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Telomere Length Correlates with Life Span of Dog Breeds

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SUMMARY

Telomeric DNA repeats are lost as normal somatic cells replicate. When telomeres reach a critically short length, a DNA damage signal is initiated, inducing cell senescence. Some studies have indicated that telomere length correlates with mortality, suggesting that telomere length contributes to human life span; however, other studies report no correlation, and thus the issue remains controversial. Domestic dogs show parallels in telomere biology to humans, with similar telomere length, telomere attrition, and absence of somatic cell telomerase activity. Using this model, we find that peripheral blood mononuclear cell (PBMC) telomere length is a strong predictor of average life span among 15 different breeds ($p < 0.0001$), consistent with telomeres playing a role in life span determination. Dogs lose telomeric DNA ~10-fold faster than humans, which is similar to the ratio of average life spans between these species. Breeds with shorter mean telomere lengths show an increased probability of death from cardiovascular disease, which was previously correlated with short telomere length in humans.

INTRODUCTION

Telomeric T_2AG_3 DNA repeats are lost in most mammalian cell types that replicate (Harley et al., 1990), and telomere length predicts the capacity of normal diploid cells in culture to replicate (Allsopp et al., 1992). Oxidative stress (Parrinello et al., 2003) and the inability of DNA polymerase to replicate the ends of linear DNA molecules (Olovnikov, 1971) play major roles in telomere loss in different species (Richter and von Zglinicki, 2007). When sufficiently short, telomeres initiate a stress response that includes activation of ATM (Vaziri et al., 1997) and p53 (Atadja et al., 1995), resulting in cell senescence (Kipling et al., 1999; Hemann et al., 2000). This can be reversed by expression of the enzyme telomerase, a reverse transcriptase that elongates telomeres (Bodnar et al., 1998; Vaziri and Benchimol, 1998).

A previous analysis of banked human blood samples uncovered a correlation between short telomere length and increased probabilities of mortality from age-associated heart disease (>3-fold) and infectious diseases (>8-fold) (Cawthon et al., 2003). Subsequent studies of both monozygotic and dizygotic twins and very elderly populations have yielded conflicting results (Bakaysa et al., 2007; Bischoff et al., 2006; Cawthon et al., 2003; Chiang et al., 2010; Kimura et al., 2008; Martin-Ruiz et al., 2005), leaving the question of whether telomere length affects life span per se, in the absence of premature mortality from age-associated diseases, unresolved. Use of the best-defined mammalian genetic model, mice, has not helped investigators to address this issue directly, because the dynamics of rodent telomeres differ significantly from that of human telomeres (for example, rodents have much longer telomeres and exceedingly short life spans). Furthermore, rodent studies have not found correlations between telomere length and senescence or life span in mice and rats that contain wild-type telomerase (Blasco et al., 1997; Hemann and Greider, 2000; Melk et al., 2003), most likely due to the predominant role played by stresses such as oxidative stress in inducing senescence in rodent cells (Parrinello et al., 2003; von Zglinicki et al., 2003), in contrast to telomeric signals, which are only seen in mice after several generations of telomere loss (Strong et al., 2011).

The domestic dog (*Canis lupis familiaris*) is a well-defined model that theoretically can reduce the effects of the intrinsic genetic diversity of the outbred human population. Dogs have 78 acrocentric autosomes, with males having XY and females having XX sex chromosomes (Parker et al., 2004, 2010). Within their genomes, dog breeds on average show an up to 100-fold greater linkage disequilibrium than humans (Sutter et al., 2004), indicating significant interbreed genetic divergence. This presents an ideal model for genetic analysis because the breeds are genetically isolated and inbred, especially compared with the relatively outbred human population. Relevant to this study, the average life span of dog breeds also ranges widely, from ~5.2 years for French mastiffs to >14 years for miniature poodles (http://users.pullman.com/lostriver/breeddata.htm). In both dogs and humans, cultured fibroblasts lose telomeric sequence (McKevitt et al., 2002), and telomere length decreases with increasing donor age. In addition, telomere length and telomerase activity in different tissues are comparable between the

