Telomere Length Measurement in Different Ocular Structures: A Potential Implication in Corneal Endothelium Pathogenesis

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Purpose. Human chromosomes are protected at their end by a long portion of hexameric tandem repeats, the telomere. In somatic cells, telomere attrition caused by endogenous and exogenous oxidative stress as well as DNA replication can threaten genomic integrity and lead to the deterioration of tissue functions and an age-related physiological decline. The human eye is a complex organ in which cells of different ocular tissues are exposed to photo-oxidation, high mitochondrial metabolic activity, and/or replicative pressure.

Methods. We employed a highly sensitive quantitative PCR technique to determine relative telomere length in different human ocular structures.

Results. The longest telomeres in all ocular structures analyzed are found in neural retina, and the shortest are in the cornea. Within the retina, retinal pigment epithelium has four times shorter telomeres when compared to neural retina. However, no age-dependent telomere attrition in the retina and no difference between telomere lengths in the macular region and the rest of the retina have been found. In the cornea, stroma has the longer telomeres. In the corneal endothelium, we found a clear age-dependent telomere shortening. Since the endothelium is one of the most metabolically active ocular structure, this result suggests that endogenous oxidative stress from high mitochondrial activity is a major determinant of telomere loss in this structure.

Conclusions. Taken together, our results imply that the aging process and telomere attrition in the different ocular structures are the result of multiple factors and could not be attributed to solely exogenous or endogenous oxidation or DNA replication.

Keywords: telomere, human eye, ocular structures, aging, corneal endothelium

Telomeres, a long portion of double-stranded hexameric tandem repeats (5′-TTAGGG-3′), are crucial for genome integrity and stability.[1] They prevent inappropriate repair and chromosome fusions, and they shelter chromosome ends from enzymatic degradation or environmental insults. During DNA replication, some nucleotides at the 3′ end of the lagging template remain uncopied, translating into progressive telomere shortening with every round of replication in somatic cells.[2] Therefore, a decline in telomeric length is observed with advancing age in replicating cells.[3]

When telomere reaches a critical level, cells lose their proliferative potential.[4,5] Thereby, they initiate growth arrest and apoptosis or permanently exit the cell cycle via the activation of a p53-mediated replicative senescence pathway.[6] This is followed by the deterioration of tissue functions and an age-related physiological decline.[7–9]

Apart from replication, oxidative stress, the most important factor of aging,[10] is also a major modulator of telomere shortening.[11–13] Over time, in nonproliferative or quiescent somatic cells, the progressive accumulation of oxidative damage in telomeres promotes their premature attrition in a replication-independent way.[14] It has been demonstrated that telomeres are hypersensitive to oxidation because of their enrichment in guanine. Thereby, due to oxidative stress, vulnerable single-stranded DNA-damaged regions occur far more often in telomeres than in any other repetitive DNA sequence.[15] Moreover, repair of breaks induced in those regions are still incomplete after 19 days in telomeres compared to complete break repair within 1 day elsewhere in the genome.[15] In addition, Kawanishi and Oikawa have shown that telomeres are hypersensitive to ultraviolet-A (UV-A)–induced 8-oxo-7,8-dihydro-2′-deoxy-guanosine (8-oxodG), the preeminent oxidized DNA lesions induced by UV-A.[12] They also found that the extent of telomere shortening correlates with the increasing amount of UV-A–induced 8-oxodG and concluded that 8-oxodG formation plays a role in the acceleration of telomere-shortening rate. In postmitotic organs, it has been reported that excessive telomere shortening can drive p53-mediated repression of the peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1), a key regulator of mitochondrial biogenesis and metabolic process.[8] This leads to

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a mitochondrial and metabolic decline consistent with the degenerative state observed in aging quiescent tissues.

For all these reasons, telomeres are considered a valid biomarker of biological aging as well as of oxidative stress–related diseases.[16] It has been widely used to investigate the rate and factors of aging in human skin,[3,17–20] a tissue subjected to the combined effects of chronological aging and photoaging.[21] Although the importance of telomere length loss in skin cells is well established and documented, the human eye has received little attention in this regard.

