

Telomeres as Sentinels for Environmental Exposures, Psychosocial Stress, and Disease Susceptibility

***A Workshop Co-sponsored by
the National Institute of Environmental Health Sciences (NIEHS) and
the National Institute on Aging (NIA)***

September 6-7, 2017

NIEHS Building 101
Rodbell Auditorium
Research Triangle Park, NC

Workshop Summary

Revised November 21, 2017



This workshop summary was prepared by Samuel Thomas, Rose Li and Associates, Inc., under contract to the National Institute on Aging. The views expressed in this document reflect both individual and collective opinions of the workshop participants and not necessarily those of the National Institute on Aging, the National Institute of Environmental Health Sciences, or any organization represented by the workshop participants. Review of earlier versions of this meeting summary by the following individuals is gratefully acknowledged: Allison Aiello, Mary Armanios, Abraham Aviv, Susan Bailey, Linda Birnbaum, Stacy Drury, Elissa Epel, Michelle Heacock, Peter Lansdorp, Rose Li, Jue Lin, Belinda Needham, Lisbeth Nielsen, Patricia Opresko, Martin Picard, Chandra Reynolds, Janine Santos, Sharon Savage, Idan Shalev, Nancy Tuvesson, Pathik Wadhwa, Nan-ping Weng, and Danyelle Winchester.

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Acronym Definitions

ALT	alternative lengthening of telomeres
ATP	adenosine triphosphate
BLSA	Baltimore Longitudinal Study of Aging
BMI	body mass index
CATSLife	Colorado Adoption/Twin Study of Lifespan Behavioral Development and Cognitive Aging
CMV	Cytomegalovirus
DKC1	Dyskerin
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
FISH	fluorescent in-situ hybridization
flow FISH	flow cytometry with fluorescent in-situ hybridization
GE	gene-environment
GTEX	Genotype-Tissue Expression Project
GWAS	genome-wide association studies
HHV	human herpesvirus 6
HIV	human immunodeficiency virus
HPV	human papilloma virus
HSC	hematopoietic stem cell
HSV	herpes simplex virus
mTAC	maximal telomerase activity capacity
mtDNA	mitochondrial DNA
NASA	National Aeronautics and Space Administration
NHANES	National Health and Nutrition Examination Survey
NIA	National Institute on Aging
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
OGG1	8-oxoguanine glycosylase
PBMCs	peripheral blood mononuclear cells
PNA	peptide nucleic acid
qFISH	quantitative fluorescent in-situ hybridization
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
SATSA	Swedish Adoption/Twin Study of Aging
SES	socioeconomic status
SNPs	single nucleotide polymorphisms
STROBE	STrengthening the Reporting of OBservational studies in Epidemiology
TERC	telomerase RNA component
TERT	telomerase reverse transcriptase
TRF	terminal restriction fragment
WGS	whole genome sequencing

Executive Summary

On September 6 and 7, 2017, the National Institute of Environmental Health Sciences (NIEHS) and the National Institute on Aging (NIA) convened an interdisciplinary workshop to explore the potential use of telomere length as a biomarker for environmental exposures, psychosocial stress, and disease susceptibility. Five sessions featured presentations on the state of the science, known effects of psychosocial stress on telomeres, critical considerations regarding telomere dynamics, the influence of genes and the environment, and the relationship between telomeres and other biomarkers. Throughout the workshop, participants discussed the challenges and opportunities of using the telomere as a biomarker of environmental exposures, psychosocial stress, and disease susceptibility. They also suggested constructive actions that would advance the field.

Challenges

Concerns about the validity and reliability of telomere length measurement methods are a key challenge. Commonly used methods to measure telomere length in large, epidemiological studies are sensitive to protocol deviations and are often associated with high measurement error. Therefore, even when consistent and of high quality, the results produced by different laboratories may not be easily compared. Although several alternative technologies exist, most are not suitable for high-throughput analysis and archived DNA samples, and the relationships between results obtained through different techniques are not always clear. Studies seeking to identify relationships between stress and environmental exposures and telomere length are further complicated by inconsistencies in approaches to measuring psychosocial stress across studies and complexities associated with isolating the effects of individual exposures. The literature on stress, environmental exposures, and telomere length is somewhat contradictory; the extent to which divergent findings are due to measurement error remains unclear.

Many unanswered questions about telomere biology and its influence on health outcomes challenge interpretation of epidemiological studies that include telomere length. The telomere system is dynamic, yet telomeric responses to stimuli remain poorly understood. Although it is well known that telomeres shorten rapidly in early life and then gradually over a lifespan, recent evidence suggests that telomeres may also shorten or lengthen significantly over a period of days and months in adults. These potential short-term variations in telomere length, if real and not an artifact of measurement error, often may be overlooked, because most studies collect samples infrequently. A further complication is that some apparent shortening or lengthening may be due to changes in the cell-type composition of collected samples, rather than real changes in telomere length in each cell. Moreover, statistically significant shortening or lengthening of telomeres is not always clinically significant. The thresholds of clinical significance require further study. Similarly, the current understanding of the mechanisms that link exposures to telomere length and telomere length to health outcomes is insufficient. Many mechanisms may theoretically contribute to observed associations, but existing evidence is inconclusive in most cases. Animal research is often an effective way to conduct mechanistic research, especially on how exposures affect telomeres; however, telomere biology differs substantially between humans and model organisms with short lifespans, complicating cross-

species comparisons. For example, rodents have long telomeres that therefore do not contribute to disease except in generational studies, when they have been shortened, or when they have been shortened genetically.

Opportunities

Despite these challenges, participants identified several promising opportunities for using telomere length as a biomarker of environmental exposures, psychosocial stress, and disease susceptibility. Interest in telomeres is growing, with many new studies published each year. Several consistent findings are emerging: on average, telomeres are longer in females than males and in blacks than whites. Telomere length is influenced by genetics as well as heritable non-genetic maternal and paternal factors and environmental factors. Extremely short telomeres are associated with a set of related medical genetic disorders, and longer telomeres may increase the risk of certain cancers. These well-defined relationships provide an opportunity for further study to understand the underlying biological mechanisms, which may, in turn, clarify other, more ambiguous findings.

Novel tools and collaborations may provide opportunities for new insights. Technological developments, such as whole genome sequencing, may provide new telomere length measurement tools and help mitigate some of the persistent challenges. Many large longitudinal cohort studies have stored biospecimens that may be available for telomere length analysis. Because these studies often measure many biomarkers, health outcomes, and lifestyle factors, they offer an ideal setting for investigating longitudinal trajectories of telomere length and their association with stress, environmental exposures, and disease. Investigators are increasingly taking advantage of natural experiments that expose some populations, but not others, to stressors that might be associated with telomere length changes. These experiments may help determine causal relationships and identify potential underlying mechanisms. Finally, telomere researchers have increasing opportunities to collaborate with experts in other disciplines. Bringing together scientists with different perspectives and expertise presents an opportunity to approach seemingly intractable questions from new angles and with new tools.

Suggested Next Steps

Workshop participants suggested several actions to help overcome the challenges and realize the opportunities of studying telomeres in the context of stress and environmental exposures. These include but are not limited to:

- **Develop consensus guidelines** for telomere length research, including biological sample collection, storage, and processing; laboratory methods; data analysis; and reporting requirements. There is an urgent need for such guidelines, and it is feasible to develop them in a short timeframe.
- **Conduct a robust methods comparison study** to determine the relationship between different telomere length assays, inter-assay variability, and the factors that influence results. A staged approach could speed availability of initial comparative results while serving as a platform to answer broader questions, such as the relationship between telomere length and certain exposures and health outcomes.

- **Support an effort to collect unpublished knowledge on best practices** for laboratory assays that would serve as a resource for the field.
- **Produce and provide standard reference samples** to calibrate telomere length measurements conducted in different laboratories using different methods.
- **Encourage more research on telomere length dynamics** including studies that take advantage of natural experiments, include repeated measures, and investigate interactions between multiple variables.
- **Focus on early life determinants** of telomere length and attrition, including mechanistic and cross-tissue studies. Existing longitudinal cohort studies with rich early life data and stored biospecimens may present an especially attractive opportunity.
- **Develop better measures of stress exposure** including both composite measures and differentiated measures of specific types of stress.
- **Foster interdisciplinary collaborations** between basic telomere biologists, exposure researchers, clinicians, epidemiologists, biostatisticians, and others.

Workshop Summary

On September 6 and 7, 2017, the National Institute of Environmental Health Sciences (NIEHS) and the National Institute on Aging (NIA) convened a workshop to discuss the potential use of telomere length as a biomarker for environmental exposures, psychosocial stress, and disease susceptibility. The workshop brought together experts in basic telomere biology, medicine, biopsychology, epidemiology, and related fields and sought to stimulate cross-disciplinary discussion. The objectives of the workshop were to:

1. Explore current and future possibilities for using the telomere as a potential biomarker of environmental and stress exposure or disease susceptibility.
2. Discuss the tractability of using telomeres as a proxy/indicator of DNA damage.
3. Consider tissue- and cell-type specific effects and how they influence the above goals.
4. Identify how telomere measurements can be integrated into and enhance epidemiological studies.
5. Develop a set of recommendations for advancing the inclusion of telomere measurement in population-based studies, including identification of both short- and long-term research needs.

Workshop participants were encouraged to suggest specific needs for research or resources to support research that could be spearheaded by NIEHS, NIA, both, or through a collaboration with other Institutes and Centers of the National Institutes of Health (NIH). Next steps following the workshop could include a white paper describing the state of the science of using telomeres in population-based research, a set of guidelines for telomere length measurement in population studies, a prioritized research agenda for addressing questions raised during the workshop, or any other concrete ideas that arise from discussion.

Session 1: State of the Science

Chair: Michelle Heacock, PhD, Hazardous Substance Research Branch, NIEHS

The purpose of this session was to provide a high-level overview of three major crosscutting themes as a foundation for the workshop: the association of telomeres and disease, the effects of environmental exposures on telomeres, and the potential role of stress.

Telomeres and Disease Causality and Association

Mary Armanios, MD, Johns Hopkins University

Telomeres define the ends of the chromosome and shorten with cell replication. At a certain threshold, telomeres become dysfunctional and activate a DNA damage response, leading to cellular senescence or apoptosis. Although telomeres shorten all the time, they are linked directly to clinical phenotypes only at certain length thresholds.

Telomerase, an enzyme that elongates telomeres, is comprised of protein and RNA components. It is upregulated in most cancers. Telomerase has sometimes been touted as a

cure for cancer or a solution for aging. Its role, however, and the health implications of short or long telomeres, are more nuanced.

Certain loss of function mutations in telomerase, most commonly mutations in telomerase reverse transcriptase (*TERT*), are heritable. Pedigrees of affected families show genetic anticipation, with symptoms having an earlier onset and greater severity in later generations. Idiopathic pulmonary fibrosis is the most common phenotype of these diseases. Yet short telomeres are also associated with risk for other lung diseases, such as emphysema. In fact, telomere defects unite a group of seemingly unrelated disorders into a single syndrome complex. These include idiopathic pulmonary fibrosis, emphysema, aplastic anemia, dyskeratosis congenita, and Hoyeraal-Hreidarsson syndrome.

In one study, mice with short telomeres that were exposed to cigarette smoke developed emphysema. Short telomeres, however, appeared to be a risk factor for environmentally induced disease rather than a marker of environmental exposure. That is, cigarette smoke caused additive genetic stress that, combined with the already short telomeres, led to the development of lung disease.

Genome-wide association studies (GWAS) provide additional evidence that telomere length is a causal risk factor for idiopathic pulmonary fibrosis. Several GWAS studies demonstrate that the same single nucleotide polymorphisms (SNPs) associated with short telomere length are also associated with development of idiopathic pulmonary fibrosis.

Although very short telomeres are clearly associated with certain diseases, the clinical significance of many studies that demonstrate statistically significant differences between telomere lengths in different groups is less clear. Many such studies show small effect sizes, and the shortening of telomeres observed may not reach a threshold sufficient to cause disease. In addition, measuring telomeres by quantitative polymerase chain reaction (qPCR) assays, as is done in many large studies, is highly variable. Better measurement methods are needed, particularly for determining whether an individual patient has a clinically significant telomere length phenotype. Rigor is also needed in interpretation of results, and current methods and available data do not support direct to consumer testing.

Just as short telomeres are associated with certain clusters of diseases, emerging evidence suggests that comparatively long leukocyte telomeres are associated with an increased risk of some cancers. For example, heritable gain of function mutations in *TERT* are associated with familial melanoma. Many common cancers, including ovarian cancer, lung adenocarcinoma, and bladder cancer, are also associated with long leukocyte telomeres.

Environmental Exposures and Telomere Effects

Patricia Opresko, PhD, University of Pittsburgh

There is growing interest in using telomere length as a biomarker in environmental and occupational exposure studies, yet the number of studies and available evidence remain limited. The most recent review on this topic was published in 2013 and covered 14 studies

published between 2009-2013.¹ Since then, at least 14 additional studies have been published on this topic. Due to the growing interest in and myriad of challenges associated with studies of telomere length as a marker of exposure, telomere length experts have an opportunity and an obligation to educate the larger research community, set standards for rigorous research, and develop guidelines to improve the reproducibility of results.

Among extant studies, many pollutants are associated with shorter telomeres, although a few are associated with longer telomeres. In addition, the quality of past studies and robustness of their results vary. Nonetheless, several case studies illustrate the importance of considering the impact of exposures on telomerase expression, age at exposure, time of exposure, and cell types measured when investigating how exposures affect telomere length.

Although studies of telomere length and environmental exposures are limited, many studies have examined the relationship between exposure to cigarette smoke and telomere length. Results of such studies are mixed, although the overall trend is toward an association between smoking and shorter telomeres. Large meta-analyses and well-planned prospective studies may help resolve disagreements between prior studies. However, challenges with these approaches remain. For example, one prospective study found a weaker association between smoking and telomere length than many previous cross-sectional studies, perhaps because smokers with shorter telomeres are more likely to be unhealthy and therefore motivated to quit smoking than smokers with longer telomeres.²

In general, the literature suggests that leukocyte telomere lengthening is associated with exposure to occupational combustion pollutants, environmental persistent organic pollutants, and arsenic. Potential mechanisms of telomere lengthening in response to these exposures may include upregulation of telomerase, changes in cell signaling, repopulation by stem cells, and killing of cells with short telomeres. In contrast, telomere shortening is associated with exposure to environmental combustion air pollutants, occupational persistent organic pollutants, radiation, and smoking. Potential mechanisms of telomere shortening in response to these exposures may include cell proliferation, DNA damage in telomeres, inflammation, and oxidative stress.

