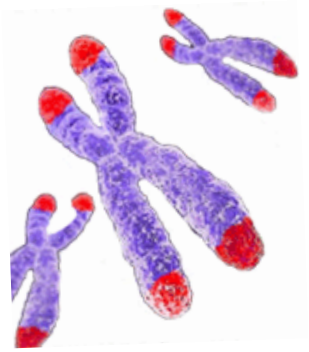
BERZELIUS SYMPOSIUM 85

TELOMERE BIOLOGY IN HEALTH AND DISEASE
– a crystal ball for the future?

25–27 May 2011 in Stockholm Sweden



- **Programme**
- **List of participants** *Not included on the web-version!*
- **General information**
- **Posters and oral abstracts**

Vetenskapsrådet

Svenska Läkaresällskapet

Introduction

The developing scientific interest in the biology of ageing and more specifically in telomere biology has contributed to our understanding of underlying mechanisms of health and disease progression. Following the 2009 Nobel Prize Award in Medicine and Physiology to three outstanding researchers in telomere biology – Elizabeth Blackburn, Carol Greider, and Jack Szostak – several new projects and publications have added to our knowledge in this expanding field of research. It is therefore timely that the Swedish Society of Medicine will organise a Berzelius symposium (nr. 85) in May 2011 dedicated to telomere biology as one important aspect of research into molecular biogerontology. This is also reflected in the EU-financed research programme "WhyWeAge" (www.whyweage.eu) and in the ambitions for the strategic research programme, including basic epidemiology, at the Lund and Uppsala universities in Sweden, Epidemiology for Health (www.med.lu.se/epihealth). The symposium aims at bringing together researchers from both sides of the Atlantic with an interest in basic science but also in clinical applications, if any, of the new developments in the understanding of regulation of telomere length and cellular senescence. There will also be rich opportunities for interactions and personal discussions, as well as the possibility for young researchers to submit abstracts for poster or oral presentations. Prizes for best abstracts will be awarded. The proceedings and abstracts of the symposium are planned to be published in a symposium booklet.

Welcome to Stockholm in May 2011!

Peter M Nilsson, Malmö-Lund (chair)
Sofie Baekert, Ghent, Belgium
Annika Dejmek, Malmö, Sweden
Frej Fyhrquist, Helsingfors, Finland
Göran Roos, Umeå, Sweden
Peter Stenvinkel, Stockholm, Sweden

ORGANISING COMMITTEE

THE SYMPOSIUM IS ARRANGED BY
THE SWEDISH SOCIETY OF MEDICINE
VETENSKAPSRÅDET, THE SWEDISH RESEARCH COUNCIL
EPIHEALTH: LUND AND UPPSALA UNIVERSITIES

IN COOPERATION WITH
THE SWEDISH TELOMERE AND TELOMERASE NETWORK
KAROLINSKA INSTITUTE
THE CANCER ACADEMY IN NORTHERN SWEDEN
LIONS CANCER RESEARCH FOUNDATION AT UMEÅ UNIVERSITY

A CONTRIBUTION FOR EDUCATIONAL PURPOSES HAS BEEN MADE BY

Programme

WEDNESDAY 25 MAY

11.30–13.00 Lunch for lecturers, mounting of posters

13.00–13.15 Welcome addresses
Margaretha Troein Töllborn, President of the Swedish Society of Medicine
Rune Toftgård, representative for the Nobel Prize Committee 2009
Peter Nilsson, chair of the organising committee

13.15–14.00 State of the Art 1: Telomere biology – a challenge for the future.
Chair: Peter Nilsson Sweden
Speaker: Elizabeth Blackburn, Nobel Laureate 2009, USA

14.00–15.30 Session 1 · Molecular biology and telomere changes
Chair: Abraham Aviv, USA

Definitions and genetics, twin studies. **Nancy Pedersen, Sweden**
How good is blood cell telomere length as a biomarker of ageing in the very old?
Thomas von Zglinicki, UK
Limiting levels of telomerase in normal human stem cells. **Peter Lansdorp, Canada**

15.30–16.00 Coffee, posters

16.00–17.30 Session 2 · Cancer and ageing: where does the telomere fit in?
Chair: Nicol Keith, UK

Alternative lengthening of telomeres in cancer and normal cells.
Roger Reddel, Australia
Telomere length as a biomarker in malignant disorders. **Göran Roos, Sweden**
Telomere mouse models of disease and ageing. **Lenhard Rudolph, Germany**

17.30–17.35 Summary of Day 1.
Annika Dejmek, Sweden

17.45–19.00 Reception at the Swedish Society of Medicine

THURSDAY 26 MAY

08.30–10.00 Session 3 · Cardiovascular disease, diabetes and life style – part 1
Chair: José J Fuster, Spain

Telomeres and coronary artery disease. **Nilesh Samani, UK**
Telomeres, tumor suppressors and cardiovascular disease. **Vicente Andres, Spain**
Observational studies in populations. The Asklepios study. **Tim de Meyer, Belgium**

10.00–10.30 Coffee, posters

10.30–12.00 Session 4 · Cardiovascular disease, diabetes and life style – part 2
Chair: Kerstin Brismar, Sweden

Observational studies, the role of lifestyle. **Katarina Nordfjäll, Sweden**
Prediction of diabetes complications. **Per-Henrik Groop, Finland**
Intervention studies in risk patients. **Frej Fyhrquist, Finland**

12.00–13.00 Lunch, posters & press conference

13.00–14.30 Session 5 · Posters, oral presentation of selected abstracts
Chairs: Elizabeth Blackburn, USA and Ernst Rietzschel, Belgium

14.30–15.00 State of the Art 2: Ageing beyond telomeres: a cardiovascular perspective
Chair: Sölve Elmståhl, Sweden
Speaker: Ed Lakatta, USA

15.00–15.30 Coffee, posters

15.30–17.00 Session 6 · Special conditions and telomere biology
Chair: Göran Roos, Sweden

Early ageing associated with renal disease. **Peter Stenvinkel, Sweden**
Molecular and cellular mechanisms of vascular ageing. **Jorge Erusalimsky, UK**
Telomeres in the hereditarian predisposition of disease. **Klelia Salpea, UK**

17.00–17.30 Award for best poster and summary of Day 2.
Chairs: Elizabeth Blackburn, USA, and Peter Nilsson, Sweden

19.00 Dinner at Nationalmuseum

FRIDAY 27 MAY

08.30–09.00 State of the Art 3: Where is research in human telomere biology heading?
Speaker: Abraham Aviv, USA

09.00–10.30 Session 7 · Psychosocial aspects of ageing and telomere attrition
Chair: Peter Nilsson, Sweden

Stress and telomere attrition. **Elissa Epel, USA**
Psychiatric disorders and telomeres. **Owen Wolkowitz, USA**
Perceived age and telomere attrition. **Kaare Christensen, Denmark**

10.30–11.00 Coffee, posters

11.00–11.30 State of the Art 4: Telomere biology in regenerative medicine.
Chair: Nilesh Samani, UK
Speaker: Calvin Harley, USA

11.30–12.30 Session 8 · Telomere-directed biomarkers for prediction in health and disease
Chair: Annika Dejmek, Sweden

The research perspective. **Nicol Keith, UK**
The clinical perspective. **Uwe Martens, Germany**
The ethical perspective. **Niklas Juth, Sweden**

12.30–12.45 Summing up and closure of the symposium
Frej Fyhrquist, Finland
Peter Nilsson, Sweden

The Society's building at
Klara Östra Kyrkogata 10 in Stockholm

The conference Hall

Nationalmuseum at
Södra Blasieholmshamnen

GENERAL INFORMATION

When & Where
May 25–27, 2011 at the Swedish Society of Medicine (SSM), Klara Östra Kyrkogata 10 in Stockholm, Sweden.

Lunches and coffee
Lunch (on Thursday) and coffee are included in the registration fee and will be served in the on-site restaurant at the SSM.

Social Program
Wednesday on 25 May
reception and the SSM at 5.45-7.

Thursday on 26 May symposium dinner at National-museum, restaurant Atrium. The symposium dinner is open to participants who have registered and payed the fee.

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Sofie Baekert, Ghent, Belgium, Annika Dejmek, Malmö
Frej Fyhrquist, Helsingfors, Finland, Göran Roos, Umeå
Peter Stenvinkel, Stockholm

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Not included in the web-version!

LECTURERS ABSTRACT

Where is research in (Human) telomere biology heading?

Abraham Aviv

The Center of Human Development and Aging UMDNJ, NJ Medical School, Newark, NJ

An expanding body of research has revealed that leukocyte telomere length, which reflects telomere length in hematopoietic stem cells, is associated with diseases of aging and with longevity in humans. Epidemiological/clinical research has not been able thus far to answer the following fundamental question: To what degree do hematopoietic stem cell telomere dynamics define and to what extent are they defined by diseases of aging and longevity? For instance, elderly persons may die with short leukocyte telomere length and not because their leukocyte telomere length is short.

The two elements of leukocyte telomere length dynamics – birth leukocyte telomere length and its age dependent shortening – define hematopoietic stem cell telomere dynamics over the human life course. But these two elements have not been examined jointly in the same individuals in relation to diseases of aging and longevity. Cross-sectional analyses, based on individuals of different ages, can cover the entire human life span. However, they relate leukocyte telomere length with diseases of aging and longevity only in middle age and elderly participants whose birth leukocyte telomere lengths are unknown. Moreover, cross-sectional analyses provide information about age-dependent leukocyte telomere length attrition for the entire sample and not for the individual. Longitudinal evaluations in the same individuals do provide information about the rate of leukocyte telomere attrition for the individual, but for obvious reasons, the duration of the follow-up is unlikely to extend over the entire life course.

Therefore, we do not know at present whether the associations of short telomere length with diseases of aging, principally atherosclerosis, and with diminished survival in the elderly, stem from relatively short hematopoietic stem cell telomere length at birth or a more rapid telomere shortening thereafter. If a relatively long leukocyte telomere length at birth explains leukocyte telomere length in exceptionally old persons or in healthy elderly individuals, it is likely that hematopoietic stem cell telomere length is a determinant in human aging and longevity. However, if short leukocyte telomere length in persons displaying aging-related diseases and diminished longevity is explained by a greater age-dependent loss of telomere repeats, it is more likely that leukocyte telomere length shortening simply registers the pace of human aging.

This presentation will offer a working model that might provide the solution about whether birth telomere length or telomere shortening afterward explains the association of short leukocyte telomere length with diseases of aging and longevity.

Definitions and genetics, twin studies

Nancy Pedersen, Karolinska Institutet, Stockholm Sweden

Telomere length may vary widely as a function of age, disease status, and other influences, including genetic variants. The importance of those genetic variants, many of which are not yet known, has been estimated in twin studies. Genetic association studies indicate that variants in genes other than the telomerase gene are important for individual differences in telomere length. Furthermore, telomere length may be seen as a biomarker of age and disease related processes. For instance, shorter telomere length is a two-fold greater risk of dying earlier, even within identical twins. This presentation will provide an overview of the ways in which epidemiological studies provide perspectives into the role of telomere length as a biomarker of disease pathogenesis and aging. It will provide a short introduction to issues raised in greater detail in many of the presentations during the remainder of the symposium.

How good is blood cell telomere length as a biomarker of ageing in the old?

Thomas von Zglinicki, Institute for Ageing and Health, Newcastle University, UK
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Since we first proposed peripheral blood cell telomere length (TL) as a biomarker of ageing and age-related disease risk, it has been associated with mortality and age-related morbidity in hundreds of studies. However, a number of fundamental questions are still open.

These include:

1. How comparable are results obtained in different labs?
2. Is there a ultimate TL threshold and is this comparable between cohorts?
3. Is TL a biomarker of ageing at the level of the single individual?
4. Is TL more informative than other biomarkers of ageing?

We addressed the first question by instigating a fully blinded comparison between nine labs, representing the main TL measurement techniques applicable to DNA samples, namely Southern blot, STELA and Q-PCR. Results, if expressed as relative TL, showed a high degree of correlation between all labs. Indications for stronger associations within a single technique than across techniques were weak. However, inter-lab coefficients of variation were much higher than those obtained in individual labs. The data obtained so far indicate that rank comparisons of TL between labs are possible, but pose severe limits to the usefulness of transferring or comparing absolute TL data generated outside a single setting.
The underlying assumption in using TL as a biomarker is that it provides a reliable measure of the distance to an ultimate threshold length at which survival or health can no longer be maintained. Comparing TL data from 8 UK birth cohorts comprising 7500 participants, we find asymmetric cohort TL distributions that strongly suggest defined lower threshold telomere lengths. However, threshold values are not the same for all UK cohorts, indicating that TL alone is not sufficient as a biomarker in an inter-cohort comparison.

A significant longitudinal correlation between TL measurements is a necessary precondition for using TL as a biomarker for individuals. Using longitudinal TL data from 5 UK birth cohorts with >4000 participants (ages between 50 and 88 years, samples taken between 1.5 and 10 years apart), we find a significant correlation only in one of the cohorts, where there was the lowest degree of TL change. Extensive verification showed that only a small part of this fluctuation can be explained by experimental measurement error.

The power of TL to predict cognitive dysfunction, multimorbidity, disability and/or short-term (18 months) mortality was compared to that of 73 other biomarker candidates in the Newcastle 85+ study, comprising >750 unselected individuals born in 1921 in Newcastle. In this age group, TL had no predictive power for any of the outcomes.

TL in peripheral blood appears largely determined by shifts between individual lymphocyte sub-populations. These shifts may become faster and more pronounced with increasing age and can severely limit the predictive power of TL. Lymphocyte population-specific TL might be a more useful biomarker in the very old.

Limiting levels of telomerase in normal human stem cells

Geraldine Aubert*[1], Gabriela M. Baerlocher* [1, 2, 3], Irma Vulto1, Steven S. Poon[1] and Peter M. Lansdorp [1, 4, 5]
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Telomerase activity is readily detectable in extracts from human hematopoietic stem and progenitor cells, but appears unable to maintain the telomere length in such cells with proliferation *in vitro* and with age *in vivo*. Because human HSCs and primitive progenitor cells are rare cells that typically reside in the bone marrow, they are difficult to study directly in large numbers of normal humans. In contrast, the various nucleated blood cells that are derived from hematopoietic stem cells are easily accessible for studies.

In order to further study the role of telomerase in hematopoiesis we performed a detailed study of the median telomere length in five distinct leukocyte subpopulations from 835 normal individuals and 89 patients with reduced telomerase activity resulting from haplo-insufficiency for either the telomerase RNA gene (*h*TERC) or the telomerase reverse transcriptase (*h*TERT) gene. The median telomere lengths in several subpopulations of nucleated blood cells over the human life span, from birth to 100 years was measured using flow FISH. Our results show that telomere length in normal individuals varies over a broad range at any given age, that telomere length on average is longer in females than in males and that the rate of telomere attrition varies with age and with cell type. Albeit with different dynamics, telomere length declined with age for each of the leukocyte subpopulations tested. These observations support differences in the turnover between circulating cell types and a limiting role for telomerase in telomere length maintenance. Our data highlight a potential important, limiting role for telomeres in the proliferation of memory T lymphocytes and NK/NKT cells in the elderly.

Strikingly, patients that are haplo-insufficient for one of the telomerase genes showed very short telomeres at all ages. These results demonstrate that normal telomerase levels are essential to limit the loss of telomere repeats in normal hematopoietic stem cells and prevent the onset of a wide spectrum of telomerase deficiency diseases such as dyskeratosis congenita, bone marrow failure and pulmonary fibrosis. Our results point to a crucial, rate limiting role for telomerase in normal HSC function supporting the concept that telomere shortening is a major factor controlling blood cell production.

Alternative lengthening of telomeres in cancer and normal cells

Roger Reddel, Jeremy Henson, Axel Neumann, Hilda Pickett
Children's Medical Research Institute, Sydney, Australia; rreddel@cmri.org.au

Populations of telomerase-null mutant yeast cells are able to generate survivors with stable telomere lengths, implying the existence of one or more mechanisms of telomere length maintenance that do not involve telomerase [1]. The existence of at least one non-telomerase telomere length maintenance mechanism in immortalized human cells was deduced from the observation that some human cell lines have stable telomere lengths over many population doublings in the absence of telomerase activity [2]. Because telomere length maintenance requires a telomere lengthening process to counteract the telomere shortening that accompanies replication of linear chromosomes, and this process appeared to be an alternative to telomerase, it was referred to as Alternative Lengthening of Telomeres (ALT).

ALT has been found in various types of cancers (reviewed in [3]). For reasons that are still unknown, it is common in cancers of mesenchymal origin such as osteosarcomas and a number of soft tissue sarcoma subtypes, and in malignant brain tumors of the astrocytic lineage including glioblastoma multiforme. Other types of cancers where it is has been found less frequently include neuroblastomas, peritoneal mesotheliomas, and carcinomas of breast, lung, stomach, and kidney. A small proportion of cancers have evidence of both ALT and telomerase activity.

The majority of human cell lines and tumors that use ALT have an unusual telomere phenotype (reviewed in [3]). Their mean telomere length is greater than in normal cells and most telomerase-positive cells, and the lengths of individual telomeres are highly heterogeneous, ranging from very short to exceptionally long. Some of the PML nuclear domains within ALT-positive cells contain telomeric DNA (which may be either extrachromosomal or attached to chromosome ends), and telomere binding proteins, together with proteins involved in DNA recombination; because these are highly characteristic of ALT cells, they are referred to as ALT-associated PML bodies (APBs). ALT cells have a high frequency of telomeric sister chromatid exchanges. Although most non-telomeric regions of the genome in ALT cells appear to be no more unstable than in other immortalized cells, some specific minisatellites are more unstable in ALT cells, for unexplained reasons. They also have abundant circles of double-stranded extrachromosomal telomeric DNA ("t-circles"), which may result from trimming of telomeres that are over-lengthened by ALT; t-circles occur in other circumstances, including where telomeres have been over-lengthened by telomerase. In addition, a highly characteristic feature of ALT cells is the presence of partially single-stranded circles of telomeric DNA where the C-rich strand is intact ("C-circles"). There appears to be a relationship between the quantity of C-circles present in a cell and the amount of ALT activity.

The nature and molecular details of the ALT mechanism are still emerging (reviewed in [4]). Telomerase-null yeast survivors are dependent on genes that encode proteins involved in homologous recombination [1]. In human cells that use ALT, DNA within a given telomere is copied to other telomeres by a process that is thought to involve homologous recombination-mediated DNA replication, i.e. one telomere can use another telomere as a template for synthesizing additional telomeric DNA [5]. Intra-telomeric replication can also occur, possibly using recombination intermediates generated as part of t-loop formation [6]. The presence of circular telomeric DNA (t-circles and C-circles) in ALT cells suggests that telomere elongation could also occur by a rolling circle mechanism as in yeast (reviewed in [7]), but this has not yet been demonstrated in human cells.

Evidence is emerging that there is ALT activity in some normal mammalian cells. Telomere lengthening was detected in proliferating lymphocytes of telomerase-null mice [8].

