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Review

Telomere Attrition as Ageing Biomarker

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Abstract. *Telomeres, the tandem-repeated hexamers at the termini of mammalian chromosomes, form protective complexes in association with specific proteins that together with telomerase, a specialised telomere-synthesizing enzyme, regulate telomere length. Telomere shortening is associated with cellular senescence and is implicated in tumorigenesis and cancer. Hence, mean telomere length has emerged as a replicative clock within each population of cells and the tissues and organs they build up in vitro and, consequently, as a biomarker for biological ageing in vivo. Chronological ageing per se does not parallel biological ageing, yet accurate and reliable biomarkers are lacking to distinguish between them. The question remains as to whether telomere dynamics is a determinant or merely a predictor of human biological age over and above chronological ageing. Although several reports have suggested a link between telomere attrition and ageing phenotypes and disorders, both reference values and a complete set of determinants are missing. Within this review, current evidence and knowledge on telomere length and telomere erosion rates reported, are summarised.*

Telomeres: gatekeepers of the genome

Telomeres are nucleoprotein structures that cap the ends of eukaryotic chromosomes, hence avoiding them being recognised as DNA breaks by the cellular checkpoint mechanisms. These specialised structures at the termini of linear chromosomes have long been an ill-defined and much neglected cytogenetic issue. It was not until the concept of chromosomes being composed of a single unbroken DNA molecule was accepted that much attention was focussed on the Achilles heel of the DNA double helix.

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The immortality dogma, stating that vertebrate cells would divide indefinitely in culture once released from the restraining multicellular *in vivo* context, was disproved only in 1961 (1). It was observed that the replicative capacity of normal human fibroblasts in culture was not infinite, but that proliferation only continued for about 40-50 population doublings (PDs), and the cells subsequently revealed senescence and eventually died, an ageing phenomenon known as the 'Hayflick-limit'. One interesting observation, when studying the mortality of cells in culture, was that the number of PDs that cells can undergo is inversely proportional to the donor age. Primary cells have a greater longevity than more differentiated cells, and ageing is clearly distinct from terminal differentiation. Moreover, when cells are reconstituted after cryogenic preservation, the remaining lifespan is determined by the total number of PDs minus the number of doublings before cryopreservation, implying a memory function of replicative age and remaining proliferative potential.

Alexei Olovnikov hypothesized, on theoretical grounds, that this Hayflick limit resulted from gradual loss of repetitive DNA sequences at the ends of linear chromosomes, because of the inability of DNA-dependent DNA polymerases to synthesize a complete replica of the template (2). Consistent with the findings of Muller (3) and McClintock (4), he defined the telomeres as the buffers that are sacrificed with every round of DNA replication. Upon exhaustion of these buffers, cells lose vitally important genes, and hence telomeres are the primary cause of cellular ageing. His theory of marginotomy would be the basis for the hypothesis that telomere shortening is the molecular clock that determines the proliferative lifespan of cells, the '*telomere hypothesis*'.

Evidence for the existence of the telomere-based mitotic clock and its implications both for ageing and cancer has since amassed. After the identification of the TTAGGG telomeric sequence (5), the mean telomere length was found to decrease progressively during serial passages of human fibroblasts (6), to be related to remaining proliferative capacity (7) and to be shorter in samples from older donors

(8, 9). In a more mathematical approach, Levy *et al.* (10) demonstrated that most if not all telomeric repeats are lost during extended growth *in vitro* and suggested that this erosion could signal cell cycle exit. Telomere shortening has been implicated with a broad range of ageing tissues and organs *in vivo* (11-13). In addition, human lymphocytes and haematopoietic stem cells were shown to lose telomere repeats with increasing age *in vivo* (14, 15). More importantly, telomere biology and telomere attrition in particular has proven to be most valuable to better understand the pathophysiology of different human diseases, including age-related disorders and cancer.

Telomere length: the two-sided medal

Actual proof for the existence of a mitotic clock based on the attrition of telomeres came with the identification of a counterbalancing mechanism by a specialised telomere synthesizing enzyme, telomerase (16). Telomerase synthesizes the required telomeric repeats onto the 3' overhangs of the telomere, thus establishing a telomere equilibrium as seen in single-celled organisms like yeast (17), in germline cells, reproductive cells, in about 90% of all tumours in humans *in vivo* (18) and established mammalian cell lines *in vitro* (19). Transient expression of telomerase prolonged the *in vitro* lifespan of retinal pigment cells and of foreskin fibroblast cells (20). Similar observations were made in normal human diploid fibroblast cells (21), providing evidence for the causal relation between telomere shortening and cellular senescence.