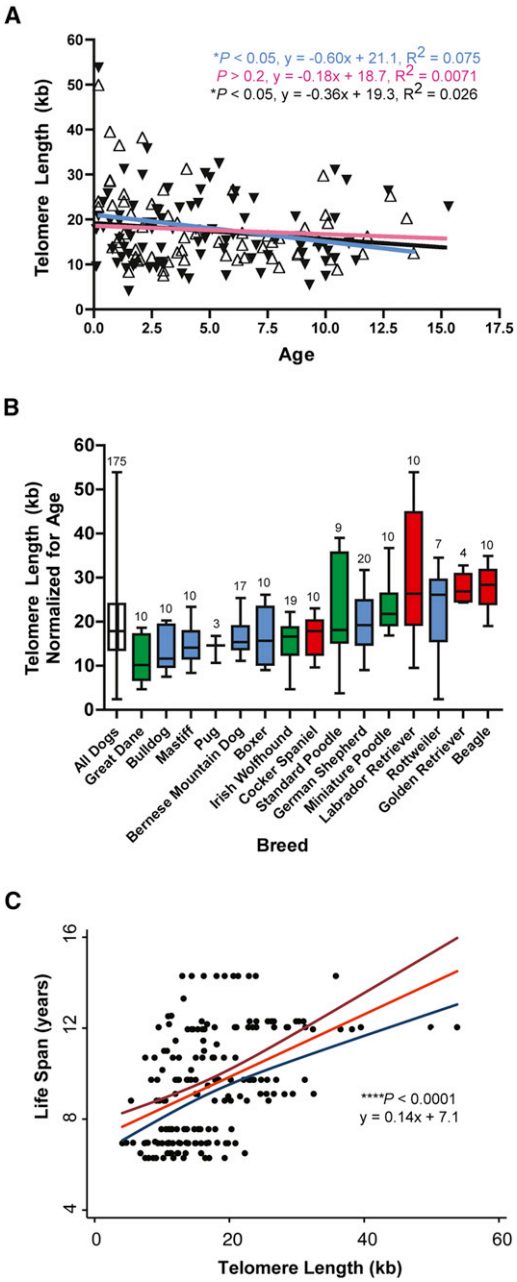

Figure 1. Telomere Length Predicts Life Span

(A) Absolute telomere length versus age was determined for 175 randomly collected healthy individuals of 26 breeds. Data are plotted for both sexes (black line), males only (open triangles, blue line), and females only (closed triangles, pink line).

(B) Telomere lengths of 15 breeds with three or more samples. Dogs are categorized into working (blue), herding (green), and hunting (red) classes, with breeds within classes being relatively more genetically similar than breeds in other classes (Sutter et al., 2004). Boxplots illustrate the distribution of the raw telomere data, with numbers above indicating the number of dog per breed.

(C) Average telomere length of breeds correlates positively with breed life span. Data are plotted as raw values with interval regression (orange) and 95% confidence intervals (brown and blue) for all 175 dogs.

two species (Nasir et al., 2001). These factors indicate that if telomere length does influence mortality and life span, as suggested by some studies of humans, long-lived dog breeds should have significantly longer telomeres.