A mouse strain was generated that has a DNA tag inserted into one of its telomeres, and it has been found that the DNA tag is copied on to other telomeres in proliferating tissues (A. Neumann and R. Reddel, unpublished data). Mouse tissues also have detectable levels of C-circles and APB-like nuclear foci (Oganesian and Karlseder, 2011).

These data suggest that more detailed investigations of the ALT mechanism are required for an understanding of both normal telomere biology and telomere maintenance in cancer cells. It is possible, for example, that some individuals with clinical features due to excessively shortened telomeres may have mutations in genes required for ALT activity. In addition, there have been a number of reports of switching between ALT and telomerase activity in human cell lines in culture, so anticancer therapies directed against telomere maintenance may need to inhibit both mechanisms.

References

1. Lundblad,V. and Blackburn,E.H. Cell 73: 347–360, 1993.
2. Bryan,T.M. et al., EMBO J. 14: 4240–4248, 1995.
3. Henson,J.D. and Reddel,R.R. FEBS Lett. 584: 3800–3811, 2010.
4. Cesare,A.J. and Reddel,R.R. Nat.Rev.Genet. 11: 319–330, 2010.
5. Dunham,M.A. et al., Nat.Genet. 26: 447–450, 2000.
6. Muntoni,A. et al., Hum.Mol.Genet. 18: 1017–1027, 2009.
7. Tomaska,L. et al., FEBS Lett. 567: 142–146, 2004.
8. Herrera,E. et al., EMBO J. 19: 472–481, 2000.
9. Oganesian,L. and Karlseder,J. Mol. Cell 42: 224–236, 2011.

Telomere length as a biomarker in malignant disorders

Göran Roos, Department of Medical Biosciences Pathology, Umeå University, Sweden

Normal somatic cells gradually shorten their telomeres with increasing number of cell divisions. Based on studies of cultured cells the telomere hypothesis of cellular aging and immortalization was proposed, including mortality stages M1 ("senescence") and M2 ("crisis"). Very rarely cells escape from crisis and become immortal, a stage associated with stabilization of the telomeres by activation of the telomerase enzyme, or rarely by the alternative lengthening of telomeres (ALT) mechanism.

Studies of human tumors have given support for the M1/M2 model and there is growing evidence that telomerase activity (TA) and TL carry information of clinical importance for cancer patients. This association between telomere biology and clinical outcome is best established for cases diagnosed with hematologic malignancies, one example of which is chronic lymphocytic leukemia (CLL) (1-5). In CLL, there is an association between short telomeres, genetic aberrations, risk of transformation and tumor progression/prognosis (6). Similar data have been shown for patients with myelodysplasia, chronic myeloid leukemia and myeloma (7). Regarding solid tumors the association between TL, TA and prognosis is less well established.

There are numerous studies indicating that blood cell TL is associated to various diseases, examples of which are diabetes, hypertension, arteriosclerosis and cancer. In general, short telomeres have been coupled to increased risk of several diseases. It has been discussed whether short TL is important for disease development, if it is a biomarker for ongoing processes leading to disease or if the disease per se, or its treatment, can cause increased telomere shortening. Factors like life style and stress have been indicated to influence the blood cell telomere attrition rate. These data are in line with the fact that the heritable impact on TL decreases by increasing age, signifying the impact of micro- and/or macro-environmental factors in determining TL (8). Regarding solid tumors there seems to be a connection between short blood telomeres and the development of some, but not other, cancer types (9).

Blood cell TL has by us been shown to represent a new and independent prognostic biomarker in newly diagnosed, untreated patients with breast and renal cell carcinoma (10,11). Patients with long blood telomeres had a significantly worse outcome compared to those with short telomeres. The individual tumor related "telomere response" can be due to factors from the tumor cells and/or their environment. Our main hypothesis is that the blood TL in cancer patients reflects clinically important events in the immune system. The collected observations regarding blood cell TL (as related to inheritance, age, various diseases and being a prognostic cancer marker) seem to be incompatible. We hypothesize that blood TL is a dynamic parameter, which can experience periods of considerable losses as well as periods of maintenance or even elongation (12), and that this feature might explain many of the observations described above. In summary, blood TL regulation through life is a complex process which we only have begun to uncover.

References

1. Bechter OE et al. Telomere length and telomerase activity predict survival in patients with B cell chronic lymphocytic leukemia. Cancer Res. 1998;58(21):4918–22.
2. Hultdin M et al. Association between telomere length and V(H) gene mutation status in chronic lymphocytic leukaemia: clinical and biological implications. Br J Cancer. 2003;88(4):593–8.

3. Damle RN et al. Telomere length and telomerase activity delineate distinctive replicative features of the B-CLL subgroups defined by immunoglobulin V gene mutations. Blood. 2004;103(2):375–82.

4. Grabowski P et al. Telomere length as a prognostic parameter in chronic lymphocytic leukemia with special reference to VH gene mutation status. Blood. 2005; 05(12):4807–12.

5. Ricca I et al. Telomere length identifies two different prognostic subgroups among VH-unmutated B-cell chronic lymphocytic leukemia patients. Leukemia. 2007;21(4):697–705.

6. Roos G et al. Short telomeres are associated with genetic complexity, high-risk genomic aberrations, and short survival in chronic lymphocytic leukemia. Blood. 2008;111(4):2246–52.

7. Svenson U & Roos G. Telomere length as a biological marker in malignancy. BBA - Molecular Basis of Disease. 2009; 1792(4):317–323.

8. Nordfjäll K et al. Large scale parent-child comparison confirms a strong paternal influence on telomere length. Eur. J. Human Gen. 2010;18(3):385–389.

9. Ma H et al. Shortened telomere length is associated with increased risk of cancer: a meta-analysis. 2011. Submitted.

10. Svenson U et al. Breast cancer survival is associated with telomere length in peripheral blood cells. Cancer Res. 2008;68(10):3618–3623.

11. Svenson U, Ljungberg B, Roos G. Telomere length in peripheral blood predicts survival in clear cell renal cell carcinoma. Cancer Res. 2009; 69(7):2896-2901.

12. Svenson U et al. Blood cell telomere length is a dynamic feature. 2011. Submitted.

Telomeres and coronary artery disease

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Coronary artery disease (CAD) has well established life-style, demographic and genetic risk factors. However, at an individual level, both susceptibility to CAD and its age of onset varies considerably, even for subjects with apparently similar risk factor profiles. CAD is an age-associated disease but not an inevitable consequence of ageing. Because of these epidemiological observations, the concept has arisen that CAD may, at least in part, represent a manifestation of premature biological ageing. Using telomere length as a potential marker of biological age, we and others have shown that mean leucocyte, and vascular wall, telomere length, is shorter in subjects with CAD compared with controls and that shorter mean telomere length in leucocytes is present before, and independently predicts, the development of clinically overt CAD. These studies have consistently shown that, except in very old subjects, mean leucocyte telomere length in subjects with (or prone) to CAD is similar to normal subjects who are chronologically 8–12 years older. Several studies have shown that risk factors for CAD such as male gender, Type 1 and Type 2 diabetes, obesity, smoking psychological stress and low socio-economic status are associated with shorter leucocyte telomeres. However, adjustment for these risk factors does not attenuate the association between shorter telomere length and CAD, suggesting that the relationship does not simply reflect the effect of these risk factors on telomere attrition. A key unresolved question therefore is whether shorter telomere length is simply a marker of biological ageing or whether telomere shortening in coronary cells itself plays a direct role in the development of CAD and is therefore a potential therapeutic target. Evidence in favour of a primary role includes pro-atherogenic changes in endothelial cells when telomeres are disrupted and reversal of vascular changes in telomerase knock-out mice when telomerase is re-activated. The "telomere hypothesis" of CAD therefore has the potential to integrate different known aspects regarding the aetiology of CAD and explain its variable age of onset and expressivity. This talk will review the evidence for this hypothesis and the gaps that still remain.

Relevant references

Samani NJ, Boultby R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. Lancet. 2001;358:472–473.

Brouilette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. Arterioscler Thromb Vasc Biol. 2003;23:842–846.

Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet. 2007;369:107.

Brouilette SW, Whittaker A, Stevens SE, van der Harst P, Goodall AH, Samani NJ. Telomere length is shorter in healthy offspring of subjects with coronary artery disease: support for the telomere hypothesis. Heart. 2008; 94: 422–425.

Mukherjee M, Brouilette S, Stevens S, Shetty KR, Samani NJ. Association of shorter telomeres with coronary artery disease in Indians subjects. Heart. 2009; 95: 669–673.

Telomeres, tumor suppressors and cardiovascular disease

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Telomere shortening and the tumor suppressors $p16^{INK4a}$, ARF and $p15^{INKb}$ encoded by the *INK4a/ARF/INK4b* locus are thought to contribute to cellular senescence, organismal aging and associated cardiovascular disease (CVD). Expression of $p16^{INK4a}$, ARF and $p15^{INKb}$ is abundant in human atherosclerotic plaque, and recent genome-wide association studies have identified several common genetic variants within a region of chromosome 9p21 near the *INK4/ARF* locus that influence the risk of coronary artery disease independently of known atherosclerotic risk factors. Remarkably, some risk alleles in human 9p21 have been associated with reduced expression of *INK4/ARF* transcripts, and deletion of the orthologous 70-kb non-coding region on mouse chromosome 4 dramatically reduce cardiac *INK4/ARF* transcript expression and increases vascular smooth muscle cell (VSMC) proliferation, a phenotypic characteristic of cells in atherosclerotic lesions. Moreover, genetic disruption of *ARF* in apolipoprotein E-null mice reduces macrophage and VSMC apoptosis and aggravates atherosclerosis in regions of the aorta that are highly prone to disease progression, but not in less atherogenic aortic segments, suggesting that *ARF* is not involved in the initiation of atherosclerosis but mediates disease progression. Interestingly, increased atherosclerosis resulting from *ARF* disruption in this mouse model is not associated with increased cell proliferation in the atherosclerotic plaque, possibly due to a compensatory up-regulation of $p16^{INK4a}$. It is also noteworthy that apolipoprotein E-null mice carrying one extra copy of *INK4/ARF* are not protected against atherosclerosis. These studies are providing novel biological insights into atherosclerosis development, however additional work is necessary to elucidate the genetic and molecular mechanisms mediating atherosclerosis susceptibility at the chromosome 9p21 locus and the exact role that each *INK4/ARF* tumor suppressor plays in atherosclerosis.

Because aging is a major cardiovascular risk factor, addressing whether age-dependent telomere exhaustion affects cardiovascular pathobiology has been the center of intensive research in recent years. Consistent with a pro-atherogenic role of telomere dysfunction, several cardiovascular risk factors (e.g, oxidative stress, hypertension, and diabetes) inactivate telomerase and provoke telomere attrition, and circulating leukocytes of coronary artery disease patients, atherosclerotic coronary endothelial cells and fibrous cap VSMCs exhibit shorter telomeres. Moreover, accelerated telomere attrition has been reported in cells from Hutchinson-Gildford progeria syndrome (HGPS) patients, who die at an average age of 13–14 year usually from precipitated myocardial infarction or stroke associated to excessive atherosclerosis, and ectopic telomerase expression rescues HGPS premature senescence through inhibition of the tumor suppressors p53, $p21^{Cip1}$ and $p16^{INK4a}$. However, evidence has been also presented suggesting a pro-atherogenic role of telomerase, since reverse transcriptase (TERT) expression is high in macrophages of human atherosclerotic lesions and telomerase is activated during atherosclerosis in low-density lipoprotein receptor-null mice. Furthermore, NFκ-B-dependent transcriptional activation of telomerase might contribute to neointimal hyperplasia by preventing senescence of macrophages and VSMCs. Consistent with this notion, atherosclerosis development is dramatically reduced in apolipoprotein E-null mice with critically short telomeres due to lack of telomerase activity. Therefore, more work is required to clarify the role of telomerase and telomeres in atherosclerosis and associated CVD.

Observational studies in populations. The Asklepios study

[1,2]De Meyer T, [3]Rietzschel ER, [3]De Buyzere ML, [1]Denil S, [4]De Bacquer D, [5]Vermeersch SJ, [5]Segers P, [6]Langlois M, [1]Van Criekinge W, [3]Gillebert TC, [7]Bekaert S

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Most knowledge on telomere length (TL) as a biological aging biomarker has been obtained by observational studies. Here, we discuss the practical implications and limitations of epidemiologic telomere biology research, with a particular focus on the Asklepios study. The Asklepios study is a longitudinal epidemiologic study on successful (cardiovascular) aging, of which the second round has recently been started. During the first round, TLs of peripheral blood leukocytes (PBL) were successfully assessed in more than 2500 subjects using the Southern Blot methodology. In light of the longitudinal character of the study, the Asklepios population was relatively young (~35 to 55 years old) and apparently healthy (no clinical cardiovascular disease, no major illness) at the time of inclusion. These population characteristics do not only provide an excellent setting for studying telomere attrition (TA) as a longitudinal feature, the presence of primordially healthy subjects might also lead to mechanistic insight in normal telomere biology.

Although age and sex dependent TL differences could clearly be confirmed, it became apparent that traditional cardiovascular risk factors, such as smoking, obesity and hypertension, were generally not associated with shorter telomeres in this relatively young and healthy population, contrasting results from previous studies. Furthermore, TL was also not associated with preclinical atherosclerosis, identified by plaque presence in left or right femoral or carotid arteries. On the other hand, we could identify significant associations with several oxidative stress and inflammation related biomarkers. In general, these results suggest major relevance of the TL attrition (TA) rate, with oxidative stress and inflammation as important drivers. Traditional cardiovascular risk factors may act via the latter or directly on TA, but undoubtedly require a minimal amount of time to have a significant effect.

This time dependency is a major drawback of epidemiologic telomere biology research, since most studies are cross-sectional in nature while investigating a longitudinal feature: telomere attrition. The selection of elderly individuals might provide a solution since the effect of TA on their TL will be more prominent than in younger individuals, although survival itself might easily affect results. The fact that significant results can point to effects of both baseline TL and TA further complicates the cause-effect relationship, which is already hard to pinpoint in observational studies. For example, several aging related diseases, e.g. atherosclerosis, are associated with oxidative stress and inflammation. But even after adjustment for markers of the latter phenomena, TL can still be significantly shorter in those affected, simply because these markers are mostly single time point indicators while TL putatively registers their more relevant cumulated effects.

The problems raised in the previous paragraph can "easily" be solved by conducting longitudinal studies that largely eliminate the TL variance associated with inheritance. Longitudinal studies, cf. the Asklepios study, are indeed becoming increasingly popular. However, there remain several concerns which should certainly be taken into account. Indeed an additional complication is the tissue which is used in the TL measurements, with the easily accessible peripheral blood leukocytes or mononuclear cells as most typical examples. As TL is largely inherited, we expect correlated TLs in the different tissues of a

single person, implying that PBL TL can be used as a proxy for the inherited component of TL in other tissue. However, it is uncertain to what extent this is also true for TA rates. Finally, known and particularly unknown confounders can have a major effect on the interpretation of results obtained by observational studies. For example, in the Asklepios study, we identified paternal age at birth to be a major determinant of offspring TL. Longer telomeres found in sperm cells of older fathers are a plausible explanation for this observation. Besides having implications on the mechanism of TL inheritance, paternal age at birth can easily become a confounding factor if not appropriately adjusted for. For example, it is known to be associated with socio-economic parameters, and tends to differ between races and nations. As the paternal age effect propagates over generations, it can at least partially explain TL variance and differences in TL study results between populations, and even have evolutionary consequences. Other unknown confounders could have important similar effects.

In summary, observational studies provide major opportunities to elucidate the value of TL as an aging biomarker. However, interpretation of cross-sectional results is often complicated due to the important role of telomere attrition. Longitudinal studies might offer a solution, but one should still take into account possible tissue dependent effects and the existence of confounders.

Selected references Asklepios Study

Rietzschel et al. Eur J Cardiovasc Prev Rehabil. 2007;14:179–191.
Bekaert et al. Aging Cell. 2007;6:639–647.
De Meyer et al. Hum Mol Genet. 2007;16:3097–3102.
De Meyer et al. Eur Heart J. 2009;30:3074–3081.
De Meyer et al. Ageing Res Rev. 2011;10:297–303.

Observational studies, the role of lifestyle

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It is a well known fact that telomere length in peripheral blood decreases with increasing age and a large interindividual variation in telomere length has also been convincingly documented. Heredity is one component determining telomere length and paternal influence seems to be of most importance.[1,2]. However, the impact of the heredity factor diminishes with age and we have calculated its effect to >50% at ages <50 years old, but <5% in individuals >70 years [3]. This gives strong support to the results shown in substantial numbers of papers published the last 5–10 years, namely that telomere length is determined by numerous factors during life, thereby overriding the hereditary component when we grow older.

Observational studies have revealed abbreviated telomeres in individuals with many types of age-related diseases i.e. cardiovascular disease,[4,5] heart failure[6] and diabetes.[7] Other conditions such as high BMI,[4,8,9] smoking,[9] psychological stress,[10, 11] low physical activity[12] as well as self-perceived early aging [13] have also shown negative influence on telomere length. It has been speculated whether short telomeres from birth are a risk factor for developing these conditions, or if the pathophysiological processes interact with the telomere machinery/telomeric DNA giving telomere shortening. The later is probably the most likely. The exact molecular mechanisms is not elucidated but oxidative stress and inflammation, both of which are important features in many of these conditions,[14, 15] have been shown to negatively influence telomere length. Moreover, short telomere length has also been demonstrated to be associated to mortality[16, 17]. This hints that lifestyle can affect both health and lifespan of an individual by regulating telomere length. However, one should bear in mind that all studies have not been conclusive on this subject and there can be many confounding factors involved.

The rate of telomere change over time in leucocytes is strongly correlated with initial telomere length,18–21 showing more rapid decrease if long telomeres at baseline. This can be explained either by increased telomerase activity in the hematopoietic stem cells acting on short telomeres or that long telomeres have higher susceptibility to harmful agents.In summary, telomere length at one time point likely reflects a cumulative result of heredity, environmental stressors and telomere regulatory mechanisms, giving an oscillating but in total decreasing telomere length pattern during life, potentially affectable by our way of living.

References

1. Nordfjäll K et al. Telomere length and heredity: Indications of paternal inheritance. Proc Natl Acad Sci U S A. 2005 8;102(45):16374–8.

2. Njajou OT et al. Telomere length is paternally inherited and is associated with parental lifespan. Proc Natl Acad Sci U S A. 2007 17;104(29):12135–9.

3. Nordfjäll K et al. Large-scale parent-child comparison confirms a strong paternal influence on telomere length. Eur J Hum Genet. 2010 18(3):385–9.

4. Fitzpatrick AL et al. Leukocyte Telomere Length and Cardiovascular Disease in the Cardiovascular Health Study. Am J Epidemiol 2007 165:14–21.

5. Brouilette SW et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet 2007 369:107–114.

6. van der Harst P et al. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J Am Coll Cardiol. 2007 3;49(13):1459–64.

7. Zee RY et al. Mean leukocyte telomere length shortening and type 2 diabetes mellitus: a case-control study. Transl Res. 2010 155(4):166–9.

8. Nordfjäll K et al. Telomere length is associated with obesity parameters but with a gender difference. Obesity 2008 16(12):2682–9.

