Comparisons of telomere lengths in peripheral blood and cerebellum in Alzheimer's disease

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Abstract

Background—Alzheimer's disease (AD) patients have been reported to have shorter telomeres in peripheral blood leukocytes (PBLs) than age-matched control subjects. However, it is unclear if PBL telomere length reflects brain telomere length, which might play a more direct role in AD pathogenesis. We examined the correlation between PBL and cerebellum telomere length in AD patients, and compared telomere lengths in cerebella from individuals with AD versus age-matched control subjects.

Methods—Mean telomere lengths were measured using quantitative telomere polymerase chain reaction of genomic DNA prepared from matched PBL and cerebellum samples from 29 individuals with pathologically confirmed sporadic AD. Telomere length was also measured in cerebellum samples of 30 AD patients versus 22 unaffected age-matched control subjects.

Results—The PBL and cerebellum telomere lengths were directly correlated in individuals with AD ($r = 0.42$, $P = 0.023$). Nonetheless, cerebellum telomere lengths were not significantly different in AD patients and age-matched control subjects.

Conclusions—Reduced PBL telomere length in AD might not reflect reduced telomere length in bulk brain tissue, but may be a marker of changes in a subset of brain tissues or other tissues that affect the pathogenesis of AD.

Keywords

Alzheimer's disease; Telomere; Cerebellum; Aging

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1. Introduction

Alzheimer's disease (AD) is the major cause of dementia in the elderly, and affects more than 40% of individuals over the age of 85 [1]. The accumulation of fibrillary proteins, including the Aβ peptide in senile plaques and tau protein in neurofibrillary tangles, appears to be central to the pathogenesis of AD [2–4]. It is nonetheless unclear why individuals with the predominant, sporadic form of AD are more prone to disease, although some genetic risk factors were identified [5,6]. Further, it is unclear why age is the major risk factor for AD; possibilities include age-related inflammatory, oxidative, and immune changes, and a time-dependent accumulation of misfolded proteins. Telomere shortening, a known age-related chromosomal change that occurs in most human tissues [7–9], emerged recently as a potential contributor to the pathogenesis of AD.

Telomeres are repeated sequences and associated proteins that protect, or "cap," the ends of chromosomes [10]. The "uncapping" of telomeres leads to the degradation and fusion of chromosome ends, and to either permanent cell-cycle arrest ("cell senescence") or death via apoptosis. Telomere length is important for capping [11]. Because the DNA-replication machinery is incapable of fully replicating the ends of linear molecules, and also because of nucleolytic degradation and oxidative DNA damage, telomeres shorten as cells divide. Some cells express high levels of the enzyme telomerase, which adds telomere repeats to existing telomere ends and thus counteracts shortening. However, there is insufficient telomerase in most human cells to prevent telomere shortening. It is clear that telomeres shorten with age in many human tissues, including skin, kidney, liver, blood vessels and peripheral white blood cells [7].

Telomere shortening limits the replicative lifespan of many different human cells in culture, and evidence is accumulating that telomeres become critically short in aging human tissues, and thus contribute to age-associated pathology. For example, individuals over age 60 years who have telomeres in the bottom half of the length distribution have 1.9-fold higher mortality rates than age-matched individuals with telomeres in the top half of the length distribution ($P = 0.004$) [12]. Further, individuals with dyskeratosis congenita, who have a 50% decrease in telomerase activity, suffer from several age-associated pathologies (e.g., bone marrow failure and osteoporosis) [13], and telomere defects may contribute centrally to the pathogenesis of the Werner premature aging syndrome [14–16]. An exponential increase with age in the frequency of uncapped telomeres and the heterochromatinization of the nuclear genome in the skin of nonhuman primates indicates an association between age-related telomere dysfunction and cellular senescence in vivo [17]. Further, telomere length may be a heritable factor correlated with lifespan [18]. Mean telomere length in a given tissue or cell type varies among individuals of the same age [19–21], and individuals born with a greater telomere reserve might be protected from the effects of age-related telomere attrition.

Although telomeres will shorten with cell division, some dividing cells express telomerase (e.g., activated lymphocytes and progenitor cells of epithelial and hematopoietic tissues), which can slow or even reverse telomere shortening. Thus in older individuals, the telomere lengths of tissues with abundant dividing cells may have a complicated relationship with inherited telomere lengths. On the other hand, the low levels of cell turnover and lack of significant telomerase in normal brains yield slow and regular age-related telomere shortening that may allow telomere length at a given age to have a simpler relationship with inherited telomere lengths. Indeed, cerebral cortex telomere lengths were found to shorten with age in a cross-sectional study of individuals up to 70–79 years of age, but were then directly correlated with age in individuals aged 80 years and older [22]. This was interpreted

as a survivor effect, where individuals who had inherited longer telomeres had a greater likelihood of living to an older age.