The eye is a highly metabolically active sense organ constitutively exposed to oxidative stress, especially because of its light-absorbance capacity. It also contains structures with highly proliferative cells. Recently, some studies focused on the implication of telomere loss in different eye pathogeneses such as glaucoma, cataract, and dry age-related macular degeneration,[22,23] but also on age-related characteristic changes exhibited by human corneal endothelial cells.[24] However, to our knowledge, no attempt to analyze and compare the aging rate across ocular structures has been made. Recently, work in our laboratory provided, we believe, the first evidence that chronic ocular exposure to UV-A wavelengths affects the corneal extracellular matrix composition and catalyzes the structural and functional changes found in aged individuals' corneas.[25] Ultraviolet-A wavelengths, a component of the sunlight, efficiently induce reactive oxygen species (ROS) and trigger oxidative stress via chromophore photosensitization reactions.[26,27] In addition, previous work in the laboratory strongly suggested that oxidizing UV and blue radiation catalyze the age-related accumulation of mitochondrial DNA mutations in the corneal stroma and in the macula region within the retina, thereby participating in ocular aging phenotypes.[28,29] Taken together, those studies suggest that cells in some ocular structures might be disposed to accelerated aging and telomere shortening because of a high cell turnover rate, endogenous source of oxidative stress (mitochondria with high energetic production), and/or exogenous source of oxidation (daily exposure to solar UV-A radiation).[30] In light of all data illustrating the importance of telomere length for cell survival and tissue integrity, telomere shortening comparison across ocular structures could provide insights into the causes and consequences of human ocular aging.

Therefore, the aim of this study was to identify any differences in telomere length and shortening with age as an indicator of accelerated aging. We focused our efforts on the cornea, the most anterior part of the eye and the most exposed to sun radiation; the iris, the structure controlling the amount of light reaching the retina; and the retina, where light is absorbed to achieve the visual cycle. Using a highly sensitive fluorescent-based quantitative real-time PCR (qPCR) technique, telomere lengths have been estimated in ocular structures from elderly subjects. We observed a great heterogeneity in telomere length across and within structures from a same eye, indicative of different rates of telomere loss in each structure. This implies that different combinations of aging factors determine the fate of each ocular structure. More strikingly, our results emphasize an age-related telomere shortening in the corneal endothelium, suggesting a potential relation with endothelial pathologies.

Materials and Methods

All experiments performed in this study were conducted in accordance with our institution's guidelines and the Declaration of Helsinki. The research protocols received approval by the Centre de Recherche du CHU de Québec - Université Laval institutional committee for the protection of human subjects.

Human Eye Structures Isolation

Human eyes were obtained from La Banque d'Yeux du Centre Universitaire d'Ophtalmologie (Québec, Canada). We used a total of 52 postmortem human eyes (corneal explants or eyeballs) from a 7-year-old subject and from 45- to 89-year-old donors, unsuitable for transplantation and without overt telomere-related diseases. Transplantation exclusion criteria are based on the tissue quality criteria (e.g., scars). All donors died from classic aging diseases such as cancer, pneumonia, or heart failure, excluding the 7-year-old subject who died of Rett syndrome, a genetic disease devoid of telomere-related disorder. Except for the 7-year-old subject, in the presence of familial history, genetic disease, or viruses (e.g., hepatitis, AIDS), the eyes were not enucleated and were thus unavailable for research purpose. The eyes were removed no later than 1 hour after death. They were dissected or frozen at −80°C for later dissection immediately upon reception 24–48 hours after enucleation.

Ocular structures were isolated as previously described.[28,29] Briefly, whole ocular globes were dissected, and cornea, iris, and retina were collected. To separate the three cellular corneal layers, the corneas were incubated in HEPES buffer (0.01 M HEPES, pH 7.45; 0.142 M NaCl; 6.7 mM KCl; and 1 mM $CaCl_2$) with 2 mg/mL of a neutral protease, grade II (Dispase II; Roche Applied Science, Branford, CT, USA) for 18 hours at 4°C, and the epithelium, the stroma, and the endothelium were mechanically isolated.[29] The retinal pigment epithelium (RPE) was mechanically separated from the neurosensory retina by manual dissection using the same treatment as previously described.[28] All structures were conserved in PBS and frozen at −20°C until used.

DNA Purification

For each ocular structure, total DNA was isolated using a DNA extraction kit (DNeasy Blood and Tissue Kit; Qiagen, Valencia, CA, USA) according to the manufacturer's protocol, with an RNase treatment. When required, melanin was extracted from melanin-containing tissues (neurosensory retina, RPE, and iris), as melanin interferes with PCR amplification.[31] Melanin extraction was conducted using a chromatography column (Micro Bio-Spin Chromatography Column with Bio-Gel P-6 in Tris buffer; Bio-Rad Laboratories, Berkeley, CA, USA) to ensure that isolated DNA is free of melanin.

Telomere Length Analysis by Quantitative Real-time PCR (qPCR)

Telomere length was quantitatively measured using a real-time thermocycler (Rotor-Gene Q; Qiagen). Fluorescent-based qPCR is a highly sensitive and reproducible method allowing a good estimation of the relative quantity of telomere repeats in a sample.[32] A relative telomere length was determined by amplifying the telomere region and the human β2-globulin (HBG) gene using specific oligonucleotide primers. *HBG* is an invariable single-copy gene and was used in this study as an endogenous control of DNA amounts.

