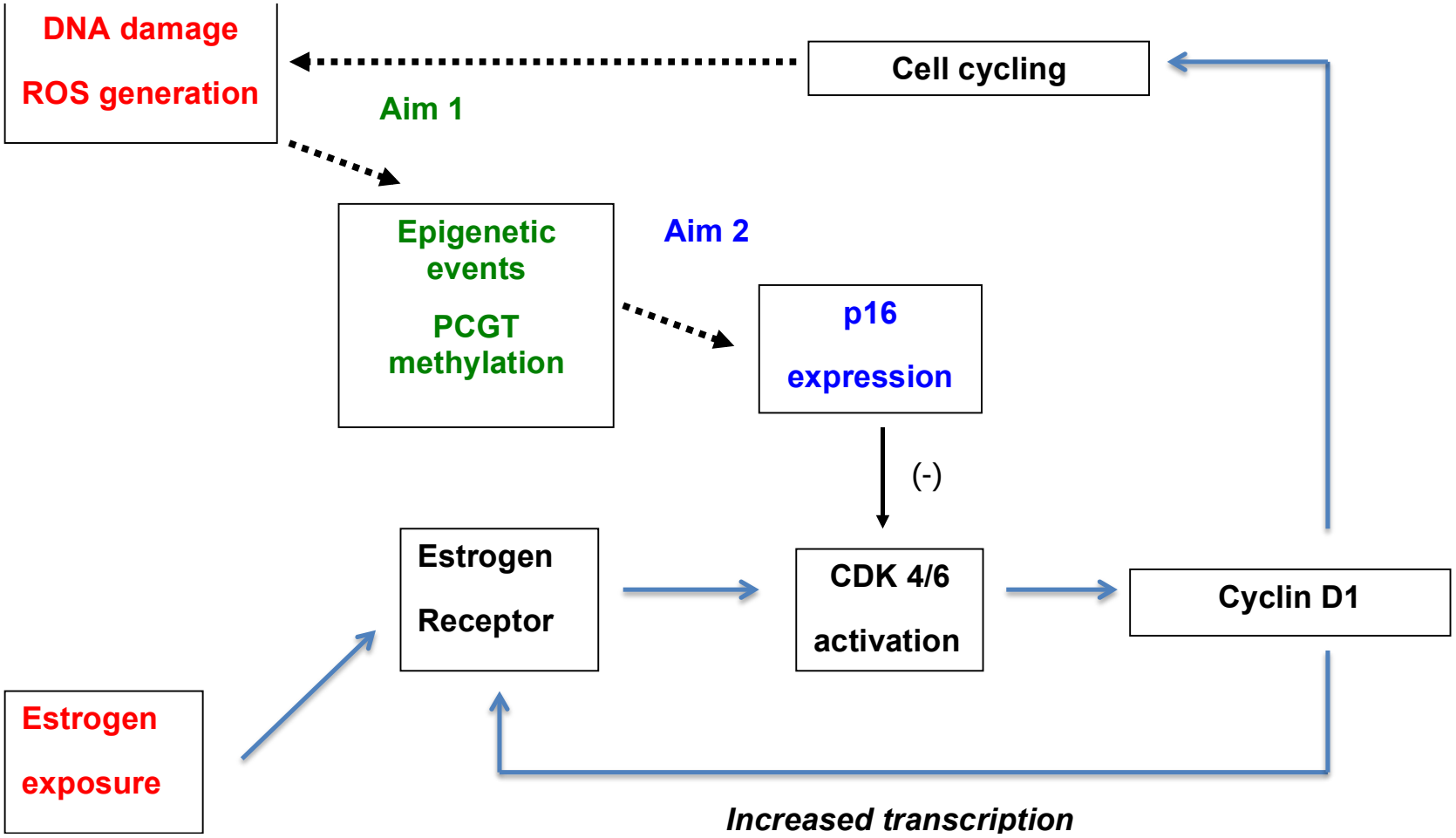
- Estrogen stimulation and chronic cell cycling
- Accumulation of DNA damage
- Generation of ROS
- Altered PCGT methylation

Potential mechanisms linking accelerated aging and breast cancer

- Dysregulation of cell cycle is a hallmark of cancer
- Genetic alterations in key cell cycle regulatory proteins occur in breast carcinogensis
- Estrogen induces proliferatio of ER-positive breast cancer cells by activation of CDK 4/6 genes
- Increased cyclin D1 signaling, Rb phosphorylation, and continued division of cells
- Cyclin D1 increases transcription of estrogen receptors in the cell

Hannahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-674.