Four studies have connected telomere length to AD, despite the lack of an apparent association in one early, small study [23]. The first study found that individuals with AD had shorter telomere lengths in peripheral blood mononuclear cells than did age-matched control subjects [24]. Further, T-cell telomere length was correlated with Mini-Mental State Examination score, and inversely correlated with serum levels of the proinflammatory cytokine tumor necrosis factor-α, suggesting that short telomeres might induce immune dysfunction and thus contribute to the pathogenesis of AD. The second study examined women with Down syndrome, and found shorter telomeres in T cells of individuals with AD-type dementia than in age-matched control subjects [25]. The third, a case control study of 257 individuals, found reduced peripheral blood leukocyte (PBL) telomere length in AD patients compared with control subjects [26]. Moreover, among individuals with AD, short telomeres were associated with higher mortality rates. A fourth study confirmed the association between short PBL telomere length and AD, and was the first to examine brain (hippocampus) telomere length in histopathologically validated AD patients [27]. Surprisingly, telomere lengths in the hippocampus were found to be 49% longer in AD patients than in control subjects ($P < 0.01$). However, because glial cells can upregulate telomerase and lengthen their telomeres upon proliferating in response to injury [28–30], the gliosis known to be associated with the most active sites of AD pathology, such as the hippocampus, likely contributed to the increased overall telomere length of cells observed in this brain region. Together, these four studies indicate that telomere length in peripheral blood cells is a marker of AD, although many questions remain about the relationship and significance of PBL telomere length to the pathogenesis of AD.

Here we examined the association between PBL and cerebellum telomere lengths in AD, and also compared telomere lengths in the cerebella of AD patients and control subjects. We chose the cerebellum because whereas it may suffer limited pathology in AD, including atrophy of the molecular and granular layers [31], it does not experience gliosis [31–33]. Thus, the measurement of cerebellar telomere length should not be complicated by telomerase expression in proliferating glial cells. We found that PBL and cerebellum telomere lengths are correlated in AD patients. However, we were unable to discern a difference in the cerebellar telomere lengths of AD patients compared with control subjects.

2. Methods

2.1. Samples

Cerebellar tissue was obtained from the Center for Neurodegenerative Disease Research Brain Bank at the University of Pennsylvania. Cases (n = 30) were selected from pathologically confirmed AD brains (n = 280), based solely on the availability of matched DNA samples from PBLs that had been collected before death (one PBL DNA sample later proved to be unavailable). Control subjects (n = 22) were selected from normal brains (n = 36), to match most closely the age and gender of the AD cases. The AD patients had an average age at death of 80 years (range, 57–92 years); 73% (n = 22) of patients were female. The mean age at death of control cases was also 80 years (range, 63–98 years); 50% (n = 11) were female. Cerebellar samples from two additional control individuals of younger ages (ages 29 and 51 years) were used for studies of the relationship between age and brain telomere length. Genomic DNA was extracted from brain tissue, using a Qiagen DNeasy kit (Qiagen, Valenica, CA).

The DNA from PBLs that had been collected and banked for *APOE* genotyping before a patient's death (mean, 3.8 years before death) was available for 29 of 30 AD patients. The

DNA from these patients was obtained from the Memory Center DNA Bank at the University of Pennsylvania Alzheimer's Disease Center. This DNA had been extracted using standard commercial methods. Unfortunately, matched peripheral blood DNA was not available for control brain cases. All studies were performed under Institutional Review Board approval.

The MRC5 cells were obtained from the Coriell Repository (Camden, NJ), and BJ-hTERT fibroblasts were a generous gift of W. Hahn (Dana Farber Cancer Institute, Boston, MA). Cells were grown in Minimal Essential Medium with Earle's salts and containing 15% fetal bovine serum and $1 \times$ penicillin/streptomycin in 5% CO_2 and 3% O_2. Genomic DNA from cultured cells was prepared as described previously [34].

2.2. Telomere polymerase chain reaction

Telomere polymerase chain reactions (PCRs) were performed exactly as described [35], except that the number of amplification cycles was increased to 25 and 35 for the T and S reactions, respectively. Briefly, two sets of PCR reactions were each carried out in triplicate in 96-well plates, and each reaction contained 35 ng of genomic DNA template. The first set amplified telomere repeat DNA (T reaction), and the second amplified a single-copy gene (ribosomal protein P0; S reaction). The PCRs were performed in an Appied Biosystems, Inc 7700 sequence detection system, and the accumulation of amplified DNA was detected via SYBR green fluorescence. Each plate contained dilutions of a reference genomic DNA sample to generate a standard curve for the relationship between C_t (cycle threshold) values and input genomic DNA mass for T and S reactions, to which the test samples were normalized to generate relative T and S values and T/S ratios. The mean coefficient of variation for triplicate T/S measurements in AD samples was 15.1% for cerebellum DNA (n = 30), and 12.5% for PBL DNA (n = 29).

