Association Between ApoE Phenotypes and Telomere Erosion in Alzheimer’s Disease

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Although several reports suggest that Alzheimer’s disease (AD) is associated with shortened telomere length, the clinical relevance of this has not yet been fully elucidated. This study was conducted to clarify the correlation of telomere length with clinical characteristics and ApoE phenotypes in 74 AD patients. Telomere length was determined from genomic DNA extracted from whole blood by quantitative real-time polymerase chain reaction. We found no significant difference in telomere length between the AD and non-dementia elderly control (n = 35) groups. Furthermore, no significant correlation was found among telomere length and the severity of cognitive decline and disease duration, age, or gender differences. However, telomere length was significantly shorter in AD patients with the ApoE4 homozygote than in those with the ApoE4 heterozygote (p < .001) and noncarriers (p < .001). These findings suggest that shortened telomere length may be associated with the ApoE4 homozygote in AD patients.

Key Words: Telomere length—Alzheimer’s disease—ApoE phenotype—Quantitative real-time PCR.

Received June 20, 2011; Accepted September 7, 2011

Decision Editor: Rafael de Cabo, PhD

ALTHOUGH Alzheimer’s disease (AD) was first described almost a century ago, the molecular mechanisms that lead to the development of its neuronal pathology are still not clear. Furthermore, there is still no causal therapy for the disease because the mechanism of neuronal cell death is also unknown. A variant of the gene apolipoprotein E (ApoE), which encodes protein apolipoprotein E, is associated with an increased susceptibility to AD (1); however, there is no consensus regarding ApoE testing for asymptomatic persons (2).

Telomeres gradually shorten with aging, and accelerated telomere shortening has been linked to several age-related disorders. Evidence suggests that AD patients have shorter telomeres in peripheral blood leukocytes than in age-matched controls (3–6), although the clinical relevance of shortened telomeres has not yet been fully elucidated.

In the present study, we set out to determine the correlation of telomere length with clinical characteristics and ApoE phenotypes in AD patients using a highly reproducible quantitative real-time polymerase chain reaction (Q-PCR) assay of telomere length from a small amount of DNA samples (7–10).

METHODS

Participants
We enrolled 74 consecutively diagnosed cases of AD from the Memory Clinic of our hospital. The age range was 60–90 years, excluding patients with familial AD. We established a diagnosis of probable AD in each patient using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria (11). All patients underwent detailed general physical, neurologic, and psychiatric examinations, extensive laboratory tests, magnetic resonance imaging, and single-photon emission computed tomography brain scanning to exclude other potential causes of dementia. Patients were excluded if they had complications from other neurologic diseases, psychiatric illness, neoplastic disorders, or immune disorders. Specimens from 84 non-dementia participants were also used in this study.

We divided non-dementia participants into three groups. Control group 1 consisted of participants 19 years or younger (mean ± standard deviation: 1.9 ± 1.3 years, n = 10), control group 2 participants were aged 20–59 years (33.4 ± 9.7 years, n = 39), and control group 3 participants were aged 60 years or older, that is, non-dementia elderly controls (NECs; 79.5 ± 6.4 years, n = 35). Group 1 participants were inpatients of a pediatric ward, group 2 participants were normal healthy volunteers, and group 3 participants were outpatients at our hospital. All control participants had normal cognitive functions and were excluded if they had complications from other neurologic diseases, psychiatric illness, neoplastic disorders, or immune disorders. Because the age of AD patients ranged...
from 59 to 94 years, we classified the NEC cohort as those aged older than 60 years (AD vs. NEC [mean ± standard deviation]: 78.5 ± 7.1 years vs 79.5 ± 6.4 years; \( p = .47 \)); thus, we considered data from the NEC group to be age-matched controls. This study was approved by the Institutional Review Board of Tokyo Medical University (no. 976: approved on July 14, 2008). Written informed consent was obtained from all patients according to the Declaration of Helsinki before specimen collection.

Telomere Length Determination by Q-PCR

Genomic DNA was extracted from 350 µL of whole blood by an automated magnetic beads system (12). Telomere length was measured on the extracted DNA in triplicates by Q-PCR as previously reported by Svenson and colleagues (9,10). The telomere and \( \gamma \)-globin primer sequences written 5′–3′ were CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGG (Tel1b), GGCTTGCCTT-ACCCTTACCCTTACCTTACCTTACCCTTACCCT (Tel2b), TGTGCTGGCCCATCACTTTG (HBG3), and ACCAGCCACCATTTCTGATA GG (HBG4). Telomere/single copy gene (T/S) values were calculated by \( 2^{-\Delta \Delta Ct} \), and relative T/S values (i.e., relative telomere length (RTL) values) were generated by dividing sample T/S values with the T/S value of a reference cell line DNA (CCRFCEM).

