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Telomere Shortening in Kidneys with Age

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Abstract. The histology and function of the kidney deteriorates with age and age-related diseases, but the mechanisms involved in renal aging are not known. *In vitro* studies suggest that telomere shortening is important in replicative senescence, and is accelerated by stresses that increase replication. This study explored the relationship between age and telomere length in surgical samples from 24 human kidneys, which were either histologically normal (17) or displayed histologic abnormalities (7). Telomere loss was assessed by two independent methods: Southern blotting of terminal restriction fragments (TRF) and slot blotting using telomere-specific probes. The results of these methods correlated with each other. The mean TRF length determined by Southern blotting in cortex was about 12 kb pairs (kbp) in infancy and was shorter in older kidneys. The slope of the regression line was about 0.029 kbp (0.24%, $P = 0.023$) per year. Telomere DNA loss in cortex by the slot blot method was 0.25% per year ($P = 0.011$). By both methods, the telomere loss in medulla was not significant and was less than in cortex. Comparisons of TRF length from 20 paired samples from cortex and medulla showed that TRF was greater in cortex than medulla, with the differences being greater in young kidneys and lessening with age due to telomere loss in cortex. These findings indicate that telomeres shorten in an age-dependent manner in the kidney, either due to developmental factors or aging, particularly in renal cortex.

The kidney develops characteristic physiologic and pathologic changes with age termed "senescence" (1–4). GFR declines by about 0.75 ml/min per yr over age 40 (2), whereas renal vascular resistance rises and the filtration fraction falls. The decline in function is variable and some healthy individuals preserve their GFR indefinitely (5), while hypertension and heart failure accelerate senescent changes (2). Pathologic changes include a 20 to 25% loss of volume, particularly in cortex, fibrous intimal thickening of arteries, loss of glomeruli due to global sclerosis (perhaps reflecting occlusion of the afferent arteriole), and patchy tubular atrophy and interstitial fibrosis. Histologic studies on autopsy kidneys indicate that aging is associated with a loss of cells and an increase in the size of the nuclei (6).

Renal senescence has many implications for nephrology, including normal aging, excess acute renal injury, increased end-stage renal disease, decreased transplant survival, and increased cancer. The usual changes of normal aging are relevant to drug dosing and render individuals more susceptible to dehydration. The older population has a high frequency of acute renal failure, reflecting reduced renal reserve, increased comorbidities, and possibly increased susceptibility to acute insults. The elderly are also up to 100 times more likely to develop end-stage renal failure than the young (7). As recently reviewed (8), donor age has become the main identifiable influence on long-term graft survival (9–12), and the pathology of chronic allograft nephropathy (CAN) (http://tpis.upmc.edu/tpis/schema/KNCode97.html) overlaps the changes of aging (13,14). Kidney transplants from older donors have higher baseline serum creatinine, more delayed graft function, and reduced long-term survival. The effect of donor age in renal transplantation may be an example of the reduced ability of aged kidneys to tolerate and recover from injuries and stress. Hypertension and heart failure accelerate the changes of renal senescence (1–3). Renal cancer is age-related, and the problem of malignant transformation is intimately related to cell senescence mechanisms (15).

In 1985, Kaysen and Myers pointed out that "the mechanisms and the full biochemical and physiologic consequences of renal senescence remain to be fully elucidated" (16), a statement that remains true. The molecular basis of senescent changes *in vivo* is not known, and many theories of aging have been proposed, including oxidative damage, genomic instability (including telomere loss), genetic programming, and cell death (17). However, considerable progress has been made in determining the mechanisms of senescence *in vitro*. Primary cultures of somatic cells complete a finite number of cycles (the "Hayflick limit") (18), which reflects the age of the cell donor and their proliferative history. As they approach this limit, they cease to replicate and enter a state of replicative senescence. Replicative senescence *in vitro* is due at least in part to telomere shortening because it can be bypassed by transfection with the enzyme telomerase (19). Telomeres are

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DNA repeats of the sequence $(TTAGGG)_n$ that protect the ends of chromosomes, and are generated mainly by the enzyme telomerase. Because telomerase is not expressed in most somatic cells, telomeres shorten with increasing age, reflecting the number of cycles that the cell has completed. The telomere hypothesis of cell aging suggests that telomere shortening in the absence of telomerase is the mitotic clock for replicative senescence in normal somatic cells (20,21). As shortening becomes critical for a telomere on a particular chromosome, that chromosome becomes unstable and the cell stops dividing. Studies on human blood cells and blood vessels suggest that chronic stresses requiring a higher replication rate increase telomere shortening *in vivo* in humans (22,23). Studies of expression of markers for mitosis suggest that the kidney is subjected to ongoing replicative stress, *e.g.*, in endothelial cells (24).

We investigated telomere length in kidneys derived from nephrectomies and autopsy specimens from individuals of different ages. We found that telomeres in human kidney cortex shorten with age, and that the shortening is faster in cortex than in medulla. These observations suggest that telomere length may reflect either developmental changes or aging. Although the causes and significance of telomere shortening in various renal cell populations will likely prove to be complex, these data are compatible with a role for telomere shortening and replicative senescence in some of the phenomena that characterize renal aging.