Studies of environmental exposures and telomere length must consider many complex factors, including exposure dose, chemical composition, duration, type of exposure, age of exposure, and indirect effects caused by mitochondria, epigenetic changes, DNA repair mechanisms, and telomerase. Tissues and cell types measured, methods of telomere length measurement, and cross-sectional versus prospective longitudinal study designs may also affect study results. Depending on these factors, telomere length could potentially serve as a biomarker of the severity of exposure or biological response to exposure, or as a predictor of disease risk or adverse events. However, causality needs to be better understood in terms of the

¹ Zhang X, Lin S, Funk WE, Hou L. Republished: Environmental and occupational exposure to chemicals and telomere length in human studies. *Postgraduate Medical Journal*. 89(1058), 722–728 (2013).

² Zhang C, Lauderdale DS, Pierce BL. Sex-specific and time-varying associations between cigarette smoking and telomere length among older adults. *American Journal of Epidemiology*. 184(12), 922–932 (2016).

consequences of shorter leukocyte telomere length on health and the mechanisms of telomere length dynamics.

Oxidative Stress and Telomeres

Opresko and colleagues have focused on oxidative stress, which is often induced by various environmental exposures, and telomere damage and repair mechanisms. Telomeres are highly prone to oxidative damage *in vitro*, and studies in humans, mice, and cell lines have shown that mild chronic oxidative stress accelerates telomere shortening. Oxidative stress has even been shown to shorten telomeres in non-proliferating cells, although the mechanism is unknown.

Oxidative stress causes DNA damage through the formation of 8-oxoguanine, which is subsequently repaired through base-excision by 8-oxoguanine glycosylase (OGG1). Knocking out *OGG1* in yeast and mice leads to longer telomeres in non-oxidative stress conditions due to 8-oxoguanine disruption of DNA structures that inhibit telomerase. Under oxidative stress conditions, however, *OGG1* knockout leads to accelerated telomere shortening. Telomere shortening may occur only once a threshold of oxidative damage has been reached.

Because oxidative stress affects many cellular processes, its effects on telomere length may be confounded. To isolate the effects of oxidative stress on telomere length, a method has been developed to induce free radical formation in specific parts of a cell or genome. This is accomplished with a peptide that is benign until activated with both a photosensitizer dye and far red light, at which point it produces singlet oxygen that forms 8-oxoguanine. These peptides can be inserted into telomeres and activated to induce oxidative damage only in the telomeric region. The Opresko lab is using this approach to examine the impact of acute and chronic oxidative damage to telomeres on telomere and cellular function in human cell cultures. The technology is now being adapted for use in living organisms (zebrafish and mice) and induced pluripotent stem cells, which will allow mechanistic study of oxidative stress and telomeres *in vivo*.

Stress Effects: Differential Lifespan Effects, Pseudo-Lengthening, and Other Complexities

Elissa Epel, PhD, University of California, San Francisco (remote)

Modern molecular science has opened the door to precision medicine; however, because half of deaths are due to behavioral factors, and because social and environmental factors shape behavior, studying internal bodily processes provides only a partial picture of human health. External factors, the “exposome,” are also important. These factors include exposure to pollution, poor nutrition, substance use, neighborhood characteristics, social relationships, trauma, psychological stress, and many others. Whether causal or not, telomere length is a correlate of these exposures.

Psychosocial stress is one component of the exposome. Yet psychosocial stress is multifaceted, and it is not straightforward to ask whether social stress is related to telomere length. One can distinguish between stress exposures (stressors) and stress responses (stress reactivity or the perception and experience of stress). Some stressors may be measured through retrospective

self-reporting, immediate self-reporting of current states such as through daily monitoring, or through monitoring of biological regulatory systems related to stress. Many studies have examined various chronic stressors, which tend to show an association between greater exposure and shorter telomeres with an observable dose response relationship. Early childhood adversity is more strongly and reliably associated with telomere length than is adult adversity, with three meta-analyses showing a negative relationship, the largest of which included 41 studies and more than 30,000 participants. Importantly, experimental studies manipulating stressor exposure in birds and other species often show effects of stress both prenatally and later in life.

Studies of the relationship between stress responses and telomere length are less clear than those of stress exposures. The literature on psychiatric disorders, however, is the most consistent: telomere shortening is associated with a range of psychiatric illnesses, including depression, anxiety, and schizophrenia. The causality of telomere shortening in psychiatric disorders, however, is not clear. Although some longitudinal studies support the hypothesis that long-term stress causes telomere shortening, the relationship is not linear. There are complex feedbacks between genetic risks, high stress exposure, depression, and telomere length, which confounds determination of causality.³

There may be different mechanisms of stress-related telomere shortening across the lifespan. Several factors influence telomere length of a fetus during pregnancy, including parental genetics, telomere length of parental gametes, and maternal stress. Epigenetic factors may also influence telomere length. Early childhood exposures clearly have a more potent effect on telomere length than do adult exposures, possibly mediated by changes in stem cell reserves. Telomere length in adolescence and adulthood is strongly influenced by telomere length in early childhood. Determining causality of various exposures on telomere length in adulthood is challenging because of a wealth of confounding factors. For example, certain medications (e.g., statins), disease states, and other factors may increase or protect telomere length.

Research on stress and telomere length and research on environmental epidemiology face similar challenges. These include (1) small relative risks with low penetrance between exposures and phenotypes combined with wide variations in individual responses to stressors, (2) multiple simultaneous exposures that may interact with each other and with individual genetics, and (3) varying exposures over time. To address these challenges, the field needs more specific markers of exposure, indices of cumulative risk, and the ability to measure sensitive periods to capture latent effects.

Several intervention studies aimed at reducing stress and modifying other health behaviors have included measures of telomerase activity or telomere length pre- and post-intervention. Although some of these studies have found telomere lengthening post-intervention, their results are not definitive and may reflect, for example, measurement error or changes in cell type composition. It is also not clear whether the effects are transient or lasting, and if they are

³ Epel, ES. and Prather, AA. Stress, Telomeres, and Psychopathology: Toward a Deeper Understanding of a Triad of Early Aging. *Annual Review of Clinical Psychology*. *In Press*. (2018).

lasting, whether they reduce health risks over time. Nonetheless, taken together, these studies do suggest that the telomeric system may be malleable even in adulthood. More research is needed to understand the mechanisms and impact for health.

Questions and Suggestions for Further Research

Many obstacles hinder research progress. Media hype and premature commercialization of telomerase activators hurt the credibility of telomere research. Causality is very difficult to establish in any telomere study. Human studies are not causal, and rodent studies are not always relevant for telomeres because of biological differences and the short lifespan of rodents compared to humans. At the same time, there is uncertainty and an excess of studies about telomeres, which makes it difficult for any one individual to be an expert in basic, clinical, and population based studies. There are few telomere experts, and many remain in disciplinary silos.

Ideally, large epidemiologic studies would be able to rule out pseudo-lengthening or -shortening of telomeres by controlling for shifts in cell-type composition or by measuring telomere length in single cell types rather than measuring whole blood samples. Better measurement of confounding and potential mediating factors, such as waist circumference, adiposity, disease, and medications would also help. Longitudinal studies with serial samples beginning at birth, measurement of non-mitotic cell types, and Mendelian randomization designs would improve the ability to distinguish mechanisms.

Questions for discussion include: What is the most important set of assays for determining cell aging in healthy humans? Are algorithmic approaches the most helpful? Should we examine telomere length in addition to other indices of cellular aging, such as inflammation, senescence-associated secretory phenotype, genetic index, epigenetic aging? In what contexts do replicative senescence and telomere length matter most? Is measuring telomere length informative early in life, when there is little senescence, and is measuring senescence-associated secretory phenotype important later in life? How important is it to have an independent review of telomerase activators?

Discussion

Flow cytometry with fluorescent in-situ hybridization (flow FISH) measures average telomere length, but the distribution of lengths typically skews toward short telomeres. Thus, average telomere length can be misleading. Cells with shorter telomeres also tend to die in culture, which further skews results. Studies that measure average length of telomeres in whole blood samples, which includes most studies of environmental exposures, are sensitive to changes in composition of blood cell types that have different characteristic telomere lengths. Differences in cell-type composition between samples collected at different points in time may lead to pseudo-lengthening, whereby the measured average length of telomeres is longer but only because of changes in the proportion of cell types being measured rather than actual lengthening of telomeres in any given cell lineage. Many studies of telomere length in whole blood attempt to control for cell-type composition using complete blood counts, and those that do not should refer to their observations as “apparent lengthening.”

Most large epidemiological studies measure telomere length using qPCR, which is highly sensitive to various assay parameters. Some of the largest Mendelian randomization studies infer telomere length based on GWAS for SNPs that were previously shown to be associated with telomere length, as measured by qPCR and Southern blots. Although they are large and therefore have high statistical power, such studies involve multiple statistical inferences that deserve thorough evaluation. Moreover, even large studies conducted in relatively homogenous populations (e.g., populations of European descent) may not be generalizable to other populations because relevant SNPs may differ across ancestral populations.

The relationships between telomerase activity, telomere length, and cancers are complex and not completely understood. Although genetically predicted long leukocyte telomeres and high telomerase activity are associated with development of several cancers, observed short leukocyte telomeres and high telomerase activity are associated with development of other cancers. The overall incidence of cancer in these populations, however, is relatively modest compared to other high-risk populations. Moreover, different studies on this topic have used different measurement techniques, which may contribute to disagreement among the results.

Session 2: Effects of Psychosocial Stress on Telomeres

Chairs: Ami Zota, PhD, George Washington University, and Max Guo, PhD, NIA

The Fetal Programming of Telomere Biology Hypothesis

Pathik Wadhwa, MD, PhD, University of California, Irvine

Telomere length, at any given age, is a joint function of the initial (newborn) setting of telomere length and the magnitude of telomere length attrition over time, which, in turn, is a function of the number of cell divisions (reflected by growth and age), exposure to oxidative and other forms of biological stress that reduce telomere length, and the counter-regulatory effect of telomerase expression and activity that attenuates telomere length reduction. If two cell populations have different initial telomere lengths but the same rate of decline, the one with the shorter initial length will reach senescence earlier. If another population has both shorter telomere lengths at baseline and reduced telomerase expression, its telomere length decline will be further accelerated.

Evidence from animal models suggests that initial telomere length and rate of attrition in early life are better predictors of lifespan than telomere length in adult life. The effects of telomere length in early life persist beyond those of other risk exposures in later life. Moreover, there are intergenerational effects of telomere length: females with chronic infection had offspring with shorter telomere lengths, whereas the offspring of males with chronic infection were unaffected. This suggests a maternally mediated environmental effect on telomere length.

There are no studies in humans of telomere length from birth to old age. One study, however, of telomere length in newborns found that those with shorter telomere length at birth had greater DNA damage at baseline and poorer response to a genotoxic challenge. Another study of early childhood found correlations between newborn telomere length and child

cardiovascular measures. Several longitudinal cohort studies show that most individuals maintain relatively constant leukocyte telomere lengths across six decades of adulthood. Similarly, twin studies suggest that early environmental factors are a major determinant of telomere length in later life. Although telomere lengths differ by tissue types, age-dependent attrition is similar across different somatic tissues. Collectively, these studies suggest that the initial (newborn) setting of telomere length accounts for the largest proportion of long-term effects.

What are the determinants of initial telomere length? Known genetic variants account for only a small proportion of variation in telomere length. Some studies indicate that telomere length of mothers appears to have a stronger effect on telomere length of offspring than does telomere length of fathers, regardless of the sex of the offspring. There is also preliminary evidence that maternal stress and nutrition-related factors during pregnancy may influence telomere length of offspring. Although the evidence suggests a large role for maternal and intrauterine effects in determining initial telomere length of infants, the clinical significance of these effects is unclear and needs to be determined through longitudinal studies.

Maximal Telomerase Activity Capacity

Telomerase expression and activity are important and complementary to telomere length. Data on basal telomerase activity, however, are difficult to interpret. Telomerase can be stimulated through a mitogen challenge in controlled *ex vivo* conditions. The ability of a cell to respond to this stimulus, or the maximal telomerase activity capacity (mTAC) may be a useful measure if it demonstrates within-subject stability and between-subject variability. Preliminary studies of mTAC in peripheral blood mononuclear cells (PBMCs) show favorable results. mTAC may therefore represent a potentially useful individual difference measure in studies of human telomere biology.

Crossing Tissues and Disciplines: Considerations of Tissues and Timing

Stacy Drury, PhD, Tulane University

Telomere lengths are usually measured in peripheral tissue samples. For studies comparing telomere length across tissue types, it is important to consider the embryonic origins of these tissues. This is especially important because telomere length phenotypes are established early in life and, although all somatic cells have the same genome, their epigenome differs. We may therefore expect different phenotypes in tissues derived from ectoderm, mesoderm, or endoderm. Interpretation of longitudinal studies considering measurements in different tissue types should consider these embryonic origins. Blood samples are particularly complex because they interact with many tissue types throughout the body.

Aging is not a linear process, and markers of aging should therefore not be linear. We should expect greater changes in biomarkers, including signals of responses to positive and negative exposures, during times when aging is accelerated. For example, telomeres shorten much faster in early life than in later life. Yet the rate of telomere shortening in early life and the timing of the relative plateau differs across cell types. Telomere lengths also differ between males and females, with females having greater telomere length on average across the lifespan. Thus, age,

sex, and cell type should each be considered when interpreting telomere length data as a biomarker.

The New Orleans Stress Physiology and Children Study comprises a sample of community-recruited African American children aged 4-16 and seeks to examine the effect of multiple stressors on telomere length. Telomere length is measured in both buccal cells and saliva samples. When controlling for sex and age, the investigators can derive an equation comparing telomere lengths from the two types of cells. The equation is different for boys and girls and changes when controlling for stress exposures.

The Infant Development Study recruited 440 mothers prenatally and is following the mother-child dyads longitudinally. Preliminary results show wide variability in individual trajectories of buccal cell telomere length. Clear differences by race (black vs. white infants) are detectable at 12 months of age, with white infants having shorter telomeres on average than black infants. Prenatal stress appears to influence telomere length in a sex-dependent way.

Placental telomere studies have found shorter telomeres in tissues from black individuals compared to white individuals, suggesting accelerated cellular aging in black mothers. The results were consistent across four placental tissues; however, results from umbilical cord blood are inconsistent, especially in samples derived from male infants, because there is far less correlation between telomere length measured in placental tissues in males compared to females.