Reproductive cells contain active telomerase and compensate for the telomere end-replication-problem, prevailing the transmission of uneroded telomeres to the offspring (22). Consequently, stable telomere lengths are maintained. This telomerase activity is down-regulated when cells differentiate. In normal somatic human cells, telomerase is repressed either completely or partially, as measured in haematopoietic cells (23-25). As a consequence, telomeric tracts are lost with every round of cell division, as evidenced in all replicating somatic cells and tissues that have been analysed, including fibroblasts and peripheral blood cells (26). At the Hayflick limit or mortality stage I, one or more telomeres become critically short, are recognised within the cell as chromosome break(s) and the cell cycle is arrested irreversibly. The signal that triggers replicative senescence is not the telomere array length *per se*, but rather the inability to form a protective higher order complex in association with telomere-specific proteins (27, 28), leading to dysfunctional telomeres (29). These activate cell cycle checkpoints and either induce replicative senescence or apoptosis. Yet, in the absence of such checkpoint systems, cells continue to proliferate and telomere erosion proceeds until nearly all telomeres reach a critical length at mortality stage II and enter crisis, resulting in chromosomal instability by erroneous DNA damage repair. At this point, the number of cell divisions is counterbalanced by an equal number of cell deaths and chromosomal end fusions and other cytological abnormalities accumulate. Rare cells can escape from this crisis by the activation of telomerase, ensuring propagation of this short telomere length and conferring immortality (30).

In human T cells, telomerase activity increases with acute antigen exposure, but decreases with repeated antigen stimulation and as cells approach senescence (31). People with dyskeratosis congenita, in which the ability to express telomerase is decreased, show shorter telomeres and die prematurely of bone marrow failure and infections (32).

Hastie *et al.* (33) measured shorter telomeres in tumour samples as compared to surrounding normal tissue cells. This provided the first evidence for a possible link between cancer and telomere biology and, since then, telomerase activity has been evidenced to be actively present in about 90% of all known human malignant tumours (34) and to maintain a stable but short telomere length equilibrium in tumour cells *in vivo* (35) and immortalised cells *in vitro* (36). As telomere shortening and, more specifically, telomere erosion rates determine the replicative capacity *in vitro* and are implicated with ageing and cancer *in vivo*, the question arose as to whether telomere length could serve as a biomarker of a cell's biological age as opposed to chronological age.

Recent observations have provided new insights into both the regulation of telomere length in normal cells and the phenotypic consequences of perturbing these processes. More specifically, recent evidence has amassed for the associations between telomere shortening (*i.e.* cellular ageing) and its implications for human health (see Figure 1); in particular, accelerated telomere attrition has been implicated in a growing list of age-related disorders from progeroid syndromes (progeria like Werner, Bloom, Hutchinson-Gilford, ...) over an increased risk for cancer (37-39), osteoarthritis (40), decreased wound healing (7) and immune function (reviewed in 41-43) to atherosclerosis (44), diabetes mellitus (45) and even Alzheimer's disease (46). This latter association is consistent with recent observation that chronic psychological life stress leads to oxidative stress and telomere shortening in peripheral blood lymphocytes (PBLs) (47). Other correlations are found in patients with chronic infections, like chronic hepatitis and liver cirrhosis (48, 49), which were ultimately linked to an 8-fold increased mortality rates in an elderly subpopulation (50) and, recently, a challenging hypothesis was formulated linking telomere attrition to organism extinction (51).

