Telomere Biology for Age Management Medicine

Joseph Raffaele, MD
PhysioAge Systems
Hey Brother, what’s in your water?

Age Reversal . . . ?
Clinical Telomere Biology Experience

• Age management medicine specialty practice for 20 years
• Telomere biology clinical research and practice for 10 years
  • Co-authored first human observational study of a telomerase activation molecule
  • Co-authored first controlled trial of telomerase activation in humans
• Reviewed over 2000 telomere length measurements in adult patients
• Followed individual telomere length measurements longitudinally for up to 10 years in over 100 patients
“You’re fifty-seven years old. I’d like to get that down a bit.”

Let’s lengthen your telomeres!
Objectives and Questions

• Basic telomere biology
• Telomere change with age: Is it important to measure and treat?
• Telomeres and uncommon genetic disorders of shortened lifespan
• Mouse models of telomere biology
• Telomeres and common diseases of aging
• Telomeres and cancer risk
• Telomere length enhancers
• Is there an optimal telomere length window?
What do Telomeres do?

**Protect**
- Serve as chromosome end-caps to protect the integrity of our genes.

**Prevent**
- Keep chromosomes from degrading to prevent fusion and massive genomic instability.

**Proliferate**
- Allow cells to replicate (cells cannot divide when telomeres get too short)

**Bottom Line:** Telomeres protect cells from DNA mutations, senescence and death.
How Do Telomeres Work?

Adapted from Neumann AA Nature Reviews Cancer 2, 879-884

Telomere binding proteins

Shelterin Complex

Adapted from Oeseburg Eur J Physiol (2010) 459:259–268

150-200 bp G-rich 3’ strand

TTAGGG
Telomeres: Length and Shortening with Age

- **Length**: 10 kb (8-12 kb) at birth
  - Accelerated loss with growth
  - 8 kb at young adulthood (4 kb variation)
- **Aging**: lose 0.05 kb per year
  - Critical TL ≈ 5 kb at end of life
  - 8-5 kb = 3 kb avg loss over adult lifetime
- **Cell division**:
  - Lose 100 base-pairs per division
  - Mostly in stem cells and highly proliferative tissues (BM, WBC, gut, skin, etc.)
- **Oxidative stress**:
  - Increases loss with each division
  - GGG portion of TTAGGG repeat very susceptible to free radicals
- **End-replication problem**:
  - Cannot fully replicate lagging (3’) strand

**Need Telomerase**

Aubert G 2014 *Prog Mol Biol Trans Sci* vol 125
Telomerase Enzyme and Shelterin Complexes

Structure: Two components
- hTERT: human telomerase reverse transcriptase, the catalytic component
- TERC: telomerase RNA template component (aka TR)

Function: Lengthen telomeres
Shelterin: Assembly of telomere binding proteins

Activation:
- Very active during embryogenesis
- Repressed before birth
- Repressed during adult life in most tissues except those with rapid turnover.
  
  Adult activity insufficient to maintain telomere length
  Birth marks beginning of telomere erosion

Reactivation:
- hTERT gene transduction
- Small molecule hTERT transcription activators

Telomere Length Determinants

Inherited Length
- “Telotype”: inherited trait
- Heritability rate 36-84% (Eisenberg D 2012)
  - Largest meta-analysis 70%
  - Maternal > Paternal (Boer L 2013)
- Race: African > Caucasian
- Gender: female > male
- Paternal age is factor: Older men pass on longer telomeres (De Meyer T 2007)

Attrition Rate
- Slowing/reversing:
  - Telomerase activity
- Increasing:
  - Proliferative activity
    - Tissue injury, chronic infections and diseases
  - Oxidative stress
    - Smoking, obesity, sedentary lifestyle, hypertension, stress, low antioxidant status
Telomere Attrition determined by balance between loss and telomerase activity

- Proliferative activity
- Oxidative stress
- Inflammation

Telomerase activity

Lifestyle
- Diet
- Stress
- Supplements
- Meds

Shorter Telomeres

Longer Telomeres
Telomere Length Measurement in Clinical Practice

$y = -0.046x + 8.78$

$R^2 = 0.32, \ p<0.001$

TL loss = 0.046 kb/yr

PhysioAge Systems 2010-2017
Unpublished data
Telomere Length Variation: Does it Matter?