In conclusion, the utility of cross-sectional telomere length data depends strongly on developmental age. Telomere length is particularly dynamic in young children. Cross-tissue correlation likely varies based on embryonic origin. It is important to consider sex and race in interpreting telomere length results. Future studies should assess factors associated with variability of telomere length and correlations with downstream health outcomes.

Social Status, the Stress Process Model, and Telomere Dynamics

Belinda Needham, PhD, University of Michigan

Individuals with lower social status (e.g., the poor and racial/ethnic minorities) tend to have poorer physical and mental health. The stress process model posits that socially disadvantaged populations are exposed to greater stress and have fewer personal and social resources to buffer the negative effects of stress on health.

While most large epidemiologic studies do not directly measure stress exposure or perceived stress, social characteristics are key drivers of exposure to stressors, and low social status may, itself, be stressful. Thus, social status indicators may be reasonable proxies for stress exposure. Socioeconomic status (SES) is a latent variable that reflects an individual's position within the class structure of a society. Indicators of SES include income, education, occupation, wealth, subjective social status, and area-based measures. In general, individuals with higher SES have better health outcomes. The explanations for this association are both material and psychosocial.

One hypothesis is that having lower SES causes more stress, which leads to shorter telomeres and increased morbidity and mortality. Several studies have reported relevant results for testing this hypothesis. The few studies that evaluated both SES and telomere length in children have found mixed results. Although there are more studies of the relationship between SES and telomere length in adults, the results are also mixed. The most consistent findings are that parental education is associated with child telomere length and that homeownership, childhood SES, and neighborhood environment are associated with telomere length in adults in the U.S. Overall, more studies supported a significant positive relationship between SES and telomere length than those that support an inverse relationship; however, most studies in adults reported null effects. Most studies also did not adjust measurements for cell-type composition.

Racial and ethnic categories may represent common geographic origins, genetic ancestry, family patterns, language, cultural norms and traditions, social history, and current social circumstances. Studies that have investigated racial and ethnic differences in telomere lengths have consistently found that U.S. blacks have longer telomeres than U.S. whites, except when measured in placenta. The underlying reason is unclear. This finding is surprising given that U.S. blacks tend to have lower social status and greater stress exposure than U.S. whites.

Key topics for further study include early life, fetal programming, and intergenerational transmission of telomere length; baseline and rate of change in longitudinal studies; cell composition effects; effect modification by race and ethnicity; and direct measurements of potential mechanisms—including stress exposure—in epidemiological studies.

Lessons for Development of Biomarkers of Aging from Telomere Research

Idan Shalev, PhD, Pennsylvania State University

Gerontology seeks to extend the lifespan while increasing the proportion of time spent healthy, the “healthspan.” To accomplish this, we need to know why some individuals age faster than others and what can be done to slow the aging process. Measures of aging are needed that can be applied early in life.

Researchers have developed several biological aging clocks to predict the age in years of a subject from a biological sample. These clocks are based on telomere length, gene expression levels, protein expression levels, epigenetic markers, or composite measures. Telomere length, which is the most studied biological aging clock, reveals four themes that can inform the design of new studies to evaluate biomarkers of aging:

1. **The biomarker function is mechanistically linked to the aging process.** For example, telomeres shorten with each cellular replication, eventually leading to cellular senescence after 50 or 60 divisions. Evidence from animal models shows that senolytic drugs that kill senescent cells can reverse the aging process.
2. **The biomarker is correlated with chronological age and predicts morbidity and early mortality.** Age is perhaps the strongest predictor of mortality, yet leukocyte telomere

length is also correlated with chronological age and predicts risk of cardiovascular disease, cancer, and early mortality.

3. **The biomarker is responsive to exposures known to increase risk for age-related disease.** Numerous studies have shown that short telomere length is related to perinatal complications, maternal stress, childhood adversity, mental health problems, and unhealthy behaviors. Conversely, health-promoting behaviors such as exercise are associated with longer telomeres.
4. **The biomarker can be measured in a precise and reproducible manner.** Precise and reproducible measurement is a key component of a reliable biomarker of aging. There are many ways to measure telomere length; development of accurate and high-throughput measures is important, particularly when considering the importance of the shortest telomere length in a sample, as opposed to average telomere length.

Algorithm-based measures of biological aging reflect a composite of different biomarkers. Such measures can predict aging, disease, and self-reported health, even among relatively young subjects. Telomere-based algorithms may be able to predict similar results. Potential components of a telomere-based composite biomarker may include telomerase activity, shelterin proteins, telomeric repeat-containing RNA, telomeric zinc finger-associated protein, mitochondrial measures, oxidative stress markers, epigenetic markers, and telomere length polygenic risk scores. Such a composite measure may give a more robust understanding of telomere biology.

Panel Discussion

Panelists: Pathik Wadhwa, Stacy Drury, Belinda Needham, Idan Shalev, and Allison Aiello

Telomere Length Measurement Methods

There are many methods for measuring telomere length. Although there is a need to measure the shortest telomere in a given sample, current techniques that allow for this lack sufficient throughput for use in large epidemiological studies, where qPCR is typically required. Further study is needed to understand the correlations between measurement methods in well-controlled environments and across tissue types to facilitate interpretation of results from larger epidemiological studies using qPCR.

Dr. Armanios noted that the measurement variability from qPCR conducted in different laboratories is as high as 20 percent, which may be higher than the effect size. qPCR is prone to several types of measurement error, including artifacts caused by different sample processing techniques. Results from different labs or even different batches from the same lab may not be comparable. Dr. Abraham Aviv noted that this is due in part to different labs having different T/S ratios (the ratio of telomere to single copy gene) without a known relationship to the mean terminal restriction fragment (TRF) length, which is directly measured in Southern blots. He suggested conducting a large-scale study to compare qPCR to other, more precise measurement methods such as Southern blots and flow FISH. Such a study would compare measurement methods to each other and to a phenotypic outcome with a known relationship to telomere length.

Dr. Peter Lansdorp agreed that a quantitative comparison of methods would be useful. He suggested that qPCR may still have utility when performed correctly, but he expressed concern that many studies have used the technique without precision, contributing to the plethora of contradictory findings in the literature.

Drs. Epel and Shalev added that qPCR remains useful in many contexts and can produce useful results if performed correctly. In addition, the results of several meta-analyses based on these data are consistent with data generated by other methods. Reviewers of peer-reviewed papers using qPCR should scrutinize the methods to ensure the robustness of published results.

Southern blots have long been the standard assay for measurement of telomere length, and they have been used in the clinical diagnosis of individual patients with diseases related to short telomeres. Dr. Armanios noted that recent studies highlight the precision of flow FISH and suggested that it may already be the new clinical standard.

Measurement of Stress

Just as the ideal measure of telomere length is unclear, so too is the ideal measure of stress. As discussed by Dr. Epel, stress and environmental exposures are multifaceted. Some of the discrepancies in the literature may be due to a lack of precision or common view in how stress is conceptualized and measured. Early life stressors are particularly difficult to measure, because they are often ascertained through retrospective self-reporting. There is also a need to distinguish between acute and chronic stressors, which require different measurement methods and may also have different effects on telomere length and health. The frequency and timing of stress and telomere length measurements must be appropriate for the type of stressor of interest.

One productive challenge is to devise a composite measure of stress that captures its complexity and is relevant for all health outcomes. Such a composite marker could be measured over time and used as an indicator of individual differences and life histories, much like cardiovascular measures. The NIA supports a network of investigators led by Dr. Epel and colleagues at UCSF, which is considering the best measures of stress and exposures for use in population-based studies.

Heritability versus Exposure

Dr. Aviv cautioned that results of studies comparing cross-sectional measures of telomere length and various indices of stress may be spurious. He noted that the great variation in telomere length at birth likely outstrips any effect of cumulative stress on telomere length in adulthood. Dr. Wadhwa suggested that genetic (DNA base pair) variation may not exert a large influence on telomere length. For example, all known SNPs associated with telomere length account for only a very modest proportion of telomere length variance. Similar effects are seen for other complex phenotypes such as body mass index (BMI), where currently identified SNPs explain only about 5 percent of the variation in BMI. Although it is likely that telomere length is influenced both by genetics and by environment or experiences, the relative contributions of these factors are unknown.

Relationship Between Telomere Length and Race

The observation that blacks tend to have longer telomere lengths than whites in the U.S. (in infancy, childhood, and adulthood) is a consistent and surprising finding. The cause of this observation is unclear. Some, but not all, studies that stratified results by SES found no differences in telomere lengths by race. Cross-tissue analysis might be a useful way to obtain more information about this question. It may also be worth evaluating how race is defined in various studies. Although SES may be a stressor that influences telomere length, other mechanisms also influence telomere length and should not be ignored.

Session 3: Critical Considerations of Assessment of Telomere Length Dynamics

Chairs: Sharon Savage, MD, National Cancer Institute, and Stacy Drury, PhD, Tulane University

Telomere Length Measurements and Cell Turnover

Peter Lansdorp, PhD, British Columbia Cancer Agency

Quantitative fluorescent in-situ hybridization (qFISH) uses fluorescently tagged peptide nucleic acid (PNA) probes that attach to denatured, single-stranded DNA. The fluorescent signals are then analyzed by digital microscopy (qFISH) to quantitatively determine the length of individual leukocyte telomeres or by flow cytometry (flow FISH), which allows higher-throughput analysis of the average telomere length by cell type. Bovine thymocytes are used in flow FISH as a control to normalize fluorescence values across assays. Results obtained using this method are highly reproducible for multiple white blood cell types.

Telomere length decreases with age in humans and other animals. The decline is rapid in early life and slows considerably during adulthood. At any age, however, there is significant inter-individual variation. This complicates the ability to use telomere length as a clock of biological aging. Studies of telomere length in cells from umbilical cord blood also show wide variation. One consistent finding is that, on a population level at any given age, females have longer average telomere lengths than males. The reasons for this observation need to be further explored.

Having only one functional copy of genes that encode for telomerase (*TERT* or *TERC*) can cause bone marrow failure in humans. Both genes are in the same pathway, and haplo-insufficiency for either gene can cause a similar phenotype.

Normal telomerase levels are required to maintain telomeres and prevent exhaustion of hematopoietic stem cell populations and bone marrow failure. The loss of some stem cells increases the number of cell divisions needed by other stem cells, which might lead to accelerated exhaustion of the entire stem cell pool, leading to various diseases associated with short telomeres.

The observed nonlinear decline in measured telomere length may reflect clonal selection of cells based on telomere length, which further complicates measurement of telomeres on an average length basis.

Germ Cells Versus Somatic Cells

Shawn Ahmed, PhD, University of North Carolina at Chapel Hill

Germ cells are transmitted across generations, whereas somatic cells are not. Various environmental factors could contribute to somatic changes or germline changes, which could be transmissible. Most somatic cells experience telomere shortening over time and eventually become senescent. Cells that become cancerous continue to proliferate and accumulate chromosomal instabilities. In a subset of cancers, the alternative lengthening of telomeres (ALT) pathway is activated, promoting telomere replication.

Studies of telomerase-deficient *C. elegans* have demonstrated progressive sterility across generations. In contrast, wildtype worms maintain fertility over time under the same conditions. Keeping *C. elegans* telomerase mutants in crowded conditions, however, leads to indefinite propagation of some individuals via upregulation of ALT. Crowded conditions apparently promote a stress response, activating ALT and leading to other stress-induced phenotypes, such as dauer larvae. Mouse models may be useful to test whether this relationship between socially induced stress, telomere maintenance in the absence of telomerase, and tumor development is evolutionarily conserved.

Telomerase RNA mutations influence several diseases, such as immunodeficiency-centromeric instability-facial anomalies syndrome and Werner syndrome, which are both characterized in part by heterochromatin defects and telomere instability. Importantly, the phenotypes of these diseases appear only in cells that lack telomerase. Thus, telomerase expression appears to dictate somatic tissue dysfunction in these cases. Small RNA pathways that promote telomere stability in the absence of telomerase may be relevant for these diseases.

Longitudinal Studies of Telomere Length: Assay Considerations and Findings

Jue Lin, PhD, University of California, San Francisco

There is an ongoing need to measure telomere length longitudinally. Past longitudinal studies have typically reported shortening of average telomere length within cohorts over time, considerable interpersonal variation, some degree of correlation between baseline and follow-up telomere length within individuals, age- and sex-dependent differential rates of telomere length change, a rate of change that is inversely associated with baseline telomere length, and a small portion of study participants exhibiting telomere lengthening. Apparent telomere lengthening could either reflect measurement error or transient lengthening that may occur over short time periods.

Because qPCR is widely used in large longitudinal studies, it should be improved as much as possible. DNA extraction is by far the most influential methods-related factor. To minimize error related to DNA extraction, all samples in a given study from both baseline and follow-up

periods should be extracted at the same time using the same kit from the same manufacturing lot. Baseline and follow-up samples from the same participant should be measured in the same batch. Each batch should also include an equal number of control and comparison samples. When feasible, triplicate samples should be measured and repeated measures from the same individual should be analyzed on the same well plate. Efforts to increase standardization and automation of assays, such as the use of liquid handling robots and automated data analysis pipelines, limit human error and bias.

Two recent longitudinal studies put these principles into practice. The first was a study of sugar-sweetened beverage consumption among pregnant women. Although there was only a marginal association between sweetened beverage consumption and leukocyte telomere length at baseline, longitudinal follow-up found significant associations between decreased consumption and increased telomere length as well as increased consumption and decreased telomere length. The second study measured telomere length in several types of T-cells and B-cells in healthy premenopausal women. Although telomere length at baseline and follow-up were highly correlated, there were significant differences in telomere length and rate of attrition between different cell types. Moreover, telomerase activity at baseline did not predict the change in telomere length over time. Telomeres in specific cell types may be more sensitive to different internal and external factors.

Key unanswered questions include: What are the mechanisms of baseline dependent telomere length change? What are the mechanisms and biological significance of telomere lengthening? What are the factors that contribute to longitudinal telomere length change? Is there a specific cell type that is a better candidate for telomere length measurement for predicting disease and health risks?

Germline Genetic Variation in Telomere Biology Genes Is Associated with a Spectrum of Phenotypes

Sharon Savage, MD, National Cancer Institute

When researchers discuss variability in telomere length at the population level, they usually mean that there are statistically significant differences in telomere lengths between the populations studied. Whether these differences are also clinically significant deserves further investigation. A theoretical framework is needed to connect what is known about telomere biology, human disease, and aging.

