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TECHNICAL NOTE

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Age estimation in dental pulp DNA based on human telomere shortening

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Abstract Age estimation based on evidence found in teeth has received considerable attention within the field of forensic science. We determined the terminal restriction fragment (TRF) length, as telomere length, to estimate age. Using dental pulp DNA we found the average TRF length showed a tendency to shortening with aging. Our findings show that telomere shortening, based on dental pulp DNA is a new and useful approach to estimate age of the subject at the time of death.

Keywords Age estimation · Telomere shortening · Dental pulp DNA · Forensic odontology

Introduction

Telomeres are specialized structures located at the ends of eukaryotic chromosomes and human telomeres are simple repeating sequences of six bases, TTAGGG [10]. Telomeres are usually replicated by telomerase, a telomere-specific reverse transcriptase that maintains the length of chromosomes. Telomere shortening occurs with each cell division, as conventional DNA polymerases cannot replicate the end of linear molecules [19]. Telomere shortening during ageing processes occurs in many cells and tissues, including fibroblasts [5], peripheral blood cells and colonic mucosa [6]. We reported that estimating the age of humans from peripheral blood samples based on telomere shortening is useful when the forensic sample carried no morphological information for estimating age [18].

Since the teeth are the hardest tissues of the human body, numerous methods for age determination from teeth under various conditions have been discussed [2, 3, 4, 7, 8, 11, 14, 16] and dental pulp is a good source of high molecular weight DNA [13, 15, 17]. In the present study we attempted to estimate the age at the time of death from dental pulp DNA based on telomere length.

Materials and methods

Genomic DNA was extracted from the dental pulp of 100 Japanese subjects according to the method described by Smith et al. [15]. All teeth were healthy molars and were extracted in the Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Sciences, Kyushu University. The age range of the subjects was 16–70 years (Table 1). As two molar teeth were obtained from each of four persons, one of the teeth was stored at room temperature (22°C) for 1 year. The research protocol was approved by the Human Subjects Committee at the Faculty of Dental Sciences of Kyushu University. All patients gave written consent to the study.

We determined the terminal restriction fragment (TRF) length as telomere length, using our method but with some modifications [18]. We used the Telo TTAGGG telomere length assay (Roche Diagnostics, Mannheim, Germany). For telomere length comparisons, 1 μg of genomic DNA was digested with *Rsa*I and *Hinf*I restriction enzymes, separated on a 0.8% w/v agarose gel, and transferred to nylon membranes in 20×SSC by Southern blotting. The blot was hybridized with a digoxigenin-labeled (DIG) probe specific for telomeric repeats and incubated with a DIG-specific antibody covalently coupled to alkaline phosphatase. Finally, the immobilized telomere probe was visualized by virtue of alkaline phosphatase metabolizing CDP-Star, a highly sensitive chemilumines-

Table 1 Number of teeth and age distribution of the subjects

Age group	Male	Female
10–20	5	14
21–30	15	33
31–40	7	7
41–50	3	1
51–60	10	1
61–70	4	0
Total	44	56

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cense substrate. The average TRF length was determined by comparing signals relative to a nuclear weight standard on X-ray film.

The telomere length was estimated according to the manufacturers instructions (Telo TTAGGG telomere length assay). Briefly, the signal from each lane was scanned using the Quantity One-package (Version 3.0.2, PDI Inc., New York) and the area divided into a grid of 20 boxes. The density reading from each individual box was then incorporated into the formula Telomere length= $\Sigma(ODi \times Li)/\Sigma(ODi)$, where ODi is the density of the box and Li is the size in bp of a band positioned at the center of the box, relative to the molecular weight markers. The straight lines were fitted by linear regression using the Microsoft Excel statistics programme (Microsoft, USA). The regression constant was accurate to within the 95% confidence limits, unless otherwise stated.

Results and discussion

We assessed the TRF length of dental pulp DNA in 100 humans and determined the peak telomeric length for each (Fig. 1). The average TRF length of dental pulp DNA clearly shortened with aging, the correlation coefficient being r=0.749 (R^2=0.562, Fig. 2). The detected average TRF length of each sample can be included in the following formula to roughly calculate the age of the subject (blind tests): as follows:

age=–0.0119y+168.0±7.52 (y: average TRF length: bp, 7.52: standard error)

The TRF length obtained from 4 teeth stored at room temperature for 1 year was 10–952 bp shorter than that of each tooth determined immediately after the extraction (Table 2). Moreover, we calculated the age of 15 forensic autopsy cases as based on their molar teeth. The calculated age of 12 cases was inside the range of standard error (S.E.), however, that of 3 cases was outside the range. The calculated age of these 3 cases was 10 years or more than the actual age. The cause of death in all cases was drowning and the postmortem periods were 6 and 7 days