Materials and Methods

Terminal Restriction Fragments

Samples were taken of kidney tissues derived from total nephrectomies or from autopsies. Whenever possible we collected cortex and medulla separately. All samples were snap-frozen in liquid nitrogen and stored at −80°C. To obtain high molecular weight DNA without degradation, we disrupted the tissue by freeze grinding. DNA was then isolated by proteinase K digestion and phenol/chloroform extraction. DNA samples were digested with the restriction enzymes *Hin*fI and *Rsa*I (Boehringer Mannheim, Mannheim, Germany) to produce TRF. Aliquots of undigested and digested DNA were resolved by 0.5% agarose gel electrophoresis (70 V, 2 h) and examined by ethidium bromide staining for the absence of unspecific degradation and complete digestion, respectively. A total of 1.5 μg of each digested sample was resolved by 0.7% agarose gel electrophoresis (40 V, 40 h). DNA was Southern-blotted onto a nitrocellulose membrane (Hybond-C Extra; Amersham) and probed as described previously (20,25) with minor modifications. The membranes were hybridized at 42°C overnight with a 5′ end-labeled ^{32}P-$(TTAGGG)_5$ oligonucleotide telomere probe in a buffer containing 25% formamide, 5× Denhardt's solution, 5× saline-sodium phosphate-ethylenediaminetetra-acetic acid, 0.1% sodium dodecyl sulfate, and 100 μg/ml denatured salmon sperm DNA. After a 15-min stringency wash at 42°C in 0.2× SSC, 0.1% sodium dodecyl sulfate, the autoradiography signal was digitized in a PhosphorImage scanner (Fuji) using ImageGauge software. All lanes were subdivided into intervals of approximately 1 to 2 mm. The mean size of the TRF was estimated using the formula $\Sigma(ODi \times Li)/\Sigma(ODi)$, where ODi is the density reading from interval I, and Li is the size in kilobase pairs (kbp) of the interval relative to the markers (20). Mean TRF length was determined over the range of 2.3 to 23.1 kbp markers (broad range) and also on the basis of the intensity of the signal (narrow range), where the intervals averaged were those intervals that were higher than 1% of the total signal in that lane. The median and mode values were also derived on the basis of the narrow range determination.

Slot Blot

Aliquots of high molecular weight, genomic DNA from cortex or medulla (described above) were slot-blotted onto a nitrocellulose or nylon membrane to determine the relative content of telomeric DNA as described previously (26) with minor modifications. A total of 3.2 μg of DNA was diluted with an equal volume of 0.5 M NaOH, 1.5 M NaCl, and denatured by boiling 5 to 10 min. Samples were placed on ice and neutralized by the addition of 0.5 M Tris, 1.5 M NaCl, pH 8.0. One microgram of DNA was slot-blotted in triplicate. A serial dilution of Jurkat DNA was used to generate a standard curve. The membrane was probed using the telomere probe described above and analyzed by phosphoimaging. The Jurkat standard curve was fitted with a polynomial equation and used to calculate the relative sample signal. After analysis, the blot was stripped and reprobed using a centromere-specific probe (GTTTTGAAACACTCTTTTTGTAGAATCTGC) and reanalyzed.

GFR

Creatinine clearance was predicted from serum creatinine in adult men, where C_{er} = (140 − Age [years]) × (wt [kg])/(72 × S_{er} [mg/100 ml]) and in adult women with a correction factor of −15% (27).

Results

Table 1 lists the clinical data of the individuals from whom the kidneys were derived. Seventeen normal samples were derived from autopsies ($n = 1$) or nephrectomies from patients with either renal cell carcinoma ($n = 11$), oncocytoma ($n = 2$), Wilms' tumor ($n = 1$), transitional cell carcinoma ($n = 1$), or severe renal artery atherosclerosis ($n = 1$). Normal renal tissue remote from the tumor was chosen for analysis. We will refer to these samples as "normal" kidneys when the histology was within the limits of changes expected for age. Three samples from the nephrectomies for renal tumors showed a small number of tumor cells representing a small minority of the cells present. Seven samples were derived from nephrectomies with histologic abnormalities such as pyonephrosis, chronic pyelonephritis, hydronephrosis, atherosclerosis, and nephrosclerosis. We will refer to those kidneys as "abnormal" kidneys. However, patients generally did not have marked renal insufficiency, as shown in Table 1, and the serum creatinine values were markedly abnormal only in the 1-mo-old infant with acute renal failure, the 9-yr-old with adult-type polycystic kidney disease (APCKD), and the 74-yr-old with chronic interstitial nephritis.

TRF in renal cortex shortened with age (Figure 1). From these blots, we plotted regression relationships of various measurements of the TRF distribution against age (Figure 2). We analyzed the mean, median, and mode of a narrowly defined TRF distribution ("narrow range"), and the mean of a more broadly defined distribution ("broad range"). All of these measurements showed a significant TRF shortening with increasing age in renal cortex. Based on the mean (narrow range) (Figure 2), the slope of the regression is 0.0293 kbp per year,

Table 1. Demographic data on the 24 individuals from whom the kidneys were derived[a]

Age (yr)	Gender	Diabetic	Hypertension	BP	Creatinine (μmol/L)	Urea	Clinical Diagnosis	Histology of Renal Parenchyma
0.1	M	No	No	58/30	143	20.7	Multiorgan failure	Normal
0.8	M	No	Yes	119/84	48	4.4	Renal dysplasia, secondary inflammation	Renal dysplasia, interstitial nephritis[b]
4	F	No	No	99/57	56	3.7	Chronic pyelonephritis, reflux	Acute on chronic pyelonephritis[b]
4	F	No	No	80/50	50	3.1	Wilms' tumor	Normal
5	M	No	No	114/66	85	NA	Hydronephrosis secondary to obstruction at uretopelvic junction	Hydronephrosis[b]
9	F	No	No	105/70	682	33.8	APCKD	Multiple cysts[b]
29	F	No	No	128/80	81	NA	Infected pyonephrosis	Infiltrate, tubular atrophy[b]
42	M	No	Yes	132/80	165	NA	RCC II, oncocytoma I	Normal
47	M	No	No	112/66	123	8.8	RCC III	Normal
50	M	No	No	150/96	114	NA	RCC II	Normal
50	M	No	No	118/70	123	NA	RCC III	Normal
51	F	No	No	118/70	82	3.4	Hydronephrosis	Hydronephrosis[b]
55	M	No	Yes	150/80	108	5	Oncocytoma II	Normal
57	M	No	No	120/70	109	4.3	RCC II/III	Normal
57	F	No	No	170/90	83	NA	RCC III	Normal
58	M	No	No	120/70	117	5.8	RCC	Mild age changes IF, TA
62	M	No	Yes	122/62	136	NA	RCC III	Mild age changes IF, TA
67	M	No	No	120/68	99	7.6	RCC II, CLL	Lymphoma infiltrate
68	F	No	Yes	140/84	137	5.5	Severe renal artery atherosclerosis	Age changes IF, TA, FIT
71	M	No	No	120/80	126	NA	RCC III	Normal
74	F	No	No	135/105	113	4.4	Papillary transitional cell carcinoma	IF, TA, FIT; compatible with age
74	F	No	Yes	150/80	189	NA	Hydronephrosis, pyelonephritis	Chronic interstitial nephritis[b]
79	F	No	No	140/84	112	NA	RCC I	Moderate TA, IF, FIT
88	F	No	Yes	180/88	96	NA	RCC II/III	Focal inflammation, IF