ApoE Phenotyping

ApoE phenotypes were determined from plasma samples by isoelectric electrophoresis and immunoblotting according to a detailed procedure described previously by Kataoka and colleagues (13).

Southern Blot Analysis

For terminal restriction fragment (TRF) assay, DNA preparation and Southern blot analysis were carried out as reported previously (14). Telomere length was detected by a DIG-labeled probe using a TeloTAGGG Telomere Length Assay (Roche Applied Science, IN), according to the manufacturer’s instruction.

Statistical Analysis

We used SPSS statistical analysis software (SPSS 10.07J, SPSS Japan Inc., Tokyo, Japan) for data analysis. The Student \( t \) test was used to determine statistically significant differences between the control and test groups, and we also used the chi-square test, when required. We performed one-way analysis of variance or analysis of covariance to test for the mean difference among the three groups of data. The Tukey honestly significant difference (HSD) test was used at the second stage of analysis of variance if the null hypothesis was rejected. Regression analyses were used to study the correlation between the T/S ratio and TRF measured by conventional Southern blot analysis. \( p \) values of less than .05 were considered to indicate statistically significant differences.

Results

Validation of Telomere Length by Q-PCR Assay

To confirm the validity of the Q-PCR assay, we compared T/S ratio and telomere length measured by conventional Southern blot analysis in various samples. We found that T/S ratio was associated with TRF (Figure 1A). To obtain reproducible results, we included a reference in every experiment. We found a linear correlation in the range of 1–8 ng of template DNA (Supplementary Figure 1). We then measured T/S ratio in the three groups of non-dementia participants: control group 1, control group 2, and control group 3 (NEC). Analysis of variance revealed a significant difference in the T/S ratio among these three groups (\( p < .001 \)). Younger controls (control group 1) had a significantly higher T/S ratio than middle-aged...
controls (control group 2; 1.076 ± 0.1391 vs 0.8966 ± 0.1171; \( p < .001 \)). Similarly, middle-aged controls (control group 2) had a significantly higher T/S ratio than NEC (control group 3; 0.8966 ± 0.1171 vs 0.7930 ± 0.1289, \( p < .001 \); Figure 1B). These findings indicate that telomere length measured by Q-PCR assay was associated with age, consistent with our previous report using TRF analysis in healthy individuals (14).

Telomere Length in AD

The clinical characteristics and telomere length of AD patients and NEC are shown in Table 1. There were no significant differences in age (\( p = .47 \)), gender (\( p = .52 \)), smoking (nonsmoker vs former and current smoker; \( p < .99 \)) or education period (\( p = .93 \)) between these two groups (Figure 2). There were significant differences in Mini-Mental State Examination scores (\( p = .13 \); Table 1), we arbitrarily divided AD patients into two groups according to the T/S ratio: a low T/S group (<0.75) and a normal T/S group (>0.75). Although there was no significant difference in cognitive severity as evaluated by Mini-Mental State Examination scores (\( p = .32 \), disease duration (\( p = .79 \)), or age at disease onset (\( p = .39 \)) between the two groups, patients with a low T/S ratio had a significantly high incidence of the ApoE4/4 phenotype according to the chi-square test (\( p = .02 \); Table 2).

Correlation Between ApoE Phenotypes and Telomere Length

We then investigated in detail whether telomere length was affected by ApoE phenotypes. We classified AD patients into three groups according to the different ApoE phenotypes: first group, AD patients without the E4 phenotype (noncarriers; \( n = 35 \)); second group, those with the E4/3 or E4/2 phenotype (\( n = 30 \)); and third group, those with the E4/4 phenotype (\( n = 9 \)). Among the clinical characteristics of the three groups, there was no statistically significant difference in age, smoking, cognitive severity, educational attainment, or disease duration (data not shown). Analysis of variance revealed a statistically significant difference among these three groups (\( p < .001 \)). The T/S ratio was significantly lower in AD patients with the E4/4 phenotype than in noncarriers or in those with the E4/3 or E4/2 phenotype (E4/4: 0.6045 ± 0.0901 vs noncarriers: 0.7623 ± 0.1238, \( p < .001 \); E4/4: 0.6045 ± 0.0901 vs E4/3 or E4/2: 0.7882 ± 0.1071, \( p < .001 \); Figure 3). There are generally two known factors that strongly affect telomere length in healthy individuals: age and smoking (14–16). We therefore replicated the association between telomere length and ApoE phenotypes in AD cases according to analysis of covariance using age and smoking (pack/year) as covariates and found no significant differences. In addition, AD cases (smoking or nonsmoking) demonstrated a significant correlation between ApoE4/4 phenotypes and telomere shortening (data not shown). The odds ratio of the ApoE4/4