Polymerase chain reactions were achieved using a qPCR sybr green mix (Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix; Agilent Technologies, Santa Clara, CA, USA). Two final reagent solutions containing 1 ng of total DNA, 200 nM of oligonucleotide primers (telomere primers pair or *HBG* primers pair), and 1X SYBR Green mix were prepared. Forward and reverse telomere-specific primers were, respec-

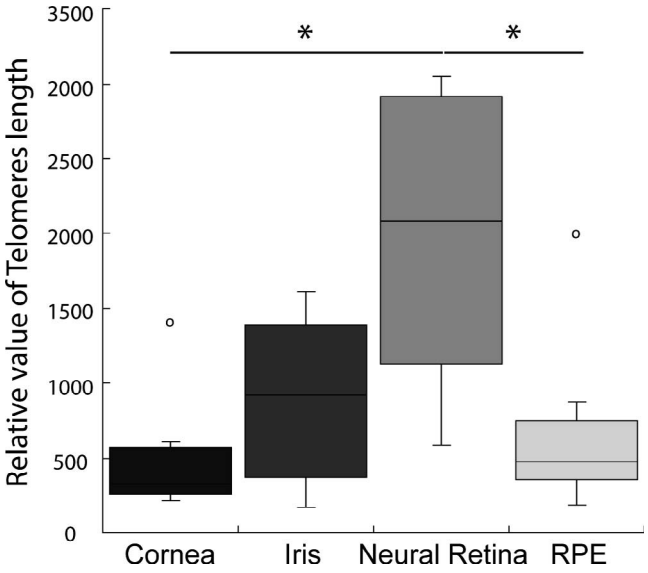

FIGURE 1. Telomere length in different human ocular structures. The cornea, iris, neural retina, and RPE were dissected, and DNA extraction was performed from each structure. Relative telomere length was determined by qPCR and estimate using the $2^{-\Delta Ct}$ method as described in Material and Methods. The experiment was performed using seven independent donors ($N = 7$), each done in quadruplicate ($n = 4$), with a median age of 65 years old. The paired-data Wilcoxon signed rank test reveals a significant difference in relative telomere length between the cornea and the neural retina and between the RPE and the neural retina ($^*P < 0.02$). No statistical differences can be found between the cornea, the iris, and the RPE.

tively, 5′-GGT TTT TGA GGG TGA GGG TGA GGG TGA GGT-3′ and 5′-TCC CGA CTA TCC CTA TCC CTA TCC CTA TC CCTA-3′. *HBG* primers were forward 5′- GCT TCT GAC ACA ACT GTG TTC ACT AGC-3′ and reverse 5′-CAC CAA CTT CAT CCA CGT TCA CC-3′. Each sample was processed in quadruplicate, and a negative control (no DNA) was included in each run. The PCR cycle was as follows: 95°C incubation for 3 minutes followed by 40 cycles of 20 seconds at 95°C, 60 seconds at 56°C for hybridization, and 20 seconds at 72°C for elongation.

Our analysis was based on the cycle threshold (Ct) value, which is the cycle number where the fluorescent signal from the PCR products crosses a set threshold of detection. This threshold has been determined on the linear portion of the amplification curves (above background levels and below saturation level) and was the same for all qPCR runs. Cycle threshold values were generated using analysis software (Rotor-Gene Q; Qiagen). The coefficient of variation (CV) of quadruplicate Ct values for telomeres and *HBG* were ≤1.5% and ≤1%, respectively. The relative telomere length in our samples was calculated as a telomere repeats copy number/*HBG* copy number ratio using the equation $2^{-\Delta Ct}$ (where $\Delta Ct = Ct^{Telomere} - Ct^{HBG}$).

Statistical Analysis

Software (KaleidaGraph, v4.5.2; Synergy Software, Reading, PA, USA) was used to perform the statistical analysis. As telomere lengths did not follow a normal distribution within eye structures, we applied the nonparametric statistical Kruskal-Wallis test to assess differences in multigroup comparisons, followed by the Wilcoxon signed rank test for pairwise comparison. When comparing only two groups, we used the nonparametric statistical Wilcoxon test for the analysis. Significance level was defined for P value ≤0.05. Data are presented as median with interquartile range of 25th-75th percentile.

RESULTS

Telomere Length in Different Human Ocular Structures

To identify differences in telomere length in different human ocular structures, the relative telomere length has been measured in corneas, iris, neural retinas, and RPE from seven different subjects with a median age of 65 years (age range, 54–89 years). The cornea, with a $2^{-\Delta Ct}$ median value of 325.7, is the structure with the shortest telomeres, whereas the neural retina presents the longest telomeres among the ocular structures analyzed, with a median of 2,073.8 (Fig. 1). Iris and RPE medians relative telomeric lengths are 921.3 and 478.5, respectively. Multigroup comparison indicates a significant difference in telomere lengths of ocular structures ($P = 0.026$). Pairwise comparisons reveal a statistically significant difference in telomere length between neural retinas and corneas ($P < 0.02$) as well as between neural retinas and RPE ($P < 0.02$). Thus, telomeres in neural retina are 5.2 (95% confidence interval [$CI_{95\%}$] = 2.4– 8.0) and 4.2 ($CI_{95\%} = 2.2$–6.1) times longer than in the cornea and the RPE, respectively. Neural retina telomeres tend to be longer than iris telomeres; however, this is not statistically significant ($P = 0.08$). No significant difference in telomere length is found between corneas, iris, and RPE.