[a] NA, not applicable; APCKD, adult-type polycystic kidney disease; RCC, renal cell carcinoma; IF, interstitial fibrosis; TA, tubular atrophy; CLL, chronic lymphocytic leukemia; FIT, fibrous intimal thickening.
[b] "Abnormal" with histology outside the limits of changes expected for age.

i.e., 29 bp per year (0.24%). Nevertheless, the outliers argue against a simple predictable annual loss. (This regression analysis was not significantly altered if samples with histologic abnormalities were excluded: The regression was still significant [data not shown]. To avoid selection bias, all samples were included in the subsequent analyses.) The Y intercept of these regression relationships provides an estimate of the TRF length at birth: 12.4 $\pm$ 0.64 kbp by the narrow range, and 11.2 $\pm$ 0.34 kbp by the broad range.

We grouped the mean cortex TRF lengths for kidneys of different ages to see if loss was accelerated in some age ranges. For this purpose, the 29-yr kidney with pyonephrosis and short TRF of 8.8 kbp was not included, as it was the only observation between 10 and 40. The groups showed mean TRF lengths as follows: age 0.1 to 9 yr, 12.2 kbp; age 42 to 50 yr, 11.5 kbp; age 51 to 58 yr, 11.5 kbp; age 62 to 68 yr, 10.6 kbp; age >71 yr, 10.1 kbp. These data do not suggest an acceleration in one age group. However, when the 17 kidneys older than 40 yr were examined by regression, the slope suggests TRF shortening of 82 bp per year ($r^2 = 0.3985$; $P = 0.0066$), indicating that there is telomere shortening in cortex in the age range in which senescence develops.

Medulla samples available on 20 specimens showed slightly shorter mean TRF length in young children (Figure 3). The

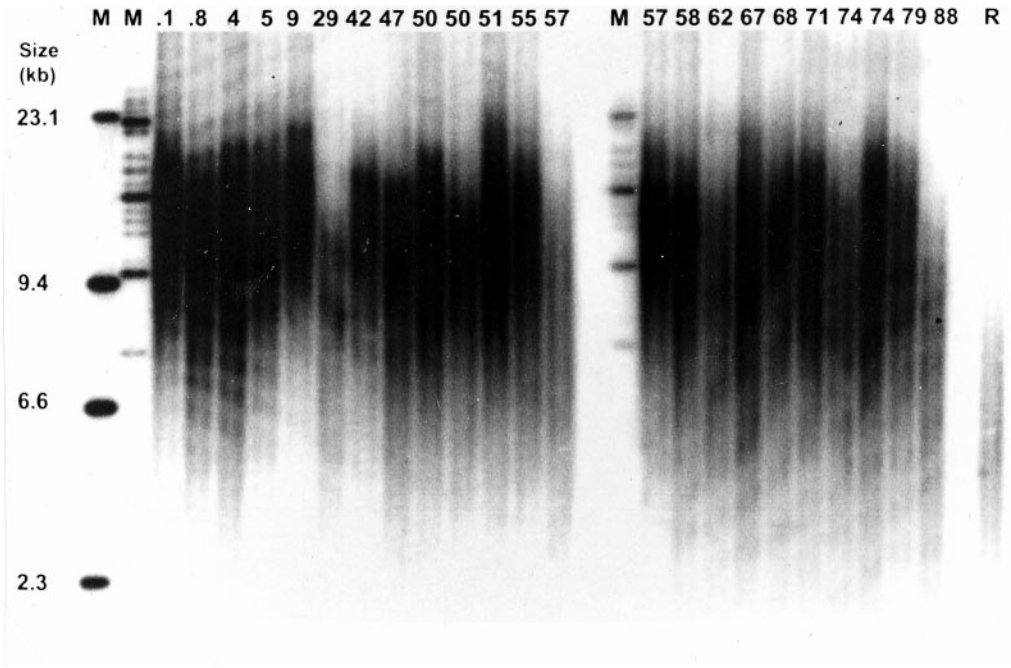

Figure 1. Telomere length in human renal cortex samples. Genomic DNA from cortex samples derived from different donors of indicated ages was prepared as described and resolved by agarose gel electrophoresis. Telomere restriction fragments were detected with a ^{32}P-labeled telomeric oligonucleotide. Size (kbp) is indicated. M, molecular weight marker; R, Raji cells.