Dyskeratosis congenita is a heritable disease associated with extremely short telomeres. Patients with dyskeratosis congenita experience a wide variety of medical problems that evolve over time. In the late 1990s, mutations in dyskerin (*DKC1*) on the X chromosome were identified as responsible for some forms of the disease. Cells isolated from patients with *DKC1* mutations have low telomerase activity and very short telomeres. This finding represented the first causal link between telomere biology and human disease.

The advent of flow FISH to measure leukocyte telomere length provided a reliable diagnostic assay for dyskeratosis congenita, with age-adjusted lymphocyte telomere lengths shorter than

the 1st percentile being more than 95 percent sensitive and specific for the disease. Dyskeratosis congenita and related disorders manifest along a spectrum, with more severe forms such as Hoyeraal-Hreidarsson Syndrome causing cerebellar hypoplasia and immune deficiency, and less severe forms causing apparently isolated diseases such as pulmonary fibrosis or liver disease. Less severe forms of dyskeratosis congenita may lack classic features, and family history may be absent.

Today, the genetic etiology of dyskeratosis congenita and related disorders is better understood. A variety of autosomal dominant and autosomal recessive mutations in more than a dozen genes related to telomere biology have been described. Each is associated with different clinical features, and the more common variants tend to be associated with more common and relatively less severe health effects. Some diseases, including susceptibility to certain cancers, are associated with abnormally long telomeres.

One recent study measured telomere length longitudinally in patients with dyskeratosis congenita, some of whom were treated with androgens for bone marrow failure. No statistically significant differences were observed over time related to androgen therapy.

A Note on Telomere Length Measurement Methods

Although qPCR is useful for well-controlled, large epidemiological studies, caution is warranted particularly in smaller studies performed by investigators who are not attuned to the potential pitfalls. Differences in DNA extraction methods lead to wide variability in relative telomere lengths measured by qPCR. Similarly, DNA purification and sample storage methods can also have significant effects on the results, although these effects likely differ by sample type.

Correlations of telomere length measured by different methods deserve close attention: a recent comparison of qPCR, flow FISH, and Southern blot measurements of telomeres derived from patients with dyskeratosis congenita yielded R^2 values between 0.4 and 0.7. Even with robust protocol controls, correlation of results across measurement methods remains an issue.

Finally, the value of qPCR as a clinical diagnostic test is limited. Although its specificity is 98 percent, its sensitivity is only 39 percent. Diagnosis of dyskeratosis congenita by qPCR is therefore prone to false-negative results.

Panel Discussion

Panelists: Peter Lansdorp, Shawn Ahmed, Jue Lin, Sharon Savage, and Sara Hagg, PhD, Karolinska Institute (remote)

Measurement of Telomere Length: Ways to Move Forward

Workshop participants broadly agreed that there are several reasons for caution when measuring telomere lengths or interpreting results of assays performed by others. Although attention to methods and standard operating procedures is important for any telomere length assay, evidence suggests that qPCR is particularly sensitive to changes in sample preparation, storage, extraction, and other assay parameters. The following concrete suggestions to move the field forward emerged from the discussion:

- **Peer-review guidelines** are needed to set expectations for details required in descriptions of study methods for both grant applications and peer reviewed manuscripts. Guidelines would help reviewers and readers judge the quality of a study.
- **Laboratories that regularly conduct high-throughput qPCR** should be used whenever possible, because they understand best how to control assay conditions. Funders should encourage or require grantees to ship samples to a core facility for analysis.
- **Sample storage and processing guidelines** should be developed. Any frozen samples should be stored at minus 80 degrees Celsius. Data are needed to understand the optimal storage method, whether immediate extraction and storage as DNA or as storage as unextracted DNA, and whether this varies based on sample type.
- **Standard reference samples** should be developed and adopted. Pooled samples might be more relevant than single sample references.
- Sample storage and DNA extraction methods must be as uniform as possible. Ideally, **all samples for a study should be extracted at the same time**, including those collected at baseline and follow-up. One caveat is that the effects of long-term storage of biosamples on telomere length assays are not fully understood and may be sample-type specific.
- **All aspects of sample preparation and assay performance should be automated** whenever possible to eliminate human error.
- Raw data from qPCR generated by different laboratories cannot be directly compared. **Comparisons of Z-scores may offer a valid comparison method** for meta-analyses or other cross-study comparisons but should be validated in collaborative studies. Alternatively, the use of standard control samples that capture the longest and shortest measurements of telomere length are needed.
- **Small studies should consider other methods** to measure telomere length; qPCR is best suited for large-scale studies where more consistent methods are impractical.

Rate of Change in Telomere Length Over Time

The factors that influence the rate of change in telomere length over time remain poorly understood. The dominant theory is that the rate of change of leukocyte telomere length reflects turnover of hematopoietic stem cells, which is primarily developmentally controlled. However, it is possible that defects in heterochromatin and other epigenetic changes may also influence leukocyte telomere shortening independent of cellular proliferation. Data on rates of telomere shortening in different cell types also cast doubt on the primary role of cell turnover, because some of the later differentiated white blood cells have faster rates of attrition than cells that differentiate earlier.

More longitudinal data are needed to definitively answer this question. It may be useful to measure the distribution of telomere lengths, rather than the average length, longitudinally in DNA preparations from health individuals. One caveat is that current measurement methods may not be sufficiently accurate to capture telomere length shortening over time at the level that would be needed to fully understand attrition dynamics. The variability within individuals is also very wide and potentially dynamic, which further complicates the question. In addition, telomere length must be measured at different intervals depending on the developmental

stage at which the trajectory of telomere length change is being measured. In other words, changes in telomere length in children should be measured more frequently than changes in telomere length in adults.

Open Discussion

Moderator: Rick Woychik, PhD, Deputy Director, NIEHS

Developing Better Measures of Stress and Exposures

In the past, NIEHS has supported research to understand the health effects of individual exposures. In the future, NIEHS is particularly interested in the effects of the exposome, that is, the combined effects of multiple environmental factors. Studying the relationship between the exposome and health will require aggregating and integrating datasets from multiple sources and harmonizing disparate data types to facilitate valid comparisons. One suggestion was for NIEHS to support the development of a large, combined, geotagged dataset of various environmental factors, such as particulate matter air pollution, solar radiation, crime data, and SES indicators. Such a dataset could potentially allow epidemiologists to enter the addresses of their study participants and easily integrate multiple types of environmental exposure data into existing cohort studies. These environmental data exist but are not always included in cohort studies because of resource constraints. Creating a central repository would facilitate integration of more environmental variables into epidemiological studies.

There was clear consensus of a need for better measures of stress exposure. These could include composite measures of multiple instruments to integrate several facets of stress. However, because different types of stress may have different health effects, there is also a need to report certain stress measures separately. Participants noted that different measures of perceived stress often provide widely divergent assessments. Moreover, perceived stress is but one type of stress, and it reflects short term states and is not always stable over time. For example, studies of frequent, real-time, repeated measures of perceived stress in single individuals—called ecological momentary assessment—show substantial within-individual variation over short periods of time. This presents a formidable challenge for assessing the validity of self-reported perceived stress.

Key challenges in stress measurement that emerged from discussion include:

- Measuring dosage or intensity
- Addressing recall bias in retrospective measures
- Distinguishing between perceived stress and exposures to potential or recent stressors and measuring the combination of the two rather than just one
- Incorporating repeated measures of exposures and responses into longitudinal studies
- Disentangling psychological and social stressors, which may have distinct effects
- Reconciling inconsistency across different instruments or measures taken at different times
- Comparing perceived stress with molecular markers of cellular stress

- Integrating a developmental model into stress assessment both in terms of the perception of stress and the potential impact of stress on biological systems that are undergoing more rapid development

Telomere Length Dynamics and Mechanisms of Change

It is known that telomeres shorten with cell divisions. Whether other factors influence telomere length, and by what mechanisms, remains less clear. Apparent lengthening of telomeres observed in some studies is particularly controversial. It may reflect true lengthening by increased telomerase activity, or it may reflect changes in cell composition or measurement error. The dynamism of telomere length needs to be further explored. Studies with very high sampling frequencies (e.g., hours, days, or weeks) and precise measurement methods that control for cell-type composition could help resolve whether short-term changes in telomere length are real and, if so, point toward the likely mechanisms.

Dr. Susan Bailey described a study of telomere length in astronauts that found significant telomere lengthening and shortening over a period of days. Telomere length was measured using multiple methods, including qPCR and a cell-by-cell FISH method, and was verified by an independent laboratory. Although the reasons for the dynamic changes in telomere length are unknown, this study demonstrates that it is possible to observe substantial changes over relatively short periods of time.

High-frequency sampling studies could also better elucidate the relationship between telomere length dynamics and various types of stress. There is disagreement over whether and how different types of stress affect telomere lengths. A study investigating the impacts of several environmental exposures on telomere lengths sampled at high-frequency may allow observation of dynamic changes correlated with different types of exposures.

Studying populations with rare telomere diseases and those exposed to extreme psychosocial stress may offer the best opportunity to understand mechanisms of telomere dynamics that may be broadly applicable. These special populations are more likely to display measurable phenotypes in which changes can be detected over time. Such studies might also help overcome several potential challenges that may arise in population-based studies, such as the potential for low-dose stresses to have beneficial effects and for certain individuals to display resilience in the face of stress.

Pediatric populations are another logical starting point for understanding the dynamics of telomere length and mechanisms of change in response to environmental exposures. Because telomeres shorten much more rapidly in children than adults, differences in telomere lengths between groups of individuals who experience different exposures are more likely to be detected in children than adults. Indeed, the relationship between early life adversity and telomere length is very consistent in the literature. For example, one study demonstrated that orphanage care is associated with longitudinal and cross-sectional decline in telomere length. Another study found that children who were exposed to violence between ages 5-10 exhibited faster telomere attrition than those who were not exposed. One evolutionary hypothesis to

explain the association between early life challenges and telomere shortening is that adversity accelerates aging to promote reproduction.

Dr. Aviv cautioned that any longitudinal study of telomere length needs to consider the interassay coefficient of variation to determine the minimum follow-up period to detect a signal that overcomes the noise of the assay variation. For example, the average telomere length of an adult is about 7kb, and the average annual telomere shortening is 20-25bp per year. Even the best telomere length assays have a coefficient of variation of about 2 percent, suggesting that the minimum follow-up period for a longitudinal telomere length study in adults should be at least 10 years.

Workshop participants suggested a need for studies that link causal relationships between exposures and telomere length with underlying biological pathways. These could include mediation studies that seek to identify variables that are independently associated with telomere regulation. It is possible that certain biological or behavioral factors may mediate the relationship between stress and telomere length or may have synergistic effects. Studies are also needed on a variety of cell types to understand effects of exposures on different biological systems. Most studies measure leukocyte telomere length, which may be more relevant for immune changes than other aspects of health.

Although most studies focus on telomere length, other aspects of the telomere system, such as telomerase expression and activity, may also be important. Telomerase activity is an acute indicator that is highly dynamic and may be difficult to incorporate into epidemiological studies in a meaningful way. Telomerase production and other measures of the telomerase system may yet prove valuable. These measures should not, however, be interpreted in isolation.

Another way to probe the mechanism of telomere shortening in response to various exposures would be to develop an alternate method of determining the number of cell divisions that have taken place to form the cells being observed. This might help determine whether observed telomere shortening is due to an increased number of cell divisions or direct damage to telomeres. Participants suggested two ways of inferring the number of divisions among stem cell populations: (1) DNA methylation signatures have been linked to the number of divisions that have occurred and (2) measurement of granulocyte telomere length by flow FISH.

Socioeconomic Status, Psychosocial Stress, and Telomere Length

Many studies have drawn connections between SES, psychosocial stress, and telomere lengths, but disagreement over whether observed associations are causal or clinically meaningful remains. Dr. Aviv commented that the association between SES and telomere shortening is well established, but he questioned the premise that psychosocial stress shortens telomeres. The first observation of a link between psychosocial stress and telomere length came from a study by Epel and colleagues that found that mothers who cared for chronically ill children (n = 39)

had shorter telomeres as measured by qPCR than those who had healthy children (n = 19).⁴ Population-based studies, he argued, have provided contradictory evidence.

Others clarified that psychosocial stress is hypothesized to be one of the primary pathways through which SES impacts health, and, at least in relation to early life stress, meta-analyses support the relationship between shorter telomeres and psychosocial adversity. Dr. Lansdorp remarked that both the initial evidence and evidence from subsequent studies, while limited, appears to be convincing, and others concurred. Dr. Lin noted that the original study showed a dose effect whereby longer durations of caregiving were associated with shorter telomere lengths. She also referenced Dr. Epel's presentation, which highlighted many studies that have investigated different types of stress that may be related to telomere length. For example, Damjanovic et al. found relatively short telomeres in caregivers using the Southern blot method.⁵ Meta-analyses of clinical and population based studies have detected a stress effect on telomere length, especially from early adversity. Consistent relationships between depression and telomere length have also been reported. The largest review of childhood adversity included 41 studies and more than 30,000 participants.⁶ Importantly, experimental studies manipulating stressor exposure in birds and other species often show effects of stress both prenatally and later in life.

Dr. Armanios suggested that studies of psychosocial stress and telomere length should be repeated using multiple methods for telomere length measurement to definitively answer the question. Samples could be blinded and shipped to multiple labs for parallel analysis. She also commented that even if psychosocial stress or other factors cause statistically significant telomere shortening, the shortening may not be clinically relevant. It is important to distinguish between telomere shortening that is significant enough to be reliably detected and telomere shortening that truly impacts health status. There is also the possibility that even if telomere length changes are not clinically relevant, they could be indicative of disease susceptibility.

Validation of Measurement Methods

Measurement error and consistency of results across telomere length measurement methods is one of the most pressing challenges in the field. For each method, concerns include absolute accuracy, within-laboratory interassay variation, across-laboratory interassay variation, and correlation of results on the same samples measured by different methods. Optimizing only one of these parameters is not sufficient; for example, it is possible to achieve good interassay variation while generating inaccurate results. Similarly, one comparative study found very consistent within-laboratory results; however, variation when pooling samples between labs

⁴ Accelerated telomere shortening in response to life stress [Internet]. Available from: <http://www.pnas.org/content/101/49/17312.long>.

⁵ Damjanovic AK, Yang Y, Glaser R, et al. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J. Immunol.* 179(6), 4249–4254 (2007).