2.3. Terminal restriction fragment analysis

The terminal restriction fragment (TRF) analysis was performed as described [36]. Briefly, 1 μg of genomic DNA was digested with *Rsa*I and *Hinf*I, and separated on a 0.5% agarose gel, which was then dried and probed using the end-labeled telomere repeat oligonucleotide $(CCCTAA)_4$, and visualized with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA). Mean telomere length was calculated as the weighted average $\Sigma(OD_i)/\Sigma(OD_i/L_i)$, where OD_i is the background-corrected intensity of telomere signal in interval i, and L_i is the average length of telomeres in interval i (each interval equal to a pixel), thus accounting for the stronger signal emitted by longer telomeres.

2.4. Statistical analyses

Statistical analyses were performed using Prism 3.0 software (GraphPad Software, Inc., San Diego, CA), except for calculation of the 95% confidence interval for the mean difference between AD and control cerebellum T/S ratios, which was performed using the method presented by Woolson [37]. Correlations were evaluated using Pearson *r*-values and two-tailed *P*-values. For pairwise comparisons of mean T/S ratios in AD patients and control subjects, a two-tailed unpaired *t*-test was applied, using Welch's correction in cases of unequal variances.

3. Results

3.1. Validation of quantitative PCR

Quantitative telomere PCR was used to measure mean telomere lengths in genomic DNA from tissue specimens. This technique requires small amounts of genomic DNA, and measures only the lengths of telomere repeat tracts. Values are expressed as T/S ratios,

where T and S are proportional to the amplification of telomere repeats or a single-copy gene, respectively. The classic method of measuring telomere length, i.e., TRF length analysis, relies on the digestion of subtelomeric DNA with restriction endonucleases to generate fragments that contain predominantly telomere repeat DNA, which are then measured by Southern blot analysis. Because the location of the terminal-most restriction site varies among chromosomes and individuals, the subtelomeric component of TRF length contributes to a variability in measurements that can be avoided using telomere PCR. To confirm that our telomere PCR measurements reflected telomere lengths, we compared them with TRF measurements of DNA samples from cultured cells (Fig. 1A) or PBLs from AD patients. Telomere PCR measurements were performed in triplicate, and T/S ratios had a coefficient of variation of 12–15% (see Methods). Comparisons among samples from a single cultured human diploid fibroblast cell line (MRC5), which had been serially passaged to effect different extents of telomere shortening, provided an excellent correlation (Fig. 1B; $r = 1.0$, $P = 0.0014$). An addition to the dataset of a sample from a cell line derived from a different individual and containing an *hTERT* transgene (BJ-hTERT), conferring high levels of telomerase and thus long telomeres, diminished the correlation slightly ($r = 0.98$, $P = 0.0035$). The PBL samples showed a reduced, but still high, correlation between TRF and telomere PCR measurements (Fig. 1C; $r = 0.77$, $P = 0.015$). It is likely that the diminished correlation in comparisons between samples from different individuals in part reflects a variability in TRF measurements caused by the polymorphic nature of subtelomeric DNA. Overall, telomere PCR provided a reproducible and accurate measure of mean telomere length.

3.2. Comparison of PBLs and cerebellum telomere lengths in AD

Genomic DNA was prepared from matched PBL and cerebellum samples from 29 individuals with sporadic AD and an average age of 80 years. Telomere PCR T/S measurements yielded nearly identical mean telomere lengths for the blood and brain samples of the group of individuals overall (means and standard deviations for PBL and cerebellar T/S were 0.778 ± 0.168 and 0.792 ± 0.209, respectively). Consistent with the similar overall T/S ratios, there was a moderate and significant correlation between PBL and brain telomere lengths (Fig. 2A; $r = 0.42$, $P = 0.023$). Because it was reported that PBL telomere length is shorter in AD patients than in control subjects, our observation that PBL and brain telomere lengths are correlated raised the possibility that brain telomere lengths might also be shorter in AD patients than in control subjects.

3.3. Comparison of cerebellum telomere lengths in AD patients and control subjects

Cerebellar telomere lengths were examined in 24 unaffected control individuals, using telomere PCR. Consistent with earlier reports of the human cerebrum and rat cerebellum [22,38], there was a weak trend toward telomere shortening with age in these control subjects (Fig. 2B; $r = -0.098$, $P = 0.64$). Next, cerebellar DNA was examined in 30 AD patients and 22 control subjects. Each group had the same mean age of death, equal to 80 years. Telomere PCR T/S ratios (means ± standard deviations) were 0.799 ± 0.210 and 0.736 ± 0.334 for the AD patients and control subjects, respectively (Fig. 2C). This mean difference of 0.063 (an 8.5% increase in patients) was not significant ($P = 0.52$), and the 95% confidence interval for the mean difference between cases and control subjects ranged from −0.09 to 0.21 (i.e., from a 12% decrease to a 28% increase). There was also no significant difference in T/S ratios when comparing patients and control subjects of the same gender (data not shown). Therefore, we were unable to detect a decrement in telomere length in the majority of cerebellar cells from these AD cases. Similar to a previous report [26], there was no association between *APOE* genotype and T/S ratio (mean ±standard deviation) in AD patients (T/S =0.793 ± 0.221 and 0.803 ± 0.209 for individuals with no, or 1 or 2, *APOE* ε4 alleles, respectively). Further, there was no relationship between cerebellar T/