RESULTS

In this study, we first confirmed that, as in humans, canine leukocyte telomere length decreases with age (Figure 1A). However, this occurred at a rate of 360 bp/year ($p < 0.05$) versus 20–40 bp/year in humans (Unryn et al., 2005). Also as in humans, we found that male dogs lose telomeric sequence slightly faster than females (Unryn et al., 2005; Figure 1A). The method we used to measure telomeres allowed us to represent the mean telomere length from quantitative PCR (qPCR) in kilobases for each dog sample, because there is an absolute quantification step inherent in the protocol (O'Callaghan and Fenech, 2011). The average telomere length determined from 175 dogs of 26 breeds varied from 11.4 kbp in Great Danes to 27.9 in beagles (Figure 1B), with variability noted between and within breeds. Some of this variability may be due to differential rates of telomere loss with age, if dog telomere attrition follows patterns similar to those observed in humans (Frenck et al., 1998; Unryn et al., 2005). Figure 1B represents the box plot distribution of the data within the 15 breeds for which we had telomere measurements for three or more animals. Determination of telomere length by the qPCR method (O'Callaghan and Fenech, 2011) was followed by age adjustment based on the telomere attrition rate determined by linear regression of telomere length versus age for all dogs in Figure 1A. These data were then subjected to both interval-regression and multiple-regression analyses of average breed life span versus average breed telomere length. Interval regression adds assumed variance into the values of mean life span, and therefore life span is represented as an interval. Interval regression relies on likelihood methodology rather than least-squares methodology, and yields regression coefficients and confidence limits that dictate the strength of the association. The interval regression introduced a variance of ±1.5 years to each breed's predicted average life expectancy. The results remained significant even after a variance of ±3 years was introduced. Multiple regression was used to rule out the potential for confounding or modifying influences of dog sex and/or age at sample collection. These rigorous analyses uncovered a very strong positive correlation between telomere length and average breed life span (Figure 1C; $p < 0.0001$), supporting the idea that telomere length is predictive of and may contribute to breed longevity. We also calculated the mean telomere lengths for the breeds using an age adjustment based on our calculated rate of telomere loss for all of the dogs. The average breed life spans and average age-adjusted telomere lengths for the 15 breeds are shown in Table 1, listed as the mean ± SEM as opposed to the median values in Figure 1B.

A comparison of mortality data from a meta-analysis of 74,556 dogs (Fleming et al., 2011) with the quantified telomere lengths of the breeds suggested that similarly to humans (Cawthon et al., 2003; Huda et al., 2007), dog breeds with shorter telomeres show a higher mortality from cardiovascular failure

Table 1. List of Average Telomere Lengths and Life Spans for the 15 Major Breeds Examined

Breed	Age-Adjusted Telomere Length (kb)	SEM	Average Life Span (Years)
Beagle	27.87	1.50	12.30
Bernese mountain dog	16.33	0.89	7.56
Boxer	16.51	2.10	8.81
Bulldog	13.45	1.47	6.29
Cocker spaniel	16.95	1.33	10.70
German shepherd	19.75	1.41	9.73
Golden retriever	27.75	1.97	12.04
Great Dane	11.39	1.59	6.96
Irish wolfhound	15.53	0.92	6.94
Labrador retriever	29.65	4.48	12.04
Mastiff	14.76	1.33	6.50
Miniature poodle	23.29	1.82	14.29
Pug	14.03	1.79	10.00
Rottweiler	25.31	2.31	9.11
Standard poodle	21.74	3.58	11.95
All 175 dogs	18.82	0.59	9.68

than breeds with longer telomeres (Figure 2A). Additional analyses suggested that shorter telomeres also correlate with increased mortality from gastrointestinal disease (Figure 2B), musculoskeletal disorders (Figure 2C), and respiratory failure (Figure 2D), but, as might be expected for tissues that do not show significant turnover, not with neurological disorders (Figure 2F). Thus, the data are consistent with short telomeres predisposing to diseases that arise in organ systems with rapidly replicating cell types that lose telomeric sequence, as has been reported for many disorders and diseases in humans (Lansdorp, 2009) and rodents (Bernardes de Jesus et al., 2011). Surprisingly, we found no relationship between telomere length and mortality due to hematopoietic disorders (Figure 2E) or to cancer (Figure 2G).