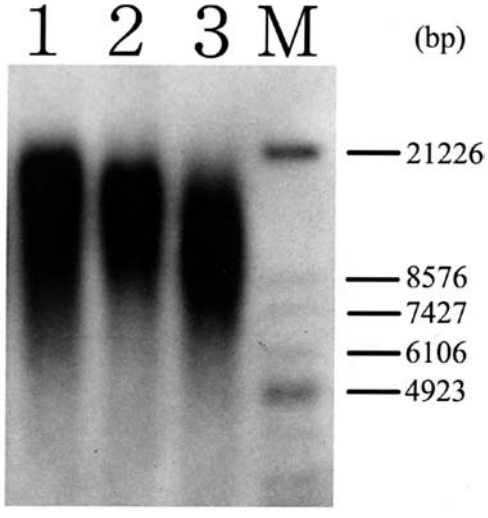

Fig. 1 A representative Southern blot analysis demonstrating length of telomeric repeats in dental pulp DNA. Telomere shortening correlates with increased chronological age: *Lane 1* 18 years old, *lane 2* 24 years old, *lane 3* 52 years old, *M* molecular weight marker

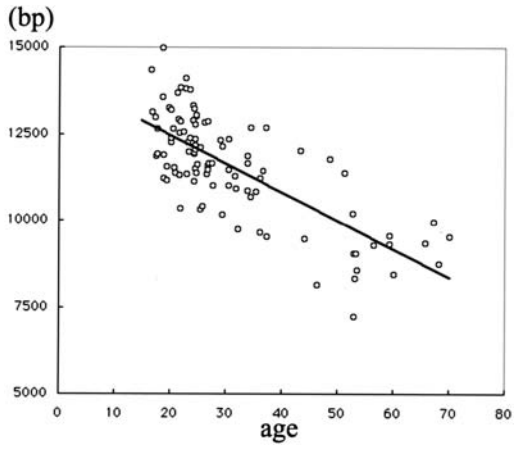

Fig. 2 Telomere lengths in 100 donors (aged 16–70 years old) declined with age. The straight line was obtained by regression analysis, the correlation coefficient being r=0.749 (R^2=0.562)

Table 2 TRF length for teeth stored at a room temperature of 22°C for 1 year, and immediately after the extraction

Case	AOS[a](bp)	IAE[b](bp)	Telomere loss (bp)
1	14090	14100	10
2	12337	12749	412
3	11355	11970	615
4	11910	12862	952

[a]*AOS* After 1 year storage.
[b]*IAE* Immediately after extraction.

Table 3 Calculated age and actual chronological age of forensic autopsied humans

Case	PMP	Calculated age	Actual age	Cause of death
1	0.5	43.5±7.52	48*	CO poisoning
2	0.5	52.0±7.52	57*	Stab wounds
3	1.0	42.6±7.52	45*	Throttling
4	1.0	48.7±7.52	53*	AMP poisoning
5	1.0	65.8±7.52	61*	Head injuries
6	1.5	37.1±7.52	31*	AMP poisoning
7	1.5	44.0±7.52	48*	AMP poisoning
8	1.5	69.7±7.52	65*	Head injuries
9	3.0	35.4±7.52	28*	Throttling
10	4.0	37.4±7.52	31*	Asthma
11	4.0	63.3±7.52	61*	Drowning
12	6.0	35.3±7.52	17**	Drowning
13	7.0	42.3±7.52	49*	Head injuries
14	7.0	76.0±7.52	51**	Drowning
15	7.0	46.3±7.52	35**	Drowning

*: Inside the range of standard error (S.E.), **: Outside the range of S.E.
PMP: Postmortem period (days), AMP: Amphetamine

(Table 3). It is considered that telomere shortening did not occur under usual dry conditions, but occurred due to immersion for at least 6 and 7 days.

In adulthood, the Gustafson's method is the most popular age estimation technique [4]. This method uses six

features of dental microstructure: gingival attachment level, transparency of the root apex, wear of occlusal surfaces, amount of secondary dentine, apposition of cementum and resorption of the root, and that led to many improvements and better results [16]. Lamendin et al. reported a simple method of age estimation which used two criteria: gingival regression and transparency of the root [8]. According to Baccino et al., Lamendin et al's technique was most effective for individuals older than 25 years [2]. On the other hand, Foti et al. stated that Lamendin et al's method could not provide reliable predictions of the age for humans with periodontal disease resulting in alterations in levels of attachment [3]. More sophisticated methods, such as the degree of aspartic acid racemization in both enamel and dentine to estimate the age of teeth, and in the femur for estimating the individual age [11, 12, 14] or measurement of the size of the pulp on dental radiographs [7], made way for a closer age estimation of the chronological age. There are certain indications that measurement of the concentrations of hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) in dentine may be a valuable tool to determine the individual age [9]. However, Acil et al. reported that this method could not be used to determine the individual age [1].

We took a different approach using dental pulp DNA to estimate age. We considered that the TRF length from inside the telomere structure to the end of the chromosome was the telomere length, and that this method is effective to obtain a rough correlation between the average TRF length and the age of the extracted teeth. Moreover, the telomere loss was 10–952 bp after 1 year of storage and the calculated age of forensic autopsy cases (postmortem period was within 1 week and the cause of death was not drowning) was inside the range of standard error. Our results show that estimating telomere shortening from dental pulp DNA is a useful method to estimate age at the time of death.

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