9. Valdes AM et al. Obesity, cigarette smoking, and telomere length in women. Lancet. 2005 20-26;366(9486) :662–4.

10. O'Donovan A et al. Childhood Trauma Associated with Short Leukocyte Telomere Length in Posttraumatic Stress Disorder. Biol Psychiatry. 2011 Apr 12. [Epub ahead of print]

11. Epel ES et al. Accelerated telomere shortening in response to life stress. Proc Natl Acad Sci U S A. 2004 7;101(49):17312–5.

12. Cherkas LF et al. The association between physical activity in leisure time and leukocyte telomere length. Arch Intern Med. 2008 28;168(2):154–8.

13. Nordfjäll K et al. Increased abdominal obesity, adverse psychosocial factors and shorter telomere length in subjects reporting early ageing; the MONICA Northern Sweden Study.Scand J Public Health. 2008 36(7):744–52.

14. Furukawa S et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004 114(12):1752–61.

15. Song Z et al. Lifestyle impacts on the aging-associated expression of biomarkers of DNA damage and telomere dysfunction in human blood. Aging Cell. 2010 9(4):607–15.

16. Cawthon RM et al. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet. 2003 1;361(9355):393–5.

17. Fitzpatrick AL et al. Leukocyte telomere length and mortality in the Cardiovascular Health Study. J Gerontol A Biol Sci Med Sci. 2011 66(4):421–9.

18. Nordfjäll K et al. (2009) The individual blood cell telomere attrition rate is telomere length dependent. PLoS Genet 5: e1000375.

19. Ehrlenbach S et al. Influences on the reduction of relative telomere length over 10 years in the population-based Bruneck Study: introduction of a well-controlled high-throughput assay. Int J Epidemiol. 2009 38(6):1725–34.

20. Aviv A et al. Leukocyte telomere dynamics: longitudinal findings among young adults in the Bogalusa Heart Study. Am J Epidemiol. 2009 1;169(3):323–9.

21. Farzaneh-Far R et al. Telomere length trajectory and its determinants in persons with coronary artery disease: longitudinal findings from the heart and soul study. PLoS One. 2010 8;5(1):e8612.

Prediction of diabetes complications

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The incidence of diabetes is increasing with epidemic proportions. In 1985 there were globally 30 million people diagnosed with diabetes, currently there are 270 million and the projection for 2025 is 370 million. Such a substantial increase will have a huge impact on the society not only from the economical point of view but also from increased burden on health care. Living with diabetes may not necessarily be associated with poor prognosis, although diabetes *per se* will have a profound influence on the patient's daily life. More importantly, the presence of diabetic complications will change this scenario dramatically. One third of patients with diabetes are at risk of diabetic kidney disease (nephropathy), which is associated with a several-fold increased risk of dying early. In patients with type 1 diabetes on dialysis or having received a kidney transplant there is an 18-fold increased risk of pre-mature mortality, in patients with macroalbuminuria there is a 9-fold increased risk and in those with microalbuminuria a 3-fold increased risk compared to the same sex and same age in the general population (standardized mortality rate). A key feature is that the patients with signs of kidney disease are also those that are likely to develop proliferative retinopathy, autonomic neuropathy, and peripheral neuropathy and likely to succumb to the effects of cardiovascular disease. The presence of kidney disease is equally devastating in patients with type 2 diabetes.

Given that diabetic kidney disease is not only a ticket to an early grave but also to the development of other diabetic complications, the key to prevention and treatment are early diagnosis and aggressive treatment. However, the question arises how can we predict diabetic complications? This presentation will discuss recent novel data from the large nationwide comprehensive Finnish Diabetic Nephropathy Study (FinnDiane), a study that is designed for the large-scale dissection of clinical, biochemical, environmental and genetic factors that predict diabetic complications in type 1 diabetic patients with and without diabetic kidney disease.

Intervention studies in cardiovascular risk patients

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Background

Several studies have shown that short leucocyte telomere length (LTL) is associated with cardiovascular (CV) risk and disease. However, some studies have failed to confirm associations with certain risk factors, notably in the very old.

Most studies have shown that LTL decreases by age although not with a constant speed, showing individual variablity when measured repeatedly in the same population. Age is the most important CV risk factor. Male gender is another CV risk factor. Accordingly, males have shorter LTL, except in the very old. A large number of cardiovascular pathologies have been linke to short LTL, e.g. hypertension, high pulse pressure, arterial atherosclerosis, myocardial infarction, and left venricular hypertrophy. Interestingly, high levels of oxidized-LDL are also linked to short LTL, as is mortality in patients with coronary artery disease. It is unclear whether and to what extent short LTL may be cause or consequence in such diseases and conditions.

Further CV risk factors are smoking and obesity, both of which are reported to be associated with short LTL, although not in all studies. Type 2 diabetes carries high CV risk and is linked to short LTL. Increased activity of the renin-angiotensin system, psychological stress and even general frailty are also reportedly associated with short LTL.

Own results

We have measured LTL by Southern blot in 1271 Finnish hypertensive patients aged 55–80 years, median age 67 year, with left ventricular hypertrophy (the LIFE study) We observed an association of short LTL with Framingham risk score, coronary artery disease, and, not reported before, transient ischemic attack . These observations were in line with results of earlier studies. A new finding was the association of shorter LTL with the D allele of the ACE insertion/deletion polymorphism, suggesting that the D allele might affect telomere attrition.

Comment

Apparent controversies generated by reports on LTL in CV risk and disease may be partly explained by differences between populations studied. In intervention studies lacking an adjudicating process, diagnostic errors may amount to 20–30%. Age and gender are important effectors which must be corrected for. At high age, a number of factors may increasingly affect LTL. Examples of such factors are decreasing telomerase and antioxidant activity, activation of P53, and genetics. Other factors affecting LTL are life style, fitness, stress, frailty, and possibly drug and hormone treatment.

References

Aviv A. Leukocyte telomere length, hypertension, and atherosclerosis. Are there potential mechanistic explanations? Hypertension 2009;53:590–591.

Fuster JJ and Andrés V, Telomere biology and cardiovascular disease. Circ Res 2006;99:1167–1180.

Fyhrquist F, Silventoinen K, Saijonmaa O, et al. Telomere length and cardiovascular risk in hypertensive patients with left ventricular hypertrophy, the LIFE study. J Human Hypertens., provisional acceptance April 22, 2011.

The stress of aging viewed from the arterial wall

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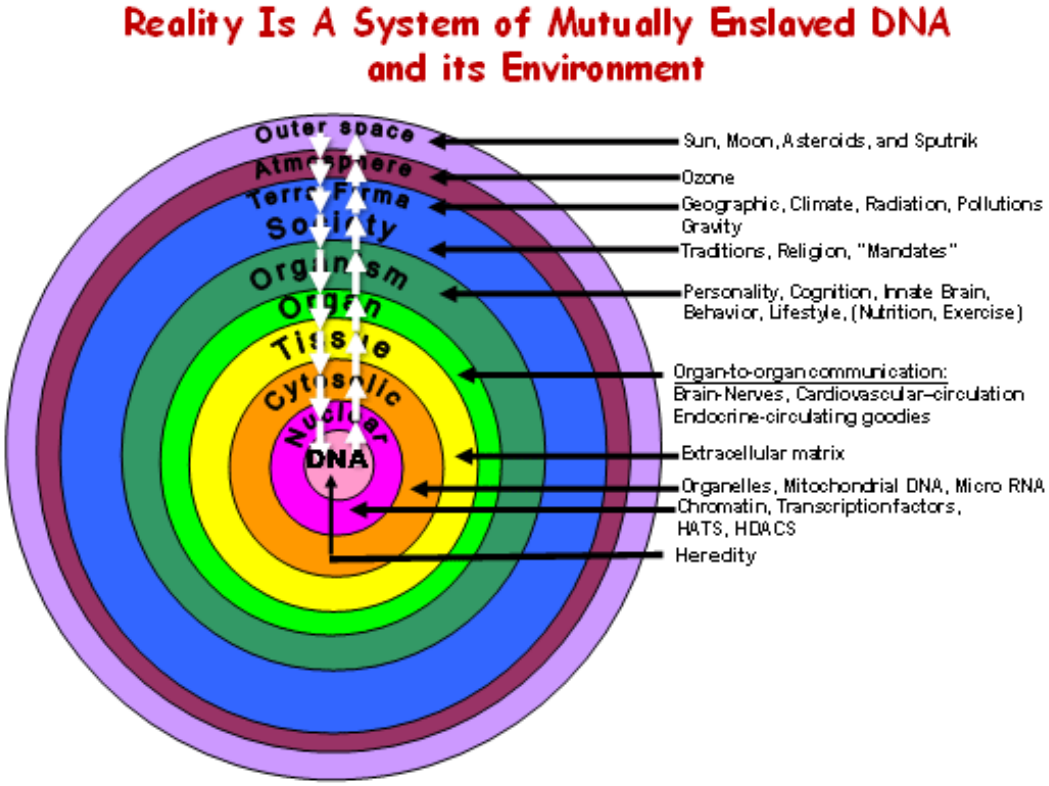
A discussion about any aspect of aging cannot beg the issue of what aging is. This is a tough question, and there are numerous perspectives regarding the answer. My view is that aging is "a shift in an organism's reality." So what's reality? This is another tough question. My view is that reality can be comprehensively defined as a system of mutual enslavement of DNA and its environment. If this appears to be a naïve assessment of reality, check out what constitutes the DNA environment (Fig. 1).



The white arrows in the figure indicate continual bidirectional signaling that must occur to sustain our existence. In this context, aging can be construed as a series of failures of the signaling within the DNA environmental system. A general description of some failing interactions in this system includes: signals, sensing of signals, signal transmission, response to signals, and proteostasis.

Viewing the stress of aging from the arterial wall beings with the realization that arterial diseases, e.g. atherosclerosis and hypertension, are rampant in Western society and increase exponentially with advancing age. Progressive changes occur throughout life in the structure and function of central arteries in numerous species. These changes include diffuse intimal and medial thickening, and enhanced stiffening (1). Since predominantly systolic hypertension is prevalent in epidemic proportion among older persons (1); it is reasonable to hypothesize that specific mechanisms that underlie alterations in the arterial substrate that accompany "aging" may be intimately linked to the age-associated exponential increase in predominantly systolic hypertension (1). Indeed, recent studies show pulse wave velocity, an index of arterial stiffness, is an independent predictor of the future increases in SBP and of incident hypertension (2).

Age-associated remodeling of the aortic wall of both animals and humans involves a proinflammatory profile of arterial cell and matrix properties (3). This profile features increased production of angiotensin II (Ang II) and downstream Ang II/AT1 receptor signaling molecules, e.g., matrix metalloproteases (MMPs), calpain-1 and monocyte chemoattractant protein (MCP-1), transforming growth factor β1 (TGF-β1) NFκb, TNFα, iNOS, and VCAM. Activation of calpain-1, MMPs, TGF-β, and NADPH oxidase within the arterial wall is increased, and nitric oxide bioavailability is reduced (1,2,4). Both invasive and proliferative capacities of early passage vascular smooth muscle cells (VSMC) isolated from the aged arterial wall are increased, and are linked to augmented Ang II signaling. This age-associated arterial proinflammatory secretory profile within the grossly appearing arterial wall and related structural/functional remodeling, is reproduced in young rats by chronic infusion of Ang-II (4).

A megacept emerges with the realization that in arteries of younger animals, in response to experimental induction of hypertension or early atherosclerosis, parts of this proinflammatory profile within the arterial wall that have been studied to date are strikingly similar to the profile that occurs with advancing age (1). Thus, "aging"-associated arterial changes and those associated with hypertension (and early atherosclerosis) are fundamentally intertwined at the cellular and molecular levels (1). In humans, other well-known risk factors (e.g., altered lipid metabolism, smoking, and lack of exercise) likely interact with this arterial substrate that has been altered during aging, and that renders the aging artery a "fertile soil" that facilitates the initiation and progression of these arterial diseases (1). Lifestyle and pharmacologic interventions have already proved to be effective in preventing or ameliorating hypertension associated with aging. The cellular/molecular proinflammatory mechanisms that underlie arterial aging are novel putative candidates to be targeted by interventions aimed at attenuating arterial aging, and thus attenuating the major risk factor for hypertension.

References:

1. Lakatta EG, Wang M, Najjar S. Arterial Aging and Subclinical Arterial Disease are Fundamentally Intertwined at Macroscopic and Molecular Levels. Medical Clinics of North America 2009; 93:583–604.

2. Najjar SS, Scuteri A, Gill V, Wright JG, Muller DC, Fleg JL, Spurgeon HP, Ferrucci L, Lakatta EG. Pulse Wave Velocity is an Independent Predictor of the Longitudinal Rise in Systolic Blood Pressure and of Incident Hypertension in the Baltimore Longitudinal Study of Aging. JACC 2008; 51:1377–1383.

3. Wang M, Zhang J, Jian L-Q, Spinetti G, Pintus G, Monticone R, Kolodgie FD, Virmani R, Lakatta EG. A Proinflammatory Profile within the Grossly Normal Human Aortic Wall Accompanies Advancing Age. Hypertension. 2007; 50:219–227.

4. Wang M, Zhang J, Spinetti G, Jiang L-Q, Monticone R, Zhao D, Cheng L, Krawczyk M, Talan M, Pintus G, Lakatta EG. Angiotensin II Activates Matrix Metalloproteinase Type II and mimics Age-Associated Carotid Arterial Remodeling in Young Rats. American Journal of Pathology 2005; 167:1429–1442.

5. Fu Z, Wang M, Gucek M, Zhang J, Wu J, Hiang L, Monticone R, Khazan B, Telljohann R, Mattison J, Sheng S, Cole RN, Spinetti G, Pintus G, Liu L, Kolodgie FD, Virmani R, Spurgeon H, Ingram DK, Everett A, Lakatta EG, Van Eyk JE. Milk Fat Globule Protein Epidermal Growth Factor-8: A Pivotal Relay Element Within the Angiotensin II and Monocyte Chemoattractant Protein-1 Signaling Cascade Mediating Vascular Smooth Muscle Cells Invasion. Circulation Research 2009; 104:1337–1346.

Early ageing associated with renal disease

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Aging is associated with impaired adaptive and homeostatic mechanisms leading to increased susceptibility to both internal and external stress and subsequent increased rate of disease. Decreased renal function leading to a toxic and unfriendly uremic milieu is an example of a clinical condition when internal stress lead tocellular senescence, which is associated with shortened or damaged telomeres and is characterized by morphological changes, and altered function. Patients with CKD have a high mortality rate primarily due to premature atherosclerotic cardiovascular disease and infectious complicationsand could, thus, be considered to a biological model of premature ageing. Indeed, the uremic phenotype is not only characterized by premature cardiovascular disease but also other features of premature ageing, such as muscle loss (sarcopenia), poor appetite, frailty, osteoporosis, cognitive dysfunction and depression. Although this patient group is exposed to a high prevalence of traditional risk factors, such as hypertension, dyslipidemia and diabetes, novel or uremia-related risk factors that may promote cellular senescence, such as persistent inflammation and oxidative stress are clustered in this patient group. Vascular calcification is another common and prominent feature of the uremic phenotype that has a multifactorial origin. Recent studiesshows that CKD patients are deficient in Klotho (a defect in the expression of this receptor for FGF23in mouse leads to a syndrome that resembles human ageing)and that a Klothodeficiency may aggravatethe vascular calcification process via not only indirect effects mediated via phosphate but also via direct effects.

Molecular and cellular mechanisms of vascular ageing: focusing on cellular senescence

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Ageing is associated with changes in the structure and function of the arterial wall. Prominent among these are endothelial dysfunction, thickening of the intima, deposition of calcium in the media, stiffening of conduit arteries and defective vascular repair. These alterations are thought to provide a propitious ground for the development age-associated cardiovascular pathologies, including hypertension, atherosclerosis, coronary syndromes, vascular dementia, heart and renal failure. Although the existence of these associations has been widely documented, a mechanistic basis for the delineation of cause-effect relationships is only now beginning to emerge. In this context, several possible underlying molecular and cellular mechanisms have been implicated. These include the oxidative damage of macromolecules and organelles, the formation of advanced glycation end products, the erosion and dysfunction of telomeres, the depletion of vascular progenitor cells and the accumulation of senescent endothelial and vascular smooth muscle cells.

One process that has been increasingly linked to both to organismal ageing and vascular pathologies is cellular senescence (1;2). Senescence is a stress and damage response phenomenon that renders mitotically competent cells in a permanent state of growth arrest. Endothelial and vascular smooth muscle cell senescence can be induced by a number of factors implicated in vascular pathologies, particularly by sustained cell replication and oxidative stress. At the molecular level senescence resulting from successive rounds of cell replication (also known as replicative senescence), has been linked to the progressive shortening of telomeres. In addition, a number of cellular insults, especially those causing oxidative stress, can induce senescence in a relatively rapid fashion. This phenomenon, often referred to as "stress-induced premature senescence", does not require extensive cell replication and although it may sometimes involve telomere damage, is generally considered to be telomere-independent.

The occurrence of senescence in cell culture is a well documented fact. In contrast, direct evidence that this phenomenon takes place also *in vivo* has been difficult to obtain. This situation changed with the advent of senescence-associated beta galactosidase, a histochemical marker of senescence (3). Using this method, senescent vascular cells were initially found in a carotid artery injury model that causes endothelial and smooth muscle cell proliferation (4), and soon after in atherosclerotic plaques (5;6) and in experimental diabetes (7); more recently stress-induced senescence has been observed in arteries of mice subjected to oxidative stress (8).

Support for the existence of vascular cell senescence *in vivo* has also been gained from assessment of telomere length in arterial tissue. These studies have shown that telomeres in both the intima and the media shorten with age and that erosion is more pronounced in atherosclerosis-prone vessels and in areas affected by disease. However, it should be emphasised that direct demonstration that telomere shortening in the vasculature eventually leads to cell senescence is still missing. Conversely, the occurrence of telomere-independent senescence could be more relevant in the context of vascular pathophysiology.

Apart from the block in cell replication senescent cells show distinct changes in gene expression and cellular function. In vascular smooth muscle cells these changes result in an inflammatory phenotype (6) that could contribute to atherosclerotic plaque rupture. In endothelial cells the most significant changes result in a phenotype that is both pro-atherosclerotic and pro-thromobotic; these include the over-expression of interleukin-1α, the leukocyte adhesion molecule ICAM-1 and the pro-thrombotic factor PAI-1. In addition,

senescent endothelial cells produce lower levels of nitric oxide suggesting that their accumulation may affect the reactivity of the endothelium (reviewed in ref. 2).

Studies with endothelial cells in culture have demonstrated that senescence can be modulated by numerous factors affecting vascular function (reviewed in ref. 2); the majority of these, act by either altering the intracellular oxidant environment and/or by modulating telomerase activity. An emerging theme in this area is the role played *in vivo* by attenuating mechanisms, such as those invoked by physical exercise (9) and dietary interventions (10). Some of these effects may also involve the activity of sirtuins (11). Thus, in endothelial cells up-regulation of sirt1 by NO was shown to prevent oxidative stress-induced senescence (8). More recently, we have obtained evidence that sirt6, which is known to modulate telomere integrity (12), also regulates senescence in endothelial cells.