S ratio and duration of disease ($P = 0.78$). There was a significant inverse correlation between cerebellar T/S ratio and age of disease onset (Fig. 2D; $P = 0.027$). In contrast, the PBL T/S ratio was not significantly correlated with age of disease onset (Fig. 2D; $P = 0.108$).

4. Discussion

We observed a significant correlation between PBL and cerebellum telomere lengths in AD patients, but no difference in bulk cerebellum telomere lengths in AD patients relative to age-matched control subjects. Although we did not have access to matched PBL samples for our control patients, previous measurements of PBL telomeres in patients with AD found decreases of 13% [24], 12% [26], and 31% [27] compared with control subjects. The 95% confidence interval for the difference between mean cerebellum telomere length in our AD cases relative to control subjects ranged from a 12% decrease to a 28% increase. Thus, if cerebellum telomeres in AD are shortened to the same extent as PBL telomeres, we should have had a reasonable chance of detecting this. However, we may have missed a small difference because of a lack of statistical power.

Our findings of correlated telomere lengths in AD PBLs and cerebella, but no apparent difference between telomere lengths in AD and control cerebella, may appear to be at odds with several reports indicating reduced telomere lengths in the PBL of individuals with AD. However, this apparent contradiction could be explained by the accelerated attrition of PBL telomeres in AD patients compared with control subjects, with a relative sparing of cerebellum telomeres from shortening in both patient populations (see hypothetical dotted line in Fig. 2A). In this case, PBL and brain telomere length would be correlated in both AD and control populations, brain telomere lengths would be the same in AD patients and control subjects, and PBL telomeres would nonetheless be shorter in AD. Unfortunately, we did not have access to matched PBL DNA samples for our control population, and so any proof of this idea will require additional studies.

Consistent with the idea that bulk cerebellar telomere length is *not* a major determinant of AD, we found an inverse correlation between age at onset of AD and cerebellar telomere length (Fig. 2D). This relationship most likely reflects a gradual shortening of cerebellar telomeres with age, rather than a pathogenic role in AD. If bulk cerebellar telomere length were a major determinant of AD risk, there should be little or no correlation between age at onset and telomere length, because individuals would develop AD as soon as their telomeres reached a threshold length. In this case, individuals who inherited short telomeres, or had accelerated cerebellar telomere attrition during their lifetime, would develop AD earlier than those with longer telomeres.

Following this logic, if an association exists between inherited or acquired changes in PBL telomere length and the pathogenesis of AD, one would not expect to see a correlation between PBL telomere length and age at AD onset. Our data (Fig. 2D, PBL data) are consistent with this hypothesis, because there was no significant correlation between PBL telomere length and age at onset (consistent with a threshold effect). However, our study may not have been adequately powered to detect such a correlation, if one exists.

How might telomere dysfunction be related to AD, and why would PBL telomere length be correlated with AD? As the least direct explanation for a correlation between short PBL telomeres and AD, excessive telomere shortening simply reflects a history of generalized tissue damage and inflammation (and thus increased cell turnover and telomere shortening), which in themselves may contribute to AD. As the most direct possibility, the telomere length of PBLs directly regulates the function of these circulating immune cells, which in

turn modulate AD pathogenesis, as suggested by Panossian et al. [24]. As a middle-ground possibility, PBL telomere length is correlated with telomere length in other tissues that play a more direct role in AD pathogenesis, and changes in telomere lengths affect the function of these tissues. For example, telomere lengths in endothelial cells in brain vasculature might decline with age, as they do in the endothelial cells of other tissues.