Conceptual Model



Project/Methods

- Collect tissue specimens from Komen Tissue Bank donated by healthy women
- Well annotated sample with survey data on medical, gynecologic and reproductive histories
- Methylation experiments on tissues (epigenome wide 450K markers)
- Compare breast and blood tissue epigenetic age
- Examine for associations between age-acceleration and cumulative estrogen exposure

Susan G. Komen Tissue Bank (KTB)

- Indiana University Simon Cancer Center
- Unique resource : healthy donors
- Goals:
  - understanding normal breast biology
  - Better understand disruption during breast carcinogensis
  - Accelerate breast cancer prevention research

KTB Longitudinal Data

- healthy women (no history of BC)
- Breast tissue
- Matched peripheral blood
- Multiple time points (at least 2)
- Extensive survey data

Survey data

- Well annotated sample
- Types of data:
  - Demographics
  - Medical history
  - Gynecologic History
  - Reproductive History
  - Medication (OCPs, HRT)

Our study sample

- 49 healthy women donors (blood and breast)
- Age range 18 – 65 years
- Grouped by menopausal status (27 pre-, 22 post-menopausal) and parity (21 nulliparous)

Participant characteristics

	Age < 50 years	Age ≥ 50 years	Overall
Participants	24	16	40
Age at first donation (mean, SD)	33.8 (8.8)	55.4 (4.3)	42.5 (12.9)
Years between first and second donatio (mean, range)	3.9 (2-7)	4.7 (3-6)	4.2 (2-7)
Pre-menopausal (n)	20	2	22

Reproductive variables

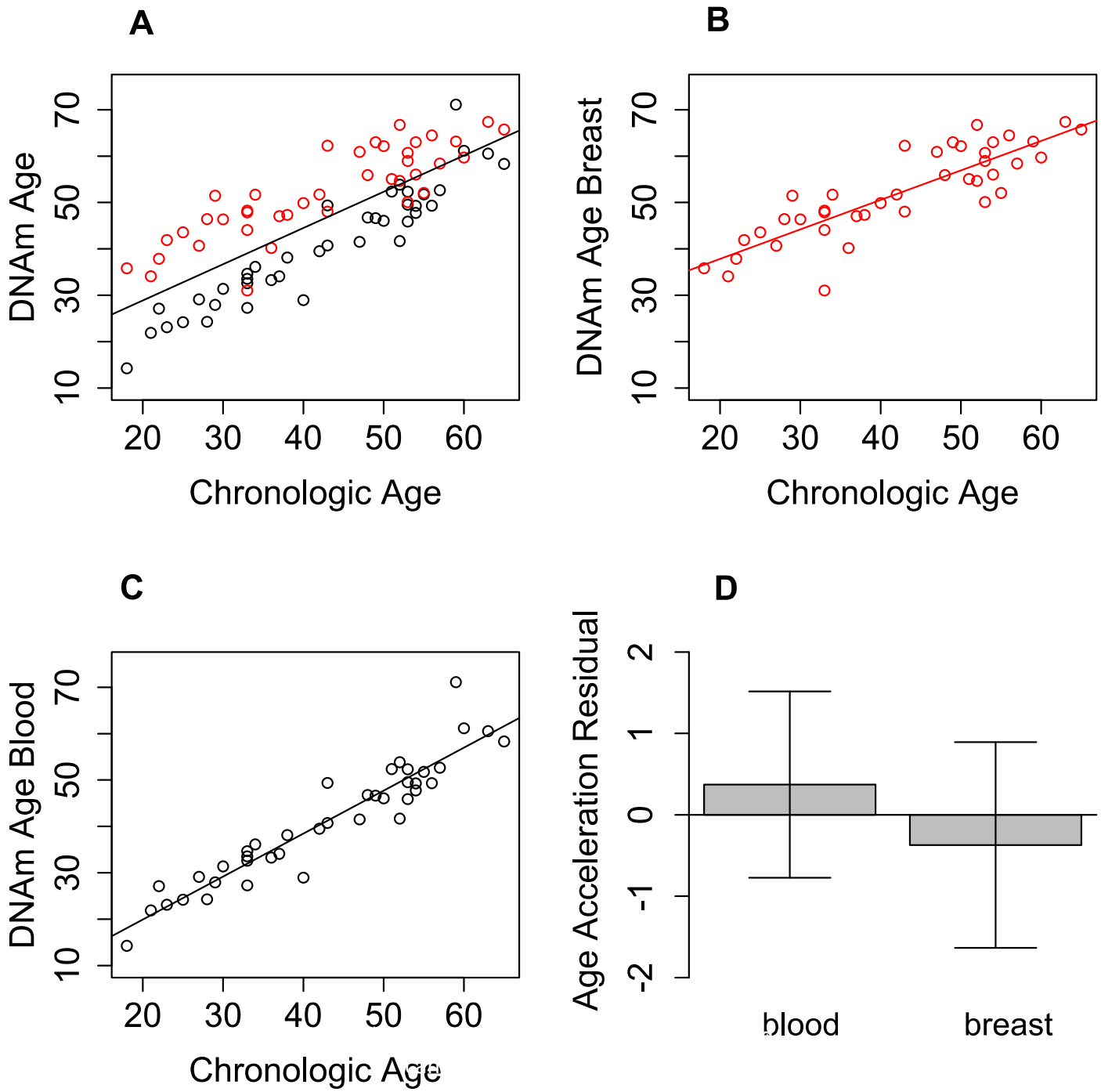
	Age < 50 years	Age ≥ 50 years	Overall
Nulliparous (n)	12	5	17
No. live births (mean, SD)	0.92 (1.0)	2.0 (1.4)	1.2 (1.2)
No. pregnancies (mean, SD)	1.0 (1.3)	2.1 (1.5)	1.5 (1.5)
Total menstrual years (mean, range)	20.2 (5-37)	32.6 (13-41)	25.3 (5-41)
Age at menopause (mean, SD)	33.0 (7.2)	45.1 (6.3)	42.5 (8.0)