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Table 1. Background Features and ApoE Phenotype in AD Patients and Non-Dementia Elderly Controls

<table>
<thead>
<tr>
<th></th>
<th>AD (n = 74)</th>
<th>Non-Dementia Elderly Controls (n = 35)</th>
<th>( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± standard deviation (y)</td>
<td>78.5 ± 7.1</td>
<td>79.5 ± 6.4</td>
<td>.47</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>24/50</td>
<td>14/21</td>
<td>.52</td>
</tr>
<tr>
<td>Smoking (non/former and current smoker)</td>
<td>55/19</td>
<td>26/9</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>MMSE scores: M ± SD</td>
<td>18.5 ± 5.4</td>
<td>27.1 ± 2.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Education period: M ± SD (y)</td>
<td>11.8 ± 2.6</td>
<td>11.7 ± 2.8</td>
<td>.93</td>
</tr>
<tr>
<td>T/S ratio: M ± SD</td>
<td>0.7536 ± 0.1259</td>
<td>0.7930 ± 0.1289</td>
<td>.13</td>
</tr>
<tr>
<td>Apolipoprotein phenotype</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Non-carriers (%)</td>
<td>35 (47.3)</td>
<td>31 (88.6)</td>
<td></td>
</tr>
<tr>
<td>E2/4 or E3/4 (%)</td>
<td>30 (40.5)</td>
<td>4 (11.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>E4/4 (%)</td>
<td>9 (12.2%)</td>
<td>0</td>
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</tbody>
</table>

Note: AD = Alzheimer’s disease; MMSE scores = Mini-Mental State Examination scores.

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Figure 2. Telomere length represented by T/S ratio in Alzheimer’s disease patients and non-dementia elderly control (aged 60+ years; age-matched control). There was no statistically significant difference in T/S ratio between these two groups.
phenotype for telomere shortening was 10.96 (95% confidence interval, 1.293–92.97), whereas the odds ratio of the phenotypes with hemiallelic and biallelic expression of ApoE4 (ApoE4/2, E4/3, and E4/4 phenotypes) for telomere shortening was 1.099 (95% confidence interval, 0.4225–2.684). Furthermore, there was no significant difference in T/S ratio between noncarriers and those with the E4/3 or E4/2 phenotype (p = .43).

**DISCUSSION**

In the present study, we investigated the involvement of telomere length in AD and determined the correlation of telomere length with clinical characteristics and ApoE phenotypes. We demonstrated that telomere length was significantly shorter in AD patients with the E4/4 phenotype than in those who were noncarriers or in those with the E4/3 or E4/2 phenotype.

Aging is the most important risk factor for the onset of AD, and telomere shortening is a marker of cellular aging (17,18). Several reports suggest that AD is associated with shortened telomeres (3–6,19). Telomere shortening is an independent risk factor in both AD diagnosis and mortality in the elderly participants, and the presence of the E4 allele reciprocally increases the mortality and risk of AD among the elderly participants with shortened telomeres (19). In the present study, the telomere length tended to be shorter in the AD participants than in the age-matched controls, but the difference was not statistically significant. This result suggests that telomere shortening in peripheral leukocytes from whole blood in general is not a diagnostic biomarker for distinguishing AD patients from age-matched controls. However, telomere length was significantly shorter in AD patients with the ApoE4 homozygote than in those with the ApoE4 heterozygote and noncarriers. Telomere shortening might be accelerated depending on the presence of the E4/4 phenotype. Previous studies have reported that the risk of AD increased and mean age at onset of AD decreased with an increasing number of the E4 allele in both sporadic and familial AD (20,21). The E4 allelic burden is linked to the production and aggregation of amyloid β peptide (Aβ) with an increasing number of the E4 allele (22), and Aβ-induced oxidative stress plays an important role in the pathogenesis of AD (23–25). Telomeres are highly sensitive to damage by oxidative stress due to their high guanine content (26,27). Furthermore, telomeric DNA lacks an efficient repair mechanism for single-strand breaks produced by reactive oxygen species, particularly the hydroxyl radical, compared with genomic DNA (28). It is well known that ApoE4 is a poorer antioxidant relative to the ApoE3 isoform (29,30). Although the mechanisms directly or indirectly involved in accelerated telomere shortening in AD patients with the E4/4 phenotype remain unknown, increased oxidative stress and decreased antioxidant levels, especially in AD patients with the E4/4 phenotype, might be associated with an accelerated pace of telomere shortening (31–33). On the other hand, in the current study, only AD patients with the ApoE4/4 phenotype showed significant telomere shortening, whereas AD patients with the ApoE4/2 or ApoE4/3 phenotype did not. This suggests that ApoE2 or ApoE3 could have a suppressive or protective effect against telomere shortening in AD patients.