⁶ Ridout KK, Levandowski M, Ridout SJ, Gantz L, Goonan K, Palermo D, Price LH, Tyrka AR. Early life adversity and telomere length: a meta-analysis. *Molecular Psychiatry.* (2017).

using qPCR was 20 percent.⁷ Not all labs use the same qPCR assay; there are many protocol variations, and results from different assays cannot easily be compared to each other. The across-laboratory variation may be even higher for studies of pediatric populations, because some, but not all studies found that the variability of qPCR is higher when measuring longer telomeres.

Telomere length measurement error may be due to minor variations in sample preparation and storage, DNA extraction, DNA purification, or measurement protocols. It may also be due to inaccurate calibration, inappropriate correction factors, or flaws in the underlying method itself. Whatever the cause, workshop participants acknowledged the urgent need to resolve the issue.

Participants offered several suggestions for steps that NIEHS, NIA, and other institutes could take toward resolving discrepancies between telomere length assays:

- **Provide a set of standards to calibrate measures.** If adopted by all laboratories measuring telomere lengths using any method, this could help encourage alignment across the research community. There are two caveats to this suggestion. First, different methods measure different parts of the telomere (e.g., Southern blots include the subtelomeric regions), which complicates interpretation of results obtained by measuring the same reference sample with different methods. Second is the need for community buy-in. NIEHS has funded development of standards for other fields, and these efforts were only successful if most laboratories adopt the new standard rather than continue to use individual protocols.
- **Fund an effort to collect unpublished knowledge on best practices.** Many laboratories have a wealth of unpublished data and institutional knowledge about best practices for various assays. This information might include effects of storage temperature, sample processing protocols, and other methodological details. Compiling a central resource of institutional knowledge across laboratories would help the field determine the best protocols and reasons why certain methods work better than others.
- **Fund a large-scale study to compare multiple methods.** Multiple laboratories would employ multiple methods (qPCR, Southern blots, flow FISH, and others) to measure the same samples, including blind duplicates. Several variations of this idea were suggested. These could be combined into a staged approach.
 1. Ship a blinded reference standard to each laboratory. One possible sample type would be cord blood from newborn boys and girls, which should show differences in telomere length. Screen laboratories for participation in the full study based on suitability of initial results.

⁷ Martin-Ruiz CM, Baird D, Roger L, Boukamp P, Kronic D, Cawthon R, Dokter MM, van der Harst P, Bekaert S, de Meyer T, Roos G, Svenson U, Codd V, Samani NJ, McGlynn L, Shiels PG, Pooley KA, Dunning AM, Cooper R, Wong A, Kingston A, von Zglinicki T. Reproducibility of telomere length assessment: an international collaborative study. *International Journal of Epidemiology*. 44(5):1673-83. (2015).

2. Send a set of blinded samples to multiple laboratories for measurement by different methods. Judge results against known variables and determine the coefficients of variation between the different assays.
3. Select several thousand samples with paired clinical data (e.g., cardiovascular measures) or genetic data (e.g., SNPs that are related to telomere length) for analysis by multiple laboratories using different methods. Judge results based on association of telomere lengths with clinical phenotypes or genetic markers, and compare the coefficients of variation between the different assays.
4. Once the differences in methods are well established, run a similar experiment using samples with paired data on psychosocial stress or environmental exposures.

Although there was consensus on the need for a comparative study across measurement methods, there was some disagreement about the best approach. For example, Dr. Lin questioned the optimal phenotype to investigate. Because the relationship between telomere length and most health outcomes is not clear, the measurement technique with the best correlation to the selected health outcome may not be the most accurate method. In addition, she cautioned that any comparative methods study should rely on fresh, rather than stored, samples to minimize variation related to sample storage parameters.

One concern is that the method determined to be the most accurate may not be suitable for high-throughput studies. Dr. Drury noted that the best method depends on the circumstances. In some cases, this may be the most accurate method. In other cases, practical considerations may prevail. There was general agreement that qPCR, even if determined not to be the most accurate, remains suitable for studies with a sufficiently large sample size. Whether a sample size is large enough can be determined by a power calculation that accounts for the sample size, measurement error, anticipated effect size, and other parameters.

New and Emerging Technologies for Telomere Length Measurement

In addition to validating existing telomere length measurement methods, it may be useful to develop new measurement technologies. Two promising technologies include NanoString and whole genome sequencing (WGS). Although using WGS data to study telomeric regions is technically possible, it is expensive and challenging to interpret. Dr. Aviv noted that an early study using WGS to study telomeres had a coefficient of variation of about 7-8 percent. It may also be possible to use off-target reads from exome sequencing data. Novel ways to combine existing knowledge about observed telomere length and genetic determinants of telomere length may also be useful.

Animal Models: Challenges and Opportunities

Studying telomere biology in animal models and extrapolating findings to humans presents both challenges and opportunities. Although animal model studies can inform mechanisms of telomere length dynamics, many common animal models have much longer telomeres and higher telomerase activity than do humans, which presents a challenge for cross-species comparisons. At least one strain of mouse, developed by Dr. Carol Greider and colleagues at

Johns Hopkins, exhibits telomerase deficits and associated telomere shortening and expression of disease phenotypes characteristic of telomere disorders in humans. Other potentially useful animal models may include various bird species and rhesus macaques. Some workshop participants maintained that human studies will provide the most useful results. Nonetheless, in addition to uncovering biological mechanisms, animal studies may help resolve controversies related to measurement methods.

Fostering Interdisciplinary Collaborations

This workshop brought together experts from a variety of disciplines and elicited productive discussions. More effort is needed to bridge the gap between molecular biologists, physiologists, epidemiologists, and others. For example, the molecular biology community may benefit from guidance on what types of cells are most relevant for researchers studying psychosocial stress and various phenotypic associations. Participants also suggested engaging biostatisticians in discussions of multidisciplinary telomere length research. Input from statisticians on study design and interpretation may help improve the robustness of results.

Session 4: Genetic Susceptibility and the Environment

Chairs: Patricia Opreko, PhD, University of Pittsburgh, and Colter Mitchell, PhD, University of Michigan

The Biological Meaning of Leukocyte Telomere Length: Is Leukocyte Telomere Length a Biomarker of Human Aging?

Abraham Aviv, MD, Rutgers University

Hematopoietic stem cells (HSCs) divide throughout life and have repressed telomerase activity. Leukocyte telomeres exhibit a high rate of shortening early in life and much slower shortening throughout adulthood. The differential rate of attrition of telomere length in leukocytes over time reflects the dynamics of the HSC pool.

HSCs can replicate symmetrically or asymmetrically. Symmetric replication results in two HSC daughter cells. It is an exponential process that rapidly grows the HSC pool. Asymmetric replication results in one HSC daughter cell and one progenitor daughter cell. It is a linear process that builds the progenitor cell pool. Adults produce 400 billion blood cells every day, yet HSCs divide only about once per year. The vast progenitor cell pool that allows for daily blood cell production was built during growth and development, and the proliferation of HSCs during that phase sets the trajectory for telomere length in adults.

The average telomere length at birth is about 9.5kb, with a variance of about 4kb. Newborn telomere length is influenced by sex, paternal age, and maternal factors. In the first two decades of life, telomeres shorten by an average of 1.5kb. In the next 80 years, telomeres shorten by an additional 2kb. Thus, throughout a lifetime, telomeres shorten by approximately the same length as the total variance in telomere length at birth. Because of the wide variation in newborn telomere length and relatively slow shortening over time, leukocyte telomere length is a poor biomarker of aging, especially in cross-sectional studies.

In a longitudinal study of adults, leukocyte telomere length after 12 years was highly correlated to the length at baseline. Cross-sectional studies of telomere length in various somatic tissues in adults illustrate that cells in different tissue types have different telomere lengths, reflecting a different number of cell divisions over time. The proportionality of telomere lengths in different tissues is, however, similar in individuals of different ages, suggesting that telomere length in somatic tissues is primarily determined in early life.

The Goldilocks Telomere Principle

A mouse weighs about 30 grams and lives about 2 years, whereas an elephant weighs about 6 tons and lives about 60 years. Yet the cancer incidence is not higher in elephants than in mice. One reason for the parity in cancer incidence despite the species' vastly different lifespans is that telomerase activity is inversely related to body size, and telomere length is inversely related to lifespan. There is a tradeoff between longer telomeres that come with a higher risk of cancer and shorter telomeres that come with a higher risk of degenerative diseases. Relative to other species, humans have repressed telomerase and very short telomeres.

Two types of disease largely define longevity in humans: cancer and cardiovascular disease. Genetic variants that are associated with longer telomeres suggest an increased risk of cancer, whereas genetic variants that are associated with shorter telomeres suggest an increased risk of cardiovascular disease. The ideal telomere length for longevity is, therefore, not too long and not too short.

Epidemiological Studies of the Association between Metal Exposure and Telomere Length

Brandon Pierce, PhD, University of Chicago

Several potential mechanisms may link chemical exposures to changes in telomere length, including oxidative stress, chronic inflammation, direct DNA damage, impaired DNA repair, and impacts on telomere maintenance processes.

Fewer than 20 epidemiological studies have investigated whether exposure to heavy metals, namely cadmium, lead, and arsenic, is associated with changes in telomere length. Available evidence suggests that exposure to cadmium and lead may be associated with shorter telomeres, whereas exposure to arsenic may be associated with longer telomeres. Extant findings are, however, inconsistent. This may be due to a lack of statistical power, measurement error associated with the use of qPCR, or study characteristics such as exposure range, timing of exposure, and mixed exposures.

Studies of exposure to cadmium (n = 5) show a trend toward shorter telomere lengths among exposed populations. In all studies, exposure levels were low to moderate. A large study conducted using the National Health and Nutrition Examination Survey data (NHANES, n = 6,796) suggested that smoking and cadmium exposure may interact to shorten telomeres.

Lead exposure studies (n = 5) also show a trend toward telomere shortening in exposed populations. Conversely, studies of arsenic exposure (n = 7) generally show a trend toward

telomere lengthening. Levels of exposure in the arsenic studies were broader than in studies of lead and cadmium. The largest study of arsenic exposure (n = 1,469) suggests that arsenic may affect telomere length in younger individuals more than in older individuals. In addition, individuals with shorter telomere lengths at baseline may be at greater risk for arsenic-induced skin lesions than those with longer telomeres at baseline.

Several studies have investigated environmental and occupational exposures to other metals, with little consistency among the findings. At least four studies have investigated associations between metal exposure and expression of genes related to telomere length, providing some evidence that arsenic exposure may be associated with increased telomerase expression.

Challenges for Studies of Metal Exposure and Telomere Length

All studies of metal exposures and telomere length have focused on accessible tissues, primarily blood. However, preliminary data from the ongoing Genotype-Tissue Expression Project (GTEx) suggest that telomere lengths vary substantially by tissue type, individual, and age. Nonetheless, telomere length measured in whole blood samples does correlate fairly well with telomere length in other tissue types.

Other challenges are that studies of exposed versus control populations are prone to several types of bias, qPCR (the measurement method used in all metal exposure studies) is prone to several types of batch effects, and several types of exposures often co-occur, which raises the possibility of unidentified causal agents and synergistic effects of certain combinations of exposures. Finally, the mechanisms of apparent telomere lengthening or shortening associated with metal exposures remain unclear.

To address some of the measurement-related issues, Dr. Pierce and colleagues have developed a Luminex-based telomere length measurement technology that offers low-cost, high-throughput analysis with only a small amount of sample. The method does not require DNA amplification and may offer advantages over existing telomere length measurement technologies.

Telomeres as Biomarkers of Psychosocial Stress and Environmental Exposures—On and Off the Planet

Susan Bailey, PhD, Colorado State University

Dr. Bailey described four natural experiments, highlighting the relationships between telomere length and psychosocial stress or environmental exposures.

Central Indian Conservation Refugees

This study examined a tribe living in a wildlife sanctuary in Central India that was separated to facilitate relocation of a population of Asiatic lions. About half of the human population was displaced from their ancestral homes and moved to a more urban setting (n = 24), while the other half remained in their now isolated wildlife sanctuary (n = 22). Several indicators of stress were measured, including salivary biomarkers, self-assessment instruments, and telomere length. Telomere length was measured using a cell-by-cell TELO-FISH assay on buccal samples

collected in the field, which yielded telomere length distributions specifically in putative stem cells (based on morphology). The displaced human population experienced higher levels of stress than their isolated counterparts, which correlated with shorter telomeres. The results were constant after controlling for demographic factors and corroborated the findings.

Honduras Cookstove Project

This epidemiological study seeks to determine whether use of cleaner-burning cookstoves will improve cardiovascular, respiratory, and metabolic health among a population that burns wood fuel for cooking indoors. An embedded pilot study sought to identify noninvasive biomarkers of health that can be measured in the field. Buccal samples were collected in the field and putative stem cells analyzed for telomere length with TELO-FISH. Micronuclei were also measured as an indicator of DNA damage caused by air pollution. Preliminary results show a positive correlation between total micronuclei and the percentage of short telomeres, suggesting that telomeres are shorter with greater exposure to indoor air pollution. Moreover, the association was stronger in older women, who have longer exposure histories.

Radiation-Exposed Wild Boar in Japan

After the Fukushima nuclear disaster in Japan, wild boar overtook evacuated exclusion zones and can therefore serve as sentinels of radiation exposure for humans returning to their homes. In this study, researchers trapped wild boar from exclusion and control zones and evaluated dicentric chromosomes (a validated indicator of radiation exposure), hair cortisol levels, and blood telomere length. Telomeres and dicentric chromosomes were measured in a combined TC-FISH assay using two fluorescent probes. Preliminary results suggest a shift toward shorter telomere lengths with increasing radiation exposure. Analysis is ongoing.

Telomeres in Space: The NASA Twins Study

This study compared extensive -omics analyses of a pair of twin astronauts, Mark and Scott Kelly, over the course of a year while Scott was aboard the International Space Station and Mark remained on Earth. The hypothesis for telomeres was that they would shorten during space flight. The investigators received whole blood samples before, during, and after flight. Samples during flight were shipped back to Earth for analysis within 48 hours. Telomerase activity was lost in in-flight samples due to uncontrollable transit conditions. In both individuals, there was little difference in telomerase activity pre- and post-flight. However, there was a dramatic change in telomerase activity in both individuals before and after a stressful family event.

Telomere length was measured in isolated PBMCs via qPCR (average) and validated with TELO-FISH (cell-by-cell analysis). The ground-twin's telomere length remained relatively constant throughout the duration of the study. In sharp contrast to expectation, the space-twin's telomere length significantly increased during flight compared to both his pre- and post-flight measures. TELO-FISH revealed a dramatic shift in the distribution toward more cells with longer telomeres, which was consistent across multiple time points during space flight. Upon return to Earth, telomere lengths shortened immediately, and the distribution of telomere lengths normalized within months. Correlations are being sought to provide mechanistic insight; for

example, with telomerase status, potential shifts in cell populations due to radiosensitivity, and enrichment of stem cells.