The study of cellular senescence in the vasculature has been hampered by the unavailability of non-invasive techniques to assess this process *in vivo*. In the future, development of new markers of senescence amenable to clinical investigation will help to ascertain if the incidence of this process can be modified by pharmaceutical or life-style interventions.

References

1. Erusalimsky,J.D., and Kurz,D.J. 2005. Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. Exp. Gerontol. **40**:634–642.
2. Erusalimsky,J.D. 2009. Vascular endothelial senescence: from mechanisms to pathophysiology. J Appl. Physiol **106**:326–332.
3. Dimri,G.P., Lee,X., Basile,G., Acosta,M., Scott,G., Roskelley,C., Medrano,E.E., Linskens,M., Rubelj,I., Pereira-Smith,O. et al 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S. A **92**:9363–9367.
4. Fenton,M., Barker,S., Kurz,D.J., and Erusalimsky,J.D. 2001. Cellular senescence after single and repeated balloon catheter denudations of rabbit carotid arteries. Arterioscler. Thromb. Vasc. Biol. **21**:220–226.
5. Vasile,E., Tomita,Y., Brown,L.F., Kocher,O., and Dvorak,H.F. 2001. Differential expression of thymosin beta-10 by early passage and senescent vascular endothelium is modulated by VPF/VEGF: evidence for senescent endothelial cells in vivo at sites of atherosclerosis. FASEB J. **15**:458–466.
6. Minamino,T., Yoshida,T., Tateno,K., Miyauchi,H., Zou,Y., Toko,H., and Komuro,I. 2003. Ras Induces Vascular Smooth Muscle Cell Senescence and Inflammation in Human Atherosclerosis. Circulation. **108**:2264–2269
7. Chen,J., Brodsky,S.V., Goligorsky,D.M., Hampel,D.J., Li,H., Gross,S.S., and Goligorsky,M.S. 2002. Glycated collagen I induces premature senescence-like phenotypic changes in endothelial cells. Circ. Res. **90**:1290–1298.
8. Ota,H., Eto,M., Kano,M.R., Ogawa,S., Iijima,K., Akishita,M., and Ouchi,Y. 2008. Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. Arterioscler Thromb Vasc Biol **28**:1634–1639.
9. Werner,C., Furster,T., Widmann,T., Poss,J., Roggia,C., Hanhoun,M., Scharhag,J., Buchner,N., Meyer,T., Kindermann,W. et al 2009. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. Circulation **120**:2438–2447.
10. Farzaneh-Far,R., Lin,J., Epel,E.S., Harris,W.S., Blackburn,E.H., and Whooley,M.A. 2010. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. JAMA **303**:250–257.
11. Haigis,M.C., and Guarente,L.P. 2006. Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. Genes Dev **20**:2913–2921.
12. Michishita,E., McCord,R.A., Berber,E., Kioi,M., Padilla-Nash,H., Damian,M., Cheung,P., Kusumoto,R., Kawahara,T.L.A., Barrett,J.C. et al 2008. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature **452**:492–496.

Telomere Length in the Hereditary predisposition to Cardiovascular Disease and Type II Diabetes

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Over recent years, with the contribution of genome-wide association studies (GWAS), the list of gene loci where common variants contribute to cardiovascular disease (CVD) and type 2 diabetes has grown considerably, however the SNPs currently identified still only explain very small portion of the heritability estimate for these clinically important disorders. Although there is robust evidence that the heritability of CVD and of T2D [1] is high -and is greater than 50% for CVD [2]-, the fact that this heritability cannot be explained by the current known genes means that the nature and number of genetic factors involved in the disease pathogenesis remains unclear. Therefore, more genetic factors need to be explored in order to have a complete picture of their genetic architecture, including rare mutations and epigenetic modifications in known genes as well as telomere length. Telomere length offers a novel insight into the genetic basis of CVD, and may prove useful in the prediction of CVD risk by reflecting an individual's genetic predisposition to premature ageing. There is strong evidence supporting the heritability of telomere length; Twin and family studies have shown that heritability of the inter-individual variation in telomere length ranges from 44% to 80% [3–5]. Quantitative trait linkage (QTL) studies have mapped several putative loci for telomere length to human chromosomes [5–7] and recent GWAS have identified SNPs affecting telomere length [8, 9]. Moreover, Graakjaer et al. [10] demonstrated that any length alteration during the lifespan impacts equally on genetically identical chromosomes. Since family history of premature CVD and T2D is a well-established independent risk factor for their development [11–14], this leads to the hypothesis that family history of these diseases may be due to a familial predisposition to short telomeres in the offspring, and through this is leading to premature development of age-related diseases.

In order to examine this hypothesis, it was investigated whether family history of premature CVD is associated with shorter leukocyte telomere length (LTL) in healthy young offspring compared to age-matched controls of the European Atherosclerosis Research Study II (EARS II, n=765). The data showed that paternal history of CHD is in part expressed through short LTL in the offspring (p=0.05), an effect which was independent of classical risk factors [15]. Although, the effect was of modest statistical significance, it is likely that the true size of the effect is larger when taking into account that the EARSII cohort consists of young healthy men of a very narrow age range (18 to 28 years) having just paternal history as discriminator between cases and controls. Moreover, the observed effect is diluted, since only half the genetic information of an offspring is inherited from the CVD prone father with the mother's non-risk telomeres contributing to 50% of the measured length. Also, worthy of remark is that most candidate gene studies with the EARS II sample failed to identify variants associated with paternal history of premature CVD [16–19], leaving telomere length and variation in the LPL gene the only genetic factors, identified so far, that discriminate between cases and controls in this offspring study. The association of CVD family history was further supported by the study of Brouilette et al. [20] and suggests that in CVD, telomere length probably contributes as a primary abnormality. Thus, the role of genetics in determining telomere length and its contribution to premature onset of age-related diseases deserves further investigation.

The short LTL found at an early age of the healthy EARSII students with family history of premature CVD, suggests that individuals genetically predisposed to develop CVD are

born with shorter telomeres. On the other hand, recent studies with follow up measurements of telomere length, including our follow up data in T2D patients (n=80) (unpublished data), support that baseline telomere length is inversely correlated to the rate of shortening [21–23]. More importantly, lengthening of telomeres has been observed in a considerable percentage of subjects with short telomeres at baseline (from 12% to 24%, and 88% in our T2D patients), supporting the existence of a negative feedback mechanism for the protection of telomeres. Thus, familial predisposition to short telomeres could be attributed mainly to genetic factors determining the rate of shortening or lengthening of telomeres during life, rather than inherited short telomeres at birth.

Since in vitro studies have shown that reactive oxygen species (ROS) cause accelerated telomere shortening during cell division [24–26], variants in genes involved in the regulation of ROS are likely to constitute some of the genetic factors determining the rate of telomere shortening during life. In order to investigate this, we employed a study sample of T2D patients (UDACS, n=569), which experience high oxidative stress, and revealed genes previously associated with CVD and T2D risk that also have a significant effect on LTL. These genes were UCP2 (-866G>A, p=0.04) and ACE (I/D, p=0.02) genes, which are both implicated in the mitochondrial production of ROS, and GPX1 (p.P200L, p=0.02) which is involved in the cell's antioxidant defense (unpublished data). In parallel, a recent GWA study by Samani et al. has shown that a locus on chromosome 3q26 which includes TERC, the gene encoding the telomerase RNA component, has a significant effect on LTL [9]. Thus individuals genetically predisposed to develop conditions which lead to faster telomere shortening such as high ROS levels, or individuals with a genetic defect in the telomere protection mechanism, such as lower telomerase activity, probably experience an accelerated telomere shortening under the burden of adverse environmental effects, giving rise to age-related pathologies.

In conclusion, telomere length constitutes a partly inherited and early-expressed risk factor for age-related diseases, however it remains to be determined whether telomere length estimation is of clinical value, meaning whether it adds information for CVD risk estimation over and above the classical risk factors. Also, information is needed on the race and ethnicity specific mean LTL, as well as on the mean length of healthy individuals in various age groups. Ultimately, telomeres may offer the molecular mechanism registering the lifelong effect of genetic factors in combination with environmental factors, and if therapeutic approaches are discovered to protect telomeres from shortening or to stimulate telomere lengthening, these may be of significant clinical value.

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References

1. Gloyn, A.L. and M.I. McCarthy, The genetics of type 2 diabetes. Best Pract Res Clin Endocrinol Metab, 2001. 15(3): p. 293–308.
2. Lusis, A.J., R. Mar, and P. Pajukanta, Genetics of atherosclerosis. Annu Rev Genomics Hum Genet, 2004. 5: p. 189–218
3. Slagboom, P.E., S. Droog, and D.I. Boomsma, Genetic determination of telomere size in humans: a twin study of three age groups. Am J Hum Genet, 1994. 55(5): p. 876–82.
4. Njajou, O.T., et al., Telomere length is paternally inherited and is associated with parental lifespan. Proc Natl Acad Sci U S A, 2007. 104(29): p. 12135–9.

5. Vasa-Nicotera, M., et al., Mapping of a major locus that determines telomere length in humans. Am J Hum Genet, 2005. 76(1): p. 147–51.
6. Andrew, T., et al., Mapping genetic loci that determine leukocyte telomere length in a large sample of unselected female sibling pairs. Am J Hum Genet, 2006. 78(3): p. 480–6.
7. Mangino, M., et al., A regulatory SNP of the BICD1 gene contributes to telomere length variation in humans. Hum Mol Genet, 2008. 17(16): p. 2518–23.
8. Mangino, M., et al., A genome-wide association study identifies a novel locus on chromosome 18q12.2 influencing white cell telomere length. J Med Genet, 2009. 46(7): p. 451–4.
9. Codd, V., et al., Common variants near TERC are associated with mean telomere length. Nat Genet, 2010. 42(3): p. 197–9.
10. Graakjaer, J., et al., The relative lengths of individual telomeres are defined in the zygote and strictly maintained during life. Aging Cell, 2004. 3(3): p. 97–102.
11. Barrett-Connor, E. and K. Khaw, Family history of heart attack as an independent predictor of death due to cardiovascular disease. Circulation, 1984. 69(6): p. 1065–9.
12. Myers, R.H., et al., Parental history is an independent risk factor for coronary artery disease: the Framingham Study. Am Heart J, 1990. 120(4): p. 963–9.
13. Evans, J.L., et al., Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev, 2002. 23(5): p. 599–622.
14. Morris, R.D., et al., Obesity and heredity in the etiology of non-insulin-dependent diabetes mellitus in 32,662 adult white women. Am J Epidemiol, 1989. 130(1): p. 112–21.
15. Salpea, K.D., et al., The association of telomere length with paternal history of premature myocardial infarction in the European Atherosclerosis Research Study II. J Mol Med, 2008. 86(7): p. 815-824.
16. De Backer, G., et al., A comparison of lifestyle, genetic, bioclinical and biochemical variables of offspring with and without family histories of premature coronary heart disease: the experience of the European Atherosclerosis Research Studies. J Cardiovasc Risk, 1999. 6(3): p. 183–8.
17. Gudnason, V., et al., C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. EARS group. Atherosclerosis, 1998. 136(2): p. 347–54.
18. Humphries, S.E., et al., Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglycerides: the European Atherosclerosis Research Study (EARS). Arterioscler Thromb Vasc Biol, 1998. 18(4): p. 526–34.
19. Poirier, O., et al., The Pro12Ala polymorphism in the peroxisome proliferator-activated receptor gamma2 gene is not associated with postprandial responses to glucose or fat tolerance tests in young healthy subjects: the European Atherosclerosis Research Study II. J Mol Med, 2000. 78(6): p. 346–51.
20. Brouilette, S.W., et al., Telomere length is shorter in healthy offspring of subjects with coronary artery disease: support for the telomere hypothesis. Heart, 2008. 94(4): p. 422–5.
21. Farzaneh-Far, R., et al., Telomere length trajectory and its determinants in persons with coronary artery disease: longitudinal findings from the heart and soul study. PLoS One, 2010. 5(1): p. e8612.

22. Epel, E.S., et al., The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. Aging (Albany NY), 2009. 1(1): p. 81–8.
23. Aviv, A., et al., Leukocyte telomere dynamics: longitudinal findings among young adults in the Bogalusa Heart Study. Am J Epidemiol, 2009. 169(3): p. 323–9.
24. Petersen, S., G. Saretzki, and T. von Zglinicki, Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. Exp Cell Res, 1998. 239(1): p. 152–60.
25. Serra, V., et al., Telomere length as a marker of oxidative stress in primary human fibroblast cultures. Ann N Y Acad Sci, 2000. 908: p. 327–30.
26. von Zglinicki, T., Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci, 2000. 908: p. 99–110.

Stress, lifestyle, and Telomere Attrition

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Eli Puterman, Ph.D., Aric Prather, Ph.D., Elizabeth Blackburn, PhD

This lecture will review the psychological and behavioral correlates of telomere length, as well as interventions that have examined changes in cell aging. There has long been a search for 'psychobiomarkers'-- measures that index psychosocial stress and well-being, and precede and predict early disease and mortality. Telomeres appear to be such a psychobiomarker. There have been many studies that link telomere shortness to psychological stress, as well as to states of severe distress, including major depression (as discussed further by Dr. Owen Wolkowitz in this Symposium). This lecture will discuss possible commonalities underlying these states of emotional distress that may be promoting telomere attrition, both psychological processes, such as threat appraisal, that promote stress arousal, as well as biochemical mediators of stress arousal that are known to affect the telomere/telomerase maintenance system.

Telomere length appears partly under personal control, as a number of health behaviors have now been linked to telomere length – including activity, nutrition, and sleep. We discuss data suggesting that exercise in particular may serve not only as a main effect but may also buffer the effect of stress on telomere attrition.

As a psychobiomarker, the telomere/telomerase system may serve as a possible barometric health marker for behavioral change interventions. Although few intervention studies have been done to date, preliminary data indicate that interventions may impact rate of telomere attrition. Human studies are needed to understand telomere dynamics over time, and modifiers, but ultimately basic in vitro and animal studies are needed to test mechanisms of telomere regulation, experimentally.

Psychiatric Disorders and Telomeres

Owen Wolkowitz, MD; Synthia Mellon, PhD, Elissa Epel, PhD, Elizabeth Blackburn, PhD, Jue Lin, PhD, Firdaus Dhabhar, PhD, Victor Reus, MD, J Craig Nelson, MD, Yali Su, PhD, Rebecca Rosser, BA, John Coetzee, BA, Heather Burke, PhD, Eve Kupferman, PhD

Many psychiatric illnesses (including major depression, schizophrenia, post-traumatic stress disorder and certain other anxiety disorders) are associated with a significantly increased risk of physical illness and early death, even after accounting for lifestyle factors. The reasons for this increased risk are unknown. The somatic illnesses that cluster with these psychiatric conditions include atherosclerosis and cardiac disease, stroke, metabolic syndrome and diabetes, osteoporosis and dementia, all conditions more typically seen in old age. Indeed, major depression has been considered as a "syndrome of premature aging." To investigate this possibility in one model system, we and others have characterized changes in leukocyte telomere length and peripheral blood mononuclear cell (PBMC) telomerase activity in individuals with psychiatric illnesses.

The possibility that mental stress is associated with cellular aging (as manifest by shortened PBMC telomeres) was first demonstrated in stressed caregivers by Epel et al., in 2004. Since that time, a number of studies have replicated this finding and have extended it to certain psychiatric illnesses. These findings are potentially important for understanding the increased medical co-morbidity in psychiatric conditions, because shortened PBMC telomeres have been associated with the development of serious medical illnesses and premature death, and because they may represent an "aging clock" of the organism as a whole. Several studies have now shown diminished PBMC telomere length in individuals with major depression; in some of these studies, this was seen only in the most chronically depressed individuals or in those with the greatest number of depressive episodes. Similarly, shortened telomere length has been observed in individuals with schizophrenia, PTSD and anxiety disorders, although in some cases, only subgroups of individuals showed this abnormality. Conflicting data do not resolve whether shortened telomeres are a pre-existing risk factor for these illnesses or whether they are the result of cumulative exposure to the illnesses. In any event, the finding of shortened PBMC telomeres across several psychiatric diagnostic categories suggests telomere shortening is not diagnostically specific, but that it may tap into an, as yet unknown, biological concomitant of these conditions.

Apart from classical psychiatric illnesses (but very often coexisting with them), childhood histories of adversity are also associated with shortened telomeres in a graded manner, in that greater adversity is associated with shorter PBMC telomeres. This suggests that even distant adversity can be reflected in adult cellular measures of "accelerated aging," perhaps by inducing subtle but long-term physiological changes. The high concordance of childhood adversity and adult psychiatric illnesses makes it difficult to tease apart their relative contributions to shortened telomeres.

There are multiple biological mediators of shortened telomeres, but prominently include oxidative stress and chronic inflammation. Interestingly, these factors are frequently elevated in the psychiatric conditions being considered. Thus, it is plausible that disease-related oxidative stress and/or inflammation contribute to the telomere shortening. Few studies have yet examined this, but preliminary data suggest significant inverse correlations between telomere length and oxidative stress or telomere length and inflammation in depressed individuals.

While most studies have focused on telomere length, fewer have focused on telomerase activity in psychiatric illnesses. The first paper to examine this in stressed caregivers found diminished telomerase activity in tandem with shortened telomeres (Epel et al., 2004), but a subsequent paper surprisingly found increased telomerase activity (Damjanovic 2007). In the only paper to date to examine telomerase activity in a major psychiatric illness (major depression), PBMC telomerase activity was also found to be significantly elevated, perhaps as a compensatory response to the leukocyte telomere shortening (Wolkowitz et al., 2011). In that study, lower telomerase activity prior to antidepressant treatment, and greater increases in telomerase activity during antidepressant treatment, predicted superior antidepressant responses. This and other findings are consistent with of the idea that telomerase activation in depression may have a beneficial effect and may highlight a new aspect of depressive pathophysiology.

Although there are several caveats in interpreting these data, and although the relationships of peripheral immune cell telomere length and telomerase activity to the same measures in the brain cells are unknown, dysregulation of these markers may provide a new and important window into the pathophysiology of serious mental disorders and may help explain the increased burden of medical illness seen with these disorders.

Perceived age and telomere attrition

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Perceived age is an easily obtained "macro biomarker" of ageing which is widely used by clinicians as a general indication of a patient's health. However, research into the validity of this approach has been very sparse, and the possible association to other important ageing phenotypes – including telomere length - has not been investigated.
In the Longitudinal Study of Aging Danish Twins (LSADT), we found that for 1826 twins aged 70+, perceived age rated from facial photographs by different groups of raters, was a robust predictor of mortality during 7 years of follow-up. The results were not sensitive to the age, sex, and professional background of the assessors.

Perceived age was influenced negatively by exposure to sun and by smoking and low body mass index, and it was influenced positively by high social status, low depression score, and by the fact of being married, although the strength of the associations varied by sex. Perceived age predicted survival, even after adjustment for chronological age, sex, and rearing environment.