More interestingly, several of these studies have demonstrated the utility of employing the more accessible PBLs rather than the more inaccessible tissues linked with the diseased status. Besides the association with atherosclerosis,

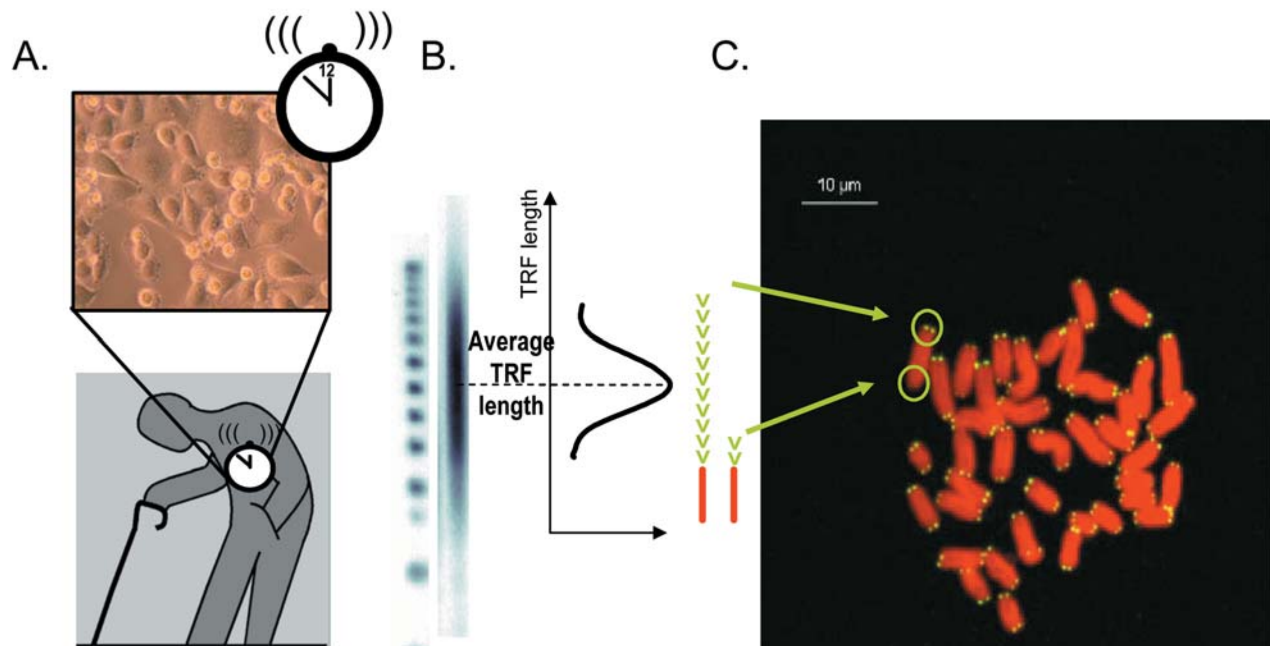

Figure 1. *Cellular ageing and the replicative clock. The wear and tear of tissues and organs in an ageing individual is reflected in the replicative potential of normal somatic cells of which they are built up (A). A cellular replicometer based on the length of the termini of linear chromosomes, the telomeres, keeps track of the number of cell divisions. Telomeres shorten with every round of cell division and under influence of environmental (oxidative) stress. Once a critically short telomere length is reached, the cell cycle is halted and the cell is signalled into replicative senescence. The average telomere restriction fragment (TRF) length is a standard measure of telomere length, reflected by the average telomere length of all chromosomes within a given population of cells (B). Whether or not one critically short telomere (green signal; chromosomes = red signal), as measured by Q-FISH-based microscopical analysis, is sufficient to elicit a senescence signal, is not known (C). Recent studies have found an intriguing link between accelerated telomere attrition in both focal (endothelial) and general (peripheral blood lymphocytes) tissues and age-related disorders like atherosclerosis, promoting telomere length not directly as a causal factor but merely as a biomarker for ageing.*

coronary artery disease and premature myocardial infarctions, Alzheimer's disease and life stress, shorter PBL telomere lengths were recently linked to an increased risk for cancer development. The latter observation was described by Wu and coworkers (52), showing that shorter telomeres were significantly associated with an increased risk for carcinoma development at positions as distinct as the neck/head, the kidney, bladder and lung. A possible explanation for the predictive value of PBL telomere length, as opposed to the more inaccessible diseased tissue, could be that ageing disorders are linked with dysregulated immune function and/or increased systemic inflammation, which is in turn reflected by the telomere attrition in PBLs.