DNA methylation studies

- Illumina 450K platform
- Epigenome-wide
- samples randomized across Illumina chip to avoid batch effects
  - Breast vs. blood
  - Young vs. old
  - Nulliparous vs. parous

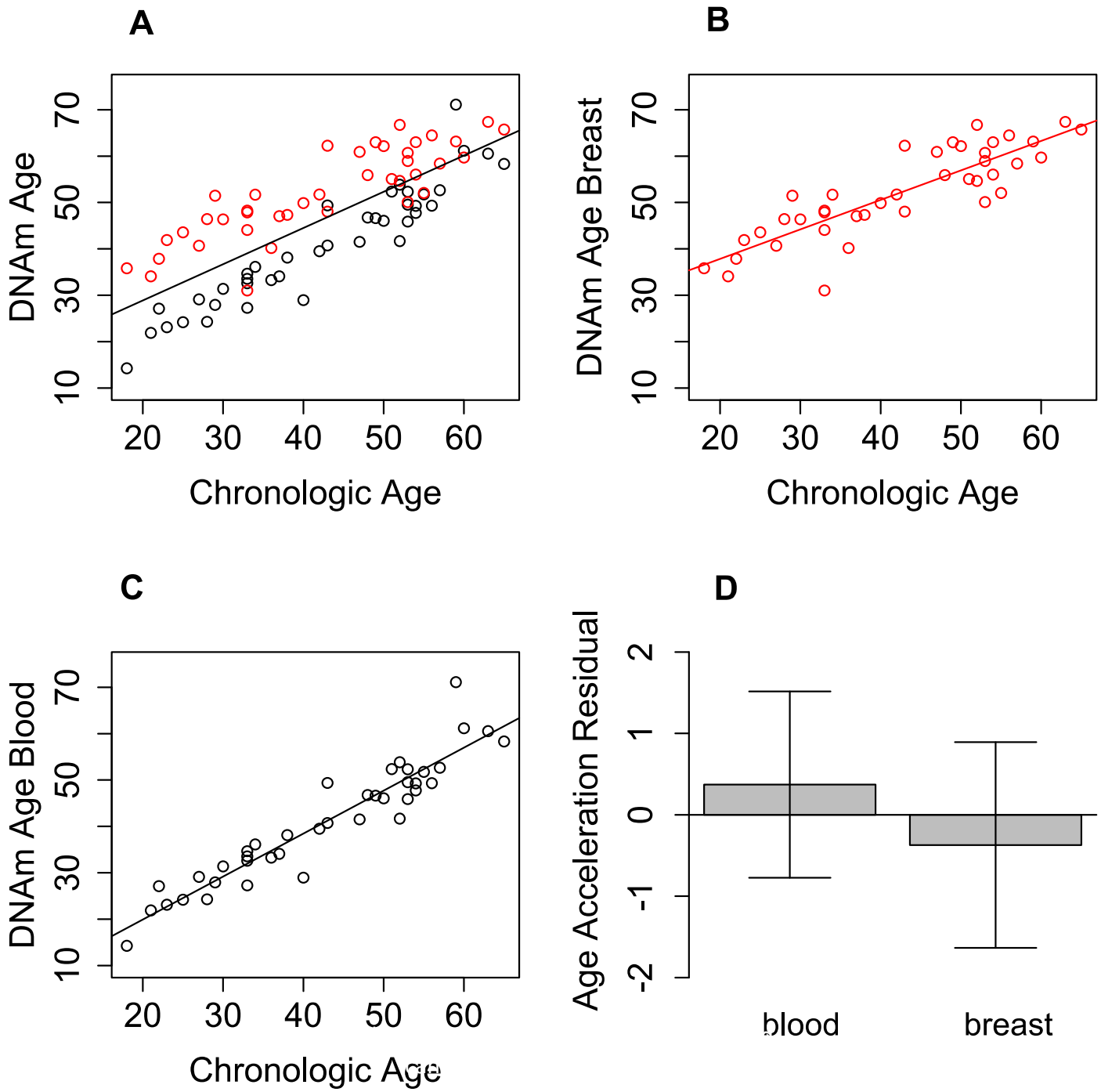
Results

- DNAm age has a strong linear relationship with chronologic age]
  - In blood ($\rho=0.94$, $p<0.0001$)
  - In breast ($\rho=0.86$, $p<0.0001$)


A
DNAm Age
Chronologic Age
B
DNAm Age Breast
Chronologic Age
C
DNAm Age Blood
Chronologic Age
D
Age Acceleration Residual
blood
breast