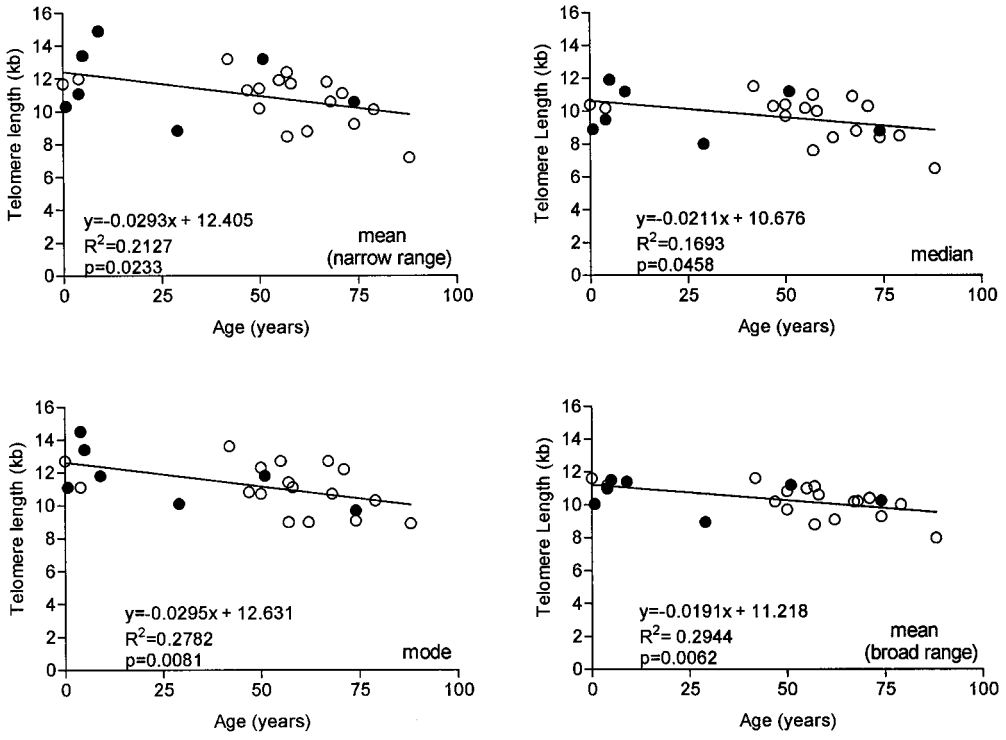

Figure 2. Regression of telomere length measurements in renal cortex by Southern blotting against age. Panels represent the mean, the median, and the mode of the telomere distribution. Filled circles represent "abnormal" kidney samples with histologic changes outside the limits of changes expected for age. (Narrow range mean was determined using values ≥1% of the total; broad range mean was determined using values over the entire molecular weight range. For details, see Materials and Methods.)

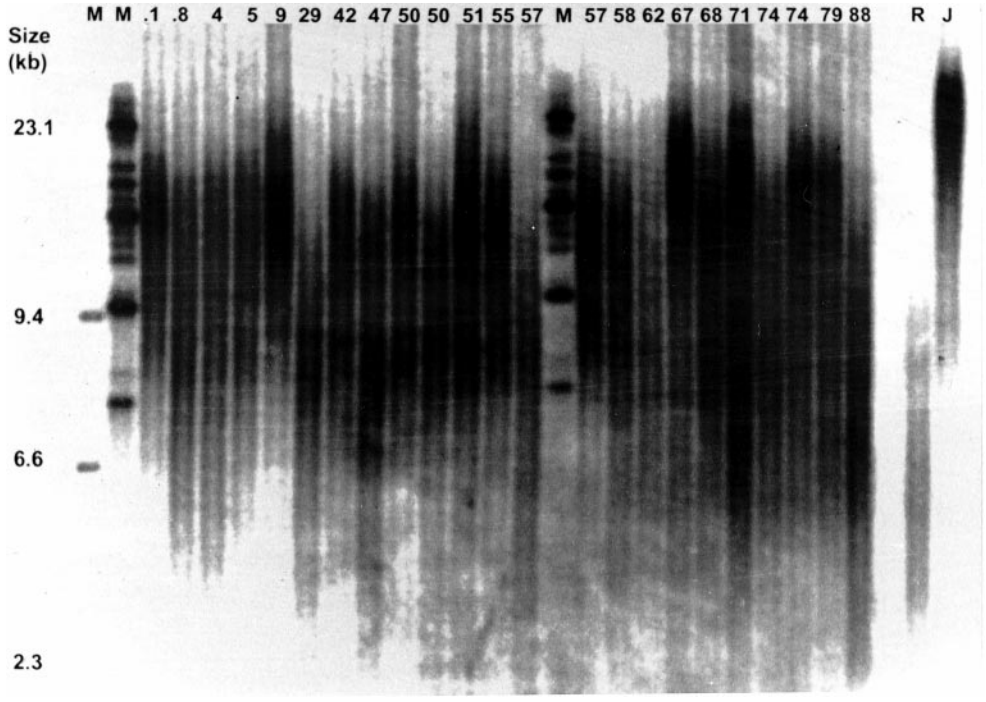

Figure 3. Telomere length in human renal medulla samples. Genomic DNA from medulla samples derived from different donors of indicated ages was prepared as described and resolved by agarose gel electrophoresis. Telomere restriction fragments were detected with a ^{32}P-labeled telomeric oligonucleotide. Size (kbp) is indicated. M, molecular weight markers; R, Raji cells; J, Jurkat cells.