DISCUSSION

Previous studies found correlations among various forms of stress, telomere erosion-dependent senescence, the development of a variety of diseases, and, ultimately, mortality. In this work, we examined the relationship between average telomere length and disease and mortality using an independent model that recapitulates human telomere characteristics and is ideally suited to address this question due to the greater linkage disequilibrium seen in dog breeds compared with human races. We find that genetically distinct breeds of dog harbor telomere lengths that are highly variable, with averages ranging from ~11 to 27 kbp, and that these averages correlate very strongly with breed life span. Given the much larger ranges in telomere length and life span among breeds of dogs compared with human races/ethnic groups, the correlation between telomere lengths and life span is clear and statistically significant using this model, with our study of 175 dogs showing an extremely strong correlation ($p < 0.0001$). We also find that the average rate of telomere loss with age (~360 bp/year) in the dog breeds examined is much higher than that seen in humans (20–40 bp/year). This ratio correlates well with the ratio seen between average canine (9.7 years) and human (82 years) life spans, further suggesting that telomere length is linked to life span in species in which telomerase activity is repressed in somatic cells. Although these correlations are very strong, it is important to note that by themselves, they do not indicate that a causal relationship exists, and the correlation could be due to currently unknown factors. For example, factors such as breed size and metabolic rate have been correlated with breed life span. The basis for such correlations is unclear but may include variations in insulin-like growth factor among breeds. However, given the congruence seen between these data and those presented in several studies of human populations, and a recent study in zebra finches in which telomere length early in life correlated well with life span (Heidinger et al., 2012), the most straightforward interpretation is that average breed telomere length is linked to and contributes to determining the average life span of different dog breeds.

The different methods used to analyze telomere length have inherent strengths and limitations (Aubert et al., 2012). The method we used here (qPCR combined with an internal oligomer standard) yields values that have been reported to correspond well with those obtained using the original method of terminal restriction fragment (TRF) analysis (O'Callaghan and Fenech, 2011). In an independent study (shown in Figure S1), we confirmed that both methods yield very similar relative values of telomere length. The qPCR method can provide more accurate results for average telomere length because TRF analysis tends to overestimate telomere length due to variations in the subtelomeric sequence and the presence of non-telomeric repeat sequences in some telomeres that, although they do not hybridize themselves, contribute to making fragments appear longer. However, qPCR does not indicate either the range of telomere length in samples or the shortest telomeres, both of which can be determined by single telomere length analysis (Baird et al., 2003). Despite this potential limitation, average telomere length seems to be more closely related to the onset of senescence than the shortest telomere, particularly in analyses of mass cultures of cells (Martens et al., 2000), as performed in this study, and thus we chose to focus on this parameter.

Combined with a previous large meta-analysis of canine diseases, our data also indicate that mortality due to diseases of replicating cell types such as respiratory and gastrointestinal epithelium and cardiovascular endothelial cells, but not of non-replicating compartments such as the central nervous system, correlates strongly with breed telomere length. This is consistent with short telomeres in canines predisposing to diseases that arise from rapidly replicating cell types that lose telomeric sequence. Given the link between oxidative stress and telomere erosion (Parrinello et al., 2003; von Zglinicki et al., 2003), this more rapid loss of telomeres in dogs predicts that canine cells may have less effective mechanisms than human cells for repairing oxidative damage, as noted previously for rodents (Parrinello et al., 2003). The lack of a relationship between telomere