There is some support for the notion that telomere shortening and cell senescence contribute to vascular dysfunction that in turn contributes to AD. For example, telomere dysfunction contributes to age-related vascular changes [39], as evidenced by 1) a shortening of telomeres with age in vascular endothelial cells [40], 2) an association between athero-sclerotic lesions and senescent cells with particularly short telomeres [41], 3) threefold higher mortality rates from cardiovascular disease in individuals with short telomeres [12], 4) the reversal by artificial telomerase expression of atherogenic gene expression patterns in senescent endothelial cells [42], and 5) the significance of mean leukocyte telomere length as a predictor of future coronary artery disease in middle-aged, high-risk men [4,43]. Further, there are indications that vascular factors contribute to AD, including 1) shared risk factors for atherosclerosis and AD (e.g., *APOE* genotype, diabetes, and hyperlipidemia), 2) the deposition of β-amyloid in brain capillaries, 3) AD risk reduction via agents (e.g., aspirin) that also reduce vascular disease, and 4) evidence that vasculature insufficiency and thrombin production upregulate amyloid precursor protein (APP) expression and the cleavage of the amyloid beta peptide from APP [44–48]. Given the small contribution of vascular cells to total brain mass (approximately 0.1%), changes in telomere length in such cells would have gone undetected in our measurements of bulk cerebellar telomeres.

Regardless of how telomere function might be related to AD, PBL telomere length could provide an independent bio-marker of AD risk that could complement other biomarkers under study. We found that PBL and brain telomere length are correlated in AD. Similar correlations were reported for telomere lengths in PBL and skin fibroblasts, and among cerebral cortex, myocardium, liver, and renal cortex, indicating correlations throughout several tissues [19,23]. Nonetheless, if there proves to be no difference between cerebellar telomere lengths in AD patients and control subjects, as our findings suggest, this would indicate that inherited telomere length may not be a major determinant of AD susceptibility, because cerebellar telomere length may be a relatively good indicator of inherited telomere length. Rather, the acquired shortening of PBL telomeres, which appears to be correlated with chronic stress or inflammation [49], might underlie the correlation of PBL telomere length with AD risk.

Further studies are required to understand the mechanistic relationship between PBL telomeres and AD, and such studies may shed new light on the mechanisms of this devastating disease.

Acknowledgments

References

1. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 2003;60:1119–22. [PubMed: 12925369]

2. Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. N Engl J Med 2003;348:1356–64. [PubMed: 12672864]

3. Forman MS, Trojanowski JQ, Lee VM. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat Med 2004;10:1055–63. [PubMed: 15459709]

4. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci 2007;8:663–72. [PubMed: 17684513]

5. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–3. [PubMed: 8346443]

6. Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 2007;39:168–77. [PubMed: 17220890]

7. Baird DM, Kipling D. The extent and significance of telomere loss with age. Ann NY Acad Sci 2004;1019:265–8. [PubMed: 15247026]

8. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 2005;120:513–22. [PubMed: 15734683]

9. Rodier F, Kim SH, Nijjar T, Yaswen P, Campisi J. Cancer and aging: the importance of telomeres in genome maintenance. Int J Biochem Cell Biol 2005;37:977–90. [PubMed: 15743672]

10. Cech TR. Beginning to understand the end of the chromosome. Cell 2004;116:273–9. [PubMed: 14744437]

11. Chan SR, Blackburn EH. Telomeres and telomerase. Philos Trans R Soc Lond [Biol] 2004;359:109–21.

12. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 2003;36:393–5. [PubMed: 12573379]

13. Bessler M, Wilson DB, Mason PJ. Dyskeratosis congenita and telomerase. Curr Opin Pediatr 2004;16:23–8. [PubMed: 14758110]

14. Du X, Shen J, Kugan N, Furth EE, Lombard DB, Cheung C, et al. Telomere shortening exposes functions for the mouse Werner and Bloom syndrome genes. Mol Cell Biol 2004;24:8437–46. [PubMed: 15367665]

15. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, et al. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. Nat Genet 2004;36:877–82. [PubMed: 15235603]

16. Crabbe L, Jauch A, Naeger CM, Holtgreve-Grez H, Karlseder J. Telomere dysfunction as a cause of genomic instability in Werner syndrome. Proc Natl Acad Sci USA 2007;104:2205–10. [PubMed: 17284601]

17. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. Science 2006;311:1257. [PubMed: 16456035]

18. Njajou OT, Cawthon RM, Damcott CM, Wu SH, Ott S, Garant MJ, et al. Telomere length is paternally inherited and is associated with parental lifespan. Proc Natl Acad Sci USA 2007;104:12135–9. [PubMed: 17623782]

19. Takubo K, Izumiyama-Shimomura N, Honma N, Sawabe M, Arai T, Kato M, et al. Telomere lengths are characteristic in each human individual. Exp Gerontol 2002;37:523–31. [PubMed: 11830355]

20. Baird DM, Britt-Compton B, Rowson J, Amso NN, Gregory L, Kipling D. Telomere instability in the male germline. Hum Mol Genet 2006;15:45–51. [PubMed: 16311252]

21. Aubert G, Lansdorp PM. Telomeres and aging. Physiol Rev 2008;88:557–79. [PubMed: 18391173]

22. Nakamura K, Takubo K, Izumiyama-Shimomura N, Sawabe M, Arai T, Kishimoto H, et al. Telomeric DNA length in cerebral gray and white matter is associated with longevity in individuals aged 70 years or older. Exp Gerontol 2007;42:944–50. [PubMed: 17606349]