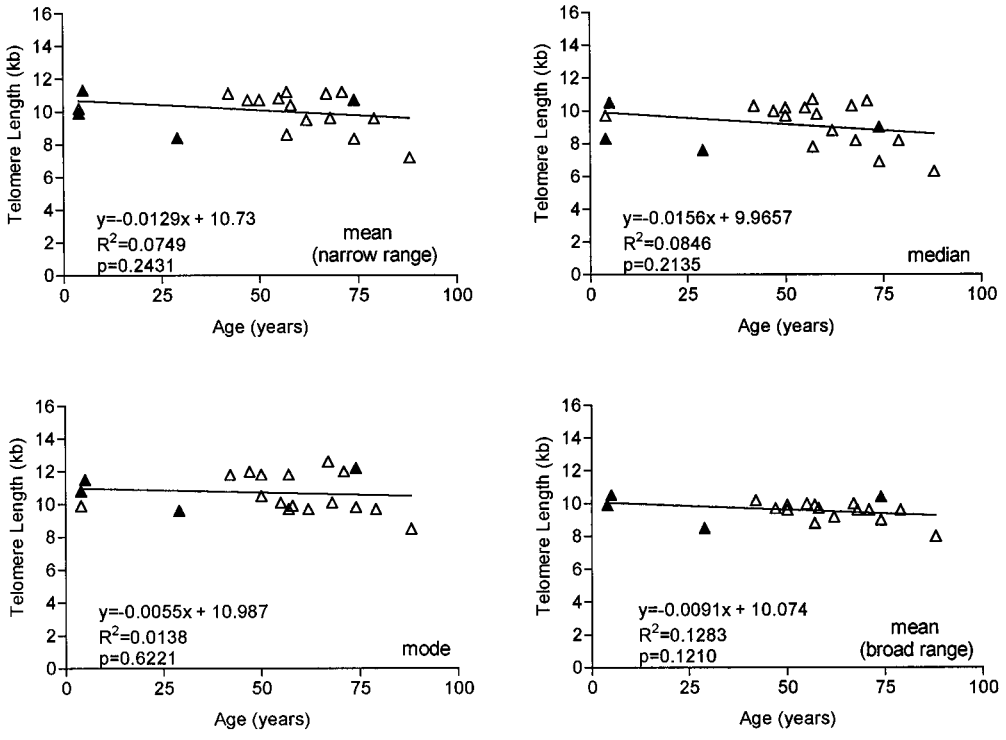

Figure 4. Regression of telomere length measurements in renal medulla by Southern blotting against age. Panels represent the mean, the median, and the mode of the telomere distribution. Filled triangles represent "abnormal" kidney samples with histologic changes outside the limits of changes expected for age. (Narrow range mean was determined using values ≥1% of the total; broad range mean was determined using values over the entire molecular weight range. For details, see Materials and Methods.)

estimated TRF lengths in medulla at birth (Y intercepts) were 10.7 ± 0.61 kbp (narrow range) and 10.1 ± 0.32 kbp (broad range), both significantly less than in cortex ($P < 0.05$). However, the medulla showed less of a tendency to TRF shortening with age. The estimates of the slopes of the mean, median, and mode of the narrow range and of the mean of the broad molecular weight range are shown in Figure 4. Mean TRF length in medulla declined slightly as a function of age by 0.0129 kbp (narrow range) and 0.0091 kbp (broad range) per year (not significant). Hereafter, the "narrow range" mean is used.

Comparing Cortex versus Medulla in Paired Samples

We compared the TRF length in cortex *versus* medulla for paired samples ($n = 20$) on which clear cortex-medulla distinctions could be made (Table 2). The mean TRF length in the cortex was longer (10.8 ± 1.6 kbp) than in medulla (10.1 ± 1.2 kbp) ($P < 0.001$). In kidneys under age 10 ($n = 3$), the TRF length in the cortex (12.2 ± 1.2 kbp) was about 1.7 kbp longer than in medulla (10.5 ± 0.74 kbp) ($P = 0.012$). In kidneys age 50 and below ($n = 8$), the TRF length in cortex was 11.4 ± 1.5 kbp *versus* 10.4 ± 0.91 kbp in medulla ($P = 0.007$). For kidneys above age 50 ($n = 12$), the mean TRF length in cortex was 10.3 ± 1.5 kbp *versus* 9.9 ± 1.2 kbp in medulla ($P = 0.014$). Above age 60 ($n = 8$), the mean TRF length was 9.9 ± 1.5 kbp for cortex *versus* 9.7 ± 1.4 kbp for medulla ($P = 0.11$), only about 0.2 kbp difference. Thus, increasing age was associated with more TRF shortening in cortex than medulla, tending to eliminate the differences between cortex and medulla (Figure 5).

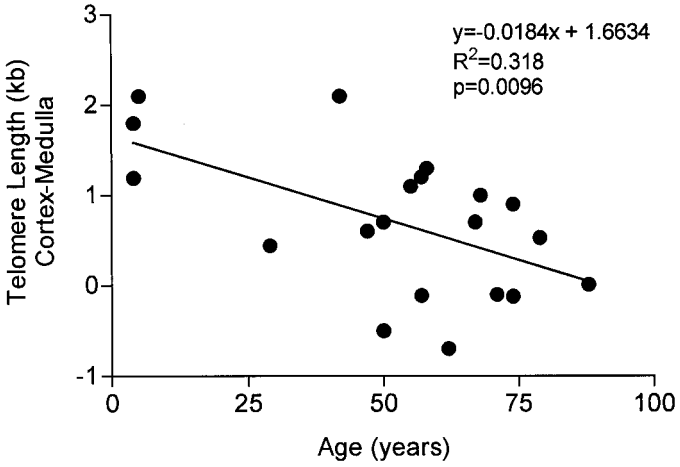

Figure 5. Regression of the difference between cortical and medullary telomere lengths against age. Telomere length in paired renal cortex and medulla samples were determined by Southern blotting as described. The difference between cortex and medulla telomere lengths from 20 different donors is plotted against age.

Assessing Telomere Length by Slot Blots

We used a second method for assessing telomere loss by measuring the relative amount of telomere DNA in slot blots, *i.e.*, the extent of binding of a telomere probe to a standard amount of DNA. We compared the amount of telomere DNA to the centromere DNA as a control, reasoning that centromere DNA would be more stable than the telomere DNA over time (Figure 6). We also calculated the ratio of the telomere to centromere DNA. We found that the telomere DNA in renal cortex as assessed by slot blotting with the telomere probe decreased with age. The slope of this relative loss was similar to the slope of the loss of cortical TRF—about 0.25% per year for slot blots *versus* 0.24% per year for TRF (Figure 7). We compared the TRF to the slot blot results (Figure 8). The TRF values correlated with the slot blot values in cortex ($r^2 = 0.6424$, $P < 0.0001$). The centromere DNA estimates tended to decline with age in cortex (not significant) (Figure 6). Thus, the telomere-to-centromere ratio did not decrease with age in cortex.