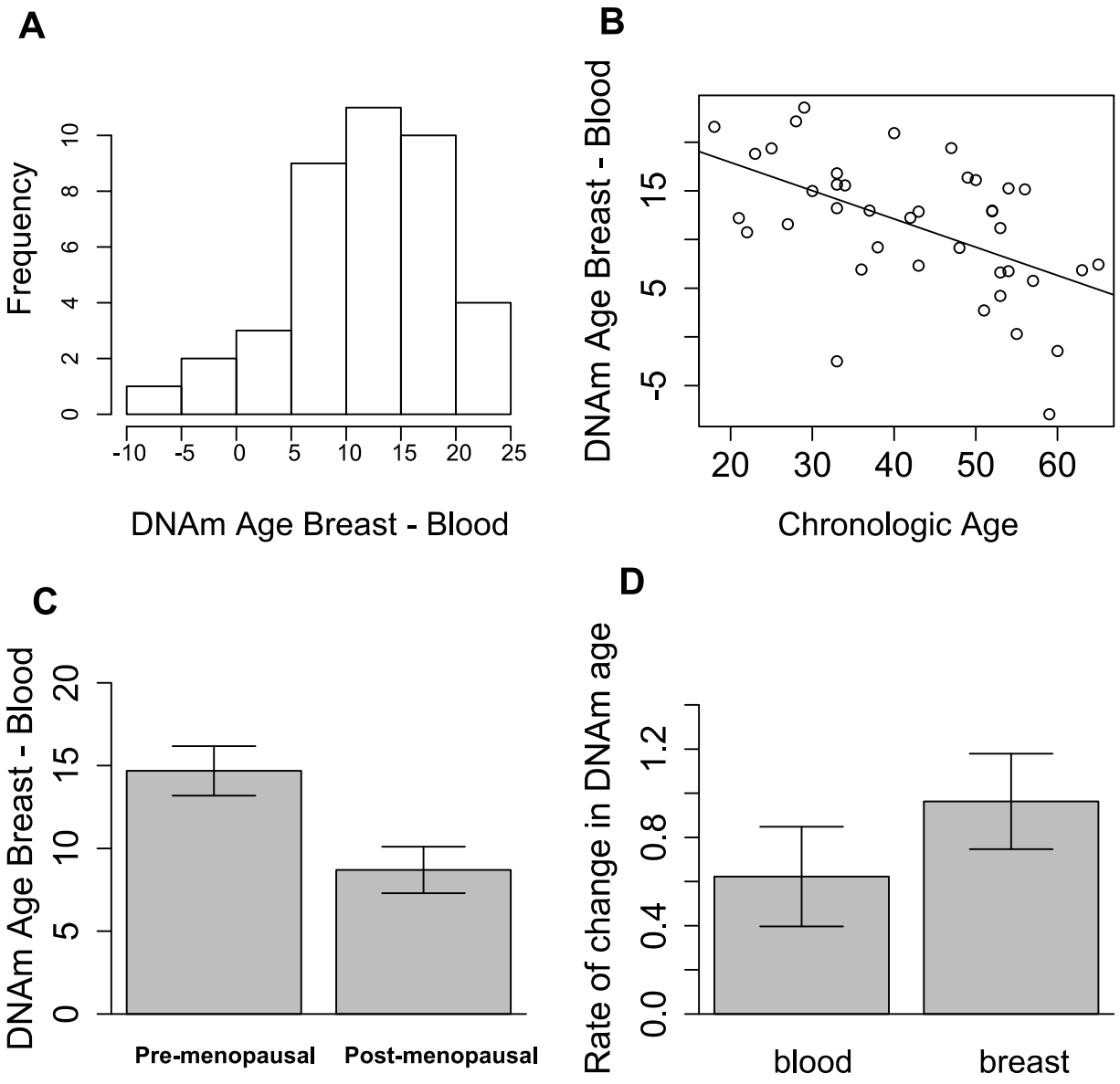
Age acceleration

- **Age acceleration residual** = residuals from linear regression of DNAm age on chronologic age
- Significantly higher in breast compared with blood


A
DNAm Age
Chronologic Age
B
DNAm Age Breast
Chronologic Age
C
DNAm Age Blood
Chronologic Age
D
Age Acceleration Residual
blood
breast