23. von Zglinicki T, Serra V, Lorenz M, Saretzki G, Lenzen-Grossimlighaus R, Gessner R, et al. Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? Lab Invest 2000;80:1739–47. [PubMed: 11092534]

24. Panossian LA, Porter VR, Valenzuela HF, Zhu X, Reback E, Masterman D, et al. Telomere shortening in T cells correlates with Alzheimer's disease status. Neurobiol Aging 2003;24:77–84. [PubMed: 12493553]

25. Jenkins EC, Velinov MT, Ye L, Gu H, Li S, Jenkins EC Jr, et al. Telomere shortening in T lymphocytes of older individuals with Down syndrome and dementia. Neurobiol Aging 2006;27:941–5. [PubMed: 16046031]

26. Honig LS, Schupf N, Lee JH, Tang MX, Mayeux R. Shorter telomeres are associated with mortality in those with *APOE* epsilon4 and dementia. Ann Neurol 2006;60:181–7. [PubMed: 16807921]

27. Thomas P, O'Callaghan NJ, Fenech M. Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease. Mech Ageing Dev 2008;129:183–90. [PubMed: 18242664]

28. Baek S, Bu Y, Kim H. Telomerase induction in astrocytes of Sprague-Dawley rat after ischemic brain injury. Neurosci Lett 2004;363:94–6. [PubMed: 15158005]

29. Flanary BE, Streit WJ. Effects of axotomy on telomere length, telomerase activity, and protein in activated microglia. J Neurosci Res 2005;82:160–71. [PubMed: 16175576]

30. Fu W, Lee J, Guo Z, Mattson MP. Seizures and tissue injury induce telomerase in hippocampal microglial cells. Exp Neurol 2002;178:294–300. [PubMed: 12504888]

31. Wegiel J, Wisniewski HM, Dziewiatkowski J, Badmajew E, Tarnawski M, Reisberg B, et al. Cerebellar atrophy in Alzheimer's disease—clinicopathological correlations. Brain Res 1999;818:41–50. [PubMed: 9914436]

32. Joachim CL, Morris JH, Selkoe DJ. Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease. Am J Pathol 1989;135:309–19. [PubMed: 2675616]

33. Li YT, Woodruff-Pak DS, Trojanowski JQ. Amyloid plaques in cerebellar cortex and the integrity of Purkinje cell dendrites. Neurobiol Aging 1994;15:1–9. [PubMed: 8159255]

34. Karlseder J, Smogorzewska A, de Lange T. Senescence induced by altered telomere state, not telomere loss. Science 2002;295:2446–9. [PubMed: 11923537]

35. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30:e47. [PubMed: 12000852]

36. Steinert S, White DM, Zou Y, Shay JW, Wright WE. Telomere biology and cellular aging in nonhuman primate cells. Exp Cell Res 2002;272:146–52. [PubMed: 11777339]

37. Woolson, RF. Statistical Methods for the Analysis of Biomedical Data. New York: John Wiley and Sons; 1987. p. 157

38. Flanary BE, Streit WJ. Telomeres shorten with age in rat cerebellum and cortex in vivo. J Anti Aging Med 2003;6:299–308. [PubMed: 15142431]

39. Edo MD, Andres V. Aging, telomeres, and atherosclerosis. Cardiovasc Res 2005;66:213–21. [PubMed: 15820190]

40. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. Proc Natl Acad Sci USA 1995;92:11190–4. [PubMed: 7479963]

41. Ogami M, Ikura Y, Ohsawa M, Matsuo T, Kayo S, Yoshimi N, et al. Telomere shortening in human coronary artery diseases. Arterioscler Thromb Vasc Biol 2004;24:546–50. [PubMed: 14726417]

42. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. Circulation 2002;105:1541–4. [PubMed: 11927518]

43. Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet 2007;369:107–14. [PubMed: 17223473]

44. de la Torre JC. Vascular basis of Alzheimer's pathogenesis. Ann NY Acad Sci 2002;977:196–215. [PubMed: 12480752]

45. Iadecola C, Gorelick PB. Converging pathogenic mechanisms in vascular and neurodegenerative dementia. Stroke 2003;34:335–7. [PubMed: 12574528]

46. Decarli C. Vascular factors in dementia: an overview. J Neurol Sci 2004;226:19–23. [PubMed: 15537513]

47. Vagnucci AH Jr, Li WW. Alzheimer's disease and angiogenesis. Lancet 2003;361:605–8. [PubMed: 12598159]

48. Zlokovic BV, Deane R, Sallstrom J, Chow N, Miano JM. Neurovascular pathways and Alzheimer amyloid beta-peptide. Brain Pathol 2005;15:78–83. [PubMed: 15779240]