Genetic and Environmental Influences on Telomere Lengths: Toward Understanding GE Interplay

Chandra Reynolds, PhD, University of California, Riverside

Twin studies offer a unique opportunity to explore the relative contributions of genetic and environmental factors on telomere length, as well as to understand gene-environment interaction effects. A large meta-analysis of cross-sectional twin studies found that leukocyte telomere length is strongly heritable, with additive genetic influences explaining about 70 percent of individual differences in telomere length. Surprisingly, the latest GWAS study identified only seven loci related to the risk of telomere shortening, which is far fewer loci than expected based on prior data.

Evidence from longitudinal twin studies paints a more nuanced picture. Data from the Danish Twins Study, which collected data in two waves 12 years apart, suggest that although genetic influences play a major role in telomere length at baseline (64 percent), environmental influences play a much stronger role in the rate of change in telomere length over time (72 percent). Data from the Swedish Adoption/Twin Study of Aging (SATSA), which has five waves of data over 20 years of follow-up, similarly show that genetic risk scores contribute to overall leukocyte telomere length, but not to differential change. The investigators detected both between- and within-pair effects among twins, suggesting a familial influence on telomere length. Although telomere length decreased in most individuals over time, apparent elongation was sometimes observed. Men had shorter telomeres than did women at all ages, but the rates of change were similar in both sexes. Growth curves were fitted to the change in telomere length and centered at age 69, which marked the age at which telomere lengths began to decline more rapidly.

Other studies using genetic risk scores and Mendelian randomization have suggested a causal association between longer telomeres and better cognitive performance as well as shorter telomeres and increased risk of Alzheimer's disease.

Genetic and environmental influences on telomere length may change in relative importance over time and may interact with each other. To probe these dynamics, the investigators fitted a two-rate biometric latent growth rate model to leukocyte telomere length data from SATSA and decomposed the variance of absolute length and rate of change before and after age 69. This enabled investigation of the relative contributions of additive genetic factors, common environment factors, and non-shared environment factors to telomere length and rate of change at different ages.

Genetic factors, and common environmental factors, and non-shared environmental factors contribute about equally to the variance in telomere length at age 69. Common environmental factors do not contribute to the variance in the rate of change of telomere length at any age. Before age 69, the variance in the rate of change is predominantly caused by non-shared

environmental factors. After age 69, variance in the rate of change is predominantly caused by additive genetic factors. However, the total variance caused by all factors declines dramatically before age 69, mainly due to declines in variance associated with genetic and non-shared environmental factors. The variance of common environmental factors is stable over time.

A subset of identical twins provides evidence of interaction between genetic and environmental factors in telomere length at age 69 and the rate of change before age 69, but not after, using a within-pair difference approach. Data suggest that genetic influences on telomere length may regulate sensitivity to environmental factors. However, genetic risk scores for telomere length were not implicated in the gene-environment interactions, suggesting that other genes may be involved.

Topics for Discussion

Telomere length dynamics are a function of genetics, environmental factors, and their interactions. The composition of these influences may change with age. However, potential selection effects and measurement effects complicate interpretation of available data and should be addressed.

Panel Discussion

Panelists: Abraham Aviv, Brandon Pierce, Susan Bailey, Chandra Reynolds, and Yie Liu, PhD, NIA

Early Life Factors

A growing body of evidence suggests a strong contribution of early life determinants on telomere length in adulthood and rate of change in telomere length over time. Similar findings connect adult cognitive function and trajectories of decline with early life factors. Dr. Nielsen asked whether it is possible to probe the relationship between early life factors, cognitive outcomes, and telomere length in twin studies of aging. Dr. Reynolds noted that although SATSA has measures of early life environments, it lacks detailed prospective information on early life exposures. Other twin studies, such as the Colorado Adoption/Twin Study of Lifespan Behavioral Development and Cognitive Aging (CATSLife), may have relevant data.

Because telomere shortening is rapid in early childhood, environmental exposures that affect telomere length may be particularly important in this life stage. However, there are too few studies of metal exposures and telomere length in early childhood to test this hypothesis in that context. More studies are needed in children, infants, and pregnant mothers. One potentially rich source of samples may be dried blood spots that are routinely taken in newborns.

Intrauterine exposures may be particularly important. Some studies have investigated fetal exposures to heavy metals, but none has also considered impacts on telomere length. The level of fetal exposure in relation to maternal factors depends on the contaminant. Although the twin studies highlight the role of genetic influences on telomere length and change over time, they do not account for nongenetic maternal effects. Dr. Reynolds explained that a twin-offspring study design would be needed to address this question, and she was not aware of any such study.

One key question for longitudinal studies of early childhood and development is whether absolute telomere length or change over time is the more important factor. If the primary concern is change over time, then these studies must consider measurement error, changes in gene expression over time, and changes in telomere length as a mediating factor between environmental exposures and health outcomes. More consideration is needed regarding the most appropriate research questions in young children.

Evidence for Heavy Metal Exposure

Dr. Aviv commented that the evidence that heavy metal exposures impact telomere lengths are conflicting. Dr. Pierce acknowledged several limitations of existing studies, including small sample sizes and potential measurement error. However, he noted that evidence from basic science studies suggest that there may be a relationship between metal exposures and telomere lengths, and that the directionality of results is consistent across studies and different for different metals. Dr. Zota added that data from NHANES show a dose response relationship between cadmium exposure and telomere length, and that these observations agree with those of *in vitro* studies. Prospective exposure studies are needed to clarify the relationships.

The mechanisms of metal toxicity remain poorly understood. There may be complex interactions between different environmental exposures and cellular processes that lead to unanticipated effects on telomere length. Finally, long telomeres may not be functional due to various types of molecular damage. Incorporating functional measures into telomere-exposure studies may yield additional insights.

Telomere Length Dynamics and Mechanisms of Change

The NASA Twin Study highlights the potential for significant short-term changes in telomere length over a period of days. This raises the question of whether longitudinal or even cross-sectional studies that measure telomere length as a snapshot in time are missing key changes. Dr. Bailey suggested that the proportion of short telomeres in an individual or population and how this proportion changes with various exposures may be more relevant than the average telomere length.

Dr. Weng commented on the potential cause of telomere length dynamics in the NASA Twin Study. This swift gain-and-loss telomere length may be result of the change of lymphocyte composition and number in blood. It is known that lymphocyte number changes in blood according to the circadian rhythm, and that different types of immune cells have different length of telomeres (e.g., B cells have the longer telomeres than other major types of cells in blood and naïve T cells have longer telomeres than memory T cells). The alteration of the circadian rhythm in space may explain the findings. Unfortunately, no measurements of lymphocyte composition and number were conducted in this study to verify this explanation.

Emerging literature suggests that several proteins associated with telomeres also have extra-telomeric functions. For example, TERT often migrates out of the nucleus and may interact with mitochondria. It is important to consider these extra-telomeric pathways when trying to determine the mechanisms by which exposures might affect telomere length, because the

effects might be indirect. Dr. Bailey commented that the NASA Twin study might shed light on such mechanisms because of the breadth and depth of the -omics data being collected.

Session 5: Combining Telomere Measurements with Other Markers

Chairs: Janine Santos, PhD, NIEHS, and Idan Shalev, PhD, Pennsylvania State University

Epigenetic Measures and Telomere Length

Colter Mitchell, PhD, University of Michigan

Epigenetics encompass heritable changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence. The epigenome is a dynamic system that is subject to both genetic and environmental influences. These mechanisms may play a key role in mediating the effects of genetic and environmental factors on health outcomes. Many epigenetic mechanisms exist, with methylation and histone modifications being among the best-studied examples.

Technologies to measure DNA methylation have rapidly proliferated in recent years and can be divided into two categories: genome-wide approaches and target region approaches. Genome-wide approaches are typically used when no candidate genes have been identified. Approaches that target specific DNA methylation sites are then used once candidate genes are known. Recent technological advances, such as Illumina BeadChips, have dramatically increased the availability of methylation data.

Both algorithm-based epigenetic measures and gene- or region-based measures may inform studies of telomere length. For example, epigenetic clocks are algorithm-based measures of DNA methylation change that can predict chronological age from tissue samples. Epigenetic clock studies have found strong correlations with age across tissue types; however, variance of epigenetic clocks in children is higher than in adults. One study that compared several epigenetic clocks to each other and to telomere length found only weak to moderate correlations. One key consideration is that epigenetic signatures are cell-type-specific. Several algorithms have been developed to estimate cell-type distributions from DNA methylation signatures, which can, in turn, be used to correct for heterogeneity in epigenetic studies performed on samples with mixed cell populations.

Epigenome-wide association studies of telomere length may be among the most relevant epigenetic measures for telomere research. Based on results of these studies, it is possible to create epigenetic scores correlated with telomere length that can be used by other research groups.

Mitochondria Are Gatekeepers at the Interface of Genome and the Environment

Martin Picard, PhD, Columbia University

Mitochondria are double-membrane organelles with their own DNA whose major function is to produce adenosine triphosphate (ATP) for cellular energy. They are dynamic and respond to stimuli. Although mitochondria are found within every cell type except red blood cells, their

relative abundance varies considerably between cell types and tissues and is higher in cells with greater energy demands. In addition to ATP synthesis, mitochondria contribute to other cellular processes, including production of reactive oxygen species, Ca²⁺ regulation, triggering of cell death, and hormone biosynthesis.

Stress responses require energy, and mitochondria are sensitive to a variety of stressors. They can communicate with each other, integrate information, and produce signals that influence cell processes. A systematic review showed that both acute and chronic psychological stress alter various aspects of mitochondrial structure and function in brains of male rodents.

Mitochondria are also involved in stress transduction and signaling, mediating the influence of psychological and other stressors on cellular processes.

Mitochondria communicate with each other and are connected through junctions. They also typically surround the nucleus and are in close proximity (~100nm) to nuclear pores, allowing metabolites to diffuse from mitochondria to the nucleus and vice versa. Many mitochondrial metabolites are substrates and co-factors for nuclear epigenetic modifications, including acetylation, methylation, phosphorylation, and others.

Mitochondrial dysfunction can affect telomere length and cell senescence. One study of patients with mitochondrial disorders showed accelerated telomere shortening compared to healthy controls. Evidence from rodent studies shows the converse: telomere dysfunction can induce mitochondrial dysfunction. The sequence of mitochondrial DNA (mtDNA) might also directly influence telomere length in humans. Preliminary studies suggest that mtDNA copy number is related to telomere length; however, mtDNA copy number varies across DNA extraction methods.

Many facets of mitochondrial health can be measured. In relation to stress and telomeres, measures of mitochondrial function are likely to be the most informative. One approach could be to conduct multi-level analyses of mitochondrial health that integrate different functional measures.

Longitudinal Studies of Telomere Length in Hiroshima Atomic Bomb Survivors and in Baltimore Longitudinal Study on Aging Cohort, and the Impact of Telomere Attrition on Immune Function

Nan-ping Weng, MD, PhD, NIA

Telomere Length in Atomic Bomb Survivors

Blood leukocytes were collected from 415 atomic bomb survivors in Hiroshima, Japan, at two timepoints: 55 years and 66 years after radiation exposure. Participants were stratified by radiation exposure dose, and complete blood counts were performed to adjust for changes in cell composition over time.

Significant differences in telomere length were observed by radiation exposure dose. The effect, however, was age dependent. Significant telomere shortening with higher radiation doses was detected in individuals who were younger than 12 years at the time of exposure, but

not in individuals who were 12 years and older. Other blood biomarkers showed differences between no-exposure controls and low- and high-dose exposed survivors. However, the observed population was likely influenced by significant selection pressures, which complicates interpretation of results in relation to other longitudinal studies of aging.

Telomere Length and Other Age-Related Biomarkers

The Baltimore Longitudinal Study of Aging (BLSA) measured longitudinal change in telomere length over a 13-year period. Females had longer average telomere lengths than males at all ages. Across the cohort, the mean telomere length shortened by about 16 bp per year. Although the average rate of telomere attrition is modest, individual trajectories differ significantly. BLSA collects a diverse set of other age-related biomarkers, such as inflammatory cytokines and anti-CMV (cytomegalovirus) antibodies; however, none of these was highly correlated with telomere shortening over time.

Telomere Length and Immune Response

BLSA participants with telomere lengths in the longest third and shortest third were selected for a sub-study of potential interactions between telomere length and immune response to influenza vaccination. Participants with long and short telomeres were generally health and more than 70 years old. All participants received an influenza vaccine. Blood samples were drawn at baseline (pre-vaccination), 21-days post-vaccination, and 84-days post-vaccination. The time points were selected to capture short- and long-term immunogenic reactions.

Anti-influenza antibody titers correlated with telomere length in B cells. In other words, B cells with longer telomeres demonstrated a more robust response to the vaccine. Telomere length also correlated with greater proliferation of influenza-specific CD8 T cells in individuals with longer telomeres at baseline. Finally, direct measurement of telomere length of influenza-specific CD8 T cells demonstrated a significant correlation of in vitro proliferation of these CD8 T cells and their telomere length.

The Impact of Persistent Stress-Related Infections on Telomere Length

Allison Aiello, PhD, University of North Carolina at Chapel Hill

Persistent herpesviruses such as CMV, Epstein-Barr virus (EBV), herpes simplex virus (HSV), and varicella zoster virus remain latent in the body and reactivate over time. Reactivation is often prompted by exposure to psychosocial stressors. Reactivated infections may be subclinical; that is, asymptomatic and detectible only through blood tests.

Several chronic diseases have been causally linked to persistent infections, including hepatocellular carcinoma and chronic hepatitis (hepatitis B virus), peptic ulcers and gastric lymphoma (*Helicobacter pylori*), and cervical cancer (human papilloma virus, HPV). Other diseases, including cardiovascular disease, dementia, and several mental health disorders, may also be influenced by herpesviruses or by an accumulation of persistent pathogen burden. For example, high pathogen burden may trigger immune responses, increased levels of circulating cytokines, and other cellular processes that increase the risk of cardiovascular disease.

An analysis of NHANES data from 1988 to 1994, led by Simanek and Aiello, found that individuals with a high number of persistent pathogens were at greater risk of all-cause mortality than those with fewer persistent infections. Certain pathogens and combinations conferred greater risk than others. Combinations that included CMV were associated with the highest risk of mortality.