Telomere length assessment

As telomere length varies considerably between chromosomes and chromosome arms, one or the few critically short telomeres are expected to play the key role in the activation of the cellular checkpoint systems (53), yet the predictive value of average telomere length of cell populations is accepted to be more valuable (54). Several methods have been developed during the last 15 years to assess telomere length both at the level of a population of cells, single cells and even single chromosomes, as summarised in Table I. Each methodology has its advantages and limitations, but the golden standard for telomere length assessment remains the Southern-blot-based telomere restriction fragment analysis (TRF) analysis which, in combination with the recent PCR-based techniques, is proving to be a most valuable tool in telomere research.

In TRF-analysis, purified genomic DNA is digested with frequently cutting restriction endonucleases, most commonly *RsaI* and *HinfI*, resulting in intact telomere restriction fragments including only limited subtelomeric tracts. The restriction fragments are separated by (preferably pulsed field) gel electrophoresis and immobilised onto a membrane by Southern blotting. The mean TRF length values are measured by densitometric analysis of membranes hybridised with labelled telomeric oligonucleotides. These mean TRF-values differ from the actual telomere lengths by the subtelomeric sequences. On conventional agarose gels,

Table I. *Methodologies to measure telomere length.*

Methodology	PCR or hyb.* - based	Study material	Amount required	Average or individual length chromosomes	Sequences detected	Major disadvantage	Ref.
TRF	hyb.- based	DNA	2-10 μg	average	a, b, c	TRF include sub-telomeres	(7)
HPA	hyb.- based	DNA or cell lysate	10 ng / 1000 cells	average	a, b, c	total lack of distribution information	(55)
direct lysate method	hyb.- based	cell lysate	>2x10^5 cells	average	a, b, c	total lack of distribution information	(56)
T-OLA	hyb.- based	DNA	5 μg	average	c	time-consuming	(57)
Q-FISH	hyb.- based	metaphase preparations	>30 nuclei	individual	b,c	labour-intensive	(58)
flow-FISH	hyb.- based	cells	>1000 cells	both	b,c	cost & complexity	(59)
Q-PCR	PCR-based	DNA	35 ng	average	b,c	total lack of distribution information	(60)
STELA	PCR-based	DNA	10-100 ng	single	b	subtelomeric sequence info. required	(61)

a=partial telomere associated sequence (TAS) domain
b=interstitial- and pseudo-telomeres
c=3' overhang
*hyb.=hybridisation

telomeric restriction fragments are revealed as heterogeneous smears in Southern analysis. In a population of telomeres a lognormal distribution of telomeric sizes is observed (62), which has clearly defined upper and lower limits. Levy and coworkers (10) were the first to propose that variation in telomere restriction fragment (TRF) lengths could not be fully explained by incomplete replication, suggesting a significant interchromosomal variation in the length of telomeric repeats.

In contrast to Southern blotting, the hybridisation protection assay (HPA) does not include subtelomeric regions. In the HPA procedure, a DNA solution or cell lysate is immediately hybridised with an acridinium ester (AE)-labelled telomere-specific probe followed by a selective hydrolysation step to remove any unbound probe. Quantification is performed by chemiluminescence. The value is normalised for the total DNA amount by measuring also an AE-labelled *Alu* probe and calculating the ratio of both (55).

In 2003, Freulet-Marriere and coworkers (56) developed a method to rapidly measure telomere length on microtitre plates without any DNA purification. The technique uses a biotin-streptavidin-based acetylcholinesterase hybridisation assay for quantification of the telomeric sequence and a DNA Sybr Green assay to correct for the total amount of DNA. The telomeric-oligonucleotide ligation assay (T-OLA) is a technique capable of measuring the length of the G-rich strand 3' overhang (57), but also gives information on the general telomere size as telomere shortening is found to be directly proportional to the length of the overhang (63). In T-OLA, [γ-^{32}P]-labelled telomeric oligonucleotides are hybridised to the undenaturated DNA and ligated. The concatenated products are released by heat denaturation, resolved by denaturating PAGE and visualised by autoradiography (57).