Cornea Analysis Reveals Shorter Telomeres in Epithelium and Endothelium Layers

We supposed that telomere length follows a gradient from anterior to posterior in aged human eyes, which would correlate with the UV wavelength penetrance in the eye.[33] Indeed, the cornea, the most UV-exposed tissue, features the shortest telomeres in the eye (Fig. 1). The cornea is organized in three main cellular layer parts (from the outer to the inner): epithelium, stroma, and endothelium (Fig. 2A). We assessed the telomere length in each cellular layer using 11 corneas from different donors with a median age of 78 years (age range, 76–85 years). Result presented in Fig. 2B shows significantly longer telomeres in the stromal layer (median of 221.6) when compared to the epithelium (136.7) or the endothelial layer (110.8) ($P < 0.01$). More precisely, telomeres in the corneal stroma are 1.8 times ($CI_{95\%}$: 1.6–2.0) higher than in the epithelium and 2.1 times ($CI_{95\%}$: 1.6–2.5) higher than in the endothelium. No statistical difference is found between epithelium and endothelium ($P > 0.4$).

We further investigated the telomere shortening with age in endothelial cells. Telomere length in human corneal endothelial cells (HCECs) from 17 donors (11 men and six women), between 45 and 75 years old was thus compared to HCECs from 15 donors (12 men, three women) between 75 and 90 years old. The average age of each group was 63.7 versus 80.0 years old, respectively. Our results show that telomere repeats are lost with age in HCECs (Fig. 2C), even if those cells are quiescent in vivo. Indeed, in the younger group (45–75 years), we found a median relative telomere length of 178.8, whereas it is 116.6 for the older group (75–90 years). Despite the close range between the two age groups, the comparison reveals a significant telomere length difference ($P = 0.0058$), with 1.53 times longer telomeres in the younger group. We have also analyzed the telomere length in HCECs from a very young donor (7 years old). We found a relative telomere length of 344.5, which is 1.9 and 3.0 times longer than in our 45–75