49. Aviv A. The epidemiology of human telomeres: faults and promises. J Gerontol [A] Biol Sci Med Sci 2008;63:979–83.
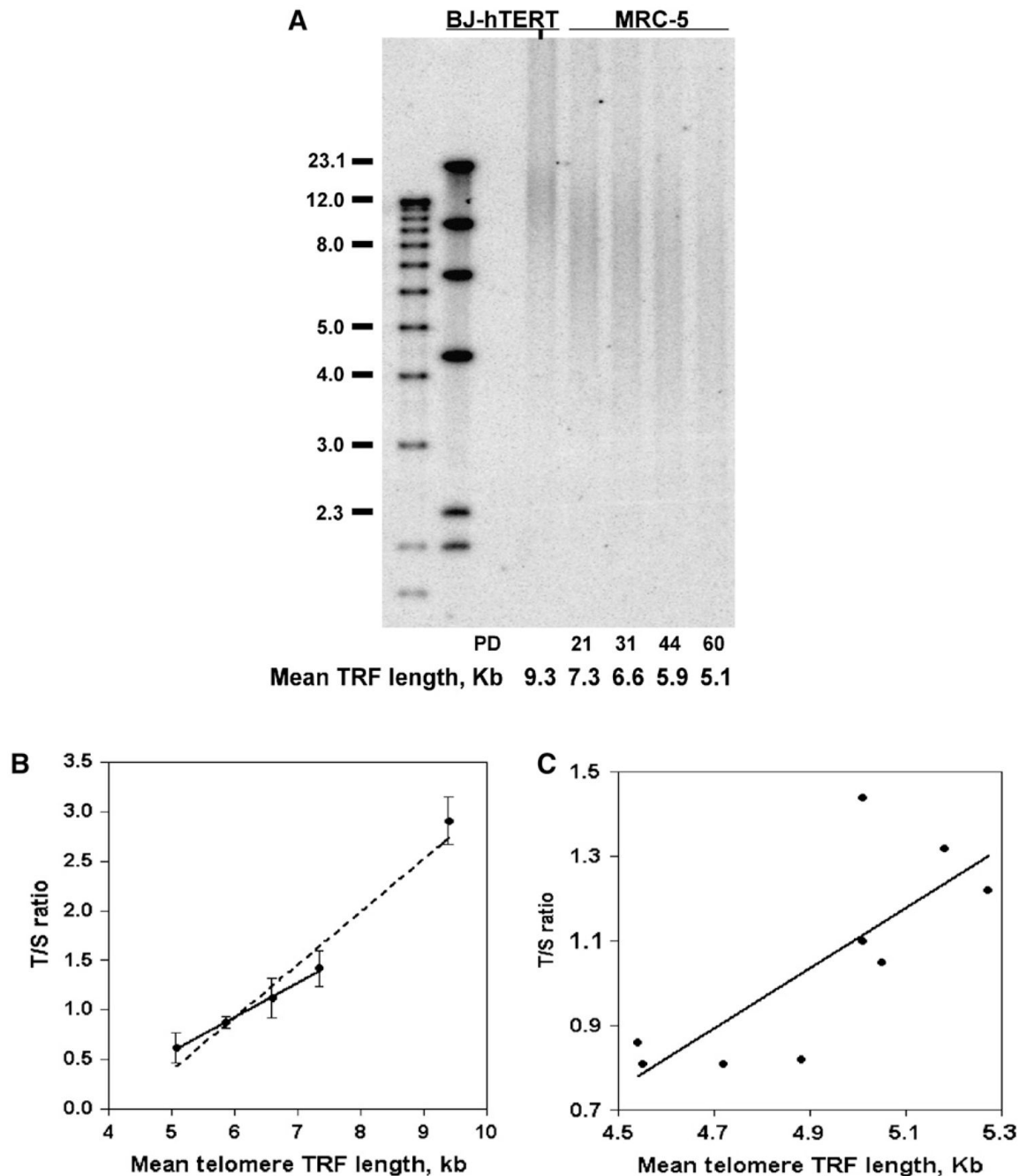
Fig. 1.
Comparison of telomere length measurements by TRF Southern blot analysis and telomere PCR. (**A**) TRF Southern blot analysis of genomic DNA from cultured fibroblasts. The DNA from primary human diploid fibroblast cell lines BJ-hTERT and MRC5 at indicated population-doubling levels (PD) was analyzed by TRF Southern blotting, as described in Methods. Mean telomere TRF lengths in kilobases (Kb) are indicated. (**B**) Comparison of telomere PCR T/S ratios and TRF measurements for cultured fibroblasts. The MRC5 samples at different PDs yielded a correlation coefficient of 1.0 (solid line; $P = 0.0014$), and inclusion of the BJ-hTERT sample yielded a correlation coefficient of 0.98 (dashed line; $P = 0.0035$). (**C**) Comparisons of telomere PCR T/S ratios and TRF measurements for PBL