Quantitative fluorescence *in situ* hybridisation (Q-FISH) is an accurate technique which permits the measurement of telomere lengths in individual cells. In Q-FISH, a fluorescent peptide nucleic acid (PNA) probe complementary to the telomeric repeats is used for hybridisation with metaphase chromosomes (58). Telomere length is generally measured as

the integrated fluorescent intensity (IFI) after counterstaining with a chromosome-specific, fluorescent dye and digital microscopy-based analysis (64, 65). The combination of Q-FISH with flow cytometry (Flow-FISH) provides more sensitivity, accuracy and speed but has a few major disadvantages, namely complexity and cost (66, 67).

Besides hybridisation-based techniques, PCR-based methodologies have been recently developed, such as quantitative PCR (Q-PCR) and single telomere length analysis (STELA). The difficulty with using PCR is the lack of suitable primer binding sites. One possible solution is the development of primers which are not perfectly complementary to the telomeric sequence, still allowing the DNA-polymerase to amplify telomeric fragments while primer-dimers cannot be amplified due to mismatch at the 3' tail. Using Q-PCR, one may especially expect the shortest possible product to be amplified at a copy number proportional to the initial number of telomeric repeats. The measure for the telomere length is then calculated based on the comparison with a Q-PCR performed on a single copy gene (50).

Another PCR-based technique is STELA. The key feature of this technique is the partial annealing of a linker with the G-rich 3' overhang followed by the ligation at the 5' end. The combination of a primer identical to this tail with a chromosome-specific primer upstream allows the amplification of chromosome-specific telomeric fragments. The individual telomere lengths are then determined by gel electrophoresis and Southern hybridisation (61).

Telomere length: reference values in different species, tissues and diseases

Telomeres are dynamic structures and telomere length determination is species- and tissue-specific. Telomere length varies greatly between different species, between tissues (to a lesser extent), between cells and even between single chromosomes (Table II).

In protozoans like hypotrichous ciliates, telomeres are paired at a specific length of, for *e.g.*, 28 bp early in macronuclear development and are strictly maintained during subsequent cell divisions (68). In yeast, telomere length is under continuous control between 300±75 bp (69). In most mammalian organisms, telomere length is not fixed and is related to cellular lifespan, senescence and immortalisation. Mice have strain-specific ultra-long telomeres (20-150 kb), which are resolved as multiple discrete restriction fragments (71, 72). Normal human somatic cells have an average telomere length between 5 -15 kb (7, 78, 79); this telomere length is shown to be synchronous among different foetal tissues (80) and those of newborns (76) and this synchrony is largely/partially maintained during extra-uterine life (75).

The presence of telomerase activity in most somatic tissues of mice explains the constant length observed during murine somatic cell divisions. It is not known why such a short-lived species should have constitutive telomerase activity and contain very long telomeres, as opposed to the shorter telomeres in human somatic cells in which telomerase is repressed. Apparently the hyper-long telomere lengths observed in *Mus musculus* strains do not seem to be essential as one particular mice species, *M. spretus*, which contains telomeres with comparable lengths to human somatic cells, has an equally long lifespan as *M. musculus*. Long-lived species may have more control mechanisms to limit cellular proliferation than short-lived species. What has been experimentally assessed are mostly associations between telomere length and population doublings *in vitro*; no information is available on reference values of telomere attrition in large groups of individuals. The reported evidence is deduced from small pilot studies.

In humans, telomere length is highly variable between individuals, and at any moment during extra uterine life it is a reflection of the mean telomere length set during intra uterine development and the telomere attrition rate, making it a valuable biomarker for human ageing. From extensive yet cross-sectional data, it has been derived that telomere length is age-dependent and that age-adjusted telomere length is to a large extent heritable and variable. Women live on average 7 years longer than men and the mortality rates beyond 60 years is about half of that of men, while no sex-related differences in mean telomere length are measured in newborn boys and girls, a discrepancy appears later in life and telomere length is shown to be longer in women compared to men (74, 50, 81, 82). Although this sexual dimorphism in telomere length reflects the gender gap in life expectancy, a complete explanation or mechanism for this sexual dimorphism has not been presented yet.

Determinants of telomere length

The primary determinant of telomere length is inheritance and mutations affecting telomere biology might ultimately affect lifespan (32). Twin studies in human have revealed that individual differences in telomere length in multiple tissues are to a large extent genetically determined (81, 83-85). Apparently the telomere length is determined in the zygote during early development and maintained during life, hence tainting the replicative lifespan of proliferative tissues. An interesting observation was made in rats relating retarded intra uterine and catch-up postnatal growth to shorter telomeres and shorter lifespan (86), in accordance with the reports on birth size and ageing disorders (87).