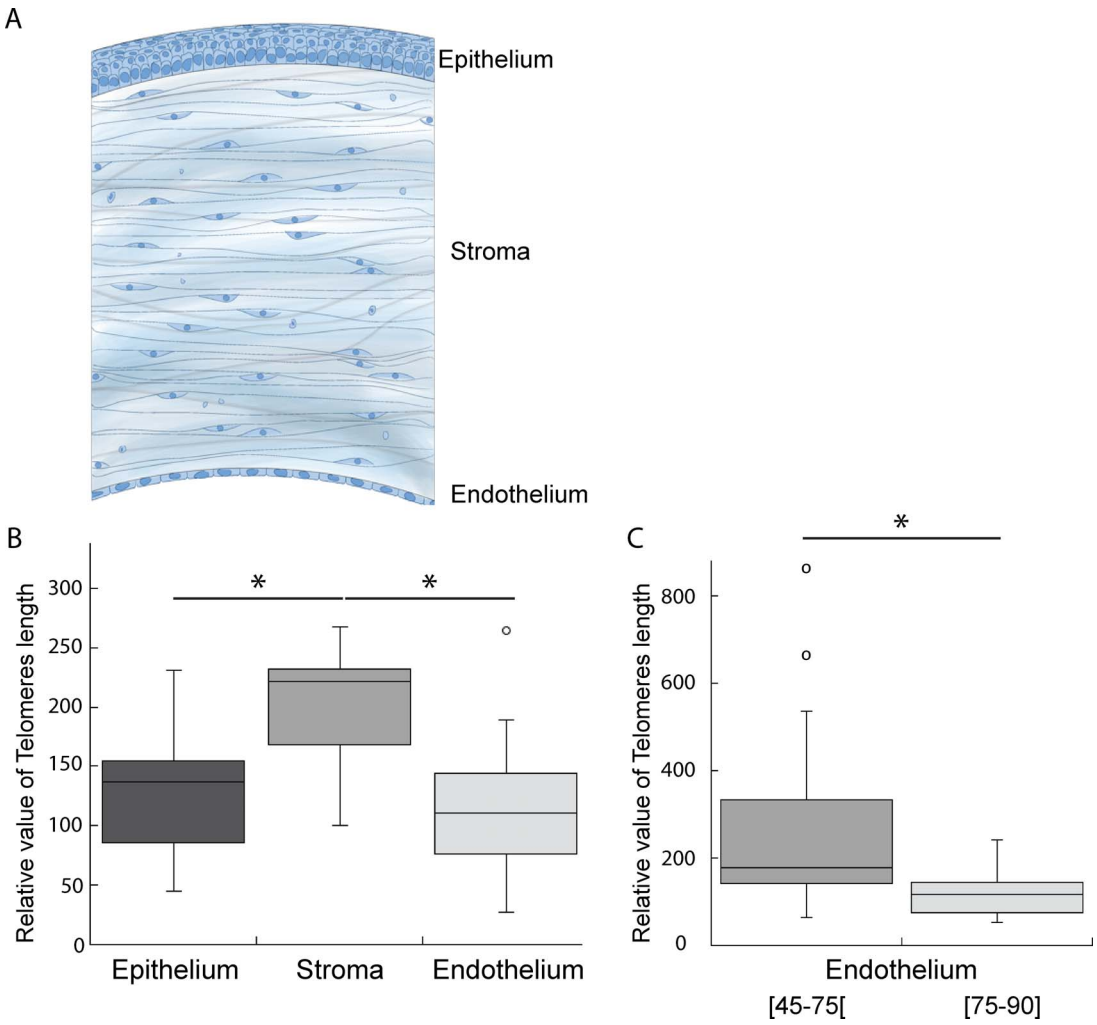

Figure 2. Telomere length in the different cellular layers of the cornea. (**A**) Schematic representation of the human cornea with its three cellular layers (from outer to inner): epithelium, stroma, and endothelium. (**B**) Corneas from 11 different donors with a median age of 78 years have been dissected to isolate the three cellular layers. DNA was extracted from each layer, and the relative telomere length within each layer has been estimated by qPCR. According to the paired-data Wilcoxon signed rank test, telomeres are significantly longer in stroma when compared to both the epithelium and endothelium. (**C**) Relative telomere length in human corneal endothelial cells (HCECs) from 17 donors aged between 45 and 75 years was compared from 15 donors aged between 75 and 90 years. The average age is 63.7 years and 80.0 years for the 45–75 and 75–90 groups, respectively. The unpaired-data Wilcoxon signed rank test reveals a statistically significant difference between the two age groups, demonstrating an age-related telomere shortening in HCECs. Each sample was tested in quadruplicate (*$P < 0.01$).

years and 75–90 years subjects, respectively. However, since donors from a young age are uncommon, it was not possible to confirm this observation with more individuals.

No Age-Related Telomere Loss in Neurosensory Retina and RPE

The posterior ocular part of the human eye, the retina, is composed of both the neurosensory retina and the RPE (Fig. 3A). While we observed the longest telomeres in the neurosensory retina, telomeres in RPE cells were significantly shorter ($P < 0.02$). Indeed, we found telomeres in RPE cells on average 4.2 times shorter than telomeres in neurosensory retina (Fig. 1). The neural retina is a highly metabolic layer, containing mainly photoreceptors (rod and cones) that convert light into nerve impulse. In humans, the macula (a 5.5-mm-diameter area of the retina, where the light focuses) is responsible for central and high-resolution vision. It contains a large concentration of cone cells, whereas the rest of the retina is concentrated in rod cells (Fig. 3A). The RPE, the outermost part of the retina, lies in direct connection with the choroid for blood supply, which makes it a highly oxygenated layer.

We first investigated whether there is a difference in telomere length between macular and nonmacular regions of the neural retina using six different donors with a median age of 60 years (age range, 45–87 years) (Fig. 3B). No significant difference in telomere length can be found between both regions of the neural retina (Fig. 3B). We also investigated the difference between macular and peripheral aged RPE from eight donors with a median age of 61.5 (age range, 45–85 years). No significant difference in telomere length can be found between macular and nonmacular RPE regions (Fig. 3C).

In an attempt to determine whether chronological aging leads to telomere shortening in human retina, we measured the telomere length in the retina of individuals of two different age groups. The first group consists of nine donors (two men, seven women) between 54 and 75 years old with an average age of 57.8 years. The second group consists of seven donors (one man, six women) between 75 and 90 years old with an