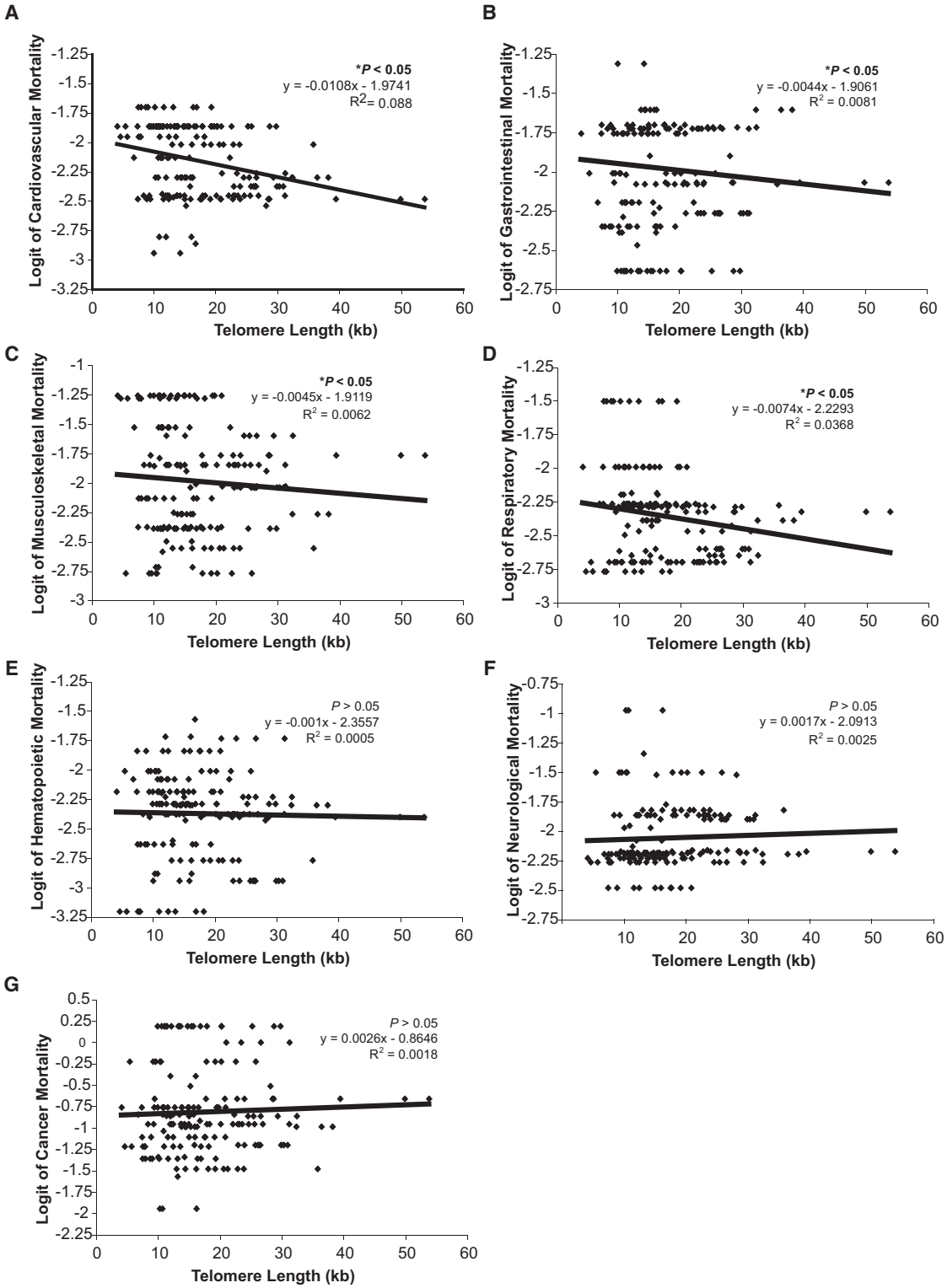

Figure 2. Telomere Length Is Associated with Organ System Mortality

(A–G) Breeds with short telomeres show susceptibility to (A) cardiovascular ($p < 0.05$), (B) gastrointestinal ($p < 0.05$), (C) musculoskeletal ($p < 0.05$), and (D) respiratory ($p < 0.05$) disorders. Telomere length does not correlate with the rates of (E) hematopoietic diseases, (F) neurological disorders, or (G) cancer. Data are plotted as the log of odds (logit) of mortality versus telomere length for all 175 dogs. The regression lines indicate the trend.

length and mortality due to hematopoietic disorders or to cancer that we find in dogs is in contrast to studies in humans, including a large longitudinal study that reported a correlation between shorter telomeres and increased cancer incidence and cancer mortality (Willeit et al., 2011). This lack of congruence may be due in part to the considerably longer life spans of dog breeds with longer telomeres, and the very strong correlation between cancer incidence and age (http://seer.cancer.gov/), which may obscure links between telomere length and cancer. Alternatively, certain inbred breeds may acquire mutations or alleles of genes predisposing to cancer that overshadow the effects of telomere length, as appears to occur in particular cancers in different races/ethnicities of humans (Haiman and Stram, 2010).