We found that perceived age adjusted for chronological age and sex correlated with physical and cognitive functioning as well as with leukocyte telomere length.
These results suggest that perceived age is a robust "macro biomarker" of ageing that predicts survival among individuals aged 70+ and correlates with important functional and molecular ageing phenotypes – including leukocyte telomere length.

Telomere biology in regenerative medicine

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In the broad fields of preventative and regenerative medicine, telomere biology is relevant to health monitoring, diagnostic tools, and therapeutics. Telomere shortening in normal ageing is associated with a wide range of diseases, and mutations in telomerase or chronic stress at the cellular level can accelerate telomere attrition, loss of regenerative capacity, and disease progression. Data show that introduction of the telomerase gene into near-senescent cells both *in vitro* and in animal models can slow or reverse the senescence process and prevent the onset of tissue degeneration and fibrosis. We believe that small molecules capable of activating or up-regulating telomerase activity will be of therapeutic value for the treatment of a broad range of degenerative diseases in humans. In addition, such molecules, if naturally occurring and safe, could be used as dietary supplements for health maintenance. Although telomerase activators may pose a potential cancer risk in humans, it is also possible that they could be tumor suppressive due to reduction in chromosomal instability and restoration of normal tumor suppressive pathways in multiple tissue and organ systems, especially in older individuals.

Reduced telomerase activity and telomere attrition play important roles in cellular replicative senescence and are associated with several age-related disorders including cardiovascular diseases, diabetes, cancer, inflammation and immunological diseases including pneumonia and arthritis, lung and liver fibrosis, CNS and neurodegenerative disorders, and skeletomuscular diseases.

Several small molecule telomerase activators have been discovered at Geron Corporation. In previous *ex vivo* studies one compound, GRN665 (TAT2), was shown to increase telomerase activity in cells of the immune system, slow telomere loss, increase replicative capacity, and importantly, enhance immune function in CD8+ T lymphocytes. A molecule (TA-65) related to GRN665and derived from a natural product was licensed to TA Sciences by Geron and is on the market as a dietary supplement. Data from a non-placebo-controlled study in users of TA-65, and independent studies in mice, suggest that it may have beneficial effects for health maintenance, and that its mechanism of action is through telomerase activation.

Recently, Geron scientists and collaborators discovered a number of proprietary analogs of GRN665, some of which have been shown to activate telomerase and alleviate or prevent bleomycin induced lung fibrosis in a telomerase heterozygous mouse model. Idiopathic pulmonary fibrosis is a debilitating and ultimately fatal disease with no effective treatment. Since telomere dysfunction has been linked to replicative senescence and apoptosis in lung epithelial cells,telomerase activation may improve the regenerative capacity of progenitor cells and slow or partially reverse disease progression. We report here new data on compounds that selectively up-regulate telomerase activity in lung airway epithelial cells and prevent senescence.

In an *in vitro* IPF model, bleomycin challenge suppressed telomerase activity in primary human small airway epithelial cell (SAEC). Treatment with a telomerase activator prevented or restored the loss of telomerase activity in bleomycin treated SAEC. In contrast, no similar effect was observed on human lung fibroblaststrain IMR-90, or primary human lung fibroblasts. The compounds increased SAEC telomerase activity ~2-4 fold at 40-100 nM concentrations. The cellular senescence markers, $p16^{INK4a}$ (p16) and $p21^{WAF1/CIP1}$ (p21), were down regulated and inversely correlated with telomerase activation in treated cells. In addition, the compounds moderately enhanced proliferation of SAEC in both short and long term culture. Our preliminary data indicate that the telomerase activators protect the small airway epithelial cells from senescence *in vitro*. Further studies will explore the potential therapeutic value of the telomerase activators as a novel approach to

the treatment of pulmonary fibrosis as well as other human diseases related to telomere dysfunction.

Collaborators

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References

1. Telomerase therapeutics for degenerative diseases.Harley CB.Curr Mol Med. 2005 Mar;5(2):205–11.
2. Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes.Fauce SR, Jamieson BD, Chin AC, Mitsuyasu RT, Parish ST, Ng HL, Kitchen CM, Yang OO, Harley CB, Effros RB.J Immunol. 2008 Nov 15;181(10):7400–6.
3. A natural product telomerase activator as part of a health maintenance program. Harley CB, Liu W, Blasco M, Vera E, Andrews WH, Briggs LA, Raffaele JM.Rejuvenation Res. 2011 Feb;14(1):45–56. Epub 2010 Sep 7.
4. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence.de Jesus BB, Schneeberger K, Vera E, Tejera A, Harley CB, Blasco MA.Aging Cell. 2011 Mar 22. doi: 10.1111/j.1474–9726.2011.00700.x. [Epub ahead of print]

Biomarker & target development opportunities in telomerase cancer biology & cellular senescence

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Telomere homeostasis through telomerase is critical for cellular immortality and tumour progression. Telomerase activity requires the catalytic subunit hTERT and the RNA subunit hTR, both of which are over-expressed in most human cancer cells and tumours. The challenge of understanding telomerase expression partly involves identification of transcription factors affecting hTR and hTERT. However, surprisingly little is known regarding the signalling contexts in which these factors operate, which has slowed development of effective telomerase therapeutics. In turn, true regulatory mechanisms remain largely untested in the human setting. Our core scientific aim is to improve this situation through (1) development of technologies for identification of immortality and senescence control pathways and biomarkers within biologically relevant models and (2) development of well designed protocols and chemical/biological probes of telomerase appropriate for use in humans. We believe that only by combining these approaches will the immortality field mature substantially.

We have built considerable understanding of the complexities of telomerase gene regulation by combining screening assays with chemical genetic/proteomic, RNAi and expression profiling/computational modelling approaches to accelerate parallel discovery of new regulatory networks, therapeutic targets, and lead compounds modulating endogenous telomerase. By integrating these approaches with focused validation technologies, relevant biological models and our informatic knowledgebase we are establishing a high resolution map of cell immortality control (see figure below).

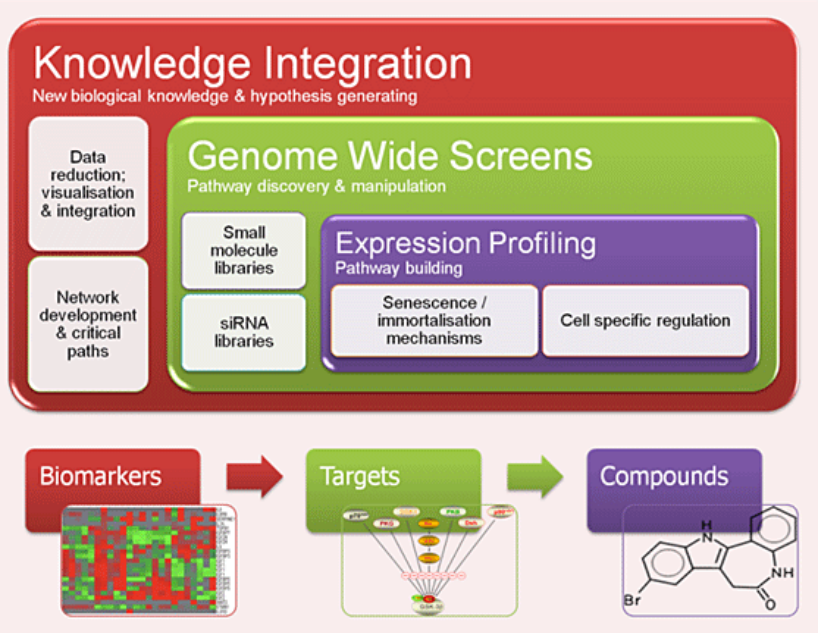

Figure 1. Integrated approach to analysis of cancer immortality

In this respect, a major outcome of our research is a growing evidence base directly linking tumour cell metabolism/redox pathways with the control of telomerase expression via regulators including GSK3. Although telomerase is an attractive therapeutic target, no small molecule leads have reached clinical development. Signal transduction inhibitors could target pathways that control over-expression of hTERT in cancer. However, druggable

upstream pathways need to be elucidated. We performed kinase inhibitor screening of the hTERT promoter, revealing suppression by diverse GSK3 inhibitors. 5-week GSK3 inhibition caused hTERT and telomerase repression with telomere shortening in several cancer cell lines. GSK3 inhibition also suppressed growth and hTERT expression in xenografts, validating our approach to identify immortality targets. Phospho-specific powerblotting confirmed the fidelity of GSK3 inhibition via several markers.

Network modelling of expression data from telomerase suppressed, GSK3 inhibited ovarian cancer cells allowed us to develop a candidate pathway for hTERT regulation involving multiple transcription factors. We performed model validation by chromatin immunoprecipitation and multiplex transcription factor binding assays, revealing extensive GSK3-dependent remodelling of the hTERT promoter involving both positive and negative regulators including c-Myc, Sp1, NF-kB, STAT3, and p53. Few studies have shown that signalling interventions affect telomere homeostasis in long term treatments. In 25-week treatments we observed dynamic regulation of hTERT expression and telomere length . Drawing on emerging concepts of network dynamics, we identified feedforward systems in the network model centred on hTERT which might explain the dynamic effects in terms of network equilibrium. We validated a p53 dependent subsystem by chromatin immunoprecipitation and showed that c-Myc, STAT3, p53, c-Jun and androgen receptor are also dynamically expressed under long term GSK3 inhibition. Finally, we performed whole-kinome RNAi screening of the hTERT promoter to provide a rational basis for selection of new targets to complement GSK3 inhibition.

This study highlights the integrative approach we have developed to identify immortalisation pathways (and so biomarkers), combining multiple screening approaches with focused validation of individual targets. This is a template for pathway deconvolution and helps move the gene regulation field toward more advanced models. GSK3 has diverse effects in multiple pathways including glycolysis and senescence. Several recent studies linked glycolysis and cellular ageing/senescence. Our results demonstrate a direct role for GSK3 in regulation of cancer cell immortality, providing the first direct link between a key metabolic regulator and telomerase expression. Our results support GSK3 inhibitor anti-cancer therapy, but also imply the possibility of stochastic treatment effects during prolonged inhibition.

Building on our successful integration of these approaches to uncover telomerase regulatory pathways we have recently adopted and developed cell-based assay to interrogate signalling pathways downstream of telomere dysfunction utilising adenoviral expression of template-mutated hTR to direct hTERT dependent synthesis of mutant telomere repeats which are incapable of binding key components of the shelterin complex. The vector thus induces rapid telomere uncapping and DNA damage signalling. Inhibition of hTERT, or downstream effectors of telomere uncapping, results in increased cell viability as shown using telomerase inhibitors BIBR1532/suramin and ATM/ATR inhibitor CGK733. Expression profiling of infected cells revealed altered regulation of multiple genes associated with G2/M arrest, senescence signalling and the DNA damage response as expected . The rapid response (observable 2 days post-infection) contrasts with the phenotypic lag associated with classical telomerase inhibition, making this an ideal model to study the pathways activated by telomere dysfunction in a screening context.

Together, these studies provide a much improved understanding of signalling mechanisms regulating immortality and senescence within cancer cells. Importantly, they reduce complex pathways to critical hubs suitable for biomarker and target development.

References

1. A. Bilsland, C.J. Cairney & W.N. Keith, Targeting the telomere and shelterin complex for cancer therapy: current views and future perspectives. J Cell Mol Med. 2010. 15(2), 179–186.

2. Lafferty-Whyte, K., A. Bilsland, C.J. Cairney, L. Hanley, N.B. Jamieson, N. Zaffaroni, K.A. Oien, S. Burns, J. Roffey, S.M. Boyd, and W.N. Keith, Scoring of senescence signalling in multiple human tumour gene expression datasets, identification of a correlation between senescence score and drug toxicity in the NCI60 panel and a pro-inflammatory signature correlating with survival advantage in peritoneal mesothelioma. BMC Genomics, 2010. 11(1): p. 532.

3. Bilsland, A.E., S. Hoare, K. Stevenson, J. Plumb, N. Gomez-Roman, C. Cairney, S. Burns, K. Lafferty-Whyte, J. Roffey, T. Hammonds, and W.N. Keith, Dynamic telomerase gene suppression via network effects of GSK3 inhibition. PLoS One, 2009. 4(7): p. e6459.

Identification of a proteomic signature of telomere dysfunction

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Telomere maintenance is essential for unlimited growth potential of tumor cells. Loss of telomere function activates a DNA damage response cascade which ultimately limits cell division and has been associated with replicative ageing. Interestingly, telomere damage may also induce mitochondrial dysfunction that is relevant to the development of common age-related diseases. Therefore, the identification of factors associated with telomere dysfunction may help to develop biomarkers for ageing and telomere/telomerase therapeutics. An efficient method to induce telomere dysfunction in human cells is the retroviral overexpression of a dominant-negative (DN) mutant of hTERT. In former proteomic profiling analyses using SELDI-TOF-MS, MS/MS and immunoblotting in various DN-hTERT clones of different tumor cell lines (lung, prostate, breast, colon) we identified discrete biomarkers related to telomere dysfunction and replicative senescence including core histones, specific keratins, and S100A6 (Zimmermann et al. 2009 Proteomics 9, 521–534; Zimmermann et al. 2009 Proteomics 9, 5175–5187). In order to study the global effects of telomere dysfunction on the proteome of tumor cells, a well-characterized DN-hTERT clone and its vector control of the HCT-116 cell line were analyzed by isotope-coded protein labeling and nanoflow HPLC MS/MS. ICPL data analysis by ICPLquant software (and by a self-made approach) revealed a list of 61 potential biomarkers of telomere dysfunction in tumor cells including many of the proteins identified in our former studies. Current studies are directed at validation of these markers in telomere damaged cells. In summary, loss of telomere function appears to result in a distinct protein signature which potentially could serve as biomarkers of replicative senescence and guide telomere/telomerase besed therapeutics.

Telomere-directed biomarkers for prediction in health and disease: the ethical perspective

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Presymptomatic genetic testing is using DNA-analysis to determine risk of future disease, so in many ways it is similar to telomere-directed biomarkers for prediction. This lecture is about what can be learned from the ethical debate regarding presymptomatic genetic testing, lessons that may be relevant for the ethics of telomere-directed biomarkers for prediction.

You can get an overview of the most important ethical issues in this area by dividing them into two basic questions:

(i) What is the value of genetic information from presymptomatic genetic testing, primarily for the person(s) tested herself (themselves)?

(ii) Do any other parties (e.g. genetic relatives, spouses, insurance companies, employers) have some kind of right to genetic information from presymptomatic genetic testing?

As regards question (i) one should distinguishing between two kinds of tests: tests of diseases for which there is some sort preventive or ameliorating measure, that is, diseases for which there are what we could call medical reasons to test in advance, and those for which there are not such reasons. Testing of the first kind is much less controversial than testing of the second kind. However, also testing for which there are medical reasons may be more or less warranted, depending on e.g. the effectiveness of preventive measures, how burdensome they are, the test reliability of the test, the probability of the disease, and the seriousness of the disease.

Other values that may be realized by presymptomatic genetic testing are psychological well-being (for instance, that the index-person does not have to suffer the anxiety of uncertainty) and autonomy (since knowledge about one's future health can increase the possibility to plan life in accordance with one's own basic desires (e.g. plans about carrier, family formation, and reproduction). However, these values may also be damaged due to presymptomatic genetic testing, since receiving information from such a test can lead to anxiety, depression, cancellation of cherished life-projects, discrimination by e.g. insurance companies and employers, survivor's guilt (due to negative results) and feeling of isolation from other family members

Moreover, it should be noticed that the idea of autonomy as a value to promote is somewhat of a novelty in the biomedical field; that is, autonomy is not only the foundation of restrictions of how health care are allowed to treat people, expressed in duties and rights, but a value the promotion of which may provide the very rationale of health care procedures, e.g. presymptomatic genetic testing. This way to think about autonomy as a value that should be promoted introduces a new goal in health care. And this goal can also be used to argue in favour of, for instance, medical enhancement, i.e. making people "more than healthy" with medical means, since exactly the same rationale can be used to justify medical enhancements: it can help people to better achieve their basic goals.

As regards question (ii), take for instance genetic relatives. The testing of the index-person can sometimes reveal genetic risk for relatives. Should they, then, be informed about this?

Here, a lot of separate questions are hidden: should they be informed before a test and, perhaps, even be allowed to veto a test that could reveal something about their genetic risk? Should index-persons (as a matter of morals) inform their relatives and then when? If we are dealing with a serious and potentially lethal disease that can be prevented, should health care personnel be allowed to override reluctant index-persons and contact the relatives anyway (as a matter of legal right)? These are different questions. In all these questions, however, the same kind of basic norms and values are relevant and potentially in conflict, for instance the autonomy, privacy, and confidentiality of the patient, and the value of promoting general health and well-being.

To some extent, the same goes for other parties, such as insurance companies, employers, researchers, or law enforcing institutions. However, as regards these third parties, often considerations of justice crop up as well. For instance, as regards insurance companies, many feel that it is unjust that you should be excluded from getting personal insurance, such as life and health insurance, due to something you obviously cannot be blamed for, namely your genes: it is like adding an insult to your bad luck.

On the other hand, insurance companies have to make risk estimations in order to be viable and they use all kind of risk information, including medical information and family history. So what is so special about genetic information? Moreover, if people are allowed to keep predictive genetic information a secret when purchasing private insurance, they can use it in order to get very beneficial insurance that they know they have a great chance of receiving. This may lead to high-risk persons buying more and more insurance and low-risk less and less, that is so called adverse selection.

To sum up, as regards predictive genetic testing, there is a host of ethical issues to consider. If telomere-directed biomarkers offer a tool for better prediction in health and disease, we should carefully ponder its consequences at an individual and societal level. Not all news is necessarily good news.

ABSTRACTS FROM OTHER PARTICIPANTS

The load of short telomeres in leukocytes from aging Danish twins

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Introduction: Telomeres shorten with age and is believed to be a strong inducer of cellular senescence. It is believed that even a single short telomere can trigger senescence. We have recently developed an assay, Universal STELA, extracting the load of short telomeres. This measure correlates strongly with the increasing number of senescent cells in a cell culture grown to senescence. We wanted to investigate if the load of short telomeres also correlates to chronological age in humans as well as to mean leukocyte telomere length (LTL) measured by TRF.

Material and Methods: We have investigated a total of 20 DZ twin pairs, 74–92 years old, with six male and 14 female pairs from the Longitudinal Study of Aging Danish Twins. LTL had been measured on previous occasion. Load of short telomeres was measured using Universal STELA. We used linear regression taking into account that twins as individuals are not independent within pairs, and adjusting for sex and age.

Results: We find a significant and positive correlation between the load of short telomeres and age (R=0.3599, p=0.0226). The number of short telomeres pr cell is very low, increasing to app. 3 telomeres below 2000bp per cell in individuals older than 90 years of age. Linear regression suggests that there is a gain of one telomere below 2000bp per cell for every 17 years of life in the studied population (p=0.026).
Mean LTL and load of short telomeres correlate in this study (R=-0.4871, p=0.0014). By linear regression we find a significant (p=0.008) association suggesting that one extra short telomere occurs per cell for every decrease in LTL of 2kb.
We have previously shown that the twin with the shortest LTL dies before the co-twin with longer LTL. We do not find such an association between load of short telomeres within pair and observed lifespan in this small study.