Although telomere length is primarily a heritable trait, it is also influenced by environmental factors like oxidative stress. The latter has been shown to accelerate telomere

Table II. *Telomere length in different species and tissues.*

Organism	Tissue/disease/age	Telomere length	Ref.
Hypotrichous ciliates		<50 bp	(68)
Saccharomyces cerevisiae		~300 bp	(69)
C. elegans		4 – 9 kb	(70)
Mus musculus		20 – 150 kb	(71)
Mus spretus		5 – 20 kb	(72)
Human	PBL		(73)
	- >10 years	- 50 kb	
	- 60-70 years	- 5 kb	
	PBL		(26)
	- newborn	- 16.4 kb	
	- 20-36 years	- 11.6 kb	
	- 42-72 years	- 9.6 kb	
	- 62-82 years	- 8.0 kb	
	PBL, age 56+/-11 years		(74)
	- male	- 8.37 kb	
	- female	- 8.67 kb	
	PBL	11.7 kb (mean)	(75)
	- 20-wk-old foetus	- 12.2 kb	
	- 72-yr-old man	- 7.2 kb	
	skin	12.8 kb (mean)	
	- foetus	- 13 kb	
	- 72-yr-old man	- 9.8 kb	
	newborn, PBL		(76)
	- male	- 10.95 kb	
	- female	- 11.07 kb	
	newborn, umbilical artery		
	- male	- 10.99 kb	
	- female	- 11.02 kb	
	naïve $CD4^+$ T-lymphocytes		(15)
	- 25 years	- 9.5 kb	
	- 70 years	- 8.0 kb	
	memory T-lymphocytes		
	- 25 years	- 8.0 kb	
	- 70 years	- 6.6 kb	
	cultured primary fibroblasts		(77)
	- 75 years old	- 5.57 kb	
	- Werner syndrome patient	- 4.67 kb	
	giant cell tumour of bone	500 bp shorter than own PBL telomeric DNA	(72)
	colorectal carcinoma	shorter than control	(33)

erosion *in vitro* (88) and *in vivo*, and antioxidant defences can in turn slow down telomere attrition (89, 90). Mild oxidative stress with homocysteine *in vitro* causes accelerated telomere erosion in vascular endothelial cells (91), and recent flow cytometric analysis demonstrated the protective effect of statins against telomere shortening (92). Differences in telomere length between men and women have been attributed to differences in hormonal status and lifestyle, linked to anti-oxidative defences. Also, body size differences (93) and different telomere dynamics on the X and Y chromosomes (94) have been proposed.

As cytokine markers of chronic inflammation, like interleukin 6 and C-reactive protein, have been associated with age-related diseases like atherosclerosis, cancer and type 2 diabetes, it is challenging to investigate to what extent telomere length is affected by their respective titre.

Telomere attrition: Application and evidence for biomarker function of telomere length

Telomere length *per se* does not predict lifespan, but the telomere rate of change (TROC) in length does (95). Likewise, the sexual dimorphism in telomere length has been attributed to a difference in attrition rates which are suggested to be slower in women than in men. The questions whether normal somatic cells beyond