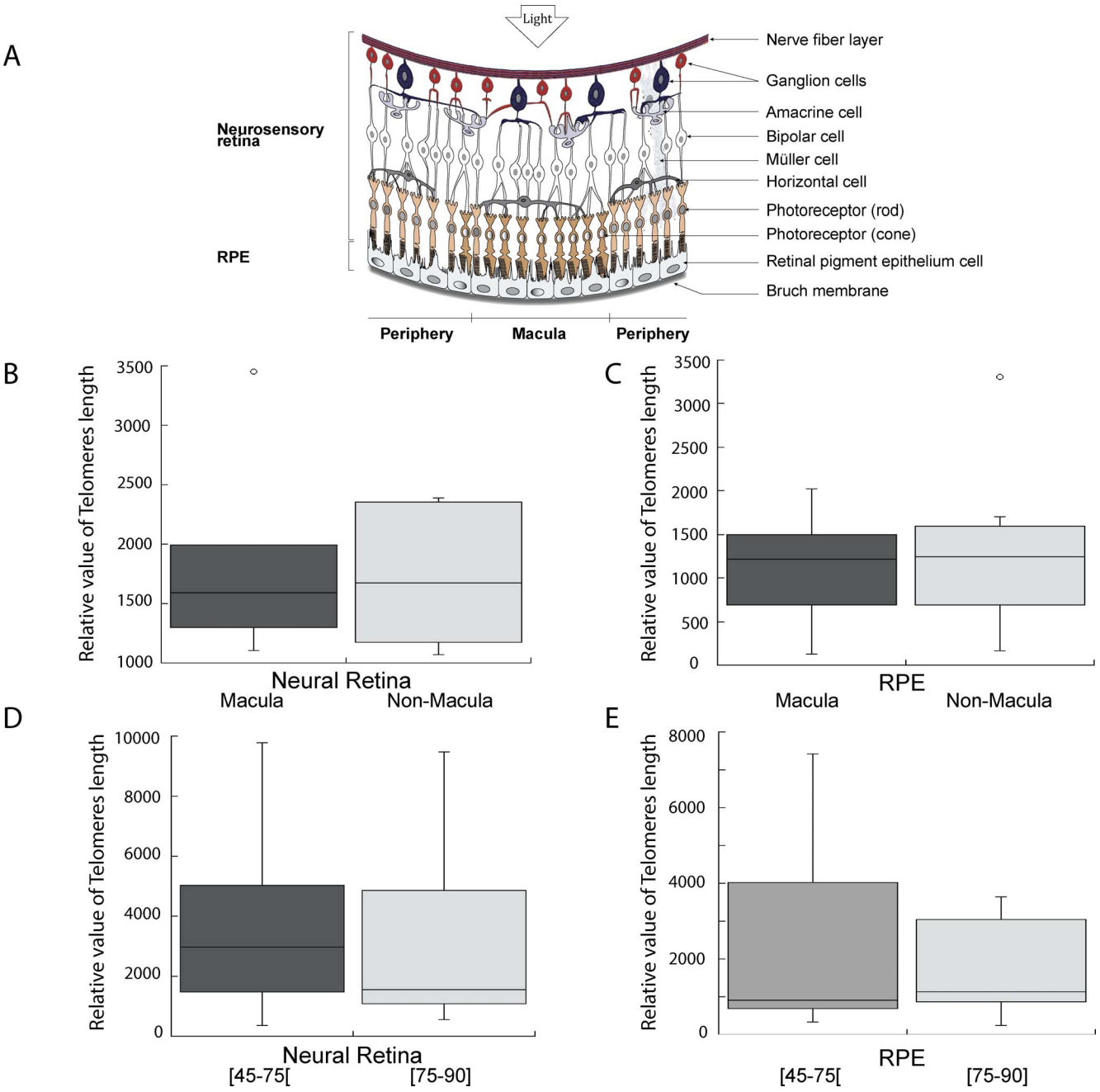

FIGURE 3. Telomere length analysis in human retina. (**A**) Schematic representation of the human retina depicting the neurosensory retina and the RPE. Macular and peripheral regions are described. (**B**) Neurosensory retinas from six donors aged between 45 and 87 years were dissected, and macular and nonmacular regions were isolated. Total DNA was extracted from each region, and relative telomere lengths in macular and nonmacular regions of neural retina were compared for each donor using the qPCR assay. No significant difference in telomere length can be found between macular and peripheral neural retina. (**C**) Telomere length in neural retina from nine donors aged 45 to 75 years old and seven donors from 75 to 90 years old were compared. The average age was 57.8 years and 83.7 years for the 45 to 75 group and the 75 to 90 group, respectively. The result shows that telomere length is not affected by age in neural retina. (**D**) Retinal pigment epithelium from eight subjects aged between 45 and 90 years were dissected, then macular and peripheral regions of RPE were separated. Total DNA was extracted from each region, and relative telomere lengths in macular and nonmacular regions of RPE were compared for each donor using the qPCR assay. No difference can be found between macular and peripheral RPE regions. (**E**) Relative telomere length in RPE from eight donors aged 45 to 75 years old and 10 donors from 75 to 90 years old were compared. The average age was 64.5 years and 81.2 years for the 45 to 75 years group and the 75 to 90 years group, respectively. Telomere length is not affected by age in RPE. The Wilcoxon signed rank test for paired data (**B**–**D**) and unpaired data (**C**–**E**) was used to assess statistical significance of results. Each sample was tested in quadruplicate.

average age of 83.7 years. Regarding the telomere length, no significant difference can be found between the two groups (Fig. 3D), suggesting that age does not influence telomere length in neural retina. Nonetheless, a great variability has been observed within each age group (Fig. 3D). Finally, we investigated telomere length in macular RPE from eight donors (two men, six women) between 54 and 75 years old and 10 donors (five men, five women) between 75 and 90 years old.