Discussion: With this study we show for the first time that the load of short telomeres correlates to age in a population of 75+ years. This is in concordance with our findings from cell culture studies. We find that at extreme ages (90+ year) humans harbor app. 3 telomeres below 2000bp per cell. An extrapolation suggests that the short telomeres will start to occur at an observable number at around 40-50 years of age. This should be explored further by including younger subjects in a later study.

We did not find that the difference within pairs in the load of short telomeres predicts which twin will die first as was previously found with mean LTL. The discrepancy could be due to the small sample size. It could, however, also be due to the origin of these short single telomeres. While mean LTL is believed to shorten almost exclusively by life-long replication in the hematopoietic stem cell pool, it can be imagined that telomeres below 2000bp are more often the result of abrupt telomere shortening. This abrupt shortening has been suggested to be caused by different mechanisms such as oxidative stress, replication fork stalling and recombination, and may therefore reflect events that have occurred more recently and perhaps even after maturation of the hematopoietic cell.

No difference in blood cell telomere length between patients with an isolated popliteal artery aneurysm and those with multiple aneurysm disease

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Objectives: Short relative telomere length (RTL) is associated with vascular ageing, inflammation and cardiovascular risk factors. The aim was to explore associations between short RTL and aneurysm disease, comparing patients with isolated and multiple aneurysms.

Design and Patients: DNA was retrieved from 183 patients with popliteal artery aneurysm (PAA). They were examined with ultrasound and had a total of 423 aneurysms (range 1–7, mean 2.3/patient).

Methods: TL was measured with Real-Time PCR, RTL was calculated by comparing with three reference populations.

Results: Patients with bilateral PAAs had a mean RTL of 0.985 versus 1.038 with unilateral PAAs (p=0.326). Patients with abdominal aortic aneurysm had RTL 1.035, versus 0.999 without (p=0.513). No difference was seen with or without femoral or iliac aneurysms. Fifty-six patients with isolated PAA at surgery and at re-examination had RTL 0.974, versus 1.033 who had >1 aneurysm (p=0.308). RTL was not associated with the number of aneurysms at re-examination (p=0.727). There was a trend towards shorter RTL among active smokers (0.93 vs. 1.04, p=0.066).

Conclusions: No association between short RTL and multiple aneurysm disease was found. The previously reported association between AAA and short RTL may be secondary to cardiovascular risk factors, rather than aneurysm disease.

Change in relative telomere length (RTL) over fifteen years is associated with markers of cardiovascular ageing in middle-aged subjects – the Malmö Diet and Cancer Study

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Background: Telomeres are located at the end of the DNA helix, protecting the end segment from degradation. After each cell division the telomeres shorten in non-germ line cells or in non-cancer cells. Telomere shortening over time has been suggested to be a marker of biological ageing and risk of cardiovascular events. Previous studies did not include a long-term follow-up for determination of changes in relative telomere length (RTL) in relation to markers of cardiovascular ageing.

Materials, methods: The study population consisted of 332 subjects from a population-based, case-control study of patients (cases) with myocardial infarction (MI) in Malmö, Sweden. We used quantitative PCR to investigate the blood RTL at baseline and after 15 years of follow-up (Cawthon RM. Nucleic Acids Res 2002), but with different subpopulations of cells used for laboratory controls when calculating RTL. Regretfully, this made it impossible to directly compare RTL values at baseline and at follow-up in individuals. Instead, z-statistics was used to compare the log-distribution of RTL at baseline and at follow-up, respectively, and the difference was calculated (Δ z-score) for each individual as a marker of relative change in telomere length over 15 years. Multiple regression analysis was used with Δ z-score as dependent variable, and age, sex, RTL at baseline, and clinical markers of cardiovascular ageing as independent variables. The z-score approach is a well recognised method to calculate the distribution of data for individual subjects in relation to the standardised mean value within a normal distribution (Printhall RC. Basic Statistical Analysis: Seventh Edition, 2003, Pearson Education Group, US).

Results: Baseline characteristics for 332 subjects (69% males) showed mean age 59.5 (SD: 5.1) years and mean RTL 0.65 (0.21). At follow-up mean RTL was 1.37 (0.40), as influenced by use of a different methodology based on a different control cell population. In all, 83 subjects had a myocardial infarction (total MI) (prevalent MI at baseline: 33; or incident MI during follow-up: 56, including 6 cases of re-infarction) and 67 subjects were treated with antihypertensive (AHT) drugs at baseline. Multiple regression analysis revealed that Δ z-score was independently associated with both total MI ($p<0.039$) and usage of AHT drugs ($p<0.015$) at baseline after full adjustment for chronological age, gender, and RTL at baseline, a well-known predictor of telomere attrition in itself. Subjects with MI or on AHT thus showed a greater reduction in the z-score for RTL during follow-up, implying a higher degree of telomere shortening.

Conclusion: Changes in blood RTL (relative shortening) is a potential marker of cardiovascular ageing, as illustrated by significant and independent associations between Δ z-score and prevalent/incident MI as well as with baseline usage of AHT drugs - a marker of longstanding hypertension in need of treatment.

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Multiple levels of deregulation during immortalization of T lymphocytes

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Cellular immortalization, a major hallmark of cancer, is a multistep process that requires numerous cell type specific changes, including inactivation of control mechanisms and stabilization of telomere length. Most of our knowledge about this process is based on analysis of human fibroblast and endothelial cell cultures immortalized by genetic manipulation, whereas less is known about this process in hematopoietic cells.

We have analyzed spontaneously immortalized human T lymphocyte cell cultures derived from five patients with Nijmegen Breakage Syndrome (NBS) and one healthy individual at increasing population doublings to identify events critical for senescence bypass and immortalization. The telomere length gradually shortened with increasing population doublings and growth crisis was associated with critically short telomeres. The clone(s) that escaped growth crisis demonstrated a logarithmic growth curve, very short telomeres, and, notably, no increase in telomerase activity or expression of the telomerase catalytic gene, *hTERT*. Instead, upregulation of telomerase activity and telomere length stabilization was late events in T lymphocyte immortalization*.

Unsupervised clustering of genome-wide gene expression, methylation (CpG site) and microRNA array data independently separated pre-immortal from immortal cell cultures. Gene expression changes in the DNA damage response (e.g *ATM, ATR*), cell cycle control (e.g *p27, p63, p15, cMyc*) and cellular senescence control (e.g *TWIST1, SYK*) pathways was identified. Furthermore, early and stepwise increased promoter methylation were identified at different stages during the process. Interestingly, applying these findings to tumors of T cell origin revealed commonly methylated CpG sites in transformed cells. Deregulated expression of polycomb complex genes might have contributed to the epigenetic changes observed.

In addition to the changes in gene expression and epigenetics, we observed that several microRNAs were commonly altered in immortal cell cultures. Ongoing studies are focusing on analyzing the function of some of these novel microRNAs.

Telomere length is associated with physical indicators of heart failure

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Objectives
The complex relation between telomere length (TL) and ageing has been demonstrated in diverse settings. In an on-going effort to improve cardiovascular disease (CVD) risk stratification, we studied the association between TL and physical indicators of heart failure. TheE/A-ratio of mitral early (E) and late (A: atrial) ventricular filling velocity is a measure of diastolic heart failure. Both high and low ratiosare associated with reduced survival. The e'/a'-ratio, arguably a more straightforward indicator derived from the movement speed of the mitral annulus during early and late filling, declines with age. Low ratios are associated with unfavourable outcomes.The E/e'-ratio can play an important role in the estimation of left ventricular (LV) filling pressures but is too limited as an independent indicator.Pulse Wave Velocity (PWV) is an established measure of arterial stiffness. It has been associated with increased risk of mortality in hypertensive patients.

Methods
Data from the Asklepios study on successful (cardiovascular) aging was used (n=2509; free from established CVD, age range 35-55 years). Telomere length was determined by TRF-length analyses performed on peripheral blood leukocytes (PBL). E, A, e', and a' were measured by Tissue Doppler Imaging. PWV was derived from time delay measurements at the left femoral and carotid arteries.

Results
We demonstrate that TL is significantly associated withage adjusted E/A-ratio before ($p<0,001$) and after ($p<0,0001$) correctionfor other known confounders (gender, heart rate, BMI, systolic blood pressure). Remarkably this relationship is very pronounced in women ($p=0,0038$;n=1284) but only borderline in men ($p=0,095$;n=1213). In light of this finding all further models were evaluated both by including a gender term and performing separate analyses for men and women. TL also correlates with the age adjusted e'/a'-ratio in woman ($p=0,047$) but not in men ($p=0,17$). Correcting for before mentioned confoundersyielded slightlylower p-values($p= 0,019$ and $p=0,11$ respectively).In a model with a gender term and these confounders, the e'/a'-ratio also decreased with declining TL ($p=0,012$). No significant associations between TL and PWV or E/e' could be detected in our data.

Discussion
Our results clearly show that TL is associated with some but not all physical indicators of heart failure. E/e' has limited use in healthy subjects, one of the inclusion criteria for the Asklepios study. This offers a likely explanation for the lack of association. PWV is influenced by the extent of atherosclerosis. Previous studies have shown a lack of association between TL and atherosclerosis.Although PBL TL is unlikely to be directly dependent onE/A or e'/a'-ratios, prolonged stress on the cardiovascular system has been suggested as acontributor to increased telomere attrition rates (TAR) in PBL. Our results thus suggest that TL could serve as an indicator of cumulative stress on the cardiovascular system. For future practical applications however it will be important to address the variability in TAR and heritability of TL as demonstrated by the gender dependent disparities in our results.

Telomere length in placentas decreases with gestational age and is delayed by multiparity: a study of third trimester live-born twin

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Background
In contrast to the postnatal period, little is known about telomere length (TL) during prenatal life. There is still debate whether TL decreases with gestational age. There appears to be a rapid decline in leukocyte TL between 27 and 32 weeks of gestation, followed by a negligible decrease between 33 and 42 weeks. Change in placental TL remains unknown. It has been argued that telomere shortening does not seem to play a role in the senescence of the placenta. However, IUGR and preeclampsia are associated shorter placental TL.

Aim
The aim of the present study was to examine whether there is a decline in placental TL during the third trimester of gestation for live-born twins. The contributions of sex, placental factors (including weight and fusion of the placentas) and maternal factors (including age, parity, smoking, alcohol use, hypertension, diabetes and SES) were taken into consideration.

Methods
The study sample consisted of 336 live-born third trimester twins (209 pairs) from the East Flanders Prospective Twin Survey. DNA was isolated from placental tissue and TL was determined using a multiplex quantitative PCR (Q-PCR) method (Cawthon, 2009) and carried out in triplicate. Reference samples with known telomere length, i.e. 5.5 kB and 14.5 kB, were included into each run to enable estimation of TL in kB. TL was log-transformed to assure normality. Next, multilevel regression analysis was conducted.

Results
Thirty five percent of the twins were born preterm and 63% twins were born at term. Placental telomeres in preterm twins were longer than in term twins: mean TL (SD) was 13.5 (6.3) kB for preterm and 12.2 (7.4) kB for term births. Median TL (IQR) was 12.0 (9.5 – 15.3) kB for preterm and 10.4 (8.9 – 13.2) kB for term births (p=0.002).
TL decreased with 0.2 kB per week of gestation (p=0.05) from 14.6 kB at 25 weeks to 10.6 kB at 42 weeks of gestation. There was no difference in TL between boys and girls. Placental weights below the 10th centile tended to be associated with longer telomeres (13.3 vs. 11.5 kB; p=0.07). Of the maternal factors, TL of a primiparous mother was shorter than TL of a multiparous mother (11.1 vs. 12.2 kB; p=0.02). The lowest SES tended to be associated with longer TL whereas alcohol use during the third trimester tended to be associated with shorter TL (both p<0.15).

Discussion
We are the first to show that placental TL decreases during the third trimester of pregnancy. Telomere shortening does seem to play a role in the senescence of the placenta and multiparity seems to delay telomere shortening.

Is BMI associated with shorter telomere length? A meta-analysis of observational studies

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Background

Shorter telomeres are associated with age-related diseases such as type 2 diabetes and cardiovascular disease. Obesity, in which oxidative stress and chronic inflammation plays a role, is a risk factor for these diseases. Increased oxidative stress and chronic inflammation are also negatively associated with telomere length (TL). Although many studies have collected information on obesity and TL, few have published on the association between TL and obesity with conflicting results. To resolve whether BMI is truly associated with TL a meta-analysis is set up. We hypothesize that BMI is associated with shorter TL independent of age.

Aim

Our main aim is to set-up a large scale powerful international study to conduct a comprehensive investigation into the effect of BMI on TL.

Methods

A meta-analysis of observational studies which collected data on BMI and TL in adult individuals.

Per study, a linear regression will be performed to test the association between BMI and TL. Study specific age- and sex-adjusted betas will be combined using a random-effects pooling. As potential sources of heterogeneity at study level are considered: (1) general factors: ethnicity, (2) factors related to TL measurement: cell type in which telomere length is determined, the technique of telomere length measurement, storage of DNA and (3) factors related to BMI: self reported or measured.

Additionally, stratified analysis will be performed by sex and by three age categories (younger than 60 years; between 60 and 75 years; older than 75 years of age).

Results

At present 55 unique cohorts are identified, of which 38 have responded in a positive way to participate. Data of 16 study cohorts are already in our possession.

We plan to present the first results at the conference.

Donor telomere length in pre-implantation biopsies is predictive for post transplant renal allograft function

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Allograft biological age, as defined by CDKN2A expression, has recently been demonstrated to be a superior pre-transplant predictive bio-marker for post-transplant function (1,2). Traditionally however, bio-ageing has been assessed through a measurement of telomere length. With age and increased environmental stress, telomere length is shortened which in turn may be adversely related to donor organ function. We measured renal pre-implantation telomere lengths and determined associations with organ function at six months post-transplant with a view to using it as a further bio-marker in kidney transplantation, which may be used in combination with CDKN2A and donor chronological age.

DNA was extracted from 'time zero' pre-implantation renal allograft biopsies Telomere length was determined by Q-PCR. Telomere length was then analysed with respect to donor age and sex, cold ischaemic time, delayed graft function and renal function 6 months post-transplant as determined by serum creatinine (SC) levels.

Donor telomere length was observed to shorten as a function of increasing chronological age ($p=0.018$). No significant difference was observed with respect to sex of the allograft, cold ischaemic time and frequency of delayed graft function. We did, however, observe significantly inferior renal function, in those who received organs with shorter telomere lengths ($p=0.025$) six months post-operatively. Linear regression analyses indicated that at 6 months post-transplant, CDKN2A levels can explain up to 25% of the variability in SC levels, donor age explains 12.0% while telomere length accounted for 7.9%.

This study confirms that measurement of donor bio-age pre-transplant can predict post-transplant function. It indicates that telomere length is inferior to donor chronological age when it is used as a bio-marker in this context. This is in keeping with previous observations indicating that CDKN2A is a superior bio-marker. Telomere length in addition to donor age and other promising biomarkers of ageing may provide a valuable pre-transplant prognostic score on organ quality, for targeted intervention strategies to preserve graft function.

1.McGlynn LM, et al Aging Cell 2008 8(1):45–51. 2.Koppelstaetter C, et al. Aging Cell 2008 7(4):491–7.

Telomere shortening in COPD and potential modulating effects of life-style factors

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COPD (Chronic Obstructive Pulmonary Disease) is a chronic disease, which not only affects the lungs but also involves systemic inflammation and oxidative stress. Accelerated telomere shortening has been associated with chronic oxidative stress, and was previously also reported in COPD patients. In addition, we observed a positive association between leukocyte telomere length and SOD activity measured in blood, suggesting that the anti-oxidant defense system and factors influencing this system may modulate telomere shortening (Houben et al., 2009).

The present study, therefore, aimed at assessing the effects of inflammatory, antioxidant and life-style factors on telomere length in a group of 89 COPD patients and 93 (ex)-smoking controls with normal lung function.
TL was measured by quantitative PCR in isolated PBMCs. In addition, plasma levels of IL-8, IL-6, TNF-α, CRP and homocysteine were determined, as well as blood SOD activity. Consumption of coffee, tea and other caffeine containing drinks, and consumption of alcoholic beverages was assessed by questionnaire.

TL was significantly shortened in COPD patients compared to age-matched (ex)-smoking controls. Plasma levels of IL-6, TNF-α as well as CRP and homocysteine were increased in COPD patients and SOD activity was decreased, indicating increased inflammation and oxidative stress in COPD patients when compared to (ex)-smokers. In both groups coffee consumption was positively, and alcohol consumption was negatively associated with TL. The potential modulating effects of coffee and alcohol consumption on TL found in this study need further confirmation in longitudinal studies and/or controlled intervention trials.

Detection of stress-induced short telomeres by Universal STELA

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Background: Gradual shortening of telomeres due to replication can be measured with the standard TRF method, measuring the mean length of all the telomeres in a cell sample. In contrast, stress-induced telomere shortening which is believed to be a just as important factor causing cellular senescence cannot be measured properly with this standard procedure. Stress-induced telomere shortening caused by e.g. oxidative damage happens in a stochastic manner leaving just a few single telomeres critically short. A few ultra-short telomeres will not have a great effect on the mean telomere length and will therefore be missed by TRF. By using the newly developed Universal STELA it is now possible to pick up these few critically short telomeres since this method is designed to measure specifically the load of short telomeres (<1500 bp), i.e. the telomere subpopulation believed to promote cellular senescence. To further support the notion that Universal STELA measures stochastic telomere breaks caused by damage we have exposed cultured cells to the oxidative stress-inducing agent H2O2, which is known to induce stochastic shortening of telomeres. Telomere lengths of the cells after exposure to H2O2 are measured with TRF and Universal STELA.

Methods: hMSC primary (p6) and hMSC telo-1 (tert positive) cells were exposed to 500 µM H2O2 for 1 h. At this time one sample was harvested. The rest of the cultures were washed, media without H2O2 was added and samples were then harvested after one, two and three cell population doublings (PD), respectively. After DNA purification TRF and Universal STELA was performed on all samples.

Results: Mean telomere length measured with TRF drops immediately after treatment with H2O2. After one PD the mean length rose again to about the same level as the non-treated control and subsequently there was only a slight and gradual shortening consistent with replicative shortening. The number of ultra-short single telomeres measured by Universal STELA decreased slightly in the sample collected immediately after treatment. However, one PD after treatment the amount of short telomeres rises significantly and then gradually decreases in the following PDs.

Conclusion: The present series confirm that telomeric DNA damage results in a rise in load of short telomeres, as detected by Universal STELA, but goes undetected by TRF.

Prematurity and lung function in relation to telomere length In 10-year old children

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Objective: To study the relationship between telomere length, prematurity and lung function in school age children.

Background: Oxidative stress, as a result of inflammation and oxygen toxicity, may affect lung growth and contribute to the development of bronchopulmonary dysplasia (BPD) in preterm infants. Preterm infants have longer telomeres than term infants at birth, but exposure tooxidative stress may accelerate telomere shortening. In adults, chronic obstructive lung disease has been related to shorter telomeres.