Top 1\textsuperscript{st} percentile

Bottom 10\textsuperscript{th} percentile

Top 10\textsuperscript{th} percentile

Bottom 1\textsuperscript{st} percentile
Telomere Associated Diseases

- Cardiovascular
- Cancer
- COPD
- Alzheimer’s
- Immunosenescence
- Degenerative Disc Disease
- Osteoarthritis
- Rheumatoid Arthritis
- Osteoporosis
- Macular Degeneration

- Muscular Dystrophy
- Cell & Tissue Transplants
- AIDS
- Dyskeratosis Congenita
- Aplastic Anemia
- Idiopathic Pulmonary Fibrosis
- Cryptogenic Liver Cirrhosis
- Down’s Syndrome
- Fanconi’s Anemia
- Progeria
- Werner’s Syndrome

• Aging Itself?

Genes load the gun. Lifestyle pulls the trigger.
Extreme cases in medicine inform more common milder dysfunction

**Genetic disorder**
- **Familial hypercholesterolemia**
  - Polygenic: one of 4 genes
  - 1:500 prevalence (heterozygote)
  - 1:1,000,00 homozygous
  - High circulating cholesterol with deposition in tendons, skin, and coronary arteries causing premature MI
    - Heterozygous MI in 40-50s
    - Homozygous MI in 20s

**Milder multifactorial disorder**
- **Hyperlipidemia**
  - 1:3 prevalence
  - Polygenic plus lifestyle/diet
  - High circulating cholesterol leads to atherosclerosis, MI, stroke, PAD

Can looking closely at the mutations, penetrance, and mechanisms of disease in rare Mendelian monogenic disorders inform our understanding of polygenic common diseases and aging?
Primary Telomeropathy: Dyskeratosis Congenita

Clinical Manifestations

- Rare childhood disorder
- High proliferative tissues
  - Abnormal pigmentation
  - Oral leukoplakia
  - Nail dystrophy
  - Aplastic anemia, BM failure
    - 80% Die of it by age 30
    - 10% get cancer
      - Head/neck squamous cell
      - AML, MDS
  - Intestinal epithelial abnormalities
- Slow turnover tissues
  - Pulmonary fibrosis
  - Cirrhosis
  - Impaired glucose tolerance
  - Insulin resistance
  - Osteoporosis

Triad of signs

Kelmenson DA N Engl J Med 2017
DKC: Disease of very short telomeres

- < 10\textsuperscript{th} percentile telomere length
- < 1\textsuperscript{st} percentile is 95\% sensitive and specific for a telomeropathy
Mutations in Telomere Biology Disorders

Adapted from Dokal I Hematology 2011;2011:480-486
Idiopathic Aplastic Anemia

• Aplastic anemia (AA): etiology multifactorial
  • Acquired is immune mediated, infectious, or environmental
  • Inherited bone marrow failure often presents as isolated AA
    • 10% with isolated AA have autosomal dominant (AD) mutations of TERT and TERC.
    • Telomere length is below 10th percentile for age
    • Presents in 20s to 40s
  • 50% telomerase activity
Idiopathic Pulmonary Fibrosis (IPF)

- Progressive, generally fatal, disease of the lungs causing scarring and loss of alveolar airspace
- Prevalence (US): 128,000
  - Incidence: 48,000
  - Mean age diagnosis: 51 years old
  - Mortality per year: 40,000
- 10% Familial
  - 8-15% of familial and 1-3% of
  - Sporadic cases with TERT and TERC mutations
- Most prevalent manifestation of a telomeropathy
- Latest presentation of a telomeropathy
- 50% telomerase activity
Cryptogenic (Non-alcoholic/infectious) Cirrhosis

- 5% of cases of cirrhosis have no risk factors
- Can accompany either AA or IPF or can be found in asymptomatic carrier family members
- Mutations in telomerase components found in TERT/TERC
How Are Slow Turnover Tissues Affected by Short Telomeres?

Telomere Mitochondria connection: PGC1α,β

↓ Mitochondria number

↓ Mitochondria efficiency

Sahin E 2012 *Nat Rev Mol Cell*
Lymphocyte Telomere Length in Telomeropathies

What’s going on here?