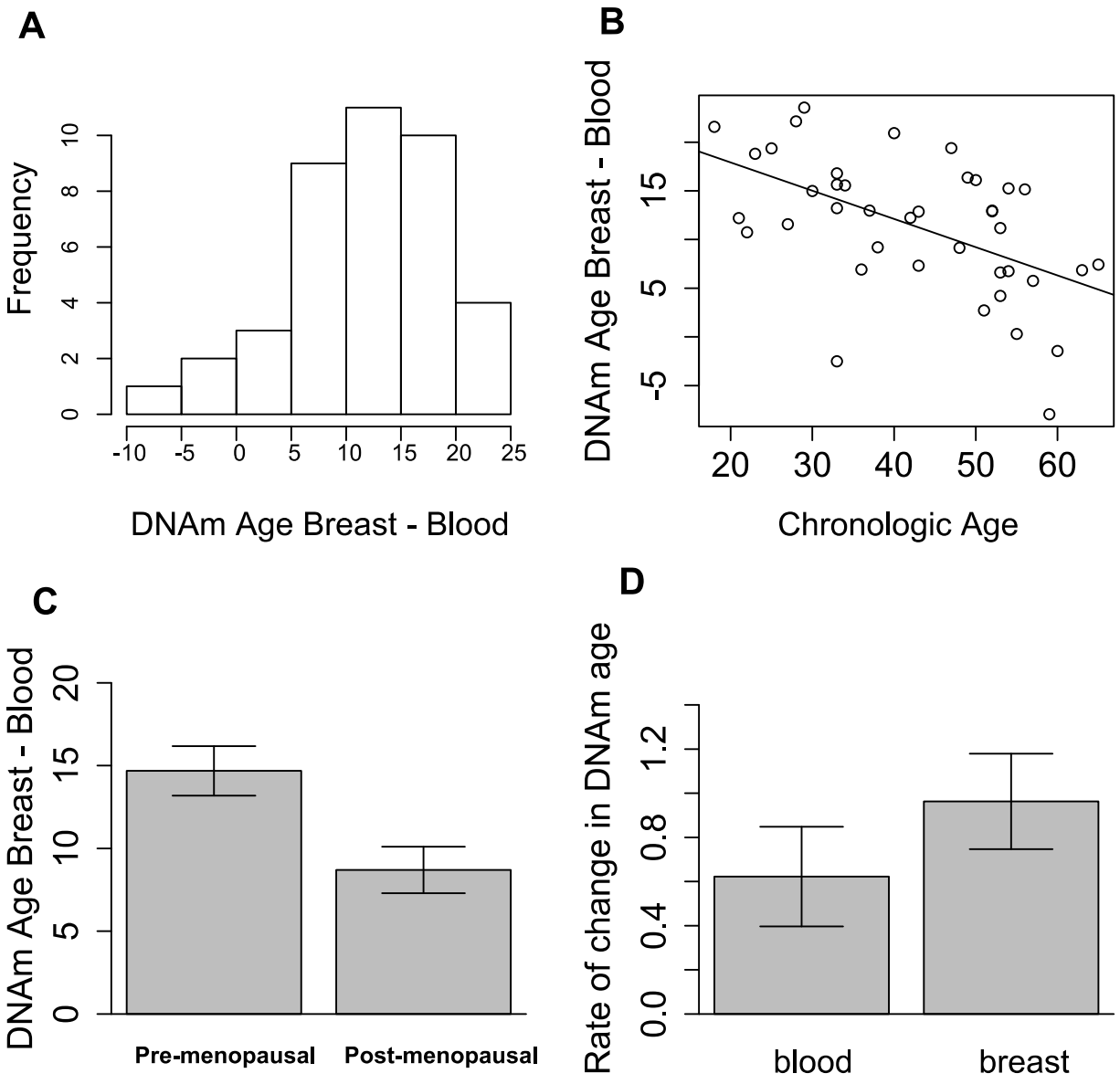
How much older is breast tissue?

- For women aged 21 years, breast 17.5 years older than blood.
- For women aged 55 years, breast 8 years older than blood.


A
Frequency
0
2
4
6
8
10
-10
-5
0
5
10
15
20
25
DNAm Age Breast - Blood
B
DNAm Age Breast - Blood
-5
5
15
20
30
40
50
60
Chronologic Age
C
DNAm Age Breast - Blood
0
5
10
15
20
Pre-menopausal
Post-menopausal
D
Rate of change in DNAm age
0.0
0.4
0.8
1.2
blood
breast

Difference between breast and blood

- Greatest at younger ages
- Convergence at typical age of menopausal transition
- The absolute difference DNAm age breast – DNAm age blood is *inversely correlated* with advancing age (r=-0.53,p=0.0004)


A
Frequency
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-5
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20
25
DNAm Age Breast - Blood
B
DNAm Age Breast - Blood
-5
5
15
20
30
40
50
60
Chronologic Age
C
DNAm Age Breast - Blood
0
5
10
15
20
Pre-menopausal
Post-menopausal
D
Rate of change in DNAm age
0.0
0.4
0.8
1.2
blood
breast