The average age was 64.5 and 81.2 years in the 45 –75 and the 75–90 years group, respectively. Similarly, no significant correlation between age and telomere length in RPE could be found (Fig. 3E).

Discussion

In this study, telomere length in cells of different ocular structures has been measured using a highly sensitive qPCR method. While telomere shortening is well established as a biomarker of chronological skin aging, it has never been used to investigate the possible aging rate differences between human eye structures. To our knowledge, this is the first report of telomere length comparison within the eye. Telomere attrition is a normal process in all somatic cells, and several factors promote its acceleration: a highly proliferative status,[5] exogenous sources of oxidation (e.g., light, especially short wavelengths),[11,18] or endogenous oxidation (e.g., from a high metabolic rate or inflammation).[30] The human eye contains highly proliferating and light-exposed cells as well as highly metabolically active structures. Thus, depending on their microenvironment, some cells within the same eye may experience a marked accentuation of their telomere attrition.

Our study was based on a cross-sectional design. To offset the limitations and discrepancies in age-related telomere shortening measurements associated with this type of design,[34,35] we estimated telomere length in relative value. Additionally, to overcome inter-individual variability associated with sex, age, or eye pigmentation, we compared different structures from same eyes and repeated this for each experiment, with comparison across structures.

Telomere Loss Is Accentuated in the Anterior Segment of the Eye and in RPE

In human adult eyes, UV wavelengths are filtered out by the cornea and the lens and do not reach the retina.[36] We observed shorter telomere in the cornea and longer in the retina, with the iris having intermediate telomere length (Fig. 1). As the role of UV-A wavelengths in telomere attrition is well established,[12,18] our gradient could be explained by UV-A penetrance in human eyes following daily exposure to sunlight.[33]

The cornea is the most anterior part of the eye, making it the most UV-A–exposed structure. It protects the eye against environmental insults. The iris is also exposed to UV-A light, but to a lesser extent than the cornea.[37] The cornea acts as a shield by filtering out more than 90% of UV-B (290–315 nm) and approximately 45% of UV-A (315–400 nm).[33,37] In this way, it protects the underlying structures against UV-induced DNA damage of the eyes.[33,38] We have also demonstrated that age-related mitochondrial DNA mutations, probably catalyzed by UV light, were concentrated in the cornea.[28,29] Our results depicting short telomeres in the cornea (Fig. 1), taken together with previous work done in our lab, support the hypothesis that sunlight exposure strongly accelerates the rate of corneal aging.[25,28,29,33,38]

The gradient of telomere length following light penetrance did not hold for the RPE, the most posterior part of the retina. Indeed, telomeres in the RPE cells are as short as in the cornea (Fig. 1). The retina (neural and RPE) is the highest metabolically active tissue in the human eye.[39] It exists under high oxygen partial pressure from the underlying choriocapillaries, an environment favoring ROS generation. Retinal pigment epithelium cells represent a major source of endogenous oxidation.[30] They are postmitotic and contain a large amount of mitochondria to sustain the energetic demand. Besides, RPE cells heterogeneously accumulate with age a large number of phototoxic chromophores in lipofuscin granules,[40] which generate oxygen-dependent damage when excited by blue light wavelengths (400–500 nm).[41–43] Photoreceptors in vertebrates' neural retina also contain potentially harmful blue light photosensitizers.[44] However, in normal conditions they do not accumulate in photoreceptor cells due to the rhythmic renewal of photoreceptors' outer segments.[45] The shortness of telomere in RPE cells might then reflect a telomere loss related to the increased lipofuscin-mediated generation of ROS with age in this part of the retina. Nonetheless, we did not find age-related decrease in telomere length in RPE from 54- to 90-year old individuals (Fig. 3E). Our age range might be too narrow to detect an age-related telomere length decrease in RPE cells.

No Age-Related or Area-Dependent Telomere Shortening in Retina

Our results indicate no age-dependent difference of telomere length in neurosensory retina (Fig. 3D). However, there was a great variability within each age group, which may account for the absence of trend toward shorter telomeres with age. It should also be noted that in neurosensory retinas our results reflected telomeres' length not only in photoreceptor cells but also in different neurons and ganglion cells with various sensitivity to oxidative stress.

We have previously observed a significant increase of mitochondrial DNA mutations in the macular region of aged neural retina and RPE when compared to peripheral regions.[28] It has been presumed that such an increase resulted from the blue light wavelengths focused throughout the eyes onto the macula. Moreover, age-related macular degeneration (AMD), the most frequent cause of irreversible blindness in western countries, affects only the macular region and is related to oxidative damage in the macula.[46–50] We found no difference in telomere length between macular and peripheral regions of both neural retina and RPE (Figs. 3B, 3C), and thus no relationship with macula-related pathogenesis could be inferred.