Is there no cross-sectional telomere length attrition with age in Telomeropathies?
Telomere Attrition Rate in Telomero-pathies

Survival Bias

Adapted from Alter BP 2012 *Haematologica*; 97(3)
Genetic Anticipation of Age of Onset and Clinical Manifestations

*Telomere length is the determinant of disease, not telomerase activity*

Progressively shorter telomere length inherited with each generation

Telomeres and Knockout Mouse Models

• Normal lab mice (*mus musculus*) have long telomeres—50-70 kb, humans 6-12 kb
• Don’t exhibit typical human aging
  • Some degenerative disease but most die of cancer,
  • Not epithelial cells, but sarcomas/lymphomas
• Telomerase knockout (KO) mice shorten telomeres over 3 generations
  • Complete telomerase knockout
Telomerase KO Mice

- TERC -/- KO mice
- Progressive decrease mean/max lifespan
- Decreased telomere length
- Premature aging pathologies worse with each generation
- Genetic anticipation similar to telomeropathies
- But, mostly high turnover tissues
  - BM, gut, germ cells

Donate LE 2011
• Homozygous TERC (TR) KO mouse model

• Significant increase in hair graying/alopecia and skin ulceration with shorter telomeres in successive generations

• Weakened immune system

• Intestinal atrophy

• Decreased spleen size

• Decreased wound healing

• Decreased lifespan

Rudolph KL 1999 Cell
**A Better Mouse Model**

- *Mus Castaneus* telomere length ≈ 15 kb
- **Haploinsufficient model:**
  - *TERC* +/-: 50% of usual telomerase activity (Similar to Telomeropathies)
  - Develop organ degeneration with short telomeres (Armanios 2009)
  - Wild type late generation littermates also have organ degeneration with age
  - Recapitulates human immunoscenesence
  - More susceptible to emphysema with cigarette smoke exposure (Alder 2011)
Further Proof of Concept: Intervention

Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer

Bruno Bernardes de Jesus, Elsa Vera, Kerstin Schneeberger, Agueda M. Tejera, Eduard Ayuso, Fatima Bosch, Maria A. Blasco

AAV wide tropism expressing mouse TERT had remarkable beneficial effects on health and fitness, including insulin sensitivity, osteoporosis, neuromuscular coordination and several molecular biomarkers of aging.

telomerase-treated mice, both at 1-year and at 2-year of age, had an increase in median lifespan of 24 and 13%, respectively.

See accompanying article
http://dx.doi.org/10.1002/emmm.201200246
First Age Reversal in a Mammal

Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice

Mariela Jaskelioff¹, Florian L. Müller¹, Ji-Hye Paik¹, Emily Thomas¹, Shan Jiang¹, Andrew C. Adams², Ergun Sahin¹, Maria Kost-Alimova¹, Alexei Protopopov¹, Juan Cadiñanos¹, James W. Horner¹, Eleftheria Maratos-Flier² & Ronald A. DePinho¹

- Telomerase Activation was used to change old mice back to young adults.
- 33% increase in telomere length
- Brain, spleen and reproductive organs were all rejuvenated;
- Resulting in increased neurons and new viable sperm cells.
- Sense of smell returned.
- None of the mice developed cancer.

2011 DePinho et al
Telomeres and Common Disease Association

• Chronic disease association
  • Hypertension
  • Atherosclerosis
  • COPD
  • Alzheimer’s dementia
  • Cancer
  • Obesity/Diabetes
  • Metabolic syndrome
  • Chronic stress

• Mortality association:
  • Cawthon 2003 Lancet: Landmark study in subjects 60 years old
    • Those with longest telomeres lived longer than shortest telomeres. Cause of death infection
    • Shortest quartile of telomere length 60% more likely to die than longest quartile. Cause again infectious
Telomere length sheds light on relationship between CVD risk factors and events

- Having shorter than average lymphocyte mean telomere length increased the risk of premature MI roughly 3-fold
- The difference in telomere length between cases and controls translates into a biological age difference of 11 years
- An example of telomere length as a modifier of disease onset

Brouilette S 2003 Arterioscler Throm Vasc Biol
Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis

Philip C Haycock postdoctoral research assistant, Emma E Heydon doctoral candidate, Stephen Kaplco senior research associate, Adam S Butterworth university lecturer, Alex Thompson senior epidemiologist, Peter Willeit research associate

1Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Strangeways Research Laboratory, Cambridge, UK; 2Medical Research Council Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol, Bristol, UK; 3Roche, Welwyn Garden City, UK; 4Department of Neurology, Innsbruck Medical University, Austria
“This meta-analysis indicates that telomere length is inversely associated with risk of coronary heart disease independently of conventional vascular risk factors.”
“Some have suggested that chronic obstructive pulmonary disease (COPD) is a disease of accelerated aging.”