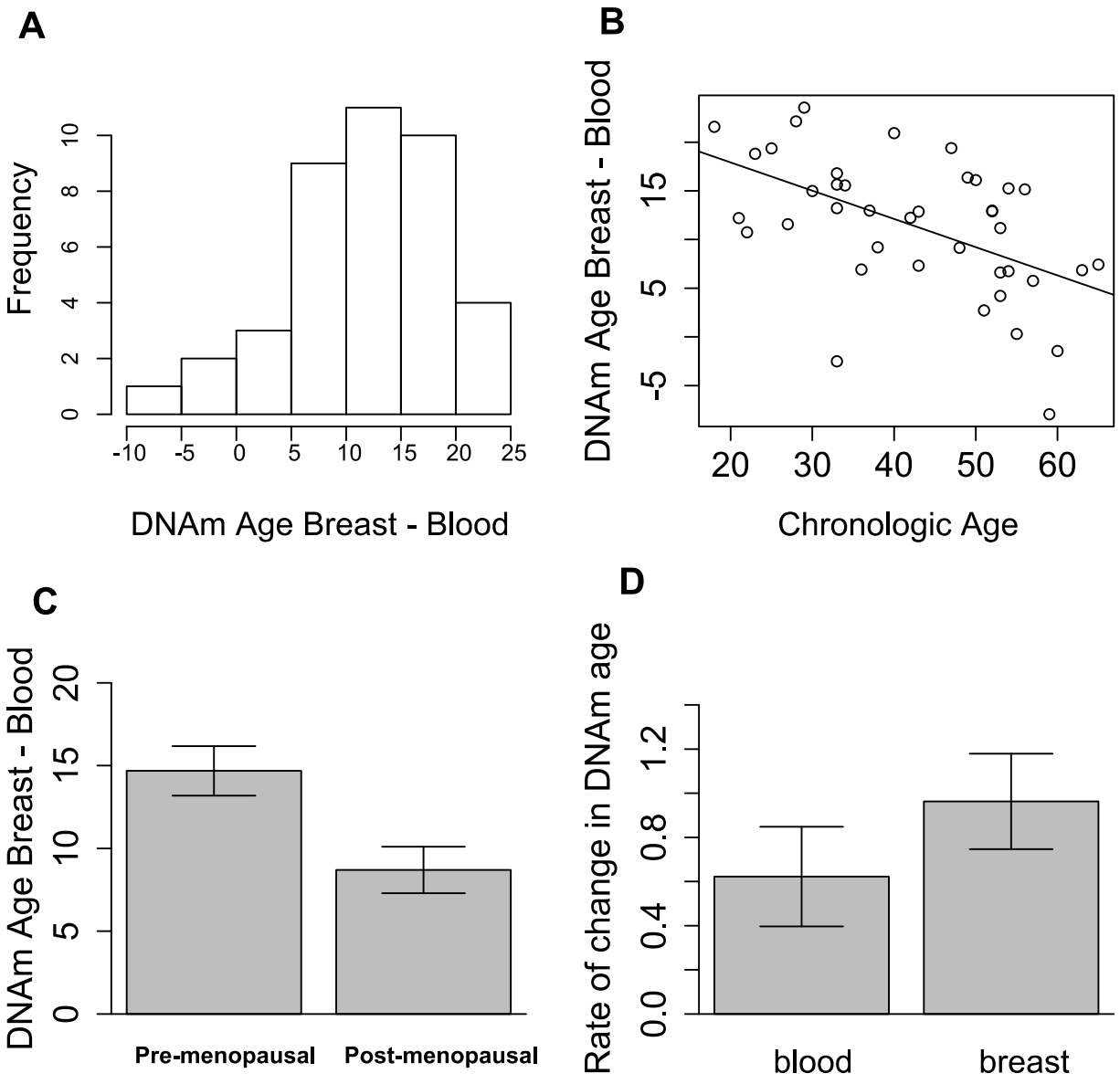
Difference between breast and blood

- Higher in pre-menopausal women compared with post-menopausal (p=0.0098)
- Not significant after adjusting for chronologic age.

Longitudinal changes in DNAm age

- Rate of change in DNAm age =

$$\frac{\text{(DNAm age visit 2 – DNAm age visit 1)}}{\text{(Age visit 2 – Age visit 1)}}$$