Pathogen burden may also impact telomere length through mechanisms including inflammation, inflammatory stress, and oxidative stress. Observational studies have found that birds with chronic malaria infections have shorter telomeres than uninfected birds. In humans, HIV infection is associated with shorter telomeres.

Using data from a large UK study, Whitehall II, Dr. Aiello and colleagues led a study that tested participants in the laboratory stressor component of the Whitehall study for CMV antibodies and connected these data with existing information on telomere length, and telomerase activity. Although CMV positivity and telomere length were not significantly associated, higher CMV antibody levels were associated with greater telomerase activity. When the investigators considered combined pathogen burden of CMV, EBV, human herpesvirus 6 (HHV-6), and HSV, they found that individuals with three or more persistent infections had significantly increased rates of telomere length shortening over a 3-year period. Individuals at greatest risk of telomere shortening were those with combined CMV and HSV or CMV and HHV-6 infections.

One theory to explain the observed telomere shortening in CMV-positive individuals, especially those who also harbor other persistent infections, is that CMV infection and the chronic stress of disease reactivation lead to cellular aging and telomere shortening. In CMV-positive adults, a large percentage of immune cells are focused on controlling CMV. Serial reactivation of CMV infections could lead to clonal accumulation of end stage, senescent T cells.

Panel Discussion

Panelists: Colter Mitchell, Martin Picard, Nan-Ping Weng, Allison Aiello, and Susan Bailey

Epigenetics: Challenges and Opportunities

Epigenetics is a quickly evolving and diverse field. At least 300 epigenetic measures have been described. Just like in telomere research, measurement error is a concern. Although many epigenetic measures are likely relevant for aging, it will take time to identify the best approaches.

The epigenetic clock correlates strongly with age in part because it was designed to correlate with age. The biological meaning of the epigenetic clock is unclear. Dr. Aviv noted that telomere lengths measured in certain cell populations correlate well with epigenetic clock measures, whereas telomere lengths in other cells do not. The epigenetic clock was designed to measure aging in adults. In contrast, the trajectory of telomere length is largely established during early childhood. Thus, one would not necessarily expect the two measures to correlate well over time.

Dr. Picard observed that the epigenetic clock, which is a composite measure, predicts aging better than individual epigenetic changes. Although biologists are good at reducing complex processes and measuring individual components, composite measures may represent a more sensitive indicator of overall health by filtering the variance of individual measures. The downside to this approach is that aggregate measures have less ability to determine biological mechanisms than do individual measures.

Mitochondria: Challenges and Opportunities

Like telomeres and epigenetics, measuring mitochondrial function is complex, and there is little agreement in the field about the best approaches. One critical challenge is that frozen tissue samples are not suitable for functional measures. Researchers must often trade ideal measures for feasibility.

Dr. Picard explained some of the complexities in functional measures of mitochondria. mtDNA copy number, which represents the number of mitochondrial genomes in a cell, can be measured as the ratio of mtDNA to nuclear DNA using qPCR but is not always easy to interpret in isolation. For example, mtDNA copy number measured in whole blood is easily skewed based on cell-type composition. The number of mitochondria in different blood cell types varies by a factor of five. Platelets have mitochondria but lack nuclei. Thus, even a small increase in the number of platelets in a sample will inflate mtDNA copy number results because they do not contribute to the denominator. Combining information on mtDNA copy number with functional measures provides more insights. For example, mtDNA copy number increases with an increased number of mitochondria. The number of mitochondria can double with exercise. Yet mitochondria also tend to proliferate in the context of mitochondrial dysfunction. More research is needed to understand the dynamics of mitochondrial function in response to stress and other stimuli.

Immune Response Dynamics

Acute stress and infections can prompt transient immune responses. If such acute responses are hypothesized to influence telomere lengths, then studies with high-frequency sampling would be needed. Most chronic, latent infections are detectable at relatively stable levels that gradually increase over a period of years or decades. Older adults typically have higher levels of circulating cytokines, and the levels typically do not decrease over time. These slowly increasing cytokine levels are related to slow and steady age-related changes.

Certain persistent viruses are found in specific cell types, whereas others—such as CMV—are found in most cells throughout the body. There is some evidence that HHV-6 integrates with telomeres and may have direct genetic effects. Overall, more research is needed to understand the pathways through which persistent herpesvirus infections may influence telomere length.

Open Discussion

Moderator: Gwen Collman, PhD, Director of Extramural Research and Training, NIEHS

NIEHS and NIA program staff set the stage for the final discussion by outlining a few potential mechanisms through which the institutes, individually or in collaboration with each other or other NIH institutes and centers, could support continued progress in research on the relationship between telomeres, environmental exposures, psychosocial stress, and disease susceptibility. These include, but are not limited to the following:

- **Trans-NIH efforts** through the NIH Common Fund or other mechanism. The NIH Common Fund supports cross-cutting, catalytic research that is unlikely to be supported by another entity.
- **Networks or consortia** to support collaborative efforts, small-scale pilot projects, mentorship programs, harmonization or standardization initiatives, and other lasting interdisciplinary efforts.
- **Regular webinars or other forums** for continued discussion on current challenges and opportunities in telomere research.
- **Collaborations with existing NIH programs**, such as the NIEHS National Toxicology Program, which maintains a vast archive of specimens from animal exposure studies and can serve as a platform a variety of assays.

Participants were encouraged to consider top research priorities and brainstorm creative solutions, even if the potential support mechanisms were not clear.

Telomere Length: Meaning and Measurement

The literature on telomere length, environmental exposures, and stress is often contradictory, in part because of the large number of underpowered studies and challenges related to measurement technologies and error. More mechanistic studies are needed to understand how various exposures effect telomere length and other aspects of telomere biology. Workshop participants noted that the goal of epidemiological studies of telomere length and exposures is not always clear and, therefore, the ideal measures are uncertain. Key remaining questions include:

- Is telomere length a biomarker of stress or environmental exposures? If so, by what mechanisms do these factors influence telomere length? Alternatively, is telomere length a marker of a health outcome?
- Should investigators focus on absolute telomere length or changes over time? If change in telomere length is more relevant, are changes at different ends of the distribution equally important? Or do changes in shorter telomeres matter more than changes in longer telomeres?
- How dynamic is the telomere system? What is the meaning of short-term changes in telomere length over hours, days, or weeks? What is the normal status of a telomere in a potentially highly dynamic system?

- What complementary biomarkers could be used to help tease out exogenous effects that may complicate interpretation of telomere length?

Answering these questions will help researchers design appropriate studies in the future.

Suggested next steps include:

- **Conducting more natural experiments** to better understand telomere dynamics and their response to various exposures.
- **Designing more studies with repeated measures** that can probe the dynamism of the telomere system and its response to various stimuli.
- **Investigating potential interactions between multiple variables**, such as environmental factors, telomere dynamics, and age-related health changes.
- **Deeper mechanistic exploration of the developmental origins** of telomere length and attrition, response to environmental exposures, and long-term health outcomes. This could include investigation of maternal and paternal influences, genetic influences, and early environmental exposures.
- **Develop a set of standards** to understand the baseline telomere length in cells that are not exposed to fluctuating environmental conditions. This could be accomplished by culturing a line of induced pluripotent stem cells in a controlled environment to determine the true telomere phenotype of an individual.

Standards and Training for Telomere Research

There was consensus that further methods development and standardization is needed for telomere research in general, and especially for telomere measurements conducted in the context of epidemiological studies. This is an urgent need: the number of publications that include telomere length measures is increasing, but, without methodological standards, the results are often dubious or difficult to interpret.

One way forward would be to codify a set of publication or peer-review guidelines, such as those issued by STrengthening the Reporting of OBservational studies in Epidemiology (STROBE).⁸ Guidelines could include a description of parameters that must be reported depending on which measurement method was used, requirements for presenting correlations with age, and other publication conventions. A companion document could be developed as a roadmap for investigators interested in conducting telomere research for the first time. This manual would describe best practices for sample preparation and storage, DNA extraction, assay protocols, and reporting results. Developing a certification program for laboratories measuring telomere length may also help improve the quality of published telomere research.

Animal Models for Telomere Research

Studying telomere dynamics in animals, such as rodents and birds, may elucidate biological mechanisms of response to environmental exposures that may be conserved in humans. Some participants, however, commented that the substantial differences in telomere biology between humans and almost any other species complicate cross-species comparisons. Humans

⁸ STROBE guidelines may be accessed at <https://www.strobe-statement.org>.

have among the shortest telomeres of any mammal, and telomerase activity is repressed in somatic cells. Mice, in contrast, have very long telomeres and high telomerase activity. Birds also have long telomeres with interstitial telomere repeats. Species with telomere lengths most like humans, such as elephants, are too long-lived for longitudinal research. Although some mouse strains have relatively short telomeres, they are still significantly longer than humans. Perhaps most critically, the high telomerase activity in rodents likely changes the way their telomeres respond to environmental exposures.

NIEHS staff encouraged workshop participants to think creatively about the potential for learning from animal studies. There are hundreds of mouse strains with vast phenotypic and genetic diversity. Some may be suitable for mechanistic telomere research. Telomere measures could be added to the NIEHS National Toxicology Program to facilitate learning about telomere dynamics in the face of diverse exposures.

Opportunities for Collaboration

Participants agreed that the diversity of experts from multiple disciplines who attended the workshop added value. Future efforts should seek to involve biostatisticians, animal model experts, and environmental exposure researchers—even those who do not typically work on telomeres.

Formalizing collaborations through the formation of a consortium or network of researchers would help maintain the momentum built during the workshop and facilitate long-term follow-up. A consortium or network could draft standards for research and peer review, conduct meta-analyses, and help integrate telomere measures into existing studies with stored samples.

Informal and ad hoc collaborations would also be useful. For example, NIEHS could connect telomere researchers with current grantees conducting exposure research. Even without a formal program to fund new applications, the institute could facilitate the formation of independent collaborations that are unlikely to arise organically. Similarly, NIA funds several large data infrastructure projects, such as the Health and Retirement Study, that are always looking for new measures to include. Some of these studies have large repositories of stored samples, and NIA staff could connect telomere researchers with the appropriate investigators.

Epilogue: Suggestions Received from Participants After the Workshop

After the workshop, participants engaged in an email discussion about suggested next steps. The key suggestions are summarized thematically below.

Develop Guidelines for Telomere Research

- The field would benefit from consensus guidelines on biological sample collection, storage, and processing; laboratory methods; data analysis; and reporting requirements.
- Guidelines should differentiate between small-scale laboratory experiments, clinical research, and population-based studies. Whether living cells are available is also an important consideration. Different methods are appropriate in different settings, and no single method is likely to meet the needs of all types of studies.

- Existing guidelines in other fields, such as STROBE for observational epidemiological studies, can serve as a model.
- The development of research and reporting guidelines is an urgent need that should begin soon. Incorporating guidelines into journal requirements and the peer-review process will have near-term influence on the quality of published telomere research.

Conduct a Robust Methods Comparison Study

Concerns and uncertainty about the accuracy of various telomere length measurement methods remain a critical barrier for the field. To address this, participants agreed that a multi-laboratory study should compare multiple telomere length measurement methods in human samples.

Basic Comparative Study Design

- Methods could include Southern blots, qPCR and related techniques, flow FISH and related techniques, and any other viable method. A funding opportunity announcement for this effort could solicit ideas for additional methods from applicants.
- At least two laboratories should measure blinded samples, including blind duplicates, using each measurement method.
- Samples should be centrally prepared and dispatched by the funder (e.g., NIEHS or NIA), and the unblinded results should be released by this entity within a prespecified timeframe.
- The study should consider both comparative assessments between methods and within-method test-retest reproducibility.

Scope of Study

The suggested scope of the comparative methods study ranged from a small-scale study of 100 samples or less to establish the relative validity and reliability of each method to a large-scale study that includes clinical correlates and longitudinal repeated measures. A staged approach could speed availability of initial comparative results while serving as a platform to answer broader questions. Additional specific considerations included:

- Use umbilical cord blood and leukapheresis samples for volunteers across a range of ages to provide (1) ample sample quantities for testing multiple techniques in multiple laboratories and (2) a variety of telomere lengths.
- Measure the types of samples most commonly used in telomere length studies, such as stored whole blood and buffy coat, buccal cells, saliva, and dried blood spots.
- Compare serial samples obtained from the same individuals longitudinally at three or four different time points. Stored samples from existing cohort studies could be used.
- Include a comparison of DNA extraction methods and batch effects.
- Include a comparison of sample storage conditions.
- Develop pre-defined criteria to judge the comparability of different methods, especially when the coefficient of correlation is not expected to equal 1.0 due to differences in the part of the telomere being measured using each method. Two ways to deal with these discrepancies are to (1) use human fibroblast cell lines harvested at different population

doublings to shorten telomeres and (2) use cells infected with the telomerase gene *hTERT* to extend telomeres.

- Consider the difference between methods that report relative measurements (e.g., qPCR) and those that report absolute measurements (e.g., Southern blots).
- Collect extra samples for non-telomere measures, which would allow investigation of correlation between telomere length and other parameters in a well-controlled, multi-method study.

Additional Suggestions

- More studies are needed that measure telomere length in a variety of tissue types in addition to peripheral blood.
- An initiative to combine existing longitudinal cohorts, particularly those with rich early life measures and stored biological samples, for telomere length measurement would be useful. Linked ancillary databases and electronic health records could shed light on the relationships between longitudinal telomere length dynamics, environmental exposures, and disease outcomes.
- In general, caution is warranted when interpreting telomere length and shortening, because many statistically significant findings may not be clinically relevant. Additional studies are needed to understand the mechanisms by which stress and exposures may affect telomere length and by which telomere length may affect health outcomes.
- Prospective measures of telomere length and shortening in early life would help better understand whether the trajectory of telomere length is established very early and to serve as a baseline for exposure studies in later life.
 - This could be accomplished through repeated measures in multiple tissue types over short timespans in newborns, infants, young children, and adolescents.
 - Assays of telomerase expression could also be informative.
 - Longitudinal studies with banked samples, such as dried blood spots collected from newborns, are also potentially valuable sources of data.

Appendix A

Workshop Agenda

Day One – Wednesday, September 6, 2017

9:00 – 9:15 a.m. Welcome Remarks and Charge for the Workshop

Linda Birnbaum, Ph.D., Director, National Institute of Environmental Health Sciences (NIEHS)

Michelle Heacock, Ph.D., Hazardous Substance Research Branch, NIEHS

Lisbeth Nielsen, Ph.D., Division of Behavioral and Social Research, National Institute on Aging (NIA)

9:15 – 10:15 a.m. Session One, State of the Science (60 min)

Goal: Provide an overview of the current research and, where applicable, point out the discrepancies in research findings and possible explanations. Provide a summary of current knowledge related to telomere changes that have been observed in response to stress and environmental exposures drawing from literature in cellular biology, population based studies, and disease-specific research in humans and animal models.