Because of their high level of metabolic activity, retina cells should be exposed to high levels of ROS and, thereby, be damaged. However, it is well established that the retina possesses efficient antioxidant mechanisms. The melatonin, a neurohormone synthesized in photoreceptors to modulate outer segment phagocytosis rates, and macular pigments (lutein, zeaxanthin, and meso-zeaxanthin), preferentially accumulated in the macula, have been found to protect the neural retina against oxidative injury.[51–55] Retinal pigment epithelium cells also have robust antioxidant protections, like eumelanin pigments (which efficiently reduce light-induced oxidation) and the nuclear factor erythroid-2–related factor 2 (Nrf2), a transcription factor essential to regulate the levels of a complex of antioxidant molecules, under oxidative stress.[56,57] However, recent studies demonstrated that in elderly persons, Nrf2 signaling in RPE is impaired,[58,59] and significant decreases in antioxidant molecule concentration have been linked to retinal diseases like AMD.[60,61] Throughout life, those defenses offset oxidation arising from both blue light–induced activation of toxic chromophores and high retina oxygen-consumption. When they become weaker, due to aging, disease, or poor lifestyle, oxidative-induced telomere shortening can be expected.

Age-Dependent Telomere Attrition in the Corneal Endothelium

Human cornea is constituted of three cellular layers, each harboring different properties: a multilayered self-renewal

epithelium with highly proliferative cells, a stroma containing quiescent fibroblastic-like cells, and a monolayered endothelium with highly metabolically postmitotic active cells. Telomere length does not follow any kind of gradient from the anterior to the posterior within the cornea (Fig. 2B). Previously, we have shown that UV absorption in cornea is mainly achieved by the epithelium.[33] Taken together with the fact that the epithelium contains mostly replicative cells, this could explain the shorter telomeres in this structure when compared to the stroma ($P < 0.02$) (Fig. 2B).

In the corneal endothelium, since HCECs are postmitotic and they receive less UV when compared to the epithelium and stroma, we were expecting to find longer telomeres. Moreover, we have previously shown that aged corneal endothelium was free of mitochondrial deletion, indicating that UV-induced oxidative stress does not play a critical role in aging phenotypes in this layer.[28,29] Surprisingly, we have measured the shortest telomere in this cellular layer when compared to other corneal layers (Fig 2B), suggesting that endothelium is subject to an accelerated telomere shortening for some reason. Furthermore, we found that HCEC telomeres were shortening with age (Fig. 2C). The corneal endothelium is one of the most metabolically active layers in the eye.[39] It is responsible for corneal stroma deturgescence (the state of relative dehydration required for corneal transparency), using sodium potassium adenosine triphosphatase (Na/K-ATPase) pumps.[62,63] Those pumps enlist 33% of endothelial cell overall ATP production.[39] As HCECs are nonproliferative in vivo, there is a progressive decline in endothelial cell density with age, but the human Na/K-ATPase activity remains constant throughout life.[64] Therefore, to compensate for the age-related cell loss, the pumps' density and/or function increase in remaining cells, and their mitochondria are thus constrained to a higher energy production pace. As a consequence, HCECs are exposed to higher oxidative stress from the enhanced respiratory process. Our results suggest that endogenous oxidative stress from a higher mitochondrial activity could be a major determinant of telomere loss of HCECs.

Age-dependent topographical differences related to cell density and senescence-like characteristics between the central and the peripheral portion of the corneal endothelium has been described in older donors.[65,66] Although we detected a significant telomere loss with age in the endothelium (Fig. 2C), no difference in relative telomere length in the central versus the peripheral portion of the corneal endothelium from four donors between 72 and 76 years has been found (data not shown). Similarly, using quantitative FISH analysis to estimate telomere length in HCECs, Konomi and Joyce found no differences in relative telomere length between cells in the central versus the peripheral endothelium.[24] We estimate that the higher oxidative burden arising from the age-related acceleration of cellular metabolism results in telomere shortening in endothelial cells, regardless of their location in the central or the peripheral endothelium, and thus might also play a role in the development of some endothelial corneal disorders arising with age.

In conclusion, our data indicate a complexity in the aging process across ocular structures, and even within a same structure, suggesting that telomere loss within the human eye is the result of multiple factors. We have observed ocular structures in which there is a constant cell replication having shorter telomeres than nondividing structures. Similarly, our results show that oxidation from exogenous (ultraviolet and blue light) or endogenous (mitochondrial activity) origin is not the sole factor in telomere shortening. There must be a combination of all those factors influencing telomere attrition rate in the different ocular structures. However, at this point, it is virtually impossible to determine the weight of each factor in the equation driving telomere-shortening rate.

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