Table III. *Telomere attrition rates.*

Tissue/disease/age	*In vivo / in vitro*	Number of subjects	Telomere attrition	Ref.
lymphocytes	*in vivo*	140	41 bp/year	(106)
T-lymphocytes	*in vivo*	30	32 bp/year no difference between naive and memory cells	(15)
B-lymphocytes	*in vivo*	121	19 bp/yr no difference between naive and memory cells	(42) (107)
whole blood leukocytes	*in vivo*			(108)
- cord blood, < 1 year		12, 18	700 bp/year	
- overall (< 31 years)		73	31 bp/year	
PBL	*in vivo*	56	27 bp/year	(109)
PBMC	*in vivo*			(103)
- 4-39 years		30	84 bp/year	
- >=40 years		50	41 bp/year	
adult bone marrow	*in vivo*	7	9 bp/year	(14)
fibroblasts	*in vitro*		+ 50 bp/PD	(7)
	in vivo	43	15 bp/year	
fibroblasts	*in vivo*	21	19.8 bp/year	(110)
fibroblasts	*in vitro*		48 bp/PD	(6)
endothelial cells: no:				(8)
- iliac artery	*in vivo*	4	102 bp/year	
- iliac vein	*in vivo*	5	47 bp/year	
- umbilical vein	*in vitro*		190 bp/PD	
proximal abdominal aorta				(111)
- intima	*in vivo*	48	15 bp/year	
- media	*in vivo*	48	6.4 bp/year	
distal abdominal aorta				
- intima	*in vivo*	47	28 bp/year	
- media	*in vivo*	47	25 bp/year	
kidney	*in vivo*	24	29 bp/year	(112)
kidney				(113)
- outer renal cortex	*in vivo*	121	21-60 bp/year	
- inner renal cortex	*in vivo*	121	14-17 bp/year	
brain cells	*in vivo*	~10	no shortening (healthy adults)	(114)
muscle cells	*in vivo*	24	no shortening (healthy adults)	(115)
respiratory chain disorders	*in vivo*	35	advanced *vs*. control	(116)

development and extra uterine regulate telomere length in an active way, or whether loss of telomere repeats occurs at a constant rate during life directly proportional to the number of cell divisions, have not been addressed yet, or only *via* cross-sectional analysis and in pilot studies. Conflicting reports exist on the rate of telomere shortening, being either gradual and constant, or increasing or decreasing with age (26, 96). The latter group described a biphasic telomere attrition, with fast attrition from birth up to the age of 4-5 and a slower and more gradual erosion onwards. Telomere shortening rates are apparently not constant but are influenced by a competing set of positive and negative regulators of telomere length.

The relevance of assessing the more practicable peripheral blood leukocytes (PBL), as opposed to the focal diseased tissue, was demonstrated in a multitude of non-neoplastic medical disorders. In patients with these disorders, increased telomere erosion of peripheral blood leukocytes was associated with cardiovascular damage in general (97) and in particular with atherosclerosis (44), hypertension (74, 81), premature myocardial infarction (98) and with hypercholesterolemia and diabetes mellitus (99, 100). Disease states featured by increased stem cell turnover like chronic myelogenous leukaemia, aplastic anaemia and recipients of allogeneic bone marrow transplantation show accelerated telomere attrition as compared to their healthy counterparts (101, 67, 102).

Telomere attrition in newborn and young individuals is shown to be significantly higher than in the older counterparts, namely up to 4-fold faster during the first 5 years of life than in adults (103, 96, 104). Clearly telomere attrition levels off to a more gradual and constant telomere

loss. As telomere length does not vary considerably between different cell populations in a given individual (105) and as a large synchrony between tissues exists within foetuses and newborns (76, 80), this difference in erosion rate can most probably be attributed to increased immune cell replication rate accompanying the characteristic changes of the immune system in newborns and infants.

In the elderly (>60), telomere attrition is significantly associated with higher mortality rates, both from infectious and cardiovascular diseases (50). If one assumes telomere shortening at approximately 50-100 bp/cell division then this corresponds with 15-30 divisions of stem cells in the first year of postnatal life and 1 stem cell division in the rest of the life. Yet, the available telomere attrition rates are calculated from cross-sectional analysis (Table III). The only 2 longitudinal studies on telomere length performed respectively in human (104) and cat (117) showed age-related telomere attrition and that telomere erosion rates are higher during early adulthood, as found in cross-sectional analyses in other studies. Yet, cross-sectional data may not fully reflect the telomere length changes within individual subjects over time. Besides the phenotypical makeup of age classes that may differ on the basis of selective mortality, environmental changes over time may be confounded by age effects. Most reports start from the hypothesis that the telomere attrition rate is constant during life and hence not age-dependent, which is clearly not the case. Thus, the identification of determinants of telomere erosion will be most difficult in cross-sectional analyses that do not provide the possibility of assessing individual telomere attrition rates. Ideally, longitudinal studies should be performed to address telomere shortening kinetics with age. We are currently finalising the first round of a longitudinal population study in a middle-aged population (35-55 years) by studying telomere length in a large population (>2,500 subjects), in an attempt to define both reference values and basic determinants of average PBL telomere length. The study will be repeated on a 4-year basis.

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