Chair: Michelle Heacock

Presentations:

- Mary Armanios, M.D., Johns Hopkins University, Causality and Association (20 min)
- Patricia Opresko, Ph.D., University of Pittsburgh, Environmental Exposures and Telomere Effects (20 min)
- Elissa Epel, Ph.D., University of California, San Francisco, “Stress Effects”: Differential Lifespan Effects, Pseudo-lengthening, and Other Complexities (20 min) (remote)

10:15 – 10:30 a.m. Break

10:30 – 11:50 a.m. Session Two, Effects of Psychosocial Stress on Telomeres (80 min)

Goal: An overview of evidence supporting how stress affects telomere length and telomerase activity and what other markers should be combined with these measurements

Chairs: Ami Zota, Ph.D., George Washington University, and Max Guo, Ph.D., NIA

Presentations: (10 min)

- Pathik Wadha, M.D., Ph.D., University of California, Irvine, The Fetal Programming of Telomere Biology Hypothesis (10 min)
- Stacy Drury, Ph.D., Tulane University, Crossing Tissues and Disciplines: Considerations of Tissues and Timing (10 min)
- Belinda Needham, Ph.D., University of Michigan, Social Status, the Stress Process Model, and Telomere Dynamics (10 min)

- Idan Shalev, Ph.D., Pennsylvania State University, Lessons for Development of Biomarkers of Aging From Telomere Research (10 min)

Panel Discussion: (40 min)

Panelists: Pathik Wadha, Stacy Drury, Belinda Needham, Idan Shalev, Allison Aiello

Questions to address:

1. How have current studies measured telomere length status and/or changes?
2. Where are there consistencies/inconsistencies in the literature?
3. What is the best way to measure telomere length changes?
4. How do telomere length measures relate to other stress biomarkers and assessments?
5. How do we, should we, parse out other effects for telomere changes?
6. Are stress effects always oxidative, or are other pathways evoked?
7. What other questions are we missing?

11:50 – 1:00 p.m. Lunch

1:00 – 2:30 p.m. Session Three, Critical Considerations of Assessment of Telomere Length Dynamics (90 min)

Goal: Provide an understanding of what is being measured in bulk cells vs single cells vs individual telomeres. Discuss the benefits and drawbacks of different approaches of measurement, cell types, and why and when rate of telomere length (i.e., following length changes over time) change should be considered

Chairs: Allison Bertuch, M.D., Ph.D., Baylor College of Medicine and Stacy Drury, Ph.D., Tulane University

Presentations:

- Peter Lansdorp, Ph.D., British Columbia Cancer Agency, Telomere Length Measurements and Cell Turnover (10 min)
- Shawn Ahmed, Ph.D., University of North Carolina at Chapel Hill, Germ Cells Versus Somatic Cells (10 min)
- Jue Lin, Ph.D., University of California, San Francisco, Longitudinal Studies of Telomere Length: Assay Considerations and Findings (10 min)
- Allison Bertuch, M.D., Ph.D., Baylor College of Medicine, The Fate of Dysfunctional Telomeres (10 min)
- Sharon A. Savage, M.D., National Cancer Institute, Germline Genetic Variation in Telomere Biology Genes is Associated With a Spectrum of Phenotypes (10 min)

Panel Discussion (40 min)

Panelists: Peter Lansdorp, Shawn Ahmed, Jue Lin, Allison Bertuch, Sharon Savage, and Sara Hagg, Ph.D. (Karolinska Institutet - remote)

Questions to address:

1. Measuring telomere length in bulk cells vs single cells, bulk telomeres vs individual telomeres, whole cell extracts versus separating leukocytes (mixed cell types) benefits and drawbacks, best approach for measurement and why
2. Sample preparation and storage considerations
3. Telomere measurement techniques (e.g., qPCR vs TRF vs flow FISH), what is being measured, what are the advantages/limitations?
4. Rate of telomere change (shortening or lengthening), how and when is it crucial to follow over time. Importance of knowing the baseline length (e.g., baseline of shorter or longer telomeres) and addressing the role of cellular context/turnover
5. Effect of SNPs on TBPs other telomere maintenance proteins?
6. Calculating effect sizes (e.g., base pairs per year) – can we arrive at a consensus?
7. Do we need, and how do we, account for cells with short telomeres being culled out of population?
8. How can some current assays be adapted to provide more high-throughput measurements? Using archived samples?
9. What kind of assays/methods offer greater sensitivity for detection of telomere length?
10. What other questions are we missing?

2:30 – 2:45 p.m. *Break*

2:45 – 3:00 p.m. *Recap – Chairs to give a re-cap to set up open discussion (15 min)*

3:00 – 5:00 p.m. *Question and Answer Session/Open Discussion to Consider Overarching Questions*

Chair: Rick Woychik, Ph.D., Deputy Director, NIEHS

Based on presentations and discussion, are there tissue-specific effects? If so, what cells can be used as a reasonable proxy (e.g., can leukocytes, buccal cells be used)? Is there a correlation between cord blood, placenta, and blood spots? Difference in length dynamics in high versus low proliferative capacity cells. If so, can a correction factor be applied so easier cells can be used?

How can epi studies benefit from telomere interrogation? What is the potential of using telomeres to understand exposure and susceptibility and what is needed to get there (e.g., assays to measure single telomere tracts, knowledge gap as to whether cells such as leukocytes can be as reliable proxies? How best should samples be harvested? Preserved? How to account for plate variability, select reference DNA (qPCR)? How big a telomere change to be to be seen in an epidemiological study?

How can basic researcher help epidemiologists/clinicians and vice versa? What can be done right now? What are the possibilities and how do we move forward?

5:00 p.m. **Remarks from day and adjourn**

Day Two – Thursday, September 7, 2017

9:00 – 10:25 a.m. **Session Four, Genetic Susceptibility and the Environment (85 min)**

Goal: Provide the current research on how mutations in telomere maintenance proteins combined with an environmental factor and or stress play a role in disease

Chairs: Patricia Opreko, Ph.D., University of Pittsburgh and Colter Mitchell, Ph.D., University of Michigan

Presentations:

- Abraham Aviv, M.D., Rutgers University, The Biological Meaning of Leukocyte Telomere Length: Is Leukocyte Telomere Length a Biomarker of Human Aging? (15 min)
- Brandon Pierce, Ph.D., University of Chicago, Epidemiological Studies of the Association Between Metal Exposure and Telomere Length (10 min)
- Susan Bailey, Ph.D., Colorado State University, Telomeres as Biomarkers of Psychosocial Stress and Environmental Exposures – on and off the Planet (10 min)
- Chandra Reynolds, M.D., University of California, Riverside, Genetic and Environmental Influences on Telomere Lengths, Towards Understanding GE Interplay (10 min)

Panel Discussion (40 min)

Panelists: Abraham Aviv, Brandon Pierce, Susan Bailey, Chandra Reynolds, Yie Liu, Ph.D. (NIA), Sharon Savage (remote)

Questions to Address: What other factors contribute to telomere shortening (and in some cases, lengthening) where it is unknown if a gene is involved (e.g., stress)

1. How is the telomere affected? At the telomere nucleotide level? By affecting telomere maintenance via binding Shelterin? Or is it damage to genome where telomeres break off?
2. What role do cell proliferation and or replication have?
3. DNA repair capacity differences? (this one might be too in the weeds)
4. Effect of SNPs on TBPs other telomere maintenance proteins?
5. What is the state of current understanding from population- based studies regarding telomeres as a read-out of environmental exposures (physical environment) and stress (psychosocial exposures and stress reactivity)?
6. What is the best way of assessing telomere biomarkers in population based studies (alone, or in relation to other biomarkers of aging)?
7. What are the other possibilities? Are the markers universal or change with different diseases?

8. What do telomere syndromes tell us about maternal effects, paternal effects, transgenerational effects on telomeres? How this be used to inform other studies?
9. Effects of secondary/mixed exposures on telomeres – what do we need to think about when we are trying to parse out the effects of mixtures?
10. What are other questions we are missing?

10:25 – 10:40 a.m. Break

10:40 a.m. – noon Session Five, Combining Telomere Measurements with Other Markers (80 min)

Goal: An overview of the power of using other biological response markers to improve the integrity of measurements to understand the impact of cumulative exposures.

Chairs: Janine Santos, Ph.D., NIEHS and Idan Shalev, Ph.D., Pennsylvania State University

Presentations:

- Colter Mitchell, Ph.D., University of Michigan, Epigenetic Measures and Telomere Length (10 min)
- Martin Picard, Ph.D., University of Pittsburgh, Mitochondria are Gatekeepers at the Interface of Genome and the Environment (10 min)
- Nan-ping Weng, Ph.D., NIA, Longitudinal Studies of Telomere Length in Hiroshima Atomic Bomb Survivors and in Baltimore Longitudinal Study on Aging Cohort, and the Impact of Telomere Attrition on Immune Function (10 min)
- Allison Aiello, Ph.D., University of North Carolina at Chapel Hill, The Impact of Persistent Stress-Related Infections on Telomere Length (10 min)

Panel Discussion (40 min):

Panelists: Colter Mitchell, Martin Picard, Nan-ping Weng, Allison Aiello, Susan Bailey, William Copeland, Ph.D. (NIEHS)

Questions to address:

1. Can the status of telomeres be used to measure cumulative exposures (i.e., stress and environmental exposures)? How can other biological response indicators be used to ground-truth the measurements? Telomerase activity measurements, how/when important?
2. What are the other ways (e.g., biological responses) can be used to measure telomere dysfunction and/or biological aging?
3. SNPs that have been identified and associated with leukocyte telomere length changes are they strong enough to be used as one of the biomarkers, as a proxy for telomere length?
4. How can epigenetics data be used to inform telomere studies?
5. Why telomeres – what advantages do they hold?

6. How are telomeres a unique biomarker in early life and childhood and not in later life?
7. What other questions are we missing?

Noon – 1:10 p.m. Lunch

1:10 – 2:30 p.m. Chair Take-aways

Lead by Gwen Collman, Ph.D., Director of Extramural Research and Training, NIEHS

Question and Answer Session/Open Discussion to consider overarching questions:

Based on presentations and discussion, are there tissue-specific effects? If so, what cells can be used as a reasonable proxy (e.g., can leukocytes be used)? Difference in length dynamics in high versus low proliferative capacity cells. If so, can a correction factor be applied so easier cells can be used?

How can epi studies benefit from telomere interrogation? What is the potential of using telomeres to understand exposure and susceptibility and what is needed to get there (e.g., assays to measure single telomere tracts, knowledge gap as to whether cells such as leukocytes can be as reliable proxies? How best should samples be harvested? Preserved? What are the best model organisms for filling the gaps to move this forward?

How can basic researcher help epidemiologists/clinicians and vice versa? What can be done right now? What are the possibilities and how do we move forward?

2:30 p.m. Meeting wrap-up

Lisbeth Nielsen and Michelle Heacock

Appendix B

List of Participants

Name	Affiliation
Ahmed, Shawn	University of North Carolina at Chapel Hill
Aiello, Allison	University of North Carolina at Chapel Hill
Armanios, Mary	Johns Hopkins University
Arnette, Robin	NIEHS
Artandi, Steven	Stanford University
Aviv, Abraham	Rutgers University
Bailey, Susan	Colorado State University
Belsky, Daniel	Duke University
Bertuch, Alison	Baylor College of Medicine
Birnbaum, Linda	NIEHS
Caglayan, Melike	NIEHS
Carlin, Danielle	NIEHS
Collman, Gwen	NIEHS
Copeland, Bill	NIEHS
Cowell, Whitney	Columbia University
Degtyareva, Natalya	NIEHS
Demanelis, Kathryn	University of Chicago
Drury, Stacy	Tulane University
Epel, Elissa	University of California, San Francisco
Factor-Litvak, Pam	Columbia University
Ferguson, Kelly	NIEHS
France, Suzanne	NIEHS contractor: MDB, Inc.
Freeman, Kenda	NIEHS Contractor: MDB, Inc.
Gaston, Symielle	NIEHS
Gordenin, Dmitry	NIEHS
Griffith, Jack	University of North Carolina at Chapel Hill
Guidry, Virginia	NIEHS
Guo, Max	National Institute on Aging
Hagg, Sara	Karolinska Institutet
Heacock, Michelle	NIEHS
Henry, Heather	NIEHS
Humble, Michael	NIEHS
Jackson, Chandra	NIEHS
Kar, Anirban	University of North Carolina at Chapel Hill
Kovi, Ramesh	NIEHS
Kresovich, Jacob	NIEHS
Lansdorp, Peter	British Columbia Cancer Agency
Lee, Mi Kyeong	NIEHS
Lin, Jue	University of California, San Francisco

Liu, Yie	National Institute on Aging
Mashal, Ahmed	NIEHS
Mathura, Emilie	NIEHS
McAllister, Kim	NIEHS
Meier, Helen	University of Wisconsin-Milwaukee
Mitchell, Colter	University of Michigan
Nadalutti, Cristina	University of North Carolina at Chapel Hill
Needham, Belinda	University of Michigan
Nielsen, Lisbeth	National Institute on Aging
Opresko, Patricia	University of Pittsburgh
Park, Yong-Moon	NIEHS
Peters, June	National Cancer Institute
Picard, Martin	Columbia University
Pierce, Brandon	University of Chicago
Reinlib, Les	NIEHS
Reynolds, Chandra	University of California, Riverside
Rodriguez, Yesenia	NIEHS
Sandler, Dale	NIEHS
Santos, Janine	NIEHS
Savage, Sharon	National Cancer Institute
Schurman, Shepherd	NIEHS
Shalev, Idan	Pennsylvania State University
Shaughnessy, Dan	NIEHS
Thomas, Duncan	Duke University
Thomas, Laura	NIEHS
Thomas, Sam	Rose Li and Associates, Inc.
Tyson, Frederick	NIEHS
Voelker, Kerri	NIEHS Contractor: MDB, Inc.
Wadhwa, Pathik	University of California, Irvine
Weng, Nan-ping	National Institute on Aging
Witt, Kristine	NIEHS/NTP
Worth, Leroy	NIEHS
Woychik, Rick	NIEHS
Xu, Miaofei	NIEHS
Zota, Ami	George Washington University