Compared with 4th quartile (longest), the other three quartiles had increased risk of cancer (1.48 HR) and total (1.29 HR) mortality.

“Accelerated aging is of particular relevance to cancer mortality in COPD.”
FREE LUNCH!

Cancer Risk?????
Telomerase is not an oncogene

- Cancer cell ≠ an immortalized cell
- Both have unlimited proliferation because of telomerase activation
- Cancer cells: oncogenic mutation
  - Lose function and control of cell cycle
  - Have altered morphology/nuclear changes
- Normal cells: without oncogenic mutations
  - Normal function and morphology
- Gene transduction with the catalytic component of hTERT on fibroblasts, epithelial cells, and keratinocytes
  - Unlimited proliferation and normal function
  - When transplanted into immunodeficient mice
    - NO altered growth and NO tumorigenesis
Theoretical Risk: Evolutionary Perspective

- ↓telomerase activation (TA) evolved as a tumor suppressor mechanism
- Telomerase suppressed at birth
- Longer lived mammals have less TA and shorter telomeres
- 95% of malignant cells expression telomerase
- Longer telomeres may allow a cell with oncogenic mutations to stay alive long enough to activate telomerase permanently
- Early life benefit (tumor suppressor) causes late life harm (degeneration and genomic instability)
Telomere Length and Cancer: Prospective Study

Study
• Bruneck Italy
• N=787
• Age 40-79
• Free of cancer
• 15 year follow up
• Tertiles of TL
• BL and 10 yr TL

Results
• Cases = 137
• Deaths = 62

The median telomere length (T/S ratio) for the shortest-length tertile was 0.81 (range, 0.19-1.04); for the middle-length tertile, 1.29 (range, 1.05-1.59); and for the longest-length tertile, 2.22 (range, 1.60-5.93). There were 137 cases of cancer incidence and 62 cases of cancer mortality. TL indicates telomere length. Y-axis shown in blue indicates range from 0 to 0.12.
“However, caution regarding this causal interpretation is warranted in light of the potential issue of pleiotropy, and a more general interpretation is that SNPs influencing telomere biology are also implicated in lung adenocarcinoma risk.”
Genome-wide Association Studies (GWAS) Linking Telomere Length and Cancer Risk

• Large GWAS find single nucleotide polymorphisms (SNPs) associated with longer telomere length
  • ACYP2, TERC, NAF1, TERT, OBFC1, ZNF208, and RTEL1 are known components of telomere maintenance genes

• Take same SNPs and associate with cancer risk to create a “Genetic Risk Score” as a substitute for telomere length measurement

• Find increase risk of melanoma and glioma as well as lung adenocarcinoma in those with higher risk scores

• Problem:
  • 1. Not a direct study of “long telomeres” and cancer risk
  • 2. SNPs only account for between 0.5% and 3% of telomere length variation
Peripheral Blood Leukocyte Telomere Length and Mortality Among 64,637 Individuals From the General Population

- Copenhagen
- 42-70 years old
- 0-22 years follow up (7 yr mean)
- qPCR TL decile comparison
- 3 SNP genetic risk score

7607 Deaths
CVD 2633
Cancer 2420

HR = 1.4 for 10th v 1st decile and similar for CVD and cancer

Rode LJ Natl Cancer Inst. 2015;107(6)

Figure 2. Risk of all-cause mortality in the 64637 participants from the general population according to telomere length deciles in age-adjusted and multivariable-adjusted Cox regression analysis. Multivariable models were adjusted for age, sex, body mass index, systolic blood pressure, smoking status, tobacco consumption, alcohol consumption, physical activity, and cholesterol level. All statistical tests were two-sided.
A Continuum from telomeropathies through the diseases of aging and aging itself?

Adapted from Changeable?

Adapted from Copyright © 2005 Nature Publishing Group
Nature Reviews | Genetics
Effective Telomere Length Enhancers?