In medulla, there was little trend toward loss of telomere DNA (Figure 8). Regression analysis estimated the loss of telomere DNA per year at 0.09% in medulla. This estimate of the loss over time was similar to that rate of TRF loss (about 0.12% per year) shown above. The telomere estimates by both methods correlated with one another ($r^2 = 0.2189$, $P = 0.0375$).

GFR versus Telomere Length

The relationship of TRF length to GFR was assessed because both of these measurements decline with age. The calculated GFR declined with age (Figure 9A) as expected, by about 1.3% per year from the third decade. This is a higher rate than in a normal population and presumably reflects the selection for renal diseases. There was a weak positive relationship

Table 2. Telomere length in paired cortical and medullary samples with age

Age	Telomere Length (kbp) (mean ± SD)		*P* Value
	Cortex	Medulla	
All paired samples ($n = 20$)	10.77 ± 1.62	10.06 ± 1.16	0.0004
Under age 10 ($n = 3$)	12.16 ± 1.16	10.47 ± 0.47	0.0120
Under 50 yr ($n = 8$)	11.43 ± 1.50	10.38 ± 0.91	0.0070
50 yr and over ($n = 12$)	10.33 ± 1.53	9.85 ± 1.23	0.0144
Over 60 yr ($n = 8$)	9.93 ± 1.46	9.65 ± 1.38	0.1144

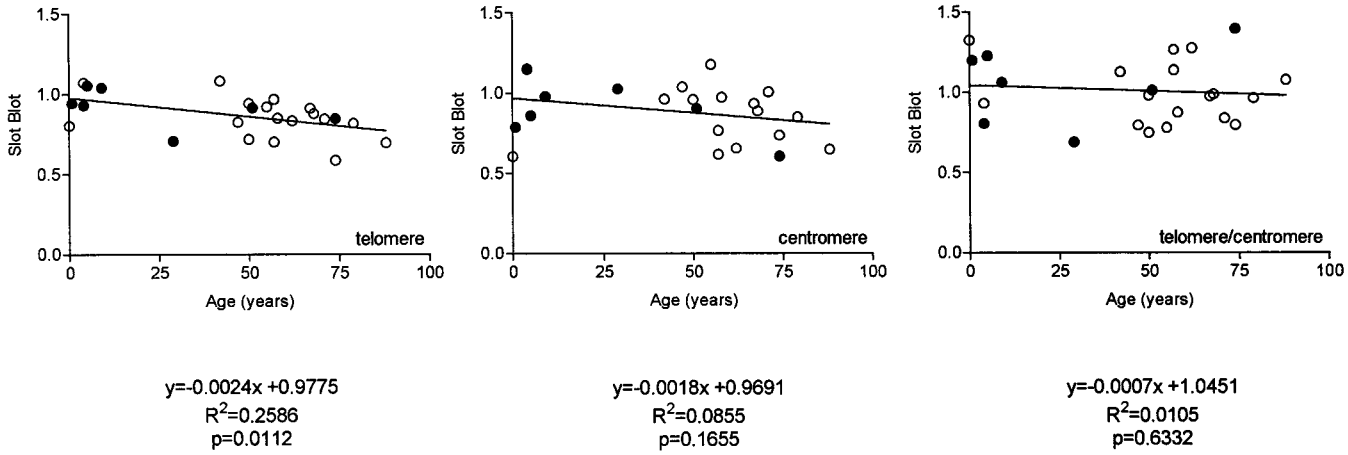

Figure 6. Regression of telomere and centromere DNA content by slot blot analysis and the telomere:centromere DNA content from renal cortex. One microgram of genomic DNA from cortex samples derived from different donors of indicated ages was prepared as described and slot-blotted in triplicate. Telomere DNA was detected with a ^{32}P-labeled telomeric oligonucleotide. Blots were stripped and reprobed with a ^{32}P-labeled centromeric oligonucleotide. Telomeric and centromeric DNA and the telomere:centromere DNA ratio is plotted against age. Filled circles represent "abnormal" kidney samples with histologic changes outside the limits of changes expected for age.

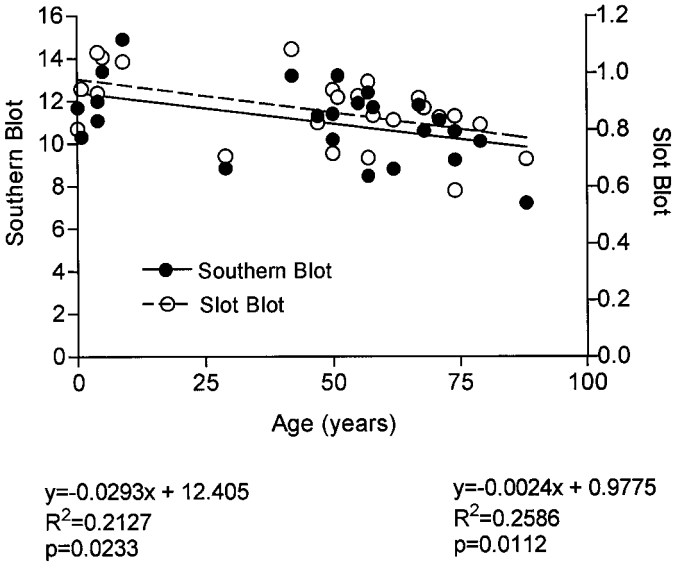

Figure 7. Comparison of the regression of telomere length by Southern and slot blot analysis in renal cortex against age. There was a significant correlation between the estimates of telomere length determined by TRF and telomere DNA by slot blot analysis ($r^2 = 0.6424$; $P < 0.0001$; paired t test). The regression of telomere length against age by Southern blot analysis estimates a 0.24% per year rate of loss of telomere length, while the analysis by slot blot estimates a rate of telomere DNA loss of 0.25% per year.

between calculated GFR and TRF length in cortex, which was not significant (Figure 9B).