The distribution of telomerase activity in different dog tissues is also similar to that seen in humans, with appreciable telomerase seen only in the male germline and in the majority of cancers (Nasir et al., 2001). Telomere length is largely inherited from parents (Chiang et al., 2010; Olsson et al., 2011; Unryn et al., 2005). Because telomere length increases with age in sperm (Allsopp et al., 1992), and in both humans (Unryn et al., 2005) and other species (Olsson et al., 2011), offspring conceived by older fathers have longer telomeres than those conceived by younger fathers, breeding practices in dogs (specifically the age of the stud males used) may be used to affect the breed life span. For example, in humans, the average telomere length in children is 22 bp longer for each year older their father was at conception; therefore, telomeres in children conceived by 50-year-old fathers are ~660 bp longer than those in children conceived by 20-year-old fathers (Unryn et al., 2005). If this relationship holds true in dogs, as suggested by their similar telomere biology, it may be possible to increase the average healthy life span significantly, particularly in shorter-lived breeds, over a small number of generations, given the observed heritability of telomere length (Chiang et al., 2010).

EXPERIMENTAL PROCEDURES

Sample Collection and DNA Isolation

Whole-blood samples were collected from registered dogs of established breeds. The age of each dog was established at the time of collection. Samples were drawn by licensed professionals through venipuncture of the cephalic vein as approved by the National Human Genome Research Institute and/or the University of Calgary Animal Care and Use Committee. In all cases, blood samples were collected into acid citrate dextrose or EDTA anticoagulant. DNA isolation was performed using standard proteinase K digestion followed by phenol/chloroform extraction.

qPCR for Telomere Length Measurement

We utilized a technique for absolute telomere length measurement based on the Cawthon method, which allows for accurate calculation of relative telomere length using known quantities of PAGE-purified standard telomere and canine ribosomal acidic protein (36B4-C) DNA oligo sequences (O'Callaghan and Fenech, 2011). The primers and oligos used are listed below:

Telo standard:
$(TTAGGG)_{14}$
36B4-C standard:
GGGTGTCCGCAACGTTGCCAGCGTATGTCTGCAGATAGGTTACCCGACTGTTGCATCAGTGCCCCATTCTATCATCAATGGATACAAGCGGGTCCTGGCTTTGTCTGTGGAAACTGA

Telo sense:
CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT
Telo antisense:
GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT
36B4-C sense:
CGTTGCCAGCGTATGTCTGCAGAT
36B4-C antisense:
ACAGACAAAGCCAGGACCCGCT

Standard reactions were set up in triplicate using six serial 10X dilutions of telo DNA standard from 60×10^{-12} g to 600×10^{-18} g, and six serial 10X dilutions of 36B4-C DNA standard from 200×10^{-12} g to 2×10^{-15} g. Sample reactions were set up in triplicate using 10 ng of template DNA. All reactions contained the Maxima SYBR Green qPCR Master Mix (2X; Fermentas). Telomere length in kilobase per reaction and diploid copy number per reaction were calculated to generate values for telomere length/diploid copy. Telomere length/diploid copy values were divided by 156 to account for the number of canine telomeres, and then by four (as there are four copies of the 36B4 gene in the canine genome) to obtain a mean value for telomere length in kilobase per sample. Additional internal controls using human cell DNA analyzed in parallel by TRF assay, as previously described (Unryn et al., 2005), showed that similar results for average telomere length were obtained with both methods (Figure S1); however, due to the inherent limitations of this analysis (Aubert et al., 2012), information regarding the range of telomere lengths or shortest telomeres in each sample was not obtained.

Statistical Analysis

The telomere attrition rate was determined by linear regression. We used interval and multiple regression to account for variability in the average breed life span predictions and to ensure that age at the time of sample donation and gender did not confound or modify the relationship between life span and telomere length. We used a linear regression of the log of odds (logit) (log[p/(1 − p)]) of mortality versus telomere length to generate trend lines for the different organ systems. Although the mortality fits are significant ($p < 0.01$–0.05), we note that variance in the probability of mortality values is not accounted for; however, this is unlikely to affect the overall interpretation. The correlation between cTERT and telomere length was determined by linear regression. All statistical analyses were performed using Stata Statistical Software (release 10; StataCorp LP, College Station, TX, USA).

Exclusion Criteria

Dogs were excluded if they exhibited any hematopoietic anomaly that could have affected measurement of telomere length from leukocytes, including chemotherapeutic treatment. No dogs were excluded in this study.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.11.021.

LICENSING INFORMATION

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