**Lifestyle**
- Stress reduction  
  Epel ES 2004 *PNAS*
- Exercise  
  Ludlow A 2011 *J Aging Res*
  - Mitigates effect of perceived stress  
    Puterman E 2010 *PloS One*
- Weight loss  
  Valdez AM 2005 *Lancet*
- Smoking cessation  
  Song Z 2010 *Aging Cell*
- Avoidance of CMV  
  Pawelec 2014 *Exp Gerontol*

**Diet**
- Omega-3 FA intake  
  Farzaneh-Far R 2010 *JAMA*
- Low fat intake  
  Ornish D 2008 *Lancet Oncol*

**Supplements**
- Vitamin D  
  Richards BJ 2007 *Am J Clin Nutr*
- Antioxidants  
  Paul L 2011 *J Nutr Biochem*
- Astragalus root  
  Harley CB 2011 *Rejuvenation Res*

**Hormones**
- Estradiol increases telomerase activation (TA)  
  Calado RT 2009 *Blood*
- Cortisol decreases TA  
  Choi J 2008 *Brain Behav Immun*
- IGF-1 increase TA  
  Moverare-Skrtic S 2009 *JCEM*
Very Short Telomeres in Healthy 39 year old woman

- Premature greying
- Increased lung age
- FHx early CAD
Lymphocytes  |  Granulocytes  |  CD45RA+ (Naïve T)  |  CD45RA- (Memory T)  |  CD20+ (B Cells)  |  CD37+ (NK Cells)
---|---|---|---|---|---
MTL | MTL | MTL | MTL | MTL | MTL |
HT | HT | HT | HT | HT | HT |
H = Very High  |  (≥ 99 percentile)  |  ≥ 99 and < 99 percentile  |  ≥ 90 and < 99 percentile  |  ≥ 1 and < 90 percentile  |  < 1 percentile |
N = Normal  |  (≥ 10th percentile)  |  ≥ 10 and < 90 percentile  |  ≥ 1 and < 90 percentile  |  < 1 percentile |
VL = Very Low  |  (≥ 1 percentile)  |  ≥ 1 and < 90 percentile  |  ≥ 1 and < 90 percentile  |  < 1 percentile |

MTL = Patient Median Telomere Length  
MTL_H = Normal MTL at age (90th percentile)  
HT = Telomere length interpretation

![Graphs of Lymphocytes and Granulocytes](image1)

![Graphs of CD45RA+ (Naïve T Cells) and CD45RA- (Memory T Cells)](image2)

![Graphs of CD20+ (B Cells) and CD37+ (NK Cells)](image3)
What can we do for her?

• Does she have a monogenic telomeropathy?
  • Genetic testing available

• Dietary and lifestyle changes
  • Definitely no smoking
  • Moderate alcohol
  • Exercise
  • Stress reduction
  • Supplements: Vitamin D, omega-3 FA, etc.

• More diligent cancer surveillance:
  • Routine, but earlier?
  • OncoBlot?

• Follow telomere length

• Telomerase activation therapy
  • 2-fold increase in telomerase activation could significantly mitigate effect if she has telomerase haploinsufficiency
Is there an Optimal Telomere Length?

**Genetic Long Telomere Syndromes?**
Adenocarcinoma lung, glioma, melanoma, ovarian cancer

**Window of Healthspan?**

- **Familial melanoma-glioma**
- Premature MI, COPD
- SCC, BCC, Lung CA

Adapted from Stanley S 2016 *Curr Opin Genet Dev*
Who do you want to be?
Do you want to know?

Top 1st percentile
- Maybe, ↓
- Denenerative dz
- ? ↑ CA

Top 10th percentile
- Yes, ↑ Healthspan
- ? ↓ CA, ↑ longevity

Bottom 10th percentile
- No, ↑ CVD, CA
- ? Earlier mortality

Bottom 1st percentile
- No, Telomeropathy
- Shortened lifespan
Telomere Length as a Biomarker versus Risk Factor

- *Macro-index* of health, disease risk, patient “resilience”
- Independent and often better than conventional risk factors

Risks for aging-related diseases
Cancer, Cardiovascular, Stroke...

Measures of the health of your Biological Retirement Plan

Genetics
Environment
Stress, diet, behavior

Telomere attrition
Arigato!

Slides available on drraffaele.com