Discussion

This report documents that both by TRF and slot blot, telomere DNA is lost with age in kidney, and that rate of loss in cortex is greater than in medulla. The TRF were longer in cortex than medulla in young kidneys, but the difference lessened with age due to greater telomere loss in cortex. Thus, the present data suggest that telomere shortening may be a phenomenon of both development and aging. Whether the extent of telomere loss in older kidneys would affect the ability of the kidney to sustain function against normal wear and tear or abnormal stresses is not known. However, given the heterogeneity of renal cell populations, and of telomere length on individual chromosomes, the present results raise the possibility that critical telomere shortening could become a limiting factor in some renal cell populations and could contribute to some of the features of the senescent kidney.

Certain caveats surround studies of telomere length. First, most studies (like these) are conducted on surgical specimens and must be confirmed on unselected normal tissues when the availability of tissue permits. Second, the critical measurements of telomere changes should be made in the population of renal cells that are likely to be limiting such as intimal cells in small arteries (22). Third, in presenting the regression between TRF or slot blots and age, we do not suggest that these are truly linear. In 24 samples, we cannot determine the shape of this relationship accurately (*e.g.*, accelerated early or late telomere loss.) Fourth, population changes could be mistaken for telomere shortening if a cell population with longer TRF was being replaced or infiltrated with a population with shorter TRF. On the other hand, if cells with short telomeres disappeared, then telomere shortening would be underestimated. Finally, the TRF determination is the gold standard method but has limitations. Specifically, because TRF are composed of telomeres plus 4 to 5 kbp of subtelomeric repeats (28), it is conceivable that differences in the subtelomeric repeats between cell populations could contribute to differences in TRF length, *e.g.*, between cortex and medulla. The slot blot is less elegant but is independent of subtelomeric influences. Telomere loss with age was significant in the renal cortex by slot blot analysis and TRF analysis (0.25% per year). The TRF difference between cortex and medulla was not paralleled by a significant difference in the slot blots (although there was a trend). Thus,

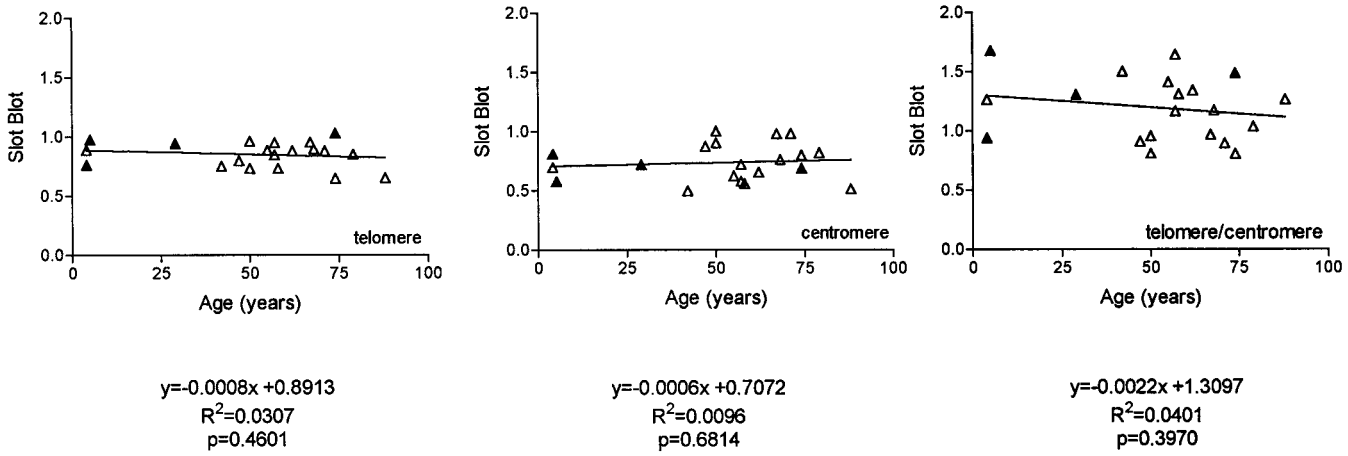

Figure 8. Regression of telomere and centromere DNA content by slot blot analysis and the telomere:centromere DNA content from renal medulla. One microgram of genomic DNA from medulla samples derived from different donors of indicated ages was prepared as described and slot-blotted in triplicate. Telomere DNA was detected with a ^{32}P-labeled telomeric oligonucleotide. Blots were stripped and reprobed with a ^{32}P-labeled centromeric oligonucleotide. Telomeric and centromeric DNA and the telomere:centromere DNA ratio is plotted against age. Filled triangles represent "abnormal" kidney samples with histologic changes outside the limits of changes expected for age.