A
Frequency
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-10
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DNAm Age Breast - Blood
B
DNAm Age Breast - Blood
-5
5
15
20
30
40
50
60
Chronologic Age
C
DNAm Age Breast - Blood
0
5
10
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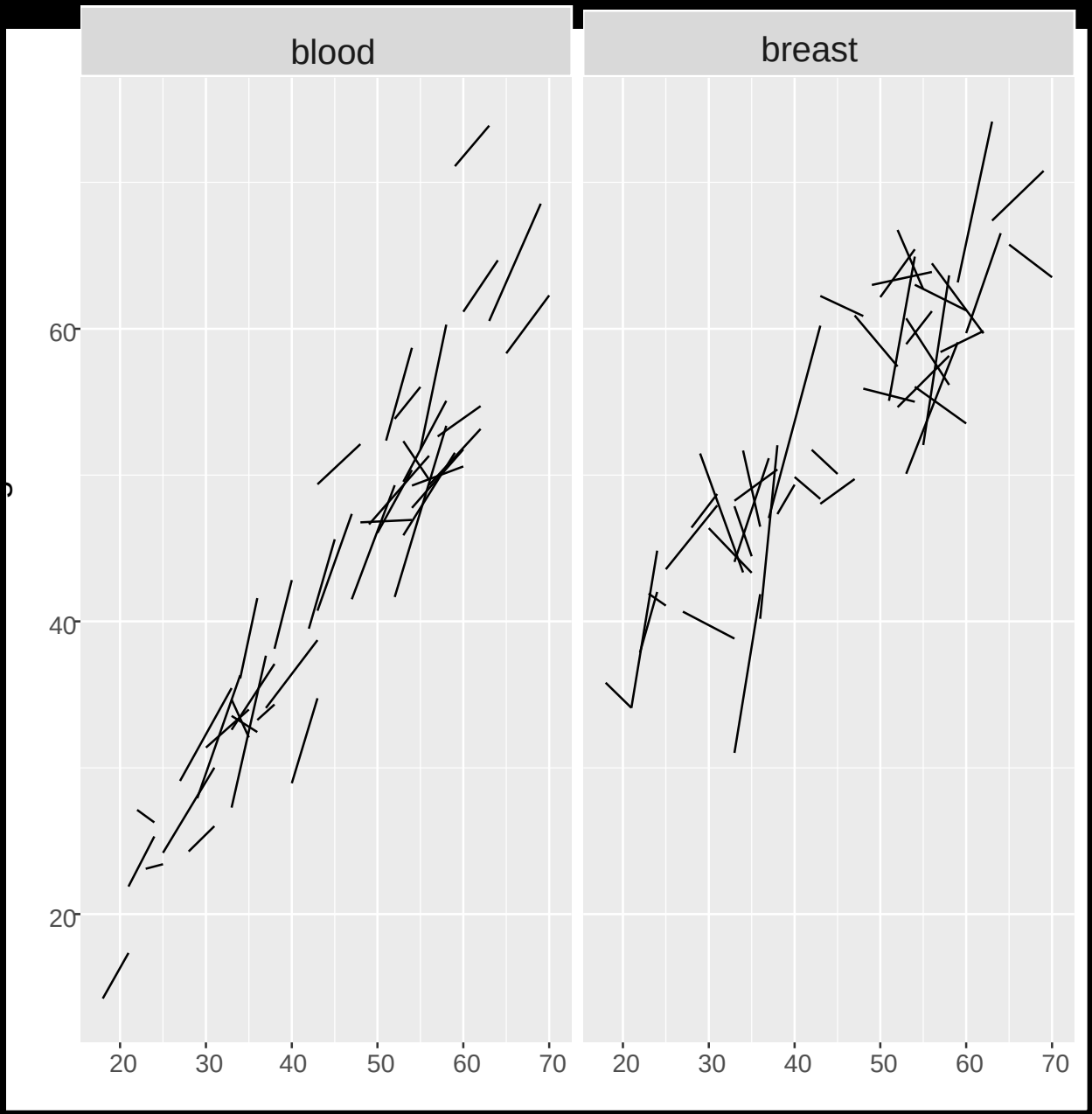
20
Pre-menopausal
Post-menopausal
D
Rate of change in DNAm age
0.0
0.4
0.8
1.2
blood
breast


blood
breast
60
40
20
20
30
40
50
60
70
20
30
40
50
60
70

Comparing age ranges

- Chronological age differences 2-7 years (mean 4.2 years)
- Biologic age difference
- -2.6 to 11.7 years (blood)
- -8.1 to 13.1 years (breast)

Factors associated with acceleration

- Difference between DNAm age breast and blood is associated with:
  - Chronologic age (β=-0.4, 95% CI -0.25 to -0.54)
  - Total menstrual years (β=0.16, 95% CI -0.02 to 0.35) (borderline)

Results Summary

- DNAm age is highly correlated with chronologic age in our sample (cor=0.94, $p<0.001$ for peripheral blood, and cor=0.88 $p<0.001$ for breast tissue).
- Age acceleration is significantly increased in breast relative to peripheral blood tissue ($p<0.001$).
- Total menstrual years borderline association with Age accleeration in breast.

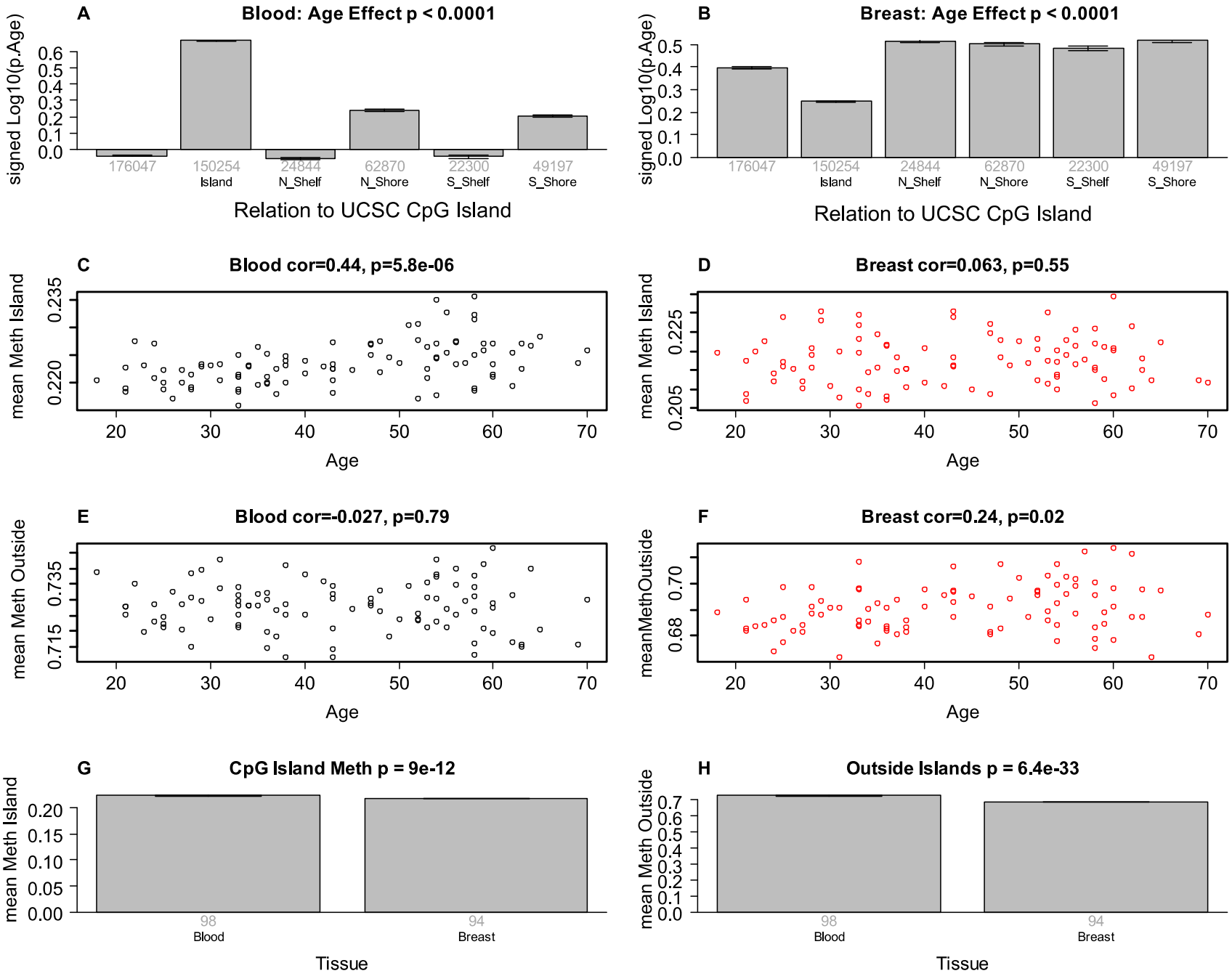
Sehl ME et al. DNA methylation age is elevated in breast tissue of healthy women. Breast Cancer Res Treat 2017;164:209-219.