![Figure 3. Telomere length represented by T/S ratio in Alzheimer’s disease patients who were classified according to apolipoprotein phenotypes. Patients with the ApoE4/4 phenotype had a statistically significantly lower T/S ratio than those who were ApoE noncarriers (p < .001) or those with the ApoE4/3 or ApoE4/2 phenotype (p < .001). In contrast, patients with the ApoE4/3 or E4/2 phenotype did not show a statistically significant difference in T/S ratio compared with those who were noncarriers (p = .43).](https://academic.oup.com/biomedgerontology/article-abstract/67A/4/330/622666)
Panossian and colleagues (6) demonstrated that telomere length in peripheral T cells was significantly shorter in AD participants than in control participants, and there was a significant correlation between telomere length and cognitive severity, as evaluated by Mini-Mental State Examination scores. They suggested that the dysfunction of T cells with shortened telomeres played an important role in the pathogenesis of AD because shortened telomeres were accompanied by a number of phenotypic and gene expression changes that result in altered function. In addition, Franco and colleagues (3) also reported that neuronal telomeres in the hippocampal neurons of AD patients were significantly shorter than neuronal telomeres in those of control participants. They also found that telomerase reverse transcriptase (TERT) immunoreactivity using antiserum against TERT, anti-K-370 (34) in the neurons of CA1 hippocampal regions was decreased significantly in AD patients compared with controls (3). More recently, Silva and colleagues (35) reported that TERT methylation frequency was associated with the aging process and differed in AD patients from controls. These results suggest that telomere shortening is partly associated with dysfunction of telomere–telomerase maintenance mechanisms in AD patients.

As for the limitations of this study, the diagnosis of AD was not pathologically confirmed and the sample size was relatively small because of the rigorous screening of AD patients. Furthermore, the results were obtained from cases in a single hospital in Japan, and thus, the possibility of the results being biased by the setting cannot be ruled out. Therefore, further multicenter and multilateral studies are required to validate the results to other groups in Japan and elsewhere. Although the number of AD patients with the ApoE4/4 phenotype was small, the frequency of this phenotype in this study was consistent with its reported frequency in AD.

Furthermore, ApoE phenotypes as assayed instead of ApoE genotypes as phenotype analysis was more readily possible in our present setting with a more practical technique using simple electrophoresis, which is routinely performed in our department. The ApoE phenotype is defined by the ApoE genotype regardless of whether a participant has AD or not by definition. The term “ApoE genotype” is used to describe the results of the analysis of DNA samples, and the term “ApoE phenotype” is used to describe the results of the analysis of plasma samples. Although the ApoE genotypes and ApoE phenotypes do not completely correspond, the applicability of the phenotypic assay in the present study as a valid indicator of genotype was based on a report by Kataoka and colleagues (1994) stating that the concordance rate between the ApoE phenotypes and the ApoE genotypes was as high as 98%. On the other hand, Q-PCR methods measure only the average telomere length, rather than the shortest telomere length, in DNA obtained from the specimens. It is possible that the shortest telomere length might better correlate with biological aging. However, we chose Q-PCR because this method has technical advantages and can easily assay large numbers of samples from a small amount of DNA samples.

In conclusion, our study demonstrated a possible association between telomere length and the ApoE4/4 phenotype, whereas the presence of the ApoE3 or ApoE2 phenotype may have a protective effect on telomere shortening in AD patients. Telomere length may be associated with the ApoE4/4 phenotype rather than with other patient characteristics, such as age, gender, smoking, disease duration, or cognitive severity in AD. To clarify the causes of accelerated telomere shortening in AD patients with the ApoE4/4 phenotype, it will be necessary to examine in detail the correlation between oxidative stress or antioxidant capacity and telomere length in AD patients in the future.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

Funding

This work was funded by the “Promotion of Science and Technology” project for private universities, with a matching fund subsidy from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), 2009–2014.

Conflict of Interest

The authors declare no competing financial interests regarding the study.

Acknowledgments

The authors are indebted to Mr. Roderick J. Turner, Assistant Professor Edward F. Barroga, and Professor J. Patrick Barron, Chairman of the Department of International Medical Communications of Tokyo Medical University, for their review of this manuscript. The authors also wish to thank Mr. Yoshinobu Kamimura and Ms. Chiaki Kobayashi for their technical assistance.

Authorship Contributions: Y.T. collected DNA samples, performed Q-PCR analysis, and wrote the paper; M.K. participated in the conceptualization of the research design and performed statistical analysis; S.K., S.S., T.U., and H.S. collected patient specimens; H.H. helped in writing the paper; T.I. coordinated the research; K.O. helped interpret the results and statistical analysis; and J.H.O. provided quality control data of telomere analysis and finalized the manuscript.

References