Method: Relative telomere length (RTL) was measured on extracted DNA, using real-time PCR, in children born preterm with a history of BPD at the age of 10 years and a control group of childrenborn at term without any need for neonatal medical care but with a history of asthma. Lung function testing with dynamic spirometry was performed in all children.

Results: Children with BPD (n= 23, examined at a mean age of 10,4 years) were born at a mean gestational age of 27 weeks with a mean birth weightof 1067 grams. Compared to children with asthma (n=18, examined at a mean age of 11,2 years) the BPD children had shorter telomeres(RTL1,43vs1,59, $p<0,05$) and reduced lung function (Forced Expiratory Fraction (FEF 25–75%, 1,55vs1,90, $p<0,05$). Lung function was not independently correlated to RTL and no correlations were found between RTL and age at testing, blood pressure and body mass index.

Conclusion: Preterm birth and lung disease in infancy resulted in shorter relative telomere length and reduced lung function at approximately 10 years of age compared to controls with asthma. This may indicate faster telomere attrition in preterm infants with BPD. The influence of oxidative stress and inflammation in the neonatal period and beyond needs to be further studied in relation withintra-individual telomere shortening rate and long-term outcome.

A role of paternal (and maternal) age at conception on offspring lifespan

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In man a few tissues are expressing telomerase. The most important of these tissues are a subset of germ precursor cells that during germ cell maturation will end up as sperm cells and egg cells containing telomeres in the range 16–18 kb. There is also some telomerase expressed in early fetal life, but later in life most somatic cells have no telomerase-expression. It can therefore be imagined that overall telomere length later in life to some degree will be determined by the telomere length in the fertilized egg and thereby by the length in germ cells. Studies in monozygotic twins support the notion that telomere length in the fertilized egg has a strong influence on telomere length later in life

A person with a lower starting length of telomeres in the fertilized egg can be thought to have slightly lower life expectancies later in life, due to short initial telomere length resulting in an increased frequency of short telomeres in the most proliferating tissues late in life. The effect on lifespan can however, be expected only to be modest, since many causes of death in man are clearly not telomere associated. In this connection it is interesting that a few publications have suggested that spermatozo telomere length increase slightly with increasing paternal age. Such a phenomenon could mean that children conceived by very old fathers would have a slightly longer lifespan compared to children with younger fathers.

On the basis of this we decided to perform a cohort study on a very large number of individuals over several generations, in order to see if parent age at conception associated with lifespan of offspring. A dataset of 184852 individuals, where birth-year, death-year and conception-age of both parents and offspring are listed, were used for this purpose. We used both Kaplan Meier statistics on cohords and a nested case-control study. After detailed normalization and data-reduction we performed two different statistical analyses on this material. In one analysis we looked at the mean age of offspring of parents defined as old or young. Here we defined old fathers as fathers above the age of 50 years and old mothers as mothers over 40 years. We found that daughters (p=0.041) and sons (p=0.050) of old fathers had better survival than offspring of young fathers. A similar tendency is seen in old and young mothers, although this is not significant for either female (P=0.443) or male (P=0.196) offspring. Kaplan Meyer analyses revealed that the difference in survival in offspring were concentrated in the higher age groups over 85 years.

In the second analysis we therefore defined two groups. One with persons alive after the age of 90 years, which was selected since it can be speculated that telomere-related death would mainly be manifested at this very old age. Furthermore we selected a control group in the age interval 70 – 85 years. We then looked at the age of their parent at the time of conception. This analysis revealed that the fathers of very old offspring (>90years) had a mean age at conception that was 0.38 years higher than the mean age of fathers of offspring in the age 70-85 years (P=0.000232). Mean conception-ages of the father in the two groups were thus 35.04 years (std. dev. 7.51 years) and 34.66 years (std. dev. 7.36 years) respectively. Furthermore the mean maternal age at birth of the offspring is 0.33 year higher (P=0.0000472) for the group of offspring with age above 90 years compared to the control group. Mean conception-ages of the mother are 30.83 years (std. dev. = 6.15 years) and 30.50 years (std. dev. = 6.07 years) respectively.

Our findings suggest a role of paternal (and maternal) age at conception on offspring lifespan. Given that older men have slightly longer telomeres than young men, these findings are compatible with the hypothesis that telomere length at conception can affect lifespan.

Telomerase activity increased by daily kirtan kriya meditation in dementia caregivers

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Objective: A simple meditation technique, Kirtan Kriya (KK), was used for stress reduction in dementia caregivers to diminish stress and improve coping ability in relation to telomerase activity in a randomized controlled study over the course of 8 weeks. Background: Meditation is a 5,000- year- old method of healing that can result in stress reduction, cognitive enhancement, and improvements in psychological well- being. Methods: Forty nine family dementia caregivers were recruited to participate. Thirty nine family dementia caregivers (mean age 60.3 y.o. (SD=10.2)) were randomized and completed either KK or listening to the relaxation music CD for 20 minutes per day for 8 weeks. Severity of depressive and anxiety symptoms, burden, and coping were assessed at baseline and over the course of the study. The mean Hamilton Depression Rating Scale (HDRS) score at baseline was 11.6 (SD=4.1). Telomerase activity was examined in the monocytes before and after the study.

Results: The severity of depressive and anxiety symptoms improved in both groups. However, improvement in cognitive performance and coping were greater among caregivers practicing KK meditation compared to the relaxation group. These improvement in coping and cognition correlated with an increase in telomerase activity in the meditation group on average by 33% compared to decrease by 8% in the relaxation group.

Conclusion: This randomized study found that KK, a simple, brief daily meditation practice by stressed family dementia caregivers can lead to stress reduction, improved coping, and cognition compared to relaxation. This improvement is accompanied by increases in the levels of telomerase signifying improvement in the stress-induced biological changes of aging. Our results need to be confirmed in a larger sample.

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Telomere length is associated with chromosomal aberrations in peripheral blood

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Background: Studies based on several European cohorts (1–2) have shown that the frequency of chromosomal aberrations (CAs) in peripheral blood is a strong predictive risk marker for cancer, regardless of cancer type or cancer site. However, the mechanisms behind this phenomenon are unknown. Exposure to genotoxic compounds, such as smoking or exposure at the workplaces, appears not to predict CAs in peripheral blood, suggesting that individual susceptibility factors may play a major role. The constitutional telomere length (TL) may be such a factor. Correlation between short telomeres and CAs has been observed in tumor cells, but it has not been studied in humans in vivo.
Materials and methods: The population consisted of 357 healthy Norwegian males cytogenetically analysed for CAs between 1974 and 1998. Information on malignant tumors diagnosed from the date of CAs testing until the end of 2008 was obtained from the national cancer registry. No difference was made for which type of malignant cancer but the individuals were characterized as either cancer cases or controls. Phytohaemagglutinin-stimulated lymphocyte cultures from heparinized whole blood were used for cytogenetic analysis and CAs were scored in 100–200 cells/subject. DNA was extracted from unstained slides of the lymphocyte cultures and relative telomere length was determined by a RT-PCR method.

Results: The median of relative TL was 0.88 for the controls and 0.80 for the cases ($p = 0.14$). TL and CAs were correlated with each other (Spearman's rho correlation $r_s = -0.158$). Also age was correlated with TL ($r_s = -0.158$), and CAs ($rs = 0.159$). TL was associated with CAs analysed by ordinal regression ($\beta = -0.88$, $p = 0.008$; $\beta = -0.76$, $p = 0.022$ after adjustments for age), which means that the shorter TL, the higher frequency of CAs. The cancer risk was significantly increased for subjects with a short TL (OR=3.2 95% CI 1.2–8.2) compared to those with long TL. A weaker increase in risk was found between CAs and cancer risk (OR=1.9 95% CI 0.76–4.9. After adjustment for age, however, this impact of TL on cancer risk was weakened to non-significant level.
Conclusions: The inverse association between TL and CAs suggests that the individual TL can explain why some individuals demonstrate more CAs in peripheral blood as compared to others. Also, the data suggest that TL in peripheral blood is a better susceptibility marker for cancer risk than CAs.

References:

1. Bonassi, S., Hagmar, L., Stromberg, U., Montagud, A.H., Tinnerberg, H., Forni, A., Heikkila, P., Wanders, S., Wilhardt, P., Hansteen, I.L., Knudsen, L.E. and Norppa, H. (2000) Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res, 60, 1619–1625.
2. Hagmar, L., Stromberg, U., Bonassi, S., Hansteen, I.L., Knudsen, L.E., Lindholm, C. and Norppa, H. (2004) Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res, 64, 2258–2263.

N–nitrosamines are associated with shorter telomere length

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Objectives: Telomeres are critical to maintain the integrity of the chromosomes, and telomere abnormalities are important features of carcinogenesis. Telomere length differs among individuals due to genetic and environmental factors. Aiming to examine the relationship between DNA-damaging agents and average telomere length in peripheral blood, we conducted a cross-sectional study among 157 workers working in the rubber industry in Sweden.

Methods: N-nitrosamines were measured in air by personal sampling on Thermosorb/N tubes and analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) for 60 individuals. Based on a similar working situation, the exposure was estimated for all workers. Polycyclic aromatic hydrocarbons (PAH) were measured as the metabolite 1-hydroxypyrene (1-HP) in urine by LC. Carbon disulphide (CS2) was measured as the metabolite 2-thiothiazolidine-4-carboxylic acid (TTCA) in urine by LC/MS/MS. Toluidines (orto-, meta-, and para-) were measured in urine by gas chromatography (GC)/MS. The average telomere length in peripheral blood was determined by quantitative polymerase chain reaction (PCR).

Results: There was a reduction in telomere length with increasing exposure to N-nitrosamines in air [measured (N=60) N-nitrosamines b-coefficient= -10, (95% confidence interval [95% CI] -17– -1.9) P=0.016; estimated (N=157) N-nitrosamines b-coefficient = -5.3, (95% CI -9.5– -0.97) P=0.016]. Also, there were negative associations between para-toluidine [b-coefficient= -0.031 (95% CI -0.055– -0.0063) P=0.014], as well as age b-coefficient= -0.005 (95% CI -0.007– -0.002) P=0.001] and telomere length. There were no strong associations between other exposures and telomere length nor did smoking modify the effect.

Conclusion: N-nitrosamines exposure may lead to telomere shortening.

A fully blinded multi-centre comparison of telomere length measurements by three different techniques

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Telomere length (TL) has frequently been proposed as biomarker of ageing and age-related disease risk in humans. A high degree of reproducibility between data obtained in different laboratories is a *conditio sine qua* non for the application of TL as biomarker on an individual level, but so far only few bilateral comparisons have been conducted. Therefore, we instigated a fully blinded comparison between nine labs with strong international reputation in the telomere field, representing the main TL measurement techniques applicable to DNA samples, namely TRF measurement by Southern blot (two labs), STELA (one lab) and Q-PCR (six labs, using three different reference genes and both single- and multiplex PCR approaches). Each lab measured the same 10 DNA samples. An independent provider coded the DNA samples independently for each lab, and codes were only broken after the last set of results was received by the provider. To enable comparison, results needed to be expressed as relative TL. Then, data show a high degree of correlation between all labs. Indications for stronger associations within a single technique than across techniques were weak. However, inter-lab coefficients of variation were much higher than those obtained in individual labs. Fully blinded tests of inter-batch variation and reproducibility in this multi-centre setting are presently underway. The data obtained so far indicate that rank comparisons of TL between labs are possible, but pose severe limits to the usefulness of transferring or comparing absolute TL data generated outside a single setting.

Telomere attrition and decreased fetuin a levels indicate accelerated biological ageing and are implicated in the pathogenesis of colorectal cancer

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Increasing chronological age is a risk factor for many types of cancer including colorectal. An understanding of the biology of ageing and factors which regulate it, may provide insight into cancer pathogenesis, as well as tools to aid in clinical prognosis and outcomes. The role of telomere biology in both the cancer and ageing process could prove useful in this regard.

Using quantitative-PCR we determined telomere length in the peripheral blood leukocytes of 64 colorectal cancer patients and 1348 controls. We also measured telomere length in colorectal tumour samples and matched normal tissue. We aimed to assess if telomere lengths were reflected in circulating mediators of inflammation and redox control factors, including Fetuin A, a circulating modulator of calcium homeostasis, whose levels have been shown previously to exhibit a dependent relationship with peripheral blood leukocyte telomere lengths.

Results

Colorectal cancer patients exhibited shorter telomeres (adjusted mean RelT/S=0.61) compared with chronologically older controls (mean age 75, adjusted mean RelT/S=0.70) (ANCOVA, p=0.004). Telomere length in tumour tissue (median=0.43, IQR=0.40) was significantly shorter than adjacent normal tissue (median=0.65, IQR=0.28) ($p<0.001$). Patients with low Fetuin A levels were shown to have significantly shorter telomeres (p=0.041). Patients with rectal tumours had significantly higher levels of Fetuin A than those with colonic tumours (p=0.045).

Conclusion

We have observed that patients with colorectal cancer display clear evidence of telomere attrition compared with controls. This is congruent with accelerated biological ageing in the pathogenesis of colorectal cancer. An imbalance in redox control mechanisms and calcium homeostasis may be a contributing factor to telomere dynamics in our patients. Furthermore, Fetuin A levels can be used distinguish between colon and rectal cancers.

Progeria mice on a telomerase deficient background

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic segmental premature ageing disorder caused most often by a single *de novo* point mutation in exon 11 of the *LMNA* gene, c. 1824C>T (G608G). This mutation activates a cryptic splice site, causing an internal deletion in prelamin A. This results in a truncated prelamin A protein known as progerin. Our mouse model based on the tet-off system, uses tetracycline controlled gene expression to epidermally express progerin and has been shown to replicate several features of the HGPS phenotype.

Telomere length in HGPS patient fibroblasts has been shown to be shorter than in age-matched controls, it has also been shown to be directly adversely affected by progerin expression, and that mutant *LMNA* expression is necessary for telomere loss. To further investigate this interaction between progerin and telomere length, we have intercrossed our c.1824C>T expressing mice with a telomerase deficient mouse, B6.Cg-Terc^{tm1Rdp}/J.

Recent results from our lab have shown that adult stem cells become exhausted in the epidermis of c.1824C>T expressing mice. To examine whether the phenotype will be improved on a telomerase deficient background, these animals will be observed and characterised by means of histopathological examination, as well as various stem cell analysis methods and qPCR for changes in signalling pathways.

Effect of regular endurance training on telomere length and telomere regulatory proteins expression in human skeletal muscle

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The length of telomeres is known to be an important indicator of the cell proliferative potential. It is likely that the regulation of telomeres *in-vitro* cannot fully mimic the behavior of telomeres in human tissues. When satellite cells, the skeletal muscle stem cells, are heavily recruited for regenerative events as in skeletal muscle of athletes, telomere length has been found to be either dramatically shortened (overtrained athletes) or maintained and even longer (healthy athletes) than in non-trained individuals (for review, see [1]). This suggests the existence of mechanisms allowing the control of telomere length *in-vivo*. Recently the expression of telomere regulatory proteins has been shown in mice cardiomyocytes and were up-regulated under the influence of physical exercise [2]. Recent findings also suggest that satellite cells express telomerase activity even in old mice [3]. The expression of telomere regulatory proteins in healthy human skeletal muscle and the effects of regular endurance training have never been addressed. The present study aims to address the question of whether the regular practice of endurance training is associated with alterations in telomere length and in the expression level of telomere regulatory proteins (telomerase, tankyrase 1, TRF2 and POT1) in skeletal muscle of healthy athletes. This was achieved by comparison of skeletal muscle (vastus lateralis) telomere length and the above cited proteins expression levels from a population of 12 healthy athletes regularly involved in endurance training (END) against those of a population of 10 healthy, active individuals (CON). Mean and minimum telomere lengths were determined using southern blot. Nuclear fractions were analyzed using western blotting. The mean telomere length in END (11.1 ± 1.0 kbp) did not differ significantly from the values collected in CON (10.5 ± 0.5 kbp). On the opposite, the minimum telomere length was significantly longer in END (5.5 ± 0.4 kbp) than in CON (4.7 ± 0.3 kbp, $P < 0.001$). All four telomere regulatory proteins were expressed in both groups. The expression levels of tankyrase 1, TRF2 were both 3-fold higher in END compared to CON ($P < 0.05$). A tendency towards higher level of POT1 expression in END was also observed ($P < 0.08$). There was no significant difference in telomerase expression level between groups. Altogether, our findings suggest a model where telomeres are dynamic structures under the influence of their environment and where telomere length is controlled *in-vivo* by several endogenous modulators of telomerase processivity. In this model, as long as the environmental disturbances do not exceed the telomere regulatory capacities, telomeres can be maintained even in well-trained skeletal muscle despite the frequent recruitment of satellite cells and/or the exposition to high levels of oxidative stress. The dynamic equilibrium can even be displaced towards longer telomeres in athletes compared to untrained individuals. However, the relationship between the protein content of telomere regulatory factors and their activity *in-vivo* requires further investigations.

1. Kadi, F. and E. Ponsot. Scand J Med Sci Sports, 2010. 20(1): p. 39–48.
2. Werner, C., et al. J Am Coll Cardiol, 2008. 52(6): p. 470–82.
3. O'Connor, M.S., M.E. Carlson, and I.M. Conboy.
Biotechnol Prog, 2009. 25(4): p. 1130–7.

Depression and telomere length: can exercise protect you?

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Introduction: Depression is a significant predictor of cardiovascular disease (CVD) development, progression and related mortality. Preliminary evidence suggests that accelerated immune cell aging, evidenced by leukocyte telomere attrition, is a likely mechanism through which this occurs. Simon and colleagues (2006) demonstrated that those with current mood disorders had shorter mean leukocyte telomere length (LTL) compared to age-matched controls. In a second study by Wolkowitz and colleagues (2011), while those with current Major Depressive Disorder did not have shorter telomeres, those participants reporting greater lifetime depression exposure had shorter telomeres compared to controls. Inconsistent findings regarding current depressive symptoms may result from poorly accounting for varied levels of physical activity, as our recent work suggests. Physical activity has previously been related to LTL (Cherkas et al., 2008) and may serve to protect the telomeres of depressed individuals. Puterman and colleagues (2010) recently demonstrated that higher levels of current psychological stress are related to short leukocyte telomeres only in sedentary post-menopausal women. In those active, stress was unrelated to LTL. These findings are the first to demonstrate the protective telomeric benefits of being physically active to individuals experiencing psychological stress. In the current study, we examine whether depressive symptomatology is related to LTL in a sample of 200 healthy midlife women, and further examine whether these associations vary by activity level.

Methods: Two-hundred and sixty three healthy, non-smoking women between the ages of 50 and 65 were recruited in the San Francisco Bay Area. Depressive symptomatology was assessed with the Patient Health Questionnaire-9 (Spitzer et al., 1999). Past three months total physical activity was assessed with the Stanford Brief Activity Scale (Taylor-Piliae et al., 2006). LTL (T/S ratio) was analyzed with quantitative polymerase chain reaction as described elsewhere (Lin et al., 2010) and converted into base pairs. Covariates included age, income, BMI, and education levels. Current analyses excluded all participants with lifetime histories of cancer and missing data on key variables, leaving 200 participants with complete data.