CpG islands

- Goal: examine difference in CpG island methylation between breast and blood tissue
- CpG islands = regions with high frequency of CpG sites located at or near the transcription start site of genes
- Within islands sites ***gain*** methylation with age
- Outside sites ***lose*** methylation with age

CpG island analysis

- Mean methylation at CpG islands
- Compare with those located in other chromosomal locations
- Separate analyses for breast and blood


A
Blood: Age Effect p < 0.0001
signed Log10(p.Age)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
176047
150254
Island
24844
N_Shelf
62870
N_Shore
22300
S_Shelf
49197
S_Shore
Relation to UCSC CpG Island
B
Breast: Age Effect p < 0.0001
signed Log10(p.Age)
0.0
0.1
0.2
0.3
0.4
0.5
176047
150254
Island
24844
N_Shelf
62870
N_Shore
22300
S_Shelf
49197
S_Shore
Relation to UCSC CpG Island
C
Blood cor=0.44, p=5.8e-06
mean Meth Island
0.220
0.235
20
30
40
50
60
70
Age
D
Breast cor=0.063, p=0.55
mean Meth Island
0.205
0.225
20
30
40
50
60
70
Age
E
Blood cor=-0.027, p=0.79
mean Meth Outside
0.715
0.735
20
30
40
50
60
70
Age
F
Breast cor=0.24, p=0.02
meanMethOutside
0.68
0.70
20
30
40
50
60
70
Age
G
CpG Island Meth p = 9e-12
mean Meth Island
0.00
0.05
0.10
0.15
0.20
98
Blood
94
Breast
Tissue
H
Outside Islands p = 6.4e-33
mean Meth Outside
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
98
Blood
94
Breast
Tissue

CpG island methylation

- Inside island methylation positively correlated with chronologic age (in blood)
- Not reproduced in breast
- Average methylation levels outside of islands correlated with chronologic age (in breast)
- Aging effects differ between these two tissues

CpG island analysis

- Mean methylation levels of CpGs lower in breast tissue compared with peripheral blood
- Particularly in CpGs located outside CpG islands

Limitations

- Whole breast tissue
  - Adipocytes
  - Epithelial cells
  - Myoepithelial cells
  - Fibroblasts
  - Inflammatory cells
  - Vascular endothelial cells
  - macrophages
- True deceleration or alterations in cell composition?

Further questions

- What factors regulate the aging process in the breast?
- Are there common mechanisms underlying accelerated aging and carcinogenesis?
- Does acceleration occur beginning at puberty? Is it linked to stimulation by estrogen, progesterone, oxytocin, and cell cycling?

Future Plans

- Expand study of KTB healthy tissue donors:
  - include larger sample of healthy women
  - examine additional factors: e.g. lactation history, exogenous hormones) that may be associated with breast age acceleration
- Continue collaboration with KTB investigators to study early molecular changes that predict risk of breast cancer
  - Subsample of KTB donors who donate tissue years before they develop breast cancer
- high risk breast clinic – identify epigenetic biomarker that predicts risk of breast cancer for persons at high risk

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- Dr. Patricia Ganz (mentor and collaborator)
- Dr. Natascia Marino (collaborator)
- Jill Henry (KTB)