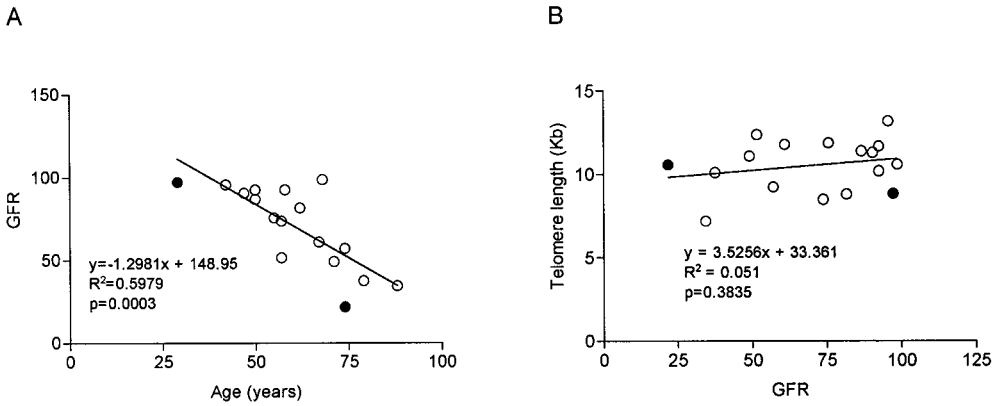

Figure 9. The relationship among GFR, telomere length, and age. GFR was calculated using the Cockcroft–Gault equation in those patients over the age of 20 from whom kidney biopsies were taken. (A) The regression between GRF and age. (B) The regression between GFR and telomere length by Southern blotting. Filled symbols represent "abnormal" kidney samples with histologic changes outside the limits of changes expected for age.

subtelomeric influences have not been excluded as an explanation of the cortical *versus* medullary differences.

Although telomere shortening with age has not previously been studied in kidney, it is known in other tissues, *e.g.*, blood cells. The rate of loss of telomere DNA in cortex is less than that reported for human lymphocytes, in which the rate of telomere loss is about 41 bp per year (29). Thus, in renal cortex telomere DNA declines at a rate intermediate between highly proliferative cells as lymphocytes and less proliferative tissues such as brain or muscle, in which TRF shortening is not detected (30,31). A recent study (32) assessed TRF length in blood and skin cells from humans of different ages, and from 15 other tissues from the fetus and eight other tissues from the 72-yr-old man. Significant differences ($P < 0.001$) were found in the shortest TRF size and in the variation of TRF length between the 20-wk fetus and the 72-yr-old man. The 72-yr-old man showed the shorter and more variable TRF for all tissues studied, but the greatest differences were observed in blood cells (*e.g.*, average TRF length was 12.2 kbp in the fetus and 7.2 kbp in the 72-yr-old man).

Although telomere regulation is complex, the principal cause of telomere loss is likely to be replication. Cell division in fibroblasts lacking telomerase shortens TRF by about 75 bp *in vivo* and 48 bp *in vitro* per population doubling (28). The observed telomere shortening in cortex with age may reflect the generation of renal cells through development and the replacement of cells lost through normal wear and tear or injury. Thus, telomere shortening reflects the replicative history of the tissue. There are also mechanisms of telomere shortening independent of proliferation. Fibroblasts *in vitro* show telomere shortening when exposed to high oxygen concentrations, even when their proliferation is inhibited, suggesting that free radical-mediated damage may shorten telomeres independent of replication (33) and may be prevented by

antioxidant strategies (34). Hemodynamic stress may cause telomere shortening in arteries, but whether the mechanism is dependent on proliferation is not known (22).

Telomere shortening may be accelerated by disease stresses, either by proliferative or other mechanisms, and could represent a mechanism of disease progression. For example, in active ulcerative colitis, mucosal cells of the affected colon show rapid turnover, and TRF length of the colonic mucosa of patients with colitis was shorter than that of the controls and of uninvolved mucosa (35). Thus, telomere shortening in the colonic mucosa may contribute to the chronic pathology of ulcerative colitis. Similarly, the stem cells of bone marrow transplant recipients show accelerated telomere shortening in the recipient compared to the donors (23). Thus, cycles of injury and repair in disease states may cause critical telomere shortening and eventually establish limits to tissue survival. In the present study, we did not find differences between tissues with histologic abnormalities and normal tissues. However, we have not sampled progressive and end-stage renal diseases adequately to answer this question.

The significance of telomere shortening in kidney over the range described is not clear. *In vitro*, telomere length predicts replicative capacity and the propensity to develop replicative senescence (28). Telomere shortening for a cell becomes critical when even one telomere reaches its threshold, because the effects of telomere shortening are dominant. Cell replication ceases and the cell expresses a new pattern of gene expression characteristic of replicative senescence (36). The mean TRF length in senescent fibroblasts is about 7 to 8 kbp but shows variation between clones. It is difficult to extrapolate from cultured fibroblasts to whole organs *in vivo*. To determine whether critical telomere shortening in the kidney contributes to renal senescence, we need new information. First, we should establish the rate of replication in renal cells at different ages and in disease states. Second, we should get estimates of telomere shortening in individual cell types, *e.g.*, intimal cells in arteries and mesangial cells. Third, we should determine whether lesser degrees of telomere shortening can induce functional changes without replicative senescence. Finally, we need to rule out the possibility that cells with short telomeres rapidly disappear, underestimating telomere shortening.

It is probable that molecular explanations will be found for normal renal aging, for the excess of end-stage disease in the elderly, for the poor performance of kidney transplants from old donors, and for the sensitivity of older kidneys to acute injuries. The development of new animal models and new technologies for studying human biopsy material would aid the identification of these mechanisms. Most common rat and mouse strains have very long telomeres, limiting their value in addressing the telomere regulation (37). Moreover, the changes in rat and mouse kidneys with age (38) differ from those in long-lived species such as the human. For example, spontaneous fibrous intimal thickening in arteries is characteristic of long-lived but not short-lived mammals (39,40). New mouse models such as the telomerase knockout mouse (41), or *Mus spretus* (37), which has telomeres resembling humans in length, should establish the significance of telomere shortening. Ultimately, we must study human kidneys to determine the mechanisms and significance of telomere shortening and other candidate molecular changes of senescence. The TRF method requires more DNA than is available from needle biopsy specimens, hampering our ability to answer such questions directly. In this regard, the slot blot adaptation described here may facilitate studies of human kidney biopsies. However, we still need methods to measure telomere changes and other candidate mechanisms of senescence in microscopic renal components (*e.g.*, arteries, glomeruli) and individual cells (*e.g.*, endothelial cells) likely to be limiting in aging, in diseases, and in transplants.

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