Results: Linear regression analyses, including covariates, indicated that while depressive symptomatology was unrelated to LTL directly (B = -13.86, SE = 9.79, p = .16), the association between depressive symptomatology and LTL was moderated by physical activity level (B = 20.18, SE = 10.18, $p < .05$). Increased levels of depressive symptomatology were significantly related to shorter telomeres only in those participants who reported being inactive (B = -61.40, SE = 26.20, p = .02) and engaging in light activity (B = -41.22, SE = 17.17, p = .02), and marginally related at moderate levels of activity (B = -21.05, SE = 10.51, p = .05). Depressive symptomatology was unrelated to LTL in those reporting vigorous activity ($p > .10$).

Conclusions: Depressive symptomatology was significantly related to LTL only in sedentary and mildly active midlife women. In moderately to vigorously active participants, there was no apparent association between depressive symptomatology and LTL. Exercise appears to be one promising way to promote resistance to cellular aging, particularly in depressed individuals. Implications will be discussed.

Mitochondrial telomerase protects brain mitochondria during dietary restriction

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Telomerase is best known for its nuclear telomere maintaining function via its enzymatic activity requiring the two major components TERT (protein) and TERC (RNA). However, telomere and TERC-independent functions for TERT have been described recently. We and others have shown previously that telomerase shuttles to mitochondria improving their function and decreasing cellular oxidative stress. Oxidative stress and mitochondrial dysfunction are well known to increase during ageing and have been implicated as a cause for age-related neurodegenerative diseases. We now demonstrate that using targeted localisation of TERT into mitochondria but not nuclei significantly decreases mitochondrial ROS levels under basal and stressed conditions.

Although telomerase activity is negligible in brain we found considerable amounts of the telomerase protein TERT in mouse and human brain. Immuno-cytochemistry showed extranuclear localisation of TERT in neurons from cortex, hippocampus and cerebellum. The protein is already enriched in mouse brain mitochondria in control animals. It becomes further increased in brain mitochondria of animals in 3 independent experiments of short term (3–6 months) dietary restriction (DR). DR is know as a condition that improves mitochondrial function, delays or prevents age related diseases and improves cognitive function. Accordingly, we could demonstrate that ROS generation is significantly decreased in brain mitochondria from DR mice.

Decreased signalling through mTOR has been described as a major mechanism of action of DR. Accordingly we found that mTor phosphorylation was significantly down regulated in brains from DR animals. To analyse whether decreased mTOR signalling causes mitochondrial redistribution of TERT, we treated cells with rapamycin. This treatment increased the extranuclear TERT amounts while decreasing intracellular oxidative stress. We conclude that down-regulation of mTor is a possible mechanism to increase TERT protein levels within mitochondria under DR.

Together, our data suggest that mitochondrial TERT can protect neurons and improve brain function.

MicroRNA-31 is secreted by senescent endothelial cells and inhibits osteogenic differentiation of mesenchymal stem cells

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The decline of physiological functions during aging results from the accumulation of damage in cells and tissues and from decline of the counteracting repair systems of organisms. Stem and progenitor cells range among such repair systems and recently their functionality has been found to depend on the systemic environment, since factors contained in the serum of elderly individuals inhibit adult stem and progenitor functions. However, knowledge about the identity and functionality of these factors is scarce.
In order to identify such factors, we have tested serum of young versus elderly healthy individuals for the presence of different miRNAs and we identified miR-31 at higher levels in the elderly donors. As a possible source of miR-31 we identified senescent endothelial cells that secrete miR-31 also in vitro to the cell supernatant, thereby miR-31 is packaged into exosomes as visualized by electron microscopy in situ hybridization. Consequently we aimed at functionally characterizing miR-31 in the context of aging and we found that exosomes of senescent cells as well as exosomes of elderly donors deliver miR-31 to adipose tissue derived mesenchymal stem cells (ASCs). Both exosomes and transfection with miR-31 alone inhibit osteogenic differentiation by downregulation of FZD3 mRNA as target in ASCs. Taken together, our data suggest that miR-31 is part of the endothelial senescence-associated secretory phenotype (eSASP), and might serve as a novel biomarker of cellular senescence and aging. In addition, it might represent a diagnostic and therapeutic target whenever osteogenesis is a limiting factor, especially in age-related diseases like osteoporosis.

Childhood adversities are associated with shorter telomere length at adult age in the population-based Health 2000 cohort from Finland

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Accelerated telomere shortening has been associated to self-perceived stress and maltreatment during childhood in several studies. However, the significance of childhood stress on adult telomere length (TL) on a population level remains to be determined.

We studied the effects of childhood and recent psychological distress on TL in a subsample from the nationally representative population-based Health 2000 Study that was carried out between 2000–2001 in Finland to assess major public health problems and their determinants. We examined the role of current psychological distress, as measured by the 12 questions of the General Health Questionnaire (GHQ-12), on telomere length. The number of childhood adverse life events was used as a measure of childhood distress. The questionnaire contained a series of 11 questions about the childhood social environment, including parents' serious illness, mental health problems, financial difficulties, serious conflicts, divorce, or own serious illness. We measured the relative TL of the peripheral blood cells by quantitative real-time PCR.
In the first phase of the study, we investigated 321 individuals with a DSM-IV anxiety disorder or subthreshold diagnosis and 653 matched (age, sex, hospital district) controls aged 30-87 years, who all had undergone the Composite International Diagnostic Interview. Self-reported current psychological distress, as measured by GHQ-12, did not affect TL. However, shorter TL was associated with a greater number of reported childhood adverse life events, among both anxiety disorder cases and controls (P=0.005). Childhood chronic or serious illness was the most significantly associated single event affecting TL at the adult age (P=0.004). These results have recently been published (Kananen et al. PLoS ONE 2010; 5:e10826).
The number of childhood adverse life events correlated significantly with the anxiety disorder diagnosis (P=5.7 x 10-14). Therefore, to assess the effect of childhood adversities on TL at adult age in a non-biased sample, we are at the moment measuring TL in the entire Health 2000 cohort (N=8028). The results regarding the effect of current stress and childhood stress on TL will be presented at the meeting.

As a conclusion, our results suggest that childhood stress might lead to accelerated telomere shortening seen at the adult age. The significance of this finding on a population level will be revealed by an ongoing analysis in a nationally representative population-based Health 2000 Study.

Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSOBID cohort

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Variation in the rate of biological ageing reflects the cumulative burden of genetic, metabolic and environmental stressors, resulting in oxidative damage and elevated inflammatory processes. This is of particular interest in Glasgow, because of the exceptional gradient of socio-economic status (SES) reflected in the large difference in life expectancy for men, between the most and least deprived areas of the city, which is 28.7 years ; a difference which is one of largest in the developed world. We investigated potential mechanisms for such gradients as part of the pSoBid study, a cross sectional population-based study to investigate the psychological, behavioural and biological determinants of ill-health. We have hypothesised that such a difference in life expectancy might be reflected in the biological age of individuals. In turn, we considered whether deprivation-related measures correlated to accelerated telomere attrition and also determined interactions with inflammatory status. It has previously been hypothesized that lower socio-economic status can accelerate biological ageing, and predispose to early onset of disease. This study investigated the association of socio-economic and lifestyle factors, as well as traditional and novel risk factors, with biological-ageing, as measured by telomere length, in a Glasgow based cohort that included individuals with extreme socio-economic differences.

Results

A total of 382 blood samples from the pSoBid study were available for telomere analysis. For each participant data was available for socio-economic status factors, biochemical parameters and dietary intake. Statistical analyses analyses were undertaken to investigate the association between telomere lengths and these afore mentioned parameters.
The rate of age-related telomere attrition was significantly associated with low relative income ($p=0.024$), housing tenure ($p=0.038$) and poor diet ($p=0.05$). Additionally, having a larger waist/hip ratio (WHR) was independently associated with greater age-related telomere attrition. Notably, telomere length was positively associated with LDL and total cholesterol levels, but inversely correlated to circulating IL-6. However, this latter association was partially attenuated with adjustment for income and diet.

Conclusions

These data suggest lower socio-economic status and poor diet are relevant to accelerated biological ageing. They also suggest potential associations of elevated circulating IL-6 and WHR, measures known to predict CVD and diabetes, with biological ageing, observations which require further study to tease out potential mechanistic links.

Relative telomere length is positively associated with blood pressure and inversely with fasting plasma glucose levels, but not to left ventricular mass in elderly subjects – The Malmö Preventive Project

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Background /Objective

Telomere length has been shown to be associated with several markers of health and illness. Previous studies have shown an inverse association between telomere length and aspects of cardiovascular ageing. Comparisons between telomere length and left ventricular mass (LVM), however, have surprisingly shown a positive correlation.

Thus, we aimed to compare relative telomere length (RTL) with LVM index (LVMI) in a large sample from the general population of Malmö, Sweden. In addition, we wanted to investigate the correlation between RTL and other markers of cardiovascular risk such as blood pressure elevation and increased pulse pressure, fasting glucose, lipid levels and smoking.

Subjects and Methods

The study population consisted of 1792 subjects (30% females, 70% males) drawn from the Malmö Preventive Project Re-Examination Study (MPP-RES) cohort examined in 2002–2006. These were mostly elderly subjects, with no previous myocardial infarction and stratified for increasing levels of hyperglycaemia and/or type 2 diabetes.

Echocardiography, TDI (Tissue-Doppler Imaging), was used to measure LVMI. DNA was extracted from peripheral blood leukocytes (PBL), and RTL was measured by using quantitative PCR as described by Cawthon *et al* (2001). RTL is defined as the relationship of telomere length (mean of triplets) to a single copy gene length (expressed as T/S). For the statistical analyses, we used linear and logistic multiple regression analysis in SPSS (version 19).

Results

We excluded subjects that were T/S outliers or had unrealistic data on blood pressure levels or LVMI, thus a total number of 1588 subjects remained. Of these, the mean age was 67.7 (SD: 5.9) years, in men 66.9 (SD: 6.02) and in women 69.5 (SD: 5.10) years, mean blood pressure 147/84 (SD: 19.8/10.3) mmHg, pulse pressure 62.8 (SD: 14.4), mean fasting glucose 6.86 mmol/L (SD: 2.08), and mean LDL-cholesterol 3.47 mmol/L (SD: 1.00). A total of 1277 subjects were hypertensives and 311 normotensives, based on a definition of hypertension as systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 mmHg, or use of anti-hypertensive treatment (AHT). In all, 570 subjects had confirmed type 2 diabetes mellitus, and 676 were current smokers.

RTL was inversely associated with age (β= -0,29, p=2.37 x 10^{-30}) and smoking (β = -0,05, p=0.048), adjusted for confounders. There was no significant difference in RTL between males and females (p=0.94).

RTL, adjusted for age, gender and use of AHT, was positively associated with levels of systolic and diastolic blood pressure (β = 0.05, p=0.045; and β = 0.06, p = 0.010, respectively). When comparing RTL in hypertensives (1.16; SD: 0.35) to normotensives (1.15; SD: 0.33) it was shown that RTL was longer in hypertensives (p=0.038), adjusted for confounders.

However, RTL was neither correlated to LVMI (p=0.94) nor to pulse pressure (p=0.33) when adjusted for age and gender. None of the associations with blood pressure, hypertension or LVMI were present in diabetic subjects. When comparing patients with diabetes to non-diabetics, RTL was longer in non-diabetics, although this association was of borderline significance (p=0.09). However, RTL was inversely correlated to fasting plasma glucose (β = -0.06, p=0.015). No association was found regarding LDL-cholesterol levels.

Conclusions

Surprisingly, our data suggests that subjects with hypertension have significantly longer RTL than normotensives. However, there was no association between RTL and LVMI or pulse pressure, both variables being influenced by elevated blood pressure levels. In subjects with established type 2 diabetes no association was found between RTL and blood pressure levels or LVMI, but RTL showed an inverse correlation with fasting plasma glucose in the whole cohort. This calls for further studies on the relationship between RTL and cardiovascular risk factors.

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The effect of the telomerase antagonist Imetelstat in esophageal cancer cells

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Telomerase is mainly active in human tumor cells, which provides an opportunity for a therapeutic window on telomerase targeting. We sought to evaluate the potential of the thio-phosphoramidate oligonucleotide inhibitor of telomerase, Imetelstat, as a drug candidate for treatment of esophageal cancer. Our results showed that Imetelstat inhibited telomerase activity in a dose-dependent manner in esophageal cancer cells. After only one week of Imetelstat treatment, a reduction of colony formation ability of esophageal cancer cells was observed. Furthermore, long-term treatment with Imetelstat decreased cell growth of esophageal cancer cells with somewhat different kinetics regarding telomere lengths. Cell cycle analysis demonstrated that short-term treatment with Imetelstat resulted in increased percentage of cells in G1 phase. Short-term Imetelstat treatment also increased γ-H2AX and 53BP1 foci staining in the esophageal cancer cell lines indicating a possible induction of DNA double strand breaks (DSBs). We also found that pre-treatment with Imetelstat led to increased number and size of 53BP1 foci after ionizing radiation. The increase of 53BP1 foci number was especially pronounced during the first 1 hour of repair whereas the increase of foci size was prominent later on. This study supports the potential of Imetelstat as a therapeutic agent for the treatment of esophageal cancer.

Key words: Telomerase, esophageal cancer, Imetelstat, DNA double strand break, γ-H2AX and 53BP1 foci

BRIEF HISTORY OF THE SWEDISH SOCIETY OF MEDICINE

Further education impossible

At the beginning of the 19th century, there were slightly more than 200 physicians in Sweden. Concerned that the radicalism of the French Revolution would spread to Sweden, King Gustav IV Adolf issued extremely strict regulations regarding the importation of books and publications. As a result, opportunities for people to keep up with scientific developments in Europe were virtually non-existent. The only publication that could be imported was a French fashion magazine read by the queen.

Growing membership

The number of physicians in Sweden and membership in the Swedish Society of Medicine has increased over time. In 1809, the Society's membership was 50, a majority of Stockholm's physicians. One hundred years later, the Society's membership was 1 039. In 2008, the Society has about 18 000 members, the majority of which live outside of Stockholm.

Reading society necessary

Many people were dissatisfied with these restrictions. In 1802, a medical lecturer named Jacob Berzelius and an instructor for the Surgical Educational Board named Eric Gadelius sought the king's permission to form a physicians' reading society. This request was justified on the grounds that foreign medical literature would result in »the promotion of true information of universal benefit to the state among virtuous and industrious citizens«. The request was denied on the grounds that »two young and already proficient physicians could be diverted from a profession that for the enlargement of their knowledge and experience requires their full attention«. The decision was made by the court chancellor Baron Christoffer Bogislaus Zibet.

Decided by fistula?

Berzelius and Gadelius, however, did not despair, and by 1807, seven physicians from Stockholm were advocating for the creation of a reading society. That same year Baron Zibet suffered an acute and very painful fistula of the urinary tract for which he was treated by Carl Fredrik von Schulzenheim, a senior surgeon at Seraphim Hospital, Zibet's personal physician, and one of the seven reading society advocates. This may have contributed to Zibet's decision on 31 December 1807 to approve another request for a reading society. On 25 October 1808, the Society held its first Tuesday Meeting.

WHO WAS BERZELIUS?

Jöns Jacob Berzelius, one of the most prominent natural scientists of the 19th century, was born in 1779 in Väversunda, in the county of Östergötland in southern Sweden, a region with rich cultural traditions.

Orphaned at an early age, he went to several foster-homes and received his schooling in nearby Linköping. After graduating in medicine at the University of Uppsala, he moved to Stockholm, where he became assistent master without pay at the so-called »Surgical School«, and earned his keep by working as a doctor for poor people. At the age of 28 he became professor of medicine and pharmacy.

In 1808 Berzelius was one of the seven men who founded The Swedish Society of Medicine »For the perfection of science through mutual mediation of knowledge and collective experience, for the promotion of friendly confidence between doctors«.

Berzelius have enriched our knowledge of nature of life phenomena, established the atomic weights of most of the known elements, presented his electrochemical theory for the understanding of the nature of chemical compounds and laid the foundation for the sciences of the chemistry of rock types.

He also found that elements combine with each other according to fixed numerical relationships. In addition to this, in his striving for order and method, with his talent for simplicity and clarity in expression, he created the chemical symbolic language in 1813, which since that time has been an essential instrument of chemistry.

With time he became a practised lecturer but preferred to express himself in writing and this he did superbly. Impressive are the great scientific works where he also demonstrated his interest and ability to spread knowledge about the latest advances of natural sciences.

Berzelius delight in research and debate was united with a great humility before the great scientific questions. Both his attitude and artistery of formulation is illustrated by the following passage in his Manual of Cheamistry (vol 3, 1818):

»All our theory is but a means of conistently conceptualizing the inward processes of phenomena, and it is presumable and adequate when all scientifically known facts can be deduced from it. This mode of conceptualization can equally well be false and, unfortunately, presumable is so frequently. Even though, at a certain period in the development of science, it may match the purpose just as well as a true theory. Experience is augmented, facts appear which do not agree with it, and one is forced to go in search of a new mode of conceptualization within which these facts can also be accomodated; and in this manner, no doubt, modes of conceptualization will be altered from age to age, as experience if broadened, and the complete truth may perhaps never be attained. But even if the goal can never be reached, let us never abondon our endeavor to get closer to it.«

Parts of this text is found in
»Berzelius – Creator of the chemical language«
by Carl Gustaf Bernhard,
The Royal Swedish Academy of Sciences

HISTORY OF THE BUILDING

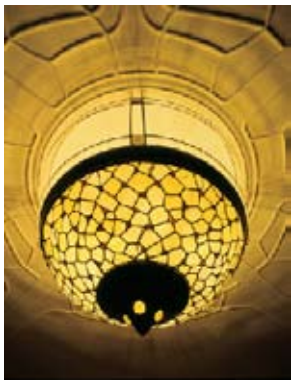
In 1879, the Swedish Society of Medicine moved from what was then the home of Karolinska Institute at Norr Mälarstrand to its own premises in Jakobsgatan in Stockholm. It soon outgrew this location and a search for new premises was resumed. On Walpurgis night in 1889, six men were inside the Katarina lift at Slussen in Stockholm.

A fault developmed in the machinery, causing the lift cage to fall. One of the passengers, Carl Westman, was injured, but a fellow passenger, Johan Rissler, a surgeon and member of the building committee of the Society of Medicine, immediately assisted him.

In 1904, the Society announced an architectural competition for a building on a site it had purchased in Klara Östra Kyrkogata.
The winner was Carl Westman, and the building was finished two years later.

The Society's building which dates from 1906, was a breakthrough for the architect Carl Westman and the national romantic style architecture he favoured.

The building itself is work of art – from its facade of handmade brick and Christian Eriksson's granite reliefs in the entrance to its mosaic floors, carved balustrades, chandeliers, and ventilation grilles – all Westman signatures. The building today is a Swedish, turn of the century architectural treasure.



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