specimens from AD patients ($r = 0.77$, $P = 0.015$). All lines were fit by linear least-squares regression.
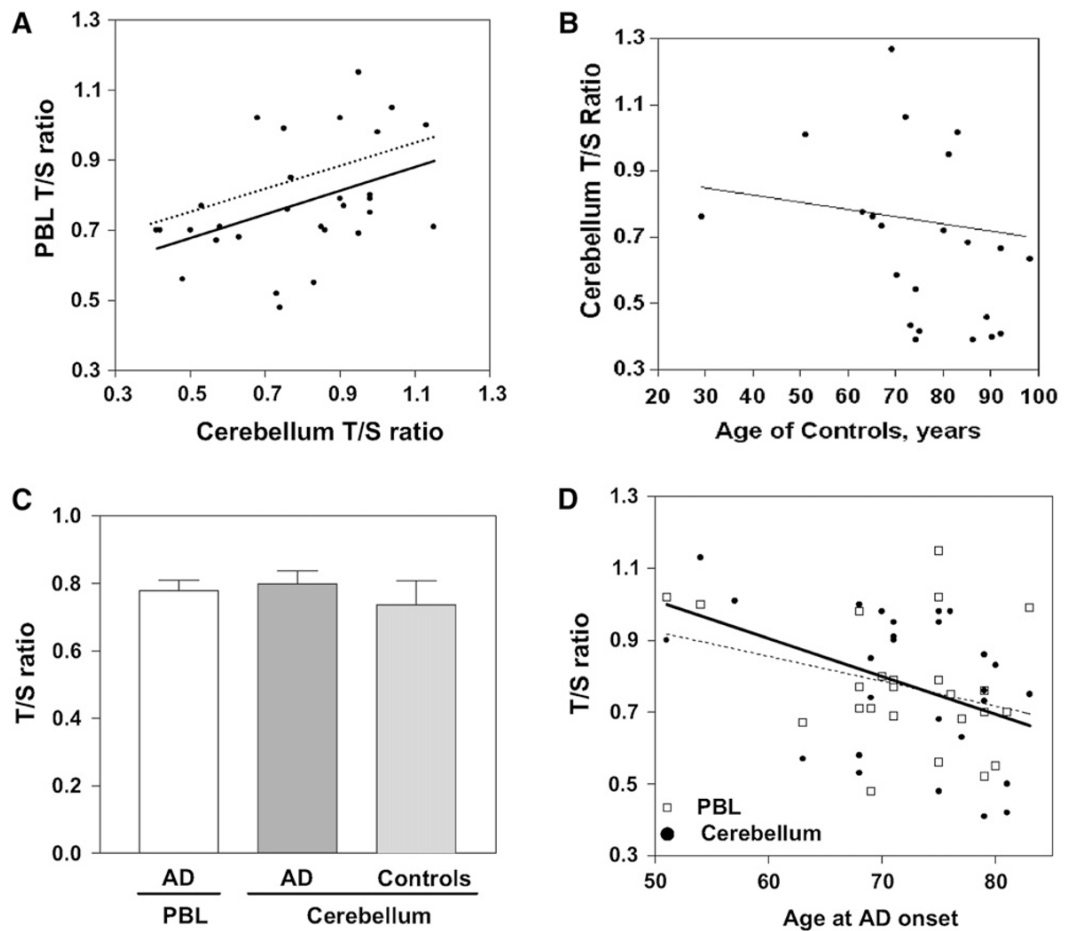
Fig. 2.
Cerebellum and PBL telomere lengths in AD cases and control subjects. (**A**) Correlation between PBL and cerebellum telomere lengths in AD patients. Mean telomere PCR T/S ratios for PBLs and cerebellum from AD patients (n =29) are shown. The correlation coefficient is 0.42 (*P* =0.023). Solid line was fit by linear least-squares regression, and dotted line represents a hypothetical control population with same mean cerebellum T/S ratio, but a higher mean PBL T/S ratio, than in the AD population (see Discussion). (**B**) Cerebellar T/S ratios for normal control subjects (n = 24) ranging in age from 29 to 98 years. Weak trend toward shortening with age is indicated by least-squares fit line (*P* =0.64). (**C**) Mean T/S ratios for PBLs from AD patients (n =29), and for cerebellum samples from AD patients (n =30; mean age, 79.5 years) and control subjects (n =22; mean age, 79.7 years). Standard errors are shown, and no significant difference between T/S ratios in the two cerebellum groups was evident (*P* = 0.52; 95% confidence interval for AD vs. controls, −0.09 to 0.21). (**D**) Relationships between age of AD onset and cerebellar T/S ratio (n = 30, solid circles and solid line; *P* = 0.027) and PBL T/S ratio (n = 29, open squares and dotted line; *P* = 0.108).